Abstract

Tiffany, Mark Elton. Cobalt requirements of growing and finishing cattle based on performance, vitamin B_{12} status and metabolite concentrations. (Under the direction of Jerry W. Spears.)

Experiments were conducted to determine cobalt requirements for growing and finishing beef cattle, compare the relative bioavailability of different cobalt sources, and evaluate the effects of cobalt on ruminal fermentation. During experiments 1 and 2 steers were fed corn-cottonseed hull-soybean meal-based growing diets, followed by high concentrate finishing diets (diets contained approximately 0.05 mg Co/kg). Dietary treatments for experiment 1 consisted of 0, 0.05, 0.10 and 1.0 mg of supplemental Co/kg DM from $CoCO_3$ or 0.05 and 0.10 mg of supplemental Co/kg DM from Co propionate (CoPr). Treatments were similar for experiment 2 with the exception that the Co supplemented at 1.0 mg/kg was as CoPr instead of $CoCO_3$. Performance was not affected by cobalt source or supplementation during the growing phase of either study. However, cobalt supplementation to the finishing diet increased feed intake, average daily gain, plasma and liver vitamin B_{12}, and plasma glucose, and decreased plasma methylmalonic acid. Supplemental cobalt increased ruminal propionate proportions during the finishing phase, and steers supplemented with CoPr had higher ruminal propionate relative to those supplemented with $CoCO_3$ during the growing phase.

During the third study the effects of supplementing cobalt to corn or barley-based finishing diets were evaluated in steers. Supplemental cobalt increased intake, gain, and vitamin B_{12} and folate status of finishing steers. Steers fed barley gained less, had lower

ruminal, plasma, and liver vitamin B_{12}, lower plasma and liver folate, and lower plasma glucose relative to those fed corn-based diets.

In the final study, in vitro fermentation characteristics of ruminal microbes fed corn-based diets supplemented with cobalt were evaluated. Within three days, cobalt supplementation resulted in a substantial increase in microbial vitamin B_{12} production. In addition, ruminal succinate concentrations of the unsupplemented control cultures increased sharply suggesting that the vitamin B_{12}-dependent enzymatic conversion of succinate to propionate had been affected. Based on performance, vitamin B_{12} status, and metabolite concentrations, 0.15 mg/kg of total dietary cobalt is required for finishing steers.

COBALT REQUIREMENTS OF GROWING AND FINISHING CATTLE BASED ON PERFORMANCE, VITAMIN B_{12} STATUS AND METABOLITE CONCENTRATIONS.

By

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DEDICATION

This dissertation is dedicated to the life and memory of Matthew Lee Tiffany, who fought the good fight, walked by faith, and now rests in the arms of our Lord.

JOHN 3:16

BIOGRAPHY

Mark Elton Tiffany was born in Boynton Beach, Florida on September 19, 1959 to Richard and Diane Tiffany. He attended grade school and high school in South Florida, and after leaving school, took a nearby job in the tool and die trade where he worked for over ten years. While working, he began night classes at Palm Beach Community College. After several years he moved to Hawthorne, Florida to begin full-time studies at Santa Fe Community College in Gainesville, where he received an Associate of Arts degree in the Spring of 1993.

Upon graduation, he began studies at the University of Florida, and in the Spring of 1996 graduated with a Bachelors degree in Animal and Poultry Science. He immediately began pursuit of a Masters degree at the University of Florida in nutrition through the Animal Science department. He received his Master of Science degree in the Summer of 1998. He then moved to Raleigh North Carolina to pursue a Doctorate in Animal Science under the guidance of Dr. Jerry W. Spears. While there he met Melissa Jennifer Croucher, and on April 6, 2002 the couple was married. While attending North Carolina State University he has conducted research in the area of mineral nutrition.

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LITERATURE REVIEW

Introduction

Cobalt (Co) is a silvery-blue, hard ferromagnetic metal, that is stable in air and water, but susceptible to attack by dilute acids (Emsley, 2001). Cobalt's atomic number is 27, atomic weight 58.9932, and its electronic configuration is $[Ar]3d^7 4s^2$. Cobalt has a melting point of 1495°C, a boiling point of 3100°C and its density is 425 (± 17) g cm^{-3} (Greenwood and Earnshaw, 1997). Cobalt comprises 0.001 to 0.002 percent of the earth's crust, and igneous rocks of the earth's surface contain approximately 10 mg Co/kg. Cobalt's concentration in soil and plants is variable, but very low, with plant concentrations rarely above 0.50 mg/kg DM and often below 0.10 mg/kg DM (McDowell, 1992; Smith, 1997). Plant Co concentrations are affected by plant species and maturity, and vary with soil conditions and Co concentration (Underwood, 1981). The total Co content of an adult man is approximately 1.1 mg, with approximately 43 percent of the total in muscle, 14 percent in bones and the rest in other tissues (Underwood, 1977). In normal humans, the Co content of liver, spleen, kidney, and heart is reported to be 0.18, 0.09, 0.23, and 0.10 mg Co/kg DM (Underwood, 1977).

Cobalt, along with iron and nickel, have several distinguishing features that separate them from other elements of the periodic table. First, these elements have a number of oxidation states and electron spin states. In addition, these elements are electron-rich, having more than five 3d electrons in their lower oxidation states. Cobalt

and nickel are truly unique in that their 3d electrons are forced into ó- or ð- orbitals due to the symmetry of their complexes when in lower oxidation states (Fraústo da Silva and Williams, 2001). These unique features enhance the reactivity of Co ions, and allow for Co insertion into molecules that participate in a number of important biological reactions.

Metabolic Aspects of Cobalt

Vitamin B_{12}

Vitamin B_{12} (Figure 1) is the generic name assigned to a complex group of molecules determined to have vitamin B_{12} activity. The empirical formula for vitamin B_{12} is $C_{63}H_{88}O_{14}N_{14}PCo$ and it contains approximately 4.5 % Co. The corrin nucleus of vitamin B_{12} contains four coupled pyrrole nuclei (tetrapyrrole), with the inner nitrogen atom of each pyrrole coordinated with a single Co atom (McDowell, 2000). Vitamin B_{12} contains a nucleotide whose base 5,6-dimethylbenzimidazole, is not found anywhere else in nature. The nucleotide is joined to the corrin ring structure in two ways; (1) an ester linkage between the nucleotide phosphate and the propionic acid moiety in the D ring of the corrin structor; and (2) a coordinate covalent linkage between Co and a nitrogen atom of benzimidazole (Lenhert and Hodgkin, 1961; Beck, 2001).

Among the many isomers of vitamin B_{12}, several are important in mammalian biological systems. Adenosylcobalamin is a vitamin B_{12} isomer in which the adenine base of the upper axial ligand is directly linked to the Co atom (Brown et al., 1998; Beck, 2001). Adenosylcobalamin is an important cofactor for reactions catalyzed by methylmalonyl-CoA mutase. Reactions involving adenosylcobalamin as an enzyme

cofactor catalyse the exchange of groups attached to carbons adjacent to one another. Methylcobalamin is an isomer of vitamin B_{12} in which the ligand attached to Co is a methyl group (Rossi et al., 1985; Beck, 2001). Methylcobalamin is a cofactor for the enzyme methionine synthase to which it is noncovalently bound. During reactions catalyzed by methionine synthase, methylcobalamin is subject to demethylization and subsequent remethylization (Matthews, 1999).

Methylmalonyl-CoA mutase

Methylmalonyl-CoA mutase is an adenosylcobalamin-dependent enzyme, located in the mitochondrial matrix. Structurally, methylmalonyl-CoA mutase is an áâ-heterodimer of 150 kDa (Mancia et al., 1996); functionally, the enzyme catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA in mammals (Figure 2). Conversion of methylmalonyl-CoA to succinyl-CoA is an important step in catabolic pathways of odd-chain fatty acids, cholesterol and some amino acids, in which catabolites can be converted to succinyl-CoA and enter the tricarboxylic acid cycle (Banerjee and Chowdhury, 1999). In bacteria such as *Propionibacterium shermanii*, methylmalonyl CoA mutase is involved in the fermentation of pyruvate to propionate (Evans and Mancia, 1998).

The generic stepwise mechanism of mutases such as methylmalonyl-CoA mutase is described by Ludwig and Matthews (1997) as follows: 1) the cobalt-carbon bond of adenosylcobalamin is cleaved, and hydrogen is transferred from the substrate to form 5´-deoxyadenosine, 2) the substrate radical rearranges to product radical with a 2,1-shift of

the R group, 3) hydrogen is transferred from 5´-deoxyadenosine to the product radical forming product and reforming the adenosylcobalamin cofactor, and 4) product is released and replaced by another molecule of substrate. This reaction is potentially very important in ruminants, for which propionate is the only gluconeogenic short chain fatty acid produced, during ruminal fermentation.

Methionine synthase

Methylcobalamin-dependent methionine synthase is active in the terminal step of *de novo* methionine synthesis (Figure 3). Methionine synthase extracted from *E. coli* is a large polypeptide 1,227 residues long and displays many sequences, properties, and activities of its mammalian counterpart (Chen et al., 1994; Drennan et al., 1998) making it a useful research tool. The work of Frasca et al. (1988) determined that the native enzyme is apparently monomeric with a molecular weight of 153,000 (determined by gel filtration). Results from this study indicate that 1 mol of vitamin B_{12} is bound per mol of protein, based on Co content and absorbance properties.

Methionine synthase participates in two methyl transfers in which enzyme bound methylcobalamin (a vitamin B_{12} isomer) is successively demethylated and remethylated (Beck, 2001), the reactions proceed as follows:

1. CH_3-cobalamin + homocysteine cobalamin + methionine
2. Cobalamin + N^5-methyltetrahydrofolate CH_3-cobalamin + tetrahydrofolate

The reaction catalyzed by methionine synthase is particularly important in mammals for which methionine is an essential amino acid. Methionine generated in this reaction is

later converted to adenosylmethionine, which is a methyl group donor to RNA and DNA bases, amino acid side chains in proteins, and involved in creatinine and epinephrine biosynthetic pathways (Matthews, 1999).

Folate is also an active participant in the reaction catalyzed by methionine synthase creating a vitamin B_{12}-folate interrelationship. Deficiency symptoms ascribed to vitamin B_{12}, such as pernicious anemia are identical to those reported during a folate deficiency (Brody and Shane, 2001). The similarity may be explained by the "methyl trap" hypothesis (Shane and Stokstad, 1985). A failure of methionine synthase would result in accumulation of 5-methyltetrahydrofolate that cannot be metabolized by other mechanisms "trapping" folate in a nonfunctional form. Accumulation of folate in its nonfunctional form would reduce the activity of other folate requiring enzymes. Studies have shown that 5-methyltetrahydrofolate is a poor substrate for the enzyme folylpolyglutamate synthetase for which tetrahydrofolate is a preferred substrate. Decreased activity of this enzyme would hinder the ability of a tissue to synthesize polyglutamates and retain folate (Cichowicz and Shane, 1987; Chen et al., 1996; Brody and Shane, 2001). The result is a vitamin B_{12}-induced folate deficiency due to lack of methionine synthase activity.

Other vitamin B_{12} dependent enzymes

Although mammals need vitamin B_{12} for only two enzymes (methionine synthase and methylmalonyl-CoA mutase), vitamin B_{12} is a cofactor for a number of bacterial enzymes. Adenosylcobalamin-dependent ribonucleotide reductases are involved in DNA

synthesis which depends on ample supply of the four deoxyribonucleotides (Reichard, 1988). Ribonucleotide reductase catalyzes the reduction of ribonucleoside diphosphates or triphosphates during deoxyribonucleotide formation (Fontecave and Mulliez, 1999). Glutamate mutase is a vitamin B_{12}-dependent enzyme that catalyzes the conversion of glutamate to (2*S*, 3*S*)-3-methyleneaspartate. This conversion represents the first step in the fermentation pathway of glutamate, in which ammonia, CO_2, acetate, butyrate and molecular hydrogen are produced (Buckel and Barker, 1974). Adenosylcobalamin-dependent glycerol dehydrase catalyzes conversion of glycerol to 1,3-propanediol during bacterial fermentation of glycerol, in which alcohols and acids are produced (Toraya, 1999).

Research has identified other bacterial-vitamin B_{12}-dependent enzymes in a number of pathways that include: ethanolamine ammonia-lyase, an adenosylcobalamin dependent enzyme active in conversion of ethanolamine and other short-chain, vicinal amino alcohols to ammonia (Bradbeer, 1965a,b); lysine 5,6-aminomutase and ornithine-4,5 aminomutase are active in conversion of lysine to acetate and butyrate, and oxidative deamination of ornithine, respectively (Frey and Chang, 1999); and isobutyryl-CoA mutase, which catalyzes the enzymatic conversion of isobutyryl-CoA to butyryl-CoA, in a reaction similar to the one catalylzed by methylmalonyl-CoA mutase (Zerbe-Burkhardt et al., 1999).

Absorption, Transport and Excretion of Vitamin B_{12}

Vitamin B_{12} is a relatively large molecule that is unable to diffuse across the intestinal wall due to its high molecular weight; therefore, specialized transporters

facilitate the absorption and transport of this cofactor. Two important proteins, haptocorrin and intrinsic factor are the main luminal vitamin B_{12} binders (Alpers and Russell-Jones, 1999). Haptocorrin and intrinsic factor are mainly produced by tissues of the foregut. Haptocorrin is secreted by many cell types, including salivary duct and acinar cells. Intrinsic factor is produced primarily in the chief cells of rats, and parietal cells of mice, monkeys and humans, (Alpers and Russell-Jones, 1999). Intrinsic factor isolated from humans is an alkali-stable protein capable of binding a variety of vitamin B_{12} isomers, and when bound to vitamin B_{12}, the intrinsic factor-vitamin B_{12} complex becomes a compact molecule that is more capable of resisting proteolysis (Beck, 2001). Haptocorrin differs from other cobalamin binders in its ability to bind corrinoids (molecules that lack vitamin B_{12} activity) as well as vitamin B_{12} (Alpers and Russell-Jones, 1999).

Although there are some species-specific differences, luminal vitamin B_{12} transport and absorption generally happens as follows (Alpers and Russell-Jones, 1999). In the stomach, dietary vitamin B_{12} is liberated by the action of HCl and pepsin. Liberated vitamin B_{12} is free to bind haptocorrin and/or intrinsic factor. It is believed that in the stomach, vitamin B_{12} binds preferentially to haptocorrin of salivary origin. This enzyme having an affinity for vitamin B_{12} some 50 times higher than intrinsic factor at low pH, likely serves to protect vitamin B_{12} from acid hydrolysis or degradation by intestinal fauna. Upon leaving the stomach, the haptocorrin-vitamin B_{12} complex is subjected to pancreatic proteases in the duodenum and vitamin B_{12} is released from haptocorrin (Allen et al., 1978a,b) and binds to intrinsic factor.

Intrinsic factor is a glycoprotein of about 60,000 Da that consists of 400 amino acids (Hewett et al., 1991; Rosenblatt and Fenton, 1999). The specificity of intrinsic factor for vitamin B_{12} is much higher than it is for cobinamide or other corrins (Kohlhouse and Allen, 1977). Upon leaving the duodenum, intrinsic factor-bound vitamin B_{12} proceeds to the ileum where it binds a specific receptor on the luminal surface of mucosal cells. Binding to the receptor is not an energy dependent process, but requires divalent cations and a relatively neutral pH. Herbert and Castle (1961) found that calcium was required for rat intrinsic factor absorption, and that magnesium could partially substitute. Their work also determined that intrinsic factor enhanced vitamin B_{12} absorption at a pH higher than 5.9, however, the effect declined steeply at pH below 5.7.

After binding to the receptor, the intrinsic factor-vitamin B_{12} complex is internalized by receptor-mediated endocytosis, which is a slow, energy-requiring process (Hines et al., 1968, and Beck, 2001). Once the intrinsic factor-vitamin B_{12} complex is internalized, intrinsic factor is cleaved by cathepsin L. Vitamin B_{12} is then released into the cell (Gordon et al., 1995), and free vitamin B_{12} is bound by transcobalamin either inside or outside the cell (Alpers and Russell-Jones, 1999). Transcobalamin is an essential plasma protein that delivers vitamin B_{12} to cells of organs and tissues. A number of tissues secrete transcobalamin including liver, ileum, spleen, kidney and heart. Transcobalamin apparently is involved in vitamin B_{12} absorption in the intestine (Hakami et al., 1971; Burman et al., 1979). Infants lacking functional transcobalamin have clinical symptoms associated with vitamin B_{12} deficiency and some fail to absorb vitamin B_{12}

from the diet. Upon leaving intestinal cells, transcobalamin-bound vitamin B_{12} is delivered to tissues where it can participate in reactions that involve methylmalonyl-CoA mutase and methionine synthase (Rothenberg et al., 1999).

Vitamin B_{12} is excreted primarily in feces (Smith and Marston, 1970a; Smith, 1997). Fecal sources of vitamin B_{12} include, unabsorbed vitamin B_{12} originating from food or bile, intestinal and gastric secretions, desquamated cells, and vitamin B_{12} synthesized by microbes in the lower gastrointestinal tract (Institute of Medicine, 1998). Small amounts of Co are also lost in sweat and hair (McDowell, 1992), however it is not known if the Co is of vitamin B_{12} origin.

There is extensive conservation of vitamin B_{12} through enterohepatic circulation (biliary excretion) and renal reabsorption. Studies utilizing non-human primates have confirmed that there is considerable enterohepatic circulation of vitamin B_{12}, and that vitamin B_{12} from bile may be more absorbable than cyanocobalamin, or that the properties of bile may enhance absorption (Green et al., 1981;1982). Results of *in vitro* work indicated that bile enhanced binding of the intrinsic factor-vitamin B_{12} complex onto ileal receptors, from receptor extracts prepared utilizing the small intestine of guinea pigs (Kanazawa et al., 1985). The majority of vitamin B_{12} secreted into bile (65 to 75 %) is reabsorbed in the ileum (McDowell, 2000).

Recent evidence suggests that the kidney plays a role in vitamin B_{12} homeostasis. Transcobalamin-bound vitamin B_{12} is filtered in kidney glomeruli (Birn et al., 2002). Moestrup et al. (1996) found that the endocytic receptor, megalin, was able to bind transcobalamin-bound vitamin B_{12} in the rabbit kidney. This receptor is located on the

apical membrane (facing the filtrate) of cells in the proximal tubule. More recently, megalin was found to be essential for renal reabsorption of transcobalamin-bound vitamin B_{12}, and that urinary vitamin B_{12} losses increased in megalin deficient rats (Birn et al., 2002). That study also determined that renal lysosomal B_{12} accumulation was dependent on vitamin B_{12} status suggesting that the kidney has a reserve function. These data suggest that vitamin B_{12} is most likely to be excreted in urine when circulating vitamin B_{12} exceeds binding capacity or if metabolic (inborn) errors were to reduce renal reabsorption of vitamin B_{12}.

Cobalt and Vitamin B_{12} in Human Nutrition

Humans require Co in its biologically active form, vitamin B_{12}. Therefore, this section will discuss the requirement, available sources, absorption, and metabolic disorders associated with vitamin B_{12}.

Requirements

The Institute of Medicine (1998) used scientific data to propose Dietary Reference Intakes for some biologically essential nutrients such as vitamin B_{12}. Dietary Reference Intakes include recommended dietary allowance (RDA), adequate intake, tolerable upper intake level, and estimated average requirement for a particular nutrient. The RDA of vitamin B_{12} for adults is 2.4 µg/d (Institute of Medicine, 1998). This RDA is based on the amount needed to maintain normal hematological status and serum concentrations of vitamin B_{12}. Growth, hypermetabolic states, and pregnancy may

increase requirements (Beck, 2001). Other factors that may affect requirements include aging, atrophic gastritis, food-bound vitamin B_{12} malabsorption, smoking, gender, nutrient-nutrient interactions, and genetic defects (Institute of Medicine, 1998).

Limited data is available on how vitamin B_{12} absorption varies with vitamin B_{12} status. When fractional absorption of radiolabeled vitamin B_{12} was studied, approximately 50% was retained at a 1 µg dose, 20 % at a 5µg dose, and 5 % an a 25µg dose (Adams et al., 1971). The assumption made by the Institute of Medicine (1998) in establishing the RDA for vitamin B_{12} is that 50 % of dietary vitamin B_{12} is absorbed by healthy adults. However, studies have shown that the percentage of vitamin B_{12} absorption varies greatly (11 to 65 %) depending on food source (Heyssel et al., 1966; Doscherholmen et al., 1975, 1978, 1981). The average daily diet in western countries contains 5 to 30 µg of vitamin B_{12}, which appears to meet requirements (Beck, 2001).

Sources

The only known origin of vitamin B_{12} in nature is microbial synthesis. Although the elaborate laboratory synthesis of vitamin B_{12} has been accomplished (Woodward, 1979; Eschenmoser, 1979), to date it is not economically feasible to produce a laboratory-created vitamin B_{12} dietary supplement that does not involve a microbial component. Commercially, treatment of bacterial vitamin B_{12} with thiocycyanate, produces cyanocobalamin, the most common form of vitamin B_{12} found in human supplements (Martens et al., 2002). Plants are practically devoid of vitamin B_{12}, and the small amount found in plants is likely absorbed from soil that that has been exposed to

vitamin B_{12} excreta of microbial origin (McDowell, 2000). Meat, liver, milk, and eggs from food animals, and seafood are generally the best sources for vitamin B_{12} in human diets (NRC, 1982; Beck 2001).

Causes, and Clinical Manifestations of Human Vitamin B_{12} Deficiency

Pernicious anemia, first described in 1836 by Thomas Addison, is the classic disease associated with vitamin B_{12} deficiency. The main cause of this disease is autoimmune atrophic gastritis, in which parietal cells break down, causing the loss of intrinsic factor in gastric secretions (Rosenblatt and Fenton, 1999). In addition, parietal cell blocking autoantibodies can bind to intrinsic factor inhibiting vitamin B_{12} binding. The net result is a vitamin B_{12} deficiency. Atrophic gastritis, which is blamed for the onset of pernicious anemia, is more common in the elderly, in whom its occurrence ranges from 10 to 30 % depending on where the study was conducted (Krasinski, et al., 1986; Johnsen et al., 1991; Hurwitz et al., 1997).

Pernicious anemia was given its name because megaloblastic anemia was among the first clinical manifestations attributed to vitamin B_{12} malabsorption. The megaloblastic anemia observed in both folate and vitamin B_{12} deficiencies are not distinguishable from one another. Treatment with folic acid can totally correct it in some, but not all cases (Stabler, 1999), hence the "methyl trap" hypothesis discussed previously in this review. Exact determination of what causes megaloblastic anemia has proven to be illusive. Not all subjects with other clinical signs of vitamin B_{12} deficiency have megaloblastic anemia. This abnormality may result from impaired DNA synthesis during

a vitamin B_{12} deficiency (Beck, 1968). Peripheral blood abnormalities include macrocytosis, anemia, hypersegmented neutrophils, and decreased white blood cells and platelets. Bone marrow abnormalities include hypercellularity, large nuclei with open chromatin pattern in erythrocytic precursors, and intramedullary cell death (Stabler, 1999).

Neurological Abnormalities

In humans, a vitamin B_{12} deficiency can have serious neurological consequences in the form of demyelinating disorders of the nervous system. Signs and symptoms include: paresthesias (abnormal sensations) in hands and feet; disorders of proprioception, and touch sensations; ataxia and abnormal gait; visual disorders and; emotional instability, depression and memory loss (Healton et al., 1991; Metz, 1992).

The main pathological lesion associated with neurological dysfunction is demyelination of nervous system tissue observed in the posterolateral collums of the spinal cord, and on occasion demyelination of cerebral white matter (Metz, 1993). Among the earliest of pathological changes observed are small vacuolated areas in myelin with focal swelling of individual nerve fibers. Eventually, individual lesions join, to form large foci involving more fiber systems. In the later stages of severe, long-term deficiency, there is posterior column, corticospinal tract, spinothalamic tract, and peripheral nerve disease (Beck, 2001). While demyelinization of nerve tissues is believed to be the cause of neurological signs during a vitamin B_{12} deficiency, the

biochemical mechanism is not clear. Impaired DNA or fatty acid synthesis may lead to the observed lesions.

Impaired Lipid Metabolism, and other Biochemical Manifestations

Lipid abnormalities have been observed in animals but there are few observations of abnormalities in humans. Children with inborn errors of propionyl and methylmalonyl-CoA metabolism have been found to have increased amounts of odd-chain fatty acids in plasma (Stabler, 1999).

