Odocoileus hemionus (hemionus) on the North Rim of the Grand Canyon:

A Study of Wildlife Nutrition,

Metabolic Response

and Interaction of the Herd

with the Winter Habitat on the North Kaibab Plateau.

by

Matthew W. Acton

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved April 2014 by the
Graduate Supervisory Committee:

William H. Miller, Chair
Ward W. Brady
Holly Huffman

ARIZONA STATE UNIVERSITY

May 2014

ABSTRACT

A mule deer herd exists on the northern rim of the Grand Canyon, located on the North Kaibab Plateau. Historical references to this indigenous mule deer herd presented reports of periodic population irruption and collapse. Partially funded by the Arizona Game and Fish Department and the Arizona Deer Association, examination of herd nutritional and metabolic status from the Fall 2005 – Spring 2008 was completed at the request of AzGFD and ADA.

Habitat analysis included forage micro-histological, protein, and caloric content plus whole blood and plasma assays gauging herd metabolic response. Modelling was completed using best management practices wildlife energy demand calculations and principal component analysis.

Forage quality analysis and modelling suggest a sufficient amount of nitrogen (N) available (DPI) to the deer for protein synthesis. Energy analysis (MEI) of forage suggest caloric deficiencies are widely prevalent on the north Kaibab plateau. Principal component analysis integrates forage and metabolic results providing a linear regression model describing the dynamics of forage utilization, energy availability, and forage nitrogen supply with metabolic demand and response of the mule deer herd.

Most of the plasma and blood metabolic indicators suggest baseline values for the North Kaibab mule deer. Albumin values are in agreement with albumin values for mule deer in the Southwest. I suggest that the agreed values become a standard for mule deer in the Southwestern U.S. As excess dietary N is converted to a caloric resource, a continual state of under-nutrition exists for the deer upon entering the N. Kaibab winter range. The population is exceeding the nutritional resource plane that the winter habitat

provides. Management recommendations include implementation of multiple small-scale habitat rehabilitation efforts over time, including invasive juniper (*Juniperous osteosperma*) and piñon *(Pinus edulis)* management, prescribed burning to control big sage *(Artemesia tridentata)* populations, and reseeding treated areas with a seed mix of native shrubs, grasses and forbs. I recommended that the population size of the North Kaibab deer herd is maintained at the current size with natural selection controlling growth, or the population be artificially reduced through increased hunting opportunities.

DEDICATION

I would like to dedicate this work to my wife Rebecca and my mother Connie, two people whose unending love and support has never let me down. You inspired me on days when I could not see the light, and gave me reason on days when reason was not found. You showed me how to eat the elephant. This dissertation is as much yours as it is mine.

ACKNOWLEDGEMENTS

A dissertation is not completed by one alone. William H. Miller for your advice, mentorship, unending knowledge and patience. I would like to acknowledge the financial support and assistance of the Arizona Game and Fish Department, Research Branch and the Arizona Deer Association. I would like to personally thank and pay respect to: Chasa O'Brien and Brian Wakeling, your patience, time, and tutelage on the phone and in the field were indispensable. Dr.'s Bateman and Young – I was always paying attention. Sue Miller, for having the patience of Job. C. Scott McAdams and the graduate and under-graduate students who "pinch-hit" for me when I was laid-up and couldn't go to the North Rim for data collections – you covered it, no questions. Ann Steffler-Chenevert for your indefatigable energy, enthusiasm and effort. You are the best lab partner a man could have. Laura Marshall, you always came through with a jug of ethanol or HCl when I was in a bind. Todd Elliot for your keys and conversation. Marjorie and Benjamin Houser for feeding a starving student and always giving a damn. Connie Acton who came out of retirement and showed everyone the right way to make a microscope slide. You got me here.

TABLE OF CONTENTS

Page

LIST OF FIGURES……..……...…ix

LIST OF TABLES……...…….....xiii

CHAPTER

I INTRODUCTION…..…...……1

II LITERATURE REVIEW……...……...……...……..…..……5

2.1 The Kaibab Plateau…….……..……6

2.2 Brief History of the Kaibab Mule Deer…….....…...8

2.3 Habitat and Diet Composition……..……...……..…….10

2.4 Diet Selection…….……..……...…….12

2.5 Diet Quality…….. ……....……..…..16

2.6 Carrying Capacity versus *Biological* Carrying Capacity……25

2.7 Nutrition versus Under-nutrition Status …….…….…...31

2.8 Metabolic Indicators ……...……...…….……..31

2.9 Conclusion…….……..…….……..50

III STUDY AREA…….……..…..…51

Location…….51

Physiography and plant communities……51

Climate ……..52

Historical and Current Resource Management……..60

Plant communities/grazing resources……61

CHAPTER Page

IV DIET COMPOSITION & SPECIES RICHNESS……..……..64

INTRODUCTION…….……...…….……..64

METHODS..…….…...……64

Statistical Analysis……....…….....67

RESULTS…..……..……67

Forage Class Composition…….68

Species Composition……..71

Species by Season by Year…….....73

Year One, Mid-winter…….....73

Year One, Early Spring…….....73

Year Two, Late Fall…….....74

Year Two, Mid-winter……...74

Year Two, Early Spring…….76

Year Three, Late Fall…….77

Year Three, Mid-winter……...……..77

Year Three, Early Spring……...77

DISCUSSION……..78

Species richness…….78

Forage Class Utilization…….……80

CONCLUSIONS…….85

V WINTER DIET QUALITY…….…88

INTRODUCTION…….88

CHAPTER Page

METHODS

Forage Quality Analysis……....88

Statistical Analysis…….91

RESULTS

Quantitative Forage Quality Analysis……91

DISCUSSION……....95

CONCLUSIONS…….109

VI METABOLIC INDICATORS……..……..……...111

INTRODUCTION……..….111

METHODS

Physiological Response……...112

Statistical Analysis……...114

RESULTS

Carbohydrate Indicators……...114

Protein Indicators…….117

Lipid Indicators……....121

DISCUSSION

Carbohydrate Indicators…….……..129

Protein Indicators…….133

Lipid Indicators……....142

Cholesterol……...151

CONCLUSIONS…….154

CHAPTER Page

VII MODELING…….157

INTRODUCTION……...157

METHODS……...……...158

RESULTS……159

DISCUSSIONS……162

CONCLUSIONS…….168

VIII MANAGEMENT RECOMMENDATIONS……..171

WORKS CITED……..175

APPENDIX

A DIET COMPOSITION AND SPECIES RICHNESS ……...192

B DIET QUALITY…….……...….200

C METABOLIC INDICATORS……203

D MODELING……...211

E PHOTOGRAPHS AND DIAGRAMS…….....215

LIST OF FIGURES

Figure Page

Figure 3.1 July 2005- June 2008 calculated normal precipitation levels for Bright Angel ranger station, AZ and Kanab, UT……..……...…53

Figure 3.2 July 2005–June 2006 recorded precipitation levels, for Bright Angel ranger station, AZ and Kanab, UT……...…….....….…...53

Figure 3.3 July 2006 – June 2007 recorded precipitation levels for Bright Angel ranger station, AZ and Kanab, UT…...…….……...….54

Figure 3.4 July 2006 – June 2007 recorded precipitation levels for Bright Angel ranger station, AZ & Kanab, UT…….....54

Figure 3.5 Calculated normal temperatures Bright Angel ranger station and Kanab, UT (NOAA 2013)……. ……....……..……..55

Figure 3.6 2005 monthly mean temperatures Bright Angel ranger station & Kanab, UT (NOAA 2013)……..…….……....……...55

Figure 3.7 2006 monthly mean temperatures Bright Angel ranger station and Kanab, UT (NOAA 2013)……...……....……..……56

Fig. 3.8 2007-2008 monthly mean temperatures Bright Angel ranger station and Kanab, UT (NOAA 2013)……..……..…….....56

Figure 3.9 Historical mean precipitation (in.) data taken from Rasmussen's 1932 dissertation, shown in Appendix E, Photograph E.1……...…..…….57

Figure 3.10 Historical mean temperature data (deg. F) taken from Rasmussen's 1932 dissertation. Original data is shown in Appendix E, Photograph E.1……...57

Figure Page

Figure 3.11 GIS-generated map showing boundaries, major drainages, forest roads, and general location of the mule deer winter range study area on the Kaibab Plateau (aka North Rim, Grand Canyon Natl. Park) in Coconino county, Arizona…..….59

Figure 3.12 General vegetation species classifications by D.I. Rasmussen (1941). Note the 1931 date in the lower left hand portion of the map legend. It is believed that this was included with his 1932 dissertation. These classifications still generally apply at the time of this study (2005-2008)……..…….……63

Figure 4.1. Mean seasonal species richness by year for female mule deer diets on the N. Kaibab winter range; November 2005 to March 2008…….....68

Figure 4.2 Percent coverage of diet mean composition/utilization, context of a Forage Class x Year x Season interaction. Results are for female mule deer on the Kaibab winter range, November 2005 to March 2008…….….72

Figure 5.1: Mean digestible protein intake (DPI) of mule deer diets interaction by season and year. Year 1 = 2005-2006, Year 2 = 2006-2007, Year 3= 2007-2008……...93

Figure 5.2: MEI interaction with the variables Season and Year. Year 1= 2005-2006, Year 2 = 2006-2007, Year 3= 2007-2008. Data points reflect seasonal changes to available MEI through the three year duration of the study…….....95

Figure 5.3 Model comparison of digestible protein intake (DPI) in N. Kaibab winter diets and the projected nutrient requirements of a given day of gestation for a pregnant doe.....99

Figure Page

Figure 5.4 Model comparison of the dietary metabolizable energy intake (MEI) in N. Kaibab winter diets and the projected nutrient requirements of a given day of gestation for a doe……....102

Fig. 6.1 Mean plasma phosphate concentration (mg/dl) observed in mule deer does during late fall and early spring from 2005 to 2008 on the North Kaibab winter range…….....116

Figure 6.2 Mean blood alkaline phosphatase concentrations (mg/dl) observed in female mule deer during late fall and early spring, 2005-2008, on the North Kaibab winter range.....117

Figure 6.3 Mean plasma urea nitrogen (PUN) concentrations observed in female mule deer during late fall and early spring, 2005-2008, on the North Kaibab winter range…….119

Figure 6.4 Mean blood creatinine concentrations (mg/dl) observed in female mule deer during late fall and early spring 2005-2008, North Kaibab winter range……120

Figure 6.5 Mean plasma urea nitrogen: creatinine ratios observed in female mule deer during late fall and early spring, 2005-2008 on the North Kaibab winter range……..……..….121

Figure 6.6 Mean blood albumin concentrations (mg/dl) observed in female mule deer during late fall and early spring, 2005-2008 on the North Kaibab winter range……123

Figure 6.7 Mean plasma cholesterol concentrations observed in female mule deer during late fall and early spring, 2005-2008 on the North Kaibab winter range.....125

Figure Page

Figure 6.8 Mean blood triglyceride concentrations (mg/dl) observed in female mule deer on the North Kaibab winter range during the late fall and early spring, 2005-2005……126

Figure 6.9 Mean blood glycerol concentrations observed in mule deer doe on the North Kaibab winter range during the late fall and early spring, 2005-2008…..…….127

Figure 6.10 Mean blood free fatty acid concentrations observed in mule deer doe on the North Kaibab winter range during late fall and early spring, 2005-2008..…..…128

Figure 6.11 Interaction of Plasma Urea Nitrogen values with variables Season and Year. Note the pincer-like movement between LF and ES to center as the study progresses in Year 3. Available plant protein decreased in the late fall of 2007-2008 (13% high protein species), but also increased more in the early spring of 2007-2008 to 20% (high protein species) of the diet, partially explaining the phenomena……..…….....135

Figure 6.12 Cholesterol hormone seasonal interaction in the designated study year. Data point units are mg/dl. Note the disparity between LF and ES of Year 2; the visual effects of the pre- and post- mid-winter diet consisting primarily of Utah juniper (Chapter IV)……..…152

Figure 7.1 Linear scree-plot of variances of the metabolic and forage quality analysis factors the north Kaibab winter range …….……160

LIST OF TABLES

Table Page

Table 4.1 Forage class composition means of female mule deer diets, organized by season and year on the N. Kaibab winter range; November 2005 to March 2008 ……..…69

Table 4.2 Percent coverage of mean diet composition for Forage Class and Year for female mule deer in the timeframe of the study……...…….……70

Table 4.3 Percent coverage of mean diet composition for Forage Class and Year for female mule deer in three seasons on the Kaibab winter range……...…….70

Table 4.4 Means of coverage by forage class, (season x year) of mule deer diets on the N. Kaibab winter range, from mid-winter 2006 to late winter 2008. (Superscript notation indicates statistical significance.)… ……..…….75

Table 5.1: Mean Dietary Protein Intake (DPI) and associated standard deviation (Sd) sorted by season and year for mule deer diets on the North Kaibab, November 2005 to March 2008……..…….....92

Table 5.2: Mean Metabolizable Energy Intake (MEI) and associated standard deviation (Sd) by season and year for mule deer diets on the N. Kaibab from November, 2005 to March, 2008. ……..……..……...….94

Table 6.1. Mean blood parameters of carbohydrate metabolism indicators observed in mule deer during late fall and early spring from 2005 to 2008 on the North Kaibab winter range…….115

Table Page

Table 6.2. Mean blood parameters of protein metabolism indicators observed in mule deer during late fall and early spring from 2005 to 2008 on the North Kaibab winter range..…….....……..……118

Table 6.3 Mean blood parameters of lipid metabolism indicators observed in mule deer during late fall and early spring from 2005 to 2008 on the North Kaibab winter range…….....122

Table 7.1 Principle component results from backward stepwise regression using Akakie's Information Citerion (AIC) to assist in deriving the model. Variances of the predictive variables are listed below…….……161

IN MEMORIUM

William W. Acton

January 21st, 1940 – April 23rd, 2001

Mark William Acton

March 25th, 1963 – October 15th, 2011

You give me courage in my life.

CHAPTER I
THE NORTH KAIBAB MULE DEER STUDY: NUTRITIONAL, METABOLIC & MODULAR INFERENCES

INTRODUCTION

The North Kaibab mule deer herd demonstrates a dynamic state of growth and development. The Arizona Game and Fish Department, the managing agency of the deer herd, recently shifted the management strategy for the Kaibab to an annual 5% growth strategy. The quality and quantity of food source (forage) on this portion of the Kaibab National Forest, is in a static or declining state (Buck 2007) with some areas undergoing experimental habitat restoration by the Kaibab National Forest, United States Forest Service. Non-governmental organizations such as the Arizona Deer Association, and the Mule Deer Foundation, along with local hunting guides have disputed the necessity of controlling herd growth in favor of increasing the number and size of the deer. This management direction has been chosen in order to provide increased trophy hunting opportunities and a more pleasurable hunting experience.

In the Southwest, resource managers and the public have considered the mule deer herd located on the North Kaibab plateau (aka the North Rim of the Grand Canyon) within the Kaibab National Forest one of the crown jewels of wildlife populations. Hunting trophies taken from of this region have set multiple Boone and Crockett and Pope and Young records (Arizona Game and Fish 2007). Resource managers across the lines of state and federal agencies have made observations of the deer population. A physiological and metabolic conundrum seems to exist – the study area's reputation for producing large-bodied trophy-class mule deer bucks is intact; animals of this size and body type have been seen by the author, alongside bucks that do not show any visible

difference in body mass than an average doe. An observed dichotomy in body condition, size and class among the male and female deer suggests a metabolic inadequacy.

Fawn to doe conception rates of 1 – 2:1 are within the expected range, while ratios of 3:1 are indicative of the conception of triplets (Acton 2006). Conception rates for the mule deer on the Kaibab – found to be 180% - are indicative of the quality of the higher altitude summer habitat than the lower altitude winter habitat. Yet the annual increase in herd numbers is not correspondent to this conception rate. Field necropsies of euthanized doe demonstrated viable fetuses, the unchanging population numbers (Wakeling 2005) are indicative of a stable population in the presence of predation.

Browse clip studies by the Arizona Department of Game and Fish demonstrate a heavy use of the browse plants on the Kaibab (Buck 2007). Two of the critical browse species utilized by deer on the Kaibab winter range Mexican cliffrose (*Purshia stansburiana*) and Apache plume (*Fallugia paradoxa*) are overtly mature, decadent and in a state of declining health. Winter forage resources sought by deer are typically young, tender, annual growth browse. On the northern many portion of the Kaibab winter range the typical morphology of Mexican cliffrose or Apache plume is more similar to that of a tree than a low-lying shrub, with total heights of three to four meters and lower canopy heights of >2 m. Each of these shrubs, part of the plant family *Rosacea* are able of nitrogen fixation, providing an excellent sources of forage protein.

Annual growth of these two shrubs provide an important protein and caloric resource for the mule deer but have become out of the reach of most deer. What is within reach is not necessarily palatable forage as the lower branches of decadent shrubs and trees may not produce the meristematic annual growth or terminal bud that is

characteristic of desired palatable browse. Drought conditions, past grazing practices, wildfire, and heavy browsing use by the deer in other areas of the study have influenced the reproductive and growth morphologies of these shrubs, stunting the palatability and nutritional quality. Active grazing by cattle in the area may even temporarily displace the deer from the cattle pasture (Miller 2013).

The pressure placed onto the shrub and understory of the shrubs (grasses, forbs, smaller shrubs) as a result of disturbance (drought or fire) or reaction to anthropogenic influence (grazing or reclamation assistance after a disturbance) can have a demonstrable effect on the palatability of a primary source of forage protein and energy. Because palatability has been affected, then availability of the nutrient resource has been affected as well – not only on what can be bitten off the plant, but on how easily the forage substrate is decomposed and the nutritional resource is obtained within the rumen. Grazing pressure can then shift to less desirable, less palatable species. On the Kaibab Plateau that means species such as big sage (*Artemesia tridentata),* rubber rabbitbush (*Ericamera nauseosa)* and Utah juniper (*Juniperous osteosperma)* are utilized on a regular basis. Browse activity has shifted from the higher energy, high protein source to the lower end of the caloric and protein (i.e. atmospheric nitrogen fixation ability) spectrum.

The Kaibab winter range has historically seen extreme fluctuations in the range's ability to support the wintering mule deer herd. During those periods when the available forage resource has been of questionable quantity and quality, the presence of large numbers of wintering deer (the result of fecundity and transient immigration into the herd) and extreme adverse climatic events have resulted in abrupt and disastrous impacts

upon the survival and number of deer that remain. The dietary intake, controlled by the hippocampus (Church 1988, Guyton and Hall 2006) of a mule deer is centered on satiation or "fullness" of the rumen and is not controlled by nutritional content. Appetite isn't increased or diminished if caloric or protein needs are being met. Satiation is a function of the volume of ingested forage held in the gastric stomach and rumen combined with rumen fluid. A rumen can be full of low-grade forage, but the time and microbial action needed to break down the forage into microbial by-products for energy is great enough that an inadequate amount of energy or protein is being provided to the deer. The ruminant would be starving with a full stomach.

There is a historical precedent on the north Kaibab winter habitat (adjacent to the Grand Canyon National Park) of a boom-and-bust population dynamic. Taken as anecdotal evidence, the diet of obvious plants combined with ocular estimation of the herd should present a quality habitat for mule deer. Historic deer population irruptions on the Kaibab suggest otherwise. The intent of this research is multi-faceted. First, to evaluate the forage resource (Early Winter /Late Fall of 2006 to Late Winter/Early Spring 2009) for energy and protein content. Second, analyze the metabolic response of the North Kaibab mule deer herd from the Late Fall of 2005 into the Early Spring of 2008 by examination of blood and plasma tests. Third, derive a model of the interaction of the Kaibab mule deer response with the available habitat forage resource. In this process, a concept of the winter nutritional ecology of the North Kaibab plateau with an understanding of the relevant historic research and prior population events will develop.

CHAPTER II
LITERATURE REVIEW

"The pyramid of science in wildlife nutrition must rest firmly on a base of studies of animal physiology and ecology, with management programs at the apex. Only then will efforts to maximize management returns by altering wildlife productivity through nutrition be rewarded."

- Charles T. Robbins, 1983

Mule deer are noted to be in an active decline throughout the American West. While exact causes are unknown, degradation and loss of habitat from energy exploration is of prime concern (Knaus 2009, Walston et al. 2009). While exact causes are unknown, degradation and loss of habitat from energy exploration is of prime concern. Habitat losses can result in such a reduction of the amount and access to critical forage resources that the population of a given species is lost, creating a "sink patch" (Dodson 1998). Habitat degradation is not limited to habitat fragmentation but can also mean overgrazing or competition from invasive species.

In the Southwest, a great deal of money, labor, and equipment are spent on wildlife habitat restoration from previous fragmentation or impact to the landscape by commercial usage. A habitat restoration project has the primary objective of altering an existing aspect of the habitat to a quantity and quality of soil, water and/or vegetation resource(s) that are more conducive to a higher quality sustainable resource. Many times this should mean a reduction of less desirable forage species and the enhancement and propagation of more desirable forage species, alteration of a fire regime or the creation of

an artificial water source where needed. Sometimes the well-intentioned public, without data to support the habitat modifications in a given area, alters the habitat without a net gain and potentially a net loss to the overall quality of the habitat. For example, the installation of water catchments may be proposed and installed as a cure-all for a habitat, on the assumption that water is *the limiting factor* for a living organism in the arid Southwest. This is an easy assumption to make when there is not a known source of water (this is a specific problem that was encountered – within the Arizona Deer Association - when beginning this study) in the area – yet the wildlife populations are not visibly dehydrated.

Without proper analyses in the context of forage quality, landscape ecology, hydrology, or at least knowing what the essential elements of a habitat for an indicator species are, a well-intentioned water catchment could be a wasted effort, a waste of finances, and not beneficial to the intended herd (Rautenstrauch and Krauseman 1989). Nutritional data may provide a more responsible direction of financial resources and physical efforts. Action based on information and fact instead of perception, with the public being further educated in good habitat management practice as an additional outcome would be the highest return on the invested dollar. Physiological assessment of the habitat conditions could be assistive for making population projections – either increases or decreases (Del Giudice et al. 1990).

2.1 The Kaibab Plateau

The Kaibab plateau, colloquially known as the North Rim (of the Grand Canyon and the Grand Canyon National Park) is not a pristine, untouched ecosystem. Since

Hispanic and Anglo exploration and settlement in the Southwest, the landscape of the Arizona Strip (including Glen Canyon National Recreation Area, Lake Mead National Recreation Area, the Grand Canyon National Park, the Kaibab National Forest) and the Colorado plateau geologic uplift shows an anthropogenic foot print. Mining, grazing, logging, rivers dammed and manipulated for the natural resources that make up the landscape for economic gain.

The mule deer of the Kaibab and the habitat they occupy are a population of animals on hectares of public land. A migratory herd that is a public resource on public land that intensively observed and studied by a number of non-governmental organizations (NGOs). These NGO's are regional to Arizona, Utah and the North American west where trophy – hunting of mule deer antler "racks" is a priority with the hunting public (ADA 2006, AzGFD 2007). The North Rim is remote, requiring a long period of travel from a major metropolitan area to access it. Until the national highway and interstate systems were improved in the 1950's and 60's, Kanab, Utah and the Kaibab Plateau, (located 48+ kilometers to the south) was considered to be "… one of the most isolated areas of the nation…". The plateau was cut-off from the east, west and south by the Colorado River (including the Grand Canyon National Park), to the north by the area now known as the Zion National Park (Kanab 2014). With modern roads, the North Kaibab is about 8 hours from Phoenix to the North Rim via Flagstaff and the Navajo Bridge at Lee's Ferry, 5.5 hours from Salt Lake City, 8 hours from Los Angeles and approximately 10.25 hours from Denver (Google Maps 2013). The nearest settlement is Fredonia, AZ, and the nearest town with medical facilities and a grocery store is Kanab, Utah.

2.2 Brief History of the Kaibab Mule Deer

A description of the relationship between a mule deer (*Odocoileus hemionus hemionus)* and the habitat that it lives in is a complex, intertwining discussion. Many factors are woven together to create an environment for the ungulate that provides a sustainable, renewable natural resource for perpetuating a mule deer herd. Rasmussen's monograph on "The Biotic Communities of the Kaibab Plateau" (1941) discusses to great extent the flora and fauna of the Kaibab, providing a historic insight into the resource management conundrum that is the Kaibab mule deer herd.

Ford's book, Modeling the Environment: An Introduction to System Dynamics Modeling of Environmental Systems (1999) introduced the history of the Kaibab mule deer herd. A rapid period of herd growth, noted in 1918, is recognized as a contributing factor to influence the declining condition of the available forage. Ford draws on John Russo's 1970 internal Arizona Game and Fish bulletin to examine the size of the predator population on the Kaibab. According to Ford, in the interval from 1907 to 1923, predator kills were estimated at 3,000 coyotes, 674 mountain lions (e.g. "puma" in older works) 120 bobcats and 11 wolves. In August of 1924, a reconnaissance party reported seeing over a hundred deer in a day's drive. Local witnesses estimated the number of deer to be no less than 50,000 (Young 2002).

Rasmussen's monograph (1941) provides corroboration to the details provided regarding the history of the population dynamics of the Kaibab deer herd, in the context of rapid removal of predation on the mule deer population. In 1941, Rasmussen estimated the 1924 - 1926 population to be at or around 50,000 deer, dropping to approximately 15,000 (Young 2002, Binkley et al. 2006). Rasmussen based his

information on the report of U.S. Forest Ranger Benjamin Swapp, who was "in charge" of the area where the deer died off (Young 2002).

A major die-off of the deer herd occurred in 1924-28, as reported by John Russo, where 75% of the previous year's fawn crop died in the winter (i.e. on the winter range). Aldo Leopold stated in his 1943 work that fawn die-off was about 60% during two successive winters, and "By then, the girdling of so much of the vegetation through browsing precluded recovery of the food reserve" (Ford 1999). To quote Caughley, "… Leopold (1943) is usually quoted as the source of these data but he has given only a generalized interpretation of estimates presented by Rasmussen (1941)." Leopold's information becomes anecdotal and unsubstantiated; Ford does not relay any information on the nutritional quality, what plants the diet consists of, meteorological conditions, weather records, or the location of the plants. Binkley et al. (2006) points out Young's (2002) statement in his work regarding the North Kaibab "…that whatever the real dynamics of the deer population might have been [during the original population crashes] the Kaibab deer story is an excellent example of the interaction of science, conservation, politics and management". Binkley's work is focused however on the historical revision of the irruptions population numbers and the re-vegetation efforts of the summer range, specifically quaking aspen (*Populus tremuloides*) growth.

Ford adds in his post script that the data on the Kaibab deer herd from 1906 to 1939 is "unreliable and inconsistent" (pg. 205), and that many factors "…that could have contributed to the upsurge in the deer population are hopelessly confounded." Ford also reinforces Caughley's deduction; found in Caughley's 1970 article from Ecology that "a change in the vegetative habitat was postulated as the cause of the initial increase in

[animal] numbers". Caughley continues with … "the willingness of one author after another to accept the previous author's account of the Kaibab situation…" when reviewing the accumulation of evidence regarding the Kaibab". Young (2002), through journalist Emerson Hough, reports the condition of deer in 1922 (shortly after the formation of the Grand Canyon National Park and adjacent Kaibab National Forest) as being plentiful and "…very low flesh". In the same paragraph Young provides Hough's statement that the cattle in the same area "…were in very poor condition" in general (Young 2002).

Young presented a convincing argument of predator control and degraded grazing conditions from overuse/misuse of the range as the causation of the sudden decline of the deer population reported in the 1920's-1930's. D.I. Rasmussen contributed his monograph regarding the Kaibab originally as a dissertation from the University of Illinois in 1932. A full record of the nutritional vegetative ecology – including energy, protein and dry matter digestibility -of the North Rim winter range has not been found to date. If these historical die-offs in the Kaibab mule deer population did occur in the magnitude that is alleged and described in the more historical literature (Rasmussen 1941); resource managers, special interests and the public at large would like to avoid this catastrophe – in terms of animal die-offs and from a public relations debacle - from occurring again (Wakeling 2005, 2010).

2.3 Habitat and Diet Composition

Provided there is adequate water, cover and space, a long held concept of wildlife habitat management is that the health and well-being of a wildlife population is directly

proportional to the quality and quantity of food resource available to the animal population (Miller 2004). A wildlife population maintained on a high nutritional plain is more productive and less susceptible to mortality factors of disease, environmental stressors and predation. If the lactating female after parturition (the time when metabolic nutritional demands are highest for the doe and thus species) is the foci of habitat management efforts, the habitat will adequately meet the needs of the designated herd size (Miller 2004).

Much of the past habitat investigations of the north Kaibab have been focused on summer range conditions and the ability of those ranges to meet the seasonal needs of the mule deer herd (Hungerford 1970, Haywood et al. 1987). , A different approach to evaluate wildlife habitat was derived from science in the past 30 to 40 years. This approach basis is the ability of a specific area to meet the nutritional and physiological requirements of the individual animal, typically an animal that would have the greatest nutritional demand from the habitat (i.e. a lactating doe with young) (Miller 2004). This approach is a keystone of nutritional ecology, the science of relating an animal to its environment through nutritional interactions with the habitat (Van Soest 1982, Robbins 1993, Parker et al. 1999).

During the late 1960s, Plummer et al. (1968) stressed the importance of good winter range for deer. Plummer and his cohorts reported on observations of winter survival of mule deer during the severe winter of 1948-49, stating that deer on poor condition winter range (where browse availability was inadequate) suffered heavy losses of up to 42%, while deer on good condition winter range during the same climatic events experienced losses only slightly higher than normal. Julander et al. (1961) reported

significant improvement in deer productivity by improving the quality and quantity of forage on both summer and winter range.

To date, conservationists, ecologists and wildlife professionals have looked at the concerns of the Kaibab herd in terms of predator/prey inter-relations, interaction with domestic grazing, availability of water sources for the deer, especially on the summer range of the deer ad nauseum. A focus that has not been considered is the forage resource of the winter range. Specifically, the energy and protein availability from the forage resources (i.e. kilocalories) and the forage usage by the deer. Forage protein resources that are available as a source for both amino acids and calories and the metabolic response of the deer to the energy content of the habitat forage resource. Assays by different common blood tests applied to a ruminant can gauge the metabolic response of the cervid to the available forage. While the forage input is converted differently than other mammals (volatile fatty acids versus large amounts of glucose) the values of protein markers, lipid analysis, steroid levels and other carbohydrate assays can provide an insight into how well the deer are utilizing the forage resource.

2.4 Diet Selection

Diet selection is "…fundamental to understanding the ecological interactions between deer and their habitat" (Hanley 1997). Ruminant animals, such as mule deer, fall into essentially three different categories, differentiated by oral structure, anatomy, and rumen capacity.

"Grazers" typically have a large rumen to facilitate the uptake of large swaths of grass and other plants that may have a comparatively low nutritional content of high

insoluble fiber and low cellulose, cellubiose and hemi-cellulose (Church 1988, Hanley 1982). This type of animal (e.g. Bison or domesticated cattle) has a large rumen capacity and a broad, coarse mouth and tongue to eat large amounts of grass. Precise selection of foodstuffs is not of concern, as the animal is concerned about *quantity*, not quality of food to maintain its nutrition.

Another type is a mixed feeder, such as an elk, utilizing both types of low quality grasses and higher quality browse. The elks oral structure is somewhere between a grazer and a browser, with a more delicate, agile tongue than a bison but lacking in the bifurcated lip of a deer.

The last type of feeder is a browser, such as a mule deer, who is selecting foodstuffs based on concentration, or *forage quality*, albeit lower in cellulose but typically lower in lignin content than a grass-grazer, such as beef cattle (*if* grass is not a significant portion of the deer's diet). Deer have a bifurcated lip, slender and agile tongue and mouth, and a smaller rumen capacity. A deer is more suited to digesting a high-quality (elevated carbohydrates and protein) diet, where a grazer is not. A grazer is better suited to digest a high-cellulose high-lignin diet. A ruminant who is a concentrate selector is not as well suited to digest lignin as a grazer because of the smaller volume (and potentially faster passage rate) of the concentrate-selector's rumen, versus the slower passage rate in the high-volume fermentation vessel of a grazers rumen. (Hanley 1997, Church 2008). While these may seem to be distinct, black-and-white categories, they instead reflect the vast majority of diet selection by the animal with enough overlap between types that distinctions become blurred. The non-differential bolus passage rates through the rumen cause this lack of definition (Robbins 1993). Deer rumen contents

taken and examined from the Kaibab during the late fall periods in the course of this study have shown presentable quantities of grass along with a notable quantity of browse from evergreen trees mixed with a sizeable acorn crop (mast) and sage browse. McCulloch (1978) supported this with similarities in rumen content data dating to 1947.

Selection of food by deer reflects not only anatomy and dentition (Church 1988) but also available browse crops. At the location of our study area, this means leaves, stems and acorns from Gambel's oak (*Quercus gambelii)* and other *Quercus* species, leaves, stems and berries from *Berberis spp.*, leaves and stems from big sage, Mexican cliffrose, Utah juniper and Piñon pine (*Pinus edulus*), plus other winter browse crops. To paraphrase Hanley (1982) "…the idea is that … (the) type of digestive system determines the overall time-energy constraints within which the ungulate must secure its food…Mouth size determines the degree of selectivity that is mechanically possible for the forager to exhibit and the time and energy costs of selectively foraging on specific plant parts…". Quality (or concentrate) feeders will require smaller anatomy to extract energy, proteins, and nutrients from smaller amounts of forage whereas grazers who focus more on high fiber, low concentration forages (i.e. bison and domestic cattle) will have a larger anatomy more suited to larger volumes of [high fiber] forage (Church 1988). The physiology of the animal will dictate the species and quantity of food that it needs; energy/nutrient demands plus rumen capacity will determine the basal metabolic rate of the ruminant, how frequently and intensely it grazes.

The bite size of a deer has been observed to be "… approximately 0.10 grams". (Hanley 1982, reporting Deschamp 1979). This seems to be in error, as Deschamp (1979) states that the bite averages were *calculated to the nearest 0.10 gram.* Deschamp

continues to provide a weight per bite estimate ranging "… from 0.02g to 1.53 grams, depending upon the species and size and form of the plant parts taken". (It is unknown if this is a wet or dry weight – Wickstrom alludes to the weights being dry, as DM listed below refers to Dry Matter.) This compares to Wickstroms time-study of g/bites per minute returns of "asymptotic grass intake of 0.15 g DM/$kg^{0.75}$ and 3.76 g ± 1.18g DM/minute of mixed diet of grasses, forbs, and browse in the understory…" of a conifer forest. Wickstrom describes a mean deer bite size in conifer understories to be at 180 ± 31mg DM/bite in a conifer forest to an overall mean for browsing mule deer ranging from 154 mg to 440 mg DM / bite (Wickstrom et al. 1984).

More time is spent in rumination than actual feeding; what a deer puts into the gastric stomach, a bolus then passed to the rumen needs to be nutritionally viable to ensure survival or promote growth. In comparison with a larger capacity Bison, it is the time in relation to rumen volume where the masticated forage is contained in the rumen liquor that determines the forage turnover rate within the rumen. Concentrate (quality) grazers though also crave coarse forage and will somewhat maintain this forage in the diet (Van Soest 1982). The time spent ruminating is "… generally proportional to the (amount of) cell wall intake". Further, "Animals with a bigger, or greater, appetite will ruminate less per gram of cell wall, resulting in a greater fecal particle size …" This is an adaptive response by the concentrate selector that will allow the consumption of more food, larger amounts of feed and thus avoid the rumen fill limit (i.e. satiation) by passing larger sized and less processed ingesta." (Van Soest 1982). Van Soest provides "… a linear relationship of the log of rumination rate and body size …" linearly modelling

Y = Rumination Rate (g Cell Wall/Min), X= Body Weight (kg) with a slope = 0.95. With the average bite size measured in hundreds of milligrams and an almost 1:1 ratio (slope =0.95) of cell wall size to time of rumination by "x" cell wall(s), it is understandable why most of the animals time is spent constantly grazing for food.

2.5 Diet Quality

Wintertime diet evaluations for North American mule deer habitats located in the south-west are infrequent in the literature. Middle Park, CO is located at the headwaters of the Colorado River (Wallmo et al. 1977). Wallmo and others conducted a study in 1977 that resembles the diet evaluations I have done on the winter habitat of the North Kaibab. The winter mule deer population, concentrating on southerly slopes in January's storms, would end up occupying as little as 0.44ha/deer of winter range (Wallmo 1977) during the winter of 1975-76. Forage resources listed are very similar to those found on the Kaibab, and provided a 1:1 relationship between the percent digestibility of dry matter (DM, or DMD) and digestible energy (DE). High cellulose content of winter diets were attributable to grasses (37 – 37%) and shrubs (19-32%). The shrubs also had a high lignin content compared to grasses (18-25 % vs 5 -6%). (While documented in a similar study to this study, these values of lignin levels are in question because of the known process of lignification of C3 and C4 grasses through winter senescence. A shrub species in the Middle Park, CO study shared with the North Kaibab winter range is big sage.

Another study in Colorado provides more insight into the forage quality that might exist in the West and Southwestern mule deer habitats. The Cache la Poudre range in Colorado was the subject of a study on chemical composition and digestibility, done

by the Colorado Department of Game and Fish in 1962 (Dietz et. al 1962). A chemical analysis was completed on the winter habitat forage to determine the percentage protein, fat, fiber and nitrogen-free extract (soluble carbohydrates). Two winter habitat locations were designated; Seven-mile Creek and Hewitt Gulch. Primarily shrubs were tested, the species included big sage, mountain mahogany, bitterbrush, Utah juniper and rabbit bush.

A difference in metrics and methodologies prevents a species-to-species comparison of the nutrient values. The Cache la Poudre study intent was to compare summer to winter evaluations of the range and forage resource. Time of use of the resources were similar; "Generally, the lowest level in protein content concurred with the peak of winter use". Mean values of percent protein during the winter were approximately 10-13% for big sage. In terms of utilization, the captive deer in the study refused to eat big sage, and on average lost "… 2.5 lbs. with a mean consumption of *Artemesia tridentata* and alfalfa-mix of 1.66 lbs. /cwt, or per hundred-weight" (paraphrase of tables and text, Dietz et al. 1962). The deer ate 1.66 lbs. of *Artemesia tridentata*/alfalfa feed for every hundred pounds (rounded) the deer weighed (paraphrase of tables, Dietz et al. 1962). Diet composition apparently can affect the appetite of mule deer and the utilization of forage by them.

What is now termed Digestible Energy is referred to in the Cache la Poudre study as "nitrogen-free extract" (NFE), and methods of proximate analysis are the study's foundation. For the 1950's-60's winter ranges, Dietz et al. 1962 is reporting NFE means for big sagebrush 56.1 - 51.5%, bitterbrush (rubber rabbitbush) 56.5 – 56.1% and Mountain Mahogany in the range of 55.5 – 53.2%. Juniper has a mean NFE of 49.3%. These numbers are stated only to present an idea of prior work. While these results were

leading edge in their day, for our purposes today the results and metrics do not have the necessary resolution for inclusive analysis within this study.

Conclusions from Wallmo et al.'s 1977 Middle Park, CO. study suggest the winter diet provides a lower level of metabolizable energy (ME) than the summer diet, and quantifies the more digestible species of the forages having provided more energy than the less digestible species. Wallmo (1977) suggests that the selection of highly digestible grass during the winter diet is possibly a reaction to the indigestible portion of the diet (and woody browse) that not only includes VOC's (volatile organic compounds) as essential oils of the sage, but also phenolic lignin compounds. Notations are made in the paper that ruminant game and domesticated livestock are "…apparently able to select plants and plant parts richer in nitrogen than those sampled by man…" reaching the conclusion that the crude protein values found might underestimate the dietary protein. Winter protein supplies though are still below "… the needed maintenance requirement" (Wallmo et al. 1977).

Rohwer's 1970 work in the Ruby Mountains of Nevada (Elko, NV) provides blood chemistry results to dietary influences of a forage mix that is very similar to the one found on the North Kaibab. Mule deer were trapped (n = 700) in order to obtain blood samples through a jugular puncture with a hypodermic needle. Some destructive sampling of the mule deer also occurred, as Rohwer provides descriptions of rumen contents in the work. The rumen contents contain Juniper, Mexican cliffrose and Antelope Bitterbrush (*Purshia tridentata).*

The primary concern for any foodstuff eaten by a ruminant is that the structural carbohydrates converts into a useable form within the rumen by fermentation. The cell

wall intake Van Soest (1982) referenced in the prior paragraphs is a structural carbohydrate that is converted via bacterial and protozoan degradation (i.e. fermentation) into a bacterial waste product of a volatile fatty acid, or VFA. These volatile fatty acids are primarily acetic, butyric and propionic acids, and include valerate, iso-butyrate and iso-valerate in smaller proportions (Allen (1977). Propionic acids become noticeably elevated when starch is introduced or increased in the diet (Beitz 2004, Goff 2004, and Leek 2004). The substrates utilized by the citric acid cycle to (eventually) formulate ATP or ADP are these same bacterial "waste products" present after a series of cellular biochemical reactions (Allen 1977, Van Soest 1982, Voet and Voet 2004).

VFA substrates can be mobilized through the bloodstream to cells in need of energy substrates or remain within the original cell, depending on where the energy demand is located within an organism. VFA production can be encouraged by the amount and type of forage ingested. Likewise, inhibition of VFA production is induced by the presence and amount of volatile organic compounds. The lack of structural carbohydrates in a ruminant's diet – provided by the forage resource – means a lack of microbial VFA by-product in the rumen liquid passing through the rumen wall and transported through the blood stream to areas within the muscles and organs that "fuel" is needed. If a forage diet falls short of these resources, the ruminant cannot exist on a dietary plane as those animals who can obtain adequate energy from their forage. "The cells of the body require a constant supply of nutrients to utilize for fuel and the synthesis of new proteins" (Goff 2012). Eventually, because the lack of energy resource of the compromises the physical integrity of the animal, the chance or risk of predation increases for the animal. Potentially predation removes the animal from the ecosystem, a

portion of the larger scale inclusive of natural selection. (Darwin 1882, Taylor 2008). Hefflefinger (2006) makes note of the evolutionary existence of the cervid ruminant since the Pleistocene era, while other conservation professionals have described the response of a ruminant species that is the product from eons of predation since their flourish in the biological world (Taylor 2008).

Dietary proteins, provided by the mastication of available forage and the activation of pepsin by hydrochloric acid within the gastric stomach, are eventually hydrolyzed in the lumen and mucosal cells of the gastrointestinal tract a number of proteases and peptidases (Bietz 2004), provide free amino acids to be transported (initiated at the liver) through the bloodstream. These forage-based amino acids are joined by the free amino acids provided by the continual catabolism of replenished tissue proteins. The values of free amino acids found in the blood "bank" of available amino acids are augmented by the protein source of expired bacteria and protozoa found within the rumen and small intestine. The microbial action within the rumen is extremely efficient at hydrolyzing dietary proteins, degrading forage nitrogen and placing nitrogen into either an –ammonia (NH_3) form or reduction into a carbon skeleton containing -N. Ammonia and the carbon skeletons are then available for re-synthesis of amino acids by the microbial population. Sources of amino acids to the ruminant animal are those proteins that escape hydrolysis by the rumen microbes and those proteins that are constituents of microbes that reach the abomasum or true stomach and small intestine for digestion.

The resources of available plant-based nitrogen and single-celled animal protein are supplemented by the ruminant's ability to recycle nitrogen found in urea in order to

extract needed nitrogen during time of dietary protein stress. This becomes especially important during times where the protein provided by microbes may be low (e.g. when the species components of the diet increase the VOC's within the rumen - as with a heavy diet of sage or Utah juniper - may have reduced the microbial population within the rumen) (Allen 1977, Owens and Zinn 1988, Beitz 2004). The ability to re-direct and de-construct ammonia at various blood concentrations from the urea cycle, to be synthesized into new amino acids or catabolize the carbon skeletons into energy products, is controlled by carbohydrate supply into the rumen, the ammonia-N concentration and the pH of the rumen liquor. Inadequate levels of structural carbohydrates and/or an acidic pH, and the rate of ammonia recycling to harvest nitrogen through the rumen wall is influenced (Owens and Zinn 1988).

Dietary protein provides a needed and necessary source of nitrogen for mRNA, RNA and DNA translation and trans-amination. An inadequate amount of dietary protein can lead to an inability to fully regulate metabolism (hormones and enzymes lacking); inability to repair structural components of bone, muscle, ligaments and tendons; or to repair cellular components damaged from disease, wear and tear or injury to the body. The inability to transport materials to the cells such as oxygen via hemoglobin, albumin for osmoregulation, and interferon or immunoglobin for defense against disease or foreign invasion to the body can result (Beitz 2004, Allen 1977). Although the ruminant is highly evolved to utilize and convert waste from catabolized amino acids and ammonia for its protein needs, the presence of a dietary source to support the symbiotic rumen population of single-celled animals is paramount to the animal's well-being. Within the scope of this study, forage supply of nitrogen is available not from supplemental feeding

of alfalfa hay (*Medicago sativa spp.*) and whole corn (Peterson 2007), but indigenous forage species of the plateau. Some species higher in nitrogen content than others would be Mexican Cliffrose and Apache plume.

Volatile Organic Compounds (VOC's) provided through "essential oils" from big sage can significantly inhibit multiple aspects of ruminant digestion. In trials with whethers, R. Ngugi (1995) determined that total Nitrogen intake (i.e. protein sources) dropped by 1+ gram(s) per day of intake. While laboratory analysis indicated high nutrient content in big sage, dry matter intake dropped within 24 hours of the introduction of big sage; the addition of 30% big sage lowered intake among the whethers from 88g/day to 23g/day. Each 1% increase in big sage provided a 2.35 g/day decrease in dry matter intake. Sagebrush levels in the diet accounted for 90% of the variation in dry matter intake. In vivo digestible dry matter dropped from 59% of grass hay to 0% with a diet of 30% big sage. The correlation with dry matter was strong, r=0.93. With big sage removed from the diet intake rose to original pretreatment levels within 24 hours for 15 of 16 whethers.

Ngugi also observed a lower water intake for animals on a high big sage diet, referencing a similar observation that co-author Powell had in 1986 regarding water output. Ngugi's conclusion was that while "… the protein source (N) is present in *A. tridentata*, it is inaccessible because of the volatile organic compounds and terpenes that inhibit the full microbial digestion of the rumen environment."

These findings add to the body of knowledge regarding the dietary efficacy of big sage, and are not a surprise. Nagy (1964) found that "…the essential oils of sagebrush inhibited all organisms tested and appeared to have a …general antibacterial spectrum for

both gram-positive and gram-negative (microbial) organisms tested." It did not take a high concentration of the essential sagebrush oils either. Tubes containing 0.04 - 0.05 mL to 0.1mL of essential sage oils inhibited all bacterial growth. Cellulose digestion was retarded but completed. By the fourth day in cultures that contained 0.002 mL to 0.006 mL of the oils. In contrast, by the third day of incubation all cellulose in the control tube was found to be digested.

Nagy also investigated the effect of big sage on VFA and gas production in a rumen. His results demonstrate a decrease in both VFA and gas (CH_4 and CO_2) production with the introduction and increase of essential oils. In a fistulated steer, by the fourth day after continued addition of sagebrush substrate via the fistula, peristaltic contractions had ceased and bloody, mucus-covered feces were recovered. Total mM/L of VFA's present in the fistulated rumen "…dropped from 123 mM to 57 mM. At the end of seven days, all muscular contraction and movement in the gut had ceased. In order to get the steer to recover from a weeklong lack of nutrition a transplant of rumen contents (including rumen fluid, similar in concept to a human fecal transplant) from cows feeding on alfalfa hay was carried out.

