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VITAMIN B(B_1) AND G(B_2) CONTENT OF COTTONSEED PRODUCTS*

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Cotton is a raw material which is more and more closely allying the agricultural and chemical industries. Because of the considerable industrial importance of cotton to the South and because of the recognized nutritional value of cottonseed meal in cattle feeding, a study of cottonseed products has been undertaken in order to learn more of their possibilities as nutrients. Stevens *(8)* in 1930 reported his finding that cottonseed meal was a rich source of both vitamins B(B)$_1$ and G(B_2). This investigation has for its object a comparative study of cottonseed meal, oil, and hulls with respect to their vitamin B(B_1) and vitamin G(B_2) content.

The rat growth methods for determination of vitamin B(B_1) as developed by Chase and Sherman *(2)* and of vitamin G(B_2) as developed by Bourquin and Sherman *(1)* were followed. The material, the vitamin content of which was to be determined, was fed to the rats receiving diets deficient in vitamin B(B)$_1$ or vitamin G(B_2). The growth produced in these rats in excess of that produced in the rats receiving only the diet deficient in vitamin B(B_1) or vitamin G(B_2) is taken as a measure of the vitamin B(B_1) or vitamin G(B_2) in the material. The conditions of the investigation were carefully controlled as to the care and selection of the animals according to the above mentioned methods.

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All of the experimental animals were bred from rats on a diet consisting of two-thirds ground whole wheat, one-third whole milk powder, and sodium chloride which was 2 per cent of the weight of the wheat *(6)*.

The cottonseed meal was fed separately from the basal diet in a weekly portion of 2.4 grams. The cottonseed oil was incorporated as the sole source of fat in the basal diets, thereby replacing the 8 per cent butter fat. The cottonseed hulls were extracted with purified 60 per cent alcohol[1], and the extract was concentrated at reduced temperature and pressure, and dried on cornstarch. This starch was incorporated in a food mixture otherwise like the basal diet, replacing an equivalent weight of ordinary cornstarch. The extract was fed at 10, 20, and 40 per cent levels. In one series the hulls were fed directly as hull bran in weekly portions of 2.4 grams.

Evidence of Third Factor of Vitamin B Complex in Cottonseed Hulls

The average results showing the measure of vitamin B(B_1) in cottonseed products are given in Table I, and Figures 1 and 2. The data clearly show that this sample of cottonseed meal is a rich source of vitamin B(B_1), that it is richer than the same weight of whole wheat *(2)* or skimmed milk powder *(7)*, and, from results to be reported later in this paper, that it is as rich as the same weight of dry baker's yeast.

In substituting the cottonseed oil for the butter fat in the basal diet, the vitamin A content was decreased, but there was little reason to doubt that sufficient vitamin A for the experimental period was supplied through storage in the animal and cod liver oil in the diet. At no time was there any indication of vitamin A deficiency. However, in order to have definite evidence that deficiency of vitamin B(B_1) and not of vitamin A was responsible for the loss in

[1]The alcohol was purified as follows: 10-20 grams potassium hydroxide were added to a liter of 95 per cent alcohol. The mixture stood several hours; 0.5 cc. saturated silver nitrate solution was slowly added. The alcohol was filtered and distilled, the first and last 10-cc. portions being discarded.

weight in the animals, four rats were given the vitamin B(B_1)–deficient diet containing the cottonseed oil with 2.4 grams a week of yeast powder. The animals within 4 weeks had more than doubled their weight. Since the yeast, according to the reported assay and from the reports of

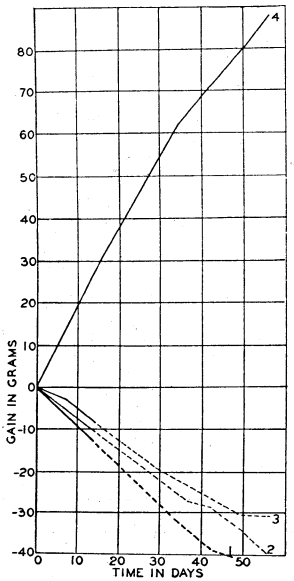

FIGURE 1. COMPARATIVE EFFECT OF COTTONSEED PRODUCTS ON RATS FED A VITAMIN B(B_1)–DEFICIENT DIET

1. Vitamin B(B_1)–deficient diet only (10 rats)
2. Cottonseed hull extract at 40 per cent level (18 rats)
3. Cottonseed oil, 8 per cent (12 rats)
4. Cottonseed meal, 2.4 grams per week (15 rats)