Vitamin B_{12} deficiency is associated with increased methylmalonic acid in serum and urine of humans (Institute of Medicine, 1998). This is an important metabolic indicator that is highly specific to vitamin B_{12} deficiency. Elevated serum MMA concentrations have been observed in many patients with neurological disorders attributed to vitamin B_{12} deficiency (Lindenbaum et al., 1988). Other studies of normal and vitamin B_{12} deficient patients (Stabler et al., 1988; Lindenbaum et al., 1990) found that approximately 95 percent of vitamin B_{12} deficient patients had elevated MMA and homocysteine concentrations in serum. In those studies 77% of patients with serum vitamin B_{12} concentrations below 200 pg/ml, had MMA and homocysteine concentrations more than 3 standard deviations above the normal mean (53 nmol/L for MMA and 4.1 µmol/L for homocysteine; in Beck, 2001).

Inborn errors of vitamin B_{12} metabolism often result in vitamin B_{12} deficiency in humans. Lack of methylmalonyl-CoA mutase, or deficiency of cytosolic reductase (reduces cobamide Co) results in methylmalonic aciduria (high MMA concentrations),

while the latter responds to vitamin B_{12} supplementation, the former does not (Chalmers et al., 1991; Raff et al., 1991). Other inborn errors cause both methylmalonic aciduria and homocysteinuria. They include conditions that decrease methionine synthase activity, and affect the normal lysosomal release of vitamin B_{12} into cells following endocytosis (Shih et al., 1989; Surtees et al.,1991).

Cobalt and Vitamin B_{12} in Ruminant Nutrition

Ruminants require Co, not preformed vitamin B_{12}, in their diets. Therefore, this section will discuss dietary Co as it relates to ruminal biosynthesis of vitamin B_{12} and the metabolic aspects of vitamin B_{12} as they relate to Co supplementation and deficiency.

Ruminal vitamin B_{12} biosynthesis

As stated above, biosynthesis of vitamin B_{12} is limited to microorganisms. Roth and others (1993) determined that synthesis of vitamin B_{12} by *S. typhimurium* required more than 30 genes, or approximately one percent of the typical bacterial genome. Relatively few studies have investigated *in vivo* vitamin B_{12} production. It was initially thought that rumen microbes utilized ingested Co very inefficiently, because a number of physiologically inactive, vitamin B_{12} analogues (corrinoids) were produced in the rumen along with vitamin B_{12} (Gawthorne, 1970). However, recent evidence suggests that a number of corrinoid proteins are utilized by acetogenic bacteria (Ragsdale, 1999).

In sheep on full feed supplemented with 1.0 mg Co/d, Smith and Marston (1970a) estimated that efficiency of Co conversion to vitamin B_{12} ranged from 1.7 to 3.1 %. In

that study, efficiency of vitamin B_{12} production in Co-depleted sheep ranged from 5 to 18%. Diet composition may also affect ruminal vitamin B_{12} production. Sutton and Elliot (1972) conducted an experiment to evaluate vitamin B_{12} production as a function of intake and forage:concentrate ratio in Co supplemented (0.5 mg/kg) sheep. They determined that vitamin B_{12} production responded linearly to increasing intake, and that ruminal vitamin B_{12} production was lower in sheep fed a 60% concentrate diet, compared to those fed a 0 or 30% concentrate diet.

Cobalt Sources, and Bioavailability

For the most part, plants and grasses are poor sources of Co. This is a particular problem for grazing ruminants. In the United States, plants (legumes) and soils are most severely deficient (< 0.07 mg Co/kg) in the lower Atlantic Coastal Plain and in most of the New England states (Kubota, 1968). In grazing systems, supplementation with Co oxide "pellets" or "bullets" administered orally is an option (Ammerman, 1969). Bullets should have a specific gravity between 4.5 and 5.0 and when correctly administered, they lodge in the rumen or reticulum and last from months to years (Underwood, 1981). In one study, ewes supplemented with Co pellets had higher vitamin B_{12} concentrations in milk and colostrum, and their lambs had higher liver vitamin B_{12} at birth (O'Halloran and Skerman, 1961). Other options for grazing animals include, free choice trace mineral salts (inorganic Co) in feeders located in pastures or Co-containing salt licks. Cattle fed in confinement, particularly those on high energy (grain) diets, are at risk of Co deficiency. Corn and particularly barley can be low in Co, depending on where they are

grown. Various studies have shown that adding Co salts to Co deficient diets improves performance and vitamin B_{12} status of cattle fed in confinement (Stangl et al.,1999, 2000; Schwarz et al., 2000).

In order for inorganic Co sources to be of nutritional value for ruminants, they must be soluble in the rumen. Few studies have investigated the relative bioavailability of different Co sources when fed at physiological concentrations. Recent advances in vitamin B_{12} and MMA determination provides for bioavailability studies based on tissue and plasma vitamin B_{12} and MMA concentrations. When fed in equal amounts per unit of Co, inorganic salts of Co ($CoCO_3$ and $CoSO_4$) had a higher nutritive value than Co oxide, based on liver uptake (Ammerman et al., 1982). Investigations of Kawashima et al. (1997) determined that relative bioavailability for Co sulfate, carbonate, glucoheptonate, and oxide were 100, 91, 84, and 0, respectively, based on in vitro vitamin B_{12} production. However, in that study, Co additions to the cultures were much higher (1 or 40 mg Co/kg) than physiological requirements. In sheep fed high concentrations of Co (20, 40 or 60 mg/kg), Co glucoheptanate was 86% as available as $CoSO_4$, based on Co accumulation in liver and kidney (Henry, 1995).

Requirements

Unlike humans and most monogastric species, ruminants require Co, not preformed vitamin B_{12}, which would not withstand the hostile environment of the rumen. Current Co requirements for ruminants, expressed as mg/kg of diet are: 0.10 for beef cattle (NRC, 1996); 0.11 for dairy cattle (NRC, 2000); 0.10 to 0.20 for sheep (NRC,

1985); and 0.10 for dairy, angora, and meat goats (NRC, 1981). Recently, the authors of several studies have suggested that the current NRC (1996) requirement of 0.10 mg Co/kg for beef cattle is low (Schwarz et al., 2000; Stangl et al., 2000). Based on performance, plasma and liver vitamin B_{12} and folate, and plasma homocysteine and MMA, authors of these studies suggest that the requirement for growing beef cattle is 0.20 mg Co/kg of diet.

Toxicity

Cobalt has a low toxicity in ruminants. Growing dairy calves were able to consume up to 50 mg Co/kg of body weight daily, without any discernable harmful effects (Keener et al., 1949). Becker and Smith (1951) concluded that yearling sheep could tolerate 160 mg Co/kg of body weight for at least eight weeks, without harmful side effects, but doses of 200 or 600 mg Co/100 lb of body weight caused depressed appetite, and weight loss. General signs of excessive Co intake include excessive urination, defecation and salivation; shortness of breath; and increased hemoglobin, red blood cell count, and packed cell volume (NRC, 1984).

Assessment of Status

Evaluation of plasma and tissue vitamin B_{12}, and plasma MMA are very useful when determining Co status of ruminants suspected of being deficient. For a long time vitamin B_{12} concentrations could only be estimated by microbiological methods, many of those utilized *Lactobacillus leichmannii* (ATCC 7830) which has proven to be the most

reliable (Thompson et al., 1950; Rosenthal and Sarrett, 1952). Since then various methods for vitamin B_{12} assay, utilizing radioisotopes have emerged. Radioassay techniques generally assess vitamin B_{12} in plasma and tissues based on the following (Beck, 2001): extraction of vitamin B_{12} from protein binders; addition of labeled ^{57}Co-vitamin B_{12} to the extract; exposure of the extract to a vitamin B_{12} binding agent; centrifugation and radioassay of the pellet. Modern radioassay methods employ use of intrinsic factor as the binding agent, ensuring binding of vitamin B_{12} and excluding binding of vitamin B_{12} analogues.

Due to the number of different assays that have been used in the past, "normal" plasma vitamin B_{12} concentrations for healthy, Co adequate ruminants have not been determined. Underwood and Suttle (1999) suggest that the marginal status for plasma vitamin B_{12} in suckling calves is 30 to 60 pmol/L, and for weaned cattle is 40 to 80 pmol/L, while the estimation for other ruminants (weaned) is 336 to 500 pmol/L.

Plasma and urinary MMA, and plasma homocysteine are useful indicators of vitamin B_{12} deficiency and are inversely related to vitamin B_{12} status (Paterson and MacPherson, 1990; Stangl et al., 2000). Methylmalonic acid and homocysteine concentrations can be determined by gas or liquid chromatography respectively (McMurray et al., 1986; Cornwell et al., 1993); however, these methods are generally not suitable for commercial diagnostic laboratories. Normal concentrations for MMA in plasma have not been established, but they should be very low (< 2.0 µmol/L), and may be more useful when used together with vitamin B_{12} concentrations.

Clinical Manifestations of Cobalt-Vitamin B_{12} Deficiency in the Ruminant

Performance

The major physiological effect of a Co-induced vitamin B_{12} deficiency is a progressive loss of appetite (Smith, 1997), which results in decreased gains, and in severe cases, weight loss, emaciation, and death (Underwood and Suttle, 1999). Numerous studies have documented decreased intakes and/or gains in sheep fed Co deficient hay or barley diets, relative to those receiving Co supplementation (Keener and Percival, 1950; O'Harte et al., 1989; Kennedy et al., 1992; Kennedy et al., 1994a).

The decline in performance attributed to low Co diets is not unique to sheep. Schwarz et al. (2000) fed growing bovine (Simmental) bulls corn silage-based diets with graduated Co supplementation. That study determined that animals consuming diets containing 0.09 to 0.69 mg Co/kg had higher intakes than those fed the basal diet (basal diet contained 0.07 mg Co/kg). It was also determined that increasing dietary Co from 0.07 mg/kg to concentrations between 0.11 and 0.69 increased daily gains. Although there are few controlled studies that involve cattle, several others have reported decreased performance of cattle consuming diets moderately deficient in cobalt (< 0.08 mg/kg), relative to those well supplemented (Kirchgessner et al., 1997; Stangl et al., 1999). The critical role of appetite during vitamin B_{12} deficiency is punctuated by the rapid reversal that occurs when vitamin B_{12} is administered (Marston et al., 1961; Smith and Marston, 1970b; Marston et al., 1972).

The decline in intake observed when ruminants are fed Co-deficient diets can be linked to a failure in propionate metabolism. The inhibitory effect of propionate on appetite of sheep was demonstrated by Farningham and Whyte (1993). Marston et al. (1972) found an inverse relationship between propionate clearance and appetite in Co-deficient sheep. A later study by Kennedy et al. (1991) found an increase in propionate half-life, when administered via jugular infusion (3.0 mmol/kg live wt) to Co-deficient sheep, relative to those supplemented (1000 and 4.2 µg Co/kg for deficient and sufficient diets, respectively).

Vitamin B_{12} Metabolism

Kercher and Smith (1956) were among the first to report lower vitamin B_{12} concentrations in blood obtained from sheep fed-Co deficient diets relative to those with Co supplementation. Since then other studies utilizing sheep (Somers and Gawthorne, 1969; Smith and Marston, 1970a) and cattle (Stangl et al., 1999) have confirmed their findings in both blood and liver. Plasma vitamin B_{12} concentrations in Co-depleted steers respond well to Co supplementation, but the magnitude of the response appears to vary with Co source and/or concentration (Paterson and MacPherson, 1990). Administration of 0.1 mg Co/d to Co-depleted sheep produced an immediate increase in serum vitamin B_{12} concentrations, which plateued in 3 to 4 weeks (Marston, 1970). In that study, liver vitamin B_{12} concentrations increased in response to Co supplementation, however, there was a negative linear correlation between the increase in liver vitamin B_{12} and the

concentration of liver vitamin B_{12} prior to repletion. This indicates that it is the capacity for storage that limits vitamin B_{12} concentrations in liver.

Energy and Lipid Metabolism

Decreased activity of adenosylcobalamin-dependent methylmalonyl-CoA mutase activity leads to dysfunction of propionyl-CoA and lipid metabolism, in Co-deficient ruminants. Various studies have shown a decrease in methylmalonyl-CoA mutase activity in the tissues of sheep (Kennedy et al., 1990; Kennedy et al., 1994b) and cattle (Kennedy et al., 1995) fed Co-deficient diets. The result of decreased methylmalonyl-CoA mutase activity is a breakdown in propionate metabolism where methylmalonyl-CoA is converted to succinyl-CoA. As a consequence, there is accumulation of MMA in blood and urine of sheep and cattle (Quirk and Norton, 1987; O'Harte et al., 1989; Stangl et al., 1999).

Accumulation of MMA in Co-deficient sheep causes other metabolic abnormalities. Kennedy et al. (1994a) reported accumulation of odd-numbered straight and branched-chain fatty acids in kidney, liver and brain tissues of Co-deficient sheep, corresponding to increased plasma concentrations of MMA. While that work suggested that methylmalonyl-CoA was misincorporated into fatty acids in place of malonyl-CoA, there were no neurological or histopathological signs attributed to such misincorporation.

In addition to increased MMA, Co-deficient ruminants fail to convert homocysteine to methionine causing increased plasma concentrations of homocysteine (Kennedy et al., 1992; Stangl et al., 2000). Kennedy et al. (1992) found that decreased

phospholipid methylation, and increased plasma homocysteine concentrations corresponded to a decrease in methylcobalamin-dependent methionine synthase activity in liver, kidney, and spinal cord of Co-deficient sheep. In addition, that study confirmed earlier findings of Gawthorne and Smith (1974) that S-adenosyl methionine concentrations in liver of Co-deficient sheep were much lower relative to those supplemented with Co. These findings are important because methylcobalamin supplies methyl groups that are utilized in a wide range of molecules including formate, noradrenaline, myelin, and phosphatidyl ethanolamine (Underwood and Suttle, 1999).

Defects in lipid metabolism that involve both of the vitamin B_{12}-dependent pathways, are used to explain the accumulation of fat (ovine white liver disease) in sheep (Richards and Harrison, 1981; McLoughlin et al., 1984; Ulvund, 1990), but not cattle liver. Methylmalonyl-CoA concentrations increase when methylmalonyl-CoA mutase activity is low due to vitamin B_{12} deficiency. The accumulating methylmalonyl-CoA inhibits oxidation of free fatty acids (FFA), which lambs mobilize from fat depot, to offset their declining intake, and the FFA accumulate in the liver. Under normal conditions the liver responds to increasing FFA by synthesis of triglycerides, which are packaged in very low density lipoproteins (VLDL) for export. Unfortunately, VLDL synthesis requires vitamin B_{12} (methylcobalamin) and methionine synthase activity. The result in the case of severe deficiency is massive accumulation of peroxidizable, unsaturated fats (summarized from Underwood and Suttle, 1999; Kennedy et al., 1994b).

Immune Function

Among the morphological abnormalities found in red blood cells of vitamin B_{12} deficient humans (but so far not ruminants) are hypersegmented neutrophils (Herbert, 1987). In Co-deficient cattle, neutrophil function is impaired, and upon infection with the internal parasite *Ostertaglia ostertagi*, Co deficient calves have a shorter prepatent period, higher worm egg output, and higher plasma pepsinogen than those receiving Co supplementation (Macpherson et al., 1987). Other work from the same laboratory noted a significant interaction between Co deficiency and *Ostertaglia circumcinta* infection in sheep (Ferguson et al., 1988.)

Conclusion and Justification for Research

In ruminants, Co is a dietary essential. When dietary Co is deficient, bacterial vitamin B_{12} synthesis is impeded, altering normal enzyme-catalyzed substrate utilization and product formation. In addition, reduced microbial vitamin B_{12} synthesis limits the concentration of vitamin B_{12} available for absorption and post absorptive metabolism in the host animal, resulting in a functional vitamin B_{12} deficiency. The result is impaired function of two important enzymes, methylmalonyl-CoA mutase and methionine synthase which function in pathways that involve carbohydrate, protein and lipid metabolism.

At the present time, Co requirements for growing and finishing cattle, consuming high energy (concentrate) diets are poorly defined. The current NRC (1996) recommendation of 0.10 mg Co/kg DM for beef cattle is based on older data and grazing

studies. In addition, few studies have been conducted to evaluate the bioavailability of different Co sources, and studies that have been conducted utilized dietary Co at high concentrations, well above what is considered physiological normals.

Given these facts, it is relevant to reevaluate Co requirements of beef cattle, particularly those fed high energy diets, as the metabolic demand for Co-vitamin B_{12} may be higher in rapidly growing animals. In addition, it is beneficial to evaluate the bioavailability of new Co sources fed at normal physiological concentrations.

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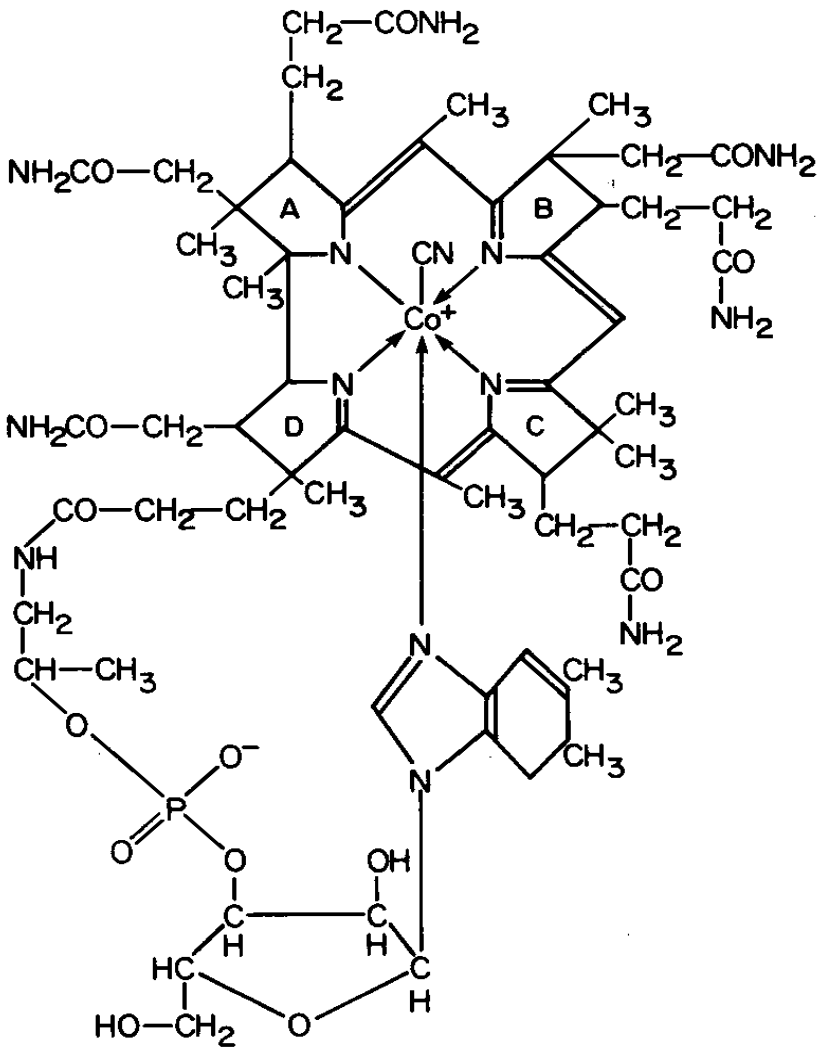

Figure 1. Vitamin B_{12} (cyanocobalamin)

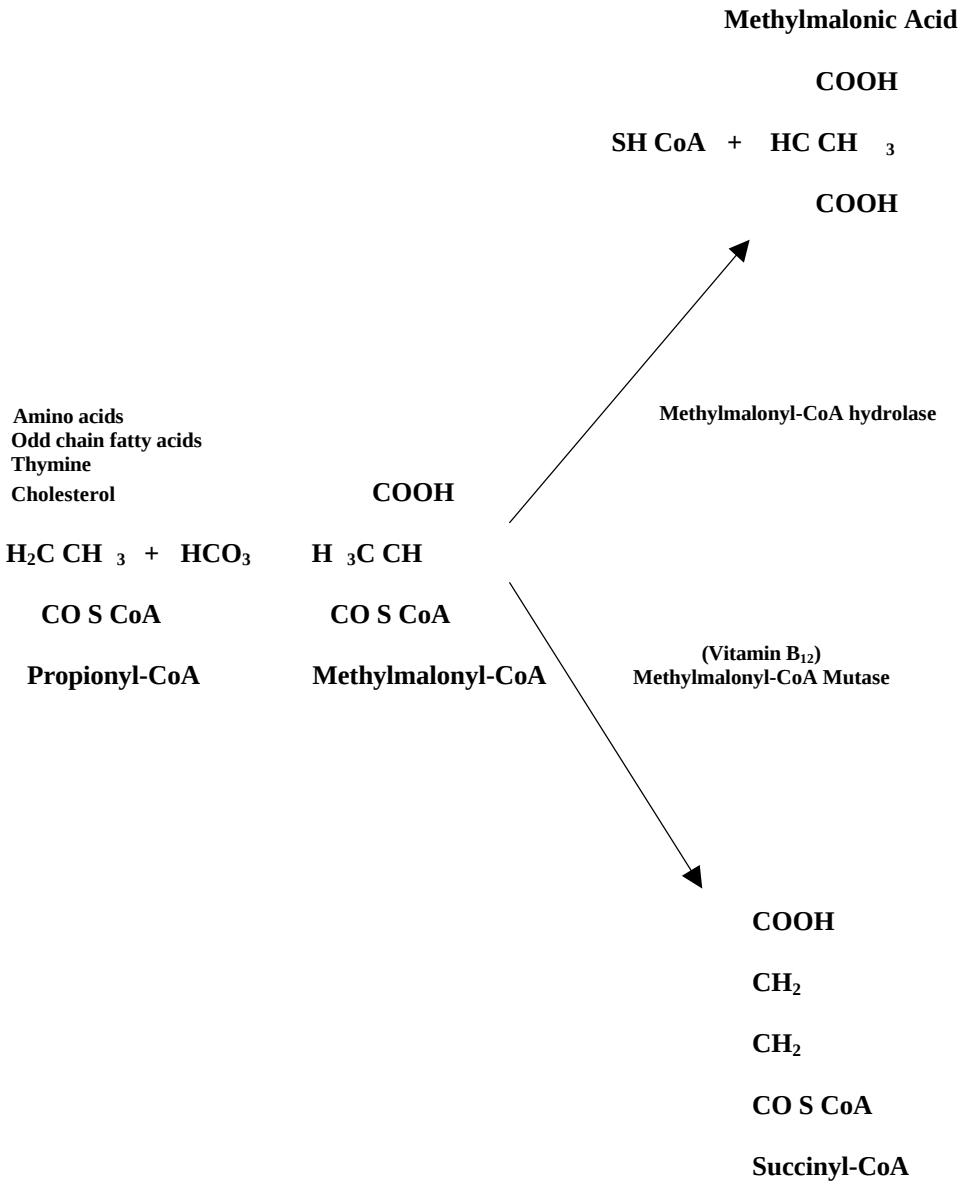

Figure 2. The pathways involving methylmalonyl-CoA

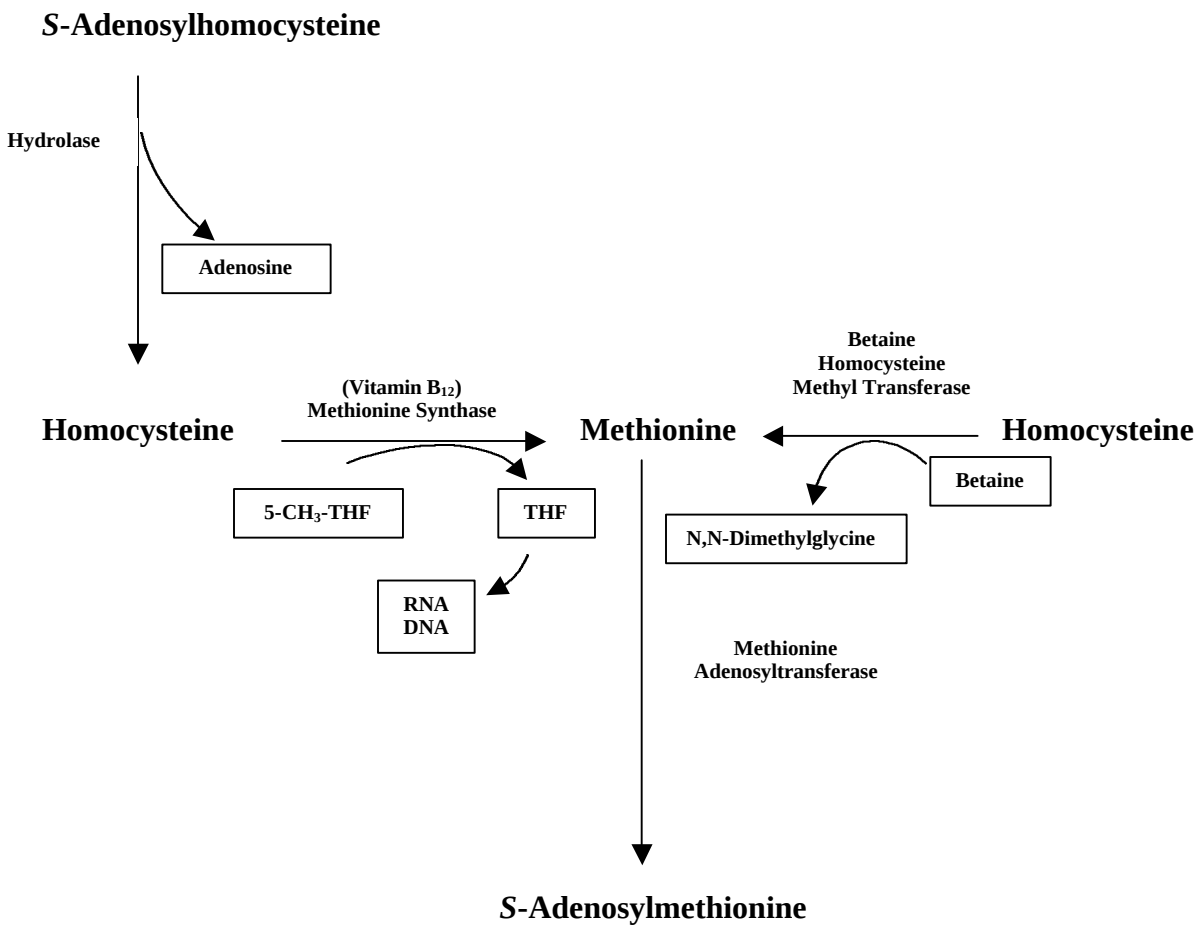

Figure 3. Pathways involving methionine, vitamin B_{12} and folate.