Essential oils and volatile organic compounds are not the only means to influence the effectiveness of a ruminant's dietary resource. A 1977 study by Wallmo and others identified lignin being present in the diet, with deciduous shrubs providing 18-25% of lignin versus grasses at 5-6% (Wallmo et al. 1977). Wallmo does not identify if sampling included only the terminal bud; secondary deciduous shrub plant parts could have been included in their analysis. Lignin is a compound with a polyphenol structure and is a component of plant cell walls. Lignin content increases as plants mature. If deer graze

second-year or older growth during times of winter stress, a higher level of lignin will be ingested. Other polyphenols can include tannins, such as those found in acorns and other nuts. Lignin and polyphenols are "…notoriously resistant to decomposition" (Brady and Weil 1999). Lignification is "…a relatively recent process that emerged about 430 million years ago … the deposition of lignin in plant cell walls was part of the mechanisms which allowed the development of upright plants …" to adapt from an aquatic to a terrestrial habitat (Boudet 2000). Lignin, accounting for nearly 30% organic carbon in the plant biomass has the function of strengthening plant tissues, augmenting and assisting turgidity in the vascular system of the plant (Boudet 2000). Lignin also provides an indirect plant defense against fungus. "Moershbacher et al. indicated that chemical inhibition of lignification in wheat result in a decreased resistance to *Puccinia graminis*…" (Boudet 2000). *P. graminis* is more commonly known as "stem rust", and is a fungal condition of cereal grains (Roelfs and Martens 1988)

Lignin is indigestible by rumen microbes, limiting digestion of carbohydrates (Hatfield et al. 2009). The molecules of the lignin phenol and polyphenols are large and complex, made of "…hundreds of interlinked phenolic ring subunits, most of which are phenyl propene-like structures with various methoxyl (-OCH_3) groups attached…" (Brady and Weil 1999). Phenols can form irreversible complexes with proteins. A basic lignin molecule contains three monomeric mono-lignol units, p-coumaryl alcohol, coniferyl alcohol and syinapyl alcohol, with the proportions of these three alcohols varying between species and response to the environment (Boudet 1998, 2000). Coniferous gymnosperm lignin are usually composed of a polymer of coniferyl alcohol

and *p*-coumaryl alcohol, while angiosperm lignin derive from combinations of coniferyl and guaiacyl and syringyl (Boudet 1998, 2000, Hatfield 2009).

Released during mastication from plant vacuoles, phenols reduce the digestibility and availability of plant protein(s) (Mould and Robbins 1981). Although lignin has "…a higher calorific value than polysaccharides …" and therefore can provide more calories (Boudet 1998), a rumen cannot break down the lignin polymer sufficiently enough for the molecule to be transferred across the rumenal wall (Mould and Robbins 1981). Although free radicals would exist after microbial degradation of the lignin (Green 2013), the lignin molecule is still too large to pass through the semi-permeable membrane of the rumen papillae wall. Too much of these substances may impede the overall bacterial action of the rumen or interfere with the absorption of VFA's and rumen liqueur by the rumen walls (Mould and Robbins 1981), essentially "clogging" the transport mechanisms via the rumenal papillae until the phenol polymers can be evacuated from the digestive system through the feces.

The result is that the combination of volatile organic compounds and lignified polymers in the ruminant diet can significantly interfere with the animal obtaining an adequate amount of nutrition from the available forage. The interference is greater when the diet approaches or exceeds a 30% content of big sage as combined with other shrub and grass species, elevating the overall amount of lignin in the rumen (Nagy et al. 1964).

2.6 Carrying Capacity versus Biological Carrying Capacity

Numerous conservationists, ecologists and wildlife professionals have looked at the concerns of the Kaibab herd in terms of predator/prey relationships, interaction with

domestic grazing and availability of water sources for the deer. Plant communities on the summer range, forage resources, habitat quality, and the carrying capacity of the Kaibab habitat (Leopold 1943) are historically a concern since President T. Roosevelt created the forest game preserve (Russo 1967, Young 2002).

Population growth is a continuation of exponential growth except with a limiting factor. Carrying capacity is often the applicable limiting factor. Mathematically this population-density logistic growth equation is expressed as: $dN/dt = r_{max} N ((K-N)/K$, where K = the carrying capacity of the habitat, N = the population density and r_{max} is a function of N. (Thornley and France 2004, Tsoularis and Wallace 2002). The limiting factor of the habitat can be many things; space, cover, water, or forage resources. In the historic and scientific literature, discussions of the concepts of carrying capacity are more thorough than the examination of the data used in the equation. Perhaps a focus on the data would be more appropriate in today's resource management paradigm.

Aldo Leopold (Leopold et al. 1943) connects the winter food supply of the North Kaibab to the concept of a nutritional limit vis-a-vis carrying capacity of the Kaibab Plateau in his report to the Wisconsin Academy of Letters and Science:

"The effect of prolonged overstocking on the winter food plants was very severe. 1931, after four/fifths of the herd had starved an only 20,000 deer were left, one investigator says "the range had been so severely damaged that 20,000 was an excessive population. The herd continued to decrease slowly until an estimated 10,000 were present in 1939…Another investigator estimates the loss in carrying capacity as high as 90% in some areas…In short, the Kaibab, by reason of the irruption, lost a large part of its deer food without any gain in deer."

And,

"…Thus on the Kaibab, deer pressure was first visible on cliffrose. As this good food became scarce, Utah juniper and finally piñon pine were taken, and fawns began to die."

Leopold also connects deer irruptions in Michigan on both the Upper and Lower Peninsulas that presented a cyclic peak in 1880/1890, and were continuing into 1943. "At present writing the Michigan herd is shrinking by starvation, and with it shrink the good foods…" connecting the forage supply to logging operations that had increased the amount of open space and fire suppression in the Michigan forests. "Good" foods are not defined for the reader in his article.

Leopold also tracked the deer population in Pennsylvania back to the days of the Revolutionary War, stating in his 1943 paper "Equilibrium between the shrinking herd and its food plants was finally reached in 1940." He noted that damage to crops in Pennsylvania was "…prevalent since 1915, and to forest and plantations since 1922. In 1938 excess deer (had) in many sections resulted in the complete overthrow of natural forest regeneration…" Hyperbole and a lack of quantified data aside, Leopold's concept of a nutritional constraint on the carrying capacity of a habitat was prescient.

Russo (1964) indicates that in the time span of post-World War II 1945 to 1964, the herd was "… on a starvation diet", with sheep grazing prior to 1945 having caused significant damage to the forage. "It was not long before the invading plants, sub-dominate forbs, annuals, undesirables and normally unpalatable plant types began to show signs of extreme use … To this day scars from early range abuse can be found on those plants not normally considered palatable food types for deer or livestock". Russo discusses a 1947 Az. Game and Fish study conducted by J.T. Wright where Wright examines the competition for forage resources between cattle and the deer. "On the whole carrying capacity of the Grand Canyon National Game Preserve is now sufficient to support the cattle and deer now using the area." Russo's work, while thorough, does

not include the quantification of the Kaibab carrying capacity, or any metric that would be included in the calculations that might describe it outside of the herd size.

Hobbs and Swift (1985) elucidated on the concept of estimating the habitat carrying capacity based on the "range food supply and animal food requirements…", pointing out that others, including Wallmo (1977), have utilized this approach to evaluate the habitat for herbivores (Hobbs and Swift 1985). The three authors advocated food-based predictive models of habitat carrying capacity needed to consider the quality of the diet obtainable by the animals utilizing the habitat. "Because the abundance of food varies as a function of its quality, food-based predictions of habitat carrying capacity must consider the quality of diets obtainable by populations of animals using the habitat in question." (Hobbs and Swift 1985)

Hanley and Rogers (1989) derived a procedure to estimate carrying capacity, based on the quantity of biomass available for forage, and the "quality" of the forage, or digestibility, calories, and protein available that would be available to the ruminant. This was an extension of Wallmo's (1977) and Moen's (1978) individual work. Within the scope of Hanley and Rogers work, they "…defined carrying capacity as the maximum density of animal having given nutritional (sic) requirements that can be supported for a given time by a given habitat. Nutritional requirements differ with species, age, sex, weight and nutritional status (for example, status of body reserves on entering the given season and costs of maintenance, production, and reproduction)." Recognizing that the quantity and quality of forage available to the animal will vary, carrying capacity is limited in its scope and meaning to only the theoretical; any practical utility is in "…

providing a quantitative measure of the productive capacity of a habitat …" (Hanley and Rogers 1989).

The carrying capacity of today's given habitat (e.g. watershed, politically defined hunt area, national park, etc.) is usually considered by a resource manager in terms of space and/or volume. How many animals of a given species can exist in an arbitrarily defined space, or area? To complicate matters, multiple species are often considered with or without overlap, or consideration for the overlap, and usually in relation to the amount of forage (metric tons/months or per year) that the range can *ideally* provide.

In the late 1960's, another concept began to emerge from studies of biology and wildlife interactions, that of a carrying capacity centered around how well the plant life of a given habitat (definition *de jour* of what a habitat is –watershed or a politically defined boundary) can support a given animal. Charles T. Robbins (1973, 1993) expanded greatly upon on this concept. Many additional researchers and authors, such as Wallmo, Seal, Hanley, Del Giudice, Moen, Hobbs and Swift added to expanded upon these concepts of the nutritional plane – dietary quality of metabolizable energy and dietary protein, or available nitrogen - of the habitat fulfilling the needs of "X" species when the metabolic demands were the greatest. Metabolic demands for a mammalian ruminant species are highest when a female is pregnant and/or is nursing its young. (Moen 1973, Wallmo 1977).

The concept of carrying capacity should be used as just that – a concept of how many animals of a given species a given habitat can hold at a given time under a given set of conditions and metrics. A guideline number, to utilize by resource managers, centered on the amount of nutrition – the nutritional plane – that a habitat can provide to the prey

species at their highest metabolic demand(s). This working concept of managing a public resource needs to be presented to the general public, so that when the nutritional plane of the habitat shifts because of a disturbance, management numbers for nutritional content and the resulting shift in carrying capacity is understandable. The carrying capacity of a habitat is not stagnant, rather it is dynamic, reactive to inputs (i.e. grazing and available biomass) and successional changes (forwards or backwards to or from a given seral state) to the plant communities.

Parker further elaborated on this approach in 1993 in her study (Parker et al. 1999). The central concept was that based on known parameters such as gender, body size, reproductive status, consumption rates, activity patterns, and climatic conditions, the daily nutrient requirements of an individual could be predicted. This data can be compared with information on diet quality, as found from diet composition and forage quality analysis, to evaluate the ability of the habitat to meet the nutritional needs of a specific individual animal on a per unit time basis. Moen (1973) and Wallmo et al. (1977) and others proposed this approach to evaluating wildlife habitat based on the ability of a specific area to meet the nutritional and physiological requirements of the individual animal. This comparison is capable of identifying to what extent the habitat was either meeting, exceeding, or deficient in providing for the needs of the individual, and when and has been put into effective use in some resource management areas. (Moen 1997, Wallmo et al.1977, Miller 1980, Nelson and Leege 1982, Holt et al., 1992; Robbins, 1993; McCall et al., 1997; Parker et al., 1999; Cook et al., 2001a,b, Miller 2013, Wakeling 2010).

2.7 Nutrition versus Under-nutrition Status

Del Guidice et al. (1980, 1990, and 2002) and Seal (1978) delved intensively into research of metabolic profiles by blood and urine analysis, utilizing captive deer, radio-collared deer, and urine deposits in snow. Data was collected on basic metabolites, including BUN (Blood Urea Nitrogen, e.g. Plasma Urea Nitrogen or PUN) and Cholesterol (Seal 1978).

The data was extrapolated and modeled for the herd, i.e. the foci of the study. Information on diet composition and individual forage species quality provides insight into what could prevent the habitat from meeting the nutritional needs of an individual animal, where a nutritional and/or ecological shortfall may occur. Enhancing this evaluation are the results of the physiological indicators, identifying the specific response of the target animal to deviations in meeting the minimal requirements (i.e. "modeled") and deciding what the nutritional status of the animal (or herd) is.

In other words, forage nutrition data combined with metabolic feedback analysis of the herd can describe the nutritional condition of the animal. The analysis will show not only the nutritional or metabolic state of the animal, but where the plant community is failing, meeting or exceeding the needs of the animal community. Further, data and feedback from predation on these ruminant prey species will also provide information on the complexity of the ecological food web, extending the reach of the analyses.

2.8 Metabolic Indicators

Animal nutrition begins with photosynthesis within the available forage. Description of the properties of sunlight properties are in the wave-like and particle-like

properties of a photon (Knight et al 2007). Resonance energy – where the excitation energy for electrons transfers from a photon of light to a neighboring chromophore via the interaction of neighboring molecular electron orbitals - is critical to the harvesting of light energy by the pigment molecules within a plant. This electron transfer initiates photosynthesis as a chlorophyll absorbs the light energy (McKee & McKee 2009). The chlorophyll is stored in chloroplasts, the plant utilizing the chloroplasts for the growth of the internal vascular system of the plant, and leaves, stems, roots, fruits (including nuts) and branches as directed by the plants genetic blueprint. The cellulose and other sugars comprising the plant becoming essentially a source of light energy converted into stored chemical energy.

This chemical energy is stored in the plant – as the plant – in the form of carbohydrates, proteins, and oils (i.e. "fats"). $C_6H_{12}O_6$ (glucose) is the simple sugar that forms carbohydrates when carbon dioxide and light interact with water (Campbell & Reese 2005). Carbohydrates, when digested and processed in an animal body as polysaccharides, are the essential powerhouse for all eukaryotic life. Without a sufficient source of carbohydrate – either in the form of simple sugars or the more complex Polysaccharides of "starch"- the terrestrial mammalian biochemical machine does not operate. Glucose is the preferred energy source for brain cells (McKee & McKee 2009). Sugars are also the "paper" that the genetic code of an organism is written on (McKee & McKee 2009) for reproduction.

Carbohydrates (including Ribose – 5 – phosphate, necessary for RNA and DNA translation) are created in and present within the ruminant, or else they would have ceased to exist long ago. However, glucose conservation is apparent in the ruminant

animal (Van Soest 1982, Church 1988). The evolutionary advantage the *Cervid* family of ruminants since arrival in the Pleistocene is the microbial environment contained within the rumenal organ (Hefflefinger 2006); similar to a monoculture of yeast microbes (used for commercial production of ethanol for industry and beverages). The rumen bacterial environment is more complex and diverse in population than a microbial monoculture (Cook 1982, O'gara and Dundas 1982).

The gastric stomach (missing a key enzyme) is limited to processing an alpha – (1, 4) glycosidic bond and cannot efficiently deal with a -beta (1, 4) polysaccharide except as dietary soluble and insoluble fiber. The ruminant's ability to break the -beta (1, 4) glycosidic bond extant in starchy polysaccharides (e.g. cellulose-based plant cell walls) provides the animal an efficient mechanism to utilize plant-based forage as an adequate energy and/or protein source (Allen 1977, Van Soest 1982, Beitz 2004, Goff 2004). The ruminant animal can utilize all of the released sugars from microbial fermentation of range forage as an energy source, whereas the monoculture of yeast is primarily limited to utilizing glucose (Hatfield 2009). Physiology and the nature of a diverse microbial population provide a means to gain energy.

Evolution by natural selection as suggested by Darwin can extend into the maximization of metabolic capacity (Darwin 1882, West 1999). West suggests in his application of fractal geometry to organisms that … "…metabolism produces the energy and materials required to sustain and reproduce life…" primarily achieved by "…increasing surface areas where resources are exchanged within the environment …" (West 1999). Mr. West continues with his explanation of allometric scaling and formulae in his 2005 article that a scale of approximately $M_b^{3/4}$ applied to metabolic rates of nearly

all organisms "…has been observed at intracellular levels…down through mitochondria to the oxidase molecules of the respiratory complex.

As pointed out by Enquist and Niklas in 2001 and reinforced by West in 2005, "… an invariance occurs in ecology …population density decreases with the individual body size as $M_b^{-3/4}$ …individual power use increases as $M_b^{3/4}$, so the energy used … in any size class in an invariant". In other words, as the population density decreases, the size of an organism increases and the amount of energy resource to maintain the organism increases. The energy resource typically is delivered by a multitude of networks (West 2005), including the metabolic network(s) inherent in a ruminant and the nutrient, energy and water networks of an ecosystem [West however did not present a metric (e.g. Watts or Joules) for his discussion of energy].

West's foci is one of vascular systems and fluid dynamics, and not necessarily applicable to the energy flow through a metabolic system of a ruminant animal or a herd. Metabolic capabilities, or the efficiency of transforming photosynthetic energy into a chemical resource of ATP by the laws of thermodynamics, can also be determinant of an organism's ultimate survival and propagation. Suarez, Darveau, and Childress (2004) responded to the simplification of life to a fractal power [derived from Kleiber's original work (Darvaeu 2002)] with the comprehension that aerobic respiration and metabolism essentially represents the sum of mitochondrial respiration rates of the various organs in the body. The effort to understand allometric scaling in the context of ATP (adenosine tri-phosphate) synthesis and utilization places the concept into an energy supply and energy demand equation, with caloric demands and supply being one focus of this study. In this concept of ATP balance for a ruminant system, the metabolic needs of not only the

ruminant are a factor; the metabolic needs of the symbiotic microbial population living within the rumen *must also* be a factor. The intermediary step that allows a ruminant to extract - what is for a human - an unobtainable energy source from a forage resource (grass or a meristematic bud) dramatically complicates West's overly simplistic answer to an extant energy equation. An evolutionary response gained in the Pleistocene and Miocene (Darwin 1882, Van Soest 1982, Hefflefinger 2006) that includes a symbiotic relationship between a complex animal and simple single-celled animals places West's equation ($M_b^{3/4}$) into doubt.

Processing the energy resource stored in plant material is relative to digestive anatomy and protein needs. A large brain that runs on glucose and demands a high-level of protein for maintenance requires a simple gastric stomach, a mid-gut (a.k.a. small intestine) and a liver that can process glucose into glucagon in order to fuel the high level of nervous tissue. Food is subject to acid at an approximate pH of 2.0 in the stomach; when the bolus has decomposed enough; it passes through the pyloric sphincter into the small intestine for the nutrients to be absorbed across the walls of the membrane in to the bloodstream (Campbell and Reese 2005). Carbohydrate metabolism, originated in the liver from dietary input is a priority for man, as the large amount of brain tissue demands it for complex thought, motion, and physiological control. (Campbell and Reese 2005).

A ruminant animal does not gain energy, protein or nutrients the same as a human. Understanding metabolic reactions and thermodynamics in the context of a ruminant animal will provide an insight into the caloric and protein demands of the Kaibab deer; especially understanding that enzymes that are present in other mammals are not present in a ruminant. The life history of a ruminant does not include the complex

thought or the creation of ideas and bringing them into being – an important portion of the human condition. "Humanizing" the animal does not advance the understanding of the animal's metabolic processes. The *Cervidae* family is an evolutionary response to the plant life (generalized as grasses, shrubs and trees) prevalent and available in the late Pleistocene era (Hefflefinger 2006) and the evolution of grasses from the Miocene (Van Soest 1982). The ability to convert forage foodstuffs into energy and protein via a symbiotic relationship in a chambered rumen establishes the means for the *Cervidae* animal family to utilize what would be, for other herbivore animal families *marginal* forage into a *required* forage source.

A ruminant's digestive anatomy and physiology consists of the mouth, esophagus, and four – chambered stomach (rumen, reticulum, omasum, and abomasum), the mid-gut small intestine and the hind gut large intestine. The size and configuration of these parts appropriately scaled to the size of the animal and the type of forage consumed. The rumen is essentially a fermentation chamber, where the forage material sorts to different chambers by size. The breakdown of forage is conducted by the bacterial and single-celled fauna that inhabits the rumenal ecosystem, an organ that maintains a pH gradient from approximately 2.0 to 7.0 via hydrochloric acid, water, bacterial residues (i.e. volatile fatty acids) and the introduction of phosphates and bicarbonates as buffers while the food source progresses through the four chambers to the mid-gut. The mid-gut or small intestine is the location of further digestion of forage particles and the single celled bacteria and protozoa of the rumen.

Typical pH of the rumen after feeding and digestion is acidic, and most of the short- chain hydrocarbon VFA's are in the –anion form rather than the free acid form

(Swenson 1977). Absorption of the VFA's by the rumen papillae, occurring as a waste product from the bacteria occurs within the rumen, where the acidic nature of the rumen fluid partially regulates and influences the rate of absorption to the bloodstream. As the VFA (most commonly butyrate, propionate and acetate) level increases the acidity, so does it increase the rate of absorption across the papillae and rumenal wall (Allen 1977, Van Soest 1982, Leek 2004, Brown et al. 2006, McKee & McKee 2009).

The nutritional and energy content of the forage the deer ingests is extracted by the bacterial and enzymatic action within the rumen on the masticated plant material. The breakdown of plant-based carbohydrate storage mechanisms of cellulose and hemi-cellulose is completed by the enzymatic activity of the rumen bacteria to dissolve the Beta (β) -1, 4 glycosidic bonds that comprise soluble fiber, something a gastric stomach lacks the necessary enzymes to do.

Rumination and further digestion of ingested plant material may take up to two full weeks (Church 1988). The ruminant gut contains bacteria to break down the β 1, 4 – glycosidic bonds of the cellulose polymer created from D-gluco-pyranose residue (McKee & McKee 2009) that a plant cell wall contains. A ruminant can utilize forage that is not as nutritionally complex or of high quality because of this trait and the biomechanics inherent in its digestive tract to handle the fermentation necessary for digestion of cellulose (Van Soest 1982, Church 1988). Glucose, essential for tissues and especially for brain function via red blood cells, is therefore at a "premium", and because of the microbial population in the rumen, the amount that reaches the mid gut never exceeds 5% of the ingested carbohydrate (Sutton 1971). The volatile fatty acids waste products "contributed" by the rumen bacteria are butyrate, propionate, acetate, valerate,

iso-butyrate and iso-valerate, generated by the bacterial TCA cycles (Church 1988, McKee & McKee 2009). The largest concentrations of VFA's are butyrate and propionate.

To paraphrase Van Soest (1982), "… glucose can only be formed from propionate or other "odd-carbon" precursors – ruminants lack not only a mechanism to convert acetate to glucose, they *also lack the citrate cleavage enzyme in the cytosol of adipocytes which bars the conversion of glucose to acetyl-CoA and to fatty acids*. (emphasis added) This is a glucose conserving mechanism". The animal runs not on glucose or glycogen as a non-ruminant does, but is energy-dependent upon the conversion of the short-chain hydrocarbon volatile fatty acids provided by symbiotic bacteria and protozoa into the energy "currency" of adenosine tri-phosphates and adenosine di-phosphates (ATP and ADP) via the Citric Acid Cycle (Van Soest 1982, McKee and McKee 2009). The ATP and ADP conversion occurs within the cells of the after active transport through the bloodstream out of the rumen to the energy-deficient cells within the *Cervidae* body.

Although the rumen has the bacterial ability to break the β-1, 4 – glycosidic bonds of the cellulose, it may not come easily. Dry matter digestibility becomes a metric for quantitative nutrition measurement – how much of a forage substance is needed to extract "x" calories of energy or "y" grams of protein from the forage during the time-dependent digestive process. Fermentation, or cellulytic breakdown of a substance via microbial action, takes time. The temporal scale has an effect on the metabolic and nutritive balance of the animal. Time translates into space, or the volume of the rumen taken up by a degrading forage substance that may not be generating the energy or protein microbial by-products of VFA's or amino acids quickly enough. Difficulty in

decomposing the cellulytic materials that create the plant cell wall means ruminants specifically need to consume more dry matter - and to keep the rumen full of forage - to obtain a needed minimal amount of energy out of the herbivorous foodstuff (Nagy and Peterson 1987). As the natural forage available is not necessarily rich in calories or protein, the animal ingests more to meet demand. However the animal cannot ingest an adequate amount of forage immediately to meet the nutritional demand because the size of the rumen will not allow it. Adequate nutrition for a Cervid can easily become a volumetric constraint.

Deer and antelope – sized ruminants, because of their digestive anatomy and physiology including rumen size, need to focus on more nutritionally concentrated forage than other ruminants (Van Soest 1982, Church 1988). A concentrate selector such as a mule deer will be more focused on foods more highly loaded in calories and protein because it has a smaller rumenal volume (~30 gal.) than a bison or beef cow (~55 gal.) that is more interested in eating grasses, hay or other forage. The beef cow, or bison, has the volume (and thus time) necessary to digest enough coarser, high-fiber grasses versus a mule deer that does not have the volume to allow a large quantity of grass to degrade for a long time. The type of forage the deer will inherently focus on will be one of a concentrated energy, protein, and nutrients that is appropriate for the volume (and thus time in the digestive system) – provided the habitat conditions provide for this.

VFA's are not only passed through the rumenal wall, but are also passed through the lipid bi-layer of the small intestine through the osmotic concentration gradient into the blood stream, ultimately delivered to the cells in its phosphorylated form for consumption (Miller 2006). The aerobic metabolism of citric acid cycle (aka Kreb's)

occurs in the mitochondria of a ruminant cell; amino acids and the volatile fatty acids enter the cycle for conversion into ATP via the ports of pyruvate (amino acids), succinate (propionate), ketone bodies from βeta-oxidation of fats (butyrate), or acetate as a precursor to Acetyl CoA (VanSoest 1982, McKee & McKee 2009). Control of the Citric Acid Cycle respondent to substrate availability, product inhibition and/or inhibition by intermediates of the TCA cycle (Voet and Voet 2004). The bacterial activity on the ingested forage – and the concentration of that bacterial "waste" of a VFA – will control the energy exchange of the Citric Acid Cycle into the rate of ATP turnover in the cell.

Accessing energy outside of the mitochondrial walls is just an equal priority to the ruminant. The chemiosmotic coupling of electrons delivered across a proton gradient by the electron transport chain allows the synthesis of ATP by a rotating molecular pump in a process known as oxidative phosphorylation (Voet & Voet 2004, McKee and McKee 2009). Oxidative phosphorylation (OXPHOS) is constant, as the thermodynamics of a living being must be in a steady state of non-equilibrium (Voet & Voet 2004, McKee & McKee 2009).

Glucose, needed for necessary biological (e.g. red blood cell creation), brain and neural functions, is formed inside the liver, kidneys and intestine through the process of gluconeogenesis. The precursors for glucose include pyruvate and lactate, formed during heavy exertion and use of muscle tissues (Allen 1977, Fahey and Berger 1988, Bietz 2004, Mithieux et al. 2004, Voet & Voet 2004, McKee & McKee 2009). Propionate is the principal substrate for gluconeogenesis in the ruminant liver (Fahey and Berger 1988). The liver of the ruminant may increase use of gluconeogenic amino acids (e.g. alanine) if the demand for glucose is increased (Overton et al. 1999). Conversion of

these precursors in the liver (in conjunction with the Cori cycle) through a process that essentially reverses glycolysis (Voet and Voet 2004). If necessary, PEP converts to pyruvate via pyruvate -kinase to re-enter the Citric Acid cycle and thus satisfy energy demands through the generation of ATP. Acetyl co-A however cannot be converted into oxaloacetate; the TCA cycle must complete the degradation of acetyl-coA into a useable substrate if additional substrate via acetyl-coA is needed for gluconeogenesis.

Lipogenesis, lipid storage, and lipid usage (beta-oxidation) in a ruminant are different from a non-ruminant. Glucose – the precursor to pyruvate in the Citric Acid Cycle - cannot be converted directly to a fatty acid in order to be used as a VFA-substrate substitute (typically derived from bacterial degradation of the forage/cellulytic material) in the Citric Acid (e.g. Kreb's) Cycle. As previously stated, a lack of a key cytosol enzyme prevents this. At the cellular level, the mitochondria of the cell take precedence in the metabolism of the ruminants' energy products of VFA's and the lesser-priority glucose. If there is an excess of ATP that is not immediately needed by the mitochondria, the now-extraneous ATP can be used to form a lipid molecule within the cytoplasm via triacylglycerol synthesis (Beitz 2004). The lipid molecule can be transported elsewhere to meet energy demands, or an adipocyte is created for lipid storage.

The main carbon source for lipogenesis in a ruminant is acetate. Regulated by the production of ATP (as excess would back up the cycle) the isocitrate cycle produces an abundance of ATP , whereas the generation of glucose through the pentose phosphate pathway results in a deficit or zero ATP being produced (Van Soest 1982, McKee and McKee 2009). Beitz (2004) expands upon this in a macro sense; "…In ruminants acetate is the major precursor of fatty acid synthesis. For some yet–to-be explained mechanism,

glucose is not a significant precursor of cytosolic acetyl CoA in ruminant lipogenic tissues, which serves as a precursor of long-chain fatty acids and steroids [i.e. cholesterol]. Acetate is activated in the cytosol, obviating the need for citrate cleavage pathway enzymes." Other reducing equivalents are needed. They can be obtained from the pentose phosphate pathway. Twenty five percent of NADPH is generated by cystolic NADP-isocitrate dehydrogenase, allowing generation of NADPH from the oxidation of acetate. Glucose is conserved, as the ruminant has systems providing both TCA substrates and NADPH for lipogenesis (Byers and Shelling 1988).

Balancing fatty acid production against the reducing equivalents is NADPH, considered the ruminant's second energy currency to ATP. Tissues most frequently and heavily involved in fatty acid and cholesterol biosynthesis include the liver, mammary gland, adipose tissue and adrenal cortex. These organs are laden with enzymes that control and contribute to the pentose phosphate pathway (Voet and Voet 2004). Cystolic NADP-isocitrate dehydrogenase generates twenty – five percent of NADPH, allowing generation of NADPH from the oxidation of acetate.

Lipids from the forage feed source typically occur from leaf or seeds. These might be in the form of a waxy coating or a triglyceride within a seed or nut (e.g. acorn or piñon pine) (Van Soest 1982). Lipolysis, metabolizing the forage lipids occurs in two parts: the microbial metabolism within the rumen of the large galactolipids found in the forage resource, and the lipids emanated from the bacteria themselves (Van Soest 1982). Hydrolysis by rumen microbes is rapid to free fatty acids, glycerol or other compounds (Byers and Shelling 1988). Bio hydrogenation within the rumen of unsaturated fatty acids facilitates the removal of excess H^+ from the rumen environment, converting

double bonds to single bonds and saturating a (the) fatty acid. A diverse end product of saturated and unsaturated fatty acids exists within the rumen after bio hydrogenation (Byers and Shelling 1988).

Lipogenesis and transport of lipids in the ruminant is radically different from the process in a human. Verme and Ozoga (1980) when studying dietary effects on growth and lipogenesis in fawns found evidence that "… lipogenesis comprises an obligatory physiological event in autumn and proceeds despite under-nutrition until a serious negative energy balance occurs". Lipogenesis does not occur in the liver within a ruminant but at the site of adipose tissue, with approximately 90% of fat synthesis occurring (Allen 1977, Van Soest 1982, Byers and Schelling 1988, Bietz 2004) at the adipose site. A triglyceride transports primary lipoproteins as a chylomicron and a very low-density lipoprotein (VLDL) (Byers and Schelling 1988). Thus, it follows that a triglyceride will not necessarily be detached within the liver, but also potentially detached at the site of adipose. The extraction of the stored chemical energy within fat usually requires an additional input of energy, a higher level of ATP than expected [especially] during an under-nutrition event if hypothyroidism has occurred in the animal (Miller 2010).

Adipocytes, forming adipose tissue, is a multi-role tissue that not only stores energy in the form of fat, but also provides thermal insulation and shock absorption to soft internal organs (Saladin 2007). They take the form of a large rounded cell filled with a triglyceride inside and the nucleus and cytoplasm occupying a thin layer beneath the plasma membrane (Saladin 2007). When dominating an area, the adipocyte becomes adipose tissue. Adipocytes range (in humans) from 70 to 120 μm in diameter. Adipose

tissue is not stagnant, with cellular turnover constant, as new triglycerides are synthesized and older ones undergoing catabolism. The process of beta-oxidation in the mitochondria allows the energy from the adipose tissue (fat) to be tapped once carbohydrate resources are no longer sufficient for energy needs. Through beta-oxidation, triglycerides are broken apart as the beta-carbon undergoes oxidation and acetyl-CoA is made available to the cells.

Hormones (norepinephrine, epinephrine, glucagon and ACTH [adrenocorticotropic hormone] activate lipase, catalyzing the reaction to severe the bonds between the alpha and beta carbons. At this point, a mono and di-glyceride exist. Lipase continues to hydrolyze the di-glycerides to fatty acid and glycerol (Swenson 1977). In energetic-currency terms, the hormones combine with receptor sites, activating adenyl cyclase, converting ATP to cyclic AMP (Allen 1977, Beitz 2004).

Transport of the fats liberated from the adipose tissue is summarized …"The free fatty acids are released in to the bloodstream, where they bind to serum albumin, a soluble 585-residue monomeric protein that comprises about half of the blood serum protein" (Voet and Voet 2004, pg. 913), travelling in concert with lipoprotein albumin (Guyton and Hall 2006). The liberation of fat is a result of insufficient carbohydrate supply and/or exertion. "Fasting and the release of many different hormones into the blood cause rapid lipolysis of stored triglycerides, releasing unesterified fatty acids for transport to other tissues" (Swenson ppg. 337 1977). Fatty Acids, after being degraded by beta-oxidation, enter into the TCA cycle to be utilized as energy substrate glyceraldehyde phosphate (glycerol) or acetyl co-A (fatty acids) (Campbell et al. 1999, Van Soest 1982).

Phosphates are mineral utilized throughout the mammal; in this context, phosphate is considered a part of phospholipids, phosphoproteins, nucleic acids of RNA, DNA, mRNA and mDNA, and part of the molecules ATP, ADP and AMP needed for energy delivery and conversion (Goff 2004). Plasma phosphorous concentrations are well correlated with dietary phosphorous absorption (Goff 2004). Alkaline phosphatase or basic phosphate, a hydrolase enzyme, removes phosphate groups from molecules including proteins and alkaloids via desphosphorylation (Goff 2004). The pentose phosphate pathway – a third method of glucose metabolism – is also directly linked to the metabolism of glucose, in which no ATP is generated (McKee and McKee, 2009) and a key process in the continuation of photosynthesis in plants when light is not present.

Phospholipids, a significant portion of plant lipids, also undergo hydrolysis and indicate microbial action within the rumen. Galactolipids are quickly degraded releasing galactose for further degradation into a VFA (Merchen 1988). Excessive amount of unsaturated fats (those typical in forage substances) and triglycerides can have the effect of suppressing methanogenic bacteria, effectively throwing the acid/base balance of the rumen out of kilter (Van Soest 1982). If neutrality is not achieved by some form of buffer (typically either a -carbonate or –phosphate) to the rumen fluid (Beitz 2004, Allen 2007, McKee and McKee 2009), degradation of plant-based fats is continued in the abomasum into the small intestine (mid-gut) with the addition of bile salts, where the fat resembles an un-esterified fatty acid (Merchen 1988).

Miller's hypotheses of an increased energy cost and DelGuidice hormone-related response to an under-nutrition situation becomes easier to comprehend *if* a ruminant has accessed some or all of its fat reserves, leading to the catabolism of muscle. The deer, if

starving, may have stored fat to utilize but be unable to access the energy reserve if hormone levels are suppressed because of the under-nutrition condition (Del Guidice 1991). This area of Del Guidice's work on under-nutrition levels needs to be further explored in the context of the north Kaibab deer herd.

Protein metabolism supports the growth and sustains the animal, while protein catabolism provides another energy pathway to sustain life. Muscle is the primary source of protein storage in a mammal. The metabolic cycle typically does not access the stored energy potential of the tissue except under in starvation conditions (Allen 1977, Beitz 2004). Protein sources can be provided from multiple vectors; dietary plant protein and non-plant protein nitrogen (Van Soest 1982), endogenous single-cell organisms that slough out of the rumen into the midgut as the microbial gut populations conduct their specific population dynamics, and urea recycling during times of low forage nitrogen/protein availability. Protein stores are difficult to store for future usage (Swenson 1977). " Intake of protein beyond daily needs results in increased urea formation accompanied by the conversion of most amino acids into carbohydrates and/or fats or in their metabolic degradation for energy purposes."

The inherent inefficiency of the mammal will cause some protein – especially in the form of single-celled animals sloughed away from the internal microbial populations - to be lost and expelled in the feces. "The pH of digesta entering the duodenum increases slowly during passage…and protease activity (pH optima >7.5) is not maximized until 7 – 15 meters past the pylorus in sheep (Merchen 1988)." A sheep is the closest analogue available to a deer in a domestic ruminant. "Consequently, active absorption of amino acids is carried on largely in the jejunum and ileum (Merchen 1988)." The digested

amino acids, having required the action of peptase activated by the acidic (HCl) gastric stomach fluids, will not be absorbed until the near the end or the end of the small intestine via the Na/K pump. They cannot be recycled; the next organ for decomposition/deposition is the large intestine/colon for water extraction and excretion. As long as amino acid requirements are met in the animal, excess nitrogen will either be utilized as an energy source or eliminated as waste.

If the energy balance is such that catabolism of proteins (i.e. muscle tissue) needs to occur for life to continue, catabolism starts with the removal of the alpha-amino groups by transamination or oxidative deamination. The alpha-keto groups can then be used in a variety of ways, including conversion to and storage of fats, formation of cholesterol, oxidative degradation or conversion to glucose (Swenson 1977)

Creatine (PCr) is a substance found primarily in muscle tissue, and is readily converted to phosphocreatine (PCr), a form of storage for high-energy phosphate in muscle tissue (especially skeletal) (Swenson 1977). "Fast-twitch" muscles maintain a large pool of PCr available for immediate, short periods of intense work – part of the "fight or flight" reflex (Wyss and Kaddurah-Daouk, 2000) and is maintained in a ready state, keeping ATP and ADP almost constant. Enzymatic pathways for the creation/regeneration of PCr from creatinine include creatine kinase, arginase, and ornithine carbmoyltransferase. Links into the "Krebs bicycle" of the citric acid cycle are present at arginino-succinate (McKee and McKee 2009). Creatinine is the chemical waste product of muscle metabolism utilizing creatine. Measurement of creatinine can be either an indicator of kidney failure or an indication of utilization of the metabolite for sustenance.

Cholesterol is a lipoprotein hormone precursor present in both forage foodstuffs and internally manufactured. Exogenous cholesterol, if it has survived bacterial breakdown in the rumen, is absorbed through the gastrointestinal tract. Endogenous cholesterol is formed within the cells of the organism; "…the cholesterol that circulates in the lipoproteins of the plasma is formed by the liver" (Guyton and Hall 2006). The sterol nucleus is synthesized entirely from acetyl-CoA (the degraded pyruvate minus pyruvate dehydrogenase) the product of fatty acid catabolism (Mckee and McKee 2009). With production controlled by feedback from the hormone insulin (Martin 1985), the lack of insulin or feedback from the thyroid hormone can increase cholesterol levels where the excess thyroid hormone levels can decrease cholesterol levels (Guyton and Hall 2006). Because acetyl-CoA is a substrate of the Citric Acid Cycle, whatever amount of acetyl CoA is necessary for cholesterol manufacture is not entering the energy redox reactions.

In females the cholesterol hormone can also be synthesized in the ovaries (Martin 1985), The exact pathway of ovarian synthesis is outside of the scope of this study, the necessity for cholesterol in life and reproduction is obvious with the location of this pathway. Multiple pathways – exogenous, if the lipid hormone is not metabolized via bacterial degradation and conversion into a VFA, endogenous from the liver and ovarian - exist for the synthesis of cholesterol and uptake via lipoproteins. Cholesterol "… loosely associated with albumin is also taken up …" within the ovaries (Martin 1985).

Cholesterol has many different roles to play in a mammal. Because of the status of cholesterol as exogenous and endogenous sources, the diversity of usage as a basic building block for cell maintenance, metabolic controls, energy conversion and

connection to the reproductive cycle, cholesterol was chosen as a hormone that 1) is easily measured and 2) is representative of the health of the deer.

2.9 Literature Review Conclusion

Not only is the overall health of a mule deer a function of the mule deer's diet, but also of how that diet interacts metabolically with the mule deer. In examining the dietary habitat of the north Kaibab winter mule deer habitat this study will also examine how well the diet is meeting the metabolic needs of the deer herd. An examination of the metabolic demands of a pregnant mule deer doe and their response via blood and plasma indicators will provide a better understanding if the north Kaibab habitat can meet the biological carrying capacity needs of the herd. The animals of the herd, being the largest trophic wild plant utilizers in the ecosystem, then define the availability of a large-bodied protein resource to the trophic levels of predators and scavengers.

This is not an exhaustive knowledge base. Rather an introduction to some of the history, biochemistry, metabolic and ecological concepts that will assist the reader in understanding the scientific methodology, results and conclusions of the forthcoming chapters. The reader is encouraged to examine the biochemical inter-changes and inter-actions, statistical and modeling processes, dietary preferences and habits of mule deer extemporaneous to what is being presented. A more complete understanding of the dietary and metabolic conundrum that exists in the habitat and understanding of the derived regression model that is presented will enrich the management decision process of the wildlife, forest and natural resource manager.

CHAPTER III
THE KAIBAB PLATEAU aka
NORTH RIM, GRAND CANYON, AZ

STUDY AREA

Location

The focus of this study is the winter range of the Kaibab mule deer herd. The study area is located in Arizona game management unit (GMU) 12A-West (Figure 1) and encompasses 46,660 ha located in Coconino County, Arizona. The borders of the study area are Snake Gulch on the north, Kanab Creek on the west, Kanab Creek and the North Rim of the Grand Canyon on the south, and Forest Service roads 425, 427 and 423 on the east. The study area is centered on 36°40' N Latitude, and 112°25'W Longitude, and is approximately 452 road miles from ASU-Polytechnic Campus via Interstate-17 and U.S. Hwy 89-A. (Google maps, 2013). The site is located approximately 193 kilometers straight-line distance (north-northwest) from Flagstaff and approximately 32 kilometers straight-line (south) from Kanab, Utah (Topo! Ver. 4.6.0, copyright 2010 National Geographic Maps. Accessed August 17, 2013.)

Physiography and plant communities

Elevation differs from 2100m (~6900 ft.) to 1500m (~4900 ft.), sloping downward from the North Rim of the Grand Canyon northward towards Fredonia, Arizona. Observations have been made of mean daily winter temperatures of approximately -10° C (15 F) to -4° C (25 ° F). Precipitation and temperature data for each year of the study is presented below.

Climate

Direct climate data for the study area was not readily available. A temperature, precipitation or stream level monitor was not found in the immediate area or at the mean altitude for the study site. Two adjacent stations were found that were functional and repeatedly reporting to the National Oceanic and Atmospheric Administration (2013). These were located in Kanab (station I.D. 44508, 4900ft elev., 37 deg. 2'N, 112 deg. 2'W) and the Bright Angel Ranger Station (station I.D. 21001, elevation 8000 ft., 36 deg. 13'N 112 deg. 4'W). Data for both stations are the closest representative of activity at the mean elevation of the study site and area surrounding the study site (Kanab). The weather activity in the immediate vicinity of the study site (Bright Angel RS) presents the weather data at a higher altitude, a direct impact and reflectance that the adiabatic lapse rate has on the study site.

Deposition of precipitation on the study site will be an estimate of somewhere between the reported levels of the two stations. The charts below, Figures 3.1 through 3.4 reflect precipitation patterns on the Kaibab during the time periods of the study. Summer precipitation levels are within normal(s) for a drought, and winter precipitation levels sometimes exceed calculated normal(s) during some winter months during the study years. There were some heavy snow events on the Kaibab. Figures 3.5 through 3.8 demonstrate temperature patterns that stayed within (or were close to) calculated normal levels during the periods of the study. Figures 3.9 and 3.10 are historical data provided for purposes of comparison to the timespan of the study.

