NUTRITIONAL COSTS OF A PLANT SECONDARY METABOLITE EXPLAIN SELECTIVE FORAGING BY RUFFED GROUSE

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Abstract. Plant secondary metabolites (PSMs) are commonly thought to deter vertebrate herbivores by being toxic or by reducing nutrient assimilation. An alternative, complementary hypothesis is that PSMs may influence herbivore forage selection at subtoxic levels by imposing high detoxication costs post absorption. Many studies of insect herbivores have been undertaken to measure the metabolic load of detoxication as it relates to host-plant specialization, but results have been equivocal and the subject of much debate. Some recent studies of vertebrate herbivores indicate that metabolism of PSMs can impose a cost by increasing nutrient losses due to conjugation of PSMs to endogenous materials, and by upsetting pH homeostasis. In this study, we demonstrate that detoxication costs in Ruffed Grouse are substantial, and are reduced by selective foraging.

In winter, Ruffed Grouse feed preferentially on quaking aspen with relatively low levels of coniferyl benzoate (CB) in stamine flower buds. We collected aspen buds with low- and high-CB levels and conducted feeding trials with captive Ruffed Grouse that had been acclimated to an aspen bud diet. We measured nutrient utilization efficiencies and excretion of detoxication conjugates. Grouse assimilated 24% less energy from high- vs. low-CB buds. Using a nutritional model, we determined that the reduction of energy utilization efficiency was mainly due to dilution of the diet by CB, and not by digestive inhibition. As CB intake increased, grouse excreted more glucuronic acid and ornithine (two major detoxication conjugates), resulting in an energetic cost of 10% to 14% of metabolizable energy intake for low- and high-CB buds, respectively. Conjugation with the amino acid ornithine increased minimum nitrogen requirement by 68% to 90% for low- and high-CB buds, respectively. Ammonium excretion also increased with CB intake, indicating an upset of pH homeostasis. Thus, detoxication costs were relatively high and increased with higher CB intake. Ruffed Grouse preference for low-CB, high-protein aspen buds in nature appears to be related to lower utilization efficiency and higher detoxication costs associated with high CB concentrations. The importance of detoxication cost to herbivores must be more thoroughly evaluated and integrated into existing models of herbivore foraging behavior.

Key words: acidosis; coniferyl benzoate; detoxication; digestion; energetics; foraging; herbivory; metabolic load; plant secondary metabolite; quaking aspen; Ruffed Grouse.

INTRODUCTION

Herbivore food preferences often cannot be predicted solely by classical nutritional measures, such as metabolizable energy or protein content of foods, and instead are related to the effects of plant secondary metabolites, PSMs (Freeland and Janzen 1974, Bryant and Kuropat 1980). In some cases, herbivores limit intake of particular PSMs in food plants to levels below those causing pathology or morbidity (Karasov 1989). However, even at subtoxic levels of intake, PSMs can exact significant nutritional costs to herbivores by affecting nutrient utilization (Glick and Joselyn 1970a, b, Lindroth and Batzli 1984, Robbins et al. 1987a, b, 1991), or by increasing the energetic cost of detoxication post absorption (Dash 1988, Remington 1990).

The metabolic load hypothesis (Dethier 1954, Whittaker and Feeny 1971) proposes that host plant specialization evolves in insects through selection for reduced costs of maintaining and fueling detoxication systems. Specialists reduce the diversity of allelochemicals they ingest and gain an energetic advantage over polyphagous species by having smaller, less expensive detoxication systems. Cost reduction in specialists is
further enhanced by target site insensitivity (Berenbaum 1986), specific excretion of major dietary allelochemicals (Maddrell and Gardiner 1976), and behavioral modification (Tallamy 1986). Studies designed to measure metabolic loads of detoxification in insects and to test the hypothesis have had equivocal results; the issue continues to be debated (Schoonhoven and Meerman 1978, Scriber 1981, Neal 1987, Appel and Martin 1992, Cresswell et al. 1992, Rausher 1992 and references therein, Berenbaum and Zangerl 1994).

The concept of metabolic load may also be useful in ecological studies of vertebrate herbivore foraging. If detoxication costs comprise a substantial portion of the herbivore nutrient budget, they may well influence foraging decisions. Measures of PSM detoxication costs may shed new light on herbivore selection within and among food plant species, and may be used to refine foraging models (Belovsky and Schmidt 1991).

In this study, we examine how detoxication costs may explain the selective foraging behavior of a vertebrate herbivore. We assess the metabolic costs of detoxifying ingested PSMs by measuring the nutrient drain associated with excretion of glucuronic acid and ornithine, two major substrates used in phase II conjugation reactions in birds (Sykes 1971). We also examine the relationship between detoxication rate and pH buffering activity. Glucuronic acid and ornithine are strong organic acids (pKₐ 3–4; Robinson et al. 1953, Smith and Williams 1966), which, along with acidic metabolites produced during phase I detoxication, may challenge pH homeostasis (Foley 1992, Foley et al. 1995). Generation of bicarbonate as a pH buffer increases ammonium (NH₄⁺) excretion, which can then be used as a simple index of systemic acid load (Okumura and Tasaki 1968, Long and Skadhauge 1983, Kurtz et al. 1990, Carlisle et al. 1991, Foley et al. 1995).

In winter, the staminate flower buds of quaking aspen (Populus tremuloides Michx.) are an important food source for Ruffed Grouse (Bonasa umbellus L.) in North American deciduous and boreal forests (Gullion 1966, Vanderschaegen 1970, Svoboda and Gullion 1972, Doerr et al. 1974, Huempfner 1981). Although grouse forage extensively on aspen flower buds, they feed only from certain trees or clones (Gullion 1966, 1970, Svoboda and Gullion 1972, Doerr et al. 1974, Huempfner 1981). Preference is correlated with the concentration of coniferyl benzoate (CB), a phenylpropanoid ester, in the flower buds (Jakubas et al. 1989, Jakubas and Gullion 1990, 1991). Field observations indicate that the mean dry mass CB concentration in aspen buds consumed by Ruffed Grouse is 10 mg/g, but buds as high in CB as 18 mg/g are eaten (Jakubas and Gullion 1990, 1991). Randomly sampled buds typically have CB levels near 25 mg/g (range 0–90 mg/g; Jakubas and Gullion 1991; C. Vispo and W. J. Jakubas, unpublished data). Grouse preference is best predicted by both the CB and crude protein content of the buds (Jakubas and Gullion 1991).

