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Walter J. Jakubas
Southern Illinois University, Carbondale, IL

Christopher G. Gulgielmo
University of Wisconsin, Madison, WI

William H. Karasov
University of Wisconsin, Madison, WI

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Dilution and Detoxication Costs: Relevance to Avian Herbivore Food Selection

Walter J. Jakubas[1], Cooperative Wildlife Research Laboratory and Department of Zoology, Southern Illinois University, Carbondale, IL 62901-6504
Christopher G. Guglielmo[2], University of Wisconsin, 226 Russell Laboratories, Madison, WI 53706
William H. Karasov, University of Wisconsin, 226 Russell Laboratories, Madison, WI 53706

ABSTRACT

Toxicity and digestive inhibition are commonly thought of as the primary postingestive consequences by which plant secondary metabolites (PSM's) limit herbivore food selection. However, food selection may also be modified by nutritional costs imposed by detoxication processes and nutrient dilution. Few studies have determined the magnitude of these costs for vertebrate herbivores and their ecological significance. Research clarifying the mechanisms by which PSM's repel animals may give new insights into the development of repellents for nuisance wildlife and improve our ability to predict ecological interactions involving herbivores. Using captive ruffed grouse *(Bonasa umbellus)*, we tested whether PSM ingestion interferes with energy and nitrogen retention and whether food selection was related to costs associated with detoxication and nutrient dilution. Two feeding experiments were conducted. In one experiment, grouse (Group 1) were fed quaking aspen *(Populus tremuloides)* flower buds with different levels of coniferyl benzoate (CB), the primary PSM in the buds. In the other experiment, birds (Group 2) were given a formulated diet treated with CB (6.5% dry wt.). We measured energy and nitrogen utilization efficiencies and the output of detoxication products. Intake of CB was associated with decreased food utilization efficiencies in both experiments. The low energy utilization efficiency of Group 1 birds consuming high levels of CB, was mainly attributed to nutrient dilution by CB. These birds retained 24% less metabolizable energy than when feeding on buds with low CB levels (P = 0.010). Group 1 birds excreted 10 to 14% of their daily metabolizable energy intake as glucuronic acid and ornithine. Excretion of detoxication conjugates and ammonium increased with CB intake in both experiments. Nitrogen excreted in the form of ornithine and ammonium accounted for approximately 30% of the daily nitrogen intake in both experiments. During the high CB trials, the excretion of ornithine conjugates alone increased Group 1 birds' minimum daily nitrogen requirement by 90.0% over that required when consuming a diet containing no

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[1] Present address: Maine Department of Inland Fisheries and Wildlife, 650 State Street, Bangor, Maine 04401.

[2] Present address: Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, V5A 1S6 Canada.

PSM's. Relative to Group 1 birds, Group 2 birds excreted a higher proportion of their daily nitrogen intake as ornithine, despite having one-forth the nitrogen intake as Group 1 birds and despite being in negative nitrogen balance. For the development of repellents to control nuisance wildlife, additional research should be conducted to determine the extent to which repellency can be increased by raising nutrient dilution costs or by manipulating detoxication pathways.

KEY WORDS

Bonasa umbellus, coniferyl benzoate, feeding behavior, plant secondary metabolite, nutrition, Populus tremuloides, quaking aspen, ruffed grouse, toxicity

INTRODUCTION

One approach for determining which physiological and chemical attributes are aversive to animals is to study chemicals in nature, which through the process of natural selection, have been refined to repel animals (e.g., Mason et al. 1990, Clark et al. 1991, Jakubas et al. 1992). One group of chemicals that is ideal for this type of study are PSM's. Although PSM's have a number of functions, they are often produced to protect plants from herbivory (Feeny 1992). These compounds not only affect herbivore feeding behavior (Janzen 1971, Freeland and Janzen 1974, Bryant and Kuropat 1980) but also their mortality rates (Haukioja 1980, Haukioja et al. 1983, Bryant et al. 1991) and range distribution (Lindroth et al. 1986, Lindroth 1989). Because herbivory is important to the organization of ecological communities (Hunter 1992, Krebs 1994), research that clarifies the mechanisms of PSM repellency not only improves our ability to devise better repellents but also increases our ability to predict ecological interactions.

