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Patrick D. French

An experiment was conducted to determine the effect of dietary copper (Cu) on Cu status and lipid metabolism in Holstein and Jersey cattle. Eight Jersey and 8 Holstein cows in mid-lactation were blocked by days in milk (169 ± 37) and assigned at random to one of two diets. Cows received either a control diet (Cu-) which contained a basal level of 8 mg Cu/kg DM or a treatment (Cu+) diet that was supplemented with 16 mg Cu/kg DM from $CuSO_4$ for a total of 24 mg Cu/kg DM. The experimental design was a 2 by 2 factorial arrangement of treatments with breed (Jersey or Holstein) and Cu supplementation (0 or 16 mg Cu/kg DM) as main effects. As expected, DM intake and milk yield were greater for Holsteins and milk fat and protein percentages were greater for Jerseys. $C_{18:0}$ was lower for both Cu+ treatment groups. $C_{18:0}$ is a precursor in the production of conjugated linoleic acid (CLA). On day 90, Cu- cows produced more CLA ($C_{18:2c9t11}$) than Cu+ cows, and Cu+ cows produced more total saturated fatty acids than Cu- cows.

THE EFFECTS OF COPPER SUPPLEMENTATION AND BREED ON MILK FATTY ACID PROFILE

by
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Jennifer M. Sumner, Author

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CHAPTER 1

INTRODUCTION

The ability to alter the fatty acid profile to increase beneficial fatty acids and decrease the secretion of detrimental fatty acids in milk has implications for human health. Bell et al. (1997) reported that short and medium chain fatty acids are digested and absorbed faster and are oxidized preferentially to long chain fatty acids. According to Ney (1991) short and medium chain fatty acids do not raise blood cholesterol levels and do not contribute significantly to body adipose tissue. However, conjugated linoleic acid (CLA; cis-9, trans-11 $C_{18:2}$), a long chain fatty acid, has been shown to have health promoting properties. MacDonald (2000) reported that CLA has anticarcinogenic activity, cholesterol lowering properties, impacts on fat metabolism, and positive effects on bone health.

Differences in the fatty acid composition of milk produced by Jersey and Holstein cattle were first reported more than 35 years ago (Stull and Brown, 1964). More recently, White et al. (2001) confirmed that Jerseys and Holsteins produce milk with different fatty acid composition. Preliminary work by Medrano et al. (1999) shows differences between breeds in the activity of the enzyme stearoyl-

Copper is recognized as an essential mineral for ruminants. It is contained in a number of enzymes and proteins and its role has been defined for many physiological functions. Du et al. (1996) showed that Cu is metabolized differently by Holsteins and Jerseys. However, National Research Council (2001) concluded that current information did not warrant different Cu requirements for Jersey cattle in the most recent revision of Nutrient Requirements of Dairy Cattle.

Research at the University of Kentucky has shown that total dietary Cu should be approximately 15 to 20 mg/kg DM for optimal immune function in lactating Holstein cows. Broaddus (2001) reported that somatic cell count was lower for Jerseys compared to Holsteins over a period of one year in a commingled herd. Immune function is generally considered the physiological process requiring the greatest level of Cu supplementation, being greater than maintenance and lactation. Therefore, Cu requirement for optimal immune function of Jersey cattle may be lower than Holsteins and similar dietary Cu for the two breeds as recommended by NRC (2001) may not be appropriate.

Copper may play a vital role in CLA synthesis and have particular importance for the Jersey breed. Thompson et al. (1973) reported increased liver stearoyl-CoA desaturase activity in pigs supplemented with Cu. The impetus of recent experiments was the hypothesis that Cu supplementation would increase CLA in milk. Engle et al. (2001) showed that Cu supplemented at 10 mg/kg DM (9 mg/kg DM in basal diet) decreased CLA content of milk from Holsteins. Morales et al. (2000) fed a Cu depleting diet or a diet supplemented with 20 mg/kg DM Cu to

Jersey and Holstein cows. Copper supplementation decreased milk CLA and CLA was lower in milk of Jerseys compared to Holsteins.

CHAPTER 2

REVIEW OF LITERATURE

Copper Distribution in Living Organisms

The overall Cu concentration in living organisms is somewhat uniform, approximately 2 mg/kg DM (μg/g) Cu (Owen, 1982b). There are a few exceptions such as in shellfish, ducks, and some microorganisms which have a greater concentration of Cu when compared to other species (Linder, 1991). In newborn and young animals, the concentration of Cu per unit of body weight is higher than in the adults (Cartwright and Wintrobe, 1964; Cymbaluk et al., 1986; Weiner et al., 1974). In the comparison of 17 tissues from each of 14 newborn calves and two mature cows, Bingley and Dufty (1972) found that the Cu concentration of all tissues except eye, tongue, skin, bone and liver are comparable.

The distribution of Cu among the tissues varies with species, age and Cu status of the animal (Owen, 1981; 1982b). Apart from the hair and nails, the highest concentrations of Cu in the adult human are in the kidney, liver, brain, heart and the skeleton (Owen, 1982a). The liver, brain, heart and hair contain the highest concentrations of Cu; the pancreas, skin, muscles, spleen, and bones have intermediate concentrations of Cu; and the pituitary, thyroid, thymus, prostrate gland, ovaries and testes contain low Cu concentrations (Davis and Mertz, 1987). In ruminants, the greatest Cu concentration is found in the liver. Generally, ruminants have a high capacity for hepatic storage. Of various tissues

analyzed for Cu in the adult bovine, the liver was found to contain approximately 80% of the total Cu (Bingley and Dufty, 1972).

Analysis of Copper Status

Copper was first suggested to be essential by McHargue (1925) in the early 1920's; however, conclusive evidence of the biological requirement for Cu was provided by Hart et al. (1928) working with anemic milk-fed rats. The anemia was not corrected either by iron supplementation alone or by liver extract alone, feeding iron and liver together caused a marked elevation in the hemoglobin and packed cell volume within 2 weeks. A bluish tinge in the ashed liver preparation was a clue to its Cu content and prompted Cu and iron supplementation to the rats. Their dramatic response in hemoglobin formation proved the essentiality of dietary Cu. Since that time Cu has proved to be a component in many essential enzymes.

The level of dietary Cu required for health is species dependent and is usually positively correlated with dietary levels of molybdenum and sulfur. Various data suggest that the Cu requirements for specific biological processes increase in the rat, for example, in order as follows: hemoglobin formation, growth, hair pigmentation, and lactation (NRC, 1980).

The liver is the primary site of Cu storage in the bovine. The liver is also the most common tissue analyzed in the determination of Cu status. Alvarez (1985) has described the U.S. Bureau of Standards reference sample of bovine liver which contains 157 µg/g Cu on a DM basis. Many factors, such as, species, age, chemical composition of the diet, and disease can affect the Cu concentration in

the liver. Most monogastric and poultry species contain between 10 and 50 µg/g of hepatic Cu on a DM basis. Ruminants have a higher storage capacity and/or capacity for excretion; and normal liver values range between 100 and 400 µg/g of Cu on a DM basis (Charmley and Symonds, 1985; Davis and Mertz, 1987). The bovine neonate stores Cu in the liver to a greater extent than the adult animal (Bingley and Dufty, 1972). In sheep, neonate liver Cu values are lower than in adults, and Cu concentration continues to increase throughout life. In most other species, including humans, neonate liver concentrations are higher than those found in adults (Linder, 1991).

Liver biopsy should give a dependable estimation of the Cu status of the animal due to the equal distribution of hepatic Cu between lobes. Buckley (1991) reported that the mean Cu concentration (mg/kg DM) of six Holstein cows in the left dorsal lobe was 421; right dorsal, 411; right ventral, 401; left ventral, 394; and caudal, 388. Hogan et al. (1971) found that good agreement was obtained between the mean Cu concentration for repeated biopsy samples of the same liver and that for replicated representative samples from the corresponding whole liver in sheep and swine. Chapman et al. (1963) found that the site of biopsy exerted a significant effect in Cu levels in liver biopsies, but that the magnitude of the range was only 36 mg/kg DM.

Most of the Cu in plasma is bound to ceruloplasmin. Plasma Cu concentration is maintained until liver Cu falls below 30 mg/kg DM. Once hepatic Cu falls below 20-30 mg/kg DM, a corresponding loss of Cu-dependent ceruloplasmin activity can be expected (Mills, 1987). It has been suggested that

plasma Cu and ceruloplasmin activity may be an appropriate indicator of Cu deficiency. However, low plasma Cu and low ceruloplasmin activity may become established months before clinical signs of Cu deficiency develop (Mills et al., 1976). To further compound the issue, both plasma Cu and ceruloplasmin activity are enhanced during the acute phase of many infections (Mills, 1987). In addition to its inadequacy in measuring Cu deficiency, plasma Cu is not a reliable indicator of Cu toxicity. Ishmael et al. (1971) reported that plasma Cu increased only days before hemolysis.

It has been suggested that superoxide dismutase (SOD) may be a more reliable indicator of Cu status in humans (Bennet et al., 1985), cattle (Masters et al., 1985), mice and rats (Paynter et al., 1979). In normal mammalian blood approximately one-half of the Cu is present in erythrocytes. Sixty percent of the Cu in erythrocytes is bound to SOD. Suttle and McMurray (1983) contend that SOD is more reliable because its activity is principally dictated by Cu status at the time of erythrocyte synthesis. During depletion and repletion with sheep and cattle, SOD declined and recovered more slowly than plasma Cu (Suttle and McMurray, 1983).

The Cu content of hair has been suggested as a possible aid in the determination of Cu status. The effect of Cu deficiency on keratinization and pigmentation of hair is well known. Kellaway et al., (1978) showed that, in cattle, Cu in hair and plasma decreased when the concentration of hepatic Cu fell below 20 mg/kg DM. However, Cu in the hair failed to show increases when the liver Cu was higher than 20 mg/kg DM. O'Mary et al. (1970) reported that dietary Cu levels

affected Cu concentrations in hair of Holstein and Hereford cattle. Van Koetsveld (1958) described that hair Cu concentrations below 8 mg/kg DM were associated with Cu deficiency. However, others have not observed changes in concentration of Cu in hair with changes in dietary or liver Cu concentrations. Increasing the Cu content of the diet resulted in higher hepatic Cu concentration and no change in hair Cu concentrations in Jersey heifers (Cunningham and Hogan, 1958). Although there was a marked effect by the addition of molybdenum to the diet, both liver and hair concentrations decreased. Cunningham and Hogan (1958) cited hair Cu values of approximately 9mg/kg. Similar values were also reported by Kellaway et al. (1978).

Metabolism

Absorbed Cu first enters the cells of the intestinal mucosa and then the blood. Some regulation of entry occurs at the serosal surface. In the blood plasma, Cu immediately binds to albumin and to transcuprein. These proteins deliver Cu to the liver and kidney, where Cu is first deposited. From there, some of the Cu enters the bile; some is used for the production of internally required liver proteins; and much of it is incorporated in ceruloplasmin for resecretion into the blood, Ceruloplamin is probably the main source of Cu for the other tissues, delivering it via specific receptors. It also serves as an extracellular scavenger for oxygen radicals to protect the outer parts of cell membranes, and it may facilitate iron transport, as well. Cu is utilized by almost all cells to form the intracellular enzymes

cytochrome *c* oxidase and superoxide dismutase. It also attaches to metallothiones. It is essential for several other enzymes, including extracellualr lysyl oxidase which cross-links elastic fibers and collagen and dopamine β-hyroxylase which is involved in catecholamine formation by the central nervous system and the adrenal medulla. It also has other less well understood functions in many tissues such as the brain and immune system. Small quantities of Cu are lost by various routes such as the urine. A larger amount in lost in the bile and it is in this form that most of the body Cu is secreted (Linder, 1991).

Absorption

In most animal species, Cu is poorly absorbed. In general, 10-15% of the Cu in the diet is absorbed the mature animals, while the young have a greater capacity of 15-30%. Absorption ranges from 26-75% in adult humans (Linder, 1991), 10-30% in rats Marceau et al., 1970), and 6.9-9.5% in dairy cattle (Buckley et al., 1985). The extent of absorption is influenced by the chemical form of the dietary Cu and the Cu status of the animal. Animals that are deficient in Cu absorb it more efficiently. Variability also exists in availability of Cu in supplements. Baker et al. (1991) found that the bioavailability of Cu as cupric oxide was near zero compared to Cu sulfate for the chick. Cupric oxide did not improve growth or increase liver Cu levels in swine (Cromwell et al., 1989). Cupric oxide was unable to increase hepatic Cu levels in dairy calves (Xin, 1990). Clark (1993) reported higher liver Cu levels when steers receive Cu sulfate as compared to cupric oxide. Liver Cu concentrations were similar for animals receiving cupric oxide or no

supplemental Cu. In addition, higher lever Cu levels were obtained by feeding either Cu sulfate or Cu proteinate compared to cupric oxide in Cu-deficient mature beef cattle. Cu chelates have a higher bioavailability that Cu sulfate in the presence and absence of high dietary iron and zinc respectively (Du, 1994).

It seems that Cu absorption can occur in all segments of the gastrointestinal tract, depending on the species. Significant absorption of Cu in the stomach of mice Van Barnefeld and Van den Hamer, (1984) has been recorded. Fields et al. (1986) failed to confirm this effect in rats. Starcher (1969) showed the majority of Cu absorption in the duodenum of the chick. In ruminants, the large intestine appears to the site of significant Cu absorption (Grace, 1975; Ivan and Grieve, 1976; and Turner et al., 1987).

There are at least two mechanisms by which Cu enters the body from the intestinal lumen (Bronner and Yost, 1985; Wapnir and Stiel, 1987). At low concentration almost all Cu is absorbed via a saturable carrier, while at higher concentrations increasing amounts appear to be absorbed by diffusion. Linder (1991) expresses concern over this assumption. The overall kinetic results are difficult to interpret and further work is needed in this area.

First, Cu passes across the brush border and enters the mucosal cells, likely in the form of Cu^{2+}. Second, it is transferred form the mucosal cells into the interstitial fluid and blood, across the basolateral surface. The uptake across the brush border is probably by simple diffusion and controlled by mass action (Fisher and L'Abbe, 1985). Once in the cell cytosol, some Cu becomes bound to metallothionein (Hall et al., 1979). Some Cu continues on to the basolateral

membrane, where it crosses to the interstitial fluid and blood by and energy-dependent, carrier-mediated, saturable process (Crampton et al., 1965; Fisher and L'Abbe, 1985). Virtually all transport at low doses entering the cell may be energy dependent. At higher doses, excess Cu crosses the basolateral surface by a mechanism that is not energy dependent and may involve diffusion down a concentration gradient (Linder, 1991).

