AN ABSTRACT OF THE THESIS OF

Lonnie J. Quinlan-Murphy for the degree of Master of Science in Animal Science presented on May 15, 1998. Title: Influences of Age, Condition, Nutrition and Season on Serum and Urine Chemistry in Rocky Mountain Elk.

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Abstract approved: _____

Timothy DelCurto

Research objectives were to establish reliable data for elk serum and urine chemistry and to evaluate the influences of age, photoperiod, condition and nutrition. An existing radioimmunoassay was validated and a pilot study on insulin-like growth factor-1 (IGF-1) response to nutritional restriction in elk calves was conducted. Elk calves subjected to a low energy diet had significantly (P = 0.0001) lower levels of IGF-1 than those on the high-energy diet during 3 separate nutrition trials. IGF-1 levels in trial #3 were significantly lower than those from trials #1 and #2 and this combined with a declining trend in IGF-1 levels over the 3 trials suggests seasonal influences as well. In the main study, 28 serum and urine variables were evaluated in a repeated measures study design to establish reference levels for elk ages 3.5-months to 4-years and to assess influences of age, body condition and nutrition. Three treatment groups consisted of 2.5 year-old female elk assigned to either a high energy ad libitum diet (HICON) year-round, a low energy restricted diet (LOCON) year-round, or a seasonally changing diet (SEACON). Six elk calves also were randomly assigned to a 4th treatment group and received the high energy ad libitum diet (JUV). The age analysis included the HICON and JUV groups only. Results indicated that 17 serum variables were significantly influenced by age. The seasonal analysis (HICON, LOCON and JUV) indicated that 8 serum variables were

strongly correlated to changing daylength. Daylength was also correlated with voluntary food intake declines in fall and winter. Reduced body condition induced by long-term nutritional restriction (HICON vs. LOCON) significantly influenced 16 variables. Of these, IGF-1 and glucose were the most sensitive and they appeared to reliably reflected body condition. Seasonally changing nutrition influenced 10 variables in the HICON vs. SEACON subset analysis and 12 variables in the LOCON vs. SEACON subset analysis. Triiodothyronine (T_3) and IGF-1 followed expected patterns and of these two, IGF-1 was more sensitive than T_3. Effects of the thermal cover and reduced body condition were also assessed in calf and yearling elk. Elk receiving the satisfactory cover treatment experienced reduced body condition compared to calves in the no cover group. Of the 5 variables that were significantly different, only IGF-1 appeared to be both reliable and sensitive to changes in body condition.

(Key Words: elk, nutrition, condition, serum chemistry, urine chemistry, IGF-1, thermal cover)

Influences of Age, Condition, Nutrition and Season on Serum and Urine Chemistry in Rocky Mountain Elk

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Lonnie J. Quinlan-Murphy

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I understand that my thesis will become part of the permanent collection of the Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Lonnie J. Quinlan-Murphy, Author

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Contribution of Authors

Dr. John Cook designed all of the studies discussed in this thesis and was responsible for data collection and experimental protocols. He also assisted with data analyses and writing of each manuscript. Dr. Steve Davis was involved with validation of the radioimmunoassay for insulin-like growth factor and provided the use of his laboratory to run the assays at Oregon State University. Dr. Tim DelCurto served as my major professor, provided guidance on laboratory procedures, data analyses, and writing of the thesis. Dr. Larry D. Bryant of the U.S. Forest Service, Pacific Northwest Research Lab and Dr. Larry Irwin of the National Council for Air and Stream Improvement provided funding for these research projects.

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Dedication

I would like to dedicate my thesis to my parents, my husband and the elk calves that gave me the desire to further my education and to pursue my dreams. My parents, Joe and Joan Quinlan, have provided me with encouragement and unconditional love as I have pursued higher education. Growing up on our family ranch fostered my interest and love for animals both domestic and wild, and I am thankful for that opportunity. My husband, Wade Murphy, deserves special mention for patiently encouraging me to finish my thesis and for being understanding of the large time commitment required to finish this thesis.

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Influences of Age, Condition, Nutrition, and Season on Serum and Urine Chemistry in Rocky Mountain Elk

Lonnie J. Quinlan-Murphy

PROBLEM DEFINITION

Scientists generally classify elk as k-selected species that are prone to density-dependent demographic changes in relation to resource availability. Resources of primary concern are food, water, cover and space. Of these, the role of the food resource is most relevant to population demographics because nutrition can exert strong influences on reproduction and survival of wild ungulates. Inadequate nutrition can negatively influence neonatal immune function (Ogra 1984), juvenile survival (Merrill and Boyce 1991), overwinter survival of adults (Guinness et al. 1978, Verme and Ullrey 1984, Hobbs 1989), and fertility (Verme and Ullrey 1984).

Still, research on large ungulates in North America has provided little insight regarding nutritional status in free-ranging settings. Nutritional requirements are often not included in management plans. Consequently resource managers and biologists continue to make management decisions about elk populations without adequate regard to nutritional status or condition of their herds (Cook et al. 1996). Managers currently rely on data such as trends in elk numbers and reproduction (e.g., pregnancy rates, calf:cow ratios) to assess herd status. Unfortunately, such information reflects a variety of confounding influences and generally is not useful for measuring nutritional status or condition of the herd. Accurate assessment of nutritional status and body condition may be necessary for maintaining the productivity of elk populations over time.

Over the last 2 decades wildlife biologists have focused considerable research on the value of serum and urine chemistry as indirect measures of nutritional status and body condition. These studies have provided insight for white-tailed deer managers, however, little information exists for elk.

Published studies on serum and urine chemistry in elk are limited to: Herin (1968), Follis (1972), Weber and Bliss (1972), Vaughn et al. (1973), Pedersen and Pedersen (1975), Wolfe et al. (1982), DelGuidice et al. (1991 *a,b)*, Garrott et al. (1996, 1997), Vagnoni et al. (1996). Several of these studies are now outdated and few were done under controlled conditions.

Accurate assessment of serum and urine chemistry requires that variables such as age and sex be controlled and that feed intake and body mass (BM) dynamics be documented. Further, studies need to be of sufficient length to provide meaningful analysis of long term and short term nutritional restrictions and to assess seasonal influences. To be useful to wildlife managers, serum or urine chemistry indices must also be reliable and sensitive to changes in nutrition. Previous wildlife studies indicated that the following variables exhibited potential as indirect indices: alkaline phosphatase (ALK), cholesterol, serum urea nitrogen (SUN), triiodothyronine (T_3), thyroxine (T_4), glucose, urinary urea nitrogen (UUN), and urinary cortisol but results were often inconsistent and sometimes controversial (DelGiudice 1995, Saltz et al. 1995, White et al. 1995).

In the late 1980's, animal scientists conducted several studies involving a peptide hormone called insulin-like growth factor-1 (IGF-1) and found that IGF-1 reliably reflected nutritional status in domestic ruminants (Breier et al. 1986, Ronge and Blum 1989, Straus 1994). In these studies, a common pattern emerged where IGF-1 levels declined under short-term protein and/or energy restrictions when compared to control animals. Recent studies also have shown that the cold environment can reduce levels of IGF-1 in domestic animals as well (Scott and Christopherson 1993, Van Kessel and Laarveld 1994). Although IGF-1 has been studied in red deer in other countries such as New Zealand (Suttie et al. 1989, Webster et

al. 1996,) and Norway (Ringberg et al. 1978), no research has yet been conducted on IGF-1 in North American wild ungulates.

STATEMENT OF PURPOSE

The primary purpose of this research was to establish reference values for selected serum and urine chemistry variables from female elk in good condition for a variety of age classes and seasons. Secondly, we wished to assess the reliability and sensitivity of serum and urine chemistry indices to changes in daylength, age, short term and long term nutritional restrictions and to changes in body condition influenced by thermal cover rather than nutrition. Thirdly, we wished to assess the potential of IGF-1 as an indirect measure of body condition or nutrition.

Because IGF-1 had not previously been measured in elk serum, an existing radioimmunoassay (RIA) procedure for bovine serum was modified and validated for use with elk serum. A preliminary assessment of IGF-1 levels in relation to nutritional status was conducted using serum collected from elk calves in a small pilot study. Following the pilot study, serum samples collected during the main blood and urine chemistry study and serum samples from a 4-year thermal cover study were assayed for IGF-1.

FUNCTION AND REVIEW OF SERUM AND URINE METABOLITES

Alanine Aminotransferase

Alanine aminotransferase (ALT) is synonymous with serum glutamic pyruvic transaminase (SGPT) (Thomas 1993). It is an enzyme present in serum and in many body tissues that acts upon

the amino acid alanine to form pyruvate during glycolysis (Newsholme and Leech 1986). Kie et al. (1983) reported that ALT levels did not show potential as an indirect measure of condition or nutritional status in white-tailed deer (*Odocoileus virginianus*). However, Seal et al. (1978*a*) found that white-tailed deer fawns subjected to a low energy diet exhibited significantly higher ALT levels when compared to fawns on a moderate energy diet and this effect was apparently independent of protein content in the diet. Knox et al. (1988) reported levels of ALT ranging from 63 to 145 IU/l in domesticated female red deer. Kie et al. (1983) reported levels of ALT ranging from 47.1 to 88.9 IU/l in adult white-tailed deer.

Albumin

Albumin is the main protein found in the blood and thus comprises 60% of the total serum proteins (Peters 1996). It influences osmotic pressure, binds calcium (Sodikoff 1995), and is the main transporter of long chain fatty acids allowing free fatty acids to be carried in the blood in a non-toxic form (Newsholme and Leech 1986). Serum albumin in young adult humans typically ranges from 3.5 to 5.0 g/dl (Peters 1996). Dehydration can cause hyperalbuminemia (abnormally high serum albumin levels), but this condition is not common (Peters 1996). During periods of prolonged starvation or malnourishment, production of this protein is decreased (Newsholme and Leech 1986), inducing a condition known as hypoalbuminemia (Peters 1996).

Although Warren et al. (1982) reported significantly higher levels of serum albumin in white-tailed deer fawns receiving low energy diets, they concluded that albumin was not a good index of nutritional status because variation of individual

animals was high in their study. Seal et al. (1972) found significantly lower albumin levels in wild white-tailed deer compared to captive white-tailed deer and attributed this to the poor dietary status of the wild deer. Reported levels of albumin in captive and free-ranging elk were 4.03 g/dl and 3.30 g/dl (Wolfe et al. 1982). Wolkers et al. (1994) reported albumin levels ranging from 3.7 to 4.6 g/dl in feed-restricted red deer compared to levels of 4.1 to 5.5 g/dl in the non-restricted control group. Knox et al. (1988) also reported albumin levels ranging from 2.3 to 3.9 g/dl in red deer.

Alkaline Phosphatase

Alkaline phosphatase (ALK) is an enzyme that plays an important role in metabolism and absorption of phospholipids, nucleotides, and carbohydrates and is involved in bone calcification (Thomas 1993). Generally, blood levels of ALK are elevated during pregnancy and levels may be up to six times higher in neonates than in adults (Thomas 1993). Further, research with cervids indicated that ALK also responds to nutritional changes.

Wolkers et al. (1994) reported that ALK levels decreased 79-86% over a 13-23 week study period in which 2-year-old red deer received 20% of the ration of the control animals. The authors attributed declining ALK levels to reduced hepatic or intestinal excretion and/or decreased osteoblastic activity. Kie et al. (1983) reported lower levels of ALK in white-tailed deer maintained at high population densities versus those occurring at lower population densities. Kie et al. (1983) also reported significantly lower levels in adult white-tailed deer compared to fawns.

ALK levels typically declined during winter months and increased in summer months for both adults and juveniles (Kie

et al. 1983, Tedesco et al. 1991, Soveri et al. 1992). Such seasonal effects, combined with effects of age, pregnancy, and antler growth may confound the use of ALK as a nutritional index in adult animals. Levels of ALK in white-tailed deer ranged from 67 to 107 IU/l in adults and from 132 to 299 IU/l in fawns (Kie et al. 1983). ALK levels ranged from 40 to 460 IU/l in red deer (Wolkers et al. 1994) and from 160 to 323 IU/l in reindeer calves (Soveri et al. 1992).

Aspartate Aminotransferase

Aspartate aminotransferase (AST) previously named serum glutamic-oxaloacetic transaminase (SGOT) is a mitochondrial-bound (Sodikoff 1995) enzyme that plays an important role in cellular metabolism (Thomas 1993). Specifically, AST is involved in the reoxidation of cytosolic NADH via the malate/aspartate shuttle between the cytosol and mitochondrion of the cell (Newsholme and Leech 1986). The malate/aspartate shuttle is important, quantitatively, for cytosilic oxidation of NADH in tissues of vertebrates (Newsholme and Leech 1986).

Wolfe et al. (1982) reported significantly lower levels of AST in captive elk (91.3 IU/l) versus free-ranging elk (134.6 IU/l) assumed to be in poorer condition. Seal et al. (1978*a*) reported no effect of dietary energy or protein content on AST levels of white-tailed deer fawns. Also, Seal et al. (1978*b*) reported no effect of age, sex or location on adult white-tailed deer AST levels. Kie et al. (1983) reported AST levels ranging from 132 to 168 IU/l in adults and 133 to 139 IU/l in fawns of white-tailed deer. Knox et al. (1988) reported a range of 59 to 150 IU/l in domesticated female red deer.

Bilirubin

Bilirubin is the orangish/yellow pigment found in bile. It is produced from catabolism of hemoglobin and is carried to the liver by the blood (Thomas 1993). The heme portion of hemoglobin is acted upon by amino acids and converted to bilirubin in the spleen, then bilirubin enters the blood and forms a complex with serum albumin (Mathews and van Holde 1990). Concentrations of bilirubin in the blood may be descriptive of the rate of red blood cell production and degradation (Mathews and van Holde 1990). Kie et al (1983) reported that total bilirubin (TB) did not vary significantly based on age, sex or condition. Levels of TB in their study ranged from 0.21 to 0.24 mg/dl in adults and from 0.29 to 0.37 mg/dl in fawns (Kie et al. 1983).

Calcium

Calcium is a mineral used for bone growth, coagulation and acid-base balance of the blood, activation of enzymes, nerve and muscle function, maintaining permeability of membranes and lactation (Thomas 1993). Calcitrol, calcitonin, and parathyroid hormone primarily control levels of calcium in the blood (Sodikoff 1995). Calcium is carried in the blood at fairly constant levels when adequately provided in the diet. Conditions that cause hemoconcentration of the blood however may lead to elevated calcium levels (hypercalcemia) and to hyperalbuminemia (Sodikoff 1995).

Kie et al. (1983) reported that levels of serum calcium in white-tailed deer varied as a function of sex, age and month but was not related to body condition. In contrast, 6 weeks of extreme nutritional restriction led to significantly elevated serum calcium levels in white-tailed deer (DelGiudice et al.

1990*b*). DelGiudice et al. (1990*b*) attributed the rise in serum calcium to dehydration due to starvation and subsequent hemoconcentration of the blood. Concentrations of proteins that bind calcium in the blood were also elevated. Reported levels of serum calcium in elk were 8.7 to 10.1 mg/dl (Wolfe et al. 1982) and white-tailed deer levels were within the range reported for elk (Seal et al. 1978*b*, Kie et al. 1983, DelGuidice et al. 1987*a*, DeLiberto et al. 1989).

Excess calcium in the blood is removed via the kidneys and excreted in the urine. Urinary calcium levels are primarily expressed in wildlife literature as the ratio of calcium:creatinine x 1000 (UCa:Cr). In terms of nutritional influences on UCa:Cr levels, DelGiudice et al. (1987*b*) did not report any significant differences based on a high protein versus low protein diet with white-tailed deer. Further research by DelGiudice et al. (1990*b*) indicated that UCa:Cr was unaffected by fasting for 6 weeks in white-tailed deer. Ratios of UCa:Cr ranged from 25 to 494 in mule deer (DelGiudice et al. 1990*a*) and from 10 to 150 in white-tailed deer (DelGiudice et al. 1987*b*).

Chloride

Chloride typically combines with sodium and becomes a salt (NaCl) in the blood (Thomas 1993). Normal values for humans range from 100 to 110 mEq/l and may fluctuate with different pathological states (Thomas 1993), but overall levels tend to be homeostatic. Kie et al. (1983) reported that chloride levels differed as a function of month of study, but not in relation to condition in white-tailed deer. Conversely, chloride levels in white-tailed deer fawns were elevated by a low protein diet and decreased by a low energy diet (Seal et al. 1978*a*). Soveri et al. (1992) also examined chloride levels

in caribou calves during winter and concluded that declining levels in late winter were likely due to nutrition, although the decline was evident at all feeding levels, including high, medium and low energy. Reported levels of chloride in the blood ranged from 100 to 117 mEq/l in caribou calves (Soveri et al. 1992) and from 91 to 105 mEq/l in white-tailed deer (Kie et al. 1983).

Cholesterol

Cholesterol is a sterol that is synthesized in the liver and is a component of bile (Thomas 1993). It plays important metabolic roles as a precursor to bile acids and steroid hormones such as the adrenal corticoids and sex hormones, is present in cell membranes, and is involved in triacylglyceol transport in the blood (Newsholme and Leech 1986). Metabolism or breakdown of cholesterol results in the biosynthesis of bile acids that promote fat digestion and absorption by emulsifying dietary lipids (Mathews and van Holde 1990). Healthy normal human values are generally < 200 mg/dl, but in other mammals plasma concentrations are generally lower than 120 mg/dl (Newsholme and Leech 1986).

Seal et al. (1978*b*) reported only moderate effects on cholesterol levels from reduced protein or energy in the diet and concluded that cholesterol was not a good index of dietary intake in white-tailed deer. Card et al. (1985) reported no significant differences in nutritionally restricted white-tailed deer versus well-fed deer. DelGiudice et al. (1987*a*) reported hypercholesterolemia in white-tailed deer after fasting for 4 weeks. According to DelGiudice et al. (1987*a*), long-term starvation induced hypothyroidism and hypercholesterolemia in association with lipolysis.

Kie et al. (1983) and Dierenfeld and Jessup (1990) reported that cholesterol levels varied mostly in relation to age, season, and sex. Seasonal changes (photoperiod) were reported in most long term studies (Warren et al. 1981, Seal et al. 1972, Soveri et al. 1992, DeLiberto et al. 1989, and Soveri et al. 1992) with lower levels typical during winter months. Levels of cholesterol ranged from 40 to 85 mg/dl in white-tailed deer (Warren et al. 1981) and from 41 to 94 mg/dl in free-ranging mule deer (Dierenfeld and Jessup 1990). Wolfe et al. (1982) reported cholesterol levels for captive elk of 89.44 mg/dl and for free-ranging elk at 111.53 mg/dl.

Serum and Urinary Creatinine

Creatinine is a creatine metabolic end product formed primarily in skeletal muscle from the generation of ATP (Mathews and van Holde 1990) that is used for skeletal muscle contractions. Creatinine is removed from the tissues and excreted via the kidneys in urine. DelGiudice et al. (1995*a*) reported that levels of creatinine excreted in the urine were proportional to muscle mass in the bodies of male white-tailed deer. Thus, one would expect juveniles to have lower levels of creatinine than adults due to smaller amounts of muscle mass. Kie et al. (1983) reported however that adult white-tailed deer had lower creatinine levels than fawns.

In terms of nutritional effects, DelGiudice et al. (1990*b*) reported slight increases in serum creatinine levels of fasted white-tailed deer after 4 weeks and attributed this to decreased excretion of creatinine. Wolkers et al. (1994) reported significantly higher serum creatinine levels in feed restricted versus control red deer, and they attributed their finding to decreased excretion during severe feed restriction.

Wolfe et al. (1982) reported significantly higher levels of serum creatinine in free-ranging elk assumed to be in poorer condition than captive elk.

In contrast, research by Kie et al. (1983) on condition parameters in white-tailed deer revealed lower serum creatinine levels in deer reported to be in poorer condition. Seasonal changes in serum creatinine levels were not reported in studies with deer and elk but Tedesco et al. (1991) reported seasonal variation in their analysis on muskoxen (*Ovibos moschatus*). Serum levels of creatinine in elk were 2.74 mg/dl and 3.35 mg/dl for captive and free-ranging animals (Wolfe et al. 1982). Reported levels of serum creatinine in white-tailed deer ranged from 1.27 to 1.44 mg/dl (Kie et al. 1983) and from 1.7 to 2.3 mg/dl (DelGiudice et al. 1990*b*).

The rate of creatinine excretion via the kidneys in humans is constant at about 0.02 gm/kg of body weight/day (Thomas 1993). DelGiudice et al. (1995*a*) reported that creatinine levels in the urine were correlated to muscle mass, not influenced by dietary intake of protein, and excretion over a 24-h period was constant in male white-tailed deer. Further research by DelGiudice et al. (1996) compared single urine samples to 24-h urine samples and found no significant differences. Therefore researchers using single urine samples rather than 24-h urine collections can still get valid results. Wildlife researchers have been using creatinine ratios to correct for potential hydration differences in animals sampled and also to compensate for dilution of urine samples collected from snow. Urinary levels of creatinine for free-ranging elk were reported by DelGiudice et al. (1991*b*) to range from 21 to 32 mg/dl.

Gamma Glutamyltransferase

Gamma glutamyltransferase (GGT) is an enzyme involved in purine nucleotide synthesis (Mathews and van Holde 1990). Apparently, no information exists about influences of age, season, nutrition or condition on GGT levels in the wildlife literature. Serum levels of GGT reported by Knox et al. (1988) for red deer ranged from 11 to 97 IU/l.

Glucose

D-glucose is an intermediate in carbohydrate metabolism in the body (Thomas 1993). From the intestines in monogastrics, glucose is absorbed and transported in the blood to the liver. Here, excess glucose is converted to glycogen via glycogenesis (Thomas 1993). In humans, glucose levels are generally maintained at about 80 to 120 mg/dl and insulin secreted by the pancreas regulates blood glucose levels (Thomas 1993).

Unlike humans, adult ruminants rely primarily on volatile fatty acids (VFAs) to meet most of their energy demands (Church 1988). However, many ruminant tissues including: muscle, adipose, mammary and fetal tissues require glucose as the primary substrate for cellular energy (Church 1988). Ruminants therefore, must convert propionic acid, lactate, amino acids and glycerol to glucose to meet certain energy demands (Church 1988). The amount of propionic acid produced in the rumen depends on the rumen microbial population present and the type and amount of feed given to the animal (Church 1988). In general, high-energy diets lead to a greater production of propionic acid and results in more substrate for glucose synthesis (Church 1988). Serum glucose levels are reflective of the microbial population in the rumen, feed quality and quantity, and the energy balance of the animal.

DelGiudice et al. (1987*a*) reported lower glucose levels in white-tailed deer when fasted compared to control animals. Seal et al. (1978*a*) also reported lower glucose levels in feed-restricted white tailed deer fawns. Levels of glucose ranged from 62 mg/dl in captive elk to 226 mg/dl in free-ranging elk (Wolfe et al. 1982). Kie et al. (1983) reported serum glucose levels ranging from 48 to 290 mg/dl in white-tailed deer fawns and from 64 to 201 mg/dl in adult white-tailed deer.

Insulin-like Growth Factor-1

IGF-1 is a peptide hormone found in vertebrate species and is comprised of 70 amino acid residues (Gluckman et al. 1987). McGuire et al. (1992) reported that IGF-1 has been detected in all biological body fluids that were examined in several species and that IGF-1 was involved in many biological processes including lactation, immune function, reproduction and postnatal growth. IGF-1 may mediate nutritionally dependent growth via the actions of growth hormone (Gluckman et al. 1987, Straus 1994). Further indicating that IGF-1 may play a key role in growth regulation are the structural and evolutionary similarities that exist between the polypeptides of insulin, proinsulin, and the insulin-like growth factors (Breier and Gluckman 1991, DeMeyts et al. 1994).

Because IGF-1 is produced both locally and in the liver, pinpointing exactly how it is involved in metabolism and growth has been difficult. At least 4 different insulin-like growth factor binding proteins (IGFBPs) have been identified and are likely involved in mediating the actions of IGF-1. The presence of binding proteins associated with peptide hormones is unique in the case of the IGFs (Gluckman et al. 1987). Researchers previously thought the binding proteins attached to the IGFs served as storage mechanisms (Gluckman et al. 1987).

Recent research suggests, however, that the IGFBPs may be controlling the actions of the IGFs (Kelley et al. 1996). A preliminary analysis of IGFBPs in elk sera was conducted and 4 different IGFBPs were observed. The relative abundance of the 4 IGFBPs was similar among neonates, calves, and adult elk except for IGFBP-5 which appeared not to be present in the sera of cows (Appendix B).

Straus (1994) reported that IGF-1 was a primary indicator of nutritional status in ruminants. Other researchers also have shown that concentrations of IGF-1 in the plasma decline under conditions of energy and/or protein restriction (Gluckman et al. 1987, Breier et al. 1988, McGuire et al. 1992, Straus 1994, and Dauncey et al. 1994). Ronge and Blum (1989) reported that restricting feed intake substantially decreased plasma concentrations of IGF-1. Breier et al. (1986) also studied the effects of plane of nutrition on IGF-1 level in steers using 3 different levels of nutrition (high, medium, and low). IGF-1 levels in steers on high feed intakes were similar to those receiving medium feed intakes, but steers receiving lower feed intakes showed significantly lower IGF-1 levels. Further research by Breier et al. (1988) again showed differences in IGF-1 between high and low levels of nutrition in steers. Webster et al. (1996) reported higher IGF-1 levels in the fed state compared to the fasted state during spring and winter in male red deer.

Effects of the thermal environment on IGF-1 levels have recently been examined in domestic ruminants. In a temperature-controlled cold environment (-17°C), plasma samples from holstein heifers showed elevated levels of insulin, glucagon, glucose, T_3, and T_4, but not GH and IGF-1 when compared to control animals (20°C) (Scott and Christopherson 1993). They attributed their findings to alterations in endocrine pathways that direct substrate production for thermogenic needs rather than for growth. Van Kessel and

Laarveld (1994) reported significantly higher serum IGF-1 levels in a group of 25-day-old lambs reared in warmer temperatures compared to cold temperatures.

Hannon et al. (1991) measured IGF-1 concentrations in liver and muscle tissue of bulls, steers, and heifers receiving an ad libitum diet prior to slaughter. They observed higher IGF-1 levels in bulls, intermediate levels in steers, and the lowest levels in heifers. Similarly, in neonatal lambs, females had significantly lower levels of IGF-1 at 4 days of age when compared to males (Scott and Christopherson 1993). Seasonal influences were suggested in data reported by Ringberg et al. (1978) between summer and winter IGF-1 levels in free-ranging semi-domesticated Norwegian reindeer (*Rangifer tarandus tarandus*). Webster et al. (1996) also examined effects of season on concentrations of IGF-1 for male red deer in New Zealand and they reported higher IGF-1 levels in spring compared to winter.

Levels of IGF-1 in red deer ranged from 221 to 651 ng/ml (Webster et al. 1996) and from 75 to 190 µg/l (Suttie et al. 1989). In domestic ruminants, Breier et al. (1986) reported IGF-1 levels in young steers ranging from 30 to 70 µg/l and McGuire et al. (1992) reported levels in mid-lactation cows from 75 to 250 ng/ml.

Phosphorus

Phosphorus (inorganic) is a mineral primarily found in bones and teeth. It plays essential metabolic roles in converting glucose into glycogen (Thomas 1993) and in the synthesis of nucleic acids (Mathews and van Holde 1990). DelGiudice et al. (1987*a*) found elevated levels of serum phosphorus in fasted deer and attributed this to a state of hyperparathyroidism related to prolonged starvation and

catabolism of muscle tissues. Wolfe et al. (1982) reported lower serum phosphorus levels in captive elk (4.4 mg/dl) compared to free-ranging elk (5.1 mg/dl) they assumed to be in poorer condition. In caribou (Messier et al. 1987) and reindeer (Soveri et al. 1992) no apparent seasonal patterns were observed. In contrast, Seal et al. (1972) reported significant seasonal variation in white-tailed deer. In mule deer, levels of serum phosphorus ranged from 5.7 to 8.4 mg/dl (DelGiudice et al. 1990*b*) and in white-tailed deer levels ranged from 9.8 to 24.5 mg/dl for adults and fawns.

In the wildlife literature, urinary phosphorus levels typically are expressed as ratios of phosphorus:creatinine x 1000. In terms of nutritional influences, DelGiudice et al. (1987*b*) reported a significant increase of urinary phosphorus ratios in white-tailed deer fasted for 24 days. DelGiudice et al. (1991*a*) reported urinary phosphorus ratios ranging from 9.7 to 30.1 in free-ranging elk and from 17 to 34 in free-ranging mule deer (DelGiudice et al. 1990*a*).

Potassium

The mineral potassium serves as the primary cation in intracellular fluids. Potassium, along with sodium and chloride, are important in the regulation of acid-base balance of blood and osmotic pressure (Thomas 1993). Excitability of muscle tissue and nerve impulses requires a proper balance of magnesium, calcium, and potassium (Thomas 1993). Hyperkalemia (highly elevated potassium levels) may occur during metabolic acidosis and may lead to paralysis and death (Thomas 1993).

During ketoacidosis and prolonged starvation, the acid-base balance of the blood is altered by increased ketone body formation. Sodium, and eventually potassium are released from intracellular proteins and are utilized to buffer the

increasing acid load (Newsholme and Leech 1986) in the blood. This leads to a depletion of potassium in the body; and this state is not readily reversed. Even after a return to normal dietary intake, supplementation of potassium ions may be required (Newsholme and Leech 1986). Depletion of potassium occurs when potassium ions and protons are excreted in the kidneys in exchange for uptake of sodium ions. This process is hormonally controlled by aldosterone (Newsholme and Leech 1986).

Potassium is also important for the hydrolysis of ATP by active transport across plasma membranes (Mathews and van Holde 1990). The sodium-potassium pump maintains a higher level of sodium outside the cell and a higher level of potassium inside the cell and consists of alpha and beta subunits, of which the alpha subunits are enzymatic and actually hydrolyze ATP thereby releasing free energy to drive transport processes (Mathews and van Holde 1990). Without the sodium-potassium pump maintaining this concentration gradient, sodium and potassium levels would come to equilibrium and the cell would no longer be able to function.

Higher levels of serum potassium were observed in captive elk (8.1 mEq/l) assumed to be in better condition than in free-ranging elk (4.6 mEq/l) (Wolfe et al. 1982). Higher levels of serum potassium in the summer compared to winter were reported in black-tailed deer (Parker et al. 1993), white-tailed deer (DeLiberto et al. 1989), and caribou (Soveri et al. 1992). In contrast, DelGiudice et al. (1990*b*) found higher levels in winter than in summer with white-tailed deer. Reported levels for cervids ranged from 4.5 to 7.0 mEq/l in mule deer (DelGiudice et al. 1990*a*) and from 7.1 to 11.2 mEq/l in free-ranging white-tailed deer (DeLiberto et al. 1989).

Urinary potassium levels in the wildlife literature are commonly expressed as the ratio of potassium:creatinine. Urinary potassium excretion may be elevated during periods of

starvation to counter-balance a decline in the pH of the blood. However, research with white-tailed deer revealed that fasting significantly lowered urinary potassium ratios but level of dietary protein had no effect (DelGiudice et al. 1987*b*). DelGiudice et al. (1991*b*) reported ratios of urinary potassium in free-ranging elk at 334 versus 423 in captive elk. Ratios in captive white-tailed deer ranged from 24.4 to 133.9 in the fasted and re-fed states respectively (DelGiudice et al. 1990*a*).

Sodium

Sodium is found in body fluids, blood and body tissues and has important functions similar to those of potassium in maintaining cell integrity and in bioelectric activity (Thomas 1993). These functions require that sodium levels in the blood be relatively homeostatic. Consequently, serum levels of sodium were not sensitive to nutrition in white-tailed deer (Kie et al. 1983, DelGiudice et al. 1987*a*, DelGiudice et al. 1990*b*), and reindeer (Soveri et al. 1992). Seasonal variations in serum sodium levels were reported for white-tailed deer (Kie et al. 1983, DeLiberto et al. 1989). Levels of serum sodium ranged from 70 to 220 mmol/l in red deer (Knox et al. 1988) and from 146 to 162 mmol/l in reindeer calves (Soveri et al. 1992). In white-tailed deer levels ranged from 137 to 143 mEq/l (DelGiudice et al. 1987*a*).

Urinary sodium levels are generally expressed as a ratio of urinary sodium:creatinine in the wildlife literature. Urinary sodium ratios ranged from 0.1 to 8.6 (DelGiudice et al. 1991*a*) for elk in Yellowstone National Park. Results from DelGiudice et al. (1987*b*) and DelGiudice et al. (1990*b*) indicated that urinary sodium, like urinary potassium, declined significantly during fasting but levels of dietary protein had no effect.

Serum Protein

Proteins found in the serum are generally weak acids that increase the buffer capacity of the blood (Thomas, 1993). For example, fatty acids must form complexes with albumin in the blood to allow transportation of the fatty acid in a non-toxic manner (Thomas 1993). In white-tailed deer (DelGiudice et al. 1987*a*) and caribou (Bahnak et al. 1979), significant differences in total serum protein levels appeared to be related to nutrition. Wolfe et al. (1982) also reported significantly higher serum protein levels in free-ranging elk in poorer condition compared to captive elk in good condition. Kie et al. (1983) reported no differences in total serum protein levels based on age, sex, condition or month of study. Several studies indicated higher levels of total serum protein in late fall and winter and lower levels in spring (DelGiudice et al. 1992, Soveri et al. 1992, Messier et al. 1987). Wolfe et al. (1982) reported total serum protein levels of 6.6 to 7.2 g/dl elk. Levels in female caribou ranged from 6.8 to 7.4 g/dl (Messier et al. 1987) and in caribou calves from 5.4 to 6.7 g/dl (Soveri et al. 1992). Levels in free-ranging white-tailed deer reported by DelGiudice et al. (1992) were similar (6.0 to 7.3 g/dl).