Previous studies of Ruffed Grouse and other birds showed that CB appears to produce few adverse toxic effects, even at high rates of intake (Jakubas et al. 1993a, b, c), and that birds can habituate to the compound's irritant properties to overcome taste deterrence (Jakubas et al. 1993b). In this study, we tested the hypothesis that the repellency of CB is related to its effect on nutrient utilization efficiency, nitrogen balance, or detoxication costs of grouse. Because utilization efficiencies were found to be different between low- and high-CB buds, we used a nutritional model to distinguish whether CB simply dilutes the diet as a nonutilizable fraction of the food, or actually inhibits nutrient assimilation.

METHODS

Grouse and diets

We conducted field studies at the Mile Lacs Wildlife Management Area (MLWMA) in east-central Minnesota (see Gullion 1981 for a description) and several locations near the town of Hayward, Sawyer County, Wisconsin (=150 km east of MLWMA). The Hayward site was dominated by second-growth aspen/birch forest, interspersed with mixed hardwoods in older stands, and pines on poorer soils.

In 1989, we captured Ruffed Grouse at the Hayward site using "clover-leaf" traps (Dorney and Mattison 1956) during fall dispersal (15 August to 15 October). Birds were housed in outdoor enclosures (Guglielmo and Karasov 1995) and fed a pelleted chow diet (50: 50 Purina gamebird maintenance chow: Purina horse chow 100). The diet contained 31% neutral detergent fiber and 12.5% crude protein, and was the same as that used by Servello et al. (1987) without 2% corn oil. Water and grit were provided ad libitum. For feeding trials, five adult grouse (two male, three female) were moved to individual cages (Guglielmo and Karasov 1993) in an environmental chamber with simulated winter conditions (0°C, 50–80% relative humidity, 9 h light:15 h dark). Animal handling and experiments were approved by the University of Wisconsin Research Animal Resources Center.

In January 1991, we located nine male quaking aspen trees in which Ruffed Grouse fed on flower buds. Using high-performance liquid chromatography (HPLC), we confirmed that CB concentration of the mixture of flower buds from these trees was relatively low (dry mass <18 mg/g). Thus, we used this mixture for a low-CB (LCB) diet.

To produce a high-CB (HCB) bud diet, we sampled flower buds at MLWMA from 13 quaking aspen that had been sampled in previous studies (Jakubas and Gullion 1991), and 20 more trees with heavy bud crops. Buds were collected from the canopy and frozen at −20°C. We made a preliminary assessment of bud CB levels using thin-layer chromatography, TLC (Jakubas et al. 1989). Ten trees with high CB levels (≥20 mg/
g) were selected for the HCB diet and three with very low CB levels were chosen for diet acclimation.

We cut down all experimental trees from 22 to 28 February 1991. Twigs with flower buds were clipped under freezing conditions in the field and stored in double plastic bags at -20°C. Flower buds were chilled while removed by hand in the laboratory. Buds from all trees within each diet classification were combined to make two homogenous diets.

Long dietary acclimation periods are required prior to feeding trials with grouse because gut morphology and microflora can change with diet (Hanssen 1979a, b, Redig 1989). We acclimated grouse to aspen buds and other woody browse over a 41-d period and housed them at 0°C to induce high levels of food intake, similar to those of wild birds (Andreev 1988). The acclimation proceeded as follows: ad lib chow (7 d); ad lib chow + 10–30 g (wet mass) aspen winter buds, AWB (10 d); 66% ad lib chow (measured days 1–7) + 30–80 g AWB (7 d); 50% ad lib chow + 60–90 g AWB (7 d); 25% ad lib chow + 90–120 g AWB + 40 g emergent willow (Salix discolor) buds, EBW (6 d); 12.5% ad lib chow + 50–140 g AWB + 40–80 g aspen catkins (AC) + 10–40 g EBW (10 d). At the final level, chow made up only 5–10% of total dry matter intake. The chow diet was removed during feeding trials and, thus, grouse ate only aspen flower buds. AC and EBW were collected near Madison, Wisconsin to make up for a shortage of low CB buds.

**Experiments and sample analysis**

We used standard mass balance trials to measure nutrient fluxes and rates of detoxication conjugate and metabolite output of grouse fed the different aspen bud diets. Because we were taking repeated measurements of the same birds, we had to consider potential negative effects of one diet (high-CB buds) on subsequent trials with that bird. We also wished to avoid exaggerated effects of increasing dietary concentration or dosage of CB that may have been related to a lack of time for induction of enzymatic detoxication systems, or for grouse to adjust to the taste of a novel food. Thus, we conducted the trials with five grouse in the order of increasing concentration of CB.

The LCB trial lasted 5 d. We then conducted a 4-d medium-CB (MCB) trial by presenting grouse with one cup containing 50% of the daily requirement of LCB buds (measured in the first trial) and a second containing an excess of HCB buds. We determined actual daily CB intake of each bird in the MCB trial by measuring CB concentration of uneaten buds by HPLC (CB Intake = LCB fed × [CB] + HCB fed × [CB] – uneaten buds × [CB]). We then conducted the HCB trial for 4 d. Tap water was provided ad libitum. Grains was removed during trials. For 2 d between each trial, we provided grit and fed grouse 12.5% ad lib chow, 75 g AWB, and 75 g AC. Following the HCB experiment, we conducted a 4-d trial with chow diet for comparison with earlier studies.

During trials, we measured water consumption (corrected for evaporation) and body mass daily. Fecal and cecal droppings were collected once daily in the morning. Microbial degradation of detoxication conjugates and ammonium was considered negligible due to low ambient temperature. Excres and uneaten food were lyophilized and stored frozen at -20°C. Samples from day 1 of each trial were omitted to allow for clearance of pretrial meals from the gut (C. G. Gugielmo and W. H. Karasov, unpublished data).

Fecal and cecal droppings were analyzed separately after they were ground in a Wiley mill (1-mm screen). Energy content (after re drying at 50°C overnight) was measured using a Phillipson Microbomb Calorimeter (Gentry Instruments). Kjeldahl and NH₄⁺ nitrogen, and acid detergent fiber (ADF) were measured at the University of Wisconsin forage and soils lab following Schulte et al. (1987). Uric acid was measured following Marquardt (1983). Neutral detergent fiber (NDF) was measured at the USDA Dairy Forage Research Center following the National Forage Testing Association (1993). Total phenols were measured following Singleton and Rossi (1965), after extracting ground buds for 3 d in 70:30 acetone : water. Total glucuronic acid was measured following Jakubas et al. (1993b) using techniques adapted from Remington (1990) and Blumenkrantz and Asboe-Hansen (1973). The assay does not distinguish between conjugated and free glucuronic acid (the latter comprised 5–51% of total in urine of common ringtail possums, Pseudocheirus peregrinus; McLean et al. 1993). Ornithine was measured following Jakubas et al. (1993b).

