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TRICHOLOMA NUDUM AS A SOURCE OF AMINO ACIDS, VITAMINS AND UNIDENTIFIED FACTORS

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ABSTRACT

Feeding trials with mice were used to evaluate the nutritional quality of dried mycelia from *Tricholoma nudum* grown in submerged culture. The material proved to be non-toxic at dietary levels as high as 50 per cent. Chemical and bio-assays indicated methionine and phenylalanine to be the first and possibly the only limiting amino acids when *Tricholoma* supplied all the dietary protein at a level of 18 per cent. The product was relatively rich in arginine and tryptophane.

Tricholoma residue, at a level of 5 per cent in the diet, provided all the necessary B-vitamins for normal growth in the mouse. Chemical or microbiological assays were obtained for riboflavin, niacin, pantothenic acid, choline and thiamin and, in all cases except niacin, the levels exceeded those reported for average Torula yeast.

Tricholoma was found to possess a factor having strong antioxidant properties. The inclusion of 10 per cent *Tricholoma* in the diet was more effective in preventing a rise in peroxide values than was 0.05 per cent DPPD.

INTRODUCTION

Previous work (6) indicated that the dried mycelium from submerged culture of *Tricholoma nudum* had potentially good feeding value. Protein and amino acid analyses and toxicity tests were reported.

This communication reports further information on the biological value of the protein and the value of the product as a source of B-vitamins and of a factor affecting the development of rancidity in dietary fat.

MATERIALS AND METHODS

Tricholoma nudum was grown in submerged culture in a molasses medium (6) and subsequently freeze-dried for use in feeding trials with mice. The material was light grey in colour, very light textured and of pleasant, mild aroma.

Chemical Analyses

The mycelium was analysed for crude protein (Nx6.25), crude fat, total ash and moisture according to standard procedures for feedingstuffs analysis. Carbohydrate content (expressed as glucose) was determined colorimetrically by the anthrone method (5). Essential amino acids were determined quantitatively by one-dimensional ascending paper chromatography (4,7). Peroxides were determined by the method of Sulley (9).

Vitamin assays were obtained for riboflavin, niacin, pantothenic acid (total and free), thiamin and choline.

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Animal Feeding Tests

The mouse experiments were conducted in two phases. The first was an attempt to evaluate the amino acid and vitamin B-complex content of the product. The second experiment was an investigation of an apparent relationship between the presence of the mycelium and the development of rancidity in the fat component of the diets since diets devoid of the mycelium proved to contain rancid fat at the end of the 14-day feeding tests.

Experiment No. 1

In this test weanling female mice of Carworth Farms No. 1 strain were allotted randomly to the various diets and were housed in individual wire-bottom, metal cages in a mobile battery. The laboratory was thermostatically controlled at about 25° C. At the time of allotment the mice weighed between 8.0 and 9.0 gm. and were under 23 days of age. Feed and water were provided *ad libitum* and accurate records of feed were kept during a 14-day growth test.

The experiment included nine diets. *Diet 1*, the stock colony diet, had proven satisfactory over several years of use in this laboratory and was, therefore, used as a positive control ration. It was composed of wheat 15.0; oat groats 20.0; wheat bran 15.0; barley 12.0; alfalfa leaf meal 5; linseed oilmeal 7.5; meat meal 15; molasses 5; brewers' dried yeast 1; lard (emulsified and stabilized) 2.5; ground limestone 1, and iodized salt 0.5 per cent, plus 10 p.p.m. of aureomycin, 250 p.p.m. manganese sulphate, 5000 I.U. vitamin A per pound of feed and 70 I.U. vitamin D_3 per pound of feed.

