Supplementation of Copper and Selenium in Molasses-Based Liquid Supplements in Growing Beef Cattle

A final report submitted to Dr. Elvin Thomas, AFIA Liquid Feed Committee

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The following information is provided as an update on the results of two studies supported by the AFIA Liquid Feed Committee and conducted at the University of Florida, Range Cattle Research and Education Center in Ona.

Introduction and Overview of Experimental Objectives

Primary and Secondary Mineral Deficiencies

Mineral deficiencies may be grouped into two broad categories depending on the characteristics of their development, these are, 1) Primary deficiency, and 2) Secondary deficiency.

Primary mineral deficiencies are the result of the consumption of feeds that are naturally low in one or more trace minerals. These deficiencies usually require an extended period of time for their development, often a year or more. The lack of supplemental mineral is a common characteristic of primary mineral deficiencies, as they are rare under normal well-managed cattle production systems.

Secondary mineral deficiencies are by far the more common of the two. Secondary deficiencies are derived from the consumption of one or more mineral antagonists that interfere with the normal metabolism of another mineral. A simple mineral evaluation of a feedstuff may suggest adequate trace mineral concentrations are present; however, the presence of a mineral antagonist will decrease the availability of the mineral, potentially leading to a deficiency.

The interactions between various minerals are numerous. A clear delineation between each mineral-mineral antagonism is impossible given our current understanding. Further, ruminant and non-ruminant animals metabolize trace minerals differently, suggesting that potential antagonisms will also vary.

The data summarized in this report will specifically focus on Cu antagonists in cattle. The importance of Cu nutrition in cattle is recognized and the influence of multiple antagonists has been widely studied. Further, following P, Cu is often the second most limiting mineral nutrient in grazing cattle.

Historical reference to Cu deficiency in Florida has been common. In fact, the first reported case of Cu deficiency in cattle was reported by researchers at the University of Florida in 1931. Copper deficiency is almost always linked to a secondary deficiency condition related to one or more Cu antagonists. Two commonly recognized Cu antagonists include molybdenum (Mo) and sulfur (S).

Confounding Research Designs. Research investigating the influence of Cu nutrition on cattle health and performance has often depended upon the experimental feeding of one or more antagonists to induce a Cu deficiency. This methodology creates confounding in the experimental design causing the investigator difficulty in separating outcomes between Cu deficiency and the potential direct effect of the antagonist. An example of this procedure relates to a common technique used in our laboratory as well as others, whereas, Mo and S are supplemented to cattle diets to induce a secondary Cu deficiency. It is difficult to separate the outcomes of this design between complications associated with the induced Cu deficiency or the direct effect of feeding high levels of Mo and S.

Molybdenum and Sulfur Antagonism

Molybdenum Antagonism. Molybdenum is an essential trace element required by all animals; nevertheless, reports of Mo deficiency are rarely recorded. In contrast, the antagonistic impact of Mo on Cu metabolism has been recognized for many years. Typically, Mo exerts its influence on Cu through the association with S in the formation of ruminal thiomolybdates (discussed below). However, strong evidence exists which shows that decreased animal performance can be related to Mo toxicity independent of decreased Cu availability. Heifers consuming supplemental Mo (dietary concentration = 5 ppm) have been shown to exhibit signs of Cu deficiency, whereas, heifers supplemented with Fe and at the same Cu status had no signs of Cu deficiency. In these studies, the signs of Cu deficiency included reduced growth and feed efficiency (Phillippo et al., 1987a) and infertility (Phillippo et al., 1987b). In another study (Gengelbach et al., 1997), calves provided diets with supplemental Mo had a lower rate of gain compared to Fe supplemented calves. Both groups of calves had an equivalent extent of Cu depletion compared to Cu-supplemented control calves. These results suggest that some conditions, which are linked to Cu deficiency, might be more accurately described as a toxicity from the antagonist (i.e. Mo toxicity).

Sulfur Antagonism. Sulfur is found naturally in nearly all feedstuffs. The form of S varies widely from an inorganic salt to organic S-containing amino acids. Recently, more evidence has been derived from commercial cow/calf production systems in Florida suggesting that S may be a primary contributor to secondary Cu deficiencies. Forage Mo is an important contributor to secondary Cu deficiencies in grazing cattle. The relationship between Cu, Mo, and S has been widely investigated in grazing ruminants. Although Mo is an essential component in this antagonism, it will seldom affect tissue Cu stores when S levels are limiting. Provided adequate dietary S, Mo combines with Cu to form an insoluble complex in the rumen, rendering Cu unavailable for absorption. We have found that a dietary concentration of S of 0.35% (total S) is sufficient for this antagonism to become a concern. The current beef cattle NRC (1996) suggests a maximum tolerable concentration of dietary S of 0.40 %.