To measure CB, we ground 5–8 lyophilized buds per sample in liquid nitrogen with a mortar and pestle and extracted 0.1 g overnight in 5 mL diethyl ether with 150 μg pinosylvin dimethyl ether (PDE) added as internal standard. For TLC, the developing solvent was toluene : chloroform : acetone (40:25:35). For rough estimation of bud CB levels, unknowns were visually compared to CB standards representing bud concentrations (dry mass) of 10.0, 15.0, 20.0, and 30.0 mg/g. For HPLC, the supernatant was concentrated to 1 mL under vacuum, rediluted to 5 mL with methanol, and filtered (0.45 micron, nylon). We injected 20 μL samples on a Waters analytical column (3.9 × 300 mm) packed with 10 μm gamma bondapak C18 reverse phase sorbant. CB standard was extracted from benzoin Siam tears (Alfred Wolff, Paris, France) following Jakubas et al. (1993b). Absorbance was measured at 295 nm.

**Data analysis**

The efficiency of mass and energy utilization, corrected for nitrogen balance, was calculated using the equations:
Table 1. Nutritional characteristics of quaking aspen staminate flower bud diets fed to Ruffed Grouse. Values are mean ± 1 se. Under N, the two numbers refer to sample sizes for LCB and HCB diets, respectively. Significance determined using two-tailed t tests.

<table>
<thead>
<tr>
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<th>N</th>
<th>LCB</th>
<th>HCB</th>
<th>P</th>
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<tr>
<td>Dry mass (% wet mass)</td>
<td>6.5</td>
<td>49.7 ± 0.1</td>
<td>49.2 ± 0.3</td>
<td>0.19</td>
</tr>
<tr>
<td>Coniferyl benzoate (mg/g)</td>
<td>3.3</td>
<td>12.8 ± 1.7</td>
<td>24.2 ± 2.0</td>
<td>0.012</td>
</tr>
<tr>
<td>Crude protein (% DM)*</td>
<td>2.2</td>
<td>13.1 ± 0.2</td>
<td>11.8 ± 0.7</td>
<td>0.22</td>
</tr>
<tr>
<td>Gross energy (kJ/g)</td>
<td>12.7</td>
<td>19.87 ± 0.22</td>
<td>20.04 ± 0.15</td>
<td>0.37</td>
</tr>
<tr>
<td>NDF (% DM)†</td>
<td>2.2</td>
<td>37.4 ± 0.7</td>
<td>39.7 ± 0.1</td>
<td>0.087</td>
</tr>
<tr>
<td>ADF (% DM)‡</td>
<td>2.2</td>
<td>33.8 ± 0.1</td>
<td>35.0 ± 0.3</td>
<td>0.0042</td>
</tr>
<tr>
<td>Total phenols (% DM)‡</td>
<td>2.2</td>
<td>6.3 ± 0.3</td>
<td>6.2 ± 0.2</td>
<td>0.89</td>
</tr>
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* DM, dry matter.
† NDF, neutral detergent fiber; ADF, acid detergent fiber.
‡ % DM in gallic acid equivalents.

$$\text{AMC}_N = \frac{Q_i - Q_s - 3.0(NQ_i - NQ_s)}{Q_i}$$ (1)

and

$$\text{MEC}_N = \frac{GEQ_i - GEQ_s - 34.5(NQ_i - NQ_s)}{GEQ_i}$$ (2)

where AMC$_N$* and MEC$_N$* are apparent assimilable mass and apparent metabolizable energy coefficients (unitless proportions of mass or energy), respectively; $Q_i$ and $Q_s$ are dry matter intake and output in grams per day; $GE_i$ and $GE_s$ are the energy contents of food and excreta in kilojoules per gram; and $N_i$ and $N_s$ are nitrogen contents of the food and excreta expressed as proportions (Karasov 1990). The numerical factors 3.0 g/g N and 34.5 kJ/g N are the dry matter and energy equivalents of nitrogen lost as urea acid, respectively (Parsons et al. 1982, Karasov 1990). Because birds in all feeding trials were near nitrogen balance, these corrections were small. Metabolizable energy intake, MEI (kJ/d), was defined as:

$$\text{MEI} = GEQ_i \times \text{MEC}_N^*.$$ (3)

To calculate energetic costs of conjugation pathways, we used energy contents of 13.5 kJ/g and 22.7 kJ/g for glucuronic acid and ornithine, respectively (measured by bomb calorimetry). We considered these conjugates in excreta as energy that had been assimilated from food. Hence, we added this loss back into the MEI before expressing the energy excreted as a percentage of daily intake. We assessed relative nitrogen cost of ornithine conjugation by expressing the gram equivalent mass of nitrogen in ornithine (0.21 g N/g ornithine) as a percentage of $E_n$, the endogenous nitrogen loss, or minimum daily requirement (0.27 g kg$^{-1}$ d$^{-1}$ in birds; Robbins 1993). This technique may underestimate the true material cost of this pathway because ornithine is derived from arginine, an essential amino acid in birds (Sykes 1971).

Differences in absolute body mass at the start of each trial were examined by comparing measurements taken on day 1 of each experiment. Rates of change in body mass were examined by normalizing mass each day to initial trial mass and using linear regression. Day 1 body masses and slopes were analyzed using a one-factor ANOVA with one repeated measure.

One-factor ANOVA with one repeated measure was used to analyze effects of diet treatments on all nutritional parameters. Proportions were arcsine square-root transformed. Differences among means were isolated using Tukey's Honestly Significant Difference (hsd) procedure (Zar 1984). Glucuronic acid, ornithine, and ammonium output as a function of CB intake were examined using standard linear regression. Nutritional characteristics of the bud diets were compared using Student's t test. We used SYSTAT statistics and multivariate general linear hypothesis modules (Wilkinson 1990) for data analysis. All values are reported as mean ± 1 se.

