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Toxicological Aspects of Activated Sludge Feeding

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Discarding activated sludge, produced in the process of municipal waste water treatment, represents a potential environmental hazard. Much research is currently underway worldwide to evaluate the advantages as well as the deleterious effects of municipal sewage sludge as a food ingredient and as a soil amendment in agriculture. The use of activated sludge (about 30% protein) as an animal feed ingredient has been the subject of research for many years, and several trials have shown that the sludge supports normal growth and development of farm animals and poultry (Pillai et al., 1967; Damron et al., 1982; Lipstein et al., 1982; Beszedits and Lugowski, 1983). One of the serious objections to the use of sludge in feed is due to the possible presence of heavy metals, pesticides and other potentially toxic chemicals (Babish et al., 1982). A survey showed that local sludge contains about 29% ash. Trials with activated sludge as a feed ingredient for broiler-type chicks showed that most of the minerals present in the sludge are excreted, but iron and zinc are accumulated to a slight extent in the chick organs (Nachtomi et al., 1984).

The objective of this study was to estimate the possible effect of feeding local activated sludge on the metabolites and enzyme activities responsible for the detoxification mechanisms in Leghorn chicks.

MATERIALS AND METHODS

The first experiment was carried with 36 male Leghorn chicks, 1100-1300 g each, in a short-term program of feeding activated sludge. The chicks were divided into three groups of 12 and starved for 48 h. They were then fed a control diet, or a diet containing 20% or 40%

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activated sludge, for 1.5 h. After 2, 4 and 24 h, four chicks of each group were sacrificed and their livers and kidneys were removed for preparation of microsomes and cytosol, for metabolite and enzyme analyses. Cytochrome P-450 was determined in the microsome by the method of Omura and Sato (1964); glutathione S-transferase (GT) activity was measured in cytosol (Habig et al., 1974), using 1-chloro-2,4-dinitrobenzene (CDNB) or 3,4- dichloro-nitrobenzene (DCNB) as substrate. The increase in absorption at 340 nm or at 345 nm was measured with a Varian DMS 90 recording spectrophotometer at 25°C. Glutathione (GSH) was determined in homogenate by the method of Ellman (1959); protein was tested by the method of nesslerization of digested samples as modified by Johnson (1941); and catalase (CAT) was determined after Hugo (1974).

In the second experiment, 24 male Leghorn chicks were divided into three groups of eight, and fed a control diet, a 20% sludge-containing diet, or a control diet plus zinc carbonate (376 mg/kg) and ferrous sulfate (1350 mg/kg), calculated to supply the elements in amounts equivalent to those in the sludge diet. The chicks were killed after one month, tissues were excised, and samples taken for mineral analysis by an atomic absorption spectrometer, Perkin Elmer Model 2380. The remaining tissues were processed as before.

RESULTS AND DISCUSSION

A 48-h period of fasting increased the sensitivity of chicks to the sludge diet. Two h after feeding an activated sludge diet to chicks, the glutathione concentration dropped in the liver and kidneys, with a significant ($P<.05$) depression in the 40% sludge fed group (Table 1). The glutathione rose significantly ($P<.05$) in the kidney after 4 h. This is a typical observation after cavaging toxic substances to rats and chicks (Nachtomi et al., 1968). Fasting prevents a rise in glutathione concentration to a maximum (Jaeger et al., 1973), and is known to enhance the lethality and the hepatotoxicity of various chemicals in animals. The activity of GT was enhanced ($P<.05$) in the liver and kidney of the sludge-fed group when examined with CDNB at 4 h. After 24 h the activity of GT declined to control level in the 20% sludge-fed group, and significantly ($P<.05$) below control level in the liver of the 40% sludge-fed group. Glutathione conjugates with many compounds with an electrophilic center, in the presence of the family of phase II enzymes. GT was concentrated in the cytosol and was responsible for the detoxification mechanism (Habig et al., 1974).

Microsomal cytochrome P-450 (phase I enzymes) did not differ from the control in the liver of the experimental groups (0.32±0.04 nmol/mg protein) at 2 and 4 h after feeding, but 24 h after feeding, a significant rise in concentration occurred: 0.50±0.05 and 0.68±0.6 nmol/mg protein, respectively, in the 20% and 40% sludge-fed groups. It was reported that glutathione S-transferase and cytochrome P-448 can be induced differently by 3-methylcholanthrene (Pickett et al., 1982).

Experiment 2 was conducted to determine whether minerals added to the diet at the levels found in sludge, are responsible for the disturbances in metabolite level and enzyme activity noted in the first experiment. Feeding for one month a control diet, a 20%

Table 1. Glutathione and glutathione S-transferase in the liver and kidney of chicks fed a control or sludge-containing diet[a]