Sulfur Antagonisms in Molasses-Based Liquid Supplements

In the southeast, and other regions of the US, the use of molasses-based supplemental feeds is common. Recently we have evaluated the absorption efficiency of supplemental Cu provided in corn- versus molasses-based supplements (Arthington and Pate, 2002). Our results indicate that the S concentration, present in cane molasses, may interfere with normal Cu absorption in cattle. In these studies, heifers receiving supplemental Cu through corn experienced a 46% increase in liver Cu concentration compared to a 9% decrease in heifers receiving supplemental Cu through a molasses supplement (Figure 1). In a following experiment a third treatment was included that provided Cu supplemented in a corn-based supplement, but also fortified with S at a level equal to the amount obtained by the molasses supplement. Increases in liver Cu concentrations were different for each treatment (155, 87, and 13 ppm for corn, corn+S, and molasses supplements, respectively (Figure 2).

In both experiments, change in heifer body weight was not affected by treatment. Effects of supplement type on heifer body weight were not expected. Normal liver Cu concentrations in Florida cattle range between 100 and 300 ppm on a DM basis (Ammerman, 1969). Liver Cu values obtained from the heifers in these studies fell within this adequacy range.

In both experiments, liver concentrations of Zn, Mn, and Fe were not affected by supplement type, suggesting that each of these elements absorb with similar efficiency when delivered in corn- and molasses-based supplements. In contrast, Mo tended (P = 0.06 and 0.10 for Experiments 1 and 2, respectively) to accumulate in the liver of heifers fed molasses-based supplements. This response is likely due to the higher amount of Mo provided by molasses- compared to corn-based supplements (3.2 vs. 1.3 mg daily for molasses- and corn-based supplements, respectively).

In both experiments, liver Cu accumulation was reduced when provided in molasses-based supplements (Figures 1 and 2). This response is most likely the result of reduced Cu absorption due to the formation of ruminal thiomolybdates. Thiomolybdates can impact Cu nutrition in ruminants by two means; 1) irreversibly bind Cu in the gut, therefore preventing absorption, and 2) post-absorption systemic depletion of Cu from tissue sites (Mason, 1990). The formation of thiomolybdates is directly dependent upon available dietary S, and indeed S intake is a major factor on influencing the sensitivity of ruminants to Mo (Mason, 1981). In the current study, the inclusion of added dietary S to a corn-based supplement (Experiment 2) resulted in the partial, but not full, inhibition of liver Cu accumulation realized with the molasses-based supplement (Figure 2). This partial inhibitory response may be the result of the rumen's inability to fully reduce the supplemental S provided in the corn supplement + S treatment. Dietary S must first be reduced to sulfide before it may interact with Mo to form thiomolybdates (Mason, 1986). Another explanation may be related to a lack of

Mo in the corn-based supplement to fully participate with S for the formation of thiomolybdate.

Activity of the Cu-transport enzyme, ceruloplasmin, was reduced in molasses-supplemented heifers in both studies. This response is also likely a result of thiomolybdate formation in molasses-supplemented heifers, as reduced ceruloplasmin activity has been shown in both sheep (Mason et al., 1986) and cattle (Lannon and Mason, 1986) infused with thiomolybdate.

Since we began reporting on our results, at least two questions continue to arise. Each of these are important considerations to the liquid feed industry's ability to formulate liquid feeds. Their investigation will likely result in liquid feed products that are more effective in their ability to deliver mineral nutrition to cattle.

1. Dietary Se is also sensitive to S antagonism. Therefore, is Se less available in molasses- versus corn-based supplements?

2. Since it appears that Cu availability is reduced in molasses-based supplements, can I simply increase the level of supplemental Cu to overwhelm the antagonism and still provide sufficient Cu for absorption?

Currently, there has been no direct research to support answers to these questions. **Therefore the objectives of these two studies were**, 1) investigate the Se status of forage-fed steers offered dietary Se in molasses- versus corn-based supplements, and 2) investigate the effect of high rates of Cu supplementation in molasses-based supplements on liver Cu accumulation, Cu-dependent enzyme activity, and forage DM intake and digestibility in pregnant heifers.

