FEED FROM SEWAGE

Activated Sewage Sludge as a Source of Vitamin B_{12} for the Pig

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Recent reports of the existence of a large potential supply of vitamin B_{12} in activated sewage sludge as well as the observation that several compounds in feces and/or rumen contents have microbiological and animal vitamin B_{12} activity, seemed to indicate the desirability of studying the vitamin B_{12} activity of sewage sludge in animals. It appears that dried activated sewage sludge fed at the 2% level is a satisfactory source of vitamin B_{12} for the baby pig. The dried sludge does not seem to have any harmful effect on the animals when fed at this level.

When fed a "synthetic milk" containing "alpha-protein" as the nitrogen source, the pig has been shown to require vitamin B_{12} (*8, 9, 13*). The quantitative requirement for crystalline vitamin B_{12} has also been established for the baby pig (*9, 13*). Recently Hoover, Jasewicz, and Porges (*4, 5*) have reported "the existence of a large potential supply of vitamin B_{12} in activated sludge" as prepared by several different treatments. Using methods of estimation based on direct assay with *Lactobacillus leichmanii*, alkaline destruction, and paper chromatographic separation, these authors report a concentration of 2.4 to 9.3γ of vitamin B_{12} activity per gram of activated sewage sludge, depending on the treatment involved in its preparation.

Because several different compounds having varying microbiological and animal vitamin B_{12} activity have been recently reported in feces and/or rumen contents (*2, 3, 11, 15*) it seemed worth while to study the vitamin B_{12} activity of sewage sludge in animals. There was also the possibility that toxic or inhibitory substances, and conversely additional unidentified growth factors, were present.

A total of 42 4-day-old cross-bred baby pigs, obtained from a commercial pig hatchery, were used in this investigation. The animals were assigned at random to their treatment groups and maintained throughout the experimental period in raised wire-bottomed cages. Data on individual *ad libitum* feed consumption as well as weekly weight gains were recorded. The method of feeding and care of the baby pigs was similar to that previously reported from this laboratory (*6*).

The synthetic milk employed in these experiments (Table I) contained alpha-protein, an isolated soybean protein which with methionine constituted the protein source. This milk was made up to contain 19.5% solids and was similar to that described by Neumann *et al.* (*14*).

Some of the animals received an aqueous solution of vitamin B_{12}, administered intramuscularly once a week at a level of 0.8γ per kg. of body weight per day (*12*). The dried activated sewage sludge was administered at a level of 20 grams per kg. of dry matter of the "milk." The sewage sludge used in these experiments contained 3.11γ of vitamin B_{12} activity per gram (dry weight) as determined by assay with a mutant strain of *Escherichia coli* (*1*).

The first experiment in this investigation was conducted with 12 animals in a nonair-conditioned laboratory in midsummer. The temperature fluctuated from 90° to 105° F. with a median of approximately 97°. As the baby pigs demonstrated very poor growth, a second experiment was conducted with more animals and under more controlled conditions.

Thirty-two animals were used in this second experiment, which consisted of two parts. Half of the animals were maintained in a constant temperature-humidity chamber in which the temperature was held constant at 85° F. and the relative humidity at 70%. The remaining 16 animals were maintained in an adjacent room where the average temperature was 86° F. and the average relative humidity was 47%. The experiment was terminated when the animals reached 35 days of age.

In the first (pilot) experiment the sewage sludge yielded gains beyond the injection of crystalline vitamin B_{12} and, therefore, appeared to be contributing some unknown factor to the ration. However, in the second experiment, in which gains were better, there was no such indication.

In order to demonstrate again that this

alpha-protein synthetic milk was deficient in vitamin B_{12}, two animals were maintained without supplementation of this vitamin until death. These two animals received a single initial injection of 0.4γ per kg. of body weight of vitamin B_{12} which allowed them to gain "normally" until the third week, at which time they began to lose weight and finally expired in the fourth and fifth weeks, respectively (Table II).

