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Free Fatty Acids and Sterols in Swine Manure

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Free fatty acids and sterols were assessed in fresh manure and anaerobic lagoon sludge from swine production facilities in North Carolina. Eight free fatty acids and five sterols were identified and quantified in both manure and sludge samples. Compound identification was performed by gas chromatography/mass spectroscopy (GC-MS), and compound quantities were determined by gas chromatography after solid phase extraction with a 50:50 mixture of diethyl ether and hexane. The free fatty acids occurring in greatest abundance in both fresh manure and lagoon sludge were palmitic, oleic, and stearic. Free fatty acid content in fresh manure ranged from approximately 3 $\mu g\, g^{-1}$ dry weight (dw) to over 45 $\mu g\, g^{-1}$ dw. In lagoon sludge, free fatty acid content ranged from about 0.8 $\mu g\, g^{-1}$ dw to nearly 4 $\mu g\, g^{-1}$ dw. Coprostanol and epicoprostanol were the sterols in largest concentrations in fresh manure and lagoon sludge samples. Total sterol content ranged from approximately 0.5 $\mu g\, g^{-1}$ dw to around 11 $\mu g\, g^{-1}$ dw in fresh manure and from 3.5 $\mu g\, g^{-1}$ dw to almost 9 $\mu g\, g^{-1}$ dw in lagoon sludge. Fresh manure and lagoon sludge both had high levels of inorganic cations (e.g., Ca, Mg, Fe) capable of binding free fatty acids and forming insoluble complexes, thereby potentially reducing fatty acid biodegradation. In anaerobic lagoons, sterols are an organic fraction of sludge that are resistant to bacterial degradation. In the case of fresh manure, fatty acids could represent a potential source of energy via the manufacture of biodiesel fuel, if efficient means for their extraction and transesterification can be devised.

Key Words: Coprostanol; Epicoprostanol; Fecal; Phytosterol; Pig.

INTRODUCTION

Lipids (glycerides and sterols) are an essential component of swine diets and are often used as dietary supplements.[1] Hydrolysis of glycerides in an animal's digestive tract frees fatty acids. These fatty acids are absorbed as micelles via co-solubilization with bile acids in the small intestine.[2] However, intestinal

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absorption of fatty acids may be affected by supplemental minerals, added to the feed to increase food palatability, and naturally occurring minerals. Mineral elements in the feed, especially calcium and magnesium, can potentially reduce fatty acid solubility and intestinal absorption.[3,4] Therefore, calcium and magnesium fatty acid salts may be poorly available for catabolism by bacteria, both enterically and after excretion into the environment.

In addition to fatty acids, swine feces contain relatively large amounts of sterols that arise from the diet (i.e., sitosterol), intestinal sloughing of cells (i.e., cholesterol), and bacterial transformation of sterols (i.e., coprostanol) from these two sources.[5] Transformation of sterols from the diet occurs in the intestinal tract of most higher animals. For instance, coprostanol is the main sterol in human feces and has been used as a biomarker of fecal contamination in water, sediments, and soils.[6,7] In swine feces, coprostanol is also the sterol found in largest amounts, but in quantities that are 10 times less than in humans.[8] Swine manure therefore, may contain lipids that could represent both a potential energy source, such as methane generation[9] or manufacture of bio-diesel fuel,[10,11] and an organic fraction that is recalcitrant (i.e., not readily bioavailable). Our study was undertaken with the objective of identifying and quantifying lipids from fresh swine manure and anaerobic lagoon sludge sources under the assumption that the different sources could have varying degrees of degradation.

MATERIAL AND METHODS

Sample Collection Sites

Swine manure and anaerobic lagoon sludge samples were collected and analyzed in triplicate between February and April 2003 from four swine farm sites in North Carolina (Table 1). Site 1, located in Duplin County, had three

Table 1: Swine manure collection site locations in North Carolina, operation type, and samples origin and form.