Two points of Cu absorption regulation are metallothionein and the basolateral surface. Dietary zinc causes an increase in the production of metallothionein in the intestinal mucosa (Richards and Cousins, 1975). Metallothionein has a higher affinity for Cu that for zinc. Copper replaces the zinc and is bound so firmly that little remains to be transferred across the basolateral membrane (Linder, 1991). The Cu bond to metallothionein is lost due to the sloughing of mucosal cells into the intestinal lumen. In addition, Cu-Zn interactions suggest regulation at the basolateral surface. Acute studies were conducted to eliminate the effects of metallothionein. Increasing the amounts to zinc in lumen of rats inhibited Cu absorption and increasing the amounts of Cu in the lumen inhibited zinc absorption (Oestreicher and Cousins, 1985). Similarly, the transfer of zinc across the basolateral membrane was inhibited by excess Cu as wall as cobalt and iron in mice (Flanagan et al., 1984). Possibly a specific carrier for both Cu and zinc may exist, and excesses of either ion displaces the other. Alternatively, specific carriers for each ion may exist, creating transport interference when large excesses of either ion exists (Linder,1991).

Other endogenous factors, in addition to metallothionein, affect Cu absorption. Those that increase Cu absorption include: Cu deficiency (Schwartz and Kirchgessner, 1974), cancer (Cohen et al., 1979), and pregnancy (Davies and Williams, 1976). Factors that inhibit Cu absorption include estradiol treatment (Cohen et al., 1979) and Menkes' disease (Danks et al., 1972).

Together with zinc, many other important exogenous factors involved in Cu absorption exist (Linder, 1991). Both molybdenum and sulfur are important when considering ruminant animals. Sulfur is implicated because it can react with Cu to form Cu sulfide, which is insoluble and unabsorbable. Molybdenum and Cu are antagonistic to each other in the animal body (Ward, 1978). Excess molybdenum increases the requirement and the amount of Cu necessary to reach a toxic state; increased dietary Cu can reduce the toxic effect of molybdenum. Molybdenum and sulfur together influence Cu absorption. In the rumen, molybdenum and sulfur form thiomolybdates that chelate Cu and render it unavailable (Mason, 1986; Price et al., 1987; Suttle, 1991).

Transport

Several extensive studies have been conducted to determine Cu transport using $^{64}Cu^{2+}Cl_2$ or $^{67}CuCl_2$ (Owen, 1965, 1971; Marceau and Aspin, 1973; Campbell et al., 1981; Weiss et al. 1985). The data for blood Cu concentration displays a biphasic pattern. They showed an initial increase in labeled Cu after administration and then a drop in isotope labeled Cu 1-2 hours after administration,

followed by a resurgence of radioactivity at 4-6 hours. Liver and kidney show maximum concentrations at 2-4 hours. The radioactivity in blood plasma was noted immediately in the transcuprein fractions (Weiss et al., 1985). The decrease in radioactivity associated with these fractions was matched by an increase in isotope activity of the liver and kidney Cu. In addition, the specific activity of ceruloplasmin increases as that of the liver declines. It is apparent from the isotope studies that the liver and kidney are the organs most abundant in Cu and they absorb the majority of the Cu that enters the blood. Initially, blood (portal and peripheral) will contain newly absorbed Cu bound to albumin, amino acids, transcuprein, and a minute fraction as Cu^{2+} (Linder, 1991). The above sources are rapidly taken up by the liver hepatocytes. Ceruloplasmin is synthesized in the liver and secreted into the bloodstream (Goode et al., 1989). Ceruloplasmin is the major mode of transport for Cu from the liver to peripheral tissues.

Excretion

The routes by which metals can be secreted from the animal body are urine, bile, salivary, gastric, pancreatic secretions, direct intestinal secretion, sloughing of intestinal cells as well as sloughing of outside body surfaces and hair loss. In all species studied, the majority of ingested Cu appears in the feces (Davis and Mertz, 1987). The Cu that appears in the feces can be from any of these routes excluding urine, and outside surface loss. The secretion of Cu in the bile is the single largest source of Cu excretion. The mechanism by which man and animals maintain zero balance of Cu is via the controlled secretion of Cu into bile

(Evans, 1973; Underwood, 1977). However, all other excretory routes taken together are at least of equal importance as compared to excretion via bile (Linder, 1991). Except for the Cu in bile, most internally excreted bile in reabsorbed. Endogenously secreted Cu in bile is largely unavailable for reabsorption in the rat (Gollan, 1975).

Gooneratne et al. (1985) found a decrease in biliary Cu excretion with increasing Cu content of sheep diets. These results agree with those Grace and Gooden (1980). Biliary Cu excretion, mg excreted/24 hours, was similar regardless of dietary Cu absorbed. This indicates that sheep are unable to increase biliary Cu excretion when excess Cu is fed in the diet. It has been suggested that this makes sheep more susceptible to Cu toxicity.

The relative concentration of Cu in bile may differ between species. Phillippo and Graca (1983) found that the total loss of bile Cu in cattle is determined by liver concentration. Biliary Cu excretion accounts for less than half of the total Cu lost from the liver in cattle during Cu depletion (Phillippo and Graca, 1983).

Biological Functions of Copper

Copper is a component of many essential enzymes and it importance is through these enzymes. Most of the Cu in circulation is bound to ceruloplasmin. Its main functions are in the transport of Cu, antioxidant defense, and it is important in the conversion of Fe^{2+} to Fe^{3+}.

Cytochome c oxidase is another important Cu containing enzyme. It is the terminal enzyme of the electron transport chain and performs one of the most essential reactions in cells, the reduction of O_2 to H_2O. In concert with the other reactions of the electron transport chain this allows for ATP synthesis.

Cu/zinc superoxide dismutase protects intracellular components from the oxidative damage of superoxide anions. It catalyzes the following reaction: $2O_2^- + 2 H^+ \rightarrow O_2 + H_2O_2$.

Lysyl oxidase is necessary fro cross-linking of elastin and collagen in connective tissue. It catalyzes the oxidative deamination of the lysine ε-amino groups in elastin and collagen to form allysine. It is particularly active in immature connective tissue, most notably in the bovine odontoblast where it is responsible for the formation of bovine teeth.

Dopamine β-hydroxylase is a cuproenzyme in the brain. It is responsible for synthesis of the sympathetic neurotransmitter, norepinephrine and is necessary for epinephrine production. Dopamine β-hydroxylase catalyzes the reaction of hydroxyphenethylamine (dopamine) with O_2 to form norepinephrine and water.

Tyrosinase is a Cu containing enzyme located in melanocytes and is responsible for the initial formation of melanin. In mammals, melanin pigments are responsible for hair, skin and eye color. They are also necessary for the normal function of the eye.

Phenylalanine hydroxylase catalyzes the conversion of phenylalanine to tyrosine, Lack of the enzyme blocks the degradation of the substrate amino acid

and results in a high blood concentration of phenylalanine which can damage brain cells especially in the early part of life.

Tryptophan oxygenase is a liver enzyme that regulates the bulk degradation of typtophan to gluco and ketogenic products and to niacin.

Another Cu containing enzyme is α-amidating enzyme which is involved in peptide hormone amidation and diamine oxidase which is responsible for histamine and polyamine inactivation.

A relationship has been shown between Cu deficieny and depressed immune function (Koller et al., 1987; Prohaska, 1991). Neutrophils collected from Cu deficient steers were shown to have decreased killing capacity of phagocytized *Staphylococcuc aureas* (Xin et al., 1991). Impairment of immune function due to Cu deficiency may be a result of reduced Cu dependent enzymes (Linder, 1991). During infection, phagocytic cells produce large quantities of superoxide ions and can be detoxified by superoxide dismutase. Likewise, ceruloplasmin functions as an extracellular scavenger of free radicals.

It has been suggested that Cu has a unique effect on long chain fatty acid metabolism. Cunnane (1989) reported that Cu supplementation in the pig and rat and Cu deficiency in the rat mouse and human indicates that inadequate Cu intake or genetic Cu deficiency impairs the ability to monounsaturate long chain saturated fatty acids and that conversely Cu supplementation (>150 mg/kg diet) usually increases monounsaturated fatty acids. Also several studies in the rat suggest changes in long chain fatty acid metabolism other than desaturation may also be affected by Cu.

Copper Toxicity

A significant time period (weeks to months) is usually required for the development of chronic Cu toxicosis sign; however, their ultimate expression is so rapid that the fatal course appears to be caused by an acute process. Calves, fed Cu as Cu sulfate, at 115 mg/kg DM (Shand and Lewis, 1957) and 300 mg/kg DM (Weiss and Baur, 1968) for up to 129 days exhibited thirst, apathy, hemolytic crises, icterus, hepatic necrosis, and death. Adult cattle are believed to be more resistant to Cu toxicosis than younger cattle. Felsman et al. (1973) have fed 1.2-5g Cu sulfate (40-500 mg/kg DM) daily for up to 16 months to cattle older than 7 months without apparent effects even in pregnant animals. Kidder (1949), however, observed Cu toxicosis in a 227 kg steer fed 5 g of Cu sulfate per day for 122 days.

Cases of acute Cu toxicosis have occurred in accidental overdosing or accidental consumption of Cu-containing anthelmintics, foot baths, and fungicides. The signs of acute Cu toxicosis include nausea, salivation, violent abdominal pain, convulsions, paralysis, collapse and death. Necropsy reveals marked gastroenteritis, necrotic hepatitis, splenic and renal congestion and evidence of antemortem intravascular coagulation.

Bovine breed differences

Few studies exist on Cu metabolism breed differences in cattle. It has been reported that the estimated true digestability of Cu was lower in Simmental cows

compared with Angus cows (Chavez et al., 1990). Gooneratne et al. (1987) reported that across treatments the bile Cu excretion of the Simmental was at least twice that of the Angus breed. This may explain the increased incidence of Cu deficiency in Simmental in comparison to other breeds.

Gibson et al. (1987) reported that Jerseys had higher blood Cu concentrations than Friesians over two lactations. Likewise, Freisians from 12-72 weeks of age had lower mean blood Cu concentrations than Jerseys of the same age (Gibson et al., 1986). In addition, Jersey cattle had a faster accumulation of Cu in the liver and higher liver Cu compared with Holsteins, while both breeds received 80 mg/kg DM Cu/kg DM (Du, 1994).

Milk Fat Synthesis and Secretion

Origins of Milk Fat

Mammary epithelial cells of lactating animals are very active in the synthesis of triacylglycerols. The mammary tissue of an average mammal can produce 1-2 ml of milk per gram of mammary tissue with a fat content that can range between species from 2-600g/l (Clegg et al., 2001). The dairy cow, producing milk with between 3.3 and 4.7g lipid /100 g milk is in the middle of this range. The biochemical pathways by which lipid is acquired and synthesized in the mammary tissue of a lactating animal are the same as those of other lipogenic tissues. Thus, fatty acids are either synthesized de novo via acetyl-CoA carboxylase and fatty acid synthase or they are supplied exogenously. Exogenous fatty acids reach the mammary epithelial cells either as non-esterified

fatty acids or esterified as the acyl groups of the triacylglycerol component of lipoprotein particles.

Non-esterified fatty acids likely enter mammary epithelial cells via one or more binding proteins or translocators that facilitate their entry into cells. Plasma non-esterified fatty acids originate primarily by lipoolytic release from adipose tissue triacylglycerol stores; the fatty acids in these stores are elaborated, for the most part , by de novo synthesis. In ruminants, the principal precursor in this synthesis is acetate, whereas glucose is the principal precursor in monogastric animals.

In the case of lipoproteins as a source of exogenous fatty acids, their triacylglycerol core must first by hydrolyzed in the capillary lumen by lipoprotein lipase. The positional specificity of lipoprotein lipase for hydrolysis of fatty acids at the sn-1(3) position of lipoprotein tryacylglycerol ensure that the predominat fatty acid donated to milk are by lipoproteins are palmitate, stearate and oleate (Clegg et al., 2001). The fatty acids liberated by lipoprotein lipase are then taken up by the mammary epithelial cells. This is not a highly efficient process. Mendelson and Scow (1972) showed that up to 40% of the fatty acid liberated by the hydrolysis of lipoprotein triacylglycerol in a single pass through the mammary gland is not taken into tissue but emerges in the venous plasma. Once generated in the capillary lumen, it has been proposed that the fatty acids molecule diffuse laterally in the outer leaflet of the plasma membrane phospholipids bilayer of capillary endothelial cells, crossing to adjacent cells at points of cell contact and ultimately reaching the basal membrane of the mammary epithelial cells. A gradient of fatty acid

concentration is required to impose directionality on this diffusion. To create such a gradient, it is necessary to postulate the presence of a fatty acid translocator or binding protein, assisting passage of fatty acids across the basal domain of the plasma membrane of mammary epithelial cells (Clegg et al., 2001). Two candidate fatty acid translocators are known to be present on the surface membrane of mammary epithelial cells. One is a fatty acid binding protein known is earlier literature as MGDI (mammary-derived growth inhibitor); (Brandt et al., 1988). The other is FAT/CD36.

There are significant differences between ruminant and monogastric animals when considering the origin of fatty acids that make up lipoprotein triacylglycerol. These differences result from differences in digestive physiology and hepatic biochemistry. Triacylglycerol-rich lipoproteins arise from the gut (chylomicrons and VLDL) and from the liver (VLDL) and, in both cases, their fatty acid profile is a reflection of the fatty acid composition of the diet. In ruminant animals, the lipoproteins secreted by the enterocytes (chylomicrons and intestinal VLDL) are very minor constituents of plasma lipoproteins because of the low lipid content of forage based diets. In the livers of monogastric animals it has been shown that exogenous fatty acids are channeled preferentially within the hepatocyte towards esterification and packaging as secreted VLDL triacylglycerol (Zammit, 1996). There is no evidence that the same system does not function in the ruminant liver. In monogastric animals the majority of the hepatic uptake of fatty acids originates from the diet via intestinal VLDL which drains first into the lymph system and then into peripheral circulation. When dietary fatty acid is

unavailable, hepatic de novo synthesis partially compensates. In ruminant animals, little fatty acid reaches the liver from the diet and hepatocytes lack the enzyme capacity for de novo synthesis. Thus the major source of ruminal hepatic fatty acid output is plasma non-esterified fatty acids. This is the reason that unlike in monogastric animals, ruminants have much smaller differences in the fatty acid compositional profiles between lipoprotein triacylglycerol and plasma non-esterified fatty acids.

Of the small quantity of fatty acid entering the gastrointestinal tract of ruminant animals distal to the rumen, the predominant molecular species to be taken- up into enterocytes is stearic acid. Enterocytes have a significant level of stearoyl CoA desaturase activity, and some conversion of stearate to oleate takes place before esterification and packaging into intestinal VLDL and chylomicrons (Clegg, 2001).

Milk Fat Secretion

There are several hypothetical pathways that have been proposed for milk lipid droplet transportation and secretion. Microlipid droplets are formed in the rough endoplasmic reticulum. They can fuse with each other or other cytoplasmic lipid droplets as they are transported to the apical cytoplasm. Alternatively, many microlipid droplets are directly transported to the apical cytoplasm with no accretion in size. Lipid droplets may be secreted form the apical plasma membrane either as microlipid droplets or larger cytoplasmic lipid droplets. Under

some circumstances lipid droplets can be secreted after secretory vesicles surround cytoplasmic lipid droplets and progressively fuse with each other to form intracytoplasmic vacuoles. These vacuoles are presumed to be transported to the apical surface and the contents released by exocytosis. A combination of both apical and secretory vesicle routes is likely (Mather and Keenan, 1998). Casein and other milk proteins are processed through secretory pathways and are secreted with the aqueous phase of milk by either compound or simple exocytosis from secretory vesicles ate the apical plasma membrane of mammary epithelial cells.