Triglycerides

The term triglyceride is synonymous with triacylglycerol and can be described as fatty acids bonded to glycerol (Thomas 1993). Triglycerides are carried in the blood as lipoproteins when they are bound to serum albumin (Mathews and van Holde 1990) and they comprise the major portion of lipid carried in

the blood (Thomas 1993). They are oxidized in order to create ATP thereby providing energy within the cell (Newsholme and Leech 1986).

In terms of nutrition effects, DelGiudice et al. (1987*a*) and DelGiudice et al. (1990*b*) reported increased triglyceride levels during fasting in white-tailed deer. Neither Kie et al. (1983) or Seal et al. (1972) reported nutrition effects in their studies with white-tailed deer, however. Likewise Messier et al (1987) concluded that triglyceride levels did not adequately reflect nutritional status in female caribou. White-tailed deer data of Kie et al. (1983) suggested higher levels in summer and lower levels observed in winter. Levels of triglycerides in free-ranging white-tailed deer ranged from 2.9 to 44.5 mg/dl (DelGiudice et al. 1992).

Triiodothyronine and Thyroxine

Triiodothyronine (T_3) and thyroxine (T_4) are thyroid hormones that play key roles in regulating energy metabolism (Newsholme and Leech 1986). T_3 is the active form of thyroid hormone created in peripheral tissues by deiodinating T_4 (Newsholme and Leech 1986). The rate of conversion of T_4 to T_3 is reduced during periods of starvation (Newsholme and Leech 1986). Metabolically, thyroid hormones are known to be involved in controlling degradation of muscle proteins as well as whole-animal energy expenditures (Newsholme and Leech 1986).

Significant nutritional influences on T_3 levels were reported for white-tailed deer by Watkins et al. (1982), Bahnak et al. (1981) Seal et al. (1978*b*) and in reindeer by Ryg and Jacobsen (1982). T_3 levels typically are greater in summer and lower in winter (Bahnak et al. 1981, Ryg and Jacobsen 1982, DeLiberto et al. 1989) suggesting seasonal effects. Reported

levels of T_3 ranged from 75 to 210 ng/dl in free-ranging white-tailed deer (DelGiudice et al. 1992), and from 70 to 135 ng/dl in free-ranging mule deer (DelGiudice et al. 1990*a*).

Fasting and low energy diets were associated with decreased levels of T_4 in white-tailed deer (Watkins et al. 1982, Bahnak et al. 1981, DelGiudice et al. 1987*a*). Seasonal changes in levels of T_3 were observed in studies with reindeer (Ryg and Jacobsen 1982) and white-tailed deer (Bahnak et al. 1981). Levels of T_4 ranged from 7.0 to 19.5 µg/dl in free-ranging white-tailed deer (DelGiudice et al. 1992).

Urea Nitrogen

Urea nitrogen is the nitrogen found in the blood and urine associated with urea rather than nitrogen associated with blood proteins or urinary proteins (Thomas 1993). Urea is formed during metabolic utilization of amino acids and provides a non-toxic form for excess nitrogen transport through the body and the kidneys (Thomas 1993) remove it. Serum urea nitrogen levels are indicative of protein consumption and protein metabolism in the body. Serum levels of urea nitrogen can be elevated by increased intake of protein in the diet, increased urea recycling (in ruminants) and by catabolism of muscle tissue.

Of the blood and urinary variables assessed in the wildlife literature, serum urea nitrogen (SUN) and urinary urea nitrogen have received the most attention. Many studies reported significant declines in SUN associated with restricted diets and in particular with reduced protein intake. Warren et al. (1981) found significantly lower SUN in nutritionally restricted white-tailed deer compared to well-fed deer. Further research by Warren et al. (1982) also indicated elevated SUN levels in fawns subjected to either low energy or

high protein diets. Kirkpatrick et al. (1975) reported significant declines in SUN associated with a low protein diet but not a low energy diet. Kie et al. (1983) reported an inverse relation between SUN levels and body mass or body fat reserves in white-tailed deer.

DelGiudice et al. (1994) found no difference between SUN levels in white-tailed deer fed a low protein, low energy diet and control animals despite mass losses of 26% (LPLE group) and 14% (control group) respectively. Wolkers et al. (1994) reported significant declines in SUN levels for feed-restricted red deer but only during the first 12 weeks of the study. In a white-tailed deer study, Bahnak et al. (1979) reported that variation observed in SUN levels were mostly related to protein content of the diet. Wolfe et al. (1982) reported SUN levels for captive elk at 20.4 mg/dl and 16.4 mg/dl in free-ranging elk. In free-ranging mule deer, SUN levels ranged from 21.7 to 35.1 mg/dl (DelGiudice et al. 1990*a*). Similarly, in white-tailed deer SUN ranged from 14 to 40 mg/dl (Bahnak et al. 1979).

Urinary urea nitrogen levels are generally expressed as a ratio of urinary urea nitrogen:creatinine (UUN:cr) in wildlife literature. Typically the ratio of urinary urea nitrogen initially declines when energy or protein is sufficiently restricted in the diet. Ruminant metabolism then increases recycling of urea to help meet protein needs in the body. However, with prolonged restriction of energy or protein, levels begin to increase as the body catabolizes tissue protein. This leads to a "U" shaped pattern of UUN:Cr ratios over time when catabolism is occurring.

In terms of nutritional influences, DelGiudice et al. (1995*a*) reported significantly lower UUN:Cr levels in white-tailed deer on low protein diets lasting 5 days. DelGiudice et al. (1990*a*) also reported significant declines in UUN:Cr levels for deer on a low plane of nutrition for 12 weeks. Severe

nutritional restriction in white-tailed deer and a body mass loss of 26% resulted in an elevated UUN:cr ratio of 33.4 compared to the control group that lost only 14% of their body mass and had a UUN:cr ratio of 6.3 (DelGiudice et al (1994). Warren et al. (1982) and Parker et al. (1993) however suggest that levels are related to immediate dietary protein intake, fat depletion and tissue catabolism, energy intake and urea recycling and may not accurately reflect body condition. In fact, research by Parker et al. (1993) indicated that UUN:Cr levels did not correspond in a consistent manner to whole body fat content in free-ranging black-tailed deer. No effects of age or season were reported for this index. Ratios of UUN:cr in free-ranging elk ranged from 0.9 to 2.2 (DelGiudice et al. 1991*a*) and in white-tailed deer fawns from 2.6 to 18.7 (Warren et al. 1982).

Urinary Cortisol

Cortisol is a glucocorticoid hormone produced by the adrenal tissues (Hadley 1996). Glucocorticoids are primarily responsible for suppressing inflammation and promoting gluconeogenesis (Mathews and van Holde 1990). Urinary cortisol is typically expressed as ratio of urinary cortisol:creatinine in wildlife literature. Saltz et al. (1991, 1992) reported that urinary cortisol:creatinine ratios were elevated in mule deer maintained at high densities compared to low densities. Parker et al. (1993) reported that higher urinary cortisol levels in black-tailed deer fawns were correlated with decreased fat stores. However in adult black-tailed deer, there was no noticeable correlation between fat stores and urinary cortisol:creatinine ratios.

Validation of an IGF-1 Radioimmunoassay and Response of IGF-1 Levels to Nutritional Restriction in Elk Calves

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ABSTRACT

Insulin-like growth factor-1 (IGF-1) shows promise as a nutritional indicator for wild ruminants. IGF-1 radioimmunoassay systems (RIA) have been used with bovine and ovine serum but none have previously been validated for use specifically with elk serum. Our goal was to validate a RIA system for measuring IGF-1 in elk using elk calf serum collected during nutrition trials in the fall of 1993. Serum samples were obtained at the end of each of 3 consecutive 18-day trials involving 2 groups of calves, one receiving feed ad libitum (HIGH) and the other receiving about 50% of the intake of the ad libitum group (LOW). Validation procedures were conducted on the RIA system prior to assaying these samples. Parallelism was achieved with the use of an acid-ethanol extraction procedure to remove IGF binding proteins prior to assay. A second antibody precipitation method was also used to separate free ^{125}I-labelled IGF-1 from antibody bound ^{125}I-labelled IGF-1. Inter- and intra-assay coefficients of variation were 11.9% and 3.9%. Recovery of added IGF-1 (0.25 ng/ml) measured over sixteen assays was quantitative (99%). Using a 2-factor ANOVA, IGF-1 levels in the HIGH calf group were significantly greater (P = 0.0001) compared to the calves in the LOW group. Also, in the HIGH group, levels of IGF-1 declined gradually over the 3 trials suggesting seasonal influences on IGF-1 levels. This decline also was accompanied by declining voluntary feed intake (HIGH group). Despite the gradual decline, however, there was no significant interaction between IGF-1 levels and trial sequence (P = 0.032). These preliminary results indicate that IGF-1 has considerable potential for being a useful indicator of nutrition in young elk calves. Further assessment of IGF-1 will be necessary to establish reference levels for IGF-1 levels in adult elk and to

examine how factors such as age, season, nutrition, and condition might influence those IGF-1 levels in adults.

INTRODUCTION

Insulin-like growth factor-1 (IGF-1) is a peptide hormone produced at multiple sites in the body (Isaksson et al. 1987) that mediates nutritionally-dependent growth (Gluckman et al. 1987, Straus 1994) through proliferation and differentiation of cells via the actions of growth hormone (Barnard et al. 1988). IGF-1 is found in all biological fluids within the body and is generally bound to IGF binding proteins in the blood (McGuire et al. 1992). Studies have shown that concentrations of IGF-1 in the blood of domestic ruminants decrease under conditions of short-term dietary protein/and or energy restrictions (Breier et al. 1986, Ronge and Blum 1989, Straus 1994). Thus, IGF-1 may have value as an indirect index of nutrition in wild ruminants but such potential has not yet been examined.

Because IGF-1 has not previously been measured in elk serum it was necessary to develop an appropriate laboratory assay to measure IGF-1 prior to widespread application for this species. An existing RIA protocol for livestock from Dr. Leon Spicer of Oklahoma State University was modified and validated prior to running actual samples. This modified RIA system was then used to measure IGF-1 levels in elk calves subjected to 2 different nutrition regimes to provide a preliminary assessment of IGF-1 response to nutrition in elk.

MATERIALS AND METHODS

Radioimmunoassay Protocol

Separation of serum binding proteins from IGF-1 in raw sera was accomplished using an established acid-ethanol procedure (Spicer 1988) to prevent interference in the assay. Aliquots of serum were diluted 1:4 with acid ethanol (12.5% of 2 N HCl:87.5% of Ethanol) in 12 x 75 mm glass tubes and vortexed for 30 seconds, then incubated for > 16 h at 4°C. Test tubes were then centrifuged at 2800 RPM for 30 minutes at (4°C), and immediately thereafter the supernatant was poured off into another tube and neutralized with 50 µl of 0.855 M Tris base. The supernatant fraction of the raw sera extracts contained the total free IGF-1 to be measured and was stored at 4°C until use. Quality control tubes of 1, 3, 6, and 8 µl of pooled sera extract were run in several assays to determine parallelism.

The assay buffer solution consisted of sodium azide (0.2 g/L), EDTA (3.362 g/L), protamine sulfate (Grade I/0.2 g/L), bovine serum albumin (RIA grade/2.5 g/L), in 1 L of ddH20, with pH of 7.5. The assay buffer was used to make working dilutions of the first and second antibodies, radioiodinated hormone, and unlabelled hormone. Anti-IGF-1/somatomedin C rabbit antiserum (UB2-495) provided by the National Hormone and Pituitary Program (NHPP), Baltimore, MD, USA, was used as the first antibody in the assay. The NHPP reported that the antiserum (UB2-495) exhibited 1.5-1.9% cross-reactivity with IGF-2. The first antibody was used at a working dilution of 1:1600 in the assay resulting in a final working dilution of 1:16,000 in each test tube.

Labeled IGF-1 (3-[^{125}I]*iodotyrosol* insulin-like growth factor-1; product no. IM-172, Amersham Corporation, Arlington Heights, IL, USA), was used in the assay system. Standards

were made from recombinant human IGF-1 (catalog no. 291-G1, R & D Systems, Minneapolis, MN, USA) rather than bovine IGF-1. Human and bovine IGF-1 are identical in structure. The second antibody consisted of anti-rabbit IgG (product no. R-0881, Sigma Chemicals, St. Louis, MO, USA) specifically made for RIA second antibody precipitation procedures to separate bound ^{125}I-IGF-1 from free ^{125}I-IGF-1.

Samples were assayed in duplicate at 4°C in 12 x 75-mm polystyrene test tubes. An existing RIA procedure for IGF-1 (Echterncamp et al. 1990) involving a second antibody precipitation method was slightly modified to achieve more accurate results with elk serum. Protocol for the modified assay was as follows: standards (0.5, 0.25, 0.125, 0.063, 0.031 and 0.015 ng/100 µl buffer solution) and unknowns (3 µl raw sera extract) were combined with 50 µl anti-IGF-1 (first antibody) in sufficient assay buffer to make the total volume in each tube 450 µl. All tubes were then vortexed and preincubated for 1 hour at room temperature. Fifty µl of 10,000 CPM labeled IGF-1 were added to each tube prior to vortexing and incubated at 4 °C overnight (12-24 h). Then 200 µl of second antibody (anti-rabbit IgG at a 1:15 dilution in assay buffer) was added prior to vortexing again and incubation continued at 4 °C for 2 h. Finally, tubes were centrifuged at 2500 RPM for 20 minutes, decanted, and the remaining precipitated radioactivity was counted using an automated gamma counter. With this procedure it was the ^{125}I-IGF-1 which is antibody bound that was counted.

Study Area

The elk calf nutrition trials were conducted near La Grande, Oregon at a 25-ha study site. This site contained 12

pens that were 0.03-ha in size and each pen had an attached barn with 3 stalls to permit individualized feeding and weighing. Pens were systematically arranged about 300 m apart throughout the study area. No forage existed within the pens during the nutrition trials. Cook et al. (1996) provided a detailed site description.

Nutrition Trials

Elk calves 4-6 months-of-age were used in 3 consecutive 18-day nutrition trials in the autumn of 1993. All calves had been captured in the wild and hand-reared prior to their use in the nutrition trials. In each trial, calves were randomly assigned to a high quality diet (HIGH) fed ad libitum and to a daily ration of 50% of the ad libitum group (LOW) (n = 6 in both treatments). Randomization was constrained so that calves were not reassigned to the same feeding levels in subsequent trials. At the end of each trial, serum samples were obtained from all calves in the HIGH and LOW groups. This study comprised a portion of a larger nutrition study described in detail by Cook et al. (1996).

Trial #1 was conducted from 17 August to 3 September 1993. Solid food (grain and pellets) and milk were fed in this trial. Calves were weaned at the end of this trial and given 2.5 weeks to adjust to solid food only before beginning trial #2. In trial #2, conducted from 21 September to 8 October and in trial #3, conducted from 12 October to 29 October, calves were fed pellets and hay only (Table 2.1). To prevent rumenitis, alfalfa hay was fed at 40% of the total solid food offered to the calves.

Calves were lightly sedated with xylazine hydrochloride (1 mg/kg administered intra-muscularly) immediately prior to blood collection. Immobilization was reversed with yohimbine

hydrochloride after blood collection (0.3 mg/kg administered intravenously). Individual blood samples were collected via jugular venipuncture and allowed to clot for 30-45 minutes prior to centrifuging. Serum was removed with pipettes and aliquoted into 1-ml storage vials and frozen at -20 C until preparation for RIA at Oregon State University.

Table 2.1: Feed ingredients, protein and energy content for solid food used in the calf nutrition trials.

Food[a]	DE (kcal/g)	CP (%)	Primary Ingredients
Grain	3.86	11.5	Rolled wheat, oats, barley, corn
Pellet-1 meal	3.23	16.9	Oats, wheat, alfalfa hay, soybean
Pellet-2	3.34	18.7	Barley, ryegrass screenings, soybean meal
Hay	2.55	16.0	Alfalfa

[a] Grain and pellet-1 rations were mixed 1:1 and fed through the end of Trial #2. Pellet-2 was fed in Trial #3. Hay was fed at 40% of total solid food offered to all calves.

Assay Validation

Estimates of assay precision and reproducibility (inter and intra-assay coefficients of variation) were calculated. The calculation of the intra-assay coefficient of variation included data obtained by measuring a single serum pool 10 times in 1 assay. The inter-assay coefficient of variation included data obtained from the same serum samples assayed in 16 assays over 7 months. Estimates of recovery were obtained by adding known amounts of added IGF-1 (0.25-1.0 ng/ml) to samples in each assay and then comparing quantities recovered versus known quantities added.

Data Analysis

Data from the calf nutrition trials were analyzed using a 2-factor ANOVA (SAS, 1988). Factors included both diet (HIGH or LOW) and trial sequence (trial 1, 2, or 3). Significant differences in the analysis were determined by examining both the main effects and the interaction effect in the ANOVA. Significant differences among trials were identified using the Waller-Duncan multiple comparison procedure (SAS, 1988). $P \leq 0.05$ was accepted as significant for all tests.

RESULTS

Inhibition curves from pooled sera extracts were parallel to the inhibition curve of the standard curve thus parallelism was achieved (Figure 2.1). The intra-assay coefficient of variation was 3.9% and the inter-assay coefficient of variation was 11.9%. Recovery of exogenous IGF-1 was quantitative (99% for 0.25 ng/ml) and correlation analysis revealed a correlation coefficient of 0.89 for the quantities added versus those recovered. Linear regression of known quantities of IGF-1 added versus those recovered revealed a y-intercept of 0.04 and a slope of 0.78 (Figure 2.2).

Intake and growth rates for the elk calves in the LOW nutrition group were lower than the HIGH nutrition group (Table 2.2). Serum concentrations of IGF-1 (Figure 2.3) reflected these differences in nutrition in all 3 trials. Significant differences were observed for the main effects, diet ($P = 0.0001$) and trial number ($P = 0.0001$), in the ANOVA. However the interaction effect between diet and trial was not significant ($P = 0.32$) in the model. The Waller-Duncan multiple comparison procedure identified trial #3 as having

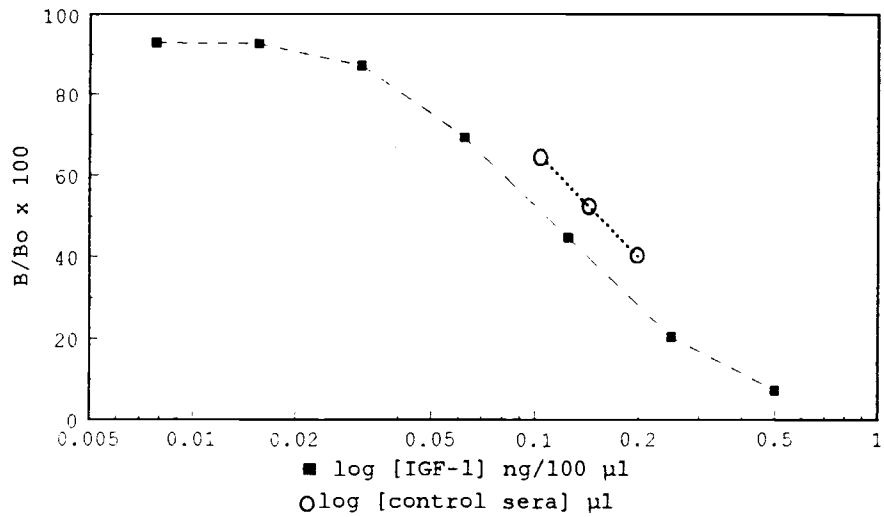

Figure 2.1: Standard inhibition curve in the IGF-1 RIA and inhibition by acid-ethanol sera extracts in the assay showing parallelism.

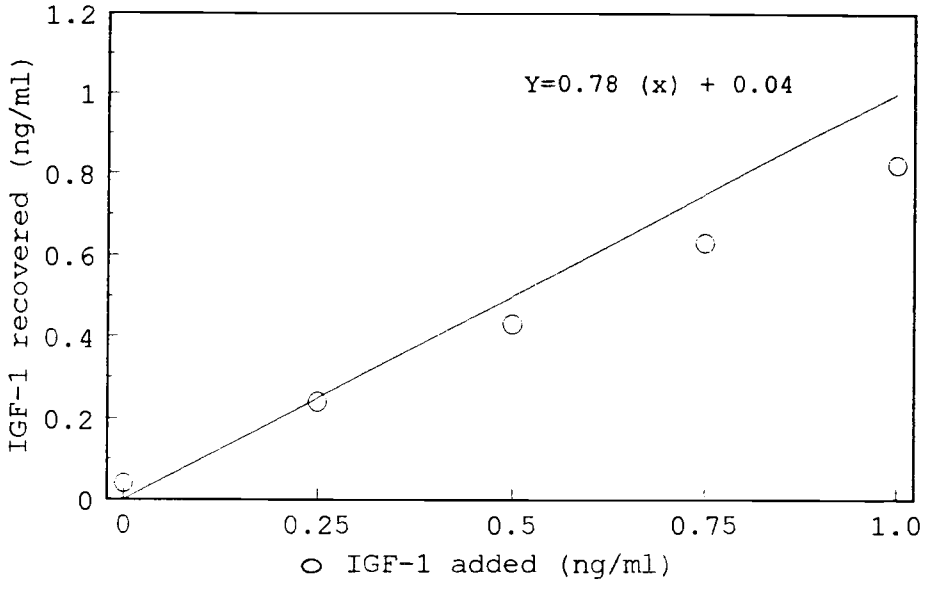

Figure 2.2: Regression model of IGF-1 assay recovery. Adjusted *R*-squared = 0.81.

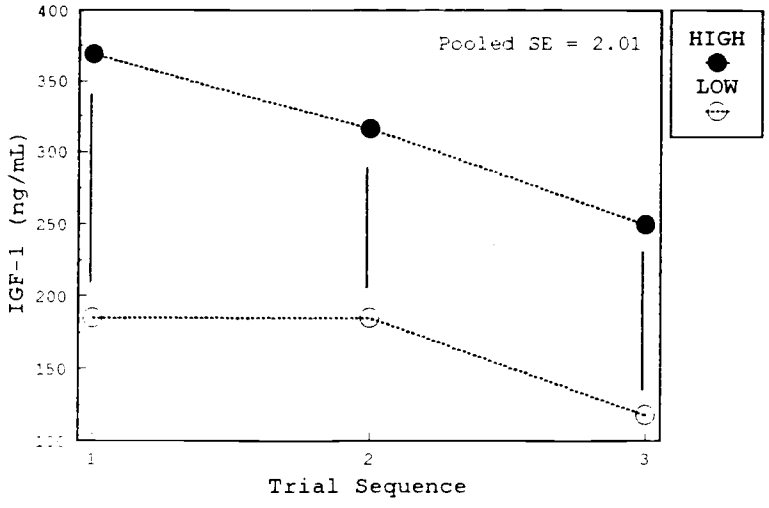

Figure 2.3: IGF-1 levels of calves in the HIGH versus LOW treatment groups. The 18-day nutrition trials were conducted between 17 Aug-29 October 1993 with captively reared elk calves. All means were significantly different (P = 0.0001) (as denoted by the bar between the means).

Table 2.2: Growth and feed intakes in calf nutrition trials. The HIGH groups were fed ad libitum and the LOW groups were fed a restricted diet during 3 nutrition trials conducted between 17-August and 30-October, 1993.

			Intake[a]	
	Group	Growth[b]	CP	DE
Trial	#1			
	High	1.53	19.7	360.1
	Low	0.48	10.5	195.2
Trial	#2			
	High	0.70	15.0	282.7
	Low	-0.14	7.5	142.9
Trial	#3			
	High	0.55	13.0	262.2
	Low	0.06	9.4	146.3

[a]Intake of crude protein (CP) (g/kg $BM^{0.75}$) and digestible energy (DE)(kcal/kg $BM^{0.75}$/day) on a dry matter basis.
[b]Growth is percent daily mass change [(mass change in kg during trial/mass in kg at the beginning of the trial)/number of days in trial * 100]

Table 2.3: Results from the Waller-Duncan multiple comparison procedure.

Waller Grouping	Mean	*n*	Trial	Dates
A	277.02	12	1	(17 Aug-3 Sept)
A	250.10	12	2	(21 Sept-8 Oct)
B	183.97	12	3	(12 Oct-29 Oct)

*Marginal means with the same letter were not significantly different
n = sample size

significantly different median levels of IGF-1 (Table 2.3). This 2-factor ANOVA model accounted for 81% of the variation in IGF-1 levels.

DISCUSSION

Previous research has shown that IGF binding proteins must be removed or treated so they cannot interfere in the RIA (Holland et al. 1988, Bang 1995). Parallelism existed in this assay system between the pooled sera extracts and the standard curve after removal of the binding proteins via acid-ethanol extraction. Recovery rates of IGF-1 in the assay system were acceptable according to the RIA laboratory techniques manual published by Chard (1987). Inter- and intra-assay coefficients of variation were within acceptable ranges for this assay as well (Chard 1987). These results indicated that the modified RIA used in this experiment produced a reasonably accurate, precise, and repeatable RIA for measuring IGF-1 in elk sera.

The overall decline in mean IGF-1 levels observed in both groups during the 3 trials suggests photoperiod effects on IGF-1 levels. Photoperiod effects on IGF-1 levels also have been reported for male red deer (Webster et al. 1996) and reindeer (Ringberg et at. 1978). The decline in IGF-1 levels probably was unrelated to the change from a milk-dominated diet to a solid food diet otherwise the decline in IGF-1 should have occurred between the 1st and 2nd trial rather than the 2nd and 3rd. Also, there was a continual decline in voluntary food intake (VFI) in the high group over the 3 trials suggesting seasonal influences on feed intake as well. Research with red deer has shown that hypophagia during late fall and early winter occurs in response to decreasing daylength (Loudon et

al. 1994). This is thought to be an evolutionary strategy for winter survival when forage quality and quantity becoming limiting in northern habitats (Loudon 1994).

Restricting nutrition (by about 50%) apparently reduced IGF-1 levels to a similar extent (42-53% lower) in the low nutrition group. These substantial declines in IGF-1 levels indicated that IGF-1 levels were sensitive to nutrition in these trials. Although photoperiod also appeared to influence IGF-1 levels, the effect did not appreciably interfere with the ability of IGF-1 levels to index changes in nutrition.

MANAGEMENT IMPLICATIONS

Based on these preliminary findings, IGF-1 appears to have considerable potential as an indirect measure of nutritional status in elk. However, inference in this study is limited to elk calves less than 8 months-of-age. Further research of longer duration and with elk of differing ages will be necessary to properly assess the response of IGF-1 to factors such as age, body condition, seasonally changing nutrition, and changes in daylength.

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Serum and Urine Chemistry Reference Values for Elk and Influences of Age and Season

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ABSTRACT

Serum and urine chemistry reference values were established for young elk (*Cervus elaphus nelsoni*), 3.5 months to 4 years of age in good to excellent condition for each season of the year. Elk were weighed twice weekly, food intake was measured daily, and blood and urine samples were collected monthly from September 1993 to June 1995. Treatment groups included cow elk held in good to excellent condition year-round (HICON), cow elk held in a reduced body condition year-round (LOCON) and calf elk held in good to excellent condition year-round (JUV). Body mass (BM) gains during the study were 27%, 4.5%, and 270% in the HICON, LOCON and JUV groups. Changing daylength was correlated to circannual rhythms observed in 8 serum variables: total bilirubin (TB), total protein (TP), alanine aminotransferase (ALT), alkaline phosphatase (ALK), triglycerides, thyroxine (T_4), insulin-like growth factor-1 (IGF-1) and serum creatinine. Seasonal changes in voluntary food intake (VFI) and BM dynamics were also observed. Significant age effects were observed for 17 serum variables and 1 urinary variable: ALK, serum creatinine, calcium, ALT, phosphorus, triiodothyronine (T_3), T_3/T_4, cholesterol, aspartate aminotransferase (AST), serum urea nitrogen (SUN), TP, triglycerides, IGF-1, chloride, gamma glutamylaminotransferase (GGT), serum albumin, TB and the urinary potassium:creatinine ratio (UK:Cr). These results indicate that the use of serum and urine chemistry as indices of nutritional status or condition must account for influences of age and season. The serum and urine chemistry reference values presented, combined with an assessment of age and seasonal influences, provide a useful guide for biologists desiring to utilize serum and urine chemistry indices in elk.

INTRODUCTION

Considerable research has assessed the value of blood and urine chemistry as indirect measures of body condition, nutrition, and indirectly, habitat quality. Results from several studies suggest that physiological indices are useful (Warren et al. 1982, Kie et al. 1983, DelGiudice et al. 1987*a,b*, DelGiudice et al. 1991, Saltz and White 1991) but warrant more investigation. Interpretation of herd nutritional status and physiological condition using blood and urine chemistry indicators requires baseline data collected from animals in which variables such as nutritional status and condition are documented (DelGiudice et al. 1990*b*). In addition, factors such as age, gender, and season have considerable potential to influence serum and urine chemistry. These factors may confound interpretation of serum and urine chemistry data, particularly for management applications.

Serum and urine studies using elk are markedly rare. The few studies that have been conducted are insufficient to establish reliable reference values for various seasons, life stages and age groups. Studies by Follis (1972), Weber et al. (1972), Herin (1968), Wolfe et al. (1982), Vaughn et al. (1973), Pedersen and Pedersen (1975), DelGiudice et al. (1991*a,b*), Garrott et al. (1996, 1997), and Vagnoni et al. (1996) constitute virtually the entire published record of serum and urine data for elk. Data from several of these studies tend to be outdated due to advances in laboratory procedures, and most were conducted opportunistically using free-ranging elk, thereby precluding controlled assessment and documentation of nutrition, condition, age, gender, and seasonal influences. Considerably more data need to be collected on elk serum and urine chemistry before it will be possible to use these indices routinely to assess nutritional status and condition of free-ranging elk.

The primary goals of this research were to: (1) develop year-round reference values for serum and urinary chemistry variables in young, non-reproducing female elk in good-excellent condition, and (2) assess the influences of age and season on serum/urine variables, VFI and BM dynamics. The study was conducted over a 22 month period using 3.5 month-old calves and 2.5 year-old cows (age at beginning of the study) fed to maintain good-excellent body condition and a third group of 2.5 year-old cows held in reduced condition.

Serum and urine variables in this assessment included a number of routinely-measured variables of diagnostic value in veterinary medicine, of value as indicators of nutrition or condition, and a variable (IGF-1) that has not yet been included in any work on North American cervids. IGF-1 was included because studies with domestic livestock and red deer have shown that IGF-1 levels decline during periods of dietary energy and/or protein restriction (Breier et al. 1986, Gluckman et al. 1987, Suttie et al. 1989, McGuire et al. 1992, Straus 1994, Webster et al. 1996). Also, based on information presented for red deer (*Cervus elaphus*) regarding VFI and BM dynamics in relation to photoperiod effects (Loudon 1994), we have included a similar assessment of those parameters in elk as well.

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Research protocol for the captive elk used in this study were in accordance with acceptable animal welfare protocol (Wisdom et al. 1993).

STUDY AREA

The study area was 30 km west of La Grande in the Blue Mountains of northeastern Oregon. It was located on elk summer range at 1325 m in elevation on a gentle (10-20%) northeast-facing aspect in the grand fir (*Abies grandis*) vegetative zone. Annual precipitation averages about 87 cm/year, with the majority falling during winter and spring. Average minimum and maximum January temperatures for this area are -5.8 and 0.1°C whereas minimum and maximum July temperatures are 11.0 and 24.7°C, based on climatological data collected 8 km from the study area at a similar elevation (NOAA 1966-78). These conditions represent typical climatological conditions during spring through autumn, and colder, wetter conditions during winter, than typically encountered by free-ranging elk in the Blue Mountains Ecoregion.

This research was conducted in a rectangular 3-ha clearcut on the northeast side of the study area. On the south side of this clearcut, 4 square 0.10-ha pens were constructed side by side. Trees occurred immediately adjacent to the south side of the rectangular complex, providing partial shade to each pen during most of the day. Barns with stalls for each elk used in the study were built adjacent to each pen and individualized feeding of pellets, and blood and urine collection occurred within the stalls. Stall floors were grated and underlaid with a support structure to hold urine collection pans made of galvanized metal. These pans were sloped such that urine drained from under the elk, thereby minimizing fouling of urine samples by hair, dirt or feces.

The barns were connected to the pens with weighing chutes, which elk passed through each day when fed. Each of the 4 pens was designed to accommodate 6 elk per pen, or a total of 24 elk (18 were used in this portion of the study).

METHODS

Two cohorts of female elk were bottle-raised for both aspects of this study, the first during summer 1991 and the second in the summer 1993 [Cook et al. (1996) presented details of capture, rearing and training]. Of the 1991 cohort, 18 were randomly selected from a total of 27 in August of 1993, when the cows were 2 years old, and randomly distributed among 3 of the 4 treatments (pens). Of the 1993 cohort, 6 elk at 3.5 months of age were randomly selected from a total of 45 and assigned to the 4th treatment (pen) in August 1993.

General Handling

Elk were fed twice daily at 0800 and 1400 hours. Each morning elk were brought into the barns, fed a pre-measured amount of pelleted food, and were held in the barns up to 3 hours each day to eat the pellets. Uneaten pellets were then weighed to measure daily pellet consumption. Alfalfa hay was fed communally in the afternoon in hay mangers located in the pens. The ratio of hay to pellets and total feeding levels of elk fed at ad libitum levels were set such that elk usually consumed all hay offered, to discourage picking through the hay. A hay manger was provided for each elk such that dominant cows could not exclude subordinates.

