

- ed., p. 469, Academic Press, New York, 1958.
- (2) Am. Oil Chemists' Soc., "Official and Tentative Methods of Analysis," 2nd ed., Chicago, 1945-50.
 - (3) Apte, S. V., Venkatachalam, P. S., *Indian J. Med. Res.* **52**, 213 (1964).
 - (4) Baliga, B. P., Bayliss, M. E., Lyman, C. M., *Arch. Biochem. Biophys.* **84**, 1 (1959).
 - (5) Barrick, E. R., in "Proceedings of a Research Conference on Processing as Related to Cottonseed Meal Nutrition," p. 42, Southern Regional Laboratory, New Orleans, La., 1950.
 - (6) Bressani, R., Elías, L. G., Jarquín, R., Braham, J. E., *Food Technol.* **18**, 1599 (1964).
 - (7) Cabell, C. A., Earle, I. P., *J. Am. Oil Chemists' Soc.* **33**, 416 (1956).
 - (8) Clawson, A. J., Smith, F. H., Barrick, E. R., *J. Animal Sci.* **21**, 911 (1962).
 - (9) Clawson, A. J., Smith, F. H., Osborne, J. C., Barrick, E. R., *Ibid.*, **20**, 547 (1961).
 - (10) Conkerton, E. J., Frampton, V. L., *Arch. Biochem. Biophys.* **81**, 130 (1959).
 - (11) Fletcher, J. L., Barrentine, B. F., Dreesen, L. J., Hill, J. E., Shawver, C. B., *Poultry Sci.* **32**, 740 (1953).
 - (12) Gallup, W. D., *J. Biol. Chem.* **77**, 437 (1928).
 - (13) Grau, C. R., Zweigart, P. A., *Poultry Sci.* **34**, 724 (1955).
 - (14) Hale, F., Lyman, C. M., *J. Animal Sci.* **16**, 364 (1957).
 - (15) *Ibid.*, **20**, 734 (1961).
 - (16) *Ibid.*, **21**, 998 (1962).
 - (17) Hale, F., Lyman, C. M., Smith, H. A., "Use of Cottonseed Meal in Swine Rations," Texas Agr. Expt. Station Bull. **898** (1958).
 - (18) Heywang, B. W., *Poultry Sci.* **36**, 715 (1957).
 - (19) Heywang, B. W., Bird, H. R., *Ibid.*, **29**, 486 (1950).
 - (20) *Ibid.*, **34**, 1239 (1955).
 - (21) Hill, F. W., Totsuka, K., *Ibid.*, **43**, 362 (1964).
 - (22) King, W. H., Kuck, J. C., Frampton, V. L., *J. Am. Oil Chemists' Soc.* **38**, 19 (1961).
 - (23) Kornegay, E. T., Clawson, A. J., Smith, F. H., Barrick, E. R., *J. Animal Sci.* **20**, 597 (1961).
 - (24) Lyman, C. M., Baliga, B. P., Slay, M. W., *Arch. Biochem. Biophys.* **84**, 486 (1959).
 - (25) Martinez, W. H., Frampton, V. L., Cabell, C. A., *J. Agr. Food Chem.* **9**, 64 (1961).
 - (26) Miner, J. J., Clower, W. B., Noland, P. R., Stephenson, E. L., *J. Animal Sci.* **14**, 24 (1955).
 - (27) Narain, R., Lyman, C. M., Deyoe, C. W., Couch, J. R., *Poultry Sci.* **39**, 1556 (1960).
 - (28) Phelps, R. A., "Cottonseed Meal for Nonruminants," "Proceedings of the Semi-Annual Meeting of American Feed Manufacturers Association," Nov. 26-27, 1962.
 - (29) Smith, F. H., *J. Am. Oil Chemists' Soc.* **40**, 60 (1963).
 - (30) Smith, H. A., *Am. J. Pathol.* **33**, 353 (1957).
 - (31) Withers, W. A., Carruth, F. E., *J. Biol. Chem.* **32**, 245 (1917).

Received for review July 6, 1965. Accepted December 27, 1965. Presented in part at the Meetings of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., 1965. Investigation supported by a grant from the W. K. Kellogg Foundation, and a grant from UNICEF. INCAP Publication I-358.