CHAPTER 2

Influence of dietary cobalt source and concentration on performance, vitamin B_{12} status, and ruminal and plasma metabolites in growing and finishing steers[1,2]

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ABSTRACT

Sixty Angus steers, averaging 274 kg initially, were used to evaluate the effects of cobalt (Co) source and concentration on performance, vitamin B_{12} status and metabolic characteristics of growing and finishing steers. Treatments consisted of 0 (control, analyzed 0.04 mg Co/kg), 0.05, 0.10 and 1.0 mg of supplemental Co/kg DM from $CoCO_3$ or 0.05 and 0.10 mg of supplemental Co/kg DM from Co propionate (CoPr). Steers were individually fed a cottonseed hull-corn-soybean meal-based growing diet for 56 d followed by a high concentrate finishing diet. Performance was not affected by Co supplementation during the growing phase. During the finishing phase ADFI and ADG were higher ($P < 0.05$) for the entire finishing phase, and gain:feed was higher ($P < 0.10$) over the first 56 d for Co-supplemented steers. Steers supplemented with 0.10 mg Co/kg as CoPr had higher ($P < 0.05$) ruminal propionate and lower ($P < 0.05$) acetate molar percentages than steers receiving 0.10 Co/kg as $CoCO_3$ during the growing phase. Supplemental Co increased ($P < 0.10$) the molar percentage of propionate during the finishing phase. Plasma vitamin B_{12} was higher ($P < 0.05$) in Co-supplemented steers by d 56 of the growing phase and remained higher ($P < 0.10$) throughout the entire finishing phase. Control steers had higher ($P < 0.05$) plasma methylmalonic acid on d 56 of the growing phase and on d 28, 56 and 112 of the finishing phase than steers receiving supplemental Co. Steers supplemented with Co had higher plasma glucose at d 56 ($P < 0.01$), 84 ($P < 0.10$) and 112 ($P < 0.01$) of the finishing phase. Steers supplemented with 0.10 mg Co/kg as CoPr had higher plasma glucose than those receiving 0.10 mg Co/kg as $CoCO_3$ at d 28 of the growing phase ($P < 0.05$) and d 28 of the finishing phase ($P <$

0.10). Final body, and hot carcass weights were lower ($P < 0.10$) in steers receiving the control diet while other carcass characteristics were not greatly affected by dietary treatment. Gain and feed efficiency for the entire finishing phase did not differ among Co-supplemented steers. However, increasing supplemental Co above 0.05 mg/kg DM (total diet Co, 0.09 mg/kg) resulted in increased (linear, $P < 0.01$) plasma and liver vitamin B_{12} concentrations and reduced (quadratic, $P < 0.10$) plasma MMA concentrations toward the end of the finishing phase. These results suggest that finishing steers require approximately 0.15 mg Co/kg DM. Vitamin B_{12} status was not affected by Co source; however, the two Co sources appeared to affect certain metabolites differently.

Key Words: Cattle, Cobalt, Vitamin B_{12}

Introduction

Dietary cobalt (Co) is required by rumen microorganisms for its addition to the corrin ring structure during the complex formation of vitamin B_{12}. Ruminant diets deficient in Co result in decreased microbial vitamin B_{12} biosynthesis in the rumen, limiting the amount of vitamin B_{12} available to microbes and the host animal. In higher animals, vitamin B_{12} is a cofactor for two enzymes, methylmalonyl-CoA mutase and methionine synthase. The former catalyzes the interconversion of methylmalonyl-CoA to succinyl-CoA (Banerjee and Chowdhury, 1999), an important step in glucogenesis, while the latter acts to remethylate homocysteine, in the terminal step of methionine synthesis (Matthews, 1999). In ruminants, a Co induced vitamin B_{12} deficiency results in reduced intake and ADG, decreased plasma and liver vitamin B_{12}, elevated plasma methylmalonic

acid (MMA) and homocysteine (Stangl et al., 1999, 2000), and decreased methionine synthase and methylmalonyl-CoA mutase activities in tissues (Kennedy et al., 1990).

Cobalt requirements of growing and finishing cattle are poorly defined, and the current NRC (1996) recommendation of 0.10 mg Co/kg DM is based on older data obtained primarily from grazing studies (Smith, 1987). Recent studies (Schwarz et al., 2000; Stangl et al., 2000) suggest that growing German Simmental bulls, fed a corn silage-based diet, require approximately 0.20 mg Co/kg DM. Limited research has evaluated the bioavailability of various supplemental Co sources (Kawashima et al. (1997a,b), and no research has compared bioavailability of different Co sources when supplemented to ruminant diets at physiological concentrations. The present study was conducted to estimate Co requirements of growing and finishing steers and to compare the relative bioavailability of Co from $CoCO_3$ and Co propionate.

Materials and Methods

General

Sixty Angus steers (274.2 kg initial BW) were utilized in this experiment. Care, handling, and sampling of the animals herein were approved by the North Carolina State University Animal Care and Use Committee. Steers were obtained from the Upper Piedmont Research Station at Reidsville, NC, or were purchased at feeder calf sales in North Carolina. After arrival, steers were ear-tagged, weighed, vaccinated with Bovashield® 4 (Pfizer Animal Health, Exton, PA) and Vision™ 7 (Bayer Corp., Shawnee Mission, KS), treated with Safe Guard® (Hoechst Roussel Vet., Clinton, NJ), and confined to fescue pasture for 53 d where they were supplemented with corn silage (2.0

kg DM/hd/d). Steers were then weighed on two consecutive days and allotted by weight and origin to one of five 12-head pens equipped with individual Calan gate feeders (American Calan, Northwood, NH). Steers were housed in covered, slotted-floor pens (5 m × 10 m) for the duration of the experiment.

Growing phase

After adjusting to the Calan gate feeding system, steers were weighed on two consecutive days, implanted with Synovex®-S (Fort Dodge Animal Health, Fort Dodge IA), bled via jugular venipuncture, liver biopsied, and randomly assigned to one of six treatments. Treatments consisted of: 1) control (no supplemental Co), 2) 0.05 mg of supplemental Co/kg DM from Co carbonate ($CoCO_3$), 3) 0.10 mg of supplemental Co/kg DM from $CoCO_3$, 4) 1.00 mg of supplemental Co/kg DM from $CoCO_3$), 5) 0.05 mg of supplemental Co/kg DM from Co propionate (CoPr; Kemin Americas, Des Moines, IA), and 6) 0.10 mg of supplemental Co/kg DM from CoPr.

During the 56 d growing phase, steers were fed a corn-cottonseed hull-soybean meal based diet (Table 1; basal diet contained 0.04 mg Co/kg DM). The diet was formulated to meet or exceed nutrient requirements for growing beef steers with the exception of Co (NRC, 1996). Diets were fed once daily in amounts sufficient to provide ad libitum access to feed. Feed offerings were recorded on a daily basis and refusals recorded every 14 d. On days where blood and ruminal fluid samples were collected, feeding times were staggered to allow samples to be obtained 2 h post feeding.

Steers were weighed prior to feeding and blood samples were collected on d 0, 28 and 56 of the growing phase. Initial and d 56 weights were the average of weights

obtained on two consecutive days. Blood samples were obtained via jugular venipuncture into heparinized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) for the determination of vitamin B_{12} and MMA, or into vacutainer tubes (Becton Dickenson) containing potassium oxalate and sodium fluoride for the determination of glucose.

On d 56, ruminal fluid was collected by stomach tube. Ruminal fluid was strained through four layers of cheesecloth and 10.0 ml added to a vial containing 2.0 ml of meta-phosphoric acid (25% wt/vol). The sample was placed on ice and transported to the laboratory where it was frozen at -70°C until analysis for VFA.

Liver biopsies were obtained initially and on d 56, through an incision made between the 11th and 12th rib on a line from the tubercoxae to the point of the shoulder. Prior to incision, biopsy sites were clipped of hair, scrubbed with Betadine (Purdue Fredrick, Norwalk, CT) and subsequently scrubbed with 70% ethyl alcohol. A core sample of liver was obtained using a modified Jan Shide bone marrow biopsy punch (0.5 cm diameter × 14 cm in length), via the true-cut technique (Pearson and Craig, 1980). Liver biopsies were rinsed without delay using 0.10 M physiological buffered saline solution (pH 7.4) and drained to remove blood. The samples were placed in acid washed polyethylene tubes, capped, placed on dry ice and subsequently transported to the laboratory where they were frozen at -70°C.

Finishing Phase

During the finishing phase, steers received the same dietary treatments as defined in the growing phase, but were switched to a high concentrate diet (Table 1) over a 7-d

period (basal diet contained 0.04 mg Co/kg DM). Feed offerings and refusals were measured and recorded as previously described for the growing phase.

Steers were implanted with Synovex-Plus® (Fort Dodge Animal Health, Fort Dodge, IA) at the beginning of the finishing phase. Steers were weighed and blood samples collected at 28-d intervals through d 112 of the finishing phase. A liver biopsy sample was collected on d 56 in the manner previously described. Equal number of steers per treatment were slaughtered after receiving the finishing ration for either 112 d (three heaviest pens, n = 36) or 127 d (2 lightest pens, n = 24). Final weights were recorded on two consecutive days and steers were slaughtered at a commercial abattoir following an overnight fast. Final liver samples were collected post-mortem and immediately placed on dry ice for transport to the laboratory where they were frozen at -70º C until time of analysis.

Analytical Procedures

Feed samples for the analysis of Co where prepared using a microwave digestion (Mars 5™, CEM Corp., Matthews, NC) procedure described by Gengelbach et al. (1994). Cobalt was determined by flameless atomic absorption spectrophotometry using a graphite furnace (GFA-6500, Shimadzu Scientific Instruments, Kyoto, Japan).

Plasma and liver vitamin B_{12} concentrations were determined using a competitive binding radioimmunoassay kit (ICN, Costa Mesa, CA) in which nonspecific vitamin B_{12} binding R-proteins were removed by affinity chromatography. Prior to liver vitamin B_{12} quantification, a tissue homogenate was prepared using a borate buffer (pH 9.2, Fisher

Scientific, Suwanee GA) and 1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO) as described by Stangl and co-workers (1999).

Plasma samples for the determination of MMA were prepared according to the method of McMurray et al. (1986) using a modified GC method. The GC (Hewlett-Packard Model 5890 Series II, Palo Alto, CA) was equipped with a 25 m × 0.32 mm × 0.17 μm methyl siloxane column (Agilent Technologies, Wilmington, DE), on which 1.0 μL of acetyl chloride-butan-1-ol derivatized sample was injected. The oven temperature program utilized was 100ºC initial followed by a temperature ramp of 10ºC/min to 155ºC for two min, followed by a temperature ramp of 10ºC/min to 215ºC where the temperature was held for 10 min to flush the column.

Ruminal fluid VFA concentrations were determined by GC (Varian Instruments Model 3800, Walnut Creek, CA) using a Nikol fused silica column, 15 m × 0.53 mm × 0.50 um (Supelco, Bellefonte, PA). The oven temperature program utilized began with an initial temperature of 80ºC, followed by a temperature ramp of 20ºC/min to 140ºC which was held for 2 min. Temperature was then ramped 30ºC/min to 175ºC where it was held for 1 min to flush the column. Plasma glucose was determined by a membrane-immobilized, glucose oxidase enzyme coupled to an electrochemical sensor (Model 27 Industrial Analyzer; Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Statistical analysis of data was performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The model for performance variables and ruminal VFA contained treatment and pen. The model for carcass data contained slaughter time, treatment and slaughter time x treatment. Liver vitamin B_{12} and plasma variables were analyzed as

repeated measures with animal within treatment as the error term for treatment effects. When a treatment x time interaction was observed, data were analyzed by sampling d and initial values were used as a covariant when appropriate. Pre-planned orthogonal contrasts were utilized to detect differences among means. Comparisons made were 1) control vs all Co supplemented treatments, 2) 0.05 mg Co/kg DM from $CoCO_3$ vs 0.05 mg Co/kg DM from CoPr, 3) 0.10 mg Co/kg DM from $CoCO_3$ vs 0.10 mg Co/kg DM from CoPr, 4) linear, and 5) quadratic effects of Co level ($CoCO_3$ treatments only). Since the supplemental Co levels were unequally spaced (0, 0.05, 0.10, 1.0, for $CoCO_3$), coefficients used to construct polynomials for linear and quadratic contrasts were calculated as described by Robson (1959).

Results and Discussion

Performance

Steer performance during the 56-d growing phase was not affected by Co source or concentration (Table 2). Cobalt addition to the control diet increased ADG ($P < 0.05$) during the first 56 d of the finishing phase (Table 2). Gain of control steers did not differ from Co-supplemented steers between d 56 and 112 of the finishing period. However, steers supplemented with 0.05 mg Co/kg DM from $CoCO_3$, gained faster than the other treatment groups during this period. The contrast comparing 0.05 mg Co/kg DM from $CoCO_3$ and CoPr was significant ($P < 0.05$). It is unclear why gains were higher in steers supplemented with 0.05 mg Co/kg DM from d 56 to 112. Average daily gain for the entire finishing phase was increased ($P < 0.05$) by Co supplementation but was not affected by Co source.

Cobalt addition to the control diet resulted in a linear ($P < 0.05$) increase in ADFI during the first 56 d and a quadratic increase ($P < 0.05$) over the total finishing phase (Table 2). Average daily feed intake was not affected by source during the finishing phase. Gain:feed was increased ($P < 0.10$) in steers supplemented with Co during the first 56 d, and responded quadratically ($P < 0.05$) to additions of Co to the control diet from d 56 to d 112 of the finishing phase. The contrast comparing the Co sources ($CoCO_3$ vs CoPr) supplemented at 0.05 mg Co/kg was significant ($P < 0.05$) during the second 56 d period and over the total finishing phase. The observed source effect corresponds to the unusual gains of steers receiving 0.05 mg Co/kg DM from $CoCO_3$ during the same time period.

A major physiologic effect of cobalt – vitamin B_{12} deficiency is loss of appetite (Smith, 1997). Early attempts to define Co requirements (Marston, 1970) and experiments that showed the detrimental effects of Co-vitamin B_{12} deficiency on intakes and liveweights, utilized hay fed sheep (Smith and Marston, 1970). More recently, Schwarz et al., (2000) conducted an experiment to determine the Co requirements of growing Simmental beef bulls consuming a corn silage rich diet (basal diet 0.07 mg Co/kg diet) fortified with graduated concentrations of $CoSO_4$. Intake of the unsupplemented control group changed little over the 266-d period, but intake was lower than for those supplemented with Co. As a result of their work, they suggested that 0.16 to 0.18 mg of dietary Co/kg is necessary to maximize intake. In the present study, intake was not affected by supplemental Co during the 56 d growing phase. Although lower than Co-supplemented groups, intake for control steers increased during the finishing

phase, even though the control diet had less Co than the diet described by Schwarz and others (2000). The degree to which storage of vitamin B_{12} in tissues and differences in diet composition contribute to the initial onset of decreased intake is uncertain. For the total finishing phase, intake increased quadratically in the present study, with feed intake being highest in steers supplemented with 1.0 mg Co/kg DM. This suggests that dietary Co concentrations above the 0.10 mg/kg DM level recommended by NRC (1996) may increase intake. These data are in agreement with the results of Schwarz et al. (2000).

Work by Schwarz and colleagues (2000) determined that diets containing 0.07 mg Co/kg resulted in lower daily gains in German Simmental bulls than those containing at least 0.11 mg Co/kg. Their analysis of data, using a broken line model, suggested that the optimum concentration of dietary Co for gain was 0.12 mg/kg DM. In the present study, steers fed the control diet containing 0.04 mg Co/kg DM, had lower gains than Co-supplemented steers during the finishing phase. The increase in gain:feed for Co-supplemented steers over the first 56 d of the finishing period diminished during the second 56 d period so that total gain:feed was not affected by Co supplementation. Performance data suggest that the control diet was only marginally deficient in Co and that the response to Co supplementation was greater early in the finishing phase. Despite the higher feed intake observed in steers supplemented with 1.0 mg Co/kg DM, the addition of 0.05 mg Co/kg DM (total dietary Co of 0.09 mg Co/kg DM) appeared to be sufficient for maximal gain and feed efficiency.

Vitamin B_{12} Status

Plasma vitamin B_{12} was affected by a treatment × time ($P < 0.01$) interaction (Table 3). By d 56 of the growing phase and at all sampling days during the finishing phase steers receiving supplemental Co had higher ($P < 0.05$) plasma vitamin B_{12} concentrations than controls. Increasing dietary Co (from $CoCO_3$) resulted in a quadratic increase ($P < 0.05$) in plasma vitamin B_{12} concentrations on d 56 of the growing phase and on d 28 and 56 of the finishing phase. Plasma vitamin B_{12} responded in a linear ($P < 0.01$) manner to increasing dietary Co on d 84 and 112 of the finishing phase. In general increasing supplemental Co from 0.05 to 0.10 mg/kg DM resulted in small changes in plasma vitamin B_{12}; however, plasma vitamin B_{12} concentrations were greatly increased when supplemental Co was increased from 0.10 to 1.0 mg/kg DM. This suggests that addition of 1.0 mg Co/kg diet DM greatly increased synthesis of vitamin B_{12} by ruminal microorganisms, compared to the low Co additions. Vitamin B_{12} concentration in plasma was unaffected by cobalt source during the growing or finishing phase.

Liver vitamin B_{12} was affected ($P < 0.05$) by a treatment × time interaction (Table 3). There was no effect of Co source or concentration on liver vitamin B_{12} during the growing phase. However, by d 84 of the finishing phase, liver vitamin B_{12} increased quadratically ($P < 0.01$) in response to Co supplementation. At d 84, liver vitamin B_{12} was not increased by 0.05 mg Co/kg DM, but addition of 0.1 or 1.0 mg Co/kg increased liver concentrations relative to controls. Final liver vitamin B_{12} increased ($P < 0.01$) in a linear manner with increasing dietary Co. Cobalt source did not affect liver vitamin B_{12} concentration.

Numerous studies in sheep (Kercher and Smith, 1956; Somers and Gawthorne, 1969; Marston, 1970) have shown that Co supplementation to Co-deficient diets greatly increases vitamin B_{12} concentrations in plasma and liver. However, relatively few studies have investigated the effects of dietary Co on blood and liver vitamin B_{12} concentrations of cattle, particularly those fed high energy diets. In agreement with the present study, Co supplementation to corn silage–based diets, containing 0.07 to 0.08 mg Co/kg DM, increased plasma and liver vitamin B_{12} concentrations in growing German Simmental males (Stangl et al., 1999; 2000). Based on samples obtained at the end of a 40–week study, Stangl et al. (2000) concluded that a dietary concentration of 0.25 mg Co/kg DM was required for maximum plasma and liver vitamin B_{12} concentrations. Plasma vitamin B_{12} concentrations increased greatly, while liver vitamin B_{12} increased slightly in the present study when supplemental Co was increased from 0.1 to 1.0 mg/kg DM. Because no dietary Co concentrations were evaluated between 0.1 and 1.0 mg/kg DM, results obtained from the present study do not allow for estimation of minimal Co requirement for maximal plasma or liver vitamin B_{12} concentrations. In humans biliary excretion of vitamin B_{12} is substantial (Castle and Hale, 1998), but little is known about vitamin B_{12} excretion in ruminants. In lactating dairy cows fed diets high in Co, urinary vitamin B_{12} concentrations were higher than vitamin B_{12} concentrations in serum or milk (Walker and Elliot, 1972). Since liver vitamin B_{12} concentrations in the present study reflected a minimal increase relative to plasma increases in steers supplemented with 1.0 mg Co/kg DM, it is likely that the excess vitamin B_{12} was excreted in bile and urine.

Plasma Methylmalonic Acid and Glucose

Plasma MMA was affected by time ($P < 0.01$) and tended ($P = 0.11$) to be affected by a treatment x time interaction (Table 4). Plasma MMA was not affected by Co source during the growing or finishing phase. Plasma MMA decreased in a quadratic manner on d 56 ($P < 0.01$) of the growing phase and d 28 ($P < 0.01$), 56 ($P < 0.10$), and 112 ($P < 0.10$) of the finishing phase in response to increasing dietary concentrations of Co. Because MMA is the substrate for the vitamin B_{12} dependent enzyme, methylmalonyl-CoA mutase, the higher plasma concentrations of MMA in steers fed the low Co diet likely reflects reduced activity of this enzyme. Kennedy et al. (1991) described the steady increase of plasma MMA, as plasma vitamin B_{12} concentrations decreased in lambs fed a barley diet low (0.04 mg/kg) in Co. High grain diets have been associated with increased urinary MMA in sheep (Lough and Calder, 1976). This could be the result of increased propionate molar proportions, when high grain diets are fed, increasing the demand on methylmalonyl-CoA mutase in propionate metabolism. This may explain the higher MMA concentrations observed in steers of all treatment groups, during the finishing phase. Studies using cattle have also been conducted to evaluate the effectiveness of utilizing MMA as a diagnostic tool for Co deficiency (Paterson and MacPherson, 1990), and to define Co requirements based on plasma MMA and other metabolites (Stangl et al., 2000). Stangl et al. (2000) reported a linear decrease in plasma MMA when dietary Co was increased from 0.07 to 0.147 mg/kg DM. Increasing dietary Co above 0.147 mg/kg DM resulted in little or no change in plasma MMA concentrations. In agreement with these findings, plasma MMA concentrations on

several sampling days in the present study tended to be slightly lower in steers receiving total dietary Co concentrations of 0.14 mg/kg (0.10 mg/kg supplemental) compared to those fed 0.09 mg Co/kg DM (0.05 mg/kg supplemental).

Plasma glucose concentrations were affected by a treatment x time ($P < 0.01$) interaction (Table 5). Cobalt addition to the control diet did not affect plasma glucose during the growing phase (Table 5). However, steers receiving supplemental Co had higher plasma glucose ($P < 0.10$) from d 56 through d 112 of the finishing phase. On d 112, glucose concentration increased in a quadratic ($P < 0.01$) manner for steers receiving supplemental Co as $CoCO_3$. Liver slices, obtained from Co-deficient lactating ewes, incorporated ^{14}C-labeled propionate into glucose at a slower rate than was observed in liver from ewes receiving injections of hydroxocobalamin (Peters and Elliot, 1983). In sheep, Co deficiency resulted in impaired propionate metabolism, and a reduction in the rate of glucose rise following propionate injection (Smith and Marston, 1971; Marston et al., 1972). Plasma glucose concentrations were higher in steers supplemented with 0.10 mg Co/kg DM from CoPr compared to those receiving a similar amount of $CoCO_3$ on d 28 of both the growing ($P < 0.05$) and finishing ($P < 0.10$) phases. The mechanism for the Co source effect on plasma glucose is not apparent, since plasma vitamin B_{12} was unaffected by Co source throughout the experiment.

