Ceruloplasmin and Copper Status in Free-Ranging Alaskan Caribou (*Rangifer tarandus tarandus*)

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Introduction

Copper (Cu) is an essential element for survival and is of particular importance to healthy immune function, reproduction and integument of ruminants. Disease and mortalities linked to deficient dietary or assimilation of Cu have been documented in captive and free-ranging ruminants including cervids and arctic species such as muskoxen. Liver analysis is considered the gold standard for evaluating copper reserves in ungulates. However, for the live ruminant, serum ceruloplasmin (Cp) can be used as non-lethal biomarker of liver copper in some species such as muskoxen, sheep and cattle. However, it must be validated for each species by age, sex, and season. A limited captive study in caribou and reindeer found liver Cu significantly related to both serum Cu and Cp in during summer or winter but not during the rut. Our aim was to confirm the validity of serum Cp or serum Cu as a biomarker of liver Cu for a larger sample of free-ranging caribou during our typical capture seasons. Additionally, we compared the different measures of Cu between genders, season, age, reproductive status, and region.

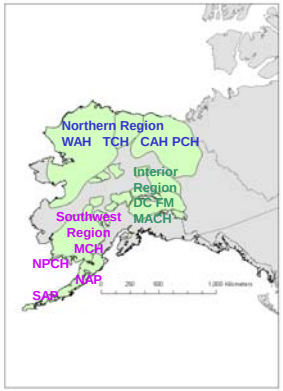

Figure 1: Caribou herds included in analysis

Methods

Liver tissue (n = 86) and serum (n = 515) samples collected from live or freshly killed caribou from 2003 and 2010 and stored frozen were analyzed for copper at ICP-MS or atomic absorption spectrometry[1,2,3]. Ceruloplasmin was determined from sera[4]. We categorized three seasons in our analysis: Winter (Nov-May) and Summer (Jun-Jul) and Fall (Sep-Oct) samples. The arctic herds, Western Arctic (WAH) and Teshekpuk (TCH), Central Arctic (CAH), and Porcupine (PCH) were pooled to establish the "northern region", North Alaska Peninsula (NAP), South Alaska Peninsula (SAP), Nushagak NPCH, and Mulchatna (MCH) were pooled to establish the "southwestern region", and Fortymile (FCH), Delta (DCH) and Macomb (MACH) were pooled as the "interior region.".

Age classes were defined as:

- Fetus
- Neonate = ≤ 1 month
- Calf = >1 month & ≤ 10 months
- Adult = > 10 months

Statistical Analysis:

- Normality was assessed using the Shapiro-Wilks Normality Test (SAS[5], PROC Univariate); data that violated the normality assumption were log-transformed prior to analysis.
- A Generalized Linear Model (PROC GLM) was used to test for significant differences in [serum Cu] between genders, age classes, seasons, and herds/regions, where $p < 0.05$.
- PROC REG was used to test for correlations between all combinations of [serum Cu], [serum Cp], and [liver Cu]
- Where model selection procedures were required, AIC was used to rank models by fit and parsimony.

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[5]SAS Version 9.2, SAS Institute Inc., Cary, NC, US

Results

- [Liver Cu] varied by age; Fetuses=Neonates> Adults ($p < 0.001$) (Figure 2), [Serum Cu] varied similarly (data not shown).
  - No significant differences were found for gender, herd, or region
- [Liver Cu] varied by season: Fall 22.3±13.4 >Summer 13.7±9.1=Winter 13.8±11.0 ($p = 0.02$)
- Mean [Liver Cu] Pregnant females < Non-pregnant females (Figure 3)
- In Adult caribou, [serum Cp] is a significant (p=0.01) predictor of [Liver Cu] but R^2=0.15 was poor (Figure 4)
  - [Serum Cu] is not a significant predictor of [Liver Cu] (p=0.13, R^2=0.06)
- [Serum Cu] & [Serum Cp] are strongly correlated in adult caribou (p<0.0001, R^2=0.67) (Figure 5)
- Regional and seasonal comparisons of [Serum Cu] were confounded by the fact that each season was dominated by samples from a single herd.
- From <5% to 22.2% of adult caribou in the herds sampled could be considered deficient for [Liver Cu] based on published reference ranges (Puls, 1994) (Figure 6)

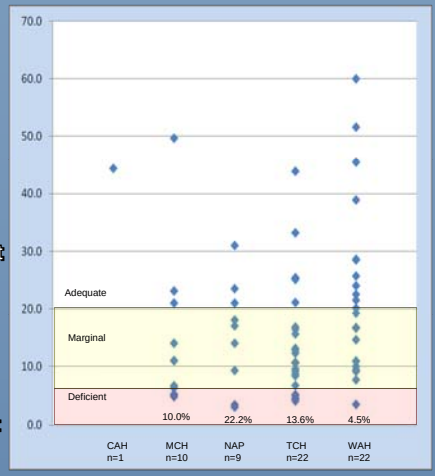

Figure 6: Adult [liver Cu] ranges between adequate, marginal or deficient based on deer (Puls, 1994)

