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The biological functions of copper in ruminant animals with emphasis on its influence on the rumen microbiota: A narrative review

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ABSTRACT

Trace elements play a significant role in the health, metabolism, and productivity of ruminants. Copper (Cu) is an essential mineral required for various biological functions in ruminant animals. The dietary requirements and the maximum tolerable concentration for Cu in ruminants are subject to variation based on multiple factors such as animal breed, production type, Cu source, and the concurrent presence of antagonistic minerals. The role of Cu within the rumen microbial ecosystem is of considerable importance in ruminant species. The source and concentration of Cu can modulate the population dynamics and metabolic activities of rumen microorganisms, particularly bacteria and protozoa. Additionally, the resulting alterations in microbial metabolite production and feed digestibility can ultimately influence ruminant production. Organic and inorganic sources of Cu have distinct effects on rumen microorganisms. An optimal Cu amount is advantageous for rumen fermentation. Conversely, elevated Cu levels, particularly those derived from inorganic sources such as oxides and sulfates, can adversely affect feed digestibility, while hydroxychloride formulations and organic amino acid chelates have lower negative impacts on this parameter at high concentrations. Primary effects of Cu on rumen microbiota include alterations in bacterial populations, particularly an increase in fiber-degrading bacteria, a decrease in protozoan communities at elevated levels of Cu, changes in the production and composition of volatile fatty acids, and the potential reduction of methane emissions. This review discusses the biological effects of Cu in ruminants, encompassing Cu requirements, deficiency manifestations, and toxicity concerns, while emphasizing the interactions between Cu and rumen microbial populations and their metabolism.

Keywords: Metabolism, Methane emission, Microorganisms, Minerals, Rumen fermentation

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Introduction

The rapid increase in the global population has resulted in a corresponding rise in the demand for food, particularly in relation to animal proteins. This population growth represents the principal driver of domestic animal population expansion, imposing immense pressure on the livestock sector. Consequently, the efficiency of animal production plays a vital role in fulfilling global food requirements (Valizadeh, 2014; Mousaie et al., 2025; Jimenez-Islas et al., 2026). To sustain the health and productive performance of animals at an optimal level, micronutrients specifically minerals, hold significant importance in ruminants. Minerals possessing structural, physiological, catalytic, and regulatory functions within biological systems, and as metabolic modifiers, they influence ruminants' metabolism, growth and production (NRC, 2005; Suttle, 2021, Mousaie et al., 2026). The Cu element is a trace mineral that is widely distributed among animal, plant, and microbial cells. In adult humans, it is present in quantities of approximately 100 milligrams, with Cu-binding proteins making up about 0.3% of bacterial proteomes (Li et al., 2019). This mineral serves a critical function in ruminant animals' physiology, as it exerts significant impacts on health status and productive performance. Copper is essential for numerous biological functions, including antioxidant system activity, wool and hair pigmentation, cellular respiration, immune system function, bone development, erythrocyte formation, and connective tissue development in ruminants (NRC, 2007; NASEM, 2021; Suttle, 2021). Both Cu deficiency and excess can result in growth retardation, lowered productive performance as well as morbidity and mortality in ruminant animals (NRC, 2005; Wang et al., 2025). Ruminants exhibit varying Cu requirements based on their productive status, breed, age, environmental conditions and nutritional plans. Furthermore, the bioavailability of Cu differs between organic and inorganic sources (Ward et al., 1996; Wang et al., 2012). However, the utilization of various Cu sources with differing concentrations, coupled with inconsistent and sometimes contradictory results reported in the scientific literature concerning the effect of this mineral on rumen microbial populations, metabolic byproducts, dietary digestibility, and ultimately livestock production performance, creates considerable uncertainty within both the farming and research communities. Therefore, this situation requires comprehensive evaluation and critical discussion of existing research evidence. The primary objectives of this review were to clarify the diverse biological functions of Cu and to assess the existing knowledge regarding Cu requirements, supplementation strategies, and their respective influences on productive performance of ruminants and rumen microbial communities. Additionally, this review aimed to address inconsistencies or conflicting results in the literature and to provide evidence-based and practical recommendations for researchers, veterinary practitioners, and livestock producers.

Biological importance of Cu in ruminant animals

Copper is essential for various biological functions in ruminants. As demonstrated in Table 1 (Aupperle et al., 2001; McDonald et al., 2010; NRC, 2005; Engle, 2011; Suttle, 2021; Postma et al., 2021; NASEM, 2021; Ranaweera et al., 2023), Cu is a component of certain enzymes and proteins such as cytochrome C oxidase (CCO), superoxide dismutase (SOD), ceruloplasmin, tyrosinase, lysyl oxidase and dopamine-β-hydroxylase that facilitate physiological and metabolic processes in these animals. This mineral is present in the environment and circulates from soil to the cells of animal bodies as illustrated in Figure 1. Most of the soluble Cu in surface soils is organically complexed, causing the total Cu in soil solution to be large, and in most cases assuming about 90% with the available Cu occupying only 10%. This element is also known to play important roles in the mineral nutrition, biochemistry, and physiology of the plant. An important function of Cu in plant metabolism is concerned with chlorophyll formation and as a component of a number of different plant enzymes