Milk Fat Composition

Lipids (3-5%) occur as globules emulsified in the aqueous phase (87%) of milk. The globules contain nonpolar or core lipids such as triacylglyhcerol, cholesteryl esters, and retinol esters (Jensen and Newberg, 1995). They are coated with bipolar materials, phosoholipids, proteins, cholesterol, enzymes, etc. into a loose layer called the milk lipid globule membrane. This membrane prevents the globules from coalescing and acts as an emulsion stabilizer.

The factors listed in Table 1 influence the lipid contents of milk from individual cows (Palmquist et al.,1993). However, production and processing practices eliminate most of these. The current tendency is to select and breed for low-fat milks, e.g., Holsteins versus Guernseys. Colastral, late and mlks from

mastitic of diseased cows and those treated with antibiotics are excluded and pooling of the remaining milk occurs.

Table 1. Factors associated with variation in milk fatty acid profile.

Animal	Feed
Genetics	Grain intake
Stage of lactation	Amount and composition of dietary fat
Ruminal Fermentation	Dietary protein intake
Udder Infections	Energy intake
Use of BST	Seasonal and regional effects

Adapted from Jensen (2001).

The average composition of milk as reported by Bitman and Wood (1990) lipids is given in Table 2. This data was obtained by densitometric analysis of separated milk lipid on thin layer chromatography plates. In processed milks the amounts of lipids will be similar to this data.

Table 2. Lipids found in milk.

Lipid Class	% of total lipid
Phospholipid	1.11
Cholesterol	0.46
Triacylglycerol	95.8
1, 2-Diacylglycerol	2.25
Free fatty acids	0.28
Monoacylglycerol	0.08
Cholesteryl ester	0.02
Hydrocarbons	Trace

Adapted from Bitman and Wood (1990).

Triacylglycerol is the predominant class of lipid present in milk. The composition of triacylglycerol is defined in terms of the kinds and amount of fatty acid present. The structure of the triacylglycerol influences the action of lipolytic enzymes and, therefore, absorption. The structure of milk triacylgycerol is

responsible for the melting points, crystallization behavior, and the properties of milk fat as globules (Jensen, 2002). As shown in Table 3 bovine milk contains 12 fatty acids in amounts greater than 1%.

Table 3. Major fatty acids found in bovine milk.

Fatty Acid common name	Fatty Acid carbon number	Average range (weight %)
Butyric	4:0	2-5
Caproic	6:0	1-5
Caprylic	8:0	1-3
Capric	10:0	2-4
Lauric	12:0	2-5
Myristic	14:0	8-14
Pentadecanoic	15:0	1-2
Palmitic	16:0	22-35
Palmitoleic	16:1	1-3
Margaric	17:0	0.5-1.5
Stearic	18:0	9-14
Oleic	18:1	20-30
Linoleic	18:2	1-3
Linolenic	18:3	0.5-2

Adapted from Kaylegian and Lindsay (1995).

Milk fatty acids and human health benefits

Milk fat has been identified as a potentially unhealthy fat due to its cholesterol content and primarily saturated fat content. However, different types of dietary saturated fats do not have equivalent effects on plasma cholesterol levels compared to polyunsaturated fats. Research indicates that the hypercholesterolemic effect of saturated fats in human diets is largely due to 12, 14, and 16 carbon chain length fatty acids. Evidence suggests that stearic acid

(C18:0) is as effective as oleic acid (C18:ln-9) in lowering plasma cholesterol when it replaces palmitic acid (C16:0) in the diet of men. Milk fat has a unique fatty acid profile as it contains approximately 10% short and medium chain fatty acids, or those having fewer than 12 carbons, and 35% of total fatty acids from stearic and oleic acids. This allows speculation that consumption of milk fat has health benefits. Another health benefit comes in the form of specific fatty acids with potent health promoting properties.

Conjugated linoleic acid is a unique compound because it is a potent anticarcinogen, occurs in ruminant products naturally, and is potent at very low levels. The term conjugated linoleic acid and its acronym CLA refer generally to mixtures of positional and geometric conjugated dieonic isomers of linoleic acid. CLA is an intermediary product of the ruminal biohydrogenation of dietary lipids. The ruminal microbiological ecosystem is a dense (approximately 1000 cells per gram of digesta) and very complex community that includes bacteria, protozoa, and fungi (Dhiman, 2000). The diversity in species and metabolic capabilities that exists in the rumen is believed to facilitate the utilization of a variety of feedstuffs by the host animal. Biohydrogenation is a component of rumen biochemistry believed to be catalyzed by rumen bacteria. The lipid fraction of the ruminant diet is derived primarily from forage and grain. When these feeds are consumed, the esterified plant lipids, or triglycerides in them, are quickly and extensively hydrolyzed to free fatty acids by microbial lipases. The unsaturated free fatty acids produced by these reactions are then rapidly hydrogenated to more saturated end products by the microorganisms of the rumen. The cis 9, trans 11, C18:2 isomer of

CLA, for example, is an intermediate product of the biohydrogenation of linoleic acid by the isomerase of *Butyrvibrio fibrisolvens* and other bacterial species (Dhiman, 2000).

CLA is found in ruminant animal products such as dairy products, beef, and lamb. It also can be found in lower levels in pork, and poultry that have been supplemented with CLA. The isomer of CLA found in the highest quantity in ruminant products is the cis 9, 11 trans isomer also called rumenic acid. Most CLA research has focused upon this isomer but other isomers have also been shown to have different biological activities. CLAs have been shown to have roles in the prevention of cancer, atherosclerosis, fat partitioning and metabolism, and bone health.

CLA and Carcinogenesis

In a 1996 National Academy of Science Publication it was concluded that conjugated linoleic acid is the only fatty acid shown to unequivocally inhibit carcinogenesis in experimental animals. There is a large and growing body of evidence indicating that free radicals and radical-mediated oxidation processes play a role in many pathological conditions, including cancer and atherosclerosis. Although the mechanism of action is not well understood, CLA has been shown to be an effective antioxidant *in vitro* and in animals.

Shultz et al. (1996) incubated human malignant melanoma, colorectal and human breast cancer cells for 12 days in a culture medium supplemented with varying concentrations of linoleic acid and CLA. CLA was shown to stimulate cell

growth initially but to inhibit after 8 and 12 days. CLA was inhibitory to cell growth at all concentrations and times tested. Cell growth inhibition by CLA was dose and time dependent. Pariza and Hargrave (1985), first reported that topical applications of a partially purified extract from grilled ground beef 5 minutes before endotoxin (7,12-dimethylbenzanthracene) treatment reduced the number of papillomas per mouse as well as the number of mice with papillomas. The anticarcinogenic factor was then isolated and identified as mixture of CLA isomers. Ip et al. (1991) reported that 1% CLA in the diet suppressed mammary carcinogenesis in rats given a high dose of endotoxin, the effect being independent of the level or type of fat in the diet. The fat was present at 10%, 13.3%, 16.7% or 20% by weight in the rat diet, representing the range of fat intake of the US diet (Figure 1).

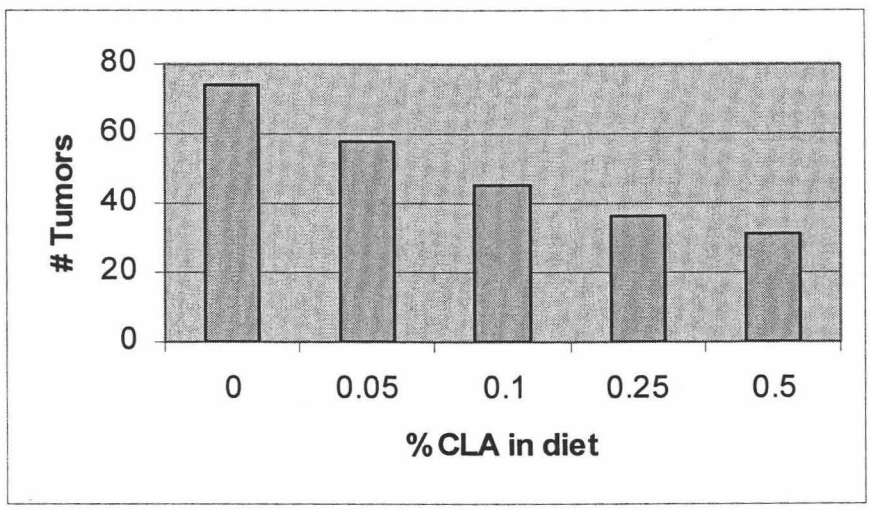

Figure 1. Response of mammary tumors in rats fed diets with varying levels of CLA (Adapted from Ip et al. 1991).

Visonneau et al. (1997) examined the effect of CLA on the growth of human breast adenocarcinoma cells in sever combined immunodeficient mice. Mice were fed a diet containing 1% CLA for two weeks prior to subcutaneous inoculation with cancer cells and throughout the study. Dietary CLA inhibited local tumor growth by 73% and 30% at nine and fourteen weeks post inoculation respectively. Moreover, CLA completely prevented the spread of breast cancer cells to lungs, peripheral tissue and bone marrow. These results indicate the ability of CLA to block the local growth and systematic spread of human breast cancer via mechanisms independent of the host immune system.

While no studies have examined the role of CLA in human treatment or prevention of cancer, epidemiologic evidence from Finland (Kneckt, 1996), demonstrated that an increase of milk consumption of two glasses per day decreased the risks of breast cancer. This study was done in Finland where milk consumption is already higher than that in the US. The increase in CLA intake that would result from American women substituting butter for margarine in their diets is nearly equivalent to the change in CLA intake examined in this study.

CLA and Atherosclerosis

Unlike linoleic acid there is very little research done on the effect of dietary CLA on plasma lipoproteins and aortic atherosclerosis. Lee et al. first tested whether CLA might affect the initiation and progression of atherosclerotic lesions in rabbits through its effect on lipid peroxidation. They reported that rabbits fed an atherogenic diet and supplemented with .5g CLA per day for 22 weeks had

significantly less plasma triglyceride, plasma LDL-cholesterol and a lower LDL-C/HDL-C ratio than control animals. CLA feeding also resulted in fewer aortic fatty lesions.

CLA's Effects on Fat Partitioning and Metabolism

Pariza et al. (1996) showed that CLA feeding is associated with a reduction in body fat mass and a slightly pronounced increase in lean body mass depending on the animal. Mice, rats, and chickens fed 0.5% CLA supplemented diet for four to eight weeks had body fat reductions of 57%, 70% and 22% respectively and lean body mass was increased by 5%, 14% and 3% respectively. These findings may have implications in the use of CLA as a feed additive to produce leaner animals and perhaps for weight loss in humans. In humans, Atkinson conducted a double blind placebo trial with 20 healthy volunteers ages 27-28, lean to modestly obese. CLA dose given was 3.6g/day. In a three-month period of study, the CLA groups experienced a 1.6 lb body weight loss and a 20% reduction of body fat while the control groups gained 1.4 lbs body weight.

CLA and Bone Health

Most studies show and increase in the percent of ash when CLA is fed to chicks. This effect is presumed to be due to protection conferred by CLA on bone loss. An increase in cytokines increases bone loss and CLA appears to counter the effect of cytokines. Rodents fed butter had a greater trabecular bone formation

(most important to prevent osteoporosis) than animals fed vegetable oil (MacDonald, 2000).

Conjugated linoleic acid is unique because unlike most naturally occurring anticarcinogenic substances found mainly in plants, it is present in food from animal sources. The only other animal source of fatty acids with similar properties is fish oil. The major difference between the two is that it is extrapolated that to get the full benefit from fish oil it would have to be consumed at levels close to 10% of the diet. CLA, however would show positive benefits at levels close to 3.5g for a 70kg person. This is significantly higher than the average consumption of 1g/person/day in the US diet but is not unrealistic. It has been shown that CLA content in foods can be manipulated with animal feeding programs and food processing techniques.

Copper, Breed, and Milk Fatty Acid Profile

Du et al. (1996) showed that Cu is metabolized differently by Holsteins and Jerseys. Jersey cows and heifers had higher hepatic Cu concentrations at similar rates of supplementation and were found to increase hepatic Cu at a more rapid rate than Holsteins. This may indicate that due to an increased sensitivity to Cu, Jersey cattle should receive a lower level of supplementation.

Differences in fatty acid composition of the milk produced by Jersey and Holstein cattle were reported more than 35 years ago (Stull and Brown, 1964).

More recently, White et al. (2001) confirmed that Jerseys and Holsteins produce milk with different fatty acid profiles.

Jersey milk was higher for short and medium chain fatty acids. Whereas, Holstein milk contained higher proportions of CLA and other long chain fatty acids. This is shown in Table 4. Morales et al. (2000) showed that Jerseys receiving tallow had higher CLA than Holsteins, but the difference was reversed for cows receiving whole soybeans. Preliminary work by Medrano et al. (1999) shows differences between breeds in the activity of the enzyme stearoyl-CoA desaturase, which is present in the mammary gland. Stearoyl-CoA desaturase oxidizes palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids to palmitoleic ($C_{16:1}$) and oleic ($C_{18:1}$) acids and is involved in CLA production (Medrano et al., 1999)

Table 4. Least squares means for fatty acid composition for each treatment and breed group.

Fatty Acid	Confine Holstein	Pasture Holstein	Confine Jersey	Pasture Jersey	SEM	Treatment	Breed	Interaction
	% of total milk fat					*P* <		
C4:0	1.05	1.06	1.09	1.08	0.04	NS	NS	NS
C6:0	1.47	1.56	1.71	1.74	0.05	NS	0.01	NS
C8:0	0.92	1.00	1.17	1.23	0.04	NS	0.01	NS
C10:0	2.00	2.22	2.68	2.89	0.10	0.01	0.01	NS
C12:0	2.34	2.65	3.14	3.48	0.12	0.01	0.01	NS
C14:0	9.43	10.22	10.44	11.47	0.24	0.01	0.01	NS
C14:1	0.59	0.80	0.60	0.82	0.04	0.01	NS	NS
C16:0	31.67	31.19	31.32	31.53	0.60	NS	NS	NS
C16:1	1.12	1.25	1.00	1.07	0.04	0.01	0.01	NS
C18:0	15.36	13.35	15.47	13.45	0.49	0.01	NS	NS
C18:1	23.28	23.09	20.87	19.44	0.53	NS	0.01	NS
C18:2	2.49	1.82	2.49	1.86	0.08	0.01	NS	NS
CLA	0.41	0.72	0.32	0.59	0.05	0.01	0.03	NS
C18:3	0.38	0.71	0.37	0.75	0.02	0.01	NS	NS
C20:0	0.17	0.13	0.17	0.11	0.01	0.01	NS	NS

Adapted from White et al. (2001).

Copper may play a vital role in CLA synthesis and have particular importance for the Jersey breed. Thompson et al. (1973) reported increased liver stearoyl-CoA desaturase activity in pigs supplemented with Cu. The impetus of recent experiments was the hypothesis that Cu supplementation would increase CLA in milk. Engle et al. (2001) showed that Cu supplemented at 10 mg/kg DM (9 mg/kg DM in basal diet) decreased CLA content of milk from Holsteins (Table 5).