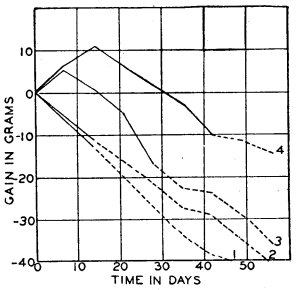

FIGURE 2. EFFECT OF COTTONSEED HULLS PLUS SMALL AMOUNT OF COTTONSEED MEAL ON RATS FED A VITAMIN B(B_1)–DEFICIENT DIET

1. Negative controls (10 rats)
2. Cottonseed hull extract at 40 per cent level (18 rats)
3. Cottonseed meal, 0.6 gram per week (6 rats)
4. Cottonseed meal, 0.6 gram per week, plus hull extract, 40 per cent (9 rats)

many workers *(4, 5)*, contained no vitamin A, the large gains made by the animals can be accounted for only through vitamin B(B_1) of the yeast. From these results it is concluded that cottonseed oil is as deficient in vitamin B(B_1) as butter fat, and it is suggested that cottonseed oil could be used instead of butter as a source of fat in the vitamin B(B_1)–deficient basal diet.

Table I. Average Results of Feeding Cottonseed Products with Vitamin B(B_1)—Deficient Diet

Rats	Supplement	Gain in Weight After: 5 Weeks GRAMS	8 Weeks GRAMS	Survival Period DAYS	Weekly Food Intake GRAMS
10	0	—21.8	—23.4	29	15
15	2.4 grams meal a week	56	88	56	50
12	Oil at 8%	—21	—24	35	16
12	10% hull extract	—27	—27	29	10
6	40% hull extract	—28	—32	29	10
9	0.6 gram meal a week	—21	—24	37	24
9	40% hull extract + 0.6 gram meal a week	— 3	—21	54	27

Eighteen rats received the vitamin B(B_1)–deficient diet in which had been included the extract from the hulls. Twelve of these animals received the extract from the hulls fed at a 10 per cent level; six were fed the hull extract at a 40 per cent level. By the fifth week only three animals from the total of both groups survived. All of them suffered from polyneuritis. The growth curve of these animals so nearly approximated that of the negative controls that no measurable amount of vitamin B(B_1) could be considered present in the hull extract.

Although it is known that in the cooking process of the cottonseed meal the gossypol seems to be changed to a less toxic form *(3)*, it was decided to determine whether or not the rapid decline of the animals receiving the hull extract could be due to some toxic substance in the extract, although it was realized from the typical polyneuritic symptoms that insufficient amount of vitamin B(B_1) was an important factor in causing the decline. As the cottonseed meal, when it was fed with the vitamin B(B_1)–deficient diet, had shown no evidence of any toxic material, it was decided to feed the extract from the hulls with the cottonseed meal, together with the vitamin B(B_1)–deficient diet.

Four rats were given the vitamin B(B_1)–deficient diet which had been made to contain hull extract at a 10 per cent level. They also received 2.4 grams of cottonseed meal each week. There was no evidence of any toxic substance. But the four animals made a greater gain on the cottonseed meal fed with the hull extract than their litter mates made on

the meal fed with the basal diet without the hull extract. Since the hulls had shown no trace of vitamin B(B_1), it was thought the additional growth brought about by the meal plus the hull extract might be an indication of some growth factor in the hulls other than vitamins B(B_1) and G(B_2).

To determine whether or not this indication was significant, a third series was started. With one group of nine animals the experiment was continued by feeding 2.4 grams of meal each week with the vitamin B(B_1)–deficient diet containing 40 per cent hull extract. With nine litter mates of the above, the effect of feeding 0.6 gram cottonseed meal a week with the vitamin B(B_1)–deficient diet containing 40 per cent hull extract was determined. Six animals, litter mates of the above groups, were given the vitamin B(B_1)–deficient diet plus 0.6 gram of cottonseed meal a week.

The results of continuing the feed of 2.4 grams of meal with the diet containing the hull extract gave no significant difference from the average gain of animals due to the 2.4 grams of meal without the hull extract.