Results

Coniferyl benzoate concentration of the LCB diet (Table 1), a mixture of buds that wild Ruffed Grouse were observed eating, was outside of a 95% CI of random quaking aspen trees sampled along transects on the Hayward site in the same winter (24.8 ± 3.97 mg/g dry matter; N = 23; C. Vispo, University of Wisconsin–Madison, unpublished data), and was within the range observed to be acceptable to wild grouse by Jakubas and Gullion (1991). The HCB diet had a greater CB concentration than the LCB diet (one-tailed t test: df = 4, P < 0.005), but was within the 95% CI of random samples of aspen trees. Mean dry mass CB concentration of the MCB diet was 18.6 ± 0.3 mg/g. The LCB and HCB buds did not differ in any measured nutritional characteristics except ADF, which was 3.6% higher in the HCB buds.

Utilization efficiencies varied among the aspen bud diets (Fig. 1a, b). AMC$_N$* decreased as CB content of aspen buds increased ($F = 11.27$; df = 2,8; $P = 0.005$). Mean values of AMC$_N$* for the LCB, MCB, and HCB diets were 0.21 ± 0.003, 0.20 ± 0.003, and 0.19 ± 0.006, respectively. AMC$_N$* of the LCB diet was greater than that of the HCB diet (df = 12, $P = 0.003$), but that of the MCB diet did not differ from the other two diets (df = 12, P > 0.07). Similarly, MEC$_N$* for aspen buds declined as bud CB concentration increased ($F =$
25.12; df = 2.8, P < 0.001). Mean values of MEC2, for the LCB, MCB, and HCB diets were 0.21 ± 0.005, 0.20 ± 0.007, and 0.16 ± 0.010, respectively. MEC2 values of the LCB and MCB diets did not differ (df = 12, P = 0.69), but both were greater than that of the HCB diet (df = 12, P ≤ 0.01).

Although AMC2 appeared to decline linearly as diet CB concentration increased (Fig. 1a), MEC2 fell more sharply (Fig. 1b) because energy content of the excreta changed, while food energy content remained constant. Fecal droppings from the HCB experiment had higher energy content (20.53 ± 0.13 kJ/g) than those from either the LCB (19.73 ± 0.17 kJ/g) or MCB trials (19.71 ± 0.20 kJ/g; F = 17.6; df = 2.8; P = 0.001). Cecal droppings energy content did not vary (mean 22.41 ± 0.14 kJ/g; F = 3.6; df = 2.8; P = 0.078).

Dry matter intake rate varied among diet treatments (F = 8.96; df = 2.8; P = 0.009; Fig. 2a). Mean dry matter intake rates for the MCB (116.1 ± 2.6 g·kg⁻¹·d⁻¹) and HCB (121.0 ± 6.2 g·kg⁻¹·d⁻¹) diets were not different (df = 12, P = 0.68), but both were greater than for the LCB diet (100.8 ± 2.0 g·kg⁻¹·d⁻¹; df = 12, P ≤ 0.05). At an ambient temperature of 0°C, grous intake of aspen buds was greater than that observed at 19–20°C (72.8 ± 2.8 g·kg⁻¹·d⁻¹; df = 6, P < 0.001; Guglielmo and Karasov 1995), and was comparable to that of wild grouse (Andreev 1988).

Metabolizable energy intake did not vary among treatments (F = 3.17; df = 2.8; P = 0.097; Fig. 2b). Mean MEI values were 428.1 ± 7.5, 463.5 ± 24.9, and 395.1 ± 37.3 kJ·kg⁻¹·d⁻¹ for the LCB, MCB, and HCB diets, respectively. Variance in MEI was not constant (Bartlett test; F = 3.5; df = 2; P = 0.03), and it appears that birds (same symbols for individuals in all figures) that ate the most food were also more able to maintain MEI as MEC2 decreased.

Glucuronic acid output increased with CB intake (F = 26.44; df = 1,13; R² = 0.67; P < 0.001; Fig. 3a), as did ornithine (F = 35.27; df = 1,13; R² = 0.73; P < 0.001; Fig. 3b). The y-intercepts of both regressions were positive (glucuronide = 7.35 ± 0.88 mmol·kg⁻¹·d⁻¹; ornithine = 5.63 ± 0.60 mmol·kg⁻¹·d⁻¹; df = 13, P < 0.001), indicating that conjugate excretion would remain elevated even if grouse ate buds with no CB, probably because of the presence of other PSMs. Although we do not have data near zero CB intake, we do not expect these relationships to be nonlinear. Glucuronic acid excretion during the chow trial was relatively low and was outside the 95% CI of the aspen diet regression. Ornithine was not detected in excrta
from the chow trial. Excretal concentrations of glucuronic acid and ornithine did not vary among the aspen diet treatments ($F = 2.11; \text{df} = 2.8; P = 0.18$), and averaged $24.3 \pm 0.5 \text{mg/g}$ and $12.8 \pm 0.2 \text{mg/g}$, respectively. Therefore, changes in total output of these conjugates were mainly a function of changes in excretal output. $\text{NH}_4^+$ output increased with CB intake ($F = 9.63; \text{df} = 1.13; R^2 = 0.43; P = 0.008$; Fig. 3c), and the y-intercept was positive ($23.0 \pm 2.6 \text{mmol-kg}^{-1}\text{-d}^{-1}; \text{df} = 13, P < 0.001$). Rates of $\text{NH}_4^+$ excretion during the chow trial fell outside the 95% CI from the aspen diet regression.

The energetic cost of detoxication, via the glucuronic acid and ornithine conjugation pathways, ranged from 10 to 14% of MEI (Table 2), and increased with bud CB concentration ($F = 37.4; \text{df} = 2.8; P < 0.001$). The energy cost did not differ between the LCB and MCB diets ($df = 12, P = 0.15$), but was greater for HCB buds ($df = 12, P \leq 0.01$). Ornithine conjugation increased the minimum daily nitrogen requirement by 68–90% above the baseline predicted for a grouse eating food without PSMs (Table 2). This cost also increased with increasing bud CB concentration ($F = 11.4; \text{df} = 2.8; P = 0.005$), and was greater on the MCB and HCB diets than on the LCB diet ($df = 12, P \leq 0.03$).

Body mass declined slowly throughout the entire series of experiments ($F = 16.83; \text{df} = 2.8; P = 0.001$; Table 2). Rate of mass loss did not vary among trials, however ($F = 1.69; \text{df} = 2.8; P = 0.24$). Nitrogen balance was highly variable and did not differ among treatments ($F = 2.47; \text{df} = 2.8; P = 0.15$; Table 3). In all trials, mean nitrogen balance did not differ from zero ($P > 0.10$). Uric acid excretion did not differ between the LCB ($4.81 \pm 0.18 \text{g-kg}^{-1}\text{-d}^{-1}$) and HCB ($4.60 \pm 0.23 \text{g-kg}^{-1}\text{-d}^{-1}$) trials ($F = 0.44; \text{df} = 1.4; P = 0.54$), indicating that increased $\text{NH}_4^+$ excretion was not compensated by decreased uric acid excretion.

