MICROBIAL PROTEINS IN DIETS FOR RATS

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SUMMARY

Two experiments involving 130 rats were conducted to evaluate the nutritive value of three microbial proteins for rats. In one of the experiments, diets containing 0, 20 and 30% microbial protein were offered for 21 days. Inclusion of up to 20% microbial protein did not significantly affect liveweight gain but a comparatively poor feed conversion efficiency was manifested at levels above 10% inclusion of the protein concentrate in the diet. In a second experiment, the microbial protein was incorporated at a level of 30% as the sole source of protein to measure the digestibility of nitrogen and RNA. The apparent digestibility of nitrogen was 80 - 89% and that of RNA was 76 - 83%.

INTRODUCTION

There is need to increase food production particularly proteins to satisfy the needs of the increasing human population. Traditional agricultural practice alone cannot meet this demand.

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In recent years, efforts have been intensified to increase the production of protein from microorganisms which can utilise organic waste. These microorganisms include bacteria, yeasts, filamentous fungi and algae (Ratledge, 1968; Calloway and Kumar, 1969; Clement, 1972). Their production involves fermentation procedure with the exception of algae which are produced photosynthetically. Substrates such as carbohydrates (cellulose, starch), petroleum waste or by-products (gas oil, n - paraffins, methane,methanol and ethanol) are being used as the carbon source - Ghose, 1969; Klug and Markovetz, 1971; Spicer, 1971; Bewersdorff and Dostalek, 1971. Apart from algae, these microorganisms derive energy from the oxidation of organic compounds. Ammonia and nitrates serve as the source of nitrogen and the medium is supplemented with minerals.

Since these products are intended for use as protein and energy sources for animals and humans in place of conventional protein supplements, knowledge of their nutritive qualities is desirable. Some nutrition experiments using chicks and pigs have been reported by Van Weerden, Shacklady and Van der Waal (1970); Lewis, Boorman and Moorgan (1971) and Shannon and McNab (1972). The objective of this study was to investigate the effect of including graded levels of three microbial proteins in the diets of rats on their growth rate and feed efficiency. The digestibility of the nitrogen and ribonucleic acid (RNA) was also determined. Two of the microbial proteins are yeasts, one was grown on n-paraffins and used as such (Yeast 1), the other was grown on crude gas oil and therefore was solvent extracted to remove residual crude oil after harvesting (Yeast 2). The third microbial protein was a filamentous fungus grown on carbohydrate waste (Filamentous fungus).

MATERIALS AND METHODS

Diets.

The effect of different dietary levels of inclusion of the microbial protein on growth rate and feed efficiency was determined by replacing 10, 20 and 30% of white fishmeal present in a basal diet (Table 1) with each of the microbial protein. Casein was added in place of glucose to raise the protein level of diets containing the microbial samples, where necessary, to the same level as that of the basal diet. The fishmeal used in this diet contains 69.11% crude protein on a dry matter basis.

A ration (Table 2) in which each of the test samples was included at a level of 30% as the sole source of protein was fed to measure the digestibility of the nitrogen and RNA. Feed and water were offered *ad libitum*.

Animals.

Weanling male Wistar rats, average weight 48.7g, were randomly allocated at the rate of 10 rats per treatment. Rats were weighed at the start of the experimen

Table 1. Composition of diets used for the growth experiment. Values expressed as percent of diet.

	1	2	3	4	5	6	7	8	9	10
Glucose	20.0	19.1	18.2	17.3	20.0	20.0	20.0	18.5	17.0	15.4
Maize starch	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3	12.3
Dextrinized starch	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4
Maize oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Cellulose powder	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
White fishmeal	32.0	22.0	12.0	2.0	22.0	12.0	2.0	22.0	12.0	2.0
Casein	-	0.9	1.8	2.7	-	-	-	1.5	3.0	4.6
Mineral mixture [a]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
B-vitamin supplement [b]	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Fat soluble vitamin [c]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Yeast 1	-	10.0	20.0	30.0	-	-	-	-	-	-
Yeast 2	-	-	-	-	10.0	20.0	30.0	-	-	-
Filamentous fungus	-	-	-	-	-	-	-	10.0	20.0	30.0

a. Mineral mixture supplied the following g/kg of diet: KH_2PO_4, 15.54; $MgSO_4$, 2.29; $CaCO_3$, 15.24; $FeSO_4 \cdot 7H_2O$, 1.08; KI, 0.03; $MnSO_4 \cdot 4H_2O$, 0.230; $ZnCO_3$, 0.020; $CuSO_4 \cdot 5H_2O$, 0.019; $CoCl_2$, 0.001; NaCl, 15.55.