RUMINANT USE OF LITTER

Nutrient Digestibility by Ruminants of Poultry Litter Containing Dried Citrus Pulp

C. B. AMMERMAN, P. W. WALDROUP, L. R. ARRINGTON, R. L. SHIRLEY, and R. H. HARMS

Department of Animal Science and Department of Poultry Science, Florida Agricultural Experiment Station, Gainesville, Fla.

Dried citrus pulp was used as the absorbent material for the droppings of broiler chicks and the digestibility of the nutrients in the resulting litter was determined with lambs. Both the nutrient digestibility and composition of the poultry litter were compared with those of the citrus pulp. On a per cent composition basis, nitrogen and ash of the combined droppings and citrus pulp were greater than in the original pulp. When compared with the citrus pulp diet, the poultry litter diet had a higher ($P < 0.01$) apparent digestion coefficient for crude protein and a lower ($P < 0.05$) digestibility of ether extract. Other nutrients were of similar digestibility for the two diets. The results suggest that dried citrus pulp and perhaps certain other feeds can be used as poultry litter and subsequently fed to ruminants.

THE value of poultry litter as a source of nutrients for plants has been known for many years, but its value as a source of nutrients for animals, particularly ruminants, has only recently been recognized. Eno (6) reviewed data relating the value of poultry litter to plant nutrition, and Chance (4) summarized research relating its value to ruminant nutrition. One of several factors influencing the nutritive value of poultry litter for ruminants is the type of absorbent material used. Generally, this material is of relatively low nutritive value and includes such materials as sawdust, wood shavings, rice hulls, sugarcane bagasse, oat hulls, peanut hulls, corncobs, and straw. The present study was designed to test the nutritive value of unsterilized poultry litter for ruminants

when dried citrus pulp, a feedstuff of relatively high nutritive value (2, 9), was used as the absorbent material.

Experimental

The poultry litter was obtained by using 50 pounds of air-dry, citrus pulp per pen of 20-day-old broiler chicks and feeding a practical broiler diet for 8 weeks. The litter was removed, dried to prevent spoilage, and stored for animal feeding.

A conventional study of digestibility and nitrogen balance was conducted using three yearling Florida native wethers averaging 45.1 kg. in body weight. They were used in a 3 × 3 Latin square design having been randomly assigned to treatment in period one. The preliminary feeding time

prior to fecal and urine collections was 21 days and the collection period was 7 days. The animals were placed in metabolism crates 2 days before starting collections.

The diets fed are shown in Table I. Either poultry litter (which included citrus pulp) or citrus pulp comprised 65% of two diets. A third diet, referred to as "basal mixture," contained the hay, corn meal, and soybean meal in a similar proportion to that of the other two diets and was used so that digestion coefficients could be calculated "by difference" for the poultry litter and the citrus pulp. The animals were fed 800 grams of feed per head daily in two equal feedings and this allowed either maintenance of body weight or slight gains by all lambs during the experiment. Water was provided *ad libitum*.

Table I. Compositions of Diets

Ingredients	Diets		
	Poultry litter	Citrus pulp	Basal mixture
Poultry litter	65.0
Dried citrus pulp	...	65.0	...
Bermuda grass hay (ground)	15.0	15.0	47.5
Corn meal	12.0	12.0	38.0
Soybean meal (50% protein)	3.0	3.0	9.5
Corn oil ^a	3.0	3.0	3.0
Salt, trace mineralized ^b	1.0	1.0	1.0
Defluorinated phosphate	1.0	1.0	1.0
Vitamins A, D, and E ^c	+	+	+
	100.0	100.0	100.0

^a Santoquin added at 0.0125% of total diet.

^b Carey Salt Co., Hutchinson, Kan. Listed minimum analysis (per cent): Fe, 0.27; Mn, 0.25; Cu, 0.033; Co, 0.01; Zn, 0.005; I, 0.007; and NaCl, 95.9.

^c 4400 I.U. vitamin A palmitate, 600 I.U. vitamin D₂, and 11 mg. DL-alpha-tocopherol added per kilogram of diet.