Ruminal VFA

Molar proportions of VFA were not affected by the addition of supplemental Co to the basal diet during the growing phase (Table 6). However, the contrast comparing the two sources supplemented at 0.10 mg Co/kg DM was significant ($P < 0.05$) for

acetate, propionate, butyrate and acetate:propionate ratio. Steers receiving supplemental Co as CoPr, had higher molar proportions of propionate ($P < 0.05$), and lower molar proportions of acetate ($P < 0.05$) and butyrate ($P < 0.05$). The increased propionate and decreased acetate resulted in a decreased ($P < 0.05$) acetate:propionate ratio.

Steers supplemented with Co had higher molar proportions of propionate ($P < 0.10$) and lower ($P < 0.10$) molar proportions of isobutyrate and isovalerate than the controls during the finishing phase. The increased molar proportion of propionate in ruminal fluid of Co-supplemented steers resulted in a decrease ($P < 0.10$) in the acetate:propionate ratio. Studies have documented the importance of vitamin B_{12} for the production of propionate by some rumen microorganisms (Chen and Wolin, 1981; Tanner and Wolfe, 1988). The higher molar proportions of propionate in Co-supplemented steers during the finishing phase may explain the higher plasma glucose concentrations observed in steers receiving supplemental Co. In other in vitro experiments, adding large amounts (5.0 or 10.0 mg/kg) of Co to cultures did not affect VFA proportions (Hussein et al., 1994). Cobalt source did not affect ruminal VFA proportions during the finishing phase.

Carcass Characteristics

Steers receiving supplemental Co had higher ($P < 0.10$) hot carcass weights than controls (Table 7). Dressing percentage, and longissimus muscle area, were not affected by Co source or concentration. As supplemental Co increased, there was a quadratic effect ($P < 0.10$) on marbling scores. Supplemental Co did not affect backfat or kidney, pelvic and heart fat (KPH); however, steers receiving supplemental Co as CoPr at 0.05

mg Co/kg had slightly lower ($P < 0.10$) backfat, KPH percentage and yield grade than those supplemented with $CoCO_3$. Dietary Co affected quality grades in a quadratic ($P < 0.05$) manner with quality grades being lower in steers supplemented with 1.0 mg Co/kg DM. Few studies have reported the effects of dietary Co on carcass characteristics. Schwarz et al. (2000) found that Simmental bulls fed a diet containing 0.07 mg Co/kg DM had lower carcass weights and less renal fat than those receiving diets containing at least 0.11 mg Co/kg DM.

Results of the present study indicate that, based on vitamin B_{12} concentrations in plasma and liver, the Co sources ($CoCO_3$ and CoPr) evaluated were similar in their ability to provide Co for microbial synthesis of vitamin B_{12}. However, certain other variables tended to be affected by Co source. Ruminal samples obtained during the growing phase indicated a lower acetate:propionate ratio in steers supplemented with 0.10 mg Co/kg DM from CoPr compared to those receiving a similar concentration of Co from $CoCO_3$. Steers supplemented with 0.10 mg Co/kg DM from CoPr had higher plasma glucose concentrations than those supplemented with $CoCO_3$ on d 28 of both the growing and finishing phase. Carcass backfat also tended to be lower in steers supplemented with CoPr (0.05 mg Co/kg DM). It is unclear why the two Co sources appeared to affect certain metabolite and carcass characteristics differently in the absence of an effect of Co source on vitamin B_{12} status.

In conclusion, the present study indicates that increasing dietary Co above the current NRC requirement of 0.10 mg/kg DM will greatly increase plasma and moderately increase liver vitamin B_{12} concentrations in finishing steers. The control diet containing

0.04 mg Co/kg DM was clearly inadequate in Co based on performance and vitamin B_{12} status. At a number of sampling dates, steers supplemented with 0.10 mg Co/kg DM or higher had lower plasma MMA concentrations then steers supplemented with 0.05 mg Co/kg DM. Although 0.05 mg supplemental Co/kg DM (total diet Co of 0.09 mg/kg) appeared to maximize gain and feed efficiency; the slightly higher plasma MMA concentrations in steers supplemented with 0.05 mg Co/kg suggest that with this level of Co, vitamin B_{12} limited conversion of methylmalonyl-CoA to succinyl CoA. Collectively, these data suggests that the dietary Co (diet plus supplemental) requirement of growing and finishing steers is approximately 0.15 mg/kg diet DM.

Implications

Results of the present study indicate that the current NRC requirement for cobalt of 0.10 mg per kilogram diet is probably marginal for growing and finishing steers. Increasing total dietary cobalt above 0.09 mg per kilogram diet increased vitamin B_{12} status and reduced plasma concentrations of methylmalonic acid. A cobalt requirement of 0.15 mg per kilogram diet is recommended based on results of the current study. Cobalt carbonate and cobalt propionate were utilized with similar efficiency by ruminal microorganisms for vitamin B_{12} synthesis. However, the two cobalt sources appeared to affect certain metabolic variables differently.

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Table 1. Ingredient composition of growing and finishing basal diets

Ingredient	Growing	Finishing
	---------- %[a] ----------	
Corn	48.76	85.80
Soybean meal	9.00	7.00
Cottonseed hulls	40.00	5.00
Urea	1.00	0.75
Calcium sulfate	0.40	0.40
Salt	0.20	0.20
Calcium carbonate	0.63	0.60
Vitamin premix[b]	0.01	0.01
Mineral premix[c]	+	+
Monensin	+	+

[a]Dry matter basis

[b]Contained per kilogram of premix: 26,432,000 IU of vitamin A, 8,811,000 IU of vitamin D, and 44,052 IU of vitamin E.

[c]Provided per kilogram of diet: 30 mg of Zn as $ZnSO_4$, 20 mg of Mn as $MnSO_4$, 10 mg of Cu as $CuSO_4 \cdot 5H_2O$, 0.05 mg of I as $Ca(IO_3)_2(H_2O)$, and 0.1 mg of Se as Na_2SeO_3.

Table 2. Effects of cobalt concentration and source on performance of growing and finishing steers

	Added Co, mg Co/kg DM							
	Control	$CoCO_3$	$CoCO_3$	$CoCO_3$	CoPr	CoPr		
Item	0	0.05	0.10	1.00	0.05	0.10	SEM	Significance[a]
Initial BW, kg	274.3	275.6	274.5	272.1	276.1	272.9	6.6	
Final BW, kg	566.3	610.3	589.3	589.4	591.7	587.9	12.7	A†
Growing Phase								
ADG, kg	2.05	2.13	2.10	2.07	2.21	2.22	0.10	
ADFI, kg	10.21	10.60	10.47	10.57	10.69	10.82	0.38	
Gain:feed	0.20	0.20	0.20	0.20	0.21	0.21	0.01	
Finishing Phase								
ADG, kg								
d 0-56	1.11	1.31	1.37	1.46	1.28	1.40	0.08	A*Q*
d 57-112	1.55	1.92	1.57	1.59	1.62	1.51	0.09	B*
Total	1.37	1.63	1.51	1.56	1.48	1.51	0.06	A*
ADFI, kg								
d 0-56	7.45	7.55	7.76	8.41	7.93	8.46	0.30	A† L*
d 57-112	8.38	9.36	9.18	9.56	9.38	9.57	0.37	A*
Total	7.99	8.54	8.58	9.08	8.69	9.12	0.31	A* Q*
Gain:feed								
d 0-56	0.15	0.17	0.18	0.17	0.16	0.16	0.01	A†
d 56-112	0.19	0.21	0.17	0.17	0.17	0.16	0.01	B** Q*
Total	0.17	0.19	0.18	0.17	0.17	0.16	0.01	B*

[a]A = control vs cobalt; B = $CoCO_3$ vs CoPr at 0.05 mg Co/kg; L = linear; Q = quadratic

†$P < 0.10$.

*$P < 0.05$.

**$P < 0.01$.

Table 3. Effects of cobalt concentration and source on plasma and liver vitamin B_{12} concentrations of steers

	Added Co, mg Co/kg DM							
	Control	$CoCO_3$	$CoCO_3$	$CoCO_3$	CoPr	CoPr		
Item	0	0.05	0.10	1.00	0.05	0.10	SEM	Significance[a]
Plasma B_{12}[b]	88.7	97.2	104.8	159.3	105.4	117.3	12.4	A^{+}B*C**L**
Growing phase								
d 28	94.3	84.8	82.2	89.8	84.4	81.8	9.0	
d 56	50.7	73.9	61.7	88.7	64.4	78.3	9.4	A* Q*
Finishing phase								
d 28	55.5	69.2	57.9	98.3	57.2	71.7	7.0	A† Q**
d 56	73.4	80.6	73.1	237.7	71.4	97.1	16.8	A† Q**
d 84	48.7	86.9	132.3	220.9	116.0	146.3	23.7	A** L**
d 112	68.7	108.1	140.9	267.6	152.8	151.2	22.6	A** L**
Liver B_{12}[c]	310.1	300.6	365.1	371.8	321.8	355.5	19.8	B*C**Q**
Growing phase								
d 56	247.1	254.0	250.1	264.7	234.9	265.6	19.1	
Finishing phase								
d 84	263.2	259.2	303.0	336.3	263.0	333.2	19.7	Q**
Final	380.1	428.8	487.1	523.6	411.8	500.4	26.9	A** L**

[a]A = control vs cobalt; B = treatment x time; C = time effect; L = linear; Q = quadratic.

[b]Expressed as pmol/L.

[c]Expressed as pmol/g wet basis.

† = $P < 0.10$.

* = $P < 0.05$.

** = $P < 0.01$.

Table 4. Effects of cobalt concentration and source on plasma methylmalonic acid concentrations of steers

	Added Co, mg Co/kg DM							
	Control	$CoCO_3$	$CoCO_3$	$CoCO_3$	CoPr	CoPr		
Item	0	0.05	0.10	1.00	0.05	0.10	SEM	Significance[a]
Plasma MMA[b]	3.30	2.80	2.29	1.99	2.92	2.60	0.19	A**B**Q**
Growing phase								
d 28	1.74	1.81	1.29	1.41	1.69	1.42	0.28	
d 56	2.79	2.02	1.79	1.35	1.66	2.11	0.30	A** Q**
Finishing phase								
d 28	4.73	3.75	3.74	2.29	3.85	4.14	0.51	A* Q**
d 56	4.26	3.04	2.20	2.59	4.16	2.68	0.50	A* Q†
d 84	4.01	4.18	2.66	2.89	4.10	3.92	0.55	
d 112	3.92	2.79	2.28	2.04	3.86	1.95	0.58	A* Q†

[a]A = control vs cobalt; B = time effect; Q = quadratic.

[b]Expressed as umol/L.

$^{\dagger}P < 0.10$.

$*P < 0.05$.

$**P < 0.01$.

Table 5. Effects of cobalt concentration and source on plasma glucose concentrations of steers

	Added Co, mg Co/kg DM							
	Control	$CoCO_3$	$CoCO_3$	$CoCO_3$	CoPr	CoPr		
Item	0	0.05	0.10	1.00	0.05	0.10	SEM	Significance[a]
Plasma glucose[b]	4.32	4.47	4.47	4.47	4.53	4.60	0.04	A**B*C**D**
Growing phase								
d 28	4.76	4.96	4.74	4.64	4.98	5.07	0.11	B*
d 56	4.61	4.80	4.71	4.77	4.75	4.86	0.13	
Finishing phase								
d 28	4.71	4.57	4.53	4.56	4.59	4.84	0.12	B†
d 56	4.07	4.44	4.51	4.46	4.51	4.50	0.12	A**
d 84	4.11	4.27	4.23	4.19	4.32	4.33	0.08	A†
d 112	4.14	4.46	4.48	4.71	4.56	4.66	0.13	A** Q**

[a]A = control vs cobalt; B = $CoCO_3$ vs CoPr at 0.10 mg Co/kg; C = treatment x time; D = time effect; Q = quadratic.

[b]Expressed as mmol/L.

$^{\dagger}P < 0.10$.

$*P < 0.05$.

$**P < 0.01$.

Table 6. Effects of cobalt concentration and source on ruminal volatile fatty acid molar proportions in steers

	Added Co, mg Co/kg DM							
	Control	$CoCO_3$	$CoCO_3$	$CoCO_3$	CoPr	CoPr		
Item	0	0.05	0.10	1.00	0.05	0.10	SEM	Significance[a]
VFA, moles/100 moles								
Growing phase								
Acetate:propionate	1.93	2.07	2.31	2.05	2.03	1.55	0.20	B*
Acetate	55.84	58.02	58.40	56.68	55.81	53.82	1.28	B*
Propionate	31.20	29.36	27.83	29.84	30.31	35.38	2.03	B*
Isobutyrate	0.50	0.46	0.45	0.57	0.52	0.42	0.04	
Butyrate	10.14	10.08	11.22	10.38	11.02	8.42	0.94	B*
Isovalerate	1.32	1.10	1.12	1.52	1.37	0.97	0.19	
Valerate	0.99	0.97	0.99	1.01	0.97	1.00	0.05	
Finishing phase								
Acetate:propionate	1.02	0.94	0.90	0.89	0.95	0.88	0.05	A†
Acetate	45.80	44.07	44.18	43.39	45.37	43.73	1.28	
Propionate	46.00	47.68	49.45	49.43	47.92	49.70	1.37	A†
Isobutyrate	0.50	0.44	0.39	0.38	0.45	0.49	0.05	A†
Butyrate	5.07	5.24	3.61	4.67	3.81	3.57	0.72	
Isovalerate	0.84	0.68	0.54	0.57	0.76	0.57	0.11	A†
Valerate	1.79	1.89	1.82	1.57	1.68	2.03	0.16	

[a]A = control vs cobalt; B = $CoC0_3$ vs CoPr at 0.10 mg Co/kg.

† = $P < 0.10$.

* = $P < 0.05$.

Table 7. Effects of cobalt concentration and source on carcass characteristics of finished steers

	Added Co, mg Co/kg DM							
	Control	$CoCO_3$	$CoCO_3$	$CoCO_3$	CoPr	CoPr		
Item	0	0.05	0.10	1.00	0.05	0.10	SEM	Significance[a]
Marbling[b]	5.9	5.9	6.3	5.3	5.7	5.6	0.3	Q†
Dressing percentage	59.0	58.7	59.9	59.5	59.5	60.1	0.5	
Hot carcass wt, kg	334.1	358.4	352.7	344.4	351.8	353.3	8.9	A†
12^{th} rib backfat, cm	1.23	1.45	1.32	1.11	1.12	1.18	0.13	B†
KPH[c], %	2.1	2.3	2.2	2.3	2.0	2.3	0.1	B†
USDA yield grade	2.57	3.11	2.74	2.36	2.59	2.68	0.21	B†
LMA[d] , cm^2	86.0	86.1	88.3	90.9	88.0	86.6	2.89	
USDA quality grade[e]	17.4	17.2	17.9	16.5	17.3	17.1	0.4	Q*

[a]A = control vs cobalt; B = $CoCO_3$ vs CoPr at 0.05 mg Co/kg; Q = quadratic.

[b]4=slight; 5=small; 6=modest.

[c]Kidney, pelvic and heart fat.

[d]Longissimus muscle area.

[e]Select + =16; choice - =17; choice = 18.

† = $P < 0.10$.

* = $P < 0.05$.

CHAPTER 3

Effects of dietary cobalt source and concentration on performance, vitamin B_{12} status, and ruminal and plasma metabolites in growing and finishing steers[1]

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ABSTRACT

One hundred twenty Angus-cross steers, averaging 279 kg initially, were utilized to evaluate the effects of cobalt (Co) source and concentration on performance, vitamin B_{12} status and metabolic characteristics of growing and finishing steers. Treatments consisted of 0 (control, analyzed 0.05 mg Co/kg), 0.05, 0.10, and 1.0 mg of supplemental Co/kg DM from Co propionate (CoPr) or 0.05 and 0.10 mg of supplemental Co/kg DM from $CoCO_3$. Steers were individually fed a cottonseed hull-corn-soybean meal-based growing diet for 84 d followed by a high concentrate finishing diet. Performance was not affected by Co supplementation during the growing phase. During the finishing phase, ADFI, ADG and gain:feed ($P < 0.01$) were higher for Co-supplemented steers. Steers supplemented with 0.10 mg Co/kg DM as propionate had higher ($P < 0.05$) molar percentages of propionate and lower ($P < 0.05$) acetate:propionate than steers receiving 0.10 mg Co/kg DM as $CoCO_3$ during the growing phase. Supplemental Co increased ($P < 0.01$) the molar percentage of propionate and decreased ($P < 0.01$) acetate during the finishing phase. Plasma vitamin B_{12} was higher ($P < 0.10$) in Co supplemented steers by d 56 of the growing phase and remained higher ($P < 0.01$) throughout the finishing phase. Cobalt supplementation increased ($P < 0.01$) liver vitamin B_{12} at the end of the growing and finishing phases. Ruminal fluid vitamin B_{12} concentration was higher ($P < 0.01$) in steers supplemented with Co, and increasing Co supplementation from 0.10 to 1.0 mg/kg DM greatly increased ($P < 0.01$) ruminal vitamin B_{12} concentration. Plasma methylmalonic acid was lower ($P < 0.05$) in Co-supplemented steers during the entire study. Cobalt supplementation decreased ($P < 0.05$) plasma succinate and increased

plasma folate and glucose ($P < 0.01$ and 0.05, respectively) in steers. Steers supplemented with 0.05 mg/kg DM as CoPr had higher plasma folate ($P < 0.01$) and glucose ($P < 0.05$) than those supplemented with 0.05 mg/kg DM as $CoCO_3$. Final body, and hot carcass weights, were lower ($P < 0.05$) in steers fed the control diet, while Co supplementation increased dressing percentage ($P < 0.10$) and longissimus muscle area ($P < 0.01$). These results combined with previous work indicate that finishing steers require approximately 0.15 mg Co/kg and that the two sources affect VFA and plasma glucose differently.

Key Words: Cattle, Cobalt, Vitamin B_{12}

Introduction

Ruminants require cobalt (Co), which is incorporated into the corrin ring structure, during microbial biosynthesis of vitamin B_{12}. In ruminants, Co-deficient diets result in a vitamin B_{12} deficiency, due to reduced microbial vitamin B_{12} biosynthesis in the rumen. Vitamin B_{12} is important for microbial growth (Tanner and Wolfe, 1988; Strobel, 1992), and the role of Co in ruminant nutrition has been reviewed (Smith, 1987; 1997). Vitamin B_{12} serves is an important cofactor for two mammalian enzymes, methylmalonyl-CoA mutase, and methionine synthase. Methionine synthase is a methylcobalamin-dependent enzyme important for methionine and tetrahydrofolate synthesis (Matthews, 1999). Methylmalonyl-CoA mutase is an adenosylcobalamin-dependent enzyme that catalyzes interconversion of methylmalonyl-CoA to succinyl-CoA, an important step in pathways that involve propionyl-CoA, such as gluconeogenesis and fatty acid metabolism (Bannerjee and Chowdhury, 1999).

When Co is deficient in ruminant diets, there is decreased methionine synthase and methylmalonyl-CoA mutase activity in tissues (Kennedy et al., 1990), increased plasma homocysteine and methylmalonic acid (Stangl et al., 2000) and altered lipid metabolism (Kennedy et al., 1994; Stangl et al.,1999b). Recent studies (Schwarz et al., 2000; Tiffany et al., 2003) suggest that current NRC (1996) requirements for Co may underestimate requirements. Tiffany et al. (2003) reported that supplementing Co propionate to steer diets affected vitamin B_{12} status similarly to $CoCO_3$, but appeared to affect certain variables differently from $CoCO_3$. This study was conducted to better define Co requirements of growing and finishing steers and to further compare $CoCO_3$ and CoPr as Co sources.

Materials and Methods

General

One hundred twenty Angus and Angus crossbred steers (279.4 kg initial BW) were utilized in this experiment. Care, handling, and sampling of the animals herein were approved by the North Carolina State University Animal Care and Use Committee. Steers were purchased at feeder calf sales in North Carolina. After arrival, steers were ear-tagged, weighed, vaccinated with CattleMaster® 4 (Pfizer Animal Health, Exton PA) and Vision™ 7 (Bayer Corp., Shawnee Mission, KS), treated with Safe Guard® (Hoechst Roussel Vet., Clinton, NJ) for elimination of internal parasites, and confined to fescue pasture where they were supplemented with corn silage (2.0 kg DM/hd/d) for 28 d. Steers were then weighed on two consecutive days, and allotted by weight to one of 24

pens. Steers were housed in covered slotted-floor pens (3 m × 4 m), equipped with concrete feed bunks, for the duration of the experiment.

Growing phase

After placement into pens, steers were implanted with Synovex-S® (Fort Dodge Animal Health, Fort Dodge IA), and pens were randomly assigned to dietary treatments within a weight block of six pens. Treatments consisted of : 1) control (no supplemental Co), 2) 0.05 mg of supplemental Co/kg DM from Co propionate (CoPr; Kemin Industries, Des Moines, IA), 3) 0.10 mg of supplemental Co/kg DM from CoPr, 4) 1.00 mg of supplemental Co/kg DM from CoPr), 5) 0.05 mg of supplemental Co/kg DM from $CoCO_3$, and 6) 0.10 mg of supplemental Co/kg DM from $CoCO_3$.

During the 84-d growing phase, steers were fed a corn-cottonseed hull-soybean meal based diet (Table 1; the basal diet contained 0.05 mg Co/kg DM). The diet was formulated to meet or exceed nutrient requirements for growing beef steers with the exception of Co (NRC, 1996). Diets were fed once daily in amounts sufficient to provide ad libitum access to feed. Feed offerings were recorded on a daily basis and refusals recorded. On days when blood and ruminal fluid samples were collected, feeding times were staggered to allow samples to be obtained 2 h post feeding.

Steers were weighed prior to feeding and blood samples were collected (three steers per pen, n = 12) on d 0, 28, 56 and 84 of the growing phase. Initial and d 84 weights were the average of weights obtained on two consecutive days. Blood samples were obtained via jugular venipuncture into heparinized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) for the determination of plasma vitamin B_{12}, folate,

methylmalonic acid (MMA) and succinate, or into vacutainer tubes (Becton Dickenson) containing potassium oxalate and sodium fluoride for the determination of glucose.

On d 84, ruminal fluid was collected from three randomly selected steers per pen (n = 12) by stomach tube. Ruminal fluid was strained through four layers of cheesecloth and 10.0 ml added to a vial containing 2.0 ml of meta-phosphoric acid (25% wt/vol). The sample was placed on ice and transported to the laboratory where it was frozen at -70°C until analysis for VFA and vitamin B_{12}.

Liver biopsies were obtained initially and on d 84 (two animals per pen, n = 8), utilizing the true-cut technique as described previously (Tiffany et al., 2003). Liver biopsies were placed in acid washed polyethylene tubes, capped, placed on dry ice and transported to the laboratory and frozen at -70°C. All blood, liver, and ruminal fluid samples were collected from the same randomly selected steers for the duration of the experiment.

Finishing Phase

During the finishing phase, steers received the same dietary treatments as described in the growing phase, but were switched to a high concentrate diet (Table 1) over a 7-d period (basal diet contained 0.05 mg Co/kg DM). Feed offerings and refusals were measured and recorded as previously described for the growing phase.

Steers were implanted with Synovex-Plus® (Fort Dodge Animal Health, Fort Dodge, IA) at the beginning of the finishing phase. Steers were weighed, and blood samples collected at 28 d intervals through d 84 of the finishing phase for the analysis of vitamin B_{12}, folate, glucose, MMA, and succinate. On d 84, ruminal fluid was collected

by stomach tube for analysis of VFA and vitamin B_{12}. Equal number of steers per treatment were slaughtered after receiving the finishing diet for either 90, 97, 111, or 118 d. Final weights were recorded on two consecutive days and steers were slaughtered at a commercial abattoir following an overnight fast. Final liver samples were collected post-mortem and immediately placed on dry ice for transport to the laboratory where they were frozen at - 70º C until time of analysis.