All other diets were semi-purified and designed to contain approximately 16 per cent protein together with adequate levels of minerals, digestible energy and vitamins in accordance with known nutrient requirements for the mouse or rat. *Diet 2* was corn starch 45; sucrose 10; cellulose[1] 10; minerals[2] 5; corn oil 5; vitamin-free casein 20 and vitamin mixture 5 per cent. The vitamin mixture contained 0.3 mg. thiamin; 0.4 mg. riboflavin; 0.9 mg. pantothenic acid; 3 mg. niacin; 10 μgm. biotin; 0.1 mg. pyridoxine; 0.5 μgm. B_{12}; 25 μgm. folacin; 0.1 mg. inositol; 85 mg. choline; 50 I.U. vitamin A; 0.5 μgm. vitamin D_2; 7 I.U.α-tocopherol and 10 mg. vitamin K_3 in 5 gm. starch. This presumably was nutritionally adequate for growth in the mouse.

Diets 3,4,5 and *6* contained 35 per cent *Tricholoma* in place of all of the cellulose, all of the vitamin-free casein and part of the starch. Calculated comparisons of the amino acid contribution of diets containing 35 per cent mycelium (16 per cent protein in the diets) with the requirements for rat growth (8), indicated that methionine and phenylalanine would be limiting amino acids. *Diets 3,4,5* and *6* were designed to test the validity of these calculations. *Diet 7* involved the further addition of vitamin-free casein to investigate the possibility of amino acid deficiencies other than the two mentioned. *Diet 8* was included to determine if a higher level (50 versus 35 per cent) of mycelium would compensate for a possible low biological value of the protein and also to test further the possibility of toxicity.

[1] Solka floc B.W.—100, Brown Corp., Montreal, Que.
[2] Bone meal 280, $CaCO_3$ 470, NaCl 250, $FeSO_4$ 20, $MgSO_4$ 0.5, $CuSO_4$ 0.5 and KI-calcium stearate 0.1 gm.

Diet 9 was similar to *Diet 2* except that 5 per cent *Tricholoma* was substituted for the vitamin mixture. The fat-soluble vitamins A, D, E and K were added separately; hence this diet was used for a gross evaluation of the B-complex content.

Diet 10 was similar to *Diet 7*, except that no amino acid supplements were added; hence it permitted a comparison of vitamin-free casein with a combination of methionine and phenylalanine sufficient to meet rat requirements for these amino acids.

Experiment No. 2

This test involved eight rations similar in formulation to synthetic *Diet 2* in the previous experiment, except that Drackett Assay Protein C-17[1] was substituted for vitamin-free casein. *Diets 11* to *14* inclusive contained 5 per cent of rancid corn oil, and *Diets 15* to *18* had fresh corn oil added. *Diets 11* and *15* were unsupplemented. *Diets 12* and *16* contained 0.05 per cent DPPD[2] (N.N′-diphenyl-p-phenylene diamine) mixed in the oil; *Diets 13* and *17* contained 10 per cent *Tricholoma* replacing 5 per cent each of starch and the vitamin mixture. *Diets 14* and *18* contained both mycelium and DPPD.

Diets 19 to *33* contained either 5 per cent fresh corn oil, 5 per cent rancid corn oil, or 5 per cent "Crisco" as the fat component. To obtain further information on the nature of the factor affecting peroxide values each of the fat treatments was further subdivided into five treatments: (a) control; (b) 10 per cent mycelium; (c) 10 per cent Skelly F solvent-

TABLE 1.—CHEMICAL COMPOSITION OF DRIED *Tricholoma nudum*

Component	Concentration
Crude protein (Nx6.25), %	44 to 50
Crude fat, %	4
Ash, %	6
Moisture, %	2
Carbohydrate, as glucose, %	24
Lysine, mg./gm.	30
Threonine, mg./gm.	17
Arginine, mg./gm.	21
Histidine, mg./mg.	13
Valine, mg./gm.	18
Methionine, mg./gm.	8
Leucine, mg./gm.	30
Isoleucine, mg./gm.	14
Phenylalanine, mg./gm.	17
Tryptophane, mg./gm.	16
Riboflavin, mg./lb.	24
Niacin, mg./lb.	68
Pantothenic acid, total, mg./lb.	66
Pantothenic acid, free, mg./lb.	54
Choline, gm./lb.	5.4
Thiamin, mg./lb.	5

[1] Drackett Products Co., Cincinnati, Ohio.
[2] Merck & Co., Ltd., Montreal, Que.

extracted mycelium; (d) 10 per cent of mycelium autoclaved 30 minutes at 15 lb. pressure, and (e) 10 per cent of mycelium heated at 105° C. for 24 hours. No B-vitamins were added to diets containing *Tricholoma*.

