Investigating the influence of copper supplementation on copper homeostatic genes in bovine liver.

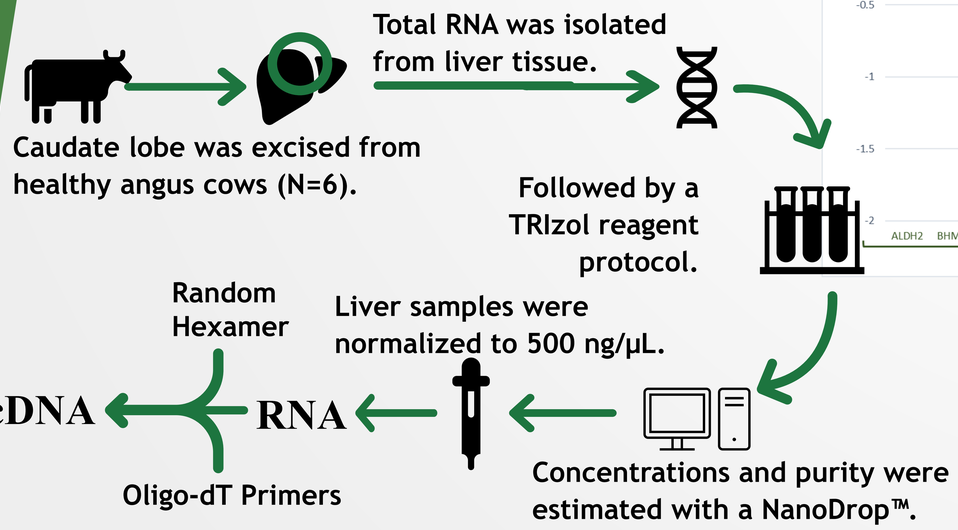
N. Tillquist, M. Thorndyke, T. Thomas, S. J. Coleman, T. E. Engle*

Colorado State University, Fort Collins, CO. Department of Animal Sciences

ABSTRACT

The objective of this experiment was to investigate the influence of copper (Cu) supplementation on Cu homeostatic genes in bovine liver. Liver samples were obtained from multiparous cows (n=3 cows per treatment) from a previous experiment. Treatments consisted of: 1) no supplemental Cu (-Cu; total diet contained 7.0 mg Cu/kg DM) and 2) 3 mg Cu/kg DM (+Cu; total diet contained 10.0 mg Cu/kg DM). Cows were fed their respective diets for 420 d. Liver samples were harvested immediately upon slaughter, rinsed with a phosphate buffered saline solution, diced into small pieces, and placed into an RNA-Later solution and frozen in liquid nitrogen. Total RNA was extracted from all samples and quantitative RT-PCR was used to determine the abundance of transcripts for genes involved in Cu homeostasis in liver tissue. The identified target transcripts were: ALDH2, APOA1, ATOX1, ATP7A, ATP7B, BHMT, BLVRB, CA2, CCS, COX17, CTR1, ELN, GAPDH, GLUD1, GSS, LOXL1, PDIA3, SOD1, SOD3. The relative abundance of APOA1, ATOX1, ATP7B, BLVRB, CTR1, GLUD1, LOXL1, and SOD3 mRNA was greater ($P < 0.05$) in cows receiving supplemental Cu when compared to cattle not receiving supplemental Cu. These data indicate possible Cu homeostatic differences in liver between +Cu vs -Cu supplemented cattle. Further investigation is warranted to determine if a difference in relative abundance of identified transcripts are related to differences in associated protein production and function.