These objectives are relevant considerations for the liquid feed industry. Recent research by Ivancic and Weiss (2001) suggested that increased dietary S resulted in reduced Se balance in dairy cows. Similar results have been reported in sheep (Hintz and Hogue, 1964; van Ryssen et al., 1998). In each of these studies, inorganic S was provided as a supplement to the diet. The total dietary level of S consumed by cows grazing bahiagrass (≈ 0.20% S) and consuming liquid molasses supplement (≈ 0.79% S) would be approximately 0.30%. This level of dietary S is similar to those used in the experiments described above.

The concept of supplementing relatively high levels of trace mineral, especially when antagonists are present, is not new. We commonly come into contact with range mineral supplements that provide many times the level of Cu required by the animal. Considering the anti-microbial characteristic of Cu, one concern of over-supplementation is its potential impact on the ruminal microbial environment. Hubert et al. (1958) suggested that relatively low concentrations of Cu were toxic to the rumen microbes contained in a culture system. In contrast, a study by Lopez-Guisa and Satter (1992) suggested that the supplementation of Cu and Co above NRC recommendations might actually improve the digestion of low quality forages. Over-supplementation of Cu to molasses-based supplements will be a likely result of the initial data produced by our group (Figures 1 and 2). A more clear understanding of how this management practice affects mineral metabolism and diet digestibility is critical.

Materials and Methods

Experiment 1

Twenty-four crossbred (Brahman x British) steers were stratified by body weight and randomly assigned to dry lot pens of equal size (114 m^2). Two supplement treatments (6 pens/treatment) were formulated using corn and cottonseed meal or molasses and cottonseed meal. Each supplement was formulated to provide 1.5 kg of TDN and 0.3 kg of CP/hd and 140, 76, and 63 mg of Cu, Mn, and Zn/hd daily. Supplemental Se (from sodium selenate) was provided at a rate of 0.30 ppm. Supplements were fed 3 times weekly. To assess the effect of supplement composition on steer performance and Se status, individual steer weights, jugular blood, and liver biopsy samples will be collected on d 0, 30, 60, and 90.

Liver tissue samples were collected, handled, and analyzed for mineral concentrations using methods previously described (Arthington et al., 1996). Plasma was harvested from blood following centrifugation at 2,400 x g for 20 min and analyzed for Se concentration using atomic absorption spectroscopy techniques.

Experiment 2

Twenty-four pregnant, yearling crossbred heifers (Brahman x British) were stratified by body weight and randomly assigned to individual pens of equal size (15 m^2, 1 heifers/pen) Four treatments consisting of a complete mineral supplement fortified with differing levels of Cu supplementation were randomly assigned to pens. The treatments provided 0, 15, 60, and 120 ppm of supplemental Cu using Cu sulfate as the Cu source. Mineral supplements were offered 3 times weekly in a molasses/cottonseed meal slurry formulated to provide 1.5 kg of TDN and 0.3 kg of CP/hd. All heifers were offered free-choice access to ground stargrass hay.

To assess the effect of treatment on heifer performance and Cu status, individual body weight, jugular blood, and liver biopsy samples were collected d 0, 42, and 84. To assess the effect of treatment on forage intake, measures of daily forage refusal were collected. The effect of supplemental dietary Cu on diet DM digestibility was estimated over a 21 d period (beginning on d 42) by the use of a sustained release chromic oxide bolus (Captec, New Zealand). During this 21 d period, seven individual fecal samples were collected on 3 d intervals. Daily fecal production was estimated by the analysis of fecal Cr concentration. Digestibility of the DM consumed was estimated by digestibility analysis of both the DM offered and refused. This amount, less the digestibility of total feces produced, was used to estimate the effect treatment on total diet digestibility.

Liver trace mineral concentration was assessed as described for Experiment 1. Plasma was harvested following centrifugation of blood at 2,400 x g for 20 min and then frozen at –20° C until later analyzed for ceruloplasmin concentration using colorimetric procedures previously described (Demetriou et al., 1974). Organic matter digestibility of forage, ort, and fecal samples was analyzed using a modification of the two-stage technique (Tilley and Terry, 1963) as previously described (Moore and Mott, 1974). Fecal Cr concentration will be analyzed using inductively coupled plasma emission spectroscopy (Fassel, 1978).

Results

Experiment 1

A pair-feeding approach was implemented throughout the study to account for lesser supplement intake in steers fed molasses- vs corn-based supplements. For the first 36 d of the study, molasses-fed steers refused 13% of the supplement offered. For the final 54 d the supplemental feeding rate for steers assigned to the corn-based supplement was decreased 15%. These changes to feeding rate resulted in a total supplemental TDN of 128 and 123 kg for molasses and corn supplemented steers over 90, respectively. Although similar intakes of TDN among treatments were achieved, steers fed corn-based supplements tended ($P = 0.07$) to have a higher ADG compared to steers fed molasses-based supplements (Table 1).

