CASSAVA AS ANIMAL FEED

Proceedings of a workshop held at the University of Guelph 18-20 April 1977

Sponsored by the International Development Research Centre and the University of Guelph

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Editors: Barry Nestel and Michael Graham

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Postal Address: Box 8500, Ottawa, Canada K1G 3H9
Head Office: 60 Queen Street, Ottawa

Nestel, B.
Graham, M.
IDRC
University of Guelph IDRC-095e

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Contents

Laboratory Animal Nutrition with Fungi Grown on Cassava

J. C. Alexander[1]

Various strains of fungi including *Aspergillus fumigatus* were grown on media based on cassava carbohydrate, and evaluated for their nutritional quality, including safety for use as an animal feed. All samples had a low sulfur amino acid level. Biological evaluations included protein efficiency ratio (PER) and net protein ratio (NPR) methods, with male weanling rats. The protein control showed significantly better results for PER, NPR, and weight gain than the fungal proteins. However, by basing the dietary protein on the α-amino acid content, and supplementing it with methionine there was much better performance by the rats. The feeding of *A. fumigatus* resulted in increased relative kidney weights, and at high levels of intake (30 and 40%) more blood urea nitrogen, and a drop in blood albumin. A deficiency in methionine, due to reduced feed consumption, may have contributed to these changes. No significant differences between the control and experimental groups were found by histopathological examinations.

Microorganisms can synthesize protein from substrates such as hydrocarbons and carbohydrates very rapidly (Snyder 1970; Spicer 1973). Hydrocarbons are still available in vast quantities, but as the cost continues to increase, renewable carbohydrate substrates may prove to be economic in certain situations. The greatest need for more food with adequate protein quality and quantity is often in densely populated tropical areas. Cassava (*Manihot esculenta*) is a starch-producing root crop cultivated extensively and almost exclusively in tropical regions of Asia, Africa, and South America as a staple foodstuff (Gutierrez and Anderson 1972). In terms of calories per hectare, its yield is among the highest of any cultivated plant (Martin 1970; de Vries et al. 1970). Because the protein content of cassava is low, its consumption has contributed to malnutrition in areas where it serves as a substantial portion of the diet (Bailey 1961). The average crude protein expressed on a dry weight basis does not exceed 3%, and the quality in terms of essential amino acids is not good (Latham 1965; Hendershott 1972).

In spite of extensive information on the use of cassava in rations for swine (Maner 1972; Gomez et al. 1977), there is still concern about toxicity attributed to its cyanogenic glucosides, particularly linamarin with its potential for production of hydrocyanic acid (Wood 1965; Oke 1969; Nartey 1973). Some studies have shown a correlation of the prevalence of tropic ataxic neuropathy, and endemic goiter with the frequency of dietary intake of cassava (Osuntokun 1973; Ekpechi 1973). In the fermentation of cassava it is detoxified by the liberation of hydrocyanic acid at a low pH (Akinrele 1964).

Gray and Abou-el-soud (1966) investigated microbial protein production from cassava, but did not study the nutritive value or safety of the fermentation products for feeding animals. Other workers (Stanton and Wallbridge 1969; Brook et al. 1969) carried out fungal fermentation of an extruded paste from flour of cassava, and with the genus *Rhizopus* showed a sevenfold increase in protein content of the product over that of the cassava substrate.

Assessment of *Aspergillus oryzae* grown on barley grain by Smith et al. (1975) indicated unsatisfactory results particularly with pig feeding tests. For this and other organisms studied, they reported non-α-amino N levels between 10 and 20% of the total N of the mycelia. Later, Smith and Palmer (1976) evaluated yeasts and bacteria as dietary protein sources for animals. Supplementation with methionine was found to be beneficial. Vander Wal (1976) has reviewed experience with SCP in animal feeding in Europe and Shenderei (1976) comments on experience in the USSR.

Nutritive Value

Studies were carried out by Khor et al. (1976) that demonstrated the nutritive value for rats of thermotolerant fungi grown on cassava. Two strains of *A. fumigatus* (I-21 and I-34), a nonreverting asporogenous mutant of *A. fumigatus* I-21 (I-21A), one strain of *Sporotrichum thermophila* (I-36), and one strain of *Paecilomyces sp.* (I-39) were included.