(Haque et al., 1993). The Cu content of pastures and forages is influenced by factors such as plant species, maturity, specific soil conditions and the type of fertilizers applied. In general, temperate grasses have been observed to contain lower levels of Cu compared to legumes when grown under identical conditions, with reported values of 4.7 mg/kg dry matter for grasses and 7.8 mg/kg for legumes, respectively (Suttle, 2010). Seeds and seed by-products are usually rich in Cu, whereas straws generally contain little amounts of this element (McDonald et al., 2010). Increased quantities of Cu have been documented as varying according to plant variety, maturity, soil conditions, fertilizer application, and environmental factors (Suttle, 2021). The consumption of feed containing Cu by ruminants leads to significant interactions with rumen microorganisms, which in turn influences its absorption from the digestive system (Zhang et al., 2007; McDonald et al., 2010; Shete et al., 2023), and has implications for the health and productivity of these animals. Copper metabolism functions through regulated pathways of Cu trafficking rather than relying on pools of Cu lability. Within the cell, Cu is transported to facilitate enzyme production, retained in metallothionein, or excreted via the Golgi apparatus into the bloodstream. In hepatocytes, the synthesis of Cu-containing caeruloplasmin allows for the systemic supply of Cu, while excess Cu is excreted through bile (Clarkson et al., 2020).

Table 1. Copper-containing proteins, as well as the biological functions of Cu and the consequences of its deficiency in ruminant animals.

Cuproenzymes and Cu-binding proteins	Biological function	Hypocuprosis/Cu-deficiency consequences	References
Cytochrome C oxidase	Cellular respiration	Anorexia Encephalomyopathy Swayback disease Reduced cellular immunity Impaired energy metabolism Reduced animal's production	Aupperle et al., 2001; McDonald et al., 2010; NASEM, 2021; Suttle, 2021
Ceruloplasmin	Cu and Fe transport, ROS scavengering	Anemia	NRC, 2005; McDonald et al., 2010; Suttle, 2021
Superoxide dismutase	Antioxidant, ROS scavenger	Increased susceptibility to oxidants Elevated risk of infection	NRC, 2005; Suttle, 2021
Tyrosinase	Tyrosin to Melanin conversion	Wool and hair depigmentation	Suttle, 2021; Ranaweera et al., 2023
Lysyl oxidase	Cross linkages in connective tissues	Bone and Joint abnormalities Reduced wool quality	Postma et al., 2021; Suttle, 2021
Dopamine-β-hydroxylase	conversion of dopamine into norepinephrine	Nervous system disorders	NRC, 2005; Engle, 2011; Suttle, 2021

The Cu deficiency has been extensively documented as an etiology of enzootic ataxia, anemia, and reduced growth performance in ruminant animals particularly in ovine species. The associated clinical manifestations are characteristically attributed to neurological degeneration, skeletal malformations, and compromised immune function, with disease severity demonstrating considerable variation across species and age groups (NRC, 2005). Cattle can experience Cu deficiency under unsuitable nutritional circumstances. A study conducted by Dargatz et al. (1999) examined 2,007 beef cows and heifers from 256 herds across 18 states, revealing that 38.9% of the subjects were identified as marginally deficient in Cu, with serum Cu concentrations equal to or less than 0.65 mg/L (Spears et al., 2022). Research has indicated that dietary Cu deficiency in cattle compromises the capacity of neutrophils to eliminate Candida albicans thereby indicating its significance in immune system function and disease prevention (Li et al., 2019). In addition, secondary Cu deficiency is primarily precipitated by antagonistic interactions with molybdenum (Mo), sulfur (S), and iron (Fe), which substantially diminish Cu bioavailability and metabolic utilization (Suttle, 2021). The manifestation of Cu deficiency observed in the small ruminants was characterized by variable severity levels of lethargy, swayback, ataxia, progressive weight reduction, emaciation, loss of hair and wool pigmentation, alopecia, marked anemia, diarrhea, reproductive anestrus, embryonic resorption, placental retention during the postpartum period, as well as extended recumbency (Almeida et al., 2022; Byrne and Murphy, 2022). The effects of Cu on livestock performance exhibit variability across studies. One investigation (Solaiman et al., 2007) indicated that increased Cu concentrations in caprine subjects improved growth performance, while another study (Zhang et al., 2008) reported that optimal growth performance and feed efficiency were achieved in goats fed diets containing 10 mg of Cu from copper sulfate ($CuSO_4$) supplementation per kg of feed.

Conversely, goats receiving diets with higher Cu concentrations of 30 mg/kg displayed the lowest weight gain, indicating the variability in outcomes based on the quantity and source of Cu supplementation. Copper, particularly from organic sources at concentrations of 10 and 20 mg/kg of feed, has been shown to reduce carcass adiposity in sheep by promoting lipolysis and inhibiting lipogenesis (Cheng et al., 2010). A study showed that elevated dietary Cu concentrations exceeding recommended amounts may enhance the digestion of low-quality forage (Lopez-Guisa et al., 1992). The evidence implies that dietary Cu has an additive effect on rumen nutrient digestion and microbial proliferation. In contrast, some findings confirmed that free Cu ions derived from rumen-soluble $CuSO_4$ may react with S, Mo, and Fe to form insoluble complexes in the rumen, which could reduce intestinal Cu absorption (Gould and Kendall, 2011; Wilk et al., 2022). Coated $CuSO_4$ has been reported to be beneficial in dairy cows by improving milk performance and feed digestion (Wang et al., 2021). However, research on dairy cows has indicated that the addition of dietary Cu did not influence feed intake or milk yield but altered the composition of milk fatty acids (Engle and Spears, 2000; Morales et al., 2000). This suggests potential variations in the responses to Cu supplementation based on different productive conditions among animals and various form and quantity of administered Cu supplements.

