RUDDED GROUSE NUTRITION AND FORAGING IN THE SOUTHERN APPALACHIANS

by

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Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Fisheries and Wildlife Sciences

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August 1994

Blacksburg, Virginia
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(ABSTRACT)

Feeding trials showed that ruffed grouse (Bonasa umbellus) performed well on diets containing 20% Christmas hollyfern (Polystichum acrostichoides) or mountain laurel (Kalmia latifolia), but diets containing 40% of these forages resulted in lower protein and energy intake and the Christmas hollyfern diet caused a loss of body mass. Grouse were not able to maintain themselves solely on evergreen leaves. Glucuronide excretion was greatest for the 40% mountain laurel diet. Ornithine conjugate excretion was greatest for a diet with 40% deciduous leaves. Sulfate excretion did not vary among diets.

Intake rate of leaves was an asymptotic function of bite size when the density of bites did not limit intake. Intake rate of leaves decreased at plants densities <322 plants/m². The maximum intake rate of leaves was 25% of the intake rate of aspen buds observed in wild grouse (Huempfner and Tester 1988). Intake rate of raisins was an asymptotic function of bite size and was 20 times greater than the intake rate of leaves. Ruffed grouse in the Southeast must forage for >100 min/day under ideal conditions to satisfy energy requirements.
Convex polygon home range size of free-ranging ruffed grouse during winter was $14.0 \pm 2.2$ ha. Activity times of ruffed grouse averaged 5-6 hours/day. These activity times are longer than those of other grouse species during winter. Unlike other grouse species, grouse in southwest Virginia were not strongly crepuscular. These high levels of activity and estimates of foraging times calculated from foraging rates of captive grouse support the hypothesis that ruffed grouse in the Southeast must spend large amounts of time foraging.

Ruffed grouse and bobwhite (*Colinus virginianus*) showed no negative effects to diets with 6% quebracho tannin. Dry matter intake was reduced in grouse consuming a diet with either 8% quebracho or 8% tannic acid. All birds excreted a larger percent of feces from the ceca when consuming diets containing tannin.

Artificial nests showed that nest predation in the Southeast is not greater than areas where ruffed grouse densities are higher, nor was nest predation density-dependent. Nest predator communities are not more diverse, nor are nest predators more abundant in the Southeast compared to areas where grouse densities are higher.
ACKNOWLEDGEMENTS

This research was funded by the John Lee Pratt Animal Nutrition Fellowship program at VPI&SU, the Department of Fisheries and Wildlife Sciences at VPI&SU, and the Ruffed Grouse Society. I would like to extend special thanks to my committee chair, Roy Kirkpatrick, for his support and encouragement. Though example, Roy extended my horizons in science, teaching, and wildlife. My graduate committee, David Bevan, James Fraser, Dean Stauffer, Michael Vaughan, and Kenneth Webb, provided valuable input and support.

Anne Robinson, Ian MacCoubrey, and Ed Mullins, technicians in the Department of Fisheries and Wildlife Sciences, provided invaluable assistance many times during this research. Nelson Lafon did a tremendous amount of work in completing the ruffed grouse rate of passage study and I appreciate his input on that aspect of the project and his willingness to let me include his work in my dissertation. I would like to thank Dr. Calvert Larsen for veterinarian services and David Gemmell for housing captive grouse in the Lab Animal Resources center. Judy Baker in the Virginia Tech Forage Testing Lab, Wendy Wark in the Dairy Nutrition Lab and Ali Battacharya in the Biochemistry Department gladly gave their time and expertise in helping with various analyses. I would like to thank Dave Stephen, Gary Norman, Bill Keffer, and The Virginia Department of Game and Inland Fisheries (VDGIF) for their support of this project. Several VDGIF and United States Forest Service personnel reported grouse nests to me and assisted me in collecting eggs. Special
thanks are due to Cecil Broce, Tom and Barbara Jones, Lucy Forrestal, and Donald
Strayley for allowing me to conduct research on their property. I would also like to
thank the following work-study students and part time technicians who assisted on this
project; their help was invaluable and their company and interest was appreciated:
Laura Krupinski, Julia Ohanesian, Terry Quesenberry, Lisa Scipioni, Jennifer Allen,
Vic Lester, Ian Nelson, and Kevin Drury.

The friendship, support, humor, and advice of the graduate students in the
Department of Fisheries and Wildlife Sciences made my time in Blacksburg
enjoyable. Special thanks are due to Bill Ensign, Mike Schrage, Alice Chung-
MacCoubrey, and Keith Grasman for help in the lab and field.

My most sincere appreciation is given to my parents, Glenn and Lee, and my
brother and sister, Mike and Julie, for their friendship, support, inspiration, and
encouragement. And finally, to my wife, Liisa, and my daughter, Nicole, thanks for
your unflattering love, support, and sacrifice that truly made this endeavor possible.
You both have added wonderful new dimensions to my life.
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CHAPTER 1

RUDDED GROUSE CONSUMPTION AND DETOXIFICATION OF EVERGREEN LEAVES EATEN DURING WINTER

INTRODUCTION

The winter diets of forest grouse often are dominated by abundant, but poor quality forages. These forages are considered poor quality because they contain high fiber, low nutrient concentrations, and high levels of secondary plant metabolites (Bryant and Kuropat 1980, Moss and Hanssen 1980, Andreev 1988, Bergerud and Gratson 1988). Secondary plant metabolites are a vast array of chemicals produced by plants that deter herbivore foraging through toxicity, decreased palatability, or reduction in nutrient availability (Rhoades 1979). Grouse, along with other herbivores, have evolved mechanisms to detoxify many of the secondary plant metabolites in their food. Recent research has focused on these adaptations in an attempt to understand forage preferences, the costs of detoxification, and possible toxicity (Remington 1990, Guglielmo 1993, Jakubas et al. 1993a, 1993b, 1993c). These studies have shown that nitrogen and energy costs of detoxification can be substantial for some winter forages (Remington 1990). Foraging patterns of ruffed grouse (Bonasa umbellus) consuming aspen (Populus tremuloides) are understood in light of coniferyl benzoate, a secondary plant metabolite found in aspen (Jakubas et al. 1989).

Ruffed grouse in the southeastern United States consume a variety of
evergreen leaves and ferns during the winter. These forages are abundant through most of the grouse’s southern range, an observation, that when considered with the lack of persistent snow cover, has caused some researchers to conclude that food is not a concern for grouse in the Southeast (Gullion 1984, Bergerud 1988). Despite the abundance of evergreen leaves and ferns, these forages rarely constitute >50% of the winter diet (Stafford and Dimmick 1979, Servello and Kirkpatrick 1987). Servello and Kirkpatrick (1987) hypothesized that high levels of plant secondary metabolites limit the amount of evergreen forages that can be consumed by ruffed grouse.

The amount of a toxic forage that a herbivore can consume may be controlled by the ability of the animal to buffer acids produced during detoxification (Foley 1992). The metabolism of plant toxins often involves conjugation with acidic molecules, such as glucuronic acid, amino acids, or mercapturic acid (through glutathione) (Sipes and Gandolfi 1991). Metabolites of the parent toxins also may contribute to the acid load (Jakubas et al 1993a). Internal pH is critical to an animal’s proper functioning and if acids resulting from secondary plant detoxification challenge the bicarbonate buffering system, consumption of forages high in toxins should be regulated (Foley 1992). Urine pH in ring-tailed possums (Pseudocheirus peregrinus) consuming toxic forages appeared to change quickly enough after the initiation of a foraging bout that the rate of foraging may be slowed or the foraging bout may be terminated, based on the acid-base balance of the animal (Foley 1992). Heavy dietary toxin loads often are associated with large increases in ammonium nitrogen in the
urine (Remington 1990, Foley 1992). Ammonium nitrogen excretion may be a
response by the animal to acidosis and an attempt to conserve bicarbonate or excrete

Two common evergreen forages eaten by ruffed grouse during the winter in
the Southeast are mountain laurel (Kalmia latifolia) and Christmas hollyfern
(Polystichum acrostichoides). These forages have high levels of phenois and tannins
(Servello and Kirkpatrick 1987) and mountain laurel contains a toxic diterpenoid,
grayanotoxin I (andromedotoxin) (Mancini and Edwards 1979). Toxins in Christmas
hollyfern have not been reported, but many fern species have high levels of tannins
and other secondary plant metabolites and appear to be avoided by herbivores
(Hendrix 1930). The objectives of this study were 1) to determine ruffed grouse
performance, as measured by changes in body weight, when consuming diets with
different levels of mountain laurel, Christmas hollyfern, or deciduous leaves, 2) to
investigate the conjugate-based detoxification strategies of ruffed grouse consuming
these same diets, and 3) to determine if ammonium nitrogen excretion is related to the
excretion of acidic conjugates, thus suggesting acidosis.

METHODS

Study Animals and Diets. Ruffed grouse were raised in captivity from eggs or chicks
collected from the wild in western Virginia, or were obtained from a commercial
breeder whose stock came from southern Ohio. Grouse were 8 months old when
trials began and were housed outdoors in covered 2x3x2 m pens at the Center Woods
Research Facility on the Virginia Tech campus. A maintenance diet and water were provided ad libitum.

The maintenance diet was prepared by grinding equal weights of Purina Game Bird Starter and Purina Horse Chow-100 in a Wiley mill, through a 1 mm screen. Two percent vegetable oil was added to increase palatability (Servello et al. 1987). The grouse consumed this diet for >6 weeks prior to the feeding trials. The diet was chosen to represent herbaceous leaves and fruits, which are the high quality forages consumed by ruffed grouse during winter in the Southeast (Servello and Kirkpatrick 1987). Metabolizable energy (ME) of the maintenance diet (Table 1.1) was similar to that of herbaceous leaves and fruits, while neutral detergent fiber was greater in the maintenance diet than most fruits and herbaceous leaves (Servello and Kirkpatrick 1987). Crude protein was similar to that of herbaceous leaves, but greater than that of fruits (Servello and Kirkpatrick 1987).

Mountain laurel, Christmas hollyfern, greenbriar (Smilax spp.), and honeysuckle (Lonicera japonica) leaves were collected near Blacksburg, VA during Dec 1992 and Jan 1993. Forages were dried under forced air at 30°C for 48 hours or to a constant weight, then ground in a Wiley mill through a 1 mm screen. Mountain laurel and Christmas hollyfern were mixed with the maintenance chow to form diets containing 10, 20 and 40% forage on a dry matter basis. Greenbriar and honeysuckle leaves were mixed together in a 40:60 ratio (w/w) to form a deciduous leaf mixture. This mixture was added to the maintenance chow at 20 and 40%.
Forty percent was chosen as the maximum level of test forage in the diet because the winter diet of grouse in the Southeast rarely contains more than 40% mountain laurel and usually contains <20% Christmas hollyfern (Nelson et al. 1938, Stafford and Dimmick 1979, Servello and Kirkpatrick 1987). All forages were tested at 40% of the diet so that animal performance and detoxification activity could be compared among forages.

**Study Design and Feeding Trials.** An incomplete block study design was used to test 6 diets: 20 and 40% mountain laurel (20% ML and 40% ML), 20 and 40% Christmas hollyfern (20% CHF and 40% CHF), 40% deciduous leaves (40% DEC), and a control (maintenance chow). For a given trial, 12 grouse (6 males and 6 females) were randomly assigned to the 6 treatments such that 2 grouse received each treatment. Three trials were completed, with the constraint during randomization that a grouse could not receive a diet it had been given previously. This study design resulted in 6 grouse on each of the 6 treatments.

Feeding trials were conducted during Jan-Mar 1993. Each trial was 10 days in duration. Carry-over effects were reduced between trials by allowing grouse to consume maintenance chow *ad libitum* for 10 days between trials. On the first day of each trial, grouse were weighed to the nearest g on an electronic balance and placed in 45X60X45 cm wire cages, contained within the outdoor pens in which the grouse were normally housed. Feces and spilled feed were collected on sheets of linoleum
placed under the cages. To help grouse acclimate to the test forage, a diet containing 10% of the test forage (20% for deciduous leaves) was offered on the first day of a trial and a diet with 20% test forage was offered on days 2 and 3. Ninety grams (fresh weight) of the treatment diet (20 or 40% forage) was offered on days 4 through 10. A sample of each diet was taken daily and dried at 100°C for 24 hours to determine dry matter content.

All feces and uneaten feed were collected on days 7-10 of each trial. Grouse were weighed on days 7 and 10 and these weights were used to calculate percent weight change. Measurements expressed on a body mass basis use the average of these two weights. Uneaten food was collected daily, dried at 100°C for 24 hours and weighed. Feces of cecal and intestinal origin were collected separately at 0800 and 1600 hours daily. Feces were frozen immediately after collection and remained frozen until freeze-dried at the end of the trial. The dried feces were weighed and ground with a mortar and pestle. A composite fecal sample was made by mixing cecal and intestinal feces from each bird in the proportions they were produced. This composite sample was used in all subsequent analyses.

**Chemical Analysis.** Nitrogen content of feed and feces and neutral detergent fiber (NDF) of the feed were determined by the Virginia Tech Forage Testing Lab using Kjeldahl and detergent fiber (Goering and Van Soest 1970) procedures, respectively. Ammonia nitrogen content of feces was determined by the Kjeldahl procedure without
the sulfuric acid digestion step (Remington 1990). Gross energy of feed and feces was determined with a Parr adiabatic bomb calorimeter. Uric acid was assayed in triplicate by the colorimetric procedure of Marquardt (1983) and repeated if the coefficient of variation (CV) > 5%. Sample absorbance at 285 nm was determined on a Hitachi 100-30 spectrophotometer. Standards of 0.006 to 0.05 μM uric acid (Sigma Chemical Co., St. Louis, Missouri) were run with every assay.

Tannin content of the feeds was measured by the radial diffusion method (Hagerman 1987). Samples (0.4 g) were extracted overnight in 70% aqueous acetone (v/v). Tannic acid was used as a standard and was purified according to Hagerman and Klucher (1986).

Glucuronic acid, sulfates, and ornithine were assayed by the methods described by Jakubas et al. (1993a). Glucuronide conjugates were extracted from a 1 g fecal sample by stirring for 30 min in 50 ml 0.01M borax buffer (pH ca. 9.5). The extract was filtered and diluted 1:10 with borax buffer. This solution was used in the colorimetric procedure of Blumenkrantz and Asboe-Hansen (1973). Each extract was run in triplicate and repeated if the CV > 5%. Glucuronic acid (Sigma Chemical Co., St. Louis, Missouri) standards of 5 to 25 mg/ml were run with each assay.

Sulfate conjugates were assayed in triplicate by mixing 200 mg of feces with 10 ml of distilled water for 1 hour with frequent stirring. The solution was centrifuged at 1500 g for 10 min and 5 ml of the supernate fraction was used in the turbidimetric procedure of Lundquist et al. (1980). Absorbance was read at 600 nm
on a Milton-Roy Spectronic 301 spectrophotometer. Standards of 0.5 to 3.0 umols SO₄ were run with all samples.

Ornithine was assayed in duplicate by extracting a 0.5 g sample of feces in 50 ml of 0.01 M borax buffer (pH ca. 9.5) for 30 min with constant stirring. The solution was centrifuged at 1500 g for 15 minutes and the supernate fraction was used in the colorimetric procedure of Jakubas et al. (1993a). Sample absorbance was determined on a Milton Roy Spectronic 301 spectrophotometer and samples were repeated if CV > 10%.

Statistical Analysis. Response variables were tested for differences among treatments using a 3-way ANOVA (SAS 1988) in which treatment, trial, and individual grouse were class variables. The F statistic and p-value from the type III sums of squares (SAS 1988) were used to determine if treatment had a significant effect on the response variable. The type III sums of squares tested the effect of treatment, controlling for trial and individual grouse. Pairwise comparisons were done with Tukey’s honest significant difference test (SAS 1988), which controls the experimentwise error rate. Variables in which Bartlett’s test (Neter et al. 1985) detected heterogeneity of variance among treatments (α=0.05) were transformed using a log, square root, or inverse transformation (Neter et al. 1985:137) before performing ANOVA. Statistical tests were considered significant at $P<0.05$. Means are presented with standard errors.
Table 1.1. Chemical composition of diets fed to captive ruffed grouse during Jan-Mar 1993 in Blacksburg, Virginia.

<table>
<thead>
<tr>
<th>Diet&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Gross Energy (cal/g)</th>
<th>Crude Protein (%)</th>
<th>NDF (%)</th>
<th>Tannin equivalents&lt;sup&gt;b&lt;/sup&gt; (mg tannic acid/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4625</td>
<td>22.8</td>
<td>36.0</td>
<td>0.00</td>
</tr>
<tr>
<td>40% DEC</td>
<td>4653</td>
<td>19.4</td>
<td>30.2</td>
<td>1.54</td>
</tr>
<tr>
<td>20% ML</td>
<td>4716</td>
<td>20.2</td>
<td>34.2</td>
<td>8.37</td>
</tr>
<tr>
<td>40% ML</td>
<td>4815</td>
<td>16.9</td>
<td>32.0</td>
<td>14.98</td>
</tr>
<tr>
<td>20% CHF</td>
<td>4587</td>
<td>20.5</td>
<td>35.8</td>
<td>8.11</td>
</tr>
<tr>
<td>40% CHF</td>
<td>4585</td>
<td>18.57</td>
<td>35.8</td>
<td>12.17</td>
</tr>
</tbody>
</table>

<sup>a</sup>The control diet (maintenance chow) was 49% gamebird chow, 49% horse chow, and 2% vegetable oil. The other diets contained maintenance chow with 20 or 40% of the following: DEC = Greenbriar/Honeysuckle, ML = Mountain laurel, CHF = Christmas hollyfern.

<sup>b</sup>Assayed by radial diffusion method.
RESULTS

The chemical composition of the diets is shown in Table 1.1. Body mass of ruffed grouse at the beginning of each trial was not significantly different among treatments except for the 40% ML diet in which grouse were an average of 20 to 30 g heavier (Table 1.2). Ruffed grouse performance, as indicated by percent change in body mass, did not differ among diets except for the 40% CHF diet which caused a larger percent body mass loss than did the control diet (Table 1.2). Dry matter metabolizability was not different for the control and 40% DEC diets and was greater than the 40% ML and 40% CHF diets (Table 1.2). Although the ME concentration of the control and 40% DEC diets was greater than the 20% ML and 20% CHF diets, grouse were able to increase intake of the latter diets to maintain daily ME intake similar to that of the control (Table 1.2). Grouse consuming the 40% ML and 40% CHF diets were unable to compensate for lower ME by increasing dry matter intake, and ME intake was lower than the control for these two diets. Lower ME intake on the 40% CHF and 40% ML diets was not due to maximum fill limitations of the digestive tracts because dry matter intake on these diets was the lowest of all treatments.

Nitrogen intake was lowest for grouse consuming the 40% ML and 40% CHF diets (Table 1.3), a result of both low nitrogen content of the diets (Table 1.1) and low dry matter intake (Table 1.2). Nitrogen balance was lowest for the 40% CHF diet, but a high variance caused nitrogen balance to differ significantly only between
Table 1.2. Body mass on the first day of the feeding trials (mean (se)), body mass change, daily dry matter consumption and metabolizability, and metabolizable energy density and intake for ruffed grouse fed 6 diets during Jan-Mar 1993 in Blacksburg, Virginia. Means in a column with different letters were different at $\alpha = 0.05$. N = 6 for all treatments.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Beginning body mass (g)</th>
<th>Mass Change (%)</th>
<th>Dry Matter Metabolizability (%)</th>
<th>Daily Intake (g/kg$^{0.75}$)</th>
<th>Metabolizable Energy Density (cals/g)</th>
<th>Daily Intake (kcal/kg$^{0.75}$)</th>
<th>Percent feces from ceca $^{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>552 B (11.9)</td>
<td>-0.5 AB (0.49)</td>
<td>43.0 AB (0.54)</td>
<td>50.5 AB (2.55)</td>
<td>2387 A (24.0)</td>
<td>120.8 A (6.70)</td>
<td>10.7 BC (0.31)</td>
</tr>
<tr>
<td>40% DEC</td>
<td>545 B (17.0)</td>
<td>-0.8 AB (0.29)</td>
<td>43.6 A (0.66)</td>
<td>52.5 AB (2.49)</td>
<td>2284 A (30.2)</td>
<td>119.8 A (5.76)</td>
<td>14.1 AB (0.39)</td>
</tr>
<tr>
<td>20% ML</td>
<td>548 B (14.9)</td>
<td>-1.1 BC (0.34)</td>
<td>40.6 BC (0.47)</td>
<td>53.2 AB (1.79)</td>
<td>2130 B (25.1)</td>
<td>113.3 AB (4.13)</td>
<td>13.3 ABC (0.38)</td>
</tr>
<tr>
<td>40% ML</td>
<td>575 A $^{b}$ (8.2)</td>
<td>-1.2 ABC (0.81)</td>
<td>37.4 CD (1.49)</td>
<td>47.8 AB (0.89)</td>
<td>1937 C (54.9)</td>
<td>92.7 BC (3.93)</td>
<td>19.5 A (2.11)</td>
</tr>
<tr>
<td>20% CHF</td>
<td>553 B (18.0)</td>
<td>0.8 A (0.32)</td>
<td>40.4 BC (0.54)</td>
<td>54.1 A (2.98)</td>
<td>2149 B (20.0)</td>
<td>116.2 A (6.10)</td>
<td>10.1 C (0.34)</td>
</tr>
<tr>
<td>40% CHF</td>
<td>548 B (18.8)</td>
<td>-4.5 C (1.69)</td>
<td>34.7 D (2.53)</td>
<td>45.5 B (1.81)</td>
<td>1922 C (94.5)</td>
<td>87.7 C (6.20)</td>
<td>10.1 C (1.32)</td>
</tr>
</tbody>
</table>

$^{a}$The control diet (maintenance chow) was 49% gamebird chow, 49% horse chow and 2% vegetable oil. The other diets contained maintenance chow with 20 or 40% of the following: DEC = Greenbriar/Honeysuckle, ML = Mountain laurel, CHF = Christmas hollyfern.

$^{b}$N = 5 due to missing data. P-value for treatment effect was 0.0629.

$^{c}$Variance was not the same between treatments and could not be stabilized by transformation.
Table 1.3. Daily nitrogen intake (mean(se)) and balance, and uric acid and ammonia nitrogen excretion for ruffed grouse fed 6 diets during Jan-Mar 1993 in Blacksburg, Virginia. Means in a column with different letters were different at $\alpha = 0.05$. $N = 6$ for all treatments.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Daily Intake (g/kg$^{0.75}$)</th>
<th>Balance (mg/day)</th>
<th>Nitrogen</th>
<th>Uric Acid</th>
<th>Ammonia Nitrogen</th>
<th>Percent Fecal Nitrogen</th>
<th>Percent Fecal Nitrogen</th>
<th>Percent Fecal Nitrogen Characterized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Excreted (g/day)</td>
<td>Percent of Fecal Nitrogen</td>
<td>Excreted (mg/day)</td>
<td>Percent of Fecal Nitrogen</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.84 A (0.093)</td>
<td>-42.7 AB (30.44)</td>
<td>2.19 A (0.136)</td>
<td>48.8 BC (1.87)</td>
<td>74.8 A (2.20)</td>
<td>6.2 C (0.20)</td>
<td>56.2 D (1.63)</td>
<td></td>
</tr>
<tr>
<td>40% DEC</td>
<td>1.62 A (0.077)</td>
<td>-8.0 AB (21.72)</td>
<td>2.03 A (0.109)</td>
<td>53.8 AB (3.17)</td>
<td>57.2 B (4.75)</td>
<td>5.5 C (0.34)</td>
<td>68.5 AB (2.66)</td>
<td></td>
</tr>
<tr>
<td>20% ML</td>
<td>1.71 A (0.058)</td>
<td>56.1 A (40.89)</td>
<td>1.73 A (0.157)</td>
<td>45.4 C (1.19)</td>
<td>74.5 A (6.23)</td>
<td>7.3 B (0.61)</td>
<td>59.4 CD (1.22)</td>
<td></td>
</tr>
<tr>
<td>40% ML</td>
<td>1.29 B (0.024)</td>
<td>-21.9 AB (41.02)</td>
<td>1.90 A (0.137)</td>
<td>59.9 A (3.49)</td>
<td>85.9 A (7.36)</td>
<td>10.0 A (0.68)</td>
<td>73.5 A (3.43)</td>
<td></td>
</tr>
<tr>
<td>20% CHF</td>
<td>1.77 A (0.097)</td>
<td>-33.5 AB (41.39)</td>
<td>2.34 A (0.174)</td>
<td>55.3 AB (2.36)</td>
<td>70.6 AB (5.86)</td>
<td>6.1 C (0.27)</td>
<td>63.7 BCD (2.17)</td>
<td></td>
</tr>
<tr>
<td>40% CHF</td>
<td>1.35 B (0.054)</td>
<td>-278 B (154.3)</td>
<td>2.34 A (0.361)</td>
<td>57.2 A (5.00)</td>
<td>85.7 A (13.86)</td>
<td>7.6 B (0.49)</td>
<td>67.2 ABC (5.52)</td>
<td></td>
</tr>
</tbody>
</table>

*The control diet (maintenance chow) was 49% gamebird chow, 49% horse chow and 2% vegetable oil. The other diets contained maintenance chow with 20 or 40% of the following: DEC = Greenbriar/Honeysuckle, ML = Mountain laurel, CHF = Christmas hollyfern.*
Table 1.4. Detoxification conjugates excreted by captive ruffed grouse fed 6 diets during January-March 1993 in Blacksburg, Virginia. Conjugate excretion from each gram of the test forage (right hand column) was calculated by subtracting the conjugates produced by the basal portion of the diet from the total conjugate production and dividing the result by the mass of test forage (e.g. mountain laurel) consumed. Means in a column with different letters were different at \( \alpha = 0.05 \). N = 6 for all treatments.