Elk were weighed twice weekly as they entered the barn for the morning feeding. Portable electronic scales were placed

underneath a floating floor in each chute to obtain body mass estimates. Doors on the chute held each elk stationary until a reliable mass estimate was obtained.

Treatment Descriptions

This experiment began in early September 1993 and continued through early summer 1995. In the 5 months prior to the beginning of this study, all adult cows were fed a high quality diet that exceeded nutritional requirements for digestible energy (DE) and crude protein (CP) of livestock (NRC 1984), at ad libitum rates. Thus all adult elk were in similar condition (good to excellent) at the beginning of the study.

Two of 3 pens of adult cow elk and the calf pen were included in the portion of the study described here. The dietary treatments for the serum and urine chemistry study began on 11 September 1993. The 2 treatment groups of adult cows included here were elk held in excellent condition year-round (HICON), and a group held in reduced condition year-round (LOCON) (n = 6 elk/group). The LOCON elk were forced to lose 10-15% of their body mass early in the study and were held at 15% below the HICON group for the remainder of the study. The LOCON treatment was induced and maintained by altering both quality and quantity of food. The HICON group was fed a high energy pellet averaging 3.2 kcal of DE/g and good quality alfalfa hay averaging 2.6 kcal of DE/g at a 60:40 ratio (pellet:hay) offered ad libitum for the entire experiment. The LOCON group was fed a low energy pellet averaging 1.96 kcal of DE/g; the pellet:alfalfa hay ratio was again 60:40. Ingredients in the high and low energy pellets are listed in Table 3.1. The amount fed varied weekly or

Table 3.1. Ingredient percentages of pelleted commercial rations. The high energy ration was fed to the JUV and HICON elk ad libitum and the low energy ration was fed to the LOCON elk in a restricted manner during the entire study.

	Experimental diet[a]	
	High Energy	**Low Energy**
Ingredient		
Soybean meal	6.70	--
Feeding oats	41.69	--
Wheat,	29.52	--
Limestone flour, 38% Ca	0.99	1.19
Biophos 18%Ca, 21%P	0.29	0.26
Magnesium oxide 54%	0.04	0.04
Salt	0.29	0.21
Selenium 200 ppm, 90.8	0.05	0.05
Alfalfa hay, 18%	18.98	--
Vitamin A,D,E -no.2	0.04	0.04
PGG Trace Mineral-no.1	0.06	0.05
Pellet stik	1.35	0.25
Sodium bentonite	--	10.56
Mollasses, cane	--	2.92
Safflower meal	--	25.29
Feathermeal	--	4.91
Ryegrass screenings	--	54.23

[a] High energy=adequate protein and high energy and Low energy=adequate protein and low energy.

biweekly as necessary to maintain the desired mass loss levels in the LOCON group. Crude protein of all rations ranged from 11-18%.

The third treatment included a group of six 3.5 month-old calves (JUV). Prior to the beginning of this experiment, these calves were fed to support rapid growth, but were relatively small (about 55 kg at the beginning of this study) due to the effects of neonatal gastrointestinal diseases (see Cook et al. 1996). They were gradually weaned from milk between 20-30 August 1993 just prior to study initiation. The type and amount of rations offered to these calves were the same as that given to the HICON treatment group (i.e., high energy pellet fed ad libitum) beginning at the end of August and continuing until the end of the study.

Blood and Urine Collection and Analysis

Blood and urine samples were collected from each elk on 2 September 1993 just prior to implementing the dietary treatments. Thereafter, blood and urine samples were taken at approximately 1-month intervals beginning 22 September 1993. Blood and urine were collected from elk in the HICON and LOCON groups through early June 1995, but collections from the JUV group continued only through March 1995.

Elk were chemically sedated using xylazine hydrochloride to obtain serum and urine samples early in the study. Until the end of November 1993, elk were brought into the barn, sedated with relatively low doses of xylazine (0.3-0.5 mg/kg), and blood samples were drawn between 5 and 30 minutes after injection. Immediately after blood was collected, sedation was reversed with yohimbine hydrochloride (0.1-0.2 mg/kg), and urine samples were collected 2-3 hours later.

During this time period, we erroneously assumed there were no chemical restraint effects on serum and urine chemistry

because xylazine had been used routinely for such work (e.g., Warren et al. 1982, DelGiudice et al. 1987*a,b,* 1990*a,b*). Further study by Cook et al. (unpublished report 1994), however, demonstrated that xylazine significantly alters several serum and most urinary variables in young elk. Therefore, beginning in December 1993, we collected urine without restraint the day before blood was sampled, and collected blood samples within 10 minutes after injection of xylazine. The 10-minute limit was based on findings by Cook et al. (unpublished report 1994) that serum, lacking xylazine effects, could be collected up to 10 minutes post xylazine injection using our doses of xylazine to sedate young elk. We used the same doses of xylazine and yohimbine with both sample collection procedures.

Blood samples were obtained by jugular venipuncture using syringes, transferred to SST vaccutainers, allowed to clot for 30-45 minutes, then centrifuged. Serum was harvested with pipettes, aliquoted into 1.8-ml freezer vials, and stored frozen at -20°C until laboratory analysis. Urine was collected from the urine-collection pans under individual stalls, pipetted into 1.8-ml freezer vials and stored frozen at -20°C until laboratory analysis. Levels of IGF-1 were determined using a double-antibody radioimmunoassay (RIA) at Oregon State University. Phoenix Central Laboratory (Tacoma, WA) assayed for total T_3 by RIA and urinary cortisol by fluorometric enzyme immunoassay. Interpath Laboratory (Pendleton, OR) conducted all other assays using a Boehringer Mannheim/Hitachi 737 analyzer and reagent systems specified by the manufacturer. Values for each urinary variable were divided by urinary creatinine levels to account for variations in urine concentrations and adjusted (see Appendix A) following DelGiudice et al. (1990*b*).

Data Analyses

Body mass data were analyzed with a univariate fixed-effects repeated measures ANOVA using PROC GLM of SAS (SAS Institute 1988). $P \leq 0.05$ was accepted as significant for all tests. Data from the HICON, LOCON, and JUV groups were included in this analysis and the LSMEANS procedure was used to identify groups with significantly different body mass estimates at each sample date. Feed intake data for the 3 groups were not analyzed with repeated measures or standard regression procedures because communal feeding of hay resulted in a lack of independence among the sampling units.

Effects of changing daylength on serum and urine chemistry were analyzed using 2 different approaches. The repeated measures design introduced a lack of independence among samples and thus precluded the use of standard regression procedures to examine relations between daylength and serum and urine chemistry variables. First the PROC MIXED procedure of SAS (SAS Institute 1989) for mixed linear models (HICON and JUV data only) was used to identify (1) variables that exhibited significant correlation with daylength over time and (2) variables with significant interactions between daylength and treatment. Data from the LOCON elk were excluded because of confounding by extreme nutritional restriction during the first 4 months of study. Two error structures were evaluated, compound symmetry (CS) and autoregressive (AR-1). Observations that were closer in time tended to be more similar, therefore the AR(1) error structure was the most appropriate for this analysis.

Next, we regressed each serum and urine variable on daylength, 1 regression per elk, using PROC REG of SAS (SAS Institute 1988). Data from the LOCON group (January 1994-June 1995) were included in this analysis. We calculated the average slope, intercept, and r^2 for each serum and urinary

variable across elk and tested for their significance (i.e., slope, intercept, and $r^2 = 0$) within each treatment group.

Photoperiod effects on VFI and BM also were examined using regressions with daylength as the independent variable. As described above, we calculated individual regressions within treatments using PROC REG of SAS (SAS Institute 1988) to obtain the average slope, intercept, and r^2 for VFI and BM. Next, we tested for significant (i.e., slope, intercept, and $r^2 = 0$) differences within each group. The LOCON group was excluded from this analysis because their food intake data was confounded by imposed nutritional restrictions and would not represent actual VFI. Three time periods were examined, Fall/Winter 1993 (31 August 1993-1 February 1994), Spring/Summer 1994 (5 April 1994-15 August 1994), and Fall/Winter 1994 (30 August 1994-15 February 1994).

Analysis of age effects analysis on serum and urine chemistry were conducted using a repeated measures fixed-effects ANOVA with PROC GLM of SAS (SAS Institute 1988). Data from the HICON and JUV groups only were included in this subset analysis. Initial data analysis indicated that the assumption of sphericity (SAS Institute 1988:605) was routinely violated for all serum and urine variables. We therefore used the Huynh-Feldt correction to the numerator degrees of freedom (SAS Institute 1988:605). The repeated measures analysis was conducted individually for each serum and urine variable; $P \leq 0.05$ was accepted as significant for all tests. The LSMEANS procedure in SAS was used to identify significant differences between the 2 groups at each sample date.

The repeated measures ANOVA identified many variables that had a significant treatment effect (i.e., age) or time by treatment interaction; but the data patterns were irrelevant in the context of age effects. Thus, before we claimed a significant age effect for any given serum or urine variable, we required that data patterns could be reasonably attributed

to age. Either of 2 patterns were required: (1) levels between the HICON and JUV groups remained significantly different over most or all of the study period, or (2) levels initially were different but showed a clear convergence over time. Although subjective, this approach resulted in identification of variables that were biologically meaningful in terms of age effects and eliminated those variables that exhibited significant but irrelevant patterns in the context of age effects.

Finally, reference values were summarized in a series of tables including means, SEs, and ranges, for elk in good to excellent condition, maintained on high quality food, by age class and season. These reference values were calculated for calves and yearlings using data from the JUV group, and for 2- to 3-year-old cows using data from the HICON group. Seasons were defined as: summer (21 June through 31 August); autumn (9 October through 4 December); winter (21 December through 28 February); and spring (8 April through 3 June). The dates for winter and summer seasons were selected to best represent periods that were stable in the context of apparent seasonal effects (based on the seasonal analyses); the dates of autumn and spring simply included the middle 2-month period of the 3-month seasonal period. Each age-season cell (e.g., yearlings in autumn) usually included 2 (1-3) sampling dates. Reference values for cells with more than 1 sampling date were estimated by first calculating the average for each elk across multiple sampling dates (i.e., within-elk averages), then calculating the among-elk average (n = 6) using the within elk averages. SEs and ranges were calculated similarly (e.g., SE of the within-elk averages and range was the smallest and largest within-elk average of the 6 elk).

RESULTS

Body mass (BM) dynamics of elk differed over time and among treatments evidenced by the significant time by treatment interaction in the repeated measures ANOVA (P = 0.0001) (Figure 3.1 a). The HICON group increased BM 27% on the high quality diet during the study (Figure 3.1 c). Elk in the LOCON treatment were induced to lose 10-15% of their mass early in the study. Inducing this decline required a moderately submaintenance diet (130-170 kcal of DE/kg MBM/day), a level that averaged about half of that consumed by the HICON elk at that time. Once the desired BM loss was achieved, DE was increased to 180-190 kcal of DE/kg MBM/day to stop the decline in BM. Body mass of the LOCON elk thereafter was held at about 15% less than that of the HICON group over the rest of the experiment (Figure 3.1 c). This required relatively small changes in feeding levels; ranging from 185-215 kcal of DE/kg MBM/day early 1994 until the end of the study (Figure 3.2 b). Over the course of the entire experiment, LOCON elk gained about 4.5% over their beginning BM (Figure 3.1 c).

Body mass increased 270% during the experiment in the JUV group. These elk were approximately as large as elk in the LOCON group but significantly smaller than elk in the HICON group at the study's conclusion (Figure 3.1 a). During the first year of the study, the calves maintained high levels of growth during fall and early winter (> 0.3 kg/day), reduced growth (about 0.2 kg/day) in mid- to late winter, then resumed rapid growth in spring (Figure 3.1 b). Seasonal changes in rates of gain were more pronounced after calves became yearlings, in the second year of the study and were similar to those of the 2-year-old cows in the HICON group (Figure 3.1 b). Voluntary intake of DM, CP, and DE on a metabolic mass basis exceeded that of adults in the HICON group by about 25% across most of this study except during the

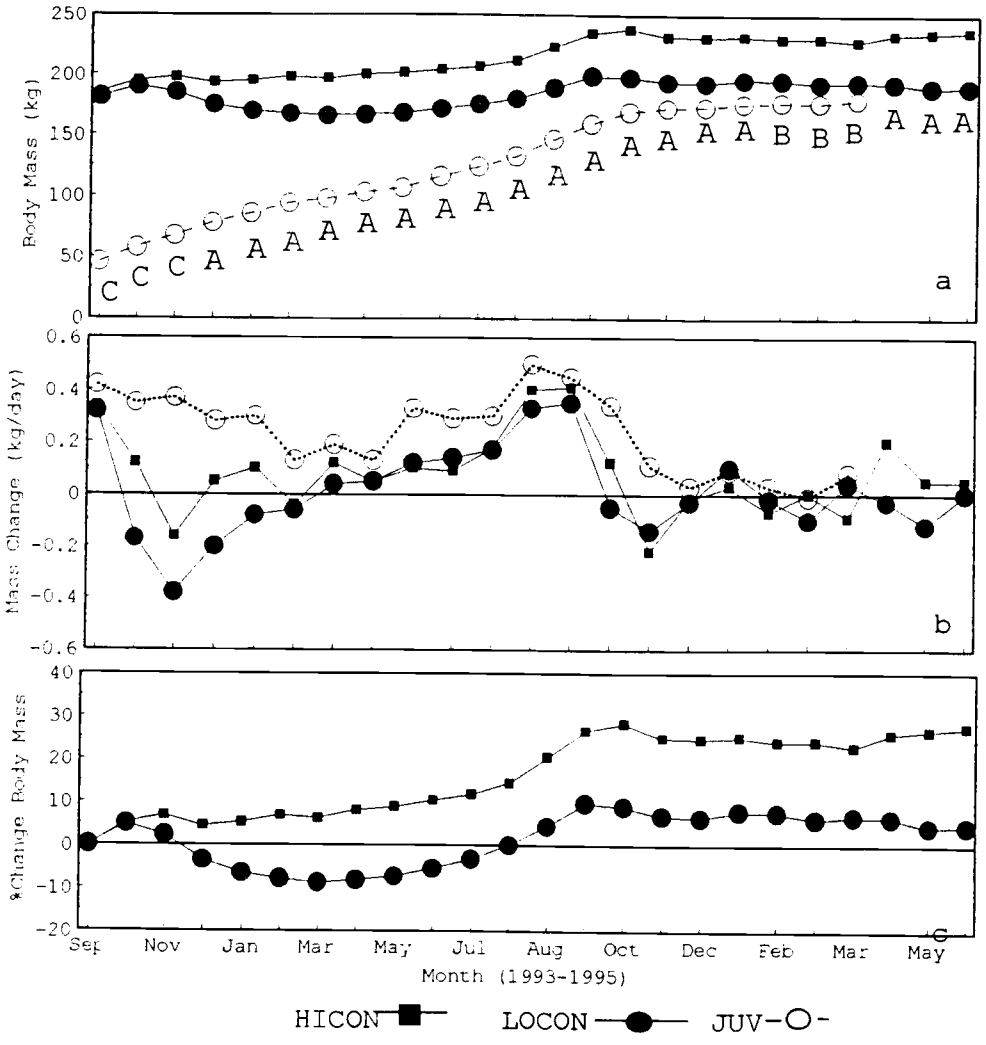

Figure 3.1 (a-c): Body mass dynamics of HICON, LOCON, and JUV elk in the study. Mass estimates were obtained twice per week for the duration of the study. Significant differences from the repeated measures ANOVA are denoted with the letters A, B, or C.

A=All means were significantly different

B=Only the Juv group was not significantly different from the LOCON group

C=The HICON and LOCON groups were both significantly different from the JUV group.

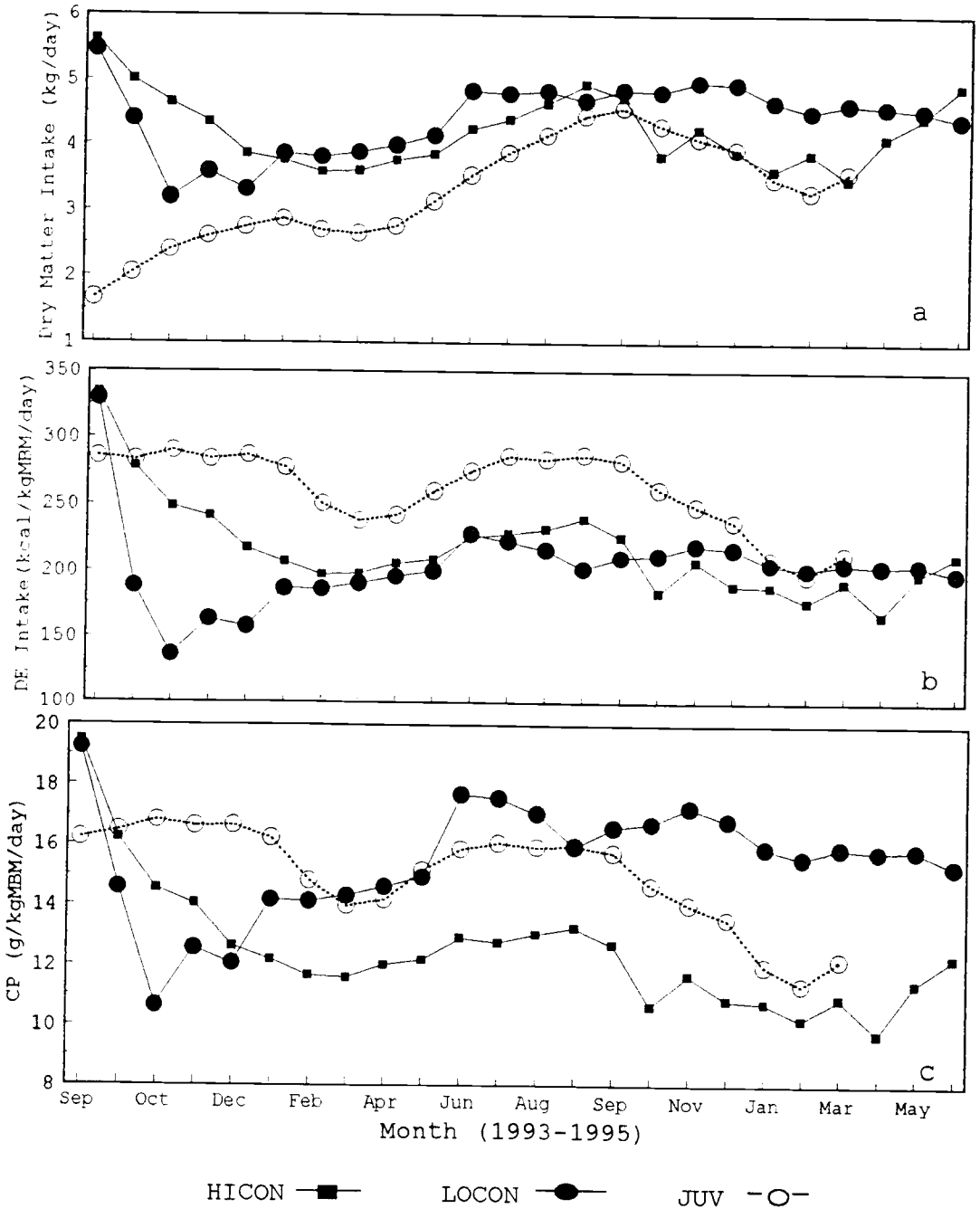

Figure 3.2(a-c): Average daily feed intakes for HICON, LOCON and JUV elk. The HICON and JUV elk received a high energy diet ad libitum year-round, and the LOCON elk received a low energy restricted diet year-round.

last few months. Seasonal patterns of VFI intakes, however, were analogous to those of adults, e.g., highest in summer, declining in autumn, and lowest during winter (Figure 3.2 a-c).

Regression analysis of VFI and BM on daylength (HICON and JUV groups) indicated that VFI was strongly correlated with changes in daylength during fall but not during summer (Table 3.2). Strong correlation between VFI and daylength were observed in the HICON group during fall 1993 and 1994 and the JUV group in the fall of 1994. Regression analysis of mean body mass on daylength showed little correlation with photoperiod effects, possibly because all animals in the study were still growing. Consequently, in growing animals, rate of body mass change rather than mean body mass may be the more appropriate index of photoperiod effects on body mass (Figure 3.1 b).

In all groups, the rate of BM gain (kg/day) exhibited seasonal patterns with peaks in rates of gain observed during late June to late August (0.3 to 0.4 kg/day) followed by a short period in which the rate of change in BM markedly declined (from about 0.35 to -0.15 kg/day) during September and October. After this sharp decline in early fall, the rate of change in BM appeared to remain relatively small and stable (-0.1 to 0.1 kg/day) until the following summer (Figure 3.1 b). Voluntary food intake (VFI) followed an analogous pattern, with greatest and lowest intakes observed in summer and winter. This pattern may be confounded in the HICON group during fall 1993 as food intake was roughly 50% greater in mid-summer just prior to the beginning of the study compared to the remainder of the study (Figure 3.2c). This probably was due to a summer compensatory growth phase induced after BM loss of 8-10% the previous winter (1992-1993) and a high quality, ad libitum diet. During the study, however, feed intake was not limited in the HICON and JUV groups and

Table 3.2: Results from regressions of VFI and BM on daylength.

Group	Season	Est. β_0	Est. β_1	Adjusted r^2	*P*-value HO: $\beta_1 = 0$
VFI(Total dry matter intake in g/day)					
JUV	FALL-93	5058.2	-259.9	0.71	0.0001
JUV	SUMM-94	-1261.1	331.1	0.13	0.1964
JUV	FALL-94	2138.5	185.7	0.37	0.0220
HICON	FALL-93	658.2	372.1	0.78	0.0005
HICON	SUMM-94	1025.3	222.8	0.06	0.1886
HICON	FALL-94	1751.4	238.9	0.69	0.0040
MEAN BODY MASS (kg)					
JUV	FALL-93	166.9	-9.3	0.66	0.0001
JUV	SUMM-94	56.9	4.9	0.02	0.1281
JUV	FALL-94	192.1	-2.6	0.75	0.0001
HICON	FALL-93	212.5	-1.8	0.01	0.2023
HICON	SUMM-94	198.1	0.9	0.00	0.7961
HICON	FALL-94	216.4	1.6	0.01	0.2367

seasonal patterns of intake and growth were consistent with expected seasonal effects and therefore assumed to be related to photoperiod.

Eight serum variables were significantly correlated to changing daylength in the PROC MIXED analysis: TB, TP, ALT, ALK, triglycerides, T_4, IGF-1, and serum creatinine (Table 3.3, Figure 3.3 a-h). Results from the regression analysis were also very similar (Table 3.4). ALT, ALK, TB, T_4, triglycerides, and IGF-1 were elevated during summer and depressed during winter months (Figures 3.3 a,c-g). In contrast, TP and serum creatinine were elevated in winter and lower in summer (Figure 3.3 b,h).

Nineteen serum variables and 2 urinary variables differed significantly between the HICON group and the JUV group (Table 3.5, Fig. 3.4 a-r) indicating appreciable age effects. Variables that exhibited significant patterns apparently not attributable to age were urinary calcium, glucose and T_4 (Appendix D). Variables that significantly differed when the JUV group were calves but converged after 1 year-of-age included SUN, cholesterol, AST, TP, and IGF-1. Levels of ALT, calcium, TB, chloride and UK:Cr were similar initially, diverged after the first couple of months, then converged again, as the calves grew older. For some of these variables, the initial similarity occurred during the period before protocol was changed to eliminate xylazine-restraint effects. If xylazine influences were absent in the first several months, a greater divergence may have been evident for ALT, calcium, and UK:Cr.

Variables that remained significantly elevated in the HICON group over the entire study period included serum creatinine and TP (Figure 3.4 b,l). Conversely, higher levels of ALK, calcium, phosphorus, T_3 and T_3/T_4 ratios were observed in the JUV group over much of the study (Figures 3.4 a,c,e-g). ALT levels were always higher in the JUV group but only significantly different during 4 sampling dates. Triglyceride levels did not follow a consistent pattern initially and after

Table 3.3: Results from the seasonal PROC MIXED analysis. (HICON and JUV data only)

Variable	Estimated (β_0)	($\beta_0 = 0$) *P*-value	Estimated (β_1)	($\beta_1 = 0$) *P*-value
T_4[c]	0.22	0.0001	1.53	0.0250
IGF-1	30.60	0.0001	27.70	0.5260
ALT[b]	1.86	0.0001	6.98	0.0628
Trig.[b]	0.61	0.0001	2.71	0.1219
Glucose[a]	-1.59	0.0199	118.30	0.0001
Calcium[a]	0.04	0.0432	9.18	0.0001
ALK	5.28	0.0017	34.8	0.2163
TB	0.02	0.0001	0.09	0.0412
TP	-0.05	0.0006	6.98	0.0001
Albumin[a]	-0.04	0.0009	4.92	0.0001
Creatinine	-0.03	0.0017	1.93	0.0001
Sodium[a]	0.20	0.0118	138.35	0.0001

[a] Variables eliminated from further examination because they did not exhibit patterns that were consistent with changes in daylength for both years of the study.
[b] Variables which were lagged because the highest and lowest levels occurred two months after other variables that closely tracked daylength cycles.
[c] T_4 was the only variable that did not have a significant daylength by treatment interaction in PROC MIXED

Table 3.4: Results from the seasonal regression analysis.

Variable	Estimated β_1	HO: β_1=0 *P*-value	Estimated r^2	HO: r^2=0 *P*-value
HICON GROUP				
Calcium	0.05	0.0003	0.06	0.0264
ALT	1.09	0.0000	0.16	0.0412
ALK	5.18	0.0010	0.19	0.0315
TB	0.02	0.0000	0.24	0.0025
Albumin	-0.01	0.0130	0.02	0.0482
Triglyc.	0.54	0.0120	0.09	0.4950
T_4	0.30	0.0000	0.27	0.0155
IGF-1	31.91	0.0000	0.45	0.0021
LOCON GROUP				
GGT	-0.06	0.0006	0.36	0.0111
ALK	10.91	0.0026	0.56	0.0022
TP	-0.09	0.0005	0.42	0.0006
ALB	-0.04	0.0018	0.15	0.0213
Cholest.	-0.20	0.0040	0.17	0.0440
T_3	0.32	0.0001	0.36	0.0005
IGF-1	38.10	0.0001	0.66	0.0001
JUV GROUP				
T_3/T_4	-3.97	0.0001	0.19	0.0046
Glucose	-2.49	0.0001	0.19	0.0087
T_4	0.29	0.0001	0.22	0.0003
IGF-1	36.43	0.0007	0.48	0.0022

Table 3.5: Results from the univariate repeated measures ANOVA age analysis (HICON and JUV groups only).

Variable	Time *P*-value[1]	Time by Treatment *P*-value[1]	Treatment *P*-value[1]
UUN:Cr[2]	0.0001	0.1257	0.1756
UNa:Cr[2]	0.0012	0.3129	0.2776
UP:Cr[2]	0.0826	0.5348	0.7527
UK:Cr	0.0001	0.0019	0.0016
UCa:Cr[3]	0.0001	0.0184	0.3453
UCor:Cr[2]	0.0001	0.5471	0.2220
T_3/T_4	0.0001	0.0003	0.0023
T_3	0.0001	0.0001	0.0005
T_4[3]	0.0001	0.0072	0.1584
IGF-1	0.0001	0.0001	0.2251
GGT[3]	0.0001	0.0068	0.2099
AST	0.0001	0.0004	0.4568
ALT	0.0001	0.0008	0.1249
ALK	0.0001	0.0001	0.0107
Calcium	0.0001	0.0003	0.0001
Phosphorus	0.0001	0.0447	0.0001
Sodium[4]	0.0001	0.1026	0.8419
Potassium[2]	0.0001	0.3826	0.1407
Chloride[3]	0.0001	0.0540	0.0014
Glucose[3]	0.0001	0.0054	0.7143
TB	0.0001	0.0015	0.5977
TP	0.0001	0.0001	0.0006
Albumin[3]	0.0001	0.0001	0.4069
SUN	0.0001	0.0001	0.0079
Creat.	0.0001	0.0049	0.0001
Cholest.	0.0001	0.0001	0.0180
Triglyc.	0.0001	0.2243	0.0351

[1] Huynh-Feldt adjusted *P*-values
[2] Variables exhibiting significant treatment effects or time*treatment interactions, but patterns of data do not correspond to predicted patterns associated with age effects.
[3] Variables that did not exhibit significant treatment effects or time*treatment interactions

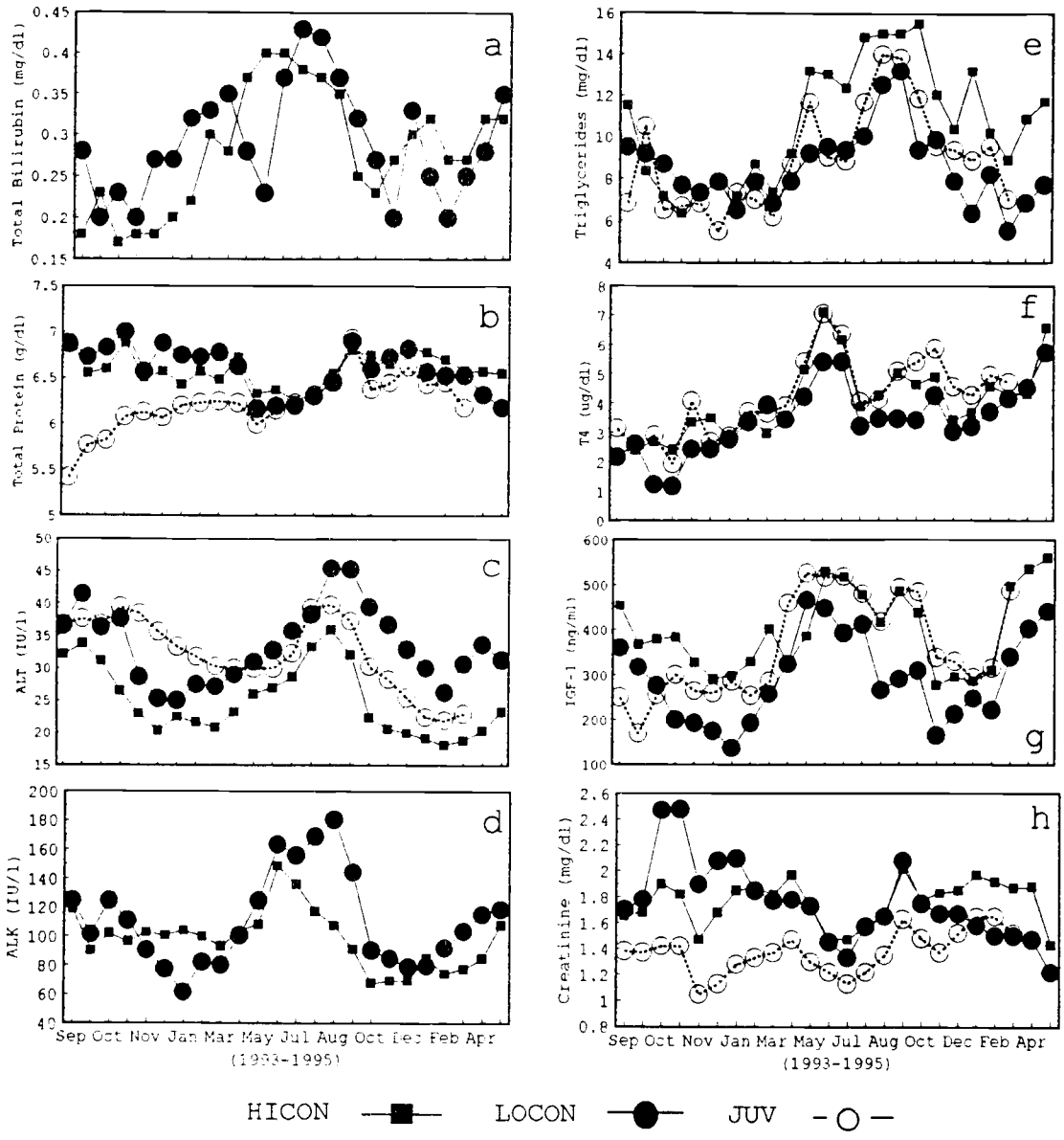

Figure 3.3: Serum and urine variables significantly correlated to daylength. All variables have slopes different from zero and visually exhibited patterns that appeared to be related to daylength during both years of the study.