Analytical Procedures

Feed samples were prepared for mineral analysis using a microwave digestion (Mars 5™, CEM Corp., Matthews, NC) procedure described by Gengelbach et al. (1994). Cobalt was determined by flameless atomic absorption spectrophotometry using a graphite furnace (GFA-6500, Shimadzu Scientific Instruments, Japan).

Prior to the determination of vitamin B_{12} concentration, ruminal fluid was centrifuged at 10,000 × g, and diluted with distilled H_2O until within reference range of the assay. Plasma vitamin B_{12} and folate, and liver and ruminal fluid vitamin B_{12} concentrations were determined using a competitive binding radioimmunoassay kit (ICN, Costa Mesa, CA), in which nonspecific vitamin B_{12} binding proteins were removed by affinity chromatography. Liver was prepared for vitamin B_{12} analysis as previously described by Stangl et al. (1999b).

Plasma samples for the determination of MMA were prepared according to the method of McMurray et al. (1986) using a modified GC method described by Tiffany et al. (2003). Ruminal fluid VFA concentrations were determined by GC as described by Tiffany et al. (2003). Plasma glucose was determined by a membrane-immobilized

glucose oxidase enzyme coupled to an electrochemical sensor (Model 27 Industrial Analyzer; Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Statistical analysis of data was performed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model for ADG, ADFI, gain;feed and VFA contained treatment, and pen. The model for carcass data contained treatment, slaughter time and slaughter time x treatment interaction. Plasma and liver variables were analyzed as repeated measures with animal within treatment as the error term for treatment effects. When a treatment x time interaction was observed, data were analyzed statistically by time. Initial values for plasma and liver variables were analyzed and found not to differ among treatments. Initial values were then used as a covariate when appropriate. Pre-planned orthogonal contrasts were utilized to test differences among means. Comparisons made were: 1) control vs all CoPr supplemented treatments, 2) 0.05 mg Co/kg from CoPr vs 0.10 mg Co/kg from CoPr, 3) 0.10 mg Co/kg from CoPr vs 1.0 mg Co/kg from CoPr, 4) 0.05 mg Co/kg DM from $CoCO_3$ vs 0.05 mg Co/kg DM from CoPr, and 5) 0.10 mg Co/kg DM from $CoCO_3$ vs 0.10 mg Co/kg DM from CoPr.

Results and Discussion

Performance

Steer performance was not affected by Co source or concentration during the 84-d growing phase (Table 2). Co supplementation of a low Co (0.04 mg/kg) corn-cottonseed hull-based diet also did not affect performance of steers during a 56-d growing phase (Tiffany et al., 2003).

Cobalt addition to the control diet increased ($P < 0.01$) ADG, ADFI, and gain:feed during the finishing phase (Table 2); however, Co source did not affect performance. Increasing supplemental Co from 0.05 to 0.10 mg/kg DM or from 0.10 to 1.0 mg/kg DM did not significantly increase ADG, ADFI, or gain:feed. In a previous study (Tiffany et al., 2003), Co addition to a control (0.04 mg Co/kg DM, no supplemental Co) diet increased ADFI and improved gain and gain:feed during the first 56 days of the finishing phase. Feed intake only tended ($P = 0.19$) to increase in the present study when supplemental Co was increased from 0.05 to 0.10 mg/kg DM and ADFI was similar in steers supplemented with 0.10 or 1.0 mg Co/kg DM. In contrast, Tiffany et al. (2003) reported that increasing supplemental Co from 0.10 to 1.0 mg/kg DM increased ADFI. Schwarz et al. (2000) evaluated ten dietary Co concentrations ranging from 0.07 to 0.69 mg/kg during a 280-d growing-finishing study with German Simmental bulls. Bulls were fed corn silage ad libitum and 2.5 kg of concentrate per d in this study. Using a broken line model they estimated that 0.12 mg Co/kg DM was required for maximum gain and 0.16 to 0.18 mg Co/kg DM was required for maximum feed intake. Early studies with high forage diets indicated that Co deficiency resulted in a gradual decline in intake followed by extreme inappetence (Smith, 1987). In agreement with the present study, recent studies with growing and finishing cattle fed corn silage (Schwarz et al., 2000) or high concentrate-based diets (Tiffany et al., 2003) in confinement, indicated that feed intake of cattle fed diets deficient, or marginally deficient in Co, did not decline with time; however, their feed intake was lower than intake in Co-supplemented animals.

Lack of a performance response to supplemental Co during the growing phase may relate to the relatively short length of time the growing diet was fed or to type of diet. Perhaps initial stores of vitamin B_{12} in liver and other tissues were sufficient to prevent vitamin B_{12} deficiency during the growing phase, but stores of vitamin B_{12} became depleted during the finishing phase. An alternate hypothesis is that cattle fed a high concentrate diet have a higher dietary requirement for Co. The higher ruminal propionate production in cattle fed a high concentrate diet may increase the amount (activity) of the vitamin B_{12}-dependent enzyme, methylmalonyl-CoA mutase, needed for the liver to metabolize propionate.

Vitamin B_{12}

Plasma vitamin B_{12} concentrations were affected by treatment ($P < 0.01$), time ($P < 0.01$) and a treatment × time interaction ($P < 0.01$; Table 3). Liver vitamin B_{12} concentrations were also affected by treatment ($P < 0.01$), time ($P < 0.01$) and treatment × time ($P < 0.09$). Steers receiving supplemental Co had higher plasma vitamin B_{12} concentrations than the controls by d 56 ($P < 0.10$) of the growing phase and at all subsequent sampling times ($P < 0.01$; Table 3). Liver vitamin B_{12} concentrations were also increased ($P < 0.01$) by Co supplementation of the control diet. This is consistent with studies in cattle where Co supplementation of control diets containing 0.07 mg Co/kg DM (Stangl et al., 2000) or 0.04 mg Co/kg DM (Tiffany et al., 2003) increased plasma and liver vitamin B_{12} concentrations. Steers supplemented with 0.10 mg Co/kg DM had higher ($P < 0.05$) plasma vitamin B_{12} concentrations on d 84 of the growing phase than steers supplemented with 0.05 mg Co/kg DM. During the finishing phase

steers supplemented with 1.0 mg Co/kg DM had higher plasma ($P < 0.10$ on d 28; $P < 0.01$ on d 56 and 84) and liver ($P < 0.05$) vitamin B_{12} concentrations than those receiving 0.10 mg Co/kg DM. A previous study (Tiffany et al., 2003) also indicated that increasing supplemental Co from 0.10 to 1.0 mg/kg DM increased plasma and liver vitamin B_{12} concentrations in cattle fed a high concentrate diet. Stangl et al. (2000) estimated that 0.257 and 0.236 mg Co/kg DM were required for maximum plasma and liver vitamin B_{12} concentrations, respectively.

Cobalt addition to the basal low Co-diet increased ($P < 0.01$) vitamin B_{12} concentrations in ruminal fluid samples obtained on d 84 of the finishing phase (Table 3). Increasing supplemental Co from 0.10 to 1.0 mg/kg increased ($P < 0.01$) ruminal fluid vitamin B_{12} concentrations by almost six fold. The large increase in plasma vitamin B_{12} concentrations observed in steers supplemented with 1.0 mg Co/kg DM corresponds well with the large increase in ruminal fluid vitamin B_{12}. Few studies have reported the effects of dietary Co on ruminal vitamin B_{12} concentrations. Smith and Marston (1970) reported lower vitamin B_{12} production and concentration in ruminal contents of sheep fed Co-deficient compared to those fed Co-supplemented diets. They also determined that most of the vitamin B_{12} found in rumen contents was contained within microorganisms. In the present study there was no attempt to release vitamin B_{12} from microorganisms. The degree to which vitamin B_{12} was released from microorganisms is uncertain. However, results obtained from the method used in the present experiment, showed a substantial increase in ruminal fluid vitamin B_{12} as Co supplementation increased.

Cobalt source did not affect plasma or liver vitamin B_{12} concentrations during the growing phase. Plasma vitamin B_{12} concentrations were higher on d 56 of the finishing phase in steers receiving $CoCO_3$ ($P < 0.05$ for 0.05 mg Co/kg DM; $P < 0.10$ for 0.10 mg Co/kg DM) than in steers receiving CoPr. Liver vitamin B_{12} concentrations at slaughter were also higher ($P < 0.05$) in steers receiving 0.10 mg Co/kg DM from $CoCO_3$ compared to those receiving a similar concentration from CoPr. Plasma vitamin B_{12} concentrations were not affected by Co source on d 28 and 84 of the finishing phase. Cobalt source also did not affect ruminal vitamin B_{12} concentrations. Previous research indicated that plasma and liver vitamin B_{12} concentrations were similar in steers supplemented with $CoCO_3$ or CoPr (Tiffany et al., 2003).

Plasma Folate, Succinate, Glucose and Methylmalonic acid

Cobalt supplementation of the control diet increased ($P < 0.01$) plasma folate concentrations (Table 4). Steers supplemented with 0.05 mg Co/kg DM from CoPr had higher ($P < 0.01$) plasma folate concentrations than those receiving 0.05 mg Co/kg DM from $CoCO_3$. Plasma folate was affected by time ($P < 0.01$) but not by a treatment × time interaction. In contrast to these results, Stangl et al. (2000) reported that plasma folate was not affected by dietary Co. In this study plasma folate was measured at the end of the 280-d study. However, liver folate concentrations were lower in cattle fed low dietary Co (0.07 mg/kg DM) and increased with increasing dietary Co up to approximately 0.19 mg/kg DM (Stangl et al., 2000). Lower plasma or liver folate concentrations in cattle fed low dietary Co may relate to decreased activity of vitamin B_{12}-dependent methionine synthase. In nonruminants vitamin B_{12} deficiency, via reduced

methionine synthase activity, is believed to result in a functional folate deficiency by trapping a larger proportion of folate as the 5' methyl derivative (Shane and Stokstad, 1985).

Plasma succinate concentrations were lower ($P < 0.05$) in Co-supplemented steers compared to the controls (Table 4). Lambs fed a barley-based diet, deficient in Co, also had higher plasma succinate concentrations than Co-supplemented lambs (Kennedy et al., 1991). Increased plasma succinate in Co-deficient lambs was related to large increases in ruminal succinate concentrations that occurred within 2-weeks after lambs were offered the Co-deficient diet (Kennedy et al., 1991). Plasma succinate was not affected by Co source, Co level, or treatment × time interaction.

Cobalt supplementation of the control diet increased ($P < 0.05$) plasma glucose concentrations (Table 4). Plasma glucose was affected by time ($P < 0.01$) but not treatment × time. Steers fed diets containing 0.08 or 0.20 mg Co/kg DM had similar serum glucose concentrations at the end of a 301-d study (Stangl et al., 1999b). However, growing-finishing steers fed a low Co diet (0.04 mg/kg DM) had lower plasma glucose concentrations than Co-supplemented steers late in the finishing phase (d 56 to 112; Tiffany et al., 2003). Plasma glucose decreased by about 40 % after dairy calves were fed a diet containing 0.04 mg Co/kg DM for 150 d (MacPherson et al., 1973). The reduced plasma glucose paralleled a decline in feed intake, and both plasma glucose and intake increased rapidly following administration of vitamin B_{12} (MacPherson et al., 1973).

Steers fed 0.05 mg Co/kg DM from CoPr had higher ($P < 0.05$) plasma glucose concentrations than steers fed a similar amount of Co from $CoCO_3$. In a previous study (Tiffany et al., 2003) steers supplemented with CoPr (0.10 mg/kg DM) had higher plasma glucose concentrations than steers receiving $CoCO_3$ on d 28 of both the growing and finishing phase.

Plasma MMA concentrations were affected by a treatment × time interaction ($P < 0.01$; Table 5). Steers consuming the control diet had higher plasma MMA concentrations throughout the growing ($P < 0.05$) and finishing ($P < 0.01$) phases than steers supplemented with Co. Previous studies have also shown elevated plasma MMA concentrations in cattle fed low Co diets (Stangl et al., 1999a; 2000; Tiffany et al., 2003). Stangl et al. (2000) reported a linear decrease in plasma MMA concentrations of Simmental males when dietary Co was increased between 0.07 and 0.147 mg Co/kg DM, but further increases in dietary Co resulted in no further decreases in plasma MMA. On d 28 ($P < 0.10$) of the growing phase and on several other sampling days in the present study, plasma MMA concentrations tended to be slightly lower in steers fed total dietary Co concentrations of 0.15 mg/kg DM (0.10 mg/kg supplemented from CoPr) compared to steers fed 0.10 mg Co/kg DM (0.05 mg/kg DM supplemental from CoPr). The higher plasma MMA concentrations in steers fed the control diet probably reflects reduced activity of methylmalonyl-CoA mutase, a vitamin B_{12} dependent enzyme. Decreased activity of methylmalonyl-CoA mutase has been reported in Co-deficient sheep (Kennedy et al., 1990). In agreement with an earlier study (Tiffany et al., 2003), plasma MMA concentrations were higher ($P < 0.01$) in all treatment groups during the finishing phase

than the growing phase. This may relate to the higher ruminal propionate production observed in ruminants fed high concentrate diets (Leng and Brett, 1966) and subsequent increased flux of propionate through the methylmalonyl CoA pathway. Increases in plasma MMA with time during the finishing phase were much greater in steers fed the low-Co control diet, suggesting an increased severity of vitamin B_{12} deficiency with time. By d 56 and 84 of the finishing phase, plasma MMA concentrations averaged over 11 mmol/L in controls, concentrations that greatly exceed the upper limit of normal for cattle of 4 mmol/L suggested by Paterson and MacPherson (1990).

Differences between Co sources in plasma MMA concentrations were inconsistent. Steers supplemented with 0.10 mg/Co/kg DM from CoPr had lower ($P < 0.05$) plasma MMA concentrations than those receiving 0.10 mg Co/kg DM from $CoCO_3$ on d 28 of the growing phase (Table 5). In contrast, on d 84 of the finishing phase steers supplemented with CoPr had higher ($P < 0.10$) plasma MMA concentrations than steers receiving $CoCO_3$ when Co was added at 0.05 mg/kg DM.

Ruminal VFA

Cobalt supplementation of the control diet decreased ($P < 0.05$) molar proportions of butyrate in ruminal fluid samples collected during the growing and finishing phase (Table 6). Isovalerate molar proportion was increased ($P < 0.10$) during the growing phase but decreased ($P < 0.05$) during the finishing phase by Co supplementation. In agreement with previous research (Tiffany et al., 2003) Co addition to the control diet did not alter molar proportion of ruminal acetate or propionate during the growing phase. However, during the finishing phase, steers receiving supplemental Co had lower ($P <$

0.01) molar proportions of ruminal acetate and higher ($P < 0.01$) molar proportions of propionate than controls, resulting in a decreased ($P < 0.01$) acetate:propionate ratio. These results confirm our earlier findings that low dietary Co reduces molar proportion of propionate and increases acetate:propionate ratios in steers fed a high concentrate diet (Tiffany et al., 2003). When lambs were changed from a Co-supplemented barley-based diet to the barley-based diet without added Co (diet contained 0.004 mg Co/kg) ruminal propionate concentrations decreased greatly while ruminal succinate concentrations increased within 4 d (Kennedy et al., 1991). In microbes, the vitamin B_{12} dependent methylmalonyl-CoA mutase catalyzes the conversion of succinyl-CoA to methylmalonyl-CoA, during the production of propionate through the dicarboxylic pathway. Decreased propionate production and increased succinate and acetate production have been observed by the ruminal bacterium *Prevotella ruminicola* when grown in a vitamin B_{12} deficient medium (Strobel, 1992).

Steers supplemented with 0.10 mg Co/kg DM from CoPr had a higher ($P < 0.05$) molar proportion of propionate and a lower ($P < 0.05$) molar proportion of butyrate than steers receiving 0.10 mg Co/kg DM from $CoCO_3$ during the growing phase (Table 6). Acetate:propionate ratio was higher ($P < 0.05$) in steers receiving $CoCO_3$ (0.10 mg/kg DM) than in those supplemented with CoPr. Similar differences between these two Co sources in VFA proportions were observed previously in steers fed a growing diet (Tiffany et al., 2003). Cobalt source did not affect ruminal VFA during the finishing phase.

Carcass Characteristics

Steers receiving supplemental Co (as CoPr) had higher hot carcass weights ($P < 0.01$) and dressing percentages ($P < 0.10$) and larger longissimus muscle areas ($P < 0.01$) than controls (Table 7). Previous studies (Schwarz et al., 2000; Tiffany et al., 2003) also reported lower hot carcass weights in steers or bulls fed diets deficient or marginally deficient in Co. Yield and quality grades were not affected by Co. Steers supplemented with 0.10 mg Co/kg DM from CoPr had lower ($P < 0.05$) backfat than those receiving 0.10 mg Co/kg DM from $CoCO_3$. Tiffany et al. (2003) reported that when Co was supplemented at 0.05 mg/kg DM, steers supplemented with CoPr had lower backfat than steers supplemented with $CoCO_3$.

Implications

Cobalt supplementation to high-energy beef cattle finishing diets, at concentrations above current NRC (1996) requirements, increased vitamin B_{12} status, and decreased plasma MMA, a key Co deficiency indicator. Based on these results and previous work from our laboratory approximately 0.15 mg Co/kg DM is required for finishing beef cattle.

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Table 1. Ingredient composition of growing and finishing basal diets

Ingredient	Growing	Finishing
	---------- %[a] ----------	
Corn	48.76	85.80
Soybean meal	9.00	7.00
Cottonseed hulls	40.00	5.00
Urea	1.00	0.75
Calcium sulfate	0.40	0.40
Salt	0.20	0.20
Calcium carbonate	0.63	0.60
Vitamin premix[b]	0.01	0.01
Mineral premix[c]	+	+
Monensin	+	+

[a]Dry matter basis

[b]Contained per kilogram of premix: 26,432,000 IU of vitamin A, 8,811,000 IU of vitamin D, and 44,052 IU of vitamin E.

[c]Provided per kilogram of diet: 30 mg of Zn as $ZnSO_4$, 20 mg of Mn as $MnSO_4$, 10 mg of Cu as $CuSO_4 \cdot 5H_2O$, 0.05 mg of I as $Ca(IO_3)_2(H_2O)$, and 0.1 mg of Se as Na_2SeO_3.

Table 2. Effects of cobalt concentration and source on performance of growing and finishing steers

	Added Co, mg Co/kg DM							
	Control	CoPr	CoPr	CoPr	$CoCO_3$	$CoCO_3$		
Item	0	0.05	0.10	1.00	0.05	0.10	SEM	Significance[a]
Initial BW, kg	278.2	279.4	279.9	279.7	279.6	279.6	3.7	
Final BW, kg	511.3	534.4	546.0	543.2	543.6	545.1	9.0	A*
Growing Phase								
ADG, kg	1.79	1.69	1.83	1.82	1.78	1.74	0.06	
ADFI, kg	10.38	10.22	10.49	10.42	10.50	10.36	0.40	
Gain:Feed	0.17	0.17	0.17	0.18	0.17	0.17	0.01	
Finishing Phase								
ADG, kg	0.79	1.06	1.10	1.11	1.10	1.15	0.05	A**
ADFI, kg	6.46	7.22	7.59	7.52	7.59	7.68	0.25	A**
Gain:Feed	0.12	0.15	0.14	0.15	0.15	0.15	0.01	A**

[a]A = Control vs cobalt.
† = $P < 0.10$
* = $P < 0.05$
** = $P < 0.01$

Table 3. Effects of cobalt concentration and source on plasma, liver and ruminal fluid vitamin B_{12} concentrations of steers

	Added Co, mg Co/kg DM							
Item	Control 0	CoPr 0.05	CoPr 0.10	CoPr 1.00	$CoCO_3$ 0.05	$CoCO_3$ 0.10	SEM	Significance[a]
Plasma B_{12}[b]	46.6	70.5	96.8	155.6	95.8	106.9	6.3	A** B** C** D** E** F**
Growing Phase								
D 28	40.1	44.7	48.1	41.6	36.8	48.8	6.6	
D 56	35.9	46.1	56.3	53.5	50.1	65.4	8.2	C†
D 84	52.7	74.3	129.3	115.3	95.0	111.4	14.7	C** D*
Finishing Phase								
D 28	58.2	73.7	99.7	134.4	96.6	110.7	13.0	C** E†
D 56	50.2	75.4	116.5	230.3	133.4	161.0	18.1	C** E**
D 84	42.6	109.5	130.9	358.7	163.2	143.4	24.7	C** E**
Liver B_{12}[c]	274.2	401.8	417.4	463.9	398.0	492.6	24.1	A** B† C** G*
Growing phase	249.5	399.0	397.3	379.3	329.7	436.1	36.3	C**
Finishing phase	298.9	407.7	439.9	542.0	466.4	550.0	29.5	C** E* G*
Ruminal fluid B_{12}[d]	2.6	3.0	4.1	24.2	3.3	4.2	0.9	C**E**

[a]A = time effect; B = treatment × time; C = control vs cobalt; ; D = 0.05 vs 0.10 mg Co/kg from CoPr; E = 0.10 vs 1.0 mg Co/kg from CoPr; F = CoPr vs $CoCO_3$ at 0.05 mg Co/kg; G = CoPr vs $CoCO_3$ at 0.10 mg Co/kg.

[b]Expressed as pmol/L.

[c]Expressed as pmol/g wet basis.

[d]Expressed as pmol/mL

† = $P < 0.10$.

* = $P < 0.05$.

** = $P < 0.01$.

Table 4. Effects of cobalt concentration and source on plasma folate, succinate, and glucose concentrations of steers

Item	Added Co, mg Co/kg DM						SEM	Significance[a]
	Control	CoPr	CoPr	CoPr	$CoCO_3$	$CoCO_3$		
	0	0.05	0.10	1.00	0.05	0.10		
Plasma folate[b]	29.6	33.4	31.9	32.1	30.5	31.8	0.6	$A^{**}B^{**}C^{**}$
Plasma succinate[c]	6.46	5.33	5.82	5.50	5.63	5.41	0.32	A^{**} B^{*}
Plasma glucose[d]	4.53	4.69	4.66	4.68	4.52	4.62	0.05	A^{**} B^{*} C^{*}

[a]A = time effect; B = control vs cobalt; C = CoPr vs $CoCO_3$ at 0.05 mg Co/kg.

[b]Expressed as nmol/L.

[c]Expressed as umol/L.

[d]Expressed as mmol/L.

* = $P < 0.05$.

** = $P < 0.01$.

Table 5. Effects of cobalt concentration and source on plasma methylmalonic acid concentrations of steers

	Added Co, mg Co/kg DM							
Item	Control 0	CoPr 0.05	CoPr 0.10	CoPr 1.00	$CoCO_3$ 0.05	$CoCO_3$ 0.10	SEM	Significance[a]
Plasma MMA[b]	6.66	2.96	2.30	2.31	2.45	2.56	0.20	A^{**} B^{**} C^{**} D^{*} $E^{\dagger}$
Growing Phase								
D 28	2.91	2.49	1.93	2.32	2.12	2.49	0.20	C^{**} $D^{\dagger}$ F^{*}
D 56	3.45	2.24	1.87	2.11	2.14	2.20	0.23	C^{**}
D 84	3.04	2.06	1.93	2.02	2.24	2.18	0.16	C^{*}
Finishing Phase								
D 28	7.84	2.85	2.16	2.23	2.62	2.71	0.44	C^{**}
D 56	11.33	3.31	2.16	2.12	2.60	2.40	0.82	C^{**}
D 84	11.41	4.84	3.71	3.08	2.98	3.38	0.78	C^{**} $E^{\dagger}$

[a]A = time effect; B = treatment × time; C = control vs cobalt; ; D = 0.05 vs 0.10 mg Co/kg from CoPr; E = CoPr vs $CoCO_3$ at 0.05 mg Co/kg; F = CoPr vs $CoCO_3$ at 0.10 mg Co/kg.

[b]Expressed as umol/L.

† = $P < 0.10$.

* = $P < 0.05$.

