A CHICK GROWTH FACTOR IN COW MANURE

I. ITS NON-IDENTITY WITH CHICK GROWTH FACTORS PREVIOUSLY DESCRIBED

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Hammond (1) reported the feeding of dried cow manure to growing chicks. He found that it improved the rate of growth when added to low grade growing diets. The addition of dried cow manure to a good diet did not make it any better. Hammond (2) reported later that dried cow manure supported growth and efficiency of feed utilization in chicks approximately as well as alfalfa leaf meal when added to a practical war time diet containing sardine fish-meal. Whitson et al. (3) showed that 8 per cent of cow manure and 3 per cent of sardine fish-meal were about equally effective in improving the growth of chicks fed a diet containing 35 per cent soy bean oil meal and no animal protein. These authors concluded that the substance contributed by cow manure was not a protein or any of the chemically characterized vitamins.

This paper shows that the growth substance in manure is not identical with any of the uncharacterized growth factors which have been reported essential for the chick; namely, the Lactobacillus casei factors, factor U, and S, vitamins B₁₀ and B₁₁, and folic acid. Hammond (4) reported that there are three Lactobacillus casei factors. Each factor has been isolated from a different source: one from liver (5), another from yeast (5), and the third from a fermentation residue (4). The evidence thus far reported (6) indicates that the recently synthesized folic acid (Lederle) is identical with the L. casei factor from liver. Vitamin B₁₂, the antianemia and chick growth factor, first reported by Hogan and Porr (7) and obtained in crystalline form by Pfiffner et al. (8), is believed by Stokstad (5) to be identical with the L. casei factor from liver.

Two of the three Lactobacillus casei factors have been shown to be required by the chick. The factor from liver has been shown to be required (7-11) for the prevention of anemia and the promotion of growth. The evidence presented for the synthetic Lactobacillus casei factor (6) shows that the prevention of anemia and the promotion of growth parallel each other when the basal diet is fed alone and supplemented with 50 γ per 100 gm. of diet. The report of Campbell et al. (12) shows that vitamin B₁₂ prevents anemia and promotes growth at the same time on a given dose of the crystalline material.
Hutchings et al. (4) stated that the *Lactobacillus casei* factor from a fermentation residue was "active in the nutrition of the chick." Scott et al. (13) reported that this factor and either α-pyracin lactone or β-pyracin lactone are required for the complete prevention of macrocytic, hypochromic anemia in chicks. The normocytic, hypochromic type of anemia developed when the *L. casei* factor was supplied without one of the pyracin lactones; the macrocytic, normochromic type of anemia occurred when β-pyracin lactone was added to the diet without the *L. casei* factor. Daniel et al. (14) have reported on the formation of folic acid (a marked increase in *Streptococcus lactis* R activity) when the *L. casei* factor from fermentation residues was incubated with α- or β-pyracin lactone in chick liver brei.

Factor U was first prepared from yeast by Stokstad and Manning (15) in 1938. They presented evidence to show that this factor, when added to their basal diet, stimulated growth of chicks. The growth factor was found to be present in large amounts in alfalfa, middlings, wheat bran, and yeast.

Factors R and S were concentrated from extracts of yeast by Bauernfeind et al. (16) in 1938. The chick's need for factors R and S was confirmed by Hill et al. (17). These workers also showed that factors R and S are not identical with folic acid, *Lactobacillus casei* factor from liver, or vitamin Bc.

Briggs et al. (18) reported the existence of two water-soluble vitamins needed by the chick. One, essential for proper feather formation, was named vitamin B12; the other, necessary for growth, was named vitamin B11. Both of these vitamins were obtained as concentrates from solubilized liver (Wilson's liver fraction L). Reporting on the distribution of these unidentified vitamins, these authors state, "liver and brewers' yeast are the best sources, adequate at 5 per cent of the diet. Linseed oil meal, soy bean oil meal, alfalfa leaf meal, and grass are comparatively good sources."