There was a significant ($P < 0.01$) sampling day x supplement source interaction for liver Se concentration. Steers fed molasses-based supplements had greater initial liver Se concentrations than steers fed corn-based supplements (Figure 3). However, steers fed corn-based supplements had a greater overall increase in liver Se accumulation resulting greater final liver Se concentrations vs molasses supplemented steers on d 90 (1.47 and 0.50 ppm; SEM = 0.18; Figure 3).

There was no sampling day x supplement source interaction for plasma Se concentration, however molasses supplemented steers tended ($P < 0.06$) to have greater plasma Se concentrations compared to steers fed corn-based supplements (0.046 and 0.038 ppm DM basis, respectively; SEM = 0.002). Similarly, there were no supplement or supplement x day interactions for plasma glutathione peroxidase (GPx) activity. However, GPx activity did increase ($P < 0.001$) over time for steers consuming both supplements (440, 833, 2813, and 3414 units of GPx activity for d 0, 30, 60, and 90, respectively). This increase in GPx activity appeared to follow increases in liver Se concentration.

Experiment 2

Heifers fed the diet without supplemental Cu tended ($P < 0.11$) to be lighter initially and at the end of the study than other treatments (Table 2). Heifers consuming the highest rate of supplemental Cu (120 mg/kg) tended ($P = 0.13$) to have a lesser ADG compared to heifers supplemented with 15 mg/kg of Cu (Table 2).

There was no significant sampling day x treatment interaction for liver Cu accumulation (Figure 4), however average liver Cu concentration did decline ($P < 0.05$) over time for all treatments combined. Observation of the general trend for liver Cu data suggest that the high rates of Cu supplementation resulted in a decline in liver Cu accumulation. Therefore, data were also analyzed combining treatments that provided 60 and 120 mg/kg of supplemental Cu into a single treatment (High Cu). This analysis revealed a tendency for a sampling time x treatment interaction ($P < 0.13$). In this analysis, heifers supplemented with 15 mg/kg Cu had higher liver Cu concentrations at the end of the study (d 84) than heifers provided no supplemental Cu. Heifers provided the high rates of supplemental Cu were intermediate to the other treatments (Figure 5).

Forage DMI was lower ($P = 0.07$) for heifers receiving no supplemental Cu compared to all other treatments (Figure 6). Interestingly, this forage intake response was detectable at its highest level in the first week of sample collection (Figure 7). Although heifer growth was reduced by the highest Cu supplementation level (120 ppm), apparent forage digestibility was not affected by Cu treatment (Figure 8).

Discussion

The data from Experiment 1 suggests that, like Cu, molasses-based supplements also antagonize Se accumulation into the liver of growing cattle. Differences in BW gain shown in this study are minimal and may likely be due to the inconsistent pattern of supplement consumption in the molasses-fed steers. Plasma Se concentrations were not decreased by molasses-supplementation, in fact they tended ($P < 0.06$) to be higher in steers provided supplemental molasses. This result is interesting and similar to previous Cu studies using antagonist feeding; whereas, Cu is absorbed and detected in the bloodstream, but unable to be properly metabolized due to the formation of large, tightly-bound molecules (i.e. thiomolybdates). A similar response may be occurring in the case of Se metabolism in cattle consuming diets containing antagonists. Another explanation may be related to the ratio of erythrocyte-bound Se. Often, measures of whole blood Se are more reliable than plasma. Whole blood Se was not measured in this study. Plasma GPx activity was not affected by treatment, but did increase over time in all steers. This increase appeared to follow increases in liver Se concentrations. Further review and interpretation of these results are currently underway. Additional studies, which investigate the influence of Se source on Se status of cattle consuming molasses diets is warranted, especially now that organic Se yeast is available in our industry.

One of the most striking results in Experiment 2 is that high levels of Cu supplementation (60 and 120 mg/kg) failed to increase liver Cu stores in this study. This refutes our earlier hypothesis, which suggested that higher levels of Cu supplementation may be needed to overcome the S antagonism found in molasses. Because these levels were quite high, this response may be the result of an adaptation reaction to protect from possible Cu toxicity. As well, these results may be quite different in cattle breeds which are more prone to signs of Cu toxicity (i.e. Jersey).