** = $P < 0.01$.

Table 6. Effects of cobalt concentration and source on ruminal volatile fatty acid molar percentage of steers

	Added Co, mg Co/kg DM							
	Control	CoPr	CoPr	CoPr	$CoCO_3$	$CoCO_3$		
Item	0	0.05	0.10	1.00	0.05	0.10	SEM	Significance[a]
VFA, moles/100 moles								
Growing Phase								
Acetate:Propionate	1.93	1.83	1.91	1.87	1.91	2.4	0.16	B*
Acetate	54.56	55.83	56.32	55.64	55.51	58.41	1.17	
Propionate	30.59	31.24	30.36	30.70	30.00	26.06	1.46	B*
Isobutyrate	0.60	0.64	0.64	0.76	0.64	0.76	0.06	
Butyrate	11.92	9.67	9.85	10.10	11.11	11.93	0.74	A* B*
Isovalerate	1.30	1.52	1.74	1.76	1.48	1.64	0.16	A†
Valerate	1.13	1.16	1.10	1.10	1.25	1.18	0.05	
Finishing Phase								
Acetate:Propionate	1.42	1.01	1.00	0.91	0.96	1.04	0.10	A**
Acetate	49.66	46.38	45.82	44.95	45.96	46.36	0.93	A**
Propionate	39.40	46.32	46.95	49.48	48.08	45.64	1.68	A**
Isobutyrate	0.56	0.49	0.56	0.48	0.47	0.55	0.05	
Butyrate	7.92	4.83	4.37	3.07	3.32	5.29	0.91	A**
Isovalerate	1.18	0.70	0.80	0.65	0.62	0.77	0.18	A*
Valerate	1.28	1.28	1.48	1.37	1.55	1.38	0.13	

[a]A = Control vs Cobalt; B = CoPr vs $CoC0_3$ at 0.10 mg Co/kg.

† = $P < 0.10$.

* = $P < 0.05$.

** = $P < 0.01$.

Table 7. Effects of cobalt concentration and source on carcass characteristics of finished steers

	Added Co, mg Co/kg DM							
	Control	CoPr	CoPr	CoPr	$CoCO_3$	$CoCO_3$		
Item	0	0.05	0.10	1.00	0.05	0.10	SEM	Significance[a]
Marbling[b]	5.4	5.2	5.3	5.3	5.6	5.6	0.2	
Dressing percentage	59.8	60.3	60.6	60.2	60.4	60.7	0.3	A†
Hot carcass wt, kg	305.7	322.1	330.9	327.4	328.2	330.4	5.6	A**
12th rib backfat, cm	0.84	1.10	0.94	0.95	1.13	1.21	0.10	C*
KPH[c], %	2.00	2.01	2.18	2.06	2.13	2.13	0.06	B†
USDA yield grade	2.45	2.68	2.53	2.46	2.76	2.84	0.15	
LMA[d] cm^2	79.4	83.1	85.4	85.2	83.8	83.4	1.7	A**
USDA quality grade[e]	16.6	16.3	16.8	16.5	17.0	16.8	0.3	

[a]A = Control vs Cobalt; B = cobalt at 0.05 vs 0.10 mg Co/kg from CoPr; C = $CoCO_3$ vs CoPr at 0.10 mg Co/kg.

[b]4=slight; 5=small; 6=modest.

[c]Kidney, pelvic and heart fat.

[d]Longissimus muscle area.

[e]Select + =16; choice - =17; choice = 18.

† = $P < 0.10$.

* = $P < 0.05$.

** = $P < 0.01$

CHAPTER 4

Effects of dietary cobalt concentration on performance, vitamin B_{12} status, and carcass characteristics of finishing steers, fed corn or barley-based diets[1]

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ABSTRACT

An experiment was conducted to determine the effects of dietary cobalt (Co) concentration on performance, carcass traits, and plasma, liver and ruminal metabolites of steers fed corn, or barley-based diets. Sixty steers were stratified by weight and randomly assigned to treatments, in a 2 × 3 factorial arrangement with factors being, corn or barley based diet, and supplemental Co added at 0, 0.05, or 0.15 mg/kg DM. Steers were fed individually using electronic Calan gate feeders. Cobalt supplementation increased ($P < 0.05$) ADFI and ADG over the total study, and was higher ($P < 0.05$) for corn vs barley-fed steers. From d 85 to finish Co supplementation increased ($P < 0.05$) ADG of steers fed corn but not barley-based diets. During the first 84 d gain:feed was higher ($P < 0.05$) for steers supplemented with Co, and over the total experiment was higher ($P < 0.05$) in steers fed corn vs barley-based diets. Supplemental Co increased vitamin B_{12} in plasma and liver ($P < 0.01$ and 0.05, respectively) and plasma vitamin B_{12} was higher ($P < 0.05$) in steers fed corn vs barley-based diets. Cobalt supplementation increased ($P < 0.05$) ruminal fluid vitamin B_{12} in steers fed corn but not barley-based diets. Folate was higher in plasma ($P < 0.01$) and liver ($P < 0.05$) of steers fed Co-supplemented diets, and barley-fed steers had lower ($P < 0.01$) plasma and liver folate than those fed corn-based diets. Increasing supplemental Co from 0.05 to 0.15 increased ($P < 0.05$) liver folate in steers fed barley but not corn-based diets. Supplemental Co decreased ($P < 0.01$) plasma MMA concentrations in steers. Plasma and ruminal fluid succinate were not affected by Co supplementation, however, steers fed barley-based diets had higher ($P < 0.05$) plasma and ruminal fluid succinate relative to those fed corn.

Addition of supplemental Co to the basal diets increased ($P < 0.01$) plasma glucose concentrations of steers, and those fed corn-based diets had higher plasma glucose than steers fed barley-based diets. Steers consuming diets with added Co had higher propionate ($P < 0.01$) and lower ($P < 0.05$) acetate and butyrate molar percentage than steers consuming the control diets. Supplemental Co increased dressing percentage ($P < 0.10$) and hot carcass weights ($P < 0.01$) at slaughter. These results indicate that feeding steers corn or barley-based diets deficient in Co, adversely affects performance and vitamin B_{12} status.

Key Words: Steers, Cobalt, Vitamin B_{12}

Introduction

Cobalt (Co) is a component of vitamin B_{12}. In mammals, vitamin B_{12} is an important cofactor for the enzymes methylmalonyl-CoA mutase and methionine synthase which are important for gluconeogenesis and methionine synthesis (Bannerjee and Chowdhury, 1999; Matthews, 1999). In cattle, a Co deficiency results in a decline in methylmalonyl-CoA mutase and methionine synthase activity (Kennedy et al., 1990), and alters lipid metabolism (Stangl et al., 1999b) and immune function (MacPherson et al., 1987).

Recent studies have investigated the effects of dietary Co on performance and metabolism of growing and finishing cattle fed high-energy corn-based diets (Tiffany et al., 2003a;b). In those studies, when steers were fed a diet moderately Co deficient, intake, gain, and gain:feed were reduced relative to Co supplemented steers. In addition, reduced plasma and liver vitamin B_{12}, decreased ruminal fluid vitamin B_{12} and

propionate, and elevated plasma methylmalonic acid concentrations, were observed.

Barley has lower ME, and higher NDF and lignin percentages than corn (NRC 1996) and ferments differently than other grains altering VFA molar percentage in ruminal fluid (Franks et al., 1972). When sheep were fed Co deficient barley-based diets, vitamin B_{12} and ruminal succinate concentrations were greatly affected (Kennedy et al., 1991a; 1994). Plasma MMA concentrations of sheep fed Co deficient barley generally exceed those of Co deficient cattle (O'Harte et al., 1989). Cobalt requirements for finishing cattle consuming high energy diets are poorly defined, and information on feeding finishing steers Co deficient barley-based diets in confinement is scarce. It is relevant to investigate the effects of feeding finishing steers supplemental Co and to evaluate the effects of Co deficiency as they relate to grain source.

Materials and Methods

General

Sixty Angus steers (316.0 kg initial BW) were utilized in this experiment. Care, handling, and sampling of the animals herein were approved by the North Carolina State University Animal Care and Use Committee. Steers were purchased at feeder calf sales in North Carolina. After arrival, steers were ear-tagged, weighed, vaccinated with Bovashield® 4 (Pfizer Animal Health, Exton PA) and Vision™ 7 (Bayer Corp., Shawnee Mission, KS), and treated for elimination of internal parasites with Safe Guard® (Hoechst Roussel Vet., Clinton, NJ). Steers were confined to fescue pasture where they were supplemented with corn silage (2.0 kg DM/hd/d) until the beginning of the experiment. Steers were then weighed on two consecutive days and allotted by weight and origin, to

one of five 12-head pens equipped with individual Calan gate feeders (American Calan, Northwood, NH). Steers were housed in covered, slotted-floor pens (5 m × 10 m) for the duration of the experiment.

After adjusting to the Calan gate feeding system, steers were weighed on two consecutive days, implanted with Synovex®-Plus (Fort Dodge Animal Health, Fort Dodge IA), bled via jugular venipuncture, and randomly assigned to one of six treatments. Treatments consisted of: 1) corn-based diet (no supplemental Co), 2) corn-based diet supplemented with 0.05 mg Co/kg DM, 3) corn-based diet supplemented with 0.15 mg Co/kg DM, 4) barley-based diet (no supplemental Co), 5) barley-based diet supplemented with 0.05 mg Co/kg DM and 6) barley-based diet supplemented with 0.15 mg Co/kg DM. Supplemental Co was provided from $CoCO_3$. One steer from the corn-based treatment without supplemental Co died after one week, due to hypertension, unrelated to treatment as diagnosed by veterinarian.

Ingredient and chemical composition of the basal diets are presented in Table 1. Diets were formulated to be similar in CP and DIP. The corn and barley-based diets analyzed 0.04 and 0.02 mg Co/kg DM, respectively. Diets were formulated to meet or exceed nutrient requirements for growing beef steers with the exception of Co (NRC, 1996). Steers were fed once daily in amounts sufficient to provide ad libitum access to feed. Feed offerings were recorded on a daily basis and feed refusals recorded.

Steers were weighed prior to feeding and blood samples were collected on d 0, 28, 56, 84, 112 and 140. On days where blood and ruminal fluid samples were collected feeding times were staggered to allow samples to be obtained 2 h postfeeding. Initial and

final weights were the average of weights obtained on two consecutive days. Blood samples were obtained via jugular venipuncture into heparinized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) for the determination of vitamin B_{12}, MMA, and folate or into vacutainer tubes (Becton Dickenson) containing potassium oxalate and sodium fluoride for the determination of glucose.

On d 84, ruminal fluid was collected by stomach tube. Ruminal fluid was strained through four layers of cheesecloth and 10.0 ml was added to a vial containing 2.0 ml of meta-phosphoric acid (25% wt/vol). The sample was placed on ice and transported to the laboratory where it was frozen at -70°C until analysis for VFA and vitamin B_{12}.

Equal number of steers per treatment were slaughtered after receiving the dietary treatments for either 146 or 160 d. Steers were slaughtered at a commercial abattoir following an overnight fast. Liver samples were collected post-mortem and immediately placed on dry ice for transport to the laboratory where they were frozen at -70ºC until analysis of vitamin B_{12} and folate.

Analytical Procedures

Feed samples for the analysis of Co where prepared using a microwave digestion (Mars 5™, CEM Corp., Matthews, NC) procedure described by Gengelbach et al. (1994). Cobalt was determined by flameless atomic absorption spectrophotometry using a graphite furnace (GFA-6500, Shimadzu Scientific Instruments, Kyoto, Japan).

Plasma, liver, and ruminal fluid vitamin B_{12} and folate concentrations were determined using a competitive binding radioimmunoassay kit in which nonspecific vitamin B_{12} binding R-proteins were removed by affinity chromatography (ICN, Costa

Mesa, CA). Prior to liver vitamin B_{12} quantification, a tissue homogenate was prepared using a borate buffer (pH 9.2, Fisher Scientific, Suwanee GA) and 1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO) as described by Stangl and co-workers (1999b). Prior to the determination of vitamin B_{12} concentration, ruminal fluid was centrifuged at 10,000 × g, and diluted with distilled H_2O until within reference range of the assay.

Plasma samples for the determination of MMA and succinate were prepared according to the method of McMurray et al. (1986) using a modified GC method. The GC (Hewlett-Packard Model 5890 Series II, Palo Alto, CA) was equipped with a 25 m × 0.32 mm × 0.17 μm methyl siloxane column (Agilent Technologies, Wilmington, DE), on which 1.0 μL of acetyl chloride-butan-1-ol derivatized sample was injected. The oven temperature program utilized was 100ºC initially followed by a temperature ramp of 10ºC/min to 155ºC for two min, followed by a temperature ramp of 10ºC/min to 215ºC, where the temperature was held for 10 min to flush the column.

Ruminal fluid VFA concentrations were determined by GC (Varian Instruments Model 3800, Walnut Creek, CA) using a Nikol fused silica column, 15 m × 0.53 mm × 0.50 um (Supelco, Bellefonte, PA). The oven temperature program utilized began with an initial temperature of 80ºC, followed by a temperature ramp of 20º C/min to 140º C, which was held for 2 min. Temperature was then ramped 30º C/min to 175ºC, where it was held for 1 min to flush the column. Plasma glucose was determined by a membrane-immobilized glucose oxidase enzyme coupled to an electrochemical sensor (Model 27 Industrial Analyzer; Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Data were analyzed statistically as a 2 × 3 factorial using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model contained cobalt, grain source, pen and cobalt × grain interaction. Degrees of freedom for cobalt were partitioned into the following single degree of freedom contrasts: 1) control vs 0.05 and 0.15 mg Co/kg DM and 2) 0.05 vs 0.15 mg Co/kg DM. Plasma variables were analyzed as repeated measures with the model containing time and all possible interactions. Initial values were used as a covariant when appropriate.

Results and Discussion

Performance

Cobalt addition to the diet increased ($P < 0.01$) ADG during the first 84 d and over the total finishing period (Table 2). Feed intake was increased ($P < 0.05$) by Co supplementation for the entire study. In agreement with these findings, Co supplementation of corn silage-based diets containing 0.08 or 0.07 mg Co/kg DM (Stangl et al., 1999b; Schwarz et al., 2000) or corn-based diets containing 0.04 or 0.05 mg Co/kg DM (Tiffany et al., 2003a,b) has increased gain and feed intake in finishing cattle. Gain:feed was increased ($P < 0.05$) by Co supplementation during the first 84 d, but not over the entire experimental period. This indicates that the gain response to Co supplementation was largely due to higher feed intake rather than improved efficiency of gain. A major consequence of Co deficiency in ruminants is loss of appetite (Smith, 1997) that may be linked to a decrease in the rate of propionate clearance from blood (Marston et al., 1972).

The present study suggests that marginal Co deficiency has a greater impact on gain and gain:feed early in the finishing period when cattle are fed high energy diets. Tiffany et al. (2003a) also reported that Co addition to a finishing diet low in Co (0.04 mg Co/kg DM) improved gain and feed efficiency for the first 56 d but not the last 56 d of the finishing period. As the BW of cattle increases, the percentage of protein in gain declines, while the percentage of fat in gain increases (NRC, 1996). This shift in protein and fat accretion may change the demand for energy derived from propionate and increase the demand for lipogenic precursors, such as acetyl-CoA, lessening the impact of Co deficiency. However, in the case of severe Co deficiency, blood propionate clearance rate can decline to a point where energy demands are not satisfied and gain decreases (Marston et al., 1972).

Increasing supplemental Co from 0.05 to 0.15 mg/kg DM did not significantly affect ADG, ADFI, or gain:feed. In previous studies (Tiffany et al., 2003a,b) with finishing cattle fed corn-based diets, low in Co (0.04 to 0.05 mg Co/kg DM) increasing supplemental Co above 0.05 mg/kg has not significantly increased gain or gain:feed. However, increasing supplemental Co from 0.05 or 0.10 to 1.0 mg Co/kg DM increased ADFI in one study (Tiffany et al., 2003a) but not in another study (Tiffany et al., 2003b). Schwarz et al. (2000) evaluated ten dietary Co concentrations ranging from 0.07 to 0.69 mg Co/kg DM during a 280-d growing-finishing study with Simmental bulls. Cattle were fed corn silage ad libitum and 2.5 kg DM of concentrate per d in this study. They estimated, using a broken line model, that 0.12 mg Co/kg DM was required for maximum gain and 0.16 to 0.18 mg Co/kg DM was required for maximum feed intake.

From d 85 until the end of the study, ADG and gain:feed were affected ($P < 0.05$) by a Co × grain interaction (Table 2). Cobalt supplementation increased ($P < 0.05$) ADG in steers fed corn-based diets but not in those fed barley-based diets. Gain:feed was not affected by Co supplementation in steers fed corn-based diets but was reduced ($P < 0.05$) by Co in animals fed barley-based diets. These responses were unexpected because the barley-based diet was lower (0.02 vs 0.04 mg/kg DM) in Co than the corn-based diet. Average daily gain, ADFI, and gain:feed for the entire study were not affected by a Co × grain interaction, indicating that responses to Co supplementation were similar for the two grain sources.

Steers fed corn-based diets had higher ADG ($P < 0.05$), gain:feed ($P < 0.01$) and final BW than those fed barley-based diets. In contrast, several studies (Owens et al., 1997; Mathison and Engstrom, 1995) have reported similar performance of finishing steers fed barley vs corn-based diets. However, other studies, in which corn was substituted for either rolled or unprocessed barley (Prichard and Robbins, 1991; Mandell et al., 1997, respectively), reported that cattle consuming barley-based diets had lower intakes and ADG relative to those consuming corn-based diets. In the present study, NDF and ADF were higher in the barley-based diets relative to the corn-based diets (as percentages of DM, NDF = 33.2 vs 24.5, ADF = 9.4 vs 6.0, for barley and corn, respectively). The higher NDF and ADF in the barley-based diets would be expected to lower the ME of these diets and can explain the decreased gain and gain:feed.

Vitamin B_{12} and Folate

Plasma vitamin B_{12} concentrations were affected by Co supplementation ($P < 0.01$), grain source ($P < 0.05$) and time ($P < 0.01$; Table 3). By d 56, and at all subsequent sampling times, steers receiving supplemental Co had higher ($P < 0.05$) plasma vitamin B_{12} concentrations than those consuming the unsupplemented control diets. Increasing supplemental Co from 0.05 to 0.15 mg/kg did not significantly increase plasma vitamin B_{12} status during any 28 d sampling period. Steers consuming the corn-based diets had higher ($P < 0.05$) plasma vitamin B_{12} from d 28 to 112, than steers fed the barley-based diets.

Numerous studies have shown decreased plasma vitamin B_{12} concentrations when sheep (Kercher and Smith 1956; Somers and Gawthorne 1969; Kennedy et al., 1991), and cattle (Stangl et al., 1999b) are fed Co deficient diets. When Simmental males were fed corn-silage-based diets with increasing concentrations of Co (0.07 to 0.69 mg Co/kg), approximately 0.25 mg of dietary Co/kg DM was required to maximize plasma vitamin B_{12} concentration (Stangl et al. 2000). In the present study increasing supplemental Co from 0.05 to 0.15 mg/kg DM only tended ($P < 0.14$) to increase plasma vitamin B_{12}. In contrast, steers fed a high concentrate diet (control diet contained 0.04 mg Co/kg DM) supplemented with 0, 0.05, 0.10 or 1.0 mg Co/kg DM showed a linear increase in plasma vitamin B_{12} concentrations with increasing dietary Co (Tiffany et al., 2003a,b).

Steers receiving supplemental Co had higher ($P < 0.05$) liver vitamin B_{12} at slaughter than those that consumed the unsupplemented control diets (Table 3). However, increasing supplemental Co from 0.05 to 0.15 mg/kg did not significantly increase liver

vitamin B_{12}. In contrast, increasing supplemental Co from 0.10 to 1.0 mg/kg DM in finishing steers fed corn-based diets, low in Co (0.04 to 0.05 mg/kg), increased liver vitamin B_{12} concentrations (Tiffany et al., 2003a,b). Based on liver vitamin B_{12} concentrations at the end of a 280-d study with growing-finishing cattle, Stangl et al. (2000) estimated that 0.236 mg Co/kg DM was required for maximum liver vitamin B_{12} concentrations. In the present study, liver vitamin B_{12} in steers consuming the corn-based diets did not differ significantly from those receiving the barley-based diets.

Ruminal fluid vitamin B_{12} was affected ($P < 0.10$) by a cobalt × grain interaction. When steers consumed the corn-based diet, ruminal fluid vitamin B_{12} was increased ($P < 0.05$) by supplemental Co and was higher ($P < 0.05$) in steers supplemented with 0.15 mg Co/kg DM compared to those fed 0.05 mg Co/kg DM. This agrees with previous research (Tiffany et al., 2003b) where Co supplementation increased ruminal fluid vitamin B_{12} concentrations in finishing steers fed corn-based diets. Ruminal fluid vitamin B_{12} concentrations were not increased by supplemental Co in steers fed the barley-based diets. When Co was supplemented at 0.15 mg/kg DM, steers fed barley-based diets had much lower ($P < 0.05$) ruminal fluid vitamin B_{12} concentrations than those fed the corn-based diets. The lack of an increase in ruminal fluid vitamin B_{12} concentrations with Co supplementation in barley-fed steers was unexpected. However, the lower ruminal vitamin B_{12} concentrations in steers fed barley-based diets is consistent with the lower plasma vitamin B_{12} concentrations observed in steers fed barley compared to corn-based diets. These results suggest that ruminal fermentation in cattle fed barley-based diets results in less vitamin B_{12} production than corn-based diets.

Plasma folate concentrations were affected ($P < 0.01$) by cobalt supplementation, grain source, time, and grain × time interaction (Table 4). Steers supplemented with 0.05 or 0.15 mg Co/kg had higher ($P < 0.05$) plasma folate concentrations on d 84 and 112 than control steers. However, plasma folate was not significantly affected by Co at other sampling times. Steers consuming the barley-based diet had lower ($P < 0.01$) plasma folate from d 28 to 112 than steers receiving the corn-based diet, but by d 140 the grain effect had diminished.

Liver folate concentrations were affected by Co supplementation ($P < 0.05$), grain source ($P < 0.01$), and cobalt × grain interaction ($P < 0.05$; Table 4). Supplemental Co did not increase liver folate concentrations of steers consuming the corn-based diets. In steers fed barley-based diets Co addition increased ($P < 0.05$) liver folate and steers supplemented with 0.15 mg Co/kg DM had higher ($P < 0.05$) liver folate concentrations than those fed diets supplemented with 0.05 mg Co/kg DM. Steers fed barley-based diets had lower ($P < 0.05$) liver folate concentrations than steers fed corn-based diets when Co was supplemented at 0 or 0.05 mg/kg DM. The lower plasma and liver folate concentrations of steers consuming the barley-based diets compared to the corn-based diets seems to mirror their lower plasma vitamin B_{12} status. When Co was supplemented at 0.15 mg/kg DM liver folate was not affected by grain source.

The role of folate during vitamin B_{12} deficiency in ruminants is poorly understood. The methyl trap hypothesis, in which the conversion of 5-CH_3-H_4-folate to H_4-folate is impaired due to decreased methionine synthase activity remains a plausible explanation (Stabler, 1999). Cobalt supplementation of diets containing 0.07 to 0.08 mg

Co/kg DM did not increase serum or plasma folate concentrations in cattle (Stangl et al., 1999a; 2000); however, in agreement with the present study liver folate was increased by Co supplementation in those studies. Plasma folate concentrations were increased by Co supplementation in steers fed a basal diet that contained 0.05 mg Co/kg DM (Tiffany et al., 2003b).

Ruminal Fluid VFA

Addition of supplemental Co to the diets decreased ($P < 0.05$) acetate and increased ($P < 0.01$) propionate molar proportions in ruminal fluid, resulting in a lower ($P < 0.01$) acetate:propionate ratio (Table 5). Acetate:propionate ratio was affected by a Co × grain interaction ($P = 0.07$). Cobalt supplementation decreased acetate:propionate ratio to a greater extent in steers fed barley-based diets. Increased ruminal propionate proportions and decreased acetate:propionate ratios in steers consuming moderately Co deficient, high concentrate finishing diets supplemented with Co has been reported previously (Tiffany et al., 2003a,b). In ruminal microbes, the vitamin B_{12}-dependent enzyme methylmalonyl-CoA mutase catalyzes conversion of methylmalonyl-CoA to succinyl-CoA (Nagaraja et al., 1997), during propionate production via the dicarboxylic pathway. The decreased molar proportion of propionate observed may reflect the lower ruminal vitamin B_{12} concentrations observed in the unsupplemented control steers, and the resultant decline in microbial methylmalonyl-CoA mutase activity.

Steers consuming corn-based diets had lower acetate and higher propionate molar proportions ($P < 0.01$) than those consuming the barley-based diets (Table 5). This resulted in a lower acetate:propionate ratio in steers consuming the corn-based diets.

When Franks et al. (1972) fed cattle diets differing in grain sources (experimental diets contained 80% of the test grain) they found that cattle consuming corn-based diets had higher molar proportions of propionate than those consuming barley-based diets.