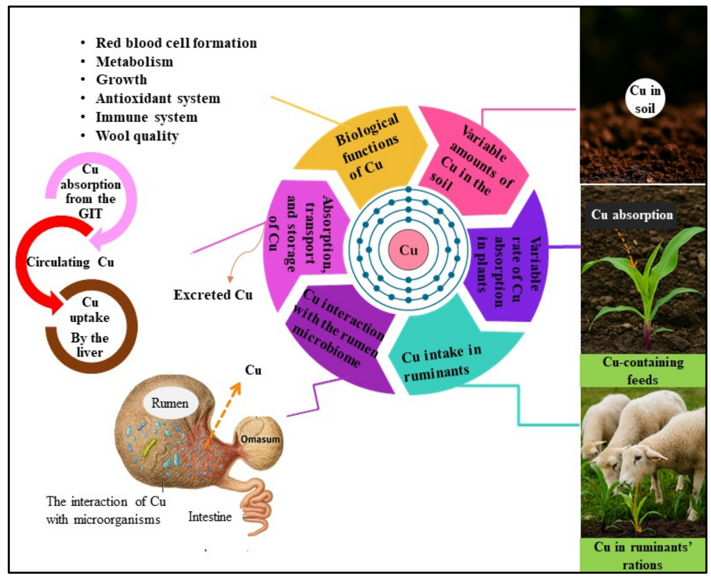

Figure 1. The circulation of Cu from the soil into plants, followed by its transfer through the digestive system of ruminants, its absorption, and subsequent entry into ruminant cells, along with its associated roles in their metabolic processes and production (Haque et al., 1993; NRC, 2005; Zhang et al., 2007; McDonald et al., 2010; Clarkson et al., 2020; Suttle, 2021; Shete et al., 2023).

Although Cu is an essential trace element for livestock, excessive concentrations in the diet can lead to toxicity (Suttle, 2021). Consequently, various organisms, depending on their species and biological conditions, have evolved specialized homeostatic mechanisms to effectively absorb, transport, and excrete Cu, while also mitigating its toxic effects (Lopez-Alonso and Miranda, 2020; Byrne and Murphy, 2022). Copper's high reactivity permits it to readily engage in redox reactions, influencing cellular processes, and this characteristic contributes to its toxicological impacts on both animal and prokaryotic cells (Solioz, 2018). Ruminants exhibit a heightened vulnerability to Cu toxicity compared to non-ruminant species, likely due to their grazing habits on pastures with low Cu content, which may also be rich in Cu antagonist minerals such as S, Mo, and Fe. Given the limited regulatory control ruminants possess over Cu absorption, they have adapted mechanisms for accumulating excess Cu in the liver by minimizing its biliary excretion (Lopez-Alonso and Miranda, 2020). The liver serves as the primary storage organ for Cu allowing for the mobilization of Cu into the bloodstream to fulfill various biochemical functions (Grace et al., 2012). When animals encounter Cu concentrations that surpass their physiological requirements, this can lead to an excessive storage of Cu in liver tissue; symptoms of Cu toxicity include diarrhea, abdominal pain, liver dysfunction, anemia, and hemolysis, with manifestations occurring either acutely or chronically based on the exposure level (NRC, 2005; Suttle, 2021; Wang et al., 2025). Instances of acute Cu toxicity are primarily linked to unintentional ingestion of Cu-containing substances, such as CuSO4 or copper chloride (CuCl2); Chronic Cu toxicity is mainly attributed to excessive dietary Cu intake and genetic predispositions linked to specific mutations (Wang et al., 2025). Research indicated that optimal Cu levels can positively affect the population of rumen microorganisms and their metabolic activities; however, the detrimental effects of Cu toxicity on rumen cellulose-degrading bacteria have been reported (Hubbert et al., 1958). Additionally, elevated levels of inorganic Cu in animals' nutrition can have negative implications for human health through increased excretion and its subsequent entry into soil and plant systems (Zhen et al., 2022).

Copper requirements of ruminants

As demonstrated in Figure 2, the Cu requirements of some industrial ruminant species such as cattle, sheep, and goats are not constant and vary according to breed, age, sex, stage of production, amount and type of production, environmental conditions, and other dietary components, particularly Cu antagonist elements such as Fe, Mo, S, and to a lesser extent Zn. Notably, liver Cu concentration serves as a superior indicator of body Cu status compared to blood Cu levels (NRC, 2005, NASEM, 2021; Suttle, 2021). However, there is limited information regarding Cu status of soils, Cu content in plants, and blood Cu concentration of ruminants in different regions of Iran. In one study, the optimal plasma Cu level was reported to be 63-127 µg/Dl (Ganjkhanloo et al., 2025). In an experiment conducted on male Kermani lambs, the blood Cu level of the control group was reported to be 58 µg/dL (Seifalinasab et al., 2022), which indicates the necessity to increase dietary Cu, particularly from organic sources, in wool-producing sheep. Additionally, the Cu concentration in the diet of fattening lambs completely mixed with alfalfa and a concentrate consisting of barley grain, soybean meal, and bran was approximately 5.8 mg/kg of dry matter, which increased to 8.2 mg with CuSO4 supplementation, thereby partially meeting livestock requirements (Mousaie, 2021). However, it seems that the Cu requirement in wool-producing sheep is considerably higher (Suttle, 2021).