Toxicity and digestive inhibition are commonly thought of as the primary postingestive consequences by which PSM's limit food selection (e.g., Clausen et al. 1990, Robbins et al. 1991, Provenza et al. 1994). It is less certain whether palatability, in the absence of toxic effects, can limit the food selection of free-ranging animals (cf. Meyer and Karasov 1989, Bernays 1991, Provenza et al. 1994). One example of a PSM that appears to repel birds in field situations but has little or no toxic effects is CB. Coniferyl benzoate is a phenylpropanoid ester and the primary PSM in the staminate flower buds of quaking aspen (concentrations range from 0 to 9% dry mass) (Jakubas and Gullion 1991, and C. Vispo, University of Wisconsin, unpubl. data). This compound is ecologically significant because it influences ruffed grouse selection of aspen flower buds (Jakubas et al. 1989, Jakubas and Gullion 1991), which are one of their important winter foods (Gullion 1966, Svoboda and Gullion 1972, Doerr et al. 1974, Huempfner 1981). Crop and fecal analyses indicate that during some years up to 66% of the winter diet of ruffed grouse is composed of these buds (Doerr et al. 1974) and that extended catkins may make up 80–100% of their spring diet (Vanderschaegen 1970). One reason why birds are repelled by CB is because it is a trigeminal irritant (Jakubas and Mason 1991). Although CB's effect on palatability may deter grouse food consumption for a short time (<24 hr), grouse habituate to CB over time and will consume 2 g $\cdot$ kg^{-1} $\cdot$ day^{-1} of this substance (Jakubas et al. 1993*b*). Even at this high rate of intake, CB appears to produce few averse toxic effects (Jakubas et al. 1993*a,c*; Jakubas et al.

1995). These studies raise the question, if CB does not produce any known toxic effect, and if birds habituate to its irritant properties, why do field observations repeatedly indicate that ruffed grouse avoid consuming aspen buds with CB concentrations >1.8% (Jakubas et al. 1989, Jakubas and Gullion 1991, Guglielmo 1993).

Another mechanism by which PSM's may repel herbivores is by imposing nutritional costs on animals through detoxication processes (Freeland and Janzen 1974) and nutrient dilution (Karasov 1990). Plant secondary metabolites dilute the nutrient content of plants by adding mass without contributing to the plant's nutritive value, similar to the way fiber dilutes the nutritive content of plants (Karasov 1990). As a result, animals must increase their food intake as dietary PSM levels increase in order to maintain the same level of nutrient intake. The nutritional costs of detoxication, for vertebrates, mainly stem from the excretion of conjugated detoxication products. Normally PSM's and other xenobiotics are detoxified in two steps. In Phase I, the structure of the PSM is modified by mixed function oxidases in the liver and gut (e.g., P-450 enzymes). These modifications make the compound more water soluble and create additional binding sites for the attachment of endogenous products in Phase II reactions. Unlike the conjugation process, the nutritional costs associated with Phase I may be relatively inexpensive for vertebrates (Brattsten 1979). In Phase II, PSM's are conjugated with endogenous products, such as glucuronic acid, glycine, or ornithine (for birds) (Sykes 1971, Scheline 1978), that enhance the renal and biliary excretion rate of the compound. The excretion of these conjugation products results in less energy and nitrogen being available for other metabolic needs (Foley 1992, Illius and Jessop 1995, Guglielmo et al. 1996). Another potential source of nitrogen loss related to the conjugation of PSM's is increased ammonium excretion (Foley 1992). Production of highly acidic detoxication products like ornithuric and glucuronic acid (Robinson et al. 1953) produces an acid load that vertebrates must buffer in order to remain in acid-base balance (Foley 1992, Jakubas et al. 1993*a*, Foley et al. 1995). The production and excretion of ammonium is directly related to renal buffering of these acid loads (Kurtz et al. 1990).

As part of a series of studies we conducted on the toxicity of CB, we tested whether PSM ingestion interferes with energy and nitrogen retention and whether food selection was related to costs associated with detoxication and nutrient dilution. We accomplished these objectives using captive ruffed grouse that were fed either aspen buds or formulated diets with different concentrations of CB. Although research on the importance of PSM detoxication costs in insects has produced equivocal results (cf. Lindroth et al. 1990, Appel and Martin 1992, Cresswell et al. 1992, Berenbaum and Zangler 1994), the studies we describe here and other recent work with mammals (Thomas et al. 1988, Foley 1992, Illius and Jessop 1995) indicate that these costs may be substantial for vertebrates and influence food selection.

METHODS

Birds

Ruffed grouse were trapped in Sawyer County, Wisconsin, or raised from eggs collected from the same region (Guglielmo and Karasov 1995). Group 1 birds, 2 male and 3 female adults were housed in individual steel mesh cages (42 cm · 42 cm) under a 9:15 (light:dark) daily light

cycle (Guglielmo et al. 1996). In order to assure that food intake would be similar to that of wild birds, we increased the birds' metabolic rates, and hence their appetites, by housing them at 0 °C. Housing for Group 2 birds (3 adult males and 3 females) was identical to housing for Group 1 birds, except that these birds were kept at 18.3 °C under a 10:14 daily light cycle (Jakubas et al. 1993*b*). Birds in this study regime will be referred to as Group 2. The two temperature and light regimes used in the above studies were considered ideal for each study and were not implemented to determine whether detoxication costs vary with temperature and light.

We conditioned Group 1 birds to an aspen bud diet over a 7-week period by gradually increasing the proportion of aspen buds in a mixed diet of aspen buds and chow (Guglielmo et al. 1996). We conditioned Group 2 birds to the base diet for up to 8 months (one bird conditioned 2 months) prior to the trials (Jakubas et al. 1993*b*). Animal care and experimental protocols were approved by the University of Wisconsin Research Animal Resources Center.