[1]Department of Nutrition, College of Biological Science, University of Guelph, Guelph, Canada N1G 2W1.

Table 1. The amino acid content of some microbial protein sources[1]

	Source of protein					
	I-21[2]	I-21A[3]	I-34[2]	I-36[2]	I-39[2]	Casein
Aspartic acid	6.0	4.3	5.6	4.7	5.0	7.2
Threonine	3.7	3.7	3.8	3.4	3.2	4.5
Serine	3.9	3.7	4.5	3.1	3.4	5.4
Glutamic acid	7.8	11.4	10.3	8.9	9.6	22.0
Proline	3.4	7.0	3.5	3.2	2.9	11.3
Glycine	5.0	4.3	4.8	3.8	3.8	1.9
Alanine	7.2	5.4	7.7	7.8	7.1	3.0
Valine	5.4	4.6	5.0	4.2	4.2	6.5
Cystine[4]	0.7	0.3	0.5	0.6	0.6	0.4
Methionine[4]	1.4	1.3	1.4	1.1	1.3	2.9
Isoleucine	4.5	3.6	4.3	3.5	3.7	5.7
Leucine	7.1	6.0	6.6	5.5	5.7	9.3
Tyrosine	2.8	2.6	2.9	2.4	2.4	5.5
Phenylalanine	4.8	3.5	4.5	3.7	3.8	5.3
Lysine	7.0	5.3	6.3	5.6	5.6	8.3
Histidine	1.3	1.5	1.5	1.2	1.2	3.0
Arginine	5.8	5.0	6.0	5.4	5.7	3.8
Tryptophan[5]	1.0	—	1.0	1.2	0.8	1.7

[1]Values are averages of duplicate analyses expressed as g/16 g nitrogen.
[2]Substrate: cassava extract medium.
[3]Substrate: whole cassava medium; product contained unfermented cassava residues.
[4]Microbiological assay.
[5]Not done for I-21A.

Gregory et al. (1976) reported the conversion of carbohydrates to protein by high temperature fungi, and the methods of Reade and Gregory (1975) involving high temperature, low pH fermentations, and a self-aspirating fermentor developed by Azi et al. (1975) were used. The medium was heated to 80 °C for 30 min during preparation. Fermentations were carried out nonasceptically at pH 3.5 and 45–50 °C for 20 h. The mould mycelia were recovered by filtration, washed once by resuspending in deionized water (0.5 × culture volume), and freeze-dried. Samples were hydrolyzed with nitrogen-saturated HCl in sealed tubes at 110 °C for 24 h. These hydrolyzates were analyzed for amino acids by ion-exchange chromatography. Values for methionine, cystine, and tryptophan were low, due to loss by this method, so alternate methods were applied. For the two sulfur-containing amino acids a microbiological technique using *Leuconostoc mesenteroides* was carried out. Tryptophan was determined by a spectrophotometric procedure involving hydrolysis by BaOH, and colour development by *p*-dimethylaminobenzaldehyde. Values for amino acid content of the microbial protein sources are shown in Table 1. The low levels of sulfur amino acids in the fungus samples are apparent. The other amino acids were in a more favourable balance. Proximate analyses are in Table 2. The first four fungi were grown on a cassava extract medium, and data were quite similar except that I-21 was higher in lipid content. For the one sample grown on whole cassava medium (I-21A), levels of lipid and crude fibre were relatively low, but the calcium content was increased.

Protein quality was evaluated by Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) techniques. The animal diet consisted of 10% corn oil, 4% mineral mixture, 1% vitamin mixture, 5% celluflour, and 80% corn starch. The level of corn starch added depended upon the amount of casein or microbial protein used at the expense of the corn starch to provide 10% protein in the diet (see Table 3 for results). For statistical analyses of the data, values with common superscripts are not significantly different ($p < 0.05$) by Duncan's multiple range test. Animals fed the supplemented casein had the most weight gain, and better average feed efficiency. Fungi I-36 and I-39 gave particularly poor results. Regarding the PER, casein and I-21 were best, but all samples produced similar NPR values.

