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The production and evaluation of protein derived from organisms grown on hydrocarbon residues

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This paper can give no more than a summary of the subject indicated in the title partly because of the range covered and partly because the work is still in progress. It is hoped to publish details of the work, as it is completed, in the appropriate journals.

The organisms with which we are now concerned are yeasts produced by fermentation on either pure n-paraffins or heavy gas oil. Because of the absence of oxygen in these substrates, it is necessary to supply this as well as a nitrogen source—usually ammonia—and an aqueous solution of the minerals required for yeast growth. Thus the mixture is one of two immiscible liquids, a gas and a solid—the yeast—which must be kept in intimate contact under carefully controlled conditions for successful fermentation.

The process is continuous, which implies that harvesting and purification are also continuous. Essentially the main difference between the two processes, i.e. n-paraffin and gas oil, is the need for a more complex purification stage in the case of the latter since only about 10% of the gas oil components are utilized in the actual production of the yeast. Our present intention is to operate the n-paraffin process at Grangemouth in Scotland and the gas oil process at Lavera in the South of France.

By the conventional $N \times 6{\cdot}25$ calculation, the protein content of n-paraffin grown yeast (BP protein concentrate) is 63–65% and that from gas oil 68–70%. But because of the different post-fermentation treatments the former contains approximately 9% of lipids, the latter about 1·5%. As might be expected, the amino acid composition of the protein is very similar in both cases and is shown in Table 1 along with that of fish meal and extracted soya-bean meal for comparison.

From this it would seem that the first limiting amino acid would be methionine and this indeed is borne out by determination of net protein utilization (NPU) and biological value (BV).

Table 1. *Amino acid composition of BP protein concentrate, fish meal and soya-bean meal*

Amino acid	Content (g/16 g N) BP protein concentrate	Fish meal	Soya-bean meal
Isoleucine	5·3	4·6	5·4
Leucine	7·8	7·3	7·7
Phenylalanine	4·8	4·0	5·1
Tyrosine	4·0	2·9	2·7
Threonine	5·4	4·2	4·0
Tryptophan	1·3	1·2	1·5
Valine	5·8	5·2	5·0
Arginine	5·0	5·0	7·7
Histidine	2·1	2·3	2·4
Lysine	7·8	7·0*	6·5
Cystine	0·9	1·0	1·4
Methionine	1·6	2·6	1·4
Cystine+methionine	2·5	3·6	2·8

*The published figures for lysine in fish meals cover a wide range. This is a mean value which also coincides with the mean value obtained in a substantial number of analyses of fish meal made by co-workers between 1961 and 1965.

Mean values for NPU (unsupplemented) for gas oil and n-paraffin grown yeasts are 41 and 48 respectively with corresponding BV of 46 and 56. Supplementation by addition of 0·3% DL-methionine to the diet raises these values to 74 and 79 for NPU and 86 and 90 for BV. The NPU and BV values for the gas oil material include some very low values from early samples and from samples where a deliberate attempt was made to apply processing conditions which might reduce them. By varying production conditions we can, in fact, halve the NPU value for gas oil grown yeast; we can also avoid doing so.

However, determinations of this nature are of limited interest unless the product is safe for use. To ascertain whether or not this was so we have carried out—in collaboration with the C.I.V.O. at Zeist in Holland (Director Dr C. Engel)—probably the most comprehensive series of toxicity tests so far undertaken on a product of this nature. These cover tests for acute (3–6 week), subchronic (90 day) and chronic (1·5–2 years) toxicity using mainly rats and mice, though guppies, chicks and quail have been used from time to time. Something like 500 or more samples have been subjected to acute toxicity tests which now consist of feeding a single 40% level of yeast in the diet and comparing the effect with that of a control. In the early stages of development we found, from time to time, growth depression and liver enlargement, but the cause of these effects has been eliminated and what was previously primarily a toxicity test is now more properly regarded as a biological confirmation of analytical figures we associate with a satisfactory product. The criteria are generally live-weight gain, feed conversion efficiency, liver and kidney weights and a general macroscopic post-mortem examination.