While maximum tolerable levels (MTL) of Cu have been established at 30, 20, and 35 mg/kg feed for cattle, sheep, and goats, respectively (Figure 3), the actual MTL for ruminants remains uncertain, and higher Cu concentrations have been documented for these animals in various productive and nutritional conditions (NRC, 2005; Solaiman et al., 2007; Marsh et al., 2026). This uncertainty arises from factors such as animal sex and species, the quantity of other dietary elements, particularly Mo, S, and Fe along with the Cu source regarding its solubility and absorption, which all influence the amount of Cu entering circulation and body stores (Suttle, 2021; Zhou and Shen, 2025). For instance, researchers have reported that Angus cattle, in the absence of Cu supplementation, demonstrate higher plasma Cu quantities and ceruloplasmin activity than Charolais and Simmental cattle, and that Angus bulls exhibit superior performance in Cu absorption and retention (Wang et al., 2025).

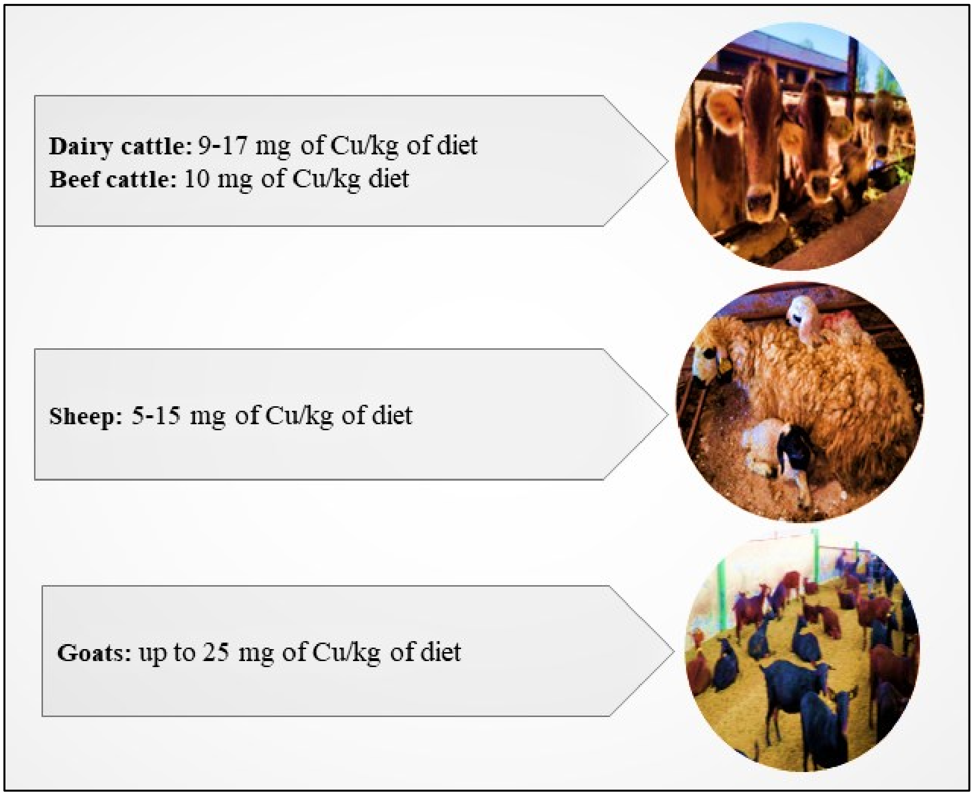

Figure 2. The Cu requirements of cows, sheep and goats (NRC, 2005, NRC, 2007; NASEM, 2021; Suttle, 2021).

Empirical evidence demonstrated that Cu tolerance varies significantly among different sheep breeds (Lopez-Alonso and Miranda, 2020). Merino sheep breed exhibits heightened resistance to elevated and toxic Cu concentrations, whereas breeds including Suffolk, Leicester, Charolais, and Texel demonstrate greater susceptibility to Cu toxicity when compared to the Cambridge breed, which shows relative resistance (Saeed et al., 2025). Nonetheless, a notable knowledge gap exists regarding the tolerable Cu threshold in indigenous Iranian sheep breeds. In comparison to bovine and caprine species, sheep display increased sensitivity to Cu toxicity. The higher sensitivity of sheep to Cu toxicity is attributed to their comparatively diminished capacity to increase bile secretion, thereby reducing Cu excretion under conditions of elevated dietary Cu intake. Owing to impaired Cu excretion through biliary pathways, sheep possess limited capacity to tolerate dietary Cu excess, resulting in hepatic Cu accumulation and subsequent hepatotoxicity (Wang et al., 2025).