Water intake varied among treatments ($F = 7.45; \text{df} = 2.8; P = 0.02$; Table 3) and was greater during the HCB trial than during the LCB trial ($df = 12, P = 0.03$), whereas intake during the MCB trial was not different from the other two ($P \approx 0.07$). Water intake did not vary when it was normalized to dry matter intake ($F = 1.36; \text{df} = 2.8; P = 0.31$). Therefore, water intake did not increase in disproportion to food intake, suggesting that there were no dose-related effects of CB on water requirements.

Utilization efficiency of the chow diet was not affected by the aspen treatments ($\text{AME}_{\text{ata}} = 0.47 \pm 0.004$ in previous studies, Guglielmo and Karasov 1995; $0.48 \pm 0.003$ in this study; $F = 1.45; \text{df} = 1.3; P = 0.32$). Thus, differences in utilization efficiencies among the aspen diets were not related to cumulative effects of CB. Mean MEI during the chow treatment ($642.3 \pm 27.1 \text{kJ-kg}^{-1}\text{-d}^{-1}$) was lower than during the three aspen trials ($df = 16, P = 0.001$).

**DISCUSSION**

**Preference behavior: taste, toxicity, or nutritional costs?**

The results of our field studies confirm earlier reports that Ruffed Grouse prefer quaking aspen flower buds with relatively low coniferyl benzoate concentrations (Jakubs et al. 1989, Jakubs and Gullion 1991). Moreover, in the winter prior to this study (1990), we collected aspen flower buds from trees used by grouse at the Hayward site, and found bud CB concentration to be within the expected range ($11.4 \pm 0.9 \text{mg/g}$; Guglielmo and Karasov 1995). Our feeding trials show that grouse assimilate dietary energy more efficiently, allocate less energy and nitrogen to detoxication, and buffer less systemic acid when eating low CB aspen buds. This is good evidence that Ruffed Grouse forage...
TABLE 2. Nitrogen and energy costs of detoxication via the glucuronic acid and ornithine conjugation pathways for five Ruffed Grouse eating staminate quaking aspen flower buds containing low, medium, and high concentrations of coniferyl benzoate (CB). Values are mean ± 1 SE.

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<th></th>
<th>LCB</th>
<th>MCB</th>
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<tr>
<td><strong>Intake</strong></td>
<td></td>
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<tr>
<td>Coniferyl benzoate (g·kg⁻¹·d⁻¹)</td>
<td>1.32 ± 0.02</td>
<td>2.21 ± 0.10</td>
<td>3.09 ± 0.10</td>
</tr>
<tr>
<td>Nitrogen (g·kg⁻¹·d⁻¹)</td>
<td>2.17 ± 0.04</td>
<td>2.37 ± 0.06</td>
<td>2.33 ± 0.12</td>
</tr>
<tr>
<td>Metabolizable energy (KJ·kg⁻¹·d⁻¹)*</td>
<td>476.3 ± 8.0</td>
<td>523.0 ± 25.1</td>
<td>459.7 ± 39.7</td>
</tr>
<tr>
<td><strong>Output</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Glucuronic (g·kg⁻¹·d⁻¹)</td>
<td>1.89 ± 0.06</td>
<td>2.33 ± 0.10</td>
<td>2.54 ± 0.14</td>
</tr>
<tr>
<td>Ornithine (g·kg⁻¹·d⁻¹)</td>
<td>1.00 ± 0.03</td>
<td>1.23 ± 0.05</td>
<td>1.34 ± 0.06</td>
</tr>
<tr>
<td><strong>Nitrogen cost</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ornithine (mg N·kg⁻¹·d⁻¹)†</td>
<td>211.29 ± 6.84</td>
<td>261.78 ± 10.12</td>
<td>283.31 ± 13.76</td>
</tr>
<tr>
<td>% of N intake</td>
<td>9.7 ± 0.2</td>
<td>11.1 ± 0.3</td>
<td>12.2 ± 0.3</td>
</tr>
<tr>
<td>% of Eₚ‡</td>
<td>67.7 ± 1.9</td>
<td>83.3 ± 3.1</td>
<td>90.0 ± 5.1</td>
</tr>
<tr>
<td><strong>Energy cost</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucuronic (kJ·kg⁻¹·d⁻¹)§</td>
<td>25.58 ± 0.82</td>
<td>31.46 ± 1.29</td>
<td>34.26 ± 1.88</td>
</tr>
<tr>
<td>Ornithine (kJ·kg⁻¹·d⁻¹)§</td>
<td>22.64 ± 0.73</td>
<td>28.05 ± 1.08</td>
<td>30.35 ± 1.48</td>
</tr>
<tr>
<td>Total (kJ·kg⁻¹·d⁻¹)</td>
<td>48.22 ± 0.85</td>
<td>59.51 ± 2.04</td>
<td>64.61 ± 2.99</td>
</tr>
<tr>
<td>% of ME intake</td>
<td>10.1 ± 0.2</td>
<td>11.5 ± 0.6</td>
<td>14.3 ± 0.7</td>
</tr>
</tbody>
</table>

* Calculated as metabolizable energy intake plus energy excreted as glucuronic and ornithine.
† Eₚ = 0.27 g·kg⁻¹·d⁻¹, the sum of endogenous urinary nitrogen (EUN) and metabolic fecal nitrogen (MFN) (Robbins 1993). It can be considered the baseline nitrogen loss of a grouse eating a plant containing no secondary metabolites.
‡ Calculated using energy contents of glucuronic and ornithine of 13.5 KJ/g and 22.71 KJ/g, respectively.

Selective on low CB buds to maximize utilization efficiency and minimize PSM detoxication costs. However, we will briefly consider the available evidence for two alternative mechanisms: taste deterrence and toxicity.

