PHYTOTOXICITY OF FATTY ACIDS PRESENT IN DAIRY AND HOG MANURE

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INTRODUCTION

Application of animal waste on farm land is currently considered as a safe method to dispose the excreta of farm animals because it provides an easy outlet to alleviate environmental dangers associated with farm animal excreta. It is generally believed by agricultural professionals that cattle, hog, and chicken waste is a valuable resource of plant nutrients. Thus land application of waste converts waste into a valuable resource by reducing the cost of fertilizers. This belief is based on concrete data collected in widely varying locations showing that farm animal excreta has the same macrocomponents, nitrogen, phosphorus, and potassium (NPK) as a traditional commercial fertilizer. The *Proceedings of the International Symposium on Agricultural Food Processing Waste* (ASAE 1990) may be referred to for most recent reviews on the subject. In most studies published during the last thirty years, farm animal waste is viewed as a good source of NPK (McCaskey et al. 1990). Little attention has been given to the minor chemical components present in the contents of excreta. The significance of minor chemical components of animal waste has been overshadowed by the desired results of the land application of farm animal waste. Information about minor chemical components present in the contents of farm animal excreta is not well documented (Marambe and Ando 1992). Minor chemical compounds of animal waste include both inorganics and organics in a wide range: from arsenic to zinc in inorganics (Sutton et al. 1990) and from an open chain to a closed chain compounds in organics (Marambe and Ando 1992). This is certainly no cause for complacency because there have been frequent errors in the past and there is still much that we do not understand (Legg 1990). Feeding of poultry excreta to ruminants was once considered safe but later was realized as a mistake and was banned in the United Kingdom in 1988 (Legg 1990). There have been many incidences of soil contamination, some from animal excreta, and whereas soil might once have been considered to have infinite buffering capacity, that is no longer the case (Legg 1990). Therefore, the crux of the whole environmental issue related with land disposal of animal waste lies in understanding the whole system. NPK present in the content of animal wastes do not comprise the whole system. Information about the minor chemical content of animal waste is scarce. Some classes of compounds which are suspected to be present in animal waste are known to be phytotoxic (Garraway Ramirez 1982; Van Sumere et al. 1973; Marambe and Ando 1992). Some animal waste compost strongly inhibits the germination of crop seeds through reduction in rate of physiological processes such as the breakdown of starch by alpha-amylase (Marambe et al. 1992) and the production of adenosine triphosphate (ATP) (Marambe and Ando 1992) in seeds. Identification and mode of action of such compounds present in the animal waste is important because agricultural practices which are considered environmentally safe today may become a hazard to agricultural crops sometimes in the future. The germinating inhibiting activity of fatty acids from plants (Butler et al. 1976; Iwanani and Iwadare 1978; Stewart and Berrie 1979; Alsasdawi et al. 1983; and Cocucci et al. 1989) and animal (Osada et al. 1990) sources have been reported. Further information is required to elucidate the inhibitory effects of fatty acid on germintation of seeds. Therefore the objectives of this study were to identify fatty acid present in the content of dairy and hog manures and to investigate their biological effects on germination of sorgham seeds.

MATERIALS AND METHODS

Hog and dairy manure samples were collected from Mississippi Agricultural and Forestry Experimental Station (MAFES-Alcorn Branch). All samples were fresh in a semi solid state. The samples were oven dried at 60oc and ground to pass through 200 mesh screen. Fatty acids were extracted with absolute methanol. Ten grams of ground manure samples were mixed with 100 ml of methanol. The extract was filtered and residue was further subjected to two more extraction with 100 ml of absolute methanol. The filterate was dried under reduced pressure. The dried extract was dissolved in 10 ml of chloroform. The aliquot was fractionated by elution through a column of silica gel. The column size was 15cms x 1 cm dia. Five formulations of mobile phases were used for elution of fatty acids: Chloroform; three mixtures of methanol. Chlorform and acetic acid 1.99:05; 5:95:05; and 20:80:0/5 v/v/v and a mixture of methanol and acetic acid 100:0.5 v/v). All five fractions of hog and dairy manure were dried with the use of rotary evaporator.