Table 5. Effects of dietary Cu on milk fatty acid composition in Holstein cows.

	Added Copper, mg/kg DM			
Fatty Acid	0	10	40	SE
$C_{16:0}$	31.44	32.45	31.19	1.01
$C_{16:1cis}$	1.31	1.38	1.4	0.09
$C_{18:0}$	12.96	12.31	11.65	0.69
$C_{18:1trans}$	2.44	1.92	1.84	0.23
$C_{18:1cis}$	20.11	20.11	19.35	0.67
$C_{18:2}$	2.93	3.01	2.46	0.13
CLA	0.37	0.25	0.24	0.04

Adapted from Engle et al. (2001)

Morales et al. (2000) fed a Cu depleting diet or a diet supplemented with 20 mg/kg DM Cu to Jersey and Holstein cows. Copper supplementation decreased milk CLA and CLA was lower in milk of Jerseys compared to Holsteins. Results from Engle et al. (2001) and Morales et al. (2000) should be interpreted with

caution for two reasons. First, milk fat was depressed in each experiment. Engle et al. (2001) fed high concentrate diets and milk fat ranged from 3.0 to 3.1%. Morales et al. (2000) fed high fat diets and milk fat ranged from 2.5 to 3.0% for Holsteins and 3.7 to 3.9% for Jerseys. Both high concentrate, low fiber diets and high fat diets can lead to milk fat depression (McGuire and Griinari, 1999). Therefore, results of the effect Cu supplementation on milk fatty acid profile may not be applicable under practical applications. Second, the level of Cu supplementation may have been too high. Thompson et al. (1973) reported an initial increase in liver stearoyl-CoA desaturase activity with Cu supplementation, then activity declined with increasing Cu.

References

Alvarez, R. 1985. The use of NBS standard reference materials in validating trace element determinations in biological materials. In: *C.F. Mills, I. Brenner and J. K. Chersters (Ed.) Trace elements in Man and Animals (TEMA-5).* pp. 655-657. Commonwealth Agri. Bureaux, Farnham Royal, U.K.

Baker, D. H., J. Odle, M. A. Funk and T. M. Wielnad. 1991. Bioavailability of copper in cupric oxide, cuprous oxide and Cu-lysine complex. J. Poultry Sci. 70:177-179

Bennett, F. I., M. H. N. Golden, and D. D. Ramdath. 1985. Red cell superoxide dismutase as a measure of copper status in man. In: *C.F. Mills, I. Brenner and J. K. Chersters (Ed.) Trace elements in Man and Animals (TEMA-5).* pp. 578-580. Commonwealth Agri. Bureaux, Farnham Royal, U.K.

Bingley, J. B., and J. H. Dufty. 1972. Distribution of copper in the tissues of the bovine neonate and dam. Res. Vet. Sci. 13:8-14

Bitman, J. and D.L. Wood. 1990. Changes in milk phospholipids during lactation. J. Dairy Sci. 73:1208-1216

Brandt, R. M. Pepperle, A. Otto, R. Kraft, F.D. Boehmer, and R. Grosse. 1988. A 13-kilodalton protein purified from milk fat globule membrane is closely related to a mammary-derived growth-inhibitor. Biochemistry. 27:1420-1425

Bronner, F. and J. H. Yost. 1985. Saturable and nonsaturable copper and calcium transport in the mouse duodenum. Am. J. Physiol. 249:G108-G112

Buckley, W. T. 1991. A kinetic model of copper metabolism in lactating dairy cattle. Can. J. Anim. Sci. 71:155-166

Buckley, W.T., S. N. Huckin, and G.K. Eigendorf. 1985. Stable isotope tracer methods for determining absorption of dietary copper in dairy cattle. In: *C.F. Mills, I. Brenner and J. K. Chersters (Ed.) Trace elements in Man and Animals (TEMA-5).* pp. 339-341. Commonwealth Agri. Bureaux, Farnham Royal, U.K.

Campbell, C. H., R. Brown and M. C. Linder. 1981. Circulating ceruloplasmin is and important source of copper for normal and malignant cells. Biochem. Biophys. Acta. 678:27-38

Cartwright, G. E. And M. M. Wintrobe. 1964. Copper metaboliam in normal subjects. Am. J. Clin. Nutr. 14:224-232.

Chapman. H. L. Jr., D. H. Cox, C. E. Haines and G.K. Davis. 1963. Evaluation of the liver biopsy technique for mineral nutrition studies in beef cattle. J. Anim. Sci. 22:733-737.

Charmley, L. L., and H. W. Symonds. 1985. A comparison of the ability of cattle and pigs to clear excess copper from the plasma and excrete it in the bile. In: *C.F. Mills, I. Brenner and J. K. Chersters (Ed.) Trace elements in Man and Animals (TEMA-5).* pp. 341-343. Commonwealth Agri. Bureaux, Farnham Royal, U.K.

Chaves, E., J. C. Laurenz, L. W. Greene, F. M. Byers and G. T. Schelling. 1990. Apparent absorption of phosphorus, magnesium, zinc, and copper in two breeds of cows. Nutr. Abs. Rev. (Series B) 60:694.

Clark, T. W. 1993. A comparison of copper oxide and copper sulfate as a copper source for the mature ruminant, the effect of copper status on immune function in the mature ruminant. Thesis. University of Kentucky, Lexington, KY.

Clegg, R.A., M.C. Barber, L. Pooley, I. Ernens, Y. Larondelle, and M.T. Travers. 2001. Milk fat synthesis and secretion: molecular and cellular aspects. Livestock Prod. Sci. 70:3-14

Cohen, D. I., B. Illowsky and M. C. Linder. 1979. Altered copper absorption in tumor bearing and estrogen treated rats. Am. J. Physiol. 236:E309-E315.

Crampton, R. F., D. M. Matthews and R. Poisner. 1965. Observations on the mechanism of absorption of copper by the small intestine. J. Physiol. 178:111-126.

Cromwell, G. L., T. S. Stahly and H. J. Monegue. 1989. Effects of source and level of copper on performance on liver stores in weanling pigs. J. Anim. Sci. 67:2996-3002

Cunnane, S. C. 1989. Modulation of long chain fatty acid unsaturation by dietary copper. In: *Copper Bioavailability and Metabolism.* Constance Kies (ed.). Plenum Press. New York

Cunningham, I. J., and K. G. Hogan. 1958. The influence of diet on the copper and molybdenum contents of hair, hoof and wool. N. Z. J. Agr. Res. 1:841-846.

Cymbaluk, N. F., F. M. Bristol, and D. A. Christensen. 1986. Influence of age and breed of equid on plasma copper and zinc concentration. Am J. Vet. Res. 47:192-195

Danks, D. M., P. E. Campbell, B. J. Stevens, V. Maynes and E. Cartwright. 1972. Menkes' kinky hair syndrome. An inherited defect in copper absorption with widespread effects. Pediatrics. 50:188-201.

Davies, M. T. And R. N. Williams. 1976. Effects of pregnancy on uptake and distribution of copper in the rat. Proc. Nutr. Soc. (Abstr.) 34:4A-5A.

Davis, G. K., and W. Mertz. 1987. Copper. In: *W. Mertz (Ed.) Trace elements in Human and Animal Nutrition – 5th ed (Vol 1)*. Pp 301-364. Academic Press, Inc. New York

Dhiman, T.R. Conjugated linoleic acid: A food for cancer prevention. Feedstuffs. 24-32, 2000

Du, Z., R. W. Hemken and R. J. Harmon. 1996. Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. J. Dairy Sci. 79:1872-1880

Du, Z. 1994. Bioavailabilities of copper in copper proteinate, copper lysine and cupric sulfate, and copper tolerances of Holstein and Jersey cattle. Ph. D. Dissertation. University of Kentucky, Lexington, KY.

Engle, T. E., V. Fellner, and J. W. Spears. 2001. Copper status, serum cholesterol, and milk fatty acid profile in Holstein cows fed varying concentration of copper. J. Dairy Sci. 84: 2308-2313

Evans, G. W. 1973. Copper homeostasis in the mammalian system. Physiol. Rev. 53:535-570

Felsman, R. J., M. B. Wise, R.W. Harvey, and E.R. Barrick. 1973. Effect of added dietary levels of copper sulfate and an antibiotic on performance and certain blood constituents of calves. J. Anim. Sci. 36:157

Fields, M. J. Holbrook, J. Scholfield, A. S. Powell, A. J. Rose, S. Reiser, and J. C. Smith. 1986. Development of copper deficiency in rats fed fructose or starch: Weekly measurements in copper indices in blood. Proc. Soc. Exp. Biol. Med. 181:120-124

Fischer, P.W. F. and M. R. L'Abbe. 1985. Copper transport by intestinal brush border membrane vesicles from rats fed high zinc of copper deficient diets. Nutr. Res. 5:759-767

Flanagan, P. R., J. Haist, I. MacKenzie and L. S. Valberg. 1984. Intestinal absorption of zinc: Competative interactions with iron, cobalt, and copper in mice with sex-linked anemia. Can. J. Physiol. Pharmacol. 62:1124-1128.

Gibson, J. P., A. C. Field and G. Wiener. 1986. Concentration of blood constituents from 12-72 weeks of age in genetically high and low milk production lines of Frisian and Jersey cattle around calving and early lactation. Anim Prod. 44:183-199

Gollan, J. L. 1975. Studies on the nature of the complexes formed by copper with human alimentary secretions and their influence on copper absorption in the rat. Clin Sci. Mol. Med. 49:237-245

Goode, C. A., C. T. Dinh and M. C. Linder 1989. Mechanism of copper transport and delivery in mammals: Review and recent findings. *In: C, F, Mills, I. Bremner and J. K. Chessters (Ed.) Trace elements in Man and Animals (TEMA-5).* Pp. 342-345. Commonwealth Agric. Bureaux, Farnham Royal, UK.

Gooneratne, S. R., D. Christensen, R. Chaplin and A. Trent. 1985. Copper: Biliary excretion in copper supplemented and thiomolbdate-treated sheep. *In: C, F, Mills, I. Bremner and J. K. Chessters (Ed.) Trace elements in Man and Animals (TEMA-5).* Pp. 342-345. Commonwealth Agric. Bureaux, Farnham Royal, UK.

Gooneratne, S. R., J.V. Bailey, H.W. Symonds, and D.A. Christensen. 1987. Effect of dietary Cu, Mo, and S levels on bile and urine copper excretion in breeds of cattle. Can. J. Anim. Sci. 67:1188

Grace, N. D. 1975. Studies on the flow of zinc, cobalt, copper and manganese along the digestive tract of sheep given fresh perennial ryegrass, or white or red clover. Br. J. Nutr. 34:73-82

Grace N. D., and J.M. Gooden. 1980. Effect of increasing intakes of Zn, Cu, and Mn of their secretion via bile and pancreatic juice and their excretion in faeces and urine in sheep fed lucerne pellets. N. Z. J. Agr. Res. 23:293-298

Hall, A. C., B. W. Young and I. Bremner. 1979. Intestinal metallothionien and the mutual antagonism between copper and zinc in the rat. J. Inorg. Biochem. 11:57-66.

Hart, E. B., H. Steenbock, J. Waddell, and C.A. Elvehjem. 1928. Iron in Nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. J. Biol. Chem. 77:797

Hogan, K. G., D. F. Money and R. S. Walker. 1971. The distribution of copper in the liver of pigs and sheep and its effect on the value of chemical analysis made on biopsy samples. N. Z. J. Agric. Res. 14:132-141

Ip C, Singh M, Scimeca JA, Pariza MW: Mammary cancer prevention by conjugated diene derivative of linoleic acid. Cancer Res 51:6118-6124, 1991.

Ishmael, J., C. Gopinath and J. McC. Howell. 1971. Experimental chronic copper toxicity in sheep. Biochemical and haematological studies during the development of lesions in the liver. Red. Vet. Sci. 13:22-29

Ivan, M, and C. M. Grieve. 1976. Effects of zinc, copper, and manganese supplementation of high-concentrate ration on gastrointestinal absorption of Copper and manganese in Holstein calves. J. Dairy. Sci. 59:1764-1768.

Jensen, R.G. and D.S. Newberg. 1995. Bovine milk lipids. Pp. 543-575. In: *Handbook of Milk Composition.* R.G. Jensen (ed.). Academic Press. San Diego

Jensen, R.G. 2002. The composition of bovine milk lipids:January 1995 to December 2000. J Dairy Sci. 85:295-350

Kellaway, R.C., P. Sitorus and J. M. L. Leibholz. 1978. The use of copper levels in hair to diagnose hypocuprosis. Res. Vet. Sci. 24:352-357.

Kidder, R.W. 1949. Symptoms of induced copper toxicity in the steer. J. Anim Sci. 8:623

Knekt, P., R, Jarvinen, R. Seppanen, E. Pukkala, and A. Aromaa. 1996. Intake of dairy products and the risk of breast cancer. Brti. J. Cancer.73:687-691

Koller, L. D., S. A. Mulhern, N.C. Frankel, M.G. Steven and J. R. Williams. 1987. immune dysfunction in rats fed a diet deficient in copper. Am J. Clin. Nutr. 45:997-1006

Linder, M. C. 1991. *Biochemistry of Copper.* Plenum Press, New York

MacDonald, H. B. 2000. Conjugate linoleic acid and disease prevention: a review of current knowledge. J AM Col of Nutr 19:11-118

McGuire, M. A., and J. M. Griinari. 1999. Trans fatty acids: Is it the butterfat switch? Proc. Pacific Northwest Anim. Nutr. Conf. pp. 145

McHargue, J. S. 1925. The association of copper with substances containing the fat-soluble A vitamin. Am. J. Physiol. 72:583

Marceau, N. and N. Aspin. 1973. The intracellular distribution of copper derived from ceruloplasmin and albumin. Biochem. Biophys. Acta. 328:338-350

Marceau, N., N. Aspin, and Sass-Kortsak. 1970. Adsorption of copper 64 from gastrointestinal tract of the rat. Am. J. Physiol. 218:377-383

Mason, J. 1986. Thiomilybdates:Mediators of molybdenum toxicity and enzyme inhibitors. Toxicology. 42:99-109

Masters, H.G., G. M. Smith and R. H. Casey. 1985 The relationships between the activity of superoxide dismutase and the concentration of copper in the erythrocytes of suckling beef calves grazing pastures with high levels of molybdenum. In: *C, F, Mills, I. Bremner and J. K. Chessters (Ed.) Trace elements in Man and Animals (TEMA-5).* Pp. 342-345. Commonwealth Agric. Bureaux, Farnham Royal, UK.

Mather, I.H., and T.W. Keenan. 1998. Origins and secretion of milk lipids. J. Mammary Gland Biol. Neoplasia. 3:259-273

Medrano, J. F., A. Johnson, E. J. DePeters, and A. Islas. 1999. Genetic modification of the composition of milk fat: Identification of poly-morphisms within the bovine stearoyl-CoA-desaturase gene. J. Dairy Sci. 82(Suppl. 1):71. (Abstr.)