Diets

Aspen bud diets consisted of male flower buds with CB concentrations of 1.28% ± 0.17, 1.86% ± 0.05, and 2.48% ± 0.40 dry mass for the low (LCB), medium (MCB), and high (HCB) diets, respectively (Guglielmo et al. 1996). We analyzed CB levels in aspen buds using high pressure liquid chromatography (Jakubas et al. 1989). The chow diet, used during Group 1's acclimation period, was made by pelleting a 50:50 mixture of Gamebird Maintenance Chow and Horse Chow (Purina Mills, Inc., St. Louis, MO) (Guglielmo and Karasov 1993). The formulated diet for Group 2 birds was made by pelleting a mixture of Purina Mazuri Pheasant Maintenance Diet, Aspen Chips (Northeastern Products, Inc., Caspian, MI), vitamins, and minerals (Jakubas et al. 1993*b*). The treatment diet for Group 2 birds consisted of the formulated diet and CB (6.5% dry mass) that had been extracted from benzoin Siam tears #3 (Alfred Wolff, Paris, France) (Jakubas et al. 1992, 1993*b*). The food restriction diet consisted of formulated diet that had been soaked in ether (the transfer medium for CB in the 6.5% CB diet) and evaporated to dryness.

Feeding Trials

Mass balance trials for all birds consisted of 4-day, no-choice feeding trials (Jakubas et al. 1993*b*, Guglielmo et al. 1996). All Group 1 and Group 2 birds were used in every trial of their respective testing regimes. We presented the aspen bud diet in order of increasing CB concentration (i.e., 1.28, 1.86, and 2.48% CB). This regime allowed birds time to acclimate to higher dietary concentrations of CB, which is similar to the way that free-ranging grouse acclimate to aspen buds in autumn (Jakubas et al. 1993*b*). We provided tap water and food ad libitum in all trials, except the food restriction trial in which only water was provided ad libitum. Daily intake and excretion measurements were made as described by Jakubas et al. (1993*b*) and Guglielmo et al. (1996). Excretal samples from day 1 of the trials were omitted because not all of the previous day's food may be excreted in 24 hr (Gasaway et al. 1975, Guglielmo, unpubl. data).

For Group 2 birds, we determined CB's effect on utilization efficiency by comparing the performance of birds during a 6.5% CB trial (treatment) to a food restriction trial (control). In the food restriction trial, birds were presented the same amount of food (no CB) they consumed during the 6.5% CB trial. We chose the 6.5% CB concentration for the treatment diet because

prior dose-response trials indicated that maximum CB intake (i.e., approx. 2 g · kg^{-1} · day^{-1}) occurred when the birds were given diets containing 6.0 or 7.5% CB (dry mass) (Jakubas et al. 1993*b*). Consequently, we expected ruffed grouse consuming a 6.5% CB diet to ingest the maximum amount of CB and that any averse effects from CB ingestion would be manifested at this concentration.

We gave birds a recovery period between each trial. Group 1 birds had 48 hr between trials, during which they received a diet of chow (5–10% of daily intake), aspen flower buds, and aspen catkins (Guglielmo et al. 1996). Group 2 birds had 10 days between the 6.5% CB and food restriction trials, during which they received a diet treated with a low concentration of CB (0.5% dry mass) (Jakubas et al. 1993*b*).

Analytical Procedures

We determined the energy content of feed and excreta using a Phillipson microbomb calorimeter (Gentry Instruments) following Jakubas et al. (1993*a*). Energy values for ornithine (23.2 kJ/g) and glucuronic acid (13.5 kJ/g) were also determined by bomb calorimetry. Total nitrogen and ammonium-nitrogen levels were determined by the University of Wisconsin's Soil and Plant Analysis Lab using standard Kjeldahl nitrogen techniques (Schulte et al. 1987). We measured the amount of glucuronic acid in excreta using techniques adapted from Remington (1990) and Blumenkrantz and Asboe–Hansen (1973), and ornithine by spectrophotometric methods (Jakubas et al. 1993*b*). We repeated ornithine and glucuronic acid analyses if the coefficient of variation exceeded 5%.

Data Analyses

Utilization efficiencies for birds are reported as apparent metabolizable energy (MEC*) or assimilable mass (AMC*) coefficients, and are calculated using the following equations:

$$AMC^* = (Q_i - Q_e) / Q_i \quad \text{(eq. 1)}$$
$$MEC^* = (GE_iQ_i - GE_eQ_e) / GE_iQ_i \quad \text{(eq. 2)}$$

where Q_i and Q_e are the rates of food intake and excretal output (g · kg^{-1} · day^{-1}), respectively, and GE_i and GE_e are the energy content of the food and excreta (kJ/g), respectively (Kendeigh et al. 1977, Karasov 1990). The coefficients are referred to as apparent because these values are not corrected for endogenous losses of mass and energy (e.g., sloughed epithelial cells, uric acid from protein catabolism).