Heifers consuming the highest level of Cu tended ($P = 0.13$) to have a lower ADG compared to heifers consuming a moderate level of dietary Cu (15 ppm). This response may be due to a Cu-toxicity at the rumen level. However, there were no differences in apparent diet digestibility between these treatments. This discrepancy makes complicates this result. In fact, the heifers may be expended additional energy to clear excessive Cu from the system. This energy drain may be responsible for the lesser ADG shown in heifers consuming 120 ppm dietary Cu. Another important finding of this study relates to the quick decline in forage DM intake in heifers receiving no supplemental Cu. This response may support the importance of dietary Cu for optimal rumen health. Nevertheless, heifers receiving no supplemental Cu still had similar apparent diet digestibility compared to those supplemented with Cu.

Implications

The results of these studies have provided further important data related to the trace mineral nutrition of molasses-fed cattle. In contrast to our previous conclusions, it appears that higher levels of dietary Cu are not effective in overcoming the S antagonism found in molasses. In fact, these high levels of dietary Cu may be negatively influencing cattle by creating an energy drain during the elevated excretion process. To date, no source or level of dietary Cu has been successful in overcoming the S antagonism found in molasses. However, in all our studies we have never found a decline in production related to the reduced Cu status of molasses-fed cattle. In almost all production situations, the likely scenario would allow molasses-fed cattle to rapidly replete lost tissue Cu stores during summer grazing months when molasses is not fed or fed at a much lower level.

New data from this research suggests that Se is also antagonized in cattle consuming molasses supplements. Further research is needed to investigate the effectiveness of the newly available organic Se yeast sources in molasses-fed cattle.

Acknowledgments

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Table 1. Effect of supplement source on performance of growing steers[a]

Item	Supplement type Molasses	Corn	SEM	P =
	------------------- kg	-------------------		
Initial BW	297	295	12.5	0.89
Final BW	306	317	11.1	0.51
ADG	0.10	0.24	0.05	0.07

[a]Total supplemental TDN = 128 and 123 kg for molasses and corn supplemented steers over 90, respectively.

Table 2. Effect of supplemental Cu level on performance of growing heifers [a]

	Supplemental Cu, mg/kg					Contrasts		
Item	0	15	60	120	SEM	1	2	3
	---------------------- kg		----------------------					
Initial wt.	365	385	392	380	9.7	0.07	0.12	0.64
Final wt.	379	401	409	383	9.5	0.10	0.10	0.19
ADG	0.17	0.18	0.20	0.05	0.06	0.67	0.92	0.13

[a]Single degree of freedom contrasts include, 1) No Cu vs all supplemental Cu treatments, 2) No Cu vs supplemental Cu at 15 mg/kg, and 3) supplemental Cu at 15 mg/kg vs 120 mg/kg.

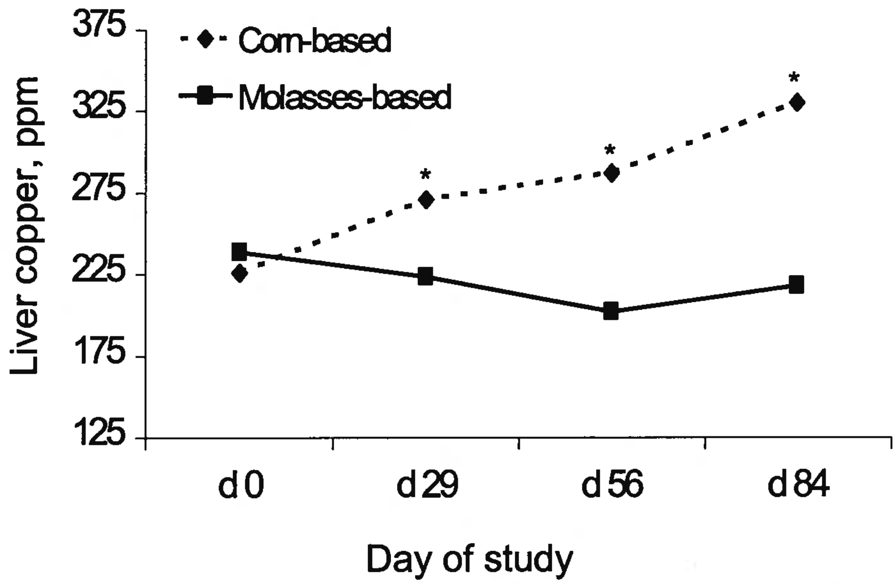

Figure 1. Effect of corn- vs molasses-based supplements on the accumulation of Cu in the liver of growing heifers. Values are provided on a DM basis. SEM = 38.0. * = P < 0.05.