Mitchell et al. (19) reported the concentration of a factor (folic acid) from spinach. This factor was active for both *Lactobacillus casei* and *Streptococcus lactis* R. Hutchings et al. (4) have reported that the three *L. casei* factors (from liver, yeast, and fermentation residue) appear to be different from folic acid obtained from spinach. They base this statement on absorption spectra of the four factors. Briggs et al. (20) showed that a concentrate of spinach folic acid obtained from Mitchell's laboratory promoted growth to some extent when added to a basal diet deficient in vitamin B11. This growth was ascribed to vitamin B11 activity in the folic acid concentrate. No information has been found in the literature to indicate that the folic acid of spinach is required by the chick. Briggs et al. (18) reported that maximum growth of chicks was obtained on a purified diet containing 17.5 γ of added folic acid per 100 gm. of diet. Hill et al. (17) indicated that the folic acid requirement appeared to be less than 15 γ per 100 gm. of diet. These two figures were not based on spinach folic acid concentrates, but on the activity of the diets for *L. casei* and *S. lactis* R.
EXPERIMENTAL

Each experimental group consisted of twenty-five cross-bred chicks. The chicks were reared in electrically heated batteries which were in an air-conditioned room. All experiments were started with day-old chicks and terminated at the end of 6 weeks. The basal diet consisted of yellow corn 38.0 per cent, barley 20.0, alfalfa leaf meal 3.0, soy bean oil meal 35.0, butyl fermentation solubles (containing 250 \( \gamma \) of riboflavin per gm.) 0.6, steamed bone meal 1.5, limestone 1.0, salt (96 per cent NaCl, 4 per cent MnSO\(_4\) \( \cdot \) 4H\(_2\)O) 0.7, and vitamins A and D feeding oil (400 A. O. A. C. units of vitamin D, 2000 U. S. P. units of vitamin A per gm.) 0.2. To each 100 gm. of this diet were added 50 mg. of choline chloride and 1 mg. of nicotinic acid hydrochloride. The various supplements tested replaced an equal weight of corn in the diet.

In order to determine the relationship between the antianemia factors reported in the literature and the chick growth factor in cow manure, a study of the hemoglobin and hematocrit values in 6 week-old chicks was made. The hemoglobin and hematocrit values were determined for twelve chicks that had been reared on the basal diet and for a similar number reared on the diet supplemented with 8 per cent cow manure. The average hemoglobin and hematocrit values for the basal group were 11.0 gm. per cent and 29.1 per cent respectively. These chicks compared favorably with the chicks fed cow manure, whose average hemoglobin and hematocrit values were 11.7 gm. per cent and 30.5 per cent respectively.

Evidently the hemoglobin and hematocrit values for the basal group were normal. The addition of 8 per cent of cow manure to the diet did not make any significant changes in these blood characteristics.

Yeast is a good source of factors U, R, and S, and vitamins B\(_{10}\) and B\(_{11}\). Butyl fermentation solubles are a good source of the characterized B vitamins and probably many of the unidentified vitamins. Experiment 1 was designed to determine how the addition of yeast or butyl fermentation solubles to the basal diet would affect the growth of chicks. Also included in this experiment were some groups fed pyracin lactone and some related compounds. Scott et al. (21) reported that pyracin lactone was essential for the promotion of growth and prevention of anemia in chicks. The supplements fed and the average weights of the chickens at 6 weeks are listed in Table I.

The results of this experiment show that yeast\(^1\) and butyl fermentation solubles do not contain any substance which is identical with the chick growth factor in cow manure. Neither pyracin lactone nor the other two

\(^1\) These yeast samples were tested for their content of vitamins B\(_{10}\) and B\(_{11}\) by Mr. Robert Lillie and Dr. G. M. Briggs, Jr., of the University of Maryland, and were found to be potent sources of both vitamins.
related compounds showed any beneficial effect on growth at a level of 1 mg. per kilo of diet.

