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RUFFED GROUSE TOLERANCE AND BIOTRANSFORMATION OF THE PLANT SECONDARY METABOLITE CONIFERYL BENZOATE[1]

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Abstract. Ruffed Grouse (*Bonasa umbellus*) consumption of quaking aspen (*Populus tremuloides*) flower buds is determined, in part, by the concentration of coniferyl benzoate (CB) in the bud. Understanding the physiological effects of this compound may clarify whether the association between annual fluctuations in CB and Ruffed Grouse population levels has a cause/effect relationship. We determined the maximum level of dietary CB that Ruffed Grouse can tolerate by feeding captive grouse a formulated diet treated with eight concentrations of CB. Loss of body mass was used to indicate toxicity. Biotransformation products from CB were identified in order to determine the detoxication mechanisms employed and the presence of potentially toxic CB metabolites. Ruffed Grouse will consume up to 2 g kg^{-1} day^{-1} of CB before rapid mass loss ensues. High dietary levels of CB were associated with decreased food intake and increased water excretion. Serum uric acid, aspartate aminotransferase, and alanine aminotransferase levels did not change significantly with increased levels of dietary CB. Mass loss that was associated with a CB intake of 2.3 g kg^{-1} day^{-1} could be explained by decreased food intake but could not be attributed to any specific toxic effect. It is uncertain whether the decreased food consumption that occurred at high CB intake levels was prompted by toxicity avoidance or decreased food palatability. Ruffed Grouse appear to use glucuronic acid, sulfate, and ornithine conjugation, along with hydrolysis, reduction, and oxygenation reactions, when detoxifying CB. Nine biotransformation products were identified, including ferulic acid and 4-vinylguaiacol. The latter compounds are known to interfere with reproduction in other animals.

Key words: Bonasa umbellus; Populus tremuloides; *allelochemical; toxicity; food selection; feeding repellent; xenobiotic metabolism; Ruffed Grouse.*

INTRODUCTION

Many studies have shown that herbivores base their food selection, in part, on the secondary metabolite content of the plant (e.g., Bryant and Kuropat 1980). Although it is generally accepted that plant secondary metabolites influence animal feeding behavior, few studies have addressed the physiological effects that these compounds have on wild animals. By studying these physiological effects, we can determine if non-lethal toxic effects (e.g., reproductive and digestive inhibition) are associated with the daily consumption of these chemicals. In addition, we can learn more about the efficacy of feeding strategies, such as dietary mixing (eating a variety of plants to avoid consuming toxic levels of a particular compound), the evolution of animal "defensive" mechanisms against plant toxins, and the reasons why animals avoid consuming particular plant secondary metabolites. It is especially important to understand the physiological effects of a plant secondary metabolite when fluctuations in its production are associated with changes in animal's population density—as is apparent for Ruffed Grouse (*Bonasa umbellus*) and the primary secondary metabolite in quaking aspen (*Populus tremuloides*) flower buds (see Jakubas and Gullion 1991). Understanding the toxic properties of this compound may be the key to determining whether such an association is purely coincidental or has a cause/effect basis.

In winter, the staminate flower buds of quaking aspen are a primary food source for northern Ruffed Grouse (Gullion 1966, Vanderschaegen 1970, Svoboda and Gullion 1972, Doerr et al. 1974, Huempfner 1981). Crop and fecal analyses indicate that up to 66% of the winter diet (i.e.,

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mean crop contents) of Ruffed Grouse can be composed of aspen flower buds (Doerr et al. 1974). In addition, the extended catkins from these buds can comprise 80–100% of the bird's diet during a brief period in the spring (Vanderschaegen 1970, Stoll et al. 1980). Although grouse may feed extensively on these buds there are two important anomalies to their use. Grouse only feed on buds from certain trees, and the overall utilization of aspen buds can vary dramatically from year to year (Bump et al. 1947:219; Gullion 1970, 1984; Doerr et al. 1974; Huempfner 1981). Annual variations in aspen bud use have been correlated to changes in grouse population densities, with high bud use coinciding with high grouse densities (Jakubas and Gullion 1991). This differential use of aspen flower buds is influenced, in part, by the concentration of coniferyl benzoate (CB) in the bud (Jakubas et al. 1989; Jakubas and Gullion 1990, 1991). In quaking aspen, CB (a phenylpropanoid ester) is produced exclusively in the flower buds (mean concentration: ca. 2.5%; range 0 to 7.2% [dry mass]) and is adversive to birds and insects (Jakubas et al. 1989, 1992; Jakubas and Gullion 1990, 1991). Field observations indicate that the mean CB concentration of aspen buds that Ruffed Grouse consume is 1% (dry mass) and that buds having CB levels as high as 1.8% (dry mass) are utilized (Jakubas and Gullion 1990, 1991). These observations suggest that there may be a threshold above which CB is toxic to grouse. This threshold may constitute some maximal rate of CB intake related to the ability of grouse to detoxify CB. Alternatively, the threshold may constitute some maximum dietary concentration related to the stimulatory effect CB has on the trigeminal senses (i.e., CB is a chemical irritant); (Jakubas and Mason 1991). Significant toxicant related effects (e.g., digestion inhibition) may also be expressed at CB intake levels below the threshold which causes food avoidance. Although there are a number of potential toxic effects associated with CB intake (Jakubas and Gullion 1990), little is known about the physiological effects of this compound.

Dose-response relationships are fundamental for evaluating the toxicity of a compound (Klaassen 1986). From dose-response studies, we can determine the amount of a compound that is needed to elicit a toxic response (e.g., mass loss, decreased egg hatchability). Additional information on toxicity mechanisms can be obtained by studying the metabolism or biotransformation of a chemical. Biotransformation is considered to be the sum of the processes that a foreign chemical (xenobiotic) is subjected to by a living organism (Sipes and Gandolfi 1986). Common biotransformations include oxidation, reduction, and hydrolysis reactions (Phase I reactions) and conjugation reactions with endogenous products such as ornithine and glucuronic acid (Phase II reactions). Phase I and II reactions generally make a xenobiotic more soluble in water and hence make it more readily excretable. Studies on xenobiotic biotransformation can reveal if metabolites more toxic than the parent compound are produced, the excretion rates of xenobiotics, and the energetic and nutritional costs of detoxication.

Identification of the biotransformation products of CB may help elucidate the mechanisms that make analogous cinnamyl compounds repellent to birds. Many naturally occurring cinnamyl compounds (e.g., methylcinnamate and 3,4-dimethoxycinnamyl alcohol) are avian feeding repellents. Consequently, recent studies have used these compounds as chemical models in order to develop alternatives to the lethal pesticides currently being used to control avian crop depredation (Crocker and Perry 1990, Jakubas et al. 1992, Avery and Decker 1992). In addition to studying structure-activity relationships of various cinnamyl compounds, Avery and Decker (1992) suggest that research on the biotransformation of cinnamyl compounds might reveal a common link between these compounds and their repellency.

The objectives of this study were threefold. First, we wanted to determine the amount of dietary CB Ruffed Grouse can tolerate. Field observations suggest that a meal of aspen buds containing more than 1.8% (dry mass) CB may be deleterious to grouse. By determining the tolerance of Ruffed Grouse for CB, we hoped to substantiate these observations with physiological data, and use these data to formulate equations to predict the amount of food available from a stand of aspen. Secondly, we wanted to determine the principle mechanisms Ruffed Grouse use to detoxify CB. Identification of these mechanisms would enable assessment of the energy and nutrient costs associated with detoxication, and with the tolerance data, indicate the detoxication capacity of Ruffed Grouse for CB and analogous phenolic compounds. Our final objec-

tive was to identify the major biotransformation products of CB in order to determine if potentially toxic products were being produced. We were particularly interested in determining if ferulic acid is a biotransformation product of CB. Ferulic acid has been shown to adversely effect reproduction in Japanese Quail (*Coturnix coturnix*) (deMan and Peeke 1982) and *Microtus montanus* (Berger et al. 1977) and is believed to affect prolactin levels in other animals (Gorewit 1983 and references therein).