While taste is apparently how Ruffed Grouse assess bud CB concentration (Jakubas and Mason 1991), it does not explain why, in nature, grouse prefer aspen buds with CB concentrations near 10 mg/g. Birds avoid CB when it is incorporated into formulated diets, in part, because it is a chemical irritant that can be sensed by the trigeminal nerves in the beak (Jakubas and Mason 1991). In a previous study, grouse did not change daily intake of a CB-treated, formulated diet until concentrations surpassed 45 mg/g, and would consume diets of up to 75 mg/g (Jakubas et al. 1993b), levels much greater than in the average aspen bud. Grouse showed no consistent preference for LCB buds (T = 2.16; df = 4; P = 0.10) in a small number of experiments conducted during the MCB trial, although individual variation was high (Guglielmo 1993). Moreover, when given only buds with CB levels near the population mean (HCB), all grouse ate as much, or more, as in the LCB trial. Thus, grouse are able to overcome any taste deterrence that may exist at the mean bud level of CB.

The role of CB toxicity in determining grouse preferences is uncertain. Grouse tend to limit intake of CB in a formulated diet to ~2 g·kg⁻¹·d⁻¹, but do not appear to suffer toxic effects beyond those caused by reduced food intake (i.e., mass loss, N imbalance, or blood toxicity parameters: Jakubas et al. 1993b). In the present study, CB intake reached 2.98 ± 0.16 g·kg⁻¹·d⁻¹ during the HCB trial, with no apparent toxicity (i.e., mass loss, N imbalance). Thus, there is no strong evidence that if grouse feed randomly on aspen buds in nature they will experience any acute toxic effects of CB.

Thus, variations in utilization efficiency and detoxication costs appear to be more important in determining preference of Ruffed Grouse for low-CB, high-protein aspen buds than either the aversive sensory properties or potential toxicity of CB. With reference to CB concentration, the LCB and HCB diets essentially represented preferred vs. random flower bud diets, respectively. HCB buds had 24% less metabolizable energy (ME = MECₚ* × GE) than LCB buds, mainly due to dilution of the diet by CB. This measure

<table>
<thead>
<tr>
<th></th>
<th>Water intake (g·kg⁻¹·d⁻¹)</th>
<th>N balance (mg·kg⁻¹·d⁻¹)</th>
<th>Body mass (kg)</th>
<th>Δ Mass (%/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCB</td>
<td>64.5 ± 1.4</td>
<td>-67.1 ± 44.1</td>
<td>0.5609 ± 0.0213</td>
<td>-0.4 ± 0.2</td>
</tr>
<tr>
<td>MCB</td>
<td>66.0 ± 3.2</td>
<td>51.9 ± 69.2</td>
<td>0.5477 ± 0.0210</td>
<td>-0.7 ± 0.02</td>
</tr>
<tr>
<td>HCB</td>
<td>75.5 ± 3.1</td>
<td>-31.6 ± 72.0</td>
<td>0.5394 ± 0.0226</td>
<td>-0.7 ± 0.1</td>
</tr>
</tbody>
</table>

TABLE 3. Water intake, nitrogen balance, and body mass measurements of five adult Ruffed Grouse fed staminate flower buds with low, medium, and high coniferyl benzoate (CB) concentrations. Values are mean ± 1 SE.
includes energy lost as detoxication conjugates. The ecological implication of this difference in ME content is that Ruffed Grouse would have to collect at least 24% more aspen buds to satisfy their energy requirements when foraging randomly rather than selectively.

The need to offset the nitrogen costs resulting from increased ornithine and NH₄⁺ excretion may explain why bud protein content is also important in determining how grouse select among aspen trees (Jakubas and Gullion 1991). Herbivores often prefer higher protein forages to meet growth and maintenance requirements (Robbins 1993). However, the high rate of ornithine conjugation, even when grouse eat low CB aspen buds, substantially increases the protein requirement for maintenance. Because ornithine is derived from dietary arginine, an essential amino acid in most birds, the actual cost of this detoxication pathway is probably higher than we have estimated. Conjugation via this route may be constrained if arginine is limiting.

Excretion of NH₄⁺ appears to add to the nitrogen cost of detoxication. In a number of vertebrates, increased NH₄⁺ excretion induced by acidosis is balanced by decreased urea excretion (Oliver and Bourke 1975, Foley 1992). We did not observe a compensatory decrease in uric acid excretion with increased NH₄⁺ output (urea excretion is minor in birds). Thus, elevated NH₄⁺ excretion appears to be a real nitrogen cost to grouse (see also Remington 1990, Jakubas et al. 1993a).

Aspen flower buds selected at random have less net nutritional value for grouse than do low-CB buds because the former have less ME, and grouse must allocate more nitrogen to detoxify them. Grouse could increase foraging time to compensate for lower nutritional quality of the diet, but might then be exposed to greater predation risk and thermoregulatory demand. Time spent out of roosts (i.e., foraging bouts) may account for the greatest proportion of daily energy expenditure above standard metabolic rate (SMR) in Ruffed Grouse (Thompson 1987). Alternatively, a grouse that feeds randomly but fails to increase food intake would have to reduce energy expenditure to maintain energy balance. Otherwise, it might compromise its physical condition and face higher risk of predation or disease (Keith et al. 1984, Temple 1987). Thus, preference for low-CB aspen buds is expected, provided the cost of searching for suitable trees is not too great. Gregarious foraging behavior of Ruffed Grouse in winter may serve an anti-predator function, but it may also relate to the patchiness of high-quality foraging sites (Doerr et al. 1974).

Metabolic load of PSM detoxication and comparison to insect studies

Ruffed Grouse clearly allocated a significant portion of assimilated energy to phase II conjugation of aspen PSMs in our study, even when eating their preferred buds (LCB). We infer from the positive intercepts of the glucuronic acid and ornithine excretion curves (Fig. 3) that much of the total cost is due to detoxication of compounds other than CB. The small amount of glucuronic acid excreted during the chow trials (95.3 ± 10.2 mg·kg⁻¹·d⁻¹) was slightly greater than that measured for a phenol-free diet (44.3 ± 4.0 mg·kg⁻¹·d⁻¹; Jakubas et al. 1993b). It may have been from conjugation with phenolics in the chow (0.61 ± 0.02% dry matter; Guglielmo and Karasov 1995) and excretion of endogenous bilirubin and steroid hormones (Jayle and Pasqualini 1966, Schmid and Lester 1966).

Glucuronic acid output rates of herbivores eating wild foods have been reported in several other studies (Table 4). Excretion rates and energetic costs of glucuronic acid conjugation appear to be comparable for Ruffed Grouse eating aspen buds and for Blue Grouse (Dendragapus obscurus, mass = 950 g) eating needles of three conifer species found normally in the diet. When Blue Grouse ate Picea engelmanni, a species not usually eaten in the field, they doubled glucuronic acid output. Hence, capacity of the glucuronic acid pathway in grouse may be higher than we observed. Remington (1990) reported low ornithinic acid excretion in Blue Grouse, but his values may have underestimated actual ornithine output because total ornithine was not measured. Alternatively, ornithine conjugation in Blue Grouse may be constrained by very low crude protein content of conifer needles (4.6–5.8%; Remington 1990).

