routine application because of the cost and storage factors. The clean-up procedure works well with both columns. The chromatograms presented are from typical analyses utilizing the pellicular columns.

Overall recovery of aflatoxins is 90% as determined from peanut samples spiked with known amounts of aflatoxin B_1. The minimum linear limits of the pellicular HPLC system are 20 ng of aflatoxin B_1, 20 ng of B_2, 30 ng of G_1, and 50 ng of G_2. For the 100-g sample, these limits, which convert to 12 ppb for aflatoxin B_1 and B_2, 18 ppb for aflatoxin G_1, and 30 ppb for aflatoxin G_2, may be further lowered by increasing the sample size or injection volume, provided there are no major interferences. The sample mass/extraction volume ratio must be retained for larger samples. A typical chromatogram obtained from a 100-g sample of naturally contaminated peanuts is presented in Figure 1.

If samples are known to have a high aflatoxin content/interference ratio, the alumina column may be omitted thereby reducing the sample preparation time from approximately 30 to 20 min. The chromatogram shown in Figure 2 was taken from a trace mineral study on aflatoxin production by NRRL no. 2999 in solid state fermentation. The 50-μL injection sample was taken directly from the 75-mL chloroform extract.

Presently the clean-up system is being enlarged to include multimycotoxin analysis which will undoubtedly require microparticulate columns and gradient elution.

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Amino Acid Composition of Dried Citrus Sludge and Its Potential as a Poultry Feedstuff

Three sludges, aerobic, anaerobic, and sun-dried, formed as citrus processing waste water by-products were analyzed for their protein and amino acid contents. Comparisons were made between amino acids present in these citrus sludges and those listed as nutrient requirements of broilers. The limiting amino acids were taken into account in considering the sludges as poultry feed supplements.

Citrus sludge is a decomposition product from plant materials discarded during the processing of citrus. An estimated 83 million lb/year of citrus sludge is produced in the United States (Ratcliff, 1976; U.S. Department of Agriculture, 1975). Managing large quantities of sludge generated by individual waste treatment systems is both expensive and technically difficult. Since excess sludge can adversely affect the efficiency of waste digesters it must be removed and discarded periodically. This sludge contains proteinaceous material formed during the digestion process involving microbial decomposition in both aerobic and anaerobic digesters. The use of citrus sludge as an animal feed supplement would eliminate a waste management problem as well as create a new product from the citrus industry. As part of investigations into the use of this by-product as a source of nutrition for animal feeds, the protein and amino acid contents need to be determined and compared with known nutrient requirements.

Some studies on the composition and use of citrus sludge as an animal feed have been reported. Dougherty and McNary (1958) analyzed a laboratory-created activated citrus sludge and speculated about its use as a poultry feed supplement. Jones et al. (1975) reported on the costs of drying a citrus sludge and suggested that dried sludge as an ingredient in a properly formulated poultry diet may be a viable means of reducing the cost of sludge handling. Damron et al. (1974, 1977) used a dried citrus sludge as a feed supplement in broiler feeding trials. None of these studies reported a detailed amino acid profile on the sludge used.

The current study reports the protein contents and amino acid profiles for three types of citrus sludges (by-products of commercial citrus processors) and compares the amino acid profiles with the nutritional requirements for poultry reported by the National Academy of Sciences (1971).

MATERIALS AND METHODS

Sludge samples were obtained from waste treatment facilities of commercial citrus processors. The following sludge samples were dried in a vacuum oven at 105 °C under 31 in. Hg for 2 days, 4 days, and 1 day, respectively.

Aerobic sludge (ca. 1% moisture) was prepared from the gelatinous material (ca. 90% moisture) separated with a "skimmer/clarifier" from the surface of an aerobic digester.

Anaerobic sludge was prepared from wet sludge (ca. 93% moisture) which had accumulated in the bottom of an anaerobic digester.