Farm site	Location	Operation type	Samples	
			Origin	Form
1	Duplin Co.	Finishing	House effluent	Slurry
			Solid-liquid separator with flocculent	Dewatered solids
			Treatment lagoons	Lagoon sludge
2	NCSU Wake Co.	Finishing	Conveyor belt separator	Fresh feces
3	Onslow Co.	Finishing	Screen separator without flocculent	Dewatered solids
4	Onslow Co.	Culling	House effluent	Slurry
			Treatment lagoon	Lagoon sludge

finishing hog production units with 4,360 animals each. Each unit consisted of six houses and one anaerobic lagoon. Hog houses had pit recharge systems to handle the liquid manure. The typical management was to weekly drain the pit content by gravity to a lagoon, and then recharge the pit with new lagoon treated liquid.[12] As part of a project to demonstrate a new technology to replace treatment lagoons, one of the three production units was retrofitted with a waste management system that combined solid-liquid separation with removal of nitrogen and phosphorus from the liquid phase.[13,14] The liquid phase of the waste stream was recycled to clean the production facilities and/or used for crop irrigation. The solid phase of the waste stream was transported off-farm for compost stabilization. Thus, dewatered manure solids were obtained from a fully automated solid-liquid separation system (Super Soil Systems USA, Clinton, NC), where the liquid manure was reacted with flocculent polymer and separated with a self-cleaning rotating screen (0.25-mm openings). Subsequently, a small filter press dewatered the manure solids. The solid-liquid separation efficiency of the system was >95%. The liquid stream was further treated for nitrogen and phosphorus removal.[14] Therefore, the sources of samples for our study at this site were house effluents, lagoon sludge from three lagoons, and dewatered solids.

Site 2 was located at the North Carolina State University Lake Wheeler Road Laboratory in Raleigh (NCSU, Wake Co.). This was a pilot demonstration finishing facility that had a conveyor belt installed below the pens in which pigs were housed. Manure drops through the pen's slatted floor onto the belt. The belt was positioned at an angle so that liquid waste flowed into a gutter alongside the belt. Thus, the belt separated the liquid and solid manure (feces) as they were deposited onto the belt. Solid manure remaining on the belt was carried to the end of the pens, where it was collected.[15]

Site 3 had a solid-liquid separation module that treated effluents from a 3,500-hog finishing farm located in Onslow Co. This separation module consisted of a 7.3-m stationary screen (0.79-mm openings), with paddle conveyor (Brome Agri, Dunham, Canada). The solid-liquid system was operated without addition of flocculent and had separation efficiencies of <30%. Separated liquid was further treated in a constructed wetland.[16] Solids samples were collected after solid-liquid separation.

Site 4 was a 200-hog culling farm located in Onslow County, with traditional house pit recharge to collect manure and an anaerobic lagoon-spray field manure treatment system. Samples were collected from the house effluents and the anaerobic lagoon.

Anaerobic lagoon sludge samples in sites 1 and 4 were obtained by scooping samples from lagoon bottom sediments (Table 1). All manure samples from houses and solid separator units were considered fresh manure and were assumed to have negligible biodegradation. All manure and sludge samples were transported and kept cool on ice (<4°C) until frozen at −55°C prior

to lyophilization using a freeze drier (Virtis Research Equipment, Gardiner, NY).

Chemical Standards

Standards of lauric (dodecanoic acid), myristic (tetradecanoic acid), palmitoleic (*Z*-9-hexadecenoic acid), palmitic (hexadecanoic acid), linolenic (*Z,Z,Z*- 9,12,15-octatrienoic acid), linoleic (*Z,Z*-9,12-octadienoic acid), oleic (*Z*-9-octadecenoic acid), and stearic (octadecanoic acid) acids were purchased from Aldrich (St. Louis, MO), as were the sterols cholesterol (3β-cholest-en-3-ol), sitosterol (3β-stigmast-5-en-3-ol), and stigmasterol (stigmasta-5,22-dien-3β-ol). Standards of coprostanol (5β-cholestan-3β-ol) and epicoprostanol (9CI(3α,5β)-cholest-3-ol) were purchased from Steraloids (Newport, RI).