Table 2. Proximate analyses (%) of some protein sources[1]

	Crude protein	Ether extract	Crude fibre	Calcium	Phosphorus
I-21[2]	40.0	12.2	25.4	0.01	0.82
I-34[2]	41.8	6.6	22.5	0.02	0.97
I-36[2]	37.1	6.6	23.4	—	—
I-39[2]	32.6	8.9	19.3	—	—
I-21A[3]	32.7	2.6	14.8	0.13	0.69

[1]Values are averages of duplicate analyses.
[2]Substrate: cassava extract medium.
[3]Substrate: whole cassava medium; product contained unfermented cassava residues.

Table 3. Fungal mycelia from cassava extract medium compared to supplemented casein as the sole sources of dietary protein based on crude protein content (average results from 10 rats per group).

	Weight gain	Feed efficiency (g feed/g gain)	PER[1] (g gain/g protein)	NPR
I-21	210^{bc}	4.2^{bc}	2.1^{ab}	3.6^{a}
I-34	180^{cd}	4.7^{ab}	1.9^{bc}	3.5^{a}
I-36	129^{e}	5.3^{a}	1.7^{c}	3.4^{a}
I-39	148^{de}	5.5^{a}	1.6^{c}	3.1^{a}
Casein[2]	315^{a}	3.4^{c}	2.5^{a}	3.4^{a}

[1]PER values were normalized relative to a value of 2.5 for the casein standard.
[2]Supplemented with 0.3% DL-methionine and 0.1% DL-tryptophan.

Table 4. Fungal mycelia supplemented with methionine compared with casein as sole sources of dietary protein based on crude protein content (average results from 10 rats per group).

	Weight gain (g)	Feed efficiency (g feed/g gain)	PER[1] (g gain/g protein)	NPR
I-21	155^{c}	7.1^{a}	1.3^{c}	2.5^{c}
I-21 + 0.6% DL-methionine	255^{b}	4.0^{bc}	1.8^{b}	3.4^{b}
I-34	140^{c}	6.6^{a}	1.2^{c}	2.7^{c}
I-34 + 0.6% DL-methionine	230^{b}	4.0^{bc}	1.8^{b}	3.4^{b}
Casein[2]	346^{a}	2.9^{c}	2.5^{a}	4.2^{a}

[1]PER values were normalized relative to a value of 2.5 for standard casein.
[2]Supplemented with 0.3% DL-methionine and 0.1% DL-tryptophan.

Analyses had shown that the microbial proteins were low in methionine, and it was considered that supplementation of the test diets with DL-methionine might improve the performance of these proteins. Beneficial effects were seen (Table 4) for *A. fumigatus* strains I-21 and I-34 as illustrated by higher PER and NPR values, as well as better weight gains and feed efficiencies. Nevertheless, in spite of the extra methionine added to the fungal proteins, the supplemented casein still performed better.

Further studies based the protein level of the diets on the α-amino acid content, because a portion of the nitrogen in microbial proteins is contributed by nonprotein sources such as nucleic acids. The results in the upper portion of Table 5 show that I-21 and I-34 as supplemented, produced comparable feed efficiency and NPR values to those for the casein. Weight gains and PER values were, however, lower. A different experiment produced the results in the lower part of Table 5. Here I-21A was grown on whole cassava medium, and the product contained unfermented cassava residues. The casein was not supplemented, but still performed relatively

Table 5. Fungal mycelia and fermented cassava compared with casein as sole sources of dietary protein based on α-amino acid content (average results from 10 rats per group).

	Weight gain (g)	Feed efficiency (g feed/g gain)	PER[1] (g gain/g protein)	NPR
I-21 + 0.6% DL-methionine	226[b]	4.8[a]	2.2[b]	3.6[a]
I-34 + 0.6% DL-methionine	210[b]	4.7[a]	2.0[b]	3.4[a]
Casein + 0.3% DL-methionine + 0.1% DL-tryptophan	392[a]	2.8[a]	2.5[a]	3.8[a]
Cassava fermented by I-21A	83	4.5	1.5	3.2
Casein	153	2.6	2.5	5.0

[1]PER values were normalized to a value of 2.5 for the casein standard.

better than the fermented cassava. Nevertheless, note the low weight gains for casein alone as the protein source.

