Using Urine in Snow to Assess Animal Condition, Nutrition, and Stress

- Nutrition – primary mechanistic thread between environmental variation and population variation.
    - How do blood and urine characteristics reflect physiological responses and adaptations of deer to nutritional restriction?
    - Winter is nutritionally challenging to survival of northern deer
        - Studies focus specifically on winter nutritional restriction and spring recovery.
- Methods used to assess winter nutritional condition of northern deer.
        - Carcass analysis for measuring body fat deposits and femur marrow fat.
        - Ground and aerial surveys to visually estimate nutritional debilitation and to determine changes in population size.
        - Weighing captured animals and collecting blood and urine samples for analyses
        - Indirect evaluations via range quality assessments.
    - Common considerations
        - Personnel and cost
        - Sample size
        - Technique sensitivity
        - Ability to collect and interpret data before gross nutritional deterioration or death occurs.
- Advantages in urinalysis for assessing nutritional restriction.
    - Kidneys, urine formation, and excretion are central to physiological mechanisms that maintain homeostatic concentrations of metabolites and constituents in blood and other bodily fluids.
    - Changes in certain urinary constituents may reflect subtle alterations in nutritional state.
    - Urine deposited in snow by ungulates can be easily collected and analyzed.
    - Avoid costs of capture and immobilization.
    - Collection of complete 24-hour urine samples from subjects for physiological assessments is optimal, though not feasible under practical field conditions.
- Urinary indicators of nutritional restriction
    - Urea nitrogen
        - Urea is end product of dietary and endogenous protein metabolism.
        - Urea nitrogen has been studied more than any other urinary characteristics with respect to its value as a nutritional index for deer.
        - Urea nitrogen constitutes 85% of urinary nitrogen.
            - WTD can conserve nitrogen when protein intake is restricted by increasing renal absorption and recycling urea, thus reducing its loss in urine.

        - Urinary nitrogen decreases associated with diminished dietary protein, specifically, and with moderate, general nutritional restriction.
            - Urinary nitrogen increases during greater restriction of dietary energy – attributable to accelerated net catabolism of body protein, which occurs simultaneously with fat mobilization.
            - Degradation of body protein is more rapid as dietary energy declines.
            - Urinary nitrogen excretion maximizes as deer consuming submaintenance levels of digestible energy deplete there fat reserves and progress toward the poor end of the tissue catabolism gradient.
    - Urinary Potassium
        - Urinary potassium excretion also a supporting indicator of progressive nutritional restriction or deprivation in deer.
        - Potassium content of browse plants is markedly higher than that of most other minerals, including during winter.
        - Urinary excretion of potassium is directly related to dietary intake.
        - Steadily declining urinary potassium reflects progressive nutritional restriction.
        - Once animal is moribund because of starvation, urinary potassium may become markedly elevated due to extensive muscle catabolism, cell destruction, and potassium release from tissue.
    - Urinary Cortisol
        - Urinary cortisol is a potential indicator of winter nutritional stress.
        - Primary function of cortisol is to mobilize energy reserves.
        - Suggested that increasing urinary cortisol reflected energy deficit of mule deer, and with extreme cases, was associated with net catabolism of protein.
- Creatinine Ratios
    - Ratios of urinary metabolites to creatinine content are calculated (assumed to represent daily values).
        - Corrects for potential differences in hydration of individual animals, which affects concentrations of urine chemicals and urine volumes, and importantly, corrects for dilution of urine constituents by snow.
        - Creatinine is excreted at a relatively constant, 24-hour rate in animals.
        - Negligible reabsorption of creatinine in the renal tubules.
        - Creatinine not affected by herbivorous diets.
        - Produced and excreted in proportion to muscle mass.
            - Allows for comparison of data from animals of varying size.
- Advantages and expectations

        - Reference values for urinary characteristics such as UN:C generated from earlier deer studies.
        - Is there sufficient information to use chemical analysis of urine in snow to directly assess winter nutritional status of deer? Yes, in select situations where researchers clearly understand the technique, recognize limitations, and design applications with realistic expectations.
        - Sequential collection and chemical analysis of urine in snow has value for general, sensitive assessments of nutritional restriction in deer and other ungulates.
    - Significant Advantages
        - Monitoring by this technique allows for detection of nutritional restriction before gross deterioration occurs.
        - Large sample sizes may be obtained for deer and other ungulates in practical, relatively time-efficient and cost-effective manner.
        - Technique is noninvasive, no associated stress and no effect of immobilizing chemicals.
        - Noninvasive nature particularly valuable in national parks due to "hands-off" management policy.
- Considerations when using urine in snow
    - Actual ratio values of each index
    - Temporal trend of the index
    - Proportion of samples indicating extreme nutritional restriction (i.e., UN:C ratios ≥3.5). Regardless of the deer's fat reserves, UN:C ratios ≥3.5 indicate severe nutritional restriction. Normal ratios will be 0.3–2.5.
    - How changes and values relate to perturbations of environmental factors.
    - Can document short-term changes in UN:C relative to winter weather extremes.
    - Increasing relationship in UN:C ratios as winter progressed in wintering areas with large snow depths (93-95 cm).
    - Once UN:C ratio approaches 23, a moribund animal is likely.
    - Values ≥23 may indicate deer with exhausted fat reserves experiencing rapid degradation of endogenous protein.
    - Nutritional surveys by snow-urine analysis could be of immediate value to managers.
        - Case: In northern MN winter 1988–1989, severe winter weather, MNDNR implemented large scale emergency feeding program.
        - However, there was little relationship between the winter weather and the deer's nutritional status.
        - Program cost $1.07 million and required >17,000 staff-hours and >100,000 volunteer hours.
        - Most of supplemental feed was unused and wasted

        - Analysis of deer urine in snow at specific locales could have helped prioritize feeding sites and manager efforts based on nutritional condition.
- Limitations and considerations
    - Cannot discriminate between sources (vegetative versus body protein) of urinary urea nitrogen (however, prolonged low UN:C ratios reflect chronic nutritional restriction and a reduced loss of urea nitrogen, regardless of the source).
    - Declining temporal trends of UN:C strongly suggest nutritional restriction and enhanced renal conservation or retention of urea nitrogen.
    - An increasing UN:C trend as winter progresses, barring supplemental feeding, indicates accelerated protein catabolism attributable to increasingly serious restriction. It is not likely that as winter progresses, deer are lactating and consuming food of significantly greater crude protein content.
    - A declining or sustained trend of low potassium:C values would lend confidence to this interpretation.
- Considerations for application
    - General knowledge of nutrient content (e.g., crude protein) of vegetation consumed by deer and availability of supplied, high quality food (including crop residue) are important.
    - Natural browse on northern winter ranges contains only 3–7% crude protein.
    - DelGiudice et al. established reference values for urinary characteristics based on diets with protein content in this range.
        - Mean UN:C ratios did not exceed 3.0 in 24-hour urine samples of captive deer fed a 6.7% crude protein diet *ad libitum* and exhibiting no change in body mass.
        - In the field, absence of sources of crude protein minimizes the chance of wrongly attributing high UN:C ratios to severe nutritional restriction.
    - Ensure statistician assistance
    - Pilot study if possible, sampling needs to represent the area.
    - Multiple collections of snow-urine samples throughout the winter.
    - Collections should be made immediately (within several days) after fresh snowfall.
    - Sampling should minimize duplicate sampling of individual deer within the same collection period.
    - Snow cover is a requisite.
    - Unknown whether sample is from doe, buck, or fawn – may limit certain ecological interpretations.