b. B-vitamin supplement supplied the following g/kg of diet; thiamine, 0.003; riboflavin, 0.01; pyridoxine, 0.004; calcium panthothenate, 0.06; nicotinic acid, 2.0; inositol, 4.0; p-aminobenzoic acid, 6.0; biotin, 0.002; folic acid, 0.05 and choline, 12.0.

c. Fat soluble vitamin mixture supplied the following g/kg of diet: retinol, 10,000 IU; ergocalciferol, 500 IU; menaphthone, 2 mg, and mixed tocopherols, 2mg.

Table 2. Composition of diet used for the digestibility experiment

Ingredient	%
Glucose	20.0
Maize starch	14.3
Dextrinized starch	19.4
Maize oil	5.0
Cellulose powder	5.0
Microbial protein	30.0
Mineral mixture [a]	5.0
B-vitamin supplement [b]	1.1
Fat soluble vitamin [c]	0.1
Methionine	0.1

a, b, c - same as for Table 1.

and after 21 days. Feed intake was measured over the 21 day period. For the determination of nitrogen and RNA digestibility, feed intake over the last 10 days of the experiment was used. Faeces and urine were collected over this period.

Collection of faeces and urine.

A total collection method was employed. Contaminants such as hairs were removed and faeces and urine were removed 12 hourly. Faeces were stored in plastic cups and kept frozen at -20°C before freeze drying. This was considered necessary to preserve the nitrogen (Shannon and Brown, 1969). Faecal samples collected over the 10 day period for each treatment were bulked, ground and thoroughly mixed before undergoing analysis. The urine was collected over toluene and sieved with glass wool to remove hairs before storage at -20°C.

Analytical Procedure.

Total nitrogen was determined by the standard Kjeldahl procedure and gross energy, by the use of an adiabatic bomb calorimeter. Dry matter and ash were determined according to methods recommended by A. O. A. C. (1970). The lipid content was determined using the ether extraction and the acid hydrolysis method (A. O. A. C., 1970) while for RNA estimation, a method of Schmidt and Tannhauser (1945) was used. Proximate analysis of the microbial proteins was carried out while diets, faeces and urine from the experiment on digestibility of nitrogen and RNA were analysed for nitrogen and RNA.

RESULTS AND DISCUSSION

The proximate composition of the three microbial proteins is listed in Table 3. No mortality was recorded throughout the course of the experiment.

Growth and Feed Conversion Efficiency.

The mean liveweight gain and feed conversion efficiency is presented in Table 4. Although, the mean liveweight gain at either 10 or 20% level of inclusion

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Table 3. Proximate Analysis of Test Feedstuffs (Dry Matter Basis)

	Yeast 1	Yeast 2	Filamentous fungus
Crude Protein %	61.45	70.60	56.00
Ether extractible fat %	7.95	0.67	4.89
Fat determined by acid hydrolysis %	9.98	1.48	7.76
Ash %	6.01	7.32	7.62
Available carbohydrate %	0.52	1.61	2.88
RNA %	8.12	12.72	9.43
Gross energy kcal/g	5.19	4.76	4.88
Dry matter %	90.91	93.46	93.80
Unavailable carbohydrate (by difference) %	22.04	18.99	25.74

did not significantly differ from the control, the results obtained when 30% microbial protein was present in the diet was significantly (P 0.05) lower than that of the control. The values obtained at 10% inclusion level of the yeast samples were in fact about 3.7% greater than those given by the rats on the control diets although this difference failed to reach significance (P 0.05). Even though the test diets were balanced for protein as the control, the liveweight progressively decreased as the level of inclusion of the microbial protein in the diet increased and the values obtained at 30% inclusion level were significantly lower than those of 10% (P 0.05). This probably suggests deficiencies in certain amino acids as the level in the diet increased. The amino acid composition of the microbial proteins and fishmeal in Table 5 shows that the test materials are lower in the sulphur containing amino acids than fishmeal.