<table>
<thead>
<tr>
<th>Diet(^a)</th>
<th>Glucuronic Acid mmol/day</th>
<th>Glucuronic Acid mg/g food</th>
<th>Sulfates umols/day</th>
<th>Sulfates umols/g food</th>
<th>Ornithine mmol/day</th>
<th>Ornithine mg/g food</th>
<th>Conjugates from forage (nmols/g)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.51 D (0.03)</td>
<td>3.1 E (0.07)</td>
<td>99.1 A (4.04)</td>
<td>3.09 AB (0.12)</td>
<td>0.17 D (0.05)</td>
<td>0.73 D (0.18)</td>
<td>0.024 D (0.001)</td>
</tr>
<tr>
<td>40% DEC</td>
<td>0.88 C (0.06)</td>
<td>5.1 CD (0.13)</td>
<td>99.9 A (12.67)</td>
<td>3.02 B (0.34)</td>
<td>1.30 A (0.56)</td>
<td>5.18 A (0.46)</td>
<td>0.135 B (0.008)</td>
</tr>
<tr>
<td>20% ML</td>
<td>0.96 BC (0.05)</td>
<td>5.5 C (0.24)</td>
<td>98.4 A (5.12)</td>
<td>2.92 B (0.17)</td>
<td>1.01 B (0.06)</td>
<td>3.96 B (0.17)</td>
<td>0.209 A (0.009)</td>
</tr>
<tr>
<td>40% ML</td>
<td>2.33 A (0.27)</td>
<td>14.7 A (1.85)</td>
<td>129.9 A (14.98)</td>
<td>4.20 A (0.50)</td>
<td>0.53 C (0.06)</td>
<td>2.28 C (0.25)</td>
<td>0.207 A (0.023)</td>
</tr>
<tr>
<td>20% CHF</td>
<td>0.81 C (0.05)</td>
<td>4.6 D (0.21)</td>
<td>99.7 A (7.00)</td>
<td>2.94 B (0.20)</td>
<td>0.34 CD (0.05)</td>
<td>1.32 CD (0.19)</td>
<td>0.086 C (0.012)</td>
</tr>
<tr>
<td>40% CHF</td>
<td>1.09 B (0.09)</td>
<td>7.8 B (0.80)</td>
<td>106.5 A (4.56)</td>
<td>3.88 AB (0.14)</td>
<td>0.31 CD (0.06)</td>
<td>1.57 CD (0.33)</td>
<td>0.104 BC (0.015)</td>
</tr>
</tbody>
</table>

\(^a\)The control diet was 49% gamebird chow, 49% horse chow and 2% vegetable oil. The other diets contained control diet with 20 or 40% of the following: DEC = Greenbriar/Honeysuckle, ML = Mountain laurel, CHF = Christmas hollyfern.

\(^b\)Variance was not the same between treatments and could not be stabilized by transformation.

\(^c\)Conjugate production from basal diet not subtracted.
the 20% ML and 40% CHF diets (Table 1.3). The amount of uric acid excreted did
not differ between treatments, although grouse on the 40% ML and 40% CHF diets
excreted a larger fraction of fecal nitrogen as uric acid than did grouse consuming the
control or 20% ML diets. Grouse on the 40% DEC diet excreted the least amount of
ammonia nitrogen. Grouse on the 40% ML diet excreted the highest percent of fecal
nitrogen as ammonia, followed by the 40% CHF and 20% ML diets (Table 1.3). The
percent of fecal nitrogen excreted as uric acid, ammonia, and ornithine ranged from
56% (control) to 74% (40% ML) (Table 1.3).

The 40% ML diet resulted in the highest excretion of glucuronides, followed
by the 40% CHF and 20% ML diets (Table 1.4). Glucuronide excretion was lowest
on the control diet, although considerable amounts of glucuronides (0.51 mmol/day)
were still produced. Daily sulfate excretion was not different among treatments. The
40% ML diet produced the most sulfate per gram of food and was significantly
greater than the 20% ML, 20% CHF, and 40% DEC diets. The daily excretion of
ornithine and the amount of ornithine per gram of food was greatest for the 40%
DEC diet, followed by the 20% ML and 40% ML diets. Ornithine excretion was not
different among the control, 20% CHF, and 40% CHF diets.

DISCUSSION

Grouse Performance. Although most grouse lost body mass, the changes were small
and not significantly different from controls on all diets except the 40% CHF diet.
Because the grouse were able to maintain or gain body mass on the control diet before
the trials and during inter-trial periods, the small loss in body mass shown by most diets was probably a response to the experimental conditions (e.g. stress of confinement) and not related to the treatments. Thus, grouse can likely maintain body mass on diets with up to 40% of greenbriar/honeysuckle or mountain laurel. The grouse performed well on the 20% CHF diet, gaining mass on average, suggesting this fern can be used to meet a portion of the bird’s energy requirements.

All of the grouse consuming the 40% CHF diet lost body mass. This was probably due to a combination of a small decrease in dry matter intake and a large decrease in dry matter metabolizability and ME concentration. Although the nitrogen balance data are equivocal, grouse consuming the 40% CHF diet had the lowest nitrogen balance, supporting the conclusion that ruffed grouse are not able to maintain themselves on moderate levels of Christmas hollyfern.

Grouse consuming the 40% ML and 40% CHF diets had the largest variation in body mass changes (Table 1.2), suggesting that individual grouse have different abilities to cope with toxic diets. One explanation for individual differences in handling toxic diets may be the bird’s ability to conjugate toxins. Grouse with a high conjugation potential would produce more conjugates per unit of diet, thus neutralizing and excreting more toxins, and would perform better. If this hypothesis is true, one would predict a positive relationship between conjugate production per unit of food eaten and performance, as measured by body mass changes. An alternative hypothesis is that grouse that produce low levels of conjugates per gram of
diet are able to handle toxins in an alternative manner (such as oxidation), and thus do not have to resort to conjugation, with its energetic and substrate costs. Grouse that perform well also may be more adept at recovering conjugates by reverse peristalsis of urine into the ceca, where microbes could metabolize both toxins and conjugates, releasing volatile fatty acids and ammonia which can be absorbed and used by the grouse (Gasaway 1976, Karawasa 1989).

Post-hoc analysis showed negative relationships between conjugates produced per gram of diet and body mass change for the 40% CHF and 40% ML diets (Figure 1.1). Thus, the grouse that performed the worst produced the most conjugates per gram of diet. These grouse must have either absorbed more toxins, handled those toxins less efficiently, or were not able to recover conjugates readily from the urine. This observation deserves more study.

Conjugate Production. Conjugate levels from this study are of the same order of magnitude as those from other studies of grouse. The highest glucuronide excretion in this study was 3 times (4.20 vs. 1.42 mmols/kg/day) the glucuronide excretion of ruffed grouse consuming high levels of coniferyl benzoate (Jakubas et al. 1993a), but only 1/3 that of ruffed grouse consuming aspen buds (ca. 14 mmols/kg/day, Guglielmo 1993). The highest levels of sulfate and ornithine excreted from a diet in this study were 1/5 and 3/4, respectively, of those excreted by grouse in the coniferyl benzoate study (Jakubas et al. 1993b). Ornithine excretion in this study was 1/5 of
Figure 1.1. The relationship between body mass change and total mmols of conjugates (glucuronic acid, ornithine, and sulfates) produced per g of food consumed for ruffed grouse consuming six diets. The control diet (maintenance chow) was 49% gamebird chow, 49% horse chow and 2% vegetable oil. The other diets contained maintenance chow with 20 or 40% of the following: DEC = Greenbriar/Honeysuckle, ML = Mountain laurel, CHF = Christmas hollyfern.
that excreted by ruffed grouse consuming aspen buds (2.4 vs. ca. 11 mmols/kg/day, Guglielmo 1993). Blue grouse consuming conifer needles excreted up to 4.5 times (65 vs. 15 mg/g feed) more glucuronic acid than ruffed grouse in this study (Remington 1990). For needles from conifer species preferred by blue grouse during the winter (Remington 1990), glucuronide excretion was 1 to 1.6 times that of ruffed grouse consuming the 40% ML diet. The amount of ornithine excreted per gram of food on the 40% DEC diet was 1.5 to 4.5 times greater than that excreted by grouse consuming conifer needles (Remington 1990). Rock ptarmigan (Lagopus mutus) (400 to 500 g body mass) consuming berries produced 0.44 to 0.65 mmols ornithine/day, or 1/3 to 1/2 of the ornithine produced daily from the 40% DEC diet (calculated from Moss and Parkinson 1975). Red grouse (Lagopus lagopus scoticus) (600 g body mass) consuming heather (Calluna vulgaris) produced 2.4 to 5 times the amount of ornithine produced daily from the 40% DEC diet (calculated from Moss and Parkinson 1972).

Control and 40% DEC Diets. The control diet had the lowest daily excretion of conjugates, but all three conjugates were still excreted in moderate amounts (Table 1.4). Jakubas et al. (1993a) detected no ornithine or glucuronides in the feces of ruffed grouse consuming a diet of aspen wood chips and commercial pheasant chow; however, daily excretion of sulfate esters averaged 0.56 mmols/kg/day (Jakubas et al. 1993a), and was not different from a diet containing 6.5% of the plant secondary
metabolite coniferyl benzoate. This level of sulfate excretion is about 3 times greater than the 0.180 mmols/kg/day excreted by ruffed grouse consuming the control diet in the present study. No ornithine was detected in the feces of rock ptarmigan consuming *Polygonum* seeds (Moss and Parkinson 1975). The sensitivity of the ornithine assay used by Moss and Parkinson (1975) was not reported, although levels of 0.2 mg/g were detected.

The cause of the high levels of conjugate excretion from the control diet is not known. Excretion of endogenous compounds, such as hormones, will result in some conjugate activity (Sipes and Gandolfi 1991). There may also have been chemicals in the maintenance diet that require conjugation. These chemicals may have been natural plant constituents of the diet, or preservatives added by the manufacturer. The Purina Game Bird Chow used in the maintenance diet contained the preservative ethoxyquin, a quinoline compound.

The 40% DEC diet was included in the experiment so that detoxification activity from higher quality winter forages could be determined. Honeysuckle and greenbriar leaves were chosen because they are a winter forage of ruffed grouse in the Southeast (Stafford and Dimmick 1979, Servello and Kirkpatrick 1987), contained moderate levels of protein and ME, and had moderate to low levels of phenols and tannins (Servello and Kirkpatrick 1987). The 40% DEC diet was similar to the control diet in dry matter digestibility, ME, and tannin content and was better quality than the 40% ML and 40% CHF diets (Table 1.1). The 40% DEC diet resulted in a
higher glucuronide excretion than the control and the highest ornithine excretion of all the diets (Table 1.3). These results indicate that conjugation activity is not limited to forages with known high levels of secondary plant metabolites and that relatively high quality forages may contain chemicals requiring conjugation. Furthermore, these results should be considered if conjugate levels in the feces of wild grouse are to be used as an index to the consumption of heavily defended, poor-quality forages (Lindroth and Batzli 1983).

Mountain Laurel. The detoxification strategy for toxins in mountain laurel changed with the level of mountain laurel in the diet. The 20% ML diet produced nearly equal molar amounts of ornithine and glucuronides whereas the 40% ML diet produced nearly 4 times the molar amount of glucuronides as ornithine (Table 1.4). The difference cannot be explained by simple saturation of the ornithine pathway because daily ornithine excretion from the 40% ML diet was half that of the 20% ML diet. A large amount of conjugation activity was transferred from the ornithine pathway to the glucuronide pathway. The nitrogen balance of grouse on the 40% ML diet was not different from zero ($t=-0.53$, df=5, $P=0.617$), despite a 25% lower nitrogen intake than the 20% ML diet (Table 1.3) and the highest level of protein binding tannins. Grouse consuming the 40% ML diet may have been conserving nitrogen by favoring glucuronide conjugation over ornithine. Evidence against this hypothesis is that ruffed grouse consuming less than half the nitrogen per day as
grouse on the 40% ML diet produced 3 times the amount of ornithine (Jakubas et al. 1993b); however, these grouse were in negative nitrogen balance. Red grouse consuming 3/4 less or the same amount of nitrogen as grouse on the 40% ML diet produced 5 to 12 times the amount of ornithine daily.

The level of conjugation activity, when compared to that of other grouse studies, suggests that grouse could handle more mountain laurel in their diet, although dry matter, ME, and nitrogen intake showed a declining trend from the control diet to the 20% ML and 40% ML diets. To the extent that increased ammonia excretion indicates acidosis, and that intake is limited by challenges to the animal’s acid-base balance, then the greater percent of fecal nitrogen excreted as ammonia suggests there was a challenge to pH homeostasis from the 40% ML diet. Other studies suggest that a diet of 40% mountain laurel is approaching the maximum amount that grouse can handle over a multi-day period. Captive grouse died when fed only mountain laurel leaves (Bump et al. 1947) and were unable to maintain body mass on a diet of 50% dried mountain laurel leaves and 50% commercial feed (Servello 1985). Captive grouse were able to maintain body mass when fed 30% dried mountain laurel leaves and 70% commercial feed (Servello and Kirkpatrick 1987).

Christmas hollyfern. The changes in conjugate ratios for the Christmas hollyfern diet suggest a simple saturation of the ornithine pathway. Ornithine conjugation is a medium capacity, high affinity pathway, whereas the glucuronide conjugation pathway
is low affinity and high capacity (Sipes and Gandolfi 1991). Thus, xenobiotics should be preferentially conjugated with ornithine until that pathway becomes saturated, at which point glucuronide conjugation will increase. The daily excretion of ornithine was essentially equal for the two Christmas hollyfern diets, whereas glucuronide excretion was about 50% greater for the 40% CHF diet (Table 1.4). This interpretation is refuted, however, in that 3 to 4 times as much ornithine was excreted on the 20% ML and 40% DEC diets as on the Christmas hollyfern diets, suggesting that excess capacity was available for ornithine conjugation.

Daily conjugate production and ammonia excretion does not suggest that intake of Christmas hollyfern was limited by conjugation activity or challenges to pH homeostasis. The intake of ME and nitrogen was lower for the 40% CHF diet than the control and 20% CHF diets. This lower intake of nutrients was not due to limits imposed by digestive tract fill, since the dry matter intake of 40% CHF was the lowest of any diet and significantly lower than the 20% CHF diet. The reason for the decreased intake of the 40% CHF diet is not clear. An overload of conjugate excretion pathways does not seem to be the cause, because at least one diet had greater excretion of each of the 3 conjugates measured. Total conjugate excretion, measured as the sum of glucuronides, sulfates, and ornithine, varied among diets (F = 72.1, d.f. = 5, \( p < 0.0001 \)). Post-hoc analysis showed that the 40% CHF diet produced fewer conjugates (1.51 mmols/day) than the 40% ML (2.99), 40% DEC (2.23), and 20% ML (2.07) diets. The lower intake for the 40% CHF diet may have
been caused by a plant toxin that was not conjugated with glucuronic acid, sulfate, or ornithine and which had physiologic consequences to which the grouse could respond. Another possible cause of decreased intake is lower palatability of the 40% CHF diet.

If one assumes that the detoxification activity for each gram of maintenance diet consumed is consistent among trials, then the conjugates produced from the basal portion of each diet can be subtracted from the total conjugate output to determine the conjugates produced from the treatment forages. These calculations show that the millimoles of conjugates (sum of glucuronides, ornithine, and sulfates) produced per gram of mountain laurel were nearly identical for the two mountain laurel diets (Table 1.4), despite the different ratios of conjugates produced. Total conjugate production per gram of Christmas hollyfern also was similar for those two diets. Thus, the total moles of toxins to be conjugated per gram of forage was the same for different levels of forage in the diet, but the ratio of conjugates changed.

If the conjugates from the control diet are subtracted and the amount of individual conjugates produced per gram of forage is considered, forages from this study result in large amounts of conjugation activity. The 40% ML diet produced 32.3 mg glucuronic acid per g of mountain laurel consumed, which is greater than the glucuronides produced by blue grouse consuming needles from preferred conifer species (13 and 24 mg/g forage), but less than that of conifer species that are not preferred (40 and 65 mg/g) (Remington 1990). Ornithine excretion was up to 10 times greater for the deciduous leaf mixture (11.9 mg/g of leaf mixture) than any of
the conifer needle diets (1.1 to 3.3 mg/g needles). The low level of ornithine excretion by blue grouse consuming conifer needles may be expected because the crude protein content of needles is low (4.5 to 5.8%) (Remington 1990) and blue grouse may switch to glucuronic acid for conjugation to conserve nitrogen.

**Nitrogen Dynamics.** Urinary excretion of ammonia nitrogen is often associated with the consumption of toxic diets (Remington 1990, Foley 1992, Jakubas et al. 1993a) and is considered an attempt by the animal to maintain pH homeostasis while producing acidic xenobiotic metabolites and conjugates (Atkinson 1992, Foley 1992). In support of this hypothesis for grouse, a close correlation was reported between the excretion of glucuronic acid and ammonia nitrogen for blue grouse consuming conifer needles (Remington 1990), and ammonia nitrogen excretion more than doubled for ruffed grouse ingesting large amounts of coniferyl benzoate (Jakubas et al. 1993a).

The amount of ammonia nitrogen excreted daily in this study, however, was not related to the level of mountain laurel or Christmas hollyfern in the diet (Table 1.3). The percent fecal nitrogen excreted as ammonia varied among diets and was greatest for the 40% ML diet and least for the 20% CHF, 40% DEC, and control diets (Table 1.3). The amount of ammonia excreted daily was only weakly related to the molar production of ornithine and glucuronides (linear regression, n=36, F = 3.67, P = 0.064, r² = 0.097). The relationship is poor in part because of the high daily output of conjugates from the 40% DEC diet, which had the lowest ammonia excretion.
Thus, the hypothesis that ammonia excretion is a response to acidosis from the production of acidic conjugates is not supported by this study.

Daily ammonia excretion in this study was about twice that of ruffed grouse consuming high levels of coniferyl benzoate and about 4 times that produced from a control diet that had no ornithine or glucuronic acid production (Jakubas et al. 1993b). The percent fecal nitrogen excreted as ammonia was similar for the 40% ML diet from this study (10%) and the coniferyl benzoate diet (11%) (Jakubas et al. 1993b). The percent nitrogen excreted as ammonia nitrogen ranged from 8 to 25% for rock ptarmigan consuming natural forages (Moss and Parkinson 1975) and was 7 to 10% for red grouse eating heather (Moss and Parkinson 1972). The highest percent of nitrogen excreted as ammonia was reported for blue grouse consuming conifer needles and ranged from 33 to 55% (Remington 1990).

The amount of ammonia excreted by grouse in this study was large; however, because the diet had high levels of protein, the total amount of nitrogen excreted was also large, and thus the percent nitrogen excreted as ammonia was similar to most other studies. The reason ammonia excretion was not related to conjugate production is not clear. One potential reason is that other acid producing metabolic processes, such as fatty acid absorption from the ceca, may have caused mild acidosis from diets with few secondary plant metabolites and thus some ammonia excretion.

The percent of fecal nitrogen that was excreted as uric acid, ammonia, or ornithine was lowest for the control diet and greatest for the 40% ML, 40% CHF,
and 40% DEC diets (Table 1.3), although even the 40% ML diet had >25% of fecal nitrogen uncharacterized. There are several other sources of fecal nitrogen that were not measured in this study. Urea is typically excreted by grouse in small amounts (Moss and Hanssen 1980), although red grouse consuming seeds excreted 13% of fecal nitrogen as urea (Moss and Parkinson 1975). Urea levels in the feces of blue grouse consuming conifer needles were at the detection limit and judged to be inconsequential (Remington 1990).

Neutral detergent fiber (NDF) bound nitrogen often is poorly digested. This class of nitrogen may be found naturally in plants or be the result of feed preparation. High levels of NDF bound nitrogen in the maintenance chow would explain the low characterization of fecal nitrogen from the control diet and the high characterization from the diets with 40% forage.

Another source of fecal nitrogen is bacterial nitrogen from the ceca. Moss and Parkinson (1972, 1975) found low levels of ammonia, urea, and urates and high levels of α-amino nitrogen and amide nitrogen in the cecal feces of red grouse and rock ptarmigan. This is not unexpected because bacteria in the ceca of galliform birds metabolize uric acid and urea rapidly (Mortensen and Tindall 1981, Karasawa 1989) and the resulting ammonia is quickly absorbed (Karasawa 1989). Much of the nitrogen in cecal feces is probably of bacterial origin. If cecal microfauna are similar to rumen microfauna in that 75% of the nitrogen from rumen microfauna is protein (calculated from Van Soest 1982, p166), then cecal feces are likely to have nitrogen
sources that were not quantified. Nitrogen from the ceca, however, does not explain why less fecal nitrogen was characterized in the control diet than the diets with 40% forage, because the percent feces that came from the ceca in the 40% ML diet was twice that of the control diet and the 40% CHF diet produced the same percent cecal feces as the control diet.

The low amount of fecal nitrogen characterized for the control diet was similar to that of rock ptarmigan eating high quality (in terms of ME and crude protein) Polygonum seeds, a diet on which the birds maintained body mass (Moss and Parkinson 1975). Ammonia, α-amino, amide, urea, urate, and ornithine nitrogen were measured and were only about 50% of fecal nitrogen. All fecal nitrogen was accounted for in diets of berries (Moss and Parkinson 1975) and 75 to 90% of fecal nitrogen was characterized for red grouse consuming heather (Moss and Parkinson 1972). Berries and heather had low levels of protein (2.5 and 7%) and grouse lost body mass while consuming them. These studies and the current study suggest that grouse on high quality diets excrete a form of nitrogen not yet recognized.

Costs of detoxification. There are a variety of metabolic costs associated with the detoxification of secondary plant metabolites. A xenobiotic may induce production of additional detoxification enzymes, thus requiring more protein and energy for protein synthesis (Thomas et al. 1988). Energy requirements may also increase because many detoxification reactions require ATP. For example, conjugation reactions
proceed through high energy intermediates, formed in ATP consuming reactions (Sipes and Gandolfi 1991).

Another potentially important, and easily quantified, cost of detoxification is the loss of energy (glucose and amino acids) and nitrogen (amino acids) as conjugates. Remington (1990) reported 1.4 to 9.8% of ME intake was excreted as glucuronic acid and 9.3 to 30.2% of nitrogen intake was lost in the form of ornithine, hippuric acid, and ammonia. Ruffed grouse consuming a diet high in coniferyl benzoate, a plant secondary metabolite, excreted about 4% and ruffed grouse consuming aspen excreted approximately 15% of apparent ME intake as glucuronic and ocnithuric acids (Jakubas et al. 1993b). Protein and energy loss as conjugates was not a significant cost of detoxification in this study. Grouse lost 0.3 to 1.5% of their apparent ME intake as glucuronides (assuming a metabolizable energy value for glucose of 2.0 kcals/g, calculated as 36 moles ATP/mole of glucose, 180 g/mole glucose, and 10 kcals/mole ATP (Hickman et al. 1979:92)). The nitrogen cost of detoxification as measured by ornithine excretion was 0.4 to 3.6% of nitrogen intake.