Alfalfa leaf meal is a good source of factor U. Solubilized liver is the material from which concentrates of vitamins B₁₀ and B₁₁ have been prepared. In Experiment 2 the basal diet was supplemented with these materials in order to determine whether they would contribute anything to the growth potential of this diet. The supplements fed and the average weights of the chickens at 6 weeks are listed in Table I.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Supplement</th>
<th>Average weight of chickens at 6 wks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>333.3</td>
</tr>
<tr>
<td></td>
<td>8% cow manure</td>
<td>427.5</td>
</tr>
<tr>
<td></td>
<td>5% butyl fermentation residue</td>
<td>311.5</td>
</tr>
<tr>
<td></td>
<td>5% yeast (Anheuser-Busch strain i)</td>
<td>299.7</td>
</tr>
<tr>
<td></td>
<td>5% &quot; (Fleischmann)</td>
<td>312.8</td>
</tr>
<tr>
<td></td>
<td>Pyridoxamine dihydrochloride (1 mg. per kilo diet)</td>
<td>289.7</td>
</tr>
<tr>
<td></td>
<td>Pyridoxal hydrochloride (1 mg. per kilo diet)</td>
<td>311.3</td>
</tr>
<tr>
<td></td>
<td>Pyraein lactone (1 mg. per kilo diet)</td>
<td>295.1</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>352.0</td>
</tr>
<tr>
<td></td>
<td>5% cow manure</td>
<td>451.2</td>
</tr>
<tr>
<td></td>
<td>5% alfalfa leaf meal</td>
<td>296.8</td>
</tr>
<tr>
<td></td>
<td>3% Wilson's liver fraction L</td>
<td>473.2</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>214.2</td>
</tr>
<tr>
<td></td>
<td>Folic acid (Lederle synthetic) (1 mg. per kilo diet)</td>
<td>215.3</td>
</tr>
<tr>
<td></td>
<td>0.1% choline chloride</td>
<td>239.9</td>
</tr>
<tr>
<td></td>
<td>Folic acid (1 mg. per kilo diet) + 0.1% choline chloride</td>
<td>248.6</td>
</tr>
</tbody>
</table>

Alfalfa leaf meal did not contribute any of the deficient growth substance to the basal diet. However, solubilized liver was a very potent source of the substance in which the basal diet was deficient. In this experiment and in all other experiments related to this problem, the feather growth of the chicks on the basal diet was normal.

During the course of these experiments the synthetic folic acid (Lederle) became available. Experiment 3 was planned to determine whether this synthetic compound would have any effect on chick growth when added to the basal diet alone or with an additional 0.1 per cent choline chloride. The supplements fed and the average weights of the chickens at 6 weeks are listed in Table I.
It is evident that synthetic folic acid (Lederle) did not improve the growth-promoting properties of the basal diet.

DISCUSSION

That the growth factor of cow manure is not identical with any of the previously reported growth factors is evident. It improves the growth-promoting properties of the basal diet, whereas the other factors and ingredients containing them do not. Deficiency of the *Lactobacillus casei* factors causes anemia in chicks, which parallels poor growth. The basal diet described here prevents anemia but does not support good growth. Furthermore, synthetic folic acid (Lederle) and pyracin lactone did not stimulate growth when added to the basal diet. These antianemia factors, therefore, cannot be identical with the growth factor of cow manure.

Yeast is a good source of factors U, R, and S, and vitamins B₁₀ and B₁₁. Two different yeasts when fed as 5 per cent of the diet failed to show the growth-promoting effect of cow manure. Alfalfa leaf meal, which is a good source of factor U and a fair source of vitamins B₁₀ and B₁₁, failed to stimulate growth in these experiments. The basal diet cannot be deficient in vitamin B₁₀ because it supports normal feather growth. Thus, the growth factor in cow manure is not identical with factors U, R, or S, or vitamin B₁₀ or B₁₁.

Solubilized liver as well as cow manure proved to be a good source of substances which promote growth when added to the basal diet, whereas butyl fermentation solubles did not.

It was reported previously from this laboratory (22) that the growth of chicks fed this same basal diet was stimulated by the addition of 0.1 per cent choline chloride in combination with either 2 mg. of calcium pantothenate or 0.4 mg. of pyridoxine hydrochloride per 100 gm. of diet, although the basal diet was calculated to contain more than adequate quantities of all three of these vitamins. That cow manure does not owe its growth-promoting properties to such a combination of known vitamins is indicated by fractionation experiments to be reported in another paper (23).

SUMMARY

Evidence has been presented to show that the growth factor of cow manure is not identical with the *Lactobacillus casei* factors (from liver, yeast, or fermentation residues), factors U, R, or S, vitamins B₁₀ or B₁₁, synthetic folic acid (Lederle), or pyracin lactone.

BIBLIOGRAPHY

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