Mendelson, C.R. and R.O. Scow. 1972. Uptake of chylomicron-triglyceride by perfused mammary tissue of lactating rats. Am. J. Physiol. 223:1418-1423

Mills, C.F. 1987. Biochemical and physiological indicators of mineral status in animals: copper, cobalt and zinc. J. Anim. Sci. 65:1702-1711

Mills, C. F., A.C. Dalgarno and G. Wenham. 1976. Biochemical changes in tissue of Friesian cattle during the induction of copper deficiency. Br. J. Nutr. 35:309-331.

Morales, S. M., D. L. Palmquist, and W. P. Weiss. 2000. Milk fat composition of Holstein and Jersey cows with control or depleted copper status and fed whole soybeans or tallow. J. Dairy Sci. 83:2112-2119

National Research Council. 1980. *Mineral Tolerance of Domestic Animals.* National Academy Press. Washington D.C.

National Research Council. Carcinogens and Anticarcinogens in the Human Diet. 1996. National Academic Press. Washington D.C.

Oesteicher, P. and R. J. Cousins. 1985 Copper and zinc absorption in the rat: Mechanism of mutual antagonism. J. Nutr. 115:159-166

O'Mary, C.C., M.C. Bell, N.N. Sneed and W.T. Butts, Jr. 1970. Influence of rations copper on minerals in the hair of Hereford and Holstein calves. J. Anim. Sci. 31:626-630

Owen, C. A. Jr. 1965 Metabolism of radio copper (Cu^{64}) in the rat. Am J. physiol. 209:900-904

Owen, C. A. Jr.1971. Metabolism of copper 67 by the copper-deficient rat. Am. J. Physiol. 221:1722-1727.

Owen, C. A. Jr. 1981. *Copper deficiency and Toxicity*. Park Ridge, New Jersey. Noyes Publication.

Owen, C. A. Jr. 1982a. *Physiological Aspects of Copper. Ccopper in Organs and Systems*. Park Ridge, New Jersey. Noyes Publication.

Owen, C. A. Jr. 1982b. *Biochemical Aspects of Copper. Copper Proteins, Ceruloplasmin, and Copper Protein Binding.*. Park Ridge, New Jersey. Noyes Publication.

Palmquist, D. L., A.D. Beaulieu, and D. M. Barbano. 1993. Feed and Animal Factors influencing milk fat composition. J. Dairy Sci. 76:1753-1771

Pariza MW, Hargrave WA: A beef derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[a]anthracene. Carcinogenesis 8:1881-1887, 1985.

Pariza M, Park Y, Cook M, Albright K, Liu W, et al. FASEB J 10: Abstract #3227, 1996.

Paynter, D. I., R.J. Moir and E.J. Underwood. 1979. Changes in the activity of the Cu-Zn superoxide dismutase enzyme in tissues of the rat with changes in dietary copper. J. Nutr. 109:1570-1576.

Phillippo, M., W.R. Humphries, I. Bremner, T. Atkinson, and G. Henderson. 1985. Molybdenum-induced fertility in cattle. In*: C, F, Mills, I. Bremner and J. K. Chessters (Ed.) Trace elements in Man and Animals (TEMA-5)*. Pp. 342-345. Commonwealth Agric. Bureaux, Farnham Royal, UK.

Phillippo, M. and D.S. Graca. 1983. Biliary copper secretion in cattle. Proc. Nutr. Soc. 42:46A.

Price, J., A.M. Will, G. Paschaleris and J.K. Chesters. 1987. Identification of thiomolybdates in digest and plasma from sheep after administration of 99-Mo labeled compounds in the rumen. Br. J. Nutr. 58:127-137.

Prohaska, J. R. 1991. Changes in the Cu,Zn-superoxide dismutase, cytochrome c oxidase, glutathione peroxidase, and glutathione transferase activities in copper-deficient mice and rats. J. Nutri. 121:355-363

Richards, M. P., and R.J. Cousins. 1975. Mammalian zinc homeostasis: Requirements for RNA and metallotionein synthesis. Biochem, Biophys. Res. Commun. 64:1215-1223.

Shultz TD, Chew BP, Seaman WR: Differential stimulatory and inhibitory responses of human MCF-7 breast cancer cells to linoleic acid and conjugated linoleic acid in culture. Anticancer Res 12(6B):2143-2145, 1992.
Shand, A. and G. Lewis. 1957. Chronic copper poisoning in young calves. Vet. Rec. 69:618

Schwartz, F.J. and M. Kirchgessner. 1974. Intestinal absorption of copper, zinc, and iron after dietary depletion. In: *W.G. Hoekstra, J.W. Suttie, H.E. Ganther, and W. Mertz (Eds.). Trace element metaboliam in animals-2*.pp. 519-522. University park Press Baltimore.

Starcher, B.C. 1969. Studies on the mechanism of copper absorption in the chick. J. Nutr. 97:321-326

Stull, J. W., and W. H. Brown. 1964. Fatty acid composition of milk. II. Some differences in common dairy breeds. J. Dairy Sci. 47:1412

Suttle N. F. 1991. The interactions between copper, molybdenum, and sulfur in ruminant animals. Annu. Rev. Nutr. 11:121-140.

Suttle, N.F. and C.H. McMurray. 1983. Use of erythrocyte copper:zinc superoxide dismutase activity and hair and fleece copper concentration in the diagnosis of hypocuprosis in ruminants. Res. Vet. Sci. 35:47-52

Turner, J.C. V. Shanks, P.J. Osborn and S.M. Gower. 1987. Copper absorption in sheep. Comp. Biochem. Physiol. 86C:147-150.

Thompson, E. H., C. E. Allen, and R. J. Meade. 1973. Influence of copper on stearic acid desaturation and fatty acid composition in the pig. J. Anim. Sci. 36:868–873

Underwood, E. J. 1977. Copper. In: *E. J. Underwood(Ed.) Trace elements in Human and Animal Nutrition 4th ed.* Pp. 56-108. Academic Press, New York.

Van Barneveld, A.A., and J. A. Van Den Hamer. 1984 Intestinal passage and absorption of simultaneously administered ^{64}Cu and ^{65}Zn and the effect of feeding in the mouse and rat. Nutr. Rep. J. 29:173-182

Van Koetsveld, E.E. 1958. The manganese and copper contents of hair as an indicator of the feeding condition of cattle regarding manganese and copper. Tijdsehr. Diergeneesk. 83:229

Visonneau S, Cesano A, Tepper SA, Scimeca JA, Santoli D, Kritchvsky D:Conjugated linoleic acid suppresses the growth of human breast cancer adenocarcinoma in SCID mice. Anticancer Res 17(2A):969-973, 1997.

Wapnir, R. A. and L. Stiel. 1987. Intestinal absorption of copper: Effect of sodium. Proc. Soc. Exp. Biol. Med. 185:277-282

Ward, G.M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: A review. J Anim Sci. 46:1078

Weiss, E. and P. Baur. 1968. Experimental studies on chronic copper poisoning in the calf. Zentralb. Veterinaermed. 15:156

Weiss, K. C., M.C. Linder and the Los Alamos Radiological Medical Group. 1985. Copper transport in rats involving a new plasma protein. Am. J. Physiol. 249:E77-E88.

Weiner, G., S. Hayter, A. C. Field and N.S.M. Macleod. 1974. Copper levels in liver and blood of sheep and dead lambs in relation to breed, age at death, and cause of death. J. Comp. Pathol. 84:27-38

White, S. L., J. A. Bertrand, M. R. Wade, S. P. Washburn, J. T. Green Jr., and T. C. Jenkins. 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84:2295-2301

Xin, Z. 1990. Copper status and luteinizing hormone secretion, copper distribution in tissues, neutrophil functions, superoxide dismutase activity and different copper sources for dairy cattle. Ph. D. Dissertation. University of Kentucky. Lexington , KY.

Xin, Z., D. F. Waterman, R.W. Hemken, and R. J. Harmon. 1991. Effects of copper status on neutrophil function, superoxide dismutase, and copper distribution in steers. J. Dairy Sci. 74:3078-3085.

Zammit, V.A. 1996. Role of insulin in hepatic fatty acid partitioning: emerging concepts. Biochem. J. 314:1-14

CHAPTER 3

EXPERIMENT AND ANALYSIS

Abstract

An experiment was conducted to determine the effect of dietary copper (Cu) on Cu status and lipid metabolism in Holstein and Jersey cattle. Eight Jersey and 8 Holstein cows in mid-lactation were blocked by days in milk (169 ± 37) and assigned at random to one of two diets. Cows received either a control diet (Cu-) which contained a basal level of 8 mg Cu/kg DM or a treatment (Cu+) diet that was supplemented with 16 mg Cu/kg DM from $CuSO_4$ for a total of 24 mg Cu/kg DM. The experimental design was a 2 by 2 factorial arrangement of treatments with breed (Jersey or Holstein) and Cu supplementation (0 or 16 mg Cu/kg DM) as main effects. As expected, DM intake and milk yield were greater for Holsteins and milk fat and protein percentages were greater for Jerseys. Plasma Cu was greater for Jerseys and hepatic Cu was greater at 90 d for Cu+ Jerseys compared to Cu+ Holsteins. $C_{18:0}$ was lower for both Cu+ treatment groups. On day 90, conjugated linoleic acid (CLA) was less for Cu+ compared to Cu-. Also on day 90, Cu+ cows produced more total saturated fatty acids than Cu- cows. These results indicate that Cu metabolism differs between these two breeds. In addition, Cu supplementation decreased CLA possibly due to inhibition of stearoyl coenzyme A desaturase, an enzyme involved in the production of CLA.

Introduction

Copper is recognized as an essential mineral for ruminants and functions in a number of enzymes and proteins (Linder, 1991). Du et al. (1996) showed that Cu is metabolized differently by Holsteins and Jerseys. However, National Research Council (2001) concluded that current information did not warrant different Cu requirements for Jersey cattle. The requirement for lactating dairy cattle is approximately 11 mg/kg DM (National Research Council, 2001). Research at the University of Kentucky has shown that dietary Cu should be approximately 15 to 20 mg/kg DM for optimal immune function in lactating cows. Broaddus (2001) reported that somatic cell count was lower for Jerseys compared to Holsteins over a period of one year in a commingled herd. Immune function is generally considered the physiological process requiring the greatest level Cu supplementation, being greater than maintenance and lactation. Therefore, Cu requirement of Jersey cattle may be lower than Holsteins and similar dietary Cu for the two breeds as recommended by NRC (2001) may not be appropriate.

Copper may play a vital role in CLA synthesis and have particular importance for the Jersey breed. Thompson et al. (1973) reported increased liver stearoyl-CoA desaturase activity in pigs supplemented with Cu. The impetus of recent experiments was the hypothesis that Cu supplementation would increase CLA in milk. Engle et al. (2001) showed that Cu supplemented at 10 mg/kg DM (9 mg/kg DM in basal diet) decreased CLA content of milk from Holsteins. Morales et al. (2000) fed a Cu depleting diet or a diet supplemented with 20 mg/kg DM Cu to

Jersey and Holstein cows. Copper supplementation decreased milk CLA and CLA was lower in milk of Jerseys compared to Holsteins.

Differences in the fatty acid composition of milk produced by Jersey and Holstein cattle were first reported more than 35 years ago (Stull and Brown, 1964). More recently, White et al. (2001) confirmed that Jerseys and Holsteins produce milk with different fatty acid composition. Preliminary work by Medrano et al. (1999) shows differences between breeds in the activity of the enzyme stearoyl-CoA desaturase, which is present in the mammary gland and other tissues. Stearoyl-CoA desaturase oxidizes palmitic (C16:0) and stearic (C18:0) acids to palmitoleic (C16:1) and oleic (C18:1) acids and is involved in CLA production (Medrano et al., 1999). The objective of this research was to determine the effect of Cu supplementation on milk fatty acid composition of Holstein and Jersey cattle.

Materials and Methods

Animals and Diets

Eight Jersey and 8 Holstein cows in mid-lactation were blocked by days in milk (169 ± 37) and assigned at random to one of two diets. Cows received either a control diet (Cu-) which contained a basal level of 8 mg Cu/kg DM or a treatment (Cu+) diet that was supplemented with 16 mg Cu/kg DM from $CuSO_4$ for a total of 24 mg Cu/kg DM. The experimental design was a 2 by 2 factorial arrangement of treatments with breed (Jersey or Holstein) and Cu supplementation (0 or 16 mg

Cu/kg DM) as main effects. An initial 7 day adjustment period was followed by a 90 day data collection period.

Cows were group housed in a free-stall barn equipped with Calan® doors (American Calan, Northwood, NH) to facilitate collection of individual daily feed consumption. Animals were fed a TMR at 0900 h and milked twice daily at 0900 and 2000 h. Diets differed only in Cu concentration and contained corn silage, grass silage, alfalfa hay, corn, barley, soybean meal, corn distiller's grain, whole cottonseed, and mineral-vitamin premix (Table 6). Ingredients were sampled monthly and composition determined by a commercial laboratory (Dairy One, Ithaca, NY). Diets were formulated to meet or exceed nutrient requirements (NRC, 2001) except for Cu, which was approximately 2 mg/kg DM deficient for the control diet and 14 mg/Kg DM in excess for the treatment diet.

Table 6. Ingredient and chemical composition of basal diet (DM basis).

Ingredient composition	%
Alfalfa hay	19.2
Corn silage	21.3
Grass silage	11.8
Barley	14.0
Corn	14.0
Whole cottonseed	9.7
Soybean meal	4.0
Corn distillers grain	4.0
Premix[12]	2.0

[1]Contained 15.0% Ca, 5.5% P, 2.0% Mg, 6.0% Na, 2.0% Cl, 6 mg Co/kg, 42 mg I/kg, 1100 mg Mn/kg, 4000 mg Zn/kg, 17 mg Se/kg, 36.3 KIU A/kg, 11.3 KIU D/kg, and 272 IU D/kg.
[2]Contained either 0 or 800 mg Cu/kg DM from $CuSO_4$.

Chemical Composition	
CP,%	15.6
NDF	34.1
NSC	40.5
Fat	4.9
Ca	0.75
P	0.44
Mg	0.26
K	1.47
S	0.20
Cl	0.40
Fe, mg/kg	269.0
Zn	75.5
Cu	9.2
Mn	50.2
Mo	<1
A, IU/kg	3284
D	1036
E	25

Sampling and Analysis

Liver and blood samples were collected via biopsy (Erwin, 1956) and jugular vein, respectively, at the beginning of the experiment and on d 45 and 90. Blood samples were centrifuged at 1000 x g for 10 min and plasma was separated and frozen for later analysis. Liver and plasma samples were analyzed for Cu by atomic absorption spectroscopy (PerkinElmer 5100, Boston, MA). Liver samples were weighed and then prepared for Cu analysis using a perchloric digestion (Boyer, 1984). Plasma samples were diluted 1:2 (vol/vol) in deionized water prior to analysis.