Table 4. **Mean Liveweight Gain and Feed Conversion Efficiency of Rats fed Various Levels of Microbial Protein.**

	Liveweight gain	Gain/Feed
Control ration	98.61 a	0.382 a
10% Test 1 in ration	102.31 a	0.375 a
20% " "	95.70 b	0.349 b
30% " "	87.33 e	0.346 b
10% Yeast 2 in ration	102.43 a	0.378 a
20% " "	92.00 cd	0.329 c
30% " "	89.33 de	0.333 c
10% Filamentous fungus in ration	97.80 b	0.385 a
20% " " "	94.52 bc	0.363 d
30% " " "	86.47 e	0.362 d

Means followed by the same letter down the column do not differ significantly (P 0.05).

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The differences between the feed conversion efficiency of rats fed the control ration and diets containing 10% of the microbial protein were not significant but thereafter, the feed efficiency declined and was significantly poorer (P 0.05) for the 20% and 30% dietary levels compared with the control diet. There were, however, no significant differences between 20 and 30% levels of inclusion for each of the test samples. The feed conversion efficiency was comparatively better for the filamentous fungus than for the two yeast samples. There was a poorer feed conversion at 20% inclusion of the yeasts in the diet even though it resulted in a comparable weight increase with the control which indicates that the rats attempted to compensate for nutrient deficiencies by increasing feed consumption.

Duthie and Edwards (1970) observed a slight growth increase when 5% of a yeast sample was included in diets for broiler chicks while 16.5% significantly depressed growth rate. Better growth rate has also been observed for turkeys when offered diets containing 5% yeast (Holmes, Holroyd and Shacklady, 1970) while Shannon and McNab (1972) reported that broiler chicks performed well when 10% herring meal was replaced by a yeast microbial protein. It is remarkable to note that even though the protein and energy contents of the filamentous fungus were slightly lower than those of the yeast (Table 3), feed conversion efficiency were better for this sample. Feed intake of rats fed diets containing the filamentous fungus was however lower than those fed yeast diets.

Table 5. Amino acid composition of microbial proteins and fishmeal. (Values are expressed in g/16gN)

Amino acid	Yeast 1	Yeast 2	Filamentous fungus	Fishmeal
Threonine	5.0	5.2	3.1	4.2
Glycine	4.8	5.0		9.5
Cystine	0.9	0.8	1.4	1.6
Methionine	1.8	1.8	2.0	2.7
Valine	5.7	5.6	6.9	5.2
Isoleucine	4.9	5.1	3.1	3.8
Leucine	6.9	7.4	8.7	6.8
Tyrosinel	3.9	3.6	3.4	2.7
Phenylalanine	4.8	5.1	3.8	4.0
Lysine	7.8	8.2	3.3	8.5
Histidine	2.3	2.1		3.0
Arginine	5.0	5.2		7.2
Tryptophan	1.6	1.5	1.5	0.9
Reference	(1)	(2)	(3)	(4)

1 & 2 Lewis *et al.*, (1971).
3 Spicer, (1971).
4 Lewis *et. al.*, (1971).

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Digestibility of Nitrogen and RNA.

The digestibility coefficients of the nitrogen were 86.7 ± 3.18%, 88.9 ± 2.7% and 80 ± 1.9% respectively for Yeast 1, Yeast 2 and the filamentous fungus. The digestibility of RNA was 81.2 ± 4.1%, 76.4 ± 3.8% and 83.1 ± 2.1% for the microbial proteins respectively. The values obtained for nitrogen digestibility of the yeasts were slightly higher than a value of 77% reported for chicks (Shacklady, 1967). Shannon and McNab (1973) however, reported a true digestibility coefficient of 90% for an n-paraffin grown yeast for chicks. The digestibility of the RNA obtained in the present study was reasonably high. RNA content of the microbial proteins was rather high varying between 8.12 and 12.72%. Generally microorganisms are much richer in nucleic acids than other feedstuffs (Miller, 1968) and as a result, 8-13% of the total nitrogen of yeasts are purines and about 4% are pyrimidines (Stokes, 1958). The effect of high dietary nucleic acid on blood levels of uric acid has been one of the factors which may limit the use of the microbial proteins as food sources for humans and animals. Although Edozien, Udo, Young and Scrimashaw (1970); Abrahamason, Hambraeus, Hofvander and Valquist (1971) showed that ingestion of high amounts of yeast resulted in a significant increase in plasma uric acid levels, the high purine base of microbial protein should not present a problem when used for animal feeding since animals can oxidise uric acid resulting from high nucleic acid intake to allantoin which is readily excreted. Birds particularly can accommodate high dietary levels of nucleic acid since the major end product of nitrogen in birds is uric acid. Shannon and McNab (1972) have reported that the inclusion of 20% yeast in a diet did not significantly affect plasma uric acid levels in 8 week-old birds.