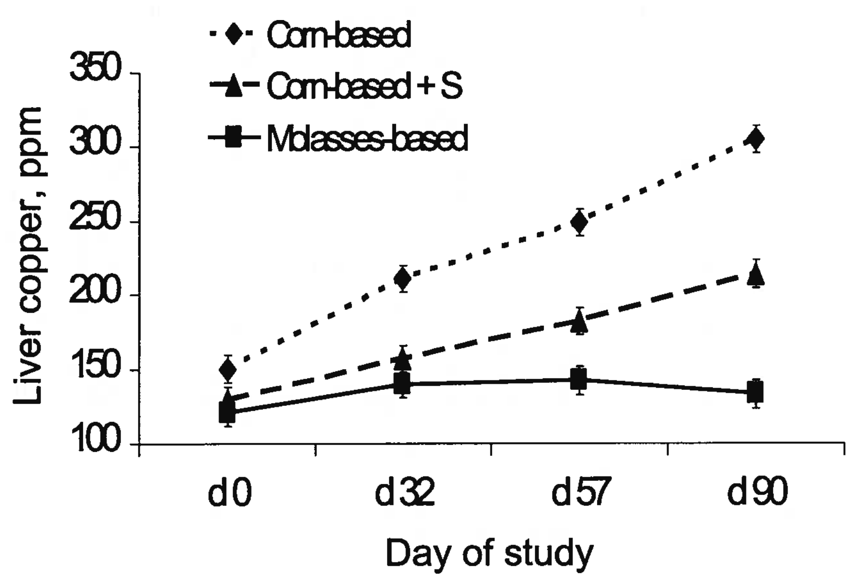

Figure 2. Effect of corn- and corn + sulfur vs molasses-based supplements on the accumulation of Cu in the liver of growing heifers. Values are provided on a DM basis. Single degree of freedom contrasts; corn- vs molasses-based supplements (P = 0.01), and corn- vs molasses-based supplement, and corn-based + S supplement (P < 0.01).

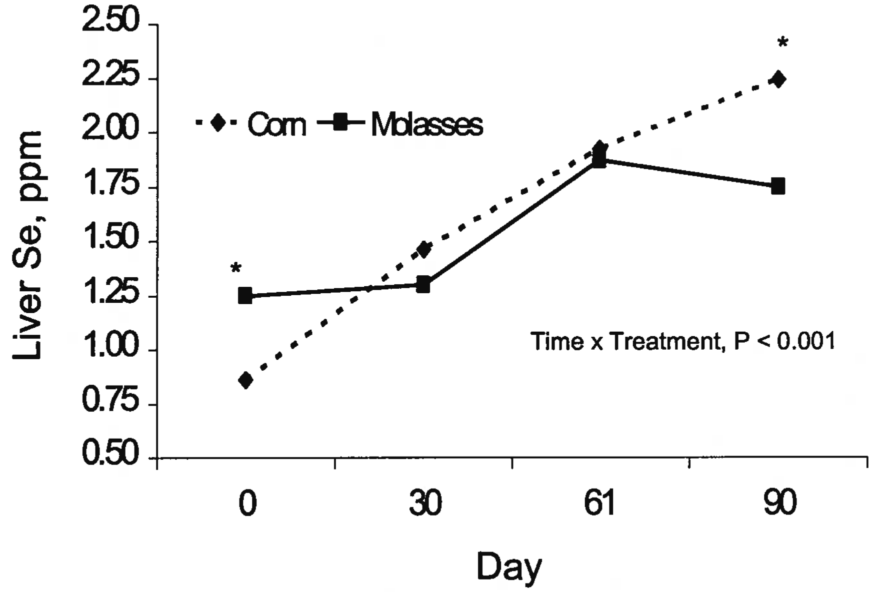

Figure 3. Effect of corn- vs molasses-based supplements on the accumulation of Se in the liver of growing steers. Pooled SEM = 0.11, * P < 0.05.