We accomplished these objectives by conducting dose-response studies with captive Ruffed Grouse using eight dietary concentrations of CB. Dietary protein and fiber levels can affect detoxication mechanisms and consequently a compound's toxicity (Boyd and Campbell 1983, deBethizy and Goldstein 1985, Sipes and Gandolfi 1986). Therefore, CB was applied to an artificial diet, formulated to mimic aspen flower buds in protein and fiber content. In birds, phenolic compounds are primarily conjugated with ornithine, glucuronic acid, and sulfate ions (Sykes 1971). Daily excretion rates for these conjugation products were determined for three diets. Biotransformation products of CB were determined from excretal extracts. All animal testing and handling procedures were approved by the University of Wisconsin's Research Animal Resource Center.

METHODS

DIET PREPARATION

Aspen Chips (Northeastern Products, Inc., Caspian, Michigan) and Mazuri Pheasant Maintenance Diet (Purina Mills, Inc., St. Louis, Missouri) were gound in an Alpine hammer mill to pass a 2 mm screen. Aspen Chips were mixed (1:2 [mass/mass]) with the Mazuri diet to increase fiber and decrease protein content. Particle size is an important factor in cecal filling (Björnhag 1989); therefore, in order to assure that natural digestion processes, rather than mechanical grinding, would determine how much fiber enters the ceca, we chose a fiber particle size that was larger than most commercial fibers. When Aspen Chips are manufactured, they are dried at temperatures that complex or degrade many naturally occurring phenolic compounds. However, to insure that phenolic compounds were not being added to the diet via Aspen Chips, the total phenolic content of Aspen Chips was measured using the Prussian blue assay (see Jakubas et al. 1989). This assay indicated that there were essentially no extractable phenolic compounds in Aspen Chips. Protein content of the diet was adjusted to approximately 10% by adding 1.311 kg of soy protein concentrate (84% protein) to every 22.7 kg (50 lbs) of Aspen Chips. Individual minerals and vitamins were added to the soy protein concentrate in order to maintain the mineral and vitamin levels offered in the Mazuri diet. Ingredients were blended in a commercial mixer and water was added to facilitate pelletization. Feed was pelleted (5 mm diameter) in a California pellet mill, and fan dried for approximately 72 hr.

The feed's nitrogen and fiber content was analyzed at the University of Wisconsin's Soil and Plant Analysis Laboratory following Schulte et al. (1987). The diet's mean crude protein and fiber levels were: crude protein (percent Kjeldahl nitrogen × 6.25) 9.7% (dry mass), range 9.5–10.1%; neutral detergent fiber (NDF) 41.3% (dry mass), range 41.0–41.6%; acid detergent fiber (ADF) 28.1% (dry mass), range 27.6–28.5%. These levels of protein and fiber are similar to those reported for quaking aspen flower buds: mean protein approximately 10% (Jakubas and Gullion 1991), NDF 42.7% (Servello and Kirkpatrick 1987), and ADF (calculated by subtraction from a mixed diet) 31.2% (Servello et al. 1987). Post-factum we determined that the metabolizable energy content of our diet (9 kJ/g) was higher than that of aspen buds (4 kJ/g), primarily because of the lower digestibility of aspen buds than our diet.

The approximate fat, mineral, and vitamin content of the diet (calculated by composition using Mazuri Pheasant Maintenance fact sheet #5643, Purina Mills) was the following: fat 2.1%, calcium 0.8%, phosphorus 0.6%, potassium 0.5%, magnesium 0.2%, sodium 0.1%, sulfur 0.1%, iron 187 ppm, zinc 90 ppm, manganese 100 ppm, copper 12 ppm, iodine 1 ppm, selenium 0.4 ppm, menadione 1 ppm, thiamin 9 ppm, riboflavin 6 ppm, niacin 93 ppm, pantothenic acid 10 ppm, choline 7,100 ppm, folic acid 3 ppm, biotin 4 ppm, vitamin B-6 6 ppm, vitamin B-12 15 μg/kg, vitamin A 6,000 IU/kg, vitamin D-3 2,250 IU/kg, vitamin E 125 IU/kg, and Carotene 4 ppm.

Coniferyl benzoate was extracted from benzoin Siam tears #3 (Alfred Wolff, Paris, France) and crystallized following the methods in Jaku-

bas et al. (1992) with the following modification. Acetonitrile and water (90:10) was used as the polar phase in the liquid/liquid extraction procedure (instead of methanol and water). This modification proved a purer crystallization liquor and increased yield. Coniferyl benzoate was applied to feed by dissolving crystalline CB in ethyl ether, thoroughly mixing the feed in the ether solution, and evaporating the ether under a fume hood. This application method allows CB to permeate the feed (determined using a phenolic indicator) and causes little if any change in the appearance of the feed pellet. Treated feed was sealed in a plastic bag and stored at −17°C until used. Base and food restriction diets were prepared by mixing ethyl ether with the feed and evaporating the ether. Coniferyl benzoate concentrations for treatment diets were 0.5, 1.5, 2.5, 3.5, 4.5, 6.0, 6.5, and 7.5 (% mass).

BIRDS

Ruffed grouse were captured in Sawyer County, Wisconsin using "lili-pad" traps (Dorney and Mattison 1956), or were raised from eggs gathered from the same area. Wild birds were acclimated to captivity in outdoor pens prior to bringing them indoors. Seven birds, four females and three males, 18+ months of age, were housed individually (indoors) in galvanized steel mesh cages (44 cm × 60 cm) with hiding boxes (42 cm × 42 cm) as described in Guglielmo and Karasov (in press). The number of birds used in these trials should have been sufficient to precisely define the amount of CB that Ruffed Grouse can tolerate (see Chan et al. 1982). Birds were kept in constant temperature (18.3°C) conditions, and under a 10:14 (light : dark) daily light cycle. Food and tap water were provided ad libitum. Prior to the dose-response trials, all birds were acclimated to indoor captivity for at least six months and, except for one bird, conditioned to the Aspen Chip/Mazuri diet for eight months (one bird conditioned two months).

DOSE-RESPONSE TRIALS

Loss of body mass was used to indicate toxicity. Pre-trial data indicated that Ruffed Grouse will lose up to 2% of their body mass per day when ill or when on a sub-maintenance level of intake (Guglielmo and Jakubas, unpubl. data). Consequently, we considered a 2% loss of original body mass per day, for three days out of a four day trial, indicative of a toxicant related response. To help insure a bird's well being, birds were immediately taken off of a CB diet when their mass loss exceeded 6%, over three days, and were given other food.