Influence of Tannins. Tannins are a common secondary plant metabolite of woody plants (Swain 1979). The digestive and physiologic effects of tannins have been studied extensively in mammals. Tannins have been shown to decrease protein digestion and dry matter intake and stimulate detoxification pathways (Robbins et al. 1987, Hagerman and Butler 1991, Chung-MacCoubrey 1993). Studying the effect of
tannins on digestion in birds is complicated by the mixing of digestive and metabolic wastes. Tannins decrease food intake, growth rates, and amino acid absorption in chickens (Armstrong et al. 1974, Nelson et al. 1975, Price et al. 1979). Quebracho and tannic acid, added to the diets of acorn woodpeckers, decreased the ME coefficient (Koenig 1991).

The winter diets of ruffed grouse are high in tannins, and the diets of grouse in the Southeast have the highest tannin levels due to the high level of evergreen leaves in the diet (Servello and Kirkpatrick 1987). Christmas hollyfern and mountain laurel had 28 and 48 mg tannic acid equivalents per gram, respectively. The maintenance portion of each diet diluted tannin to the levels shown in Table 1.1. Although this study was not designed to investigate the effects of tannins directly, there are two observations regarding tannins that deserve mention.

If significant amounts of protein were bound by tannins and thus made undigestible, then the amount of protein nitrogen in the feces should increase. Protein nitrogen was not measured specifically, but its level should be reflected in the percent of uncharacterized fecal nitrogen (see section on nitrogen dynamics) and one would expect an increase in uncharacterized fecal nitrogen with an increase in tannin bound protein. In fact, the opposite occurred, with the control diet, which had the lowest tannin levels, having the largest amount of uncharacterized fecal nitrogen, and the 40% ML diet having the least amount of uncharacterized fecal nitrogen. This analysis, however, cannot be used as an argument against tannin binding of protein.
Because uncharacterized nitrogen represents many different forms of nitrogen in the feces, changes in uncharacterized nitrogen due to tannin-bound protein could be masked by larger changes in other types of uncharacterized nitrogen, such as bacterial nitrogen or fiber-bound nitrogen.

Chapter 2 suggests that the ceca may play a role in handling dietary tannins in that a larger fraction of fecal matter is excreted from the ceca when tannins are in the diet. This observation was supported in these trials in that the percent of feces excreted from the ceca varied among treatments (Table 1.2) and was nearly twice as much on the 40% ML (19.5±2.12%) diet as the control (10.7±0.31%). However, despite high levels of protein binding tannins in the 40% CHF diet, there was no effect on fecal partitioning (10.1±1.32%).

Ecological Significance. This study shows that ruffed grouse are able to consume small amounts of Christmas hollyfern and moderate amounts of mountain laurel daily. If the non-evergreen portion of the diet of a wild grouse has a lower ME concentration than the maintenance portion of the diets in these trials, then the percent of mountain laurel and Christmas hollyfern in the diet is likely to be lower than reported here, assuming intake of evergreen leaves is limited by toxins. The ability to consume evergreen plants is important for ruffed grouse in the Southeast for two reasons. First, the abundance of evergreen plants and their large leaf size allows high intake rates and grouse can significantly decrease their daily foraging times by
incorporating some evergreen leaves in their diet (Chapter 3). Lower foraging times will decrease energy expenditure and exposure to predation. Second, evergreen forages may be the only forage available to grouse during short periods during the winter, such as during snow storms. Although grouse are not able to meet their nutrient requirements solely on evergreen leaves, consuming these leaves will result in smaller nutrient deficits than would occur otherwise.

Food habits studies of ruffed grouse in the Southeast indicate that evergreen leaves and ferns are <50% of the diet during the winter (Stafford and Dimmick 1979, Servello and Kirkpatrick 1987). This pattern of food selection has been attributed to the high level of secondary plant metabolites in evergreen leaves and ferns (Servello and Kirkpatrick 1987), which limit intake through toxicity. Evidence for this hypothesis from this study is that conjugation activity was higher from the evergreen leaves than the control diet. However, conjugation levels were not as great as those reported for grouse consuming other forages (Remington 1990, Guglielmo 1993, Jakubas et al. 1993a). Metabolizable energy and protein intake was lower for the diets with high levels of evergreen leaves, although it is not clear if this response was due to toxicity or palatability.

The amount of variation in many of the response variables was greater for grouse consuming evergreen leaves or ferns than for the control diet (Tables 2-4). This variation suggests that not all grouse are able to handle toxic forages to the same extent. Chung-MacCoubrey (1993) likewise found great variability in gray squirrels
consuming acorns high in tannins. The consequences for grouse in the wild may be significant when conditions require the consumption of evergreen forage to survive. Grouse that are unable to consume evergreen forages are likely to die during some winters. Grouse able to consume evergreen leaves and ferns would maintain body condition and would support the view that grouse do not experience nutrient stress during winter (Bergerud 1988). It should be noted, however, that the presence of significant variation suggests that selective pressures enabling grouse to consume toxic forages have not been intense.

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CHAPTER 2

EFFECT OF CONDENSED TANNINS ON RUFFED GROUSE AND THE ROLE OF THE CECA IN HANDLING SECONDARY PLANT METABOLITES

INTRODUCTION

Ruffed grouse (*Bonasa umbellus*) consume diets high in tannins during the winter throughout their range (Servello and Kirkpatrick 1987). Tannins are a widespread secondary plant metabolite, found in 79% of deciduous woody perennials and 87% of evergreen woody perennials (Rhoades and Cates 1976). Tannins are functionally defined by their ability to bind and precipitate protein (Hagerman and Butler 1991). They are divided into 2 groups based on their chemical composition and susceptibility to hydrolysis. Condensed tannins are composed of oligomers of flavan-3-ols and flavan-3,4-diols and are less susceptible to hydrolysis in the digestive tract than hydrolyzable tannins. Hydrolyzable tannins are composed of gallic acid or hexahydroxydiphenic acid esterified to glucose or other polyols and are more likely to be degraded in the digestive tract and absorbed.

Tannins have a wide variety of effects in herbivores. Tannins decrease dry matter intake in both birds and mammals (Mole and Waterman 1987, Servello and Kirkpatrick 1989, Chung-MacCoubrey 1993). Protein digestibility in mammals is decreased by tannin (Robbins et al. 1987, Robbins et al. 1991). The effect of tannins on protein digestibility in birds are difficult to study; however, amino acid availability was lower in varieties of sorghum with high tannin levels than varieties with low
tannin levels (Nelson et al. 1975). Growth rates of chicks were lower when tannins were included in the diet (Elkin et al. 1978, Rogier et al. 1985). Recent studies have shown that both condensed and hydrolyzable tannins can be absorbed, causing a reduction in dry matter intake and perhaps toxicity (Clausen et al. 1990, Robbins et al. 1991, Chung-MacCoubrey 1993). Evidence indicates that toxicity resulting from absorption of tannin and its components is a more significant deterrent to herbivores than is digestion inhibition (Mole et al. 1990, Clausen et al. 1990, Bryant et al. 1992).

Despite high levels of tannins in the diet of ruffed grouse and the potentially negative effects of those tannins, the effect of tannins on ruffed grouse has not been studied. Servello and Kirkpatrick (1989) noted that grouse decreased dry matter intake and were in negative nitrogen balance when consuming red oak acorns which had high levels of tannins relative to acorns from other oak species. Jakubas et al. (1989) concluded that ruffed grouse choice of aspen (Populus tremuloides) buds and catkins was influenced more by other secondary plant metabolites than tannin levels.

Three studies were conducted to investigate the effect of tannins on ruffed grouse. The objective of the first 2 studies was to determine the effect of a condensed tannin on dry matter intake, nitrogen balance, and body weight of bobwhite (Colinus virginianus) and ruffed grouse. Based on the observation from these first two studies that cecal function was affected by dietary tannins, a third study was conducted to determine the influence of tannin on cecal fill and rate of passage in
ruffed grouse. This third study was done jointly with Nelson Lafon who conducted the research as part of the John Lee Pratt Senior Scholarship program at VPI&SU.

METHODS

Bobwhite Tannin Trials. The 12 adult male bobwhite quail used in this experiment were obtained from a commercial breeder and were housed individually in wire cages in the animal room of Cheatham Hall on the Virginia Tech campus. The quail were maintained on Southern States XLA poultry chow (6% crude fiber and 21% crude protein) and were provided water ad libitum.

The control diet was prepared by grinding the maintenance chow in a Wiley mill over a 1mm screen. Two tannin diets were prepared by adding either 3 or 6% (dry weight) crude quebracho tannin to the ground maintenance chow. Quebracho tannin (provided by A. E. Hagerman, Miami Univ., OH) contained 82% condensed tannin and 18% non-tannin phenolics (Chung-MacCoubrey 1993).

Four bobwhite were randomly assigned to each of the 3 treatments. Trials were conducted in July 1992 and began with a 4-day pretrial period in which quail were allowed to acclimatize to their treatment diets. On day 5 of the trial period, all quail were weighed to the nearest 0.1 g and a 5-day collection period began. Each bird was offered 30 g (as fed) of feed daily and all feces and uneaten food were collected twice daily from wax paper placed under each bird’s cage. A sample of feed was dried daily at 100° C for 24 hours to determine dry matter content. Uneaten feed was dried at 100° C for 24 hours and weighed. Feces of cecal and
intestinal origin were collected separately and immediately frozen. At the end of the trial all quail were weighed to the nearest 0.1g. Feces were freeze-dried, weighed, ground with a mortar and pestle, and stored at -15° C until analyzed.

Cecal and intestinal feces were analyzed separately. Nitrogen content of the feed and feces was determined by the Kjeldahl technique in the Virginia Tech forage testing lab. Tannin was extracted from a 150 mg sample of feces using four rinses of 70% acetone (v/v) and 1% sodium dodecyl sulfate (Robbins et al. 1991). Tannin in the extract was quantified using the acid-butanol assay (Porter et al. 1986). This assay was >90% efficient at extracting tannins from the feces of ruminants and >84% efficient for black bears (Robbins et al. 1991). Crude quebracho powder from the same batch used to prepare the diets served as a standard.

Nitrogen balance was the difference between daily nitrogen intake and nitrogen excretion. Apparent dry matter metabolizability was calculated by subtracting the mass of feces produced from the mass of food consumed and dividing by the mass of food eaten.

The effect of diet on response variables was determined using a one-way ANOVA (SAS 1988). Tukey’s honest significant difference (SAS 1988) was used for pairwise comparisons for variables in which the overall ANOVA was significant. Alpha=0.05 was used for all statistical tests and means are reported with standard errors.
Ruffed Grouse Tannin Trials. One male and 3 female subadult ruffed grouse were raised in captivity from eggs or chicks collected from the wild in western Virginia. Grouse were housed indoors in metal cages in the Lab Animal Resources facility on the Virginia Tech campus. The grouse were maintained on Southern States XL-A poultry chow and were provided water ad libitum. A control and 6% tannin diet were prepared as described in the quail experiment.

Trials were done in October 1992. A replicated latin square experimental design was used in which two grouse were randomly assigned to each treatment for a 9-day trial, then the grouse switched treatments and completed a second 9-day trial. Each trial had a 5-day pretrial period in which grouse acclimated to the diet, and a 4-day collection period in which all feces and uneaten feed were collected. Each grouse was offered 100 g of the treatment diet daily. Fecal collection and handling procedures were the same as those described for the quail experiment. Grouse were weighed to the nearest gram on days 5 and 9 of the trial.

Nitrogen of feed and feces was determined by the Kjeldahl technique and nitrogen balance and apparent dry matter metabolizability were calculated as in the quail trials. The effect of diet on response variables was determined by a 3-way ANOVA (SAS 1988) in which treatment, individual grouse, and time period were class variables. The type III sums of squares from the GLM procedure of SAS (SAS 1988) were used to determine if treatment had a significant effect, controlling for period and grouse.
Rate of Passage Study. Eleven adult ruffed grouse (5F, 6M) were raised in captivity from eggs or chicks collected from western Virginia or were obtained from a commercial breeder whose stock came from southern Ohio. Grouse were held outdoors and maintained on a 50:50 (w/w, dry matter basis) mixture of Purina game bird flight conditioner and Purina horse chow-100, ground over a 1mm screen in a Wiley mill. Crude fiber and crude protein levels of this diet were 21 and 14.5%, respectively, according to analysis by the manufacturer. Two percent vegetable oil was added to increase palatability.

This diet served as a control in the experiments. Treatment diets were formed by mixing 8% tannic acid (Sigma Chemical Co., ST. Louis, MO) or 8% quebracho tannin on a dry matter basis with the maintenance diet. Quebracho was from the same batch as used in the two previously described experiments.

During September 1993 ruffed grouse were placed in wire cages outdoors on the Virginia Tech campus. Four birds were randomly assigned to each tannin treatment and 3 birds to the control diet. Grouse were given 100 g (as fed) of food daily. During the first 3 days of the trial, tannin diets contained 4% tannin to allow grouse to acclimate. During the remainder of the trial tannin diets contained 8% tannin. On the morning of the sixth day of the trial, grouse were weighed and given an oral dose of 0.2g cobalt-ethylenediamine tetraacetic acid (Co-EDTA, prepared after Uden et al. 1980) with an intubation needle to mark the liquid phase of the digesta. Chromium-mordant was used to mark the particulate phase of the digesta and was
given orally to the grouse in gelatin capsules (0.2 g total, 54.7 mg Cr/g fiber). The Cr-mordant was provided by J. H. Herbein (Virginia Tech) and had been prepared from grass fiber. The mordanted fiber had been fed to a cow and collected from the feces. The fibers were washed, dried, and sieved through a 1000 um screen and over a 500 um screen to provide a known range of particle sizes.

Sheets of linoleum were placed under each cage to allow collection of feces and uneaten food. Feces of cecal and intestinal origin were collected separately and frozen immediately after collection. Feces were collected every 2 hours for 24 hours following dosing, every 4 hours during the second day, and every 12 hours for the final two days of the trial. The time of collection, grouse number, and fecal type were recorded for each collection.

Uneaten food was dried at 100° C for 24 hours and weighed. Feces were freeze-dried and each individual collection weighed. Fecal samples were then ground with a mortar and pestle and stored at -15° C.

Cobalt and chromium analyses were done in the Dairy Nutrition Lab at Virginia Tech. Fecal samples were analyzed in duplicate according to the procedures of Berzaghi (1993:31). Briefly, 300 mg of feces were digested for 24 hours in nitric, perchloric, and sulfuric acid and hydrogen peroxide. This solution was diluted with distilled water and Cr and Co concentrations were measured with a Varian AA-475 atomic absorption spectrophotometer. Standards were prepared from commercial solutions.
Maximum cecal fill was calculated by assuming all the Co recovered from the cecal feces entered the ceca and was mixed with cecal contents before the first cecal feces were produced (Gasaway et al. 1975). With this assumption and a known concentration of Co in the first cecal feces, the total amount of dry matter in the ceca could be calculated. Passage rate was represented by the time necessary for 50% and 95% of the marker to be excreted. When cumulative marker recovery was <50% (or 95%) for one collection and >50% (95%) for the next collection, a linear relationship between cumulative marker recovery and time since dosing was assumed in calculating the time when 50% (95%) of the marker passed.

Two-sample t-tests were used to determine significant differences between the control and the quebracho treatments. The tannic acid treatment was not analyzed because 3 of the 4 birds had to be removed from the trials because of low intake rates and subsequent emaciation.

RESULTS

Bobwhite Tannin Trials. One quail on the 6% tannin diet ate no food during the first 3 days of the pre-trial and was removed from the experiment on day 4. The body mass of birds at the beginning of the collection period was not different among treatments (Table 2.1). The percent change in body mass was not different among treatments and was not different from zero for any treatment. Dry matter intake, apparent metabolizability, and nitrogen balance also were not different among treatments (Table 2.1). The proportion of feces that came from the ceca increased
Table 2.1. Body mass at the beginning of the collection period (mean(se)), body mass change, dry matter metabolizability and intake, and nitrogen balance for bobwhite fed a control diet and diets containing different amounts of quebracho tannin. Means in a column with a different letter are different at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Body Mass</th>
<th>Dry Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial (g)</td>
<td>Change (%)</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>187 A</td>
<td>-0.8 A</td>
</tr>
<tr>
<td>3% tannin</td>
<td>4</td>
<td>183 A</td>
<td>3.1 A</td>
</tr>
<tr>
<td>6% tannin</td>
<td>3</td>
<td>185 A</td>
<td>-0.8 A</td>
</tr>
</tbody>
</table>
Table 2.2. The percent of feces that came from the ceca (mean(se)), the percent of tannin recovered from the feces that came from the ceca, and the percent of tannin consumed that was recovered in the feces for bobwhite fed a control diet and diets with different amounts of quebracho tannin. Means in a column with a different letter are different at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Feces from ceca (%)</th>
<th>Percent in cecal feces</th>
<th>Percent of consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>10.9 C</td>
<td>---$^a$</td>
<td>---$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% tannin</td>
<td>4</td>
<td>20.3 B</td>
<td>58.9 A</td>
<td>52.6 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.05)</td>
<td>(4.99)</td>
<td>(1.09)</td>
</tr>
<tr>
<td>6% tannin</td>
<td>3</td>
<td>36.9 A</td>
<td>76.0 A</td>
<td>60.1 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.02)</td>
<td>(4.47)</td>
<td>(5.32)</td>
</tr>
</tbody>
</table>

$^a$Tannin analyses were done on the feces from one control grouse and no tannin was detected.
significantly with increasing amounts of tannin in the diet (Table 2.2). The percent of ingested tannin that was recovered in the feces was not different between diets and averaged 56%. Of the tannin recovered in the feces, 59±5.0% and 76±4.5% was in the cecal feces of the 3% and 6% tannin diets, respectively, although this difference was not significant (Table 2.2).

The mass of tannin detected in the cecal feces was calculated to determine if this tannin accounted for the increase in cecal feces noted from tannin containing diets. The 3% tannin diet had 0.52±0.07 g of crude tannin in the cecal feces. The difference in the amount of cecal feces produced from the control and 3% tannin diet was 2.13 g. The amount of crude tannin in the cecal feces from the 6% tannin diet was 1.44±0.05 g. The difference in the amount of cecal feces from the control and 6% tannin diets was 5.45 g. The difference in the amount of cecal feces produced from the 3% and 6% tannin diets was 3.32 g while the difference in tannin from the ceca was 0.92 g. Thus, in all of these comparisons, the difference in cecal feces produced from different diets was >3 times the difference in tannin found in the cecal feces. This analysis suggests that the additional material coming from the ceca was not only tannin.

**Ruffed Grouse Tannin Trials.** The body mass of ruffed grouse on the first day of the collection period was not significantly different between diets (Table 2.3). The apparent dry matter metabolizability of the tannin diet was lower than the control diet,
Table 2.3. Initial body mass, body mass change, dry matter metabolizability and daily intake, nitrogen balance, and the percent of feces that came from the ceca for ruffed grouse fed a control diet and a diet containing 6% quebracho tannin. N = 4 for both treatments.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Tannin</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Initial body mass (g)</td>
<td>572</td>
<td>16.6</td>
<td>549</td>
</tr>
<tr>
<td>Body mass change (%)</td>
<td>-0.5</td>
<td>0.36</td>
<td>-1.0</td>
</tr>
<tr>
<td>D. M. metabolizability (%)</td>
<td>65.2</td>
<td>0.22</td>
<td>62.2</td>
</tr>
<tr>
<td>Dry matter intake (g/kg$^{0.75}$)</td>
<td>40.7</td>
<td>2.01</td>
<td>35.4</td>
</tr>
<tr>
<td>Nitrogen balance (mg/day)</td>
<td>-128</td>
<td>35.2</td>
<td>-81</td>
</tr>
<tr>
<td>Percent feces from ceca</td>
<td>4.9</td>
<td>0.26</td>
<td>16.1</td>
</tr>
</tbody>
</table>
but body mass change, dry matter intake, and nitrogen balance were not different between treatments (Table 2.3). Ruffed grouse, like bobwhite, excreted a larger percentage of feces from the ceca when consuming a tannin containing diet.

**Rate of Passage Study.** One grouse from both tannin treatments was removed from the study after the pretrial period because of reduced intake and large weight loss. Two other grouse consuming tannic acid were removed from the experiment after 1 day of fecal collection due to large weight loss. Grouse consuming the tannic acid diet lost 32.7±3.2% of body mass before being removed from the trial. Dry matter consumption of the tannic acid diet was about 30% of the control diet and <50% of the intake of the quebracho diet. The tannic acid treatment was not analyzed further.

Dry matter intake of the quebracho diet was 66% of the control diet. The quebracho diet had a lower apparent dry matter metabolizability and birds consuming the quebracho diet lost over 5% of body mass, which was a greater loss than the control birds, which maintained body mass (Table 2.4). Grouse on the quebracho diet excreted 20% of feces as cecal feces, which was twice that of the control.

An average of 93±4.7% of chromium given to the grouse was recovered in the feces (Table 2.5). Cobalt recovery averaged 72±8.4%. An average of 81±9.4% of recovered cobalt was in the cecal feces while only 7±1.2% of the recovered chromium was in cecal feces (Table 2.5). If bird number 5 is excluded, 90±3.3% of recovered cobalt came from the cecal feces.
Table 2.4. Body mass change, daily dry matter intake and metabolizability, percent of feces that was excreted as cecal feces, and the number and average size of fecal droppings excreted by ruffed grouse consuming a control diet and a diet with 8% quebracho tannin. N = 3 for both treatments.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th></th>
<th>Tannin</th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td></td>
<td>Mean</td>
<td>SE</td>
<td></td>
</tr>
<tr>
<td>Body mass change (%)</td>
<td>0.5</td>
<td>1.3</td>
<td></td>
<td>-5.6</td>
<td>0.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Dry matter intake (g/kg$^{0.75}$)</td>
<td>51.4</td>
<td>3.6</td>
<td></td>
<td>33.9</td>
<td>5.0</td>
<td>0.047</td>
</tr>
<tr>
<td>D. M. metabolizability (%)</td>
<td>48.8</td>
<td>1.5</td>
<td></td>
<td>40.1</td>
<td>1.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Percent feces from ceca</td>
<td>9.5</td>
<td>0.5</td>
<td></td>
<td>20.2</td>
<td>0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Number of cecal feces</td>
<td>8.0</td>
<td>0.6</td>
<td></td>
<td>6.7</td>
<td>1.2</td>
<td>0.42</td>
</tr>
<tr>
<td>Average cecal dropping (g)</td>
<td>0.79</td>
<td>0.07</td>
<td></td>
<td>1.59</td>
<td>0.36</td>
<td>0.16</td>
</tr>
</tbody>
</table>
Table 2.5. Percent of elemental markers given to ruffed grouse that were recovered in the feces, the percent of the marker recovered that was in the cecal feces, the concentration of cobalt in the first cecal feces, the estimated dry matter fill of the ceca, and the time required for passage of 50% and 95% of the recovered marker. Dry matter cecal fill was calculated from the concentration of cobalt in the first cecal dropping and the total amount of marker recovered. N = 3 for both control (maintenance diet) and tannin (maintenance diet with 8% quebracho tannin) treatments.

<table>
<thead>
<tr>
<th></th>
<th>Control Diet</th>
<th>Tannin Diet</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Cobalt recovery (%)</td>
<td>81.0</td>
<td>15.6</td>
<td>62.7</td>
</tr>
<tr>
<td>Chromium recovery (%)</td>
<td>91.7</td>
<td>8.5</td>
<td>94.3</td>
</tr>
<tr>
<td>Cobalt in cecal feces (%)</td>
<td>87.3</td>
<td>4.9</td>
<td>75.7</td>
</tr>
<tr>
<td>Chromium in cecal feces (%)</td>
<td>6.7</td>
<td>1.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Cobalt in 1st cecal feces (ug/mg)</td>
<td>8.6</td>
<td>1.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Dry matter cecal fill (g)</td>
<td>2.23</td>
<td>0.16</td>
<td>3.67</td>
</tr>
<tr>
<td>Chromium 50% passage time (hr)</td>
<td>4.1</td>
<td>0.5</td>
<td>10.6</td>
</tr>
<tr>
<td>95% passage time (hr)</td>
<td>20.1</td>
<td>5.1</td>
<td>25.0</td>
</tr>
<tr>
<td>Cobalt 50% passage time (hr)</td>
<td>23.1</td>
<td>0.3</td>
<td>28.0</td>
</tr>
<tr>
<td>95% passage time (hr)</td>
<td>51.7</td>
<td>6.6</td>
<td>74.5</td>
</tr>
</tbody>
</table>
Table 2.6. Percent of elemental markers given to ruffed grouse that were recovered in the feces, the percent of the marker recovered that was in the cecal feces, the concentration of cobalt in the first cecal feces, the estimated dry matter fill of the ceca, and the time required for passage of 50% and 95% of the recovered marker. Dry matter cecal fill was calculated from the concentration of cobalt in the first cecal dropping and the total amount of marker recovered. Values are given for individual grouse, of which 3 were fed a maintenance diet and 3 were fed the maintenance diet with 8% quebracho tannin.

