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Added dietary sulfur and molybdenum has a greater influence on hepatic copper concentration, intake, and performance in Holstein-Friesian dairy cows offered a grass silage- rather than corn silage-based diet

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ABSTRACT

To test the hypothesis that the metabolism of Cu in dairy cows is affected by basal forage and added S and Mo, 56 dairy cows that were 35 (standard error ± 2.2) days postcalving and yielding 38.9 kg of milk/d (standard error ± 0.91) were offered 1 of 4 diets in a 2 × 2 factorial design for a 14-wk period. The 4 diets contained approximately 20 mg of Cu/kg of dry matter (DM), and had a corn silage-to-grass silage ratio of 0.75:0.25 (C) or 0.25:0.75 (G) and were either unsupplemented (−) or supplemented (+) with an additional 2 g of S/kg of DM and 6.5 mg of Mo/kg of DM. We found an interaction between forage source and added S and Mo on DM intake, with cows offered G+ having a 2.1 kg of DM lower intake than those offered G−, but no effect on the corn silage-based diets. Mean milk yield was 38.9 kg/d and we observed an interaction between basal forage and added S and Mo, with yield being decreased in cows offered G+ but increased on C+. No effect of dietary treatment on milk composition or live weight was noted, but body condition was lower in cows fed added S and Mo irrespective of forage source. We found an interaction between forage source and added S and Mo on milk somatic cell count, which was higher in cows offered G+ compared with G−, but not in cows fed the corn silage-based diets, although all values were low (mean values of 1.72, 1.50, 1.39, and 1.67 $\log_{10}$/mL for C−, C+, G−, and G+, respectively). Mean plasma Cu, Fe, and Mn concentrations were 13.8, 41.3, and 0.25 μmol/L, respectively, and were not affected by dietary treatment, whereas plasma Mo was 0.2 μmol/L higher in cows receiving added S and Mo. The addition of dietary S and Mo decreased liver Cu balance over the study period in cows fed either basal forage, but the decrease was considerably greater in cows receiving the grass silage-based diet. Similarly, hepatic Fe decreased more in cows receiving G than C when S and Mo were included in the diet. We concluded that added S and Mo reduces hepatic Cu reserves irrespective of basal forage source, but this decrease is considerably more pronounced in cows receiving grass silage- than corn silage-based rations and is associated with a decrease in intake and milk performance and an increase in milk somatic cell count.
Key words: copper, corn silage, dairy cow, grass silage, liver

INTRODUCTION

It has long been recognized that Cu is an important trace element for normal health and performance in dairy cattle, principally due to its requirement in approximately 300 different proteins with functions ranging from efficient iron metabolism, hair pigmentation, antioxidants, release of hormones, and synthesis of connective tissue (Suttle, 2010). As a consequence, Cu-responsive disorders result in production and economic losses due to effects on fertility, performance, and health (NRC, 2005). Clinical signs in dairy cows can be caused by a dietary deficiency of Cu, but often are related to interactions with dietary antagonists such as S and Mo, Fe, and Zn that inhibit Cu absorption or metabolism (Suttle, 2010), with S and Mo receiving the most research attention. It has been proposed that dietary sulfates present in feed or water are reduced in the rumen to sulfides, which then react with molybdate to form thiomolybdates (Dick et al., 1975). Gould and Kendall (2011) discussed that thiomolybdates may be present in the rumen as di-, tri-, or tetrathiomolybdates, with trimolybdate predominant at a ruminal pH of 6.5, whereas tetrathiomolybdate is most prevalent at lower pH values. Thiomolybdates form insoluble complexes with Cu, rendering it unabsorbable (Suttle, 1991), resulting in Cu-responsive disorders. At high

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Mo intakes (e.g., >8 mg of Mo/kg of DM) and very low Cu:Mo ratios (less than 1:1), thiomolybdates may also leave the rumen and be absorbed (Suttle, 2010), subsequently binding to Cu-containing enzymes such as ceruloplasmin (**Cp**), impairing their function (Gould and Kendall, 2011).