The results of the present study suggest that the microbial proteins can be satisfactorily incorporated in diets as a source of protein at 10% and up to a level of 20% can even be tolerated in rats.

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LOCAL PEPPERS AS SOURCES OF VITAMIN A AND COLOUR IN THE EGG YOLK

By

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ABSTRACT

Two varieties of peppers, Capsicum frutescence and Capsicum annum were included in rations for laying hens at 2%, 4% and 6% levels. Egg production was not affected by these levels of the peppers but larger egg sizes were obtained. The annum produced more carotene in the egg yolk than the frutescence.

Similar results were obtained for the yolk vitamin A. Corresponding levels of the two peppers produced similar colours in the egg yolk and the dark-yolked eggs preferred by many consumers can be obtained with 4% and 6% levels of either peppers.

INTRODUCTION

Egg yolk colour plays a prominent role in egg acceptance by consumers. In Nigeria, intensive poultry production is one of the quickest approaches to alleviating low levels of animal protein consumed by the population. The Joint Planning Committee of the Federal Ministry of Agriculture and Natural Resources (1974) set the target for poultry production in 1976 at over 100 million birds.

If a quarter of this number are laying birds, then a substantial amount of eggs should be available in Nigeria.

Eggs are needed not only for direct consumption but also for the hatchery operation. Many Nigerians prefer dark pigmented egg yolks and as such consumers have preference for guinea fowl eggs which are available in appreciable quantities at limited periods of the year. Egg yolk colour had been adjusted in different parts of the world to suit local demands. A review of products so used as well as study of some Nigerian pasture species that pigment egg yolk had been made by Ogunmodede and Wogar (1971). As forage production throughout the year is still not practised widely in Nigeria, the possibility of inadequate supply and competition between herdsmen and poultry feed manufacturers for these pasture species had been envisaged. Consequently, other pigmenters for yolk are being investigated.

Furthermore, carotenoids and vitamin A are accumulated in the egg yolk in attempts to adjust the yolk colour. This is useful for the growth of chicks subsequently hatched from the eggs. Vitamin A plays a role in the rate of growth of animals and the relationship between liver reserves of retinol in chicks and protein metabolism was shown by Stoewsand and Scott (1964). The storage of vitamin A in the liver of chicks is important in areas where the rate of growth of intensively reared chicks is relatively low. Thus the accumulation of nutrients with vitamin A activities in the egg yolk is the first step in ensuring adequate store of the vitamin in the liver of newly hatched chicks.

Vitamin A and Egg Colour in Relation to Dietary Pepper Levels.

MATERIALS AND METHODS

Two varieties of peppers namely "Ata Were" *Capsicum frutescens* and "Shombo" *Capsicum annum* were obtained from the Federal Department of Agricultural Research, Moor Plantation, Ibadan. The peppers were dried, milled and incorporated into the rations, shown in Table 1 after the proximate analysis had been done.

Table 1. Percentage Composition of Experimental Rations.

Components	Rations			Number			
	1	2	3	4	5	6	7
Maize	61	59	57	55	59	57	55
Groundnut cake	18	18	18	18	18	18	18
Groundnut oil	3	3	3	3	3	3	3
Blood meal	2	2	2	2	2	2	2
Fish meal	3	3	3	3	3	3	3
Dried yeast	2	2	2	2	2	2	2
C. annum	-	2	4	6	-	-	-
C. frutescens	-	-	-	-	2	4	6
Rice bran	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Dicalcium phosphate	4	4	4	4	4	4	4
Oyster shell	3	3	3	3	3	3	3
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
A-D vitamins* (for layers)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
D. L. Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Antibiotics	+	+	+	+	+	+	+

* A synthetic vitamin and mineral mixture that supplied minimum requirement of the laying hen.