Table II. Nutrient Composition of Dried Citrus Pulp, Poultry Litter, and Mixed Diets^a

	(Per Cent)			
	Ash	Nitrogen	Ether Extract	Crude Fiber
Dried citrus pulp	5.5	1.18	3.9	11.4
Poultry litter	9.5	4.08	3.0	11.8
Mixed diets				
Dried citrus pulp ^b	6.1	1.34	5.8	13.3
Poultry litter ^c	8.9	3.18	4.6	13.3
Basal mixture	4.6	1.89	4.1	16.9

^a Expressed on moisture-free basis.

^b Containing 65% dried citrus pulp.

^c Containing 65% poultry litter.

Table III. Average Apparent Digestion Coefficients for Lambs Fed Dried Citrus Pulp, Poultry Litter, or Basal Mixture

	Organic Matter	Nitrogen	Ether Extract	Crude Fiber
Mixed diets				
Dried citrus pulp	75.8 ± 2.4 ^a	51.3 ± 8.3 ^c	85.2 ± 0.9 ^a	59.6 ± 2.2 ^a
Poultry litter	74.0 ± 0.2 ^a	79.1 ± 4.6 ^a	79.2 ± 1.3 ^{a,b}	56.5 ± 3.3 ^a
Basal mixture	61.8 ± 6.8 ^b	64.8 ± 7.1 ^b	74.4 ± 5.8 ^b	34.1 ± 8.9 ^b
Calculated by difference				
Dried citrus pulp	83.3 ± 1.8 ^a	41.1 ± 10.6 ^b	101.4 ± 5.4 ^a	79.7 ± 3.5 ^a
Poultry litter	80.7 ± 3.3 ^a	82.0 ± 5.2 ^a	85.6 ± 3.4 ^b	72.8 ± 10.4 ^a

Figures represent means with standard deviations. Means in same column within each set of data with different superscripts are significantly ($P < 0.05$) different according to Duncan's (5) multiple range test.

Table IV. Nitrogen Balance Data

Nitrogen	Diets		
	Poultry litter	Citrus pulp	Basal mixture
Intake, grams/day	21.90	8.90	13.03
In feces, grams/day	4.53 ± 0.74 ^a	4.30 ± 0.56 ^a	4.63 ± 1.22 ^a
Digested, grams/day	17.37 ± 2.19 ^a	4.60 ± 1.10 ^c	8.40 ± 0.44 ^b
In urine, grams/day	12.63 ± 2.25 ^a	3.07 ± 0.31 ^c	6.33 ± 0.41 ^b
Net retained, grams/day	4.74 ± 1.66 ^a	1.53 ± 1.03 ^b	2.07 ± 0.32 ^b
Net retained, %	21.64 ^a	17.19 ^b	15.89 ^b

Figures represent means with standard deviations. Means in same line with different superscripts are significantly ($P < 0.05$) different according to Duncan's (5) multiple range test.

All chemical determinations on the feeds, feces, and urine were made by AOAC (3) methods. The data were subjected to analysis of variance to facilitate the application of Duncan's (5) multiple range test as a measure of significance for mean differences.

Results and Discussion

The nutrient composition of the citrus

pulp, poultry litter, and resulting diets is shown in Table II. The poultry litter was higher in ash, considerably higher in nitrogen, similar in crude fiber, and lower in fat than the citrus pulp. These differences resulted in similar differences in the composition of the mixed diets containing either 65% poultry litter or dried citrus pulp. The poultry litter diet contained 19.9% crude protein

(N × 6.25) compared with 8.4% crude protein in the citrus pulp diet.

Average apparent digestion coefficients (Table III) for organic matter, nitrogen, ether extract, and crude fiber in the poultry litter diet were 74.0, 79.1, 79.2, and 56.5, respectively. When compared with the citrus pulp diet, the poultry litter diet had a significantly higher ($P < 0.01$) digestion coefficient for nitrogen and a significantly lower ($P < 0.05$) digestibility of ether extract. Apparent digestibility for the other nutrients was similar for the two diets. The coefficients of digestibility for the nutrients in either the citrus pulp portion of the diet or the poultry litter portion as calculated "by difference" emphasized, in particular, the greater digestibility of the nitrogen in the poultry litter. Total organic matter and crude fiber were similar in digestibility for the two ingredients, while ether extract from the citrus pulp was more digestible. The coefficients obtained for citrus pulp were somewhat lower for nitrogen and higher for ether extract and crude fiber than reported previously (7, 7).