Free Fatty Acid and Sterol Analyses

Two hundred mg of the freeze-dried manure or sludge was suspended in 4.0 ml of 50:50 methanol:water containing 0.5% hydrochloric acid. After 10 min of sonication, the solution was extracted with 10 ml of a 50:50 mixture of diethyl ether and hexane, followed by 10 ml hexane. These solutions were combined, dried with anhydrous Na_2SO_4, and concentrated to dryness on a centrifugal concentrator with an explosion-proof vacuum pump at 40°C (Labconco Corp., Kansas City, MO). The residue was suspended in 950 μl of high-purity n-octane (Acros Organics, Fisher Scientific, Atlanta, GA) and derivatized with 50 μl *N,O-bis*-(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (Pierce Biotechnology Inc., Rockford, IL).

Compounds were quantified by injection onto a Varian model 3800 GC (Varian Associates, Walnut Grove, CA) equipped with a flame ionization detector (FID) and a 30 m $\times$ 0.32 mm DB-1 column with a film thickness of 0.25 μm (J&W Scientific, Folsom, CA). Injections were made in splitless mode for 1 min with an injector temperature of 260°C, column initial temperature of 60°C for 2 min, and column oven programming at 4°C min^{-1} to 100°C. The column oven was then programmed at 6°C min^{-1} to 260°C and held for 32 min. Other GC operating conditions were: FID 260°C, airflow rate 300 ml min^{-1}, hydrogen 30 ml min^{-1}, helium linear flow velocity 17 cm sec^{-1}, and an injector split ratio of 75:1. Response factors of individual compounds were calculated by injection of authentic samples of the individual compounds. Total content of free fatty acids and sterols was determined as the sum of the GC peak area.

Compound Identification

Gas chromatography/mass spectroscopy (GC-MS) was performed on a gas chromatograph equipped with a 30 m $\times$ 0.25 mm HP-5 column (Hewlett-Packard, Palo Alto, CA) interfaced to a Hewlett-Packard GCD Plus mass selective detector. Injections were made onto the GC in splitless mode for 1 min, and the mass ion detector used a scanning range of 40–450 amu. Chromatograph

operating conditions were: helium linear flow velocity 21 cm sec^{-1}, injector 220°C, column oven 80°C for 1 min then programmed at 3°C min^{-1} to 140°C, held for 2 min then programmed at 5°C min^{-1} to 240°C. Compounds were identified by comparisons of mass spectral data and retention time matches with those of authentic samples of compounds on the GC equipped with the DB-1 column.

Moisture, Carbon, Nitrogen, and Cations Analyses

Sample moisture was determined in fresh manure and sludge using a microwave moisture analyzer (Omnimark Instrument Corp., Tempe, AZ). Carbon and nitrogen contents were determined in freeze-dried manure and sludge samples by dry combustion with a LECO C/N-analyzer (Leco Corp., St. Joseph, MI). Manure and sludge samples were digested using a nitric acid with hydrogen peroxide block digester method prior to determination of inorganic cations by inductively coupled plasma spectrometry (ICP).[17]

RESULTS AND DISCUSSION

Fatty Acids and Sterols of Fresh Manure

Eight fatty acids were identified as major components of fresh swine manure (Table 2). These fatty acids are presumed to arise largely from the swine or

Table 2: Mean concentration of fatty acids and sterols identified in slurries and fresh swine manure samples.

	Compound Yield (ng g^{-1} dry weight)				
	House		Solid Separator		
Compound	Culling Onslow Co.	Finishing Duplin Co.	0.25-mm Screen Duplin Co.	0.79-mm Screen Onslow Co.	Belt NCSU Wake Co.
Fatty Acids					
Lauric	446	621	750	81	547
Myristic	1,217	1,495	1,102	91	779
Palmitoleic	307	218	162	11	70
Palmitic	11,289	7,817	9,068	1,221	11,814
Linolenic	1,169	422	1,008	197	2,037
Linoleic	2,411	498	1,237	373	3,044
Oleic	6,905	5,522	9,461	652	9,962
Stearic	15,244	6,543	9,594	1,195	19,891
Sterols					
Coprostanol	7,147	812	2,184	154	456
Epicoprostanol	1,612	603	904	146	132
Cholesterol	1,329	196	541	65	179
Sitosterol	254	136	761	38	107
Stigmasterol	697	156	566	98	278