Molar proportion of butyrate in ruminal fluid was affected by a Co × grain interaction ($P < 0.05$; Table 5). Cobalt supplementation decreased ($P < 0.01$) molar proportion of butyrate in steers fed barley, but did not significantly affect butyrate in those fed corn-based diets. Molar proportions of isobutyrate and valerate were reduced ($P < 0.05$) by Co supplementation (Table 5).

Steers fed barley-based diets had higher ($P < 0.01$) molar proportions of butyrate and isobutyrate relative to those fed corn-based diets (Table 5). Acetate and butyrate formation through pyruvate in microbes may be linked since acetate formation requires condensation of two acetyl-CoA molecules (Williams and Coleman, 1997). Valerate molar proportion was lower ($P < 0.05$) in steers fed barley-based diets compared to those given corn-based diets (Table 5).

Methylmalonic Acid, Succinate, and Glucose

Plasma MMA concentrations were not affected by time or a Co × time interaction (Table 6). Cobalt addition to the control diet decreased ($P < 0.05$) plasma MMA concentrations and increasing supplemental Co from 0.05 to 0.15 mg/kg DM further decreased ($P < 0.01$) plasma MMA regardless of grain source. Cobalt deficiency results in elevated plasma MMA concentrations due to decreased activity of methylmalonyl-CoA mutase, a vitamin B_{12} dependent enzyme (Kennedy et al., 1990). In previous studies (Tiffany et al., 2003a,b) Co supplementation of low Co diets decreased plasma

MMA concentrations in finishing steers and increasing supplemental Co from 0.05 to 0.10 mg/kg DM further decreased MMA concentrations. Diagnostic criteria for the determination of vitamin B_{12} deficiency based on plasma MMA (and vitamin B_{12}) have been proposed ((McMurray et al., 1985; O'Harte et al., 1989). However, those criteria were based on data from sheep, not cattle.

Steers fed the barley-based diets had lower ($P < 0.05$) plasma MMA concentrations than those fed corn-based diets (Table 6). Lower MMA concentrations in steers fed barley-based diets may be explained by less propionate being available for metabolism in these animals. Molar proportion of propionate was much lower ($P < 0.01$) in steers fed barley compared to those fed corn-based diets (Table 5).

Plasma succinate was affected by time ($P < 0.01$) but not by Co or a Co × time or Co × grain interaction (Table 6). Steers supplemented with 0.05 mg Co/kg DM had higher ($P < 0.10$) ruminal fluid succinate concentrations than those supplemented with 0.15 mg Co/kg DM. However, ruminal succinate in steers fed the control diets did not differ from Co-supplemented steers. Kennedy et al. (1991b) reported a sharp increase in ruminal fluid succinate and a corresponding increase in plasma succinate when sheep were fed a low Co (0.004 mg/kg DM) barley-based diet. In contrast to the present study, Tiffany et al. (2003b) found higher plasma succinate concentrations in steers fed a low Co diet compared to Co-supplemented steers. The discrepancy between that study and the current study may relate to the degree of Co deficiency. In the present study control steers were fed the low Co diet for a shorter time period. Steers in the present study were also less Co deficient at the end of the study based on plasma and liver vitamin B_{12}

concentrations and plasma MMA concentrations than in the previous study (Tiffany et al., 2003b).

Steers fed barley-based diets had higher ($P < 0.05$) plasma and ruminal succinate concentrations than those fed corn-based diets (Table 6). These findings are consistent with the lower ruminal vitamin B_{12} concentrations observed in barley-fed steers relative to those corn-fed (Table 3). Reduced ruminal vitamin B_{12} production in barley-fed steers, relative to those corn-fed, may have decreased microbial methylmalonyl-CoA mutase activity, resulting in accumulation of ruminal succinate and decreased ruminal proportions of propionate.

Plasma glucose increased ($P < 0.01$) in response to supplemental Co ($P < 0.01$; Table 6). The slightly lower plasma glucose concentrations in steers fed a low Co diet is consistent with the findings of recent studies (Tiffany et al., 2003a,b). The lower plasma glucose observed in the control steers may relate to the lower molar proportions of propionate in the ruminal fluid observed in those animals. In addition, the lower plasma vitamin B_{12} concentrations observed in the control steers, may have reduced conversion of methylmalonyl-CoA to succinyl-CoA in the liver due to decreased methylmalonyl-CoA mutase activity, limiting the availability of gluconeogenic precursors. Plasma glucose was lower ($P < 0.01$) in steers fed the barley-based diets relative to those fed corn-based diets (Table 6). This is consistent with the much lower molar proportions of ruminal propionate in the steers fed the barley-based diets, limiting the concentration of propionate available for post-absorptive metabolism and gluconeogenesis.

Carcass Characteristics

Steers receiving supplemental Co had higher hot carcass weights ($P < 0.01$) and dressing percentages ($P < 0.10$) than control steers (Table7). Previous studies (Tiffany et al., 2003a,b) have reported higher hot carcass weights when supplemental Co was added to Co deficient diets, however, dressing percentage was increased in the latter, but not the initial study. Other carcass measurements were no affected by dietary Co.

Steers fed the corn-based diets had higher ($P < 0.01$) hot carcass weights, dressing percentages, backfat, and yield grades than steers fed barley-based diets. Marbling scores also tended ($P < 0.10$) to be lower for steers finished on barley-based diets. In contrast to carcass results obtained in the present study, Mathison and Engstrom (1995) reported similar carcass characteristics in steers fed barley or corn-based diets.

Implications

These results suggest that ruminal fermentation differs according to grain source when steers are fed corn or barly-based diets. Furthermore, Co supplementation evokes a greater response in the vitamin B_{12} status of steers fed corn-based diets relative to those fed barley-based diets.

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Table 1. Composition of basal diets

	Corn-based	Barley-based
Ingredient	——————% DM——————	
Corn, ground	84.00	-
Barley, ground	-	85.00
Soybean meal	6.75	6.75
Cottonseed hulls	7.00	7.00
Urea	1.00	-
Salt	0.20	0.20
Calcium sulfate	0.40	0.40
Calcium carbonate	0.63	0.63
Vitamin premix	+	+
Trace mineral premix[1]	+	+
Chemical composition		
CP, %	14.5	14.6
NDF, %	24.5	32.2
ADF, %	6.0	9.4
Co, mg/kg	0.04	0.02

[1]Provided per kilogram of diet: 30 mg of Zn as $ZnSO_4$, 20 mg of Mn as $MnSO_4$, 0.5 mg of I as $Ca(IO_3)_2(H_2O)$, 0.1mg of Se as $NaSeO_3$, and 10 mg Cu as $CuSO_4$.

Table 2. Effects of dietary cobalt and grain source on performance of finishing steers

	Added Co, mg/kg				Grain source		
Item	0	0.05	0.15	SEM	Corn	Barley	SEM
Initial BW, kg	317.7	314.4	315.8	2.6	319.9	312.0	2.1
Final BW, kg[a,b]	532.9	552.3	560.0	6.5	561.5	535.3	5.3
ADG, kg							
d 0-84[c,d]	1.62	1.86	1.95	0.07	1.91	1.70	0.06
d 85-finish[e]	1.12	1.21	1.19	0.05	1.18	1.16	0.04
Corn[a]	1.00	1.27	1.26	0.08			
Barley	1.24	1.14	1.11	0.08			
Total[c,d]	1.42	1.57	1.61	0.04	1.60	1.48	0.03
ADFI, kg							
d 0-84[f]	8.45	9.07	9.23	0.25	8.92	8.92	0.21
d 85-finish[a]	8.70	9.60	9.79	0.29	9.18	9.55	0.23
Total[a]	8.53	9.31	9.48	0.21	9.04	9.21	0.17
Gain:Feed							
d 0-84[a,b]	0.19	0.20	0.21	0.01	0.21	0.19	0.01
d 85-finish[e]	0.13	0.13	0.12	0.01	0.13	0.12	0.01
Corn	0.12	0.13	0.13	0.01			
Barley[a]	0.14	0.12	0.11	0.01			
Total[b]	0.17	0.17	0.17	0.01	0.18	0.16	0.01

[a]Control vs Co ($P < 0.05$).

[b]Grain source effect ($P < 0.01$).

[c]Control vs Co ($P < 0.01$).

[d]Grain source effect ($P < 0.05$).

[e]Cobalt × grain interaction ($P < 0.05$).

[f]Control vs Co ($P < 0.10$).

Table 3. Effects of dietary cobalt and grain source on plasma, liver and ruminal fluid vitamin B_{12} concentrations in steers

	Added Co, mg/kg				Grain source		
Item	0	0.05	0.15	SEM	Corn	Barley	SEM
Plasma B_{12}[a,b,c,d]	68.1	94.2	104.3	4.8	96.0	81.7	3.9
d 28[c]	71.3	76.7	81.9	6.3	85.8	67.5	5.2
d 56[e,f]	54.3	77.0	84.7	8.0	89.4	54.6	6.5
d 84[e,f]	53.3	75.6	77.7	8.9	85.6	52.1	7.3
d 112[b,c]	58.2	93.2	96.3	9.1	93.9	71.3	7.4
d 140[b]	70.5	115.4	127.0	11.4	114.1	94.5	9.3
Liver B_{12}[e,g]	527.6	643.2	672.6	41.5	652.0	576.9	33.8
Ruminal B_{12}[c,h,i]	1.35	1.40	2.28	0.33	2.15	1.21	0.27
Corn[e,j]	1.37	1.70	3.37	0.47			
Barley	1.34	1.10	1.18	0.47			

[a]Expressed as pmol/L.

[b]Control vs Co ($P < 0.01$).

[c]Grain source effect ($P < 0.05$).

[d]Time effect ($P < 0.01$).

[e]Control vs Co ($P < 0.05$).

[f]Grain source effect ($P < 0.01$).

[g]Expressed as pmol/g wet basis.

[h]Expressed as pmol/ml.

[i]Cobalt × grain interaction ($P < 0.10$).

[j]0.05 vs 0.15 mg Co/kg DM ($P < 0.05$).

Table 4. Effects of dietary cobalt and grain source on plasma and liver folate concentrations in steers

Item	Added Co, mg/kg			SEM	Grain source		SEM
	0	0.05	0.15		Corn	Barley	
Plasma folate[a,b,c,d,e]	26.2	29.6	27.8	0.5	29.9	25.9	0.4
d 28[c]	28.3	29.9	28.9	1.1	31.8	26.2	0.9
d 56[c]	25.9	27.7	26.3	1.0	28.3	25.0	0.8
d 84[b,c]	26.9	30.4	30.6	1.0	33.0	25.6	0.8
d 112[b,c]	23.0	29.7	27.9	1.4	29.9	23.9	1.1
d 140	30.5	32.9	31.6	1.5	32.8	30.5	1.2
Liver folate[c,f,g,h]	36.2	40.8	43.1	2.1	43.6	36.7	1.6
Corn	42.0	47.4	42.6	3.0			
Barley[g,i]	30.3	34.2	43.5	3.0			

[a]Expressed as nmol/L.

[b]Control vs Co ($P < 0.01$).

[c]Grain source effect ($P < 0.01$).

[d]Time effect ($P < 0.01$).

[e]Grain source × time interaction ($P < 0.01$).

[f]Expressed as nmol/g wet basis.

[g]Control vs Co ($P < 0.05$).

[h]Cobalt × grain interaction ($P < 0.05$).

[i]0.05 vs 0.15 mg Co/kg DM ($P < 0.05$).

Table 5. Effects of dietary cobalt and grain source on ruminal fluid volatile fatty acid proportions of steers

Item	Added Co, mg/kg				Grain source		
	0	0.05	0.15	SEM	Corn	Barley	SEM
	moles/100 moles						
Acetate[a,b]	49.0	44.9	44.0	1.4	43.2	48.7	1.2
Propionate[b,c]	32.0	40.5	43.0	1.9	43.2	33.8	1.5
A:P[b,c,d]	1.73	1.20	1.10	0.11	1.05	1.64	0.09
Corn	1.20	1.00	0.95	0.15			
Barley[c]	2.25	1.40	1.26	0.15			
Isobutyrate[a,e]	1.04	0.72	0.53	0.15	0.54	0.99	0.13
Butyrate[b,c,f]	14.6	10.0	9.3	0.9	9.5	13.1	0.8
Corn	10.9	8.0	9.5	1.3			
Barley[c]	18.3	12.1	9.0	1.3			
Isovalerate[b]	1.47	1.24	1.15	0.19	0.95	1.62	0.15
Valerate[a,b]	3.42	2.67	2.10	0.36	3.69	1.77	0.29

[a]Control vs Co ($P < 0.05$).

[b]Grain source effect ($P < 0.01$).

[c]Control vs Co ($P < 0.01$).

[d]Cobalt × grain interaction ($P = 0.07$).

[e]Grain source effect ($P < 0.05$).

[f]Cobalt × grain interaction ($P < 0.05$).

Table 6. Effects of dietary cobalt and grain source on plasma methylmalonic acid and glucose concentrations and plasma and ruminal succinate concentrations in steers

	Added Co, mg/kg				Grain source		
Item	0	0.05	0.15	SEM	Corn	Barley	SEM
Plasma MMA[a,b,c,d]	3.45	3.15	2.31	0.14	3.18	2.76	0.11
Plasma glucose[b,e,f,g]	4.31	4.37	4.45	0.03	4.48	4.26	0.03
Plasma succinate[a,d,g]	4.90	5.39	4.96	0.18	4.86	5.30	0.15
Ruminal succinate[a,d,h]	150.6	183.3	62.0	49.5	59.1	204.8	40.3

[a]Expressed as μmol/L.

[b]Control vs Co ($P < 0.01$).

[c]0.05 vs 0.15 mg Co/kg DM ($P < 0.01$).

[d]Grain source effect ($P < 0.05$).

[e]Expressed as mmol/L.

[f]Grain source effect ($P < 0.01$).

[g]Time effect ($P < 0.01$).

[h]0.05 vs 0.15 mg Co/kg DM ($P < 0.10$).

Table 7. Effects of cobalt concentration and grain source on carcass characteristics of finished steers

Item	Added Co, mg/kg				Grain source		
	0	0.05	0.15	SEM	Corn	Barley	SEM
Marbling[a,b]	5.5	5.5	5.4	0.13	5.6	5.3	0.11
Dressing percentage[c,d]	56.9	57.5	57.6	0.30	58.4	56.2	0.20
Hot carcass wt, kg[d,e]	302.5	317.6	322.7	4.0	327.9	300.7	3.3
12th rib backfat, cm[d]	0.71	0.80	0.83	0.07	0.90	0.67	0.06
KPH[f], %	2.46	2.48	2.50	0.08	2.51	2.45	0.07
USDA yield grade[d]	2.41	2.46	2.71	0.10	2.69	2.36	0.08
LMA[g,h] cm^2	78.6	82.2	79.1	1.4	82.0	77.9	1.2
USDA quality grade[i]	17.1	17.1	17.0	0.2	17.2	17.0	0.1

[a]4=slight; 5=small; 6=modest.

[b]Grain effect ($P < 0.10$).

[c]Control vs Co ($P < 0.10$).

[d]Grain effect ($P < 0.01$).

[e]Control vs Co ($P < 0.01$).

[f]Kidney, pelvic and heart fat.

[g]Longissimus muscle area.

[h]Grain effect ($P < 0.05$).

[i]Select + =16; choice - =17; choice = 18.

CHAPTER 5

Influence of cobalt concentration on vitamin B_{12} production, and fermentation of mixed ruminal microorganisms grown in continuous culture flow-through fermenters, fed a high concentrate diet[1]

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[1]Use of trade names in this publication does not imply endorsement by the North Carolina Agric. Res. Serv. or criticism of similar products not mentioned

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ABSTRACT

An experiment was conducted to determine the effects of dietary concentrations of cobalt (Co) on vitamin B_{12} production and fermentation of mixed ruminal microbes grown in continuous culture fermenters. Four fermenters were fed 14 g DM/d of a corn-cottonseed hull-based diet. Dietary treatments were: 1) control (contained 0.05 mg Co/kg DM), 2) 0.05 mg supplemental Co/kg DM (as $CoCO_3$), 3) 0.10 mg supplemental Co/kg DM and 4) 1.0 mg Co/kg DM. Following a 2-d adjustment period, fermenters were sampled over a 3-d sampling period. This process was repeated two additional times for a total of three runs. Ruminal fluid vitamin B_{12} concentrations were affected by Co supplementation ($P < 0.01$) and treatment × day interaction ($P < 0.01$). By d 3, cultures fed the basal diet supplemented with 0.10 mg Co/kg had higher ($P < 0.05$) vitamin B_{12} concentrations than those supplemented with 0.05 mg Co/kg DM, and increasing supplemental Co from 0.10 to 1.0 mg/kg DM increased ($P < 0.01$) ruminal fluid vitamin B_{12}. Ruminal fluid succinate was affected ($P < 0.10$) by a treatment × day interaction. Cobalt supplementation to the control diet greatly decreased ($P < 0.05$) succinate in ruminal cultures on d 3 but not on d 1 or 2. Molar proportions of acetate, propionate, and isobutyrate, and acetate:propionate ratio were not affected by the addition of supplemental Co to the basal diet. However, molar proportions of butyrate, valerate, and isovalerate increased ($P < 0.05$) in response to supplemental Co. Cultures fed diets supplemented with Co had higher ($P < 0.10$) percentages of C18:0 fatty acids in ruminal cultures relative to the control, other long-chain fatty acids were not affected by Co supplementation. Methane, ammonia, pH and apparent digestibility were not greatly

affected by Co supplementation. These results indicate that fermenters inoculated with mixed ruminal microbes respond rapidly to Co supplementation which increased ruminal vitamin B_{12} and decreased succinate concentrations.

Key Words: Ruminal Cultures, Vitamin B_{12}, Succinate,

Introduction

Cobalt is required by ruminal microorganisms for the complex synthesis of vitamin B_{12} (McDowell, 2000). Various studies have determined that vitamin B_{12} is an important growth factor for some ruminal microorganisms (Tanner and Wolfe, 1998; Strobel, 1992) and is utilized by others in pathways that produce propionate (Chen and Wolin, 1981). In ruminal microbes, vitamin B_{12} functions as a cofactor for methylmalonyl-CoA isomerase which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA during propionate formation (Nagaraja et al., 1997). Adding supplemental Co to high-energy diets moderately deficient in Co increased ruminal fluid vitamin B_{12} concentrations and molar proportions of propionate concentrations in finishing steers,(Tiffany et al., 2003b). Feeding sheep diets severely deficient in Co caused an immediate (within 3 d) and dramatic increase in ruminal fluid succinate, and a decline in ruminal propionate concentrations (Kennedy et al., 1991). Tiffany and Spears (2004) found that ruminal fluid succinate was lower in steers fed diets supplemented with 0.15 mg Co/kg than in steers supplemented with 0.05 mg Co/kg after consuming a high concentrate finishing diet for 84 d. The present study was conducted to determine the effects of dietary Co on ruminal vitamin B_{12} production and fermentation of mixed ruminal microorganisms, cultured in continuous dual-flow fermenters.

Materials and Methods

Fermenter Conditions

Whole ruminal contents were collected from a nonlactating, ruminally-fistulated Holstein cow fed a diet consisting of fescue hay fed ad libitum, and 4.5 kg of cracked corn daily. Ruminal contents (approximately 4.0 L) were placed in preheated vacuum containers, transported to the laboratory, and strained through double-layered cheesecloth prior to fermenter incubation. Approximately 700 mL of the filtered ruminal fluid was placed into each of the four vessels. This study utilized all glass, closed system fermenters, which allowed continuous independent flow of liquid and particulate matter. Anaerobic conditions of the cultures were maintained by sealing the fermenter openings with rubber and providing a continuous flow of CO_2 (20.0 mL/min) to maintain a positive internal pressure.

Artificial saliva was prepared as described by Slyter et al. (1966), and delivered by precision pump at a flow rate of 0.73 mL/min. Over a 24-h period 1.1 L of artificial saliva was delivered to each ruminal culture to yield a fractional dilution rate of 6.8 %/h. Temperature of the cultures was maintained at 39ºC by a circulating water bath and ruminal contents were continually stirred at 10 rpm as described by Fellner et al. (1995).

Diets and Treatments

Feed totaling 14.0 g DM was added to each fermenter daily in equal portions delivered at 0800 and 1500 h. Dietary treatments were: 1) control (Table 1; corn-cottonseed hull-based diet containing 0.05 mg Co /kg DM, no supplemental Co), 2) control supplemented with 0.05 mg Co (as $CoCO_3$)/kg DM, 3) control supplemented with

0.10 mg Co/kg DM and 4) control supplemented with 1.0 mg Co/kg. The control diet was formulated to meet or exceed requirements for finishing beef cattle (NRC, 1996) with the exception of Co. Following introduction of ruminal fluid to the fermenters there was a 2-d stabilization period, when cultures were fed dietary treatments and pelleted alfalfa in a 50:50 or 75:25 ratio on d 1 and 2, respectively. Following the stabilization period, ruminal cultures were fed the dietary treatments exclusively for a 3-d period. The above procedure was repeated three times so that this experiment utilized four continuous culture fermenters on three separate 5-d runs (2-d adjustment period followed by a 3-d sampling period).

Sample Collection and Analytical Procedures

Feed samples for the determination of Co concentration were prepared using a microwave digestion (Mars 5™, CEM Corp., Matthews, NC) procedure described by Gengelbach et al. (1994). Prior to and 2 h post-feeding, 10 µL of headspace gas samples were collected from each fermenter using a gas tight syringe (Hamilton Co., Reno, NV) and analyzed for methane using gas chromatography (model CP-3800; Varian, Walnut Creek, Ca). At the same time pH of the cultures was recorded. Subsequent to thorough mixing of the ruminal cultures, 5 mL was removed and analyzed for VFA by GLC (model CP-3380; Varian, Walnut Creek, CA), and for NH_3-N using a colorimetric assay (Beecher and Whitten, 1970), 2-h post afternoon feeding on each sampling d. At the same time the 5 mL was obtained for VFA and NH_3-N analysis, two 1.0 mL aliquots of the mixed ruminal fluid were obtained for the determination of succinate and vitamin B_{12}. Ruminal fluid samples for the determination of ruminal succinate were prepared

according to the method of McMurray et al. (1986) using a modified GC method described by Tiffany et al. (2003a). Ruminal fluid was prepared for the determination of vitamin B_{12} as described by Tiffany et al. (2003b), and vitamin B_{12} was determined using a competitive binding radioimmunoassay kit (ICN, Costa Mesa, CA), in which nonspecific vitamin B_{12} binding proteins were removed by affinity chromatography. On the final day (d 3) a separate 5 mL sample was collected 2 h after the final afternoon feeding for analysis of long-chain fatty acids. Samples for the determination of long-chain fatty acids were methylated as described by Kramer et al. (1997) and analyzed for fatty acid composition by GLC.

Calculations and Statistical Analysis

Substrate used for VFA production (VFA apparent) was calculated according to stoichiometrical equations (Wolin, 1960; Eun 2002), and percent apparent digestibility was determined as described by Eun (2002). Statistical analysis of data was performed using the Proc Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The model for fermentation variables and vitamin B_{12} contained treatment, run, day and all possible interactions. The model for VFA and long-chain fatty acids contained treatment, run, and treatment × run interaction. Pre-planned orthogonal contrasts were utilized to detect differences among means. Comparisons made were 1) control vs all Co supplemented treatments, 2) 0.05 mg Co/kg vs 0.10 mg Co/kg, and 3) 0.10 mg Co/kg vs 1.0 mg Co/kg.

Results and Discussion

Ruminal Fluid Vitamin B_{12}

Vitamin B_{12} concentration in continuous cultures was affected by Co supplementation ($P < 0.01$) and there was a treatment × day interaction ($P < 0.01$; Table 2). Vitamin B_{12} concentrations in cultures increased as Co addition to the control diet increased from 0.05 to 0.10 mg/kg DM or from 0.10 to 1.0 mg/kg DM ($P < 0.05$ and 0.01, respectively). Cobalt supplementation to the experimental diet did not affect vitamin B_{12} concentrations on d 1 or 2. However, on d 3 cultures fed diets supplemented with Co had higher ($P < 0.01$) ruminal fluid vitamin B_{12} concentrations. Increasing supplemental Co from 0.05 to 0.10 or from 0.10 to 1.0 mg/kg DM increased ruminal vitamin B_{12} concentrations ($P < 0.05$ and 0.01, respectively) on d 3.