<table>
<thead>
<tr>
<th></th>
<th>Control Diet (Grouse Number)</th>
<th>Tannin Diet (Grouse Number)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Cobalt recovery (%)</td>
<td>83</td>
<td>107</td>
</tr>
<tr>
<td>Chromium recovery (%)</td>
<td>76</td>
<td>105</td>
</tr>
<tr>
<td>Cobalt in cecal feces (%)</td>
<td>97</td>
<td>81</td>
</tr>
<tr>
<td>Chromium in cecal feces (%)</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Cobalt in 1st cecal feces (ug/mg)</td>
<td>9.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Dry matter cecal fill (g)</td>
<td>2.01</td>
<td>2.55</td>
</tr>
<tr>
<td>Chromium 50% passage time (hr)</td>
<td>5.1</td>
<td>3.5</td>
</tr>
<tr>
<td>95% passage time (hr)</td>
<td>11.0</td>
<td>28.6</td>
</tr>
<tr>
<td>Cobalt 50% passage time (hr)</td>
<td>23.1</td>
<td>22.6</td>
</tr>
<tr>
<td>95% passage time (hr)</td>
<td>46.8</td>
<td>43.4</td>
</tr>
</tbody>
</table>
The low cobalt recoveries are not readily explainable. Some cobalt may have been lost in fecal samples that were too small to analyze. While absorption of Co-EDTA from the digestive tract is unlikely, it has been noted in rabbits (Uden et al 1980). However, cobalt absorbed by rabbits was excreted in the urine and because urine in birds is excreted with the feces, absorbed cobalt would have passed more slowly, but would still have been recovered in fecal collections. Graphs of cobalt concentration vs. time since dosing show that cobalt concentrations were approaching zero 96 hours after dosing for all grouse except number 8, which still had 1.4 ug/mg of cobalt in the cecal feces (Figure 2.1). Because cobalt concentrations in the feces indicated that nearly all the cobalt had passed out of the digestive tract, the amount of cobalt recovered, not the amount of cobalt given, was used to calculate dry matter fill of the ceca and passage rates.

Visual inspection of cumulative recovery curves showed no consistent differences between rate of passage of the control and tannin diets; however, grouse 5 and 8, both consuming tannin diets, had altered passage rates (Table 2.6, Figure 2.2). Over 60% of recovered cobalt was excreted in the intestinal feces of grouse 5, and therefore cobalt in this grouse passed quickly. This grouse also reduced dry matter intake after dosing, which slowed the passage of fiber and therefore the passage of chromium. Passage of cobalt in grouse 8 was slower than other grouse (Table 2.6) and not complete after 96 hours (Figure 2.1).

Chromium rate of passage was rapid with 50% of recovered chromium passing
Figure 2.1. Concentration of cobalt in cecal and intestinal feces of ruffed grouse relative to the time since the birds were dosed with Co-EDTA. Grouse 1, 4, and 10 were fed a control diet and grouse 5, 8 and 9 were fed a diet containing 8% quebracho tannin.
Figure 2.2. The cumulative recovery of chromium (fed as Cr-mordanted fiber) and cobalt (fed as Co-EDTA) from the feces of ruffed grouse versus the time after markers were given. Cumulative recovery was calculated as the amount recovered at that time divided by the total amount of marker recovered. Grouse 1, 4, and 10 were fed a control diet and grouse 5, 8, and 9 were fed a diet containing 8% quebracho tannin.
in <5.5 hours and 95% of chromium passing in <29 hours for all birds except grouse number 5 in which chromium passed slowly (Table 2.6). Cobalt excretion was dependent on patterns of cecal evacuation and showed a distinctive stairstep pattern (Figure 2.2). Cobalt excretion in the first 20 hours was generally low and the result of low levels of cobalt in the intestinal feces. The first cecal feces containing cobalt were passed the morning following dosing. Grouse number 9 produced cecal feces 3.8 hours after dosing with Co-EDTA, but no cobalt was detected.

Four of 6 grouse excreted 60 to 67% of the cobalt recovered from the ceca in the first cecal dropping. Assuming complete mixing of Co-EDTA with cecal contents, 60 to 67% of cecal contents were then evacuated with this cecal movement. Grouse 5 and 8 excreted 8.2 and 29.6% of cecal cobalt in the first cecal dropping, respectively. Cecal fill was about 60% greater in grouse consuming tannin than the control grouse, but the difference was not significant (t = 2.05, df = 4, P = 0.1098) (Table 2.5). The number of cecal feces produced by each bird was not different between treatments (Table 2.4). The average size of fecal droppings was twice as large for grouse consuming tannin (Table 2.4), although the difference was not significant (P = 0.16) because of the large variation in cecal dropping size of tannin diet birds.

DISCUSSION

Performance of Birds on Tannin Diets. Dietary tannin ≤6% of the diet had little effect on bobwhite or ruffed grouse, other than changes in the proportion of feces
coming from the ceca. However, ruffed grouse consuming 8% dietary quebracho reduced dry matter intake, had lower apparent dry matter metabolizability, and lost more body mass than grouse consuming the control diet. The response of ruffed grouse to dietary tannin in the rate of passage study may have been influenced by the higher fiber and lower protein levels of the basal diet used in that study. If tannins (or nontannin phenolics) are absorbed and cause malaise or toxicity in a dose dependent manner, then a high fiber diet may exaggerate the toxicity because more diet must be consumed to meet energy requirements and therefore more toxins also would be consumed. Because crude fiber levels were greater in the rate of passage diet (21% vs 6%), and apparent dry matter metabolizability was lower (48.8% vs. 65.2%), grouse in the rate of passage study had to consume 25% more dry matter to meet their energy requirements. Despite a reduction in dry matter intake of the 8% tannin diet relative to the control, total tannin intake was still greater for the 8% tannin diet (1.61g/day) than the 6% tannin diet (1.36g/day). These levels of tannin intake begin to define the upper level of crude quebracho tannin that ruffed grouse can handle.

Tannic acid depressed intake in ruffed grouse to a greater extent than quebracho tannin. Tannic acid is a hydrolyzable tannin and more likely to be degraded and absorbed (McArthur et al. 1991, Hagerman et al. 1992). Glucuronide excretion of gray squirrels (Sciurus carolinensis) was 10 times greater on a diet containing tannic acid than either a control diet or a diet containing 6% quebracho
tannin (Chung-MacCoubrey 1993), indicating that tannic acid or its metabolites had been absorbed.

**Cecal Response to Dietary Tannin.** Results from all 3 studies suggest that grouse and quail respond to dietary tannin by increasing the proportion of feces excreted from the ceca. This response could result from 2 different processes. First, the ceca may fill quicker and evacuate more often, or second, the ceca may fill quicker, but evacuate at the same rate. If the first process occurs, then maximum cecal fill may not change with dietary tannins. If the second process occurs, then maximum cecal fill must increase. Evidence from the rate of passage study is not conclusive, but suggests that the second process occurs. Maximum cecal fill may have been greater for grouse consuming tannins ($P = 0.1098$), the number of cecal droppings did not vary between treatments, and there was weak evidence ($P = 0.16$) that cecal droppings may have been larger from grouse consuming tannin. The significance of more digesta entering the ceca is not clear, but 4 hypotheses could be proposed concerning the processes causing greater cecal fill.

The first hypothesis is that the ceca continue to function normally by selectively allowing only soluble and fine particulate matter to enter. Because tannin diets in these studies had up to 8% soluble dry matter added in the form of quebracho tannin, this addition may account for the increased proportion of feces coming from the ceca. The second hypothesis is that the ceca may respond to dietary tannin by
becoming less selective in the size of particles allowed into the ceca. By allowing a wider range of particle sizes into the ceca, digesta that would normally be excreted from the intestine would pass into the ceca. The third hypothesis is that the gizzard may respond to dietary tannins by grinding feed more finely, resulting in an increased proportion of small particles that would be allowed to pass into the ceca. The final hypothesis is that cecal fill may be influenced by the rate at which urine is brought by reverse peristalsis from the cloaca to the ceca. This increased amount of liquid may wash more material into the ceca than would enter otherwise (Akester et al. 1967).

Evidence against the first hypothesis is that the amount of tannin recovered from the ceca accounted for only about a third of the increased cecal material. However, if tannin were bound to protein, each unit of tannin could represent a larger amount of dry matter than simply the tannin measured. A testable consequence of hypothesis 1 is that the amount of intestinal feces produced per gram of basal diet consumed [where basal diet consumed = dry matter consumed X (1 - tannin content of the diet (either 0.03 or 0.06))] should not change if this hypothesis were true. To the contrary, the amount of intestinal feces produced per g of basal diet for the quail trials decreased with increasing tannin in the diet (ANOVA, $F = 8.60$, $df = 5, 2$, $P = 0.0102$) from $0.337 \pm 0.018$ g for the control diet to $0.305 \pm 0.008$ g for the 3% tannin diet and $0.260 \pm 0.001$ g for the 6% tannin diet. This result suggests that material that would normally be excreted from the intestine entered the ceca instead.

The second and third hypotheses are consistent with the observation that
digesta enters the ceca that would normally be excreted in the intestinal feces. To differentiate these hypotheses, it would be necessary to determine the range of particle sizes entering the ceca. While particle size was not determined, if the size of the largest particles in the ceca does not increase with tannin in the diet, then hypothesis 2 would not be supported.

The final hypothesis is based on the studies of Björnham (1989) who showed that more urine was transported from the cloaca to the ceca in chickens on low protein diets compared to high protein diets. Retrograde movement of urine into the ceca has been proposed as a means to recycle nitrogen (Björnham 1989, Karasawa 1989). If tannin binds protein and makes it less digestible, and if the birds were not absorbing enough protein to meet their requirements, then retrograde urine flow may have increased. Akester et al. (1967) suggested that urine flow may help transport digesta from the intestine into the ceca. Thus, if more urine was transported to the ceca in birds consuming tannin diets, then more digesta could potentially have been washed into the ceca with the urine.

What is the practical significance of greater cecal fill for birds consuming tannins? This is also not certain, but there are a couple of possibilities. First, the ceca may be less selective in particle size to ensure that large tannin-protein complexes enter the ceca where symbiotic microorganisms could metabolize the protein and perhaps the tannin (McArthur et al. 1991). In doing so, volatile fatty acids and ammonia may be released, which could be absorbed by the bird and used as
energy or as a nitrogen source (Braun and Campbell 1989, Karasawa 1989).

If retrograde transport of urine from the cloaca to the ceca occurs, it may function to retrieve conjugates (from the urine) used in detoxifying absorbed tannin or tannin metabolites. While conjugates could be retrieved in this manner, the toxins would be released and available for reabsorption if not metabolized by the microfauna. Studies have provided evidence that condensed tannin, including quebracho tannin, may be absorbed (Clausen et al. 1990, Robbins et al. 1991).

**Cecal function.** The rate of passage study supports the hypothesis that the galliform ceca is selective in allowing only soluble and fine particulate matter to enter (Fenna and Boag 1974, Remington 1989). Evidence to support this hypothesis is that >80% of Co-EDTA (the marker of soluble material) entered the ceca while <11% of chromium marked fibers entered the ceca. Thus, the purpose of the ceca apparently is to salvage unabsorbed nutrients that reach the lower tract because of the high rate of passage of grouse. This high rate of passage allows grouse to consume large volumes of poor quality forage, while not adding significant weight that would restrict flight.

The maximum cecal fill of ruffed grouse was 4 to 5 times greater than that reported for captive rock ptarmigan (*Lagopus mutus*, body mass = 425 g) (0.66 to 0.70 g) (Gasaway et al. 1975), but was less than that reported for wild rock ptarmigan (ca. 5 to 10 g) (Gasaway 1976). The proportion of cecal material excreted
with each cecal evacuation averaged 55% in rock ptarmigan (Gasaway et al. 1975), and was similar to the 60 to 67% calculated for most birds in this study.

CONCLUSION

Winter diets of ruffed grouse are high in tannins. Because tannins could potentially decrease dry matter intake at a time when energy intake is critical to survival, selective pressures should favor individuals that could cope with tannins. Quebracho, a condensed tannin, had no effect on dry matter intake or body mass at levels ≤6% of the diet. However, a high fiber diet with 8% quebracho did result in lower dry matter intake and body mass loss. A high fiber diet containing 8% tannic acid, a hydrolyzable tannin, resulted in a 50% decrease in dry matter intake and large body mass losses. Thus, grouse are more susceptible to tannic acid than to quebracho tannin.

Bobwhite and ruffed grouse responded to dietary condensed tannin by increasing the proportion of digesta that entered the ceca. The significance of this change in digesta partitioning is not certain, but may help grouse salvage tannin-bound protein or it may be the result of increased retrograde flow of urine from the cloaca to the ceca. Elemental markers showed altered cecal function of 2 of 3 grouse consuming quebracho tannin, but changes were not consistent and may have been influenced by low dry matter intake. In most grouse, the liquid phase marker entered the ceca and required 43 to 68 hours for 95% of the marker to be excreted. Chromium marked fiber was excreted in the intestinal feces and passed from most
birds in 20 to 30 hrs. These results support the hypothesis that the ceca selectively maintain soluble and fine particulate digesta while fibrous material is excreted quickly in the intestinal feces.

LITERATURE CITED


CHAPTER 3

FACTORS AFFECTING FORAGE INTAKE RATES OF RUSSIAN GROUSE AND
SOME ECOLOGICAL IMPLICATIONS

INTRODUCTION

A fundamental aspect of the ecology of a species is the rate at which food is captured and processed. Forage intake rates determine the amount of time an animal must devote to foraging and thus influence other aspects of the species' ecology, including exposure to predation, time available for other activities, and energetics (Newton 1980, Stephens and Krebs 1986). Avian foraging, primarily that by insectivores and frugivores, has received a great deal of attention (e.g. Snow and Snow 1988, Morrison et al. 1990), but foraging research of avian folivores is limited. Bite rates of herbivorous birds have been measured and used as an index to intake rates (Murton et al. 1966, Doerr et al. 1974, Savory 1978, Bland and Temple 1990). Murton (1968) applied Holling's (1959) disk equation to the type II functional response described for wood-pigeons (Columba palumbus) foraging on different densities of grain.

Ruffed grouse (Bonasa umbellus) are herbivorous as adults and their distribution and abundance may be related to forage resources (Bump et al. 1947, Servello and Kirkpatrick 1987). Ruffed grouse, like many grouse species, rely on poor quality forage during the winter (Bergerud 1988a). Grouse compensate for poor quality forage by consuming large amounts. Because winter forages appear to be

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abundant, some researchers have concluded that food resources are not important in the ecology of grouse (Bergerud 1988b). However, since large amounts of food must be consumed daily, foraging times can vary significantly depending on the intake rate attainable from a forage. Ruffed grouse densities are greatest in the range of quaking aspen (*Populus tremuloides*) and grouse foraging behavior in aspen trees has been studied (Svoboda and Gullion 1972, Doerr et al. 1974, Huempfner and Tester 1988). These studies concluded that aspen buds are an abundant, easily harvested resource that can be consumed at high rates. Outside of the range of quaking aspen, ruffed grouse densities are lower and other forages are important during the winter. Intake rates of these forages have not been measured and it is not clear what attributes of a forage influence the rate at which it can be harvested.

Spalinger and Hobbs (1992) recently proposed models describing intake rates of mammalian herbivores. They hypothesized 3 patterns of foraging that differ in the density and apparent density (visibility) of bites. A bite is defined as the amount of forage removed by a herbivore in a single cropping motion. Pattern 1 foraging occurs when bites are not apparent and the density of bites is low, such that one bite is completely processed (chewed and swallowed) before the next bite is encountered. Pattern 2 foraging has the same constraint on bite density, but subsequent bites are apparent. Pattern 3 foraging occurs when bite density is high enough that the processing time for one bite is greater than the time necessary to find the next bite. In this study, models describing intake rates for pattern 2 and 3 foraging were applied to ruffed
grouse. While free-ranging ruffed grouse are likely to engage in pattern 1 foraging, this study considered pattern 2 and 3 foraging because tests of pattern 1 foraging were difficult given the study animals and facilities, and an understanding of pattern 2 and 3 foraging will provide maximum intake rates possible for a type of forage. Also, during the winter ruffed grouse often consume poor quality, but abundant forages that likely meet the bite density constraints of pattern 3 foraging.

Pattern 2 foraging occurs when bites are apparent, but bite density is low enough that one bite can be processed before the next is encountered (Spalinger and Hobbs 1992). Because mammalian herbivores can process one bite while traveling to the next, the rate at which bites are encountered determines bite rate. To the extent that birds are able to process food while traveling from one bite to the next, the mammalian model should be directly applicable to avian herbivores. Spalinger and Hobbs (1992) begin their model development by defining intake rate as

$$I = B \times S$$

(1)

where $I$ is the intake rate (g/min), $B$ is the bite rate (bites/min), and $S$ is the bite size (g/bite). Bite rate in this foraging pattern is equal to the rate at which bites are encountered. Encounter rate is the product of the velocity of foraging and the distance between bites. Assuming bites are randomly or uniformly distributed, the average distance between bites is $D^{\frac{1}{2}}$ (Pielou 1977). Spalinger and Hobbs (1992) show that realized velocity of travel ($V$, m/min) can be described by

$$V = V_{\text{max}} / (1 + q \times D^{\frac{1}{2}})$$

(2)
where $V_{\text{max}}$ (m/min) is the maximum velocity of foraging in the absence of cropping, 
$q$ (m/bite) is the decrease in velocity due to cropping, and $D$ is the density of bites 
(bites/m$^2$). Intake rate then is predicted from
\[ I = [(V_{\text{max}} \times D^{\frac{q}{2}}) / (1 + q \times D^{\frac{q}{2}})] \times S. \]  
(3)

Equation 2 predicts an asymptotic decrease in realized foraging velocity as 
plant density increases. For a given bite size, equation 3 predicts an increasing intake 
rate with increasing plant density, up to the point where intake rate will be limited by 
pattern 3 foraging processes, which are described next.

Pattern 3 foraging occurs when the density of bites is great enough that the 
herbivore is in continuous contact with the forage and handling time, not search time, 
limits intake rate. Spalinger and Hobbs (1992) propose that in this situation, 
mammalian intake rates can be predicted from the maximum rate of food processing 
(chewing), the bite size of the forage, and the average time required to crop a bite.

This model, however, is not directly applicable to birds since food is not 
chewed, but is swallowed whole and processed at a later time in the gizzard. To 
derive a model of intake rates for avian herbivores, the logic of Spalinger and Hobbs 
(1992) was followed by defining intake rate as in equation 1 above. For pattern 3 
foraging, bite rate is the inverse of handling time ($H$, min/bite), the time required to 
harvest and swallow one bite. Handling time can be described as the sum of cropping 
and swallowing time
\[ H = T_c + T_s. \]  
(4)
where $T_c$ (min/bite) is the time required to crop one bite and $T_s$ (min/bite) is the time required to swallow one bite. Observations of foraging grouse indicate that the time required to swallow a bite increases as bite size increases (D. Hewitt, pers. observ.) because more time is required to position a large bite in the beak, draw it into the esophagus, and swallow it. Thus,

$$T_s = S/A$$

(5)

where $A$ (g/min) is a constant relating swallowing time to bite size. Substituting equation 5 for $T_s$ in equation 4, taking the inverse, and rearranging terms, bite rate can be described by

$$B = 1/H = A/(S + T_c \times A)$$

(6)

Substituting equation 6 for $B$ in equation 1

$$I = A \times S/(S + T_c \times A)$$

(7)

This model of intake rate for avian herbivores is in the same mathematical form as the model proposed by Spalinger and Hobbs (1992) for mammalian herbivores. The models differ in the meaning of the parameter $A$, which determines the asymptote of the relationship between intake rate and bite size. In the avian model, $A$ represents the relationship between bite size and handling time. In the mammalian model, $A$ is the maximum rate at which food can be chewed and swallowed, independent of bite size.

The relationship between bite size and handling time, i.e. the value of the parameter $A$, is likely to vary for different forage classes depending on the ease with
which the forage can be swallowed. One factor likely to affect the ease of
swallowing is the shape of the bite. For a bite to be swallowed by a bird, it must not
only fit into the buccal cavity, but because birds do not chew their food, bites must be
able to fit into the esophagus. Leaf material is distributed primarily in 2 dimensions
and as the bite size of leaf material increases, the average width and length of the bite
become greater. The grouse must invest increasingly more time to fold the leaf into a
shape that can be swallowed. By contrast, the biomass of fruits and buds is
distributed over 3 dimensions and therefore the shape changes less dramatically with
increasing bite size. Thus, handling time is likely to be less for the same sized bite,
and the maximum intake rate should be greater.

This foraging model has 2 important implications, that can also serve as tests
of the model’s validity (Gross et al. 1993). First, equation 6 predicts that bite rate
should decrease asymptotically with bite size. Second, equation 7 predicts that intake
rate should increase asymptotically with bite size. These predictions were found to be
true for 12 species of mammalian herbivores (Gross et al. 1993).

The objectives of this study were 1) to test the ability of foraging models
adapted from Spalinger and Hobbs (1992) to predict intake rates of ruffed grouse
foraging in plant assemblages with known characteristics, 2) to compare the foraging
efficiency of ruffed grouse with similar sized mammalian herbivores, and 3) to apply
the results of these trials to the foraging ecology of ruffed grouse in different portions
of the species’ range.
METHODS

Ruffed grouse were raised in captivity from eggs or chicks collected in western Virginia, or were obtained from a commercial breeder whose stock came from southern Ohio. Three males and 2 females were used for trials in which bite size varied and 2 males and 2 females were used for trials in which plant density varied. Grouse were 12 to 19 months old and weighed approximately 550 g. Birds were housed individually on the Virginia Tech campus in outdoor cages (2X2X3 m) made of 2.5X5 cm welded wire. Purina game bird chow (flight conditioner) and water were provided ad libitum except as noted below. All procedures were approved by the Virginia Tech Animal Care Committee (approval # A-3208-01).

Trials using leaves were conducted during Jul-Aug 1993 and trials using raisins during Jan-Mar 1994. Four trials using whole honeysuckle leaves were done in Mar 1994 to determine if leaf intake rates were similar to those done in the summer of 1993, thus allowing comparison of raisin and leaf intake rates. All trials were done between 08:30 and 10:30. To ensure a high level of interest in foraging, food was removed from the bird’s cage the night before each trial and replaced at the conclusion of the trial. Trials were conducted in each bird’s cage and each grouse was used in only one trial per day.

Raisins, which served as a model for fruits and buds, were bought commercially. Leaf forages were collected locally the night before or the morning of the trial. For each trial the following was done. The forage to be offered was
weighed and a grab sample was dried at 100° C to a constant weight to determine dry matter content. Forages were presented to the grouse and a stop watch was activated when the bird began feeding. A trial was concluded when the bird lost interest in foraging or all the forage had been consumed. A trial was considered valid if it was at least 60 seconds in duration. Successful bites, defined as cropping attempts in which leaf material was removed and swallowed, were counted. Forage not consumed was dried at 100°C to a constant weight and weighed. For each trial, dry matter consumed (g) was calculated by subtracting the dry weight of forage remaining at the end of the trial from the weight of forage offered, average bite size (g) as the weight of forage consumed divided by the number of bites counted, bite rate (bites/min) as the number of bites divided by the duration of the trial, and intake rate (g/min) as dry matter consumed divided by the duration of the trial. For trials in which bite density varied, the distance each bird walked was measured to allow calculation of average travel velocity, and the number of unsuccessful cropping attempts, defined as motions in which grouse struck at the forage with their beaks but failed to remove plant material, were counted.

