# Effect of Vitamin $B_{12}$ and Folic Acid on Utilization by Rats of Soybean Protein and Amino Acids

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The effect of vitamin $B_{12}$ and folic acid on utilization of soybean protein by rats was investigated. Addition of vitamin $B_{12}$ and folic acid to the soybean diets increased the growth rate as well as the carcass nitrogen retention of rats, which had been depleted of these vitamins. Vitamin $B_{12}$ and folic acid supplementation increased the availability of methionine for rats fed soybean protein. Deficiency in either vitamin $B_{12}$ or folic acid or a combination of these vitamins increased the excretion of urea and decreased the excretion of allantoin in the urine of rats. The ratio of urinary allantoin to urea in rats was the highest when vitamin $B_{12}$ and folic acid were included in the diets.

Many investigators have been searching for an explanation of the effect of vitamin $B_{12}$ in promoting the growth of the animals fed diets containing vegetable protein. Studies have reported that vitamin $B_{12}$ plays a possible role in nucleic acid metabolism (*14*), in the metabolism of methyl group (*15*), and in the formation of enzymes in the liver (*22*).

Vitamin $B_{12}$ has an effect on protein metabolism through its influence upon the synthesis of methionine; thus $B_{12}$ would be expected to improve protein utilization of diets deficient in this amino acid (*7*). Evidences have been recorded regarding the interdependence of vitamin $B_{12}$ and folic acid on methylation reaction as well as in other metabolic processes (*10*, *11*, *13*, *18*). The interrelationship of vitamin $B_{12}$ and folic acid on utilization of protein and amino acids is not thoroughly understood. The object of the present study was to investigate the influence of vitamin $B_{12}$ and folic acid on utilization by rats of soybean protein and amino acids. Four amino acids—lysine, methionine, leucine, and valine—were selected for this investigation. The effect of vitamin $B_{12}$ and folic acid on the excretion of urinary urea and allantoin was also investigated.

## *Methods*

Forty-eight weanling albino rats of the Sprague and Dawley strain were divided into four groups with 12 animals in each group. Four diets were used: Diet 1 contained vitamin $B_{12}$ and folic acid; diet 2 contained neither vitamin $B_{12}$ nor folic acid; diet 3 contained vitamin $B_{12}$ but without folic acid; diet 4 contained folic acid but not vitamin $B_{12}$. The four diets consisted of the following ingredients (in grams per 100 grams of diet): soybean protein, 18; hydrogenated fat, 8; corn starch, 69; salt mixture (USP 14), 4; and vitamin mix in sucrose, 1. The vitamin mix supplied (in milligrams per kilogram of ration): thiamine, 5; pyridoxine, 5; riboflavin, 10; *p*-aminobenzoic acid, 10; nicotinic acid, 20; Ca pantothenate, 50; D-$\alpha$-tocopheryl succinate, 75; biotin, 0.3; inositol, 400; choline chloride, 1000; vitamin A, 5000 USP units; and vitamin D, 500 USP units. The vitamin $B_{12}$ supplemented to diets 1 and 3 was 100 $\mu$g. per kg. of diet. The folic acid supplemented to diets 1 and 4 was 5.5 mg. per kg. of diet. The soybean protein used in this experiment was a purified protein purchased from Nutritional Biochemical Corp., Cleveland, Ohio.

The animals were initially depleted of their vitamin $B_{12}$ and folic acid reserves by maintenance on a diet devoid of these vitamins but with addition of 0.15% iodinated casein for 2 weeks. Thereafter, the animals were placed on the four experimental diets. Body weight and food consumption of the rats were recorded weekly. At the end of 12 weeks, the animals were placed in metabolic cages; feces and urine were collected for 5 days. The method for collection and treatment of the fecal samples was described in a previous paper (*4*). Fecal amino acids—lysine, methionine, leucine, and valine—were determined microbiologically (*8*). Urine samples were analyzed for allantoin according to the method of Young and Conway (*23*) and for urea according to the method of Engel and Engel (*9*). At the end of 13 weeks, the animals were anesthetized by injection of sodium amytal. The body nitrogen of each rat was determined according to the method of Miller and Bender (*17*). The total carcass nitrogen in 10 weanling rats, killed at the start of the experiment, was determined by the same method. Differences in means were tested, using Duncan's multiple-range test (*21*).

## *Results and Discussion*

**Growth and Nitrogen Retention.** The growth rates of rats fed the various diets are shown in Table I. The mean weight gain of rats fed diet 1 was the highest. The mean weight gain of the animals singly deficient in vitamin $B_{12}$ or folic acid was higher than those of the doubly deficient rats.