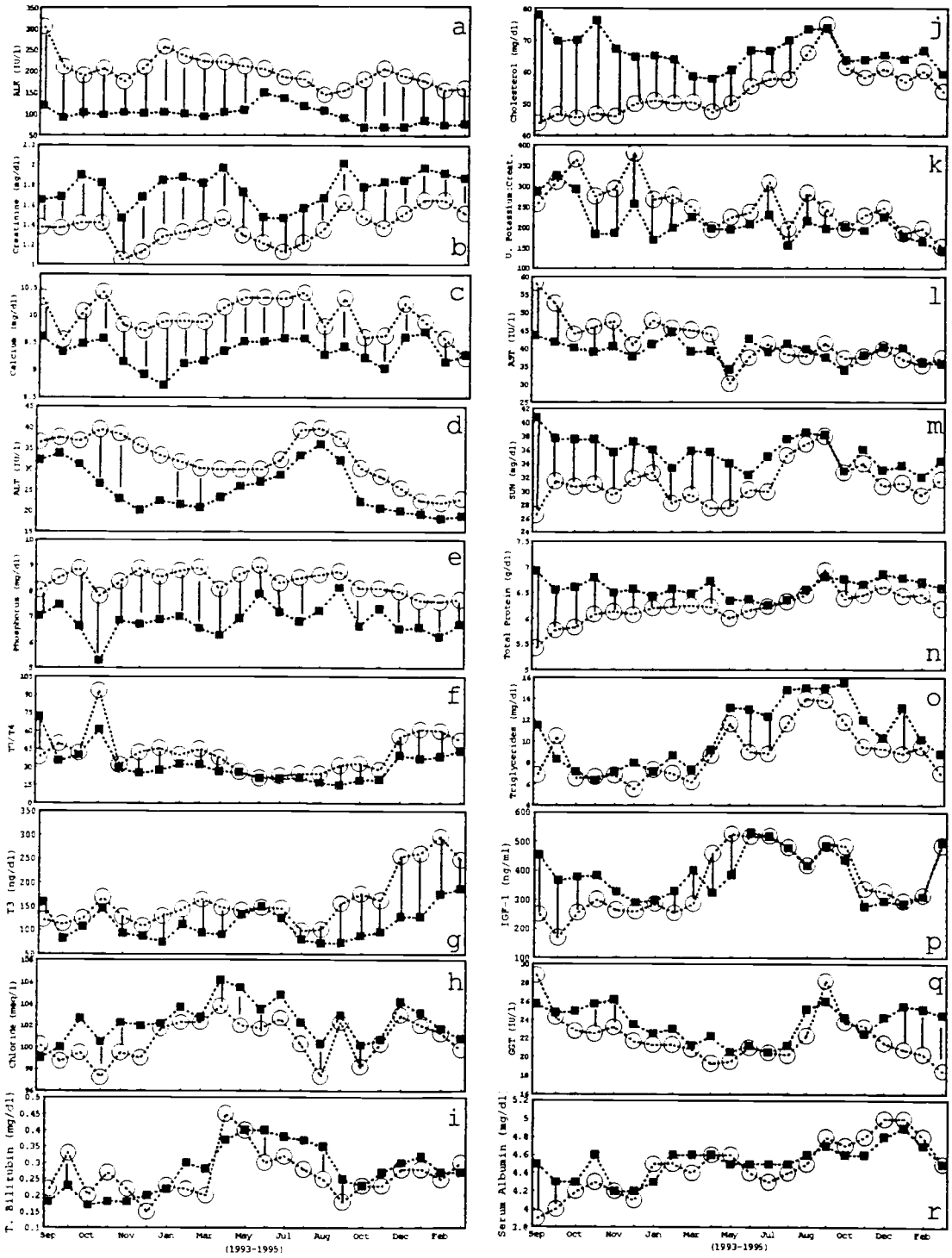

Figure 3.4 a-r: Serum and urine variables influenced by age. Bars between the data at specific sampling dates indicate significant differences ($P < 0.05$).

the first year of study levels were continuously elevated in the HICON group but significantly so only on 4 sampling dates. GGT levels were elevated in the HICON group during the fall 1993 and winter 1994 only. Serum albumin levels were initially lower in the JUV group, but levels quickly converged to be similar to those observed in the HICON group.

In Tables 3.7-3.8, we present summaries of serum and urine chemistry reference levels for the elk in this study held in good to excellent condition (the HICON and JUV groups). Because of significant age and seasonal effects, the reference serum and urine chemistry values are presented for elk of various ages from 3.5 months to 4 years and for each season of the year.

DISCUSSION

Circannual cycles in VFI and BM

Elk in this study exhibited circannual rhythms in VFI correlated with season. The general pattern observed was increasing VFI during summer (nearly 2-fold) followed by a gradual decline in fall and a trough over winter and spring. This was identical to the pattern reported for red deer (Loudon et al. 1989) except that peak intake was reached in late summer rather than mid-summer. Expression of seasonal appetite changes were clearly visible during the fall 1994 for the HICON and JUV groups on ad libitum diets but not in the LOCON group on the restricted diet in 1994 or in the JUV group fall 1993 (Figure 3.2a). This most likely indicates that expression of seasonal effects on VFI and rates of gain depends on the physiological needs of the animals. Heydon et al. (1993) also reported that expression of VFI cycles in red deer hinds depends on the availability of food.

Table 3.6: Reference values for selected serum chemistry variables in young cow elk. Data are means, SEs, and range (minimum and maximum values connected by hyphens) for 6 animals within each season-age category maintained on high quality rations fed ad libitum.

	Calves			Yearlings		
	Autumn[a]	Winter	Spring	Summer	Autumn	Winter
Var.	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE	Mean SE
GLU	115.2 9.07 93.7-157.7	103.5 4.41 93.0-123.0	91.3 5.60 71.0-109.0	81.9 2.53 73.3-91.7	94.2 3.80 84.5-107.0	97.1 2.58 90.5-106.0
GGT	22.8 0.63 20.7-24.7	21.3 0.88 18.0-24.5	19.5 1.06 16.0-24.0	21.0 1.34 16.0-25.7	23.5 1.23 20.5-28.0	20.6 0.81 17.5-23.0
CAL	10.1 0.11 9.8-10.5	9.9 0.12 9.3-10.2	10.3 0.11 10.0-10.6	10.2 0.09 9.9-10.5	9.6 0.08 9.4-9.9	9.7 0.13 9.3-10.2
PHO	8.4 0.23 7.4-9.2	8.6 0.28 7.3-9.1	8.7 0.37 7.7-10.2	8.5 0.16 8.0-9.0	8.1 0.14 7.7-8.7	7.6 0.28 6.8-8.8
ALK	189.7 25.67 133.7-300.3	247.6 24.22 196.0-352.5	210.3 23.47 152.0-309.0	169.9 29.50 104.3-293.3	192.8 36.33 106.5-336.0	167.0 22.04 102.5-239.5
AST	45.9 2.58 38.3-53.7	46.8 3.28 38.5-56.5	30.2 2.24 23.0-38.0	39.2 3.49 31.3-55.0	37.5 3.08 28.5-49.5	36.2 2.94 26.5-44.5
ALT	38.3 3.70 28.0-49.0	32.8 4.15 19.5-46.0	30.0 3.25 19.0-41.0	37.2 4.31 26.7-54.7	29.3 3.78 18.0-44.0	22.3 2.46 13.5-30.5
TB	0.2 0.02 0.2-0.3	0.2 0.03 0.2-0.3	0.4 0.03 0.3-0.5	0.3 0.02 0.2-0.3	0.2 0.01 0.2-0.3	0.3 0.02 0.2-0.4
TP	6.0 0.05 5.8-6.1	6.2 0.07 5.9-6.5	6.0 0.12 5.6-6.5	6.3 0.10 6.0-6.8	6.4 0.07 6.2-6.7	6.4 0.12 5.9-6.7
ALB	4.2 0.05 4.1-4.4	4.5 0.06 4.3-4.7	4.6 0.09 4.4-4.9	4.4 0.06 4.3-4.7	4.7 0.04 4.6-4.9	4.9 0.05 4.7-5.1
BUN	30.3 1.28 26.7-36.0	30.7 1.51 25.5-36.0	27.5 1.15 23.0-31.0	33.9 0.94 29.7-36.7	33.3 1.30 28.0-36.5	30.3 1.12 27.0-35.0
CRE	1.3 0.04 1.2-1.4	1.3 0.04 1.2-1.4	1.3 0.04 1.2-1.4	1.2 0.02 1.2-1.3	1.4 0.03 1.3-1.6	1.7 0.04 1.6-1.8
SOD	138.7 0.43 137.7-140.7	141.3 0.92 137.0-143.5	142.8 0.95 140.0-146.0	139.2 0.44 138.0-140.7	139.9 0.51 138.5-142.0	141.0 0.29 140.0-142.0
POT	4.4 0.10 4.2-4.7	4.0 0.09 3.9-4.4	4.1 0.06 3.8-4.2	4.1 0.05 4.0-4.3	4.1 0.03 4.0-4.2	4.2 0.12 3.9-4.7
CHL	98.7 0.54 97.3-100.7	101.8 0.62 99.0-103.5	102.0 0.97 99.0-106.0	100.1 0.45 99.0-101.7	99.3 0.40 98.0-101.0	101.7 0.44 100.0-103.0
CHO	46.1 2.84 38.3-56.3	50.9 3.56 44.0-64.0	50.2 2.77 43.0-58.0	60.6 3.14 53.0-73.0	60.0 3.91 52.0-73.0	58.8 4.06 49.5-73.5
TRI	6.7 1.01 4.7-11.3	7.3 0.66 5.0-9.0	11.7 0.80 9.0-14.0	11.5 0.73 9.0-13.7	10.7 1.11 8.5-15.0	9.2 0.68 7.0-11.5
T_3	140.6 13.64 98.7-181.0	135.2 6.87 106.0-152.0	140.8 11.43 106.0-191.0	115.7 9.21 80.3-145.3	170.0 7.75 152.0-195.0	279.3 23.02 223.0-370.0
T_4	3.0 0.21 2.3-3.6	3.3 0.18 2.5-3.8	5.4 0.28 4.6-6.4	4.9 0.15 4.1-5.1	5.7 0.22 5.0-6.5	4.6 0.15 4.2-5.1
T_3/T_4	55.7 5.54 41.8-73.0	42.8 2.81 33.1-51.9	26.3 2.44 19.3-34.1	23.9 1.40 20.3-29.2	30.5 1.73 25.1-36.4	61.0 5.33 47.3-80.3
IGF-1	273.7 9.72 229.0-291.4	283.2 32.22 210.1-384.8	526.4 48.96 369.8-658.3	472.3 19.23 404.5-530.6	411.3 21.38 334.3-465.9	305.1 16.44 254.3-376.1

[a] These data likely reflect effects of xylazine hydrochloride sedation.

Table 3.6 (Continued): Reference values for selected serum chemistry variables in young cow elk. Data are means, SEs, and range (minimum and maximum values connected by hyphens) for 6 elk within each season-age category maintained on a high quality ration fed ad libitum.

	Cows: age 2			Cows: age 3			
	Autumn[a]	Winter	Spring	Summer	Autumn	Winter	Spring
Var.	Mean /SE	Mean /SE	Mean /SE	Mean /SE	Mean \SE	Mean\SE	Mean\ SE
GLU	124.3 5.32 112.7-150.0	93.6 2.88 84.5-103.5	83.2 2.96 75.0-95.0	80.9 2.26 76.3-90.3	104.3 6.09 80.0-121.0	109.0 3.50 96.5-120.5	97.2 2.52 89.0-105.0
GGT	25.6 0.78 22.7-28.3	22.8 1.18 18.5-26.5	20.5 0.81 17.0-22.0	22.3 1.35 16.3-25.3	23.4 1.00 20.0-26.5	25.3 1.32 20.5-28.5	24.5 1.52 20.0-30.0
CAL	9.4 0.07 9.1-9.5	8.9 0.14 8.4-9.3	9.5 0.11 9.1-9.8	9.5 0.05 9.3-9.7	9.1 0.07 8.9-9.4	9.4 0.06 9.3-9.7	9.2 0.07 9.0-9.4
PHO	6.3 0.09 6.0-6.5	6.9 0.18 6.2-7.3	6.9 0.32 5.7-8.0	7.1 0.37 5.5-8.0	7.0 0.23 6.2-7.6	6.4 0.18 5.7-7.0	6.6 0.35 5.8-8.1
ALK	100.4 16.37 72.3-181.0	102.0 15.90 68.5-174.0	108.2 16.96 74.0-186.0	120.2 23.62 78.3-227.3	69.0 14.73 44.0-134.0	79.4 24.72 49.0-201.0	84.5 18.75 38.0-168.0
AST	39.9 1.65 32.3-44.0	42.8 2.58 32.5-49.0	34.2 1.45 30.0-39.0	40.1 2.54 27.7-45.0	35.9 2.66 25.5-41.5	38.1 3.23 23.0-44.5	40.2 2.37 29.0-46.0
ALT	26.9 1.77 20.7-32.3	22.1 1.94 13.0-26.0	26.0 2.77 14.0-32.0	32.7 3.14 17.3-37.7	21.5 1.88 13.5-26.0	18.7 2.17 8.5-24.0	20.3 1.61 13.0-24.0
TB	0.2 0.01 0.1-0.2	0.3 0.04 0.1-0.4	0.4 0.06 0.2-0.6	0.4 0.03 0.2-0.4	0.3 0.02 0.2-0.3	0.3 0.03 0.2-0.4	0.3 0.05 0.2-0.5
TP	6.7 0.03 6.6-6.8	6.5 0.08 6.4-6.9	6.3 0.10 6.0-6.7	6.4 0.06 6.2-6.5	6.7 0.11 6.3-7.0	6.7 0.10 6.5-7.1	6.6 0.11 6.3-7.1
ALB	4.4 0.06 4.2-4.5	4.4 0.04 4.3-4.6	4.5 0.06 4.3-4.7	4.5 0.04 4.4-4.7	4.6 0.12 4.0-4.9	4.8 0.10 4.5-5.1	4.3 0.08 4.0-4.5
BUN	36.9 1.38 32.7-40.3	34.7 1.12 29.5-37.0	34.0 0.58 32.0-36.0	37.0 1.37 34.3-43.3	34.4 1.03 31.0-38.0	32.8 0.78 31.0-35.0	34.0 1.13 32.0-38.0
CRE	1.7 0.07 1.4-1.9	1.9 0.06 1.7-2.1	1.7 0.06 1.5-1.9	1.6 0.07 1.3-1.7	1.8 0.07 1.6-2.0	1.9 0.04 1.8-2.1	1.9 0.06 1.7-2.1
SOD	140.0 0.32 138.7-141.0	139.9 0.79 137.5-142.5	144.3 0.21 144.0-145.0	140.6 0.63 138.3-142.7	139.3 0.28 138.5-140.5	141.3 0.53 139.0-142.5	140.8 0.17 140.0-141.0
POT	4.1 0.07 3.8-4.3	4.0 0.06 3.8-4.2	3.9 0.08 3.7-4.2	4.0 0.07 3.6-4.1	4.2 0.08 4.0-4.6	4.2 0.08 3.9-4.4	4.0 0.07 3.8-4.3
CHL	101.8 0.17 101.3-102.3	102.9 0.61 101.5-105.5	105.5 0.34 105.0-107.0	102.5 0.51 100.7-104.3	100.4 0.27 99.5-101.5	102.4 0.44 101.5-104.0	101.8 0.31 101.0-103.0
CHC	71.2 3.84 56.7-86.0	64.6 4.11 54.5-82.0	60.7 3.59 50.0-73.0	70.1 4.08 60.7-84.7	63.8 3.21 52.5-71.0	65.4 4.79 50.0-78.5	59.3 4.07 46.0-71.0
TRI	6.9 0.34 5.3-7.7	7.9 0.52 6.0-9.5	13.2 0.70 11.0-15.0	14.1 1.10 11.7-19.0	13.8 1.16 10.5-18.0	11.7 0.88 9.5-14.5	10.8 1.22 8.0-16.0
T_3	115.8 7.52 84.0-138.0	93.6 3.90 80.0-108.0	133.8 9.77 104.0-174.0	92.9 3.94 77.7-101.0	92.6 9.21 56.0-125.5	152.7 11.95 119.5-201.0	160.0 12.79 119.0-200.0
T_4	2.8 0.20 2.1-3.5	3.2 0.20 2.6-3.9	5.2 0.27 4.5-6.1	4.8 0.13 4.5-5.3	4.8 0.27 3.7-5.4	4.1 0.33 2.5-4.6	4.3 0.27 3.4-5.0
T_3/T_4	43.7 2.54 34.6-51.6	29.9 2.09 23.3-37.6	26.1 1.72 18.9-30.4	19.4 0.73 17.0-21.5	19.3 1.51 15.4-25.9	38.0 3.32 27.9-50.0	37.4 2.92 29.8-48.2
IGF-1	363.4 28.67 289.4-489.6	313.5 19.33 250.5-376.0	385.1 25.23 303.6-466.9	470.6 17.34 419.6-530.7	356.8 22.80 258.1-411.4	298.1 19.77 227.8-374.9	535.0 34.98 382.4-626.4

[a] These data likely reflect effects of xylazine hydrochloride sedation.

Table 3.7: Reference values for selected urine chemistry variables in young cow elk. Data are means, SEs, and range (minimum and maximum values connected by hyphens) for 6 elk within each season-age category maintained on high quality rations fed ad libitum.

	Calves			Yearlings		
	Autumn[a]	Winter	Spring	Summer	Autumn	Winter
Var.	Mean/SE	Mean/SE	Mean/SE	Mean/SE	Mean/SE	Mean/SE
UUN:Cr	12.4 0.63 10.3-14.5	10.1 0.50 8.2-11.4	9.5 0.40 7.7-10.5	11.4 0.34 10.4-12.5	10.2 0.35 8.7-11.0	8.2 0.39 7.1-9.8
UNa:Cr	43.8 15.92 11.7-115.7	7.9 1.52 5.1-15.1	5.8 0.43 4.3-6.9	14.3 4.60 6.9-36.6	8.2 0.46 7.2-10.2	11.0 0.80 8.7-13.5
UK:Cr	311.5 22.57 240.8-393.8	272.3 12.99 222.5-309.6	225.1 24.04 139.4-304.7	263.1 10.66 227.3-303.4	212.4 9.68 180.3-245.8	191.4 6.64 177.6-216.6
UP:Cr	24.5 7.35 7.6-54.7	6.2 1.47 3.2-12.5	3.9 0.43 2.7-5.3	19.3 13.45 2.3-85.9	22.2 15.84 2.1-100.7	21.5 15.49 2.5-98.4
Uca:Cr	87.4 14.32 48.0-139.9	40.0 17.33 4.0-106.4	3.4 1.73 0.6-11.5	57.9 9.67 25.6-89.6	18.8 6.61 0.6-37.4	36.0 5.29 17.5-50.9
Ucor:Cr	0.9 0.22 0.4-1.9	0.5 0.08 0.2-0.7	0.5 0.13 0.1-1.1	0.6 0.09 0.2-0.8	0.6 0.08 0.3-0.8	0.6 0.05 0.4-0.7

	Cows: age 2			Cows: age 3			
	Autumn[a]	Winter	Spring	Summer	Autumn	Winter	Spring
UUN:Cr	11.9 0.33 10.5-12.9	8.8 0.29 7.7-9.7	8.9 0.48 7.6-10.9	9.9 0.34 9.0-11.2	8.9 0.32 8.0-9.8	7.5 0.20 6.9-8.3	8.1 0.15 7.7-8.5
UNa:Cr	25.6 2.73 19.7-38.2	4.0 0.21 3.2-4.6	6.8 0.52 4.8-8.1	8.5 0.98 6.0-11.7	10.9 1.50 6.3-15.8	15.9 2.04 8.6-21.6	6.3 0.54 4.9-7.8
UK:Cr	220.3 12.55 172.4-259.5	183.6 4.80 170.9-205.1	194.5 9.70 157.5-228.3	200.3 8.17 174.3-222.5	196.1 13.69 150.7-249.5	172.3 8.06 151.4-206.8	166.7 12.11 128.6-193.9
UP:Cr	25.4 4.33 7.0-37.4	3.6 0.86 1.9-7.6	10.8 4.56 2.2-25.9	24.4 11.95 3.4-81.7	5.6 1.57 2.8-12.7	5.9 1.65 1.5-12.8	3.4 0.44 2.2-5.3
UCa:Cr	78.8 6.73 61.5-104.7	16.5 5.79 3.2-39.6	12.7 6.48 2.0-44.1	35.8 7.32 12.9-61.6	7.7 3.93 0.6-24.6	26.0 3.79 17.5-40.0	29.6 4.09 13.4-38.9
Ucor:Cr	0.7 0.08 0.5-1.0	0.3 0.03 0.2-0.4	0.3 0.03 0.1-0.4	0.6 0.03 0.5-0.7	0.6 0.13 0.4-1.0	0.6 0.08 0.3-0.9	0.4 0.10 0.1-0.8

[a] These data likely reflect effects of xylazine hydrochloride sedation.

Although there was no substantial correlation between daylength and BM, a marked change in the rate of BM gain was observed. This coincided with the onset of hypophagia in the HICON and JUV groups. Rapid changes in rate of BM gain partly reflect changes in gut fill. The decline in dry matter intake, however, continued well into the winter months, while the rate of gain (kg/day) rapidly declined from September to mid-October, then stabilized. This pattern suggests that elk have the ability to rapidly cease energy expenditure for growth and switch to an energy conservation mode that prevents significant body mass loss despite declining VFI during winter months. This is most likely an adaptation that allows wild ruminants to survive winters in northern latitudes when forage quantity and quality often become very limiting.

These voluntary changes in VFI and associated BM cycles are probably related to changes in daylength and altered secretions of melatonin via the pineal gland (Loudon 1994). Further, researchers have determined the circannual rhythms of VFI and BM changes in wild ruminants are endogenous and cannot be eliminated by environmental changes (Loudon 1994). In contrast, expression of VFI and BM declines or increases are species specific, and timing of those events may be altered by environmental changes (Loudon 1994). These alterations in timing have allowed each species to optimize growth, reproductive opportunities, and possibly prevented hybridization as conditions vary within any ecological or climatalogical setting (Loudon 1994).

Enzymes

Season and age significantly influenced ALT (previously known as SGPT) levels. Kie et al. (1983) also reported that ALT levels varied significantly over time, by age and by sex in white-tailed deer. In terms of seasonal influences ALT

levels lagged 2 months behind other serum variables in the timing of the cycle of the peak and trough each year. The amplitude of change in ALT levels was nearly 2-fold in September compared to February and that pattern was consistent in all groups during the study. Levels of ALT in our study were much lower (about 50%) than those reported previously for red deer (Knox et al. 1988) and white-tailed deer (Kie et al. 1983).

ALK levels typically were greater in juveniles in this study, as would be expected due to increased osteoblastic activity in rapidly growing animals, and levels declined with increasing age. Age effects for ALK also have been reported for white-tailed deer (Kie et al. 1983, Seal et al. 1978*b*). There was a slight decline in ALK levels for the JUV group at study initiation (Figure 3.4a) probably due to post-weaning nutritional stress. Levels of ALK in our calves were similar to levels reported by Soveri et al. (1992) for reindeer calves. Reported levels for deer however were lower than those seen in elk and reindeer (Seal et al. 1978*b*, Kie et al. 1983).

Age but not photoperiod significantly influenced levels of AST (previously known as SGOT). In the JUV group, AST levels were significantly elevated only during the first 4 sampling dates but then converged to levels similar to the HICON group. Thus, age effects were observed only in elk < 7 months of age. Baseline levels of AST were much lower than levels reported previously for white-tailed deer (Seal et al. 1978*b*, Kie et al. 1983), red deer (Knox et al. 1988) and elk (Wolfe et al. 1982).

Changes in GGT levels were significantly different in the age analysis but only at 5 sample dates. GGT levels appeared to be cyclic with all groups exhibiting declining GGT levels until late summer 1994 after which a peak occurred in September, followed by another gradual decline. Data from the HICON group indicated that GGT levels during fall 1994 did not

respond in the same manner as they had in 1993. As with AST, levels of GGT were lower than previously reported levels for red deer (Knox et al. 1988).

Serum Proteins

Levels of serum albumin were significantly higher in the HICON group but only during the first 2 sampling dates and there were no consistent seasonal effects (Appendix C). Levels in our study were within ranges previously reported for elk (Wolfe et al. 1982) but greater than those reported for captive red deer (Knox et al. 1988). Levels of TB were influenced by season but not by age in this study. Kie et al. (1983) previously reported significant age effects in white-tailed deer. However, we speculate that observed differences attributed to age by Kie et al. (1983) were probably due to season rather than age. Total bilirubin was consistently influenced by season, with nearly a 2-fold increase observed in summer compared to winter. Levels of TB were similar to those reported for white-tailed deer (Kie et al. 1983).

Age and season significantly influenced TP levels. In terms of age effects, TP levels were higher in the HICON group until about 1-year of age when levels converged. Similar age effects have previously been reported for white-tailed deer (Kie et al. 1983). From December to July, TP levels declined about 6% (or 1 g/dl). Data presented by Soveri et al. (1992) from caribou showed a similar pattern with higher levels in November and lower levels in April, but the authors attributed this to protein deficiency in the diet rather than season. Protein levels in this study met or exceeded NRC guidelines in our study, therefore we attribute these consistent yearly changes in TP to changes in daylength. Levels of TP in our

study were similar to levels reported for elk (Wolfe et al. 1982), caribou (Messier et al. 1987), and red deer (Knox et al. 1988).

Electrolytes and Minerals

Calcium is a mineral carried in the blood with primary roles in bone growth and maintenance of bones (Thomas 1993). Serum calcium levels were significantly greater in the JUV group, however, as the elk approached 2 years of age, calcium levels declined to levels similar to those observed in the HICON group. Age effects also were reported for white-tailed deer (Kie et al. 1983, Seal et al. 1978*b*). Although calcium levels differed over time, the changing levels did not consistently correspond to changes in daylength in our study. Fluctuations in calcium levels may have been related to hemoconcentration of serum or recent dietary intake instead. Levels of calcium were within the ranges reported previously for elk (Wolfe et al. 1982) and were similar to those reported for white-tailed deer (DeLiberto et al. 1989, Seal et al. 1978*b*, DelGiudice et al. 1990*b*, Kie et al. 1983).

The JUV group also had significantly higher serum phosphorus levels during most of the study, probably due to elevated bone growth. This effect also was reported for white-tailed deer (Kie et al. 1983). Serum phosphorus levels were not significantly influenced by season. Levels of inorganic phosphorus were nearly double the levels reported by Wolfe et al. (1982) for elk but were very similar to levels reported for white-tailed deer (DelGiudice et al. 1992, Kie et al. 1983, DeLiberto et al. 1989, Seal et al. 1978*b*) and mule deer (DelGiudice et al. 1990*a*).

Sodium, along with potassium and chloride are functionally important in maintaining cell integrity through sodium-potassium ion pumps, maintaining acid-base balance of the

blood and in nerve functions (Thomas 1993). Therefore, levels of these cations in the serum were expected to be homeostatic in healthy animals and in fact there were no detectable influences of age or photoperiod on serum sodium and potassium levels. Levels of chloride were observed to be slightly lower in the JUV group and significantly so on 7 sampling dates. Baseline normals for sodium, potassium and chloride were similar to levels reported for caribou (Soveri et al. 1992) and white-tailed deer (Kie et al. 1983, Seal et al. 1978*b*).

Cholesterol and Triglycerides

The JUV group exhibited significantly lower cholesterol levels than adults during the first year of life; their levels then converged with those of the HICON group. Age effects reported by Kie et al. (1983) for white-tailed deer were opposite our findings, with fawns having higher serum cholesterol levels than adults.

Seasonal influences on cholesterol levels have previously been reported in white-tailed deer (Warren et al. 1981, Seal et al. 1972, DeLiberto et al. 1989) and reindeer calves (Soveri et al. 1992). Although levels of cholesterol fluctuated during the study such that early fall levels were elevated compared to winter levels, no significant relation with daylength was identified. Levels of cholesterol were slightly lower than levels reported previously for captive and free-ranging elk (Wolfe et al. 1982) but similar to levels reported for mule deer (Dierenfeld and Jessup 1990, DelGuidice et al. 1990*b*) and white-tailed deer (Kie et al. 1983, Coblentz 1975, Seal et al. 1972, DelGiudice et al. 1990*a*).

Significant age effects on triglyceride levels were observed only during 3 sampling periods. This lack of consistency, combined with significant photoperiod effects, indicates that photoperiod may have confounded the age

analysis. The peak and trough in triglyceride levels lagged about 2 months behind other variables that tracked actual daylength patterns closely. Triglyceride levels were 2-times greater in early fall compared to early spring. Levels of triglycerides were much lower in our study than levels previously reported for white-tailed deer (DelGiudice et al. 1992, Kie et al. 1983, Seal et al. 1972, DeLiberto et al. 1989).

Thyroid Hormones

In terms of age effects, the JUV group exhibited significantly higher T_3 and T_3/T_4 ratios during most of this study probably due to a higher metabolic rate related to rapid growth. Ryg and Langvatn (1982) also reported higher levels in red deer at 1-2 years of age compared to older animals. Although seasonal variation has previously been reported for T_3 (DeLiberto et al. 1989, Ryg and Langvatn 1982, Ryg and Jacobsen, 1982), we did not observe patterns consistent for each year with changes in daylength. Levels of T_3 were highly variable but similar to levels reported previously for white-tailed deer (DeLiberto et al. 1989, DelGiudice et al. 1992, Bahnak et al. 1981) and mule deer (DelGuidice et al 1990*a*).

T_4 levels were not influenced by age, but were correlated to changes in daylength. Levels of T_4 increased substantially in summer (2-fold) compared to winter. Seasonal influences on T_4 levels have previously been reported for white-tailed deer (Bahnak et al. 1981). Levels of T_4 were lower than those previously reported for white-tailed deer (DelGiudice et al. 1992, Bahnak et al. 1981, Seal et al. 1972, DeLiberto et al. 1989) and mule deer (DelGiudice et al. 1990*a*).

Serum Creatinine

Creatinine is a creatine metabolic end product excreted by the kidneys (Thomas 1993) and thought to be directly related to muscle mass in the body. Both age and photoperiod significantly influenced creatinine levels. Creatinine levels were elevated in the HICON group compared to the JUV group over the entire study. This result was expected if in fact creatinine levels are directly related to muscle mass of the body. Increasing daylength was inversely correlated to levels of serum creatinine. Seasonal variation has not previously been reported for cervids, but Tedesco et al (1991) reported seasonal variation in their analysis of serum chemistry in muskoxen (*Ovibos moschatus*). Levels of creatinine in serum were lower than levels reported previously by Wolfe et al. (1982) for elk but were similar to levels reported for white-tailed deer (DelGiudice et al. 1992, Kie et al. 1983).

Serum Urea Nitrogen

Serum urea nitrogen levels differed between age groups but were similar among seasons. Although age effects have not previously been reported for SUN in cervids, we documented a distinct difference between age classes early in the study. This difference decreased and disappeared after 1 yr. of age. Levels of SUN were slightly greater than levels previously reported for adult elk (Wolfe et al. 1982) and white-tailed deer (Warren et al. 1981, DelGuidice et al. 1992, Kie et al. 1983, Seal et al. 1978*b*).

Glucose

Age or season did not significantly influence serum glucose levels. Increased glucose levels at the beginning of the study probably were related to sedation protocol (Cook et al. 1994). Protocol for blood collection was changed in December of 1993; consequently, glucose levels declined in the study groups. Reference levels of glucose were within levels reported previously for elk (Wolfe et al. 1982) and white-tailed deer (Kie et al. 1983). Several other authors reported higher levels of serum glucose in white-tailed deer (Seal et al. 1978*b*, DelGuidice et al. 1987*a*, DeLiberto et al. 1989) however.

IGF-1

Both age and season significantly influenced IGF-1 levels. In terms of age, the JUV group exhibited lower levels of IGF-1 compared to the HICON group until reaching 1 year of age, when levels converged and then remained similar. IGF-1 levels were greater in summer (2.5 times) compared to winter. Seasonal changes of similar magnitude and timing have been reported for male red deer in New Zealand (Webster et al. 1996, Suttie et al. 1989). Our reference levels of IGF-1 were higher than levels reported by Webster et al. (1996) and Suttie et al. (1989) for male red deer in New Zealand, however red deer are of smaller body size than elk.

Urine Chemistry

Levels of UCa:Cr were not significantly influenced by age or photoperiod. We observed high variability between sampling dates and large standard errors associated with the

measurement of UCa:Cr in our study. This has been reported in other studies as well (DelGiudice et al. 1990*b*, DelGiudice et al. 1987*b*). This large variability precludes the potential use of this index. Ratios of UCa:Cr were similar to those reported for white-tailed deer (DelGiudice et al. 1990*b*, DelGiudice et al. 1987*b*). Urinary phosphorus:creatinine (UP:Cr) ratios were not significantly influenced by age or photoperiod. Ratios of UP:Cr were slightly lower than levels reported for free-ranging elk, (DelGiudice et al. 1991) but similar to mule deer (DelGiudice et al. 1990*a*) and white-tailed deer (DelGiudice et al. 1990*b*, DelGiudice et al. 1987*b*).

Analysis of age effects indicated that UK:Cr levels were significantly elevated in the JUV group but converged with time to be similar to levels observed in the HICON group. No significant seasonal effects were observed for UK:Cr ratios. Ratios of (UK:Cr) were higher than those reported for white-tailed deer (DelGiudice et al. 1987*b*) but similar to ratios reported for black-tailed deer (Parker et al. 1993). Ratios of UNa:Cr were not significantly influenced by age or season. Ratios of UNa:Cr were higher than those reported for elk (DelGiudice et al. 1991) but similar to levels observed in mule deer (DelGiudice et al. 1990*a*) and white-tailed deer (DelGiudice et al. 1990*b*).

Levels of UCor:Cr were not significantly affected by age or season. Ratios of UCor:Cr were similar to levels reported for black-tailed deer (Parker et al. 1993, Saltz and Cook 1993) but were greater than levels reported for mule deer (Saltz and White 1991, Saltz et al. 1992). Levels of UUN:Cr also were not significantly influenced by age or photoperiod. Ratios of UUN:Cr were greater than those reported previously for free-ranging elk (DelGiudice et al 1991), mule deer (Saltz et al. 1992), and similar to ratios reported for white-tailed deer (DelGiudice et al. 1995, Warren et al. 1981) and black-tailed deer (Parker et al. 1993).