Whole and partial white clover (Trifolium repens) and honeysuckle (Lonicera sp.) leaves provided a variety of potential bite sizes. Clover leaves were presented to the grouse by securing the petioles between two 2X3.5X30 cm pieces of wood which were fastened together with bolts. Approximately 30 clover leaves were placed in each of 3 of these wooden binders. Honeysuckle leaves were offered to the grouse by
hand. When feeding whole honeysuckle leaves, which were large enough to contain several bites each, we held the petiole of each leaf and allowed the grouse to determine how much leaf material was removed with each bite. Partial honeysuckle leaves were prepared by cutting each leaf into 2 to 5 approximately equal sized pieces. When the grouse attempted to bite the leaf, we released it and allowed the piece to be consumed whole. Pattern 3 foraging was assured by having at least 2 bites available to the grouse at all times.

Raisin bite size was varied by using different sized raisins and by cutting raisins into 2, 4, or 8 equal parts. To reduce stickiness, raisins were lightly coated with corn starch and excess corn starch was removed using forced air. Raisins were offered to the grouse by placing them on the tips of 6.7 cm long wooden sticks (toothpicks), of which 20 were secured in each of the wooden binders described previously.

Bite density was varied by offering whole white clover leaves to the grouse through the wire of their cage. Cage limitations required that grouse forage back and forth over an 80 cm long path. The distance between the plants in a given trial was 5, 10, 20, 40, or 80 cm. In addition to the bite being consumed, at least one bite was always visible to the grouse. Trials in which whole clover leaves were offered in the wooden binders described above were included in the analysis of bite density trials to provide bites that were 1 cm apart. The term "plant density" will be used for "bite
density” in pattern 2 foraging trials since grouse sometimes took >1 bite from each clover plant.

Leaf and raisin trials were analyzed separately. Dry weights are reported throughout. Equations for pattern 2 and 3 foraging were fitted to the appropriate data using nonlinear regression (SAS 1988). Corrected sums of squares were used to calculate an F-statistic to test the significance of each regression and to calculate r² values.

RESULTS

Ruffed grouse adapted quickly to all experimental protocols and foraged actively. Leaf bite size was varied in 19 clover and 22 honeysuckle trials. Bite sizes ranged from 0.001 g for partial clover leaves to 0.024 g for partial honeysuckle leaves. Intake rate ranged from 0.056 to 0.399 g/min. As predicted by equation 7, intake rate increased asymptotically with increasing bite size (Figure 3.1). The pattern 3 foraging model provided a significant fit to the data (P < 0.001), although only about a third of the variation in intake rate was explained by the model (r² = 0.38). The parameter A, and thus the average maximum intake rate, was 0.326 g/min. Cropping time (Tc) was 0.009 min/bite. Grouse were reluctant to consume bites of leaves larger than 0.025 g. When given the opportunity to choose bite size, as when whole honeysuckle leaves were offered, grouse selected average bites >0.01 g in only 3 of 14 trials (Figure 3.1). As predicted by equation 6 of the foraging model, bite rate
Figure 3.1. Ruffed grouse dry matter intake rate of a) whole (x) and partial (*) clover and whole (+) and partial (□) honeysuckle leaves and b) raisins as a function of bite size at forage densities high enough that bite density did not limit intake. Lines fitted from nonlinear regression of the pattern 3 foraging model. Trials were conducted during July-Aug 1993 and Jan-Mar 1994 in Blacksburg, VA.
decreased asymptotically with bite size (Figure 3.2). The average intake rate of honeysuckle leaves from 4 trials done in March 1994 was 0.337 ± 0.043 g/min and was not significantly different (t-test, P = 0.17) from 0.260, the intake rate predicted from the pattern 2 foraging model using the average bite size of the trials.

Thirty-three trials were completed in which the bite size of raisins varied. Bite sizes ranged from 0.05 to 0.48 g and intake rates from 1.98 to 6.86 g/min. The pattern 3 foraging model provided a significant fit to the data (P < 0.001) and explained over half of the variation in intake rate (r² = 0.53). As predicted by equation 7, intake rate increased asymptotically with increasing bite size, although intake rates appeared to decline at the largest bite size (Figure 3.1). Asymptotic intake rate was estimated by the model at 6.61 g/min and cropping time was estimated at 0.013 min/bite. Bite rate also declined asymptotically with increasing bite size (Figure 3.2), as predicted by equation 6. The asymptotic intake rate was over 20 times greater than that possible when foraging on leaves.

Forty-five trials were completed in which plant density varied. The pattern 2 foraging model provided a significant fit to the data (P < 0.001) and explained over half of the variation in intake rate (r² = 0.55). As predicted by equation 3, intake rate increased with increasing plant density. Intake rate at 6.25 plants/m² was underestimated by the model (Figure 3.3). The maximum foraging velocity was estimated by the pattern 2 foraging model at 14.65 m/min. Foraging velocities decreased in a non-linear manner with increasing bite density, as predicted by
Figure 3.2. Bite rates of ruffed grouse as a function of bite size for a) whole (x) and partial (*) clover and whole (+) and partial (□) honeysuckle leaves and b) raisins. Lines fitted from nonlinear regression of the pattern 3 foraging model in which forage density does not limit bite rate. Trials were conducted during July-Aug 1993 and Jan-Mar 1994 in Blacksburg, VA.
Figure 3.3. Ruffed grouse a) dry matter intake rate and b) travel velocity as a function of clover leaf density. Different symbols represent different grouse and lines were fitted from non-linear regression of the pattern 2 foraging model. Trials were conducted during July-Aug 1993 in Blacksburg, VA.
equation 2 (Figure 3.3). The decrease in velocity due to cropping \( q \) was 0.390 m/bite. Grouse successfully harvested plant material in 80.7 ± 1.3% of their cropping attempts.

The average bite size of clover leaves in pattern 2 foraging trials was 0.007 ± 0.0003 g. The intake rate for this bite size using the pattern 3 foraging model is 0.230 g/min. This intake rate was attained at 322 plants/m². Thus, for an average bite size of 0.007 g, intake rate is not controlled by plant density at densities > 322 plants/m².

DISCUSSION

Model Performance. The relationship between plant density and intake rate as described by the pattern 2 foraging model was statistically significant (Figure 3.3). A similar type II functional response was reported for wood-pigeons foraging on different densities of clover and grain (Murton 1968), but was explained in terms of the disk equation with searching and handling times (Holling 1959). Two discrepancies were noted between the pattern 2 foraging model and the data. First, intake rates were underestimated at 6.25 plants/m². Intake rate at this density had a lower variance (F-test, \( P < 0.1 \) for all tests) than the other densities. This low variance appears to be related to a loss of the lower tail of the distribution, i.e. of lower intake rates at that density (Figure 3.3). This lower variance was partially the result of one grouse, which often had low intake rates, not having a successful trial at that density. Second, foraging velocities were asymptotically related to plant density,
but the average velocity at 1.5 plants/m² was less than that predicted by equation 2 (Figure 3.3). This resulted in part from a decreased enthusiasm for foraging at this low plant density. Grouse appeared reluctant to walk from one bite to the next.

There likely is a density of plants below which a herbivore is reluctant to forage, and it appears this density was being approached at 1.5 plants/m².

The relationship between bite size and intake rate in pattern 3 foraging, as described by equation 7, was significant (P < 0.001) for both leaf and raisin trials. Both sets of trials showed an asymptotic decrease in bite rate with increasing bite size (Figure 3.2). The apparent decrease in intake rate for raisins with the largest bite size was a result of bite rates for large raisins that were lower than the bite rate predicted by the model (Figure 3.2). The time required to crop a bite did not appear to change with bite size, but the time required to swallow a bite was affected in 2 ways. First, larger bites appeared to take slightly longer to position in the mouth and swallow, a relationship noted in tropical birds consuming various sized fruits (Wheelwright 1985). Although a large increase in pre-swallowing handling times for grouse consuming whole raisins was not noted, a behavior that contributed significantly to a lower bite rate was noted. Grouse consuming whole raisins would stop foraging every 3 to 5 bites and sit still for approximately 5 seconds while muscles in the crop region appeared to contract and relax. A hypothesized function for this behavior is that large bites need to be positioned in the crop to make room for subsequent bites. Additional positioning may not be necessary for smaller bites,
which pass easily into the crop. This result suggests that the model may not adequately describe intake rate at large bite sizes.

As hypothesized, the parameter A (constant relating swallowing time to bite size) was greater for raisins than leaves. Because the value of A can vary by forage class, the generality of the model is reduced. There are at least 2 forage attributes that likely influence the value of A. The 3-dimensional distribution of the biomass in a bite accounts for part of this difference (see introduction). Another factor that influences the difference in intake rate is the dry matter content of the two forage classes. Clover averaged approximately 15% dry matter, honeysuckle 24%, and raisins 65%. Thus, a given weight of fresh leaf material contained approximately 1/3 of the dry matter that the same weight of fresh raisins contained. These two factors determined the maximum leaf bite size that could be handled, the dry matter weight of which was less than half of the smallest raisin bite size tested. Despite these different characteristics, the relationship between bite rate and bite size was similar for the two forage classes (Figure 3.2). Both had maximum bite rates near 60 bites/min and declined asymptotically toward 12 bites/min.

Intake rates of wild ruffed grouse foraging on aspen buds and twigs were compared with intake rates estimated by the pattern 3 foraging model. Huempfner and Tester (1988) observed average bite rates of 25.1 bites/min and measured average bite size from crop contents as 0.053 g/bite for an estimated intake rate of 1.33 g/min. The intake rate predicted by the model for a bite size of 0.053 g was 2.52
g/min (A = 6.61 and T_e = 0.013). The discrepancy between observed and predicted intake rates for grouse foraging on aspen buds may result from reasons other than an incorrect model. The average bite rate assumed above was from 181 observations of grouse foraging in which bite rate ranged from 7 to 50 bites/min. Thus, grouse with low bite rates were included in this average. Grouse in two-bird flocks had greater bite rates (29.2) than those feeding alone (23.1 bites/min), suggesting that vigilance behavior may have affected feeding intensity (Huempfner and Tester 1988). Ruffed grouse foraging in aspen trees in Alberta spent 14% of the time in nonforaging behavior, such as walking and looking around (Doerr et al. 1974). The model may also have overestimated intake rates because not all grouse were meeting the assumptions of pattern 3 foraging. The distribution of aspen buds may be such that grouse could finish consuming one bite before arriving at the next. Evidence for violation of pattern 3 foraging assumptions is that grouse foraging alone had greater bite rates early in the winter than later, suggesting that food depletion may have occurred (Huempfner and Tester 1988). The highest bite rates observed by Huempfner and Tester (1988) and by Svoboda and Gullion (1972) were 50 and 47.4 bites/min, respectively. A bite rate of 47.4 bites/min gives an intake rate of 2.51 g/min which is similar to that estimated by the model.

The models explained only ⅛ to ½ of the variation in intake rate. Two aspects of grouse foraging are likely to cause high variation in intake rates. First, not every cropping motion resulted in plant material being harvested. In trials using whole
clover leaves, 19.3 ± 1.3% of the cropping attempts were unsuccessful. The maximum and minimum percent failed attempts were 39.7 and 4.2%, so that significant variation is added to intake rates. Second, grouse have only marginal control over the size and shape of the bite attained in a cropping attempt. Grouse control where on a plant the beak strikes, but not the amount of material removed. The ease with which a bite can be swallowed is only partially determined by the dry matter weight. The shape of the bite also influences how easily it can be handled and swallowed and because the shape of bites varies, intake rates become more variable.

A consequence of this model with implications for other foraging research deserves recognition. Bite rates have been used in avian studies as an index to intake rates (Murton et al. 1966, Savory 1972, Bland and Temple 1990). If birds at different times or locations are consuming the same types of forages with the same bite size, then bite rates should adequately predict intake rates. However, if bite size differs between locations or places, and assuming forage density is not limiting, then bite rate will be inversely related to intake rate. Bite rates would be even more biased if birds of one population (or time period) were consuming small leaves (high bite rate, low intake rate) and birds in another population were consuming large fruits (low bite rate, high intake rate). Studies using bite rate as an index to intake rate need to demonstrate that forages and bite sizes are similar between the populations being compared.
Efficiency of Grouse Foraging Relative to Mammalian Herbivores. Maximum intake rates of mammalian herbivores in pattern 3 foraging are determined by the rate at which chewing reduces food particles to a size suitable for swallowing (Gross et al. 1993). Since birds do not chew their food, but swallow it in the form it is cropped, one may predict that birds could attain a higher intake rate than a similar sized mammal for a forage of a given bite size. This prediction was tested by comparing my pattern 3 foraging trials with those of Shipley et al. (1994).

Shipley et al. (1994) investigated intake rate as a function of bite size for 12 species of mammalian herbivores. Alfalfa clippings of various size were offered to 11 of the species on a foraging board, with the distance between plants varying between 4.5 and 18 cm, depending on the size of the herbivore. A twelfth species, collared lemmings (Dicrostonyx groenlandicus), was offered alfalfa in plastic clips, with plants 1 cm apart. Other than the manner in which plants were offered to the animals and the species of forage used, the trials in this study are similar to those done by Shipley et al. (1994). The different manner in which forage was presented should not preclude comparisons since all trials were done with bite densities high enough to ensure pattern 3 foraging. The difference in forage species also should not preclude comparisons since alfalfa, clover, and honeysuckle are all high quality, leafy forages.

Shipley et al. (1994) found that the maximum processing rate (A) scaled to body size according to the equation $\log_e(A) = -0.342 + 0.70 \times \log_e(\text{body mass})$
The asymptotic intake rate for ruffed grouse consuming leaves in this study (0.326 g/min) was lower than the intake rate predicted for a 0.550 kg animal (0.467 g/min). Another allometric relationship of maximum processing rate as a function of body mass derived by Shipley and Spalinger (1992) predicted an asymptotic intake rate of 0.420 g/min for a 0.550 kg animal. Thus, the prediction that grouse have a greater intake rate than a similar sized mammal is not supported, at least for herbaceous forage.

Differences in the efficiency of harvesting forage may help explain why grouse have lower maximum intake rates than mammals, despite not having a chewing constraint. Mammalian herbivores have morphologic adaptations that enable them to efficiently harvest forages (Hilderbrand 1982). Prehensile lips bring forage into the mouth where incisors, often with the assistance of small motions of the head, remove a bite. In comparison, ruffed grouse have a rather undifferentiated beak that is used to not only harvest leaves, but to consume such varied items as seeds, buds, insects, and grit (Bump et al. 1947). When cropping leaves, grouse use a quick motion of their head and neck to strike at the forage, attempting to tear off a bite with their beak. There are at least 2 inefficiencies associated with this manner of cropping forages. First, in contrast to mammals which can maintain nearly continuous contact with their forage, grouse must pull their head away from the forage to initiate each cropping motion. One may predict intake rates would decrease due to the time needed to break contact with the forage repeatedly, although $T_c$’s from this study
(0.009 and 0.013 min/bite) were similar to those for mammals (0.014; Gross et al. 1993). A second inefficiency in ruffed grouse foraging occurs because forage is not harvested in every cropping attempt. The proportion of cropping motions that are unsuccessful has not been reported for mammals; however, in this study grouse were unsuccessful in 19.3 ± 1.3% of their attempts to crop clover leaves. Unsuccessful cropping attempts resulted from the grouse missing the forage altogether or forage slipping out of the grouse’s beak before being cropped.

Although chewing is a constraint to mammalian intake rates, chewing allows mammals to reduce large bites in a systematic way to a particle size suitable for swallowing. Birds, while not having to chew their food, are constrained by their ability to make large bites fit into their esophagus. If grouse can attain higher intake rates, it should occur with forages which are easily swallowed, such as fruits and buds. Gross et al. (1993) did not determine intake rates for mammals feeding on forages other than alfalfa leaves. Shipley and Spalinger (1992) measured intake rates of mammals feeding on red maple (Acer rubrum) stems and on leaves of red maple or clover. For ungulate species, intake rates were lower for maple stems than for maple leaves. Snowshoe hares (Lepus americanus) (0.8 to 1.8 kg) had similar intake rates for both maple stems and clover leaves. Grouse, in contrast, had a nearly 20-fold increase in the asymptotic intake rate when foraging on raisins compared to leaves. Snowshoe hares consuming maple stems with an average bite size of approximately 0.05 g were able to attain intake rates of approximately 0.4 to 0.6 g/min (Shipley and
Spalinger 1992, Figure 3.1). Ruffed grouse in this study consuming raisins weighing 0.05 g had an average intake rate of 2.43 g/min, and estimates of intake rates for ruffed grouse foraging on aspen buds with a similar average bite size were 1.33 g/min (Huempfner and Tester 1988). Thus, grouse appear to be able to attain higher intake rates for some types of forages than a similar sized mammal.

Ruffed Grouse Foraging Ecology. Ruffed grouse densities are greater in central portions of the species' range than along the southern margins (Bump et al. 1947). The winter diets of grouse in the central part of the range are dominated by buds and twigs whereas diets of grouse in the Southeast are dominated by leaves and fruits (Svoboda and Gullion 1972, Doerr et al. 1974, Stafford and Dimmick 1979, Seehorn et al. 1981, Servello and Kirkpatrick 1987). The nutritional consequences of these different diets have been explored (Servello and Kirkpatrick 1987); however, the consequences for foraging times, energetics, and exposure to predation have received only limited attention (Thompson and Fritzell 1989).

Foraging behavior of wild ruffed grouse in aspen trees has been intensively studied (Svoboda and Gullion 1972, Doerr et al. 1974, Huempfner and Tester 1988) resulting in estimates of intake rates and daily foraging times. These studies have led to the conclusion that buds, especially aspen buds, are an abundant, easily harvested resource that support high intake rates and short foraging times. Svoboda and Gullion (1972) estimated that a ruffed grouse could fill its crop in 16.2 min of foraging.
Observations of wild ruffed grouse have shown that the duration of foraging periods averaged 12.2 to 16 min in the morning and 15.5 to 24 min in the evening [Svoboda and Gullion 1972, Doerr et al. 1974, Brander 1965 (cited by Svoboda and Gullion 1972), and Huempfner and Tester 1988].

The duration of foraging also can be estimated by the time necessary to harvest forage sufficient for the grouse's energy requirements. Daily energy expenditure (DEE), the sum of energy for basal metabolism, activity, thermoregulation, and heat increment of feeding (Robbins 1993), has not been measured for free-ranging ruffed grouse. The standard metabolic rate (SMR, energy expenditure while resting in a post-absorptive state and a thermoneutral environment) of a 607 g ruffed grouse was estimated at 66.1 kcals/day (Thompson and Fritzell 1988). Daily energy expenditure of free-ranging blue grouse (Dendragapus obscurus) during the winter in Utah was estimated at 1.4 times the SMR (Pekins et al. 1992).

Assuming the same relationship, DEE for ruffed grouse would be 93 kcals/day. Captive ruffed grouse held outdoors during the winter in Virginia consumed 121 kcals metabolizable energy (ME)/kg body weight\(^{0.75}\)/day (Chapter 1). At this rate a 600 g grouse held in captivity consumes 82 kcals ME/day. Allowing 11 kcals/day for additional activity and thermoregulation of wild grouse, then an estimate for DEE of 93 kcals/day seems reasonable. Assuming 1.38 kcals ME/g for aspen buds (Servello and Kirkpatrick 1987), a bite rate of 25.1 bites/min, and a bite size of 0.09 g (Svoboda and Gullion 1972, Huempfner and Tester 1988), grouse could meet their
daily ME requirements in 30 min. If a bite size of 0.053 g is assumed (Huempfner and Tester 1988), 51 min of foraging are required. In summary, observations of wild grouse and calculations of foraging times necessary to meet energy requirements suggest that ruffed grouse foraging on aspen buds can meet their daily energy requirements in 30 to 50 min of foraging.

Grouse in the Southeast use predominately 3 forage categories during the winter (Nelson et al. 1938, Harlow and Guthrie 1972, Smith 1977, Stafford and Dimmick 1979, Seehorn et al. 1981, Servello and Kirkpatrick 1987). Evergreen forages [e.g. mountain laurel, (Kalmia latifolia), and Christmas hollyfern (Polystichum acrostichoides)] are abundant and available during winter, but are often only 30% of the diet, presumably because of their toxic properties (Servello and Kirkpatrick 1987, Hewitt et al. 1994). Herbaceous and deciduous leaves (e.g. Smilax sp. and Lonicera sp.) and fruits are high quality, but often have a low biomass from Jan-Mar (Hewitt et al. 1994). These high quality leaves and fruits make up 30 and 40% of an average diet, respectively (Nelson et al. 1938, Harlow and Guthrie 1972, Smith 1977, Stafford and Dimmick 1979, Seehorn et al. 1981, Servello and Kirkpatrick 1987). Metabolizable energy concentration of evergreen leaves, herbaceous leaves, and fruits are 2.11, 2.74, and 2.50 kcals/g, respectively (Servello and Kirkpatrick 1987). The intake rate of evergreen leaves is estimated at 0.326 g/min since their abundance is likely to result in pattern 3 foraging. The highest biomass of herbaceous leaves during the winter on a study site in southwest Virginia
was estimated by Hewitt et al. (1994) to be 1.5 kg/ha, which is 18.8 plants/m² if plants are 0.008 g each, the average size of herbaceous leaves found in 3 grouse crops (D. Hewitt, unpubl. data). From the pattern 2 foraging equation, an intake rate of 0.189 g/min was estimated. The biomass of fruits is low during late winter in the Southeast (Hewitt et al. 1994), but because a pattern 2 foraging relationship has not been established for fruits and because their patchy distribution may allow for pattern 3 foraging when a patch is found, an intake rate of 3.38 g/min was estimated for fruits. This intake rate assumed a bite size of 0.09 g, the average weight of wild grapes (Vitis spp.) (D. G. Hewitt, unpublished data). Assuming these values, a grouse in the Southeast would have to forage for 100 min to consume 93 kcats ME/day.

This estimate is likely to be low for 3 reasons. First, no allowance has been made for vigilance behavior. The intake rates used to calculate this foraging time were derived from captive grouse in a situation designed to maximize intake rates. Vigilance behavior was accounted for in the intake rate estimates of grouse foraging on aspen buds (Svoboda and Gullion 1972, Huempfner and Tester 1988). Second, daily foraging times of grouse in the Southeast assume forages are apparent which may not be true for forages at ground level where leaf litter may be heavy. Finally, a maximum intake rate was assumed for fruits, which are often scarce and patchy in late winter (Hewitt et al. 1994). This daily foraging time may be an overestimate
because grouse can search for and consume different forage classes at the same time, thus reducing slightly the time necessary to harvest herbaceous leaves.

The consequences of greater foraging times during the winter for ruffed grouse in the southern portions of the species’ range are not clear. Ruffed grouse in Missouri had lower survival rates during seasons when their daily movements were greatest (Thompson and Fritzell 1989). Since predation is a major mortality factor for most ruffed grouse populations (Bergerud 1988a, Small et al. 1991), any factor that increases exposure to predation is likely to be important in the species’ population dynamics. Predation rates for grouse in the Southeast should be studied.

CONCLUSION

Dry matter intake rates for ruffed grouse are influenced by forage density, bite size, and forage type. The maximum intake rate of leaf material is ≤25% of intake rates observed for aspen buds and only 5% of the intake rate possible for fruits and buds. Because diets of grouse in the Southeast have a high proportion of leaves during the winter, daily foraging times are likely to be greater than for grouse in the central portions of the species’ range. High levels of daily activity for radio tagged grouse in southwest Virginia support this conclusion (Chapter 4). Longer foraging times may increase predation risks and energy expenditures.

Ruffed grouse appear to have intake rates for leaf material similar to those of a comparable sized mammal, despite not having a chewing constraint that limits intake
rate in mammals. When consuming raisins, grouse have maximum intake rates 16
times greater than predicted from an allometric mammalian equation.