Three types of feeding trials were used to assess dose-response relationships. Each type of trial was conducted using identical methods, except as noted. (1) Mass-balance trials were conducted with feed containing 0, 0.5, 1.5, and 2.5 (% CB [dry mass]). The purpose of these trials was twofold. Body mass changes were measured for the dose-response study, and energy and nutrient utilization efficiencies were determined for another study (i.e., Jakubas et al., in press a). (2) Immediately following the mass-balance trials, sequential toxicity threshold trials were conducted with feed containing 3.5, 4.5, 6.0, and 7.5 (% CB [dry mass]). (3) Paired intake trials were conducted, after determining the approximate CB intake that appeared to cause a toxic response (i.e., following the 7.5% CB trial). These trials were conducted in order to distinguish between the physiological effects associated with CB toxicity and decreased food intake. Paired intake trials (i.e., 6.5% CB and food restriction [0% CB] trials) were conducted following the standard routine for mass-balance trials, with the exception that the food restriction trial consisted of limiting the consumption of the base diet to the amount of food individual birds consumed during the 6.5% trial. Consequently, the food restriction trial was used as the primary control for the 6.5% CB trial. In total, 10 feeding trials were conducted over a three month period (July through mid-October). During this time, there were no apparent changes in the birds' physiological condition as indicated by feeding rates on the base diet or by feather replacement (i.e., the birds did not molt). All trials (i.e., 0–7.5% CB) prior to the paired intake trials were conducted with the same seven birds (four females, three males). During the paired intake trials one female died; therefore, the 6.5% CB and food restriction trials were conducted with only six birds.

Between trial carry-over effects from lower CB doses were not a concern. Our objective was to try to determine the level of CB that grouse might tolerate under natural conditions. Grouse in the field feed on aspen buds from late fall to early spring, and hence acclimate to CB. Similarily, we presented our birds with sequentially higher doses of CB. Furthermore, birds in the field are exposed to other plant secondary metabolites and

have higher energetic costs than our captive birds. Thus, any physiological effects associated with 0.5–3 month exposure to CB in our study design should be conservative to those physiological effects that may occur under field conditions.

All trials consisted of a four day treatment period, during which the bird's sole food source was feed treated with a given concentration of CB. Food and tap water were provided ad libitum except during the food restriction trial. Food was presented in a single bowl, and water presented in a drink cup that filled automatically. Food and water consumption were measured at 15:00 hr each day for all trials. For mass-balance trials, ort (spilled food) and total collections of excreta were made at 08:00 and 15:00 hr each day, and bird mass measurements were taken at 08:30 hr (days 1–4 [3 days]). Bird mass measurements were taken without physically handling the birds (to reduce disturbance), by persuading them to enter a dark weighing box. For threshold trials, feeding bowls were checked at 08:30 each day (to insure that all the food hadn't been spilled), and bird masses were measured at 15:00 hr (from the start of the trial [time-0] through day-4 [4 days]). Blood samples (1–2 ml) were taken from the jugular vein, at 08:00–09:00 hr on day-5, for all mass-balance trials and for the 7.5% threshold trial. Following blood collection, birds were allowed to feed on the base diet a minimum of 55 hr before the next trial. If the start of the next feeding trial was delayed more than 55 hr, birds were given a 0.5% CB diet until the trial could begin.

Exceptions to the above routine were the 0.5% CB and food restriction trials. Prior to the 0.5% trial, birds were acclimated for 10 days to a 0.5% CB diet. This was done to allow the gastrointestinal microflora time to adjust to potentially toxic metabolites from CB, and to allow time for the induction of detoxication enzymes (e.g., P-450 enzymes). Preceding the 6.5% CB trial, birds were kept on a 0.5% CB diet for approximately one month, during which time they had the opportunity to regain any mass lost during earlier trials. Following the 6.5% CB trial, birds were given the base diet for 10 days before the start of the food restriction trial. This allowed the birds to regain mass lost during the 6.5% CB trial and time to recover from any acute effects of CB ingestion.

Excreta were collected twice a day during the mass-balance trials in order to limit ornithine decomposition by bacteria (see Baldwin et al. 1960). Excreta were immediately frozen at −17°C and lyophilized within 10 days after collection. All lyophilized samples were ground in a Wiley mill to pass a 20 mesh screen. Morning and afternoon collections (days 2–4) were, respectively, combined for each trial, with the exception of the base (0% CB) and 0.5% CB diets (days 1–4 combined). Past studies indicate that not all of the previous day's food may be excreted in 24 hr (Gasaway et al. 1975; Guglielmo, unpubl. data). Therefore, samples from day-1 were omitted in order to exclude excreta originating from food consumed prior to the start of the trial. Day-1 samples were not omitted from the 0 and 0.5% CB trials because the birds had been feeding on the same feed prior to these trials. Biotransformation products from CB were determined from total collections of excreta made on day-4 of the 6% CB trial. Samples were immediately frozen and stored at −17°C until extracted. Excretal moisture content (wet mass − freeze dried [fd] mass) was determined from excreta collected in the afternoon. Afternoon samples were used for moisture determinations because they were exposed to ambient room conditions for a shorter period of time.

CB METABOLISM STUDIES

Excretion of uronic acids (principally glucuronic acid) was measured using techniques adapted from Remington (1990) and Blumenkrantz and Asboe-Hansen (1973). Briefly, 1 g of ground, lyophilized, excreta was vigorously mixed with 100 ml of 0.01 *M* borax buffer (pH ca. 9.5) for 30 min. After filtering (#4 followd by #1 Whatman filter paper), 20 μl of the solution was diluted with distilled water to 200 μl and analyzed for uronic acids following Blumenkrantz and Asboe-Hansen (1973). Photometric absorbance (515 nm) was determined for all samples on a Beckman DU-64 spectrophotometer. Analyses were done in duplicate and repeated if the coefficient of variation (CV) was above 5%. Glucuronic acid was used to develop a standard curve.

A spectrophotometric method for analyzing conjugated ornithine in excreta was developed based on the acid ninhydrin methods of Chinard (1952) and Troll and Lindsley (1955). Briefly, 0.5 g of ground, lyophilized, excreta (from afternoon collections only [0–5 hr old]) was extracted (as in the glucuronic acid procedure) and frozen (−17°C) until analyzed. Urea and protein were

precipitated with 20% trichloroacetic acid (4:1, sample solution : acid) and removed by filtering the solution through a 0.2 μm filter overlain with Celite 545 (Fisher Scientific, Pittsburgh, PA). Conjugated ornithine was hydrolyzed by adding 0.5 ml of the protein free solution and 0.5 ml 6 *M* hydrochloric acid to a culture tube, topping the tube with nitrogen (2 min), and heating the sealed tube in an oil bath at 105–110°C for 20 hr. Following hydrolysis, the sample was evaporated using a Savant Speedvac Concentrator (Model SVC-200H), and re-diluted with 3 ml 0.01 *M* borax buffer. From this solution, 0.5 ml was analyzed for ornithine, 0.5 ml was reserved as a sample blank, and 1.5 ml was used to correct for proline interference. Proline and ornithine when reacted with acidic ninhydrin produce nearly the same molar adsorption curves (Chinard 1952). Therefore, proline interference was corrected for by removing ornithine, using a weakly acidic cation exchange resin; determining sample absorbance due to proline; and subtracting proline absorbance from total sample absorbance (ornithine and proline). Briefly, 1.5 ml of hydrolyzed sample was diluted with ≥1.5 ml of buffer to raise the pH of the solution above 9. The diluted sample (2 ml) was mixed with 0.2 g of Amberlite CG-50 (Sigma Chemical Co., St. Louis, Missouri) for 5 min and allowed to settle. Two 0.5 ml aliquots of the supernatant were removed for blank and proline determinations. Interference by other amino acids was determined not to be significant based on molar adsorption curves (Chinard 1952) and levels of interfering amino acids in excretal extracts from birds fed a 2.5% CB diet (samples analyzed by an automated amino acid analyzer, Brad Ricker, U.S. Dairy Forage Research Laboratory, Madison, Wisconsin). Blanks, ornithine, and proline samples were processed according to Chinard (1952) (reagent volumes adjusted for 0.5 ml samples) and absorbances determined on a Beckman DU-64 spectrophotometer. Ornithine hydrochloride (Sigma, St. Louis, Missouri) was used to develop calibration curves. Analyses were done in duplicate and repeated if the CV was above 5%. Analytical standards consisting of 40, 80, 120, 160 μg/ml of ornithine hydrochloride and 24.4 μg/ml of proline were processed with the samples starting at the hydrolysis step. Sample ornithine concentrations were adjusted according to the amount of ornithine recovered from the analytical standards.