Sun-dried sludge was obtained from the waste spray field of a processor whose waste treatment system con-

Table I. Amino Acid and Protein Composition of Dried Citrus Sludges Compared with Amino Acid Requirements for Broilers (%)

Amino acids	Requirement[a] (pullets 0-6 wks)	Aerobic	Anaerobic	Sun
Arginine[b]	1.4	1.32	1.18	0.42
Glycine and/or	1.15	1.62	1.66	0.57
Serine		0.92	0.92	0.41
Histidine[b]	0.46	0.37	0.31	0.10
Isoleucine	0.86	1.13	1.02	0.42
Leucine	1.6	2.11	1.85	0.76
Lysine	1.25	1.25	1.30	0.38
Methionine[b] or	0.86	0.67	0.54	0.12
Cystine and	0.40	NC	NC	NC
Methionine	0.46	0.67	0.54	0.12
Phenylalanine or	1.5	1.44	1.24	0.43
Tyrosine and	0.7	0.80	0.88	0.27
Phenylalanine	0.8	1.44	1.24	0.43
Threonine	0.8	1.34	1.18	0.51
Tryptophan	0.23	0.36	0.39	0.10
Valine	1.0	1.73	1.51	0.57
Alanine	N[c]	2.61	2.34	0.92
Aspartic	N	2.74	2.65	0.99
Glutamic	N	3.61	3.10	1.08
Proline	N	1.10	1.03	0.45
Total available amino acids		25.45	23.8	8.65
Protein	23	42.01[d]	34.25[d]	20.94[d]
Ash		0.5	12.4	11.7

[a] From National Academy of Sciences (1971). [b] Limiting amino acid in aerobic and anaerobic sludge samples. [c] N = not required for broilers. [d] Kjeldahl nitrogen × 6.25.

sisted of a holding tank and spray field. Residue accumulated in lower, less-permeable areas of the spray field where the sun eventually dried it to ca. 10% moisture.

Protein Determinations. The crude protein content of each dried sludge sample was calculated by multiplying the Kjeldahl nitrogen, as determined by the method of Blaedel and Meloche (1963), by 6.25.

Amino acid analyses were carried out by WARF Institute, Inc. (Madison, Wis.) by use of an autoanalyzer incorporating the method of Spackman et al. (1958). Prior to being introduced into the autoanalyzer, samples (1 g) of each sludge were hydrolyzed by heating for 18 h with 40 mL of 3 N HCl in an autoclave at 120 °C. The mixture was then adjusted to the appropriate pH. For analysis of tryptophan a 1-g sample of each sludge was heated at 120 °C for 18 h with 20 mL of 5 N NaOH, followed by neutralization to pH 6.8. Methionine was determined by microbiological assay (Henderson and Snell, 1948).

Ash was determined by Standard Methods (1965).

RESULTS AND DISCUSSION

In considering the aerobic, anaerobic, and sun-dried citrus sludges for use in poultry feeds, the percentages of amino acids found in the sludges are compared in Table I with the nutrient requirements for broilers as reported by the National Academy of Sciences (1971). Laying and breeding hens require significantly less metabolizable energy, amino acids, and protein than do broilers. The authors site amino acid requirements for broilers because those requirements are the highest listed. Table I also lists the amino acids found in the dried sludges but not itemized in the nutrient requirements for broilers.

The differences in amino acid composition of the three dried citrus sludges indicate the variations in composition that were found in sludges obtained from different sources. Qualitatively, the three sludges were similar; quantitatively the dried aerobic and anaerobic sludges were similar to each other but quite different from the sun-dried sludge sample. Sun-dried sludge had a lower amino acid content than either aerobic or anaerobic sludges because it had not undergone aerobic or anaerobic digestion. Microbial digestion lowers the quantity of decomposable organic matter while building a quantity of protein. Since the sun-dried sludge was not exposed to digestive processes, it had a lower protein content and therefore was considered less desirable as a poultry feed supplement.

The percentages of required amino acids found in aerobic and anaerobic sludges in less than the recommended quantities necessary to maintain desired growth rates are indicated by footnote *b* in Table I. In these two sludges, arginine, histidine, and methionine–cystine are limiting amino acids with respect to amino acid requirements for broilers. [The term "required amino acids" (National Academy of Sciences, p 2, 1971) refers to "The minimum protein requirement for *maximum* growth" whereas the essential amino acids are those which cannot be synthesized by the animal rapidly enough to meet demands for *normal* growth and body maintenance.] The utilization of dried citrus sludge protein could be limited by insufficient quantities of required amino acids. In particular, the sulfur-containing amino acids, cystine and methionine, are very important nutrients for poultry. Since cystine can partly replace methionine in the diet of poultry, the two are combined in this table.

The crude protein content in two of the three sludge samples exceeds the required level of ca. 23% for optimum growth of chickens (Table I). The value for crude protein is higher than that for total available amino acids. Some possible reasons for this difference are the presence of nonprotein nitrogen which would contribute to the crude protein value and destruction of a portion of the amino acids during the hydrolysis step which would produce a lower value for available amino acids.