Recently, Tiffany et al. (2003b) determined that adding supplemental Co to a low Co diet (control diet contained 0.05 mg Co/kg DM) increased vitamin B_{12} concentration in ruminal fluid obtained from steers on d 84 of the finishing period. In that study, increasing supplemental Co from 0.10 to 1.0 mg/kg greatly increased vitamin B_{12} concentration of ruminal fluid. A subsequent study (Tiffany and Spears, 2004) found that vitamin B_{12} concentration in ruminal fluid was higher in steers supplemented with 0.15 mg Co/kg than for those fed the control diet, or the control diet supplemented with 0.05 mg Co/kg. The present work demonstrates that vitamin B_{12} production of mixed ruminal microbes grown in continuous culture fermenters increases substantially in response to increasing dietary Co. Previous in vitro experiments conducted by McDonald and Suttle (1986) utilized concomitant cultures of RUSITEC (Czerkawski and Breckenridge, 1977)

with inoculum obtained from sheep well supplemented with Co. They found that ruminal fluid vitamin B_{12} concentrations declined greatly between d 3 and 5 when the cultures were fed a hay diet very low (0.54 μmol/kg DM) in Co. In contrast, during the present study ruminal vitamin B_{12} concentration of cultures fed the control diet that was moderately deficient in Co (0.05 mg/kg DM) did not decline over the 3-d sampling period. However, ruminal vitamin B_{12} concentrations of the unsupplemented control were significantly lower than those fed diets supplemented with 0.10 or 1.0 mg Co/kg DM by d 3. These results indicate that microbial vitamin B_{12} production responds rapidly to Co supplementation in cultures fed high-energy corn-based diets, grown in continuous flow fermenters.

Ruminal Fluid Succinate

Ruminal fluid succinate concentrations (Table 2) were affected by Co supplementation ($P < 0.05$) and a treatment × day interaction ($P < 0.10$). Ruminal fluid succinate concentrations increased across all treatments ($P < 0.05$) from d 1 to 3, but the increase was dramatic for the unsupplemented controls on d 3 which had higher succinate concentrations ($P < 0.05$) than all other treatments. In some bacteria, methylmalonyl-CoA isomerase (mutase) works in the reverse metabolic direction than its mammalian counterpart (Bannerjee and Chowdhury, 1999). Early work by Swick and Woods (1960) elucidated the pathway of propionate to succinate through methylmalonyl-CoA in the bacterium *Propionibacterium shermanii*. Later, Allen et al. (1964) purified methylmalonyl-CoA isomerase from *Propionibacterium shermanii* and evaluated the enzymes kinetic properties. Tiffany et al. (2003b) reported that steers fed moderately Co-

deficient corn-based diets had plasma succinate concentrations higher than steers consuming Co-supplemented diets, which is consistent with increased succinate absorption from the rumen. A subsequent study (Tiffany and Spears, 2004) found that ruminal fluid succinate concentrations where higher in steers consuming a corn-based diet supplemented with 0.05 mg Co/kg DM than for those supplemented with 0.15 mg Co/kg DM. Previous observations of Strobel (1992) utilizing continuous and batch cultures of the rumen inhabitant *Prevotella ruminicola*, found that succinate concentrations increased in response to vitamin B_{12} limitation. When Kennedy et al. (1991) fed sheep a Co-sufficient (1.0 mg Co/kg DM) barley-based diet for over 2 mo, then abruptly switched to a severely Co-deficient (0.0042 mg Co/kg DM) diet, ruminal fluid succinate concentrations increased greatly. In that study, within 4 d of consuming the Co-deficient diet, ruminal succinate increased from approximately 0.13 to 50.4 mmol/L. The sharp and immediate increase in ruminal succinate concentrations observed in that study agree with the findings of the current study utilizing continuous culture fermenters. However, much higher ruminal succinate concentrations were observed by Kennedy et al. (1991). This probably relates in part to the much lower Co concentrations of the basal diet in that study compared to the present work (0.004 vs 0.05 mg/kg).

Ruminal Fluid VFA and Long-Chain Fatty Acids

Cultures fed the control diet supplemented with 0.10 mg Co/kg DM had lower ($P < 0.05$) total VFA concentrations than those supplemented with 0.05 or 1.0 mg Co/kg DM (Table 3). Molar proportions of acetate tended ($P < 0.12$) to be lower in cultures fed diets supplemented with Co (Table 3). However, increasing level of Co supplementation

from 0.05 to 0.10 or from 0.10 to 1.0 mg/kg DM did not affect acetate molar proportions. Propionate molar proportions, and acetate:propionate ratios were not affected by Co addition to the control diet. Kennedy et al. (1991) found that propionate concentrations in ovine ruminal fluid decreased substantially within 4 d of consuming a low Co diet. The lack of propionate response to supplemental Co in the current study when compared to the work of Kennedy et al. (1991) may be due to the higher Co concentration in the basal diet. Previous studies with steers (Tiffany et al., 2003a,b) fed low Co (approximately 0.05 mg/kg), high concentrate finishing diets reported a decrease in the molar proportions of ruminal propionate relative to those consuming Co-supplemented diets at 84 d. Results of those studies suggest that long-term consumption of high concentrate diets, moderately deficient in Co, may decrease microbial methylmalonyl-CoA isomerase activity, reducing the conversion of succinate to propionate. In contrast to those studies, molar percentages of propionate were not affected by supplemental Co in the present study, in spite of the observed increase in succinate. This can be explained in part by the existence of an alternate mechanism for propionate production that has been observed in the ruminal microorganisms *M. elsdenii* and *B. ruminocola* (Baldwin et al., 1963; Baldwin and Allison,1983). The acrylate pathway involves the conversion of lactate to acrylyl-CoA via phospholactyl-CoA and reduction of acrylyl-CoA to propionyl-CoA (Baldwin and Allison, 1983), thus eliminating the need for a vitamin B_{12}-dependent enzyme-catatyzed reaction.

Addition of supplemental Co to the control diets increased ($P < 0.05$) molar proportions of butyrate in continuous cultures (Table 3). In contrast, Tiffany et al.

(2003a,b) reported that steers fed high concentrate diets supplemented with Co had lower ruminal molar proportions of butyrate than unsupplemented controls. The high proportions of butyrate observed in the present study are more representative of in vivo butyrate proportions observed in ruminal fluid of steers during the growing phase of previous studies (Tiffany et al., 2003a,b). The growing diets of those studies contained a lower percentage of corn than the present study (48.8 vs 80.0 % DM respectively). The higher percentage of butyrate may be the result of a microbial population that did not fully adapt to the high grain diet fed during the present study. Isobutyrate molar proportions were not affected by addition of supplemental Co to the control diet. Molar proportions of valerate and isovalerate in ruminal cultures increased ($P < 0.05$) in response to Co supplementation of the control diet. Increasing supplemental Co from 0.05 to 0.10 mg/kg DM increased ($P < 0.05$) the molar proportion of isovalerate in ruminal cultures.

There were no major effects of Co supplementation on the percentages of long-chain fatty acids in continuous ruminal fluid cultures (Table 4). However, cultures fed the control diet supplemented with Co had a higher ($P < 0.10$) percentage of C18:0 fatty acids than those fed the control diet. The increased percentage of C18:0 fatty acids in mixed cultures fed diets supplemented with Co suggests that Co alters biohydrogenation by rumen microbes. However, the mechanism is unclear given that concentrations of C18:1 and C18:2 fatty acids were not affected by dietary Co.

Methane, Ammonia, pH, and Apparent Digestibility

Methane production and ammonia concentration in continuous ruminal cultures were not affected by the addition of supplemental Co to the basal diet (Table 5). Ruminal fluid pH was affected by Co supplementation ($P < 0.10$), however the effect was not consistent with increasing dietary additions of Co to the control diet (Table 5). Ruminal fluid pH was similar between cultures supplemented with 0.05 or 1.0 mg Co/kg DM but tended ($P < 0.05$) to be higher in cultures supplemented with 0.10 mg Co/kg DM. Previous research by Jenkins et al. (2003) utilizing continuous flow fermenters determined that a diet with a 70:30 concentrate to forage ratio (concentrate portion contained 70 % corn and 27 % soybean meal DM) resulted in a ruminal fluid pH of 6.14. Other research with continuous ruminal cultures found that when dietary corn was increased from 12.6 to 54.6 % DM in gammagrass silage-corn based diets that the pH dropped from 6.1 to 5.9 (Eun, 2002). As expected the range of pH in the present work (5.43 to 5.59) was lower than that observed in these studies, reflecting the higher percentage of concentrate that provides a rapidly fermentable substrate.

Apparent DM digestibility of the control diet did not differ from diets supplemented with Co (Table 5). However, mixed ruminal cultures supplemented with 0.10 mg Co/kg had lower apparent digestibility than cultures fed diets supplemented with either 0.05 or 1.0 mg Co/kg DM. This effect corresponds to the lower concentrations of acetate and propionate in the ruminal fluid of the cultures supplemented with 0.10 mg Co/kg, and to acetate and propionate concentrations being part of the calculation used to calculate apparent digestibility. Previously, when 5.0 or 10.0 mg Co/kg were added to

substrates (alfalfa, orchard grass or corn) adequate in Co, and fermented in vitro for 24 or 48 h, supplemental Co did not affect digestibilites of OM, DM, or NDF (Hussein et al., 1994). However, that study supplemented Co at concentrations well above current recommendations (0.10 mg Co/kg DM; NRC 1996) and may not be comparable to the current study. Other studies found that adding supplemental Co to diets of sheep, not deficient in vitamin B_{12}, had no effect on digestibility (Smith and Marston, 1970a,b). Further research is warranted to determine the effects of Co supplementation at physiological concentrations on digestibilities.

Implications

The results of this study suggest that diets marginally deficient in Co result in a rapid increase in succinate concentrations in ruminal fluid of mixed microbes of bovine origin grown in continuous dual flow fermenters. Furthermore, the increase in succinate can be linked to a decline in microbial methylmalonyl-CoA isomerase activity since succinate concentrations decreased with the addition of supplemental Co to the basal diet. In addition, Co supplementation to diets that are marginally Co-deficient, results in a rapid increase in ruminal vitamin B_{12} production.

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Table 1. Ingredient composition of the basal diet

Ingredient	Percent dry matter
Corn	80.00
Soybean meal	6.16
Cottonseed hulls	12.00
Urea	0.60
Calcium sulfate	0.40
Salt	0.20
Calcium carbonate	0.63
Vitamin premix[a]	0.01
Mineral premix[b]	+

[a]Contained per kilogram of premix: 26,432,000 IU of vitamin A, 8,811,000 IU of vitamin D, and 44,052 IU of vitamin E.

[b]Provided per kilogram of diet: 30 mg of Zn as $ZnSO_4$, 20 mg of Mn as $MnSO_4$, 10 mg of Cu as $CuSO_4 \cdot 5H_2O$, 0.05 mg of I as $Ca(IO_3)_2(H_2O)$, and 0.1 mg of Se as Na_2SeO_3.

Table 2. Effects of cobalt concentration on vitamin B_{12} and succinate concentrations in continuous cultures of ruminal microbes.

	Added Co, mg/kg DM				
Item	0	0.05	0.10	1.0	SEM
Vitamin B_{12}, pmol/mL[a,b,c,d]	1.02	1.18	1.66	2.33	0.13
d 1	0.53	0.53	0.65	0.66	0.10
d 2	1.16	1.11	1.30	1.58	0.14
d 3[a,c,d]	1.14	1.91	3.02	4.74	0.29
Succinate, µmol/L[e,f]	28.82	6.68	4.82	4.67	7.08
d 1	3.17	1.97	2.06	1.35	1.06
d 2	5.42	5.33	5.93	4.17	1.58
d 3[e]	77.87	12.74	6.50	8.48	21.62

[a] Control vs cobalt ($P < 0.01$).

[b]Treatment × day ($P < 0.01$).

[c]0.05 vs 0.10 mg Co/kg DM ($P < 0.05$).

[d]0.10 vs 1.0 mg/Co kg DM ($P < 0.01$).

[e]Control vs cobalt ($P < 0.05$).

[f]Treatment × day ($P < 0.10$).

Table 3. Effects of cobalt concentration on VFA concentrations and molar proportions in continuous cultures.

	Added Co, mg/kg DM				
VFA	0	0.05	0.10	1.0	SEM
Total, mM[a,b]	68.9	69.8	54.7	68.9	6.1
Individual, mol/100 mol					
Acetate	54.7	48.2	48.1	50.2	4.6
Propionate	32.2	34.1	31.9	31.0	5.6
Butyrate[c]	11.9	16.1	17.4	16.4	3.7
Valerate[c]	0.32	0.56	0.62	0.62	0.16
Isobutyrate	0.23	0.40	0.32	0.35	0.05
Isovalerate[a,c]	0.73	0.91	1.77	1.47	0.28
Acetate:propionate	1.95	1.57	1.53	1.81	0.46

[a]0.05 vs 0.10 mg Co/kg DM ($P < 0.05$).

[b]0.10 vs 1.0 mg Co/kg DM ($P < 0.05$).

[c]Control vs cobalt ($P < 0.05$).

Table 4. Effects of cobalt concentration on major fatty acids in continuous cultures of ruminal microbes.

	Added Co, mg/kg DM				
Fatty acid, g/100 g	0	0.05	0.10	1.0	SEM
C14:0	0.72	0.89	0.67	0.69	0.08
C16:0	16.96	16.87	16.35	17.05	0.48
C18:0[a]	18.74	20.18	19.97	21.87	0.70
C18:1 total	33.17	33.00	34.92	33.85	0.74
trans-C18:1	15.76	16.32	17.18	15.28	0.92
cis-C18:1	17.41	16.68	17.74	18.57	0.59
C18:2	17.21	18.48	18.72	18.10	2.27
Conjugated 18:2					
*cis*9,*trans*11	0.78	0.77	1.91	0.58	0.89
*trans*10,*cis*12	0.28	0.39	0.20	0.25	0.11
C20:0	0.38	0.81	0.36	0.53	0.19

[a]Control vs cobalt ($P < 0.10$).

Table 5. Effects of cobalt concentration on methane, ammonia, pH, and digestibility in continuous cultures of ruminal microbes.

	Added Co, mg/kg DM				
Item	0	0.05	0.10	1.0	SEM
Methane, mmol/d	10.3	10.7	12.4	13.4	1.9
Ammonia-N, mg/dl	22.8	22.8	23.2	23.0	1.3
pH[a,b,c]	5.58	5.43	5.59	5.44	0.03
Digestibility, %[d,e,f]	50.9	52.8	43.0	53.2	5.2

[a]Control vs cobalt ($P < 0.10$).

[b]0.05 vs 0.10 mg Co/kg DM ($P < 0.01$).

[c]0.10 vs 1.0 mg Co/kg DM ($P < 0.01$).

[d]Grams of feed used for VFA + methane per 100 g of feed added per day.

[e]0.05 vs 0.10 mg Co/kg DM ($P < 0.05$).

[f]0.10 vs 1.0 mg Co/kg DM ($P < 0.05$).

CHAPTER 6

Summary and Conclusions

Three experiments were conducted with a total of 240 Angus steers to evaluate response to supplemental Co in control diets moderately deficient in Co. Experiments 1 and 2 were designed to evaluate the effects of supplemental Co concentration and source on performance, vitamin B_{12} status, and plasma and ruminal metabolites of growing and finishing steers. During the growing phase (56 or 84 d for experiment 1 and 2, respectively) a corn-soybean meal-cottonseed hull (40 %)-based diet was fed. During the finishing phases a high concentrate (86 % corn and 7 % soybean meal) diet was fed. The experimental growing and finishing diets were formulated to meet or exceed current NRC requirements with the exception of Co (diets contained 0.04 and 0.05 mg Co/kg DM for experiment 1 and 2, respectively). Dietary treatments for experiment 1 consisted of 0, 0.05, 0.10 and 1.0 mg of supplemental Co/kg DM from $CoCO_3$ or 0.05 and 0.10 mg of supplemental Co/kg DM from Co propionate (CoPr). Treatments were similar for experiment 2 with the exception that the Co supplemented at 1.0 mg/kg was as CoPr instead of $CoCO_3$. Sixty Angus steers were utilized in experiment 1, and 120 Angus-cross steers in experiment two. Steers were housed in covered, slotted-floor pens for the duration of the studies.

Experiment 3 was designed to evaluate the effects of dietary Co on performance and vitamin B_{12} status of steers fed high concentrate corn or barley-based diets. The experimental diets contained 0.04 and 0.02 mg Co/kg DM for corn and barley-based diets, respectively. Experimental diets were fed to 60 Angus steers for for at least 140 d,

and steers were randomly assigned to treatments, in a 2 × 3 factorial arrangement with factors being, corn or barley-based diet, and supplemental Co added at 0, 0.05, or 0.15 mg/kg DM. Steers were individually fed and housed in covered slotted-floor pens for the duration of the experiment.

The final experiment was conducted to evaluate the effects of dietary Co on in-vitro fermentation and vitamin B_{12} production of mixed rumen microbes cultured in continuous culture flow-through fermenters. After a 2-d adjustment period, cultures were fed a high concentrate diet for three d. Dietary treatments were: 1) control (contained 0.05 mg Co/kg DM), 2) 0.05 mg supplemental Co/kg DM (as $CoCO_3$), 3) 0.10 mg supplemental Co/kg DM and 4) 1.0 mg Co/kg DM. The experiment consisted of three separate runs.

Results of the growing-finishing studies (experiment 1 and 2) determined that performance was not affected by dietary Co during the growing phase. This may be the result of decreased molar proportions of propionate produced in cattle fed growing diets lessening the demand for post-absorptive conversion of propionate to succinate, via vitamin B_{12}-dependent methylmalonyl-CoA mutase. An alternate hypothesis would suggest that vitamin B_{12} deficiency is slow to occur when diets are marginally Co-deficient due to extensive enterohepatic recycling and renal resorption of vitamin B_{12}.

Dietary Co increased performance during the finishing phase during both these studies and during the subsequent finishing study (experiment 3). The increased intake of steers receiving dietary Co during the finishing phase suggests that Co addition to diets, moderately deficient in Co, increases methylmalonyl-CoA activity and conversion of

propionate to succinate. Gain:feed was not affected by dietary Co in the initial study, however, in experiment 2 and the subsequent finishing study (experiment 3) dietary Co improved the efficiency of gain suggesting that the increased molar percentages of ruminal propionate in Co-supplemented steers was more effectively utilized as gluconeogenic precusor. In experiment 3 steers fed corn had higher ADG and gain:feed relative to those fed barley-based diets, however intake was not affected by grain source suggesting that these findings may relate in part to factors other than vitamin B_{12} availability. These factors include the lower ME of the barley-based diets relative to the corn-based diets due to the increased percentage of fiber in barley, and the decrease in propionate proportions observed in the barley-fed steers.

Increased plasma vitamin B_{12} concentrations in steers receiving dietary Co became significantly higher than controls by d 56 of the growing phase of both growing-finishing studies, and by d 56 of the finishing study and remained higher for the duration of these studies. The lack of dietary Co response on plasma vitamin B_{12} concentrations early in these studies bolsters the hypothesis that the failure of supplemental Co to affect performance during the growing phase of experiments 1 and 2 is due in part to efficient vitamin B_{12} conservation. During experiment 1, the quadratic increase in plasma vitamin B_{12} over the first 56 d of the finishing phase gave way to a linear increase over the final 56 days, and results of both growing-finishing studies determined that the large increase in plasma B_{12} observed when Co was supplemented at 1.0 mg/kg did not improve performance and had little effect on plasma MMA or succinate accumulation. It is likely that the excess vitamin B_{12} observed in the plasma of steers supplemented with 1.0 mg

Co/kg DM would be excreted in feces, and if concentrations exceed the binding capacity of renal receptors vitamin B_{12} would be excreted in urine.

Supplemental Co increased liver vitamin B_{12} concentrations of steers during the finishing phase in all experiments. The linear increase in liver vitamin B_{12} observed in experiment 1 suggests that the liver does have a storage capacity, however, this storage capacity may be limited since the large increase in plasma vitamin B_{12} observed when Co supplementation was increased from 0.10 to 1.0 mg/kg was accompanied by a much lesser percent increase in liver.

Cobalt supplementation increased in vivo (experiments 2 and 3) and in vitro (experiment 4) microbial vitamin B_{12} production and increasing supplementation from 0.01 to 1.0 mg Co/kg (experiment 2) greatly increased ruminal vitamin B_{12}. The results of the in vitro study demonstrated that microbial vitamin B_{12} production responds rapidly (within 3 d) to Co supplementation. Perhaps the most interesting finding occurred in experiment 3 where dietary Co increased ruminal vitamin B_{12} in steers fed corn but not barley-based diets. This observation is consistent with the lower plasma vitamin B_{12} in the barley fed-steers. This finding remains unexplained, but may indicate a shift in microbial populations away form starch fermenters toward cellulose digesters.

Ruminal fluid propionate increased in response to dietary Co when finishing diets were fed (experiments 1-3). This important finding indicates that moderately Co-deficient diets decrease microbial conversion of succinate to propionate via bacterial methylmalonyl-CoA mutase. However, it is noted that this affect may be lessened due to the existence of the acrylate pathway which provides an alternative route for bacterial

propionate production through lactate. Steers consuming the barley-based diets had much lower molar proportions of propionate relative to those fed corn. This is consistent with the higher starch and lower fiber content of corn which favor propionate production.

Plasma MMA concentrations decreased in response to dietary Co during the finishing periods of experiments 1 and 2 and during the finishing study (experiment 3). This indicates that Co supplementation increased the activity of methylmalonyl-CoA mutase in the liver and is consistent with the increased ruminal and plasma vitamin B_{12} concentrations observed in Co-supplemented steers. However, plasma MMA concentrations of cattle fed conrol diets did not approach the high concentrations that have been reported in the plasma of sheep during previous studies. This may relate to several factors. The diets fed to sheep in previous studies were much lower in Co which would have a greater effect on methylmalonyl-CoA mutase activity due to decreased vitamin B_{12}. There is also the suggestion that the demand for vitamin B_{12} is greater in sheep than cattle because wool production may increase the need for sulfur amino acids, particularly methionine which is synthesized via cobalamin-dependent methionine synthase.

Plasma (experiments 2 and 3) and liver (experiment 3) folate concentrations increased in response to increasing dietary Co. Although these increases were small these findings suggests that diets moderately deficient in Co decrease the conversion of 5-CH_3-tetrahydrofolate to its active form tetrahydrofolate via the vitamin B_{12}-dependent enzyme methionine synthase. Folate concentrations were also affected by grain source in experiment 3 with steers fed barley-based diets having lower plasma and liver folate

relative to those fed corn-based diets. These findings are consistent with the lower vitamin B_{12} concentrations of steers fed barley relative to those fed corn. The degree to which dietary folate concentration affected these results is not clear since dietary folate was not quantified, however, it is generally assumed that most of the water soluble vitamins of dietary origin are degraded in the rumen.

Cobalt supplementation resulted in a small but significant increase in plasma glucose relative to controls in all three animal experiments. These findings are consistent with the increased propionate molar percentages observed in Co supplemented steers relative to the controls. It is also possible that the increased ruminal propionate, when absorbed, is more efficiently converted to gluconeogenic precursors via methylmalonyl-CoA mutase in Co-supplemented steers, this would agree with previous studies which found that Co-deficient sheep had lower plasma propionate clearance rates than Co-supplemented sheep. When steers were supplemented with CoPr they had higher plasma glucose at several times than those supplemented with $CoCO_3$. This likely relates to increased ruminal propionate observed in CoPr supplemented steers particularly in the growing phase, not vitamin B_{12} which was relatively unaffected by Co source.

In general carcass characteristics were not greatly affected by Co supplementation. However, dietary Co increased final BW, and hot carcass weights of steers in all three studies and increased dressing percentage in experiments 2 and 3. The lower carcass weights and dressing percentages of control steers is consistent with the lower ADFI, ADG, and gain:feed observed in these animals. Steers supplemented with 0.05 mg/kg (experiment 1) or 0.10 mg/kg (experiment 2) as CoPr had less backfat than

those supplemented with equal amounts of $CoCO_3$ but the explaination for this effect can not be explained by the concentrations of metabolites evaluated in this study.

In conclusion, vitamin B_{12} production responds rapidly to Co supplementation, and Co supplementation improves overall vitamin B_{12} status based on vitamin B_{12} concentrations in rumen, plasma and liver as well as other metabolites. Feeding corn vs barley-based diets resulted in differing responses to Co supplementation and further research may reveal that Co requirements differ based on the type of diet fed. Based on these results it is estimated that the total dietary Co requirement for finishing cattle fed corn-based diets is 0.15 mg/kg DM.