their diet rather than from bacterial sources because bacteria are known to synthesize a number of branched-chain, hydroxy-, and odd-carbon numbered fatty acids.[18,19] These types of fatty acids, such as margaric (heptadecanoic), were present only in trace amounts (<30 $ng\,g^{-1}$ dw) relative to the identified fatty acids. In addition, two typical swine fatty acid compounds, arachidic (eicosanoic) and arachidonic (*Z,Z,Z,Z*-5,8,11,14-eicosatetraenoic) acids, were detected in trace amounts.

Palmitic, oleic, and stearic acids constituted the greatest yield of free fatty acids in all the fresh wastes (Table 2). Much lower levels of free fatty acids were isolated from samples of the solid separator in Onslow County finishing farm than from the screen separator in Duplin County or the belt separator at NCSU (Table 4). We observed that solids obtained from the separator in Onslow County contained appreciable amounts of hair, which is an indication of low solid-liquid separation efficiency (<30%). Thus, low separation efficiency was the cause of low fatty acids and sterols contents in separated solids produced at the Onslow County finishing farm.

The highest quantities of free fatty acids were extracted from fresh feces obtained from the NCSU facility. (Table 4). In this facility, a convexly sloped conveyor belt was used to separate urine from feces. We concluded that fatty acids partition more to solids and may have less bacterial degradation in a drier environment than in liquid manure samples.

Five sterols were identified in fresh manure samples (Table 2). Of these five sterols, stigmasterol and sitosterol are typical plant constituents used in the animal feed. The sterols coprostanol and epicoprostanol occurred in the largest concentrations (35% to 65%) among identified sterols of fresh manure samples. Total sterols in fresh manure ranged from about 11 $\mu g\,g^{-1}$ dw from the culling farm in Onslow County to approximately 0.5 $\mu g\,g^{-1}$ dw in dewatered solids from the finishing farm in Onslow County (Table 4). As previously encountered at the finishing farm in Onslow County, the lowest total sterol content found in solid samples was due to low separation efficiency.

Free Fatty Acids and Sterols in Lagoon Sludge

Fatty acid levels in anaerobic lagoon sludge were an order of magnitude less than in fresh manure (Table 3). In general, the fatty acid profiles in lagoon sludge samples were similar to those of fresh manure, in that palmitic and stearic acids were predominant. Lagoon 1 sludge, however, had significantly lower fatty acid concentrations than did the other three anaerobic lagoons (analysis of variance, $P < 0.05$) because it had been converted to a clean water reservoir. Due to the conversion, this lagoon stopped receiving raw house effluents three months prior to collection of sludge samples. Therefore, considerable reductions in fatty acids levels had occurred in lagoon 1 sludge. It is

Table 3: Mean concentrations of fatty acids and sterols identified in anaerobic swine lagoon sludge samples.

	Compound yield (ng g^{-1} dry weight)			
	Finishing Lagoon, Duplin Co.			Culling Lagoon Onslow Co.
Compound	1	2	3	
Fatty Acids				
Lauric	281	59	77	tr†
Myristic	114	279	238	81
Palmitoleic	33	32	74	103
Palmitic	78	839	778	1,202
Linolenic	49	59	38	129
Linoleic	24	106	106	245
Oleic	137	173	213	464
Stearic	60	552	683	1,777
Sterols				
Coprostanol	3,403	2,012	2,144	3,392
Epicoprostanol	2,348	1,365	870	2,761
Cholesterol	334	107	142	494
Sitosterol	480	57	198	1,225
Stigmasterol	197	98	185	990

†Yield less than 30 ng g^{-1} dry weight.

interesting to note that linolenic and linoleic, both polyunsaturated fatty acids susceptible to oxidation,[20] were present in sludge of all four lagoons. Although their levels were still an order of magnitude lower than in fresh manure samples, it is most probable that the lagoon's anaerobic environment preserved these two polyunsaturated fatty acids.