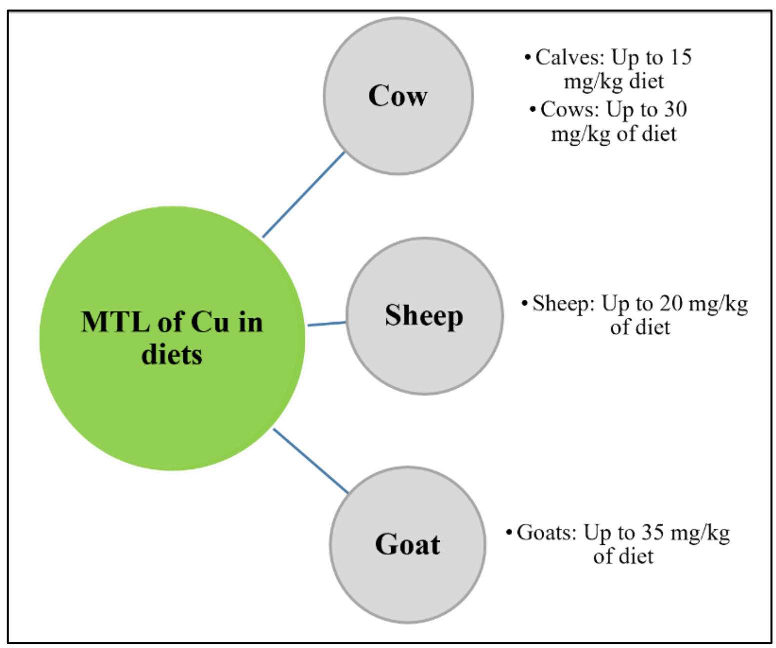

Figure 3. The maximum tolerable level (MTL) of Cu in the diet of ruminants (NRC, 2005; Solaiman et al., 2007; Lopez-Alonso and Miranda, 2020; Suttle, 2021; Marsh et al., 2026).

Copper absorption and bioavailability of different Cu sources in the digestive tract

Ruminants possess a distinctive digestive system that differentiates them from other mammalian species which consequently influences Cu metabolism. In these organisms, Cu metabolism occures in the rumen, and the tripartite interaction among Cu, Mo, and S represents one of the most significant physiological distinctions between ruminants and monogastric animals, thereby affecting animal health and productivity. This noncompetitive interaction mechanisms result in diminished intestinal absorption of this mineral, ranging from less than 1 to 10%, when compared with nonruminant and suckling calf species, which demonstrate absorption rates reaching up to 70%; however, the degree of absorption varies substantially based on the relative concentration of Cu antagonistic compounds. Although there is some Cu absorption within the rumen environment, intestinal absorption demonstrates greater bioavailability efficiency (Lopez-Alonso and Miranda, 2020; Byrne and Murphy, 2022). Inorganic and organic S compounds undergo microbial metabolism in the rumen, resulting in sulfide production, as illustrated in Figure 4. Concurrently, S and Mo undergo a chemical reaction to form thiomolybdates, which exist in mono-, di-, tri-, and tetrathiomolybdate forms. These compounds exhibit strong binding affinity to Cu, with tri- and tetrathiomolybdates demonstrating irreversible binding capacity, thereby forming Cu-thiomolybdate complexes. Copper bound to thiomolybdates exhibits low solubility and remains unabsorbed in the intestinal tract. In the absence of Cu in the rumen environment, thiomolybdates are absorbed through the rumen epithelium and, at a reduced rate, through the small intestinal mucosa. Following absorption into the circulatory system, these compounds may bind to Cu present in biological compounds (Lopez-Alonso and Miranda, 2020; Zhou and Shen, 2025). Ceruloplasmin, a glycoprotein, functions as the primary Cu carrier in blood plasma, transporting approximately 90% of circulating Cu, and serves an essential role in facilitating Fe utilization for hemoglobin synthesis (NRC, 2005; Suttle, 2021). The liver is the primary storage site for Cu, and this mineral can be readily mobilized from hepatic tissue into the bloodstream to meet the biochemical requirements of the animal. The liver Cu quantity is greater in newborn ruminants than adults with developed rumen. Depending on hepatic Cu concentrations and the level of dietary Cu antagonists, liver Cu reserves can sustain normal plasma Cu levels for extended periods, even in ruminants consuming Cu-deficient diets (Spears et al. 2022).