We corrected for endogenous losses resulting from protein catabolism by first determining the nitrogen balance (N_b) of the animal ($N_b = Q_iN_i - Q_eN_e$), where N_i represents the nitrogen content (g) of the food and N_e the nitrogen content (g) of the excreta. We then converted nitrogen losses to moles of uric acid to calculate mass and energy losses associated with uric acid excretion (Sibbald 1982). The mass correction for uric acid nitrogen is 3 g/g N, and the energy correction is 34.5 kJ/g N (Karasov 1990). Given these values, the equations for nitrogen correction would be:

$$AMC_N^* = 1 - ([Q_e + 3(N_b)] / Q_i) \quad \text{(eq. 3)}$$

$$MEC_N^* = 1 - ([GE_eQ_e + 34.5(N_b)] / GE_iQ_i) \qquad \text{(eq. 4).}$$

Metabolizable energy intake (MEI), or the kJ/day available to the bird from the diet, was defined as:

$$MEI = GE_iQ_i \cdot MEC_N^* \qquad \text{(eq. 5)}$$

For Group 2 birds, we adjusted AMC_N^* and MEC_N^* values for CB dilution of the nutrient content of the diet by correcting for CB mass and energy,

$$AMC_{NS}^* = 1 - ([Q_e - Q_iS_i + 3(N_b)] / [Q_i - Q_iS_i]) \qquad \text{(eq. 5)}$$
$$MEC_{NS}^* = 1 - ([GE_eQ_e - Q_iS_iGE_S + 34.5(N_b)] / [Ge_iQ_i - Q_iS_iGE_S]) \qquad \text{(eq. 6)}$$

where S_i is the percent dietary concentration of a secondary metabolite (e.g., CB) expressed as a decimal, and GE_S is the energy content of a secondary metabolite (energy content of CB = 30.096 kJ/g; Jakubas et al. 1993*a*). For Group 1 birds, we determined the degree that PSM's in aspen diluted the nutritional content of the buds based upon a model which uses various food and physiological measurements to predict MEC* (Karasov 1990). Briefly, our model predicts the difference in MEC* between LCB and HCB diets by accounting for the dilution effect of dietary fiber, CB, and detoxication conjugate output (Guglielmo 1993, Guglielmo et al. 1996).

For Group 1 birds, means were compared among trials using 1-factor analysis of variance (ANOVA) with one repeated measure (trials). Tukey's Honestly Significant Difference procedure (Tukey's HSD) was used to isolate significant differences among means (Montgomery 1984). Paired-tests were used to compare trial means for Group 2 birds. Standard t-tests were used to determine if nitrogen balance within a trial was different from zero. We examined whether outputs of glucuronic acid, ornithine, and ammonium were a function of CB intake using standard linear regression techniques. In all cases, differences were considered significant if $P \leq 0.05$. Means are presented with standard errors ($\pm$ SE). Additional clarification of the methods used in these studies can be found in Jakubas et al. (1993*a,b*) and Guglielmo et al. (1996).

RESULTS

Food Utilization Efficiency

Intake of CB was associated with decreased food utilization efficiencies for birds in Groups 1 and 2. Both AMC_N^* and MEC_N^* decreased with increased CB content of the diet (Tables 1 and 2). Birds feeding on aspen buds (Group 1) retained less food mass when feeding on HCB buds (Table 1). Postfactum analyses indicated that AMC_N^* values were lower during the HCB trial than during the LCB trial; however, AMC_N^* values for birds during the MCB trials did not differ from those during either HCB or LCB trials. Higher CB levels in aspen buds also affected energy utilization efficiencies. For MEC_N^*, postfactum analyses indicated that energy utilization during the HCB trial was lower than during either the MCB or LCB trials, and that energy utilization was similar during the latter two trials.

Table 1. Mean Mass and Energy Assimilation Coefficients for Five Ruffed Grouse Feeding on Quaking Aspen Flower Buds with Low (L), Medium (M), and High (H) Coniferyl Benzoate (CB) Levels. Included are Supporting Data on Intake, Excretion, and Changes in Body Mass

	Feeding Trials						
	LCB		MCB		HCB		
Parameter	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	*P*
Mass assimilation:							
Intake ($g \cdot kg^{-1} \cdot day^{-1}$)	103.4	1.9	118.7	3.2	123.3	6.4	0.009
Excreta ($g \cdot kg^{-1} \cdot day^{-1}$)	81.7	1.7	95.2	2.3	100.5	4.6	0.002
AMC_N*[a]	0.21	0.003	0.20	0.003	0.19	0.006	0.005
Energy assimilation:							
MEI ($kJ \cdot kg^{-1} \cdot day^{-1}$)	428.1	7.5	463.5	24.9	395.1	37.3	0.222
MEC_N*	0.21	0.005	0.20	0.007	0.16	0.010	<0.001
Body mass:							
Mean (g)	561	21	548	21	539	23	0.001
Percent change (%/day)	-0.4	0.2	-0.7	0.02	-0.7	0.1	0.240