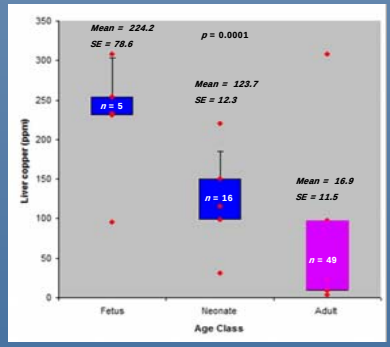

Figure 2: Comparison of [liver Cu] between age classes.

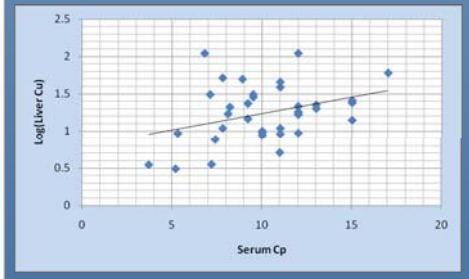

Figure 4: Log [Liver Cu] = Intercept +Serum Cp
Log [Liver Cu] = 1.65 + (Serum Cp * 0.104)

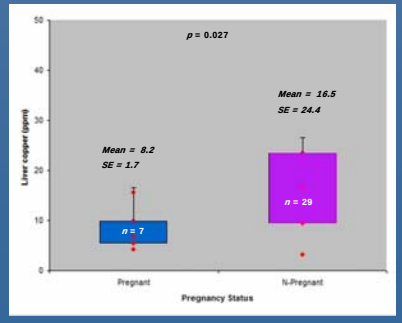

Figure 3: Comparison of [liver Cu] between pregnant females and non-pregnant females.

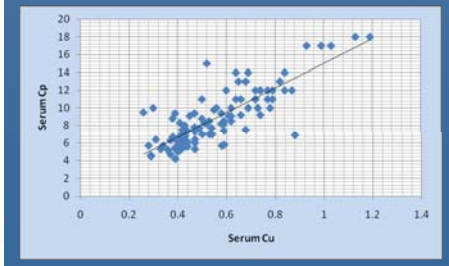

Figure 5: [serum Cu] = 0.134+(0.04839*Serum Cp)

Discussion

- Our results confirm the conclusions of Barboza and Blake (2001), that in *Rangifer,* Cp is provides a better predictive measure of [liver Cu] than [serum Cu]. However, we did not demonstrate a similar strong correlation between [liver Cu] and Cp in summer and winter. Thus, if Cp is to be used for trend analysis or comparisons between herds, timing of sample collection must be consistent and uniform over time and outside of the rut period when Cp is most variable and [liver Cu] is highest.
- Our observation of high fetal [liver Cu] and low pregnant female [liver Cu] supports the conclusion that there is mobilization of copper stores from the cow to the fetus, as in cattle (Puls, 1994). Neonates had similar values of copper in serum as those published for cattle between the ages of 1-7days, consistent with copper requirements prior to weaning (Puls, 1994).
- A Cp value below 6 appears to be a threshold that indicates a caribou is likely to be Cu deficient, however, a value above 6 doesn't assure adequate copper reserves unlike the findings in red deer (Laven & Lawrence 2010).
- The southwestern NAP caribou herd had a significant percentage of adults with inadequate copper reserves to maintain health during the years ('05-'06) sampled coinciding with the nadir of a population decline.

References

Barboza, P.S. & Blake, J.E. 2001. Ceruloplasmin as an indicator of copper reserves in wild ruminants at high latitudes. Journal of Wildlife Diseases 37(2): 324-331.

Laven, R.A. & Lawrence, K.E. 2010. Analysis of the value of measurement of the activity of caeruloplasmin as an alternative to measurement of the concentration of elemental copper in plasma and serum of farmed red deer. New Zealand Veterinary Journal 58(4): 207-212.

O'Hara, T.M., George, J.C., Blake, J., Burek, K., Carroll, G., Dau, J., Bennett, L., McCoy, C.P, Gerard, P., & Woshner, V. 2003. Investigation of Heavy Metals in Larger Mortality Event in Caribou of Northern Alaska. The Arctic Institute of North America. 56 (2): 125-135.

Puls, R. 1994. Mineral levels in animal health. Diagnostic Data. 2nd edition. pp83-90

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