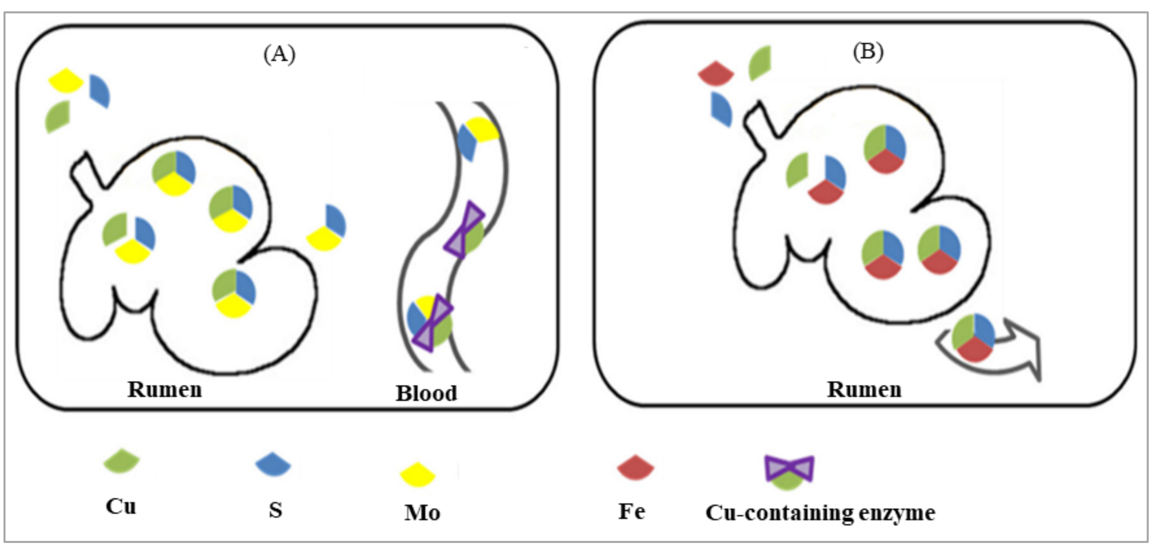

Figure 4. The interaction of Cu with S and Mo in the rumen and blood (a), and with Fe and S in the rumen (b) of ruminants (Lopez-Alonso and Miranda, 2020).

Dietary Cu absorption in adult dairy cattle ranges from 1 to 5 percent (Wang et al., 2021). Copper sulfate represents the predominant Cu supplement employed within the livestock industry. Although Cu absorption primarily occurs in the small intestine, $CuSO_4$ demonstrates solubility in the rumen environment and may establish insoluble complexes with S, Mo, and Fe, thereby hindering Cu absorption (Wang et al., 2021). Although elevated concentrations of elemental Zn may potentially diminish Cu absorption, a study in lambs indicated that utilizing three Zn sources—nano-zinc oxide (25 mg of Zn/ kg of diet), zinc-methionine (50 mg of Zn/ kg of diet), and zinc oxide (100 mg of Zn/ kg of diet)—produced no adverse effects on Cu absorption or blood Cu concentrations (Hashemzaei et al., 2025). Among inorganic Cu formulations, sulfate and chloride forms exhibit greater bioavailability than carbonate and oxide forms. Organic Cu supplements, including amino acid chelates and Cu-proteinate, demonstrate enhanced intestinal bioavailability compared to inorganic Cu supplements due to absorption via specific transporters and hydrochlorides, attributed to diminished rumen interactions, particularly with Mo and S (Byrne and Murphy, 2022). Additionally, using organic Cu supplement (Cu-methionine) in sheep revealed superior Cu absorption and reduced gastrointestinal excretion compared to $CuSO_4$ (Pal et al., 2010). Conversely, a study reported equivalent bioavailability between Cu-proteinate and $CuSO_4$ at low Mo concentrations (Ward et al., 1996). However, sulfate supplements, encompassing $CuSO_4$, have been documented to exert negative effects on feed cell wall digestibility within the ruminant animals' gastrointestinal tract (Arce-Cordero et al., 2020). Therefore, the source and concentration of Cu as well as the presence of other antagonistic minerals, significantly influence its absorption.

The effect of Cu on microbial fermentation in the rumen

The impact of Cu on microbial community in the rumen of cattle has produced inconsistent findings. Some studies have reported negative effects of dietary Cu supplementation at levels ranging from 5 to 25 mg/kg of diet on the feed digestibility (Genther and Hansen, 2015; Faulkner et al., 2017; Caldera et al., 2019). In a study, decreased neutral detergent fiber (NDF) and crude protein digestibility was observed throughout the entire gastrointestinal tract of cross-bred bulls administered an intraruminal tablet containing 12.5 g of Cu-oxide (Arthington, 2005). This particular study documented mean liver Cu concentrations exceeding 600 mg/kg in bulls receiving these tablets, substantially higher than concentrations observed in cattle receiving $CuSO_4$ through dietary supplementation, indicating that the Cu tablet formulation produces elevated rumen Cu levels with greater effects on nutrient digestibility. These findings suggest that adverse effects manifest at markedly high Cu concentrations, particularly when derived from inorganic sources such as oxide and sulfate, whereas hydroxychloride formulations and organic chelate sources do not adversely affect digestibility. Conversely, it has been reported either an absence of Cu effects on fermentation or improvements in fermentation parameters when Cu-supplemented diets were provided. Research indicated that molar ratios of volatile fatty acids (VFA) in the rumen were not affected by Cu supplementation, and supplemental Cu at 10 or 20 mg of Cu/kg of high-concentrate diet containing 4.9 mg of Cu/kg of dry matter, produced no effect on rumen fermentation (Engle and Spears, 2000). However, in vitro investigations using culture media containing rumen microorganisms demonstrated that different Cu sources at varying concentrations affected microbial fermentation, with in vitro dry matter degradability and microbial amylase activity being elevated with Cu-methionine compared to $CuCl_2$ and tribasic $CuCl_2$ (Zhao et al., 2022). In addition, ammonia nitrogen concentration, microbial protein, and propionate were higher in the Cu-methionine treatment relative to the tribasic $CuCl_2$ treatment. In a study, the Cu supplementation at 10 mg/kg of feed from $CuSO_4$, with a basal diet containing approximately 7.5 mg of Cu/kg, reduced rumen pH in goats while enhancing microbial fermentation, resulting in increased total VFA concentration, acetate ratio, and NDF digestibility throughout the gastrointestinal tract (Zhang et al., 2007). Similarly, lambs receiving 10 or 20 mg of Cu from an organic source, specifically Cu-proteinate, demonstrated improved NDF and acid detergent insoluble fiber (ADF) digestibility in the gastrointestinal tract relative to those receiving an inorganic source (Dezfoulian et al., 2012). In another research, cattle receiving 7.5 mg of Cu/kg of feed exhibited higher apparent digestibility of dry matter, organic matter, crude protein, NDF, and ADF compared with control animals (Wang et al., 2021). Similarely, bulls provided with a diet containing 7.68 mg of supplemental Cu from CuSO4, showed increased total rumen VFA, particularly acetate, with concurrent improvements in dry matter, organic matter, protein, and NDF digestibility (Shang et al., 2020), while application of 15 mg of Cu as coated CuSO4 within a continuous culture system showed that microencapsulation of Cu reduced the molar ratio of acetate and increased the butyrate ratio, though feed nutrient digestibility remained unaffected (Arce-Cordero et al., 2020). These researchers proposed that CuSO4 may modify the metabolism and production of VFA and their molar ratios in the rumen without altering nutrient digestion, thereby increasing the butyrate molar ratio relative to acetate.