[a]Abbreviations:
AMC_N* –apparent assimilable mass coefficient corrected for protein catabolism.
AMC_{NS}* –the preceding acronym corrected for dilution by CB.
MEC_N* – apparent metabolizable energy coefficient corrected for protein catabolism.
MEC_{NS}* –the preceding acronym corrected for dilution by CB.
MEI –metabolizable energy intake

Although food utilization efficiency decreased, MEI did not vary among trials for either bird group (Tables 1 and 2). Ruffed grouse responded to lower food utilization efficiencies during the HCB trial by increasing their dry matter intake (Table 1). Postfactum tests indicated that mean intake during the MCB and HCB trials was higher than during the LCB trial. When compared to the intake of LCB buds by birds housed at room temperature (72.8 ± 2.8 $g \cdot kg^{-1} \cdot day^{1}$) (Guglielmo and Karasov 1995), Group 1 birds ate more ($P<0.001$) LCB buds per day. This higher intake rate by Group 1 birds was similar to that of free-ranging grouse (Andreev 1988).

Detoxication Products

Intake of CB was associated with higher excretion rates of detoxication products for Groups 1 and 2 (Table 3). For Group 1 birds, CB intake was positively correlated to the molar excretion rates of ornithine ($r^2 = 0.74$, $P<0.001$), glucuronic acid ($r^2 = 0.68$, $P<0.001$), and ammonium ($r^2 = 0.41$, $P<0.003$) (Guglielmo et al. 1996). However, at 0 CB intake, the Y-intercepts of the regressions on glucuronic acid and ornithine excretion rates did not pass through 0 but rather were 7.35 ± 0.88 and 5.63 ± 0.60 mmol $\cdot kg^{-1} \cdot day^{-1}$, respectively. This indicates that the majority of

Table 2. Mean Mass and Energy Assimilation Coefficients for Six Ruffed Grouse Feeding on Formulated Diets. Included are Supporting Data on Intake, Excretion, and Changes in Body Mass

	Feeding Trials				
	6.5% CB[a]		Food Restriction		
Parameter	$\bar{x}$	SE	$\bar{x}$	SE	*P*
Mass assimilation:					
Intake ($g \cdot kg^{-1} \cdot day^{-1}$)	35.4	1.7	33.8	2.1	0.454
Excreta ($g \cdot kg^{-1} \cdot day^{-1}$)	20.0	1.3	18.3	1.0	0.233
AMC_N*	0.44	0.006	0.47	0.008	0.029
AMC_{NS}*	0.47	0.006	0.47	0.008	0.721
Energy assimilation:					
MEI ($kJ \cdot kg^{-1} \cdot day^{-1}$)	301.1	16.1	313.7	30.2	0.538
MEC_N*	0.46	0.01	0.49	0.02	0.038
MEC_{NS}*	0.47	0.01	0.49	0.02	0.260
Body mass:					
Mean (g)	578	39	575	39	0.489
Percent change (%/day)[b]	-0.8	0.2	-0.5	0.1	0.257

[a] Abbreviations:
AMC_N* – apparent assimilable mass coefficient corrected for protein catabolism.
AMC_{NS}* – the preceding acronym corrected for dilution by CB, CB—coniferyl benzoate.
MEC_N* – apparent metabolizable energy coefficient corrected for protein catabolism.
MEC_{NS}* – the preceding acronym corrected for dilution by CB.
ME – metabolizable energy intake.
[b] Percent change per day is for the last 3 days of the 4-day trial.

the conjugates excreted were not associated with CB ingestion but with other PSM's in aspen buds. Group 2 birds, excreted more glucuronic acid ($P<0.001$), ornithine ($P<0.001$), and ammonium ($P<0.002$) during the 6.5% CB trial, than during the food reduction trial.

Groups 1 and 2 differed in detoxication product excretion. Group 1 birds had substantially higher outputs of detoxication products than Group 2, and Group 2 birds excreted a higher ratio of ornithine to glucuronic acid than Group 1 (Table 3). Although glucuronic acid excretion by Group 1 birds was correlated to their CB intake ($r^2 = 0.68$), glucuronic acid excretion by Group 2 birds, during the 6.5% CB trial, varied considerably with CB intake ($r^2 = 0.18$). Despite the lack of correlation between glucuronic acid excretion and CB intake during the 6.5% CB trial, ornithine output of Group 2 birds during the same trial was highly correlated ($r^2 = 0.90$) to CB intake.