Differences in dietary nutrients result in a difference in body composition. In some cases, weight gain may depend more on a deposition of body fat than an increase of body protein (*1*). In order to assure that increase in body weight indicated an increase in tissue protein, the per cent of carcass nitrogen retained in rats fed the various diets was determined by subtracting the carcass nitrogen at the beginning of the experiment from the carcass nitrogen at the end and dividing this result by

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the total nitrogen consumed. Table I shows that the carcass nitrogen retained in rats of the vitamin $B_{12}$ and folic acid supplemented group and of the singly deficient groups was higher than that in rats of the doubly deficient group. This study indicated that the weight gain of rats under the influence of either $B_{12}$ or folic acid or the combination of the two was a gain in tissue protein rather than in body fat. Also, the effect of vitamin $B_{12}$ and folic acid on carcass nitrogen retention appears to be additive.

**Excretion of Urinary Nitrogen Compounds.** UREA. The measurement of urea excretion has been employed by many investigators as an index of the quantity and quality of protein ingested as well as the effects of other nutrients on protein utilization (*12*). In recent studies, Kiriyama and Ashida reported that rats fed gluten as a source of protein excreted more urea than rats fed a casein diet (*16*). Schimke found that starvation was associated with an increase in urea excretion, whereas a protein-free diet resulted in a decrease in urea excretion (*20*). In order to ascertain whether vitamin $B_{12}$ and folic acid would exert an effect on protein utilization, the urea excretion of rats fed the various diets was determined (Table II). The ratio of urea nitrogen to total nitrogen of rats fed a diet supplemented with vitamin $B_{12}$ and folic acid is significantly lower than that of rats fed a diet devoid of these two vitamins. There were no significant differences in the ratios of urea nitrogen to total nitrogen in rats among the other groups. Charkey *et al.* have reported that vitamin $B_{12}$ deficiency results in high serum levels of amino acids in the chick (*5*). In this study, the high urea excretion in rats fed a diet deficient in vitamin $B_{12}$ and folic acid may have been related to the high serum levels of amino acids. The inverse relationship of urea excretion with vitamin $B_{12}$ and folic acid supplementation suggests that these vitamins may function in the utilization of free serum amino acids for the synthesis of tissue protein thus reducing renal wastage.

ALLANTOIN. The excretion of urinary allantoin as well as the ratio of allantoin-urea is related to the biological value of dietary protein (*16*). Kiriyama and Ashida found that when rats were fed a casein diet, they exhibited a higher ratio of allantoin-urea compared with those rats fed a wheat gluten diet (*16*). These authors suggested that the allantoin-urea ratio could be used as a measure for the evaluation of the biological value of protein. Following such assumption, the effect of vitamin $B_{12}$ and folic acid on the excretion of allantoin in the urine of rats was determined (Table II). The allantoin excreted by rats in the vitamin $B_{12}$ and folic acid–supplemented group was the highest, while the allantoin concentration was similar in the urine of rats fed the other experimental diets. Since allantoin is the end product of metabolism of the purine base derived from ribonucleic acid, an increase in excretion of allantoin would indicate the increased turnover rate of this nucleic acid.

The ratio of allantoin-urea for rats fed the various diets was calculated (Table II). The vitamin $B_{12}$ and folic acid supplemented rats showed a significantly higher value in allantoin-urea ratio than the singly or doubly deficient rats.

As shown in Table II, vitamin $B_{12}$ and folic acid do

**Table I. Effect of Vitamin $B_{12}$ and Folic Acid on the Growth Rate and Nitrogen Retention in Rats[a]**

| Supplements | Total Gain, Grams | Carcass[b] N, Grams | Carcass N at Start, Grams | Carcass N Retained, Grams | Total N Consumed, Grams | N[c] Retained, % |
|---|---|---|---|---|---|---|
| Vitamin $B_{12}$ and folic acid | 159a ± 7.4 | 4.93 | 1.56 | 3.36 | 21.42 | 15.70f ± 0.2[d,e] |
| None | 93b ± 6.4 | 3.94 | 1.56 | 2.47 | 20.53 | 12.00g ± 0.4 |
| Vitamin $B_{12}$ | 133c ± 5.8 | 4.71 | 1.56 | 3.15 | 23.37 | 13.47h ± 0.1 |
| Folic acid | 128c ± 3.4 | 4.66 | 1.56 | 3.10 | 22.85 | 13.55h ± 0.1 |

[a] Twelve animals in each group.
[b] Carcass nitrogen at the end of 13 weeks.
[c] $\% \text{ N retained} = \frac{\text{carcass N retained}}{\text{total N consumed}} \times 100$.
[d] Mean ± standard error.
[e] Means having the same letter are not significantly different ($P < 0.05$).