Copper and the activity of rumen bacteria

The mechanism by which Cu exerts its effects on rumen bacteria remains incompletely understood. Approximately 18 to 27% of the supplemented Cu was analyzed in a bacteria-enriched fraction, indicating that Cu might be assimilated by rumen bacteria (Vigh et al., 2023). This mineral influences the activity of numerous rumen bacteria through its participation as a cofactor for essential enzymes, including CCO, NADH dehydrogenase, and SOD (Kenney and Rosenzweig, 2012). In one study (Shang et al., 2020), male cattle receiving diets supplemented with 7.68 mg of Cu from CuSO4 demonstrated increased activity of microbial enzymes, namely carboxymethyl cellulase, cellobiase, xylanase, and pectinase, as well as elevated total bacterial populations, particularly Ruminococcus albus, Ruminococcus flavefaciens, Fibrobacter succinogenes, Butyrivibrio fibrisolvens, Prevotella ruminicola, and Ruminobacter amylophilus. The enhancement of bacterial populations, especially cellulose-degrading bacteria, resulted in increased feed digestibility and daily weight gain in studied animals. In another study (Wu et al., 2021), the administration of 10 mg of Cu/kg of feed from a protected CuSO4 source in Holstein bulls increased cellulase and protease activities while decreasing alpha-amylase activity in rumen microorganisms. Additionally, coated CuSO4 increased populations of Butyrivibrio fibrisolvens and Ruminococcus flavefaciens while decreasing populations of Prevotella ruminicola and Ruminobacter amylophilus. The researchers reported that these alterations in microbial populations enhanced feed digestibility and VFA production, particularly the molar ratio of acetate. In this regard, it has been documented that among rumen bacteria, Butyrivibrio has greater sensitivity to CuSO4 compared to other rumen microorganisms and can make changes in VFAs molar ratios without exerting substantial effects on substrate fermentation (Arce-Cordero et al., 2020). Copper has the ability to diminish methanogenic microorganisms by adversely impacting rumen protozoa (Shete et al., 2023). In addition, elevated Cu concentrations, particularly from inorganic sources, may produce negative effects on certain rumen bacterial species. In one investigation (Hernandez-Sanchez et al., 2019), high Cu levels (80 and 100 μg of Cu/g dry matter) reduced bacterial populations. The researchers attributed this effect to intracellular mineral accumulation. Fluctuations in cytoplasmic Cu between oxidized ($Cu2+$) and reduced ($Cu1+$) states can convert it into a toxic substance. Gram-negative bacteria exhibit heightened sensitivity to Cu due to membrane permeability characteristics; consequently, Cu accumulation in the periplasm results in bacterial lysis and cellular death. Bacterial capacity to tolerate high quantities of Cu correlates with bacterial homeostatic systems, and specific genes within bacterial cells mediate Cu resistance; furthermore, proteins associated with Cu-efflux pumps function within bacterial cells. Moreover, Cu resistance has been documented through plasmid DNA transfer among rumen bacteria (Hernandez-Sanchez et al., 2019).

The interaction of Cu with rumen protozoa and fungi

Rumen protozoa constitute approximately 50% of the microbial biomass. Nonetheless, their functional role within this ecosystem remains poorly understood. Initial studies indicated a potential involvement of protozoa in carbohydrate and protein metabolism, however, due to their close association with bacteria, has made it difficult to conclusively assign these functions to protozoa (Williams et al., 2020). The existing literature on the effects of Cu on rumen protozoa reveals contradictory results. In one investigation, supplementing the diet of bulls with CuSO4 increased the total rumen protozoa population (Shang et al., 2020), whereas in another study, elevated Cu levels (100 and 200 mg of Cu per day) reduced rumen protozoa populations in goats, with researchers attributing improved growth performance to this reduction (Solaiman et al., 2007). The antiprotozoal effects of Cu have been demonstrated to a certain extent. Nanocopper administration has been shown to eliminate rumen ciliated protozoa, consequently reducing methanogen populations and methane production (Shete et al., 2023). Consistent with these findings, another experiment indicated that the application of 10 and 20 mg of nanocopper/kg of feed reduced rumen protozoa in cattle (Palangi et al., 2024). Notably, a foundational study conducted approximately 40 years ago, addressed the protective role of protozoa in mitigating Cu toxicity in livestock (Ivan et al., 1986), a topic that has received limited attention in subsequent research.