Table 3. **Mean Intake of Coniferyl Benzoate (CB) and Nitrogen (N), Plus the Excretion Rates of the Principal Detoxication Conjugates and Ammonium for Ruffed Grouse Feeding on Quaking Aspen Flower Buds with Low (L), Medium (M), and High (H) Levels of CB and on Formulated Diets. Excretion Rates Are Expressed in Terms of Mass, N Intake, and Energy**

	Feeding Trials									
	LCB		MCB		HCB		6.5% CB		Food Restriction	
Parameter	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Intake										
CB ($g \cdot kg^{-1} \cdot day^{-1}$)	1.32	0.02	2.21	0.10	3.09	0.10	2.3	0.11	0	
Nitrogen ($g \cdot kg^{-1} \cdot day^{-1}$)	2.17	0.04	2.37	0.06	2.33	0.12	0.51	0.04	0.51	0.04
Output										
Glucuronic acid ($g \cdot kg^{-1} \cdot day^{-1}$)	1.89	0.06	2.33	0.10	2.54	0.14	0.28	0.02	<0.01	<0.01
Ornithine ($g \cdot kg^{-1} \cdot day^{-1}$)	1.00	0.03	1.23	0.05	1.34	0.06	0.42	0.02	0	
Ammonium ($mg \cdot kg^{-1} \cdot day^{-1}$)	478.8	8.4	591.4	16.8	579.5	37.4	75.9	4.6	32.1	3.4
Nitrogen cost										
Ornithine (% N intake)	9.7	0.2	11.1	0.3	12.2	0.3	17.7	0.4	0	
Ammonium (% N intake)	17.5	0.1	19.8	0.2	19.6	0.8	11.9	0.7	4.9	0.5
Balance ($mg \cdot kg^{-1} \cdot day^{-1}$)	-67.1	44.1	51.9	69.2	-31.6	72.0	-70.6	23.3	-30.4	22.3
Energy cost										
Glucuronic acid[b] ($kJ \cdot kg^{-1} \cdot day^{-1}$)	25.58	0.82	31.46	1.29	34.26	1.29	3.73	0.25	0.54	0.12
Ornithine[b] ($kJ \cdot kg^{-1} \cdot day^{-1}$)	22.64	0.73	28.05	1.08	30.35	1.48	9.64	0.51	0	
Total ($kJ \cdot kg^{-1} \cdot day^{-1}$)	48.22	0.85	59.51	2.04	64.61	2.99	13.37	0.68	0.54	0.12
% of MEI[a]	10.1	0.2	11.5	0.6	14.3	0.7	4.27	0.17	0.17	0.04

[a] MEI (metabolizable energy intake), in this case, was calculated as MEI (Tables 1 and 2) plus the energy excreted as ornithine and glucuronic acid.
[b] Calculated using energy contents of glucuronide and ornithine of 13.5 kJ/g and 22.71 kJ/g, respectively.

Nutrient Dilution

Our digestive model indicated that lower food utilization efficiencies during the HCB trial, as compared to the LCB trial, could be explained primarily by nutrient dilution rather than by preabsorptive digestive interference (e.g., protein precipitation). Dilution effects explained 0.03 of the 0.05 MEC* unit differences between the LCB and HCB trials. Once dilution effects were accounted for, the MEC* for the LCB and HCB trials did not differ (P = 0.15). Coniferyl benzoate dilution accounted for the largest amount of difference in metabolizability (0.02), followed by fiber (0.01), and detoxication conjugates (0.003). If dilution effects alone were responsible for lowering MEC_N* during the HCB trial, our model predicted that 29% of the energy content of aspen buds would have to come from the PSM's in aspen buds. Using a similar model to predict the difference in AMC* between the two diets, we found that nutrient dilution could explain all of the difference. Likewise, equations 5 and 6 indicated that the lower AMC_N* and MEC_N* for Group 2 birds during the 6.5% CB trial could be explained by the CB diluting the energy and nutrient content of the diet (Table 2). In the 6.5% CB trial, 10% of the gross food energy came from CB.

Energy and Nitrogen Costs

For Group 1 birds, the energetic cost of detoxication via the glucuronic and ornithine conjugation pathways ranged from 10 to 14% MEI, and increased with CB concentration ($P<0.001$). Postfactum tests indicated that energy costs were higher during the HCB trial than during the LCB or MCB trials. Energetic cost of detoxication, via the glucuronic and ornithine conjugation pathways was low for Group 2 birds relative to Group 1 (Table 3). Using an equation to calculate the endogenous excretion rate of nitrogen (Robbins 1993), we estimated, for Group 1 birds, that ornithine conjugation increased their minimum daily nitrogen requirement by 67.7% $\pm$ 1.9 (LCB) to 90.0% $\pm$ 5.1 (HCB) over that required when consuming a diet containing no PSM's. Although we did not measure every nitrogen output related to detoxication processes, the amount of nitrogen used for ornithine and ammonium production was approximately one-third of the daily nitrogen intake (N_i) for both Groups 1 and 2 (Table 3). Group 2 birds feeding on the 6.5% CB diet maintained a high level of nitrogen excretion related to detoxication despite having an N_i less than one-fourth that of Group 1 and despite being in negative nitrogen balance. Nitrogen balance for Groups 1 and 2 did not differ among trials within each group (P = 0.15 and P = 0.12, respectively) (Table 3). However, Group 2 birds during the 6.5% CB trial were in negative nitrogen balance (P = 0.013) but remained in balance during the food restriction trial (P = 0.120).