	Groups		
	Control	Sludge 20%	Sludge 40%
		Liver	
Time (2h)			
GSH (µmol/g)	4.0±0.2[b]	3.6±0.3	3.2±0.1[c]
GT[d] (nmol/min/mg prot)	211±25	220±11	236±22
Time (4h)			
GSH (µmol/g)	4.8±0.2	4.8±0.1	4.8±0.4
GT (nmol/min/mg prot)	297±19	465±21[c]	452±60[c]
Time (24h)			
GSH (µmol/g)	3.3±.02	3.2±0.1	3.5±0.2
GT (nmol/min/mg prot)	254±27	253±35	180±8[c]
		Kidney	
Time (2h)			
GSH (µmol/g)	3.6±0.1	3.4±0.1	2.9±0.1[c]
GT (nmol/min/mg prot)	226±11	217±10	250±13
Time (4h)			
GSH (µmol/g)	4.5±0.1	5.0±0.2	5.2±0.1[c]
GT (nmol/min/mg prot)	294±16	363±13[c]	361±15[c]
Time (24h)			
GSH (µmol/g)	3.7±0.2	3.9±0.1	4.1±0.2
GT (nmol/min/mg prot)	372±11	295±12	312±11

[a] Chicks were starved for 48 h before being fed for 90 min, and killed at different times thereafter.
[b] Values are means ± SE of four chicks.
[c] Significantly different (P<.05)from the control.
[d] 1-chloro-2,4-dinitrobenzene was used as substrate.

Table 2. Body and organ weights, glutathione level, and enzyme activities of Leghorn chicks fed the experimental diets

Parameters	Groups		
	Control	Sludge(20%)	Fe+Zn (1350+376ppm)
Body Weight (g)	1025±53[a]	1033±47	1001±78
Liver " (g%)	2.33±0.3	2.63±0.2	2.3 ±0.2
Kidney " "	0.86±0.1	0.95±0.2	0.86±0.2
Liver homogenate			
Catalase (unit)[b]	50.0±7.5	84.1±6.2[c]	49.8±5
Glutathione (µmole/g)	4.7±0.4	5.4±0.5	5.1±0.5
Microsome			
Cytochrome P-450 (µmol/mg pro)	0.39±0.07	0.67±0.08[c]	0.39±0.06
Cytosol			
GT(CDNB)[d]	224±32	401±45[c]	360±44
" (DCNB)[e]	18±4.6	23±4.5	24±3.3

[a] Values are means ± S.E. of eight chicks.
[b] µmol of H_2O_2 utilized per min per mg protein.
[c] Significantly ($P<.01$) different from the control.
[d] nmol of 1-chloro-2,4-dinitrobenzene (CDNB) utilized per minute per mg protein.
[e] nmol of 3,4-dichloronitrobenzene (DCNB) decomposed per minute per mg protein.

Table 3. The mineral concentration (ppm/dry wt.) in the liver and kidney of chicks

Groups	Liver		Kidney	
	Fe	Zn	Fe	Zn
Control	465±20[a]	95±5	230±12	89±5
Sludge 20%	720±31[c]	91±4	275±15	93±6
Minerals[b]	880±33[c]	89±6	310±14	93±5

[a] Values are means ± S.E. of eight chicks.
[b] Fe (1350 ppm) and Zn (376 ppm) added to the diet.
[c] Significantly ($P<.05$) different from the control.

sludge-containing diet, or a diet incorporating iron and zinc in amounts equivalent to those in sludge, did not affect body weight (Table 2). A slight increase was noticed in liver and kidney weights in 20% sludge-fed groups. In liver homogenate a significant ($P<.01$)

increase of catalase activity was observed in the sludge-fed group. Catalase is the enzyme responsible for destroying H_2O_2 formed in oxidative stress and in the free radicals scavenging process. The concentration of microsomal cytochrome P-450 in the sludge-fed group was significantly ($P<.01$) augmented. Microsomal cytochrome P-450 is part of the mixed function oxidase system responsible for oxidative metabolism of hydrophobic compounds such as steroids, insecticides and hydrocarbons (Conney, 1967). Glutathione concentration rose only slightly in the sludge-fed groups. Cytosolic GT activities were enhanced to different degrees, depending on the substrate used; This was significant with CDNB as the substrate.

Addition of ferrous sulfate and zinc carbonate to the diet, in amounts equivalent to the metals found in the sludge, did not affect the parameters examined in the livers except for the slight enhancement of the activity of GT in liver cytosol.

The concentration of iron in the liver was significantly higher than the control, in the sludge-fed diet and in the added-mineral diet groups (Table 3). No apparent increase in zinc levels occurred in the experimental groups. The results of this experiment confirmed the effect of sludge found in the first experiment, on phase I and phase II enzymes. The presence of a high concentration of iron in the diet is apparently not enough to explain the enhanced activity of catalase.

The present results point to the possible presence of organic toxins in the sludge that could induce the hepatic cytochrome P-450 required in their oxidative detoxication, leading to oxidative stress and H_2O_2 formation. Catalase activity was elevated for protection before toxic oxygen formed. The presence of iron, specifically the nature of the ligation state of iron (ferric or ferrous), in turn could potentiate this process by formation of free radicals. The presence of ferrous and H_2O_2 is of prime importance in the formation of reactive oxygen species (Floyd, 1979).

The municipal waste water was analyzed for different chlorinated hydrocarbons (S. Kary, personal communication). The concentration range was generally 0-5 µg/liter, except for methoxychlor, which was found at a level of 50 µg/liter. The latter may be partly responsible for the metabolic changes found in the present study.
Low concentration of sludge can be useful in animal diets, but care should be taken to analyze each batch

of sludge before use.

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