**Table II. Vitamin $B_{12}$ and Folic Acid on Excretion of Urea and Allantoin in the Urine of Rats[a,b]**

| Supplements | Urea N, Mg./Day | Total N, Mg./Day | Urea N/Total N, % | Urea, Mg./Day | Allantoin, Mg./Day | Allantoin-Urea, % |
|---|---|---|---|---|---|---|
| Vitamin $B_{12}$ and folic acid | 123.6 | 188.8 | 65.5a ± 2.8[c,d] | 264.9 | 30.2 | 11.4c ± 1.0 |
| None | 142.2 | 177.1 | 80.3b ± 1.0 | 304.9 | 25.7 | 8.4d ± 0.3 |
| Vitamin $B_{12}$ | 143.5 | 179.4 | 80.0b ± 0.7 | 307.7 | 26.1 | 8.5d ± 0.2 |
| Folic acid | 150.0 | 189.6 | 79.1b ± 1.4 | 321.1 | 26.4 | 8.2d ± 0.3 |

[a] Twelve rats per group.
[b] 5-day collection period.
[c] Mean ± standard error.
[d] Means having the same letter are not significantly different ($P < 0.05$).

Table III. Vitamin $B_{12}$ and Folic Acid on the Apparent Availability of Amino Acids for Rats[a,b]

| Supplements | Leucine, % | Lysine, % | Methionine, % | Valine, % |
|---|---|---|---|---|
| Vitamin $B_{12}$ and folic acid | 93.16 ± 0.76 | 94.24 ± 0.32 | 94.82a ± 0.62[c,d] | 94.30 ± 0.41 |
| None | 93.60 ± 0.29 | 94.70 ± 0.39 | 91.54b ± 0.71 | 93.97 ± 0.81 |
| Vitamin $B_{12}$ | 95.50 ± 0.80 | 94.17 ± 0.44 | 93.29a,b ± 0.56 | 93.39 ± 0.21 |
| Folic acid | 92.55 ± 0.74 | 93.64 ± 0.43 | 92.56a,b ± 0.97 | 92.48 ± 0.72 |

[a] Twelve rats per group.
[b] 5-day collection period.
[c] Mean ± standard error.
[d] Means having the same letter are not significantly different ($P < 0.05$).

not seem to affect the amount of total nitrogen excretion; however, they apparently affect the distribution of the urinary nitrogen compounds such as urea and allantoin. If the excretion of such compounds is related to the nutritive value of dietary protein as has been suggested by previous investigators (*16*), a decrease in excretion of urea or an increase in the allantoin-urea ratio would, therefore, indicate an increase in nutritive value of dietary protein. Apparently, then, the growth-promoting effect through vitamin $B_{12}$ and folic acid supplementation may have resulted from the improved biological value of soybean protein.

**Availability of Amino Acids.** The availability of lysine, methionine, valine, and leucine for rats as affected by vitamin $B_{12}$ and folic acid was determined (Table III). The apparent availability of amino acids was calculated as follows: From the total amino acid intake subtract the fecal amino acid excreted; then divide this result by the total amino acid intake. Table III shows that the availability of leucine, valine, and lysine for rats of the various dietary groups is practically identical. However, methionine is less available when rats are fed a diet devoid of vitamin $B_{12}$ and folic acid. Previous experiments in the authors' laboratory indicated that vitamin $B_{12}$ and folic acid did not affect the excretion of metabolic fecal amino acids. If the fecal amino acids excreted by rats are derived from exogenous amino acids and metabolic amino acids, the lower availability of methionine for doubly deficient rats would be due solely to the rats' slower release of methionine from soybean protein. This finding is in accord with the in vitro studies of Baliga, Bhagavan, and Rajagopalan (*2*). These authors found that during digestion of soybean protein with trypsin, the release of methionine from raw soybean protein is higher in the presence of vitamin $B_{12}$. Since methionine is less available only in the absence of vitamin $B_{12}$ and folic acid, folic acid may have a sparing effect along with vitamin $B_{12}$ on the release of methionine from soybean protein. In the doubly deficient rats, their slow growth rate and lower carcass nitrogen retention may be due to lower methionine availability from the soybean.

Reports in the literature concerning the effect of vitamin $B_{12}$ on protein utilization are somewhat contradictory. Baliga and Rajagopalan found that vitamin $B_{12}$ increased the biological value of protein (*3*). Fatterpaker *et al.* found that vitamin $B_{12}$ and folic acid increased the protein content and decreased the fat content in the liver of rats (*10*). In contrast to the above reports, Chow and Barrows (*6*) did not obtain better nitrogen retention with vitamin $B_{12}$ for the rats on a deficient diet. Rupp, Paschkis, and Cantarow (*19*) failed to prove the beneficial effect of vitamin $B_{12}$ on protein utilization. The discrepancies among different workers may be due to the difference in experimental diets or the different methods which were chosen by the investigators for the evaluation of protein utilization. Data obtained in this paper demonstrate clearly that vitamin $B_{12}$ and folic acid influence protein utilization and thereby increase the biological value of protein when excretion of urinary nitrogen compounds, such as urea and allantoin, is used as a measure for protein utilization. The same conclusion can be made when the availability of methionine is used as a measure of protein utilization.

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