MANAGEMENT IMPLICATIONS

Reference values presented in this study may be used to assess nutritional status of elk herds. Comparisons between young female elk in good to excellent condition (our study) and free-ranging elk female elk of similar ages are possible. Accurate comparisons, however, require that cow elk be sampled during fall, at the end of lactation and prior to breeding for the most accurate results. Further, influences such as age and season have confounding effects on many variables, so care must be taken to make only appropriate comparisons. Planning to sample free-ranging elk at appropriate times and determining and documenting age are important when assessing serum and urine chemistry.

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Influences of Nutrition and Condition on Serum and Urine Chemistry in Elk

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ABSTRACT

We assessed the reliability and sensitivity of serum and urinary chemistry variables as indices of nutrition and body condition in young elk (*Cervus elaphus nelsoni*). Nutrition was used to manipulate body condition in Experiment I, and in Experiment II, the thermal environment influenced body condition. Experiment I lasted for 22 months and included 18 young cow elk randomly assigned to treatment groups that induced (1) good-excellent body condition year-round (HICON), (2) reduced body condition year-round (LOCON) or (3) seasonally fluctuating body condition (SEACON) similar to that of free-ranging elk (n = 6 elk/group). Experiment II was conducted over 4 winters and included 25-36 elk/year that were randomly assigned to 4 different thermal cover treatments. Significant body mass (BM) differences occurred only between the NC (no cover) group and the SC (satisfactory cover) group during each year of Experiment II, therefore only data from these 2 groups were included in our analyses. Changes in BM were used as a relative measure of body condition in both studies. Serum and urine samples were collected monthly during Experiment I, and 5 times during each winter of Experiment II. Elk in both experiments were weighed twice per week and feed intakes were recorded daily. Results from the suboptimal condition analysis (Expt. I) indicated that UUN:Cr, UK:Cr, UCa:Cr, glucose, ALT, SUN, cholesterol, triglycerides, T_3, T_4, IGF-1, and creatinine differed significantly at several sampling dates. In the seasonal nutrition analysis (Expt. I-HICON vs SEACON), total bilirubin (TB), IGF-1, T_3/T_4, glucose, creatinine and T_3 differed significantly, and for the LOCON vs SEACON analysis, cholesterol, IGF-1, TB, T_3, SUN, T_4, creatinine and T_3/T_4 were significantly different at several sampling dates. In Experiment II, variables that were significant and exhibited

relationships with BM loss included: serum potassium, glucose, IGF-1, T_4, and UUN:Cr. Variables that responded as predicted based on food intake and body mass dynamics included: glucose, T_3, T_4, T_3/T_4, cholesterol, triglycerides, and IGF-1. Of these however, only IGF-1 appeared to be consistently sensitive and reliable for indicating body condition or nutrition status. Therefore, further assessment of IGF-1, for elk of different ages and reproductive stages may be of value to wildlife managers.

INTRODUCTION

Influences of nutrition and condition on serum and urine chemistry variables have not been assessed in elk under controlled experimental conditions except for several recent urinary assessments in free-ranging (e.g., DelGiudice et al. 1991, Garrot et al. 1996) and captive elk (e.g., Vagnoni et al. 1996, Garrott et al. 1997). Thus, there are few studies that elk biologists might use to select appropriate variables to assess nutritional status and condition of elk herds. Elk biologists must therefore extrapolate from studies of serum and urine chemistry in deer. Moreover, most studies of urinary and serum indices in deer only focused on winter and adult age classes. There are numerous conditions for which little is known about serum and urine indices.

Hobbs (1987) noted that the value of indices as measures of nutrition largely depends on sensitivity to changes in the attributes they are intended to index. Indices that perform well are those that change significantly and consistently in response to relatively small changes in the attributes they index. Indices that are effective only for identifying large differences in nutrition or condition often indicate what is already obvious to field biologists. Assessments of serum and

urine chemistry indices often involved experimental approaches that compared indices between groups of animals offered markedly different nutritional regimes (e.g., Seal et al. 1978*b*, Bahnak et al. 1981), or included treatment groups wherein animals were starved for several weeks (e.g., DelGiudice et al. 1987*a*, 1990*b*). Such assessments provided valuable initial indications of the value of serum and urine indices. However, with regard to elk, additional work needs to be included to assess sensitivity of serum and urine indices to small but significant changes in animal nutrition or condition.

Our primary goal for this research was to compare and contrast differences in serum and urine chemistry of groups of elk held at different levels of condition, induced by either nutrition or thermal cover, to identify indicator variables most sensitive to differences in condition. This goal was based on analysis of data collected in 2 different experiments conducted under markedly different protocols.

The National Council of the Paper Industry for Air and Stream Improvement, Northwest Forest Resource Council, Boise Cascade Corporation, U.S. Forest Service-Pacific Northwest Forest and Range Experiment Station, National Fish and Wildlife Foundation, Rocky Mountain Elk Foundation, and Oregon Department of Fish and Wildlife provided logistic and financial support. We are indebted for the assistance and contributions of B. Johnson, R. Riggs, C. Bowers, M. Buhler, S. Clark, D. and J. Hengel, and V. Walker. T. McCoy and S. Parish provided guidance on raising and training elk calves. Research protocols involving captive elk were in accordance with accepted animal welfare protocol (Wisdom et al. 1993).

STUDY AREA

The study area located near La Grande in northeast OR (see Chapter 3) was partitioned into 9, 2.3-ha square treatment units, 3 of which were clearcut, 3 were selectively cut such that 40-60% canopy cover remained, and 3 were left unharvested by random assignment (Figure 4.1). The latter provided dense cover ranging from 70-90% canopy cover. In addition, a fourth treatment was integrated into the study area in which elk had access to both dense forest cover and clearcut units. The treatments were referred to as the no-cover (NC), marginal cover (MC), [e.g. Thomas et al. (1988)], satisfactory cover (SC) [e.g., Thomas et al. (1988)], and combination cover (CM) treatment units.

An 8 x 25-m elk holding pen was located at the center of each treatment unit. The 3 pens for the CM units measured 7 x 70 m, with 50 m extending into SC and 20 m extending into the NC. A small barn (3 x 4 m) with 3 stalls was located adjacent to each pen, and was connected to each pen with a weighing chute (Figure 4.1). The barns were used for weighing, feeding, and collecting blood and urine samples. Barn stalls were equipped with a grated floor and a supporting structure to hold urine collection pans under the floor as described in Chapter 3. This portion of the study area, constructed in 1990-91, consisted of a total of 12 pens with barns and accommodated a total of 36 elk that were used in Experiment II.

One of the clearcut areas was elongated to accommodate construction of 4 additional pens in 1993 (described in Chapter 3). These pens were constructed specifically for conducting Experiment I, and were 0.10 ha in size, square, and arranged side by side in a rectangular complex on the south side of the clearcut (Figure 4.1). Trees provided shade on the south side of each pen, and barns were constructed on the north side for feeding, weighing, and collecting physiological samples. This

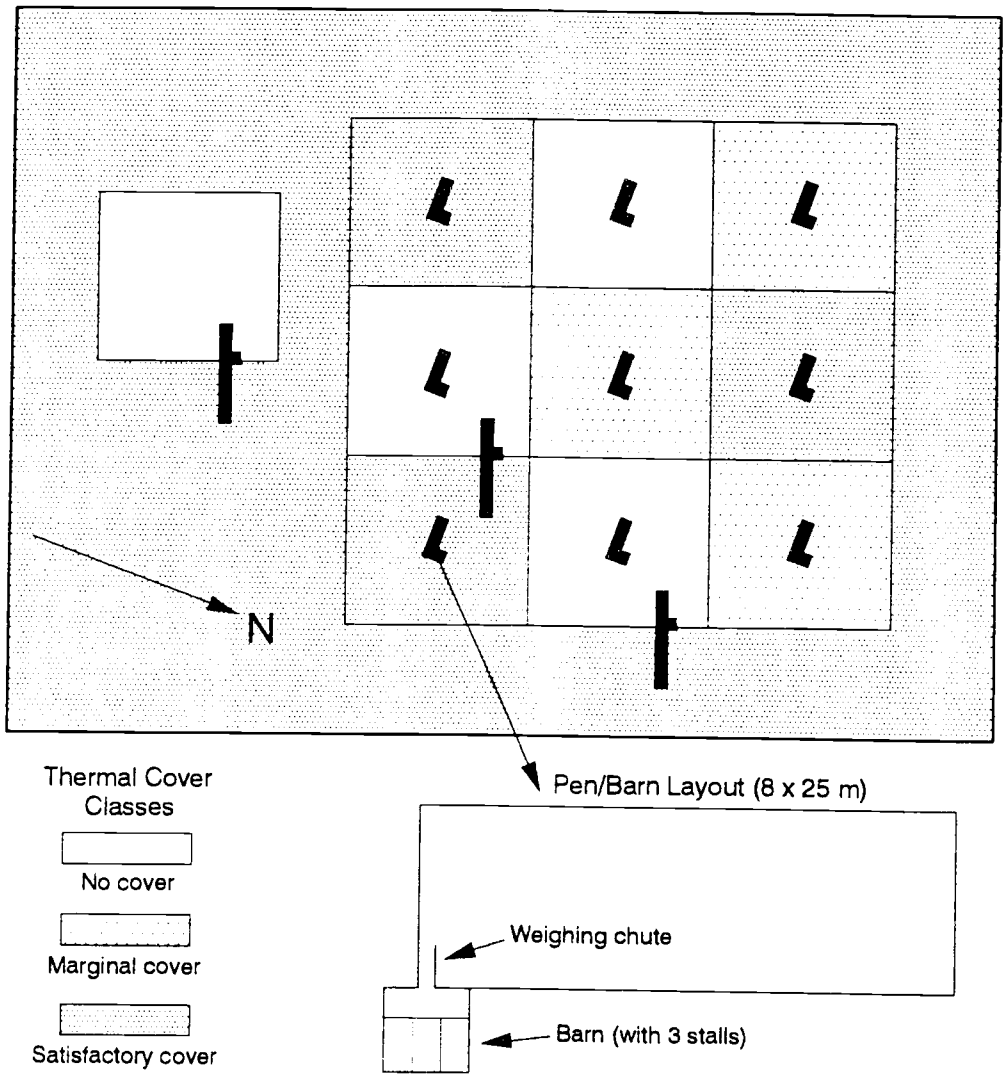

Figure 4.1: Layout of 2.3 ha-forest cover treatment units and elk holding pens in Northeast Oregon. The 3 elongated pens represent the combination cover treatment units, where elk had access to both the no-cover and satisfactory cover areas.

portion of the study area was designed to accommodate 6 elk per pen for a total of 24 elk. Climate and vegetative descriptions of the study were presented in Chapter 3.

METHODS

Experiment I (partially described in Chapter 3) was conducted over 22 months beginning in early September 1993 and ending June 1995 (see Chapter 3). Eighteen cows, all 2.5 years-old at the beginning of the study, were divided into 3 treatment groups to provide 3 body condition levels: (1) cows in good-excellent condition year-round maintained on high quality rations fed ad libitum (HICON group), (2) cows in reduced condition year-round induced by feeding a moderate quality ration at restricted levels (LOCON group), and (3) cows in reduced condition in winter and good condition in summer/autumn induced by feeding reduced quality and reduced quantity in late autumn and winter in high quality ad libitum in other seasons (SEACON group). Serum and urine samples collected at 1-month intervals were used to compare and contrast serum and urine chemistry among these 3 treatment groups.

Experiment II comprised a portion of a larger study (Cook et al. in press) conducted over 4 winters from December 1991 through March 1995 that assessed effects of thermal cover on condition of young elk. In this study, differences in thermal environments among the NC, MC, SC, and CM forest treatments induced moderate, but significantly different over-winter body mass (BM) and fat catabolism dynamics in calves and yearlings despite identical, highly controlled nutritional regimes. The resulting differences in condition in animals on identical

diets provided a unique basis to assess relationships between relatively small differences in condition and serum and urine chemistry.

Experimental Animals

Two cohorts of female elk were bottle-raised during the summers of 1991 and 1993. Cook et al. (1996) presented details of capture and training. The 1991 cohort consisted of 27 elk that were used in the 1991-92 winter trial as calves and again in the 1992-93 winter trial as yearlings (for Experiment II). Then, 18 of the 1991 cohort were randomly assigned to 3 treatment groups and used in Experiment I on 27 August 1993 (i.e., 6 elk per pen). The 1993 cohort consisted of 45 elk calves of which 6 were randomly assigned to a fourth pen for Experiment I. The remaining 36 were used during the 1993-94 winter trials as calves and in the 1994-95 winter trials as yearlings. Data were collected year-round from September 1993 through early summer 1995 for Experiment I and data were collected for 2 winters using calves and 2 winters using yearlings for Experiment II.

General Handling

Elk generally were handled and fed as described in Chapter 3. Briefly, elk received their pellets individually in the barns at 0800 hours, and hay was fed communally in the pens at 1400 hours. Elk were held in the barns up to 4 hours each day in Experiment I and up to 1 hour in Experiment II to eat pellets. Uneaten food (normally pellets only) was weighed each day. Elk were weighed twice weekly as they entered the barn for the morning feeding.

Blood and Urine Collection and Analysis

Detailed methods for blood and urine collections relevant for both experiments were presented in Chapter 3, except for some differences in sedation with xylazine hydrochloride in Experiment II. In Experiment II during the winters of 1991-92 and 1992-93, elk were brought into the barns, injected with xylazine hydrochloride, and blood was sampled within 5-30 minutes. Immediately thereafter, sedation was reversed with yohimbine hydrochloride, and urine was collected from the urine collection pans within 1-3 hours after yohimbine reversal. During the winters of 1993-94 and 1994-95, urine was collected the day before blood collection without the use of any chemical restraint, and blood was collected within 10 minutes after xylazine injection. This 10-minute limit should have removed all substantial drug effects on serum (Cook et al. 1994). Justification for changes in blood and urine collection protocol was presented in Chapter 3.

Blood samples were obtained by jugular venipuncture, and harvested serum was frozen in 1.8-ml aliquots until laboratory analysis. Likewise, urine was aliquoted into 1.8-ml freezer vials and stored frozen until laboratory analysis. In Experiment I, individual serum and urine samples were obtained. In Experiment II, serum and urine samples were pooled using equal amounts of serum and urine from each of either 2 or 3 elk within a pen, thus providing 1 sample per pen and 3 samples per treatment. Levels of IGF-1 were determined by radioimmunoassay as described in Chapter 2. Measurement of T_3 and urinary cortisol were conducted at Phoenix Central Laboratory (Tacoma, WA) and all other assays were conducted at Interpath Laboratories in Pendleton, Oregon.

Treatment Descriptions

Experiment I--During the 5 months prior to the beginning of this experiment in September 1993, all cows were fed ad libitum high quality feed that exceeded nutritional requirements for digestible energy (DE) and crude protein (CP). Thus, all cows were in good to excellent condition at the beginning of the experiment. Blood and urine were collected on 2 September 1993, prior to implementing the dietary treatments on 11 September 1993. This facilitated before and after comparisons of variables and allowed us to examine variation among the treatment groups prior to the experiment.

We continued to feed elk in the HICON group the high quality ration ad libitum through the end of the study to maintain their good to excellent condition. Elk in the LOCON group were forced to lose 10-15% of their body mass early in the study and held at 15% below that of the HICON group thereafter. The feeding regime used to induce this loss was presented in Chapter 3. The SEACON group of elk were forced to lose 10-15% of their body mass over winter (from late November through mid-March) and fed to permit recovery of condition the following spring and summer. The feeding regime for these 2 seasons was identical to that of the HICON group during late-March through mid-September, to coincide with the period when native vegetation is of good quality for free-ranging elk. After mid-September, dietary quality and quantity were reduced for SEACON elk by mixing a low energy (1.96 kcal of DE/g) and a high energy (3.2 kcal of DE/g) pelleted ration in different ratios, such that pellet DE declined from 3.2 kcal in mid-September to 1.96 kcal by late November. Through the rest of autumn and winter, pellet quality was 1.96 kcal, and feeding levels were adjusted weekly or biweekly to gradually achieve

the desired weight loss (10-15%) by early March. Throughout the year, alfalfa hay was fed in a 60:40 (pellet:hay) ratio to all elk. CP of all rations ranged from 15-18%.

Experiment II--Each of the 4 winter experiments began in early December and terminated mid-March. Elk were fed a high quality diet (the same as fed to the HICON group) during fall prior to the beginning of each experiment. Beginning 10-14 days before each experiment began, elk were randomly assigned to the 12 pens used in this portion of the study, and dietary quality and feeding level were reduced to those of the winter experiment. This change in diet prior to the beginning of the experiment was intended to minimize dietary-change effects on the first serum and urine samples collected during the experiments. Serum and urine samples were collected during the first 2-3 days after experiment initiation, and thereafter collected at 3- to 4-week intervals, 5 times per experiment.

The winter feeding regime was designed to provide submaintenance levels of DE and to induce average body mass losses of about 5% in calves and 10% in yearlings. Animal-specific feeding levels were set at the beginning of each winter trial and held constant through winter. Elk in each experiment received identical amounts of food, on a metabolic body mass basis (e.g., grams of dry matter/kg of $BM^{0.75}$/day). They were fed a pellet of moderate energy content (2.7 kcal of DE/g) and 11-12% crude protein and alfalfa hay from the same source as that for experiment I (2.6 kcal of DE/g and 16-18% CP) (Table 4.1). The pellet:hay ratio averaged about 60:40 over these winter experiments. Restricting daily feeding levels to 55-59 g of dry matter/kg MBM or 152-155 kcal of DE/kg MBM achieved BM loss goals.

Data Analysis

Experiment I--Body mass data and serum and urine chemistry data were analyzed as described in Chapter 3 using a univariate fixed effects repeated measures ANOVA with the Huyhn-Feldt correction to the numerator degrees of freedom to account for violations of the sphericity assumption (SAS Institute 1988:605). $P \leq 0.05$ was accepted as significant for all tests. For each of the subset ANOVAs and the body mass ANOVA, least significant difference (LSMEANS) were used to identify sampling dates when significant differences occurred.

We conducted the repeated measures analysis on serum and urine chemistry in 2 stages. First, using all data collected from the 3 treatment groups, we identified variables that were not significant for the treatment effect or time by treatment

Table 4.1. Ingredients of rations fed during the thermal cover study. Crude protein (CP), gross energy (GE), *in vitro* digestible dry matter (DDM) digestible energy (DE) are expressed on a dry matter basis

Season/ Year	Ration	CP %	GE (kcal/g)	DDM %	DE (kcal/g)	Primary Ingredients
Wtr. 91-92	Pellets	12.3	4.34	63.5	2.76	Oats, ryegrass screenings
	Hay	18.1	4.48	63.0	2.82	Alfalfa
Wtr. 92-93	Pellets	10.5	4.42	61.8	2.73	Oats, oat hulls[a]
	Hay	17.9	4.50	59.1	2.66	Alfalfa
Wtr. 93-94	Pellets	11.5	4.27	59.7	2.55	Oats, ryegrass screenings
	Hay	17.2	4.50	58.7	2.64	Alfalfa
Wtr. 94-95	Pellets	12.1	4.35	62.2	2.71	Oats, ryegrass screenings
	Hay	18.2	4.40	57.9	2.56	Alfalfa

[a] Ryegrass screenings were commercially unavailable when this ration was produced and oat hulls were used as a substitute.

interaction and eliminated those variables from all subsequent analyses. Second, for all of the significant serum or urine variables, we conducted subsets of repeated measures ANOVA to assess several specific relationships: (1) relations between different levels of long-term condition (i.e., between the HICON and LOCON groups), and (2) relations between seasonally variable condition and serum or urine variables (i.e., between HICON versus SEAOCN and LOCON versus SEACON).

Experiment II--In all 4 winters, Cook et al. (in press) found that cover generally had consistent and significant effects on BM (Figure 4.2) and body fat dynamics (i.e., measures of condition). Elk retained in dense forest cover (SC) lost BM relatively quickly in early winter and ended the winter with greater mass loss than elk in other treatments. Elk held without forest cover (NC) generally lost the least amount of BM. Body mass responses of elk in the MC and CM treatments usually fell between that of elk in the SC and NC treatments. Typically however, significant differences in BM and body composition changes existed only between elk in the NC and SC treatments. Therefore, our data show a consistent treatment effect for the NC and SC groups only.

We conducted 2 sets of analyses to identify those variables that were sensitive to BM loss. For both analyses, data from only the SC and NC treatments were used. The first analysis involved assessing differences in serum and urine chemistry for each year of data. A 1-way repeated measures ANOVA was used for this analysis. The second analysis involved assessing serum and urine differences using data from all 4 experiments. A 2-way repeated measures ANOVA was used; year and cover-treatment (NC or SC) were the 2 factors. Differences among means within time periods (sampling dates) were examined with protected least significant differences (LSDs) comparisons. The multivariate mode of ANOVA in PROC GLM (SAS Institute 1987:605) was used to conduct the repeated measures analysis.

The assumption of sphericity was tested routinely (in contrast to Experiment I, the sphericity assumption often was not violated). We used the normal univariate F-test if sphericity was satisfied and the Huyhn-Feldt adjustment to the numerator degrees of freedom when the sphericity assumption was rejected (SAS Institute 1987: 605). Pens (n = 3), rather individual elk, comprised the experimental unit for analysis of data generated in Experiment II.

During the third winter experiment of 1993-94, 9 calves either died or had to be removed from the study to prevent death. "Mortality" was due to very low body condition. When the calves became too weak to stand, they were removed from the study and treated as dead for the purposes of the study (3 actually died). Five, 3, and 1 elk were removed in the SC, MC, and NC treatments, respectively. This "mortality" confounded the repeated measures ANOVA particularly because all elk from one of the SC pens (referred to as the "lower" SC (LSC) pen) were removed after the third serum and urine sampling period. Only those data collected prior to the beginning of the majority of calf attrition (3 sampling periods) were used for the 1-way ANOVAs for the winter of 1993-94.

For the 2-way ANOVAs, 2 subsets of analyses were conducted, 1 in which the data from the LSC pen was eliminated and another in which missing data from the final 2 time periods were estimated by extrapolating from the other 2 SC pens. Thus we were able to include data from the LSC pen in the second subset of analyses. The average of each serum and urine variable from the LSC pen in the second and third time periods divided by the average of that in the other 2 pens in the same time periods provided an adjustment factor for extrapolation. This adjustment factor was multiplied by the average of the other 2 pens in the fourth time period, and was multiplied by the average of the other pens in the fifth time period to estimate the missing value in the fifth time period. For all ANOVAs the

effect of primary interest was the time (sampling date) by treatment interaction effect, because elk in all treatments would be expected to start in equivalent condition and deviate as the winter experiment proceeded.

A final set of analyses was conducted for the serum and urine variables that showed reasonable evidence of a treatment or interaction effect based on the 1- and 2-way ANOVAs. "Reasonable" included those serum and urine variables that had a significant ($P < 0.05$) interaction effect in at least 2 winter experiments, based on 1-way ANOVAs, or a significant interaction effect in either of the 2-way ANOVAs. Serum and urine variables considered to be key indicators by wildlife biologists, whether or not they satisfied the criteria of "reasonable" also were included. These were glucose, SUN, T_3, T_4, UCor:Cr, and UUN:Cr.

This final analysis was conducted using linear regression and included serum and urine data collected from all 4 treatment groups. Linear regression was used to examine the relations between body mass loss each winter and serum/urine chemistry levels. The regressions were run for data collected after mid-January, the time period in which body mass differences among treatments were apparent, and thus were intended to assess relations when significant differences in body mass occurred. Data for all 4 winters were combined in this analysis. Data collected from the SC treatment during winter trial of 1993-94 were excluded due to confounding introduced by calf attrition from the SC treatment. Each data point was based on the average for all animals within a treatment group in each winter, providing a total of 15 data values (4 each winter, 1 per treatment group, excluding the one sample from the SC treatment in 1993-94).

These regression analyses potentially suffer from lack of independence due to greater similarity within winters versus among winters resulting from (1) influences of xylazine

sedation in 2 of the 4 winters, (2) differences attributable to age (yearlings versus calves), and (3) differences due to winter weather severity. Adjustments (see below) were used to minimize the potential for this lack of independence due to among-year differences. The NC treatment was selected as a base of comparison and used to convert serum and urine estimates from the other treatments as a percent of those measured for elk in the NC treatment:

$VAR\%_i = (VAR_i - VAR_{NC})/VAR_{NC} \times 100$, where

$VAR\%_i$ = level of serum or urine variable expressed in percent, for the i^{th} treatment group (either NC, MC, CM, or SC),

$VARi$ = level of serum or urine variable in units originally measured for the i^{th} treatment group, and

VAR_{NC} = level of serum or urine variable in units originally measured for the NC treatment group.

This approach standardized serum and urine estimates in the various treatments relative to those estimated for the NC treatment. For example, if SUN averaged 35 mg/dl in the NC treatment from mid-January to mid-March, and averaged 40 mg/dl in the SC treatment over the same period, then the estimate of $VAR\%_{SC}$ would be 14.3%. Estimates of $VAR\%_{NC}$ always equaled zero using this approach.

Body mass change was calculated slightly differently:

$MD_i = MC_i - MC_{NC}$, where

MD_i = mass change differences for the i^{th} treatment in percentage point units,

MC_i = percent mass change from the beginning of the winter experiment (same units as presented in Fig. 2) for the i^{th} treatment, and

MC_{NC} = percent mass change from the beginning of the winter experiment for the NC treatment.

The different approach was used because average mid-January to mid-March percent mass loss in the NC treatment was close to zero in several winters. Dividing the difference between MC_1 and MC_{NC} by MC_{NC} and converting to percent would result in highly variable and large estimates of MD_1 that would be biologically unreasonable.

RESULTS

Experiment I

Body mass and Feed Intake Dynamics--Body mass (BM) differed over time and among treatments, evidenced by the significant treatment effect ($P = 0.0001$) and time by treatment interaction ($P = 0.0001$) in the repeated measures 1-way ANOVA. Body mass of the 3 groups prior to study initiation was not significantly different. Body mass increased in all groups during the study. However, the amount of increase differed among them: the HICON group gained 27%, the LOCON group gained 4.5%, and the SEACON group gained 9.9% over initial body mass (Figure 4.2 c).

Body mass of the LOCON group (Figure 4.2 a) was significantly different than the HICON group after December 1993 (Figure 4.2 a). Beginning at this time, BM of the LOCON group averaged about 15% less than the HICON group (Figure 4.2 c). Seasonal manipulation of diet in the SEACON group resulted in BM profiles that were similar to that of the HICON group during summer but closely resembled BM of the LOCON group during winter (Figure 4.2 a-c) as would be expected for free-ranging elk. Each fall a noticeable decline in the rate of BM change and a slight decline in actual BM (Figure 4.2 a-b) occurred beginning in late August in all treatment groups. In the case of the HICON group on the ad libitum diet, these

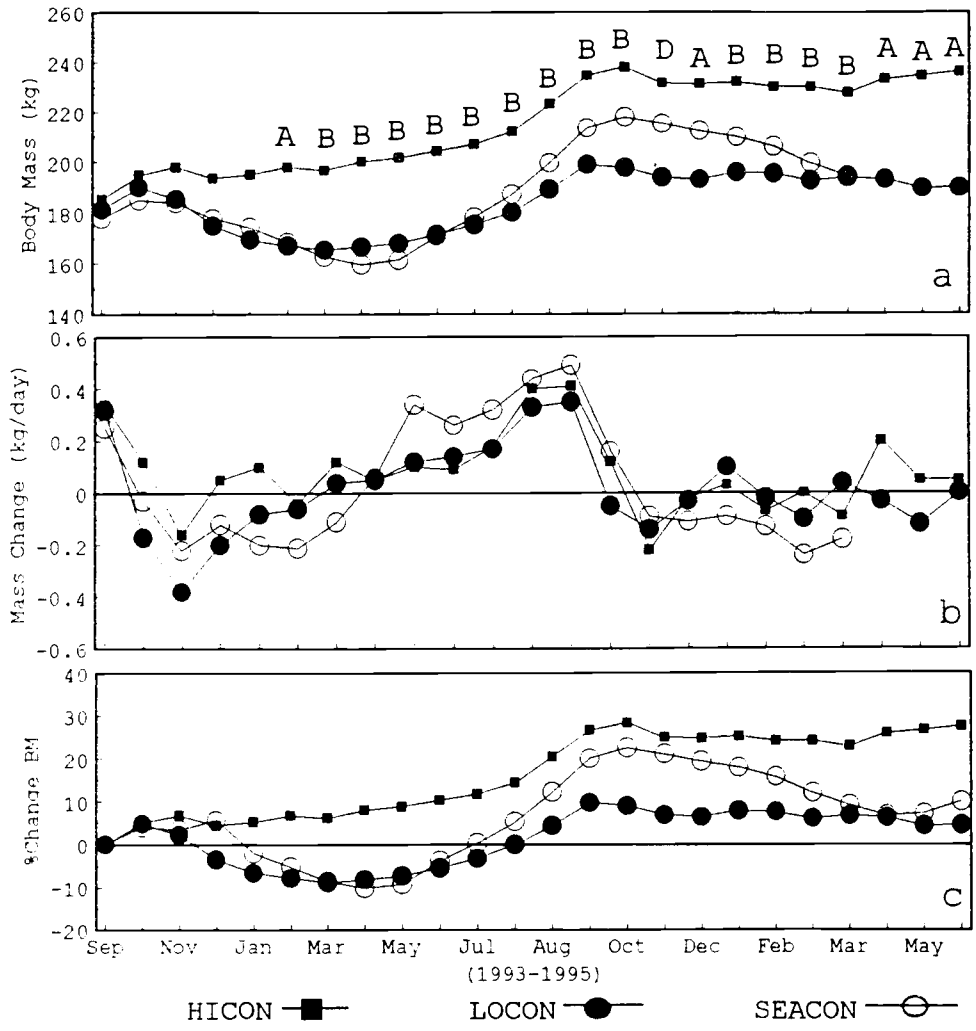

Figure 4.2 (a-c): Body mass dynamics for elk in Expt. I. Mass estimates were obtained twice weekly during the study. Significant differences are denoted with letters A, B, or C.

A=HICON differed significantly from the LOCON group

B=HICON differed significantly from the LOCON and SEACON groups

C=LOCON differed significantly from the HICON and SEACON groups

changes corresponded to voluntary reductions in food intake (Figure 4.3 a) and correlated to declining daylength (see Chapter 3 for analysis and discussion).

Indicators of Condition --Results from the overall analysis of serum and urine variables (all groups included) indicated that the following variables did not show significant treatment effects or time by treatment interactions: ALK, calcium, potassium and serum albumin (Table 4.2). These 4 variables were eliminated from further analysis. Comparisons between the HICON and LOCON groups indicated 21 remaining variables with significant treatment effects or time by treatment interaction effects (Table 4.3). Graphical profiles of those significant variables from the ANOVA revealed that 5 variables exhibited significant fluctuations unrelated to differences in body condition (Appendix E). These variables were GGT, phosphorus, chloride, TB and TP. Thus, 16 serum and urinary variables were considered significant and meaningful in the suboptimal condition analysis: UUN:Cr, UK:Cr, UCa:Cr, UNa:Cr, UCor:Cr, AST, sodium, T_3, T_4, IGF-1, ALT, glucose, SUN, creatinine, cholesterol and triglycerides (Figure 4.4).

The LOCON elk had elevated UUN:Cr, UK:Cr, UCa:Cr, UNa:Cr UCor:Cr, ALT, AST, and SUN and reduced glucose, sodium, cholesterol, triglyceride, T_3, T_4, and IGF-1 levels. Serum creatinine was elevated in the LOCON group early in the study then merged to be similar to the HICON group. During the second winter however, levels of creatinine were elevated in the HICON group. These results (Figure 4.4) suggest that the 16 variables had considerably different potential as reliable indicators of body condition. We would predict that the best indicators of body condition would show a divergent pattern between the HICON and LOCON groups in the first 2-4 months of the study when rapid BM loss occurred in the LOCON

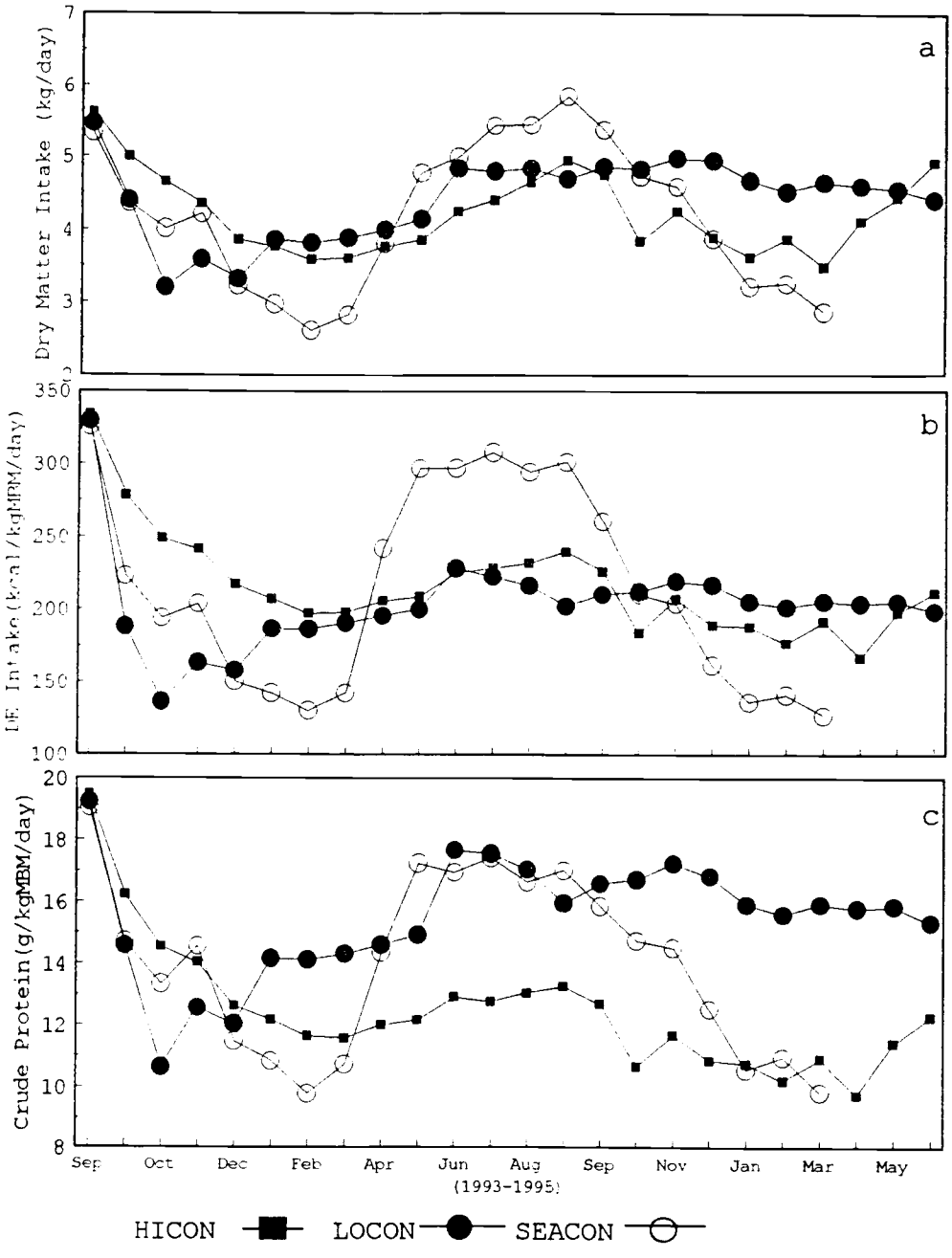

Figure 4.3(a-c): Average daily feed intakes for Expt. I. The HICON elk received an ad libitum diet year-round, the LOCON elk received a restricted diet year-round, and the SEACON elk received a seasonally changing diet.