Six fatty acid were the subjects of this study: two short chain acids, two medium chain acids, and two long chain acids. Short chain acids were acetic and butyric acid. Medium chain acids were caproic and caprylic acids. Long chain acids were palmitic and linoleic acids. The quantitative analysis of fatty acids were conducted using a gas chromatograph (SRI-Model 8610). Flame ionization mode was used for detection and quantitation. Two capillary columns were used: DB-FFAP of J. & W. Scientific for short chain acids and DB 225 of J. & W. Scientific for medium and long chain acid. The direct injection method was used for quantitation of short chain fatty acids. Medium and long chain acids were derivatized to form esters. A flame ionization detector was used in both analyses. Hydrogen was used as the carrier gas. Both chromatographic runs were isothermic at 250oC. Pure acids supplied by Sigma Chemical Company were used as standards. Sorghum seeds from a commercial source were used in the bioassay experiments. Commercially available fatty acids were also used as control in bioassay experiments. In treatments, extracts of hog and dairy manures were used. These fatty acids were processed by the same methods as manure samples. Manure samples and commercially available fatty acid were dissolved in a methanol/water (1:9 v/v) matrix. All bioassay trial had five replicates. Three tests were included in bioassay trials: germination, imbibition, and a-amlylase activity.

The germination trials were conducted in petri dishes. One gram of sorgham seeds was soaked in 3 ml of test or standard solution of fatty acids for 4 hours at 28oC + 1. The seeds were transferred to petri dishes containing two discs of #2 filter paper moistened with 3 ml of respective solution. The petri dishes were incubated at 28oC for 48 hours. Seeds with prominent radicle growth were considered to have germinated. The imbibition trial was based on the total uptake of water by the seeds. It was a comparison of two sets of seeds: viable and non-viable. The batch of non-viable seeds were generated with the exposure of seeds to 600C for 36 hours under humid conditions according to the method of Marambe and Ando (1993). The water uptake by non-viable seed represented total physical uptake of water, while the water soaked by viable seed was physiological and physical uptake. The difference in two rates of uptake of water was physiologically mediated.

Alpha - amylase activity of seeds determined as per procedure described by Marambe et al. 1992. The seeds were soaked in hog manure or standard fatty acids solution. Crude extracts of amylase were obtained in ice cold buffer by crushing the seeds with mortar and pestle in succinate buffer. Alpha amylase activity was recorded as ug of starch degraded by one ml of extact in 30 minutes at 30 C. The duration of imbibition of seeds for physiological water uptake test and a-amylase activity was 24 + 1 hours.

RESULTS AND DISCUSSION

All six targeted fatty acids were found in the content of dairy and hog manures. The fatty acid content was more than double in the hog manure as compared to dairy manure, being 8.5mg/g of dry weight in hog manure and 3.53mg/g of dry weight in dairy manure (Table 1). A similiar pattern was observed in the content of palmatic acid which constituted approximately 1/4 the bulk of six fatty acids (Table 1). A very interesting correlation was noticed in the number of carbon atoms present in fatty acid and concentration of respective fatty acid in both manures: the concentration of all fatty acid proportionally increased with the number of carbon atoms present in the fatty acids except for linoleic acid (Table 1).

Peaks of medium and long chain fatty acids were associated with more than one secondary peak. The number of secondary peaks increased with the number of carbon atoms present in the chain (Figure 1). It is suspected that secondary peak and/or peaks associated with the medium and long chain fatty acids might be of the isomers of respective fatty acid. This assumption was further supported by the patterns of secondary peaks. The retention time for n-butyric was 9.2 minutes. Yet another peak at 8.9 minutes was confirmed as isobutyric acid. Two similiar peaks pattern was also found with caproic acid. It was assumed that the secondary peak associated with caproic acid's peak may also be an isomer of caproic acid. No attempt was made to identify these peaks because they were not included in the objective of this study. However, it implies that the effect expressed in the physiological tests of seed might not be attributed only to the six fatty acids selected for this study but possibly, in part, to other unidentified fatty acids. There was evidence of other fatty acids and also isomers of the six fatty acids.

It has been mentioned elsewhere that five fractions of isolates of fatty acids were collected. All of the fatty acids were detected in the first fraction only. No significant amount of fatty acid was detected in practice 2, 3, 4 and 5; therefore, for physiological studies, only the first fraction was used and the remaining were discarded. Germination inhibitory effect trials, a-amalyse activity test, and the physiological water uptake experiment had two controls: water and methanol (9:1v/v) (control I), 40mg/liter-1 solution of six fatty acid (control II) and two treatments fraction I of dairy and hog manures extracts which was equivant to 40mg of total fatty acids in the content of the respective fraction. Total fatty acid content of hog and dairy manure was known after gas chromatographic analysis (Table 1). The rate of germination of sorgham