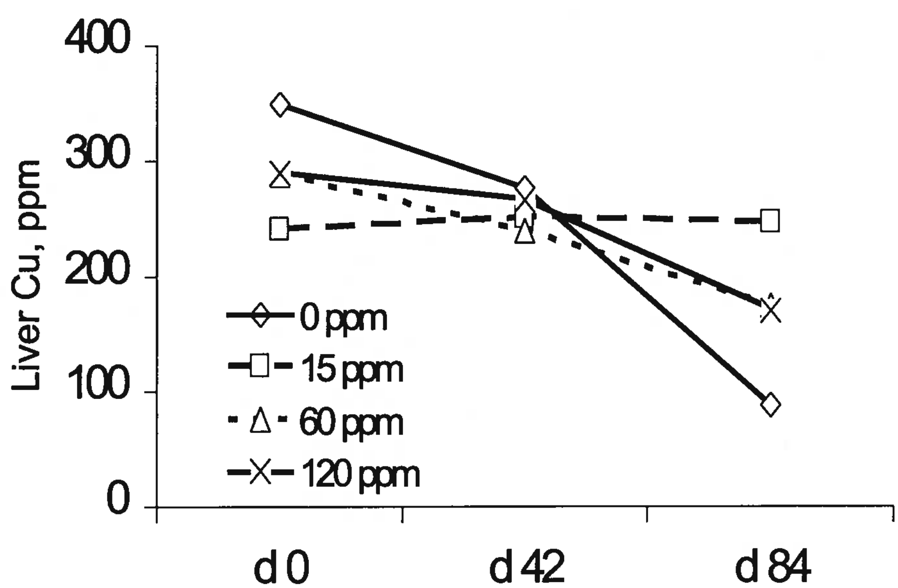

Figure 4. Effect of supplemental Cu level on liver Cu accumulation in growing heifers. Pooled SEM = 61.3. Time x treatment interaction; P = 0.30.

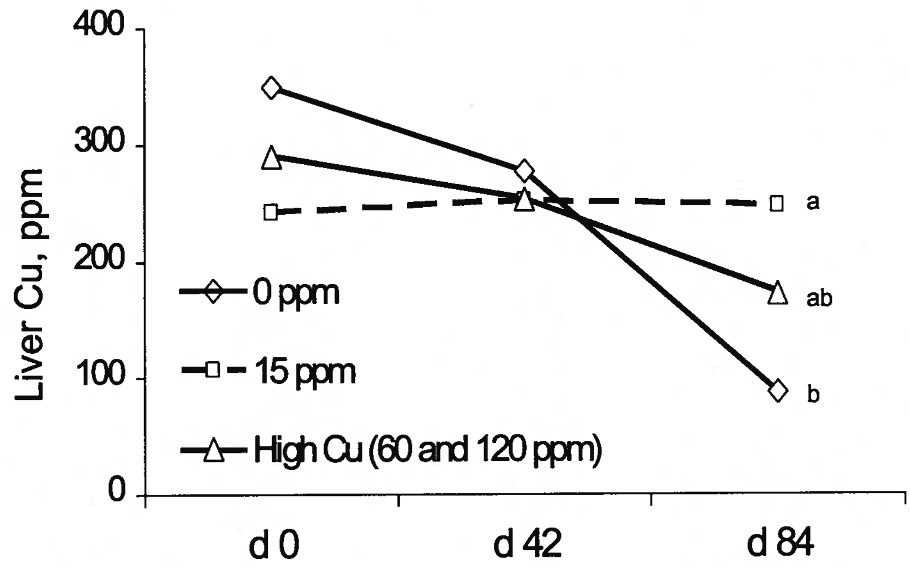

Figure 5. Effect of supplemental Cu level on liver Cu accumulation in growing heifers; high Cu rates are pooled. SEM = 58.1, 41.1, and 31.5 for d 0, 42, and 84, respectively. Time x treatment interaction; $P < 0.13$. ab = means differ, $P < 0.05$.

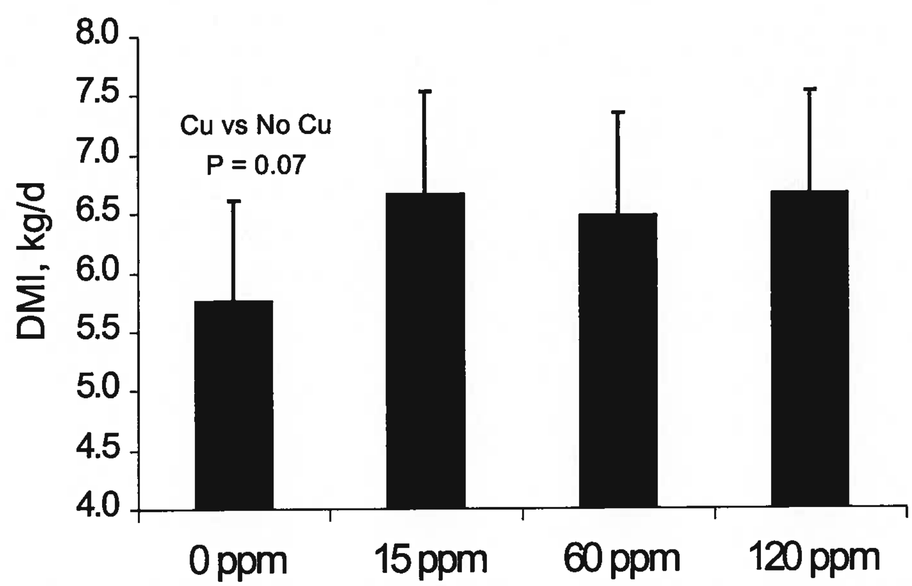

Figure 6. Effect of supplemental Cu level on DMI of growing heifers. Single degree of freedom contrast (Cu vs No Cu), $P = 0.07$.