DISCUSSION

Our study demonstrates that nutrient dilution by PSM's and the energy and nitrogen costs of detoxication can be substantial for an avian herbivore. Together, these effects may alter food preference even when other averse toxic or digestive effects appear to be absent (also see Jakubas et al. 1993*b,c*). Nutrient dilution by PSM's appeared to be the primary factor lowering energy

utilization efficiencies, while excretion of ornithine conjugates and ammonium increased minimum nitrogen requirements. Although our study was limited to studying detoxication and dilution costs in only one avian species, other research indicates that some of these costs may be substantial in other species of grouse (Remington 1990) and mammals (Illius and Jessop 1995). Furthermore, our results suggest that if other vertebrate herbivores have nutrient dilution and detoxication costs similar to those in our study, then these costs may be one of the fundamental reasons why herbivores select foods having low PSM levels. This would especially be true for plants normally consumed by a herbivore. Although toxicity may be one primary reason why herbivores do not typically consume some species of plants, toxicity may not be as critical a factor in the selection of customary foods. Food selection by generalist herbivores has been hypothesized to be a two-step selection process in which free-ranging animals normally exclude plants with relatively toxic compounds from their diet and primarily consume plants having PSM's of low toxicity (Marquis and Batzli 1989).

Decreased efficiency in energy utilization may be one of the primary reasons why free-ranging grouse select aspen buds having CB concentrations $<1.8\%$ dry mass. In our study, ruffed grouse feeding on randomly collected buds (HCB) retained 24% less metabolizable energy than when feeding on LCB buds. Ecologically, these results imply that if free-ranging birds randomly fed on aspen buds rather than selecting specific trees to feed from, they would need to consume 24% more aspen buds to satisfy their energy requirements. Birds exhibiting this behavior may also incur greater predation risks and higher thermoregulatory costs because of increased foraging time. Therefore, it is conceivable that natural selection may favor birds that are able to associate the irritant properties of CB with poor food quality. The low energy utilization efficiency of birds feeding on HCB buds compared to LCB buds was mainly attributed to nutrient dilution by CB. There was no reason to invoke preabsorptive interference with digestive processes as a mechanism contributing to the low efficiency of energy utilization during the HCB trial. Although detoxication costs were not the major reason why birds during the HCB trial were less efficient in their energy utilization, these costs, nevertheless, accounted for the use of a substantial portion of Group 1's daily MEI. Ornithine and glucuronic acid excretion accounted for 14% of the bird's daily MEI during the HCB trial.

This study shows that much of the energy excreted as conjugation products resulted from the detoxication of PSM's other than CB. At least 53% of the glucuronic acid excreted and 58% of the ornithine excreted by birds feeding on HCB buds may be attributed to other PSM's in the buds. Although evidence is strong that birds are adverse to CB, it is less clear to what extent other PSM's are involved in deterring grouse from feeding on aspen buds. There appeared to be no difference in total phenolics between buds selected by ruffed grouse and randomly collected buds in this and earlier studies (Jakubas et al. 1989, Guglielmo and Karasov 1995). However, Jakubas et al. (1989) noted that the total phenolic assay does not detect different concentrations of CB and may not be a reliable assay for total phenolics in aspen. Despite a greater proportion of the detoxication conjugates being associated with the ingestion of other PSM's, it was CB intake that was highly correlated to the excretion of detoxication products. This indicates that grouse which avoid high concentrations of CB should be able to minimize their detoxication costs when feeding on aspen, irrespective of the detoxication costs associated with other PSM's.

Glucuronic acid excretion rates for Group 1 birds were similar to those for blue grouse *(Dendragapus obscurus)* eating needles from three conifer species (Remington 1990). Excretion

of glucuronic acid accounted for 7.5% of the daily MEI of Group 1 birds feeding on HCB buds. Although this appeared to be a substantial amount of energy devoted to 1 detoxication pathway, Remington (1990) found that glucuronic acid excretion for blue grouse doubled when birds fed on *Picea engelmannii* needles (a nontypical food). At this level of glucuronic acid excretion, 15.9% of the birds' daily MEI was being lost as glucuronic acid alone (Guglielmo 1993). The high excretion rates of glucuronic acid by blue grouse suggest that birds in our trials may have had additional capacity to conjugate PSM's with glucuronic acid and that the energetic costs of this detoxication pathway may be higher under some circumstances. Conversely, Group 2 birds, which had a 26% lower CB intake than Group 1 birds feeding on HCB buds, only excreted one-tenth as much glucuronic acid as Group 1. When Group 1's glucuronic acid excretion rate is corrected for the other PSM's in aspen buds, they still excreted three times the amount of glucuronic acid as Group 2 in order to detoxify an equivalent amount of CB. We cannot explain the low excretion rate of glucuronic acid excretion by Group 2 birds but suspect the answer lies in the regulation of the different detoxication pathways in grouse. It would be interesting to determine the relationship between the rate of glucuronic acid output and the level of induction of this detoxication pathway.