However, the two groups receiving 0.6 gram of meal a week with and without hull extract gave very different results. Of the animals receiving 0.6 gram of meal with the vitamin B(B_1)–deficient diet only, all but one had either died following development of severe polyneuritis or were suffering from the disease by the fifth week. One lived through 8 weeks; polyneuritis developed the fifty-third day. The average loss in weight at the end of the fifth week was 21 grams. The nine litter mates of these rats receiving 0.6 gram of meal a week and the vitamin B(B_1)–deficient diet containing the hull extract showed at the end of 5 weeks an average loss of only 3.3 grams. All but two of the animals survived the 8-week period but showed alternately the onset and partial or complete recovery of polyneuritis. At the end of 8 weeks the average loss in weight was 21 grams. The average weekly food intake of the two groups showed no significant difference.

The hull extract seemed to be the cause of this less rapid loss in weight, yet it apparently had nothing to do with

the partial recovery from polyneuritis, as the diet containing the hull extract was always accessible to the animal; but it was only after feeding the weekly portion of meal that there was improvement in the conditions brought on by polyneuritis. The earlier results have shown that in the extract of hulls there is no vitamin B(B_1); therefore, it seems that there must be some growth factor in the hull extract other than vitamin B(B_1) or G(B_2).

The animals receiving the larger quantity of cottonseed meal with or without the hull extract made such large and rapid gains that probably they were not sensitive to the effect of the hulls.

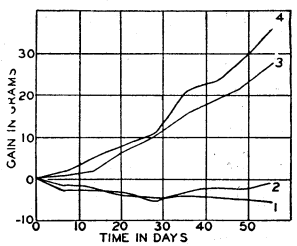

FIGURE 3. COMPARATIVE EFFECT OF COTTONSEED PRODUCTS ON RATS FED A VITAMIN G(B_2)-DEFICIENT DIET

1. Vitamin G(B_2)-deficient diet only (11 rats)
2. Cottonseed oil (13 rats)
3. Cottonseed meal, 2.4 grams per week (15 rats)
4. Cottonseed hulls, 2.4 grams per week (8 rats)

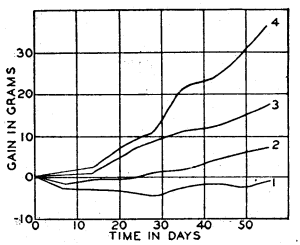

FIGURE 4. EFFECT OF COTTONSEED HULLS ON RATS FED A VITAMIN G(B_2)-DEFICIENT DIET

1. Vitamin G(B_2)-deficient diet only (11 rats)
2. Extract of hulls fed at 10 per cent level (16 rats)
3. Extract of hulls fed at 20 per cent level (8 rats)
4. Hulls unextracted fed at 5 to 7 per cent level (8 rats)

Evidence of Vitamin G(B_2) in Cottonseed Hulls

The average results showing the measure of vitamin G(B_2) in cottonseed products are given in Table II and in Figures 3 and 4. Cottonseed meal is shown to be a good source of vitamin G(B_2), but it is richer in vitamin B(B_1) than in vitamin G(B_2). The cottonseed oil showed slightly less vitamin G(B_2) than butter fat. By means of autoclaved yeast it was established that the decline in weight of the animals receiving the basal diet containing cottonseed oil was not due to vitamin A deficiency. This oil could be con-

veniently substituted as a source of fat for the butter in the vitamin G(B_2)–deficient basal diet.

The results of feeding the hull extract at a 10 per cent level gave evidence of some vitamin G(B_2), and because of this evidence a further investigation of the hulls was undertaken. In the second series the hull extract was incorporated with the vitamin G(B_2) basal diet at a 20 per cent level. Eight rats were given this diet. To eight litter mates of these, carefully matched as to weight and sex, were given

Table II. Average Results of Feeding Cottonseed Products with Vitamin G(B_2)–Deficient Diet

Rats	Supplement	Gain in Weight After: 5 Weeks GRAMS	8 Weeks GRAMS	Weekly Intake of Food per Rat GRAMS
7	0	3	3	25
13	8% cottonseed oil	—3	—6	23
15	2.4 grams cottonseed meal per week	15	28	30
16	10% hull extract	2	7	23
8	20% hull extract	11	17	22
8	2.4 grams hull bran per week	23	32	30

2.4 grams a week of the unextracted hull bran, which amount was between 5 and 6 per cent of the food intake. At the end of 5 weeks the first group (receiving the hull extract at 20 per cent level) made an average gain of 10 grams. The second group (receiving hull bran) at 5 weeks had made an average gain of 23 grams; at the end of 8 weeks the gain was 32 grams.

The cottonseed hulls are as good a source of vitamin G(B_2) as the cottonseed meal.

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