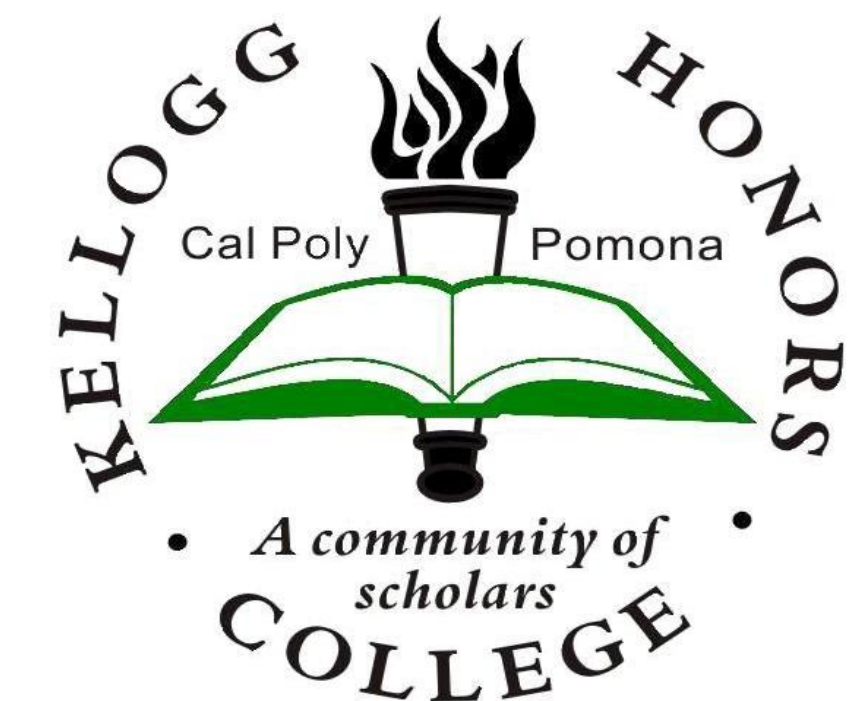


Influence of parturition as a stress-inducing event on the blood circulation levels of copper in ewes

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Abstract

Copper toxicity can be very dangerous in sheep because they are more susceptible than any other food-producing animal. When stress levels in sheep increase, it is believed that large amounts of stored copper can be released from the liver into the blood, potentially leading to copper toxicity and death. Evidence of this occurring in a research setting has never been published. In order to see the correlation, a stressful event was necessary for observation in an experimental setting. Lambing is one of the most natural stress inducing events, and is easily observed in sheep since they are a food animal species. The goal of the experiment was to determine if lambing induces enough stress to cause an increase in circulating blood copper levels. Ten (10) pregnant ewes were used in the experiment. All ewes were penned in a large group and provided normal levels of mineral supplementation in the feed and ad libitum as a trace mineral block. Each ewe was sampled for blood via jugular venipuncture twice before lambing and again after lambing. Blood serum was extracted and analyzed for blood copper levels using a copper assay kit. If an increase in copper levels is observed following lambing then it can be safe to assume a positive correlation between stress and copper release. The results from the experiment will show a definitive relationship of stress to circulating copper levels, which can lead to further research to better understand mineral supplementation methods to reduce copper toxicity concerns in sheep.

Background

- Sheep are the most prone domestic animal to chronic copper poisoning. Rather than absorbing copper according to the body's needs, their bodies absorb the amount of copper that is offered. The excess copper builds up in liver cells until they reach toxic levels, and are finally released in a stressful event. Copper buildup in the liver occurs because sheep do not efficiently excrete copper from the body as well as other animals^[1]. The liver cells rupture to release copper into the blood stream. Once the copper levels in the blood are high, the breakdown of red blood cells occur^[2]. Nutritional copper management is extremely important with sheep because the range for healthy copper levels is very narrow.
- We wanted to observe a stressful event that was common for this species that has not been extensively studied in relation to copper. Gestation and parturition involve physical and hormonal changes to the body causing stress and discomfort. For this reason, parturition was chosen as a possible high stress event.
- **Goal of Study:** To determine if parturition induces enough stress to cause an increase in blood copper levels