Sulfate ester excretion was quantified using a turbidimetric procedure adapted from Sperber (1948), Lundquist et al. (1980), and Sörbo (1987). Briefly, 100 mg of ground, lyophilized excreta was vigorously mixed with 5 ml of deionized water for 1 hr. The supernatant was decanted and 1 ml of acidic barium chloride solution (Sperber 1948) added to remove inorganic sulfate. After 5 min the solution was centrifuged at 3,000 rpm for 10 min. The supernatant was decanted and 1 ml of a 5% (mass/v) solution of sodium carbonate added to precipitate any excess barium ions. After centrifuging at 3,000 rpm for 10 min, 3 ml of the supernatant was pipetted into a culture tube, 1 ml of 10% (v/v) hydrochloric acid added, the tube topped with nitrogen, and the sealed tube heated in an oil bath at 100°C for 30 min. After the hydrolysate had cooled to room temperature, 2 ml was combined with 1 ml of Ba-PEG-reagent (Lundquist et al. 1980), and the remainder of the hydrolysate combined with 1 ml of polyethylene glycol (PEG) solution (150 g of PEG 8000/l of water) to serve as a sample blank. After mixing, samples were allowed to sit for a minimum of 5 min, and were vortexed immediately before reading their absorbance at 600 nm. A standard curve was developed using sodium sulfate as described in Lundquist et al. (1980). Samples were analyzed in triplicate.

Unconjugated biotransformation products of CB were identified by comparing extracts of excreta from male and female birds, respectively, that had fed on 0 and 6% CB diets. Frozen excreta were thawed slightly and extracted twice (1:10, sample : solvent [mass/v]), at room temperature, with either acetone and water (50:50) (Cork and Krockenberger 1991) or ethyl ether. Extract solutions were stirred vigorously for 30 min in darkened containers and filtered (#1 Whatman filter paper overlain with Celite 545). Acetone was evaporated from the acetone/water solution under an air stream, and the aqueous layer extracted (3×) with an equal volume of methylene chloride. The resulting ether and methylene chloride extracts were dried with sodium sulfate and stored at −17°C until analyzed. Samples were analyzed with a Shimadzu GC-14A gas chromatograph coupled to a Finnigan Mat 800 series ion trap detector. Chromatographic specifications included: (30 m × 0.25 mm i.d.) DB-1 column (Durobond), split injection, column flow rate—1.22 ml min^{-1}, temperature program: 60°

(0.5 min) to 280° at 10° min^{-1} and held at 280° for 15 min. Compounds were identified by comparing mass spectra to Ralph and Hatfield (1991) and were confirmed with known standards.

Serum samples were analyzed for uric acid, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) by the University of Wisconsin's Veterinary Teaching Hospital.

DATA ANALYSIS

Percent mass loss (over 3 days) during mass-balance trials was calculated by subtracting the bird's mass on day-4 from its day-1 mass (day-1 mass was measured 17.5 hr after being presented the treatment diet). Percent mass loss (over 4 days) during the toxicity threshold trials (and 0% CB trial) was calculated by subtracting the bird's mass on day-4 from its mass immediately prior to the start of the trial (time-0). In cases where data from threshold and mass-balance trials were combined (e.g., Fig. 2b), only data from days 1–4 were utilized from the threshold trials. Mean ornithine, glucuronic acid, and sulfate ester production were estimated by multiplying the bird's mean daily excreta production (0%, 6.5%, and food restriction trials) by the mean excretal concentration (fd mass) of the compound. Mean CB intake was calculated by multiplying a bird's mean daily feed intake, for a given trial, by the CB concentration of the feed. Mean trial body mass was used to calculate the amount of a substance excreted per gram of body mass (e.g., mg/kg), while intake rates were normalized using daily measurements of body mass. Means were examined in a 1-factor analysis of variance (ANOVA) with one repeated measure (i.e., trials). Tukey's honestly significant difference tests were used to isolate significant differences among means (Montgomery 1984). In all cases, differences were considered significant if $P \leq 0.05$. Means are presented with standard errors (±SE).

RESULTS

TOXICITY

Food consumption, during the trials, remained relatively constant (46 to 49 g kg^{-1} day^{-1}) until CB concentrations surpassed 4.5% (Fig. 1a). Coincident with this, CB intake increased linearly with CB concentrations up to the 4.5% CB diet. Above this concentration of dietary CB, food intake decreased such that CB intake essentially reached a plateau at ca. 2.1 ± 0.09 g kg^{-1} day^{-1} (Fig. 1a). The slightly lower consumption of food and the accompanying loss of body mass during the base (0% CB) trial is believed to be due to the initial disturbance of the new handling routine. As according to trial design, food consumption did not differ significantly between the 6.5% and food restriction trials (Table 1).

In contrast to mean four day consumption rates, day-1 food consumption was inversely related to low concentrations of CB (Figs. 1a, b). Day-1 food consumption rates should be a more sensitive indicator of food palatability, because of the lower hunger stress associated with rejecting unpalatable food over a 24-hr period as compared to 96 hr. Day-1 food consumption was highly correlated to CB concentrations ($r^2 = 0.94$, $P < 0.0001$) over all dietary levels of CB (Fig. 1b). Day-1 food consumption of the base trial was measured prior to the birds experiencing the afternoon handling routine (i.e., the most disturbing part of the new handling routine), whereas the 4-day intake of the base trial (Fig. 1a) reflects the apparent "disturbance effect" of the new handling routine.

Analysis of body mass loss as a function of CB intake indicated that consumption of more than 2 g kg^{-1} day^{-1} of CB was associated with a significant loss of body mass ($F = 52.34$; 5, 15 df; $P < 0.0001$) over a 4-day period (Fig. 2). Mean loss of body mass over the 7.5% trial (CB intake = 2.06 g kg^{-1} day^{-1}) was 6.5% ± 0.48, exceeding the toxic end-point (2% day^{-1} for 3 day) for most birds. Three birds were taken off the 7.5% diet after three days, because of rapid mass loss, and were allowed to feed on the base diet. One bird during the 7.5% trial apparently regurgitated some of its stomach contents on day-3 of the trial.

The amount of body mass lost during the 6.5% trial appeared to be primarily due to decreased food intake. Loss of body mass over the 6.5% trial was not significantly different than during the food restriction trial (Table 1).

Excretal moisture content differed significantly ($F = 10.75$; 4, 20 df; $P < 0.0001$) among mass-balance trials. Post-factum analysis indicated that excreta from the 6.5% CB trial had a significantly higher moisture content (61.5% ± 1.7) than all other trials (0%, 55.6% ± 1.7; 0.5%, 57.3% ± 1.4; 1.5%, 54.6% ± 1.6; 2.5%, 57.7% ± 1.1). The greatest difference ($F = 30.4$; 1, 5 df; $P = 0.003$) among trials in excretal moisture content occurred between the 6.5% and food restriction trials (Table 1). Another apparent difference in excreta

TABLE 1. Comparison of intake and physiological measurements for Ruffed Grouse ($n = 6$) during the 6.5% coniferylbenzoate (CB) and food restriction trials. P-values (two-tailed) are from repeated measure comparisons of these two trials.