Glucuronic acid excretion, and the associated energy cost, was 4–10 times greater in the two grouse species than in three marsupial folivores and two voles eating natural forages (Table 4; values were 2.5–7.5 times greater when corrected for mass-specific metabolic rate). Glucuronic acid is the dominant conjugation substrate used by most mammals, which are not known to excrete ornithine conjugates, but may use other amino acids (Schelvine 1978, Baudinette et al. 1980). The apparent lower cost of conjugation in mammals may reflect more economical metabolism of PSMs, but could also result from: (1) differences in the specific PSMs ingested; (2) greater conjugation via other, unmeasured pathways (sulfate, hippuric acid); or (3) more complete phase I metabolism of PSMs, resulting in hydrophilic metabolites (this also requires energy).

The high energy cost of excreting glucuronic acid and ornithine conjugates demonstrates that PSM detoxication imposes a significant metabolic load on Ruffed Grouse. Although we did not measure energy losses at other stages of detoxication, there are some indications that they may be relatively low. Brattsten (1979) argued that the cost of maintaining polysubstrate monoxygenases (PSMOs), the major phase I detoxication enzymes (Ahmad et al. 1986, Brattsten 1988), may be negligible in animals larger than insects, and that operating costs (in NADPH, etc.) may be as low as 20 J·mmol toxin. Thus, for example, CB would
Table 4. A summary of studies of two avian and five mammalian herbivores eating natural forages, in which glucuronic acid output has been measured. The energetic cost of this detoxication pathway may be greater in grouse than in the mammals studied.

<table>
<thead>
<tr>
<th>Species</th>
<th>Diet*</th>
<th>Glucuronic acid output† (g·kg⁻¹·d⁻¹)</th>
<th>Energy loss (kJ·kg⁻¹·d⁻¹)</th>
<th>% MEI‡</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Birds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ruffed Grouse (Bonasa umbellus)</td>
<td>1.28% CB Aspen Bd.</td>
<td>1.89</td>
<td>25.58</td>
<td>5.4</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>1.86% CB Aspen Bd.</td>
<td>2.33</td>
<td>31.46</td>
<td>6.0</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>2.42% CB Aspen Bd.</td>
<td>2.54</td>
<td>34.26</td>
<td>7.5</td>
<td>This study</td>
</tr>
<tr>
<td>Blue Grouse (Dendragapus obscurus)</td>
<td>Pseudotsuga menziesii Fl.</td>
<td>2.4</td>
<td>32</td>
<td>4.0</td>
<td>Remington 1990§</td>
</tr>
<tr>
<td></td>
<td>Abies lasiocarpa Fl.</td>
<td>2.2</td>
<td>29</td>
<td>7.7</td>
<td>Remington 1990§</td>
</tr>
<tr>
<td></td>
<td>Pinus contorta Fl.</td>
<td>1.1</td>
<td>15</td>
<td>2.4</td>
<td>Remington 1990§</td>
</tr>
<tr>
<td></td>
<td>Picea engelmanni Fl.</td>
<td>4.7</td>
<td>64</td>
<td>16</td>
<td>Remington 1990§</td>
</tr>
<tr>
<td><strong>Mammals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brushtail possum</td>
<td>Eucalyptus melliodora Fl.</td>
<td>0.20</td>
<td>2.6</td>
<td>1.2</td>
<td>Dash 1988‖</td>
</tr>
<tr>
<td>(Trichosurus vulpecula)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common ringtail possum</td>
<td>Eucalyptus radiata Fl.</td>
<td>=0.5</td>
<td>=6</td>
<td>1.5</td>
<td>Foley 1992‖</td>
</tr>
<tr>
<td>(Pseudocheirus peregrinus)</td>
<td>Eucalyptus ovata Fl.</td>
<td>=0.2</td>
<td>=2</td>
<td>0.6</td>
<td>Foley 1992‖</td>
</tr>
<tr>
<td>Koala (Phascolarctos cinereus)</td>
<td>Eucalyptus sp. Fl.</td>
<td>=0.3</td>
<td>=4</td>
<td>...</td>
<td>Hinks and Bollinger 1957</td>
</tr>
<tr>
<td></td>
<td>Eucalyptus p. puncata Fl.</td>
<td>=0.4</td>
<td>=5</td>
<td>...</td>
<td>Eberhard et al. 1975</td>
</tr>
<tr>
<td>Meadow vole</td>
<td>Andropogon gerardii. Lespedeza cuneata, Penstemon digitalis</td>
<td>=0.6</td>
<td>=7</td>
<td>...</td>
<td>Lindroth and Batzli 1986#</td>
</tr>
<tr>
<td>(Microtus pennsylvanicus)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prairie vole</td>
<td>Medicago sativa</td>
<td>=0.4</td>
<td>=5</td>
<td>...</td>
<td>Lindroth and Batzli 1986#</td>
</tr>
<tr>
<td>(Microtus ochrogaster)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Bd., Buds; Fl., Foliate.
† In cases where body mass data were unavailable, values are estimates using literature values.
‡ MEI, metabolizable energy intake.
§ Calculated from Remington (1990: Table 3.2), using 13.5 kJ/kg of glucuronide and adding this energy loss to metabolizable energy intake.
‖ MEI estimated from Foley and Hume (1987).
¶ Using a mean body mass of 0.75 kg.
# Using ocular estimation from Lindroth and Batzli (1986: Fig. 6) and a vole body mass of 30 g.

only require ≈0.08–0.2 kJ·kg⁻¹·d⁻¹ to be metabolized by PSMDs (0.02–0.05% of MEI).