Materials and Methods

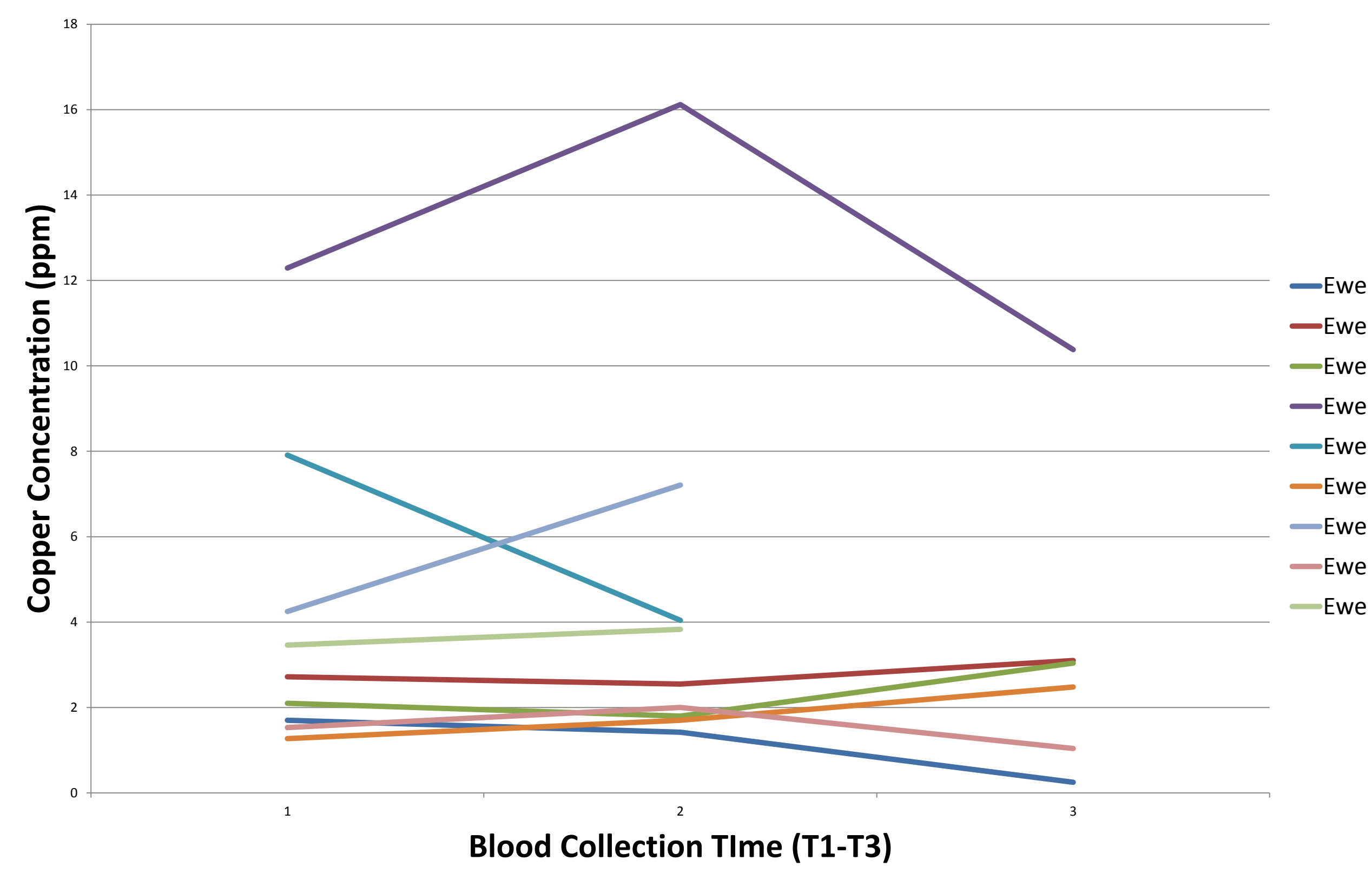
- **Blood Collection & Processing**
 - 10 Dorper-Katahdin Pregnant Ewes
 - Blood collected from jugular vein 3 times (T1-T3) using serum separators (Vacutainer) and a 20 gauge needle
 - T1 (April 7): before lambing
 - T2 (Varied): 16-20 hours after lambing
 - T3 (Varied): 7 days after lambing
 - Samples were centrifuged for 3 minutes at 6000 RPM and serum was extracted
 - Serum was placed into labeled tubes with ewe ear tag ID and collection time (T1-T3)
 - Tubes kept frozen until analysis
- **Copper Assay**
 - Assay Kit used: QuantiChrom™ Copper Assay Kit (DICU-250)
 - quantitative colorimetric copper determination at 359nm
 - Kit included Copper Standard, trichloroacetic acid (TCA), Reagents B & C
 - Mixtures made:
 - Working Standard (WS) = 40µl copper standard + 160µl dH2O
 - Working Reagent (WR) = 10µl B + 300µl C (for each tube)
 - Final mixtures in cuvette:
 - **Blank:** 70µl TCA + 200µl dH2O + 300µl WR
 - **Standard:** 70µl TCA + 200µl WS + 300µl WR
 - **Samples:**
 - Mixed 70µl TCA + 200 µl Sample in Eppendorf tube
 - If ppt formed, pun in centrifuge for 2 min at 14,000 rpm
 - Transferred 200µl of supernatant from Eppendorf tube to cuvette
 - Final mixture: 200µl Sample Supernatant + 300µl WR
 - All samples were ran in duplicates
 - UV Spectrophotometer
 - Optical density (OD) values taken at 359 nm
 - Calculation: $[(OD_{\text{sample}} - OD_{\text{blank}}) / (OD_{\text{standard}} - OD_{\text{blank}})] * 300\mu\text{l/dL}$
 - Conversion: 100µl/dL = 1 ppm
 - To convert optical density to parts per million (ppm)