Feeding and management procedures were similar to those in Experiment 1, except that male mice were used.

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition relative to the major constituents, amino acids and B-vitamins is given in Table 1. As mentioned previously, a comparison of the amino acid distribution in this material with the growth requirements of the rat indicated methionine and phenylalanine as the first and possibly the only limiting acids. It is perhaps significant that no essential amino acids were grossly deficient and none was present in great excess. However, there was a surplus of both arginine and tryptophane, relative to rat requirements, when the mycelium supplied the entire protein at a 16 per cent protein level in the diet.

The protein content of dried *Tricholoma* was in the range of 44 to 50 per cent. The fat and ash contents were typical of many common feedstuffs but it will be noted that approximately one-fifth of the material was unaccounted for in the analysis. According to Foster (3) materials of this kind have been reported to contain appreciable quantities of pentosans, hemicellulose, "cellulose" and "lignin" as well as glycerol, none of which was investigated here.

Among the vitamins assayed, all except niacin proved to be higher than the amounts contained in average quality Torula yeast (2).

TABLE 2.—DIETS USED IN EXPERIMENT 1 FOR EVALUATING THE AMINO ACID AND GENERAL B-VITAMIN CONTENT OF *Tricholoma*, WITH FEEDING TRIAL RESULTS

Diet No.	Description	Gains[1]	Feed intakes
		gm.	gm.
1	Stock colony diet. Positive control	9.5	54
2	Synthetic (semi-purified) basal diet	—	—
	Synthetic basal containing no protein, no vitamins		
3	Basal + 35% *Tricholoma* + vitamins	8.5	44
4	Basal + 35% *Tricholoma* + vitamins + 0.13% l-methionine	9.7	45
5	Basal + 35% *Tricholoma* + vitamins + 0.30% l-phenylalanine	8.1	44
6	Basal + 35% *Tricholoma* + vitamins + 0.13% l-methionine + 0.30% l-phenylalanine	10.6	46
7	As in diet 6 + 4% vitamin-free casein	11.2	45
8	Basal + 50% *Tricholoma* + vitamins	10.2	50
9	Basal + 20% vitamin-free casein + 5% *Tricholoma*	10.7	44
10	Basal + 35% *Tricholoma* + 4% vitamin-free casein + vitamins	10.4	45

[1] Necessary difference is 0.6 gm. (P=.05).

Experiment No. 1

The results of this trial are shown in Table 2. The basal diet (No. 2) proved to be deficient in potassium; however it was shown (1) that 5 to 10 per cent of *Tricholoma* added to the diet would provide the necessary potassium.

With respect to the amino acids, the inclusion of *Tricholoma* as the only source of protein and with a 16 per cent level of protein did not permit normal growth as compared to the stock diet. The addition of methionine brought growth rates to normal, whereas phenylalanine alone failed to do so. Thus methionine is the first limiting amino acid. A combination of the two amino acids resulted in a significant improvement over methionine alone and also over the stock diet, which, of course, may have resulted in part from the relatively high digestible energy content of the synthetic diet. The feeding trials thus confirmed the calculations based on amino acid analysis in relation to rat requirements.