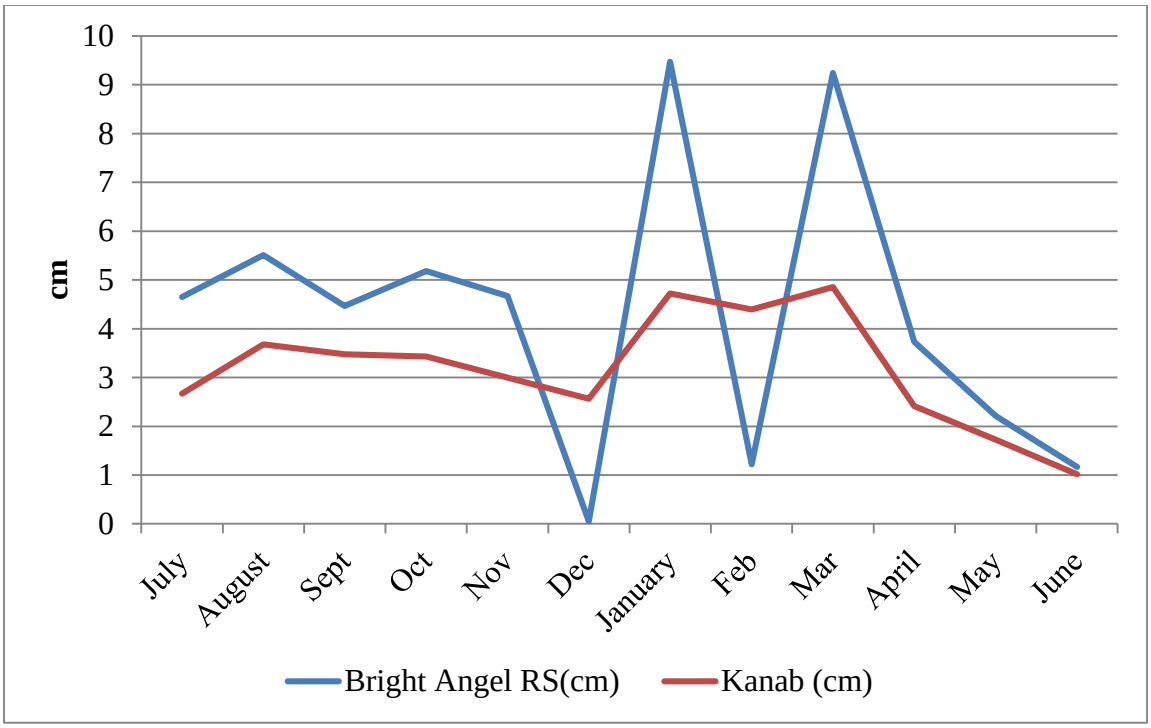


Figure 3.1 July 2005- June 2008 calculated normal precipitation levels for Bright Angel ranger station, AZ and Kanab, UT

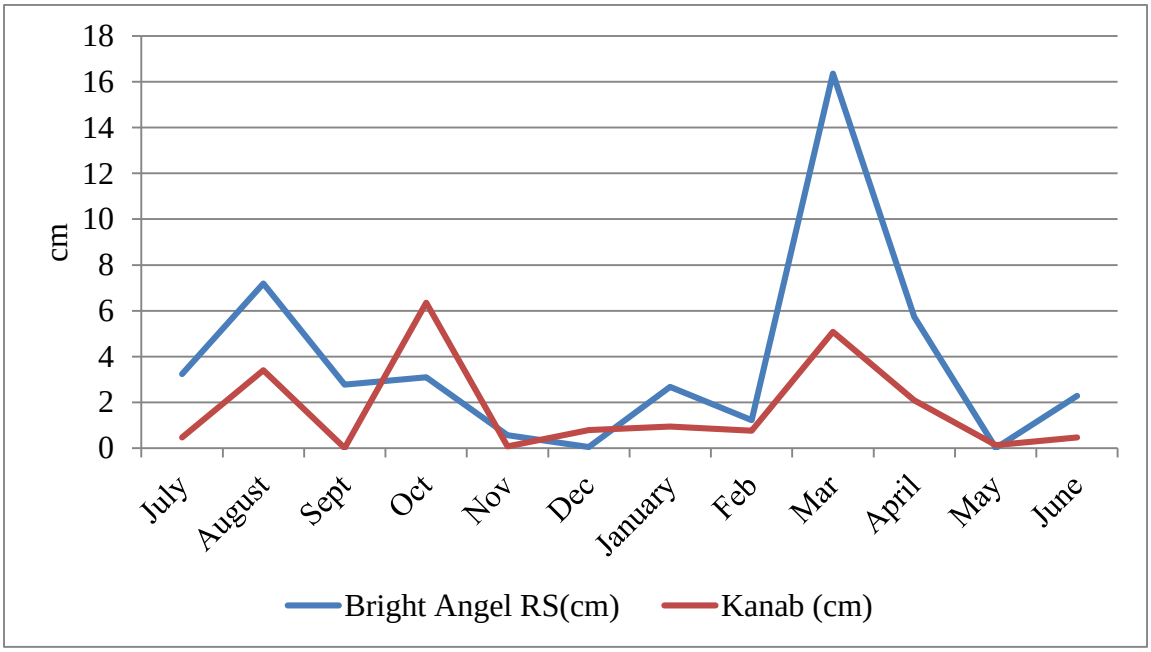


Figure 3.2 July 2005–June 2006 recorded precipitation levels, for Bright Angel ranger station, AZ and Kanab, UT

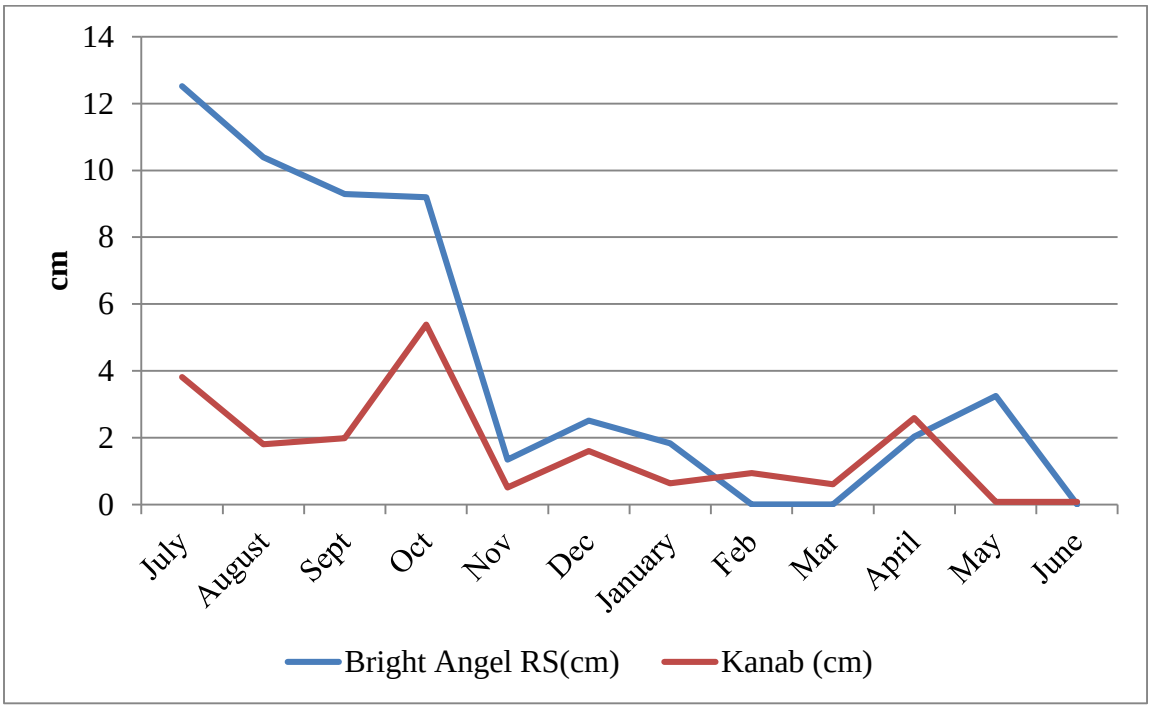


Figure 3.3 July 2006 – June 2007 recorded precipitation levels for Bright Angel ranger station, AZ and Kanab, UT

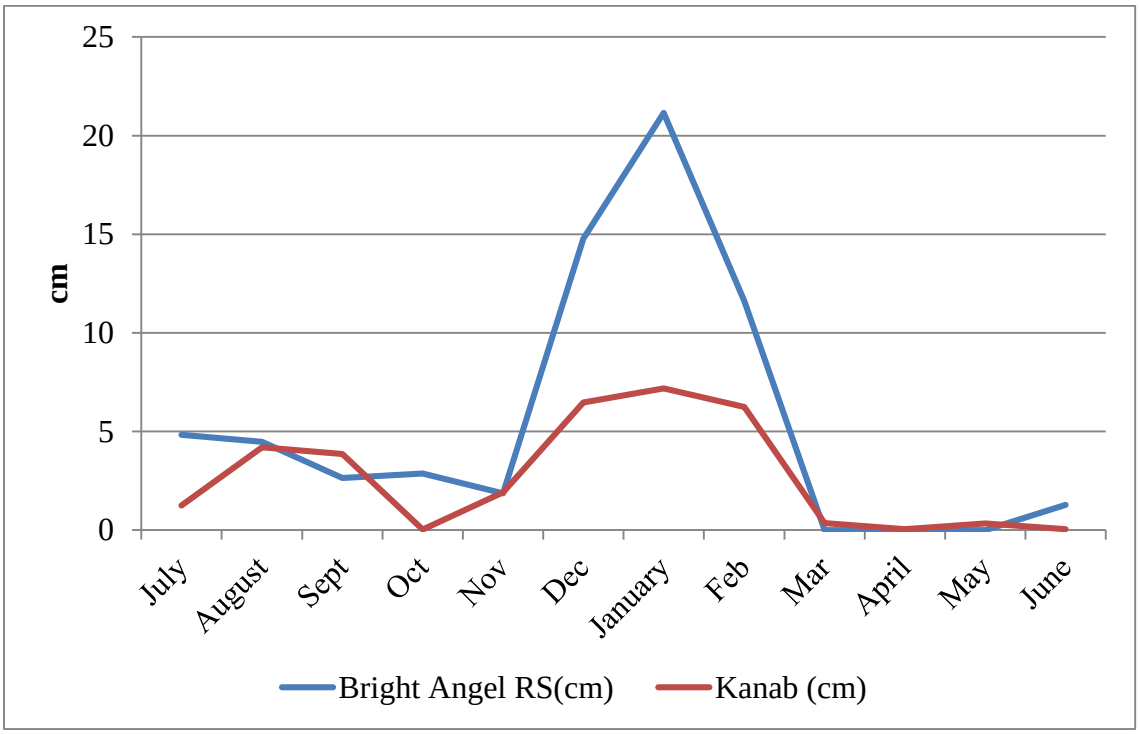


Figure 3.4 July 2007 – June 2008 recorded precipitation levels for Bright Angel ranger station, AZ & Kanab, UT

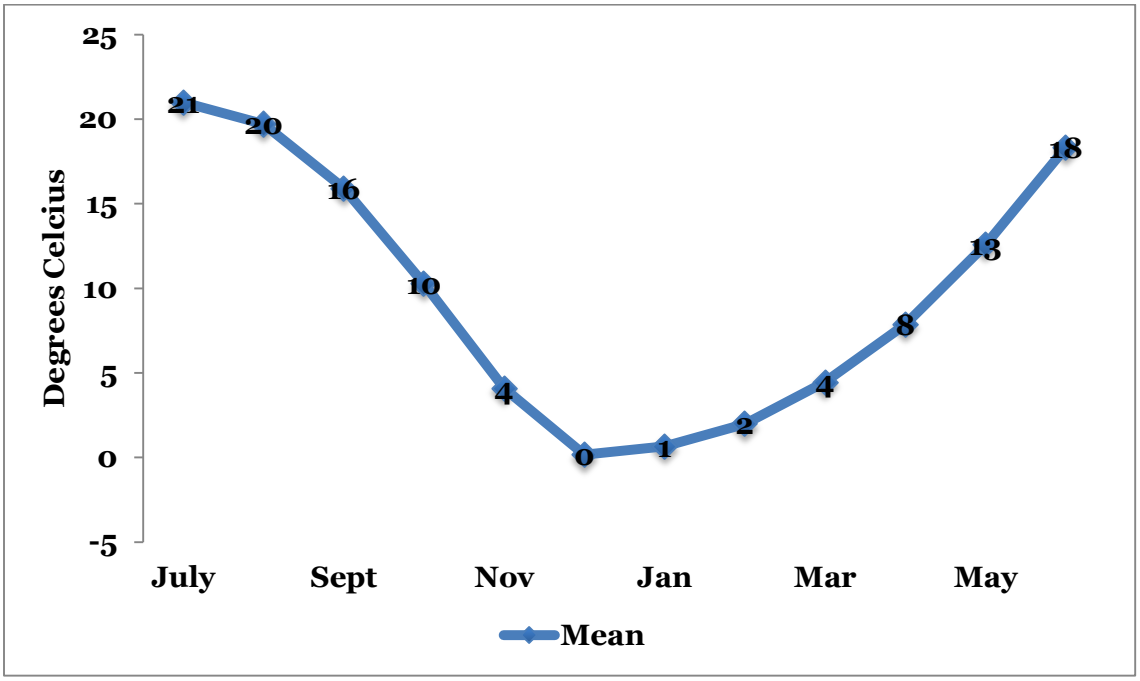


Figure 3.5 Calculated normal temperatures Bright Angel ranger station and Kanab, UT (NOAA 2013)

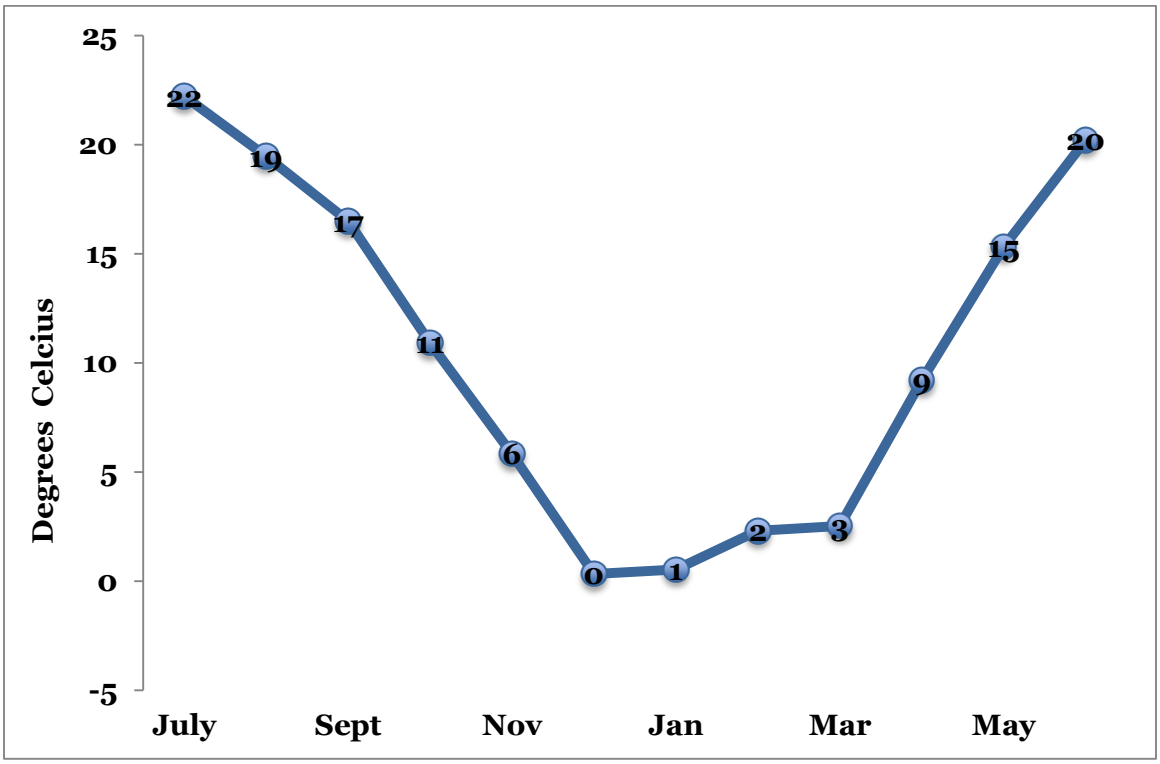


Figure 3.6 2005-2006 monthly mean temperatures Bright Angel ranger station and Kanab, UT

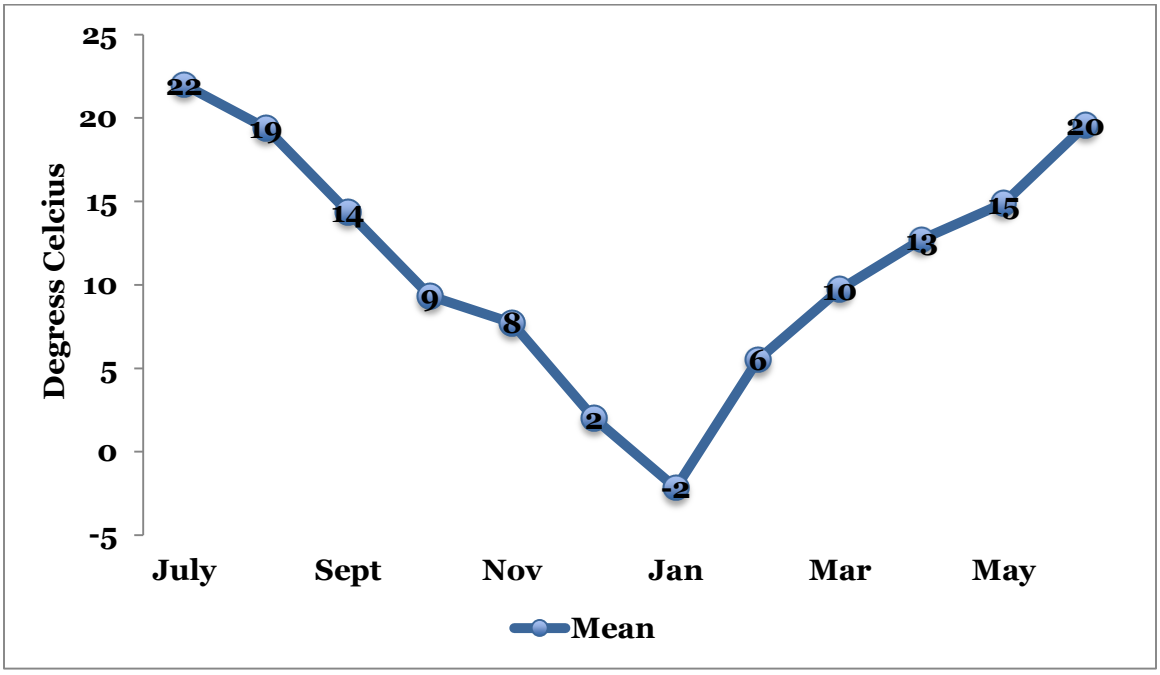


Figure 3.7 2006-2007 Monthly mean temperatures Bright Angel ranger station and Kanab, UT

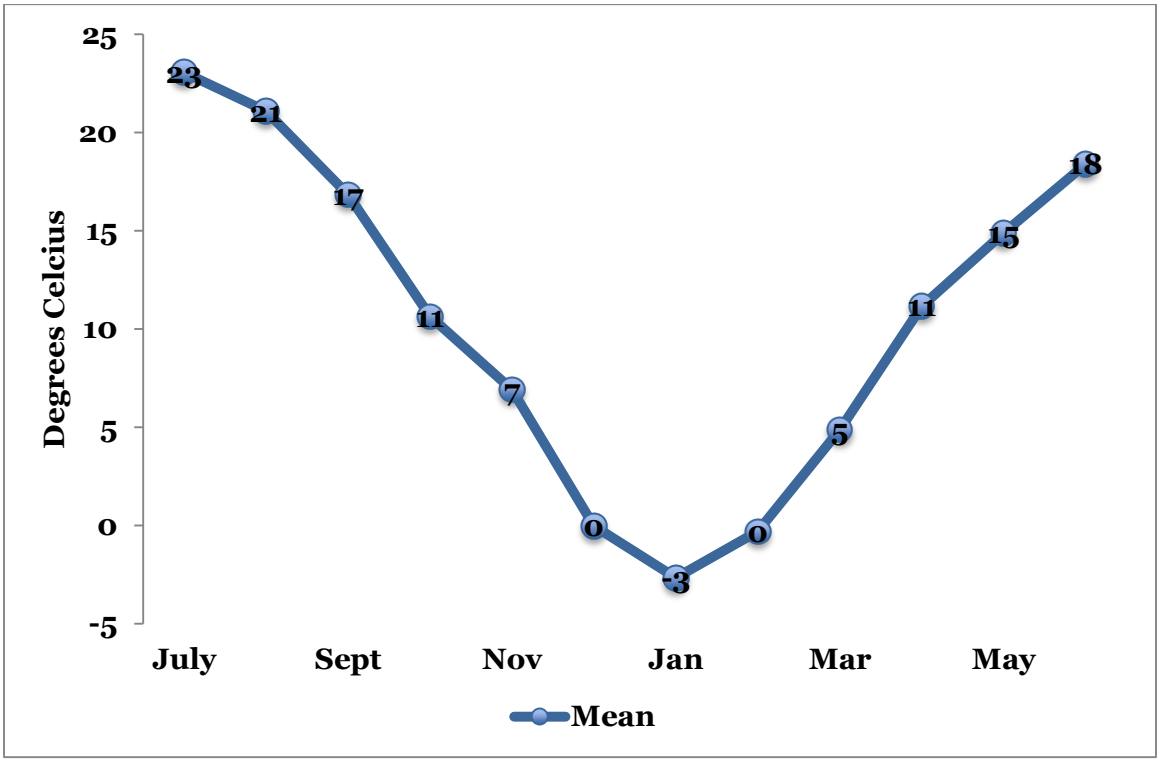


Figure 3.8 2007-2008 monthly mean temperatures Bright Angel ranger station and Kanab, UT

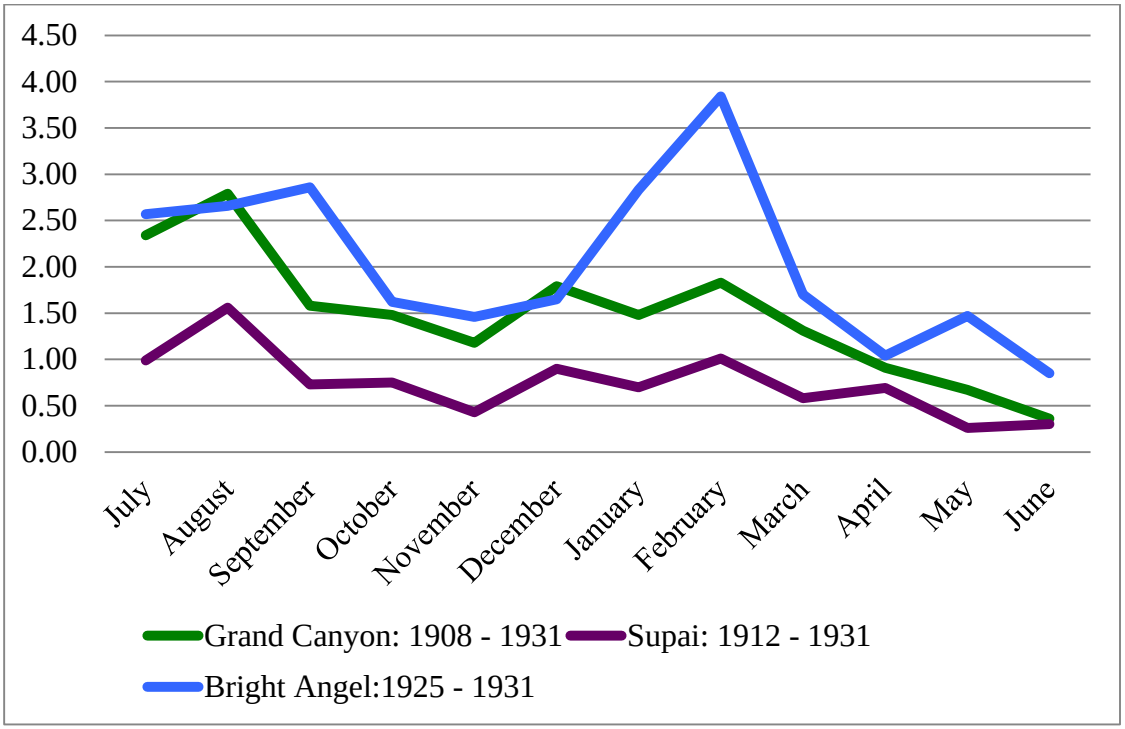


Figure 3.9 Historical mean precipitation (in.) data taken from Rasmussen's 1932 dissertation, shown in Appendix E, Photograph E.1.

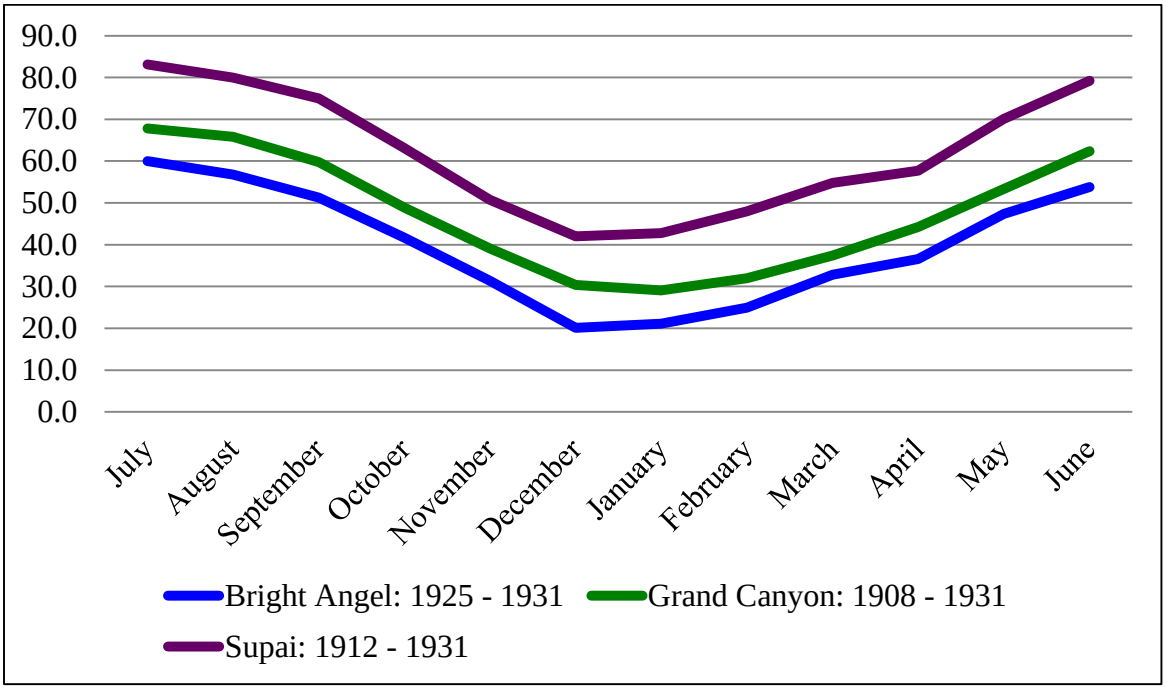


Figure 3.10 Historical mean temperature data (deg. F) taken from Rasmussen's 1932 dissertation. Original data is shown in Appendix E, Photograph E.1.

The last two figures shown, Figures 3.9 and 3.10, are provided for historical context and comparison to modern levels only. This data was taken from Rasmussen's 1932 dissertation and provides an accurate depiction of the early 20th century climatology of the Plateau. Note the significant precipitation events recorded from January to March at Bright Angel Ranger Station between 1925 -1931. Comparison of the precipitation events noted in Figures 3.2 and 3.4 and the calculated normals in Figure 3.5 describe the adiabatic lapse rate that occurs on the north Kaibab. Figures 3.2, 3.3, and 3.4 demonstrate how the adiabatic lapse rate can affect the habitat at the lower elevations (~6,500 ft. / ~2000 meters) directly below the ranger station.



Figure 3.11 GIS-generated map showing boundaries, major drainages, forest roads, and general location of the mule deer winter range study area on the Kaibab Plateau (aka North Rim, Grand Canyon Natl. Park) in Coconino County, Arizona.

Historical and Current Resource Management

Anglo settlers seeking privacy and independence in the latter 19th century (Ford 1999) typically piggybacked agricultural methods of cultivating and irrigating crops upon ancient Native American irrigation systems. The mining for mineral resources, and most importantly, grazing in what is now the Grand Canyon National Park and the Kaibab National Forest was a financial staple of Gilded Age pioneerism (Ford 1999, Russo 1964). The Kaibab plateau has a grazing and mining history dated by either anecdotal or government (U.S.D.A. and U.S. Forest Service) historical records back at least to the 1870's. The area was originally settled by homesteading by Latter Day Saint (aka Mormon) pioneers, evidenced by photographs hanging in the Jacob Lake Lodge and local oral histories gathered at the counter of the only gun store/pawn shop in Kanab (Rassmussen 1941, Russo 1964, Ford 1999).

Typical of the period known as the raid on the resources of the Golden Age, grazing practices by homesteaders were uncontrolled. Implementation of the United States Forest Service in the early 1900's and the creation of the National Parks helped to bring control to the extraction and usage of the regional resources, the keys being Grand Canyon National Park, by Theodore Roosevelt in 1906. The Kaibab National Forest, created at approximately the same time, being "… set aside for the protection of game animals and birds…and is a breeding place therefore."(Kaibab 1975). The period known as the "Golden Age" and the corresponding "raid on the resources" affected the Kaibab as much as anywhere else (Holecheck et al. 2000).

Anthropogenic alterations of the landscape led to alterations of plant communities and landscapes through accelerated natural forces, the same forces of wind and water that

have and continue to carve out the Grand Canyon and every small side arroyo that descends downward from the volcanic uplift. The effects of uncontrolled grazing, the alteration of the plant communities by domestic livestock (cattle, horses, sheep, and other domestic herds), the suppression of fire in the area as a management strategy and uncontrolled hunting in the late 19th and early 20th centuries led to a "boom and bust" cycle of the mule deer population on the Kaibab over time (Ford 1999), and helped to define current species richness of the available grazing resources.

Plant Communities/Grazing Resources

The mixed Shrub/Grass/Piñon pine-Juniper plant community on the Kaibab Plateau extends from the edge of Snake Gulch and Kanab Creek until the bottleneck at Jump Up Point/Indian Hollow. Along the northern and eastern edge of the plateau (Snake Gulch and Kanab Creek), the plant community is more shrub-oriented, with inter-spaces filled with low shrubs and native and non-native grasses. Piñon pine and Utah junipers are present here, but infrequent. Big sage and other shrubs, including Mexican cliffrose and Apache plume define the shrub plant community.

Moving into the interior of the plateau, multiple smaller fire scars are evident and shrubs with grass and forb filled inter-space gives way to more open spaces (or small plain) at Slide Tank that are filled with grass communities that include crested wheatgrass (*Elymus cristatum*), sideoats grama *(Bouteloua curtipendula),* cheatgrass *(Bromus tectorum)* and red brome grass *(Bromus rubens)*, blue grama, and Arizona fescue (*Festuca arizonica*).

Large fire scars from the Bridger Fire of the 1980's are evident in the heart of the Plateau, where thickets of Gambel's oak are intermixed with shrubs such as four-wing

salt bush (*Atriplex canadens)*, utah serviceberry (*Amelanchier utahensis*), smooth brome and squirrel tail *(Elymus elymoides*). Again grasses are also present and include re-seeded crested and western wheat grass [*Agropyron smithii* (aka *Elymus smithii)*]. Moving from the north to the south towards the North Rim of the Grand Canyon (i.e. Crazy Jug Point), these shrubs and grasses fill in the interspace between the increasing numbers of piñon pine and Utah juniper as the elevation of the plateau increases. Juniper, two-needle piñon pine *(Pinus edulis)* and some limited numbers of singleleaf piñon pine [*(Pinus monophyllia)*, collectively and colloquially known as "piñon pine"] are dominant in the middle of the study area, some thickets with density so heavy that experimental Utah juniper control plots have been created where any and all trees have been removed in a 0.5 to 1 ha area in the White Rock/Jump Up Point.

Ponderosa pine *(Pinus ponderosa*) begin to transition into the Piñon pine-Juniper community, with pine forests extending to the edge of the North Rim. As altitudes increase to the East, ponderosa pine communities give way to those of a mixed spruce/fir community.

Forbs can include lupines (*Lupinus spp*.), sticky geranium *(Geranium viscosissiumum)* and white sage (*Artemesia ludvisiana)* and are inter-mixed throughout the different plant communities on the plateau. Forbs are not prevalent in the winter months, but can easily be found in a mid-winter warm-up on the plateau. Rassmussen's 1941 general classifications of the plant communities (from his 1932 dissertation) seen in the Figure 5 graphic still apply and have been verified by both geographic information systems data and ocular estimation in the field.

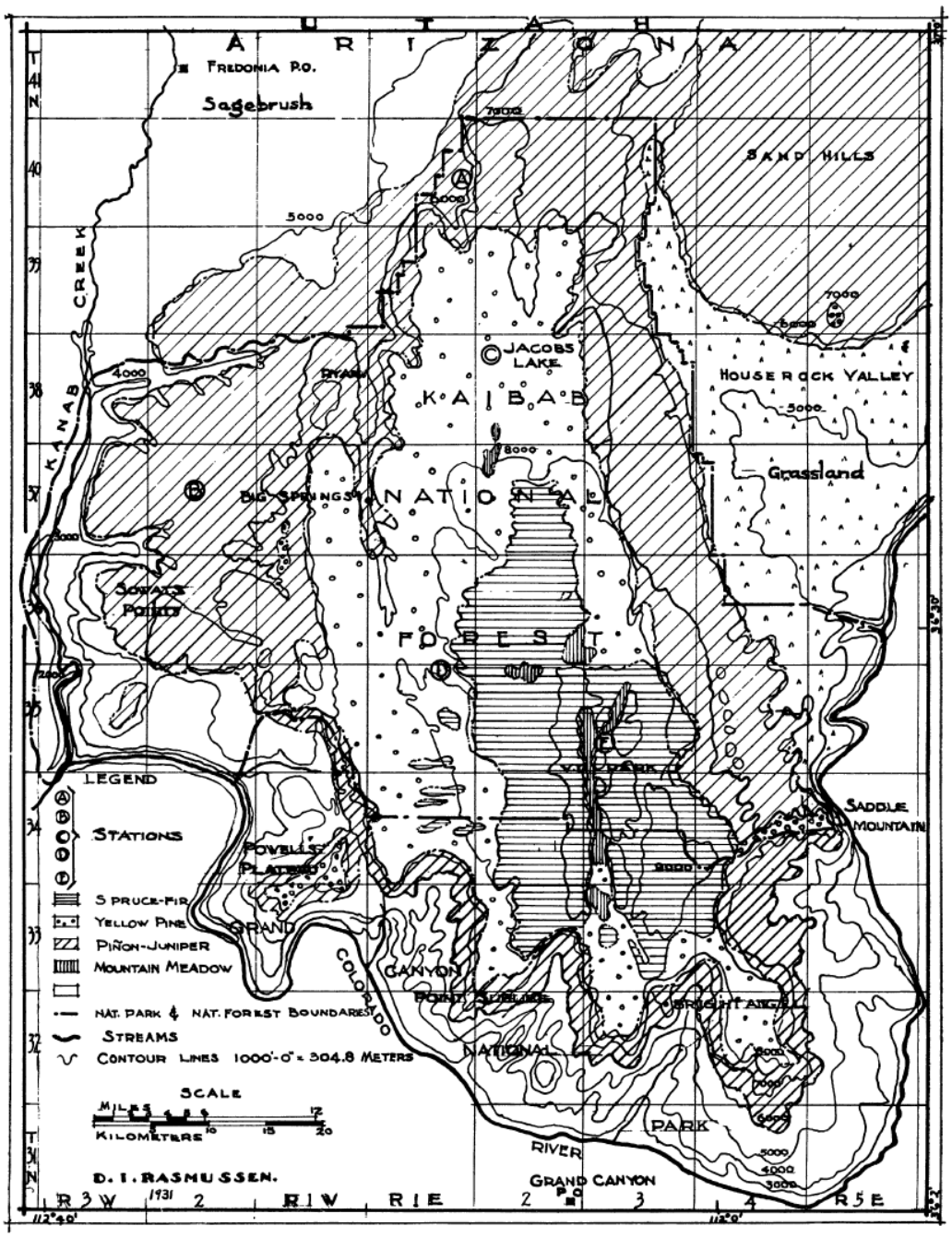


Figure 3.12 General vegetation species classifications by D.I. Rassmussen (1941). Note the 1931 date in the lower left hand portion of the map legend. It is believed that this was included with his 1932 dissertation. These classifications still generally apply at the time of this study (2005-2008).

CHAPTER IV

DIET COMPOSITION & SPECIES RICHNESS

INTRODUCTION

It is important to be able to qualify the diet in terms of species and quantify the diet in terms of utilization in order to gain a fuller insight into the interaction of the mule deer with its habitat. All previous historical studies have centered on either the summer range forage, or unsophisticated, baseline identification of forage species from rumen contents.

The objectives of this chapter are three-fold. One objective is to describe and investigate the species richness (i.e. the number of plant species utilized) of the north Kaibab mule deer winter diets using micro-histological techniques combined with standard ecological fieldwork. Another objective is to categorize the utilized species into forage classes of Grasses, Forbs, Shrubs, and Trees. The last objective is to determine the level or amount of utilization of the described forage classes. All of these objectives share the same priority in the scope of this study. The amount or percentage of slide field coverage (XX% out of 100 %) will be described for the largest forage class - species contributors for the given time frame of Year and Season. This information will then be used to further evaluate diet quality, metabolic response to the diet, and provide a foundation for modeling the dietary habitat.

METHODS

Fecal pellet collections were made during three time periods starting in late fall, and reoccurring at six to seven week intervals (Late Fall –last week of November, Mid-Winter – mid to late January, Early Spring – mid to late March). Fecal material came

from three different sources: Youth-harvested mule deer doe in November (Late Fall ’06 and ’07, n=28); ASU collected pellet groups in late December through Mid-February (Mid-Winter ‘06, 07, and ’08, n=69) and AzGFD harvested deer in mid to late March (Late Winter ’06, ‘07, and ’08, n=89), Figure 2. Criteria for the late fall and early spring collection was a minimum of 10 g from as many doe as were harvested, with a minimum of 16 doe harvested per sample period. Samples were placed in breathable paper bags and burlap sacks in the field to deter mold, fungus and other forms of sample degradation. Fecal samples were dried in a convection oven for 48-72 hours at 50° Celsius.

Fecal pellet collection dates and the number of pellet groups collected are described in Appendix A, Table A.1. Site elevation and precipitation events were correlated with the pellet group locations; pellet groups were not found in locations that experienced heavy snow events, especially from mid-winter onward. The Indian Hollow portion of the study area – increasing in elevation as the proximity of the North Rim increased - did not contain many pellet groups and was regularly checked when weather permitted entry. Deep snow events often prevented access by vehicle or on foot and it is deduced that access was inhibited/prohibitory for mule deer.

Four to five diets (dependent on the number of pellet groups found) for each sample period were established out of the collected pellet groups. Whole pellet group samples for each sample period were divided into four replicates. Three to five pellet groups were placed into each replicate. Three to five grams from each pellet group were added to each replicate, or diet. The combined pellet groups (diets) were then placed into a commercial blender with tap water and blended. Composite fecal replicates were

processed for analysis in accordance to Davitt and Nelson (1980). Six to eight slides were created from sub-samples of the processed fecal material and fixed onto a glass plate using a solution of glycerin and phenol.

The slides of each diet were then sampled. Quantification of the diet composition from each fecal replicate was made using a cover-based measurement under a 10X10 grid mounted under the ocular of a microscope. A total of 300 fields were randomly located using a random number generator in Excel configured in an X-Y matrix. A minimum of 50 fields (six different slides per replicate) that held identifiable epidermal fragments at 100X magnification (Holt et al., 1992) were located and used.

Concurrent with each of the fecal collection periods (except late fall 2005) samples of all available forage species were collected from the north Kaibab winter range. Availability is defined as those plant species accessible by mule deer as a function of the morphology/growth form of the plant and snow cover conditions at the time of collection.

Voucher samples of all available forage species were collected from the north Kaibab winter range concurrent with the fecal collections (except late fall 2005). Availability is defined as those plant species accessible by mule deer as a function of the morphology/growth form of the plant and snow cover conditions at the time of collection. Plants were identified using either the Plants National Database (http://plants.usda.gov), Kearny or Peebles (1979), and/or Epple & Epple (1995). Voucher slides of the plant material were processed using the same protocol utilized to process fecal samples and fixed in a glycerin/phenol medium. These voucher slides were used to create

classification keys based on epithelia characteristics, training and conformation when necessary during the diet determination process.

Statistical Analysis

The study design for this phase of the study was a completely random two or three factor factorial analysis of variance (ANOVA). For species richness, the factors were season and year, for forage class analysis the factors were forage class, season and year, and finally for species composition the factors were plant species, season and year. All analysis were performed at the P> 0.1 using R® statistical software version 3.02.

RESULTS

Figure 4.1 describes the richness of the amount of species utilized as a forage resource by the mule deer population on the North Kaibab plateau. Mean species richness of the winter mule deer diets on the North Kaibab winter range varied from a mean during the Late Fall (2006-2007, or Yr. 2) of 31 and 28.5 (SE=2.4) of the species utilized in the late fall of 2007-2008 (Yr. 3). Mid-winter diets gave a mean of 30 species in 2005-2006(Yr. 1), 17 species in 2006-2007, and 30 different species utilized in 2007-2008. Early Spring diets demonstrated a utilization of 21 different species in Yr. 1, 28 in Yr. 2, and 28 different species in Yr. 3.

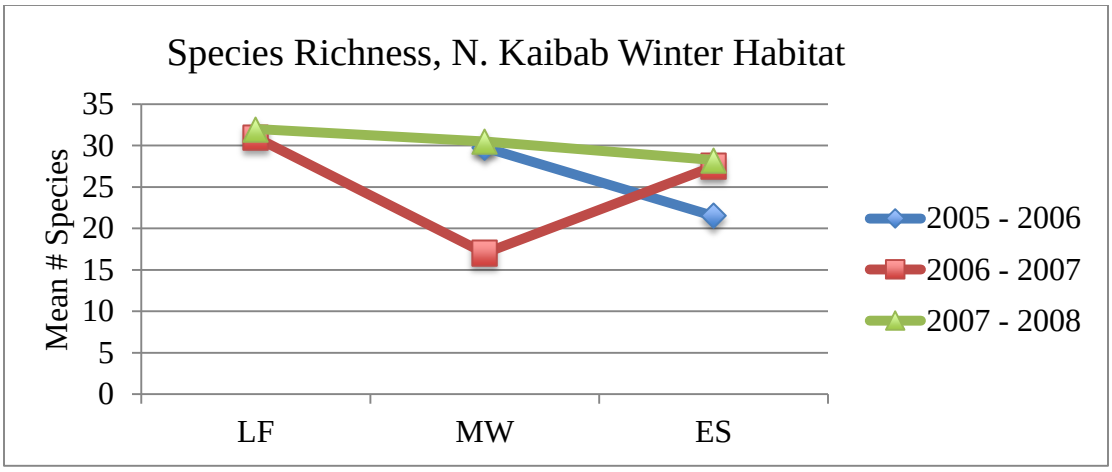


Figure 4.1. Mean seasonal species richness by year for female mule deer diets on the N. Kaibab winter range; November 2005 to March 2008.

An analysis of variance of the species richness (Appendix A, Table A.3) demonstrated a significant difference between the species richness in the variable study years ($P \geq 0.0441$, $F = 4.478$). A Bonferroni means separation test (Appendix A, Table A.3) revealed a significant difference ($P \geq 0.082$) in the species richness between years 2 and 3. This significant difference is graphically demonstrated in Figure 4.1.

Forage Class Composition

An overall summary of forage class, season, year and interactions with statistical significance is provided in Table 4.2.

Year	Season	Forage Class			
		Grass	Forb	Shrub	Conifers
2005 -2006	Late-Fall	NA	NA	NA	NA
	Mid-winter	20%	5%	61%	14%
	Early Spring	20%	1%	59%	19%
2006 - 2007	Late Fall	25%	7%	54%	13%
	Mid-winter	21%	6%	29%	43%
	Early Spring	25%	6%	55%	14%
2007 - 2008	Late Fall	26%	16%	37%	21%
	Mid-winter	12%	5%	60%	23%
	Early Spring	27%	5%	54%	14%

Table 4.1 Forage class composition means of female mule deer diets, organized by season and year on the N. Kaibab winter range; November 2005 to March 2008.

Forage Classification of the utilized species fell into four groups: Grasses, Forbs, Shrubs and Conifers. Classification was made based on plant morphology, fruit (if available), inflorescence (if available), comparisons to Kearney and Peebles (1979), the Plants National Database (2005 – present) and lab voucher samples. (Refer to Appendix A, Table A.2 for a full description of the species that comprise these forage classes.) Experimental design for this phase of the study was a completely random three-factor factorial of Forage Class, Season, and Years. The dependent variable was the amount of slide coverage of plant cells sorted by forage class.

Analysis of Variance (Appendix A, Table A.5) was use for the factors Season, Year and Forage Class. Plots of the un-transformed coverage data resembled a Poisson distribution. Data was transformed using a square root transformation (Sokal and Rohlf 1969, Steele, Torrie, and Dickey 1997, Zar 1999) of $\sqrt{(x)} + \sqrt{(x+1)}$. Results of the statistical analysis indicate that the variable Forage Class is significant ($P \geq$ <2e-16, F=512.894), while Year and Season were not statistically significant. The interaction of Season x Year x Forage Class are also significant ($P \geq 2.97e\text{-}4$ & $P \geq 2.99e\text{-}8$).

Tukey's HSD (Appendix A, Table A.6) multiple comparison of the individual factors provides significance between all four different forage classes ($P \geq 0.000$) *except* the interaction between the classes of Grasses x Trees ($P \leq 0.760$, $\alpha = 0.10$). The multiple comparison analysis does not show any significant differences between the compared means for the variable Year. The interaction is significant because of the effect of Forage Class.

Forage Class x Year				
	Shrub	Conifers	Grass	Forb
2005-2006	60.10%	16.76%	20.15%	2.99%
2006-2007	45.97%	23.58%	23.72%	6.74%
2007-2008	50.42%	19.06%	21.64%	8.89%

Table 4.2 Percent coverage of mean diet composition for Forage Class and Year for female mule deer in the timeframe of the study.

The interactions of Forage Class x Year is significant at $P \leq 0.040$ (rounded), F=2.312 (Table 4-3; Appendix A, Table A.5). Differences in the percent utilization between forage classes Shrubs/Grasses and Conifers/Forbs explain the statistical significance in the three years of the study.

Forage Class x Season				
	Shrub	Conifers	Grass	Forb
Late Fall	46%	17%	26%	12%
Mid-winter	50%	27%	18%	5%
Early Spring	56%	16%	24%	4%

Table 4.3 Percent coverage of mean diet composition for Forage Class and Year for female mule deer in three seasons on the Kaibab winter range.

The interaction of Season x Forage Class is significant at $P \geq 2.97e\text{-}4$, F=4.721 (Table 4.4; Appendix A, Table A.5). Shrubs again are the largest category utilized. The utilization of Shrubs & Forbs oscillates with the utilization of Conifers and Grasses

exchanging relative levels of usage among the seasons. Levels of Forbs in the diet composition are low and significant in comparison because of the low percentage of utilization.

The three-way interaction of Year x Season x Forage Class is highly significant ($P \geq 2.99e\text{-}8$, $F=7.512$; Appendix A, Table A.5), with change in percentage utilization occurring most notably in the mid-winter of Year 2. The shift in diet composition/utilization of shrubs from the Late Fall of Year Two into conifers during the Mid-winter of '06-07 is notable, as is the change back into shrub and grass utilization (e.g. utilization) from conifers in the Early Spring of '06-07.

Species Composition

A graphical breakdown of the species composition of the diet in the context of forage-class mean percent coverage versus season and year is demonstrated in Figure 4.3. Forty-six different species, including those both unknown and of such a small amount they were lumped under the general heading of their respective Forage Class (i.e. "Grass" or "Shrub") comprised the diets, or list of species, utilized by the mule deer as determined by micro histological study. The experimental design for this phase of the study was a completely-random three-factor factorial on Species, Season and Year. The dependent variable was X-percent slide coverage based on species. The analysis of variance provided results where all three variables of Species, Season and Coverage were significant (Appendix A, Table A.7). Additionally, all interactions of these three variables were found to be significant.

Multiple Comparison tests of the variable Season (Appendix A, Table A.8) provided none of the seasons were significantly different when compared against each other. Multiple comparison tests of the variable Year showed a significant difference

between Years one and three (P ≥ 4.30E-05) and Years two and three (P ≥ 2.00E-05). Multiple comparison tests (Tukey's HSD or Bonferroni) of the 44 sub-variables of species and different interaction of species became very complex and difficult to reify. Figure 4.2 adequately describes the utilization and species composition of the diet of the mule deer by year, then forage class, and then season, the top three species and percent coverage have been described. The individual species composition by season and year is presented in Appendix A, Table A.2.

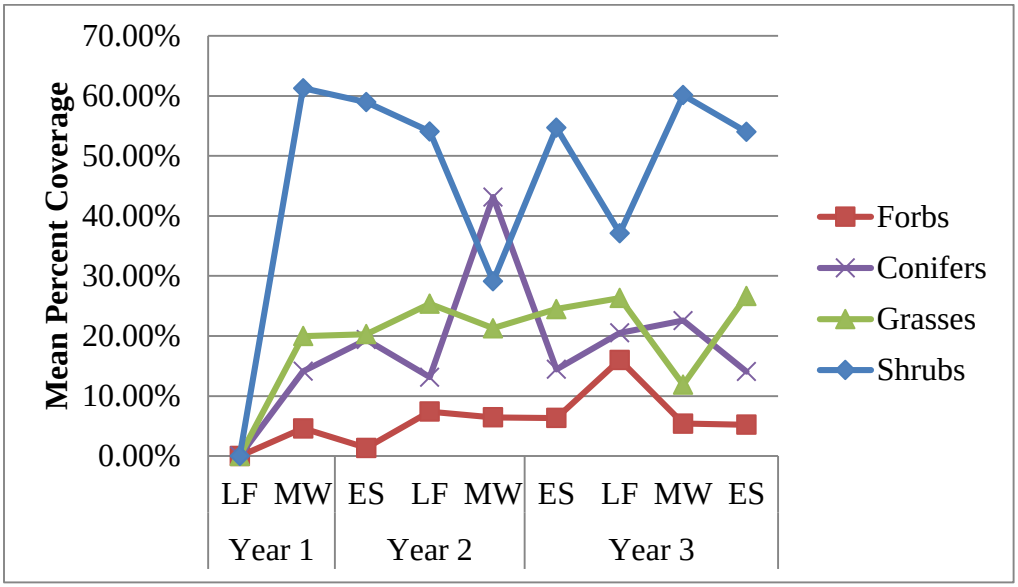


Figure 4.2 Percent coverage of diet mean composition/utilization, context of a Forage Class x Year x Season interaction. Results are for female mule deer on the Kaibab winter range, November 2005 to March 2008.

Species by Season by Year:

Year One, Mid-winter:

Year 1 of the study (2005 – 2006), the forage classes Shrubs were the majority of the diet composition (61% [MW 2005] and 59% [ES 2006], followed by Grasses (20% for both seasons) and Conifers (14% and 19%) (Appendix A, Table A.2).

The shrubs big sage *(*24%) and Mexican cliffrose (12% and 13%) were utilized by the mule deer during the mid-winter of the first year (Year 1). Each of these shrubs made up 24% and 12% each of the mule deer mid-winter diet.

Grasses comprised 20% of the mid-winter diet for year one. The grass species sampled and the percentage found included crested wheatgrass at 5%, bottlebrush squirreltail (*Elymus elymoidies*) at 5%, and Arizona fescue at 6%. Utah juniper was utilized at 7% of the diet in the midwinter of year one. Piñon pine comprised 6% of the diet (MW) and Ponderosa pine was found at 5% in the mid-winter of 2005.

Year One, Early Spring:

In the early spring of year one big sage made up 26% of the diet and Mexican cliffrose held 13% of the mule deer diet. Four-wing saltbush (*Atriplex canascens)* also was included in the early spring of ’05-’06, comprising 8% of the diet. Forb utilization during Year One was ≤5% for the Mid-winter. Desert trumpet (*Eriogonum inflatum)* (~5%) was the most utilized forb during mid-winter of the first year. Utilization of trees in the early spring of year one showed Utah juniper was utilized at 8% in of the diet. Piñon pine rose to 11% (ES) from 6% in MW. Utilization of ponderosa pine dropped to 0-1% throughout the remainder of the study. Grasses comprised 20% of the early spring (ES) diet for year one (‘05-’06). This included 9% crested wheatgrass and 4%

bottlebrush squirreltail. Early spring of year one also included the use of blue grama and western wheatgrass (*Elymus smithii*) at 3% each. Forb utilization during Year One was ≤5% for the Early Spring. All species of forbs in ES of year one were <1% utilized.