Several reports documented that sterols from sewage or animal waste sources, such as coprostanol, accumulate in the environment[8,21,22] and are known to have adverse reproductive effects on fish[23,24] and other aquatic animals.[25] Unsurprisingly, coprostanol and epicoprostanol levels were the highest (70% to 94% of identified sterols) in lagoon sludge samples (Table 3). The other three identified sterols (cholesterol, sitosterol, and stigmasterol) constituted the remaining 6% to 30% portion of the identified sterols. Relatively minor sterol components, such as cholestanol and cholesterone, were tentatively identified on the basis of mass spectral fragmentation data in both fresh feces and lagoon wastes. Some other sterols detected in trace amounts were not identified due to difficulty in mass spectral interpretation or lack of suitable reference compounds. Nevertheless, it seems that little further structural modification occurred to the sterols after excretion because the sterol composition of fresh feces and lagoon solids was qualitatively similar.

The level of sterols from swine lagoon sludge was similar in all lagoon samples (Table 4). Total sterol content ranged from about 9 $\mu g\, g^{-1}$ dw from the lagoon in Onslow County culling farm to about 3.6 $\mu g\, g^{-1}$ dw from lagoon 2 in

Table 4: Total concentration of fatty acids and sterols from fresh swine manure and lagoon sludge.

Source	(ng g^{-1} dry weight) Fatty acids	Sterols	FA/S*
Fresh Swine Manure			
Finishing house effluent, Duplin Co.	21,226 ± 2,536	1,814 ± 483	11.7
Culling house effluent, Onslow Co.	38,978 ± 3,498	11,136 ± 1,720	3.5
Screen separator, Duplin Co.	32,045 ± 1,366	4,773 ± 414	6.7
Screen separator, Onslow Co.	3,824 ± 134	493 ± 42	7.8
Belt separator NCSU, Wake Co.	47,730 ± 7,591	1,095 ± 137	43.6
Lagoon Sludge			
Finishing, Duplin 1	825 ± 178	6,763 ± 2,388	0.12
Finishing, Duplin 2	2,081 ± 90	3,637 ± 449	0.57
Finishing, Duplin 3	2,633 ± 593	3,500 ± 822	0.75
Culling, Onslow	3,992 ± 817	8,863 ± 513	0.45

*FA/S = fatty acid/sterols content ratio.
†Mean and standard error of the mean.

the Duplin County finishing farm. A large reduction in waste volume occurred during the anaerobic digestion process in the lagoon, causing an accumulation of sterols in lagoon sludge. Table 4 shows that lower fatty acid:sterol (FA/S) ratios were found in lagoon sludge (FA/S < 1.0) than in fresh manures (>3.5). Lower FA/S ratios indicated that sterols accumulated in the sludge because fatty acids were more available or structurally amenable to degradation by anaerobic lagoon bacteria than sterols.

Carbon, Nitrogen, and Cation Analyses

Fresh manure samples had combustible C contents averaging 32.6% in effluents from finishing and culling houses, while solid separators ranged from about 31% to over 40% C for manure collected from the belt separator at NCSU (Table 5). Lagoon sludge samples had an average combustible C of 29.3%, which was lower than the average of 35.6% found in fresh wastes. This undoubtedly is a result of carbon losses as CO_2 during the anaerobic lagoon digestion process. In addition, higher mean total P content in sludge samples (4.9%) than in fresh manure (2.6%) is an additional rough indicator of biodegradation of lagoon solids.

Both fresh manure and lagoon sludge had high concentrations of polyvalent cations (Ca, Mg, Zn, Cu, Mn, Fe, Al) capable of binding fatty acids and forming water-insoluble complexes. On the other hand, both fresh wastes and lagoon sludge samples had high levels of the monovalent cations K and Na that form soaps with fatty acids. Further research will be required to understand how the absolute levels of the various cations may affect fatty acid solubility and degradation in swine manure.

Table 5: Percentage moisture and elemental content on dry-weight basis of swine manure and lagoon sludge.