The rats fed I-21A grown on the whole cassava medium had very poor growth during the first week of the experiment (Fig. 1). Because feed intake was comparable to that for the casein-fed animals the poor growth performance resulted from inefficient utilization of the feed. For example, the feed efficiency during the first week was 2.4 for the casein group, and 10.7 for the I-21A. During this time there was considerable variation in weight gain response by individual animals within the I-21A dietary group, indicating that the rats did not adapt readily to the diet. After the initial lag period, the growth rate improved greatly (Fig. 1).

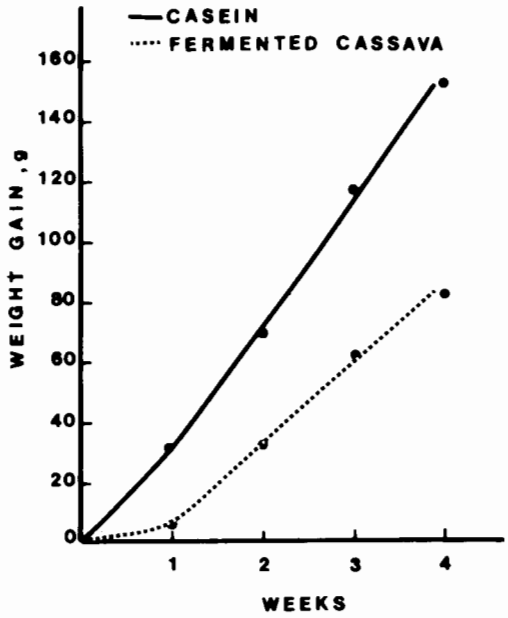

Fig. 1. Weekly weight gains by rats fed casein or cassava fermented by A. fumigatus I-21A.

Safety Evaluation

It was recognized that the fermentation products from fungi grown on cassava should be subjected to rigorous toxicological examination prior to feeding them to farm animals. The Protein-Calorie Advisory Group of the United Nations System (PAG) has issued official guidelines for systematic clinical testing of new protein sources (PAG Guideline No. 6 1970; PAG Statement No. 4 1970; PAG Guideline No. 15 1974). The *A. fumigatus* I-21 studied for nutritive value (Khor et al. 1976) was subjected to a safety evaluation for use as an animal feed (Khor et al. 1977).

There were 10 male weanling rats per group and all diets and water were offered ad libitum. The test groups received the fungus sample at levels of 20, 30, or 40% of the ration, whereas the control group was provided with soybean oil meal as the sole source of protein. The protein contents of the test diets were brought to the same level as that in the control diet (18% crude protein) by addition of soybean oil meal (Table 6). The rats were maintained on these rations for 90 days, then killed, and autopsied. Body weight and feed consumption data were obtained, and extensive clinical and histopathological examinations were carried out.

Table 6. Composition of diets (g/kg) for the subchonric toxicity study of *A. fumigatus* I-21.

	Control diet	Fungus diets		
		20%	30%	40%
Soybean oil meal	408	263	190	117
Fungus I-21	—	200	300	400
Mineral mixture	40	40	40	40
Vitamin premix	20	20	20	20
Corn oil	60	60	60	60
Celluflour	10	10	10	10
Corn starch	458	403	376	349
DL-methionine	4.0	4.0	4.5	4.5

The average body weights were consistently higher for the control diet than for the fungus diets (Table 7). However, there was no statistical difference in weight gain throughout the experiment between the rats fed 30 and 40% fungus, but both of these groups were lower than the 20% fungus group. The control animals ate more feed but there were no important differences in feed efficiency data.

In general, none of the organ weights, except for the kidneys, showed significant differences between fungus-fed groups and the control group (kidney weights (g/100 g body weight) were: soybean oil meal 0.81; 20% I-21 0.95; 30% I-21 1.01; 40% I-21 0.98). The blood glucose and glutamic-pyruvic transaminase concentrations were not altered by feeding the fungus diets, but urea nitrogen levels tended to rise at the higher levels of intake (soybean oil meal 20; 20% I-21 20; 30% I-21 22; and 40% I-21 24 mg urea N/100 ml). The glutamic-oxaloacetic transaminase, and alkaline phosphatase showed no relationship to dose level. Rats fed 30 and 40% of *A. fumigatus* I-21 had a significant drop in serum albumin (Table 8) but other serum protein values were not changed.