Milk samples were collected on four consecutive milkings at the beginning of the experiment and on days 45 and 90. Milk samples were analyzed for fat and protein by Willamette DHI (Salem, OR). Milk fatty acid profile was determined by gas chromatography. Milk samples were prepared for extraction by mixing four 4 mL samples from consecutive milkings and then taking duplicated 3 mL samples from the pooled milk. Lipid extraction was accomplished via a rapid extraction modification of the chloroform:methanol procedure of Folch et. al. (1999). This involved measuring 3 mL samples into 50 mL screw-capped tubes. Then 18 mL of Folch solution (2:1, chloroform:methanol) was added, tubes were then immediately vortexed for 30 seconds. Tubes were then held for 6 hours at room temperature. After 6 hours, 4 mL of 0.88% NaCl was added and tubes were inverted to mix. Tubes were then centrifuged at 2000 x g for 10 min. The aqueous layer was then aspirated to waste and the chloroform or bottom layer containing the lipid extract

was transferred to a scintillation vial for storage (-20°) until methylation. Following extraction, the lipids were dried under nitrogen. Methyl ester derivatives of the fatty acids were prepared in duplicate using a boron triflouride (BF_3) reagant as described by Cherian et al. (2002). Fatty acid compositon of milk was determined via gas chromatography (Agilent 6890, Wilmington, DE) in splitless mode equipped with a fused silica capillary column: (Supelco #SP-2560, 100m x 0.25mm i.d.) and a flame ionization detector. The initial oven temperature was set at 60° C and held for 4 min, then ramped at 4° C/min to 165° C, and held for 2 min. The temperature was then increased by 15° C/min to 200° C, held for 30 min, ramped up again by 15° C/min to 215° C, held for 15 min and finally increased by 15° C/min to 230° C and held for 3 min. Inlet and detector temperatures were 250° C. Helium was the carrier gas.

Statistical Analysis

The experimental data were analyzed using the mixed models procedure of SAS® (Littell et al., 1996) for analysis of variance with breeds (Holstein or Jersey) and Cu supplementation (0 or 16 mg/kg DM) as main effects in a 2 x 2 factorial block arrangement of treatments. In addition, the model included all interactions and replication. Initial measures (0 d) of liver Cu and milk fatty acids were used as a covariate to give appropriate adjusted means. Daily milk yield and DMI were averaged by week prior to analysis. Differences were considered significant at $P < 0.10$. The linear model used was:

$$y_{ijklmn} = \mu + \beta_o + \rho_i + \alpha_j + \tau_k + \alpha\tau_{jk} + c(\alpha\tau_{ij})_l + \gamma_m + \alpha\gamma_{jm} + \tau\gamma_{km} + \alpha\tau\gamma_{jkm} + \varepsilon_{ijklm}$$

where $\mu =$ overall mean;

$\beta_o =$ regression coefficient for initial hepatic Cu, and milk fatty acid composition;

$\rho_i =$ effect due to i-th block;

$\alpha_j =$ effect due to j-th level of Cu;

$\beta_k =$ effect due to k-th breed;

$\alpha\beta_{jk} =$ interaction of the j-th level of Cu with the k-th breed;

$c_{ijkl} =$ effect of the l-th cow within the i-th block, j-th level of Cu, and k-th breed;

$\gamma_m =$ effect of the m-th day;

$\alpha\gamma_{jm} =$ interaction of the j-th level of Cu with the m-th day;

$\beta\gamma_{km} =$ interaction of the k-th breed with the m-th day;

$\alpha\beta\gamma_{jkm} =$ interaction of the j-th level of Cu with the k-th breed with the m-th day;

$\varepsilon_{ijklm} =$ residual error.

Results and Discussion

Data for DM intake, milk yield, and milk composition are shown in Table 7. As expected, DM intake and milk yield were greater ($P<0.01$) for Holsteins and

milk fat and protein percentages were greater (*P<0.01)* for Jerseys. Copper supplementation increased (*P<0.10)* DM intake, but did not affect milk yield or composition. The increase in DM intake in response to Cu supplementation is unexplainable and was due mainly to Cu+ Holsteins, although breed by treatment interaction was not significant. Energy balance was not calculated in the current study, but it was assumed that cows were in positive energy balance based on the constant intake and declining milk yield (linear and quadratic, *P<0.01)* over the 90 d data collection period. Mid-lactation cows were chosen in this study to minimize the contribution of body lipid stores to milk fat.

Cu concentrations in plasma and liver tissue are shown in Table 7. Plasma Cu concentrations were not indicative of levels provided in the diet and suggest that plasma concentrations are a poor indicator of Cu status. Plasma Cu concentrations ranged from 0.65 and 1.10 μg/ml and are similar to concentrations that have previously been reported for ruminants (Auza et al., 1999, Dargatz et al., 1999). Jersey plasma Cu concentrations were higher (*P<0.10)* than that of Holstein cows. The breed effect in plasma Cu concentration supports a genetic difference in Cu metabolism as previously reported by Du et al. (1996).

Initial hepatic Cu concentrations ranged from 325 to 450 mg/kg DM. These values ranged from normal to slightly high. The range considered normal for ruminants is 100-400 mg/kg DM (Charmley and Symonds, 1985; Davis and Mertz, 1987). The Cu- groups exhibited the expected drop in hepatic Cu levels from d 45

(376 mg/kg DM) to 90 (276 mg/kg DM), since the diet was slightly deficient in Cu. The Cu+ Jersey group had hepatic Cu levels of 403 and 397 μg/g DM at 45

Table 7. Effect of supplemental Cu on feed intake, milk yield, milk composition, plasma Cu, and hepatic Cu.

	Jersey		Holstein			*P* <		
	-Cu[1]	+Cu	-Cu	+Cu	SE	B[2]	Cu	B x T
DMI, kg/d	19.3	19.5	21.6	24.0	0.7	0.01	0.08	0.15
Milk yield, kg/d	21.8	23.5	32.5	31.8	2.4	0.01	0.86	0.65
Milk fat, %	4.77	4.61	3.78	3.93	0.25	0.01	0.99	0.56
Milk protein, %	4.06	3.92	3.35	3.50	0.13	0.01	0.99	0.29
Plasma Cu, ug/ml	0.88	0.84	0.78	0.70	0.06	0.07	0.51	0.85
Hepatic Cu, ug/g DM	328	400	323	391	47	0.88	0.26	0.96

[1] –Cu = no supplemental Cu, +Cu = 16 mg Cu/kg DM
[2] B = effect of breed, Cu = effect of Cu

and 90 d, respectively. However, the Cu+ Holstein group had an unexpected decline from 45 to 90 d, 436 and 345 mg/kg DM, respectively. The Cu+ Jersey group did not decrease at the same magnitude as did the other treatment groups presumably due to the sensitivity of Jersey cattle to Cu supplementation. The decrease in the Cu+ Holstein group cannot be explained.

Hepatic Cu differences for the Cu+ groups indicates that the Jersey breed metabolizes Cu differently compared to Holsteins. The genetic difference may be related to the efficiency of dietary Cu absorption, the excretion of endogenous Cu, or the amount of feed intake relative to body weight. In the Simmental breed of cattle, high biliary excretion of Cu cause Cu deficiency more frequently than in any

other breed of cattle (Gooteratne, 1989). This illustrates that genetic differences in Cu metabolism exist within other breeds of cattle and that more research into the mechanism that causes this difference in Cu metabolism in the Jersey breed is warranted as it could play a role in modulating feeding recommendations for the breed.

Fatty acid profile for each treatment and breed group is shown in Table 8. $C_{18:0}$ was lower for both Cu+ treatment groups ($P<0.05$). $C_{18:0}$ is involved in the synthesis of CLA. Engle et al. (2001) also observed a decrease in $C_{18:0}$ as Cu supplementation level was increased. The decrease in $C_{18:0}$ could be due to an inhibitory effect of Cu on biohydrogenation of polyunsaturated fatty acids (PUFA) to $C_{18:0}$ in the rumen. Engle et al. (2001) speculated that Cu has an effect on the microbial population of the rumen, thereby altering the overall process of fatty acid biohydrogenation. Inhibition of biohydrogenation induced by ionophores results in a decrease in $C_{18:0}$ and an increase in trans $C_{18:1}$ (Fellner, 1997). Contrary to expectations, this study, however, did not observe any change in trans $C_{18:1}$. A change in the ruminal output of fatty acids can be observed by assessing milk fatty acid concentrations, but any effect has a much smaller magnitude in milk analysis compared to direct ruminal output measures.

Jersey cows produced more $C_{8:0}$ ($P<0.01$), and $C_{16:0}$ ($P<0.02$) than Holstein cows. While this study did show a breed difference in specific short to medium chain fatty acids, there were no differences in the total percentages of short and

medium chain fatty acids. This contradicts Palmquist and Beaulieu (1992) who reported that Holstein cows produced from 8% to 42% more short and medium chain fatty acids. This study also contradicts White et al. (2001) and Capps et al.

Table 8. Least–squares means for fatty acid composition from each treatment and breed group expressed as a % of total fatty acids.

	Holstein		Jersey			P <		
Fatty Acid	-Cu[1]	+Cu	-Cu	+Cu	SEM	B[2]	C	B x C
$C_{6:0}$	3.19	2.69	2.94	2.93	0.25	0.97	0.29	0.30
$C_{8:0}$	1.63	1.77	1.86	1.89	0.05	0.01	0.11	0.28
$C_{10:0}$	3.84	4.91	4.36	4.33	0.33	0.95	0.17	0.15
$C_{11:0}$	0.28	0.35	0.36	0.37	0.03	0.11	0.16	0.33
$C_{12:0}$	4.22	4.91	4.75	4.73	0.12	0.21	0.03	0.02
$C_{14:0}$	12.80	13.32	13.27	13.28	0.24	0.39	0.36	0.32
$C_{15:0}$	1.10	1.25	1.15	1.16	0.03	0.58	0.06	0.08
$C_{16:0}$	30.31	30.89	32.39	32.19	0.48	0.02	0.72	0.40
$C_{17:0}$	0.63	0.67	0.67	0.66	0.01	0.03	0.09	0.01
$C_{18:0}$	13.32	11.81	13.21	11.79	0.56	0.93	0.04	0.95
SFA[3]	70.82	73.02	73.31	71.58	1.01	0.71	0.79	0.05
$C_{16:1}$	1.91	2.02	1.71	1.86	0.08	0.21	0.11	0.79
$C_{18:1}$	1.48	1.80	1.30	1.44	0.16	0.19	0.20	0.56
$C_{18:1n9}$ trans	0.64	0.64	0.61	0.55	0.03	0.06	0.36	0.39
$C_{18:1n7}$ cis	19.34	19.41	18.96	18.64	0.55	0.41	0.82	0.70
$C_{20:1}$	0.33	0.34	0.30	0.31	0.01	0.02	0.36	0.76
MUFA[4]	23.98	24.00	23.29	23.38	0.56	0.39	0.92	0.95
$C_{18:2}$	2.64	2.84	2.53	2.57	0.08	0.05	0.20	0.38
$C_{18:2c9t11}$[5]	0.34	0.37	0.36	0.33	0.01	0.71	0.87	0.26
$C_{18:3}$	0.34	0.34	0.34	0.37	0.01	0.18	0.12	0.37
PUFA[6]	3.32	3.39	3.26	3.09	0.10	0.13	0.65	0.27
Tn6 PUFA[7]	2.65	2.85	2.49	2.58	0.08	0.04	0.21	0.57

[1] –Cu = no supplemental Cu, +Cu = 16 mg Cu/kg DM
[2] B = effect of breed, Cu = effect of Cu
[3] SFA = Total saturated fatty acids
[4] MUFA = Total mono-unsaturated fatty acids
[5] $C_{18:2c9t11}$ = CLA isomer
[6] PUFA = Total poly-unsaturated fatty acids
[7] Tn6 PUFA = Total Tn6 poly-unsaturated fatty acids

(1999), which reported that Jerseys have higher concentrations of short and medium chain fatty acids when compared to Holsteins. Holstein cows produced more $C_{18:1n9}$ (*P<0.10*), $C_{18:2}$ (*P<0.10*), $C_{20:1}$ (*P<0.10*), and Tn6 PUFA (*P<0.10),* than Jersey cows. These breed differences have not been shown in similar studies.

Several breed x Cu interactions were observed. The Jersey Cu- group had a higher level of C12:0 and C17:0 than the Holstein Cu- group (*P*<0.05). The Holstein Cu+ group had a higher level of C15:0 than the Jersey Cu+ group. The Jersey Cu- group had a higher level of total saturated fatty acids the Holstein Cu- group (*P*<0.05). Since Jersey cattle have a higher concentration of plasma Cu this breed may have less stearoyl coenzyme A desaturase activity than Holstein cows at the same supplementation level, resulting in greater concentration of saturated fatty acids.

The means for each treatment and breed group by day are shown in Table 9. On day 90, Jersey cows produced more C11:0 (P<0.10) and total saturated fatty acids (P<0.05) than Holstein cows. On day 45, Holstein cows produced more C16:1 (P<0.10) than Jersey cows. On day 90, Cu- cows produced more C18:2c9t11 (P<0.10) than Cu+ cows. On day 90, Cu+ cows produced more total saturated fatty acids (*P<0.10)* than Cu- cows. These interactions can be explained by assuming that Cu supplementation over time inhibits the activity of stearoyl coenzyme A desaturase, which would reduce the production of $C_{18:2c9t11}$ and increase total saturated fatty acids secreted in milk. The increase in total saturated

Table 9. Least–squares means for fatty acid composition for each treatment and breed group by day as expressed as a % of total fatty acids.

Fatty Acid	Holstein				Jersey					P[1] <		
	-Cu1		+Cu		-Cu		+Cu					
Day	45	90	45	90	45	90	45	90	SEM	BxD	TxD	BxTxD
$C_{6:0}$	3.69	2.69	2.47	2.91	2.69	3.20	2.69	3.16	0.36	0.17	0.20	0.18
$C_{8:0}$	1.61	1.64	1.86	1.67	1.91	1.80	1.84	1.94	0.06	0.37	0.97	0.02
$C_{10:0}$	3.83	3.85	5.92	3.89	4.55	4.17	4.33	4.34	0.43	0.17	0.16	0.05
$C_{11:0}$	0.30	0.26	0.44	0.27	0.36	0.36	0.36	0.39	0.04	0.06	0.38	0.22
$C_{12:0}$	4.24	4.19	5.30	4.52	5.01	4.49	4.82	4.64	0.16	0.75	0.37	0.03
$C_{14:0}$	12.99	12.61	14.12	12.51	13.72	12.81	13.32	13.23	0.32	0.27	0.64	0.04
$C_{15:0}$	1.12	1.08	1.33	1.18	1.21	1.10	1.19	1.13	0.04	0.81	0.58	0.16
$C_{16:0}$	31.52	29.11	31.94	29.81	33.17	31.62	32.31	32.08	0.65	0.15	0.39	0.57
$C_{17:0}$	0.65	0.61	0.67	0.66	0.67	0.68	0.67	0.65	0.01	0.30	0.83	0.11
$C_{18:0}$	13.49	13.16	11.03	12.59	12.81	13.63	11.15	12.43	0.68	0.62	0.19	0.41
SFA[3]	73.13	68.51	74.64	71.40	74.41	72.21	69.25	73.90	1.40	0.02	0.06	0.18
$C_{16:1}$	1.91	1.92	2.10	1.95	1.65	1.76	1.85	1.87	0.10	0.10	0.13	0.67
$C_{18:1}$	1.70	1.26	1.59	2.03	0.94	1.67	1.38	1.49	0.24	0.23	0.71	0.05
$C_{18:1n9}$ trans	0.64	0.65	0.64	0.64	0.62	0.59	0.53	0.56	0.04	0.64	0.35	0.29
$C_{18:1n7}$ cis	19.00	19.69	18.13	20.70	18.36	19.57	19.23	18.05	0.66	0.10	0.78	0.04
$C_{20:1}$	0.31	0.34	0.32	0.35	0.29	0.32	0.28	0.33	0.02	0.64	0.86	0.89
MUFA[4]	24.02	23.94	22.36	25.64	22.48	24.10	23.94	22.81	0.68	0.13	0.73	0.01
$C_{18:2n6}$	2.64	2.64	2.80	2.87	2.51	2.54	2.53	2.61	0.10	0.88	0.57	0.92
$C_{18:3n3}$	0.34	0.33	0.33	0.36	0.33	0.35	0.39	0.34	0.02	0.53	0.69	0.13
$C_{18:2c9t11}$[5]	0.34	0.33	0.38	0.37	0.35	0.37	0.36	0.29	0.03	0.49	0.06	0.11
PUFA[6]	3.37	3.26	3.26	3.52	3.21	3.31	3.15	3.02	0.12	0.53	0.64	0.05

[1] –Cu = no supplemental Cu, +Cu = 16 mg Cu/kg DM
[2] B = effect of breed, D = effect of Day, C = effect of Cu supplementation
[3] SFA = Total saturated fatty acids
[4] MUFA = Total mono-unsaturated fatty acids
[5] $C_{18:2c9t11}$ = CLA isomer
[6] PUFA = Total poly-unsaturated fatty acids

fatty acids found in Jersey cows at day 90 would also be explained by their increased sensitivity to the Cu supplementation effect on stearoyl coenzyme A desaturase.