Source/Location	Moisture	C	N	P	K	Ca	Mg	Zn	Cu	Mn	Fe	Na	Al
						Fresh manure %							
Finishing house, Duplin Co.	96.0	31.8	3.50	2.50	6.30	1.69	0.94	0.12	0.09	0.04	0.38	0.92	0.07
Culling house, Onslow Co.	99.4	33.3	3.7	2.99	2.00	2.44	1.38	0.18	0.04	0.04	0.30	0.60	0.10
Screen separator, Duplin Co.	81.9	38.5	4.8	2.9	0.89	2.00	1.59	0.17	0.17	0.06	0.38	0.19	0.09
Screen separator, Onslow Co.	80.8	30.9	1.5	2.42	0.39	4.24	0.69	0.03	0.09	0.03	0.13	0.11	0.07
Belt separator, NCSU	60.0	43.3	3.7	2.31	1.13	2.96	0.70	0.13	0.15	0.04	0.15	0.51	0.11
						Lagoon sludge %							
Duplin Co. 1	91.4	26.0	4.5	5.56	1.54	4.96	2.70	0.28	0.10	0.09	0.59	0.33	0.59
Duplin Co. 2	98.5	34.3	5.0	4.39	2.20	3.45	2.13	0.22	0.12	0.06	0.39	0.49	0.33
Duplin Co. 3	99.5	29.4	5.2	5.54	3.57	3.89	2.56	0.27	0.09	0.09	0.48	1.70	0.05
Onslow Co.	96.0	27.4	3.7	4.27	9.91	5.40	1.58	0.24	0.31	0.04	0.29	0.52	0.09

Energy Production

Swine manure is a biomass source that has the potential to be converted into energy through biological and physicochemical processes such as anaerobic digestion, thermochemical conversion, or aerobic thermophilic digestion.[26,27,28] As an alternative, the lipids in swine manure could be used for biodiesel production. However, excessive water contents in feedstocks are a major problem in biodiesel production.[10] For instance, the screen separator at the finishing farm in Duplin County produced solids with 81.9% moisture, while the NCSU belt separator produced solids with much lower moisture content (60%). The belt separation technology could be more attractive for obtaining manure solids for biodiesel production. Thus, the economic feasibility of animal manure as feedstock for biodiesel production will depend on having efficient processes for both removal of moisture from manure and transesterification of free fatty acids.

CONCLUSION

In summary, both fresh swine wastes and anaerobic lagoon sludge contain substantial levels of free fatty acids and sterols. The free fatty acids occurring in greatest abundance in both fresh manure and lagoon sludge were palmitic, oleic, and stearic. Identified free fatty acid content in fresh manure ranged from approximately 3 $\mu g\,g^{-1}$ dw to over 45 $\mu g\,g^{-1}$ dw. In lagoon sludge, free fatty acid content ranged from about 0.8 $\mu g\,g^{-1}$ dw to nearly 4 $\mu g\,g^{-1}$ dw. Coprostanol and epicoprostanol were the sterols in largest concentrations in fresh manure and lagoon sludge samples. Total sterol content ranged from approximately 0.5 $\mu g\,g^{-1}$ dw to around 11 $\mu g\,g^{-1}$ dw in fresh manure and from 3.5 $\mu g\,g^{-1}$ dw to almost 9 $\mu g\,g^{-1}$ dw in lagoon sludge. Fresh manure and lagoon sludge both had high levels of inorganic cations (e.g., Ca, Mg, Fe) capable of binding free fatty acids and forming insoluble complexes, thereby reducing fatty acid biodegradation. In anaerobic lagoons, sterols are an organic fraction of sludge that are resistant to bacterial degradation. Lower fatty acid:sterol (FA/S) ratios in lagoon sludge (FA/S < 1.0) than in fresh manure (>3.5) indicated that sterols accumulated in lagoon sludge because fatty acids were more available or structurally amenable to degradation by anaerobic lagoon bacteria than sterols. In the case of fresh manure, fatty acids could represent a potential source of energy via the manufacture of biodiesel fuel, if efficient means for their extraction and transesterification can be devised.

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