The other major cost of detoxication apparent from our study was the excretion of nitrogen in conjunction with the elimination of amino acid conjugation products and ammonium. Ornithine and ammonium excretion were significantly related to CB intake for both groups of birds. The excretion of ornithine conjugates alone during the HCB trial increased Group 1's minimum daily nitrogen requirement by 90% over that required when feeding on a diet with no PSM's. These results offer one explanation for field observations that indicate ruffed grouse select aspen buds based upon their CB and protein content rather than just on CB alone (Jakubas and Gullion 1991). In a broader sense, our results support other studies that suggest dietary nitrogen may limit detoxication capacity (e.g., Illius and Jessop 1995). However, it is unclear at what dietary level of nitrogen intake detoxication may be limited or whether limitation may be related more to the intake of specific amino acids, such as arginine (needed by birds to synthesize ornithine). Group 2 birds did not appear to be limited in their ornithine production, despite having a negative nitrogen balance and a daily nitrogen intake one-fourth that of Group 1 birds during the HCB trial. We calculated that if Group 2 birds consumed 10.9 mmol of CB $\cdot$ day^{-1}, they would have excreted 4.3 mmol of ornithine, which is similar to the ornithine excretion (4.5 mmol) of Group 1 birds at that level of CB intake (other PSM's subtracted).

One cost for maintaining pH homeostasis while producing acidic detoxication products is increased ammonium excretion (Foley 1992, Foley et al. 1995). Ammonium excretion was significantly related to CB intake for both Groups 1 and 2. Although some mammals, when challenged with an acid load, may remain in nitrogen balance by proportionally excreting less urea as their ammonium excretion increases (Oliver and Bourke 1975, Foley 1992), we did not observe a concurrent decrease in the excretion of uric acid with ammonium in our studies (Jakubas et al. 1993*a,b*; Guglielmo et al. 1996). Therefore, it appears that nitrogen excreted as ammonium is not compensated for by a decrease in other nitrogenous excretory products and could potentially affect nitrogen balance in ruffed grouse. Even though ammonium and ornithine production increased with CB intake in our studies, nitrogen balance was not related to CB intake for Group 1 birds. Group 2 birds, which had a much lower nitrogen intake, did not statistically differ in nitrogen balance between trials, despite a two-fold increase in nitrogen imbalance during the 6.5%

CB trial. However, during the 6.5% CB trial, birds were in negative nitrogen balance, while they remained in balance during the food reduction trial. Together, these studies suggest that detoxication may adversely affect nitrogen balance if grouse cannot find high protein foods in the wild. We do not know whether the acid load from detoxication products is sufficient to affect systemic pH levels (Foley et al. 1995) or whether averse effects are limited to increased ammonium excretion. One possible averse effect from acidosis is increased sodium excretion. However, for ruffed grouse, acid loads from detoxication products do not appear to influence sodium excretion rates (Jakubas et al. 1995).

RESEARCH IMPLICATIONS

Research on the detoxication costs associated with PSM ingestion by vertebrates is still in its early stages. If we are to fully understand the implications of our studies, additional research will have to be conducted on mammalian and avian herbivores. Studies should focus on (1) the relationship between detoxication and dilution costs and the amount of energy and nutrients in a diet and (2) determining whether animals select foods that minimize these costs. With an improved understanding of the fundamental physiological consequences of PSM ingestion, we may be better prepared to address the impacts of herbivory on ecosystems and predict the effects of anthropogenic disturbances on herbivore populations.

For the development of repellents to control nuisance wildlife, our studies indicate that more research is warranted to determine the extent to which repellency can be increased by raising detoxication or nutrient dilution costs. The addition of inert substances to avian diets has already been shown to suppress feeding in birds; however, nutrient dilution was not considered as a possible physiological mechanism causing this food aversion (Mason and Clark 1994). One topic that may be worth investigation is whether detoxication costs can be substantially increased by the induction of UDP-glucuronyltransferase and UDP-D-glucuronic acid in mammals and birds. Many chemicals can cause prolonged induction of this detoxication system (Dutton 1980, Watkins and Klaassen 1983); however, we do not know what costs are associated with this induction or whether a high level of induction would increase the detoxication costs of PSM's in an animal's diet. The detoxication cost model proposed by Illius and Jessop (1995) predicts that nitrogen may be more important in determining tolerance to PSM's than energy. Therefore, the manipulation of amino acid conjugation pathways and acid-base balance may also be fruitful areas of investigation. Hopefully, further research on this topic will clarify the ecological importance of detoxication and dilution costs and potential applications for this research.

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