was significantly reduced with the exposure to solution of all six fatty acids (Table 2). The rate of reduction was least in short chain fatty acids and was highest in long chain fatty acids. The rate of reduction increased with the number of carbon atoms present in the chains of the fatty acids. The only exception in this regard was linoleic acid which has 18 carbon atoms. Its rate of reduction in germination was 17 percent as compared to the rate of reduction in palmitic acid which was higher than linoleic acid: it was almost twice in palmatic acid than that of linoloic acid. These results support the results of Bulter et al. (1976) and Cocucci et al. (1989) whose work was related with the short chain fatty acids. According to the results, the exposure to fatty acids was deterimental to the germination of sorghum seeds and the severity of effect increased with the increase in the length of carbon atoms present in the chain of fatty acids. This assumption is further confirmed by the rate of reduction in germination of seeds exposed to hog and dairy manures. Hog manure had higher levels of long chain fatty acid than dairy manure so exhibited a more severe effect on sorghum seed germination. Germination is a process of reactivition of the metabolic machinery of the seed and the emergence of the radicle. In the metabolic machinery imbibition is the first step. As is evident from the result of the physiological water uptake experiment, the germination processes reacted to the exposure of fatty acid at the very beginning of the processes. The reduction in water uptake had the similiar pattern as the emergence of the radicle. Alpha-amlyse activity is a process between imbibition and emergence of the radicle. Therefore, the pattern of a-amylase activity was in correlation with the other two activities (Table 2).

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Table 1. **Composition of Six Fatty Acids Present in the Content of Hog and Dairy Manures.**

Fatty Acids	Hog Manure	Dairy Manure
Acetic Acid (2)b	0.11a (0.003)c	0.03 (0.001)
n-Butyric Acid (4)	0.23 (0.009)	0.02 (0.003)
n-Caproic Acid (6)	1.82 (0.01)	0.92 (0.003)
n-Caprylic Acid (8)	1.67 (0.12)	0.65 (0.008)
Palmitic Acid (16)	2.32 (0.13)	1.10 (0.003)
Dinoleic Acid (18)	2.10 (0.13)	0.81 (0.02)
Total	8.25	3.53

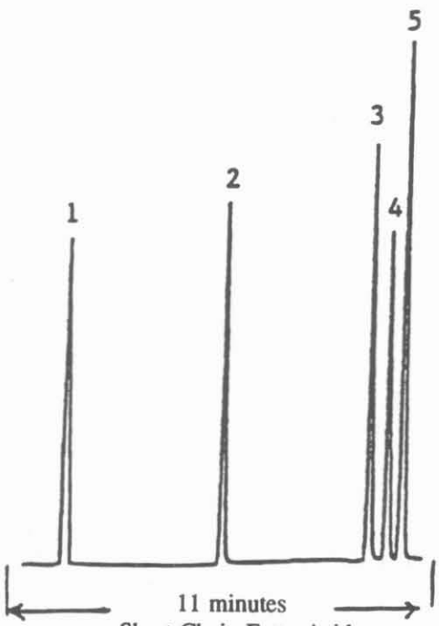
a = mg/g of dry weight of manure
b = number of carbon atoms
c = standard deviation

Table 2. **Effect of Six Fatty Acids and Extracts of Hog and Dairy Manures in % on Germination Rate, a-Amylase Activity and Physiological Water Uptake of Sorgham Seeds.**

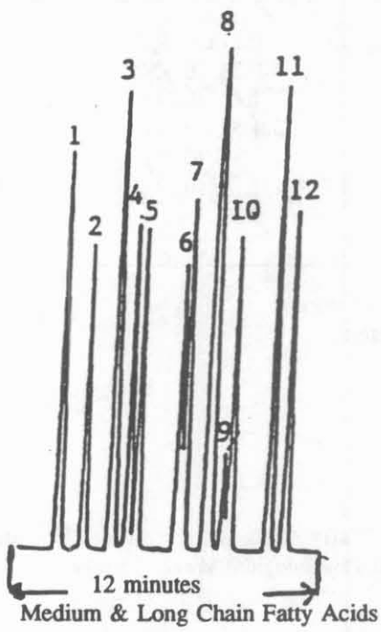
Treatment	Germination Rate	a-amylase activity	Physiological Water Uptake
Control (Matrix)	100	100	100
Acetic Acid	94(10)	98(3)	91(6)
Butyric Acid	91(15)	96(7)	93(6)
Caproic Acid	89(6)	91(8)	88(7)
Caprylic Acid	88(8)	83(12)	86(10)
Palmitic Acid	68(3)	69(15)	57(7)
Linoleic Acid	83(7)	58(7)	57(7)
Hog Manure Extracts	63(4)	52(9)	68(10)
Dairy Manure Extract	73(12)	68(12)	76(11)

Numbers in parentheses indicate S.D.

Figure 1. **Chromatograms of short, medium and long chain fatty acids showing secondary peaks associated with butyric, caproic, caprylic, palmitic and linoleic acids present in the content of hog manure.**



1. acetic acid
2. unknown
3. butyric acid
4. isobutyric acid
5. unknown



1. caproic acid
3. caprylic acid
8. palmitic acid
11. linoleic acid

2, 4, 5, 6, 7, 10, 12 unknown but suspected to be isomers of respective fatty acid