When it became clear that we could produce consistently good material we extended the toxicity studies to 3 months and finally to 2 years. At the same time we started an investigation into the effect on reproduction in rats and carcino-

genicity in rats and mice. Yeast was included in the diet at the 10, 20 and 30% level and the experimental procedure followed internationally recognized practice being similar in most respects to that described by Whitehair of the US Atomic Energy Authority in determining the safety of irradiation pasteurized foods.

The first of our 2-year tests will end in January 1969, but a complete histopathological report on a sample number of rats killed after 1 year has indicated no treatment-related abnormalities whatsoever.

Assuming, as we do, that these tests will show the material to be safe, we are still far from the end of its nutritional evaluation since it could well be safe without being a particularly attractive proposition as a food or feed ingredient. We have explained elsewhere why we took the decision to concentrate in the first place on our product as an ingredient of animal feeds and, to avoid misunderstanding, may I stress that this is a question of priorities only, not the rejection of an alternative use. Without knowing the content of Mr McKenzie's paper—other than from the summary—it looks as though he may lend support to our decision if the rapidity of application of this product to nutrition in the broadest sense is considered to be of importance.

Our intention was to evaluate our material as a component of pig and poultry feeds when it was used at what we considered would be the normal commercial maximum level. The scheme envisaged its use in short-, medium- and long-term experiments—some of which are still in progress. Finally, as an indication of the margin of safety we might expect, we incorporated rations containing twice this 'commercial maximum' level in cases where we had enough animals to give valid results.

Considering first poultry, we used 7·5 and 15% of yeast in the rations of broilers to replace some or all of the fish meal or fish–soya mixtures. The reasons were that these are the two protein sources in poultry feeds and that their amino acid composition—bearing in mind the now commonplace use of DL-methionine in feeds—was, or could be made, reasonably similar. Furthermore our determination of the metabolizable energy of the yeast at 2550 kcal/kg was in between the values reported for fish and soya meals. All the work with farm animals is being done at the I.L.O.B., Wageningen, Holland (Director Dr Ir. P. van der Wal), Dr Ir. E. van Weerden being the head of the poultry department.

Most of the broiler trials have been performed on birds in cages, fifteen per group, and have ended after 5 weeks because of this, although on one or two occasions we have gone up to 8 weeks.

The treatments were:

Group 1	Control ration with 10% fish meal
Group 2	Ration with 7·5% LL360 plus 2·5% fish meal
Group 3	Ration with 10% LL360, no fish meal
Group 4	Ration with 15% LL360, no fish meal
Group 5	Ration with 7·5% BRG 3053 plus 2·5% fish meal
Group 6	Ration with 15% BRG 3053, no fish meal

Tables 2 and 3 give results that are quite typical of these experiments. The only

significant difference is in group 4 which was below the others from the point of view of feed conversion ($P=0.05$).

Table 2. *Average weights of birds at 3 and 5 weeks of age*

	3 weeks		5 weeks	
Group no.	g	As % of control	g	As % of control
1 (control)	315	100	739	100
2	327	103·8	748	101·2
3	316	100·3	730	98·8
4	314	99·7	715	96·8
5	320	101·6	742	100·4
6	316	100·3	714	96·6

Table 3. *Feed conversion and mortality of birds at 5 weeks*

	Feed conversion		Mortality
Group no.	kg feed/kg wt gain	As % of control	No. of deaths
1 (control)	1·79	100	1
2	1·80	100·6	3
3	1·81	101·1	1
4	1·86	103·9	4
5	1·81	101·1	4
6	1·80	100·6	3

Medium-term and long-term experiments are virtually the same in that the first consist of evaluating the effect of 10 and 20% of yeast in the rations during the rearing and laying period of pullets and the second consist in measuring this effect over two or three generations.

Our first experiment was with broiler breeders in which levels of 10 and 20% of yeast were used in the experimental rations replacing appropriate quantities of fish and soya meals. Details of this experiment are given by Shacklady (1967). In summary, however, we found that the only differences between the treatments were in terms of egg weight which was 1·7% lower in the yeast groups than in the controls. The 20% yeast group produced 6·9% fewer eggs than its control group which was said not to be significant because of an interaction effect. None the less we should be reluctant to claim equality of performance between these two rations in this experiment.