Blood values obtained at termination of the feeding study were not remarkable except for small changes in leukocyte counts, and urine samples showed normal colour, and tests for glucose, acetone, bilirubin, protein, pH, and blood. Kidney function tests revealed that rats in all groups were able to concentrate their urine when deprived of water. Histopathological examinations of many tissues indicated no significant differences between the control and test groups.

Discussion

Microbial proteins in general have a low methionine content, and supplementation with this amino acid is necessary to obtain a protein quality approaching those of animal sources. Early studies (Skinner and Müller 1940; Klose and Fevold 1945) revealed that

Table 7. Body weight, feed consumption, and feed efficiency of rats fed soybean oil meal or *A. fumigatus* I-21 at different dietary levels.

	Body weights (g)			Feed consumption (g/rat/day)			Feed efficiency (g feed/g gain)		
	4 wk	8 wk	12 wk	4 wk	8 wk	12 wk	4 wk	8 wk	12 wk
Soybean oil meal	247[a]	335[a]	357[a]	17.3[a]	19.2[a]	18.7[a]	2.8[b]	6.3	21.9
20% I-21	211[b]	272[b]	277[b]	14.3[b]	14.8[b]	13.8[b]	2.9[b]	7.2	34.8
30% I-21	190[c]	243[c]	245[c]	13.3[b,c]	13.9[b]	12.2[b]	3.2[a]	8.1	34.2
40% I-21	176[c]	241[c]	241[c]	12.5[c]	13.6[b]	12.5[b]	3.5[a]	10.3	38.9

Table 8. Plasma protein values for rats fed soybean oil meal or *A. fumigatus* I-21 at different dietary levels for 90 days.

	Total serum proteins (g/100 ml)	Serum albumin (g/100 ml)	Serum globulin (g/100 ml)	Albumin / Globulin
Soybean oil meal	6.0	4.2[a]	1.9	2.2
20% I-21	6.0	3.9[a,b]	2.0	2.0
30% I-21	5.9	3.7[b]	2.1	1.8
40% I-21	5.6	3.6[b]	2.0	1.8

both moulds and yeast gave much improved performance as protein sources for rats if supplemented with methionine. Bressani (1968) reported a dramatic improvement in PER when 0.5% DL-methionine was added to torula yeast. Also, *Candida lipolytica* grown on alkanes had a higher biological value when supplemented with 0.3% DL-methionine (Shacklady and Gatumel 1972).

In the study of Khor et al. (1976) the strains of *A. fumigatus* tested produced better results if supplemented with methionine and evaluated by weight gain of rats, feed efficiency, PER and NPR values. There was, however, considerable variation in feed intake among rats maintained on diets containing the fungi. When rations are offered ad libitum, many factors contribute to the amounts of feed actually consumed. The flavour, dry texture, and nutritive balance of the microbial proteins could have adversely affected the intake of feed. When growth is to be compared, a difference in feed intake makes conclusions regarding composition of the rations more difficult. Consequently, PER calculated from the results of ad libitum feeding at a 10% level tends to penalize the less palatable protein source.

With a substantial portion of the microbial nitrogen in the form of nonprotein nitrogen, unless the dietary protein level is based on α-amino nitrogen, the fungal proteins are penalized further.

The increase in kidney weights relative to body weights of rats fed *A. fumigatus* I-21 was not accompanied by pathological changes. Therefore, this increase was considered to be a response to metabolic requirements. The tendency toward an elevation in blood urea nitrogen with an increase in the fungus level in the diet could indicate an imbalance of amino acids in these rats as a result of decreased feed intake. Thus, excess amino acids would have to be deaminated. Nitrogenous substances in the fungi such as glucosamine in the cell wall, and nucleic acids would also contribute to urea nitrogen.

Selye (1950) has explained that many types of dietary deficiencies could act as stressor agents in exerting effects upon blood components. The highest levels of fungus could have resulted in some measure of stress, related to the decreased feed intake and possible deficiency of methionine. This might have depressed the albumin synthesis, and exerted a physiological influence on the ACTH to bring about small changes in the leukocyte counts. Volesky et al. (1975) fed diets containing fungus grown on natural gas to rats for 5 months. They found growth depression, decreased leukocyte counts, and increased kidney weights, but no pathological changes were observed at autopsy.