Year Two, Late Fall:

Shrubs in Year 2 composed the majority of the diet (54%) in the late fall (LF), '06-'07. Consumption of big sage was 14% of the diet and Mexican cliffrose comprised 11%. Forage kochia (*Bassia prostrata)* was utilized at 8%. The diet composition was dispersed more evenly through the remainder of the shrub species in the late fall. Grasses in the Late Fall of study year two (2006-2007) were utilized at 25 percent and 14 percent. The grass species utilized most in this time frame were crested wheatgrass and bottlebrush squirreltail each providing approximately 8% and 5% of the composite for the diet. Late fall of year two utilization of trees (conifers), consisted primarily of Utah juniper and Piñon pine (*Piñon pine).* Juniper was 9% of the diet and Piñon pine provided 3% of the diet. Ponderosa Pine was also present but not in a significant amount, providing ≤1% of the diet.

Year Two, Mid-winter:

In the mid-winter of year two ('06-'07) the shrubs big sage and Mexican cliffrose comprised 24 % and 12% of the utilized species in the diet. Grasses were utilized at 21 percent in the mid-winter of year two. Bottlebrush squirreltail (4%) and Arizona fescue (6%), with the remainder of utilization more or less evenly distributed throughout the *Bromus* species. The forage class of (coniferous) trees demonstrated a

Table 4.4 Means of coverage by forage class, (season x year) of mule deer diets on the N. Kaibab winter range, from mid-winter 2006 to late winter 2008. (Superscript notation indicates statistical significance.)

Season	Grass	Forb	Shrub	Conifers
LF	$1142.125^{e,h}$	$0.13^{g,j}$	$2145.5^{c,m}$	$792^{e,o}$
MW	$767.25^{b,i}$	$0.07125^{g,j}$	$2102^{c,m}$	$1547.875^{d,n}$
ES	$1177.375^{e,h}$	$0.0675^{g,j}$	$2465.72^{a,k}$	$671.625^{e,p}$
Forage Class Means	$1028.92^{f,r}$	$0.09^{g,s}$	$2237.74^{b,l,q}$	$1003.83^{f,r}$
n=95	EMS=8.69E+5			
	$P_{(FC)}$=2.e-16	$P_{(FC:Season)}$=2.97e-04	$P_{(FC:Year)}$=.0397	$P_{(FC:Season:Year)}$=2.99e-8
	$^{a\ldots g}$ Means with the same letters are not significantly different at			
Forage Class	P≤0.10			
FC:Season	$^{h\ldots p}$ Means in column with the same letters are not significantly different at P≤0.10			
FC:Season:Year	$^{q\ldots r}$ Means in the row with the same letters are not significantly different at P≤0.10			

utilization in the Mid-winter 2007 of 43%, reflecting the sole use of Utah juniper. Early spring of year two (’06-’07), big sage and Mexican cliffrose were heavily utilized by the mule deer at 26% and 13%. Utah serviceberry was also present in the diets at 5% in the early spring. Early spring of year two 25 percent respectively of the diet composition for the forage class Grasses in both seasons. This included western wheatgrass, (4% ES’07) bottlebrush squirreltail and 4% ES ’07). Early spring of year two also included the grass species smooth brome (4%), cheatgrass (3%) and Western wheatgrass at 5% utilization. Grasses were utilized at 21%, with usage of blue grama *(5%),* smooth brome (6%), cheatgrass (6%) and bottlebrush squirreltail *(*4%) distributed more or less evenly. Shrub usage during this winter period was 29% overall, primary utilization to Mexican cliffrose (13%) and willow (*Salix spp.*) (7%); four-wing saltbush was browsed at a 4% utilization.

Year Two, Early Spring:

Utilization of trees in the diet for the early spring of year two demonstrated 14% Juniper and 1% Piñon pine. The forage class Forbs comprised less than 10% of the species utilized across all three seasons in year two, 2006-2007. This forage class included species such as white sage *(Artemesia ludoviciana*) scarlet globe mallow *(Sphaeralcea ambigua*) and mullen (*Verbascum thapsus*). Forb usage in the mid-winter of 2007 was less than 10%, however scarlet globe mallow and mullen were utilized at <2%. The diet composition for the late fall (LF) of 2007 – 2008 (year three of the study) held grasses at 26%. Crested wheatgrass provided 6% of the diet in late fall. Arizona fescue (5% LF) and smooth brome (2% LF) were also utilized.

Year Three, Late Fall:

Shrub utilization during the late fall, of year 3 of the study (2007 – 2008) provided a diet composition of 37% (LF) for this forage class. Mexican cliffrose (8%), wild rose (*Rosa woodsii)* (5%), and rubber rabbitbush (6%) were the primary shrub species that were utilized. Forbs were utilized at 16% of the diet in the late fall of year 3. White sage provided for 8% combined with rose pussytoe (*Anternnaria microphylla)* (5%) for utilization in the late fall. Scarlet globe mallow, mullen and white sage each provided ≤ 2% of the diet. Piñon pine and Utah juniper provided 21% (10.5% Piñon pine, 9% Utah juniper; LF) and 14% (13% Utah juniper; ES) of the diet during late fall of year three.

Year Three, Mid-winter:

Grasses were utilized in Mid-winter of 2007-2008 (year three) at 12 percent of the diet (bottlebrush squirreltail 4%, crested wheatgrass 2%, and blue grama 2%). Forbs were utilized at 5 percent of the diet (no species > 2%) during the same Mid-winter time frame. Shrubs composed 60 percent of the diet (Mexican cliffrose 18%, Apache plume 12%, and big sage 6% and rubber rabbitbush 5%) and coniferous trees 23 percent (Utah juniper) in the Mid-Winter of year 3. Forbs were not utilized in any meaningful amount in the mid-winter of Year 3.

Year Three, Early Spring:

Diet composition for the early spring (ES) of year three of the study (’07 – ’08) was similar to the prior years. Shrub utilization during the early spring of year 3 of the study (2007 – 2008) provided a diet composition of 54% (ES) for this forage class. The shrubs big sage (9%), Mexican cliffrose (11%), Rubber rabbitbush (8%) and Apache plume (8%) represent the

composition of the diet in the early spring of 2008. Forbs were utilized at 5% in the early spring of year 3 (’07-’08). Amounts of the individual species used were not notable.

Early spring of 2007 – 2008 (year three of the study) demonstrated a consumption of grasses at 27%. Crested wheatgrass provided 6% of the diet in early spring (ES). Utilization of Arizona fescue (2%ES) and smooth brome (6% ES) provide a more comprehensive understanding of grass utilization in the composited diets for early spring of year three. Trees provided 14% (13% Utah juniper, 1% Piñon pine) of the diet during early spring seasons of year three, 2007-2008.

DISCUSSION

Species richness

The description of species richness of the diets (i.e. how many species were compose the total diet) was acceptable within the historical context provided by Rassmussen (1941) and Russo (1964). The species listed as potential forage within the historic works are consistent with findings in the 21st century.

The *species richness* found in the diet are also a function of the precipitation and climate data presented in the Site Description of this work. Species richness at any given moments in the season x year interaction is a reflection of the pattern of migratory and grazing behavior for the mule deer during warmer temperatures, colder temperatures without a snow layer (e.g portions of Late Fall), without a significant precipitation event (mid-winter ’05-06, and ’06-’07), and sub-freezing temperatures that included at least two noted significant precipitation events during the three study years.

The ANOVA for Species Richness (Table A-x) showed that there was a statistical difference ($P \geq 0.0441$), and that a means separation test showed ($P \geq 0.082$) a significant difference between years two and three. Reviewing the precipitation data presented in the Site Description showed a significant lack of a winter time large precipitation events during the second year of the study. Temperature trends for the second year of the study (Figure 3-3) show temperatures stayed within the mean and median temperatures for the Bright Angel Ranger Station circa 1920's. Both of these factors could be influencing the vegetation response and availability of species for grazing for mule deer on the Kaibab.

The level of species richness found in the diets is also a function of time and past anthropogenic activity. Rassmussen (1932/1941), Russo (1964), and Young (2002) specifically referred to sheep grazing prior to 1924 removing all of the available desirable forage and leaving only the sagebrush as winter forage for the mule deer prior to the 1920's deer irruption. If a significant amount of damage was inflicted on the range then the ecological bottle-neck of available species before and after the reported deer population irruption has helped to define the available species mix on the range today. Lacking photo-point documentation or other pre- and post-disturbance vegetation studies to say otherwise, there is no reason to not to take the comments by the three prior researchers at their anecdotal face value.

Man's influence on species richness is obvious in the species list describing the grasses and the number of conifers present. Non-native, introduced grass species including western wheatgrass and crested wheatgrass. Species including cheatgrass and smooth brome are prolific and invasive. Grasses are a high fiber, low protein resource in comparison to shrubs, have a higher amount of lignin by volume than shrubs, and have become an important part of the

Kaibab mule deer diet, albeit a high-fiber, moderate concentrate one that includes orchard grass. Ingestion of a volume of grass potentially high in lignin that can't be digested removes an available volume of rumen capacity from the digestion of a higher concentrate/more nutritious type of forage.

Casual observation of the plant communities indicate typical fire response/suppression of natural fire events through most of the late 19th and 20th centuries. The intensive fire response of suppression by the land management agencies has encouraged the growth and encroachment of low-growing conifer trees (Utah juniper and juniper sp., Piñon pine, and other unknown seedlings*)* throughout the study area, helping to redefine what was known as a sagebrush shrub community (Rassmussen 1941, Russo 1964).

Forage Class Utilization

Forty-nine species found on the North Kaibab are divided into four separate and distinct forage groups from the efforts of fieldwork and laboratory micro-histological analysis. Late-Fall dietary samples were not available during the first year of the study due to budgetary and logistic concerns. The three-way ANOVA for forage class utilization demonstrates a lack of statistical significance between the variable Year and the variable Season for the percent coverage of the species presented.

The type of species utilized and the percentage of coverage found in the micro histological analysis in the early spring of all three years alludes not only to the degree of senescence and degradation the long winters have on the forage species, it also describes the habituation of the mule deer herd to where the forage is located. Assume that the forage is depleted in the locations that the deer regularly browse. Habitual use of depleted forage plants in

the regularly browsed areas during the winter and periods of intense winter storms (i.e. "yarding up" in a familiar copse of Piñon pine and Utah juniper, big sage, and Apache plume), when an adjacent, unfamiliar clearing or flat might have a more robust forage source that has not been heavily grazed by wildlife or livestock. This re-occurring phenomena of instinct was observed during the study more than once. *The frequently changing botanical and nutritional composition of their food intake* has not been investigated … (McCullough and Smith 1991, italicized emphasis added). The concept of forage class and diet composition is available to the immediate natural resource managing agency (i.e. the Kaibab National Forest, the adjacent National Park Service and Bureau of Land Management) and perhaps known, but the knowledge about the diet composition has not been known or available.

The diet – judged by the percentages of species used in a forage class(es) does not greatly shift and appears stable during the year. The completed ANOVA (Appendix A, Table A.3) in relation to Table A.2 may add some insight.

An analysis of variance in the context of species richness (Appendix A, Table A.3) demonstrates that different types of utilized forage (i.e. classes) were important enough ($P \leq 0.00E+0$, F=2.165E+2) within the context of an interactions between a) the temporal value of a season ($P \leq 1.66E-07$, F= 9.252E+0) and b) the forage class with the temporal values of a season and a specific year ($P \leq 6.31E-10$, F= 1.304E+1). These interactions are relevant; it didn't matter what season was chosen, the forage classes of shrubs and coniferous trees were the main food source utilized. The study year was insignificant, as was the season. The year in the context of (i.e. the interaction between) the forage class was significant. The year and the season, when considered in the context of the forage class, was significant (Appendix A, Table

A.3). The lack of statistical significance between seasons (Appendix A, Table A.3) helps to explain the low level of differences in diet composition between seasons in the winter habitat of the Kaibab mule deer.

Tukey's HSD multiple comparison was utilized to adequately control the family wise error rate, also known as the Sidak phenomena within the three-way ANOVA analysis (Sidak 1967, Shaffer 1995, Herv'e 2007, Hair 2010). This less-conservative multiple comparison test provided the latitude needed in analyzing a natural system while still assisting in the control of the family-wise error rate.

Tukey's HSD results demonstrates the statistical significance of Forage Class. The significance of Forage Class provides the basis of statistical significance in the two-way and three-way interactions contained in the analysis of variance; the significance of Forage Class is robust and provides significance to the interactions, when the other variables are not significant.

From Appendix A, Table A.2 the species mix per season changed, but not at levels that would be statistically significant. The Tukey's HSD tests (Append. A, Table A.5) provided a method for means separation tests that not only describe the significance of the forage classes, but the lack of significance of the seasons. Multiple comparisons between forage classes (Table A.5) support the statistical significance of the forage class comparison of means ($P \leq 0.00$, MSE=95620). Utilization of forbs, although low in usage (Append. A, Table A.2) can be interpreted as being low, yet a distinct enough difference between the other forage classes that a statistical significance is demonstrated. The phenomena becomes readily apparent when the contents of the diet for Forbs was usually below 10%, or 10% utilization is remembered. It is

hypothesized that the low level of Forb utilization may be connected to precipitation levels during the study.

The lack of statistical significance between seasons (Append. A, Table A.3) helps to explain the low level of differences in diet composition between seasons in the winter habitat of the Kaibab mule deer.

Grasses are significant compared to shrubs ($P \leq 0.00$) but not significant when compared to trees (conifers) ($P \leq 0.994$). The means belonging to the shrubs were large enough compared to the means of the grasses to be statistically significant. Utilization of shrubs is significant when compared to trees ($P \leq 0.00$); a higher percentage of coverage (i.e. utilization) was found among the shrubs than the trees. More shrubs than grasses or trees were utilized, but the same level of grasses as trees were also utilized.

The species utilization that defines the forage classes described within these results are as much a function of the anthropogenic influences on the land as it is not. Where man's influence on the plant communities is most evident is perhaps in the species list describing the grasses and the conifers. Western wheatgrass, crested wheatgrass, the species of cheatgrass and smooth brome are not native to the area but introduced and prolific. Grasses are a high fiber, low protein resource in comparison to shrubs. The grasses have become an important part of the diet, albeit a high-fiber, moderate concentrate one (i.e. the presence of orchard grass in the diet).

The consistent usage of smooth brome throughout the study may belie the species importance to the diet of the mule deer. The introduction of these grasses to the habitat is as much a function of the anthropogenic influences on the land as it is not. These grass species are a product of wind-born, vehicular and animal deposition and re-seeding by the U.S. Forest

Service in response to the multiple fire scars and needed post-fire ground coverage and transient invasion. The consistent and "even" usage of smooth brome by the mule deer throughout the study may belie the species importance to the diet of the mule deer.

Utilization of grasses in some time periods of this study approach and exceed 50%. In comparison, Deitz (1976) (referring to Kufeld 1973) states that only one species of grass was heavily utilized in the winter, crested wheatgrass. The heavy use of grass on the Kaibab in this study is in stark contrast to Dietz's statement. Dietz also lists usage of a similar composition of shrubs and trees in the Black Hills (South Dakota), listing "common juniper" (*Juniperus communis)*, and creeping barberry (low Oregon grape; *Mahonia repens)* from Schneeweis et al. 1972."

Shrubs in our study may be statistically significant because of the reconstructive effects of the fires (Bridger Knoll [1980's] and Slide Tank [late 2000's] having cleared out the some conifers in these areas. That trees (conifers) were significant could be construed as the presence of Piñon pine and Utah juniper trees in locations where earlier (1890's to 1990's) over-grazing of livestock and fire suppression efforts were in full force. Forbs are significant within the context of the Tukey's multiple comparison simply because of the low number of forbs comprising the diet compared to the level of percent composition of the other forage classes.

The presence of Ponderosa pine in the late fall of '06-'07 demonstrates the migration from higher altitudes or utilization close to the North Rim, altitudes where the species occurs regularly. It is thought to be a remnant in the diet from the summer range or the transition to the winter range, as the majority of the pellets constructing the diets were collected well below the elevation containing Ponderosa pine.

Low levels of utilization of willow in the Mid-Winter of 2007 may infer a southern migration by some individual deer or smaller segments of the herd down into the bottom of Snake Gulch/Kanab Creek, or the Grand Canyon where this species is more prevalent in the riparian areas, and then travelling back up to the Plateau. Willow (*Salix spp).* was not prevalent or obvious around any water catchments visited or discovered on the Kaibab plateau winter range.

Sudden mid-winter precipitation and unseasonable warming midwinter of '06-early spring '07 instigated a late December-early January sprouting and utilization of grasses (i.e. *Bromus spp.*) and forbs that normally would not have been available on the range. This additional, unexpected source of energy and protein - while it provided a reprieve from the metabolic realities of a cold winter - were not lasting as cold temperatures and frozen precipitation soon settled in again before the completion of Early Spring. The heavy utilization of Utah juniper in this time frame indicates an isolation of the mule deer on to the elevation of the plateau where Utah juniper is most prevalent; located at approximately 1500m - ~2000m (5000-6500 ft., + or – 20 ft.)

CONCLUSIONS

With dentition of a cervid, a highly flexible upper lip and prehensile tongue, dependence on shrubs by mule deer was expected. Utilization by forage class reveals a heavy dependence on trees and grasses that was not expected during a winter-time study. This dependence demonstrates an effect of the past range and fire management efforts guided more by commercial grazing interests than forest health or habitat quality.

The number of species available for utilization by foraging mule deer on the North Kaibab winter range is limited. The species richness, or number of species utilized by the mule deer are not necessarily an original observation as the same or similar species names are referred to by Rasmussen in his 1941 article referencing his 1932 dissertation work. Hanley - who reviewed forage species preferences in his 1997 work, McCulloch in 1978 who reviewed statewide utilization from archival records and McCulloch and Smith in 1991 in their work on the Kaibab also alluded to these species being utilized. The identified species used by the mule deer as a diet has not been fully quantified or qualified past the casual observation of rumen findings (McCulloch 1978) until now.

The species richness found in the diets in this study has not substantially increased over what species were described growing on the Plateau in 1931/1942. Increased species richness on the winter habitat, with increases to the available number of shrub species combined with invasive juniper control, will help to provide a diet that is more diverse for the mule deer in most forage classes.

Plant community diversity and age structure on the north Kaibab needs to be increased. Many of the *Rosacea* – family shrubs are nitrogen-fixing and contribute to the high protein curve of the forage. The height and quality of the available annual growth on located decadent stands needs to be placed into a management plan. Juvenile and adolescent plants of the *Rosacea* family – found in areas where large-scale forest fires have occurred - need to be propagated along with other types of shrubs to encourage a quality forage resource that is accessible to the mule deer.

The forage habits of the deer do not seem to change from generation to generation. Depleted forage in given locations that have become a habit for the deer to use during the periods of "yarding up" during the winter and winter storms (i.e. Piñon pine and Juniper, big sage, Mexican cliffrose and Apache plume) are returned to even if an unused adjacent clearing or "flats" might have a better source of forage. This re-occurring phenomena was described by Russo (1964), and later Haywood (1987) in a technical report by Arizona Game and Fish. Consideration needs to be given to range remediation efforts that help to restore native shrubs and grasses and increase the diversity of species on the Kaibab plateau. As changing the behavior of a returning deer herd would be difficult, a series of small controlled burns applied to selected portions of the winter range, throughout the study area would force the mule deer to forage elsewhere on the winter range while providing an opportunity to reseed with a diverse mix of native species.

CHAPTER V

WINTER DIET QUALITY

INTRODUCTION

The objective of chapter III is to evaluate the known diet as determined in Chapter II for quality. Diet quality is defined as meeting or exceeding metabolic needs for available protein (N), and energy (calories). This chapter evaluates the known diet for excesses or deficiencies, modeling calculated baseline levels against known values for energy and protein from forage supplies obtained from the North Kaibab winter habitat. These known values for energy and protein were determined in the laboratory using common, accepted best practices and laboratory techniques. Previous published studies using similar habitat conditions are used for comparative values. These derived values are to be used as part of the foundation for further habitat modeling in the study.

METHODS

Forage Quality Analysis

Prior to the initiation of sampling, a general visual survey of plant species present on the winter range was conducted to develop a list of potential forage species (Appendix A. Table 2A). Concurrent with each of the fecal collection periods (except late fall 2005) samples of all available forage species were collected from the north Kaibab winter range. Availability was defined as those plant species accessible by mule deer, and was a function of the growth form of the plant and snow cover conditions. Collection of forage species/material was restricted to the terminal bud and annual growth, i.e. those portions of the plants that were erect and when possible green, indicting active photosynthesis. For shrub species, collection was concentrated

on 2.5 to 5 cm of the current annual leader growth at heights less than 1.5 m above the ground. In addition to current annual growth, emphasis was given to any actively photosynthesizing leaves. To limit bias in estimation of the nutrient content of the plants as a result of soils or micro-site factors, individual species samples were collected from at least five individual plants at five separate locations throughout the study area and combined into a single representative sample by species, sampling period, and year. The total number of species collected in any year by sampling period combination was dependent on climatic conditions.

All forage samples were oven dried at 50ºC for 48 -72 hours (A.O.A.C., 2004), ground in a Wiley mill™ to a uniform particle size of 1mm, and stored in an air tight container for later analysis. Forage quality analysis consists of three procedures: crude protein (CP) determination (PerkinElmer PE2400 elemental CHN analyzer, PerkinElmer Inc.); gross energy (GE) assessment (Parr 6400 Calorimeter, Parr Instrument Co.); and in vitro dry matter digestibility (DMD) (Goring and Van Soest 1971, Tilley and Terry 1963). All analysis were performed on a by-species / by-sampling period / by-year basis in triplicate.

The four measures of forage quality (DP_i, DP, ME_i, and ME) were used to determine diet quality in terms of digestible protein by species (DP_i), digestible protein of the diet (DP), metabolizable energy of the i^{th} species (ME_i), and metabolizable energy (ME) of the diet as defined by:

$$DP_i = CP_i \times DMD_i \quad ,(i)$$

Where: DP_i is the digestible protein content of the i^{th} plant / forage species, CP_i is the crude protein content of plant species i, DMD_i is the dry matter digestibility of plant species i;

$$DP = \sum (DP_i \times DC_i), \text{ and (ii)}$$

$$DPI = DP \times DMI \quad \text{(iii)}$$

where: DP is the digestible protein content of the diet, DP_i is the digestible protein content of the i^{th} plant / forage species and DC_i is the proportion of plant species i (i.e. percentage) in the diet.

Similarly Metabolizable Energy of the i^{th} species is defined as,

$$ME_i = GE_i \times DMD_i \times MCoef \quad \text{(iv)}$$

where: GE_i is the gross energy content of the i^{th} plant species, DMD_i is the dry matter digestibility of the i^{th} plant species, MCoef is the ability to be effectively metabolized by the ruminant animal, a coefficient of 0.84 (Robbins 1993). DC_i is the proportion of plant species i in the diet. Thus,

$$ME = \Sigma (ME_i \times DC_i), \text{ and} \quad \text{(v)}$$

$$MEI = ME \times DMI \quad \text{(vi)}$$

The previous four examinations were integrated into a single evaluation of the adequacy of the Kaibab winter range to meet mule deer nutritional requirements at any point in time during their occupation of the winter range (Moen 1973, Wallmo et al.1977, Miller 1980, Nelson and Leege 1982, Holt et al. 1992, McCall et al. 1997, Parker et al., 1999; Cook et al. 2001, Drake 2009). The central concept of this approach is historically based on site specific parameters such as gender, body size, reproductive status, consumption rates, activity patterns, and climatic conditions, to predict the daily nutrient requirements of an individual for digestible protein and metabolizable energy.

Daily nutrient requirement values were compared with information on dietary nutrient intake as determined by the product of the DPI or MEI times the daily dry matter intake

(BW * DIR), to assess the ability of the habitat to meet the nutritional needs of a specific individual at any point in time during the time period covered by the forage quality data (Moen 1973, Wallmo et al. 1977, Miller 1980, Nelson and Leege 1982, Holt et al. 1992, Parker et al. 1999).

Statistical Analysis

A two-way Analysis of Variance was applied to the calculation results for the Digestible Protein Intake (DPI) and the Metabolizable Energy Intake (MEI) using R (3.0.0 & 3.0.2). Factors were Season and Year. Analysis of Variance was applied to the calculated results of the DPI and MEI levels. Statistical significance (i.e. alpha) level was placed at $P \geq 0.10$. Tukey's HSD or Bonferroni's post-hoc multiple comparison tests were utilized to assist in the control of the family-wise error rate (Herv'e 2007, Sidak 1967, Shaffer 1995, Hair 2010) of these analyses.

RESULTS

Quantitative Forage Quality Analysis

Seasonal means for Digestible Protein Intake (DPI), Metabolizable Energy Intake (MEI) and the associated standard deviations for DPI and MEI found in the mule deer diets from November 2005 to March 2008 are reported in Tables 4.1 and 4.2 below. (Seasonal means for crude protein (CP), gross energy (GE), and dry matter digestibility (DMD) are reported in Appendix B, tables B-1 through B-3 for reference.) These values begin a conversation of how well the forage species discussed in Chapter 1 provide for the protein and energy needs of the north Kaibab mule deer herd.

The seasonal mean DPI (Table 5.1) available for the mule deer from the north Kaibab plateau ranged from a low of 33 grams of dietary protein in the mid-winter '06-'07 to a high of 61 grams in the early spring of '05-'06. Standard deviation (SD) of these measurements were within 7 and 3 g/day of digestible protein. Analysis of variance of the Digestible Protein Intake (Appendix B, Table B.1) levels demonstrated that season ($P \leq 0.0647$, $F = 3.076$), alpha (α) = 0.10, year ($P \leq 3.82e\text{-}06$, $F = 21.937$), and the season x year ($P \leq 0.0407$, $F = 3.217$, $\alpha = 0.10$) interaction were significant.

Table 5.1: Mean Dietary Protein Intake (DPI) and associated standard deviation (Sd) by season and year for mule deer diets on the N. Kaibab, November 2005 to March 2008.

	Season		
	Late-Fall	Mid-Winter	Early-Spring
Year		DPI (g/day)	
2005 – 2006	NA	54	61
2006 – 2007	45	33	37
2007 – 2008	35	40	43
		DPI Sd (g/day)	
2005 – 2006	NA	7	3
2006 – 2007	10	3	3
2007 – 2008	6	3	8

Analysis of variance (ANOVA) permits the study of the variance between factors or variables. A mean separation test is necessary to investigate the differences between the levels of a factor. Tukey's HSD (Appendix B, table B-2) applied to the variable Season demonstrated significant differences between late fall and early spring ($P \leq 0.066$). Tukey's HSD when

applied to the variable Year had significant differences between the study years 2005-2006 and 2006-2007 (P≤ 1.87e-5) and between 2005-2006 and 2007-2008 (P≤ 4.25e-5). The ANOVA interactions between the seasons and years were also significant. These results are graphically displayed in Figure 5.1.

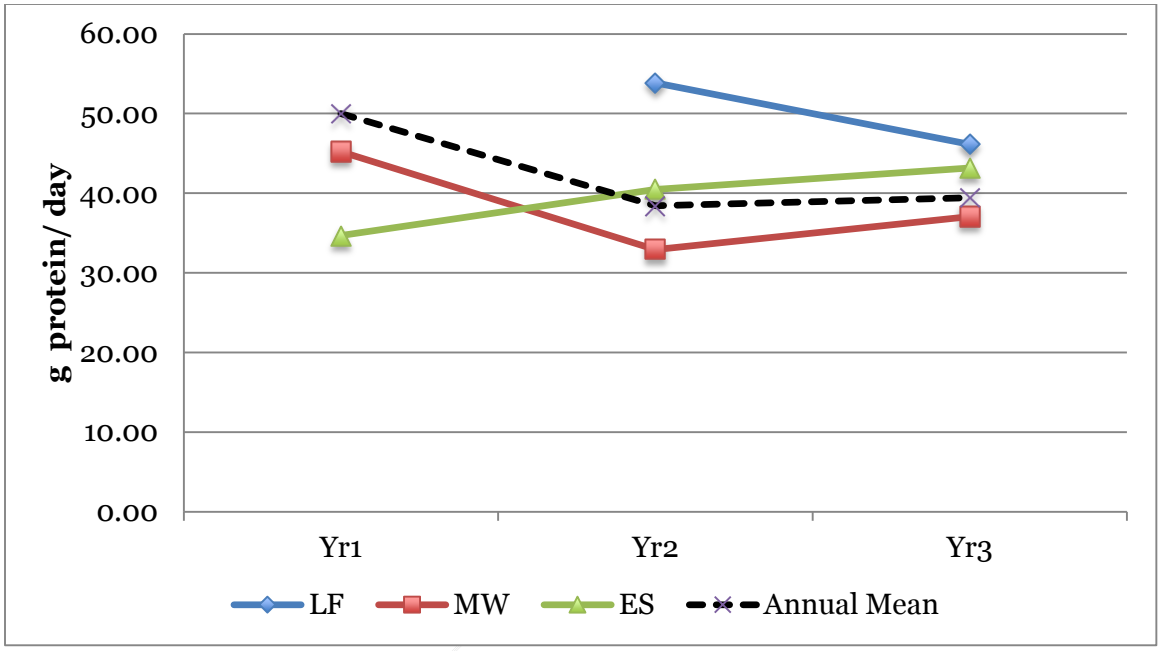


Figure 5.1: Mean digestible protein intake (DPI) of mule deer diets interaction by season and year. Year 1 = 2005-2006, Year 2 = 2006-2007, Year 3= 2007-2008.

Seasonal Dietary Metabolizable Energy means (Table 5.2) ranged from a high of 2266 kcal/day in the Early Spring of '05-'06 (SD 204kcal/day to a low of 1210 kcal/day (SD 261 kcal/day) in the late fall of '07-'08. Results of the ANOVA for the values of Metabolizable Energy Intake (MEI) (Appendix B, Table B.3) demonstrated that season ($P \leq 2.75e\text{-}4$, $F = 11.761$), year ($P \leq 0.017$, $F = 4.827$), and the interaction of the variables Season and Year ($P \leq 4.846e\text{-}3$, $F = 5.55$), were significant.

Table 5.2: Mean Metabolizable Energy Intake (MEI) and associated standard deviation (Sd) by season and year for mule deer diets on the N. Kaibab from November, 2005 to March, 2008.

	Season		
	Late-Fall	Mid-Winter	Early-Spring
Year	MEI (kcal/day)		
2005 – 2006	NA	2105	2266
2006 – 2007	1752	1969	1541
2007 – 2008	1210	2015	1877
	MEI Sd (kcal/day)		
2005 – 2006	NA	305	204
2006 – 2007	447	126	245
2007 – 2008	261	161	9

Tukey's HSD multiple comparisons were applied (Appendix B, Table B.4) to the MEI values to test between the means of the factors year and season and the interaction between the two. The multiple comparison tests for Season provides a significant difference in the MEI between late fall and early spring ($P \leq 4.04e\text{-}3$) and mid-winter and late fall ($P \leq 2.17e\text{-}4$). The multiple comparison tests for Year provides a significant difference in MEI differences between the means of year two when compared to year one at $P \leq 6.78e\text{-}2$. A comparison between the

means of 2005-2006 (year one) and 2007-2008 (year 3) were significant, P≤ 2.5e-2. These results are graphically displayed in Figure 4.2. Note the differences in values between Year 1, Year 2, and Year 3. The differences between years, when considered by season, can be correlated back to the precipitation records (Figures 3-1 through 3-4) in the description of the Study Area.

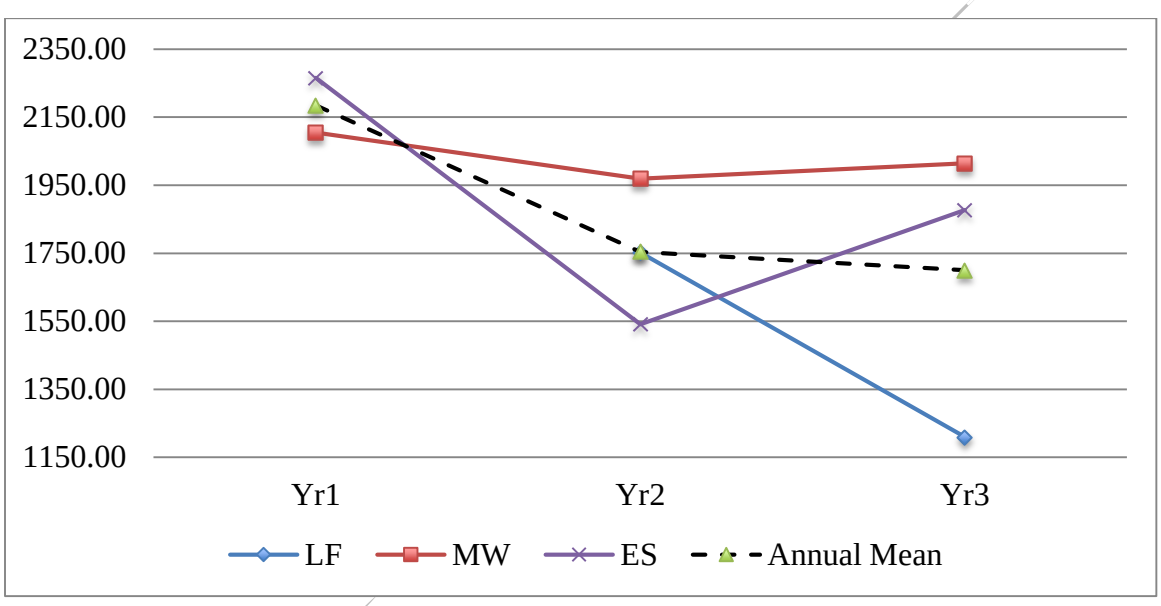


Figure 5.2: MEI interaction with the variables Season and Year. Year 1= 2005-2006, Year 2 = 2006-2007, Year 3= 2007-2008. Data points reflect seasonal changes to available MEI through the three year duration of the study.

DISCUSSION

To facilitate ease of analysis and adequately include the first year forage quality results, the experimental results were left as an unbalanced experimental design. Differences among the means of the study years (’05-06, ’06-07, ’07-08) allowed the examination of the available

dietary protein resource on an annual and three year temporal scale. The first year of the study provided the highest levels of the available dietary protein during the duration of the study (Table 5.1). Standard deviation values for stated means provide the accuracy of the dietary data collection during the study. The resulting statistical significance in the analysis of variance and the mean separation test (i.e. Bonferroni) are comprehendible in this context.

Three different seasons were also examined – late fall, mid-winter and early spring. Digestible protein intake values were not significantly different alone. Six out of the eight seasonal means for DPI (’06-07, ’07-08) were similar; the differences were not great enough to generate a calculated F value of significance. Examination of Tukey’s HSD helps to explain why; the statistical significance between the late fall and early spring exists, but is countered by the lack of significance between the comparisons of late fall - mid-winter and mid-winter - early spring.

MEI values were significantly different alone. MEI values reached a mid-point high in mid-winter in Years 2 and 3 (figure 5.2), with energy levels lower in the mid-winter for years two and three in the late fall, and depressed again for years two and three in the early spring. Year one is the only year that escalates the available metabolizable energy into the early spring. A correlation with the amount and timing of precipitation deposited on the plateau (Figures 3-1 through 3-4, 3-9) and the vegetative response to the precipitation is coincident with the spring warm-up that occurs (Figure 3-5 through 3-8, 3-10) in the months of February and March.

Comparative DPI values were extrapolated from reported intake data of Wallmo et al. (1977) study. Middle Park, CO. mule deer had 27 g DPI in the early winter (our late fall) and 29 g DPI for consumption. Wallmo et al. (1977) also reported ME values for carrying capacity vis-

à-vis the Middle Park, CO. study to be at 1,530kcal in the Early Winter (late fall in this study) and 1,377kcal in the Late Winter (early spring). Miller (1980) reported an approximate level of 31g dietary protein in the late fall and approximately 62g in early spring. Metabolizable energy for a single does was graphed at ~ ≤ 4,100 kcal/day in the late fall and ~≤ 3,600kcal/day for Bailey Creek in North Central Washington.

Examining the diet of Arizona mule deer on the Three Bar experimental area located on the Four Peaks Mountain on the Tonto National Forest (Tonto Basin), Urness et al. (1971) reported protein levels of 10 – 13 percent of the late fall to early spring diets for mule deer and Coues white-tail deer. The diets were accompanied with a 34 – 43% digestibility. Micro histological methods of diet identification were utilized for diet identification and were similar to the methodology used in this study (Urness et al. 1971).

This study on the North Kaibab was conducted during the breeding cycles of the mule deer. A precept of wildlife management is to manage the resource for the resources highest metabolic demand (Robbins 1973 and 1993, Wickstrom et al. 1984). The benefits of the diet to the ruminant is linked to the digestibility (i.e. Dry Matter Digestibility) and the caloric and nitrogenous protein amounts the diet can provide (Page and Underwood 2006). In the temporal scope of this study the largest demand for protein by a mule deer on the plants of the Kaibab plateau would be during the gestational cycle, where energy and protein demands of the doe and fawn would need be supported.

Figure 5.3 reflects equation (iii), modeling the decline in DPI in the temporal scope of the three seasons examined. Metabolic requirements for DPI were modeled after Robbins (1973, 1993), Parker et al. (1984, 1999), Handley (1982, 1997), Handley et al. (1989, 1985) and Hobbs

and Swift (1985). The tested data was assembled into a calculated model of the DPI versus the nutritional needs during the same time-frame for a mule deer doe. There is an adequate amount of protein in the diet including an excess of 12 g/day DPI at the beginning of the gestation cycle. Pregnancy with twin fawns may places the mule deer into a dietary deficit for protein (Miller 1980). The reported fawning rate at the time of the study was 180% (Buck 2007, Miller 2013). By the date of parturition, the metabolic needs of the maternal doe is 41 grams of DPI, a shortfall of at least 4 grams DPI per day. This shortfall in protein initiates and/or accelerates a cycle of urinary nitrogen recycling and protein catabolism (Allen 1977, Guyton 2006).

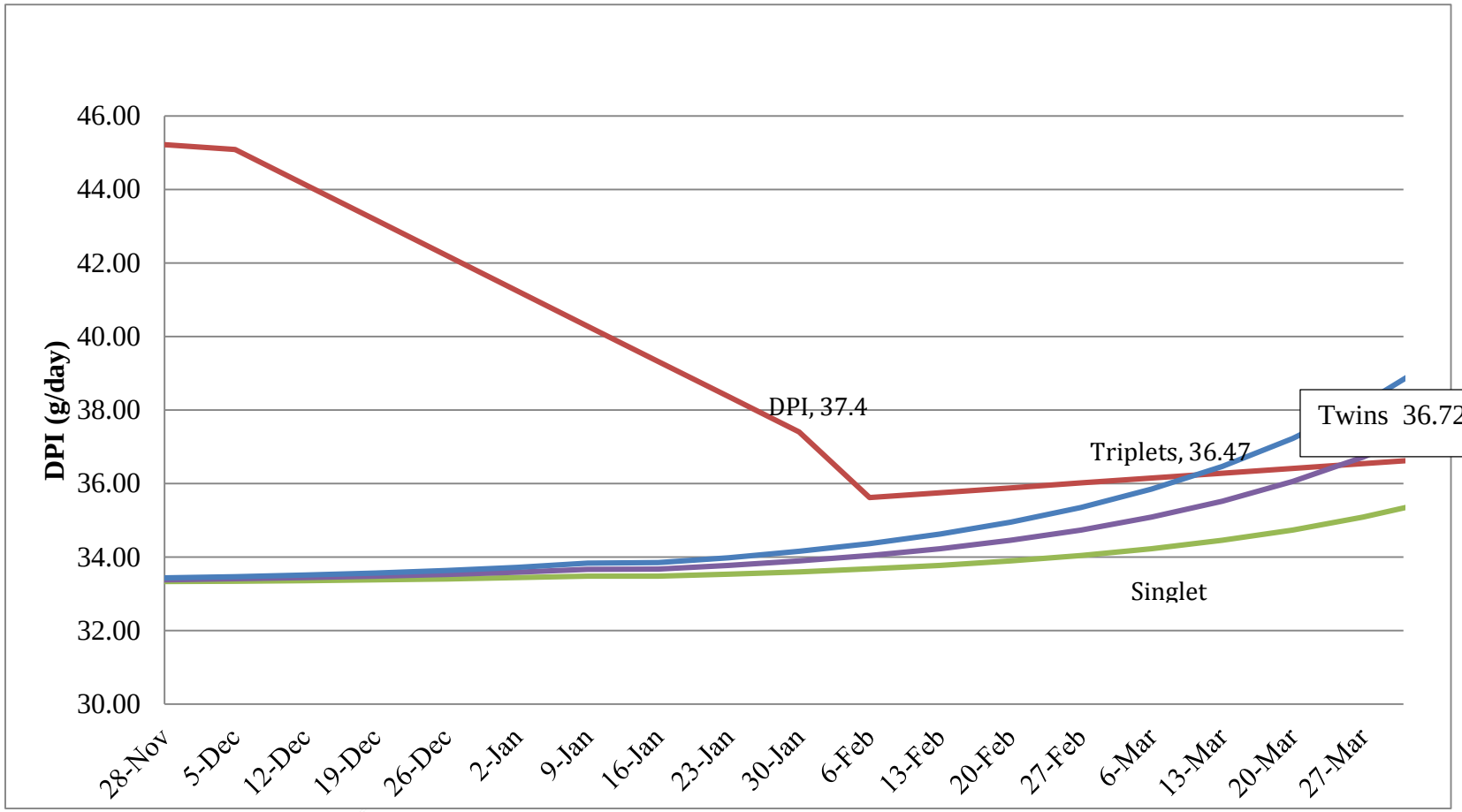


Figure 5.3 Model comparison of digestible protein intake (DPI) in N. Kaibab winter diets and the projected nutrient requirements of a given day of gestation for a pregnant doe.

If the largest demand for protein (DPI) is during the gestational cycle, then it would follow largest demand for energy (calories) is also during the gestational cycle (Robbins 1993, Drake 2009, Tluczek 2012). Page and Underwood (2006) explain that "Energy is typically the most critical dietary component for deer during the winter, with other nutrient needs being satisfied while foraging for energy".

Figure 5.4 demonstrates the digestible metabolic energy needs calculated from equation (vi). Metabolic requirements for MEI were modeled after Robbins (1973, 1993), Parker et al. (1984, 1999), Handley (1982,1997), Handley et al. (1989, 1985) and Hobbs and Swift (1985).The tested data was assembled into a calculated model of the Digestible Metabolizable Energy needs versus the gestation cycle of the mule deer. The model expressed by the equation within the temporal scope of the study. While protein needs are met by the forage resource until approximately the middle of March (about the last date of data and sample collections per year), the MEI needs are no longer being met by the first week of January.

These basic metabolic needs are compensated using the excess protein available from the Kaibab plateau forage. The energy needs are met until approximately the middle of January. The decrease in MEI available from the forage drops steadily from 1800 kCal (adjusted for metabolizable energy contributed by the excess DPI) to approximately 1350 kCal in the early part of February, increasing to approximately 1550 (adjusted for metabolizable energy from excess DPI) kCal/day in the middle of April. Examining the model, for approximately four months in the gestation cycle the doe is dependent upon catabolism of fat and protein resources for daily basic metabolic needs

and activity (Warren et al. 1982, Parker 1999). Derived from work with domestic livestock (when used for the comparison of nutrient quality) in habitats for whitetail deer, blacktail and/or mule deer (Moen 1973, Robbins 1993) it begins to explain the intricacies of the metabolic shortfalls affecting one of the most publicized deer herds of the western U.S.

It becomes a problem of volumetrics, dry matter digestibility and turnover rate (Ammann et al. 1973, Wallmo 1977, Wickstrom et al. 1984, Ngugi et al. 1995, Miller 2013). Wallmo has stated it succinctly "...deer apparently have limited ability to increase forage intake after the rumen reaches a threshold capacity … food intake and passage rates of mule deer remained essentially constant as fiber content of forage increased. This was attributed to the relatively small size of the digestive organs, suggesting that deer are not well adapted for using high fiber forage" Short's and Robbins's research (two separate studies reflected in Robbins's 1973 dissertation) provide that adult-proportion of the rumen size and volume are reached when a white-tail deer reached a body weight of 25 Kg. This allows yearling deer the volumetric ability to forage ad libitum in the same physiological constraints of a fully mature mule deer. With the exception of fawns – which were not taken or utilized for this study – this provides a physiological level measure of response of the deer to appetite and satiation when caloric needs are not being met (Moen 1973).

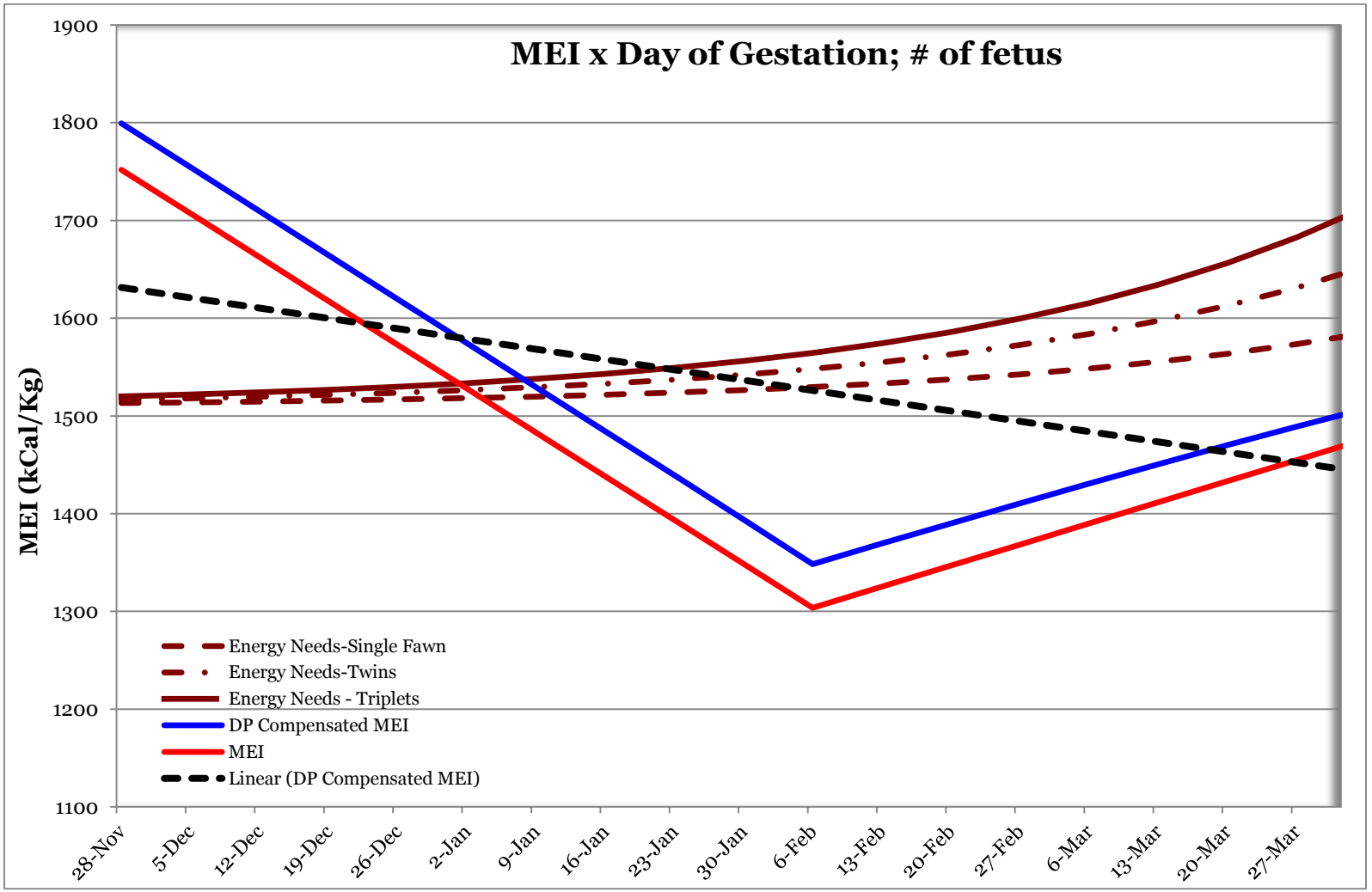


Figure 5.4 Model comparison of the dietary metabolizable energy intake (MEI) in N. Kaibab winter diets and the projected nutrient requirements of a given day of gestation for a pregnant doe.