These researchers reported that introducing rumen ciliated protozoa into defaunated sheep reduced Cu absorption and hepatic accumulation. Consequently, the prevalence of chronic Cu toxicity is elevated in protozoa-depleted ruminants. Limited information exists regarding potential effects of Cu on rumen fungi. Although fungi represent a modest proportion of total rumen microbiota, accounting for approximately 8% of rumen biomass, they play a significant role in feed degradation; the composition of ruminant diets, particularly elevated fiber content, may promote rumen fungal population development (Krol et al., 2023). A study reported that adding CuSO4 to the diet increases anaerobic rumen fungi populations in cattle (Shang et al., 2020). Similarly, it was found that using organic Cu sources (up to 15 mg of Cu/kg of diet) positively influenced rumen fungal populations and fiber digestion (Adedeji et al., 2024). In contrast, no significant effect of Cu on rumen fungal populations of cattle was reported (Hanauer, 2017), suggesting that the source and concentration of dietary Cu may influence fungal response. Nevertheless, additional research is necessary to elucidate the relationship between Cu intake and rumen fungi. In general, the potential effects of Cu on the population and activity of rumen microorganisms can be summarized as presented in Figure 5.

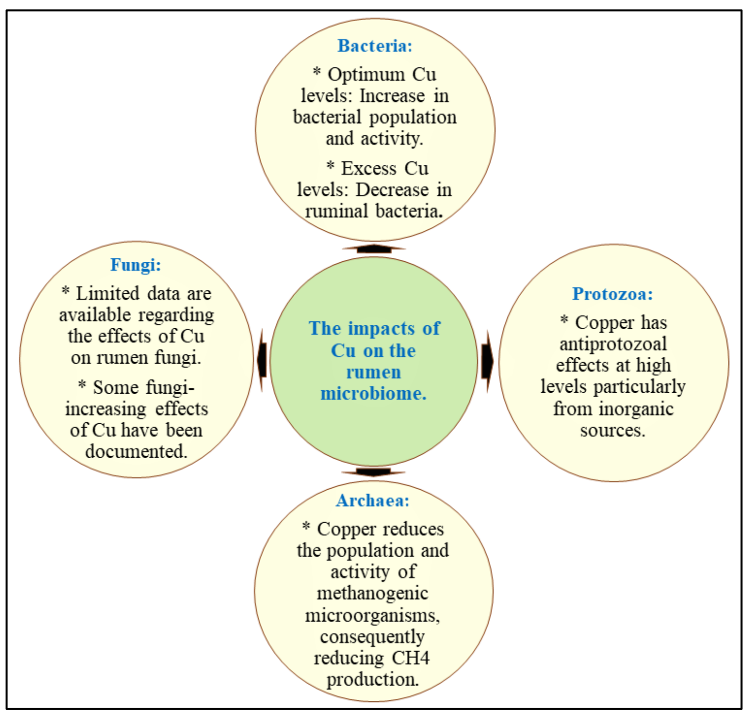

Figure 5. A summary of the effects of Cu on rumen microbiota, including bacteria, protozoa, fungi, and archaea (Hernandez-Sanchez et al., 2019; Shang et al., 2020; Wu et al., 2021; Shete et al., 2023; Palangi et al., 2024; Adedeji et al., 2024).

Conclusion

The findings of this review demonstrated that the administration of Cu to ruminants at appropriate levels is positively associated with their health and productive performance. This mineral is crucial for the optimal functioning of antioxidant and immune systems, nutrient metabolism, nervous system activity, as well as skin and wool health. A deficiency of Cu can lead to disease occurrence and diminished productive performance in ruminants. Conversely, excessive Cu intake, especially in sheep, can result in severe toxicity. The absorption rate of organic Cu supplements is higher than that of inorganic forms. The use of organic Cu supplements, particularly amino acid chelates at recommended concentrations, may yield beneficial effects on feed digestibility and reduce methane production while enhancing energy efficiency in ruminants by decreasing protozoa and certain methanogenic species. However, elevated levels of Cu, particularly from inorganic sources, may adversely affect specific microbial species and decrease their populations; hence, the incorporation of CuSO4 supplement in ruminants' diets should be minimized. It is therefore recommended that during drought conditions and thermal stress, when animals may not receive adequate nutrition, or in circumstances where dietary Cu levels are insufficient or S and Mo concentrations are high, Cu supplements should be added to diets, particularly in the form of organic compounds such as Cu-methionine and Cu-proteinate, or mineral forms such as Cu-hydroxychloride. In addition, the MTL for ruminant breeds should be taken into account to avoid Cu toxicity. Nevertheless, a more comprehensive understanding of Cu's effects on rumen microbiota necessitates further research, particularly utilizing genomics-based and metabolomics technologies to elucidate the potential roles of Cu in influencing the population and activity of rumen microorganisms. Moreover, further studies may be required to revise the optimal dietary Cu concentrations for ruminants when employing Cu sources with greater bioavailability than inorganic alternatives, such as amino acid-chelated and nanoparticle formulations of Cu.

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