The nitrogen deposited in the litter, primarily through the droppings, was well utilized by the animals (Table IV). Even though twice as much nitrogen was ingested daily from the poultry litter diet than from the citrus pulp diet, the amount of nitrogen excreted in the feces was essentially the same (4.53 vs. 4.30 grams). Since the protein requirement for the yearling wether is approximately 10% of the diet (8) and the poultry litter diet contained 19.9% crude protein, it is not surprising that the major portion of the apparently absorbed nitrogen from this diet was eliminated through the urine. In spite of the urinary loss, the average net nitrogen retained daily by the animals consuming the poultry litter diet was 4.74 compared with 1.53 grams ($P < 0.05$) for those animals consuming the citrus pulp diet.

Acknowledgment

The authors thank the Florida Citrus Processors Association in cooperation with the Florida Citrus Commission, for funds in support of this study; Dawes Laboratories, Inc., Chicago, Ill., for supplying vitamins A and D; and the Monsanto Chemical Co., St. Louis, Mo., for supplying Santoquin; and acknowledge the technical assistance of Nawal N. Masri and P. A. Hicks.

Literature Cited

- (1) Ammerman, C. B., Arrington, L. R., Florida, Univ. Agr. Expt. Sta., Gainesville, Proceedings of Florida Nutrition Conference, p. 20, 1961.
- (2) Ammerman, C. B., van Wallegghem, P. A., Palmer, A. Z., Carpenter, J. W., Hentges, J. F., Arrington, L. R., Florida, Univ. Agr. Expt. Sta., Gaines-

- ville, Animal Science, Mimeo. Rept. AN64-8 (1963).
- (3) Assoc. Offic. Agr. Chemists, Washington, D. C., "Official Methods of Analysis," 9th ed., 1960.
- (4) Chance, C. M., Maryland, Univ. Agr. Expt. Sta., College Park, Proceedings of Maryland Nutrition Con-

- ference, p. 8, 1965.
- (5) Duncan, D. B., *Biometrics* **11**, 1 (1955).
- (6) Eno, C. F., Florida, Univ. Agr. Expt. Sta., Circ. S-140 (1962).
- (7) Keener, H. A., Colovos, N. F., Eckberg, R. B., New Hampshire, Univ. Agr. Expt. Sta., Bull. 438 (1957).

- (8) National Research Council, Washington, D. C., Publ. 1193 (1964).
- (9) Peacock, F. N., Kirk, W. G., Florida, Univ. Agr. Expt. Sta., Bull. 616 (1959).

Received for review August 6, 1965. Accepted March 7, 1966. Florida Agricultural Experiment Stations, Journal Series No. 2183

CARBARYL METABOLITES

Metabolites of Carbaryl (1-Naphthyl Methylcarbamate) in Mammals and Enzymatic Systems for Their Formation

NORMAN C. LEELING¹ and JOHN E. CASIDA²

Department of Entomology, University of Wisconsin, Madison, Wis.

Ether-extractable carbaryl (1-naphthyl methylcarbamate) metabolites found in the urine of treated rabbits and formed by enzyme preparations from rat, mouse, and rabbit liver are tentatively identified as follows: 1-naphthyl *N*-hydroxymethylcarbamate; 4-hydroxy-1-naphthyl methylcarbamate; 5-hydroxy-1-naphthyl methylcarbamate; 5,6-dihydro-5,6-dihydroxy-1-naphthyl methylcarbamate; 1-hydroxy-5,6-dihydro-5,6-dihydroxynaphthalene; and 1-naphthol. Additional unidentified metabolites are also present in ether and butanol extracts of the urine. Each of the ether-extractable metabolites formed by liver enzymes is of reduced biological activity compared with carbaryl. Optimum conditions are given for metabolism of carbaryl by rat liver microsomes plus soluble fractions; inhibition of the metabolism system by five insecticide synergists is demonstrated. Hydroxylated carbaryl metabolites are probably conjugated, in part, as glucuronides and ethereal sulfates, based on *in vitro* studies with conjugation systems.