TABLE 3.—THE EFFECTS OF *Tricholoma* PREPARATIONS AND OF DPPD ON RANCIDITY IN DIETS HELD AT 25° C. FOR 3 WEEKS

Diet No.	Ration description	Mouse gains	Peroxide values		Final
			Initial	Mid-test	
	Synthetic basal plus:	gm.		(m.e./kgm. fat)	
11	5% rancid corn oil	—			551
12	5% rancid corn oil + 0.05% DPPD	—			151
13	5% rancid corn oil + *Tricholoma*	11.5			28
14	5% rancid corn oil + *Tricholoma* + DPPD	13.1			16
15	5% fresh corn oil	—			604
16	5% fresh corn oil + 0.05% DPPD	—			49
17	5% fresh corn oil + *Tricholoma*	12.2			9
18	5% fresh corn oil + *Tricholoma* + DPPD	11.7			6
19	5% fresh corn oil	—	247	265	90
20	5% fresh corn oil + *Tricholoma*	12.9	6	7	8
21	5% fresh corn oil + extr. *Tricholoma*	10.1	9	7	6
22	5% fresh corn oil + autoclaved *Tricholoma*	4.4	6	5	6
23	5% fresh corn oil + heated *Tricholoma*	10.2	5	5	7
24	5% rancid corn oil	—	441	227	108
25	5% rancid corn oil + *Tricholoma*	16.2	35	17	20
26	5% rancid corn oil + extr. *Tricholoma*	10.1	37	31	40
27	5% rancid corn oil + autoclaved *Tricholoma*	4.7	17	19	19
28	5% rancid corn oil + heated *Tricholoma*	10.1	7	28	27
29	5% Crisco	—	13	82	104
30	5% Crisco + *Tricholoma*	12.2	3	4	5
31	5% Crisco + Extr. *Tricholoma*	11.7	5	7	8
32	5% Crisco + autoclaved *Tricholoma*	5.0	8	5	4
33	5% Crisco + heated *Tricholoma*	11.5	2	3	5

The addition of 4 per cent vitamin-free casein to the amino acid-supplemented diets resulted in further increases in growth and feed efficiency. This finding was not explored further to determine its nutritional significance, but the results from feeding 50 per cent of *Tricholoma* to increase the protein intake and to provide about as much of the two limiting amino acids as required indicates that protein level *per se* was not too low. It is thus possible that, if higher levels of one or both of the supplementary amino acids had been fed, growth rates would have been greater.

The results from *Diet 9* indicate that the use of 5 per cent of *Tricholoma* in place of the synthetic B-vitamin mixture allowed normal growth. This confirms and extends the conclusions based on vitamin assays to the effect that *Tricholoma* is an excellent source of B-vitamins.

Experiment No. 2

The results of this test are shown in Table 3. The gains obtained in the growth studies are not shown for those diets which were deficient in potassium (*Diets 11, 12, 15, 16, 19, 24, 29*), but it may be stated that normal growth was obtained in later work in which similar diets contained at least 0.2 per cent potassium (1). A marked depression in gains was evident with *Diets 22, 27* and *32* which contained autoclaved *Tricholoma*. Supplementary thiamin was shown later to restore the ability of these diets to support normal growth. The same applied to the dry-heated *Tricholoma* treatments but it is not evident what dietary factor was affected by solvent extraction.

Of most interest in the experiment was the finding that *Tricholoma* treated or untreated, was associated with low peroxide values. The peroxide titres were 50 and 4 m.e./Kgm. fat for rancid and fresh corn oils, respectively, before they were incorporated in the rations. The values for diets containing fresh oil remained near the initial value over the 21-day period in the presence of the mycelium but showed marked rancidity in its absence. There was a suggestion of an additive effect between *Tricholoma* and DPPD (*Diets 13, 14, 17, 18*).

The peroxide values in diets to which rancid oil had been added were consistently lower than the initial values and the diets remained relatively free of the rancid odours characteristic of the control rations. It is noted that the peroxide data are not related to the growth rates of the mice; hence the treatments of the *Tricholoma* had no obvious effect upon the peroxide responses.

No explanation can be offered at this time for the apparent effects of *Tricholoma* on peroxide values but the finding is of special interest with regard to the incorporation and utilization of fats in feeds. Beyond this it is noteworthy that *Tricholoma nudum* residue was found to be approximately equal to Torula yeast in B-vitamin content and to be a relatively good source of arginine and tryptophane. The first limiting amino acid was methionine.

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