The diets and forage samples were collected during a time frame of plant and fruit/nut maturity to declining senescence of the forage resources in the habitat. Not only is an aging temporal aspect influencing the quality of forage, but so are temperature fluxes and precipitation changes. The forage is already mature by the entry of the mule deer into the defined habitat by the October-November start of late fall. Unless an unorthodox warming trend with precipitation occurs during the winter, the remaining available forage will continue to decline in quality as it ages (e.g. levels of lignin and polyphenols (Brady and Weil 1999), entering or continuing senescence. Protein and caloric resources become constricted as the available shrub and tree-classed forage age becoming increasingly unpalatable (Moen 1973, Pederson 1978). Selection and utilization of the standing crop of forage species such as big sage, Mexican cliffrose, Apache plume and the opportunistic utilization of new growth and sprouts as new, green plants and buds became available are part of the survival and reproductive strategy of mule deer, as is locating available grass for utilization.

Additionally, there is a problem in metabolizing protein when caloric needs are not met. Although the amino acid resource of nitrogen is present, the remaining carbon structure is deficient; "…animals consuming protein in excess of their nitrogen requirement, but deficient in energy, may not retain dietary nitrogen as efficiently, because sufficient carbon substrates would not be available for microbial amino acid synthesis" (Belasco 1954, Mould and Robbins 1981).

Females – during the breeding season - will have a steadily increasing, more demanding rate of forage intake and caloric response versus males who are active during the rut. Metabolic demands of ovulation/pregnancy/parturition are higher than that of a

sexually reproductive male (Robbins 1973). Feedback for appetite control through the hypothalamus will be affected by the volume of the rumen-reticulum, the rate of passage of the forage particles, the rate of digestion of the particles, and the single-celled micro-organisms sloughing off of the rumen bacterial environment into the mid-gut (Robbins 1973, Ammann 1973, Bryant 1977, Klem 1977, Merchen 1977, Phillipson 1977, Van Soest 1982, Church 1988, Fahey 1988, Hoffman 1988, Owens 1988, Argenzio 2004, Leek 2004).

A hypothesis arises that there may be an inherent animal instinct that limits the diet of the Kaibab mule deer to no more than 30% concentration of big sage. In interpreting Swank's 1958 works, McCullogh and Smith (1991), when examining rumen samples, state that "Sage brush may provide more nutritious food for deer during the latter part of winter for another reason. Spring growth increases the nutritive content, especially that of protein and phosphorus, in shrubby foods of deer." and that dormancy in sagebrush may end in March or sometimes February, "… a month or so before growth starts on cliffrose at the same site … sage brush may be the higher quality food at that time."

This may be an optimistic view of the nutritive qualities of sagebrush. While nitrogen content of diets increased by 0.06% with increasing levels of big sage in the diet vs. dry grass hay, digestibility declined with a 30% big sage diet (R. Ngugi, 1995). These conclusions coincide with findings by Nagy (1964), where "essential oils" in sage brush – specifically big sage, have an anti-bacterial capability, along with being capable of essentially bringing rumenal digestion to a halt. Combining the effect of a moderate – level big sage diet (i.e. a diet comprised of 10% – 30% big sage) with the potential lignin

content of a winter – time diet in a ruminant, the need for the constant turnover of dry matter and substrate that Van Soest specified (1982) becomes apparent and comprehensible. Dietz (1976) corroborates Nagy's findings regarding the inhibitory effects of big sage on rumen microbial activity and rumen muscular function.

Lignin, previously described as having phenolic and polyphenol compounds that are not available for microbial breakdown, prevents the animal from extracting an adequate level of energy from shrubs and especially trees (Brady and Weil 1999), a forage class whose means in this study were not different in utilization from grass (Tables A-2, A-3). This translates as the same amount of forage intake occurred with the species of trees as was done to species of grasses. The higher lignin amount in the tree forages (Utah juniper berries and twigs, Piñon pine nuts and twig growth) provides a lower digestibility of the forage resource and places into question the digestibility and quality for annual and perennial grasses the herd may be consuming (Boudet 1998 and 2000, Hatfield 2009).

Grasses, because of the difference in volume versus surface area, typically contain more lignin per unit of measure than shrubs. Brome grass has a distinct carbohydrate structure for the cell wall (Hatfield 2009) that may permit easier acylation by enzymatic activity, leading to a more complete (yet imperfect) break down of the lignin compounds (Hatfield 2009), potentially offsetting the higher per volume lignin content. However, unless the polyphenol molecule is fully reduced with the assistance of aerobic fungi, the lignin molecule will be partially reduced in size by bacterial action in an anaerobic rumen medium with free radicals being released into the rumen liquor, the rest of the molecule

being evacuated in excrement (Green 2013). Adequate energy levels are still elusive for a ruminant under these dietary conditions.

Knowing the consist of the winter diet that the Kaibab mule deer utilize (as specified in Chapter 4) places the calculated metabolic energy shortfall in perspective. A 30% (mid-winter and early spring, Year 2) make-up of big sage (with "essential oils" [i.e.VOC's] and lignin present); 23-27% of their extant diet consists of conifer trees with their inherent volatile organic compounds and lignin; and grasses exceeding 50% of the deer diet places the digestible functionality of the rumen at a reduced level, if not at risk of failure (Nagy 1964).

It is not enough that the mule deer are not receiving a high enough level of calories by the mid-winter as demonstrated in Figure 5.4. Forages in the available diet to the mule deer have been demonstrated to inhibit digestion in a multitude of methods; the "essential oil" of sage inhibiting rumen movement and having anti-bacterial properties (Nagy 1964) and diminishes dry matter intake of other species (Ngugi 1995). The heavy utilization of grass does not compliment the small volume of the deer rumen (Phillipson 1977, Ferrell 1988). A large enough quantity of substrate and liquor are not held of the grass for fermentation/energy extraction into microbial byproducts of VFA's to meet energy needs to be sustainable (Moen 1978, Merchen 1988, Owens and Goetsch 1988, Orsov 1990).

Substrate passage and turnover then, in the context of a rumen full of low-energy grass, big sage and other lignified tissue that is reduced or inhibited (Nagy 1964, Ngugi 1995) then becomes an exacerbating factor to the overall energy – acquisition constricts facing this herd. The composition of the diet is directly affecting the ability of the mule

deer to digest and utilize the diet. Appetite determines the amount of substrate available for fermentation and is reflective of the metabolic demands for energy and protein (Leek 2004). The deer are eating, but not getting enough energy out of the substrate. The substrate is being inhibited from passing through the rumen by the big sage oil content (Nagy1964, Ngugi 1995), and interrupting (in some fashion) the motor functions of the rumen muscle structure (Argenzio 2004, Leek 2004). Additional time in the rumen (i.e. a retarded passage rate) won't necessarily assist fermentation either – unless the amount of sage oil and other volatile organic compounds are reduced. The substrate lingering within the rumen won't assist the appetite control centers of the brain activate if passage of substrate through the rumen is slowed. The instinct to select forage in a timely manner will be suppressed (Argenzio 2004, Leek 2004) by a rumen that takes longer to empty.

Examination of the diet composition and characteristics of the forage classes in Chapter IV shows a reliance on grasses during the study years, between 20 and 27% for each season. Knowing the vertical utilization of a forage class allows us to understand the level of energy and protein provided to the animal. Comprehension of the problem can then extend from a three-dimensional construct into a fourth dimension with time as a variable. The new variable encompasses both long-term seasonal and annual usage by study year (Robbins 1973) and short-term turnover rate of forage substrate within the rumen of anywhere from 24-48 hours to two weeks (Allen 1977, Van Soest 1982, Hoffman 1988, Beitz 2004) dependent upon the digestibility of the plant particles and particle size. The amount of time spent digesting a diet that is increasingly indigestible translates into more time and energy spent foraging for food that *is* digestible. *If* the diminishing energy supply provided by the poor quality forage in the rumen provides

enough energy to forage and the rate of passage through the rumen allows new substrate to be introduced; *if* the appetite is triggered to forage.

The adjective "digestibility" encompasses any problems the animal might have with particle size, microbial decomposition, volatile organic compounds (i.e. essential oils), or lignification/lignifaction. Grasses as a forage class are generally a high-fiber, low protein resource with an amount of cellulose that is provided – as an annual plant, or annual growth on a perennial base (i.e. *Bouteloua* spp.) which, by volume of individual particle, is low in lignin. Plants do not need to be large, woody and multi-stemmed to introduce lignin into the diet. Grasses can also provide more lignin by "x" unit of volume because of the higher amount of overall surface area (Miller 2013) of smaller plants multiplied by the number of grass blades/plants eaten. This amount of lignin introduced into the rumen would decrease the digestibility of grasses along with the shrubs that would also present lignin. Because more grass particles are consumed, more lignin is being delivered to the digestive system of the deer.

The monthly precipitation records have provided an unintentional "smoothing" effect to the data and chart lines (NOAA 2013); deep snow events such as those experienced by the researcher in the mid-winter and early spring of 2006-2007 and the mid-winter of 2007-2008 were of magnitude to be memorable and expensive to equipment. Similar to other southwestern deep snow events, they did not linger past a few weeks. These deep snow occurrences provided observations during the study of "yarding" of the deer underneath low-lying Utah juniper branches and *Piñon pine* with active foraging on these plants. These precipitation events severely limited the mobility

to any locomotive animal on the Kaibab (Moen 1973, Robbins 1973, Parker 1984, Wickstrom 1984, Parker et al. 1999,) while they existed.

The statistical significance reported between the seasons Late Fall and Early Spring may be interpreted as the continued reliance on mast crops such as the nuts of the Piñon pine (*Pinus edulis*) and the intact Utah juniper berries as the winter progressed and the availability of grasses diminished

The dynamics of the rumen and biochemical inputs and feedbacks from the forage resources, precipitation and terrain become incredibly complex, more than the referenced equations can precisely depict. It has also been suggested the mule deer on the Kaibab have intuitively found a forage mix that will provide a measure of energy and protein during poor foraging conditions, and can distinguish between plants when a botanist cannot (McCullogh and Swank 1991), within the limits of the volume capacity of the rumen. My data, the data utilized in this study and others that have been referenced, does not support that hypothesis.

CONCLUSIONS

The forage resource on the North Kaibab deer range provides an adequate protein resource that is in excess of protein requirements. This is directly related to the shrubs that are a member of the Rosacea family and the protein provided from the single-celled animals sloughing off within the rumen environment. The high level of protein more than adequately meets the needs of the animal, and the excess level of protein can be utilized by the animal as an additional source of energy. This high level of plant protein

liberates the animal from extensive urinary recycling to meet protein needs, and helps to provide for the caloric demands of the deer.

The forage resource on the North Kaibab deer range provides an insufficient amount of energy for a pregnant mule deer doe. This caloric deficit (including the extraneous dietary protein) becomes apparent by the mid – winter (January). The limited number of species available (e.g. the truncated level of species richness) becomes a limiting factor in the nutritional ecology. In addition, the species composition places limitations on the ability of the animal to extract needed VFA's from the diet. The amount of volatile organic compounds introduced to the rumen by foraging on big sage and coniferous trees (Utah juniper and Piñon pine) inhibits the digestion of an already calorically deficient diet. The amount of lignin in the diet, while not quantified, is suspected to exacerbate this deficiency.

In order to provide an adequate amount of energy the species composition of the available forage (i.e. the North Kaibab winter range) would need to be altered. To do so would mean fundamentally changing the species composition available for forage. This is not necessarily realistic. Applying a program of mechanical rehabilitation to selected areas of known conifer-encroachment, small scale controlled burns, and reseeding efforts with native plant life may help to alleviate the caloric deficiency encountered.

CHAPTER VI
METABOLIC INDICATORS

INTRODUCTION

Previous works on mule deer blood and plasma metabolic profiles are infrequent in the literature. Available reference literature regarding ecological work in understanding deer or elk interaction with the habitat has been typically completed in controlled trials with captive deer or elk, and pelletized food collected from the available forage resource. Little work has been done with wild populations with data taken from live or fresh-killed mule deer. This chapter examines the metabolic indicators provided by blood and plasma analysis. Samples were provided directly from the north Kaibab winter range via doe-only hunts in the fall using check-stations and hunter-collected samples, or herd culls by the Arizona Game and Fish Department in the spring and samples collected from the fresh kills directly off the multi-thousand hectare study area. Blood and plasma samples were collected from the fall of 2005 through the spring of 2008, concurrent with fecal and plant samples described in the previous chapters.

The objectives for examination of the blood and plasma indicator values in the categories of Carbohydrates (Energy), Protein, and Lipids are multiple. First, gain an understanding of how the mule deer on the north Kaibab winter range are responding to the available forage resources. Second, determine what metabolic systems are responding to known dietary stressors from Chapter IV and Chapter V, and find out how the metabolic systems are responding. Third, attempt to understand why the metabolic systems are responding in the fashion they are. Baseline levels of some blood and plasma

metabolic indicators are set and/or refined in this chapter of the study. A detailed description of the metabolic response to the diet and nutrition conditions modeled in Chapter V is provided.

METHODS

Physiological Response:

Beginning with the late fall youth hunt and early spring Arizona Game and Fish Department (AZGFD) doe collections, a minimum 5 mL sample of blood was taken from each animals collected from November, 2005 to March, 2008. The total number of samples varied between hunts with a minimum of 16 samples available for analysis for each year by season combination with the exception of the late fall collection of year three, when only 11 females were harvested. Blood samples were initially collected in Falcon tubes, transferred and stored in purple-topped EDTA treated tubes to be centrifuged. Blood was allowed to clot prior to cold storage during transit (Waid and Warren 1984, Cunningham 1992). Severely hemolyzed samples were discarded as a potential bias to results (Waid and Warren 1984, Cunningham 1992). Plasma was separated from other blood components as soon as possible after the initial collection. Early spring samples were typically centrifuged and separated within three hours of collection. Late fall samples were collected by junior hunters and their responsible adult chaperones. The time from collection to separation varied because of hunter response and hunter-check station protocol. Usable serum was separated from all available samples. All plasma and/or whole blood samples were kept chilled and transported back

to the laboratory where serum was stored at -5°C and whole blood at 2°C until further analysis could resume.

Analysis of physiological indicators fell into three categories: carbohydrate metabolic indicators, protein metabolic indicators, and lipid metabolic indicators. Three separate measures of carbohydrate metabolism were selected via spectrophotometry: plasma glucose concentrations (GLU) (BioAssay, 2006), alkaline phosphatase (AP) (Bio Assay, 2006), and plasma phosphate (PP) (BioAssay, 2006).

The protein metabolism indicators selected were designed to provide information of the overall condition and performance of the body, and the nutrient status of the animals, i.e. was the forage and habitat placing them into or perpetuating a stressed metabolic state? The protein indicators selected were: plasma urea nitrogen (PUN) (BioAssay, 2006), plasma creatinine (CR) (BioAssay, 2006), total protein (TP) (BioAssay, 2006) and the Creatinine: PUN ratio.

The third set of metabolic indicators are indicative of lipid metabolism. The primary lipid indicators measured were plasma albumin (PA) (BioAssay, 2006.), plasma triglycerides (TAG), glycerol (GLY) (BioVision, 2005), plasma free fatty acid concentrations (FFA) (BioAssay Cit.), and plasma cholesterol levels (PC) (BioVision, 2005). The steroid cholesterol permitted an indication of overall herd health through examination of the values.

All analyze were performed in triplicate using a using a Biotek µQuant MQX200 spectrophotometer. The methodology for the metabolic assays paralleled Seal's prior 1970's efforts (Seal 1978) but also allowed the use of up-to-date technology, knowledge and best practices.

Statistical Analysis

The data for the various metabolic panels were examined for normality. The experimental design for the study was a completely random two factor factorial ANOVA. If the data of an analysis did not approach a distribution of normality, a square root transformation of $[\sqrt{(X)} + \sqrt{(X+1)}]$ was utilized on the selected metabolic data prior to analysis (Baker 1930, Sokal and Rolf 1981, Zar 1999). Consideration of statistical significance (i.e. the alpha) was maintained at $\alpha = 0.10$. Bonferroni's or Tukey's Honestly Significant Difference (HSD) post-hoc multiple comparison tests were utilized to assist in the control of the family-wise error rate (Sidak 1967, Shaffer 1995, Neter et al. 1996, Howell 2002, Meyers and Arnold 2003, Crawley 2007, Herv'e 2007, Hair 2010, Logan 2010).

RESULTS

Carbohydrate Indicators

Mean blood concentrations of carbohydrate metabolites for mule deer does on the Kaibab winter range by season and year are presented in Table 6.1. Glucose values ultimately were rejected and not utilized (see Chapter III Discussion) because of the range of values obtained from laboratory analysis. Stabilized glucose levels that were repeatable were not obtainable. Thus, glucose values taken during the study were not utilized. (Cunningham (1992) expressed similar concerns regarding glucose values.)

Table 6.1. Mean blood parameters of carbohydrate metabolism indicators observed in mule deer during late fall and early spring from 2005 to 2008 on the North Kaibab winter range.

Blood Parameter	Season	Study Year 1	2	3	Period Mean
Glucose (mg/dl)					
	Late Fall	187.0 (±27.8)	114.9[a] (±9.1)	195.7† (±0.3)	NA
	Early Spring	NA†	89.8[b] (±0.3)	196.6 † (±0.1)	NA
	Yearly Means	NA	NA	NA	
n=28	df = 21	t-value = 2.4446	P = 0.0232		
Alkaline Phosphatase (mg/dl)					
	Late Fall	39.2 (±4.4)	25.8 (±4.9)	49.2 (±12.2)	38.1(±7.2)
	Early Spring	51.4 (±5.5)	25.0 (±4.8)	40.2 (±8.2)	38.9(±6.1)
	Yearly Mean	45.3(±4.9)	25.4(±4.9)	44.7(±10.2)	
n = 72	EMS = 1,156.2	$P_{(Season)}$ = 0.818	$P_{(Year)}$ = 0.680	$P_{(Season: Year)}$ = 0.422	
Phosphate (mg/dl)					
	Late Fall	925.8 (±4.9)	921.3 (±5.3)	916.0 (±7.4)	921.5(±5.9)
	Early Spring	913.7 (±4.3)	907.5 (±2.3)	913.3 (±4.2)	911.7(±3.6)
	Yearly Mean	919.8 (±4.6)	914.4(±3.8)	914.7 (±5.8)	
n = 74	EMS=300.806	$P_{(Season)}$ = 0.173	$P_{(Year)}$ = 0.2861	$P_{(Season: Year)}$ = 0.984	

† Data not available or compromised due to hemolyzed samples preventing accurate glucose determination.

( ) Values within ( ) are the standard error of the means.

[ab] Means with the same letter within carbohydrate parameter are not significantly different at $P \leq 0.1$.

The analysis of variance (Appendix C, Table C.1) of the transformed blood phosphate concentration data provides a statistical significance between seasons ($P \leq 0.018$, $F = 5.92$). Tukey's HSD (Appendix C, Table C.2) multiple comparison tests demonstrate a significant difference between the means for the seasons late fall and early spring ($P \leq 0.018$). The seasonal means for phosphate levels (Figure 6.1) in blood plasma

ranged from a late fall high of 926 mg/dl in 2005 to a low of 916 in 2007. Early spring values ranged from 907 mg/dl in the early spring of 2007 to 913 in 2006 and 2008. The duplication of values in is notable.

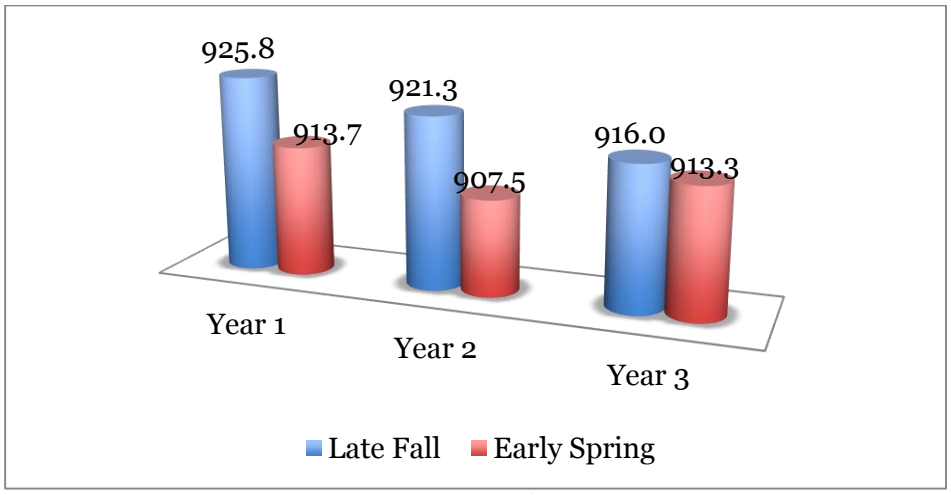


Figure 6.1 Mean plasma phosphate concentration (mg/dl) observed in mule deer does during late fall and early spring from 2005 to 2008 on the North Kaibab winter range.

The analysis of variance of the transformed alkaline phosphatase (Appendix C, Table C.3) data did not provide any statistical significance for the factors season, year, or the interaction of season and year. Multiple comparison tests were not applicable (Appendix C, Table C.4) because of the lack of significant differences among the variances of the means. Seasonal levels of Alkaline Phosphatase (Figure 6.2) ranged from an early spring 2006 high of 51.15 to an early spring 2007 low of 17.9 in 2007. Late fall values ranged from approximately 26 mg/dl to 37 mg/dl in 2007.

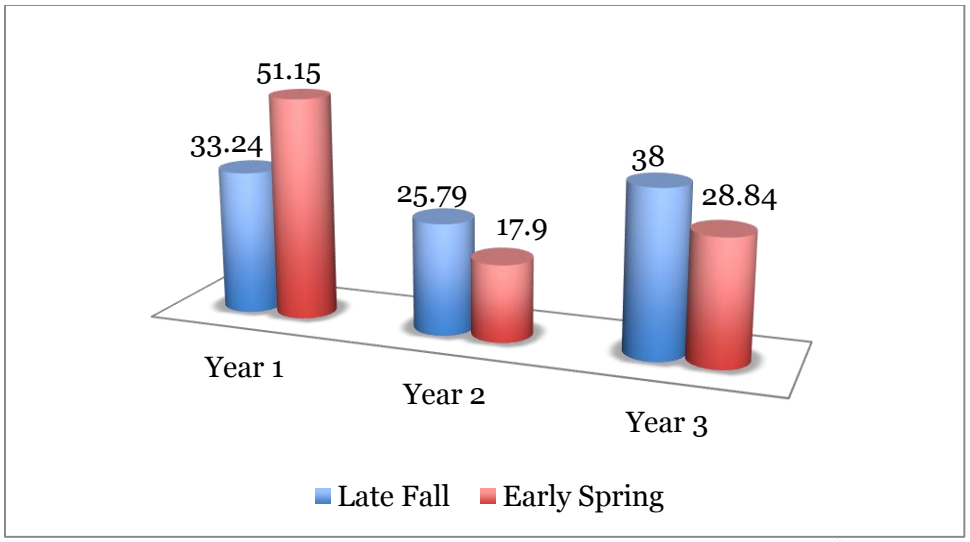


Figure 6.2 Mean blood alkaline phosphatase concentrations (mg/dl) observed in female mule deer during late fall and early spring, 2005-2008, on the North Kaibab winter range

Protein Indicators

Mean blood concentrations of protein metabolites for mule deer does on the Kaibab winter range by season and year are presented in Table 6.2. An analysis of variance on the transformed plasma urea nitrogen (PUN) data (Appendix C, Table C.8) demonstrated a statistically significant difference between seasons ($P \leq 1.41e\text{-}15$, $F = 92.242$) for levels of PUN. The interaction of season by year was also significant ($P \leq 0.07$, $F = 2.737$). Tukey's HSD (Appendix C, Table C.9) provided a significant difference between the late fall and early spring seasons, $P \leq 0.0$.

Plasma urea nitrogen values (Figure 6.3) ranged from a low of 87.6 mg/dl in the late fall of 2007 to a late fall high of 144.1 mg/dl in the late fall of 2005. Early spring values ranged from a low in the early spring of 2007 of 21.33 mg/dl (Year 2) to a high of 38.7 mg/dl in the early spring of 2008. The early spring of 2006 falls in the middle of these two values at 27.9 (mg/dl).

Table 6.2. Mean blood parameters of protein metabolism indicators observed in mule deer during late fall and early spring from 2005 to 2008 on the North Kaibab winter range.

Blood Parameter	Season	Study Year 1	2	3	Period Mean
Plasma Urea Nitrogen (mg/dl)					
	Late Fall	27.9^{g} (±5.9)	21.3^{g} (±4.6)	33.7^{g} (±11.8)	29.3^{a} (±7.4)
	Early Spring	144.1^{f} (±22.9)	153.4^{f} (±22.3)	87.6^{g} (±9.1)	128.3^{b} (±18.11)
	Yearly Mean	86.0(±14.4)	87.4(±13.5)	63.1 (±10.5)	
n = 98	EMS = 4,6946	$P_{(Season)}=4.8e^{-11}$	$P_{(Yr.)}=0.18$	$P_{(Season: Year)} = 0.086$	
Creatinine (mg/dl)					
	Late Fall	0.6 (±0.1)	0.7 (±0.1)	1.7 (±0.4)	1.0 (±0.2)
	Early Spring	1.3 (±0.2)	0.5 (±0.1)	3.1 (±0.3)	1.6^{b} (±0.3)
	Yearly Mean	1.0^{d} (±0.1)	0.6^{e} (±0.3)	2.3^{c} (±0.3)	
n = 98	EMS= 1.0574	$P_{(Season)} = 0.002$	$P_{(Year)} = 3.4e^{-6}$	$P_{(Season: Year)} = 0.406$	
PUN:CreatinineRatio(mg/dl)					
	Late Fall	44.0 (±3.6)	40.92 (±4.6)	19.5 (±2.1)	34.8^{a} (±3.5)
	Early Spring	135.4 (±12.2)	397.1 (±36.5)	29.1 (±1.3)	187.2^{b} (±16.7)
	Yearly Mean	87.7 (±7.9)	219.0 (±20.6)	24.3 (±1.7)	
n = 98	EMS=19,644.0	$P_{(Season)} = 1.38e^{-7}$	$P_{(Year)} = 0.1045$	$P_{(Season: Year)} = 0.374$	

( ) Values within ( ) are the standard error of the means.

[ab] Means with the same letters in blood parameter by season are not significantly different at $P \leq 0.1$.

[cde] Means with the same letters in blood parameters by years are not significantly different at $P \leq 0.1$.

[fg] Means with the same letters in blood parameter by season and year are not significantly different at $P \leq 0.1$.

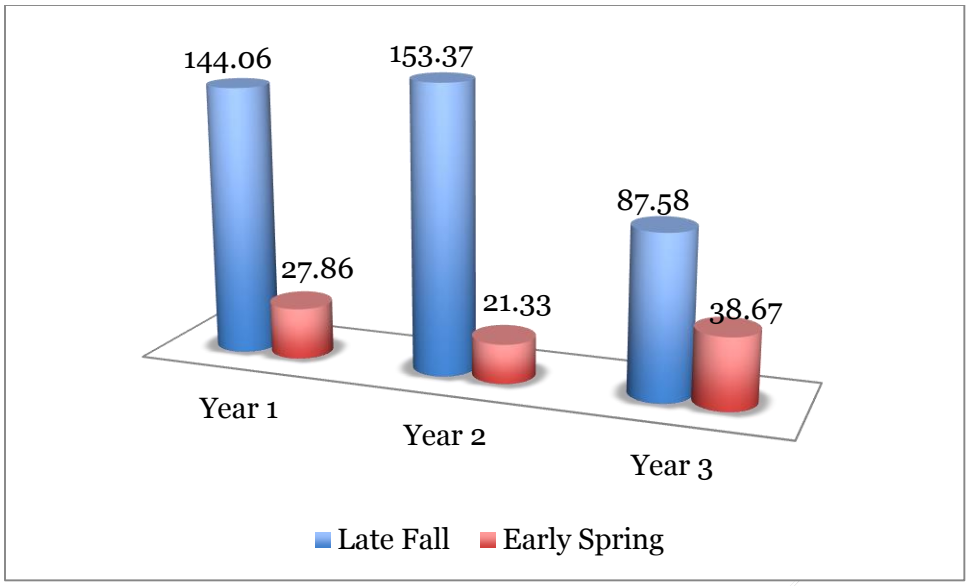


Figure 6.3 Mean plasma urea nitrogen (PUN) concentrations observed in female mule deer during late fall and early spring, 2005-2008, on the North Kaibab winter range.

Analysis of variance applied to the transformed values for creatinine (Appendix C, Table C.5) provided a statistical significance for season ($P \leq 2.66e\text{-}4$, $F = 14.377$), year ($P \leq 9.21e\text{-}16$, $F = 51.405$), and the interaction of season x year ($P \leq 1.35e\text{-}3$, $F = 7.122$). Tukey's HSD multiple comparison did not provide an adequate level of information (Appendix C, Table C.6) regarding the significance of the means; the returned data was liberally distributed. Bonferroni's multiple comparison (Appendix C, Table C.7) was then applied to the transformed data of variables Season and Year. A significant difference exists between the means of all three years of the study. The multiple comparison also provided a significant difference between the two seasons, $P \leq 0.011$.

Creatinine means (Figure 6.4) for the late fall were distributed between 0.57 mg/dl in the late fall of 2005 and 1.7 mg/dl in the late fall of 2007. Early spring values ranged between 0.45 in the early spring of 2007 and 3.09 mg/dl in the early spring of

2008. Creatinine levels differed by ±0.20 mg/dl in the late fall of 2006 and the early spring of 2006 (Figure 6-4).

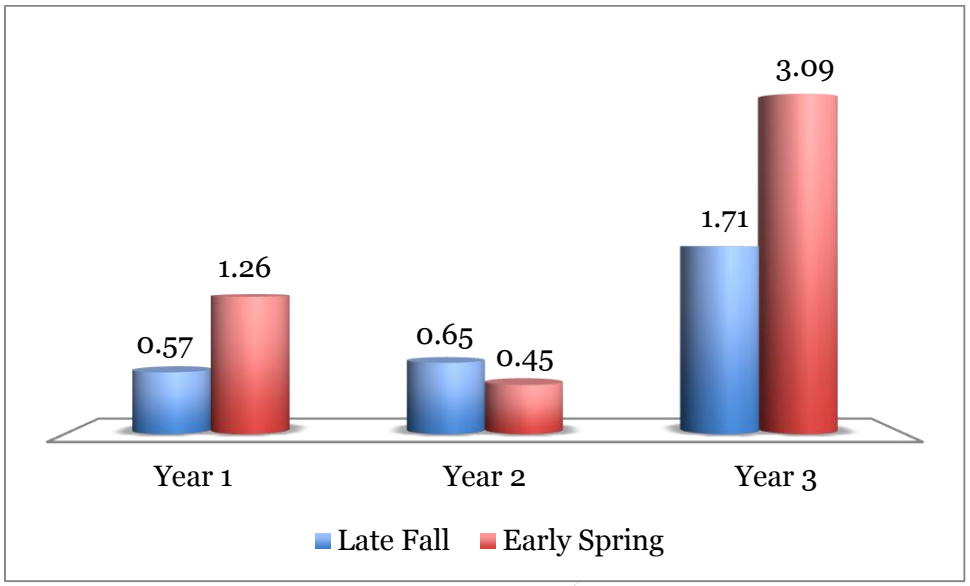


Figure 6.4 Mean blood creatinine concentrations (mg/dl) observed in female mule deer during late fall and early spring 2005-2008, North Kaibab winter range.

PUN: Creatinine (PUN: C) ratios are an indicator of total protein activity within the metabolic systems of an ungulate (DelGuidice et al., 1987, 1988, 1989; Page and Underwood, 2006; Warren et al., 1982, DelGuidice 1991). Analysis (Appendix C, Table C.10) of the variances of the PUN: C ratios provided a significant difference between the seasons ($P \leq 1.45e-07$), between the years ($P \leq 4.25e-09$), and the interaction of season by years ($P \leq 1.18e-05$) with alpha set at 0.10. Tukey's HSD multiple comparisons (Appendix C, Table C.11) of the variable Season provide a significant difference between the mean ratios of late fall and early spring ($P \leq 1.0e-7$). The variable Year provides a significant difference between the mean ratios in all years.

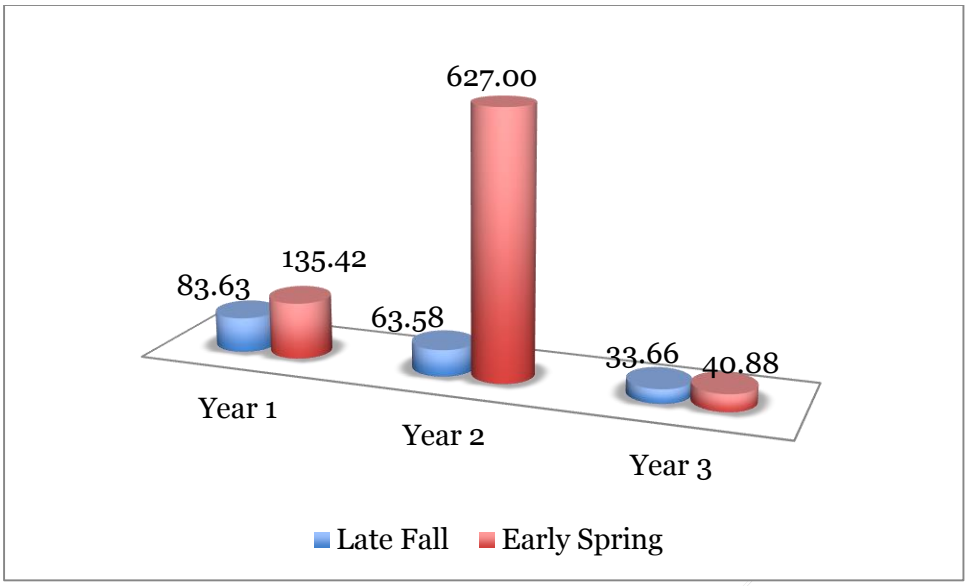


Figure 6.5 Mean plasma urea nitrogen: creatinine ratios observed in female mule deer during late fall and early spring, 2005-2008 on the North Kaibab winter range.

The means of the PUN: C ratios (Figure 6.5) varied from 83.63 mg/dl in the late fall of 2005 (Year 1) to 33.66 mg/dl in the late fall of 2007. Early spring values of the PUN: C ratios were 135.42 mg/dl in the early spring of 2006 to 40.88 mg/dl in the early spring of 2008. The early spring of 2007 demonstrated a ratio value of approximately 627 mg/dl.

Lipid Indicators

Mean blood concentrations of lipid metabolites for mule deer does on the Kaibab winter range by season and year are presented in Table 6.3. Triglycerides in a ruminant are not formed until the excess energy source – e.g. volatile fatty acids, glycogen, phosphates, or ATP/ADP molecules - have been transported out of the cytoplasm of the cell and have reached the site of adipose tissue. The presence of triglycerides when caloric needs are not being met by the diet in a ruminant animal indicates the body fat

resource has been mobilized in response to a condition of "under nutrition" (DelGuidice 1991, Allen 1977, Beitz 2004, Orskov and Ryle 1990).

Table 6.3. Mean blood parameters of lipid metabolism indicators observed in mule deer during late fall and early spring from 2005 to 2008 on the North Kaibab winter range.

Blood Parameter	Study Year			
Season	1	2	3	Period Mean
Albumin (g/dl)				
Late Fall	2.4 (+0.2)	2.1 (+0.1)	N.A	2.2[a] (+0.2)
Early Spring	2.6 (+0.2)	2.5 (+0.7)	N.A	2.8 [b] (+0.3)
Yearly Mean	2.6(+0.2)	2.3 (+0.4)	N.A	
n=76 EMS=0.864	$P_{(Season)} = 0.0035$	$P_{(Year)} = 0.992$	$P_{(Season: Year)} = 0.243$	
Cholesterol (g/dl)				
Late Fall	6.3 (+0.05)	6.5 (+0.01)	5.5 (+0.07)	6.1[a] (+0.04)
Early Spring	6.4 (+0.06)	5.6 (+0.12)	5.4 (+0.03)	5.8[b] (+0.07)
Yearly Mean	6.4[c] (+0.06)	6.0[d] (+0.07)	5.4[e] (+0.05)	
n = 92 EMS= 0.128	$P_{(Season)} = 8.76e^{-5}$	$P_{(Year)} = 2.31e^{-16}$	$P_{(Season: Year)} = 0.548$	
Triglycerides (mg/dl)				
Late Fall	6.2 (+0.8)	4.8 (+0.6)	5.4 (+1.2)	5.5 (+0.9)
Early Spring	8.4 (+1.0)	5.6 (+0.6)	5.8 (+0.6)	6.6 (+0.7)
Yearly Mean	7.3 (+0.9)	5.2 (+0.6)	5.6 (+0.9)	
n = 92 EMS = 10.63	$P_{(Season)} = 0.2011$	$P_{(Year)} = 0.230$	$P_{(Season: Year)} = 0.753$	
Glycerol (mg/dl)				
Late Fall	7.8 (+1.5)	5.4 (+0.9)	5.5 (+1.2)	6.2 (+1.2)
Early Spring	9.3 (+1.1)	5.6 (+0.6)	5.6 (+0.6)	6.9 (+0.8)
Yearly Mean	8.5[c] (+1.3)	5.5[d] (+0.7)	5.7[d] (+0.9)	
n = 92 EMS = 16.460	$P_{(Season)} = 0.500$	$P_{(Year)} = 0.017$	$P_{(Season: Year)} = 0.852$	
Free Fatty Acids (mg/dl)				
Late Fall	0.8 (+0.1)	1.4 (+0.0)	2.2 (+0.4)	1.5 (+0.1)
Early Spring	1.4 (+0.2)	0.9 (+0.1)	2.9 (+0.5)	1.7 (+0.3)
Yearly Mean	1.1[c] (+0.1)	1.1[c] (+0.1)	2.6[d] (+0.4)	
n = 92 EMS = 1.475	$P_{(Season)} = 0.186$	$P_{(Year)} = 4.25e^{-5}$	$P_{(Season: Year)} = 0.991$	

() Values within ( ) are the standard error of the means.

[ab] Means in columns with the same letters by blood parameter are not significantly different at $P \leq 0.1$.

[cde] Means rows with the same letters by blood parameter are not significantly different at $P \leq 0.1$.

Albumin levels in blood panels can be utilized as an indicator of lipid mobilization in the catabolic process of energy resource delivery to the cells, specifically the mitochondria (McKee and McKee 2009, Voet and Voet 2004). Statistical testing (Appendix C, Table C.12) of blood albumin concentrations did not show a significant

difference between the variances of the seasons, years, or within the interaction of season x years. The means of the Albumin levels were counted for the first two years of the study. Plasma samples for Albumin testing were not available for the third year as a result of inadequate sample substrate. Albumin levels varied from 2.45 mg/dl in the late fall of 2005 (Year 1) to 2.64mg/dl in the late fall of 2006. Early spring values of the albumin ratios were similar in range, from 2.11 mg/dl in the early spring of 2006 to 2.51 mg/dl in the early spring of 2007 (Figure 6.6).

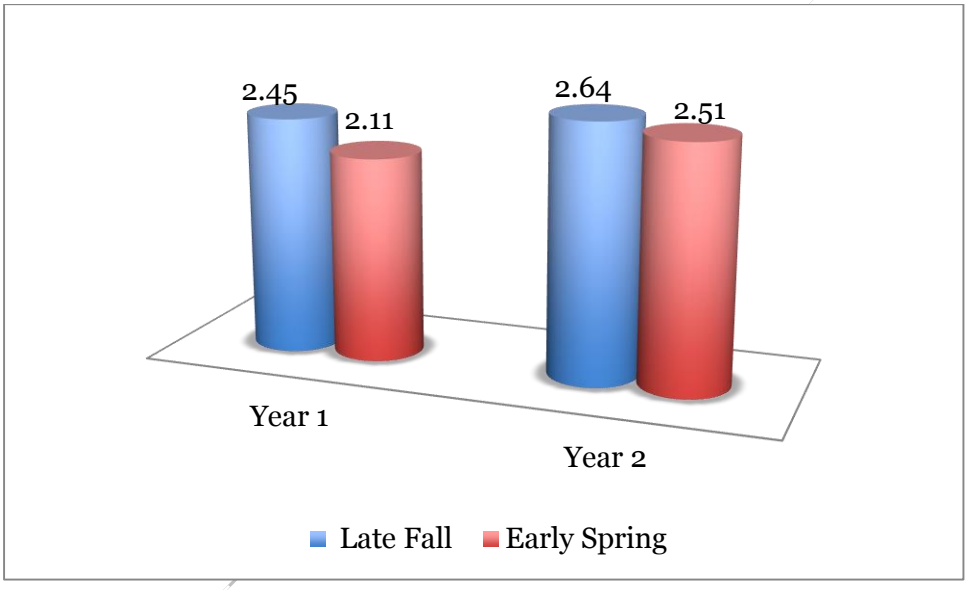


Figure 6.6 Mean blood albumin concentrations (mg/dl) observed in female mule deer during late fall and early spring, 2005-2008 on the North Kaibab winter range

Cholesterol levels in the blood stream can play multiple roles. Cholesterol is a precursor for many important steroid hormones within the endocrine system (Allen 1977). The hormone cholesterol derived from both alpha-keto groups and lipids can be used in multiple roles within a ruminant organism, including the building and repair of nervous tissue (Allen 1977, Klemm 1977). A precursor to progestrogens, estrogens, androgens, glucocorticoids and mineralocorticoids the level of cholesterol and the

changes in those levels over time can affect the corpus luteum, the ovaries, testis, and adrenal cortex of an animal (Beitz 2004). Cholesterol can be a good indicator of the functionality of the endocrine system of an animal and overall herd health because of the diversity of steroid hormones it is a precursor for (Beitz 2004).

Examining the variances of blood cholesterol concentrations (Appendix C, Table C.19) found seasonal differences were significant ($P \leq 1.64$ E-07), yearly differences were also significant ($P \leq$ <2E-16), as was the season x year interaction ($P \leq 7.88$E-12).

The Tukey's HSD comparison of the means between late fall and early spring was significant at $P \leq 2$E-07 (Appendix C, Table C.20). Comparisons between Year One and Year Two are significant ($P \leq 7.8$E-06). Comparison of the means between Year One - Year Three, and, Year Two - Year Three, are significant ($P \leq 0.0$E+00). There was a significant difference in the cholesterol levels of the deer between every year, every season, and every season in every year.

The means for all years and seasons were within 11.3 g/dl for each season and each year of this study. Late fall had an increase in year two of 1.9 g/dl. From 63.1 g/dl in the late fall of year 1 to 65 g/dl, decreasing to 54.7 g/dl in the late fall of year three. Early spring saw a steady decrease in values from 64.2 g/dl (Year 1), 55.9 g/dl (Year 2), and 53.7 g/dl (Year 3). Values decreased from Year one to Year three, with the exception of the elevated level in the late fall of year 2 (Figure 6.7).

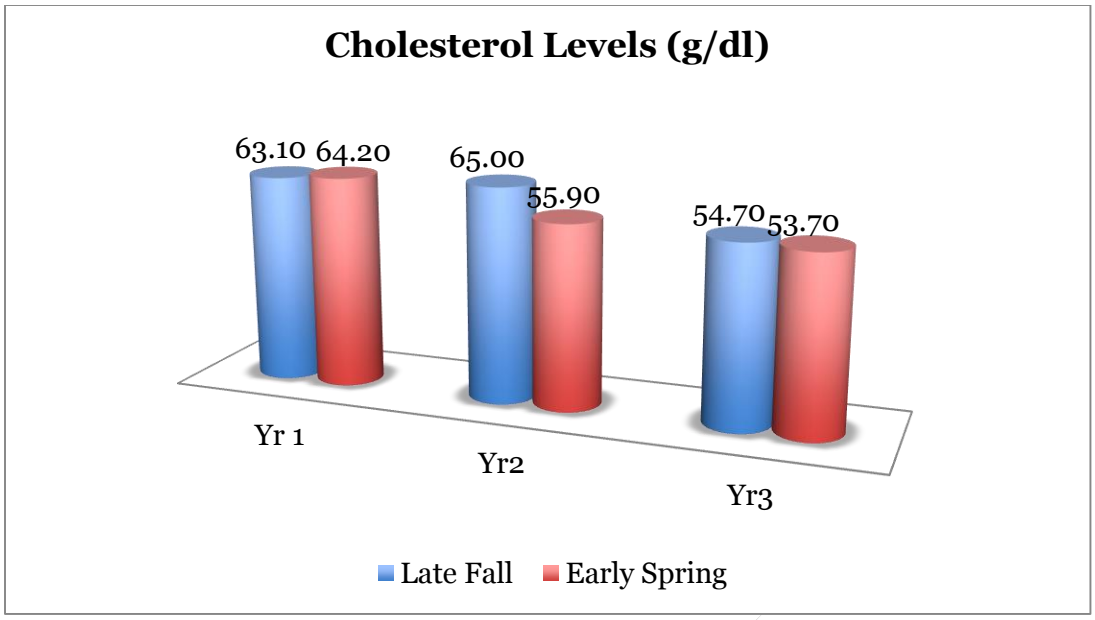


Figure 6.7 Mean plasma cholesterol concentrations observed in female mule deer during late fall and early spring, 2005-2008 on the North Kaibab winter range.

An analysis of the variance (Appendix C, Table C.15) of the triglyceride means (Figure 6.8) showed that the differences between the means between years were statistically significant ($P \leq 0.032$). Seasons were also significant ($P \leq 0.1085$) if the P-value was not rounded and our alpha is extended past 0.10. Tukey's HSD (Appendix C, Table C.16) provides a significant difference between years one and two ($P \leq 0.29$) and years one and three ($P \leq 0.09$). Tukey's HSD also provides that seasons were significant at $P \leq 0.106$ if the same latitude with alpha is extended to the multiple comparison test.

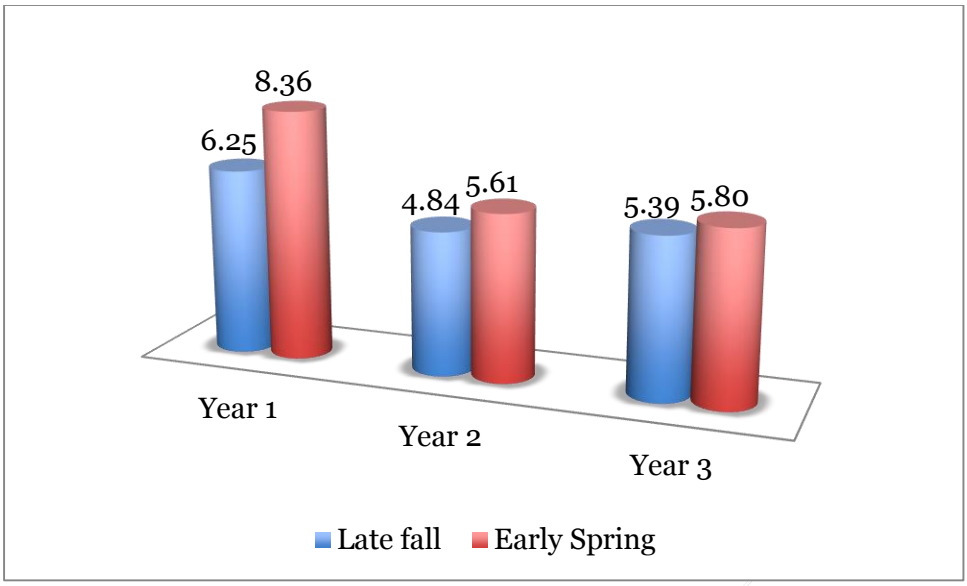


Figure 6.8 Mean blood triglyceride concentrations (mg/dl) observed in female mule deer on the North Kaibab winter range during the late fall and early spring, 2005-2008.