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CHAPTER 4

ACTIVITY TIMES AND HOME RANGES OF RUFFED GROUSE
IN SOUTHWEST VIRGINIA

INTRODUCTION

Densities of ruffed grouse are lower in the southern Appalachians than in the central portions of the species’ range. (Bump et al. 1947). Servello and Kirkpatrick (1987) hypothesized that densities were lower in part because the biomass of high quality forage (e.g. herbaceous leaves and fruits) was insufficient in late winter for grouse to subsist. Furthermore, abundant evergreen forage (e.g. mountain laurel (Kalmia latifolia)) and ferns are likely toxic to grouse when eaten in large quantities (Bump et al. 1947, Servello 1985). Thus, ruffed grouse in the southern Appalachians may have to eat as much evergreen material as they can detoxify, then search for herbaceous leaves and fruits to meet the remainder of their nutrient requirements. A consequence of this hypothesis is that widely dispersed food resources should cause ruffed grouse in the southern Appalachians to have larger home ranges and greater activity times than grouse in central portions of the range. The objective of this study was to determine activity times and home range sizes of ruffed grouse in southwest Virginia during winter.

METHODS

This study was conducted on 3 areas in Montgomery and Giles counties in southwest Virginia. Elevations ranged from 650 to 900m. The Buckeye study area

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was second growth, mixed hardwoods, interspersed with pasture and pasture reverting to eastern red cedar (Juniperus virginiana) and black locust (Robinia pseudoacacia). Hardwood areas had an open understory whereas reverting areas had a thick growth of honeysuckle (Lonicera spp.), greenbriar (Smilax spp.), coral berry (Symphoricarpus sp.), multiflora rose (Rosa spp.), and blackberry (Rubus sp.). The Norris Run study area was oak (Quercus spp.)-hickory (Carya spp.) forest with yellow poplar (Liriodendron tulipifera) and northern red maple (Acer rubrum) also common in the overstory. A majority of the study area had burned 20 years previous. The burned areas had high densities (12,184±701 stems/ha, D. G. Hewitt, unpublished data) of small diameter (<10cm) trees and an open understory with scattered vaccinium (Vaccinium sp.), greenbriar, and grape (Vitis sp.). The Lick Run study area was oak-hickory forest. A portion had burned 20 years previous and had heavy regeneration of overstory species. The unburned portions of the Lick Run area were second growth forest with a well developed understory of mountain laurel, vaccinium, and rhododendron (Rhododendron sp.).

Ruffed grouse were trapped from December 1991 to February 1992 on the Norris Run and Buckeye study areas and from November 1992 to January 1993 on all 3 areas. Grouse were captured using lily-pad traps (Gullion 1965) and fitted with 15g radios (Advanced Telemetry Systems, Isanti, MN), which were held in the crop region of the bird by harness straps around the neck and body. The radios were battery powered and contained real time tip switches that changed the pulse rate from
45 to 75 pulses/min when the plane of the radio against the bird's breast tipped past 25° (1992) or 45° (1993) from horizontal. All birds were released immediately within 50 m of their capture site. Grouse were flushed within 5 days of being trapped to ensure their flight was normal. The birds were then located an average of 3 times a week by triangulation or homing using a Telonics (Mesa, AZ) radio receiver and H-antennae. Harmonic mean and convex polygon estimates of home range size were determined from program HOME RANGE (Ackerman and Samuel 1990).

To determine if an adequate number of locations had been obtained to describe home range size, the number of locations for each grouse was plotted vs. home range size. A bootstrapping procedure in program HOMERANG (Raphael and Brink 1988) was also used, which took multiple subsamples of various sizes from the location data of a single bird. Minimum convex polygon home range was then determined for each of these subsamples and the relationship between home range size and number of locations was plotted.

Three to 5 radios identical to those used on free ranging grouse were placed by an assistant on each study area, each year, to determine accuracy of triangulation. Accuracy was recorded as the distance between true and estimated radio locations.

Survival rates were estimated as described by Hisey and Fuller (1985) using program MICROMORT. The date of death, if not known exactly was considered to be the mid-point between the last date the grouse was known alive and the date the mortality was noted. The period over which survival rates were calculated was from
the date when the first grouse was captured to March 15. Both years of data were combined.

During January-March 1992, 2 grouse from one of the study areas were monitored for activity on 2 predetermined days each week. The following week, 2 grouse on the other study area were similarly monitored. The radio signals from the 2 grouse were monitored in alternating 2-minute time blocks from 15 minutes before sunrise to 15 minutes after sunset. Changes in signal strength or pulse rate were recorded for each 2-minute interval. The same person monitored all grouse and was within 500 m of the birds while doing so. A grouse was classified active during a time block if any change in pulse rate or signal strength was noted. The percent time of active was calculated as the percent of 2-minute blocks classified active. A test of the accuracy of these activity estimates is given in Appendix B.

During January-March 1993, radio signals were monitored using an omnidirectional antennae, radio receiver, and data processor (Telonics, Mesa, AZ), and a two-channel data recorder (Rustrak RangerII, model RR2-1200, East Greenwich, RI), which recorded both signal strength and pulse rate every 0.5 seconds. This system was set up within 250 m of the grouse and the antenna was secured to prevent signal changes from movement of the receiving antenna. Each radio tagged grouse was monitored from 30 minutes before sunrise to 30 minutes after sunset on one predetermined day each week. Data files were transferred to a micro-computer and software from Rustrak was used to plot signal strength and pulse rate
vs. time of day.

Periods of activity and inactivity at least 2 minutes in duration were delineated based on the frequency of changes in signal strength or pulse rate (Figure 4.1). A period was always classified as inactive if neither signal strength nor pulse rate varied and as active if both signal strength and pulse rate varied. Periods in which only signal strength or pulse rate varied were classified as active or inactive according to which of two methods was being used. The "single criteria method" required changes in either signal strength or pulse rate to classify a period as active, while the "dual criteria method" required changes in both signal strength and pulse rate to classify a period as active. Time active was calculated using both of these methods. A test of the accuracy of these activity estimates is given in Appendix B.

Percent activity for both years was the percent of the trial duration that a grouse was active. Daily activity patterns were determined using the time active for each successive 60-minute period from the beginning of a trial (referred to as dawn) until a period that contained 1200 hours and from the end of the trial (referred to as dusk) backwards to a period that contained 1259 hours. We calculated the percent of each 60-minute period that the grouse was active and the percent of the total activity for that day that occurred in each 60-minute period. Any 60-minute period in which the signal was lost >20% of the period was discarded from this analysis.

For both years, data from a day were not used if the radio signal was lost for >10% of the potential monitoring time of that day. Temperature and rainfall data
Figure 4.1. Plots of radio signal data interpreted to determine activity of radio-tagged ruffed grouse. The top line is signal strength and the lower line is pulse rate. The vertical dotted lines are interpreted changes between activity and inactivity. The top row of letters are classifications of activity (A) or inactivity (I) according to the single criterion method. The lower row of letters are activity classifications according to the dual criterion method (See methods section for explanation).
were obtained from National Oceanic and Atmospheric Administration (1992, 1993) records from Blacksburg, VA which was <30 km from all study sites. At least 6.4 mm of rain had to be reported for a day to be considered rainy. Snow conditions and cloud cover were recorded at the study site.

Standard errors are given with all means. Least-squares regression was used to determine the relationship between activity and temperature. The effect of snow cover and rain on activity was tested with a 2 sample t-test. The effect of cloud cover on activity was tested by ANOVA, with cloud cover categories of 0, 50, and 100%. All statistical tests were conducted using SAS (1988) software.

RESULTS

During the winter of 1991-92, 2 grouse were caught on Buckeye Mountain in 129 trap days and 4 grouse on Norris Run in 295 trap days. During 1992-93, 1 grouse was caught on Buckeye Mountain in 264 trap days, 2 grouse on Lick Run in 338 trap days, and 3 grouse on Norris Run in 242 trap days. One bird from each study area in 1992-93 was killed by predators or lost its radio before usable data were gathered.

Activity. Activity was determined exclusively from signal strength changes during 1992 because tip switches in the radios were not at the proper angle for frequent pulse rate changes. Twenty-two days of activity estimates were obtained on 6 grouse.

Four grouse had ≥4 days of activity monitoring each with average percent activity
Table 4.1. The percent time active from 15 minutes before sunrise to 15 minutes after sunset of free ranging ruffed grouse in southwest Virginia during January-March 1992. On a given day, radio signals from two grouse were monitored in alternating 2-minute periods. If any signal strength change was noted, that 2-minute period was considered active.

<table>
<thead>
<tr>
<th>Grouse Number</th>
<th>Date</th>
<th>Total Time Hr</th>
<th>Total Time Min</th>
<th>Percent Time Active</th>
<th>Percent time signal lost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1202</td>
<td>20 Jan</td>
<td>10</td>
<td>36</td>
<td>45.2</td>
<td>9.4</td>
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<td>24 Jan</td>
<td>10</td>
<td>36</td>
<td>50.4</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>3 Feb</td>
<td>10</td>
<td>44</td>
<td>45.3</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>6 Mar</td>
<td>12</td>
<td>04</td>
<td>54.7</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Avg(SE)</strong></td>
<td><strong>11 00</strong></td>
<td><strong>48.9 (2.28)</strong></td>
<td><strong>Percent</strong></td>
<td><strong>3.8 (1.87)</strong></td>
<td></td>
</tr>
<tr>
<td>1020</td>
<td>20 Jan</td>
<td>10</td>
<td>32</td>
<td>56.3</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>24 Jan</td>
<td>10</td>
<td>40</td>
<td>43.1</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>3 Feb</td>
<td>11</td>
<td>00</td>
<td>46.7</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>7 Feb</td>
<td>11</td>
<td>04</td>
<td>40.3</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>17 Feb</td>
<td>11</td>
<td>28</td>
<td>34.2</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Avg(SE)</strong></td>
<td><strong>10 57</strong></td>
<td><strong>44.1 (3.67)</strong></td>
<td><strong>Percent</strong></td>
<td><strong>3.8 (1.75)</strong></td>
<td></td>
</tr>
<tr>
<td>4020</td>
<td>31 Jan</td>
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<td>56</td>
<td>42.7</td>
<td>3.0</td>
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<tr>
<td></td>
<td>10 Feb</td>
<td>11</td>
<td>12</td>
<td>39.8</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>28 Feb</td>
<td>11</td>
<td>52</td>
<td>57.4</td>
<td>0.0</td>
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<td></td>
<td>9 Mar</td>
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<td>70.3</td>
<td>0.5</td>
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<tr>
<td></td>
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<td>12</td>
<td>20</td>
<td>56.3</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Avg(SE)</strong></td>
<td><strong>11 42</strong></td>
<td><strong>53.3 (5.52)</strong></td>
<td><strong>Percent</strong></td>
<td><strong>0.9 (0.56)</strong></td>
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</tbody>
</table>

Table 4.1 (con’t)

<table>
<thead>
<tr>
<th>Grouse Number</th>
<th>Date</th>
<th>Total Time</th>
<th>Percent Time Active</th>
<th>Percent time signal lost</th>
</tr>
</thead>
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<td>4100</td>
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<td>11 00</td>
<td>21.1</td>
<td>2.4</td>
</tr>
<tr>
<td>10 Feb</td>
<td>11 12</td>
<td>33.2</td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>28 Feb</td>
<td>11 52</td>
<td>43.8</td>
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<td>0.0</td>
</tr>
<tr>
<td>9 Mar</td>
<td>12 12</td>
<td>37.1</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
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<td>12 20</td>
<td>34.1</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Avg(SE)</strong></td>
<td>11 43</td>
<td><strong>33.9 (3.69)</strong></td>
<td><strong>0.6 (0.46)</strong></td>
<td></td>
</tr>
<tr>
<td>4294</td>
<td>2 Mar</td>
<td>11 56</td>
<td>60.8</td>
<td>0.0</td>
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<tr>
<td>6 Mar</td>
<td>12 04</td>
<td>80.7</td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>4274</td>
<td>2 Mar</td>
<td>11 52</td>
<td>52.2</td>
<td>0.0</td>
</tr>
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</table>
for an individual ranging from $34 \pm 3.7$ to $53 \pm 5.5\%$ (Table 4.1). Two grouse had 1 and 2 days of monitoring. Percent activity of these birds ranged from 52 to 81\% (Table 4.1). The amount of time active per day averaged $327 \pm 21.5$ min.

Signal strength and pulse rate changes were used to determine activity for 16 days on 3 grouse in 1993. Average percent activity ranged from $48 \pm 5.1$ to $60 \pm 6.2\%$ with the single criteria method of classifying activity and from $37 \pm 6.5$ to $52 \pm 5.8\%$ with the dual criteria method (Table 4.2). The difference in these two estimates of activity is the percent time that pulse rate and signal strength were in conflict as to the bird’s activity status. On average, the two methods of determining activity were in agreement $90 \pm 1.8\%$ of the time. The amount of time active per day averaged $376 \pm 26.5$ min for the single criteria method and $300 \pm 31.0$ min for the dual criteria method.

Patterns of daily activity were similar whether presented as the percent of each 60-minute period a grouse was active or as the percent of total daily activity that occurred in each 60-minute period. Only the former is presented here. For grouse from both years with $\geq 3$ days of activity data, there were no strong trends in daily activity patterns consistent to all birds (Figs. 4.2 and 4.3, Appendix C). There was a tendency for grouse to show a peak in activity 2 to 3 hours before dusk and for activity to decrease in the last hour before dusk. This pattern was most evident for the 4 grouse from the Norris Run area. These grouse showed moderate levels of activity throughout the rest of the day, although grouse 1202 and 0112 showed peaks
Table 4.2. The percent time active from half hour before sunrise to half hour after sunset of free ranging ruffed grouse in southwest Virginia during January-March 1993. Active periods were determined when signals from radio tags varied in either strength or pulse rate or when both signal strength and pulse rate varied. The radio tags contained a mercury tip switch that altered the pulse rate of the radio signal between 45 and 75 pulses/minute as the radio tipped past 45 degrees from horizontal.

<table>
<thead>
<tr>
<th>Grouse Number</th>
<th>Date</th>
<th>Total Time</th>
<th>Percent Time Active</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>Hr  Min</td>
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<td>19 Jan</td>
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<td>35.8</td>
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<td></td>
<td>3 Feb</td>
<td>11 24</td>
<td>42.7</td>
</tr>
<tr>
<td></td>
<td>9 Feb</td>
<td>11 36</td>
<td>47.1</td>
</tr>
<tr>
<td></td>
<td>16 Feb</td>
<td>11 42</td>
<td>30.6</td>
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<td>12 10</td>
<td>59.8</td>
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<td>12 22</td>
<td>57.1</td>
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<td>Average</td>
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<td><strong>5.10</strong></td>
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<tr>
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<td>31 Jan</td>
<td>11 18</td>
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<td>Single Criteria</td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>0091</td>
<td>29 Jan</td>
<td>11 14</td>
<td>62.4</td>
</tr>
<tr>
<td></td>
<td>4 Feb</td>
<td>11 27</td>
<td>52.6</td>
</tr>
<tr>
<td></td>
<td>18 Feb</td>
<td>11 56</td>
<td>40.8</td>
</tr>
<tr>
<td>Average</td>
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<td>11 32</td>
<td>51.9</td>
</tr>
<tr>
<td>SE</td>
<td></td>
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<td>6.24</td>
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</tbody>
</table>
Figure 4.2. Mean percent time active during successive hours after dawn and before dusk for radio tagged ruffed grouse during Jan-Mar 1992. Each symbol represents a different grouse.
Figure 4.3. Mean percent time active during successive hours after dawn and before dusk for radio tagged ruffed grouse during Jan-Mar 1993. Each symbol represents a different grouse.
in activity 3 and 5 hours after dawn, respectively.

Grouse 4020 from the Buckeye study area showed a broad peak in activity during the middle of the day, with moderate levels of activity most other times. Grouse 4100 of the same area had a period with little activity during the second hour after dawn. The grouse on the Lick Run study site had relatively constant and high levels of activity throughout the day.

Rain and cloud cover did not have a significant effect on activity ($t=1.08$, $df=36$, $P=0.29$ and $F=0.46$, $df=2,35$, $P=0.63$, respectively). There was weak evidence that activity was lower when snow cover was present ($t=1.6$, $df=36$, $P=0.12$). Activity levels were positively related to daily high temperature ($r^2=0.13$, $n=38$, $P=0.02$) and daily low temperature ($r^2=0.10$, $n=38$, $P=0.04$).

**Home Range and Survival.** The average distance between radios placed in the field and our estimate of their position using telemetry ranged from $36\pm7.9\,m$ for the Lick Run study area to $138\pm56.2\,m$ for the Norris Run area. The average distance between actual and estimated radio positions on the Buckeye Mountain area was $48\pm16.7\,m$.

Nine grouse had an average of $25\pm3.1$ locations/grouse (Table 4.3). Both methods for describing the relationship between the number of locations and home range size suggested that home range may have been underestimated for some birds (Figures 4.4 and 4.5). Therefore, the term minimum home range will be used. The
Table 4.3. Harmonic mean (95%) and convex polygon (100%) home range size of radio tagged ruffed grouse during the winter (from date bird was radio tagged to March 15) in Southwest Virginia during 1992-93.

<table>
<thead>
<tr>
<th>Grouse ID</th>
<th>Area</th>
<th>Location Dates</th>
<th>Number of Locations</th>
<th>Home Range (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First</td>
<td>Last</td>
<td>Harmonic Mean</td>
</tr>
<tr>
<td>4100</td>
<td>Buckeye</td>
<td>2 Jan 92</td>
<td>14 Mar 92</td>
<td>36</td>
</tr>
<tr>
<td>4020</td>
<td>Buckeye</td>
<td>2 Jan 92</td>
<td>13 Mar 92</td>
<td>36</td>
</tr>
<tr>
<td>1020</td>
<td>Norris</td>
<td>9 Jan 92</td>
<td>18 Feb 92</td>
<td>27</td>
</tr>
<tr>
<td>1202</td>
<td>Norris</td>
<td>9 Jan 92</td>
<td>14 Mar 92</td>
<td>35</td>
</tr>
<tr>
<td>4274</td>
<td>Norris</td>
<td>18 Feb 92</td>
<td>15 Mar 92</td>
<td>12</td>
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<td>4294</td>
<td>Norris</td>
<td>18 Feb 92</td>
<td>15 Mar 92</td>
<td>16</td>
</tr>
<tr>
<td>0091</td>
<td>Norris</td>
<td>12 Dec 92</td>
<td>26 Feb 93</td>
<td>23</td>
</tr>
<tr>
<td>0112</td>
<td>Norris</td>
<td>7 Dec 92</td>
<td>14 Mar 93</td>
<td>23</td>
</tr>
<tr>
<td>0012</td>
<td>Lick</td>
<td>6 Jan 93</td>
<td>15 Mar 93</td>
<td>16</td>
</tr>
</tbody>
</table>
Figure 4.4. Home range size as a function of the number of locations for ruffed grouse during the winter in southwest Virginia. Convex polygon (□) and harmonic mean (■) home ranges are shown with least-squares regression lines which had $P$-values = 0.0352 and 0.0847, respectively.
Figure 4.5. The relationship between home range size and number of locations for ruffed grouse during the winter in southwest Virginia. For each grouse, multiple samples ranging in size from 3 to the number of locations for that grouse were taken from the grouse’s total locations. A convex polygon home range was calculated for each sample and the average home range of all samples of given size was plotted. Only grouse with >20 locations are shown. (Note different scales).
average minimum home range was 14.0±2.2 ha and 23.1±3.9 ha for convex polygon
and harmonic mean estimates, respectively (Table 4.3). For grouse with >20
locations (N = 6), the convex polygon and harmonic mean minimum home range
averages were 15.4±2.6 ha and 25.1±5.4 ha, respectively.

Eleven grouse lived >14 days after being tagged and were used in survival
analysis. Survival rates from 14 Nov to 15 Mar were 0.577 (95% CI = 0.309 -
1.00). This estimate included one bird that was caught on 14 Nov and was found
dead on 6 Dec, 1992. If this bird is dropped from the analysis, the survival period
becomes 1 Dec to 15 March and the survival rate becomes 0.723 (95% CI = 0.461 -
1.00). Both of these estimates also include a grouse which lost its radio 36 days after
capture. Since no evidence of predation was noted and the bird was alive the day
before the radio was found, the radio days for this bird were included, but a mortality
was not added to the survival calculations.

DISCUSSION

Activity. Using changes in radio signal strength to determine activity is controversial.
The technique has been used with a variety of species including capercaillie (Tetrao
urogallus) (Gjerde and Wegge 1987), beaver (Castor canadensis) (Lancia et al. 1980),
roe deer (Capreolus capreolus) and mountain hare (Lepus timidus) (Cederlund and
Lemnell 1980). None of these studies reported the accuracy of their methods.
Gillingham and Bunnell (1985) reported that signal strength from captive black-tailed
deer (Odocoileus hemionus) varied least when animals were inactive and most when
animals were active. They cautioned that the amount of signal strength change varied between days and with distance between the transmitter and receiver. Beier and McCullough (1988), while testing their ability to estimate activity of white-tailed deer (O. virginianus) using radio signals, reported 29 of 30 observations of deer bedded or standing alert had constant signal strength. However, 72 of 193 (37%) observations of deer active also had constant signal strength. These data suggest an underestimation of activity when using only changes in signal strength.

In this study, not only did tests with captive grouse show a reasonable relationship between changes in signal strength and activity (Appendix B), but field data from 1993 showed 90% agreement for activity classification between changes in signal strength and pulse rate of radios with activity sensors. In addition, the 2 points from the 1993 captive grouse activity data that are far below the x=y line (Appendix B) result from the dual criteria method of estimating activity in which pulse rate did not change when grouse were active but signal strength did. Thus, activity estimated by the single criteria method for these 2 trials was closer to observed activity because of changes in signal strength.

Estimates of activity from changes in signal strength and pulse rate have been tested in large mammals and it has been shown that this technique can differentiate between periods of activity and inactivity (Gillingham and Bunnell 1985, Beier and McCullough 1988). Similar tests have apparently not been conducted for grouse. The regression of percent activity estimated and observed from the accuracy tests of
this study suggest that the 1993 estimates of activity may be overestimates (Appendix B). A portion of this overestimation may be due to activity from radio signals being estimated in blocks of time (>2 minutes) whereas observed activity was done instantaneously every 10 seconds. In this way, a period in which activity was interspersed with short intervals (a few seconds) of inactivity would be estimated from radio signals as entirely active, but would be observed as active only a portion of the time. These short periods of inactivity should not be considered separate from active periods for field data because the grouse is still more vulnerable to predation and expending more energy during these periods than if it was roosting.

The average time active for grouse in this study (5 to 6 hours/day) was greater than activity times reported for other grouse species during winter. Capercaillie in Norway were active an average of 3 hours/day (Gjerde and Wegge 1987), black grouse (*Tetrao tetrix*) in Finland, 90 min/day (Marjakangas 1992), willow ptarmigan (*Lagopus lagopus*) in British Columbia, 52 minutes/day (Mossop 1988), and white-tailed ptarmigan in Colorado, approximately 3 hours/day (Braun and Schmidt 1971).

Activity times for ruffed grouse in the central part of the species’ range have not been reported. Huempfner and Tester (1988) discussed unpublished data suggesting that during periods of deep, powdery snow in Minnesota, ruffed grouse had three peaks in activity at dawn, dusk and for 1 to 2 hours around midday. Arboreal feeding was the primary activity during crepuscular periods. When snow crusted, midday activity increased as grouse searched for food at ground level. Bump
et al. (1947) remarked that, during winter, ruffed grouse in New York certainly had feeding periods in the morning and evening, but that tracks in the snow and flushing records indicated that grouse may feed at any time during the day.

**Daily Foraging Times.** Long periods of foraging are not necessary in areas where grouse feed extensively on buds and catkins. Data from ruffed grouse feeding in quaking aspen (*Populus tremuloides*) trees show that grouse can meet their energy requirements in 30 to 50 min of foraging (Chapter 3). Arboreal feeding behavior of ruffed grouse in aspen trees has been intensively studied and these studies show that foraging bouts of grouse in aspen trees are short. Morning foraging bouts in Alberta and Minnesota averaged 12.2 to 16.0 minutes and evening bouts 17.6 to 24.0 minutes (Doerr et al. 1974, Huempfner and Tester 1988). Midday arboreal feeding was infrequent in Minnesota but averaged 31.3 minutes and was characterized by frequent pauses and less intense foraging than morning or evening periods (Huempfner and Tester 1988). Aspen, while important, is rarely >50% of ruffed grouse food habits (Servello and Kirkpatrick 1987, Huempfner and Tester 1988, Doerr et al. 1974). Intake rates have not been reported for other tree species in which ruffed grouse feed but are likely greater than intake rates possible with leaves because buds and catkins have a larger bite size, compact shaped bite, and greater dry matter content (Chapter 3). Compared to aspen, grouse intake rates may be lower when feeding in birch (*Betula* spp.) or cherry (*Prunus* spp.) trees because the thin branches will not readily
support a grouse, which must frequently stop foraging and flap its wings to maintain its position in the tree (Bump et al. 1947, Svoboda and Gullion 1972).