Previous work has shown that citrus sludge is, under certain conditions, a suitable feed supplement for broilers. Damron et al. (1977) used a dried citrus sludge similar to the aerobic and anaerobic sludges studied here as a supplement in broiler feed trials. They reported that weight gain in broilers fed diets which included from 0 to 20% dried citrus sludge was acceptable when diets contained 7.5% or less of this sludge. At 10% or more of the diet, weight gain in broilers decreased even though total

protein content of the sludge was near 40%.

The results of Damron et al. (1977) and the amino acid composition suggest that dried citrus sludges can be utilized as a protein source in broiler diets on a limited basis when supplemented with arginine, histidine, methionine, and cystine.

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Microsynthesis of [*ring*-^{14}C]Diuron (3-(3,4-Dichlorophenyl)-1,1-dimethylurea) from 3,4-[^{14}C]Dichloroaniline and Dimethylcarbamoyl Chloride

[*ring*-^{14}C]Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) was synthesized from 3,4-[^{14}C]dichloroaniline (DCA) and dimethylcarbamoyl chloride. After preparative thin-layer chromatography, radiochemical purity of [*ring*-^{14}C]diuron was greater than 99%. Radiochemical yield was 71% from 89 μCi or 3.2 mg of [^{14}C]DCA.

Ring-labeled substituted ureas are required for studying the environmental fate and bound residue formation of these herbicides (Hsu and Bartha, 1976). Multistep syntheses of [*ring*-^{14}C]diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and other substituted ureas have been published, but the methods require relatively large amounts of starting materials (Tanaka, 1970; Attar et al., 1973).

A single-step conversion of 3,4-dichloroaniline (DCA) to diuron was desired because [^{14}C]DCA was commercially available. [*ring*-^{14}C]Monuron (3-(4-chlorophenyl)-1,1-dimethylurea) has been synthesized from 4-[^{14}C]-chloroaniline and dimethylcarbamoyl chloride, but details of the synthesis were not published (Swanson and Swanson, 1968). This communication reports a one-step, microsynthesis of [*ring*-^{14}C]diuron from [^{14}C]DCA and dimethylcarbamoyl chloride.

MATERIALS AND METHODS

Unlabeled reference compounds were purchased or synthesized, and structures were confirmed by mass spectroscopy. Pyridine and acetonitrile were distilled. Acetonitrile was dried with phosphorus pentoxide before distillation.

Dimethylcarbamoyl chloride was purchased from Eastman. (Caution: Dimethylcarbamoyl chloride may be a carcinogen and causes skin, eye, and respiratory irritation (*Chem. Eng. News*, 1976; Van Duuren et al. 1972; Hey et al., 1974).

Labeled DCA (ring-U-^{14}C, 4.5 mCi/mmol) was purchased from California Bionuclear Corporation. Radioactivity was measured with a Model 2002 Packard Tri-Carb liquid scintillation spectrometer. Aquasol, from New England Nuclear, was added to solutions. Methanol and toluene scintillation solution were added to thin-layer chromatography segments. The toluene solution contained 5 g/L of 2,5-diphenyloxazole (PPO) and 0.3 g/L of 1,4-bis(2-(4-methyl-5-phenyloxazolyl))benzene (dimethyl-POPOP). Quenching was corrected by the channels ratio method.

In preliminary experiments to optimize reaction conditions, tracer [^{14}C]DCA was added to an amount of unlabeled DCA equivalent to the total amount of [^{14}C]-DCA. Reaction yields of the preliminary experiments and final reaction were measured by liquid scintillation and thin-layer chromatography.

[^{14}C]DCA had been stored in benzene to reduce radiolysis. Forty-five milliliters of the benzene solution was evaporated to about 0.1 mL. One milliliter of acetonitrile, 0.2 mL of pyridine, and 0.3 mL of dimethylcarbamoyl chloride were added and kept in the dark at 20–22 °C for 3 days.

The reaction mixture was diluted with 50 mL of 2 N hydrochloric acid. The product was extracted with three 10-mL portions of dichloromethane. The product was purified by double development with benzene–acetone (80:20, v/v) on a silica gel 60, F-254, 0.5 mm thick thin-layer plate from EM Reagents.

Identity and purity of ^{14}C compounds were confirmed by cochromatography on thin-layer sheets of silica gel F254, layer thickness 0.25 mm on aluminum support from EM Reagents. Developed strips were cut into 2-mm segments for zonal-profile analysis (Snyder, 1970). The developing solvents were benzene–acetone (2:1, v/v),