Parameter	6.5% CB trial	Food restriction trial[a]	P-value
Food intake (g kg^{-1} day^{-1})	20.1 ± 1.4	19.2 ± 1.3	0.546
CB intake (g kg^{-1} day^{-1})	2.3 ± 0.11	0	NA[b]
Pre-trial body mass (g)	605 ± 43	596 ± 42	0.057
Percent body mass loss (4-day)	2.37 ± 0.55	1.63 ± 0.36	0.336
Percent water in excreta	61.5 ± 1.7	48.7 ± 3.7	<0.0001
Water intake (g kg^{-1} day^{-1})	60.6 ± 3.6	48.6 ± 1.9	0.002
Serum uric acid (mg/dl)	3.3 ± 0.64	4.8 ± 0.62	0.025
Serum ALT[c] (units/l)	5 ± 0.82	3 ± 0	0.058
Serum AST[d] (units/l)	528.7 ± 32.5	524.8 ± 15.9	0.872
Glucuronic acid excreted (mmoles kg^{-1} day^{-1})	1.42 ± 0.1	0.19 ± 0.03	NA
Ornithine excreted (mmoles kg^{-1} day^{-1})	3.14 ± 0.17	0	NA
Sulfate esters excreted (mmoles kg^{-1} day^{-1})	0.72 ± 0.11	0.57 ± 0.08	0.165

[a] Food restriction trial consisted of limiting the consumption of the 0% CB diet to the amount of food individual birds consumed during the 6.5% CB trial.
[b] NA = No statistical analysis attempted.
[c] Alanine aminotransferase.
[d] Aspartate aminotransferase.

among trials was the general yellow color of feces or the excretion of bright yellow crystals from birds feeding on diets having CB concentrations ≥6%. Water intake varied directly with food intake; therefore, except for the 6.5% and food restriction trials (equal food consumption), water intake, per se, could not be compared between the other trials. Mean 4-day water intake during the 6.5% CB trial was significantly higher than during the food restriction trial (Table 1). For comparing water intake among other trials, daily water intake was first normalized for food consumption by dividing water intake by daily food intake. A comparison of normalized water intake levels revealed a significant ($F = 4.13$; 4, 20 df; $P = 0.013$) difference among the other mass-balance trials (0, 0.5, 1.5, 2.5, and 6.5 [% CB]). Post-factum analysis indicated that significantly more water was consumed with food during the 6.5% trial (1.71 ± 0.04 g water/g of food) than during the 2.5% trial (1.34 ± 0.04 g water/g of food).

Blood analyses following the mass-balance (0, 0.5, 1.5, 2.5 [% CB]), and 7.5% trials indicated that serum uric acid levels did not differ significantly ($F = 1.97$; 4, 24 df; $P = 0.131$) among trials. Serum uric acid levels were statistically different between the 6.5% and food restriction trials (Table 1). However, in terms of indicating protein catabolism, the difference between the means was not biologically significant. Okumura and Tasaki (1969) reported that plasma uric acid levels of chickens increased an order of magnitude when the birds were fasted for 72 hr. Stable serum uric acid levels throughout the trials suggest that any body mass lost over the trials was primarily due to catabolism of fat rather than protein. Likewise, mean serum AST and ALT levels did not differ significantly among trials (0%–7.5%) ($F = 0.70$, 4, 24 df; $P = 0.600$ and $F = 0.12$, 4, 24 df; $P = 0.975$, respectively) (Table 1). Ranges for mean AST and ALT levels for all trials were 524 ± 30 to 573 ± 25 (units/l) and 3 ± 0 to 5 ± 3 (units/l), respectively.

DETOXICATION

Concentrations of uronic acids in the excreta, measured as glucuronic acid, were more than an order of magnitude higher in the 6.5% trial (14.74 ± 1.0 mg/g of fd feces) than from the 0% (0 mg/g) or food restriction (1.41 ± 0.48 mg/g) trials. Correspondingly, total daily excretion of glucuronic acid was markedly higher during the 6.5% trial as compared to the food restriction trial (Table 1). Ornithine was not detected in feces from the 0% or food restriction trials. However, high concentrations (20.86 ± 0.35 mg/g) were present in excreta from the 6.5% trial (also see Table 1). Mean ornithine recovery for all analytical standards was 97% ± 0.03.

Excretal sulfate content, from sulfate esters, differed significantly ($F = 3.99$; 2, 10 df; $P = 0.053$) among trials. Concentrations were higher in the 6.5% trial (35.1 ± 3.3 μmoles/g) than in

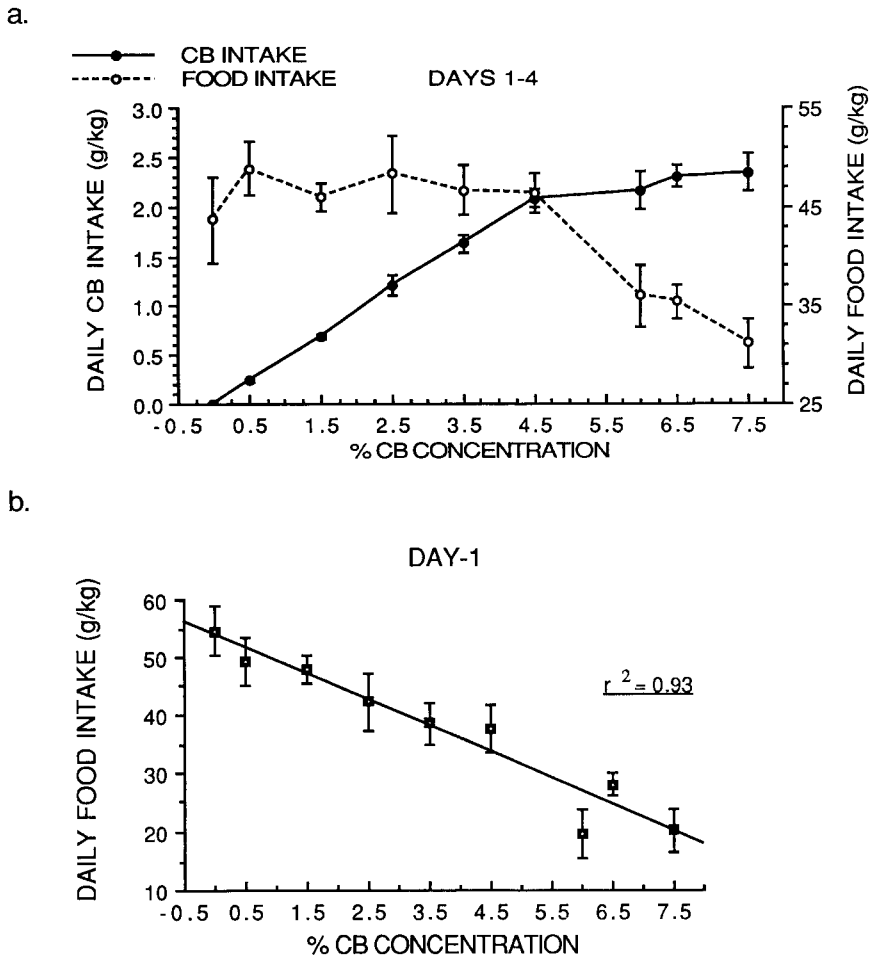

FIGURE 1. (a.) Dietary coniferyl benzoate (CB) concentrations compared to food consumption of Ruffed Grouse (±SE) and daily intake (±SE) of CB. (b.) Linear regression of mean day-1 food consumption (±SE) compared to dietary CB concentrations.