Many studies designed to test the metabolic load hypothesis in insects have manipulated dietary allochemical content and then monitored indirect measures of detoxification cost, such as changes in relative growth rate (RGR), efficiency of conversion of digested food to biomass (ECD), proportion of assimilated food allocated to respiration, respiration rate, or PSMD activity (Schoonhoven and Meerman 1978, Scriber 1978, 1981, Neal 1987, Appel and Martin 1992, Cresswell et al. 1992, Berenbaum and Zangerl 1994). These measures may be problematic because one cannot be sure that changes in allocation of energy or space on membranes (Diamond and Hammond 1992) to detoxication are not offset by negative changes in other unmeasured systems. For example, induction of gut PSMD activity to high levels may not affect respiration rate (Neal 1987, Appel and Martin 1992, Cresswell et al. 1992), but does this mean there is no cost or that there has been a compensatory down-regulation of some other capacity, such as the immune system? One would conclude that there is no cost of inducing PSMDs when, in fact, there may have been a reduction in herbivore fitness that was overlooked. We suggest that assessing activity of phase II detoxication by measuring conjugate excretion (glucosides, amino acids, mercapturic acids; Dauterman 1986) may make interpretations of cost less ambiguous. One caution is in order: changes in conjugate excretion may be manifest as changes in apparent utilization efficiency, but one should be able to account for this effect.

**Detoxication and pH homeostasis**

Ruffed Grouse appear to face an acid load as an indirect cost of detoxifying PSMDs in aspen buds. Glucuronic acid output is not a good index of acid load because of phylogenetic variation in detoxication pathways (Baldwin et al. 1960, Scheline 1978, Baudinette et al. 1980). NH₄⁺ is a better index because it is excreted in 1:1 stoichiometry with net metabolically generated bicarbonate, the main physiological buffer (Halperin and Kamel 1990, Carlisle et al. 1991, Foley et al. 1995). In common ringtail possums, an increase in NH₄⁺ excretion of ≈20 mmol·kg⁻¹·d⁻¹ above baseline was correlated with increased urine titratable acid and phosphate, and lowered urine pH (Foley 1992). In our study, NH₄⁺ excretion during the LCB and HCB treatments, respectively, was elevated 20.8 ± 0.7 mmol·kg⁻¹·d⁻¹ and 26.4 ± 2.3 mmol·kg⁻¹·d⁻¹ above levels on the chow diet. Measuring urine pH and titratable acid directly would be very intrusive in birds, but we infer from the
NH₄⁺ data that the relative acid load in Ruffed Grouse eating aspen buds is comparable to, or slightly higher than, that in ringtail possums eating Eucalyptus. Remington (1990) found a direct relationship between NH₄⁺ and glucuronic acid excretion in Blue Grouse, but did not associate it with acid-base regulation. Future studies should seek to confirm whether or not metabolic acidosis can occur as a result of detoxication of PSMS, and to determine how the defense of pH homeostasis may influence foraging ecology in herbivores (Foley et al. 1995).

Effects of CB on utilization efficiency

We used a simple nutritional model to determine if dilution effects alone explained the observed difference in MECₙ* between the LCB and HCB diets, or if CB had some negative, pre-absorptive effects on digestion (e.g., complexing with nutrients or reducing absorption). The model was adapted from Karasov (1990: Eq. 6) and predicts the difference in MEC* between two diets based on dilution due to fiber, PSMS, and detoxication conjugate output. The model is:

\[
\Delta \text{MEC}^* = \text{GE}_n \Delta R_n/\text{GE}, + 34.5 \Delta N_n/\text{GE}, + \text{GE}_n \Delta S_n/\text{GE}, + \text{GE}_n (Q_o D_n - Q_o D_r)/[\text{GE}_n Q_o D_2].
\]

where \(\Delta \text{MEC}^*\) is the predicted difference in MEC*; \(\text{GE}_n\) is energy content of refractory material in the diet (usually insoluble fiber); \(\Delta R_n\) is difference in refractory proportion of the diets; \(\Delta N_n\) is difference in nitrogen content (N) of the food (assuming N for each equals or exceeds the minimum requirement); \(\text{GE}_r\) is energy content of the PSMS (CB = 30.1 kJ/g); \(\Delta S_n\) is the difference in PSM proportion of the diets; and \(\text{GE}_n\) and \(D_r\) are energy content (kJ/g) and output (g) of the excreted conjugates, respectively (subscripts 1 and 2 indicate the two diets being compared). The model assumes that fiber digestion (negligible in our experiments; C. Guglielmo, unpublished data) and the rates of endogenous energy loss were the same in both experiments. A complete derivation is given in Guglielmo (1993).

We retained \(\Delta R_n\) in our analysis because ADF differed significantly between the two diets, and we used 16.7 kJ/g for \(\text{GE}_n\) (Karasov 1990). We dropped \(\Delta N_n\) because \(N_n\) of the diets was the same. Small values of \(\Delta \text{MEC}^*_n\) compared with the observed \(\Delta \text{MEC}^*\) of 0.05, would indicate that the model variables did not account for the difference in MEC* between the two diets, and that other pre-absorptive mechanisms were important.

We found that dilution effects explained 60% of the 0.05 MEC* unit difference in metabolizability between the LCB and HCB aspen bud diets. The remaining 0.02 unit difference was not significant (\(P = 3.08; df = 1,4; P = 0.15\)). CB dilution accounted for the largest amount of the difference in metabolizability (0.02), followed by that accounted for by fiber (0.01) and the detoxication conjugates (0.003). Using a similar model, we found that dilution effects completely explained \(\Delta \text{AMC}^*\). Jakubas et al. (1993a) were able to account entirely for \(\Delta \text{AMC}^*\) between a control diet and a diet of 65 mgCB/g, based on dilution by CB. We conclude that CB in quaking aspen buds does not have a significant pre-absorptive effect on digestive function in Ruffed Grouse.

Conclusion

The deterrence of coniferyl benzoate to Ruffed Grouse does not appear to be a function of its toxicity, but rather of its negative effect on the nutrient budget. Coniferyl benzoate dilutes the diet as any indigestible material might (e.g., cellulose or lignin), resulting in a significant energetic benefit to grouse that avoid the compound. Moreover, after absorption from the gut, CB is metabolized and conjugated to endogenous glucuronic acid and ornithine, resulting in a significant loss of energy and nitrogen. Finally, a further nitrogen drain results from the excretion of ammonium as a secondary response to imbalance in systemic pH. Thus, grouse will maximize utilisable energy and nitrogen intake by feeding on the lowest CB, highest nitrogen aspen buds available.

It is clear that plant secondary metabolites do not have to be toxins or digestive inhibitors to deter herbivory by vertebrate herbivores. There is growing evidence that the costs of detoxifying plant secondary metabolites post absorption can be substantial, and that these costs have the potential to influence forage selection. Moreover, dilution of utilisable nutrients by PSMS may also be important as a mode of overall digestibility reduction of plant foods. The importance of detoxication costs and dilution effects to herbivore foraging behavior needs to be more thoroughly evaluated.

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