Winter diets of ruffed grouse in the Southeast are dominated by leaves and fruits (Smith 1977, Stafford and Diimmick 1979, Seehorn et al. 1981, Servello and Kirkpatrick 1987). Leaves, even when abundant, are harvested at 25% of the rate estimated for quaking aspen (Chapter 3). If leaf forage is abundant, ruffed grouse consuming only leaves must forage for at least 100 min to meet their energy requirements (Chapter 3). Fruits, if abundant, accessible, and providing a large bite size, could be harvested at rates similar to aspen buds, and the mass of fruits needed to meet a grouse’s energy requirements would be less than the mass of aspen buds because fruits have a higher metabolizable energy content (Servello and Kirkpatrick 1987).

The biomass of herbaceous leaves and fruits in southeast grouse habitats is often low during late winter (Harlow et al. 1975, Hewitt et al. 1994). A grouse foraging on clover sized leaves would have to forage continuously for 3 hours when herbaceous leaf biomass is 1.5 kg/ha (Chapter 3), the highest biomass reported by Hewitt et al. (1994) from a wildlife management area in southwest Virginia. This foraging time will be greater if grouse have to search for leaves, which is likely during the winter when ground forage is covered by leaf litter. A more realistic foraging pattern is for grouse to consume a mixture of herbaceous leaves, fruits, and evergreen leaves (Servello and Kirkpatrick 1987). For a 30%, 40%, and 30% diet of
these forages, respectively, assuming biomass does not limit the intake of fruits and evergreen leaves, a grouse would have to forage for $>100$ min to meet its energy requirements (Chapter 3). A notable exception to this foraging pattern will occur when acorns are abundant. Acorns have characteristics of a forage that can be harvested quickly and it is likely that grouse could meet their daily energy requirements in a short time when acorns are abundant.

If foraging is the primary activity of grouse during winter and if ruffed grouse in the Southeast must forage for longer periods to meet their energy requirements, then the daily activity times of grouse in the Southeast are going to be greater than those of grouse in the central portion of the range. Greater periods of activity for grouse in the Southeast also may result from a lesser need for thermoregulatory behavior, such as snow roosting, that is common in northern climates (Bump et al. 1947). Longer periods of activity expose grouse to greater predation risks. Ruffed grouse in Missouri had lower survival rates when daily movements were greatest (Kurzejeski and Root 1988, Thompson and Fritzell 1989). Since predation is the greatest cause of mortality, excluding hunting, for full-grown ruffed grouse (Bergerud 1988, Small et al. 1991), an increase in predation risks may have consequences for population dynamics and possibly densities.

Predation rates have not been reported for grouse in the southeast. Male grouse in Missouri had a survival rate of 0.486 between the beginning of October and the end of March (Thompson and Fritzell 1989). Survival rates of ruffed grouse in
Wisconsin between September and December ranged from 0.19 to 0.52 (Small and Rusch 1985).

Daily Activity Patterns. Daily activity patterns of grouse in this study differ from those of other grouse species. Capercaillie, black grouse, willow ptarmigan, and white-tailed ptarmigan were all crepuscular during winter (Braun and Schmidt 1971, Gjerde and Wegge 1987, Mossop 1988, Marjakangas 1992). Not only did ruffed grouse in the present study have >30% probability of being active during the middle of the day, but most birds were less active during the final hour before dusk than during the preceding 2 to 3 hours. Although published data are lacking, observations suggest that ruffed grouse in the central part of the range have short periods of intense feeding at dawn and dusk, but are also active periodically during the middle of the day (Bump et al. 1947, Doerr et al. 1974, Huempfner and Tester 1988).

One explanation for the pattern of ruffed grouse activity in this study is that grouse consuming foods with low harvest rates would have to be active for a large proportion of each day, resulting in activity throughout the day. The absence of significant crepuscular activity in Virginia ruffed grouse may be related to a number of factors. First, preferred forages of grouse in the southeast are often small, located on the ground, interspersed with leaf litter, or widely dispersed during winter. Ambient light levels may be too low near dawn and dusk for grouse to efficiently search for these forages. In the central portion of the grouse’s range, buds and
catkins are relatively large and located on shrubs and trees above ground. These forages can be easily located and harvested even in dim light, allowing grouse to forage when predators that rely on vision to locate prey are less effective (Doerr et al. 1974, Bergerud and Gratson 1988). Second, the northern goshawk (Accipiter gentilis), an important diurnal predator of grouse, is more abundant in central and northern portions of the species’ range than in southern portions. Predation pressure from goshawks may force northern ruffed grouse to forage when low light levels decrease that predator’s effectiveness (Bergerud and Gratson 1988). Two significant groups of predators of grouse in the Southeast are likely to be mammalian predators and owls. Grouse can most easily avoid mammalian predation by foraging when light levels allow detection of a stalking or ambushing predator. Owl predation could be reduced by avoiding crepuscular activity. Finally, nights are longer in northern areas during winter. Grouse in the north may forage intensively at dawn and dusk to ensure a full crop when entering their night roost and to meet energy deficits accrued during the night. Shorter nights in the south would decrease these pressures for grouse in Virginia.

Minimum Home Ranges. Convex polygon estimates of ruffed grouse minimum home range size in this study (14.0 ± 2.2 ha) were similar to winter home range estimates from Tennessee (mean = 8.9 ha for males and 20.4 ha for one female) (Boyd 1990) and smaller than those from Missouri (mean = 84 ± 12.2 ha) (Thompson and Fritzell
1989). Harmonic mean estimates of home range (23.1 ± 3.9 ha) were also smaller than those from Missouri (mean=109±15.9 ha) (Thompson and Fritzell 1989). Home range size estimates for ruffed grouse during winter in the central portion of the range have not been published. Home range size of ruffed grouse in Missouri during spring and summer was larger than that of grouse in the central portions of the range and may be due to more widely dispersed resources (Thompson and Fritzell 1989).

CONCLUSION

This study has shown that ruffed grouse in Virginia during the winter are active an average of ≥5 hours/day. This level of activity is greater than that reported for other grouse species. There are no comparable published data available from ruffed grouse in other portions of the species’ range, however, observed feeding rates suggest that ruffed grouse in the central portions of the range can meet their energy requirements in less than 1 hour when foraging on quaking aspen buds. Ruffed grouse consuming an average diet of leaves and fruits in Virginia must forage for ≥100 min to meet their energy requirements. High levels of activity increase exposure to predation and may have consequences for population density. Minimum home range sizes averaged 14.0 and 23.1 ha for convex polygon and harmonic mean estimates, respectively. These estimates are slightly greater than those reported in Tennessee and less than half those reported from Missouri. No home range data could be found from the central portion of the ruffed grouse’s range. This study
provides evidence in support of the hypothesis that the low density of high quality food increases activity levels of ruffed grouse during the winter in the Southeast.

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CHAPTER 5

GROUND NEST PREDATION AND RUFFED GROUSE DENSITIES IN SOUTHWEST VIRGINIA

INTRODUCTION

Densities of ruffed grouse (Bonasa umbellus) are substantially lower in the southern Appalachians than in central portions of the species' range (Bump et al. 1947, Atwater and Schnell 1988). Bergerud (1988) suggested these low densities were due to nest predation. He argued that the greater diversity and abundance of nest predators at lower latitudes results in higher rates of nest predation and lower recruitment. Furthermore, he contends that nest predation is density-dependent so that the proportion of nests destroyed by predators increases as the density of nests increases. Because of greater nest predation, ruffed grouse in the Southeast must space their nests further apart than grouse in the north to achieve nesting success sufficient to maintain a stable population. Thus, Bergerud (1988) hypothesized that nest predation is the cause of low ruffed grouse densities in the Southeast.

The objectives of this study were to use artificial nests 1) to test whether ground nest predation in the southern Appalachians is density-dependent and 2) to obtain an estimate of the extent of ground nest predation in the Southeast. Artificial nests were used because of the difficulty in locating adequate numbers of ruffed grouse nests and because nest densities could be manipulated.
METHODS

Artificial Nest Predation. Three study sites were selected on the Jefferson National Forest in Montgomery and Craig counties, Virginia. All 3 sites were within the ridge and valley province of the Appalachian hardwood subregion (Smith and Linnartz 1980) and were characterized by oak (Quercus sp.)-Hickory (Carya sp.) forests with pine (Pinus sp.), red maple (Acer rubrum), and yellow popular (Liriodendron tulipifera) also common in the overstory. Mountain laurel (Kalmia latifolia), vaccinium (Vaccinium sp.), flowering dogwood (Cornus florida), rhododendron (Rhododendron sp.) and saplings of overstory species were common in the understory. All 3 sites contained small (<5 ha) clear-cuts less than 25 years of age with heavy regeneration of overstory species. Ruffed grouse were observed and heard drumming on or near all sites.

Two areas were delineated at each of the 3 study sites and were randomly assigned high and low nest densities. High density areas were 500 X 500 m and low density areas were 500 X 2000 m. High and low density areas were separated by at least 1 km and were >100 m from any road open to vehicular use. Abandoned logging roads and foot trails traversed most areas.

Nests were located using 2 random numbers; 1 was the number of meters along a transect that bisected the area (ranged from 1 to 500 for high density areas and 1 to 2000 for low density areas) and the other was the number of meters perpendicular to this baseline transect (range 1 to 250 for both areas). The direction
perpendicular to the baseline transect was randomly determined. To aid in relocating
the nests, colored flagging was placed in trees at approximately 50 m intervals along
the baseline transect and on the perpendicular lines leading to the nests. Flagging was
placed 8 to 15 m from the nest and explicit directions from this flagging to the nest
were recorded. Twenty nests were placed in each of the 6 areas, resulting in nest
densities of 0.8 and 0.2 nests/ha for the high and low density areas, respectively.

Nests consisted of 5 fresh brown chicken eggs placed in a small depression in
the leaf litter at the base of a tree, stump, or log. To minimize human odors at the
nest site, eggs were handled with plastic or rubber gloves and all personnel wore
rubber boots when constructing and checking nests. In addition, eggs were placed in
the nest using a 2.4 m pole with a shallow dish at the end so that field personnel
remained $>2$ m from the nest.

Nests were constructed in mid-April to coincide with laying and incubating of
grouse in the area (Servello and Kirkpatrick 1988). Nests were placed on the John's
Creek study site on 13 and 14 April 1992, on the Poverty Creek site on 15 and 17
April, and on the Craig Creek site on 21 and 22 April. A random draw determined
which nest density at each site was constructed first. All nests were checked 10 and
20 days after being constructed and any disturbance was noted. A nest was
considered depredated if $\geq 1$ eggs were broken or missing. Predation was classified
as avian or mammalian according to Rearden (1951). Nests were classified as
disturbed if they had been depredated as described above or if $\geq 1$ eggs, although
intact, had been displaced from the nest. After checking the nests the final time, all eggs were broken and any indication of spoilage was noted.

**Nest Predators.** The diversity and abundance of mammalian nest predators was compared between 4 northern states with high densities of ruffed grouse (New York, Minnesota, Michigan, and Wisconsin) and 4 southern states with low densities of ruffed grouse (West Virginia, Virginia, North Carolina, and Tennessee). The species used in the analysis are shown in Appendix D and were chosen because they were potential nest predators (Bump et al. 1947, Leimgruber et al. 1994). Diversity of mammalian nest predators was estimated by determining the number of species in each of the 8 states. The distribution of furbearing species was determined from Novak et al. (1987) and that of fox (*Sciurus niger*) and grey squirrels (*S. carolinensis*) was determined from Burt (1976). An index to the abundance of mammalian nest predator species was developed from furbearer trapping data (Novak et al. 1987). Novak et al. (1987) gave up to 5 levels of harvest for each furbearing species. The greatest harvest level was given a value of 1 and the lowest harvest level a value of 4 or 5. Average abundance of mammalian nest predators for a state was the average of the abundance indices for species located in that state. Abundance and diversity was calculated for all mammals in Appendix D and separately for medium sized mammalian predators, who are responsible for a large portion of ruffed grouse nest predation. Species considered medium sized mammalian nest predators were red fox
(Vulpes vulpes), grey fox (Urocyon cinereoargenteus), raccoon (Procyon lotor),
Virginia opossum (Didelphis virginiana), spotted skunk (Spilogale putorius), and
striped skunk (Mephitis mephitis).

Unpublished breeding bird survey data from 1966-89 (data files provided by J.
L. Waldon, Fish and Wildlife Information Exchange, Blacksburg, VA) were used to
compare densities of avian nest predators between northern and southern portions of
the ruffed grouse’s range. The average number of blue jays (Cyanocitta cristata),
common ravens (Corvus corax), and American crows (Corvus brachyrhynchos) seen
per breeding bird survey route was recorded for each of the 8 states used in the
mammalian analysis. Only routes from the following physiographic regions were
included because these regions were likely to contain ruffed grouse habitat: Great
Lakes plain, Cumberland plateau, Blue ridge mountains, Adirondack mountains,
Northern spruce-hardwoods, Great Lakes transition, and Ridge and valley (Robbins et
al. 1986).

Paired t-tests were used to compare the percent of nests depredated and
disturbed between high and low density areas and between the first 10 days and the
second 10 days of the study, pairing on sites. The test for the effect of density was
one sided because density-dependent nest predation capable of controlling the
population would only be supported if predation rates on the high density area were
greater. Alpha = 0.05 for all statistical tests.
RESULTS

Predation rates for all 6 areas combined averaged 10.0% \( \pm 2.9 \) (mean \( \pm \) SE) after 10 days and 19.2% \( \pm 4.2 \) after 20 days. Nests on the high density areas had greater predation rates after 10 days of exposure (\( t=3.46, P=0.037 \)), but there was no difference in predation rates after 20 days (\( t=1.00, P=0.211 \)) (Table 5.1). The percentage of nests classified as disturbed averaged 15.8% \( \pm 4.0 \) after 10 days and 25.8% \( \pm 7.0 \) after 20 days (Table 5.1). There was no difference in the percentage of nests disturbed between high and low density areas at either 10 or 20 days (\( t=1.75, P=0.111 \) and \( t=0.96, P=0.219 \), respectively). The difference between the number of nests classified as depredated and disturbed was due to nests in which one or more eggs had been displaced from the nest but were found intact near the nest. The 12 nests in this category had an average of 3.0 \( \pm 0.43 \) eggs displaced an average of 29 \( \pm 3 \) cm. The percentage of nests depredated during the first 10 days was not significantly different from the percentage depredated during the second 10 days for either the high or low density areas (\( t=1.66, P=0.239 \) and \( t=-1.65, P=0.241 \), respectively).

Sixty one percent (14 of 23) of nest predation was attributed to mammals. No avian predation was noted. The class of predator was not evident in 39% of the depredated nests. This category included nests in which only a small number of fragments were found (7 nests), the eggs were entirely missing (1 nest), or the eggs
Table 5.1. Percent (number of nests) of artificial nests depredated and disturbed after 10 and 20 days of exposure at 2 nest densities on 3 study sites during April and May 1992 in Montgomery and Craig counties, Virginia.

<table>
<thead>
<tr>
<th>Area</th>
<th>Days</th>
<th>Depredated</th>
<th></th>
<th>Disturbed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>John's Creek</td>
<td>10</td>
<td>15 (3)</td>
<td>10 (2)</td>
<td>15 (3)</td>
<td>10 (2)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>15 (3)</td>
<td>20 (4)</td>
<td>15 (3)</td>
<td>20 (4)</td>
</tr>
<tr>
<td>Poverty Creek</td>
<td>10</td>
<td>10 (2)</td>
<td>0 (0)</td>
<td>15 (3)</td>
<td>10 (2)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>15 (3)</td>
<td>5 (1)</td>
<td>20 (4)</td>
<td>15 (3)</td>
</tr>
<tr>
<td>Craig Creek</td>
<td>10</td>
<td>20 (4)</td>
<td>5 (1)</td>
<td>35 (7)</td>
<td>10 (2)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>35 (7)</td>
<td>25 (5)</td>
<td>60 (12)</td>
<td>30 (6)</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>15 (9)</td>
<td>5 (3)</td>
<td>22 (13)</td>
<td>10 (6)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>22 (13)</td>
<td>17 (10)</td>
<td>32 (19)</td>
<td>22 (13)</td>
</tr>
</tbody>
</table>
were broken but the contents not eaten (1 nest). None of the eggs remaining after 20
days of exposure showed any sign of spoilage.

There were no large differences between northern and southern states in the
diversity or abundance of mammalian nest predators (Table 5.2). American crows
were more abundant in the southern states, common ravens were more abundant in
northern states, and blue jays were similarly abundant in northern and southern states
(Table 5.2). The only difference in diversity of medium sized mammalian nest
predators was that spotted skunks were not found in the north. The abundance of
medium sized predators in southern states was generally lower (index ranged from 2.0
to 3.5) than in the northern states (range 1.0 to 2.0).

DISCUSSION

Predation Rates. The ground nest predation rates reported here are lower than those
reported in other studies in which brown chicken eggs were used in artificial nests.
Forests in southeastern Tennessee had predation rates of artificial ground nests of
30% after 21 days of exposure (Matschke 1965) and 56% after 43 days of exposure
(Henry 1969). Artificial nests exposed for 5 to 6 days in central Pennsylvania had
predation rates of 83, 25, and 32% in intensively managed aspen (Populoides sp.)
stands (Yahner and Wright 1985, Yahner and Scott 1988, Yahner et al. 1989) and
80% in irrigated oak-red maple stands (Yahner and Morrel 1991).

The Barrens Grouse Habitat Management Study Area (BGMA), where the nest
predation studies of Yahner and his colleagues were conducted, allows comparison of
Table 5.2. The number and density index of trappers (Kovac 1987), the diversity and average abundance of mammalian nest predators, and the average number of 3 avian nest predator species seen per breeding bird survey route in states with high and low ruffed grouse densities. Trapper density and mammalian abundance indices are 1 = greatest and 5 = lowest density. See Appendix D for original mammal data, and references.

<table>
<thead>
<tr>
<th>High grouse densities</th>
<th>Low grouse densities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NY</td>
</tr>
<tr>
<td>Trappers (Density)</td>
<td></td>
</tr>
<tr>
<td>20,000</td>
<td>(1)</td>
</tr>
<tr>
<td>Diversity</td>
<td></td>
</tr>
<tr>
<td>All Mammals</td>
<td>13</td>
</tr>
<tr>
<td>Medium sized mammals*</td>
<td>5</td>
</tr>
<tr>
<td>Abundance*</td>
<td></td>
</tr>
<tr>
<td>All Mammals</td>
<td>2.4</td>
</tr>
<tr>
<td>Medium sized mammals*</td>
<td>1</td>
</tr>
<tr>
<td>American Crow</td>
<td>10.0</td>
</tr>
<tr>
<td>Common Raven</td>
<td>0.1</td>
</tr>
<tr>
<td>Blue Jay</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*Gray fox, red fox, raccoon, opossum, spotted skunk, and striped skunk.

*The average abundance index from Appendix D for species present in each state.
the current study with an area where grouse are more abundant. Ruffed grouse densities on the BGMA during the 1980's ranged from 3.1 to 6.8 breeding males/100 ha and spring flush rates were 0.10 to 0.40/km (Storm et al. 1993). No population estimates are available from my study sites. As indicators of grouse densities on my study areas, 8 grouse were flushed during approximately 90 hours of field work conducting this study (D. Hewitt, unpubl. data), and transects walked in similar habitat within 30 km of the study sites had winter flush rates of 0.11/km (Hewitt et al. 1994). Oak-hickory forests in Tennessee support grouse densities of 0.2 to 2.7 breeding males/100ha (Pelren 1991). Assuming artificial nest predation rates are correlated to actual nest predation pressure, the low grouse densities in my study area as compared to those on the BGMA do not appear to be the result of more intense nest predation.

**Density-Dependent Nest Predation.** The results of this study favor density independent nest predation but are not conclusive. Density-dependent predation was significant after 10 days of exposure when considering nests classified as depredated. There was no significant effect after 20 days of exposure or for nests classified as disturbed at either 10 or 20 days. Given that the biologically important parameter is the number of nests that survive laying and incubating, the results after 20 days of exposure are more relevant than those at 10 days, thus arguing against density-dependent nest predation. The power (Cohen 1988) of the nonsignificant tests was
low (<30% for 20 days of exposure) and due in part to the small magnitude (5 to 12%) of the mean difference in predation rates between the high and low density areas. The biological significance of this magnitude of difference is questionable, supporting the conclusion that density-dependent nest predation did not occur.

Several other studies have investigated density-dependent nest predation using artificial nests. Studies that demonstrated a positive relationship between predation rates and nest density have several factors in common (Table 5.3). These studies generally had high predation rates (>50%), short nest exposure times, high nest densities, primarily avian predators, and habitats with low structural diversity. Structural diversity is negatively associated with ground nest predator efficiency (Bowman and Harris 1980). In contrast to these studies, significant density-dependent nest predation was not shown where predation rates were low (generally <50%), birds were not the primary predator, and the habitat was structurally diverse (Table 5.3).

For density-dependent nest predation to occur, the average inter-nest distance must be less than the radius of the area searched by a predator after depredating a nest (Andren 1991). Inter-nest distances are not known for ruffed grouse in the Southeast, but nest densities can be estimated using the estimate of breeding male ruffed grouse in Tennessee referred to previously (0.2 to 2.7 /100 ha, Pelren 1991). Liberally assuming 4 males/100 ha to account for nondrumming males, and equal sex ratios, a nest density of 4 nests/100 ha results. Andren (1991) used Monte-Carlo
Table 5.3. Artificial nest predation studies that investigated density-dependent nest predation.

<table>
<thead>
<tr>
<th>Density depend.</th>
<th>Habitat</th>
<th>Percent predation</th>
<th>Nests per ha</th>
<th>Days of exposure</th>
<th>Primary predator</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Deciduous forest</td>
<td>85-95</td>
<td>&lt; 1 - 44</td>
<td>7</td>
<td>Avian, squirrel</td>
<td>Andersson and Wiklund (1978)</td>
</tr>
<tr>
<td>Yes</td>
<td>Heath</td>
<td>72-98</td>
<td>1-4</td>
<td>1-3</td>
<td>Avian</td>
<td>Goransson et al. (1975)</td>
</tr>
<tr>
<td>Yes</td>
<td>Dry lake</td>
<td>9/13 graphs &gt;50%</td>
<td>0.17-6.7</td>
<td>5</td>
<td>Avian</td>
<td>Page et al. (1983)</td>
</tr>
<tr>
<td>Yes</td>
<td>Pasture</td>
<td>45-91</td>
<td>1.5-102</td>
<td>6</td>
<td>Avian</td>
<td>Sugden and Beyersbergen (1986)</td>
</tr>
<tr>
<td>Yes</td>
<td>Shrub and grass</td>
<td>22-100</td>
<td>156-40,000</td>
<td>&lt; 0.25</td>
<td>Avian</td>
<td>Tinbergen et al. (1967)</td>
</tr>
<tr>
<td>No</td>
<td>Woodland</td>
<td>0-7</td>
<td>2-6</td>
<td>15</td>
<td>Rat</td>
<td>Amarasekare (1993)</td>
</tr>
<tr>
<td>No</td>
<td>Reverting Field</td>
<td>24-31</td>
<td>4.7-12.7</td>
<td>6</td>
<td>Snakes and Avian</td>
<td>Gottfried (1978)</td>
</tr>
<tr>
<td>No</td>
<td>Pine Forest</td>
<td>67%</td>
<td>0.25-1.0</td>
<td>22</td>
<td>Mammal</td>
<td>Boag et al. (1983)</td>
</tr>
<tr>
<td>No</td>
<td>Shrub and tundra</td>
<td>31-77</td>
<td>0.27-1.0</td>
<td>28</td>
<td>Mammal</td>
<td>O'Reilly and Hannon (1989)</td>
</tr>
<tr>
<td>No</td>
<td>Deciduous Forest</td>
<td>17-22</td>
<td>0.2-0.8</td>
<td>20</td>
<td>Mammalian</td>
<td>This Study</td>
</tr>
</tbody>
</table>
simulations to show, for different nest densities, the proportion of nests that would have a nearest-neighbor within a given distance. These simulations showed that with 4 nests/100 ha, approximately 1%, 5%, and 17% of nests would have a nearest-neighbor within 25 m, 50 m, and 100 m, respectively. These percentages assume a random dispersion of nests and would be lower if grouse spaced their nests in a uniform pattern (Andren 1991). Uniform nest spacing has been documented in blue (Dendragapus obscurus) and spruce grouse (D. canadensis) and may occur in ruffed grouse (Bergerud and Gratson 1988).