Table 4.2: Overall results from the repeated measures ANOVA (HICON, LOCON, SEACON groups included)-Experiment #1.

Variable	Time *P*-value[a]	Time*Treatment *P*-value[a]	Treatment *P*-value[a]
UUN:Cr	0.0001	0.0001	0.0329
UK:Cr	0.0001	0.0001	0.0016
UP:Cr	0.0001	0.0166	0.1833
UCa:Cr	0.0001	0.0016	0.1088
UCor:Cr	0.0001	0.0632	0.0052
T_3/T_4	0.0001	0.0216	0.0611
T_3	0.0001	0.0001	0.0085
T_4	0.0001	0.0073	0.0001
IGF-1	0.0001	0.0001	0.0001
GGT	0.0001	0.0014	0.8175
AST	0.0001	0.0001	0.3573
ALT	0.0001	0.0001	0.0609
ALK	0.0001	0.0636	0.9127 [b]
Calcium	0.0001	0.0988	0.3763 [b]
Phosphorus	0.0001	0.0001	0.9690
Sodium	0.0001	0.0161	0.5828
Potassium	0.0001	0.0557	0.0936 [b]
Chloride	0.0001	0.0001	0.2486
Glucose	0.0001	0.0001	0.0001
TB	0.0001	0.0001	0.0673
TP	0.0001	0.0001	0.7092
ALB	0.0001	0.3245	0.6780 [b]
SUN	0.0001	0.0031	0.0163
Creat.	0.0001	0.0001	0.9327
Cholest.	0.0001	0.0001	0.4019
Triglyc.	0.0001	0.0111	0.0373

[a] Huyhn-Feldt adjusted *P*-values

[b] Variables that were not significant for treatment effects or time by treatment interactions

Table 4.3: Results from the repeated measures ANOVA examining influences of body condition (HICON and LOCON groups only).

Variable	Time *P*-value[b]	Time*Treatment *P*-value[b]	Treatment *P*-value[c]
UUN:Cr	0.0001	0.0001	0.0001
UNa:Cr	0.0001	0.0731	0.0126
UK:Cr	0.0001	0.0001	0.0001
UP:Cr	0.0002	0.0652	0.1275 [a]
UCa:Cr	0.0082	0.0450	0.0045
UCor:Cr	0.0001	0.5418	0.0141
T_3/T_4	0.0001	0.2581	0.4486 [a]
T_3	0.0001	0.0003	0.0116
T_4	0.0001	0.0006	0.0094
IGF-1	0.0001	0.0005	0.0001
GGT	0.0001	0.0088	0.4872
AST	0.0001	0.0001	0.1842
ALT	0.0001	0.0001	0.0276
Phosphorus	0.0001	0.0233	0.8899
Sodium	0.0001	0.0189	0.2315
Chloride	0.0001	0.0448	0.9208
Glucose	0.0001	0.0001	0.0001
T. Bilirubin	0.0001	0.0001	0.8419
T. Protein	0.0001	0.0001	0.8650
S. Urea Nit.	0.0001	0.0024	0.0224
Creat.	0.0001	0.0001	0.6546
Cholest.	0.0001	0.0001	0.1705
Trigylyc.	0.0001	0.0497	0.0055

[a] Variables that did not have significant treatment effects or time by treatment interactions.

[b] Reported *P*-values are Huyhn-Feldt adjusted p-values.

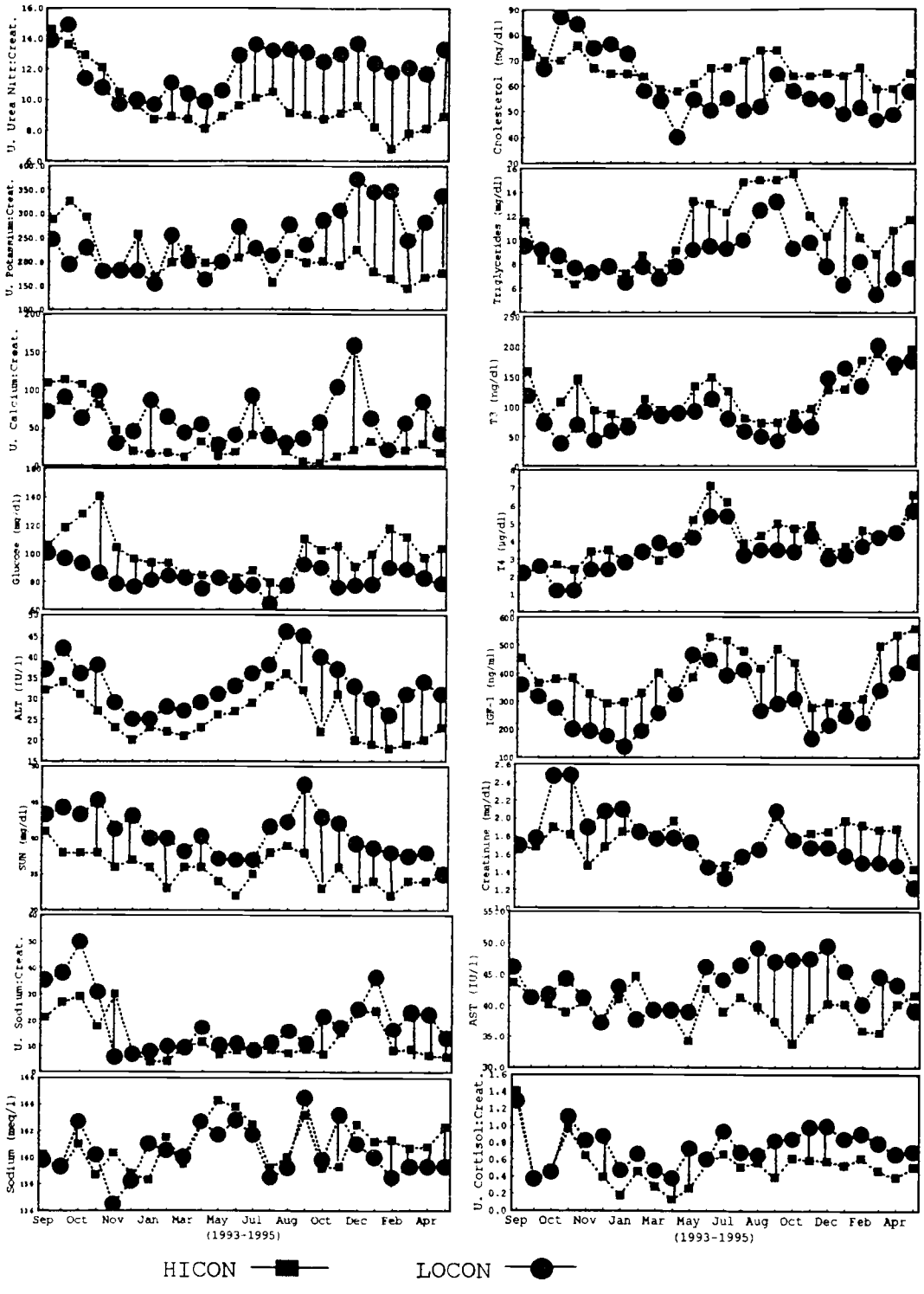

Figure 4.4: Serum and urinary variables significantly influenced by reduced body condition. Bars between data from both groups indicate significant differences at specific sampling dates ($P < 0.05$).

group and that those differences would have remained relatively constant throughout the study. Most of these variables however, did not perform well given this basis for assessment. Urinary Urea N and SUN were higher in the LOCON elk, a result in direct contradiction of expectations (unless they were rapidly catabolizing muscle tissues over the course of the experiment, which is unlikely). Cholesterol and triglycerides did not exhibit differences until the summer of 1994 and those differences appeared sporadically. ALT levels also became elevated in the LOCON group during the summer of 1994 but levels remained elevated throughout the remainder of the study. Only IGF-1 exhibited differences that were consistent and of relatively large magnitude (approximately 50% higher in the HICON group throughout much of the study).

Comparisons between the HICON and SEACON treatment groups indicated 10 serum and urinary variables differed significantly (Fig. 4.5). Four serum variables were not included because they exhibited irrelevant patterns or were only significantly different at 1-2 sampling dates (Appendix F). We predicted the better indicators of condition would show, after the first 2-3 months of the experiment, significant differences from mid-fall through early spring and little or no significant differences in summer and early fall (i.e., divergence in winter and convergence in summer). However, these patterns might not hold for variables that change in response to compensatory growth phases. Such variables would be expected to differ primarily during spring and early summer.

Several of the variables did not follow either of these predictions, however. Total bilirubin, creatinine, and chloride tended to be elevated in summer and depressed in winter in the SEACON group relative to levels in the HICON group. T_3 and the T_3/T_4 ratio tended to diverge in winter and converge in summer among the 2 groups as predicted, but the divergence appeared mostly limited to the first winter. Levels

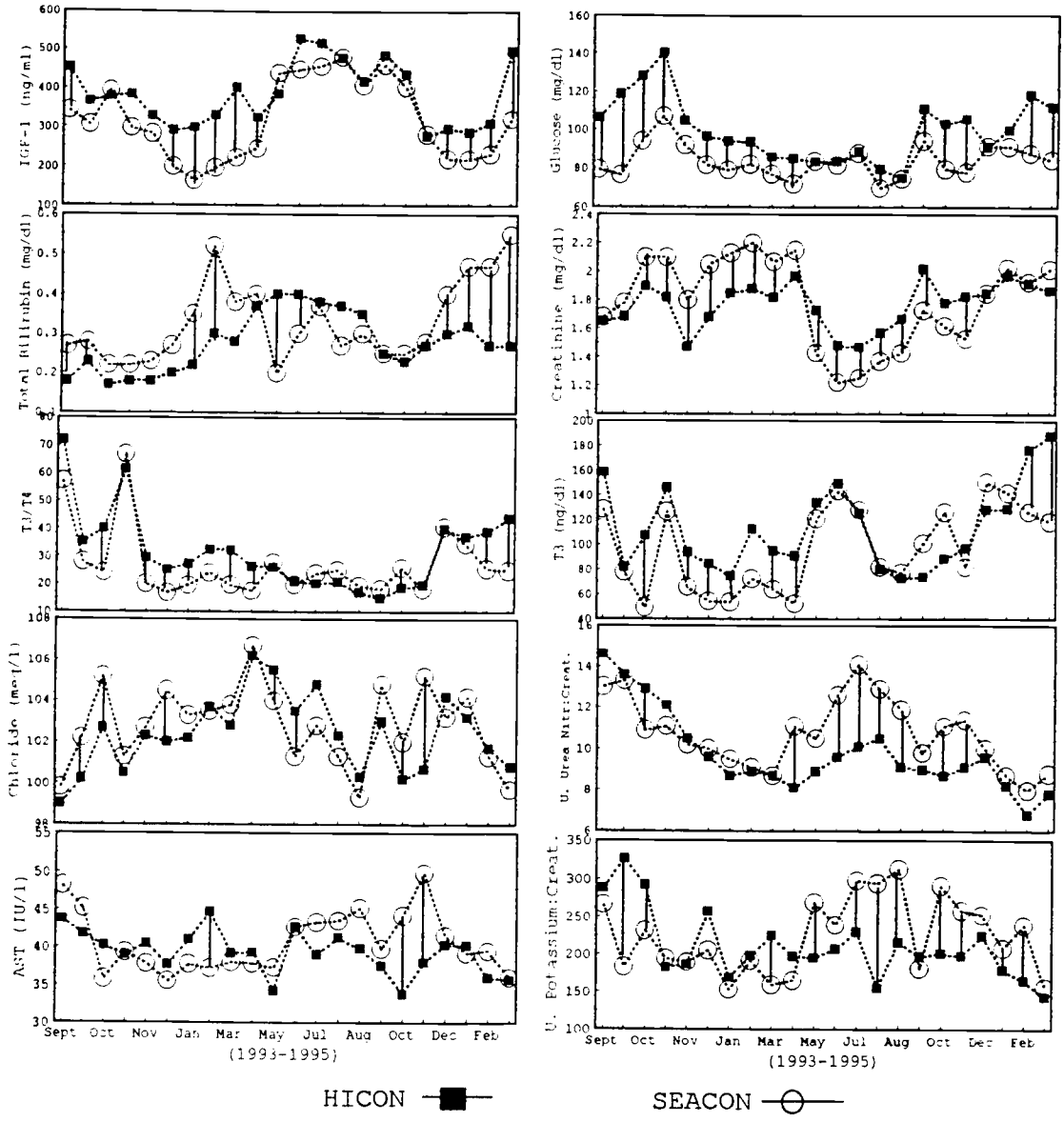

Figure 4.5: Serum and urine variables significantly influenced by seasonal nutrition (HICON vs. SEACON) ($P < 0.05$). Significant differences at sampling dates are indicated with bars.

of UUN:Cr and UK:Cr, and AST were elevated in the SEACON group during the fall of 1994 only. Only IGF-1 and glucose closely followed the predicted pattern of winter divergence and summer convergence over the course of the study.

Comparisons between the LOCON and SEACON treatment groups indicated 12 variables with significant differences (Figure 4.6). Eight of these exhibited irrelevant patterns or were significant at only 1-2 sampling dates (see Appendix G). We predicted that indicators of condition would converge in winter and diverge in summer, because the 2 treatment groups would be in similar condition during winter and in different body condition states in summer (dietary regimes, particularly food intake, deviated from this pattern slightly and may have confounded this prediction). This prediction generally held for IGF-1, T_3, and to some extent for cholesterol. Urinary urea nitrogen, SUN, and ALT levels in the SEACON group were significantly lower in the SEACON group at several sampling dates during the study and data patterns deviated from our prediction. Total bilirubin and creatinine levels were greater in the SEACON group in winter and lower in summer than the LOCON group. Urinary cortisol levels were significantly elevated in the LOCON group during the fall of 1994 only. Of these 12 variables, only IGF-1 and T_3 followed the predicted patterns reliably.

Experiment II

Weather and Body Mass Dynamics-- Weather during the 4 winters of this study ranged from relatively mild to relatively severe in relation to long-term averages of the Blue Mountains Ecoregion (Cook et al. in press). Temperature was mildest during the first winter, coldest during the second winter, and intermediate during the third and last winter (Table 4.4).

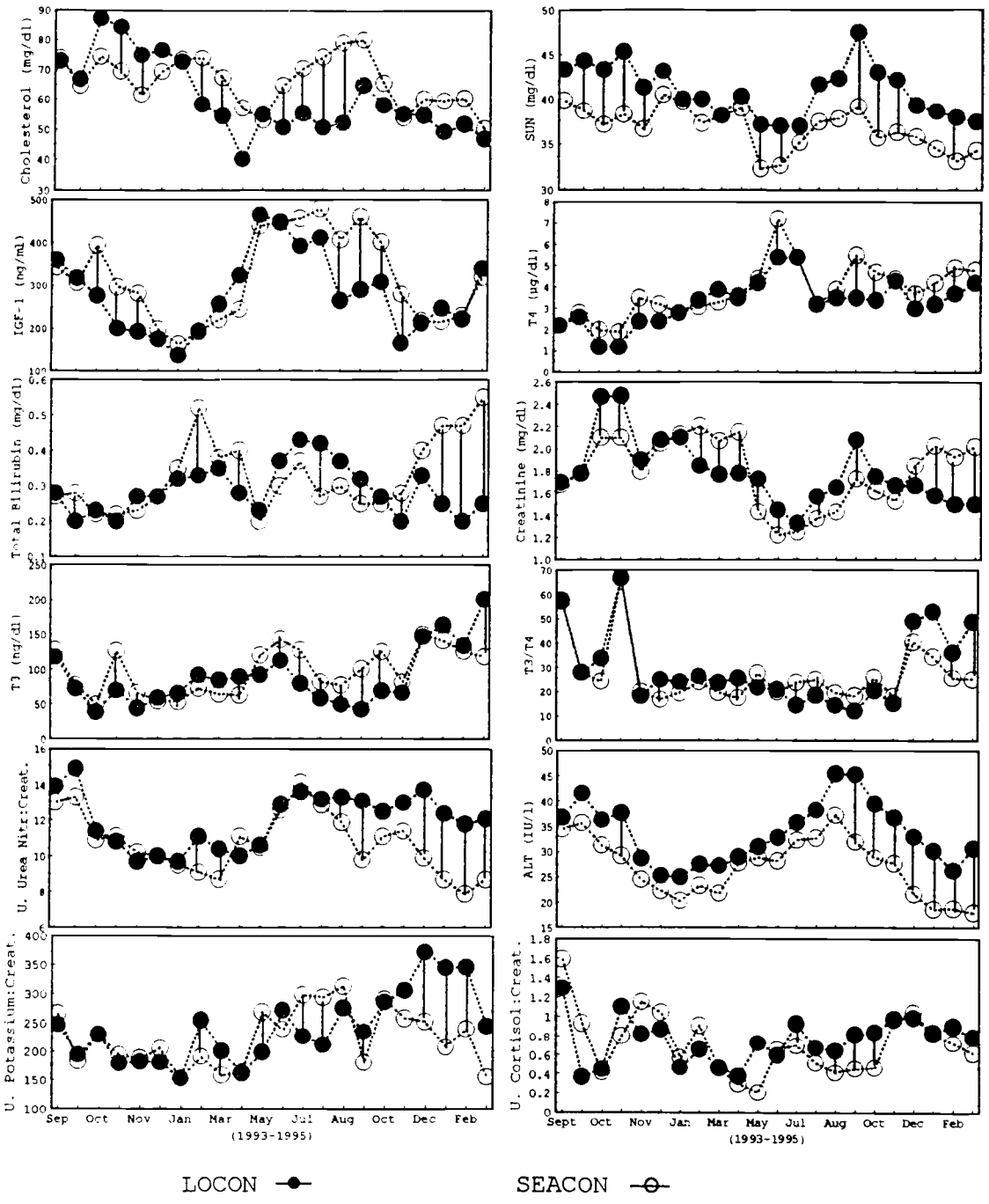

Figure 4.6: Serum and urinary variables significantly influenced by seasonal nutrition (LOCON vs. SEACON)($P < 0.05$). Means that differed significantly between the two groups are indicated on each graph with a bar.

Table 4.4: Winter weather summary for Expt. II. Average minimum, maximum temperatures (°C) are given along with average windspeed (m/s) for each winter of the thermal cover experiment. Within months, means with different letters are significantly different ($P < 0.05$) based on analyses by Cook et al. (in press).

	December		January		February		March		Winter Avg.	
Year	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Minimum Temperatures										
1991-92	-2.7a	0.9	-3.9a	0.7	-2.1a	0.6	-0.3a	0.4	-2.4a	0.4
1992-93	-7.6b	0.7	-9.6b	0.8	-8.3b	1.1	-2.3bc	0.6	-7.1b	0.5
1993-94	-4.7ac	0.6	-3.3a	0.6	-6.6b	1.0	-3.4b	0.8	-4.6c	0.4
1994-95	-6.3bc	0.9	-4.1a	0.8	-3.4a	1.1	-0.8ac	1.2	-4.2c	0.5
Maximum Temperatures										
1991-92	3.9a	0.4	3.1a	0.6	8.0a	0.8	10.8a	1.0	6.3a	0.5
1992-93	-1.0b	0.6	-2.5b	0.8	1.1b	0.8	6.3b	0.6	0.8b	0.5
1993-94	1.8ac	0.6	4.2a	0.7	2.9b	0.5	8.2ab	1.1	3.9c	0.4
1994-95	0.5bc	0.9	4.1a	0.8	6.0a	1.1	7.9ab	1.2	3.6c	0.6
Average Windspeed										
1991-92	1.6	0.2	1.5	0.1	1.2	0.1	1.2a	0.1	1.4ab	0.1
1992-93	1.6	0.1	1.4	0.1	1.4	0.1	1.3ab	0.1	1.4a	0.1
1993-94	1.4	0.1	1.3	0.1	1.3	0.1	1.1a	0.1	1.3b	0.1
1994-95	1.4	0.1	1.5	0.1	1.1	0.1	1.6b	0.2	1.3ab	0.1

Colder temperatures likely accounted for the relatively greater body mass loss during the second winter compared to that during the other winters (Figure 4.7). We removed elk from the study, as a result of poor body condition, during the third winter (Figure 4.7) in an attempt to prevent death. Greater mass loss during this winter was attributed to 2 periods of cold, rainy weather occurring during the first half of the study. The affects of forest cover on solar radiative flux undoubtedly accounted for differences in elk body mass dynamics among treatments (i.e., better performance of elk in the NC treatment-see Figure 4.7). The trend of mass attributed to substantial increases in total daily solar radiation flux was mostly due to increasing daylength and to moderating weather severity (Cook et al. in press).

Cook et al. (in press) concluded that BM dynamics under the conditions of this study provided a useful index of changes in body condition, a key assumption for the serum and urine analyses included here. Their estimates of body composition data for the 3 winters the data were collected indicated that patterns of fat catabolism and losses of total endogenous energy mirrored patterns of BM dynamics. Additionally, they noted that the elk removed from the winter experiment (1993-94) had lost the greatest amount of BM of any animals in the study at the time of removal. This supports our contention that BM served as a useful index of body condition in this study.

Effects of time and year on serum and urine chemistry are briefly presented (Tables 4.5-4.8), followed by results regarding the treatment by time interactions of the 1- and 2-way ANOVAs and results of the body mass dynamics-serum/urine chemistry regression analyses.

Sampling Period (Time) Effects on Serum and Urine Chemistry--Significant differences in serum and urine chemistry across winter experiments (main effect of time) probably were indicative of either photoperiod effects or within-treatment

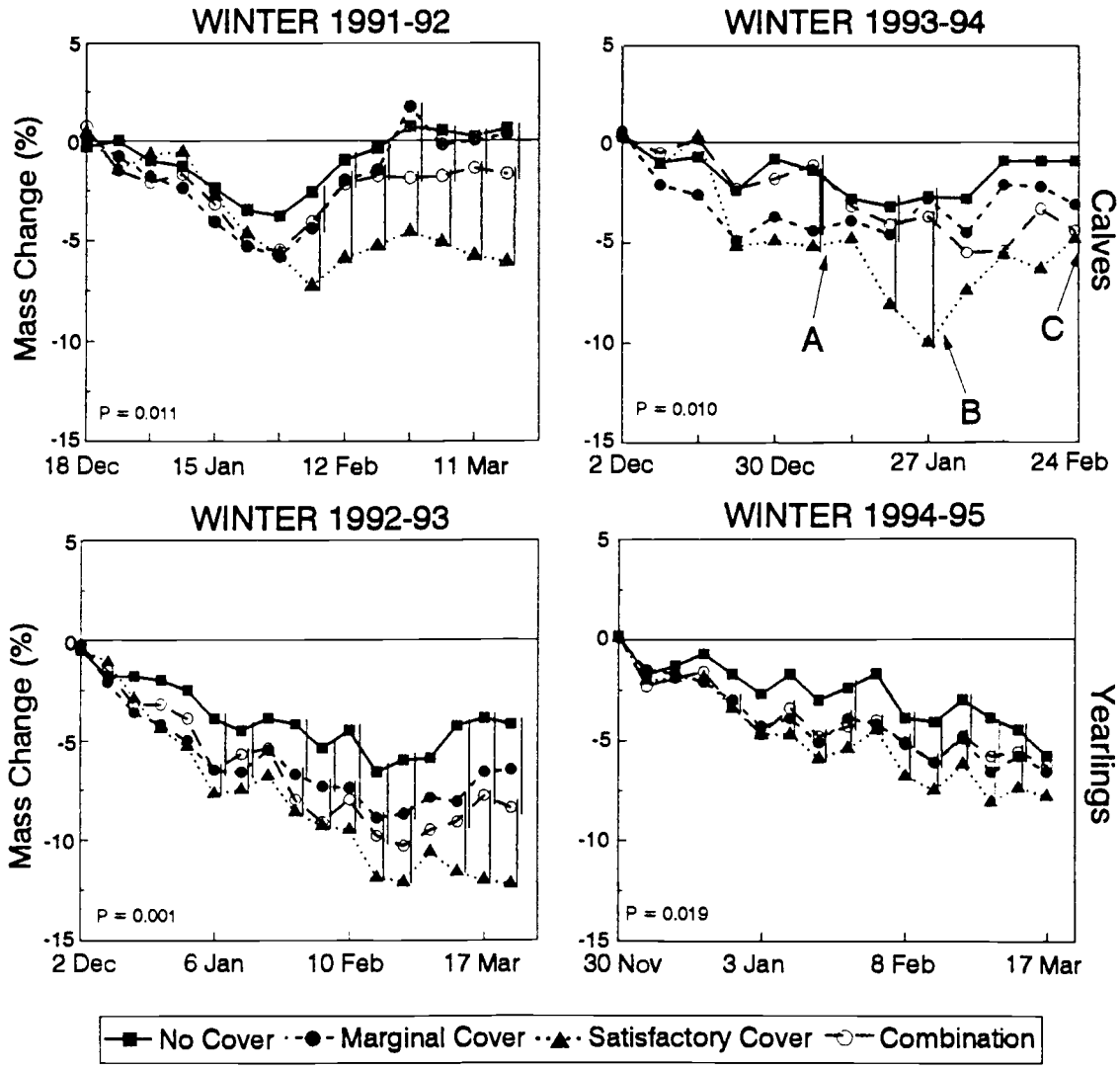

Figure 4.7: Body mass dynamics of female elk held in 4 forest cover treatments during winter in Northeast Oregon. Data values not connected by vertical lines differ significantly ($P < 0.05$) within weekly periods. The P values are the significance levels of the time by treatment interaction effect based on repeated measures ANOVA. In the graph for the calves during winter 1993-94, all elk were in the study prior to "A", 3 and 1 elk were removed from the satisfactory and marginal cover treatments between "A" and "B". Two elk also were removed from the satisfactory cover treatment between "B" and "C" (graphs modified from Cook et al., in press).

Table 4.5: Significance levels from the 1-way ANOVA-Expt. II. *P* -values for treatment (trt) and time main effects and treatment by time interaction (inter.) effects for serum chemistry values presented in Figure 4.8.

	Calves Dec-Mar 1991-92			Calves Dec-Mar 1993-94			Yearlings Dec-Mar 1992-93			Yearlings Dec-Mar `994-95		
Serum Variable	trt	time	inter.	trt	time	inter.	trt	time	inter.	trt	time	inter.
Creatinine	0.265	<0.001	0.900	0.680	0.203	0.042	0.958	0.006	0.202	0.741	<0.001	0.601
TB	0.016	0.003	0.530	0.715	0.313	0.611	0.903	0.078	0.726	0.213	0.001	0.004
GGT	0.417	0.277	0.343	0.915	0.099	0.269	0.015	0.250	0.154	0.187	0.023	0.057
ALT	0.679	0.001	0.072	0.634	0.665	0.825	0.979	0.421	0.422	0.896	0.384	0.402
AST	0.944	0.487	0.375	0.427	0.954	0.288	0.323	0.630	0.412	0.260	0.436	0.401
Calcium	0.958	0.144	0.947	0.622	0.031	0.137	0.413	0.001	0.148	0.814	0.205	0.528
Chloride	0.369	0.003	0.433	0.123	0.251	0.819	0.906	0.004	0.728	0.808	0.608	0.422
Phosphorus	0.868	<0.001	0.136	0.017	<0.001	0.160	0.296	0.009	0.593	0.272	0.012	0.351
Potassium	0.946	<0.001	0.003	0.690	0.192	0.395	0.216	<0.001	0.923	0.663	0.531	0.154
Sodium	0.806	<0.001	0.354	0.276	0.533	0.192	0.684	<0.001	0.119	0.612	0.487	0.394
Glucose	0.320	0.005	0.325	0.084	0.278	0.636	0.079	0.007	0.424	0.250	0.014	0.505
SUN	0.087	0.002	0.108	0.328	0.070	0.536	0.424	0.359	0.484	0.021	0.196	0.220
Albumin	0.912	0.024	0.213	0.593	0.853	0.747	0.201	0.001	0.549	0.347	0.121	0.105
TP	0.632	0.207	0.881	0.761	0.172	0.351	0.122	0.197	0.628	0.264	0.511	0.792
Cholesterol	0.592	<0.001	0.354	0.177	0.085	0.687	0.466	0.013	0.841	0.168	0.001	0.477
Triglycerides	0.123	0.045	0.392	0.116	0.045	0.392	0.074	<0.001	0.786	0.645	0.002	0.741
ALK	0.866	<0.001	0.355	0.559	0.444	0.476	0.645	0.029	0.947	0.023	<0.001	0.669
IGF-1	0.483	0.076	0.386	0.243	<0.001	<0.001	0.038	0.164	0.010	0.033	0.018	0.565
T_4	0.185	0.265	0.631	0.165	0.074	0.937	0.111	0.023	0.265	0.484	0.028	0.072
T_3	--	--	--	0.150	0.277	0.735	--	--	--	0.476	<0.001	0.334

Table 4.6: Significance levels from the 1-way ANOVA-Expt. II. *P* -values for treatment (trt) and time main effects and treatment by time interaction (inter.) effects for urine chemistry values presented in Figure 4.9.

	Calves Dec-Mar 1991-92			Calves Dec-Mar 1993-94			Yearlings Dec-Mar 1992-93			Yearlings Dec-Mar `994-95		
Urine Variable[a]	trt	time	inter.	trt	time	inter.	trt	time	inter.	trt	time	inter.
UCa:Cr	0.688	0.780	0.019	0.818	0.037	0.258	0.309	0.213	0.333	0.419	0.006	0.735
UP:Cr	0.137	0.084	0.715	0.494	0.159	0.950	0.825	0.098	0.199	0.465	0.101	0.392
UK:Cr	0.553	<0.001	0.366	0.239	<0.001	0.528	0.092	0.037	0.358	0.233	0.363	0.061
UNa:Cr	0.357	<0.001	0.994	0.419	0.199	0.150	0.166	0.268	0.496	0.216	0.261	0.382
UUN:Cr	0.348	<0.001	0.854	0.253	0.224	0.294	0.070	0.140	0.419	0.361	<0.001	0.250
UCor:Cr	--	--	--	0.129	0.257	0.264	--	--	--	0.446	0.011	0.281

[a] Urine values are presented as ratios with urinary creatinine and adjusted as described in Appendix A.

Table 4.7: Significance levels from the 2-way ANOVA-Expt. II. *P* -values for treatment (trt) and time main effects and treatment by time interaction (inter.) effects for serum chemistry values presented in Figure 4.8.