The performance of the animals in the second experiment is tabulated in Table II. Thirty-two animals were randomly assigned to the treatment groups originally, but two of them are excluded from the analysis: One animal did not learn to eat and was sacrificed during the first week just prior to starvation, while the second gained only 0.39 kg. during the 5-week experimental period.

Analysis of covariance showed that there was no significant difference in average final weight and feed consumption between treatments, each group being adjusted to equal initial weights. In this experiment the effect of variation in original weight on final weight was less, when the conditions of temperature and humidity were kept constant, by an amount significant at the 5% level.

From these results it appears that dried activated sewage sludge fed at the 2% level is a satisfactory source of vitamin B_{12} for the pig. When fed at this level, dried activated sewage sludge does not appear to have any harmful effect on the animals over a 5-week period and in a pilot experiment carried out under adverse conditions gave some evidence of growth stimulation above that due to vitamin B_{12}.

Baby pigs, maintained on a synthetic milk shown to be deficient in vitamin B_{12} and containing alpha-protein as the nitrogen source, made gains over a 5-week period which were not significantly different when supplemented either intramuscularly with crystalline vitamin B_{12} at a level of 0.8γ per kg. of body weight per day or orally with 20 grams of dried activated sewage sludge shown to contain 3.11γ of vitamin B_{12} activity per gram (dry weight). The effect of variations in initial weight on final weight was shown to be reduced when temperature and humidity were kept constant.

Table I. Composition of Basal Ration

Constituents[a]	%, Dry Basis
Alpha-protein (isolated soybean protein)[b]	29.4
DL-Methionine[c]	0.6
Sucrose	30.9
Lard	30.8
Mineral salts[d]	8.3
	Mg./Liter Milk
Water-soluble vitamins[e]	
Thiamine	0.65
Riboflavin	1.30
Calcium pantothenate	7.80
Nicotinic acid	2.60
Pyridoxine hydrochloride	1.30
Choline chloride	130.00
p-Amino benzoic acid	2.60
Pteroylglutamic acid	0.052
Ascorbic acid	16.00
Biotin	0.01
Inositol	26.00
Fat-soluble vitamins	
Vitamin A	2000 I.U.
Vitamin D	200 I.U.
α-Tocopherol acetate	1
2-Methyl-1,4,-naphthoquinone	26

[a] Homogenized into a synthetic milk containing 19.5% solids and 6% fat.
[b] Obtained from Glidden Co., Chicago, Ill.
[c] Supplied by Dow Chemical Co., Midland, Mich., through courtesy of Julius Johnson.
[d] For composition of mineral mixture, see (7).
[e] Thiamine hydrochloride riboflavin, pyridoxine hydrochloride, calcium pantothenate, biotin, nicotinic acid, ascorbic acid, and α-tocopherol acetate were generously supplied by Merck and Co., Inc., Rahway, N. J., through the courtesy of H. H. Draper. Pteroylglutamic acid was supplied by the American Cyanamid Co., Pearl River, N. Y., through the courtesy of T. H. Jukes.

Table II. Effect[a] of Sewage Sludge and Crystalline Vitamin B_{12} on Vitamin B_{12}-Deficient Baby Pig

(5-Week experimental period)

Treatments	1 Basal Ration	2 Basal Ration + Sewage Sludge	3 Basal + Vit. B_{12}	4 Basal Ration + Sewage Sludge + Vit. B_{12}
No. of pigs	2	10	10	10
Average initial wt., kg.	3.21	1.88	1.88	1.85
Average final wt., kg.	5.68	8.74	8.99	8.02
Average total gain, kg.	...	6.86	7.11	6.16
Average daily gain, kg.	0.079	0.196	0.203	0.176
Average total dry matter consumed, kg.	7.48	7.74	7.59	6.52

[a] None of the differences indicated between groups 2, 3, and 4 are statistically significant.

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Correction

In the article on "Malodorous Fermentation. Acidic Constituents of Zapatera of Olives" [Delmouzos, J. G., Stadtman, F. H., and Vaughn, R. D., J. AGR. FOOD CHEM., **1,** 333 (1953)] the fourth line should read: "fermentation also sometimes found in storage fruit."