There were several significant three-way interactions for several of the fatty acids examined in this study. These were unexplainable with our current level of understanding of this system and may warrant further research in the future. These interactions are shown in Table 9.

Breed differences in fatty acid production could have implications on milk and dairy product consumption. Most milk in the United States is produced by Holsteins. However, some farms process and distribute their own branded milk products. More research is needed to confirm and quantify differences in milk fatty acid profiles between breeds and management systems. Likewise, more research is also needed to determine the effects of Cu supplementation on lipid metabolism, mammary stearoyl coenzyme A desaturase, and milk fatty acid profile.

In conclusion, feeding a diet containing 24 mg/kg DM of Cu decreases the production of $C_{18:0}$, a fatty acid used in the production of CLA. The proposed mode of action is through inhibition of the biohydrogenation process in rumen. Copper also increased the total saturated fatty acids in milk and decreased the secretion of the $C_{18:2c9t11}$, an isomer of CLA, possibly by inhibiting the activity of stearoyl coenzyme A desaturase. This study also confirmed that Jersey and Holstein cows metabolize Cu differently based upon differences in plasma Cu concentrations.

References

Auza, N.J., W.G. Olson, M.J. Murphy, and J.G. Linn. 1999. Diagnosis and treatment of Cu toxicosis in ruminants. JAVMA 214:1624-1628

Boyer, K.W. 1984. Metals and other Elements at Trace Levels in Food: "*Official Methods of Anaysis*. Association of Official Analytical Chemists. Pg. 444

Capps, V.A., E.J. DePeters, S.J. Taylor, H. Perez-Monti, J.A. Wyckoff, and M. Rosenberg. 1999. Effect of breed of dairy cattle and dietary fat on milk yield and composition. J. Dairy Sci. 82(suppl. 1):45

Charmley, L. L., and H. W. Symonds. 1985. A comparison of the ability of cattle and pigs to clear excess copper from the plasma and excrete it in the bile. In: *C.F. Mills, I. Brenner and J. K. Chersters (Ed.) Trace elements in Man and Animals (TEMA-5)*. pp. 341-343. Commonwealth Agri. Bureaux, Farnham Royal, U.K.

Cherian, G., R.K. Selvaraj, M.P. Goeger, and P.A. Stitt. 2002. Muscle fatty acid composition and thiobarbituric acid-reactive substances of broilers fed different cultivars of sorghum. Poultry Sci. 81:1415-1420

Dargatz, D.A., F. B. Garry, G.B. Clark, and P.F. Ross. 1999. Serum Cu concentrations in beef cows and heifers. JAVMA 215:1828-1832

Davis, G. K., and W. Mertz. 1987. Copper. In: *W. Mertz (Ed.) Trace elements in Human and Animal Nutrition – 5th ed (Vol 1)*. Pp 301-364. Academic Press, Inc. New York

Du, Z., R. W. Hemken and R. J. Harmon. 1996. Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or Cu proteinate. J. Dairy Sci. 79:1872-1880

Engle, T.E., V. Fellner, and J.W. Spears. 2001. Copper status, serum cholesterol, and milk fatty acid profile in Holstein cows fed varying concentrations of Cu. J. Dairy Sci. 84:2308-2313.

Erwin, E.S., I. A. Dyer, T. O. Meyer, and K.W. Scott. 1956. Uses of aspiration biopsy technique. J. Anim. Sci. 15:428-434

Fellner, V., F.D. Sauer, J.K. Kramer. 1997. Effect of nigericin, monensin, and tetronasin on biohydrogenation in continuous flow-through ruminal fermenters. J Dairy Sci. 80:921-928

Folch, J.M., P. Dove, J.F. Medrano. 1999. Differential expression of bovine beta-lactoglobulin A and B promoter variants in transiently transfected HC11 cells. J Dairy Res. 66:537-544

Gooneratne, S.R., and D.A. Christensen. 1989. A survey of maternal Cu status and fetal tissue Cu concentrations in Saskatchewan bovines. Can J. Anim. Sci. 69:141

Linder, M. C. 1991. *Biochemistry of Copper.* Plenum Press, New York

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® System for Mixed Models. SAS Inst., Inc., Cary, NC.

Medrano, J. F., A. Johnson, E.J. DePeters, and A. Islas. 1999. Genetic modification of the composition of milk fat: Identifiction of polymorphisms within the bovine stearoyl-Co-A-desaturase gene. J. Dairy Sci. 82(suppl. 1):71

Morales, S. M., D. L. Palmquist, and W. P. Weiss. 2000. Milk fat composition of Holstein and Jersey cows with control or depleted Copper status and fed whole soybeans or tallow. J. Dairy Sci. 83:2112-2119

Palmquist, D.L., and A. D. Beaulieu. 1992. Feed and animal factors influencing milk fatty acid composition. J. Dairy Sci. 75(suppl. 1):292

White, S. L., J. A. Bertrand, M. R. Wade, S. P. Washburn, J. T. Green Jr., and T. C. Jenkins. 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84:2295-2301

BIBLIOGRAPHY

Alvarez, R. 1985. The use of NBS standard reference materials in validating trace element determinations in biological materials. In: *C.F. Mills, I. Brenner and J. K. Chersters (Ed.) Trace elements in Man and Animals (TEMA-5).* pp. 655-657. Commonwealth Agri. Bureaux, Farnham Royal, U.K.

Auza, N.J., W.G. Olson, M.J. Murphy, and J.G. Linn. 1999. Diagnosis and treatment of Copper toxicosis in ruminants. JAVMA 214:1624-1628

Baker, D. H., J. Odle, M. A. Funk and T. M. Wielnad. 1991. Bioavailabilitty of Copper in cupric oxide, cuprous oxide and Cu-lysine complex. J. Poultry Sci. 70:177-179

Bennett, F. I., M. H. N. Golden, and D. D. Ramdath. 1985. Red cell superoxide dismutase as a measure of Copper status in man. In: *C.F. Mills, I. Brenner and J. K. Chersters (Ed.) Trace elements in Man and Animals (TEMA-5).* pp. 578-580. Commonwealth Agri. Bureaux, Farnham Royal, U.K.

Bingley, J. B., and J. H. Dufty. 1972. Distribution of Copper in the tissues of the bovine neonate and dam. Res. Vet. Sci. 13:8-14

Bitman, J. and D.L. Wood. 1990. Changes in milk phospholipids during lactation. J. Dairy Sci. 73:1208-1216

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Pysiol. 37:911-917

Boyer, K.W. 1984. Metals and other Elements at Trace Levels in Food: "*Official Methods of Analysis.* Association of Official Analytical Chemists. Pg. 444

Brandt, R. M., Pepperle, A. Otto, R. Kraft, F.D. Boehmer, and R. Grosse. 1988. A 13-kilodalton protein purified from milk fat globule membrane is closely related to a mammary-derived growth-inhibitor. Biochemistry. 27:1420-1425

Bronner, F. and J. H. Yost. 1985. Saturable and nonsaturable Copper and calcium transport in the mouse duodenum. Am. J. Physiol. 249:G108-G112

Buckley, W. T. 1991. A kinetic model of Copper metabolism in lactating dairy cattle. Can. J. Anim. Sci. 71:155-166

Buckley, W.T., S. N. Huckin, and G.K. Eigendorf. 1985. Stable isotope tracer methods for determining absorption of dietary Copper in dairy cattle. In: *C.F. Mills, I. Brenner and J. K. Chersters (Ed.) Trace elements in Man and Animals (TEMA-5)*. pp. 339-341. Commonwealth Agri. Bureaux, Farnham Royal, U.K.

Campbell, C. H., R. Brown and M. C. Linder. 1981. Circulating ceruloplasmin is and important source of copper for normal and malignant cells. Biochem. Biophys. Acta. 678:27-38

Capps, V.A., E.J. DePeters, S.J. Taylor, H. Perez-Monti, J.A. Wyckoff, and M. Rosenberg. 1999. Effect of breed of dairy cattle and dietary fat on milk yield and composition. J. Dairy Sci. 82(suppl. 1):45

Cartwright, G. E. And M. M. Wintrobe. 1964. Copper metabolism in normal subjects. Am. J. Clin. Nutr. 14:224-232.

Chapman. H. L. Jr., D. H. Cox, C. E. Haines and G.K. Davis. 1963. Evaluation of the liver biopsy technique for mineral nutrition studies in beef cattle. J. Anim. Sci. 22:733-737.

Charmley, L. L., and H. W. Symonds. 1985. A comparison of the ability of cattle and pigs to clear excess Copper from the plasma and excrete it in the bile. In: *C.F. Mills, I. Brenner and J. K. Chersters (Ed.) Trace elements in Man and Animals (TEMA-5)*. pp. 341-343. Commonwealth Agri. Bureaux, Farnham Royal, U.K.

Chaves, E., J. C. Laurenz, L. W. Greene, F. M. Byers and G. T. Schelling. 1990. Apparent absorption of phosphorus, magnesium, zinc, and Cu in two breeds of cows. Nutr. Abs. Rev. (Series B) 60:694.

Cherian, G., R.K. Selvaraj, M.P. Goeger, and P.A. Stitt. 2002. Muscle fatty acid composition and thiobarbituric acid-reactive substances of broilers fed different cultivars of sorghum. Poultry Sci. 81:1415-1420

Clark, T. W. 1993. A comparison of copper oxide and copper sulfate as a copper source for the mature ruminant, the effect of copper status on immune function in the mature ruminant. Thesis. University of Kentucky, Lexington, KY.

Clegg, R.A., M.C. Barber, L. Pooley, I. Ernens, Y. Larondelle, and M.T. Travers. 2001. Milk fat synthesis and secretion: molecular and cellular aspects. Livestock Prod. Sci. 70:3-14

Cohen, D. I., B. Illowsky and M. C. Linder. 1979. Altered Copper absorption in tumor bearing and estrogen treated rats. Am. J. Physiol. 236:E309-E315.

Crampton, R. F., D. M. Matthews and R. Poisner. 1965. Observations on the mechanism of absorption of Copper by the small intestine. J. Physiol. 178:111-126.

Cromwell, G. L., T. S. Stahly and H. J. Monegue. 1989. Effects of source and level of copper on performance on liver copper stores in weanling pigs. J. Anim. Sci. 67:2996-3002

Cunnane, S. C. 1989. Modulation of long chain fatty acid unsaturation by dietary copper. In: *Cu Bioavailability and Metabolism.* Constance Kies (ed.). Plenum Press. New York

Cunningham, I. J., and K. G. Hogan. 1958. The influence of diet on the copper and molybdenum contents of hair, hoof and wool. N. Z. J. Agr. Res. 1:841-846.

Cymbaluk, N. F., F. M. Bristol, and D. A. Christensen. 1986. Influence of age and breed of equid on plasma copper and zinc concentration. Am J. Vet. Res. 47:192-195

Danks, D. M., P. E. Campbell, B. J. Stevens, V. Maynes and E. Cartwright. 1972. Menkes' kinky hair syndrome. An inherited defect in copper absorption with widespread effects. Pediatrics. 50:188-201.

Dargatz, D.A., F. B. Garry, G.B. Clark, and P.F. Ross. 1999. Serum copper concentrations in beef cows and heifers. JAVMA 215:1828-1832

Davies, M. T. And R. N. Williams. 1976. Effects of pregnancy on uptake and distribution of copper in the rat. Proc. Nutr. Soc. (Abstr.) 34:4A-5A.

Davis, G. K., and W. Mertz. 1987. Copper. In: *W. Mertz (Ed.) Trace elements in Human and Animal Nutrition – 5th ed (Vol 1).* Pp 301-364. Academic Press, Inc. New York

Dhiman, T.R. Conjugated linoleic acid: A food for cancer prevention. Feedstuffs. 24-32, 2000

Du, Z., R. W. Hemken and R. J. Harmon. 1996. Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. J. Dairy Sci. 79:1872-1880

Du, Z. 1994. Bioavailabilities of copper in copper proteinate, copper lysine and cupric sulfate, and copper tolerances of Holstein and Jersey cattle. Ph. D. Dissertation. University of Kentucky, Lexington, KY.

Engle, T. E., V. Fellner, and J. W. Spears. 2001. Copper status, serum cholesterol, and milk fatty acid profile in Holstein cows fed varying concentration of copper. J. Dairy Sci. 84: 2308-2313

Erwin, E.S., I. A. Dyer, T. O. Meyer, and K.W. Scott. 1956. Uses of aspiration biopsy technique. J. Anim. Sci. 15:428-434

Evans, G. W. 1973. Copper homeostasis in the mammalian system. Physiol. Rev. 53:535-570

Fellner, V., F.D. Sauer, J.K. Kramer. 1997. Effect of nigericin, monensin, and tetronasin on biohydrogenation in continuous flow-through ruminal fermenters. J Dairy Sci. 80:921-928

Felsman, R. J., M. B. Wise, R.W. Harvey, and E.R. Barrick. 1973. Effect of added dietary levels of copper sulfate and an antibiotic on performance and certain blood constituents of calves. J. Anim. Sci. 36:157

Fields, M. J. Holbrook, J. Scholfield, A. S. Powell, A. J. Rose, S. Reiser, and J. C. Smith. 1986. Development of copper deficiency in rats fed fructose or starch: Weekly measurements in Copper indices in blood. Proc. Soc. Exp. Biol. Med. 181:120-124

Fischer, P.W. F. and M. R. L'Abbe. 1985. Copper transport by intestinal brush border membrane vesicles from rats fed high zinc of Copper deficient diets. Nutr. Res. 5:759-767

Flanagan, P. R., J. Haist, I. MacKenzie and L. S. Valberg. 1984. Intestinal absorption of zinc: Competative interactions with iron, cobalt, and Copper in mice with sex-linked anemia. Can. J. Physiol. Pharmacol. 62:1124-1128.