In a ruminant glycerol molecules are formed when either there is an excess of glucose to begin the energy storage process (lipogenesis), or the reserve energy source of adipose has been accessed because of a dietary energy deficit and the complete hydrolysis of triglycerides (beta-oxidation) has been carried out. The resulting glycerol molecule is absorbed into the blood and transported to tissue that possess glycerol kinase (Allen 1977) to form alpha – glycerol phosphate. The α – glycerol phosphate molecule, when needed for energy, is reduced with NADH to dihydroxy-acetone phosphate. Glycerol molecules are the result of the long-chain triglyceride being disassembled; glycerol is the "backbone" holding the three fatty acids in the -triacyl configuration (Allen 1977). The presence of glycerol is a result of the three fatty acids being detached in the process of beta oxidation and becomes a reliable indicator of the level of fat catabolism as "…virtually no monoglycerides are absorbed into the bloodstream from the intestine" (Merchen 1988).

An analysis of the variance (Appendix C, Table C.17) of the mean blood glycerol concentrations (Figure 6.9) showed that the differences between year means were statistically significant ($P \leq 0.04$, $F = 8.748$). Tukey's HSD (Appendix C, Table C.18) demonstrates a significant difference between the glycerol means of years one and two ($P \leq 0.009$) and years one and three ($P \leq 0.013$). Tukey's also provides that Season is significant at $P \leq 0.106$ if the same latitude with alpha is extended to the multiple comparison test.

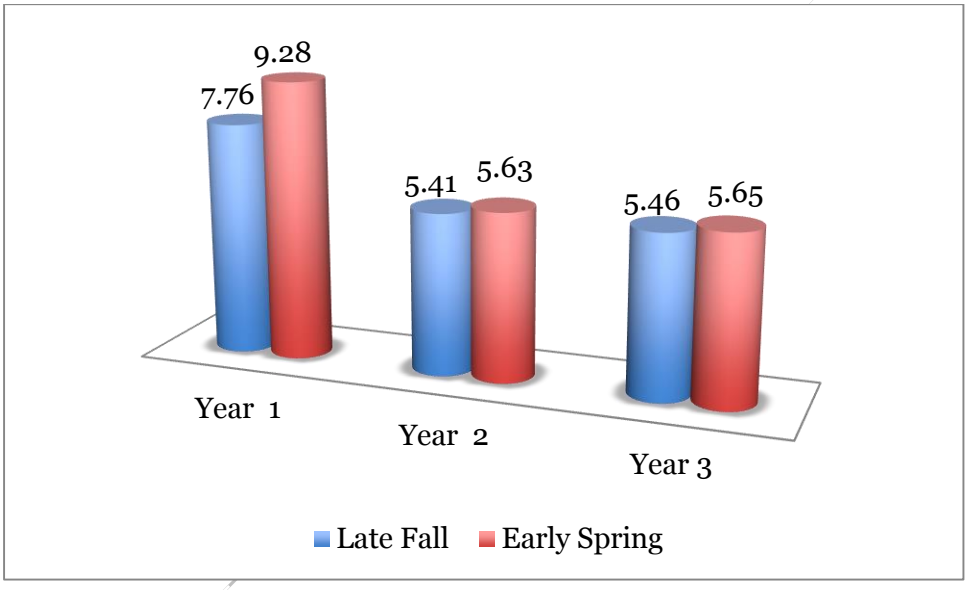


Figure 6.9 Mean blood glycerol concentrations observed in mule deer doe on the North Kaibab winter range during the late fall and early spring, 2005-2008.

Free Fatty (Non-Esterified Fatty Acids, or NEFA's and/or FA's) in the bloodstream are a prime indicator of lipid mobilization and catabolism in the metabolic process of mammals. (Allen 1977, Beitz 2004). Statistical testing (Appendix C, Table C.13) of the variances for Fatty Acids showed a significant difference between the variances of the study years ($P \leq 0.00$, $F=17.93$). Bonferroni's multiple (Appendix C, Table C.14) comparison tests were completed for the variable Year. A statistically

significant difference (P ≤ 3.7e-6) between the means of years one and three of the study (’05-06 vs. ’07-‘08) and years two and three (’06 – ’07 vs ’07-‘08) of the study (P ≤ 6.3e-06) exists.

Free fatty acid levels of the mule deer does on the North Kaibab winter range varied from 0.78 mg/dl in the late fall of 2005 (Year 1) to 2.23 mg/dl in the late fall of 2007. Early spring values of the albumin ratios were ranged from 1.39 mg/dl in the early spring of 2006 to 2.93 mg/dl in the early spring of 2008. Early spring 2007 provided a value of 0.87 mg/dl (Figure 6.10).

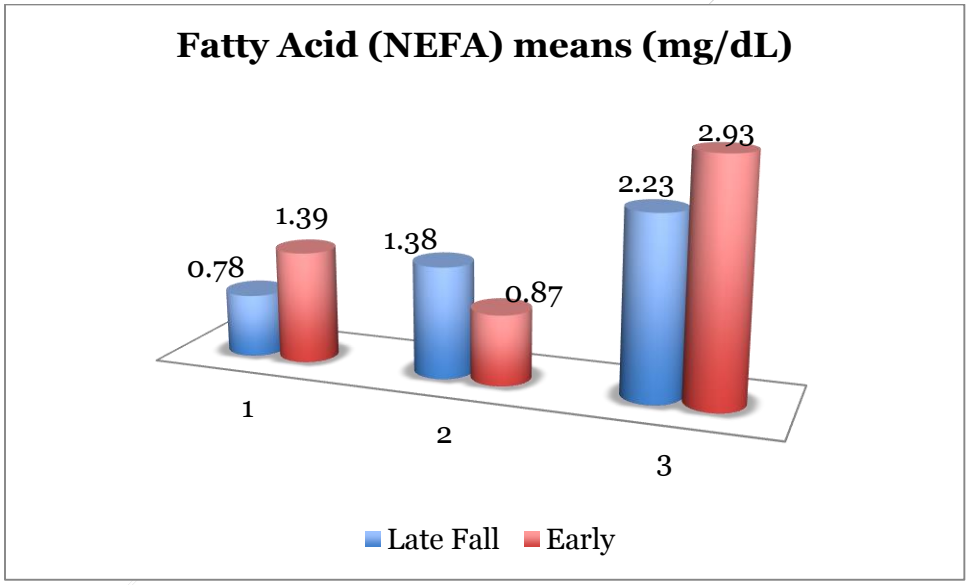


Figure 6.10 Mean blood free fatty acid concentrations observed in mule deer doe on the North Kaibab winter range during late fall and early spring, 2005-2008.

DISCUSSION

Mean levels of blood serum chemistry are an insightful indicator of a herd's health, as the means are composited from the individual animal values (Cunningham 1992).

Carbohydrate Indicators

Phosphates are a carbohydrate intermediary in the glycolytic/glycolysis pathway (Campbell and Reese 2005). Phosphates are an indicator of the carbohydrate resource available to be metabolized into pyruvate and a precursor to the TCA Cycle (Voet and Voet 2004). Phosphate transport is controlled by the plasma phosphate level and excretion of hormones by the parathyroid (Reece 2004). The significant differences reported in the ANOVA and the Tukey's multiple comparison tests between seasons demonstrates the relevance of a declining carbohydrate resource provided by the available forage. The values I found for phosphates in the female Kaibab mule deer herd ranged from 907.5 mg/dl (Year Two, Early Spring) to 925.8 mg/dl (Year One, Late Fall) (Table 6.1). Two values in the early spring and late fall of year three are within 4 mg/dl of each other. Similarly, within the season of late fall the values for all years are descending and consecutively within 5 mg/dl of each other. In the early spring, all values are within 5 mg/dl also, with the lowest value in year 2.

Plasma phosphorous concentrations are normally between 1.2 and .6mmol/L, or 4 and 8 mg/dl (Goff 2004). Smith reported a mean phosphorus value in a similar unit that equivocates to 10.29 mg/dl, and an equivalent range of 6.9 – 14.0 mg /dL). Kie (1983)

reports phosphorous values of 8.2 mg/dl (Nov), 13.7 mg/dl (Jan), and 9.4 mg/dl (March). Using captured deer held in pens and fed a low-energy diet, phosphorus levels ranged from 6 – 12 mg/dl at the Kingsville Station, Texas A&M University (Brown 1995). Rohwer's (1970) findings of all the mature female levels being the lowest phosphorous readings at all locations/groupings suggest a sex-based difference in energy processing versus males.

The phosphate levels in findings by Rhower of 4.6 mg/100 mL (South Ruby) – 6.5 mg/100 mL (Cherry Creek)) do not correspond with those of this Kaibab study. The values of this Kaibab study are elevated when placed in comparison with all other discussed reported values. A conclusion can be reached that there is not an issue in mobilizing Phosphorous from either bodily stores or available forage for the phosphorylation of AMP and ADP within the mule deer physiology. An observation can also be made that the forage resource is laden with readily available phosphorous; the inorganic source of phosphorous may be an area of interest for future study.

Alkaline phosphatase is an enzyme that provides the ability to divest a phosphate group from a molecule and transport the divested phosphorylated sugars (Cunningham 1992). This assists in the formation of other phosphate groups in the glycolytic pathway and ultimately in the formation and regeneration of ATP and ADP from AMP (Harper 1979).

Kie reported alkaline phosphatase levels of 105 units/liter, circa 1976 for pregnant females. Kie (1983) differentiated his reported values for deer inside the exclosure at 35 units/liter (November) and 38 units/liter for January during his mid-1970's Texas white tail study.

Reported values from hunter-collected samples from the Kaibab state an alkaline phosphatase mean level as 53.5 μg /mL and a range of 25 – 123 μg/mL for an equally distributed population of bucks and does (Smith 1976). Smith's reported alkaline phosphatase levels are closer to Brown's extrapolated 1995 reported means of 55 – 70 μg/mL. There is an obvious differentiation between μ g/mL and units of mg/dl. 1000 micrograms to a milligram places Smith's values at an equivalency of 0.053mg/dl, and equivocates Brown's extrapolated findings at 0.055-0.070mg/dl. The levels reported in Table 6.1 are well below these sample means.

Statistical significance was not found in the oak/juniper/mesquite rolling hills and flats of the YO ranch (Lubbock, TX). Alkaline phosphatase values of 62 IU/L (Oct.), 61.7 IU/L (Jan), and 68.2 IU/L (March) were found in 1981-82 (Waid and Warren 1984) in a study similar to the design of the metabolic portion of this study for the North Kaibab.

As enzymatic alkaline phosphatase may be elevated in pregnancy and adolescence (Smith 1976), the reported low alkaline phosphatase values (with the exception of the early spring - year 1 of the study) indicate that virtually no phosphate is being excreted from the deer when there is a low phosphate load (Reece 2004). As most of the does examined and necropsied during sample collection were pregnant (often with twins), higher alkaline phosphatase levels were to be expected. Considering the stressor and trauma induced by being shot, levels were considerably lower than expected for both pregnancy and the level of tissue damage inherent of a bullet (Waid and Warren 1982).

My values for alkaline phosphatase in this study ranged from a low 25.0 mg/dl (Year Two, Early Spring) to a high 51.4mg/dl (Year One, Early Spring) (Table 6.1). The

lack of statistically significant differences between either the seasons or years for alkaline phosphatase implies that the metabolic systems of the deer are consistent in their ability to cleave a phosphate group from a molecule – the physiology of the Kaibab mule deer is intact to continue production of the enzyme throughout the study period. The metabolic process of energy delivery of phosphates to the cells for renewal of ATP and ADP levels is intact and consistent.

The phosphate results for this study suggests a recurring phenomenon of a forage resource that is unable to provide for the energy resource (carbohydrate) needs of the animal. Phosphate levels for the herd from the winter habitat are stable and consistent, as were Waid and Warren's (1982). The phosphate levels are elevated from the values used for comparison. This may be a difference in test sensitivity or methodology (i.e. the use of highly sensitive spectrophotometry) versus the undisclosed methodologies and less refined technology used in comparative studies from the 1970's and 1980's.

In the context of the endocrine system, the parathyroid hormone would not be "… Promoting excretion" (Reece 2004). While abnormal levels can indicate functional impairment of the liver (Cunningham 1992) or a hormonal imbalance, dietary deficiencies are described by plasma phosphorus levels of 2 - 4 mg/dl and lower (Goff 2004). The symptomology for these conditions were not observed in the deer at any time during the three years that this study was conducted. From these blood and plasma results, while the diet is not deficient in phosphorus, it is not necessarily phosphorous-rich either. While enzymatic alkaline phosphatase levels are low in comparison, they are also not deficient and are stable. There is an adequate ability within the herd to

metabolize the available forage. The carbohydrate values demonstrate a diet that has every indication of being energetically deficient.

However, neither is the available forage necessarily providing phosphorous in excess. The animal is mobilizing minimal levels of phosphorous as reflected by the levels of alkaline phosphatase. Tangentially I have to wonder what the level of phosphate resource is in the soil, to be able to impart such a high level of phosphate into the mule deer of the north Kaibab. Placed against comparative values, the phosphate values found in the blood and plasma samples for the north Kaibab mule deer herd were *consistently* ten to one hundred times the expected value. Test procedures, samples and testing results (including calculations) were checked and re-checked in light of this fact. The values are legitimate within the scope of this study and timeframe. What is the mineral composition of the soil that it provides such a phenomenal phosphate resource, and how does it interplay with the dimension and quality of the antlers of the North Kaibab mule deer? I can hypothesize that perhaps the excess phosphate is being used as buffering compounds against unfavorable rumen environments aggravated by the level of mono-terpenes and essential oils vis-à-vis ingestion of sage and Utah juniper.

Protein Indicators

Plasma urea nitrogen (PUN) and creatinine concentrations can be effective to evaluate protein metabolism, as they are proportional to amino acid metabolic activity (Cunningham 1992). The blood pool serves as a major source of amino acids for synthesis of amino compounds and catabolization for fuel, or energy (Beitz 2004). The values from the N. Kaibab winter study can be compared to reported means for PUN of

14.0 mg/dl in deer for all seasons (Seal 1978) and reported means for blood urea nitrogen (BUN) of 13 mg (±7%) (Seal and Erickson 1969). A more accurate comparison for PUN could be attributed to Seals' grouping of pregnant females; 18 %mg (±6 %mg) (Seal and Erickson 1969). Plasma urea nitrogen examines the level of urea in the blood as an indicator of renal function (Guyton and Hall 2006, Harper et al. 1979); ruminants have the ability to recycle their urea for nitrogen and amino acid production and/or catabolism. Serum urea nitrogen is a common, reliable and effective method to gain insight into the status of wild ungulates and the use of available dietary protein in the habitats forage resource (Brown 1995). Waid and Warren (1982) utilized blood urea nitrogen (BUN) to detect seasonal protein changes in the diet of white-tailed deer.

Cunningham (1992) determined that serum urea nitrogen (SUN) could change seasonally with changes in dietary protein levels. In an under-nutrition condition described by DelGuidice (1995), the final source of available energy is endogenous protein present as muscle; an energy source catabolized only if absolutely needed. Mobilization of this source of energy would be expressed as an increased amount of PUN in the bloodstream. Mean levels between the North Kaibab winter habitat study and other studies referenced differ in value and metric (i.e. Seal and Erickson 1969) and can be attributed the high protein value of the protein-rich nitrogen-fixating forage referenced in Chapter IV.

Figure 6.11 demonstrates the pattern of PUN concentration between seasons. In each year PUN concentrations increased from the fall levels (season 1) lows to early spring (season 2) highs, demonstrating annual catabolic activity. The decrease in the levels between Year a (2005 – 2006, or Year 1) and b (2006 – 2007, or Year 2) suggest

that either the summer range, or the winter range upon entering, or both were highly productive in forage. The third year of our study (2007-2008) demonstrates early catabolic activity in the fall and a decrease in catabolic activity in the early spring. Years 1 and 2 (’05-06 and ’06-07) had large depositions of snow in late January or February that would have prevented an early spring budding/green-up.

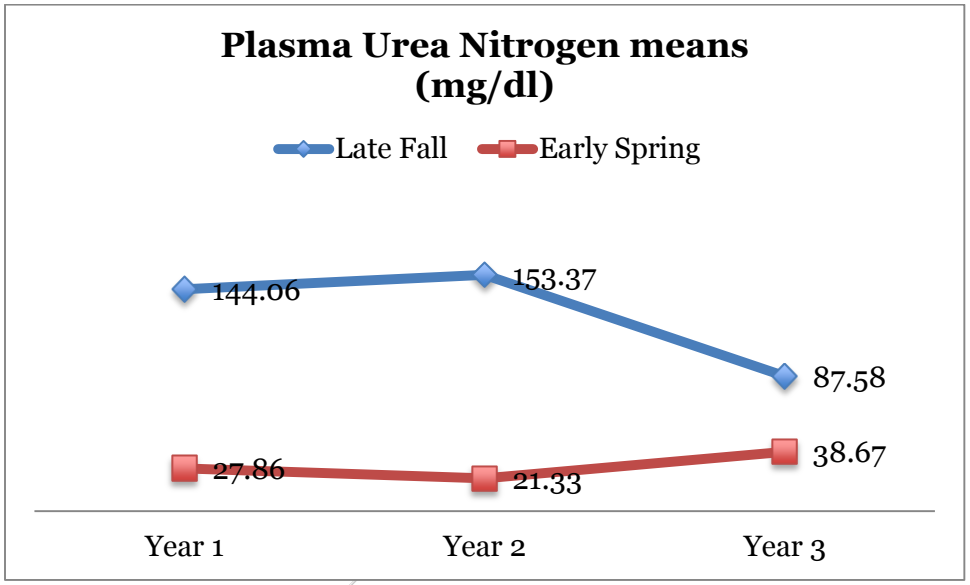


Figure 6.11 Interaction of Plasma Urea Nitrogen values with variables Season and Year. Note the pincer-like movement between LF and ES to center as the study progresses in Year 3. Available plant protein decreased in the late fall of 2007-2008 (13% high protein species), but also increased more in the early spring of 2007-2008 to 20% (high protein species) of the diet, partially explaining the phenomena.

Differences in protein levels can be related to differences in the protein content of available food resources (Seal 1978). The diet content of the deer did not change from season to season in this study. Migration from the higher elevations to the winter range had already occurred prior to the November timeframe for the junior doe-only hunts, providing the two-week window for the turn-over of forage material within the rumen and a full reflection of the winter range diet in the PUN blood panels.

Kirkpatrick (1975) noted that "…the doubling of the protein intake resulted in an approximate doubling of the BUN (Blood Urea Nitrogen aka Plasma Urea Nitrogen) level." Kopf et al. (1984) experienced similar phenomena with free-ranging white-tailed deer measured in Texas, utilizing studies by Kirkpatrick et al. (1975) and Bahnak et al. (1979) to explain the increase of BUN (e.g. PUN) and Morgan (1979) for "…mobilizing endogenous protein for use as energy." Kie et al. (1983) found mean BUN values of 21.1 mg/dl (Nov), 30.1 mg/dl (Jan) and 24.8 mg/dl (Mar) in Texas white-tail, circa 1976. Feeding penned deer a high-protein, low-energy diet Brown et al. (1995) determined SUN levels of 35.4 mg/dl.

These comparative values, while similar to our late fall values in all three study years, diverge greatly from our determined values in the early spring in all three study years. In comparison, Smith (1976) reported mean BUN values (mg/100mL, or mg/dl) of 8.69 for the Kaibab herd with a range of 3-15 mg/100mL in the 1971 hematology study.

The divergent seasonal pattern demonstrated in the above study year x season interaction is a reflection of the large amount of forage protein available as a resource (late fall, blue line) and the catabolic activity within the mule deer to obtain protein from available muscle tissue (early spring, red line) to facilitate necessary metabolic processes in the early spring. The significant difference between seasons in each year tested (confirmed by the multiple comparison, Appendix C, Table C.9) demonstrates that by the early spring the mule deer on the Kaibab winter range are catabolizing not only the protein resource available from the forage and the gut micro-organisms, but also bodily muscle tissue. Catabolism of bodily tissues are being conducted in order to meet their energy requirements for life. Early spring in Year 3 has an increase in mean PUN as a

response to the decrease in mean PUN in late fall of Year 3. Table 5.1 provides mean DPI of 35 g/day in the late fall of year 3 (2007 – 2008) and a MEI, or caloric resource of 1210 kcal/day. The response of the PUN levels indicates that there could be an insufficient level of calories (i.e. needed carbon structures for construction) to facilitate effective amino acid formation (Belasco 1954, Mould & Robbins 1981). If the herd has increased significantly in size since the 1971 Kaibab study reported by Smith (1976), the caloric resource available could be over-taxed so that the caloric resource vis-à-vis available carbon structure is insufficient.

Another source of energy to muscle cells is creatine - phosphate. Creatine - phosphate, typically a readily available energy source reserved in muscle tissue, has a significantly higher energy potential than ATP, and in muscle cells acts as an energy storage mechanism that will quickly transfer its stored chemical energy to adenosine-diphosphate (ADP) for the purpose of reconstituting the ATP concentration and restoring the depleted chemical energy source. This energy resource is typically utilized "… for short periods of fast work …" (Wyss and Kaddurah-Daouk 2000). For a ruminant this can translate into the fight – or –flight reaction that is driven with epinephrine and endorphin by reaction and fear (Harper et. al 1979, Wyss and Kaddurah-Daouk 2000, Miller et al. 2009). The by-product of this process is creatinine, which is transferred from the cell into the blood to be filtered in the kidneys as a waste product. In an unstressed animal, this concentration of creatine-phosphate and creatine are proportional to the muscle mass of the individual (Cunningham 1992), ideally making the amount of available creatine independent of dietary influence and relatively constant in the individual animal.

Ruminant bodily energy reserves are in a dynamic state of flux; carbohydrates, muscle and fat stores are normally accessed and replenished continuously (Orskov and Ryle 1990). Daily excretion of creatine is rarely influenced by ordinary exercise or by urine volume as cellular levels are constantly maintained (Guyton 1976, Allen 1977, Miller et al. 2009). An organism that feeds on a nutritionally balanced diet should not have a large increase in creatinine levels as the resource can be linked to short-duration heavy work by smooth muscle tissue and/or to the release of the hormone epinephrine (Wyss and Kaddurah-Daouk 2000, Voet and Voet 2004). Increases in creatinine concentrations can be a reliable indicator of bodily muscle resource catabolism.

The metabolic resource of creatinine is intended to be a quick fix of energy upon demand. The levels found in this study (Table 6.2) ranged from 0.5 mg/dl (MW, Yr. 2) to 3.1 mg/dl (ES, Yr. 2). Compared to those reported by Cunningham (1992) in Zion National Park of 1.2 mg/dl, the levels for this study indicate a depleted level of muscle mass through all study years and seasons, with the exception of the early spring of year 3 (3.1 mg/dl).

In feeding penned deer a high-protein, low-energy diet Brown et al. (1995) determined creatinine levels of 1.28 mg/dl. The creatinine levels determined for the North Kaibab in this current study are within those reported by Kie et al. (1983) for a high-density population (1.27 mg/dl) versus a low-density population (1.44 mg/dl [Cunningham 1992]). The numbers reported by Cunningham may in fact reflect juvenile fawns – which were purposely excluded from this study of the North Kaibab as we were targeting reproductive females. Specifically for adult white tail outside of the exclosure, Kie et al. (1983) reports circa 1976 creatinine values ranging from 1.76 in November to

1.36 in March. Seal and Erickson (1969) provide creatinine values of 2.1 mg% (± 0.3) for adult female white-tail deer. (Although Seal and Erickson's metric values are difficult to matriculate into my study, numerically they are similar and it is doubtful that the metric is significantly different from mg/dl.)

Accessing this metabolic resource on a regular basis – as the biochemical and statistical analysis indicate – demonstrate that muscle catabolism for an energy resource is taking place seasonally and annually. The interaction of season by year indicates this catabolism is a reoccurring phenomenon that has a potential lasting physiological effect on the organism; one that the deer may not be able to recover from (DelGuidice 1990). Year three of this study amplifies this point; with late fall values of 1.71 and early spring values of 3.09 describing an extreme catabolic use of creatinine.

The results of the creatinine assays provided a significant difference between seasons, years and the interaction of seasons and years. This indicates that the mule deer herd are having to catabolize muscle tissues for energy from the date of entry into the winter range to the date of exit (e.g. late fall to early spring), and are doing so on an annual basis.

The ratio of PUN: C to has been used as a measure of renal function in a wide range of mammals. PUN:C has been suggested as a measure of nutritional status in caribou (*Rangifer tarandus tarandus*, Säkkinen et al. 2001), white-tailed deer (*Odocoileus virginianus*, DelGuidice et al., 1987, 1988, 1989, Wade and Warren 1982, Warren et al., 1982, Page and Underwood 2006). The work of these researchers suggest that a PUN: C ratio $\geq$ 20 appeared when either dietary protein intake is greater than requirement or when energy intake was restricted. Low ratios occur when protein intake

is low and energy intake adequate (Warren et al., 1982, DelGuidice et al., 1987, 1988, 1989, Säkkinen et al. 2001, Page and Underwood, 2006). The PUN: C ratios for my study range from a low of 19.5 mg/dl (ES, Yr. 1) to a high of 397.1 mg/dl (MW, Yr. 2).

The PUN: C ratio showed complex dynamics that were affected by both season and intake of digestible protein in forage. The fact that all of the mean levels of the PUN: C ratios are elevated at or above 20 mg/dl correlate with the forage quality findings presented in Chapter IV. An excess amount of available protein from the forage and an insufficient amount of calories available from the forage provide the elevated mean ratios, especially in the mid-winter of Year 2. The significant differences between the means of the study years indicates the levels found in the late fall and early spring are a recurring phenomenon, while the interaction of season and years combined with the Tukey's HSD results show it is the seasonal differences that are driving the annual re-occurrences of the elevated PUN:C ratios. The significant differences between seasons demonstrate the declining quality of the forage in calories and protein as time progresses from late fall to early spring, inducing catabolism (DelGuidice et. al. 1994). A deeper comprehension of the forage quality on the north Kaibab winter habitat is provided by the variable interaction, that the species composition of the diet can be an explanatory vector for these numbers, validating the calculations from the habitat model of chapter IV and supporting a conclusion that the forage resource is insufficient for the deer herd.

Dominant forages in Del Guidice's 1994 work with bison and under-nutrition included (but was not limited to) "…western sage (*Artemesia tridentata*)", a species of significant proportion in the Kaibab diet and a mix of different grass species, similar in composition to what has been described on the Kaibab winter range in Chapter IV.

Grasses as previously discussed are, as a forage class a high(er) fiber – low(er) concentrate form of forage. Grasses are not ideal for a deer to use as forage as the microbial population of the deer gut as the physiological structure of a grass is not as complex of a carbohydrate as a forb or shrub bite is. Glucose, acetate or propionate are not as generated in large proportions from the simpler, less energetically – dense carbohydrate structure of the grass. More grass by volume and surface area is needed than is a shrub to reach an equal stored or chemical potential energy of carbohydrates. The elevated PUN: C ratio levels in the late fall indicate that the deer are walking into a deficient, dietary - stressed situation upon entering the winter habitat. The annual growth (e.g. terminal bud) of big sage and the annual and perennial forage grasses are beginning to senesce for the fall during the time period of entrance onto the winter range.

The early spring PUN: C ratio indicates that the most expedient way of alleviating the low calorie / high protein situation is to migrate back to the summer habitat as soon as possible, provided the caloric resource can be obtained for locomotion to a higher elevation (Parker 1984). The significant differences between the means of the study years indicates the levels found in the late fall and early spring are a recurrent phenomenon, while the interaction of season and years combined with the Tukey's HSD results help to understand that it is the seasonal differences driving the annual re-occurrences of the elevated PUN: C ratios.

Page and Underwood (2006) found that whitetail fawns, darted at selected sites in the state of New York, had a high level of creatinine in blood samples also had the least amount of body fat. Elevated levels of creatinine confirmed the ocular field estimation (conducted remotely with field glasses) of little to non-existent fat stores left in the

carcasses of the necropsied mule deer doe. White-tail apparently deposit fat even during situations of low digestible energy (Waid and Warren 1982), confirming Verme and Ozoga's 1980 finding that lipogenesis is an "obligatory event". Beitz (2004) and Allen (1977) confirm this phenomenon. Zero rump or omental fat deposits were found on the carcasses of the north Kaibab deer in this study. If there is any excess energy, fat anabolism will occur, which is not the situation on the North Kaibab (Reference Chapter V).

Lipid Indicators

Unlike a mammal with only a simple gastric stomach, in the ruminant the majority of lipogenesis is not conducted in the liver, rather it is conducted at the site of adipose tissue where triglycerides are formed (Allen 1977, Bietz 2004). The major precursors for fatty acid synthesis are found in the VFA's generated by the rumen (Beitz 2004, Church 1988).

As adipose tissue is the primary site where lipogenesis occurs, the majority of triacylglycerol synthesis begins with substrates of acetate and lactate being converted into acetyl CoA and long-chain fatty acids via the citric acid cycle (Beitz 2004). If there is a low demand for energy by the animal and an excess of VFA or substrate remains circulating in the blood, fatty acids can be metabolized from the volatile fatty acid substrate acetate (Allen 1977, Beitz 2004) provided by the bacterial digestion of forage resource in the rumen. Excess fatty acids can then be formed into a triglyceride via the VFA substrates provided by the TCA cycle and shuttled to the cellular cytosol in the adipose tissue (Allen 1977, Beitz 2004).

Lipolysis is actively occurring within the animal at two different places during an under-nutrition condition; 1) to divest any plant resource of its fatty acid components for further degradation into substrates for VFA's – all within the rumen and mid-gut, and 2) within the cytoplasm of the cell. At the site of adipose the intent is to "harvest" the stored energy within the fat cell. Plant-based lipids are hydrolyzed via bacterial and protozoan lipase within the rumen. Little, if any plant-based lipids then enter into the bloodstream. If plant lipids are not fully hydrolyzed, lipase activity from bile and pancreatic secretions in the small intestine provide a means for the ruminant to absorb and hydrolyze any triglycerides that have managed to bypass fermentation in the rumen (Van Soest 1982, Beitz 2004). Lipase activity also initiates beta-oxidation on the triglyceride molecule located in the animal adipose tissue.

Because triglycerides are formed at the site of adipose and are not transported from the liver, triglycerides (triacylglycerol) found in the bloodstream of a ruminant are indicative of beta-oxidation, the enzymatically-driven catabolic activity applied to the adipose tissue in order to withdraw the energy resource from bodily reserves.

During ruminant triacylglycerol catabolism, once the TAG molecule is detached from the adipose tissue, lipolysis will continue to break the bonds of the alpha and beta-carbons with the released fatty acids being used in-situ or transported by an albumin-fatty acid complex to the site of metabolic demand, typically a muscle cell (Allen 1977, Beitz 2004, Church 1988, Van Soest 1982). In a situation where a known caloric deficit exists for the animal, the elevated levels of triglyceride, glycerol, albumin and free fatty acids are evaluated singularly and in combination, the larger the amount of beta-oxidation is occurring in the animal. Triacylglycerol (TAG) levels become an indicator of energy

withdrawal from the bodily reserves induced by a lack of forage-based ingested energy from foraging and grazing.

The statistical tests of the triglyceride (TAG) levels demonstrate that this mobilization was an annual occurrence in the population, with years being significant. The progressive deficit of calories available from forage throughout the seasons provided a significant difference between the triglyceride seasonal means. On the north Kaibab winter habitat, the animal described by the MEI model in Chapter V is consistently catabolizing triacylglycerol reserves for their caloric value (9 kcal/gram) in order to meet the energy deficit left by the forage resource (Allen 1977, Van Soest 1982, Church 1988, Orskov and Ryle 1990, Beitz 2004).

The results of Tukey's HSD applied to the triglyceride level measurements provide that the significant differences are between years one and two and years one and three. The difference between year two and year three isn't large enough to define a significant event. That the variance between the means of year two and year three are similar (insignificant) suggests that the amount of triglyceride mobilized in study year two and year three is at a consistent rate; conditions from year to year were similar enough that a comparative level of fat tissue was mobilized in each year. Caloric forage resource deprivation did not worsen between the two years, however neither was it alleviated. The deer hadn't found a calorically adequate forage to alleviate the under-nutrition condition (Del Guidice et al. 1990, 1991, 1994, Seal et al 1969, 1978, Wallmo 1977, Robbins 1993, Parker 1993).

Glycerol molecules and free fatty acids- the result of a triglyceride having been catabolized by lipolysis and converted into a free fatty acid (FFA) via the lipase enzyme

also demonstrate the degree of TAG catabolism from the site of adipose. The fat cell has been diminished to glycerol (for transport to the liver) and free fatty acids for further beta-oxidation.

Non-esterified fatty acids (i.e. NEFA's, or Free Fatty Acids) are then either converted into the energy substrate acyl – CoA for transport by carnitine transferase into the mitochondria, or further transported through the vascular system to meet energy demands elsewhere in the animal (Allen 1977, Beitz 2004, McKee and McKee 2009). The linkage between accessing triglycerides, conversion into fatty acids and delivery of the energy source to muscle tissue via fatty acid oxidation is adequately demonstrated in a graphic included by Bauchart (1993, Appendix E.2), assisting in comprehension of the statistical analysis.

The low-level increases in the amounts of NEFA's/FFA's and glycerol found between seasons in each year of this study explain the lack of statistical significance found between seasons. This correlates with results from Warren et al. (1982). Yet, this also indicates that fat catabolism may be occurring as deer migrate downward from higher elevations into the winter range in late October – mid November (late fall) and the calorically-deficit forage is encountered and utilized.

Decreasing dietary energy as the seasons changed from late fall to mid-winter into early spring places reliance for meeting daily energy demands on the endogenous bodily fat stores (Seal 1978). The statistical difference between the years indicates reoccurrence of the catabolism and the inability of the range to consistently deliver energy-rich forage. This could potentially be evidence of the long-term effects of a continuous drought the Southwest has been experiencing. Year three had very high metabolic demands placed

on the fat resources. Further, Verme and Ozoga (1980) when studying dietary effects on growth and lipogenesis in fawns found evidence that "… lipogenesis comprises an obligatory physiological event in autumn and proceeds despite under-nutrition until a serious negative energy balance occurs". Verme and Ozoga (1980) also state that Seal (1978) found elevated NEFA's (FFA's) among fawns receiving inadequate forage energy in the autumn.

If the energy balance is negative with the forage resource, the reduction of lipogenesis in relation to the acceleration of beta-oxidation can indicate a severe under-nutrition or starvation condition. This under-nutrition condition can have immediate effect, especially on a younger deer. With bodily fat stores not being able to be maintained or renewed as the seasons advance into the min-winter months, concerns for maintaining a body core temperature and survival arise as additional homoeothermy is added to the energy demands of the ruminant (Parker et al. 1984, 1999). If the forage resource (vis-à-vis the interior rumen temperature for fermentation) is heavily laden with big sage, (Rassmussen 1941, Nagy 1964, Russo 1964, Ngugi et al. 1995) thus decreasing or ceasing rumen activity, and insulation from fat reserves has also been exhausted, catabolism of body mass (protein) will occur next. A low or non-existent fat reserve plus a low energy forage and a younger deer without a significant body mass of muscle can easily be an equation that leads to death for the animal.

The method of transport for the FFA's and glycerol through the bloodstream to the mitochondria of the cells where energy is needed, is via albumin. Albumin is a protein utilized for fatty acid transport to the liver and a key indicator of the lipid-energy transport mechanism (McKee and McKee 2009, Voet and Voet 2004, Beitz 2004).

Albumin concentrations also reflect liver and kidney functions. Low levels can indicate liver disease or malnutrition (Cunningham 1992). Warren (1982) found that white-tail fawns that were fed a low energy diet exhibited greater serum albumin concentrations than those fed high energy diets, and goes on to explain that conflicting information in the literature can confound the interpretation of albumin results. In years one and two, the levels of albumin were relatively constant, and did not demonstrate any statistically significant differences between the means. This same condition applied to Waid and Warren's 1982 work, where albumin was used more as a protein indicator, rather than as an indicator of fat mobilization.

Page and Underwood (2006) cite Sam's 1998 study where "… albumin levels in deer increased as population densities were reduced and protein status improved." Smith (1971) provides an albumin "average" for female deer (doe) of 2.89 g/100mL (g/dl) (n=7) on the Kaibab. Smith also provides other values from other locations in the state of Arizona.

Three Bar game reserve, located on the northwestern slopes of the 4 Peaks mountain adjacent to Roosevelt Lake, provides an albumin level of 2.982 g/100mL for mule deer does (n=4). The Santa Rita Mountains are approximately 65 miles south east of Tucson, AZ (U.S.F.S. 2013). Smith provides albumin values for mule deer bucks (n=10) of 2.560 g/100 mL. The season of the year for Smith's studies is the fall and collection procedures were left to the discretion of hunter in the field. Smith reports white tail values for Arizona at 2.969 g/100 mL (1971) and 3.51 g/100 mL (1972).

Kie (1983) provides albumin ranging of 2.65 g/dl (Nov), 2.45 g/dl (Jan), and 3.45 g/dl (Mar) from his circa 1976 Texas white tail deer studies. Del Guidice (1987) reports

albumin levels for deer that have been fasting for four weeks at 3.8 g/dl in a controlled feeding trial. Cunningham reports a mean serum albumin level for the Zion Canyon mule deer of 3.0 g/dl (1992). Rowher, in a study on mule deer in Nevada's Ruby Mountains, provided albumin values ranging from 3.98 – 4.40 g/100mL. At the time of the study (late 1960's), the significant differences between the groups of his findings were attributable to "…possible lower planes of nutrition…" at the four sites sampled. Rohwer's sites were primarily defined by altitude at the study location.

The values for this study are comparable to Smith's 1971 findings. Albumin levels for the north Kaibab mule deer herd during this study period of 2005 – 2008 did not differ; fat transport was occurring in years one and two at a consistent rate from the beginning of the late fall period to the end of the early spring season. Unlike the situation described by Sams (1998), the north Kaibab population densities were not decreasing (Wakeling 2005, 2010) and the condition of the forage protein resource was stable and not in flexion throughout the scope of the study.

Rohwer's rationale of a lower plane of nutrition can be discarded for the context of this study; the Kaibab mule deer have a more than adequate level of accessible nitrogen. An energy deficiency of carbohydrate exists. The albumin levels tell us that the energy transport mechanism was working on a comparable level with other mule deer herds in the state. That albumin values were within the range of other sampled mule deer herds in Arizona indicates that once the triglyceride was metabolized via beta-oxidation the fatty acid was used in the TCA cycle at the cellular level as an energy substrate, not needing to be transported to the liver for conversion into glucose (Allen 1977, Van Soest 1982, Byers 1988, Ferrell 1988, Bauchart 1993, Beitz 2004). "When a carbohydrate

deficiency exists, hormone sensitive lipase activity continues, resulting in increased amounts of NEFAs being transported to the liver *and other tissues* (emphasis added)… (Beitz 2004). While most released fatty acids are transported in large part to the liver as albumin–NEFA complexes, the long-chain free fatty acid that may remain in the outer mitochondrial membrane is converted to acyl CoA by a thiokinase (dependent upon the length of the carbon chain), which is further reduced via oxidation and thiolysis via CoA to produce acetyl CoA. At that point, the oxidized fatty acid now converted and reduced to acetyl CoA may be directly used by the tissue in need via the citric acid cycle (Allen 1977). The process of beta oxidation may be repeated ad infinitum until the original even-carbon fatty acid has been completely degraded to acetyl CoA (Allen 1977). Albumin levels were fairly even and constant during the timeframe of the study. Statistically albumin was not significant, indicating a consistent source of beta oxidation and transport of NEFA's was in place. NEFA levels fluxuate and reached a high during the third year of the study.

The metabolic demand of the female deer dictated that the albumin-NEFA complex be shunted away from the liver and towards immediate cellular need. I suggest that the capability of the animal to reduce long chain fatty acids in the mitochondrial space (away from the liver) into acetyl-CoA via beta oxidation, thiokinase and thiolysis was active and providing needed energy to the cells. The utilization of this metabolic pathway, bypassing the liver, indicates a significant under nutrition condition.

The deer herd may have been catabolizing fat constantly from the beginning of its entry onto the winter habitat until it left for the return to summer habitat. The results from this study can be considered to be in the range of the other studies. Albumin results,

while comparable, are inconclusive as indicating the degree of fat catabolization yet we know from ocular estimates and measurements that the fat reserves of the deer in the early spring collections were decimated or non-existent.

It is proposed that, in the Southwest and within the biological and ecological scope of this study and site conditions, the albumin indicators for a population of ruminants in an under-nutrition situation where fat stores are being catabolized do not move upward, indicating increased activity. It is suggested that the albumin indicators for ruminants experiencing an under-nutrition condition where the forage resource does not meet their metabolic demands decrease in relation to the other metabolic indicators. Albumin levels would decrease if there is no longer a large amount of glycerol or non-esterified fatty acid (NEFA/FFA) to mobilize to the liver. This would be indicative of a fat resource that is either not being utilized, or has been exhausted and there is nothing left of the adipose tissue to metabolize.

Cholesterol

Serum cholesterol has been examined as a potential indicator of nutritional condition for mule deer in the past (Smith 1976, Warren 1981, Waid and Warren 1982, Del Guidice et al. 1987, Cunningham 1992). Del Guidice and others found (1987, 1990, and 1991) while conducting feeding and blood plasma trials with deer that mean cholesterol levels increased with 4 weeks of fasting. Cholesterol levels returned when normal feeding was resumed (Cunningham 1992).

Seal et al. (1972) noted "... greater cholesterol concentrations in fawns fed LP (low protein) diets than HP (high protein) diets. Volgelsang (1977) reported lower cholesterol concentrations in does subjected to a 50% energy restriction during a 12 week study. Cholesterol concentrations generally declined during the first 16 weeks and were higher at week 32. Seal et.al (1972) also noted seasonal elevations in cholesterol concentrations of pregnant does in April when compared to December or March. Waid and Warren reported values of 75.7 mg/dl (Oct), and 58.9 mg/dL (Jan and Mar) in their 1982 work on the YO ranch, Texas.

Smith (1976) reports 1971 mean values for females on the Kaibab at 85.5 mg/100 mL (n=7), a mean of 100.6 mg/100 mL (n=4) for the Three Bar Game Reserve (Four Peaks Mtn.), Az., and a mean of 88.40 mg/100 mL (n=10) for mule deer (sex unknown) in the Santa Rita mountains south-east of Tucson. Smith continues with 1971 mean whitetail cholesterol results from unknown locations in the state of Arizona of 120.8 mg/100 mL for bucks (n=13), and 1972 white tail results for bucks (n = 22) of 80.3mg/100 mL.

By comparison, the values demonstrated in Table 6-3, Figure 6-10 and 6-12 are lower. The lower cholesterol amount in this study however corresponds with what is considered to be a "normal" value by Del Guidice of 40 to 70 mg/dl (Cunningham 1992).

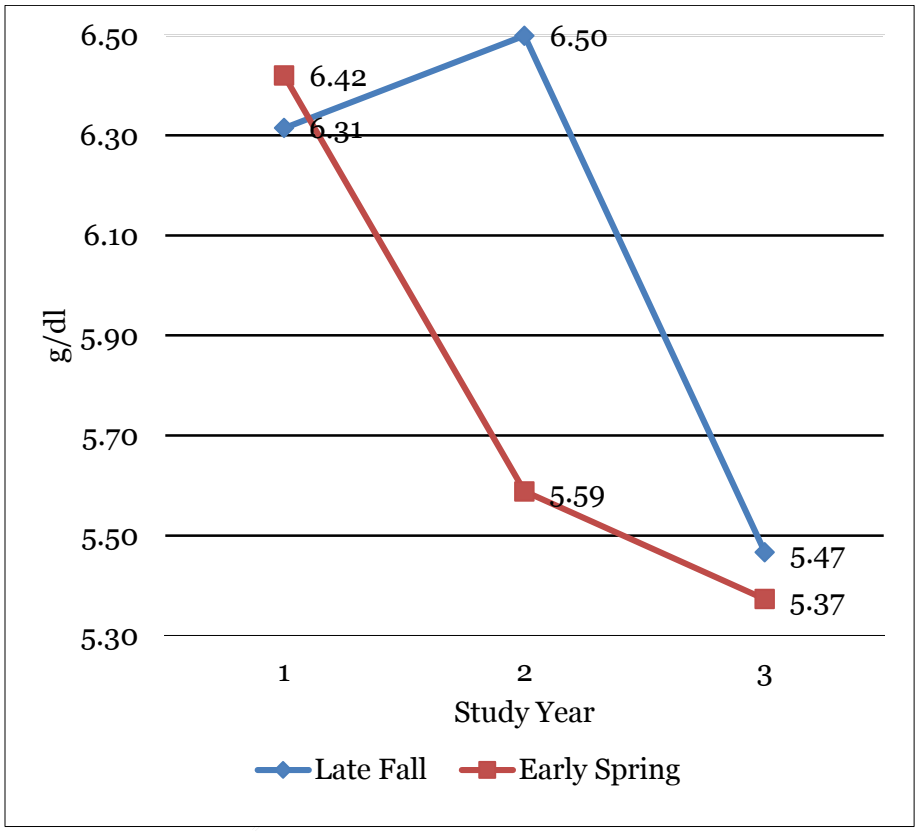


Figure 6.12 Cholesterol hormone seasonal interaction in the designated study year. Data point units are mg/dl. Note the disparity between LF and ES of Year 2; the visual effects of the pre- and post- mid-winter diet consisting primarily of Utah juniper (Chapter IV).

Our values align with Waid and Warren's (1982), are higher than Cunningham's 43.5 mg/dl (1992), and Smith's 1976 cholesterol values were obtained while looking for vectors of disease and infection among deer herds in the state of Arizona. "Cholesterol is of little diagnostic value as it exhibits only a secondary response to disease. … This factor responds to diet, stress, anemia and starvation. It is noteworthy that these values

for deer are lower than those for most domestic animals" (Smith 1976). The value of Smith's work is that it is within the ecological biome of our North Kaibab study. Smith's "Kaibab" is not defined as to whether or not it is on the North rim (this study site) at the altitude of this study, above the altitude and in the summer range of this study, or on the Kaibab National Forest on the South Rim. Next to Cunningham's reported values of Zion National Park, the deer sampled by Smith from a location that provides the most similar diet to the diet used by the deer on the North Kaibab, provide insight into the ability of the North Kaibab herd to synthesize a basic corticosteroid needed for primary biochemical synthesis (i.e. "life").

The statistical significance of the analysis of variance and multiple comparison tests highlight the diminished amount of mean plasma cholesterol in the early spring of all years. The large discrepancy between the late fall and early spring of year two demonstrate the inhibited ability of the mule deer to synthesize a basic hormone in the early spring because of a decrease in available forage resource and (most likely) resulting acetyl CoA.

The fact that cholesterol levels were consistent in Year 3 may indicate a correlation to precipitation cycles. Although a large precipitation event occurred in the mid-winter/early spring of year two (2006-2007), the precipitation record (Figure 1-C and 1-D) show an annual precipitation shortfall. This precipitation shortfall suggests that overall forage growth on the north Kaibab winter *and summer* ranges may have been stunted, allowing the deer to enter into the winter range in year 3 of the study (2007-2008) while in an existing state of under-nutrition. As evident from the precipitation profile, the mule deer may not have been able to fully recover from the harsh winter of

year two where forage was limited to low-quality and digestion-inhibiting juniper and piñon, as suggested by the low level of cholesterol in year 3.

Cholesterol, NEFA/Free Fatty Acid, and TAG levels, along with Creatinine and PUN levels, along with alkaline phosphatase levels are all higher in late fall and early spring of year three. I suggest that not only are the mule deer responding to the lack of available energy in the forage resource on an annual basis, but specifically responding to the lack of significant summertime precipitation in the summer of 2006-2007.

