Assessing blood serum copper levels in hair sheep grazing alfalfa pasture before & after supplementation

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Abstract
Copper toxicity is potentially very dangerous for sheep as they can tolerate very low levels of dietary copper compared with other animals. It is widely believed that when sheep are stressed, copper stores from the liver are released into the blood and cause severe tissue damage, leading to death, but data to show this is very limited. The objective of this study was to see if blood serum copper levels would change after supplementation and after a mildly stressful event. Twenty-five hair sheep were used which had been grazing alfalfa pasture for over 75 days prior to the first blood collection. The stressor event was the act of hoof trimming, which involves flipping the animal onto their back and restraining them for 2 minutes. Blood was collected four times: before and after supplementation with minerals; and before and after the stress event. Blood samples were processed, and the serum was extracted and stored at -70ºC. To measure copper levels, we used a copper assay kit and spectrophotometer. Results show that the copper assay kit we used was able to detect serum copper in sheep. Serum copper levels decreased (P<0.05) in lambs after they were stressed, which did not support our hypothesis. This may be due to the fact that hoof trimming may not have been a true stressor for this group of lambs. This study shows that serum copper levels do fluctuate and can be used for further research in assessing copper status in sheep.

Introduction
Sheep are the highly susceptible to Copper toxicity but still require it as an essential mineral in their diet. There are two types of Copper toxicity: acute and chronic. Acute occurs after eating a large amount of copper in feed, chronic occurring after ingesting larger amounts of copper in the diet over a period of time [1]. The copper is then bound in the liver where it continues to build up as sheep cannot excrete copper as efficiently as other species. While stored in the liver, the copper bound in the lysosomes pose no threat to the sheep, but when put under stress (shearing, transport, or extreme weather), these copper stores can be released into the blood stream, causing severe tissue damage throughout the body [2]. When this happens, there are no clinical signs: just dead sheep. There is little one can do to determine copper toxicity if it’s acute, but when it’s chronic, one of the most trusted ways to determine copper toxicity is by doing a liver biopsy. However, liver biopsies are time consuming and expensive, and are not a practical approach to managing copper toxicity in the field. Very little research has been done to assess how fast copper is being released from the liver or how much stress it will take to elicit an elevated copper response that can be detected in blood serum.

The goal of this study was to try and assess the effects of stress on sheep by using a copper assay kit to measure serum copper in sheep before and after mineral supplementation.

Materials and Methods

Study Design:
• 25 Hair sheep (Dorper-Katahdin cross breed)
• Blood collected from jugular vein 4 times (T1-T4) using serum separators (Vacu-tainer) and a 20 gauge needle
• T1 (March 3) = (+) stress / (+) supplement
• T2 (March 5) = (+) stress / (-) supplement
• T3 (April 4) = (-) stress / (+) supplement
• T4 (April 7) = (+) stress / (-) supplement

Blood Processing:
• After blood was collected, it was spun down and stored in a cooler
• Stressor: the act of hoof trimming
  • Flipped on their backs and restrained for 2 minutes each
  • After stressing, sheep were allowed to rest for 2 hr before collecting blood
• When not on mineral supplement (T1 & T2) sheep were on a strict alfalfa diet and kept at Spadra Farm
  • T1 and T2 were collected 1 day apart
  • Sheep were moved back to the Sheep unit at Cal Poly Pomona to be fed mineral supplement (T3 & T4)
  • T3 and T4 were collected 2 days apart
• Initial mineral supplement amount given: 50 lbs
• Were on mineral supplement for 1 month after collecting on T1 and T2

Serum Copper Assay:
• Spectrophotometer used: Shimadzu UV 160v (cm/Vissible recording
• Located in Bldg 2, room 101 (Nutrition Lab)
• Read absorbance at 359 nm
• Kit used: Quantichrom™ Copper Assay Kit (DUEU-250)
• Quantitative colorimetric copper determination at 359 nm
• Kit included TCA, Standard, Reagent B & C
• Mixtures made:
  • Working Standard (WS) = 20µl x 80µl dH2O
  • Working Reagent (WR) = [10µl B + 500µl C] # tubes needed
• Final mixtures in cuvette: (according to kit instructions)
  • Blank: 70µl TCA + 300µl Working Reagent (WR)
  • Standard: 70µl TCA + 200µl WS & 300µl WR (60µl)
  • Samples procedure:
    • Mix 70µl TCA + 200µl Sample in Eppendorf tube
    • Spin for 2 min at 14,000 rpm if pps form
    • Extract 200µl from Eppendorf tube to cuvette
  • Final mix: 200µl Sample + 300µl WR
• Calculation: ([ODsample-ODblank]/ODRefrigerated centrifuge) x 400µl
• Conversion: 100xµlL = 1 ppm

Discussion

• Why did the copper levels decrease between T1 & T2?
  • Stressor wasn’t stressful enough for the animal
  • Sheep had been grazing alfalfa pasture for over 75 days prior to the first blood collection. The stressor event was the act of hoof trimming, which involves flipping the animal onto their back and restraining them for 2 minutes. Blood was collected four times: before and after supplementation with minerals; and before and after the stress event. Blood samples were processed, and the serum was extracted and stored at -70ºC. To measure copper levels, we used a copper assay kit and spectrophotometer. Results show that the copper assay kit we used was able to detect serum copper in sheep. Serum copper levels decreased (P<0.05) in lambs after they were stressed, which did not support our hypothesis. This may be due to the fact that hoof trimming may not have been a true stressor for this group of lambs. This study shows that serum copper levels do fluctuate and can be used for further research in assessing copper status in sheep.

Conclusions
• Even though we saw an increase in serum copper levels between T3&T4, we can’t attribute it all to the stress event of trimming hooves due to the decrease in serum copper from T1 to T2
• Weather events, which were out of our control, may have had an effect on our serum copper levels
• This work showed that measurable differences in serum copper can be detected in hair sheep

References

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Figure 1: The hair sheep used in the study. The blue markings designate if they had been sampled

Figure 2: Blood was collected from each sheep via jugular venipuncture

Figure 3: After blood was collected, it was spun down. The top, clear half (serum) was used for the copper assay

Graph 1: This graph shows what happened between the first two collection times. The general trend of this graph is that the copper concentration decreased (pH=0.02) when sampled after the stress event. (T1= no stress, T2= stress)

Graph 2: This graph shows what happened between the 3rd and 4th collection times. The general trend of this graph is that the copper concentration increased (pH=0.01) when sampled after the stress event. (T3= no stress, T4= stress)