Folch, J.M., P. Dove, J.F. Medrano. 1999. Differential expression of bovine beta-lactoglobulin A and B promoter variants in transiently transfected HC11 cells. J Dairy Res. 66:537-544

Gibson, J. P., A. C. Field and G. Wiener. 1987. Concentrations of blood constituents in genetically high and low production lines of British Frisian and Jersey cattle around calving and in early lactation. Anim. Prod. 44:183-199

Gibson, J. P., A. C. Field and G. Wiener. 1986. Concentration of blood constituents from 12-72 weeks of age in genetically high and low milk production lines of Frisian and Jersey cattle around calving and early lactation. Anim Prod. 44:183-199

Gollan, J. L. 1975. Studies on the nature of the complexes formed by Copper with human alimentary secretions and their influence on Copper absorption in the rat. Clin Sci. Mol. Med. 49:237-245

Goode, C. A., C. T. Dinh and M. C. Linder 1989. Mechanism of Copper transport and delivery in mammals: Review and recent findings. *In: C, F, Mills, I. Bremner and J. K. Chessters (Ed.) Trace elements in Man and Animals (TEMA-5).* Pp. 342-345. Commonwealth Agric. Bureaux, Farnham Royal, UK.

Gooneratne, S. R., D. Christensen, R. Chaplin and A. Trent. 1985. Copper: Biliary excretion in Copper supplemented and thiomolbdate-treated sheep. *In: C, F, Mills, I. Bremner and J. K. Chessters (Ed.) Trace elements in Man and Animals (TEMA-5).* Pp. 342-345. Commonwealth Agric. Bureaux, Farnham Royal, UK.

Gooneratne, S. R., J.V. Bailey, H.W. Symonds, and D.A. Christensen. 1987. Effect of dietary Copper, Mo, and S levels on bile and urine Copper excretion in breeds of cattle. Can. J. Anim. Sci. 67:1188

Gooneratne, S.R., and D.A. Christensen. 1989. A survey of maternal Copper status and fetal tissue Copper concentrations in Saskatchewan bovines. Can J. Anim. Sci. 69:141

Grace, N. D. 1975. Studies on the flow of zinc, cobalt, Copper and manganese along the digestive tract of sheep given fresh perennial ryegrass, or white or red clover. Br. J. Nutr. 34:73-82

Grace N. D., and J.M. Gooden. 1980. Effect of increasing intakes of Zn, Copper, and Mn of their secretion via bile and pancreatic juice and their excretion in faeces and urine in sheep fed lucerne pellets. N. Z. J. Agr. Res. 23:293-298

Hall, A. C., B. W. Young and I. Bremner. 1979. Intestinal metallothionien and the mutual antagonism between Copper and zinc in the rat. J. Inorg. Biochem. 11:57-66.

Hart, E. B., H. Steenbock, J. Waddell, and C.A. Elvehjem. 1928. Iron in Nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. J. Biol. Chem. 77:797

Hogan, K. G., D. F. Money and R. S. Walker. 1971. The distribution of Copper in the liver of pigs and sheep and its effect on the value of chemical analysis made on biopsy samples. N. Z. J. Agric. Res. 14:132-141

Ip C, Singh M, Scimeca JA, Pariza MW: Mammary cancer prevention by conjugated diene derivative of linoleic acid. Cancer Res 51:6118-6124, 1991.

Ishmael, J., C. Gopinath and J. McC. Howell. 1971. Experimental chronic Copper toxicity in sheep. Biochemical and haematological studies during the development of lesions in the liver. Red. Vet. Sci. 13:22-29

Ivan, M, and C. M. Grieve. 1976. Effects of zinc, Copper, and manganese supplementation of high-concentrate ration on gastrointestinal absorption of Copper and manganese in Holstein calves. J. Dairy. Sci. 59:1764-1768.

Jensen, R.G. and D.S. Newberg. 1995. Bovine milk lipids. Pp. 543-575. In: *Handbook of Milk Composition.* R.G. Jensen (ed.). Academic Press. San Diego

Jensen, R.G. 2002. The composition of bovine milk lipids:January 1995 to December 2000. J Dairy Sci. 85:295-350

Kellaway, R.C., P. Sitorus and J. M. L. Leibholz. 1978. The use of Copper levels in hair to diagnose hypocuprosis. Res. Vet. Sci. 24:352-357.

Kidder, R.W. 1949. Symptoms of induced Copper toxicity in the steer. J. Anim Sci. 8:623

Knekt, P., R, Jarvinen, R. Seppanen, E. Pukkala, and A. Aromaa. 1996. Intake of dairy products and the risk of breast cancer. Cancer. 73:687-691

Koller, L. D., S. A. Mulhern, N.C. Frankel, M.G. Steven and J. R. Williams. 1987. immune dysfunction in rats fed a diet deficient in Copper. Am J. Clin. Nutr. 45:997-1006

Linder, M. C. 1991. *Biochemistry of Copper.* Plenum Press, New York

MacDonald, H. B. 2000. Conjugate linoleic acid and disease prevention: a review of current knowledge. J AM Col of Nutr 19:11-118

McGuire, M. A., and J. M. Griinari. 1999. Trans fatty acids: Is it the butterfat switch? Proc. Pacific Northwest Anim. Nutr. Conf. pp. 145

McHargue, J. S. 1925. The association of Copper with substances containing the fat-soluble A vitamin. Am. J. Physiol. 72:583

Marceau, N. and N. Aspin. 1973. The intracellular distribution of Copper derived from ceruloplasmin and albumin. Biochem. Biophys. Acta. 328:338-350

Marceau, N., N. Aspin, and Sass-Kortsak. 1970. Adsorption of Copper 64 from gastrointestinal tract of the rat. Am. J. Physiol. 218:377-383

Mason, J. 1986. Thiomilybdates:Mediators of molybdenum toxicity and enzyme inhibitors. Toxicology. 42:99-109

Masters, H.G., G. M. Smith and R. H. Casey. 1985 The relationships between the activity of superoxide dismutase and the concentration of Copper in the erythrocytes of suckling beef calves grazing pastures with high levels of molybdenum. In: *C, F, Mills, I. Bremner and J. K. Chessters (Ed.) Trace elements in Man and Animals (TEMA-5).* Pp. 342-345. Commonwealth Agric. Bureaux, Farnham Royal, UK.

Mather, I.H., and T.W. Keenan. 1998. Origins and secretion of milk lipids. J. Mammary Gland Biol. Neoplasia. 3:259-273

Medrano, J. F., A. Johnson, E. J. DePeters, and A. Islas. 1999. Genetic modification of the composition of milk fat: Identification of poly-morphisms within the bovine stearoyl-CoA-desaturase gene. J. Dairy Sci. 82(Suppl. 1):71. (Abstr.)

Mendelson, C.R. and R.O. Scow. 1972. Uptake of chylomicron-triglyceride by perfused mammary tissue of lactating rats. Am. J. Physiol. 223:1418-1423

Mills, C.F. 1987. Biochemical and physiological indicators of mineral status in animals: Copper, cobalt and zinc. J. Anim. Sci. 65:1702-1711

Mills, C. F., A.C. Dalgarno and G. Wenham. 1976. Biochemical changes in tissue of Friesian cattle during the induction of Copper deficiency. Br. J. Nutr. 35:309-331.

Morales, S. M., D. L. Palmquist, and W. P. Weiss. 2000. Milk fat composition of Holstein and Jersey cows with control or depleted Copper status and fed whole soybeans or tallow. J. Dairy Sci. 83:2112-2119

National Research Council. 1980. *Mineral Tolerance of Domestic Animals.* National Academy Press. Washington D.C.

National Research Council. Carcinogens and Anticarcinogens in the Human Diet. 1996. National Academic Press. Washington D.C.

National Research Council. Nutrient Requirements of Dairy Cattle 7th revised ed. 2001. National Academic Press. Washington D.C.

Oesteicher, P. and R. J. Cousins. 1985 Copper and zinc absorption in the rat: Mechanism of mutual antagonism. J. Nutr. 115:159-166

O'Mary, C.C., M.C. Bell, n.N. Sneed and W.T. Butts, Jr. 1970. Influence of rations Copper on minerals in the hair of Hereford and Holstein calves. J. Anim. Sci. 31:626-630

Owen, C. A. Jr. 1965 Metabolism of radio copper (Cu^{64}) in the rat. Am J. physiol. 209:900-904

Owen, C. A. Jr.1971. Metabolism of Copper 67 by the Copper-deficient rat. Am. J. Physiol. 221:1722-1727.

Owen, C. A. Jr. 1981. *Copper deficiency and Toxicity.* Park Ridge, New Jersey. Noyes Publication.

Owen, C. A. Jr. 1982a. *Physiological Aspects of Copper. Copper in Organs and Systems.* Park Ridge, New Jersey. Noyes Publication.

Owen, C. A. Jr. 1982b. *Biochemical Aspects of Copper. Copper Proteins, Ceruloplasmin, and Copper Protein Binding .* Park Ridge, New Jersey. Noyes Publication.

Pariza MW, Hargrave WA: A beef derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[a]anthracene. Carcinogenesis 8:1881-1887, 1985.

Pariza M, Park Y, Cook M, Albright K, Liu W, et al. FASEB J 10: Abstract #3227, 1996.

Palmquist, D. L., A.D. Beaulieu, and D. M. Barbano. 1993. Feed and Animal Factors influencing milk fat composition. J. Dairy Sci. 76:1753-1771.

Paynter, D. I., R.J. Moir and E.J. Underwood. 1979. Changes in the activity of the Cu-Zn superoxide dismutase enzyme in tissues of the rat with changes in dietary Copper. J. Nutr. 109:1570-1576.

Phillippo, M., W.R. Humphries, I. Bremner, T. Atkinson, and G. Henderson. 1985. Molybdenum-induced fertility in cattle. In*: C, F, Mills, I. Bremner and J. K. Chessters (Ed.) Trace elements in Man and Animals (TEMA-5).* Pp. 342-345. Commonwealth Agric. Bureaux, Farnham Royal, UK.

Phillippo, M. and D.S. Graca. 1983. Biliary Copper secretion in cattle. Proc. Nutr. Soc. 42:46A.

Price, J., A.M. Will, G. Paschaleris and J.K. Chesters. 1987. Identification of thiomolybdates in digest and plasma from sheep after administration of 99-Mo labeled compounds in the rumen. Br. J. Nutr. 58:127-137.

Prohaska, J. R. 1991. Changes in the Cu,Zn-superoxide dismutase, cytochrome c oxidase, glutathione peroxidase, and glutathione transferase activities in Copper-deficient mice and rats. J. Nutri. 121:355-363.

Richards, M. P., and R.J. Cousins. 1975. Mammalian zinc homeostasis: Requirements for RNA and metallotionein synthesis. Biochem, Biophys. Res. Commun. 64:1215-1223.

SAS. 1988. SAS GLM, ed. 6.03. SAS Inst. Cary, NC

Shultz TD, Chew BP, Seaman WR: Differential stimulatory and inhibitory responses of human MCF-7 breast cancer cells to linoleic acid and conjugated linoleic acid in culture. Anticancer Res 12(6B):2143-2145, 1992.

Shand, A. and G. Lewis. 1957. Chronic Copper poisoning in young calves. Vet. Rec. 69:618.

Schwartz, F.J. and M. Kirchgessner. 1974. Intestinal absorption of Copper, zinc, and iron after dietary depletion. In: *W.G. Hoekstra, J.W. Suttie, H.E. Ganther, and W. Mertz (Eds.). Trace element metaboliam in animals-2.*pp. 519-522. University park Press Baltimore.

Starcher, B.C. 1969. Studies on the mechanism of Copper absorption in the chick. J. Nutr. 97:321-326.

Stull, J. W., and W. H. Brown. 1964. Fatty acid composition of milk. II. Some differences in common dairy breeds. J. Dairy Sci. 47:1412.

Suttle N. F. 1991. The interactions between Copper, molybdenum, and sulfur in ruminat animals. Annu. Rev. Nutr. 11:121-140.

Suttle, N.F. and C.H. McMurray. 1983. Use of erythrocyte Copper:zinc superoxide dismutase activity and hair and fleece Copper concentration in the diagnosis of hypocuprosis in ruminants. Res. Vet. Sci. 35:47-52.

Turner, J.C. V. Shanks, P.J. Osborn and S.M. Gower. 1987. Copper absorption in sheep. Comp. Biochem. Physiol. 86C:147-150.

Thompson, E. H., C. E. Allen, and R. J. Meade. 1973. Influence of Copper on stearic acid desaturation and fatty acid composition in the pig. J. Anim. Sci. 36:868–873.

Underwood, E. J. 1977. Copper. In: *E. J. Underwood(Ed.) Trace elements in Human and Animal Nutrition 4th ed.* Pp. 56-108. Academic Press, New York.

Van Barneveld, A.A., and J. A. van den Hamer. 1984 Intestinal passage and absorption of simultaneously administered ^{64}Cu and ^{65}Zn and the effect of feeding in the mouse and rat. Nutr. Rep. J. 29:173-182.

Van Koetsveld, E.E. 1958. The manganese and Copper contents of hair as an indicator of the feeding condition of cattle regarding manganese and Copper. Tijdsehr. Diergeneesk. 83:229.

Visonneau S, Cesano A, Tepper SA, Scimeca JA, Santoli D, Kritchvsky D:Conjugated linoleic acid suppresses the growth of human breast cancer adenocarcinoma in SCID mice. Anticancer Res 17(2A):969-973, 1997.

Wapnir, R. A. and L. Stiel. 1987. Intestinal absorption of Copper: Effect of sodium. Proc. Soc. Exp. Biol. Med. 185:277-282.

Ward, G.M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: A review. J Anim Sci. 46:1078.

Weiss, E. and P. Baur. 1968. Experimental studies on chronic Copper poisoning in the calf. Zentralb. Veterinaermed. 15:156.

Weiss, K. C., M.C. Linder and the Los Alamos Radiological Medical Group. 1985. Cu transport in rats involving a new plasma protein. Am. J. Physiol. 249:E77-E88.

Weiner, G., S. Hayter, A. C. Field and N.S.M. Macleod. 1974. Cu levels in liver and blood of sheep and dead lambs in relation to breed, age at death, and cause of death. J. Comp. Pathol. 84:27-38.

White, S. L., J. A. Bertrand, M. R. Wade, S. P. Washburn, J. T. Green Jr., and T. C. Jenkins. 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84:2295-2301.

Xin, Z. 1990. Cu status and luteinizing hormone secretion, Cu distribution in tissues, neutrophil functions, superoxide dismutase activity and different Cu sources for dairy cattle. Ph. D. Dissertation. University of Kentucky. Lexington , KY.

Xin, Z., D. F. Waterman, R.W. Hemken, and R. J. Harmon. 1991. Effects of Copper status on neutrophil function, superoxide dismutase, and Copper distribution in steers. J. Dairy Sci. 74:3078-3085.

Zammit, V.A. 1996. Role of insulin in hepatic fatty acid partitioning: emerging concepts. Biochem. J. 314:1-14.