CONCLUSIONS

As all indicators demonstrate, including examination of field dressed carcasses of multiple doe, beta-oxidation and mobilization of energy stored in fat is occurring; a conclusion that a significant under-nutrition condition is in place from the late fall to early spring on the north Kaibab winter range is warranted. This is unusual because an assumption of the public (hunters or recreationists) is that deer fatten up in the fall on the mast crop and summer's additions of photosynthetic forages (browse, fruits, nuts and forbs, etc.). The lipid panel blood work is demonstrating that this does not happen on the north Kaibab. As soon as the deer are entering that habitat, their fat resources are being taxed.

Physiological changes of pregnancy can alter and affect blood values (Kie 1983), if the conception rate is 180%, then the blood values of a herd that is seemingly prolific should be analyzed on the most metabolically - stressed condition of pregnancy; parturition and lactation. The scope of this study covers the blood values during conception and pregnancy leading up to parturition. While not timed during parturition

and lactation, this study provides the most complete view into the window of metabolic exchanges for the reproducing north Kaibab mule deer to date.

Although the albumin levels are within the range of other studies and do not appear to be elevated, we know from the measurement of the elevated fat catabolism by the triglycerides, the free fatty acids and the glycerol levels that lipid catabolism is occurring. Cunningham's 1992 reported levels for Zion Canyon, UT were done in a known condition of under-nutrition. Smith's 1976 values (circa 1971) for the Kaibab were done during the fall. Smith's 1971 work on the Three Bar Game reserve notes a problem with fawn survival; it is unknown if this is from suspected disease or possibly from a poor forage resource (Smith 1976). The values are from the same species of mule deer and very close to the longitude, latitude and altitude of the Kaibab study site.

Smith's and Cunningham's reported levels for the "Kaibab" (Smith 1976) provide the most direct comparison for a metabolic response to the habitat that is available. The levels reported here and compared to other metabolic panel levels reported from other researchers in the Western U.S. sets a baseline level for albumin as a marker for elevated levels of albumin in a deer. These levels, when taken in total with the other blood and plasma indicators, provide definition to albumin levels of mule deer during an under-nutrition condition and act as an indicator of nutritional stress in a deer.

Likewise, the cholesterol levels and protein levels provide baseline amounts for the deer of the north Kaibab winter habitat in an under-nutrition condition. These are within the stated norms utilized by Del Guidice and other researchers. The condition of under-nutrition for the Kaibab mule deer has been defined by the dietary work provided in Chapters IV and V.

Protein profiles of PUN, Creatinine, PUN: C ratio, and albumin (if albumin is utilized as a protein; we did not for our interpretation) provide a useable protein profile for the deer of the north Kaibab winter habitat in an under-nutrition condition. Perhaps most interesting is the metabolic response of these indicators is to a diet exceedingly rich in plant protein.

Incidental to all prior conclusions; when the deer were culled and the carcasses processed at the Ryan Station, post mortem examinations were conducted. No omental fat was noted in any carcass in 2008 and 2007. Kidney fat was minimal to non-existent. Rump fat was minimal to non-existent, with emphasis on non-existent. The layer of fat important to maintenance of homoeothermic within a young ruminant animal (Beitz 2004, Cannon and Nedergaard 2004, Saladin 2009) had been metabolized and was visibly absent. This is a significant visible extent of the fat metabolism seen within the animals of the herd.

CHAPTER VII

MODELING

INTRODUCTION

Historical examination of previous ecological work on the north Kaibab plateau is an effective, thorough descriptive work on the flora and fauna of the entire plateau. Little if any modeling was done in the early years of research. More recent works by the Az. Game and Fish Department (circa mid-1970's-late 1990's) provide more technical, science-oriented results of specific aspects of the north Kaibab (i.e. weather effects, available water, etc.) and typically focus on the entire plateau as a whole. The scope of this study has been confined to the winter range as defined in the Site Description, and the interaction of the mule deer with the forage provided on the winter range and the quality of that forage.

A model of these interactions is made using the derived data from the diet quality (protein and energy factors) and the metabolic indicators. A principal components analysis was generated using this derived data set. Recognizing the potential non-orthogonal approach that correlated nutrition, blood and plasma measures could present to the variances of the data, a PROMAX rotation was applied to the data. A simple, additive linear regression was derived from the components using a stepwise process to statistically and mathematically describe the interaction of the mule deer with the nutritional plane of their habitat. The eigen-values of the components provide an indication of the direction and magnitude of the effect on the variances of the data set.

METHODS

Metabolic and Nutritional Modeling

Data collected from the individual metabolic assays and the forage quality indicators of Dietary Protein Intake (DPI) and Metabolizable Energy Intake (MEI) used in the previously discussed ANOVA's were assembled into a data frame. This data frame was the basis for a correlation matrix (Appendix D Table D.1), a Principal Component Analysis (Appendix D Table D.2) and a linear regression model applied to the components derived from the Principal Components Analysis via the statistical program R (Appendix D Table D.3). These three sets of tabular data were compiled to model the response of the mule deer to their dietary inputs and habitat conditions. Selection of metabolic and nutritional components compositing the PCA was accomplished by first applying a non-orthogonal promax rotation to the PCA analysis in response to the correlation of metabolic processes evident in Appendix D, Table D.1. As the data are measurements of the simultaneous metabolic processes of a natural, living organism and life itself is a dynamic continuum of change, the promax rotation was intended to permit any inherent, inseparable correlation within the variables that compose the data set to apply. Allowing for the dynamic state of life by including and permitting correlation in the results was felt to be highly appropriate.

Derived components were excluded after approximately 70% (rounded) of the variance had been explained (Joliffe 2002, Hair et al. 2010, Everett and Hothorn 2011). Eigen-values and scree-plots were utilized to discriminate relative importance of the factors (Hair et al. 2010). Five factors, at or greater explaining approximately 70% of the variance were utilized for fitting the linear model.

The scores of the five components were then ex-generated into another data frame to be used with R. Using the -lm function a linear model was set up using the five components. The –step function provided a step-wise regression with an accompanying AIC-indictor score. The resulting four-predictor (i.e. component) model provided the fit necessary for the model when residuals and communalities were examined. Utilizing the principles of parsimony and Occam's razor pervasive in statistical analysis this model provides an explanatory insight into the metabolic response of the Kaibab mule deer herd within the three dimensions suggested by Robbins (1973), and presents a possibility of a presence of a conceptual fourth dimension of time passage to the equation. (Steele et al. 1997, McGarigal et. al 2000, Everitt and Hothorn 2001, Joliffe 2002, McCune and Grace 2002, Abdi 2007, Crawley 2007, Hair et al. 2010, Zar 2010);

RESULTS

Original variable data were transformed prior to analysis using log(x+1) to equalize the multiplicative variances (Zar 2010). The Principal Components Analysis (Appendix D, Table D.2), having had a promax rotation applied, was delivered using the –princomp command of R ver. 3.0.2 (Crawley 2007). A Scree-plot of the variances based on eigen-values was returned, forming a heuristic "broken stick" chart to assist in determining the number of applicable components (Figure 7-1) (McGarigal et al. 2000, McCune & Grace 2002). Cumulative explained variance and sum of square loadings in the context of a natural system were also considered before the five components were chosen.

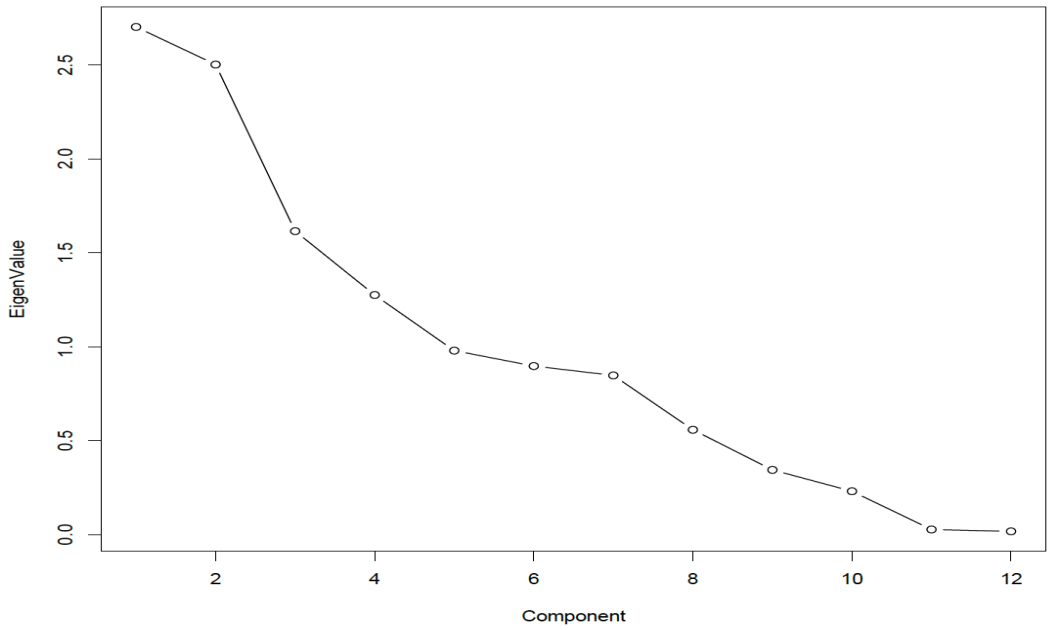


Figure 7.1 Linear scree-plot of variances of the metabolic and forage quality analysis factors the north Kaibab winter range.

The scree-plot indicates a perceptible inflection point at the fifth component, indicating a change in eigen-values (vis-à-vis variances less the component). Appendix D, Table D.3 demonstrates the variance explained by five components. The first five components derived from the PCA analysis explain up to 70% of the variance for the eigen-value loadings of the Kaibab study.

Utilizing the component scores from the promax-rotated PCA analysis (Hair et al. 2010, Everitt and Hothorn 2001), an exploratory factor analysis seeking a descriptive model of how the mule deer's metabolism interacts with its forage and other habitat resources was created.

The five components were subjected to a step-wise linear regression via R. Principle component two was excluded and removed. The resulting model is:

$$Y = PC4 + PC5 + PC1 + PC3;$$

where Y = the metabolic response, or status, of the Kaibab mule deer to their forage and habitat during the time frame of this study (Late Fall 2005 – Early Spring 2008).

The model specifically is expressed as:

$$Y = PC4 + PC5 + PC1 + PC3; \text{ (Table 7.1)}$$

Table 7.1 Principle component results from backward stepwise regression using Akakie's Information Criterion (AIC) to assist in deriving the model. Variances of the predictive variables are listed below.

	PC Four	PC Five	PC One	PC Three
AlkP	-0.23	-0.21	0.03	-0.36
Phos	0.13	-0.29	0.09	-0.56
Alb	0.02	0.91	0	-0.11
Chol	0.12	0.09	-0.5	-0.48
FA	-0.16	0.21	0.54	-0.01
Gly	0.07	0	-0.06	0.14
TAG	0.08	0.08	0	0.18
C	0.13	-0.07	0.96	0.14
PUN	0.03	-0.21	0.17	0.79
PUN_C	-0.04	-0.13	-0.76	0.63
DDP	0.92	-0.11	0.18	-0.08
DME	0.9	0.13	-0.03	0.03

$P \geq 3.3E\text{-}29$, $R^2 \sim 83\%$. $a_{incept} = -3.460E\text{-}14$

Where the components (Eigen vectors expressed) equate to variances of the sum of each of the four PCA outputs (Table 7.1). The intercept, in the interest of parsimony, was deleted from the equation because of the small, insignificant size of the number. The intercept ($a_{incept} = -3.460E\text{-}14$) has become meaningless and irrelevant, and as such is not stated within the equation.

DISCUSSIONS

When examining the model, to suggest an application of the dimension of time to the model by scrutinizing the quantity of the individual eigen-values and the contributions to the variances of the eigen-vectors would be erroneous. The eigen-value is a description of the portion of the total variance corresponding to the eigen-vector and the dimension, or principal component (McCune and Grace 2002). As the eigen-vectors form a mathematical matrix, the eigen-values of the eigen-vectors represent the contribution of each variable to the axis (principal component) and as such, after the application of a promax rotation, the eigen-vectors/principal components theoretically do not have any (i.e. zero) correlation with each other (McCune and Grace 2002). While mathematically this is applicable, one can also examine the components and the eigen-values that construct them in the context of a metabolic sequence or nutritional resource supply/demand response. A researcher could then hypothesize and deduce the environmental and habitat conditions that would (or did) exist within the scope of the study and elicit a mathematical response of the variances of the means as demonstrated by the eigen-values of the variables within the individual principle components.

With the promax rotation applied to allow for systemic physiological correlation, Principal Component Four takes on the most important, significant loadings in the linear model and reflects the forage resources available. Component 4 demonstrates an equal, initial loading of 0.9 and 0.92 eigen-values in the factors of MEI and DPI.
The eigen-values reflect an animal that is receiving an adequate level of nutrition upon entering the winter range. In response to these eigen-vectors of ingestible nutrition, the eigen-values of the enzyme of Alkaline phosphatase are decreasing; phosphates are

present and adding energy in the form of ATP to the muscle tissues, Albumin is slightly active but not elevated (o.02) as a carrier protein in the blood stream. Cholesterol is present and being formulated as a base -sterol for other hormones (0.12) (Martin 1985) in the background, demonstrated by an eigenvalue of 12/100ths or 12 percent. Fatty acids are in decline and not actively transported (eigen-value -0.16). Glycerol and Triglycerides are present and show a low portion of the variance (0.07 and 0.08), demonstrating a low amount of deposition (perhaps in response to maintenance activities (Saladin 2007)). Muscle tissue is intact and under anabolic/catabolic maintenance, not being mobilized for energy. The high protein levels endemic of the forage of the North Kaibab (see Chapter II) is evident in the slightly depressed eigenvalue of the PUN_C ratio (-0.04). Slightly elevated PUN eigen-values (0.03) and the elevated Creatinine eigen-value of 0.13 describe a very small portion of the variance. This could be indicative of the digestion of heavily nitrogen-laden mast (Gambel's oak) and shrubs such as Mexican cliffrose and Apache plume while mobility is not constrained by deep winter snows - yet.

Principal Component Five demonstrates an eigenvalue for the carrier protein Albumin (Alb, 0.91). This large proportion of the variance could demonstrate catabolic activity on the adipose tissue and utilization of phosphates. Protein metabolic variance indicators have shifted, perhaps demonstrating movement away from the easily obtained forages of Mexican cliff rose and Apache plume. Increases in the amount of variances explained by the eigen-values of triglycerides (TAG 0.08) and Fatty Acids (FA 0.212) are evident in principal component five. It is suggested that this proportion of the variance may demonstrate catabolic activity on the adipose tissue in conjunction with Albumin.

Decreases in PUN (-0.21), Creatinine (-0.07), and the PUN: C ratio (-0.13) eigen-values combined with decreased eigen-values for AlkP (-0.21) and Phosphates (-0.29) lend understanding to the shift in variances that would follow if the immediately available muscular energy source of creatine is being heavily utilized. This shift in variance is understandable when the difficulty of restoring these energy sources within muscle tissues when attempting to use much needed but absent phosphate groups is realized. Eigen-vector values of 0.13 for MEI, and a negative influence from the eigen-vectors for DPI (-0.11) describe a shift in dietary variance as the mast, grasses and fruits of shrubs available on the autumnal habitat of the winter range changes to one that is perhaps more senescent and available in winter. Utilization of the immediate forage resource does not meet the dietary needs for long-term seasonal protein maintenance and or caloric demands.

Principal Component one and Principal Component three are factors of the equation that load the distribution of variance on protein catabolism and utilization. Where PC One loads on creatinine, PC Three loads on PUN and the PUN: C ratio. PC One presents eigen-values for creatinine (0.96) and PUN (0.17) that have increased and eigen-values for the corresponding PUN: C ratio has decreased (-0.76), demonstrating the PUN: C ratio has moved closer to 1:1. The component PC1 of the linear regression equation is loading the variances on muscle catabolism and the urea-nitrogen recycling occurring within the animal, as the negative MEI variance loading and decreased loading of DDP (0.18) indicates a lack of ingestion of quality forage.

Protein catabolism is occurring because the available forage is not meeting their basic metabolic needs, any available protein forage resource is being catabolized for

energy. A lack of eigen-values for TAGs and depressed eigen-values for Glycerol and Cholesterol but increased eigen-values for fatty acids suggest a lack of mono-, di-, and tri-glycerides to metabolize further, while the increase in the eigen-values for Fatty Acids (0.54) indicate a near-completion of the beta-oxidation of available fat resources – at least as described by the indicators of variances. Albumin has dropped also, to a zero level – no net effect by Albumin on the eigen-values in this principal component. There is a positive effect by the variables Alkaline Phosphatase (0.03) and Phosphorous (0.09), on the principal component 1, indicating that the immediately available energy stores in smooth muscle tissue have not been fully utilized yet.

The final component of the equation, Principal Component 3, provides a minimal effect by MEI on the variance with eigen-values reaching 0.03. Dietary protein is demonstrating a small negative influence on the variance of -0.08 eigen-values on the component – to reiterate, ingestion of available forage is not meeting their metabolic needs. The protein metabolic markers contain values for 0.63 eigen-values (PUN-C ratio), PUN with 0.79 eigen-values, and C (Creatinine) at 0.14 eigen-values indicates an increase in endogenous protein levels, although this is belied by the DPI eigenvalue amounts. Glycerol and TAGs are providing positive loadings of 0.14 and 0.18, along with Creatinine having a positive loading on the component of 0.14. These variance loadings by the eigen-values, including a slight negative loading of Fatty Acids (-0.01) describe internal energy stores being catabolized as a supplement to the energy provided by the forage resource, including catabolism of creatine, elevating the creatinine levels. Negative loadings by AlkP (-0.36), Phos (-0.056), and Albumin (-0.11) demonstrate an absence of easily available energy to reload the AMP into ADP and ATP. The negative

loading of cholesterol eigen-values (-0.11) helps to demonstrate the herd's state of under - nutrition, as synthesis of the steroid cholesterol is not a priority within the herd at this point of time.

The model and its principal components is an additive linear model, so the effect of each component (as described) will be at the very least additively cumulative on the physiological condition of the deer. Additive accumulation suggests the passage of time, or the sequence of seasons upon each other, which could be re-defined as study years. The decline of forage quality throughout the eigen-vectors of the principal components, PC 4 in series to PC3, suggests progression of the senescent state of the available forage. In the R psych package documentation, "…the eigen-vectors are rescaled by the square root of the eigen-values to produce the component loadings …" (Revelle 2013), so direct comparison with the eigen-vectors comprising a linear model provided by a different software package (such as SPSS, SAS, or other packages available within R) may not reproduce the same exact numbers. The same net effect by the principal component analysis or a linear regression applied in a step-wise fashion (including AIC criteria of selection) should be trended and commonalities between responses noted.

A varimax rotation for the factor analysis (versus a non-orthogonal promax rotation) was attempted. Cross-loading of the Fatty Acid variables between factors one and two emerged after originally applying the log(X+1) transformation suggested by Hair et al. 2010. The procedural suggestion by Hair et al. to then remove the variable FA was ignored, because of the consistently low correlations that Fatty Acid enjoys with the other variables (Table D.1) except TAG, Gly (cerol) and C (reatinine).

Cross-loadings existed after application of transformations (log(x+1)) and applying both Varimax and Promax rotations. During each trial with either the Varimax or Promax rotations, cross-loadings would then exist on variables that had not been previously eliminated during the process of extracting a factor analysis. Variables were eliminated until the data set was no longer a comprehensive explanation of the metabolic processes examined or involved, and still cross-loadings persisted. Confidence in an exploratory factor analysis model at that point was fully eroded; a model existed but it mathematically meant nothing. The application of a linear additive model to explain what was metabolically occurring within the North Kaibab mule deer between the Late Fall of 2005 and the Early Spring 2008 was found to be most appropriate in response to this trial and error.

The best explanation that can be provided is for the simultaneous (perhaps that is the essence of deriving this theoretical model) metabolic processes occurring within the temporal contexts of year and season as described within the scope of this study. These metabolic processes are intuitively included in the animal's DPI and MEI response curves of the fitted Forage Quality model in Chapter Five. Elimination of any of the metabolic responses would therefore be outside of the holistic, systemic view already presented.

A data set comprised of the metabolic response variables and available forage variables suggest a systemic theory of the process of the Kaibab mule deer herd entering a metabolic state of moderate to severe under-nutrition (Del Guidice et al. 1990, 1991, 1994, Seal 1969, 1978). Eliminating a variable for cross-loading meant eliminating a natural metabolic response by the animal to the habitat in that temporal context. That is not a realistic analysis, to ignore a known blood response because it doesn't fit a

mathematical constraint. Doing so places a natural world phenomena occurring within its natural habitat into an unnatural vacuum. The inverse of this argument explains the inherent level of correlation between the variables and justifies the utilization of an approach to modeling with a Principal Components analysis. Factor reduction (12 individual factors reduced to a more manageable four) justifies the use of PCA and linear regression on to PCA; a holistic viewpoint of examining the response of the whole organism to the habitat provides an equal amount of justification.

CONCLUSIONS

Combining the metabolic response variables found in the blood plasma of the Kaibab mule deer with the forage analysis data of Digestible Protein Index and Metabolizable Energy Index provides a unique data set reflective of the response by the animal to its winter habitat.

A minimum of twelve distinct yet inherently correlated variables emerge to describe the response of the Kaibab mule deer herd to their winter environment. Because many of the metabolic processes that the variables measure are sequential and/or simultaneous, it is logically obvious that a typical linear regression or log-linear regression model would not be appropriate. Principal component analysis with a non-orthogonal promax rotation was utilized to assemble the variables into components that are inherently responsive to a temporal scale of the study. These components were then subjected to a step-wise regression using AIC-scoring criteria. Parsimony was sought in the final descriptive model (Hair et al. 2010).

The loadings of the principal components were centered on ingestible forage, catabolism of fats, and the catabolism of proteins within the animal. Two components of

the models four are fully centered on protein catabolism. This indicates that fat catabolism – while occurring – is less significant than protein catabolism to the under-nutrition status of the animal. Principal component eigen-vector indicators of fat and protein catabolic activity confirm the model predictions of Chapter II. These indicators of catabolic activity describe not only the catabolic sequence of the animal, but provide an indicator of severity of under-nutrition through the numerical value of the loadings for eigen-vectors (Everitt and Hothorn 1997, Crawley 2007, Hair et al. 2010), (which in turn describe the effect on the variances from the means) but the ordination of the components. Protein resources are a last resource an organism that is starving turns to for survival.

Resource planning does not necessarily follow anthropogenic "common sense"; public sentiment from environmental organizations such as The Sierra Club or the Arizona Deer Association may push management emphases away from scientific reasoning and input. In creating a sustainable environment for a renewable mammalian resource, the best course of action may be to maintain a flexible resource management posture that is responsive to changing conditions. In a winter range situation, "…the optimal balance of grazing and browsing depends on an individual's current stores of energy and protein and the trade-off in nutrient harvest and safety when grazing or browsing under predation risk" (Christianson 2007). Where there is an active large predator population, maintaining enough immediately - available energy stores in muscle tissues and bodily reserves for a fight-or-flight response to a predator during the winter would be difficult for any species. Regaining that energy spent by the deer requires additional energy feeding and ruminating, with forage taking up to two weeks to fully

digest (Church 1988) and discharged muscle-tissue phosphoryl creatine, glucagon and mitochondrial ATP levels to be restored (Wyss and Kaddurah-Daouk 2000).



CHAPTER VIII
MANAGEMENT RECOMMENDATIONS

Hanley (1997) provides that diet selection can be an overwhelming influence on all factors describing the animal (physiological condition, growth rate, reproduction and survival rates), and can be considered on the individual and for the total effect on the herd. Hanley's words are prescient when the results of the dietary modeling in chapter five are aligned with the results of the species richness forage classification in chapter two. Many of the Rosacea – family shrubs present on the Kaibab Plateau can fixate nitrogen into the soil. This ability, with the high level of protein available within the plant contribute to the high protein curve of the available forage. These same plants - perhaps the most redeeming plant group available to the deer as forage - are observed to be decadent in growth and morphology and in a state of decline.

Forage needs to not only to be nutritious for a ruminant but also accessible. The ability of the mule deer to reach the annual growth of a decadent stand of shrubs and trees becomes questionable with a known energy deficiency in place. Serious consideration should be given to juniper and piñon control efforts – both mechanically and via controlled burning, in order to create and increase the plant inter-space between the shrubs and trees. Replanting efforts need to involve Mexican cliffrose and Apache plume but also more of the other identified shrubs and forbs. Grasses need to be minimized, encouraging more calorically-dense plant matter such as that found in the annual growth of a forb, a shrub bud, twig or stem.

The metabolic values found in the scope of this study provide for multiple comparisons within the literature. Of these, Albumin is notable as other comparative

values are available and are within range. Cunningham (1992) laments that standard albumin and other blood values were not available; upon reflection on the values presented in her work, with the addition of the values found in this work, an Albumin standard for *O. hemionus* in the Western U.S. is apparent. Under-nutrition levels for cholesterol and other protein indicators are within the norms reported by Del Guidice, with these levels becoming apparent as baselines when placed alongside other reported levels (i.e. Smith's) or as a stand-alone value.

The loadings of the principal components are centered on ingestible forage, catabolism of fats, and the catabolism of proteins within the animal. Two components of the models four are fully centered on protein catabolism. Principal component eigen-vector indicators of fat and protein catabolic activity confirm the model predictions of Chapter V.

These indicators of catabolic activity describe not only the catabolic sequence of the animal, but provide an indicator of severity of under-nutrition. Severity is described not only through the numerical value of the loadings for eigen-vectors (which in turn describe the effect on the variances from the means) but through the ordination of the components (Crawley 2007, Everitt and Hothorn 1997, Hair 2010). Protein resources are a last resource an organism that is starving turns to for metabolic survival. None of the principal components suggest that the herd needs to be grown larger. On the contrary, a very different picture has emerged.

All available indicators direct to a vector where maximum growth may have been reached. The Kaibab mule deer have entered a severe state of under-nutrition when wintering on the Kaibab winter range as defined by our study area. If a mule deer is

entering a winter habitat in a compromised state, the predatory natural selection process is unbalanced in favor of the predator, whether the predator is four- or two-legged (Darwin1882, Hefflefinger 2006, Taylor 2008). The ability to leave this state of under-nutrition and re-enter a metabolic state where all demands are being met and fat deposition is occurring may or may not be energetically possible by the beginning of spring green-up, or when the return migration to summer grazing areas has begun. Whether or not the mule deer, after surviving a winter in this metabolic state, have the caloric resources and bodily energy reserves to complete the journey back into the Buckskin Mountains is a serious question left for another study.

Habitat management and restoration efforts are typically directed at the limiting resource of a given habitat (Wallmo 1977, Robbins 1993, DelGuidice 2001, Moen 1973 and 1997). Regaining that energy spent by the deer requires additional energy feeding and ruminating, with forage taking up to two weeks to fully digest (Church 1988) and discharged muscle-tissue phosphoryl creatine, glucagon and mitochondrial ATP levels to be restored (Wyss and Kaddurah-Daouk 2000). The limiting resource on the North Kaibab is the caloric content of the forage supply. Any reclamation effort by a resource management bureau or private company would need to be comprehensive in either mechanically removing large shrubs or coniferous trees or a series of prescribed burns to remove fuel and undesirable forage.

Comprehensive is a term used sparingly; in the process of increasing shrub inter-space for reseeding of forbs and other shrubs, adequate amounts of cover for concealment against predation needs to be maintained in the winter range. In order to minimize the amount of disturbance the animal populations are exposed to and facilitate the safety of

cover against predation, plot sizes during habitat restoration efforts via mechanical removal or a controlled burn need to be small and multiple, measured in the 10's to 100's of square hectares (or even square meters) rather than 1000's of hectares. This is especially important around the known water sources on the north Kaibab plateau.

The size of the herd either needs to be maintained by natural selection via predation, disease and other ecological factors, or the size of the herd needs to be reduced through hunting. A course of irresponsible management would be the sustained growth of the herd where the primary objective is to facilitate increased hunting opportunities, when the food supply of the habitat simply won't support the increase in the size of the herd.

WORKS CITED:

Abdi, H. 2007. The Bonferonni and Šidák corrections for multiple comparisons. Pages 104-108 *in* The encyclopedia of measurement and statistics. Sage Publications via ASU Electronic Library http://knowledge.sagepub.com.ezproxy1.lib.asu.edu/view/statistics/n60.xmL

Acton, M.W. Lab and field notes from North Kaibab data collection. Jan. 2006, March 2007, March 2008-present. *Unpublished*

Allen, R.S. 1977. Metabolism. Pages 327–335; Lipid metabolism. Pages 336 – 345; Protein metabolism. Pages 346 – 356 *in* M.J. Swenson, editor. Duke's physiology of domestic animals 9th ed. Comstock Publishing Associates, Cornell University Press Ithaca, NY

Ammann, A.P., R.L. Cowan, C.L. Mothershead, and B.R. Baumgardt. April 1973. Dry matter and energy intake in relation to digestibility in white-tailed deer. Journal of wildlife management.37:195-201.

Argenzio, R.A. 2004. Gastrointestinal motility. Pages 391-404 *in* W.O. Reese, editor. Duke's physiology of domestic animals 12th ed. Comstock publishing associates. Cornell University Press Ithaca, NY.

Arizona Deer Association (ADA). 2006 – present; http://www.azdeer.org/

Arizona Game and Fish Department. 2007. http://www.azgfd.gov/h_f/hunting_units_12a.shtmL

Baker, G.A. Nov. 1930. The transformation of bi-modal distributions. The annals of mathematical statistics. 1:334-344.

Batzli, G.O., S.J. Harper, Y.K. Lin, and E.A. Desy. 1999. Experimental analyses of population dynamics: scaling up to the landscape. Page 124 *in* Landscape Ecology of Small Mammals. Springer-Verlag. New York U.S.A.

Bauchart, D. 1993. Lipid absorption and transport in ruminants. Journal of Dairy Science. 76:3864-3881.

Belasco, I.J. 1954. New nitrogen feed compounds for ruminants – A laboratory evaluation. Journal of animal science. 13:601-610.

Bergman, E.N. 1977. Disorders of carbohydrate and fat metabolism. Pages 357-367 *in* M.J. Swenson, editor. Duke's physiology of domestic animals 9th ed. Comstock publishing associates. Cornell University Press. Ithaca, NY.

Beitz, D.C. 2004. Lipid Metabolism. Pages 517 – 534 Protein and amino acid metabolism. Pages 535-552 *in* W.O. Reese, editor. Duke's physiology of domestic animals 12th ed. Comstock publishing associates. Cornell University Press. Ithaca, NY.

Binkley, D., M.M. Moore, W.H. Romme, and P.M. Brown. 2006. Was Aldo Leopold right about the Kaibab deer herd? Ecosystems. 9:227-241

BioAssay Systems. 2006. Alkaline Phosphatase Assay Kit DALP-250. BioAssay System. Haywood Ca.

_____. 2006. Albumin Assay Kit DIAP-250. BioAssay System. Haywood Ca.

_____. 2006. Creatinine Assay Kit DICT-500. BioAssay System. Haywood Ca.

_____. 2006. Glucose Assay Kit DIGL-200. BioAssay System. Haywood Ca.

_____. 2006. Phosphatase Kit POPN-500. BioAssay System. Haywood Ca.

_____. 2006. Protein Assay Kit QCPR-500. BioAssay System. Haywood Ca.

_____. 2006. Urea Assay Kit DIUR-500. BioAssay System. Haywood Ca.

_____. 2006. Protein Assay Kit QCPR-500. BioAssay System. Haywood Ca.

BioVision. 2005. Cholesterol/Cholesteryl Ester Quantification Kit K603-100. BioVision Research. Mountain Vista, CA.

Boudet, A.-M. 2000. Lignin and lignification: Selected Issues. Plant physiology and biochemistry. 38.5:81– 96

1998. A new view of lignification. Trends in plant science. 3:67-71

Buol, S.W., F.D. Hole, R.J. McCracken, and R.J. Southard. 1997. Soil genesis and classification 4th ed. Iowa State University Press. Ames, USA.

Brady, N.C. and R.R. Weil. 1999. Pages 449 – 451 *in* The nature and properties of soils. Prentice-Hall, Upper Saddle River, New Jersey.

Brown, R.D., E.C. Hellgren, M. Abbott, D.C. Ruthven III, and R.L. Bingham. 1995. Effects of dietary energy and protein restriction on nutritional indices of female white-tailed deer. The journal of wildlife management. 59:595–609.

Brown, T.L., H.E. LeMay Jr., B.E. Bursten, and C.J. Murphy. 2006. Acid-base equilibria. Pages 669–719 *in* Chemistry: the central science 10th ed. Pearson Education, Inc. Upper Saddle River, NJ.

Bryant, M.P. 1977. Microbiology of the rumen. Pages 287–304 *in* Melvin J. Swenson, editor. Duke's physiology of domestic animals 9th ed. Comstock publishing associates. Cornell University Press. Ithaca, NY.

Buck, T. 2007. Wildlife Manager, Arizona Game and Fish Department Management Unit 12. *Personal conversation.*

Byers, F.M. and G.T. Schelling. 1988. Lipids in Ruminant Nutrition. Pages 298–312 *in* D.C. Church, editor. The ruminant animal; digestive physiology and nutrition. Prentice-Hall, New Jersey.

Campbell, N.A., J.B. Reece, and L.G. Mitchell. 1999. Pages 1-488 *in* Biology 5th ed. Benjamin/Cummings. Menlo Park, CA.

Cannon B., and J. Nedergaard. 2004. Brown adipose tissue: function and physiological significance. Physiol. Rev. 84:277-359.

Caughley, G. 1970. Eruption of ungulate populations with emphasis on Himalayan Thar in New Zealand. Ecology. 51:53 – 72.

Christianson, D.A. and S. Creel. 2007. A review of environmental factors affecting elk winter diets. The journal of wildlife management. 71:164–176.

Church, D.C. Ph.D. Prof. Emeritus, editor. 1988. Pages 1–14 *in* The classification and importance of ruminant animals. The ruminant animal; digestive physiology and nutrition. Prentice-Hall, New Jersey.

Coleman, J.E. 1992. Structure and mechanism of alkaline phosphatase. Annual review of bio-physics and bio-molecular structures. 21:441-483.

Cook, J.G. 1982. Nutrition and Food. Pages 259 - 351 *in* D.E. Toweil and J.W. Thomas, editors. North American Elk; ecology and management. Smithsonian Institution Press. Washington, D.C. U.S.A.

Crawley, M.J. 2007. Analysis of variance. Pages 449 – 526. Multivariate statistics. Pages 731–747. The R Book. Wiley-Blackwell. Hoboken, NJ USA.

Cunningham, B. 1992. Diet and diseases of Zion Canyon mule deer, Zion National Park, Utah. Thesis. Dept. of Biological Sciences. University of Nevada-Las Vegas, USA.

Dale, H.H. 1977. Energy metabolism. Pages 368–377 *in* M.J. Swenson, editor. Duke's physiology of domestic animals 9th ed. Comstock publishing associates. Cornell University Press. Ithaca, NY.

Danielson, B.J. and G.S. Anderson. 1999. Habitat selection in geographically complex landscapes. Page 89 *in* landscape ecology of small mammals. Springer-Verlag, NY.

Darwin, C. 1882. Natural Selection; or Survival of the Fittest. Pages 169-281 *in* On the Origin of the Species 6th ed. iBooks public domain. https://itunes.apple.com/us/book/on-the-origin-of-species/id395536758?mt=11

Darveau, C-A., R.K. Suarez, R.D. Andrews, and P.W. Hochachka. 2002. Allometric cascade as a unifying principle of body mass effects on metabolism. Nature. 417:166-170.

Del Guidice, G.D., D.L. Mech, and U.S. Seal. 1990. Effects of winter under-nutrition on body composition and physiological profiles of white-tailed deer. The journal of wildlife management.. 54:539-550.

Del Guidice, G.D., D.L. Mech, U.S. Seal and P.D. Karns. 1987. Effect of winter fasting and re-feeding on white-tailed deer blood profiles. The journal of wildlife management.. 51:865-873.

Del Guidice, G.D., R.A. Moen, F.J. Singer, and M.R. Riggs. 2001. Winter nutritional restriction and simulated body condition of Yellowstone elk and bison before and after the fires of 1988. Wildlife monographs. 147:1-60.

Del Guidice, G.D., F.J Singer, and U.S. Seal. 1991. Physiological assessment of winter nutritional deprivation in elk of Yellowstone National Park. The journal of wildlife management.. 55:653-664.

Del Guidice, G.D., F.J. Singer, U.S. Seal.and G. Bowser. 1994. Physiological responses of Yellowstone bison to winter nutritional deprivation. The journal of wildlife management.. 58:24-34.

Deschamp, J.A., P.J. Urness, and D.D. Austin. 1979. Summer diets of mule deer from lodge pole pine habitats. The journal of wildlife management.. 43:154-161.

Deitz, D.R. and J.G. Nagy. 1976. Mule deer nutrition and plant utilization. Proceedings, Mule deer decline in the West. http://www.muledeerworkinggroup.com/Docs/Proceedings/1976-Mule%20Deer%20Decline%20in%20the%20West/ *via* Western Association of Wildlife Agencies (http://www.muledeerworkinggroup.com/index.htm) Accessed on 11/05/2013 and 11/26/13

Deitz, D.R., RH. Udall, and L.E. Yeager. December 1962. Chemical composition and digestibility by mule deer of selected forage species, Cache La Poudre range, Colorado. Colorado Game and Fish Department. Technical publication number fourteen.

Dodson, S.I. editor. 1998. Ecosystem ecology. Pages 123-163 *in* Ecology. Oxford University Press. New York USA.

Doman, E.R., and D.I. Rasmussen. 1944. Supplemental winter feeding of mule deer in northern Utah. The journal of wildlife management.. 8:317-338.

Dougherty, R.W. 1977. Physiopathology of the ruminant digestive tract. Pages 305-314 *in* M.J. Swenson, editor. Duke's physiology of domestic Animals 9th ed. Comstock publishing associates. Cornell University Press. Ithaca, NY.

Drake, M.L. 2009. Pronghorn antelope food habits and diet quality on Anderson Mesa and Garland Prarie, Arizona. Thesis. Arizona State University. Tempe, USA.

Epple, A.O. and L.E. Epple. 1995. A field guide to the plants of Arizona. The Globe Pequot Press. Guilford, Connecticut USA.

Everitt, B. and T.Hothorn. 2001. Principal components analysis. Pages 61–105 Exploratory factor analysis. Pages 135–163 *in* An introduction to applied multivariate analysis with R. Springer. New York USA. Kindle edition.

Fahey Jr., G.C. and L.L. Berger. 1988. Carbohydrate nutrition of ruminants. Pages 269-298 *in* D.C. Church, editor. The ruminant animal; digestive physiology and nutrition. Prentice-Hall. New Jersey, USA.

Fennessey, P.F. and K.R. Drew. 1985. Biology of deer production: Proceedings of an international conference held at Dunedin, New Zealand, 13-18 February 1983. Bulletin/Royal Society of New Zealand. The Royal Society.

Ferrell, C. L. 1988. Energy Metabolism. Pages 250-268 *in* D.C. Church, editor. The ruminant animal; digestive physiology and nutrition. Prentice-Hall. New Jersey, USA.

Folin, O. 1905. A theory of protein metabolism. American journal of physiology. 13:117-139

Ford, A. 1999. Ppg183 – 211 *in* Modeling the environment: An introduction to system dynamics modeling of environmental systems. Island Press. Washington, D.C. USA.

Goff, J.P. 2004. Disorders of carbohydrate and fat metabolism. Pages 575-599 Minerals. Pages 575-599 *in* W.O. Reese, editor. Duke's physiology of domestic animals 12th ed. Comstock publishing associates. Cornell University Press. Ithaca, NY.

Google maps. ASU Polytechnic practice fields to Grand Canyon National Park – North Rim, AZ. https://maps.google.com/maps?hl=en&authuser=0 Accessed August 17, 2013.

Green, Dr. D.M. November 25, 2013. Personal conversation re: lignin, carbon sequestration and carbon sinks

Guyton, A.C., and J.E. Hall. 2006. Secretory functions of the alimentary tract. Pages 794-807, 841, 847 – 848 *in* Textbook of medical physiology. Elsevier Saunders. Philadelphia, PA USA.

Hair, J.F. Jr., W.C. Black, B.J. Babin, and R.E. Anderson. 2010. Principal components and common factor analysis. Page 17. Exploratory factor analysis. Pages 383-458. Multivariate data analysis-6th edition. Pearson Prentice Hall. Upper Saddle River, NJ. USA.

Hanley, T.A., A.E. Hagerman, O. Hjelhord, D.L. Baker, and C.C. Schwartz. and W.W. Mautz. 1987. Role of tannins in defending plants against ruminants: Reduction in protein availability. Ecology. 68:98-107.

Hanley, Thomas A. 1982. The nutritional basis for food selection by ungulates. Journal of range management. 35:146-151.

- 1997. A nutritional view of understanding and complexity in the problem of diet selection by deer (Cervidae). Oikos. 79:209-218.

Hanley, T.A. and J.J. Rogers. 1989. Estimating carrying capacity with simultaneous nutritional constraints. United States Dept. of Agriculture Research Note. PNW-RN-485.

Hanley, T.A. and J.D. McKendrick. 1985. Potential nutritional limitations for black-tailed deer in a spruce-hemLock forest, southeastern Alaska. The journal of wildlife management. 49:103-114.

Harper, Ph.D. H.A., V.W. Rodwell, Ph.D., and P.A. Mayes, Ph.D. 1979. Review of physiological chemistry 17th ed. Lange medical publications. Los Altos, CA USA.

Haywood, D.D., R.L. Brown, R.H. Smith, and C.Y. McCulloch. Migration patterns and habitat utilization by Kaibab mule deer: a final report. Az. Game and Fish Dept. Research Branch, Federal Aid in Wildlife Restoration Project W-78-R.

Hatfield, R.D., J.M. Marita, K. Frost, J. Grabber, J. Ralph, F. Lu, and K. Hoon. 2009. Grass lignin acylation: *p*-coumaroyltransferase activity and cell wall characteristics of C3 and C4 grasses. Planta. 229:1253-1267

Hefflefinger, Jim. 2006. Pages 117 – 142 *in* Deer of the Southwest: A complete guide to the natural history, biology, and management of Southwestern mule and White-tail deer. Texas A&M University press.

Hervert, J.J. and P.R. Krausman. 1986. Desert mule deer use of water developments in Arizona. Journal of wildlife management. 50:670-676.

Hobbs, N.T. and D.M. Swift. 1985. Estimates of habitat carrying capacity; incorporating explicit nutritional constraints. Journal of wildlife management. 49:814-822.

Hofmann - Med. Vet., Dr. R.R. 1988. Anatomy of gastro-intestinal tract. Pages 14-44 *in* D.C. Church, editor. The ruminant animal; digestive physiology and nutrition. Prentice-Hall. New Jersey USA.

Holecheck, J.L., R.A. Cole, J.T. Fisher, and R. Valdez. 2000. Pages 45-50 *in* Natural resources: ecology, economics and policy. Prentice-Hall. New Jersey, USA.

Holt, B. S., W. H. Miller, and B. Wakeling. 1992. Composition and quality of Desert Bighorn sheep diets in the Superstition Mountains, Arizona. Desert Bighorn Council proceedings. 36:25-30

Howell, David C. 2002. Multiple comparison among treatment means. Pages 369-415. Statistical methods for psychology. Wadsworth Group. Belmont, CA USA.

Hungerford, C.R. 1970. Response of kaibab mule deer to management of summer range. The journal of wildlife management.. 34:852-862

Ingle, D.L., D.E. Bauman, and U.S. Garrigus. 1972. Lipogenesis in the ruminant: in-vitro study of tissue sites, carbon source and reducing equivalent generation for fatty acid synthesis. Journal of nutrition. 102:609-616.

Jolliffe, I. 2002. Principal components analysis. Ed. Springer-Verlag. Secaucus, NJ USA.

Julander, O. 1962. Range management in relation to mule deer and habitat and herd productivity in Utah. Journal of Range Management. 15:5 ppg. 278 – 281.

Julander, O., W.L. Robinette, and D.A. Jones. 1961. Relation of Summer Range Condition to Mule Deer Herd Productivity. The Journal of Wildlife Management. 25:1 ppg. 54 – 60

Julian day calculator. National Atmospheric and Space Administration (NASA). http://landweb.nascom.nasa.gov/browse/calendar.htmL Accessed on August 13, 2013.

Kanab Utah, A Western Classic. About Kanab – History. 2014 http://kanab.utah.gov/resources.cfm

Kaibab National Forest map. 1975. U.S. Department of Agriculture, Forest Service.

Kearney, T.H., R.H. Peebles, J.T. Howell, and E. McClintock. 1979. Arizona flora. University of California Press. USA.

Kie, J.G., M. White, and D.L. Drawe. 1983. Condition parameters of white-tailed deer in Texas. Journal of wildlife management.47:583 – 594.

Kirkpatrick, R.L., D.E. Buckland, W.A. Abler, P.F. Scanlon, J.B. Whelan, and H.E. Burkhart. 1975. Energy and protein influences on blood urea nitrogen of white-tailed deer fawns. The journal of wildlife management. 39:692 – 698.

Klem, W.R. 1977. Design and basic functions of the nervous system. Pages 531-548 *in* M.J. Swenson, editor. Duke's physiology of domestic animals 9th ed. Comstock publishing associates. Cornell University Press. Ithaca, NY.

Knight, R.D., B. Jones, and S.Field. 2007. Wave optics. Page 552 *in* College physics: a strategic approach. Pearson Education. San Francisco, CA USA.

Koenen, K.K.G., and P.R. Krausman. 2002. Habitat use by desert mule deer in a semi-desert grassland. The southwestern naturalist. 47:353-362.

Kopf, V., R.D. Brown, and D.L. Drawe. 1984. Prediction of Protein Intake in White-Tailed Deer in South Texas. The journal of wildlife management. 48:518-526.

Kuenzi, A.J., R.C. Etchberger, K.R. Rautenstrauch, L.L. Ordway, and J.J. Hervert. 1997. Diets of desert mule deer. Journal of range management. 50:513 – 522.

Krausman, P.R., and R.C. Etchberger. 1995. Response of desert ungulates to a water project in Arizona. Journal of wildlife management.59:292-300.

Lancia, R. A., Pollock, K.H., Bishir, J.W. , and M.C. Conner. 1988. A white-tailed deer harvesting strategy. The journal of wildlife management. 52:589-595.

Leek, B. 2004. Digestion in the Ruminant Stomach. Pages 438 - 475 *in* William O. Reese, editor. Duke's physiology of domestic animals 12th ed. Comstock publishing associates. Cornell University Press. Ithaca, NY USA.

Leopold, A., E.F. Bean, and N.C. Fasset. 1943. Deer irruptions. Natural Resources Committee, Wisconsin Academy of Sciences, Art and Letters.

Logan, M. 2010. Factorial ANOVA. Pages 334-376 *in* Biostatistical design and analysis using R: a practical guide. Wiley-Blackwell. Hoboken, NJ USA.

Martin, PhD. C.R. 1985. The endocrine pancreas. Pages 168-170 *in* Endocrine physiology. Oxford University Press. New York USA.

McGarigal, K., S. Cushman, and S. Stafford. 2000. Ordination: principal components analysis. Pages 19-81 *in* Multivariate statistics for wildlife and ecology research. Springer. New York

Mckee, T., and J.R. Mckee. 2009. Carbohydrates. Pages 227-258, 308, 316, 477-479 *in* Biochemistry; The molecular basis of life 4th ed. Oxford University Press. New York USA.

McCulloch, C.Y. 1978. Statewide deer food preference. Research Division, Az. Game and Fish Department internal report.

MuCulloch, C.Y. and R.H. Smith. 1991. Relationship of weather and other variables to the condition of the Kaibab deer herd. Research Division, Az. Game and Fish Department internal report.

McCune, B. and J.B. Grace. 2002. Principal components analysis. Rotating ordination axes. Pages 122-125 *in* Analysis of ecological communities. MjMSoftware Design. Gleneden Beach, Oregon U.S.A.