MATERIALS AND METHODS



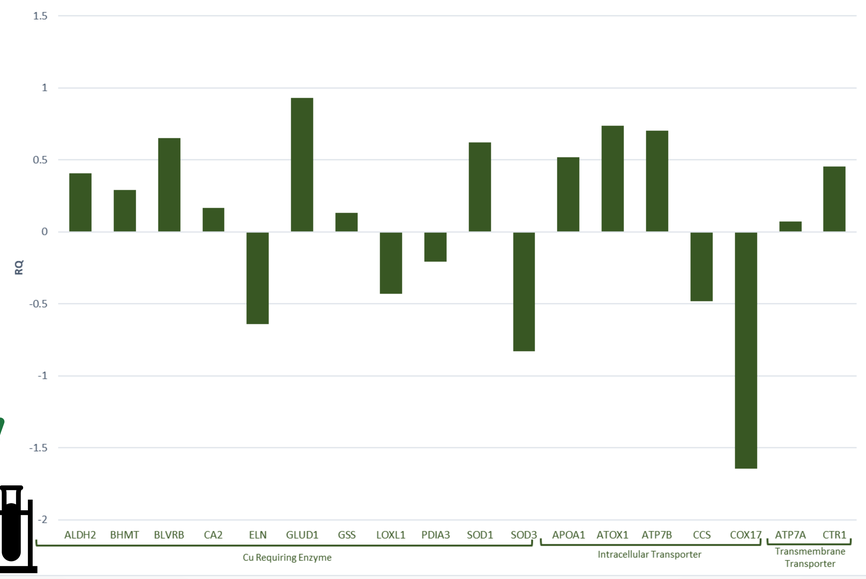
(Endogenous control ACTB was selected from a previous experiment)

RESULTS

Table 1. The effects of copper (Cu) supplemented liver copper concentration on change (Δ) in threshold (C_T)

Item	Treatment 1 No supp. Cu ΔC_T Mean	Treatment 2 3mg Cu/kg DM ΔC_T Mean	SEM	P<
Liver Cu Concentrations, mg/kg DM	13.40	16.10	1.23	0.091
Aldehyde dehydrogenase (ALDH2)	-0.74	-1.06	0.216	0.16
Apolipoprotein A-1 (APOA1)	1.57	1.15	0.144	0.01
Antioxidant 1 copper chaperone (ATOX1)	1.13	0.52	0.252	0.03
ATPase copper transporting alpha (ATP7A)	7.22	7.23	0.096	0.92
ATPase copper transporting beta (ATP7B)	4.17	3.5	0.198	0.005
Betaine homocysteine methyltransferase (BHMT)	0.62	0.34	0.241	0.26
Flavin reductase (BLVRB)	1.44	0.84	0.224	0.02
Carbonic anhydrase II (CA2)	8.10	7.94	0.250	0.51
Copper chaperone for superoxide dismutase (CCS)	9.15	9.63	0.416	0.26
Cytochrome c oxidase copper chaperone (COX17)	2.28	3.93	1.012	0.12
Copper transporter I (CTR1)	2.34	1.92	0.197	0.03
Elastin (ELN)	10.23	10.90	0.298	0.05
Glutamate dehydrogenase (GLUD1)	1.76	0.83	0.273	0.003
Glutathione synthetase (GSS)	5.53	5.40	0.185	0.49
Lysyl oxidase (LOXL1)	5.06	5.50	0.163	0.02
Protein disulphide isomerase A3 (PDIA3)	1.05	1.26	0.295	0.49
Superoxide dismutase [Cu-Zn] (SOD1)	0.79	0.17	0.205	0.008
Extracellular superoxide dismutase (SOD3)	5.81	6.63	0.338	0.02

Figure 1: Log transformed relative gene expression between bovine liver fed Cu compared to a negative control



DISCUSSION

- **ATOX1 shuttles Cu to ATP7B.**
  -Possibly upregulated because that pathway is responsible for Cu excretion.
- **CTR1 brings Cu into cell**
  -Possibly upregulated to allow more Cu from the diet in to metabolize quicker.
- **LOXL1 is involved in ELN production**
  -Both were down regulated, so perhaps production was stopped to deal with other stress from increase Cu.
- **BLVRB, GLUD1, and SOD1**
  -Possibly upregulated to produce more antioxidants to counter the influx of Cu+ in it's reactive state.
- **SOD3 is an extracellular enzyme**
  -Normally shown to have a dependent relationship with Cu content. In this case, it was down regulated.

CONCLUSION

- Some relative expression values for Cu homeostatic genes differed in Treatment 2 from Treatment 1.
- These results indicate that livers of cattle supplemented additional Cu in their diet differ from those not supplemented additional Cu.
- Mineral analysis indicated no difference in liver Cu concentration: cattle were on feed for 419 days and samples were collected after they had been off treatment for 121 days.

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