	ANOVA excluding data from 1994 LNC trt group							ANOVA with extrap. data for 1994 LNC trt group						
Serum Variable	trt	year	ty	time	tm	my	tmy	trt	year	ty	time	tm	my	tmy
Creatinine	0.541	0.001	0.826	0.102	0.085	0.001	0.197	0.315	<0.001	0.547	0.067	0.042	<0.001	0.086
TB	0.164	0.003	0.431	<0.001	0.468	0.004	0.052	0.199	0.002	0.288	<0.001	0.473	0.002	0.063
GGT	0.017	0.012	0.017	0.358	0.128	0.142	0.062	0.010	0.008	0.012	0.314	0.097	0.112	0.046
ALT	0.442	0.043	0.917	0.818	0.087	0.085	0.624	0.413	0.031	0.890	0.779	0.066	0.064	0.506
AST	0.886	0.948	0.267	0.751	0.242	0.703	0.726	0.794	0.963	0.237	0.778	0.191	0.673	0.646
Calcium	0.809	0.207	0.617	0.368	0.332	0.001	0.541	0.991	0.079	0.782	0.187	0.174	<0.001	0.479
Chloride	0.864	0.097	0.753	0.315	0.404	0.047	0.842	0.763	0.057	0.621	0.216	0.365	0.029	0.763
Phosphorus	0.026	<0.001	0.377	<0.001	0.596	<0.001	0.357	0.032	<0.001	0.453	<0.001	0.565	<0.001	0.226
Potassium	0.249	0.872	0.741	<0.001	0.030	<0.001	0.127	0.334	0.922	0.683	<0.001	0.096	<0.001	0.048
Sodium	0.542	0.225	0.896	0.471	0.785	0.268	0.367	0.391	0.292	0.837	0.420	0.757	0.205	0.282
Glucose	0.045	<0.001	0.754	<0.001	0.979	<0.001	0.161	0.022	<0.001	0.814	<0.001	0.739	<0.001	0.334
SUN	0.027	0.004	0.702	<0.001	0.176	0.037	0.375	0.013	0.003	0.895	0.002	0.054	0.005	0.575
Albumin	0.712	<0.001	0.802	0.682	0.425	<0.001	0.568	0.419	<0.001	0.774	0.529	0.289	<0.001	0.044
TP	0.861	<0.001	0.359	0.630	0.620	0.080	0.839	0.878	<0.001	0.333	0.524	0.524	0.030	0.711
Cholesterol	0.284	0.429	0.652	<0.001	0.868	<0.001	0.442	0.323	0.368	0.659	<0.001	0.774	<0.001	0.399
Triglycerides	0.887	<0.001	0.047	<0.001	0.622	<0.003	0.960	0.761	<0.001	0.044	<0.001	0.563	0.002	0.351
ALK	0.289	0.038	0.106	<0.001	0.565	<0.001	0.741	0.166	0.025	0.124	<0.001	0.431	<0.001	0.700
IGF-1	0.003	<0.001	0.255	<0.001	0.041	<0.001	0.206	0.001	<0.001	0.284	<0.001	0.027	<0.001	0.147
T_4	0.581	<0.001	0.016	0.001	0.214	<0.001	0.446	0.343	<0.001	0.056	0.008	0.195	<0.001	0.394
T_3	0.996	<0.001	0.236	<0.001	0.218	<0.001	0.842	0.776	<0.001	0.302	<0.001	0.086	0.001	0.858

Table 4.8: Significance levels from the 2-way ANOVA-Expt. II. *P* -values for treatment (trt) and time main effects and treatment by time interaction (inter.) effects for urine chemistry values presented in Figure 4.9.

	ANOVA excluding data from 1994 LNC trt group							ANOVA with extrap. data for 1994 LNC trt group						
Urine Variable[a]	trt	year	ty	time	tm	my	tmy	trt	year	ty	time	tm	my	tmy
UCa:Cr	0.528	0.001	0.586	0.071	0.366	0.008	0.006	0.463	0.004	0.567	0.060	0.329	0.002	0.004
UP:Cr	0.722	<0.001	0.467	0.454	0.772	0.001	0.253	0.712	<0.001	0.438	0.389	0.726	<0.001	0.202
UK:Cr	0.186	<0.001	0.331	0.001	0.044	<0.001	0.354	0.103	<0.001	0.145	<0.001	0.055	<0.001	0.242
UNa:Cr	0.128	0.034	0.434	0.083	0.105	0.179	0.101	0.077	0.045	0.262	0.032	0.025	0.084	0.010
UUN:Cr	0.084	<0.001	0.821	0.147	0.184	<0.001	0.961	0.052	0.002	0.399	0.114	0.069	<0.001	0.711
UCor:Cr	0.126	0.048	0.252	0.038	0.183	0.001	0.513	0.083	0.041	0.137	0.127	0.151	0.072	0.352

[a] Urine values are presented as ratios with urinary creatinine and adjusted as described in Appendix A.

changes in BM and condition. Based on the within winter, 1-way ANOVAs, time was significant in at least 2 of the 4 winters for: serum creatinine, TB, calcium, chloride, phosphorus, potassium, sodium, glucose, albumin, cholesterol, triglycerides, ALK, IGF-1, T_4, T_3, and for UCa:Cr, UK:Cr, UUN:Cr, and UCor:Cr, the majority of serum and urine variables measured (Fig. 4.8-9; Tables 4.5-6). Based on the among-winter 2-way ANOVAs, there were significant time effects and significant interactions between time and year for virtually all of these serum and urine variables, indicating the time effect depended on year of study (Tables 4.7-8; Figures 4.8-9). These significant interaction effects apparently resulted from inconsistent trends among winters, particularly for TB, creatinine, calcium, chloride, sodium, albumin, T4, and all urinary variables. Nevertheless several trends were apparent.

Serum phosphorus, potassium, glucose, and cholesterol tended to decline across winter. Serum triglycerides, ALK, and IGF-1 tended to decline until mid-winter, then increased in late winter. Of these serum variables, triglycerides, ALK, and IGF-1 exhibited patterns consistent with photoperiod effects (see Chapter 3). Several of the urinary variables tended to increase in early winter and decline in late winter (e.g., potassium, urea N, cortisol), but these trends were inconsistent among years. Season analysis (Chapter 3) did not indicate significant relations with changing daylength for urinary variables. Therefore we believe inconsistent trends in serum phosphorus, potassium glucose, cholesterol, and urinary variables may reflect recent dietary intake, xylazine effects or changes associated with reproductive hormones.

Year Effects on Serum and Urine Chemistry--Significant year effects, based on the 2-way ANOVAs, may be indicative of xylazine effects (relevant to the winter of 1991-92 and 1992-93), age of elk, differences in weather severity among years, and, in turn, differences in BM/condition dynamics among years.

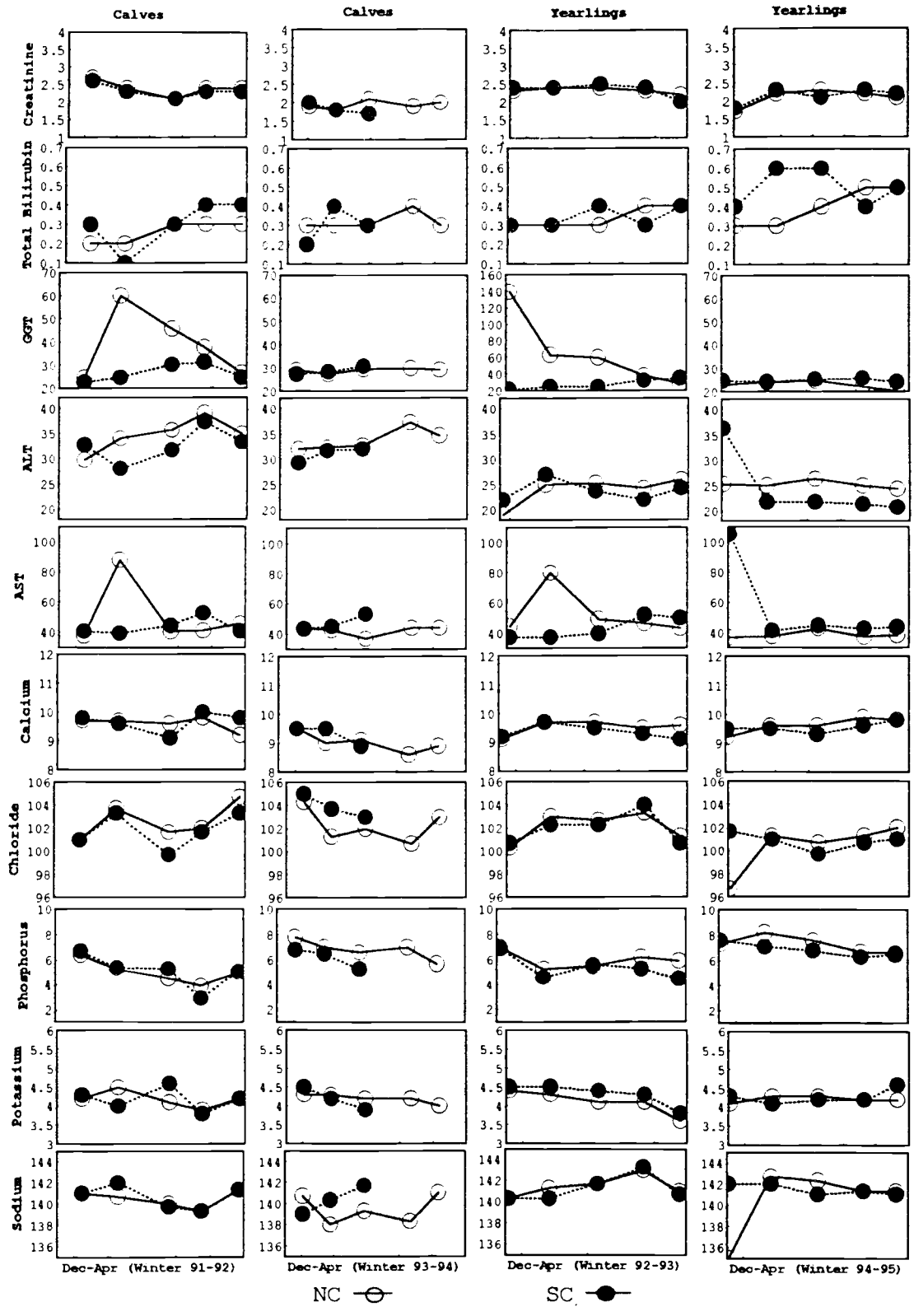

Figure 4.8: Levels of serum variables for young elk held in no-cover (NC) or satisfactory cover treatments (SC)-Expt. II.

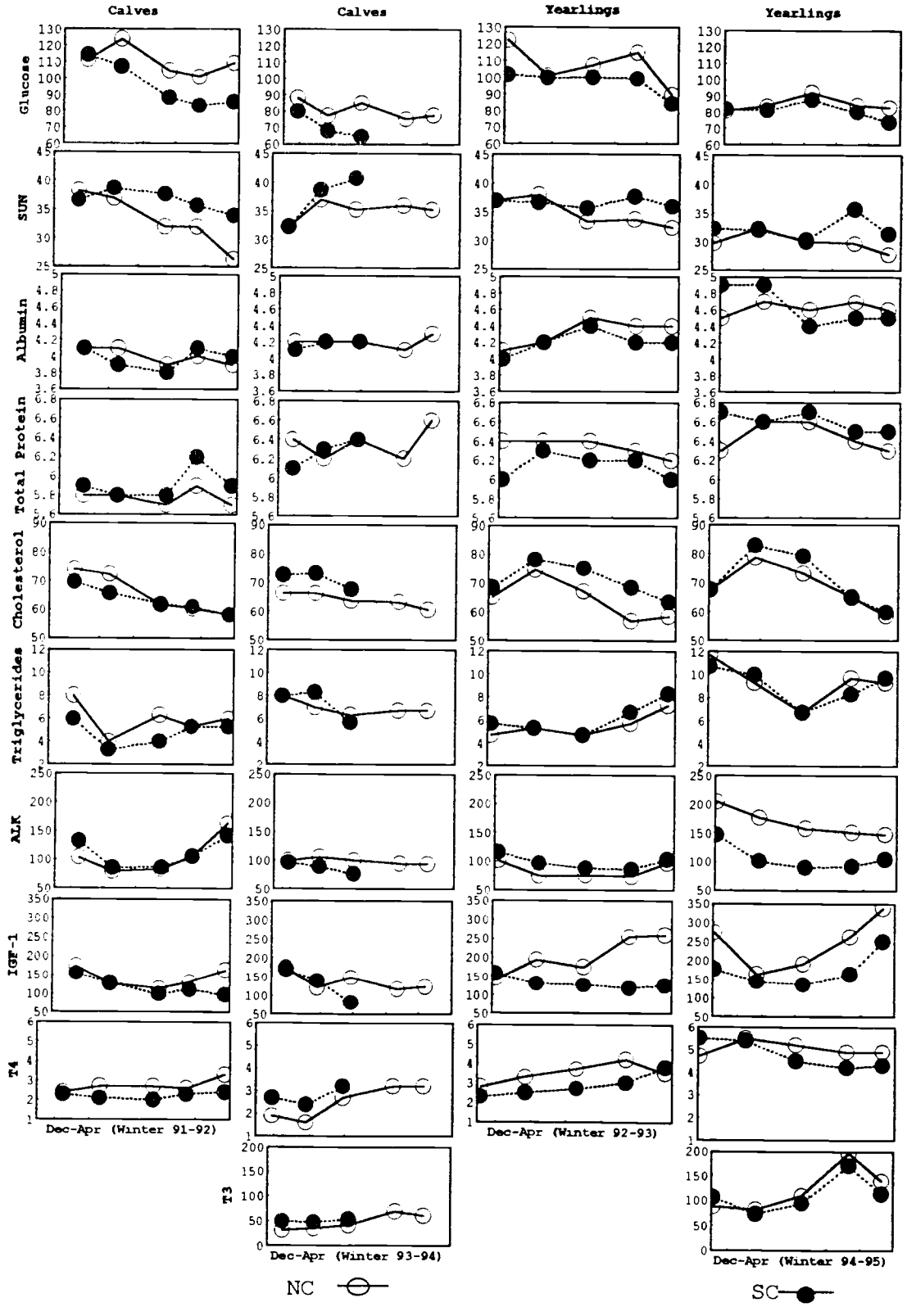

Figure 4.8 (Ctd.): Levels of serum variables for young elk held in no-cover (NC) or satisfactory cover treatments (SC)-Expt. II.

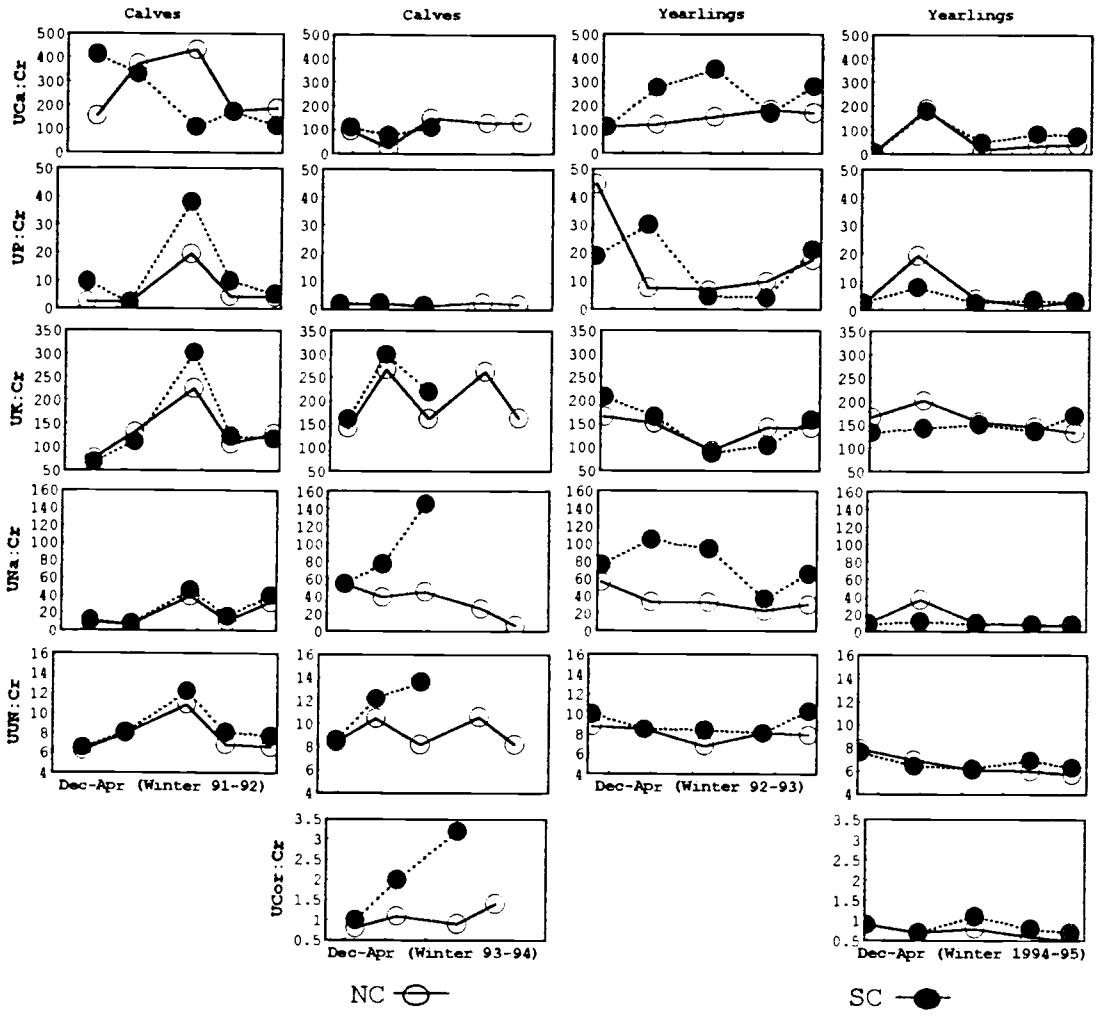

Figure 4.9: Levels of urinary variables for young elk held in no-cover (NC) or satisfactory cover treatments (SC)-Expt. II.

The year effect was significant for all serum and urine variables except serum AST, calcium, chloride, potassium, sodium, and cholesterol (Fig. 4.8-9; Tables 4.7 and 4.8). The year by time interaction effect also was significant for all serum and urine variables that exhibited a significant year effect, indicating that the effect of year depended on time, e.g., sampling period. General trends suggest age effects (i.e., similar levels within age groups and different levels between age groups) for ALT, albumin, TP, IGF-1 and T_3. General trends also suggest xylazine effects (i.e., different levels between the first 2 winters versus the last 2 winters) for serum phosphorus, glucose, albumin, TP, triglycerides, UCa:Cr, and UP:Cr.

Serum/Urine Chemistry and Body Mass Relations--The primary test statistic of interest regarding serum/urine chemistry-BM relations in both sets of ANOVA is the treatment by time interaction effect. Although the 3-way interaction between treatment, time and year also is relevant, virtually none of these 3-way interactions were significant (Tables 4.7-8) (exceptions included TB based on the 2-way ANOVA without data from the 1994 LSC treatment group and GGT and serum potassium based on the 2-way ANOVA with the extrapolated data for the LSC group.

Many of the serum and urine variables showed no significant time by treatment interaction effect in any of the 1-way and 2-way repeated measures ANOVAs (Fig. 4.8-9; Tables 4.5-8). These included serum GGT, ALT, AST, calcium, phosphorus, sodium, glucose, albumin, TP, cholesterol, triglycerides, ALK, T_4, T_3, and UP:Cr, and UCor:Cr. Difference in ALT approached significance ($P \leq 0.10$) in the 1-way ANOVA for the winter calf trial of 1991-92 and in both 2-way ANOVAs, and so was included in the regression analysis.

Serum ALT --Although the time by treatment interaction effect approached significance only in the first winter trial (Fig. 4.8, Table 4.7), there was a tendency for ALT levels of elk in the NC treatment to exceed those of elk in the SC treatment after early winter in 3 of the 4 winter experiments. This trend probably accounts for the near-significance ($P \leq$ 0.087 and 0.066) in both sets of 2-way ANOVAs (Table 4.7). However, the regression analysis that included data from all treatments indicated no relation between mass loss and ALT (Fig. 4.10; Table 4.9).

Serum Creatinine --Serum levels of creatinine differed significantly (treatment by time interaction) among the NC and SC treatments only during the winter calf trial of 1993-94 (Table 4.5). The treatment by time interaction was significant in the 2-way ANOVA that included the extrapolated data for the LSC pen (Table 4.7). However, the lack of definitive trends suggestive of differences in 3 of the 4 winters (Fig. 4.8) cast doubt regarding the value of this variable as a condition indicator. In the regression analysis using data from all 4 treatment groups (Fig. 4.10, Table 4.9), no significant relationship was found between mass loss and creatinine ($P \leq$ 0.180).

Serum Potassium --The treatment by time interaction effect was highly significant ($P = 0.003$) for the calf 1991-92 experiment (Fig. 4.8), but was not significant for any of the winter trials (Table 4.5). Moreover, there seemed to be no clear trends among winters regarding potassium levels in the 2 treatment groups (Fig. 4.8). Even so, the treatment by time interaction effect of the 2-way ANOVA without extrapolated data was significant ($P = 0.03$). In addition, the regression analysis (Fig. 4.10, Table 4.9) indicated a highly significant relation ($P \leq 0.001$, $r^2 = 0.59$) between BM dynamics and serum potassium. These analyses indicate that elevated potassium levels are associated with increasing BM loss.

Table 4.9: Significance levels, coefficients of determination, and simple linear regression equations for selected serum and urine variables in Figure 4.10.

Variable	*P*-value	r^2	Equation[a]
Serum			
ALT	0.899	0.00	--
Creatinine	0.180	0.13	--
SUN	0.112	0.18	--
Potassium	<0.001	0.59	y = -0.1 - 0.89x
Glucose	0.011	0.40	y = 2.3 + 2.25x
IGF-1	<0.001	0.66	y = 6.7 + 7.21x
T_3	0.718	0.03	--
T_4	0.012	0.39	y = -0.9 + 2.73x
Urine[b]			
UCor:Cr	0.348	0.18	--
UUN:Cr	0.027	0.32	y = -4.3 - 2.85x
UK:Cr	0.107	0.19	y = -1.6 - 2.26x
UNa:Cr	0.063	0.24	y = 1.0 - 13.5x

[a] Regression equations are omitted for serum or urine variables with $P > 0.10$.
[b] Urine variables are presented as creatinine ratios and adjusted as described in Appendix A.

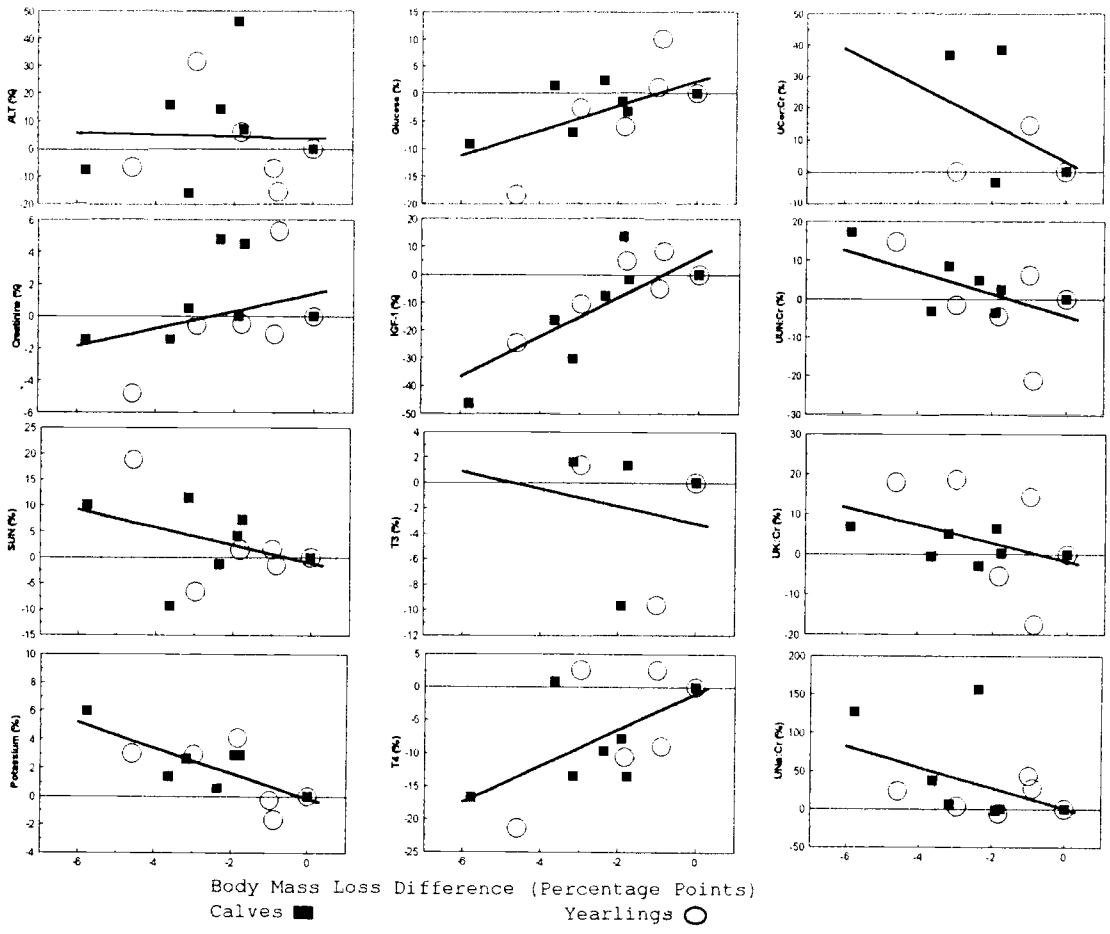

Figure 4.10: Relations between selected serum and urine chemistry levels and percent mass loss, during mid to late winter. Data for the 4 winters have been combined. Within-winter serum and urine levels of elk in each treatment were calculated as a percent of that of elk in the no-cover treatment; within-winter mass loss of elk in each treatment was calculated as the difference (in percentage points) with that of elk in the no-cover treatment group (see text for details).

Serum Glucose --The treatment by time interaction effect for glucose was not significant for any individual winter trial nor for either of the 2-way ANOVAs (Fig.4.8; Tables 4.5 and 4.7). But, there were trends indicative of such an interaction effect particularly in both calf trials. There also were significant treatment effects in both 2-way ANOVAs that would make sense if forest cover affected glucose levels over the 10-14 days of habituation to the pens prior to collecting the first blood sample. The regression analysis indicated a significant relation ($P = 0.011$, $r^2 = 0.40$) between BM dynamics and glucose (Fig. 4.10; Table 4.9). Yet the lack of significant treatment by time interaction effects in any of the ANOVAs and inconsistent significance levels associated with treatment effects indicate glucose may be a weak indicator of condition.

SUN --There were no significant treatment by time interactions in any of the ANOVA results (Tables 4.5 and 4.7), although the interaction effect approached significance ($P = 0.054$) for the 2-way ANOVA with extrapolated data. Serum urea N levels of SC elk tended to exceed that of elk in the NC treatment in most winters after the first 2 sampling periods, thus general patterns are congruent with the prediction that SUN levels increase as condition declines (Fig. 4.8). The regression analysis (Fig. 4.10; Table 4.9) depicted trends that support this prediction, but the relation between BM dynamics and SUN were not significant ($P = 0.112$).

Serum IGF-1 --The treatment by time interaction effect for IGF-1 was significant in 2 of the 4 winters and was significant in both 2-way ANOVAs ($P \leq 0.041$) (Fig.4.8; Tables 4.5 and 4.7). The interaction effect was significant during the winters (1992-93 and 1993-94) in which the greatest differences in BM occurred between the NC and SC treatment groups (Fig. 4.7). Although not statistically significant, different levels of IGF-1 among treatments also suggested a treatment by time

interaction during the first winter experiment (1991-92). In the last winter experiment (1994-95), large differences existed in IGF-1 among the 2 treatment groups at the beginning of the experiment, perhaps masking a potential interaction. In this winter, IGF-1 levels of elk in the NC treatment group averaged significantly higher ($P = 0.033$, treatment effect, Table 4.5) than those of the elk in the SC group.

Regression analysis (Fig.4.10; Table 4.9) indicated a moderately strong relation ($P \leq 0.001$, $r^2 = 0.66$) between BM dynamics and IGF-1, providing the highest r^2 of any variable included in this analysis. In addition, the slope of the regression line was the steepest of any of the variables with significant correlation. A relatively steep slope is indicative of a relatively high level of sensitivity of IGF-1 to differences in BM.

Serum T_4 --None of the treatment by time interaction effects from the ANOVAs for T_4 were significant (Fig.4.8; Table 4.5 and 4.7), although the interaction effect approached significance ($P = 0.072$) during the 1994-95 winter experiment. The regression analysis however, indicated a significant relation ($P = 0.012$, $r^2 = 0.39$) between BM dynamics and T_4 (Fig. 4.10; Table 4.9).

Serum T_3 --There was little indication from the 2 winters of data that T_3 provided a useful index of body mass and condition (Fig.4.8). The interaction effect from either sets of ANOVAs (Tables 4.5 and 4.7) and regression results (Fig. 4.10; Table 4.9) were not significant, although the treatment by time interaction effect from the 2-way ANOVA using the extrapolated data approached significance ($P = 0.086$).

UK:Cr --The treatment by time interaction for urinary potassium was not significant during any of the winter experiments (Fig. 4.9; Table 4.6), although the treatment by time interaction approached significance ($P = 0.061$) during the winter of 1994-95. The treatment by time interaction effect

was significant for the two-way ANOVA without extrapolated data ($P = 0.044$), and approached significance for the 2-way ANOVA with extrapolated data ($P = 0.055$) (Table 4.8). Regression analysis on urinary potassium was not significant ($P = 0.107$), however (Fig.4.10; Table 4.9).

UUN:Cr --None of the interactions of any of the ANOVAs were significant for UUN:Cr (Fig. 4.9, Table 4.6 and 4.8), although the 2-way ANOVA with extrapolated data approached significance ($P = 0.069$). As with the urinary potassium data, urea N patterns were consistent with expected responses during the 2 calf experiments, i.e., similar UUN:Cr levels among the 2 treatments early in the experiments and elevated levels in the SC treatment in mid-winter. The regression analysis indicated a significant relation between body mass dynamics and urinary urea N ($P = 0.027$, $r^2 = 0.32$) (Fig. 4.10; Table 4.9).

UCor:Cr --None of the ANOVA treatment by time interactions were significant for urinary cortisol (Fig. 4.9; Tables 4.6 and 4.8) based on the 2 winters of available data. During the second calf experiment (1993-94), the winter in which calf "mortality" occurred, there was a substantial divergence of urinary cortisol between the treatment groups that is consistent with predicted patterns of cortisol response, but the treatment by time interaction was not significant ($P = 0.264$). The regression analysis indicated no significant relation ($P = 0.348$) between BM dynamics and UCor:Cr (Fig. 4.10, Table 4.9).

DISCUSSION

Of the serum enzymes (GGT, AST, ALT, ALK), only ALT and AST differed among nutrition and condition groups. Levels of ALT were significantly different in the suboptimal condition analysis (HICON vs. LOCON), in the seasonal analysis (LOCON

versus SEACON) and differences approached significance in Experiment II. In general, levels of ALT and AST were lower in animals in good condition. AST plays an important role in cellular metabolism (Thomas, 1993). ALT is also functionally important to the metabolic process of glycolysis (Newsholme and Leech 1986) and increased levels may reflect increased gluconeogenesis or catabolism of amino acids.

Levels of SUN and AST were elevated at all sampling dates when ALT levels were significantly elevated in the LOCON group compared to the HICON group. Those sampling dates, however, do not correspond to time periods when significant BM loss occurred in the LOCON group (catabolism of fat and muscle). Further, there was no evidence of elevated ALT and AST in Experiment II when substantial body mass losses occurred. Consequently, we speculate that the elevated ALT and AST levels in the LOCON and SEACON groups were not due to catabolism of tissue. Newsholme and Leech (1986) reported that key enzymes associated with amino acids such as alanine and glutamine are known to increase when protein intake is high.

In Experiment I, TB was the only serum protein which differed significantly among treatment groups. In the seasonal analysis, a similar pattern of TB levels was observed when comparing the HICON and SEACON and the LOCON and SEACON groups. That is, levels of TB were elevated both winters in the SEACON group compared to the LOCON or HICON group and were lower during summer than the LOCON or HICON group. Hemoconcentration of serum has previously been used as the explanation for increased levels of serum proteins (DelGiudice et al. 1987*a*, Seal et al. 1972), however other serum proteins were not significantly different in our study. Thus we conclude that observed differences in TB were not related to hemoconcentration of the serum. Measurement of bilirubin in the serum is however indicative of red blood cell (RBC)

degradation (Mathews and van Holde 1990). Therefore fluctuations in TB levels may have been a result of changes in RBC production and degradation.

In Experiment II, virtually no relevant significant results were observed for all of the serum proteins (TP, ALB, TB). All serum proteins measured in this experiment showed inconsistent trends among winters and between treatment groups, indicating these variables were not sensitive or reliable indices of animal condition in this experiment. Kie et al. (1983) found no significant differences in TB and TP levels for white-tailed deer at high and normal densities, but ALB was significantly lower in the high density group. Reduced levels of ALB attributed to reduced body condition were reported by Wolfe et al.(1982) in elk, Wolkers et al. (1994) in red deer, and Seal et al. (1972) in white-tailed deer. In contrast, increased serum albumin levels were reported in fasted (DelGiudice et al. 1987*a*) and energy restricted (Warren et al. 1982) white-tailed deer. Levels of serum albumin in our studies, however, were not consistent with respect to nutrition or condition experiments and did not appear to be reliable or sensitive indices of condition.

General trends of calcium, phosphorus, sodium, potassium, and chloride in Experiment I were mostly unrelated to body condition based on serum levels. In Experiment II, only serum potassium was identified as being significantly related to BM dynamics. Although general trends were inconsistent among winters, the regression analysis indicated a highly significant inverse relation between declining BM and increasing serum potassium levels, in turn suggesting hyperkalemia. In contrast, DelGiudice et al. (1987) previously described declining levels of potassium (hypokalemia) during fasting in white-tailed deer. Decreased serum potassium levels associated with reduced condition were also reported by Wolfe et al. (1982) in elk and by Seal et al. (1972) in white-tailed deer.

Decreasing levels of serum calcium were reportedly related to reduced condition in caribou calves (Soveri et al. 1992) and to a low energy diet in white-tailed deer fawns (Seal et al. 1978*b*).