Merchen, N.R. 1988. Digestion, absorption and excretion in ruminants. Pages 172-201 *in* D.C. Church, editor. The ruminant animal; digestive physiology and nutrition. Prentice-Hall. New Jersey, USA.

Meyers, Jerome L., and A.D. Well. 2003. Contrasts among means. Pages 233-267 *in* Research design and statistical analysis 2nd Ed. Lawrence Erlbaum Associates, Publishers. Mahwah, N.J. USA.

Miller, W. H. 1980. Food habits and diet quality of deer on Bailey Creek, north central Washington. Thesis. Washington State University, Pullman, WA. USA.

Miller, Dr. William H. 2004. Lecture notes, animal nutrition. *Unpublished.*

April 7, 2010. August 26, 2013. October 29, 2013. *Personal conversations*.

December 2006 – January 2007. Field notes and personal conversation.

Miller, William H., M.W. Acton, A.M. Chenevert-Steffler, and R.E. Williams. 2009. Nutritional status of mule deer on the north Kaibab winter range. Revised final report to the Az. Game and Fish Department, ResearchBranch.

Mithieux, G., F. Rajas, and A. Gautier-Stein. 2004. A novel role for glucose phosphatase in the small intestine in the control of glucose homeostasis. The journal of biological chemistry. 279:44231–44238

Moen, N. 1973. Physiological, behavioral, and genetic responses to the the environment. The organization of energy and matter in plant and animal communities. A biological basis for the calculation of carrying capacity. Mathematical analyses of factors affecting carrying capacity. Population requirements for protein and energy.
Pages 273-389, 400 *in* Wildlife Ecology. W.H. Freeman and Co., San Francisco, USA

Moen, A. N. 1978. Seasonal changes in heart rates, activity, metabolism and forage intake of white-tailed deer. Journal of wildlife management.42:715 – 738.

Moen, A. N. and Del Guidice, G.D. 1997. Simulating nitrogen metabolism and urinary nitrogen:creatinine ratios in ruminants.
The Journal of wildlife management. 61: 881-894.

Moen, R. and G. D. DelGiudice. 1997. Simulating nitrogen metabolism and urinary nitrogen:creatinine ratios in ruminants. Journal of wildlife management. 61:881-894.

Mould, E.D. and C.T. Robbins. 1981. Nitrogen metabolism in elk. Journal of wildlife management. 45:323 – 324.

Nagy, J.G., H.W. Steinhoff, and G.M. Ward. 1964. Effects of essential oils of sagebrush on deer rumen microbial function. Journal of wildlife management. 28:785 – 790.

Nagy, K.A., and C.C. Peterson. 1987. Water flux scaling. Pages 131-140 *in* P. Dejours, L. Bolis, C.R. Taylor and E.R. Weible, editors. Comparative physiology: life in water and on land. Fidia Research Series, Volume 9. Liviana Press. Springer-Verlag. Pavoda.

Nelson, J. R. and T. A. Leege. 1982. Nutritional requirements and food habits. Pages 323-267 *in* J. W. Thomas and D. E. Toweill, editors. *in* Elk of North America, ecology and management. Smithsonian Institution Press. Washington, D.C. USA.

Neter, J., M.H. Kutner, C.J. Nachtsheim and W.Wasserman. 1996. Analysis of factor level effects. Pages 710 – 756 *in* Applied linear statistical models 4th ed. Times Mirror Higher Education Group. Chicago, IL USA.

Ngugi, R.K., F.C. Hinds, and J. Powell. 1995. Mountain big sagebrush (*Artemesia tridentata)* decreases dry matter intake, digestibility, and nutritive quality of sheep diets. Journal of range management 48:487-492

NOAA climatic data center (weather data). http://www.ncdc.noaa.gov/IPS/cd/cd.htmL;jsessionid=B1FC29D87F8AD2ADD2%09A659CD7779D15 Accessed March 2013.

O'gara, B.W. and R.G. Dundas. 1982. Distribution: past and present. Pages 67-121 *in* D.E. Toweil and J.W. Thomas, editors. North American Elk ecology and management. Smithsonian Institution Press. Washington, D.C. U.S.A.

Orskov, E.R. and M. Ryle. 1990. Energy nutrition in ruminants. Elsevier Science Publishers. New York, New York USA.

Overton, T. R., J. K. Drackley, C. J. Ottemann-Abbamonte, A. D. Beaulieu, L. S. Emmert and J. H. Clark. 1999. Substrate utilization for hepatic gluconeogenesis is altered by increased glucose demand in ruminants. Journal of animal science. 77:1940-1951.

Owens, F.N.and A.L. Goetsch. 1988. Ruminal fermentation. Pages 145 – 172 *in* D.C. Church, editor. The ruminant animal; digestive physiology and nutrition.. Prentice-Hall. New Jersey, USA.

Owens, F. N. and R. Zinn. 1988. Protein metabolism of ruminant animals. Pages 227-249 *in* D.C. Church, editor. The ruminant animal; digestive physiology and nutrition. Prentice-Hall. New Jersey, USA.

Page, B. D. and B. H. Underwood. 2006. Comparing protein and energy status of winter-fed white-tailed deer. Wildlife society bulletin. 34:716 – 724.

Parker, K. L., C.T. Robbins, and Thomas A. Hanley. 1984. Energy expenditures for locomotion by mule deer and elk. The journal of wildlife management. 48:474 – 488.

Parker, K.L., M.P. Gillingham, T.A. Hanley, and C.T. Robbins. 1999. Energy and protein balance of free-ranging black-tailed deer in a natural forest environment. Wildlife monographs. 143:3 – 48.

Pederson, J.C. and K.T. Harper. 1978. Factors influencing productivity of two mule deer herds in Utah. Journal of range management. 31:105-110.

Peterson, C. and T.A. Messmer. 2007. Effects of winter-feeding on mule deer in northern Utah. The journal of wildlife management.. 71:1440 – 1145.

Phillipson, A.T. 1977. Ruminant Digestion. Pages 250-287 *in* Melvin J. Swenson, editor. Duke's physiology of domestic animals 9th ed. Comstock Publishing Associates. Cornell University Press. Ithaca, NY USA.

Pierce, B.M., T.R. Bowyer, and Vernon C. Bleich. 2004. Habitat Selection by Mule Deer: Forage Benefits or Risk of Predation?. Journal of wildlife management.68:533-54.

Plants National Database. http://plants.usda.gov/ . Accessed 2005-2013.

Plummer, A. P., D. R. Christensen, and S. B. Monsen. 1968. Restoring Big Game Range In Utah, Utah Div. of Fish and Game. Pub. No. 68-3. 183 pp.

Rasmussen, D.I. 1932. Biotic Communities of the Kaibab Plateau. Thesis. University of Illinois. Urbana, USA.

Rasmussen, D.I. 1941. The Biotic Communities of the Kaibab Plateau. Ecological Monographs. 11:229-275.

Rautenstrauch, K.R. and P.R. Krausman. 1989. Preventing Mule Deer Drownings in the Mohawk Canal, Arizona. Wildlife Society Bulletin. 17:280-286.

Reece, William O., editor. 2004. Kidney Function in Mammals. Pages 73-106 *in* Duke's Physiology of Domestic Animals, 12th ed. Comstock Publishing Associates, Cornell University Press. Ithaca, NY USA.

Revelle, William. 2013. Procedures for Psychological, Psychometric, and Personality Research. Package 'psych", R-depository, cran.Rproject.org, ver. 1.3.10.12. http://cran.r-project.org/web/packages/psych/psych.pdf . Accessed October 12th, 15th 2013.

Robinette, W. L., O. Julander, J.S. Gashwiler and Justin G. Smith. 1952. Relation of summer range condition to mule deer productivity. The Journal of Wildlife Management. 16:3 ppg. 289-299.

Robbins, C. T. 1993. Wildlife Feeding and Nutrition, Second Edition. Academic Press. New York USA. 457 pp.

1973. The Biological Basis for the Determination of Carrying Capacity. Dissertation. Cornell University. Ithaca, NY USA. Proquest Dissertation and Theses. University Microfilms.

Roelfs, A.P. and J. W. Martens. 1988. Special Topics: An International System of Nomenclature *Puccinia graminis* f. sp. *tritici*. Phytopathology. 78:526 – 533.

Rohwer, G.L. 1970. Blood composition of wintering Nevada mule deer (*Odocoileus hemionus hemionus)*. University of Nevada, Reno. Thesis.

Russo, J.P. 1964. The Kaibab North Deer Herd: Its History Probems and Management (A Research and Management Study). Wildlife Bulletin No. 7. State of Arizona Game and Fish Department. Phoenix, Arizona USA.

Säkkinen, H., A. Stien, Ø. Holand, K. Hove, E. Eloranta, S. Saarela, and E. Ropstad. 2001. Plasma urea, creatinine, and urea: creatinine ration in Reindeer (*Rangifer tarandus tarandus)* and in Svalbard Reinddeer (*Rangifer tarndus platyrhynchus*) during defined feeding conditions and in the field. Physiological and biochemical zoology. 74:907-916.

Saladin, K.S. 2007. Anatomy and Physiology: The Unity of Form and Function, 4th ed. McGraw – Hill. New York, New York USA.

Seal, U.S. and A.W. Erickson. 1969. Hematology, blood Chemistry and Protein Polymorphisms in the White-Tailed Deer (*Odocoileus Virginianus)*. Comparitive biochemistry and physiology. 30:695-713

---, M.E. Nelson, D.L. Mech and Reed L. Hoskinson. 1978. Metabolic indicators of habitat differences in four minnesota deer populations. Journal of wildlife management.42:746 – 754.

Shaffer, J.P. 1995. Multiple Hypothesis Testing. Annual Review of Psychology. 46:561-584.

Shingoethe, David J. 1988. Nutrient needs during critical periods of the life cycle. Pages 421 –437 *in* D.C. Church, editor. The ruminant animal; digestive physiology and nutrition. Prentice-Hall. New Jersey, USA.

Sidak, Z. 1967. Rectangular Confidence Regions for the Means of Multivariate Normal Distributions. Journal of the American Statistical Association. 52:626 – 633.

Smith, R.H. 1976. Hematology of Deer: A Final Report. Az. Game and Fish Dept. Research Branch. Federal Aid in Wildlife Restoration Project W-78-R, Work Plan 3, Job 4.

Sokal, R.R. and J.F. Rohlf. 1969. Single Classification Analysis of Variance. Regression. Pages 226-249, 458 *in* Biometry. W.H. Freeman and Company. San Francisco, CA. USA

Steel, R.G.D., J.H. Torrie and David A. Dickey. 1997. Multiple Comparisons. Pages 178 – 204 *in* Principals and Procedures of Statistics: A Biometrical Approach. McGraw-Hill. San Francisco, CA. USA.

Stevens, C.E. 1977. Comparative Physiology of the Digestive System. Pages 216-232 *in* M.J. Swenson, editor. Duke's physiology of domestic animals 9th Ed. Comstock publishing associates. Cornell University Press. Ithaca, NY. USA.

Stevens, C.E. and A.F. Sellers. 1977. Basic characteristics of the digestive system. Pages 210-216 *in* M.J. Swenson, editor. Duke's physiology of domestic animals 9th Ed. Comstock publishing associates. Cornell University Press. Ithaca, NY. USA.

Suarez, R.K., C.-A. Darveau and J.J. Childress. 2004. Metabolic scaling: a many-splendored thing. Comparative biochemistry and physiology, part B. 139:531 – 541.

Sutton, J. D. 1971. Carbohydrate digestion and glucose supply in the gut of the ruminant. Proc. Nutr. Soc. 30:243.

Switalski, A. 2013. The effects of artificial water sources on small mammal communities. Thesis. Arizona State University. Tempe, USA.

Taylor, M.E. Personal conversation. Deer evolution, feeding behavior and fright/predation response. December 2008

Thornley, J.H.M. and J. France. 2004. An open-ended logistic-based growth function. Ecological Modelling. 184:257 – 261

Tluczek, M. 2012. Diet, nutrients, and free water requirements of pronghorn antelope on Perry Mesa, Arizona. Thesis. Arizona State University. Tempe, USA.

Topo! Ver. 4.6.0, copyright 2010 National Geographic Maps. Accessed August 17, 2013.

Tsoularis, A. and J. Wallace. 2002. Analysis of logistic growth models. Mathematical Biosciences. 179:21-55

Turner, M. 1998. Landscape Ecology. Pages 77–122 *in* Stanley I. Dodson, editor. Ecology. Oxford University Press. New York, NY USA.

United States Forest Service (U.S.F.S.), U.S. Dept. of Agriculture. 2013. Draft record of decision and finding of non-significant forest plan amendment for the Rosemont Copper Project. http://rosemonteis.us/files/final-eis/rosemont-feis-draft-rod.pdf. Accessed on December, 2013, February 2014.

Utah History Encyclopedia-Kanab. http://www.uen.org/utah_history_encyclopedia/k/KANAB.htmL Accessed on April 7, 2010.

Utah, Kanab. information. http://kanab.utah.gov/index.cfm Accessed on March 29, 2010.

Urness, P.J., W. Green and R.K. Watkins. 1971. Nutrient intake of deer in Arizona chaparral and desert habitats. Journal of wildlife management. 35:469-475.

Van Soest, P.J. 1982. Rumen anatomy and function. Intermediary metabolism. Limitations of ruminants. Pages 178-194, 249-259, 325-344 *in* nutritional ecology of the ruminant. O & B Books. Corvallis, Oregon USA.

Verme, L.J. and J.J. Ozoga. 1980. Effect of diet on growth and lipogenesis in deer fawns. Journal of wildlife management. 44:315 – 324.

1980. Influence of protein-energy intake on deer fawns in autumn. The journal of wildlife management.. 44:305 – 314.

Voet, D. and J.G. Voet. 2004. Other pathways of carbohydrate metabolism. Pages 844, 862 – 870 *in* Biochemistry 3rd ed. John Wiley and Sons, Inc. U.S.A.

Young, C.C. 2002. In the absence of predators: conservation and controversy on the Kaibab plateau. University of Nebraska Press USA.

Waid, D.D. and R.J. Warren. 1984. Seasonal variations in physiological indices of adult female white-tailed deer in Texas. Journal of wildlife diseases. 20:212 – 219.

Wakeling, B.F. 2005. Wildlife management decisions and Type I and Type II errors. 6th western states and provinces deer and elk workshop proceedings. Reno, NV USA.

Personal Communication/Telephone interview. Fall 2010.

Wallmo, O.C., Carpenter, L.H., Regelin, W.L., Gill, R.B., and D.L. Baker. 1977. Evaluation of deer habitat on a nutritional basis. Journal of range management. 30:122 – 127.

Warren, R.J., R.L. Kirkpatrick, A. Oelschlaeger, P.F. Scanlon, and F.C. Gwazdauskas. 1981. Dietary and seasonal influences on nutritional indices of adult male white-tailed deer. Journal of wildlife management. 45:926 – 936.

Warren, R.J., R.L. Kirkpatrick, A. Oelschlaeger, P.F. Scanlon, K.E. Webb Jr., and J.B. Whelan. 1982. Energy, protein, and seasonal influences on white-tailed deer fawn nutritional indices. Journal of wildlife management.46:302-312.

Welch, B.L., D. McArthur, and J.N. Davis. 1983. Mule deer preference and monoterpenoids (essential oils). Journal of range management. 36:485- 487.

West, Geoffrey B., J.H. Brown and B.J. Enquist. 1999. The fourth dimension of life: fractal geometry and allometric scaling of organisms. Science. 284:1677 – 1679.

--- and J.H. Brown. 2005. Review: The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. The journal of experimental biology. 208:1572 – 1592.

Wickstrom, Mark L., C.T. Robbins, T.A. Hanley, D.E. Spalinger and S.M. Parish. 1984. Food intake and foraging energetics of elk and mule deer. Journal of wildlife management.48:1285 - 1301

Wyss, M. and R. Kaddurah-Daouk. 2000. Creatine and creatinine metabolism. Physiological Review. 80:1107 – 1183.

Yokayam, M.T. and K.A. Johnson. Microbiology of the rumen and intestine. Pages 125 – 145 *in* D.C. Church, editor. The ruminant animal; digestive physiology and nutrition. Prentice-Hall. New Jersey, USA.

Zar, J.H. 2010. Biostatistical analysis 5th ed. Simon and Schuster N.J., U.S.A.

Zen-Bio. 2008. Triglyceride assay kit TG-1-NC. Zen-Bio, Inc.
Research Triangle Park, NC USA.



APPENDIX A
DIET COMPOSITION AND SPECIES RICHNESS



Table A.1. Collection dates and sample size (n= number of pellet groups/samples) for mule deer fecal materials from the North Kaibab winter range (GMU 12A West).

Collection Year	Late Fall	Mid-Winter	Late Winter
2005-06	No Collection	Jan 7-10, 2005 (n=18) Feb 6-11, 2006 (n=18)	Mar. 28-29, 2006 (n=30)
2006-07	Nov. 22-25, 2006 (n=15)	Dec. 28-31, 2006 (n=9) Feb 5-12, 2007 (n=8)	Mar. 21-22, 2007 (n=30)
2007-08	Nov.9-11, 2007 (n=13)	Jan 10-15, 2008 (n=16)	Mar. 18-19, 2008 (n=29)

Table A.2: Forage species utilized by N. Kaibab mule deer sorted by year, season, and forage class.

Species	Forage Class	Mid-winter 2005	Early Spring 2006	Late Fall 2006	Mid-winter 2007	Early Spring 2007	Late Fall 2007	Mid-winter 2008	Early Spring 2008
Blue grama	1	2%	3%	3%	5%	4%	4%	2%	6%
Smooth brome	1	0%	n-a	n-a	6%	4%	2%	2%	7%
Cheatgrass	1	1%	1%	2%	6%	3%	2%	2%	4%
Crested wheatgrass	1	5%	9%	8%	n-a	4%	6%	2%	6%
Bottlebrush squirreltail	1	5%	4%	5%	4%	4%	4%	4%	2%
Western wheatgrass	1	3%	3%	3%	1%	5%	2%	1%	3%
Festuca arizonica	1	6%	0%	2%	n-a	n-a	5%	n-a	2%
Grass spp.	1	1%	0%	2%	1%	1%	1%	0%	0%
Forage Class	Grass = 1	20%	20%	25%	21%	25%	26%	12%	27%
Rose pussytoe	2	0%	n-a	n-a	1%	1%	5%	1%	n-a
White sage	2	1%	1%	3%	2%	3%	8%	1%	2%
Mojave thistle (*Cirsium mohavense)*	2	0%	n-a	n-a	n-a	n-a	1%	n-a	n-a
Bull thistle *(Cirsium vulgare)*	2	n-a	n-a	0%	n-a	n-a	0%	1%	1%
Desert trumpet	2	5%	0%	1%	n-a	1%	1%	2%	0%
Forb (unidentified)	2	0%	0%	0%	n-a	0%	0%	n-a	0%
Sticky geranium	2	0%	n-a	0%	n-a	n-a	0%	n-a	2%
Lupine	2	2%	0%	6%	0%	0%	1%	0%	0%
Penstemon *(Penstemon spp.)*	2	n-a	n-a	1%	n-a	n-a	1%	n-a	n-a
Scarlet globe mallow	2	1%	0%	1%	2%	2%	1%	1%	1%
Mullen	2	1%	1%	2%	2%	0%	1%	1%	2%
Forb spp.	2	n-a	n-a	n-a	n-a	n-a	n-a	n-a	n-a
Forage Class	Forb = 2	5%	1%	7%	6%	6%	16%	5%	5%

Table A.2 (Cont.)

Species	Forage Class	Mid-winter	Early Spring	Late Fall	Mid-winter	Early Spring	Late Fall	Mid-winter	Early Spring
Utah serviceberry	3	0%	1%	0%	n-a	5%	2%	n-a	n-a
Artemesia nova	3	1%	n-a	n-a	n-a	n-a	n-a	n-a	n-a
Manzanita *(Arctostaphylos pungens spp.)*	3	0%	0%	0%	n-a	4%	1%	2%	1%
Big sage	3	24%	26%	14%	3%	2%	2%	6%	9%
Four-wing saltbush	3	1%	8%	1%	4%	2%	2%	1%	2%
Deerbrush *(Ceanothus integerrium)*	3	2%	0%	1%	n-a	0%	1%	1%	0%
Rubber rabbitbush	3	2%	2%	0%	4%	4%	6%	5%	8%
Mormon tea (*Ephedra viridis)*	3	1%	1%	3%	n-a	1%	1%	0%	1%
Apache plume	3	3%	3%	1%	n-a	8%	3%	12%	8%
Broom snakeweed *(Gutierreza sarothrae)*	3	3%	0%	1%	1%	1%	4%	0%	0%
Bassia prostrata	3	2%	6%	9%	1%	3%	2%	3%	3%
Krascheninnikovia Lanata	3	1%	0%	2%	4%	2%	1%	2%	0%
Wolfberry (*Lycium torreyi)*	3	0%	0%	0%	0%	0%	0%	2%	0%
Freemont holly (*Mohonia freemonti)*	3	0%	0%	0%	n-a	n-a	2%	0%	n-a
Creeping barberry	3	1%	0%	1%	n-a	n-a	4%	2%	2%
Mexican cliffrose	3	12%	13%	11%	13%	9%	8%	18%	11%
Gambel's oak	3	3%	1%	3%	1%	2%	2%	3%	1%
New Mexico locust *Robinia neomexicana)*	3	1%	0%	1%	0%	2%	0%	1%	2%
Wild rose *(Rosa woodsii)*	3	1%	2%	6%	n-a	8%	5%	1%	3%

Table A.2 (Cont.)

Species	Forage Class	Mid-winter	Early Spring	Late Fall	Mid-winter	Early Spring	Late Fall	Mid-winter	Early Spring
Willow	3	6%	3%	3%	7%	3%	1%	3%	4%
Utah elderberry (*Sambucas Mexicana)*	3	2%	n-a	n-a	n-a	1%	n-a	0%	n-a
Unk 1	3	0%	n-a	n-a	n-a	1%	n-a	1%	n-a
Shrub spp.	3	1%	2%	1%	1%	5%	3%	1%	3%
Forage Class	Shrub = 3	61%	59%	54%	29%	55%	37%	60%	54%

Species	Forage Class	Mid-winter	Early Spring	Late Fall	Mid-winter	Early Spring	Late Fall	Mid-winter	Early Spring
Utah Juniper	4	7%	8%	9%	43%	14%	9%	22%	13%
Piñon pine	4	6%	11%	3%	n-a	1%	11%	0%	1%
Ponderosa pine	4	5%	0%	1%	n-a	0%	1%	n-a	0%
Forage Class	Conifer = 4	14%	19%	13%	43%	15%	21%	23%	14%

Table A.3 Analysis of Variance, Species Richnesss

Variable	Df	Sum Sq	Mean Sq	F-Value	Pr(>F)
Year	1	124.4	124.39	4.478	0.0441
Season	2	130.9	65.44	2.356	0.1148
Year:Season	2	37.4	18.68	0.672	0.5191
Residuals	26	722.2	27.78		

Table A.3 Bonferonni Multiple Comparison, Species Richness for Year.

	1	2
2	1	-
3	0.206	0.082

Table A.5 Analysis of Variance for Forage Class Composition
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	2	99	50	0.503	0.606445
Year	2	2	1	0.01	0.989786
FC	3	151574	50525	512.894	< 2e-16
Season:Year	3	97	32	0.329	0.804382
Season:FC	6	2791	465	4.721	0.000297
Year:FC	6	1366	228	2.312	0.039667
Season:Year:FC	9	6660	740	7.512	2.99E-08
Residuals	96	9457	99		

Table A.6. Tukey HSD for Forage Class Composition
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

Season				
	diff	lwr	upr	p adj
MW-LF	1.5734354	-3.8188670	6.9657380	0.7671859
ES-LF	2.2545079	-3.1377950	7.6468110	0.5815981
ES-MW	0.6810724	-4.1419500	5.5040950	0.9396469
Year				
	diff	lwr	upr	p adj
MW-LF	0.2996794	-5.0926230	5.6919820	0.9903972
ES-LF	0.1030059	-5.2892970	5.4953090	0.9988605
ES-MW	-0.1966735	-5.0196960	4.6263490	0.9948182
FC				
	diff	lwr	upr	p adj
G-F	60.6180130	54.1304190	67.1056100	0.0000000
S-F	95.9497660	89.4621720	102.4373600	0.0000000
T-F	58.1817390	51.6941450	64.6693300	0.0000000
S-G	35.3317530	28.8441600	41.8193500	0.0000000
T-G	-2.4362730	-8.9238670	4.0513200	0.7600987
T-S	-37.7680270	-44.2556210	-31.2804300	0.0000000
	G = Grass	F= Forbs	S = Shrubs	T = Trees

Table A.7 , Analysis of Variance, Species Composition
Transformation: : $Y = \sqrt{x} + \sqrt{(x+1)}$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
SPP	44	137440	3124	42.369	< 2e-16
YEAR	2	6673	3336	45.255	< 2e-16
SEASON	2	1204	602	8.167	0.000302
SPP:YEAR	88	61095	694	9.417	< 2e-16
SPP:SEASON	88	23303	265	3.592	< 2e-16
YEAR:SEASON	3	4790	1597	21.658	1.26E-13
SPP:YEAR:SEASON	132	44302	336	4.552	< 2e-16
Residuals	1080	79623	74		

Table A.8 , Bonferonni Multiple Comparison, Species Composition
Variable: Season Transformation: : $Y = \sqrt{x} + \sqrt{(x+1)}$

	LF	MW
MW	1	n/a
ES	0.81	0.22

Table A.9 , Bonferonni Multiple Comparison, Species Composition
Variable: Year Transformation: : $Y = \sqrt{x} + \sqrt{(x+1)}$

	YR1	YR2
YR2	1	n/a
YR3	4.30E-05	2.00E-05

APPENDIX B
DIET QUALITY



Table B.1. Analysis of Variance for Forage Quality, DPI

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	2	270.8	135.4	3.076	0.0647
Year	2	1,931.3	965.7	21.937	3.82E-06
Season:Year	3	424.8	141.6	3.217	0.0407
Residuals	24	1,056.5	44.0		

Table B.2. Tukey HSD for Forage Quality, DPI

Season				
	diff	lwr	upr	p adj
LF-ES	-7.166667	-14.729369	0.3960353	0.0656003
MW-ES	-4.666667	-11.430953	2.0976196	0.2174396
MW-LF	2.500000	-5.062702	10.0627020	0.6911350
Year				
	diff	lwr	upr	p adj
YR2-YR1	-17.38889	-24.951591	-9.826187	0.0000187
YR3-YR1	-16.38889	-23.951591	-8.826187	0.0000425
YR3-YR2	1.00000	-5.764286	7.764286	0.9278472

Table B.3. Analysis of Variance for Forage Quality, MEI

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	2	1498930	749465	11.761	0.000275
Year	2	615144	307572	4.827	0.017307
Season:Year	3	1062205	354068	5.556	0.004846
Residuals	24	1529399	63725		

Table B.4. Tukey HSD for Forage Quality, MEI

Season				
	diff	lwr	upr	p adj
LF-ES	-413.8750	-701.6166	-126.1334	0.0040368
MW-ES	134.8333	-122.5305	392.1972	0.4044002
MW-LF	548.7083	260.9668	836.4499	0.0002172
Year				
	diff	lwr	upr	p adj
YR2-YR1	-270.77778	-558.5193	16.96379	0.0678403
YR3-YR1	-324.44444	-612.1860	-36.70288	0.0250116
YR3-YR2	-53.66667	-311.0305	203.69721	0.8620059

APPENDIX C
METABOLIC INDICATORS



Table C.1. Analysis of Variance for Carbohydrates, Phosphates
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	1.891	1.8908	5.902	0.0177
Year	1	0.315	0.3145	0.982	0.3252
Season:Year	1	0.265	0.2648	0.827	0.3664
Residuals	70	22.428	0.3204		

Table C.2. Tukey HSD for Carbohydrates, Phosphates
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

Season				
	diff	lwr	upr	p adj
LF-ES	0.3196999	0.05723162	0.5821682	0.0176965

Table C.3. Analysis of Variance for Carbohydrates, Alkaline Phosphatase
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1.000	5.400	5.450	0.205	0.652
Year	2.000	102.300	51.160	1.926	0.154
Season:Year	2.000	39.700	19.860	0.748	0.477
Residuals	66.000	1752.600	26.550		

Table C.4. Tukey HSD for Carbohydrates, Alkaline Phosphatase
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

Season				
	diff	lwr	upr	p adj
LW-LF	-0.5578991	-3.017339	1.901541	0.6521058
Year				
	diff	lwr	upr	p adj
Yr2-YR1	-2.803537	-6.230390	0.6233168	0.1298904
YR3-YR1	-1.364412	-5.039303	2.3104794	0.6483481
YR3-YR2	1.439125	-2.235766	5.1140161	0.6177688

Table C.5. Analysis of Variance for Proteins, Creatinine
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	4.69	4.691	14.377	0.000266
Year	2	33.54	16.772	51.405	9.210E-16
Season:Year	2	4.65	2.324	7.122	0.001325
Residuals	93	30.34	0.326		

Table C.6. Tukey HSD for Proteins, Creatinine
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

Season				
	diff	lwr	upr	p adj
LF-ES	-0.4380546	-0.6674752	-0.2086341	0.0002658
Year				
	diff	lwr	upr	p adj
YR2-YR1	-0.3782155	-0.6949853	-0.06144574	0.0150068
YR3-YR1	1.1032456	0.7546822	1.45180911	0.0000000
YR3-YR2	1.4814611	1.1252022	1.83772009	0.0000000

Table C.7. Bonferroni multiple comparisons for Creatinine

Creatinine and YEAR
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

	YR1	YR2
YR 2	0.065	-
YR 3	3.0E-10	2.6E-14

Creatinine and SEASON

	LF
ES	0.011

Table C.8. Analysis of Variance for Proteins, PUN

Proteins, PUN, ANOVATransformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	3455	3455	92.242	1.41E-15
Year	2	82	41	1.096	0.339
Season:Year	2	205	103	2.737	0.070
Residuals	93	3483	37		

Table C.9. Tukey HSD for Proteins, PUN
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

Season				
	diff	lwr	upr	p adj
LF-ES	-11.88836	-14.34643	-9.430297	0
Year				
	diff	lwr	upr	p adj
YR2-YR1	-0.1324858	-3.526430	3.261458	0.9952457
YR3-YR1	-2.1429781	-5.877567	1.591611	0.3624982
YR3-YR2	-2.0104924	-5.827532	1.806548	0.4244610

Table C.10. Analysis of Variance for Proteins, Creatinine PUN Ratio
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	4717	4717	32.41	1.45E-07
Year	2	6953	3477	23.89	4.25E-09
Season:Year	2	3742	1871	12.86	1.18E-05
Residuals	93	13536	146		

Table C.11. Tukey HSD for Proteins, Creatinine PUN Ratio
Transformation: $Y = \sqrt{x} + \sqrt{(x+1)}$

Season				
	diff	lwr	upr	p adj
LF-ES	-13.89107	-18.73667	-9.045466	1E-07
Year				
	diff	lwr	upr	p adj
YR2-YR1	11.56012	4.869611	18.250629	0.0002446
YR3-YR1	-10.01417	-17.376200	-2.652149	0.0046852
YR3-YR2	-21.57429	-29.098856	-14.049732	0.0000000

Table C.12. Analysis of Variance for Lipids, Albumin

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	1.28	1.2791	0.976	0.328
Year	1	0.83	0.8286	0.632	0.430
Season:Year	1	0.15	0.1526	0.116	0.734
	5				
Residuals	1	66.86	1.3110		

Table C.13. Analysis of Variance for Lipids, Fatty Acids

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	2.61	2.610	2.058	0.1551
Year	2	44.26	22.129	17.448	4.36E-07
Season:Year	2	6.99	3.497	2.758	0.0691
Residuals	86	109.07	1.268		

Table C.14. Bonferroni multiple comparison for Free Fatty Acids
(Bonferrono Pairwise comparisons using t tests with pooled SD)

Fatty Acid and Year		
	YR1	YR2
YR 2	1	-
YR 3	3.7E-06	6.3E-06

Fatty Acid and Season	
	LF
ES	0.23

Table C.15. Analysis of Variance for Lipids, Triglycerides

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	27.3	27.32	2.630	0.1085
Year	1	49.2	49.20	4.735	0.0322
Season:Year	1	9.4	9.40	0.904	0.3442
Residuals	88	914.4	10.39		

Table C.16. Tukey HSD for Lipids, Triglycerides

Season				
	diff	lwr	upr	p adj
LW-LF	1.090194	-0.2382566	2.418645	0.1064628
Year				
	diff	lwr	upr	p adj
YR2-YR1	-2.0782979	-3.987350	-0.1692459	0.0295229
YR3-YR1	-1.7538178	-3.729874	0.2222388	0.0923932
YR3-YR2	0.3244801	-1.651576	2.3005367	0.9190210

Table C.17. Analysis of Variance for Lipids, Mono-glycerides

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	7.6	7.56	0.470	0.49488
Year	1	140.7	140.73	8.748	0.00398
Season:Year	1	5.3	5.29	0.329	0.56798
Residuals	88	1415.6	16.09		

Table C.18. Tukey HSD for Lipids, Mono-glycerides

Season				
	diff	lwr	upr	p adj
LW-LF	0.5735006	-1.079531	2.226532	0.4922451
Year				
	diff	lwr	upr	p adj
YR2-YR1	-3.001148859	-5.376640	-0.625658	0.0094239
YR3-YR1	-2.993187648	-5.452054	-0.534321	0.0128996
YR3-YR2	0.007961211	-2.450905	2.466828	0.9999671

Table C.19. Analysis of Variance for Cholesterol

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Season	1	2.169	2.169	32.26	1.64E-07
Year	2	14.887	7.443	110.70	<2E-16
Season:Year	2	4.629	2.315	34.42	7.88E-12
Residuals	90	6.051	0.067		

Table C.20. Tukey HSD for Cholesterol

Season				
	diff	lwr	upr	p adj
LW-ES	-0.300625	-0.4057803	-0.1954697	2E-07
Year				
	diff	lwr	upr	p adj
YR2-YR1	-0.3253125	-0.4797997	-0.1708253	7.8E-06
YR3-YR1	-0.9490625	-1.1035497	-0.7945753	0.0E+00
YR3-YR2	-0.6237500	-0.7782372	-0.4692628	0.0E+00

APPENDIX D
MODELING



Table D.1 Correlation Matrix, North Kaibab mule deer Metabolic and Dietary Variables

	AlkP	Phos	Alb	Chol	FA	Gly	TAG	C	PUN	PUN:C	DDP	DME
AlkP	1.000	0.041	0.019	0.183	0.160	0.156	0.142	0.021	-0.005	-0.116	-0.029	-0.020
Phos	0.041	1.000	-0.077	0.236	-0.217	-0.045	-0.109	-0.043	-0.259	-0.177	0.142	0.030
Alb	0.019	-0.077	1.000	-0.023	0.241	0.063	0.145	0.156	0.034	-0.139	-0.193	-0.036
Chol	0.183	0.236	-0.023	1.000	-0.246	0.191	0.134	-0.391	-0.214	0.113	0.173	0.302
FA	0.160	-0.217	0.241	-0.246	1.000	0.384	0.468	0.476	0.137	-0.361	-0.223	-0.246
Gly	0.156	-0.045	0.063	0.191	0.384	1.000	0.964	0.012	0.306	0.243	0.150	0.078
TAG	0.142	-0.109	0.145	0.134	0.468	0.964	1.000	0.067	0.339	0.221	0.136	0.074
C	0.021	-0.043	0.156	-0.391	0.476	0.012	0.067	1.000	0.430	-0.567	-0.141	-0.183
PUN	-0.005	-0.259	0.034	-0.214	0.137	0.306	0.339	0.430	1.000	0.462	-0.083	0.035
PUN_C	-0.116	-0.177	-0.139	0.113	-0.361	0.243	0.221	-0.567	0.462	1.000	0.083	0.250
DDP	-0.029	0.142	-0.193	0.173	-0.223	0.150	0.136	-0.141	-0.083	0.083	1.000	0.633
DME	-0.020	0.030	-0.036	0.302	-0.246	0.078	0.074	-0.183	0.035	0.250	0.633	1.000

Table D.2 Principle Component Analysis, North Kaibab mule deer Metabolic and Dietary Variables

LOADINGS

	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10	Comp.11	Comp.12
AlkP	-0.104		-0.35	-0.221	-0.309	0.781		0.321				
Phos	0.201		-0.406		-0.294	-0.379	0.621	0.304	0.279			
Alb	-0.205		-0.121	-0.102	0.821	0.101	0.376	0.265	-0.153			
Chol	0.208	-0.302	-0.354	-0.26		0.105	0.195	-0.731		0.268		
FA	-0.492		-0.206				-0.288		0.607	0.494		
Gly	-0.358	-0.446	-0.153	-0.106		-0.244			-0.164	-0.284	0.678	
TAG	-0.402	-0.424	-0.136			-0.22			-0.115	-0.187	-0.728	
C	-0.379	0.269		0.469	-0.137		0.268	-0.27	-0.156	-0.133		-0.59
PUN	-0.319	-0.177	0.448	0.184	-0.186	0.208	0.46	-0.137		0.218		0.525
PUN_C		-0.414	0.5	-0.232			0.142	0.194	0.209	0.216		-0.604
DDP	0.191	-0.328	-0.171	0.565			-0.202	0.242	-0.384	0.509		
DME	0.202	-0.363		0.476	0.268	0.252			0.518	-0.431		

	Comp.1	Comp.2	Comp.3	Comp.4	Comp.5	Comp.6	Comp.7	Comp.8	Comp.9	Comp.10	Comp.11	Comp.12
Standard Deviation	1.644	1.582	1.271	1.129	0.990	0.947	0.921	0.747	0.587	0.481	0.166	0.133
Variance Proportion	0.225	0.209	0.135	0.106	0.082	0.075	0.071	0.047	0.029	0.019	0.002	0.001
Cumulative Variance	0.225	0.434	0.568	0.675	0.756	0.831	0.902	0.948	0.977	0.996	0.999	1.000

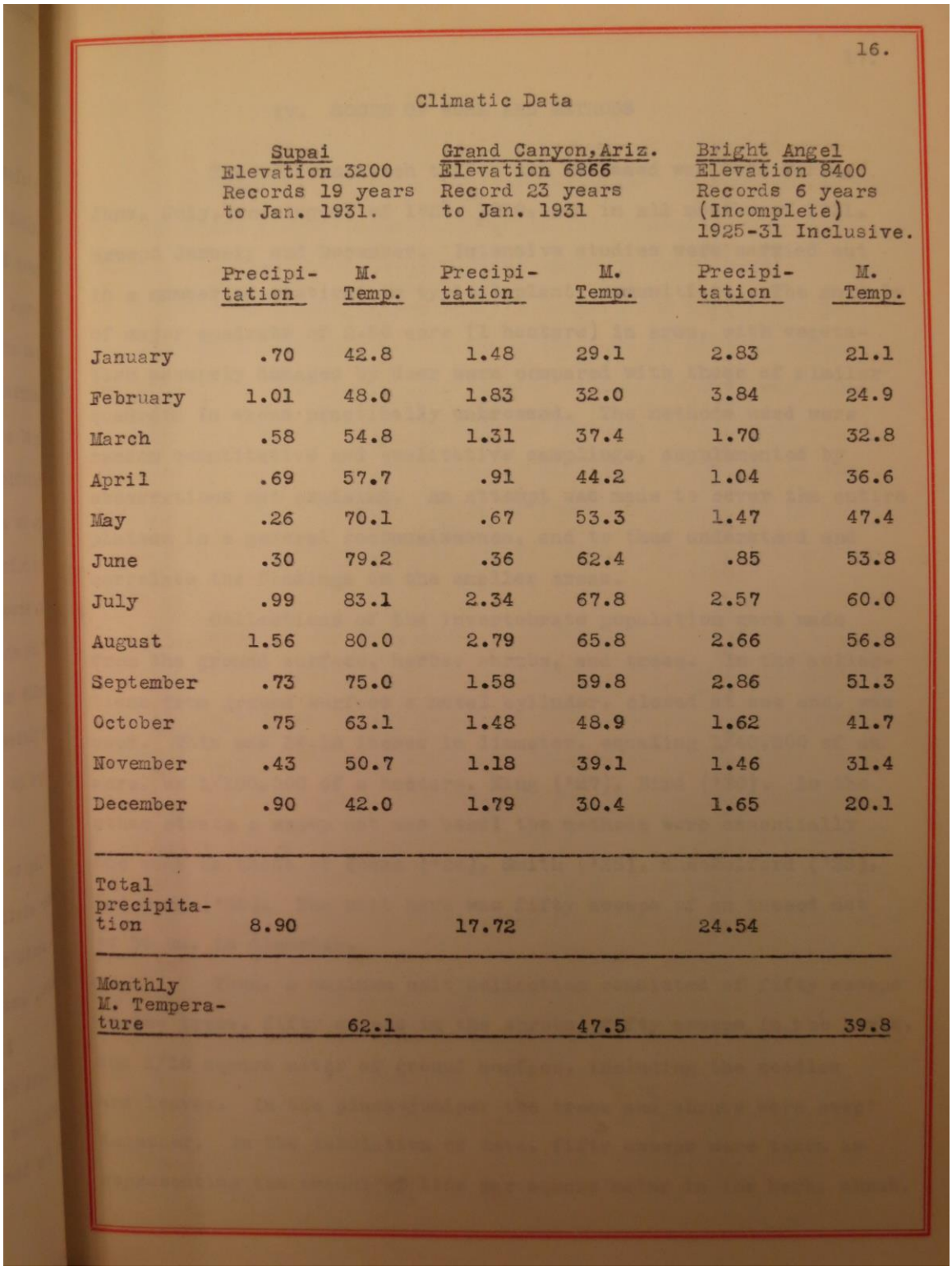
Note: Missing values in Loadings are not shown as the values were too small, insignificant, and close to zero for R to display.

Table D.3 Stepwise Regression Results North Kaibab mule deer Metabolic and Dietary Variables

Y = PC4 + PC5 +PC1 +PC3

	PC4	PC5	PC1	PC3
AlkP	-0.23	-0.21	0.03	-0.36
Phos	0.13	-0.29	0.09	-0.56
Alb	0.02	0.91	0	-0.11
Chol	0.12	0.09	-0.5	-0.48
FA	-0.16	0.21	0.54	-0.01
Gly	0.07	0	-0.06	0.14
TAG	0.08	0.08	0	0.18
C	0.13	-0.07	0.96	0.14
PUN	0.03	-0.21	0.17	0.79
PUN_C	-0.04	-0.13	-0.76	0.63
DDP	0.92	-0.11	0.18	-0.08
DME	0.9	0.13	-0.03	0.03

APPENDIX E
HISTORICAL AND CONTEMPORARY PHOTOGRAPHS AND DIAGRAMS NORTH KAIBAB WINTER RANGE



16.

Climatic Data

	Supai Elevation 3200 Records 19 years to Jan. 1931.		Grand Canyon, Ariz. Elevation 6866 Record 23 years to Jan. 1931		Bright Angel Elevation 8400 Records 6 years (Incomplete) 1925-31 Inclusive.	
	Precipitation	M. Temp.	Precipitation	M. Temp.	Precipitation	M. Temp.
January	.70	42.8	1.48	29.1	2.83	21.1
February	1.01	48.0	1.83	32.0	3.84	24.9
March	.58	54.8	1.31	37.4	1.70	32.8
April	.69	57.7	.91	44.2	1.04	36.6
May	.26	70.1	.67	53.3	1.47	47.4
June	.30	79.2	.36	62.4	.85	53.8
July	.99	83.1	2.34	67.8	2.57	60.0
August	1.56	80.0	2.79	65.8	2.66	56.8
September	.73	75.0	1.58	59.8	2.86	51.3
October	.75	63.1	1.48	48.9	1.62	41.7
November	.43	50.7	1.18	39.1	1.46	31.4
December	.90	42.0	1.79	30.4	1.65	20.1
Total precipitation	8.90		17.72		24.54	
Monthly M. Temperature		62.1		47.5		39.8

Photograph E.1: Photographic copy of climate data from Rassmussens's 1932 dissertation. These numbers were utilized to create Figure 3 – 9. Digital photograph has been provided by the library of the University of Illinois – Champaign.

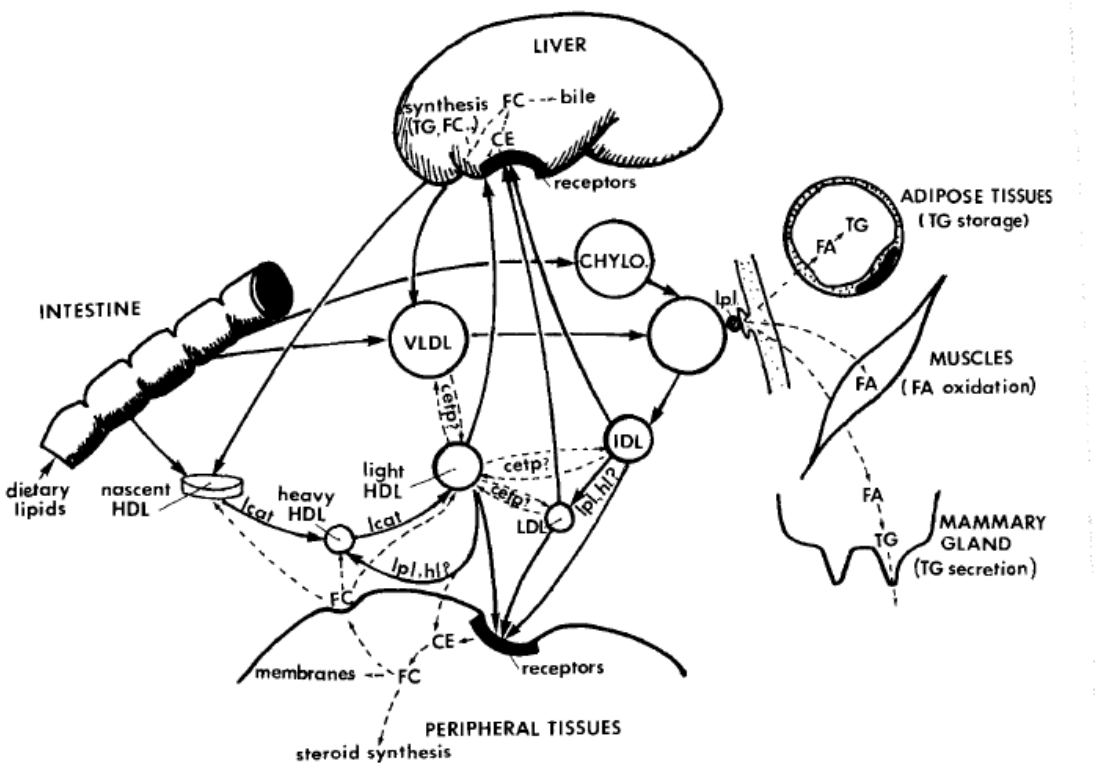


Figure 1. The tissue origins and postsecretory metabolic transformation of lipoproteins within the plasma compartment in ruminants. HDL = High density lipoproteins, LDL = low density lipoproteins, TG = triglyceride, CE = cholesterol ester, cetp = CE transfer protein, lcat = lecithin:cholesterol acyltransferase, CHYLO = chylomicron, VLDL = very low density lipoproteins, lpl = lipoprotein lipase, hl = hepatic lipase, FA = fatty acid, FC = free cholesterol.

Journal of Dairy Science Vol. 76, No. 12, 1993

Illustration E.2: Diagram illustrating the conversion of dietary lipids into cholesterol (HDL, High Density Lipoproteins; LDL, Low Density Lipoproteins; VLDL, Very Low Density Lipoproteins) and other artifacts of lipid metabolism within a ruminant animal. Transfer from the digestive tract can become complex, including peripheral tissues (i.e. muscle or organs) to the liver for cholesterol synthesis. Transfer of fatty acid can also be to or from adipose for lipolysis or lipogenesis, or secretion through the mammary gland for neonatal metabolic needs. Taken from the Bauchart, 1993.