0% (28.6 ± 2.7 μmoles/g) or food restriction (26.2 ± 3.4 μmoles/g) trials. Daily excretion of sulfate esters did not differ significantly ($F = 2.69$; 2, 10 df; $P = 0.116$) among trials. Mean daily sulfate ester excretion was similar for the 0% CB (0.56 ± 0.09) and food restriction trials but appeared to be slightly higher during the 6.5% CB trial (Table 1). Only duplicate analyses, instead of the planned triplicate runs, were used to calculate these data. Data from the first run were not used due to storage problems (malfunctioning freezer).

Nine biotransformation products from CB were identified from ether extracts of excreta (Fig. 3). No major qualitative differences existed between the acetone : water and ether extracts; therefore, ether extracts were used in all subsequent analyses. There were no qualitative differences in biotransformation products among sexes. Major biotransformation products were benzoic acid, eugenol, acetovanillone, ferulic acid, and 4-hydroxybenzenepropanoic acid. The structure of the latter compound, especially in regards to the position of the phenyl group, was not confirmed because we lacked a standard. We suspect that the phenyl group may be located at the meta position, since Scheline (1978:209) reports that one of the major biotransformation products from ferulic acid metabolism in rats is 3-hydroxybenzenepropanoic acid. 4-vinylguaiacol was present principally as an artifact from the thermal degradation of ferulic acid during gas chro-

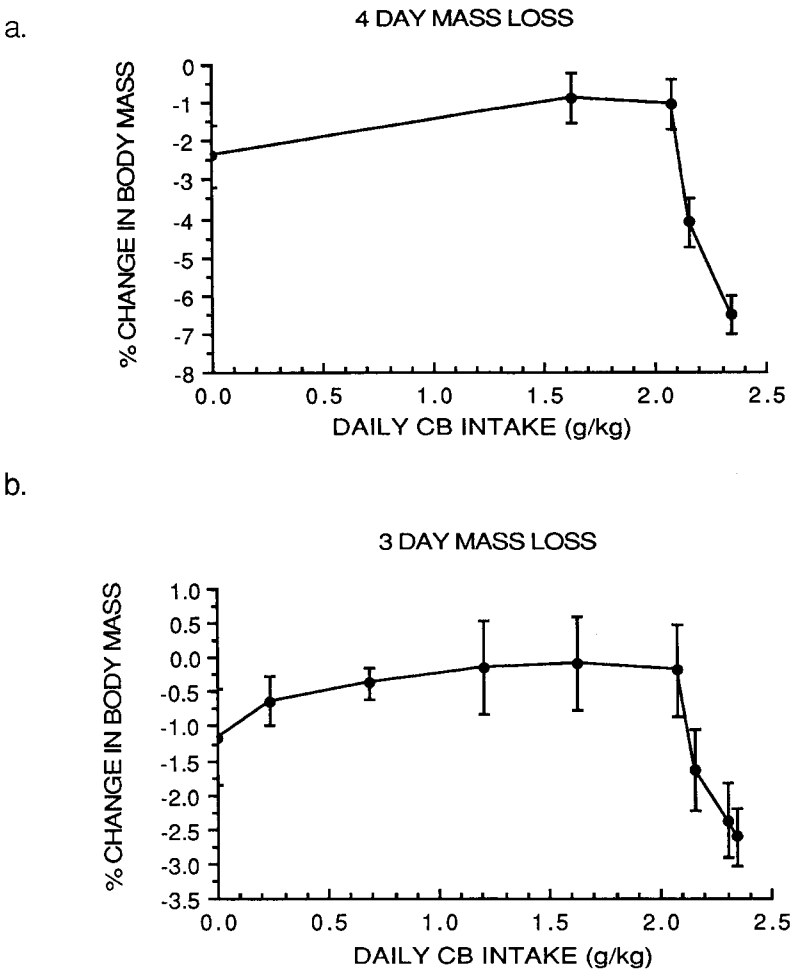

FIGURE 2. (a.) Percent change in Ruffed Grouse body mass (±SE) (time-0 to day-4) compared to coniferyl benzoate (CB) intake during CB tolerance and 0% CB trials. (b.) Percent change in body mass (±SE) (day-1 to day-4) compared to CB intake during all trials.

matography. To stop the thermal degradation of ferulic acid, ether extract was silylated by evaporating the extract, dissolving the residue in dichloromethane, adding BSTFA (Alltech Associates, Deerfield, Illinois), and heating the sample at 70°C for 15 min. Analysis of the silylated extract by GC indicated that 4-vinylguaiacol was present in trace amounts.

DISCUSSION

CB TOLERANCE AND TOXICITY

Ruffed Grouse restricted their intake of CB in our experiments to about 2 g kg^{-1} day^{-1}; however, it is uncertain whether toxicity was the primary factor limiting CB intake. Toxicant related effects did not cause birds to lose body mass when CB consumption rates approached 2 g kg^{-1} day^{-1}. This was evident from the equivalent amount of body mass lost during the paired intake trials. If CB toxicity, per se, was responsible for mass loss, the percentage of body mass lost in the 6.5% CB trial should have been greater than in the food restriction trial. Rather, it appears that the decreased food consumption associated with high CB intake was responsible for the observed mass loss.

The tolerance that Ruffed Grouse had for CB in our laboratory trials closely corresponds to field data which suggest that Ruffed Grouse avoid consuming over 2 g of CB kg^{-1} day^{-1}. To cal-

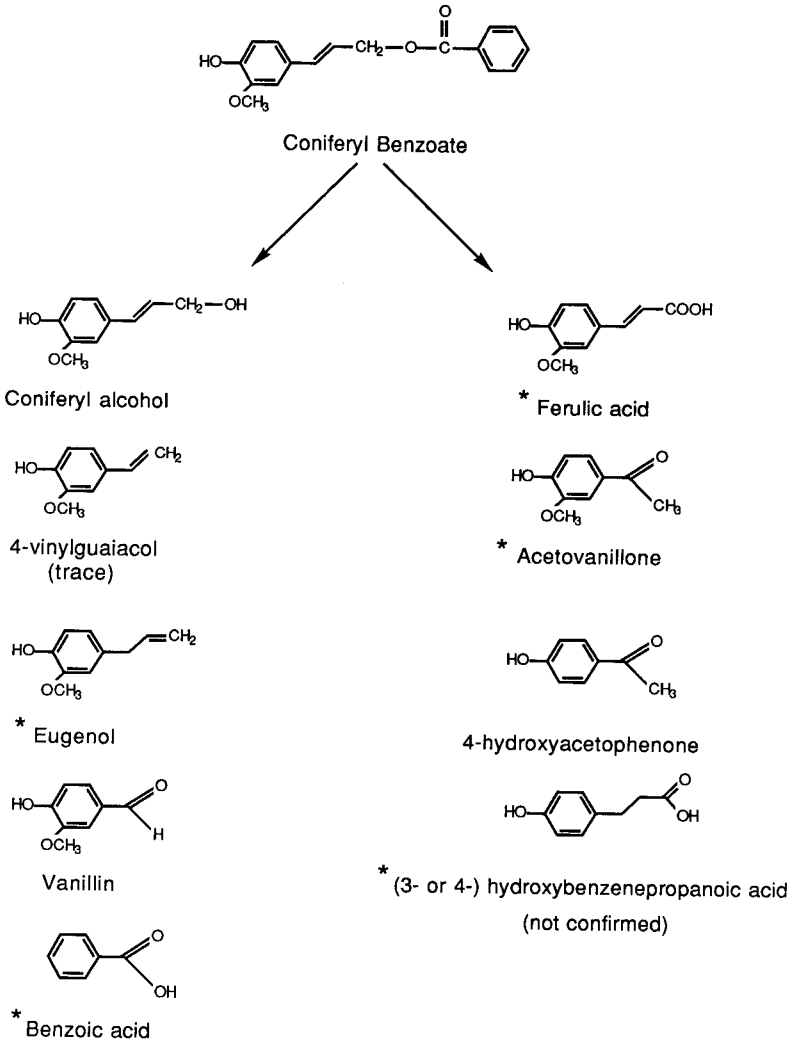

FIGURE 3. Coniferyl benzoate (CB) and its biotransformation products. Biotransformation products were identified from excreta of Ruffed Grouse feeding on a 6% CB diet. Asterisks indicate primary excretal products.