Results

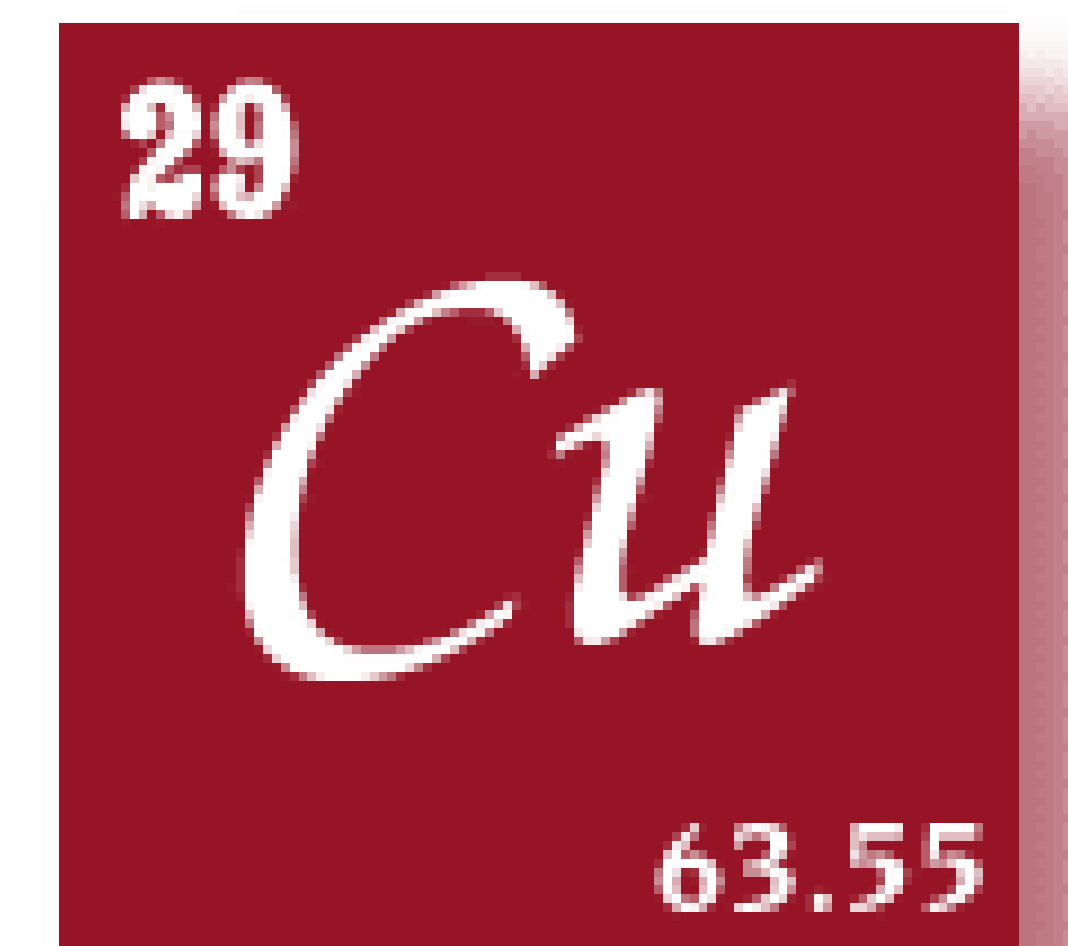
- 9 ewes were used for data analysis
 - 6 of the 9 ewes were sampled 3 times (T1-T3)
 - 3 of the 9 ewes were sampled only twice (T1&T2) because they lambing late in the study
 - 10th ewe did not lamb in time to be included in the study
- Copper concentration was graphed in relation to the time sampled (T1-T3) in Graph 1
- Copper levels did not show changes
 - No significant change with a p-value= 0.46, where $P < 0.10$ is considered to be significant
 - Ewe 4 was an outlier with much higher copper levels than the other ewes



Cu²⁺ Serum Concentration vs. Blood Collection Time



Graph 1: Measure of copper concentration in blood serum at the 3 collection times. Ewes 5, 7, and 9 only had 2 data points. Copper levels did not make any significant changes (P-value= 0.46; t-test)



Discussion

- Possible explanations for the unchanged copper levels:
 - **Time of sampling:** Samples after parturition (T2) were taken 16-20 hours after lambing. It could have been that the increase in copper levels happened a few hours after lambing so by the time blood was taken, the copper levels had returned to normal.
 - **Parturition is a low stress event:** As production animals, lambing is a common occurrence, usually happening in high numbers. These animals are bred for characteristics favoring reproductive success with traits such as ease of lambing. None of the ewes in the study experienced difficulty when lambing so it is possible that parturition in sheep is not as stressful of an event as originally thought.

Conclusion

- In this study, parturition did not show increases in blood copper levels of sheep. It can be due to the times of blood collection being too late to pick up the increase or because parturition in sheep is not a high stress event. I hope this project can be used to highlight the importance of balanced mineral supplementation in sheep and as a baseline for future projects.

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