METABOLISM of carbaryl (1-naphthyl methylcarbamate) by mammals includes both hydrolytic and nonhydrolytic pathways. Plasma albumin acts as an enzyme in effecting carbaryl hydrolysis (5). Hydroxylation of the ring at the 4- and 5-positions and of the *N*-methyl group results from incubation of carbaryl with rat liver microsomes fortified with reduced nicotinamide-adenine dinucleotide phosphate (NADPH₂) (7, 8). An additional metabolite formed by microsomes *in vitro* and found in the milk and urine of a treated goat is assumed to be 3,4-dihydro-3,4-dihydroxy-1-naphthyl methylcarbamate based on analogy with the metabolic pathway for naphthalene (7). 1-Naphthol derived from carbaryl metabolism is assumed to be conjugated and excreted in the urine as 1-naphthyl glucuronide by humans, rats, and guinea pigs (7, 4, 25), and 1-naphthyl sulfate is known to be a carbaryl metabolite in cow urine (25). One half to two thirds of the radiocarbon from carbaryl-carbonyl-C¹⁴ administered to rats and a goat is elimi-

nated in the urine (7, 15), indicating that no more than half of the dose is hydrolyzed under these conditions. Several conjugates of carbaryl metabolites, as formed in isolated systems and as excreted in the urine of treated animals, were recently identified by Knaak *et al.* (14) in a report which appeared after the present investigation was accepted for publication.

The present investigation supplements the available information on the nature of carbaryl metabolites (7) and gives a more complete picture of the nonconjugated metabolites excreted in urine and, particularly, the enzymatic systems for their formation.

Methods and Materials

Chemicals and Apparatus. Carbaryl-naphthyl-1-C¹⁴ was synthesized by reaction of 1-naphthol-1-C¹⁴ (Nuclear-Chicago Corp., Des Plaines, Ill.) with a slight excess of methyl isocyanate in a sealed ampoule in the dark for 48 hours at 25° C. The excess methyl isocyanate was evaporated and the product was purified on a Florisil column (16). The radiochemical purity of each batch was greater than 99.9%, as determined by cochromatography with authentic carbaryl (analytical purity, Union Carbide Chemicals Co., New York, N. Y.) on Silica Gel G chromatoplates.

The specific activity of carbaryl-naphthyl-1-C¹⁴ was adjusted to 1.0 mc. per mmole (approximately 2500 c.p.m. per μ g.).

Carbaryl and its metabolites were resolved by chromatography, detected by radioautography or chromogenic agents, and quantitatively determined by scintillation counting. Florisil (60- to 100-mesh) for column chromatography was obtained from the Floridin Co., Tallahassee, Fla., and was used without activation (16). Thin-layer chromatography (TLC) utilized Silica Gel G or aluminum oxide G (Brinkman Instruments, Inc., Great Neck, N. Y.) chromatoplates. To detect fluorescent regions corresponding to certain carbaryl metabolites, the silica gel plates were examined under long-wavelength ultraviolet light (Model SL 3660 Minera-light, Ultra-Violet Products Inc., South Pasadena, Calif.). Carbaryl, 1-naphthol, and certain metabolites were detected by spraying the chromatoplate with 15% aqueous potassium hydroxide followed by 0.1% Gibbs' (*N*,2,6-trichloro-*p*-benzoquinoneimine) reagent (7, 16). Carbamates were detected by spraying with 1% ninhydrin in pyridine followed by a 30-minute period of heating at 100° C. (7); pyridine without ninhydrin was used as a control. Radioactive compounds on the thin-layer chromatograms were detected by radioautography using medical "no screen"-type x-ray film (Eastman Kodak Co., Rochester, N. Y.).

¹ Permanent address, Department of Entomology, Michigan State University, East Lansing, Mich.

² Permanent address, Division of Entomology and Acarology, University of California, Berkeley, Calif.