Search patterns and decision criteria used by nest predators will determine the inter-nest distance necessary for density-dependent nest predation to occur. Although these factors are poorly understood, the low probability of finding a second nest, even within 100 m, reduces the likelihood of density-dependent nest predation. Furthermore, the low predation rates of this study and the fact that nest predation was independent of density at 20 and 80 nests/100 ha suggests that the probability of density-dependent nest predation limiting grouse in the Southeast is low.

The finding of this study that mammals are the primary ground nest predators in southeastern forests is supported by another study. Using photography, Leimgruber et al. (1994) found that raccoons, striped skunks, white-footed mice (Peromyscus leucopus), and grey squirrels were predators at 67% of unsuccessful artificial nests in northern Virginia. Overall, 91% of the predators visiting nests were mammals and 9% were birds. No snake predation was detected. Eggs in my study
that were displaced from nests but not broken may have been moved by small mammals who were not able to break the shells, as this was observed in the study of Leimgruber et al. (1994) (W. J. McShea, pers. comm.). In New York, Bump et al. (1947) attributed 88% of ruffed grouse nest predation to mammals, and 78% of nest predation to foxes, weasels, skunks, and raccoons. Yahner and Morrell (1991) found 98% of their artificial nests in central Pennsylvania were disturbed by non-avian predators, primarily raccoons. On the BGMA study area, 19, 71 and 91% of artificial nests were disturbed by avian predators (Yahner and Wright 1985, Yahner and Scott 1988, Yahner et al. 1989).

Bergerud's Hypothesis. Bergerud's (1988) argument that nest predation limits ruffed grouse densities in the Southeast requires greater nest predation pressure in the Southeast than in northern areas where grouse densities are greater. He supports this contention with 2 relationships (Bergerud 1988, Fig. 15.7): 1) nesting success, when considered across North American grouse species, decreases with decreasing latitude and 2) the diversity and abundance of predators increases at lower latitudes. The first relationship is determined in part by the high nest predation rates of steppe grouse species (Bergerud 1988, Table 5.2), which are more common at lower latitudes. The relationship, therefore, may be heavily influenced by habitat, in which case Southeastern ruffed grouse could be considered outliers for their latitude because their habitat is more similar to northern forest grouse than steppe grouse. In addressing the
second relationship, nest predator diversity differs little between 4 northern states, where ruffed grouse densities are high, and 4 southern states where densities are low (Table 5.2). Furthermore, there is no evidence that mammalian nest predators are more abundant in the Southeast than in northern states. Although large differences in predator abundance could likely be detected with the data in Table 5.2, these data are inferior to actual density estimates because they are based on trapping records that do not incorporate trapping effort, which may vary between states. However, the number and density of trappers in West Virginia are similar to northern states, and the abundance indices are also similar (Table 5.2). Density estimates for raccoons are available. In a removal experiment, Bump et al. (1947) estimated raccoon densities of 0.015/ha in New York. Raccoon density estimates from upland habitats in the Southeast are <0.01 to 0.03/ha in Western Tennessee (Leberg and Kennedy 1987, 1988), 0.04 to 0.06/ha in eastern Tennessee (Minser and Pelton 1982), and 0.01 to 0.02 in Kentucky (Fredrick et al. 1986). The significance of higher densities of American crows in the Southeast is not apparent since avian predation does not appear to be significant for ruffed grouse nests (Bump et al. 1947) or for ground nests in the Southeast (Leimgruber et al. 1994, this study).

CONCLUSION

There are a number of arguments against nest predation as a primary factor limiting ruffed grouse in the southern Appalachians. First, the low nest predation rates of this study suggest low predation pressure in early to mid-successional stage
forests in the Southeast. Second, nest predators do not appear to be more diverse or exceptionally more abundant in the Southeast compared to northern portions of the species’ range. Third, the densities of grouse in the Southeast appear too low for density-dependent nest predation to develop, especially compared to nest densities in which density-dependent predation has been demonstrated (Table 5.3). In a study of nest success in the Southeast, Hardy (1950, as cited in Bergerud 1988) reported that only 3 of 12 ruffed grouse nests hatched in Kentucky. Obviously, more study of ruffed grouse nest predation in the southern portions of the species’ range is required before definitive conclusions can be drawn.

LITERATURE CITED


CHAPTER 6

CONCLUSION: RUFFED GROUSE NUTRITION AND ITS ROLE IN LIMITING GROUSE DENSITY IN THE SOUTHEAST

The ruffed grouse has one of the largest ranges of any nonmigratory bird in North America, extending across the northern United States and southern Canada from New England to Alaska, and reaching south along the Appalachian, Rocky, and Pacific Coast mountain ranges. Ruffed grouse densities are highest in the central portions of the range, generally in the upper midwestern United States from Minnesota to New York. Progressing southward in the Appalachians, grouse densities decrease south of Pennsylvania. The southern edge of the range is in northern Georgia and Alabama.

One hypothesis about why ruffed grouse densities are low in the Southeast is that winter food resources are inadequate (Servello and Kirkpatrick 1987a). The argument is based on the difference in food habits between grouse populations in different portions of the range. Grouse in the central portion of the species' range consume primarily buds, twigs, and catkins during winter (Doerr et al. 1974, Servello and Kirkpatrick 1987a, Huempfner and Tester 1988). These forages are generally abundant, quickly harvested, and have adequate protein and energy levels (Servello and Kirkpatrick 1987a, Huempfner and Tester 1988). Ruffed grouse in the Southeast eat primarily leaves and fruits during the winter (Stafford and Dimmick 1979, Norman and Kirkpatrick 1984, Servello and Kirkpatrick 1987a). There are 3
categories of leaves that are commonly eaten: herbaceous, deciduous, and evergreen. Evergreen leaves, including mountain laurel (*Kalmia latifolia*), winter green (*Gaultheria procumbens*), and fern fronds, are abundant throughout winter, but contain high levels of secondary plant metabolites. Herbaceous and deciduous leaves along with fruits are considered high quality forage because of high levels of protein or energy and low levels of secondary plant metabolites (Servello and Kirkpatrick 1987a). These forages, however, are generally scarce during late winter (Servello and Kirkpatrick 1987a, Hewitt et al. 1994). Thus, the foraging choices available to grouse in the Southeast during late winter are evergreen leaves, which are abundant but potentially toxic, and fruits and high quality leaves, which are not toxic and have high levels of nutrients, but are scarce during winter.

Given these forage choices, the following foraging pattern for ruffed grouse in the Southeast is proposed. Grouse probably consume fruits and high quality leaves when they can be harvested at rates above some minimum rate. This minimum rate is probably determined by energy, time, and predation risk constraints. As winter progresses and the biomass of high quality forages declines, the intake rate of these forages also declines. Grouse begin to consume evergreen leaves to minimize foraging time, predation risk, and energy expenditure. During some winters and in some areas, the biomass of high quality leaves and fruits drops too low for these forages to be harvested efficiently. Again, this critical biomass is probably determined by energy, time, and predation risk constraints. In these situations,
grouse must rely heavily on evergreen leaves, supplementing their diet with any high quality forages they can find. Diets in these situations may be submaintenance. Grouse in areas where high quality foods are depleted before the end of winter are less likely to survive.

There are 2 ways in which this foraging pattern could result in lower grouse densities in the Southeast. First, grouse living in areas with abundant high quality forage and good cover will be least susceptible to predation and nutrient shortfall. In habitats where these forages are scarce or where cover is inadequate, grouse must spend more time foraging or may forage in less protected areas and thus will be more susceptible to predation or nutrient deficiencies. If areas containing the best habitat are limited and if some grouse are excluded from these areas, then grouse relegated to poor habitat are more likely to die during winter. In this manner, the proportion of the landscape in these high quality habitats will determine the density of grouse. A model describing how landscape diversity can influence population regulation has been proposed by Pulliam (1988). Similar mechanisms have been proposed for regulation of red grouse (Lagopus lagopus) in Scotland (Watson and Moss 1980) and ruffed grouse in Wisconsin (Cary et al. 1992)

High quality habitats may be less common in the Southeast than in central portions of the range. Areas most useful to grouse are early-mid successional habitats with heavy cover and a variety of herbaceous plants and fruits. Succession moves these habitats toward the mature oak (Quercus spp.)-hickory (Carya spp.) forests
common to the southern Appalachians. These forests typically have a closed canopy and a well developed shrub layer containing evergreen plants. High quality leaves and fruits are less abundant in mature habitats and are often depleted by late winter (Hewitt et al. 1994). Thus, there is a window of opportunity after a disturbance and before succession makes habitat unsuitable, when grouse can thrive. Before exploitation of the eastern forest by Europeans, grouse likely colonized patches of suitable habitat created by fire, wind throw, ice storms, and other phenomena that opened the forest canopy. Reproduction in these areas was probably high and offspring would disperse in search of other suitable habitat. The rate at which these areas formed, their size, and the rate at which succession reclaimed them may have determined the original density of ruffled grouse.

By contrast, northern forests contain tree species in which ruffed grouse feed during the winter. The most prominent of these tree species is quaking aspen (Populus tremuloides), but birch (Betula spp.), bigtooth aspen (P. grandidentata), and cherry (Prunus spp.) are other important forage species common in northern forests. Even these trees may be succeeded by a beech (Fagus sp.) - maple (Acer spp.) climax, but they remain a part of the landscape for a much longer period than do the early successional habitats preferred by ruffed grouse in the Southeast. Evidence in support of this hypothesis is that grouse densities become lower south of the range of quaking aspen.

A second manner in which ruffed grouse densities in the Southeast could be
limited by winter foods was also discussed by Servello and Kirkpatrick (1987a). Winter diets of ruffed grouse in the Southeast have low protein levels and low protein:energy ratios (Servello and Kirkpatrick 1987a). Tannins, which are found in high levels in evergreen leaves, may exacerbate the low levels of protein by binding protein and making it unavailable for digestion (Nelson et al. 1975, Hagerman and Butler 1991). Low levels of protein while the hen is laying can reduce clutch size and chick viability (Beckerton and Middleton 1982). It is not known if low protein diets in the winter could reduce reproductive success of ruffed grouse in the spring. Clutch size of ruffed grouse in the Southeast is the same as that in other portions of the ruffed grouse's range (Handley 1932, Bump et al. 1947). Chick viability has not been studied in the Southeast. If chick viability is affected by the protein intake of the hen, and if habitats differ in the level of protein attainable from the diet, then grouse densities could again be a function of the amount of high quality habitat.

A mechanism quite similar to this has been proposed for regulation of red grouse (Watson and Moss 1980). In this hypothesis, clutch size, egg quality, and chick survival are a function of the quality of the laying hen's diet. In addition, Watson and Moss (1980) proposed that chicks produced by a hen whose diet was poor quality would become more aggressive and defend larger territories. In this manner, the density of grouse in high quality habitats would decline, and because survival in poor habitats is low, population densities overall would decrease.

The studies conducted for this dissertation have implications for these
hypotheses of limiting factors for ruffed grouse in the Southeast. An assumption of these hypotheses is that grouse cannot subsist solely on evergreen leaves. Feeding trials showed that grouse perform well with low levels of Christmas hollyfern or mountain laurel in the diet, but that energy and nitrogen intake were reduced when 40% of the diet was Christmas hollyfern or mountain laurel (Chapter 1). Grouse were unable to maintain body mass when 40% of the diet was Christmas hollyfern. Ruffed grouse cannot maintain body weight when consuming diets of 50% mountain laurel (Servello 1985). Grouse consuming these evergreen leaves excreted more detoxification conjugates than control grouse, suggesting that secondary plant metabolites were being absorbed and may have contributed to low levels of protein and energy intake by limiting dry matter intake (Chapter 1).

Because grouse cannot subsist solely on evergreen leaves, grouse must consume enough fruits and high quality leaves to meet their energy requirements. However, these forages likely have low intake rates during the winter because they are often inconspicuous, widely dispersed, and covered with leaf litter. Foraging trials with captive ruffed grouse show that leafy forages are harvested at only 25% of the rate of aspen buds (Chapter 3). The difference is probably greater for wild grouse because plants in the foraging trials were conspicuous and surveillance behavior was not included as it was for grouse foraging on aspen buds. The low biomass of herbaceous leaves during winter would further depress intake rates. Fruits, if abundant, could be harvested at rates comparable to aspen buds. These
varying harvest rates mean that a grouse consuming a mixed diet of leaves and fruits would have to forage at least twice as long and possibly 3 to 4 times as long as grouse foraging in aspen trees to consume the same amount of metabolizable energy (Chapter 3).

A consequence of the food limitation hypothesis is that grouse in all but the best habitats in the Southeast should be active for longer periods each day than grouse in central portions of the species' range. Estimates of activity times of ruffed grouse in the Southeast (Chapter 4) show that grouse were active 5 to 6 hours/day. Comparable data are not available from ruffed grouse in other areas, but most feeding activity of grouse in the central portions of the range occurs at dawn and dusk (Huempfner and Tester 1988). Grouse in Virginia were not strongly crepuscular since they were less active in the final hour before dusk than during the preceding hour (Chapter 4). Other grouse species are active only 1 to 3 hours/day in the winter. Thus, ruffed grouse in Virginia are active a large portion of each day, which supports the hypothesis that winter food resources limit ruffed grouse in the Southeast.

There have been no published reports of starvation or nutrient stress for ruffed grouse in the Southeast. Late winter carcass fat of ruffed grouse from North Carolina and Virginia suggest that grouse have adequate energy intake during winter (males = 5.0 to 11.3%, females = 9.8 to 22.4%, Servello and Kirkpatrick 1987b, 1988). These figures should be considered with some caution, however, because grouse
living in high quality habitat are most likely to be available for collection in late
winter, while grouse living in poor habitat and containing the lowest carcass fat may
have already died. It is also possible that good body condition is maintained at the
expense of long foraging times and greater predation risk.

Winter diets of ruffed grouse in all parts of the species’ range contain forages
that are considered poor quality due to high levels of fiber and secondary plant
metabolites (Servello and Kirkpatrick 1987a). Poor quality forages are often the only
foods available for ruffed grouse during the winter and the bird’s survival depends on
being able to consume these forages. While many adaptations of grouse to poor
quality foods are not understood, some are becoming clear.

There are two possible digestive strategies for herbivores consuming high fiber
diets. The herbivore can either rely on microbial fermentation of fiber to release
energy or the herbivore can meet nutrient requirements by consuming large volumes
of forage (Remington 1989). Fermentation of fiber requires that forage be maintained
in the digestive tract for long periods of time to accommodate the slow rate of energy
release typical of fermentation. This in turn requires that a large pool of fermenting
forage be maintained in the digestive tract, an option not practical for an avian
herbivore that relies on flight for escape and travel and therefore must minimize body
mass. Instead, grouse have adapted to consume large amounts of poor quality forage.
A problem with consuming large volumes of forage, however, is that the rate of
passage must be increased to make room in the digestive tract for the additional
material. Because digestive efficiency is an inverse function of the rate of passage (Robbins 1993), potentially useful nutrients may pass through the small intestine without being absorbed. Grouse "recover" these nutrients from the large mass of fiber also present at the end of the small intestine by selectively squeezing soluble material from the digesta into the ceca (Fenna and Boag 1974, Gasaway et al. 1975, chapter 1). This material can be absorbed directly or can be metabolized by microbes, which release volatile fatty acids to be absorbed by the bird and used as an energy source (Gasaway 1976).

The mechanisms by which ruffed grouse cope with secondary plant metabolites are only beginning to receive attention. Ruffed grouse metabolize these chemicals using phase I and phase II detoxification reactions typical of most vertebrates (Sipes and Gandolfi 1991, Jakubas et al. 1993, chapter 1). A unique adaptation of grouse to toxic diets may be the recovery of some conjugates from the urine by retrograde movement of urine from the cloaca to the ceca (Akester et al. 1967). In the ceca, microbes may cleave toxins from conjugates and metabolize the conjugates and possibly the toxins. This mechanism could provide energy in the form of volatile fatty acids and nitrogen in the form of ammonia. This mechanism would be counterproductive, however, if toxins were reabsorbed from the ceca and required additional conjugation to excrete. This potential adaptation deserves more study.

An important group of secondary plant metabolites common in grouse forages are tannins (Servello and Kirkpatrick 1987a). Ruffed grouse showed no significant
negative effects from diets containing 6% condensed tannin; however, 8% condensed
tannin in a high fiber, low protein diet resulted in lower dry matter intake and loss of
body mass (Chapter 2). Grouse were more susceptible to 8% hydrolyzable tannin and
responded by drastically reducing dry matter intake. Condensed tannin caused an
increase in the amount of material entering the ceca (Chapter 2). The reason for this
partitioning of digesta is not certain, but may be related to recovering either tannin
bound protein or conjugates from the urine.

Two final observations concerning sex and age ratios of grouse in the
Southeast deserve comment. The average ratio of males: females in the hunter harvest
in Virginia is 65:35 (Norman 1991), compared to approximately equal sex ratios in
northern states (Bump et al. 1947, Rusch and Keith 1971). Assuming equal sex ratios
at birth and that both sexes are equally vulnerable to hunter harvest, females must
experience greater mortality than males in the Southeast. Predation pressures may be
greater for females because of nesting and brood rearing. In addition, because
females maintain higher fat levels than males during winter (Servello and Kirkpatrick
1987b, 1988), females may have to forage more and thus experience greater predation
risks. Why these factors affect females in the Southeast more than females in the
North is not known.

The percent of juveniles in the hunter harvest is lower in Virginia and other
southern states than in northern states (45 to 55% vs. 65 to 75%) (Davis and Stoll
1973, Norman 1991). Assuming juvenile and adult grouse are equally susceptible to
harvest, it appears that production is lower in the Southeast than in northern states. Lower production is consistent with there being a smaller percentage of females in the population, as noted above. Production could also be decreased due to greater chick mortality or a smaller percentage of hens nesting (Watson and Moss 1980). These aspects of ruffed grouse biology also deserve further study.

Lower production of young in the Southeast could also be the result of increased nest predation. Nest predation was proposed as a factor limiting ruffed grouse in the Southeast (Bergerud 1988). This hypothesis assumed density-dependent nest predation and a more diverse predator community in the Southeast (Bergerud 1988). Predation of artificial nests in southwest Virginia suggested nest predation is density-independent and that nest predation rates are not excessive in the Southeast (Chapter 5). Furthermore, nest predator communities do not appear more diverse, nor are nest predators more abundant in the Southeast. Finally, a review of studies investigating density-dependent nest predation indicated that predation is most likely to be density-dependent when habitat is simple, nest densities are high, predation rates are high, and birds are the primary predators (Chapter 5). None of these conditions apply to ruffed grouse in the Southeast.

The specific causes of low ruffed grouse densities in the Southeast are not understood. A hypothesis involving heterogenous habitat, increased foraging times, and increased mortality due to predation has been proposed. Future research should concentrate on winter predation, especially mortality rates of grouse in different
habitats, the percentage of hens that attempt nesting, and the survival of chicks during the summer and early fall.

LITERATURE CITED


APPENDIX A

Values for Bartlett's test of homogeneity are shown for untransformed, and if necessary, transformed variables. A Bartlett's value < 11.07 ($X^2_{5, \alpha=0.05}$) was considered evidence that variances were equal among treatments and that the homogeneity assumption of ANOVA was satisfied. If a transformation was necessary, the transformation type is shown. Type III mean square values from the GLM procedure of SAS are shown, along with the error mean square and the p-value for the treatment effect. Type III mean squares is the variance attributed to that class variable, controlling for all other class variables in the model.

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<th>p-value</th>
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<tr>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
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<td>Conjugates/g forage</td>
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</table>
APPENDIX B

The accuracy of using signal strength and pulse rate to estimate activity was investigated by placing radios identical to those used in the field on captive grouse held in 2X3X2m pens. For methods used in 1992, an observer recorded the grouse’s activity during 1 minute intervals while the radio signal was simultaneously monitored from a distance by the same person who monitored the signals of wild grouse. All pulse rate and signal strength changes were recorded. A bird was considered active if it walked, fed, or flew during the 1 minute period. A bird was estimated as active if any pulse rate or signal strength changes were heard. Accuracy was estimated as the percent of 1 minute periods correctly classified as active or inactive.

The accuracy of our 1993 activity estimates was determined using 2 methods. First, an observer recorded the activity of a captive grouse every 10 seconds for a period of 30 to 60 minutes. The monitoring system described above simultaneously recorded radio signals from the bird. The percent time actually active was determined as the percent of the observations in which the bird was active (walking, flying, or feeding). The estimated percent time active was determined as it was for field data and compared to the observed percent time active by least squares regression.

In the second method of evaluating the relationship between radio signals and activity, observations taken every 10 seconds were used to classify a grouse as active or inactive during successive 1-minute periods of each trial. If a bird walked, flew,
or fed during a minute period, that period was considered active. Radio signals from
the same 1-minute periods were independently classified as active or inactive by the
following criteria: signal strength was classified as active if a 0.075mA change was
noted over the entire 1-minute period or there was an instantaneous change of
0.025mA. Pulse rate was considered active if the pulse rate remained constant for
<95% of the period. These criteria were established by comparing graphs of radio
signals with observations of grouse.

Using signal strength changes, captive grouse in 1992 were accurately
classified as active or inactive in 158 of 180 (87.8%) 1-minute periods. Of the 22
misclassifications, 13 resulted from classifying the grouse as active when it was
inactive.

Eleven tests of activity were conducted in 1993 using 4 captive grouse. Using
our first test of accuracy, a significant regression ($P<0.001$, $r^2 = 0.87$) was found
between percent activity observed and estimated using the single criteria method
(Figure A.1). Percent activity was overestimated by an average of 10.8 percentage
points. The dual criteria method of estimating activity was also significantly
correlated with observed activity ($P = 0.006$), but had a smaller correlation
coefficient ($r^2 = 0.59$, $n = 11$), and underestimated activity by 1.4 percentage points
(Figure A.1). The second test of accuracy for 1993 methods showed signal strength
correctly classified 91.3% of 278 minute long periods as active or inactive.
Figure A.1. Percent activity of captive grouse observed and estimated from radio signals. Least-squares regression lines are shown for the single (□) and dual (+) criteria methods of estimating activity.
APPENDIX C

Plots of percent activity for hour-long periods from dawn to a period that contained 1200 hrs and from dusk backwards to a period that contained 1259 hrs. Each point represents the percent of that hour the bird was active on a given day. The line connects the hourly averages.
APPENDIX D
Abundance index of mammalian nest predators from trapping data (1983-84) and presence of other important mammalian nest predator species in northern states where ruffed grouse are abundant and southern states where ruffed grouse are relatively scarce. The abundance index is 1 = greatest and 5 = lowest density. A "0" indicates the species is not found in that state and "+" indicates the presence of a species for which density estimates are unavailable. See original reference for actual density estimate associated with each abundance index.

<table>
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<tr>
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<tr>
<td>Fisher</td>
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<td>Opossum</td>
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<td>Red Squirrel</td>
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<td>4</td>
</tr>
<tr>
<td>Least Weasel</td>
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</table>
Long-tailed Weasel

Short-tailed Weasel

Literature Cited for Appendix D.


VITA

David Glenn Hewitt was born in Salt Lake City, Utah on September 24, 1964. He attended public schools in Greeley, Colorado and graduated from Greeley Central High School in 1983. David graduated with a Bachelor's degree in Wildlife Biology with Highest Distinction and Honors from Colorado State University in 1987. In 1989, he completed a Master's degree in Wildlife Biology at Washington State University. David was married to Liisa Ruminski in 1989 and worked for a year as a research associate at the Texas Agriculture Experiment Station in Uvalde, Texas. In 1990, he began a doctoral program in the Department of Fisheries and Wildlife Sciences at Virginia Polytechnic Institute and State University. David and Liisa's daughter, Nicole, was born in October, 1992. David completed requirements for his doctoral degree in August 1994.

David G. Hewitt