Seal et al. (1978*b*) also reported elevated serum phosphorus and chloride and decreased sodium levels in relation to a low energy diet in white-tailed deer fawns. We also observed significantly lower serum sodium levels in the LOCON group compared to the HICON group during the winter of 1994-95. In the seasonal analysis, serum chloride levels in the SEACON group were elevated in winter on the low energy diet but then declined during summer when elk consumed a high energy diet. These differences were not apparent in the suboptimal condition analysis. Therefore, we conclude that none of the electrolytes or minerals adequately reflected condition of elk in this study.

DelGiudice et al. (1990*b*) reported that elevated cholesterol levels (hypercholesterolemia) in nutritionally restricted white-tailed deer were indicative of lipolysis due to undernutrition. Early in our study, there was an increase in cholesterol and triglycerides in the LOCON group during the rapid BM loss (autumn 1995), but levels were not significantly different from the HICON group. Levels in the LOCON group began to decline in late winter (1994) and became significantly lower than levels observed in the HICON group during summer 1994 and winter 1995. This pattern possibly indicates that initially the LOCON elk relied more on lipolysis and mobilization of fat reserves to meet energy demands; however, after energy levels were increased to stabilize BM (winter 1993-94), these animals probably no longer relied on catabolism to meet energy demands.

In the seasonal analysis, cholesterol levels were greater in the LOCON group compared to the SEACON group during fall 1993. This phenomenon did not occur during the second fall and

winter of the study, thus providing further evidence that the LOCON elk briefly experienced a period of hypercholesterolemia during the fall of 1993. Although not significant in the thermal cover analysis, levels of cholesterol tended to be elevated in the SC treatment group compared to the NC group except during the first winter, the mildest of the four. Wolfe et al. (1982) reported reduced cholesterol levels in free-ranging elk assumed to be poorer condition than captive elk in good condition. Triglyceride levels did not follow a consistent pattern during any of the winters.

Cholesterol is functionally important as a precursor to bile acids and steroid hormones, is present in cell membranes, and is involved in triglyceride transport in the blood (Newsholme and Leech 1986). Triglycerides are carried in the blood as lipoproteins when they are bound to serum albumin (Mathews and van Holde 1990). Functionally, they are oxidized to ATP to provide energy for cellular activities (Newsholme and Leech 1986). Cholesterol and triglycerides are both important in fatty acid metabolism and levels in the serum are typically related to energy demands of the animal. Collectively, our data combined with the literature, suggest that slight to moderate submaintenance energy intake lowers cholesterol and triglyceride levels, but moderate to severe energy restriction that induces substantial fat catabolism elevates cholesterol and triglycerides.

In Experiment I, both T_3 and T_4 levels generally responded as expected based on feeding regimes. However, T_3, T_4, and the T_3/T_4 ratio were not reliable or sensitive enough to be used accurately as indirect measures of nutrition or condition in our study. In Experiment II, there was little evidence that T_3 or T_4 were significantly influenced by body condition. However, T_4 showed a significant correlation with BM loss. The general trend for T_4 was lower levels in the SC group compared to the NC group except during the winter of 1993-94. T_3 was

only measured during the last 2 years of the study, providing minimal data for proper assessment of this variable. In reindeer calves subjected to nutritional restrictions, changes in T_4 (not T_3) correlated to feed intake and weight gain (Ryg and Jacobsen 1992).

Both thyroid hormones are known to play important roles in energy metabolism, and their role of T_4 in maintaining basal metabolism has been well established (Newsholme and Leech 1986). The active thyroid hormone is actually T_3, made from the deiodination of T_4 (Newsholme and Leech 1986). Research has shown that the rate at which T_4 is converted to T_3 is generally reduced during periods of starvation (Newsholme and Leech 1986). Observed patterns for T_3 in the LOCON and SEACON groups supports those findings. DelGuidice et al. (1994) reported that declining levels of T_3 are indicative of diminishing energy in the diet and of dwindling fat concentrations in animals. Sensitivity of T_3 to short-term changes in energy was previously reported by Seal et al. (1978*b*).

In Experiment I, levels of serum creatinine differed significantly among treatment groups in the suboptimal condition and seasonal analyses. General trends indicated creatinine levels responded to body mass dynamics with increased creatinine levels observed during periods of body mass loss and decreased levels observed when body mass increases occurred. In the thermal cover analysis, creatinine levels differed significantly during one winter, with no clear trends observed in other winters.

Creatinine is a creatine metabolic end product that is excreted by the kidneys (Thomas 1993), and levels are thought to be directly related to mass of muscle in the body. However, in this study we observed increased creatinine levels in elk during periods of body mass loss. Creatinine levels responded quickly to changes in nutritional intake as observed in the

seasonal analyses. Wolkers et al. (1994) reported similar findings in feed restricted red deer. They attributed an initial increase in serum creatinine to reduced excretion by reduced glomerular filtration. After 12 weeks of feed restriction, however, creatinine levels declined, possibly due to reduced mass of muscle (Wolkers et al. 1994). DelGiudice et al. (1990*b*) also reported slight increases in serum creatinine associated with nutritional deprivation in white-tailed deer and attributed this to reduced glomerular filtration.

Serum urea nitrogen levels were elevated in the LOCON group compared to the HICON group in the suboptimal condition analysis. Likewise, levels were elevated in the LOCON group compared to the SEACON group during several sampling dates. The elevated levels in the LOCON group compared to the SEACON group during each fall and similar levels in spring did not follow expected patterns based on feed intakes and BM dynamics. We would have predicted that SUN levels should have declined initially, then increased in response to declining body mass (catabolism) in both groups during fall and winter of 1993-94. Patterns in serum urea nitrogen levels instead tracked crude protein intake and was not elevated when the ratio of CP:DE was highest (e.g., the LOCON group in the later part of Experiment I). When CP intake is high relative to DE intake, dietary CP is utilized more effectively, and urea formation is reduced.

In Experiment II, when dietary CP:DE ratios were constant among treatment groups, SUN levels tended to be greater in elk that lost the most BM, suggesting elevated SUN in response to elevated catabolism. These varying patterns in SUN between the 2 experiments complicate the interpretation of SUN data as noted by Saltz et al. (1995).

In Experiment I, glucose levels in the LOCON group declined initially during the first fall/winter of the study and remained about 10% lower than the HICON groups blood glucose levels. The HICON group ended the study with blood glucose

levels similar to those established prior to study initiation. However, glucose levels did fluctuate in the fall of 1993 possibly due to xylazine effects. Levels in the SEACON group also were lower than that of the HICON group during much of the study. Trends in glucose levels suggested that elk subjected to the low energy diet experienced lower serum glucose levels. Microbial populations in ruminant animals are known to differ based on the type of diet and intake levels (Church 1988). More efficient microbes in the HICON cows on a high energy pellet may have produced more propionic acid which can be efficiently converted to glucose (Church 1988). In contrast, SEACON cows in winter and the LOCON cows may have produced more acetic acid which is not convertible to glucose when subjected to the low energy pellet in this study. Blood glucose in ruminants is maintained by converting propionate and lactate to glucose (Church 1988). Glucose levels tend to be higher in animals fed a high energy diet because a higher proportion of propionate is produced (Newsholme and Leech 1986).

General trends in Experiment II indicated lower levels of serum glucose in the SC treatment group compared to the NC treatment group. Also, regression analysis indicated a significant correlation between glucose levels and BM loss. Since elk in both of these treatments were receiving the same diet, we can attribute changes in serum glucose to declining condition across winter or to a faster rate of glucose utilization because animals encountered a greater energy deficit.

Significant results for the serum and urine variables were observed in both the suboptimal condition analysis and the seasonal analyses in Experiment I. IGF-1 levels declined quickly when dietary energy was restricted in the LOCON and SEACON groups during this study. General trends suggested that IGF-1 levels closely tracked DE intake and BM dynamics in Experiment I. In Experiment II, IGF-1 levels also were

significantly correlated to BM loss. Lower levels of IGF-1 were consistently observed in the SC treatment group compared to the NC treatment group. Research with domestic ruminant animals has shown that IGF-1 appeared to be a primary indicator of nutritional status (Straus 1994, Breier et al. 1986, McGuire et al. 1992, Gluckman et al. 1987). In red deer, Webster et al. (1996) reported significantly lower levels of IGF-1 in fasted animals, and concluded that responses of IGF-1 to declining intakes were rapid (within 48 hours). Suttie et al. (1989) reported that IGF-1 levels were positively correlated to liveweight gain and antler development in red deer.

Overall there was no consistent trend for UCa:Cr that could be related to the 3 treatments in Experiment I. Likewise, UCa:Cr did not differ significantly in Experiment II. Levels of UK:Cr were significantly elevated in the LOCON group compared to the HICON group during the latter part of Experiment I. Urinary potassium levels also were significantly different in both subsets of seasonal anlayses. In the HICON vs. SEACON subset, urinary potassium exhibited a pattern similar to UUN:Cr, both of which closely followed patterns of dietary protein intake. In the LOCON vs. SEACON subset levels were elevated in the SEACON group during summer and lower than the LOCON group during the winter of 1994-95. In Experiment II, there was a consistent trend of elevated UK:Cr levels in the SC calf groups during mid-winter both years. However, in the yearlings, this trend was not consistent. Increased urinary potassium excretion can be related to elevated levels of fatty acids and ketones in the blood; this is associated with increased protein catabolism (Newsholme and Leech 1986). Essentially, the increased levels of ketones and fatty acids decrease the pH of the blood causing acidosis which is then counteracted by increased excretion of protons in the urine to alleviate toxicity (Newsholme and Leech 1986). The elevation of UK:Cr levels may therefore be indicative of increased

catabolism in these groups. Increased levels of urinary potassium may also reflect recent dietary intake of potassium (DelGiudice et al. 1990*b*). In our study, patterns suggested that levels may have been elevated due to increased levels of fatty acids and ketones in the blood, particularly in experiment I, but corresponding significant body mass losses did not occur. In Experiment II, the calves did experience significant mass losses and hence this explanation seems more plausible.

In Experiment I, significant differences occurred for UUN:Cr ratios in the suboptimal condition analysis and in both seasonal subset analyses. In the suboptimal condition analysis, the general trend was an initial decline in both groups, followed by a gradual increase in the LOCON group mid-way through the study. Overall, however the LOCON group ended the study with no change in their UUN:Cr levels, while the HICON group declined about 38%. In both seasonal analyses, changes in levels of UUN:Cr closely followed dietary protein intakes for the HICON, SEACON, and LOCON groups. In Experiment II, results were similar to those of urinary potassium in that trends for the calves were as expected for increased protein catabolism mid-winter, but results for the yearlings were inconsistent. The overall meaning of changing UUN:Cr ratios in this study were difficult to interpret because the ratios reflect multiple metabolic responses including protein catabolism, protein intake, urea recycling, energy intake, and fat depletion (Parker et al. 1993).

Levels of UCor:Cr were elevated in the LOCON group compared to the HICON group during the first winter and remained elevated throughout the rest of Experiment I. Significant differences were observed at 6 of the 24 sampling dates in the suboptimal condition analysis of Experiment I. Also, significant results were observed in the seasonal analysis (LOCON vs. SEACON group) during 4 of 22 sampling dates. In

Experiment II however, the response from calves during the winter of 1993-94 was substantial with the SC levels rising to be 3 times greater than the NC group. In the last year of the thermal study with yearlings, the response was minimal in the SC group. Saltz and White (1991) previously reported significantly elevated urinary cortisol levels in mule deer maintained at higher densities compared to lower densities. In black-tailed deer, Parker et al. (1993) reported elevated urinary cortisol levels in fawns which coincided with declining fat stores; however, effects in adults were not significant. Urinary cortisol:creatinine levels were only measured during 2 of the 4 years of the thermal cover analysis, thus it is difficult to predict the reliability of sensitivity of this index from our data. The lack of significance in the suboptimal condition analysis during the first fall of Experiment I is surprising. However, it is possible that a xylazine effect masked potential results. Our data and that of Parker et al. (1993) perhaps indicate that UCor:Cr levels of younger animals are more sensitive to nutrition than those of adults.

MANAGEMENT IMPLICATIONS

Responses of variables related to protein metabolism in the body (SUN, ALT, AST, UUN:Cr and UK:Cr) to the various treatments were difficult to interpret. Protein metabolism in ruminant animals is quite complex; it involves influences such as anabolism or catabolism, dietary protein intake, energy intake, and urea recycling and excretion. When comparing the overall patterns of these variables to body mass data in Experiment I, we observed that levels responded primarily to dietary protein intake rather than increased catabolism. In Experiment II, dietary protein intake was much lower and the

levels of these variables appeared to be correlated to increased catabolsim, however trends between winters were still inconsistent with BM dynamics.

Variables that responded as predicted under the conditions of both experiments included glucose, T_3, T_4, T_3/T_4, cholesterol, triglycerides, and IGF-1. IGF-1 in particular appeared to be very sensitive to changes in condition and was consistent during all experiments. To be useful as an index of condition or nutritional status, serum and urine chemistry variables must be sensitive to slight changes in the factors they index. Our findings indicate that further research on IGF-1 may be particularly useful in search of variables that provide good indicators of nutrition or condition.

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Summary of Serum and Urine Chemistry in Elk and Management Implications

Lonnie J. Quinlan-Murphy

REFERENCE VALUES FOR SERUM AND URINE CHEMISTRY

In general, reference values for serum and urine chemistry were similar to levels previously reported for other cervids. Levels of AST, ALT, GGT, triglycerides and T_4 were lower than previously reported, while levels of SUN and IGF-1 were slightly higher than levels previously reported. However, levels of IGF-1 were similar to those reported for domestic animals of similar body size and ages. Chemical immobilization and other stress associated with capture can alter many serum and urine chemistry variables. Therefore we caution that serum and urine collection protocol that differ appreciably from ours may confound comparisons to our data. Such is the nature of serum and urine chemistry data, and considerably more research needs to be conducted to find ways to minimize or account for the effects of animal capture on serum and urine chemistry.

AGE EFFECTS

Age effects were significant in 18 of 28 variables assessed. Therefore the potential of age effects to confound serum and urine chemistry data is quite large. Fortunately, age determination between calf, yearling, and adult elk is generally not a problem in the field. Our data suggest that age effects for most variables were unimportant as long as sampled animals were > 1.5 years-old. However, indiscriminate samples in which calves and adults are mixed will be confounded by age; care should be taken to stratify samples at least by these 2 age classes. This may be difficult when collecting samples from snow.

SEASONAL INFLUENCES

Cyclic patterns in variables related to daylength were examined in Chapter 3. Eight variables exhibited significant and consistent variation between winter and summer levels. The majority (6 of 8) of these variables exhibited nearly a 2-fold increase in summer levels compared to winter levels and included TB, ALT, ALK, triglycerides, IGF-1, and T_4. Voluntary feed intake also exhibited strong correlation with declining daylength during late summer and fall but not during the spring and early summer when daylength was increasing. Feed intake and daylength patterns in the JUV group indicated that during the first year of the life growth processes to some extent override the circannual cycle of VFI. By the second year of life however, the VFI cycle was highly correlated (r^2 = 0.75) to daylength. Seasonal influences have potential to confound data of serum variables that may be used to assess condition or nutritional status in elk. Our research has identified variables that were consistently influenced by changing daylength and provides a guide to the response of the variable to changes in daylength.

SUBOPTIMAL CONDITION EFFECTS

Elk in the LOCON group were subjected to long-term nutritional restriction to induce suboptimal body condition. This state had significant influence on 18 of the 28 serum and urine variables measured. Assessment of the reliability and sensitivity of each variable to suboptimal condition indicated that many variables were inconsistent. Variables that exhibited significant differences in at least half of the 24 monthly sampling dates included: UUN:Cr, glucose, and IGF-1. Elevated UUN:Cr levels of the LOCON group during the last 2/3

of the study did not correspond to BM loss and probably resulted from a high CP intake in relation to DE intake. Glucose levels may have been elevated in the HICON group due to the higher quality or higher dietary energy content of the pellet fed. IGF-1 levels consistently were different after the first 3 sampling dates and remained different except during late spring the 1st year of the study. However, changing daylength strongly influenced IGF-1 levels too. Thus seasonal effects must be accounted for if this variable is used as a condition or nutrition index. Our data suggest suggest that seasonal effects are most confounding in late spring and early summer and that during the rest of the year, IGF-1 appeared to be a reliable and sensitive index.

SEASONALLY CHANGING NUTRITION EFFECTS

In the HICON/SEACON subset analysis 10 variables showed significant differences. Based on feeding regimes, we expected indicator levels to be similar in summer and significantly different in winter if they were reasonably sensitive and reliable. Glucose, IGF-1, T_3 and T_3/T_4 were the only variables that consistently followed this expected pattern. The most sensitive of these variables was IGF-1 with differences of 30-50% observed between the 2 groups during winter. In the LOCON/SEACON subset analysis 12 variables showed significant differences. The predicted pattern in this analysis was similar levels in winter and different levels in summer. T_3 and IGF-1 followed this pattern most consistently and again IGF-1 exhibited the greatest differences between the 2 groups indicating sensitivity as well as reliability.

THERMAL COVER/BODY CONDITION EFFECTS

In Experiment II, (Chapter 4) we assessed effects of body condition on serum and urine chemistries. Thermal cover rather than nutrition in this case, induced different levels of body condition. Elk were held in pens with thermal cover (50-50% canopy closure) or in pens with no cover (clearcut areas). In all 4 winters of the study, elk in the pens with thermal cover lost significantly more BM than elk in the clearcut pens [see Cook et al. (in press) for more details]. This provided a unique assessment for serum and urine chemistry in young elk.

Variables that were significantly affected by treatments and exhibited a correlation with BM dynamics included serum potassium, glucose, IGF-1, UUN:Cr. and T_4. Of these, consistent and expected patterns between winters were observed in IGF-1, glucose and UUN:Cr. The variable that exhibited the most sensitivity in this analysis was IGF-1.

CONCLUSION

Results of our research indicate that age and season have the potential to confound serum and urine chemistry data. To minimize potential effects, data should be collected in a standardized manner similar to the protocol used in this study and information on age and sampling dates must be taken into account when results are interpreted. IGF-1 appeared to be a primary indicator of nutrition and condition. Since IGF-1 is known to exist in all body fluids, it may be possible to measure IGF-1 in urine samples as well, thus making the use of this index more desirable to wildlife researchers and managers.

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Appendices

Appendix A: Master list of serum and urine variables. All variables were measured in Rocky Mountain elk from 1991-1995 in Northeastern Oregon. Common abbreviations and associated units of measure are listed for each variable.

Serum

Alkaline Phosphatase, (ALK), (IU/l)[b]
Alanine Aminotransferase, (ALT), (IU/l)
Gamma Glutamyltransferase, (GGT), (IU/l)
Serum Albumin, (mg/dl)
Total Protein, (TP) (g/dl)
Total Bilitrubin, (TB) (mg/dl)
Triglycerides, (mg/dl)
Cholesterol, (mg/dl)[b]
Urea Nitrogen, (SUN) (mg/dl)[b]
Calcium, (Ca), (mg/dl)
Sodium, (Na), (meq/l)
Potassium, (K), (meq/l)
Inorganic Phosphorus, (P), (mg/dl)
Chloride, (Cl), (meq/l)
Triiodothyronine, (T_3), (ng/dl)[b]
Thyroxine, (T_4), (µg/dl)[b]
Glucose, (mg/dl)[b]
Insulin-like Growth Factor-1, (IGF-1), (ng/ml)[b]

Urine

Creatinine, (Cr), mg/dl
Urea Nitrogen, (UUN), mg/dl)[b]
Cortisol, (UCor), (µg/dl) [a b]
Potassium, (UK), (meq/l)[a]
Sodium, (UNa), (meq/l)[a]
Phosphorus, (UP), (mg/dl)[a]
Calcium, (UCa), (mg/dl)[a]

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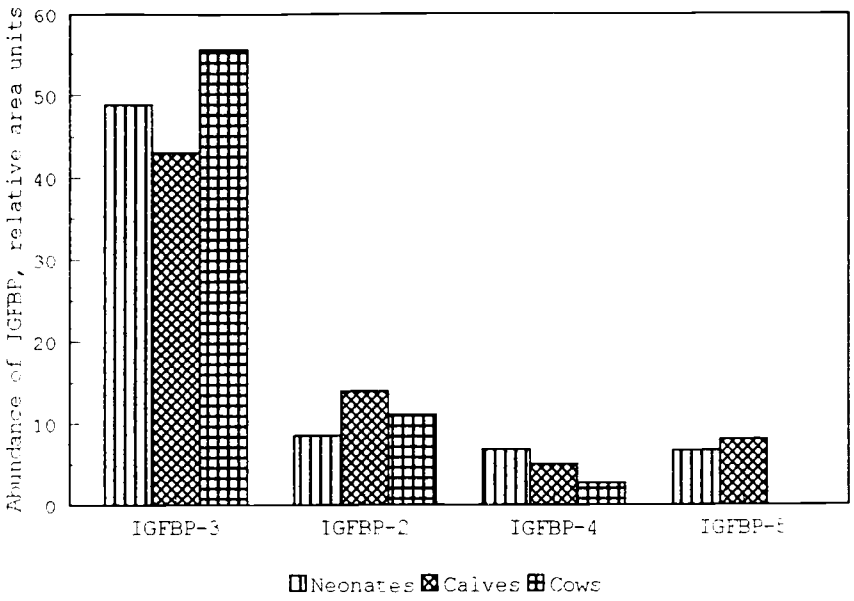
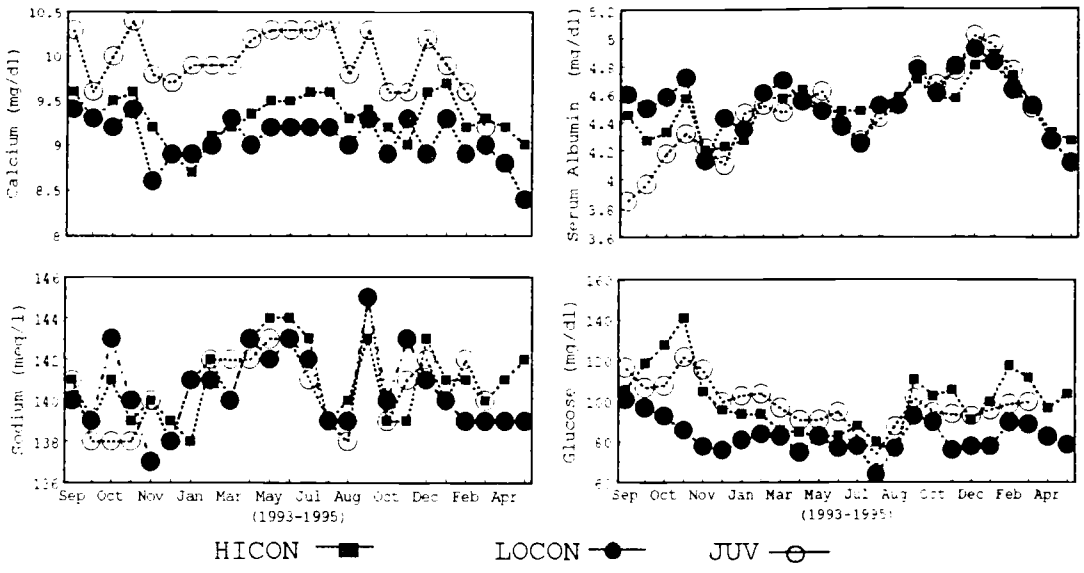
[a] Values for these urinary variables were divided by creatinine values to account for urinary dilution. Then, ratios were adjusted to allow comparison with published literature as follows: Potassium:Creatinine*100 (UK:Cr); Sodium:Creatinine*100 (UNa:Cr); Phosphorus:Creatinine*1000 (UP:Cr); Calcium:Creatinine*1000 (UCa:Cr); and Cortisol:Creatinine*100 (UCor:Cr).

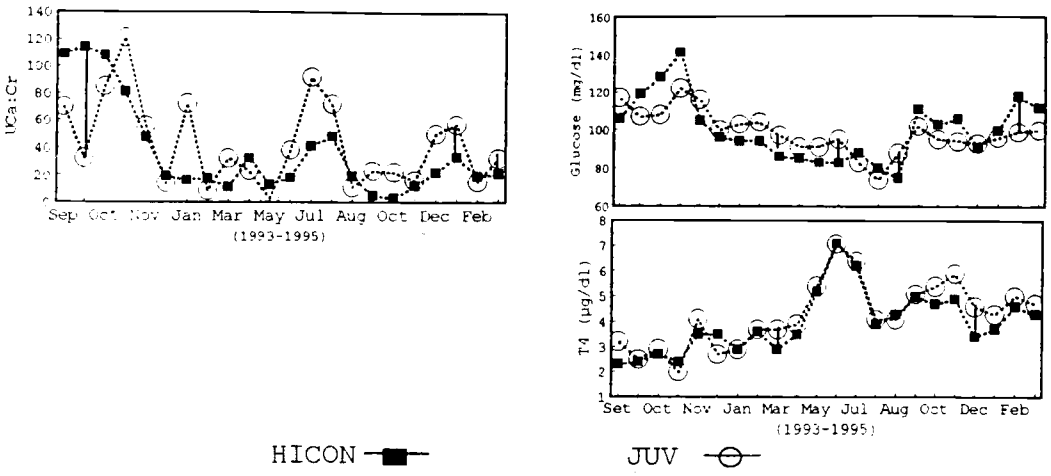
[b] Variables recognized in wildlife literature as having potential for indicating condition or nutritional status in wild ungulates



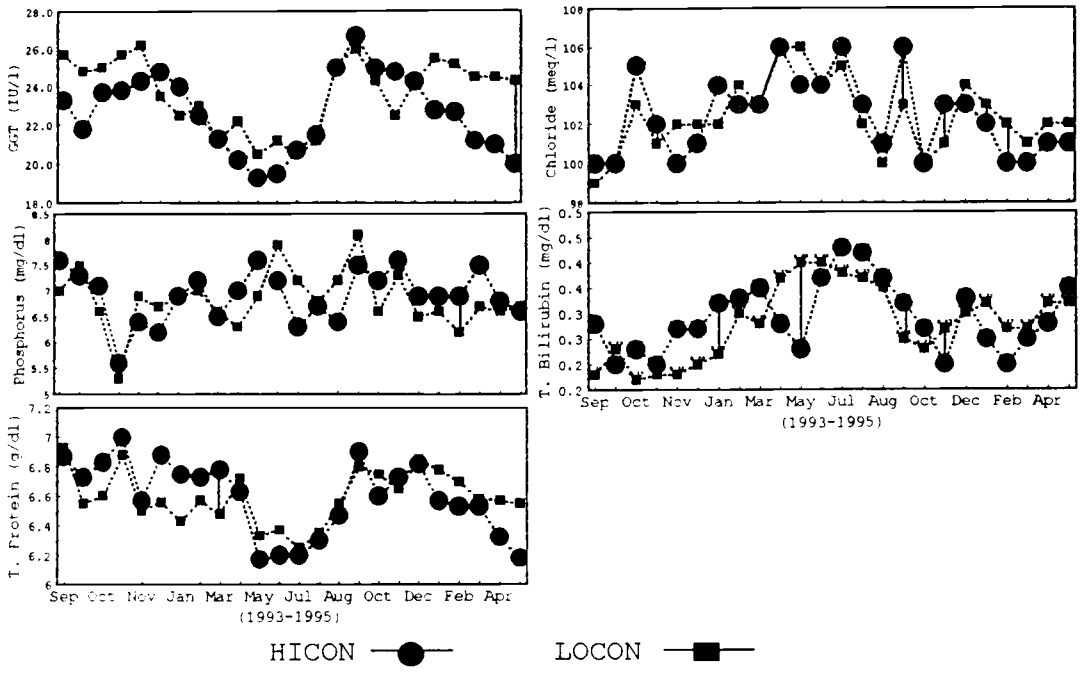
Appendix B: Levels of Insulin-like Growth Factor Binding Proteins (IGFBP) in elk serum (neonates, calves and cows).



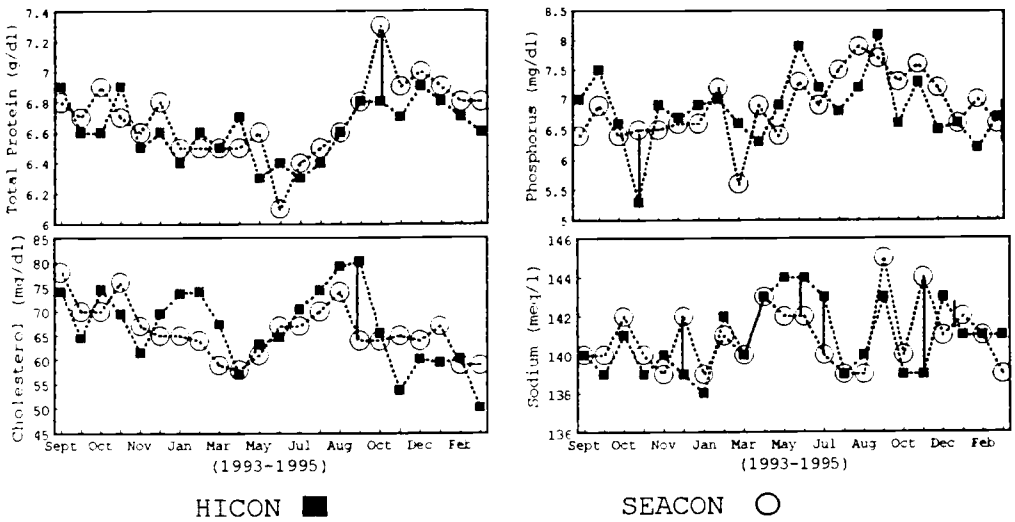
Appendix C: Variables that did not show consistent patterns likely related to season in both years of this study.



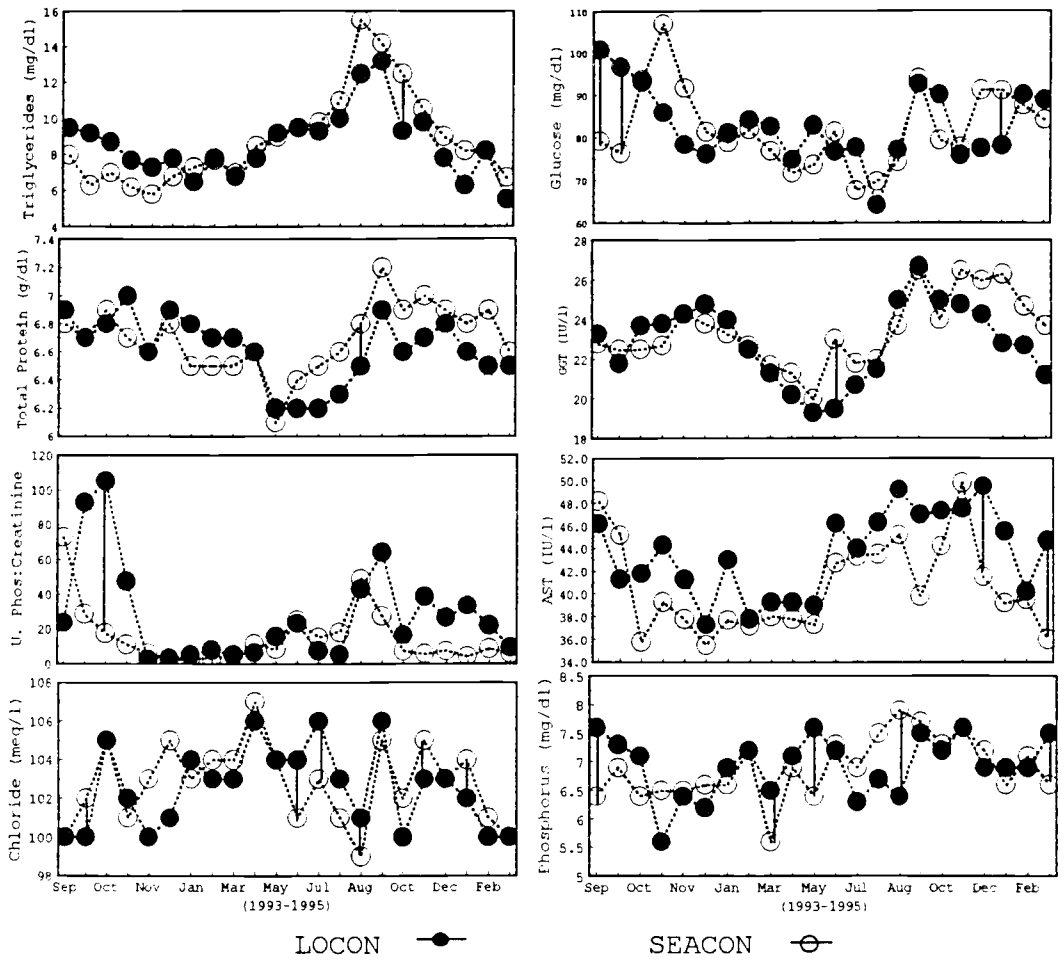
Appendix D: Variables that did not show predicted patterns based on age effects. All variables were however, significant ($P < 0.05$) for treatment or time by treatment interactions in the repeated measures ANOVA ($P < 0.05$). Vertical lines between sampling dates indicate significant differences at specific sampling dates.



Appendix E: Variables with patterns not attributable to reduced body condition. All variables, however, were significant in the repeated measures ANOVA ($P < 0.05$).



Appendix F: Variables with patterns not attributable to changes in seasonal nutrition. All variables, however, were significant in the repeated measures ANOVA ($P < 0.05$).



Appendix G: Variables with patterns not attributable to changes in seasonal nutrition. All variables, however, were significant in the repeated measures ANOVA for treatment or time by treatment interactions ($P < 0.05$).