It is recognized that the degree of thiomolybdate formation in the rumen can also be affected by the basal forage and method of preservation (Suttle, 1974, 1983, 2010), although our understanding of the mechanism remains poor. For example, in grass hays, the inhibitory effect of Mo on Cu absorption is less than that of S, whereas in fresh grass Cu absorption is greatly affected by small additions of S and Mo, with semipurified diets being intermediate (Suttle, 1983). A large body of literature exists comparing the effect of grass silage with corn silage on dairy cow intake and performance (e.g., Phipps et al., 1995; Hart et al., 2015), and, in general, replacing grass silage with corn silage results in an increase in DMI, milk yield, and milk protein content. However, little information is available on the relative effects of either of these forages on Cu metabolism in Holstein-Friesian dairy cows, despite their importance in contemporary dairy cow rations. A lack of understanding of the influence of S and Mo on Cu metabolism in dairy cows fed different forages may be contributing to the unnecessary oversupplementation of Cu. Indeed, recent surveys of commercial trace element feeding rates in the United States and United Kingdom (e.g., Castillo et al., 2013; Sinclair and Atkins, 2015) have reported that dietary Cu is frequently fed at levels well above that recommended by national feed standards, such as ARC (1980) or NRC (2001). Feeding Cu above nutritional requirements can result in chronic Cu poisoning, whereby a gradual increase in hepatic Cu concentrations occurs, ultimately leading to rupture of lysosomes, hepatic necrosis, hemoglobinuria, methemoglobinemia, and rapid death (Bidewell et al., 2000). The objectives of our current study were to determine the effect of level of inclusion of corn silage and grass silage fed either without or with added sulfur and molybdenum on indicators of copper status, performance, and health in Holstein-Friesian dairy cows.

MATERIALS AND METHODS

Animals, Management, and Treatments

The procedures involving animals were conducted in accordance with the UK Animals (Scientific Procedures) Act (UK Parliament, 2012), and were approved by the Harper Adams Animal Welfare and Ethical Review Board. Fifty-six Holstein-Friesian dairy cows (8 primiparous and 48 multiparous) that were 35 (SE ±2.2) d postcalving and yielding 38.9 kg/d (SE ±0.91) of milk were used. From calving until wk 5 of lactation the cows were group housed and fed a diet containing 95 g/kg of DM of grass silage, 90 g/kg of DM of alfalfa silage; 324 g/kg of DM of corn silage; 20 g/kg of DM of chopped wheat straw; 100 g/kg of DM of urea-treated wheat; 80 g/kg of DM of soy hulls; 50 g/kg of DM of molasses; 66 g/kg of DM of soybean meal; 64 g/kg of DM of rapeseed meal; 64 g/kg of DM of distillers grains; 18 g/kg of DM of palm kernel meal; 14 g/kg of DM of protected fat; and 15 g/kg of DM of minerals and vitamins. Based on recordings taken in wk 4 of lactation the animals were blocked and allocated to 1 of 4 dietary treatments according to lactation number (primiparous or multiparous), calving date, milk yield, milk composition, BCS (using a 1–5 scoring system on a quarter point scale; Lowman et al., 1976), and live weight. Cows remained on study for 14 wk.

Based on the mineral analysis of the forages (Table 1) and NRC (2001) values for the other feeds, 4 diets were formulated to contain approximately 20 mg of Cu/kg of DM and a corn silage-to-grass silage ratio of 0.75:0.25 (**C**) or 0.25:0.75 (**G**; DM basis; Table 2). To evaluate the effects of dietary antagonists on Cu metabolism, the diets were either unsupplemented (−) or supplemented (+) with additional S and Mo, to result in a total dietary concentration of approximately 3.5 g of S/kg of DM or 7.5 mg of Mo/kg of DM [an increase of approximately 2 g of S/kg of DM (+160%) and 6.5 mg of Mo/kg of DM (+500%)]. Therefore, we used 4 dietary treatments: C− [0.75:0.25 corn silage:grass silage (DM basis), no additional antagonists]; C+ (0.75:0.25 corn silage:grass silage, with additional S and Mo); G− (0.25:0.75 corn silage;grass silage, no additional antagonists); and G+ (0.25:0.75 corn silage:grass silage, with additional S and Mo). Additional Cu was supplied as $CuSO_4 \cdot 5H_2O$, sulfur as ammonium sulfate (TG Tennants, West Bromwich, United Kingdom), and molybdenum as sodium molybdate (Acros Organics, Geel, Belgium). Feed-grade urea was added to G− and C− to provide an equivalent amount of rumen-degradable N as supplied by the ammonium sulfate. The diets were supplemented with other feed ingredients to support a milk production of approximately 38 kg/d according to Thomas (2004; Table 2). All dietary ingredients were mixed and fed as a TMR using a forage mixer calibrated to ±1 kg, and fed through roughage intake feeders (Hokofarm Group BV, Insentec, Marknesse, the Netherlands) fitted with an automatic animal identification and forage weighing system calibrated to ±0.1 kg (Sinclair et al., 2005). Fresh feed was offered daily at 105% of ad libitum intake with refusals collected twice