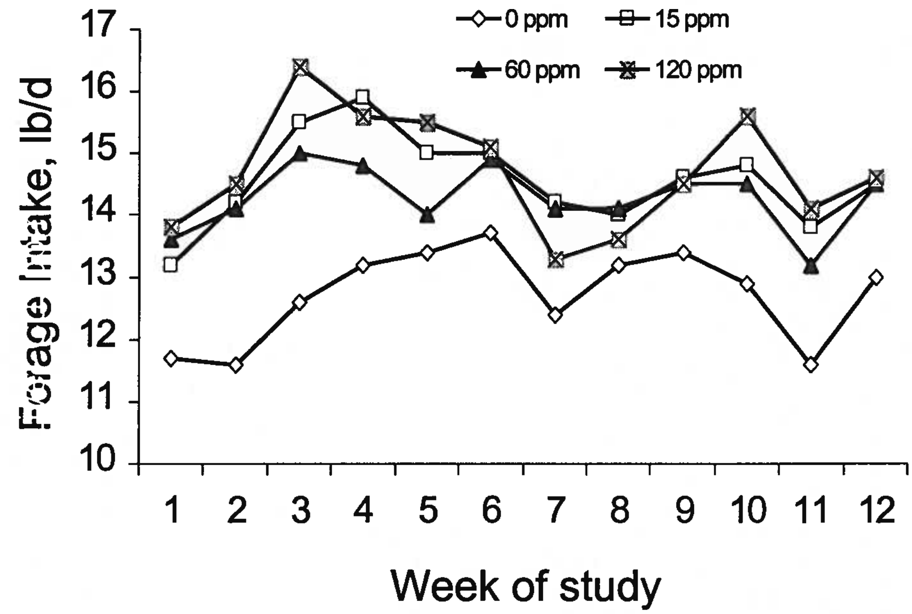

Figure 7. Effect of supplemental Cu level on DMI of growing heifers. Single degree of freedom contrast (Cu vs No Cu), P = 0.07; SEM = 1.0.

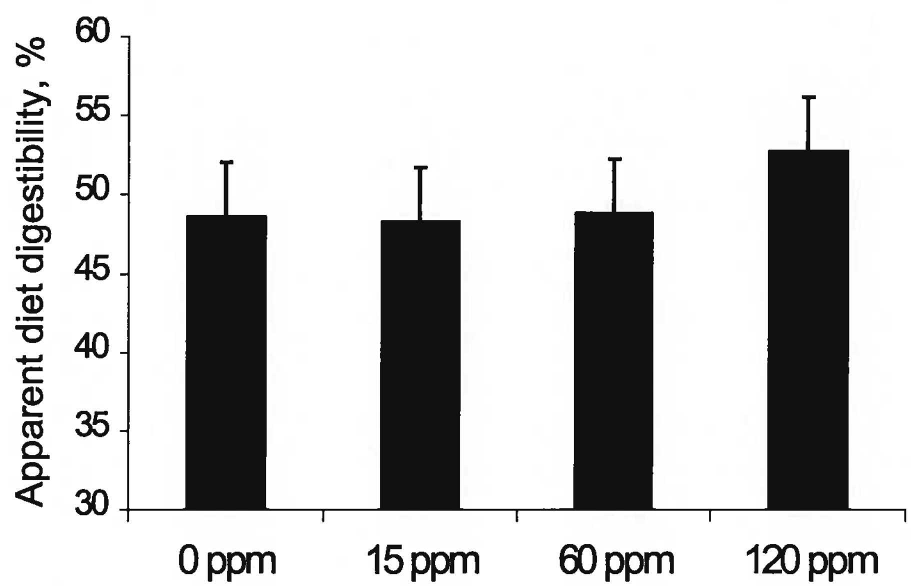

Figure 8. Effect of supplemental Cu level on apparent diet digestibility of growing heifers. Single degree of freedom contrast (15 vs. 120 ppm Cu), P = 0.34.