culate the maximum CB intake observed in the field, we used 100 g as the maximum amount of vegetative material Ruffed Grouse could hold in their crop or consume in a feeding bout (two feeding bouts per day) (Svoboda and Gullion 1972). We assumed that the highest percentage of aspen buds in a bird's crop would be between 70 and 80% (to allow for other food items, twigs, and leaf buds that are consumed with the flower buds [see Svoboda and Gullion 1972, Doerr et al. 1974]), a bud moisture content of 44% (Jakubas, unpubl. data), a mean CB concentration of 1% in buds that Ruffed Grouse select (Jakubas and Gullion 1990), and a mean bird mass of 550 g. Using these figures, we estimate that a typical grouse would consume between 1.7 and 1.9 g of CB kg^{-1} day^{-1}. The availability or suitability of aspen buds can vary both temporally and spatially. Annually, the percentage of suitable aspen feeding trees in a population may vary from ca. 0 to 30% (Jakubas and Gullion 1991). Quantification of the amount of CB Ruffed Grouse can tolerate makes it possible to develop equations to predict the amount of food available for Ruffed Grouse from a stand of aspen. Predictions of aspen suitability may especially be useful when

selecting new (i.e., non-traditional) relocation sites for grouse.

We are left with the question of why Ruffed Grouse decreased their food consumption. Was it simply to avoid eating highly unpalatable food, even if it meant starvation; was it to avoid the toxic effects that may occur at higher levels of CB intake; or was it because of a combination of aversive and post-ingestive effects? If grouse decreased their food intake to avoid the toxic effects of CB, then toxicity avoidance may partially explain behavioral and evolutionary questions such as: why is it advantageous for grouse to feed only on aspen buds having low CB levels; does natural selection favor birds that can detect small changes in CB concentrations (cf. Fig. 1b); and why do aspen buds make up a small component of the diet of Ruffed Grouse during some years? If grouse reduced their food intake to avoid food that was simply unpalatable, it would raise a larger evolutionary question of whether natural selection would favor the production of a plant defensive compound that was unpalatable but not toxic. Or, can a plant secondary metabolite, that simply makes a food unpalatable, be a sufficient deterrent to keep an animal from utilizing one of its primary winter foods?

Evidence supporting the hypothesis that food palatability limited food intake in our experiments comes from several sources. First, lower food consumption on day-1 of the trials (as compared to mean food intake) indicates that palatability played, at least, a temporal role in regulating food intake at all CB concentrations (i.e., birds appeared to habituate to food taste and usually increased food consumption towards the end of the trial) (cf. Figs. 1a, b). Additionally, day-1 food consumption was highly correlated to dietary CB concentrations, again indicating that palatability was likely a factor in regulating food intake (Fig. 1b). These observations are further supported by previous work that indicates that Ruffed Grouse can inherently detect CB, and that CB is sensed by the trigeminal nerves in a bird's beak (Jakubas and Gullion 1990, Jakubas and Mason 1991). Other studies also indicate that reduced food consumption by grouse on high CB diets may not be strictly related to the toxicant effects associated with CB intakes near 2 g kg^{-1} day^{-1}. Guglielmo (1993) observed that Ruffed Grouse housed at 0°C and fed aspen buds (2.4% CB) consumed ca. 3 g of CB kg^{-1} day^{-1}, thus indicating that a CB intake of 2 g kg^{-1} day^{-1} is not an absolute upper limit for Ruffed Grouse. Although most birds in that study could not maintain body mass or increased food intake in order to maintain mass, the higher maximum intake of CB, at a lower dietary concentration, raises the question of the importance of the interaction between CB intake and dietary concentration in determining CB toxicity or the upper tolerance limit of Ruffed Grouse for CB.

Evidence that CB toxicity may limit food intake and result in loss of body mass at CB concentrations >2.0 g kg^{-1} day^{-1} was seen in a companion study on the reproductive effects of CB (Jakubas et al., in press b). Over a 13-day feeding trial, female Japanese Quail given a 1.5% CB diet lost a significant amount of body mass ($P = 0.009$) from pre-trial levels, while pair-fed control birds, laying 15% more eggs did not lose a significant ($P = 0.899$) amount of mass (Jakubas et al., in press b). This mass loss by birds feeding on the 1.5% CB diet clearly suggests that post-ingestive effects were involved. As in the Ruffed Grouse feeding trials, quail, on average, limited their intake of CB to ca. 2.2 g kg^{-1} day^{-1}.

One mechanism by which CB may limit food intake is by challenging the pH homeostasis of Ruffed Grouse. Ornithine and glucuronide conjugation products are highly acidic (Robinson et al. 1953). The production of acidic conjugation products may result in increased generation of bicarbonate ions to control an animal's pH homeostasis (Foley 1992). Increased generation of bicarbonate ions and a concomitant increase in ammonium excretion has been observed in mammals excreting high levels of glucuronic acid (Foley 1992). Foley (1992) proposed that an animal's capacity to buffer acidic loads may limit the amount of xenobiotics they can consume. In our studies, birds during the 6.5% trial secreted higher ($P < 0.0001$) levels of ammonium than during the baseline or food restriction trials (Jakubas et al., in press a). This higher level of ammonium excretion was likely associated with maintaining acid-base balance (see Long and Skadhauge 1983, Atkinson 1992) while producing acidic biotransformation products (e.g., glucuronic, ornithuric, ferulic, and benzoic acids). However, the excretion of ammonium by grouse feeding on the 6.5% CB diet was not as pronounced as ammonium excretion reported in other studies where birds and mammals were fed a natural diet high in plant secondary metabolites (e.g., Remington 1990, Foley 1992). We know

from our companion studies, in which grouse were fed aspen buds containing 2.4% CB (and other naturally phenolic compounds), that daily glucuronic acid excretion was an order of magnitude higher than in the 6.5% CB trial (Guglielmo 1993). Therefore, if the detoxication capacity of Ruffed Grouse is determined, to an extent, by their ability to maintain pH homeostasis, the other phenolic compounds in aspen buds should further tax the bird's ability to maintain acid-base balance and lower the bird's capacity for consuming aspen flower buds.

Studies, to date, do not indicate that CB is toxic to Ruffed Grouse. Unchanged serum AST and ALT levels indicate that acute to subchronic exposure to CB does not result in necrotic injury to the liver. However, hepatotoxicity cannot be ruled out until additional factors are studied (e.g., hepatoexcretatory functions and hepatic lipid content). The increased water excretion associated with increasing CB concentrations in this study does not indicate a specific toxic effect. Increased water elimination may indicate a decreased ability to concentrate urine (nephrotoxic effect), a disruption in gastrointestinal absorption, or may simply be due to the larger volume of water needed to void the high concentration of CB metabolites. Although the amount of energy and nutrients grouse obtain from their food (i.e., aspen buds) may be diluted by high concentrations of CB, this compound does not appear to affect utilization efficiencies by decreasing the physiological ability of grouse to absorb and retain nutrients (Jakubas et al., in press a). Biotransformation processes may enhance the biological activity of CB by creating metabolites that have a greater biological activity than CB itself. The excretion of free ferulic acid and other related biotransformation products such as acetovanillone, (3- or 4-) hydroxybenzenepropanoic acid, and 4-vinylguaiacol raises the possibility that CB ingestion may inhibit reproduction similar to ferulic acid or 4-vinylguaiacol (but see Jakubas et al., in press b).

At this point, the mechanism by which Ruffed Grouse perceive that they have consumed enough CB is a matter of conjecture. We cannot say that rejection of CB treated food is necessarily a learned response due to post-ingestional effects, because naive grouse will avoid CB treated food when first exposed to it (Jakubas and Gullion 1990). Habituation to CB treated food was apparent in this study and has been noted for other birds (Jakubas and Mason 1991); therefore, it seems logical that wild grouse will eventually consume enough CB to eventually "learn" what its toxic effects are. Compounds exhibiting a toxic or emetic effect have been shown to be superior feeding deterrents over strictly unpalatable substances (Alcock 1970, Rogers 1974). The apparent emetic effect that CB had on one bird, at high CB concentrations, suggests that this compound produces some type post-ingestional malaise that may enhance its deterrent properties.

There is no direct evidence that Ruffed Grouse limit their CB intake (i.e., g kg^{-1} day^{-1}) due to toxicant related effects. However, toxicant related effects are strongly suggested by the consistent upper limit (i.e., ca. 2 g kg^{-1} day^{-1}) to CB intake observed for grouse in our feeding trials, for grouse in the field, and for Japanese Quail (toxicant related effects seen). In each case, maximum CB intake occurred at different CB concentrations (1.5% to 7.5% CB), indicating that the mechanisms causing food rejection were not solely dependent on CB concentration or palatability. Alternatively, a growing body of literature suggests that a number of plant secondary metabolites that are effective repellents do not exhibit any post-ingestional toxicity (Bernays 1991). Certainly there are many examples in nature of animals that defend themselves by appearance (i.e., Batesian mimicry), or objectional odors without truly being toxic or dangerous to their predators. Likewise, it would seem plausible that plants could defend themselves with compounds that had sensory characteristics that were extremely objectional to certain herbivores or mimicked other toxic compounds. However, for Ruffed Grouse, CB tolerance would appear to be dependent on a combination of the compound's sensory and toxic properties.

CB DETOXICATION

Coniferyl benzoate biotransformation in Ruffed Grouse appears to be very similar to the biotransformation of analogous cinnamyl compounds in mammals. The following biotransformation processes are based on mammalian metabolism of cinnamyl compounds (Scheline 1978), and are not meant to be conclusive but rather illustrative of the types of reactions involved (Fig. 3). Coniferyl alcohol and benzoic acid would likely result from the hydrolysis of coniferyl benzoate. Oxidation of coniferyl alcohol by oxidoreductases in the liver could yield

ferulic acid, while reduction of coniferyl alcohol could produce eugenol. Acetovanillone is likely a product from ferulic acid β-oxidation, and vanillin may be produced from the β-oxidation of eugenol. (3- or 4-) hydroxybenzenepropanoic acid is likely a product of double bond reduction and subsequent demethylation of ferulic acid by gut microflora (Scheline 1978:209). Acetovanillone could undergo demethylation by intestinal microflora to produce 4-hydroxyacetophenone. Finally, 4-vinylguaiacol may result from the metabolism of ferulic acid by intestinal microflora (Scheline 1978:48).

Ruffed Grouse appear to utilize all of the major conjugation pathways (glucuronic acid, ornithine, and sulfate) when detoxifying CB. Utilization of high capacity conjugation systems, such as ornithine and glucuronic acid conjugation, would seem to be a necessity, considering the large amount of CB Ruffed Grouse must detoxify when they feed on quaking aspen in the winter. The large amount of conjugated ornithine excreted when grouse feed on foods with phenolic based plant defenses, such as aspen buds, can result in biologically significant losses of nitrogen and energy (e.g., 5% of the daily metabolizable energy intake [Guglielmo 1993]). In addition, large ornithine losses may result in an arginine (the precursor of ornithine) deficiency (Nesheim and Garlich 1963). The relatively small increase in sulfate ester excretion that occurred when birds were fed the 6.5% CB diet was not unexpected. Sulfate conjugation is rate limited by the amount of endogenous inorganic sulfate available and is considered to be a low capacity, conjugation mechanism (Scheline 1978). Involvement of cytochrome P-450 enzymes in the detoxication of CB is not certain. The oxidation of coniferyl alcohol to ferulic acid could be accomplished by oxidoreductases in the liver (see Scheline 1978: 13). However, in some instances alcohol oxidation may involve cytochrome P-450 enzymes (Andrews and Snyder 1984:650). The production of 4-vinylguaiacol by bacterial decarboxylation of ferulic acid (Scheline 1968, 1978) may be important if significant quantities of 4-vinylguaiacol are reabsorbed. Orally administered 4-vinylguaiacol is known to interfere with reproductive functions in *Microtus montanus* (Berger et al. 1977).

Given the number of detoxication systems involved in metabolizing CB, and the potential for overloading these systems due to high CB intake, it may be interesting to determine if CB potentiates the toxicity of other secondary metabolites common in the diet of Ruffed Grouse. If CB does significantly decrease a bird's phenolic detoxication capacity, this raises the question of whether grouse can avoid the toxicant related effects of one phenolic compound by simply switching to another dietary item having a different suite of phenolic compounds. The toxic properties of the other phenolic compounds may be expressed at relatively low levels of intake because the bird's detoxication pathways have already been swamped by CB (or analogous compounds), prior to these compounds causing significant food avoidance.

CONCLUSIONS—CB TOLERANCE

Ruffed Grouse, if not given an alternative choice, will consume food having CB concentrations (i.e., 2.5–4.5% dry mass) well above the mean concentration of CB in aspen buds (i.e., 1% dry mass) that they select for food in the wild. This indicates that the rejection of high CB (2–7%) aspen buds in the wild is not strictly linked to the aversive properties of CB, which are concentration dependent. However, Ruffed Grouse do have the ability to detect small changes in CB concentrations. They appear to utilize their sensory abilities, along with post-ingestional effects, to limit their intake of CB to approximately 2 g kg^{-1} day^{-1}, possibly to protect themselves from the toxicant related effects of this compound. Possible effects of high CB consumption on wild Ruffed Grouse include: (1) negative nitrogen balance associated with ornithine and ammonium excretion (Jakubas et al., in press a), (2) decreased efficiency in energy utilization (Jakubas et al., in press a; Guglielmo 1993), and (3) a decreased capacity to maintain acid-base balance because of the production of acidic detoxication products. Whether Ruffed Grouse in the field limit their CB intake because of its toxicity, the dilution effect it has on the nutrient content of aspen buds, or a combination of palatability and toxicant related effects, the end result is that this compound appears to have deterrent properties that are sufficient to (1) restrict which quaking aspen grouse can feed on, and (2) limit the overall consumption of aspen flower buds when CB levels are high in the general population of aspen. A decrease in the suitability of aspen buds may force grouse to use alternative foods which they feed on (or utilize) less efficiently, thus raising

the bird's energetic costs and predation risks (Jakubas and Gullion 1991).

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