The hybrid nature of these birds precluded our continuing the experiment into the next generation so we started again with day-old Rhode Island pullets and cockerels. These were reared on rations again containing 10 and 20% yeast and the pullets were caged individually at point of lay. They were then taken through a complete laying cycle of 12 months on the same rations as the previous year's birds. In this instance we achieved somewhat better performance in both the 10 and 20% yeast groups than in their corresponding controls.

Egg production in the 10% yeast group was 3% above that of the control and that in the 20% yeast group 13·7% above its control. This latter result is some-

what distorted owing to an unaccountable fall in production in the control group during the 8th month.

The kg feed required per kg eggs produced was 96·6% of the control for the 10% yeast group and 91·3% of the control for the 20% yeast group. There is no significant difference in the first instance but the performance of the 20% yeast group was significantly better ($P=0{\cdot}001$) than that of the control.

In all the rations containing yeast DL-methionine was added to equalize the level in control and experimental diets. This meant adding 0·053% to the 10% and 0·07% to the 20% yeast rations.

Towards the end of the laying cycle we hatched enough eggs to provide chicks in order to repeat the experiment on the offspring generation. These chicks have been reared as before and are now in their 5th month of lay following the same treatments as described previously. Cumulative percentage egg production at the end of the 4th month was 69·2% for the 10% yeast group and 71·1% for its control, 69·6% for the 20% yeast group and 67·1% for its control.

We had noticed that fertility of the eggs on both levels of yeast was below that of the controls though hatchability of fertile eggs was not. There is now some evidence that this was—to a large extent—a result of the artificial insemination technique we were employing. This is being investigated in the present laying flock.

Experiments with pigs have followed a similar pattern in that short-, medium- and long-term effects are being studied. Short-term experiments are those on growing pigs from weaning to slaughter, medium-term involve the gestation, farrowing and suckling period of the sows and the extension of these experiments to two generations beyond the parent generation constitutes the long-term trial. We have extended our original concept of producing two litters in each generation to producing three, the only reason for doing so being to prove the boars of the 'a' litter before using them to inseminate gilts of the 'b' litter. It is from this mating that the 'a' and 'b' litters of the next generation are obtained.

We used a single level of 10% yeast in the sow feed, 15% in the creep feed and two levels, 7·5 and 15%, in the growing pig rations. Two groups each of sixteen sows formed the parent generation and after insemination sixteen control and thirteen yeast-fed sows conceived. The average litter size and weight of the F1a generation were 10·25 and 1244 g for the control and 10·85 and 1231 g for the experimental group.

After the piglets were weaned, the sows were inseminated again and the corresponding figures for the F1b litter were for the control 11·3 pigs per litter at 1326 g, and for the experimental group 12·4 and 1271 g. An F1c litter was produced by mating F1a boars with the parent generation sows and gave 12·4 pigs at 1191 g in the control group as against 13·7 at 1164 g in the experimental group. All of these litters were taken up to weaning but only the F1a and F1b litters were taken through to slaughter. The control creep feed was based on fish and soya meal, the experimental one contained 15% yeast which had replaced all the fish (12·5%) and 3·0 out of the 4·5% of soya-bean meal. Birth weights of the yeast-fed F1a, b and c litters were 90·6, 88·2 and 94·6% respectively of their controls and weaning weights

Table 4. *Mean daily live-weight gain of pigs (litter F1a) over 8- and 17-week feeding periods*

	0–8 weeks		0–17 weeks	
Group	g/day	% of control	g/day	% of control
Control	623	100	679	100
7·5% BP protein	643	103·2	691	101·8
Control	620	100	670	100
15% BP protein	645	104·0	695	103·7*

*Significant at $P=0.05$.

Table 5. *Feed conversion efficiency of pigs (litter F1a) over 8- and 17-week feeding periods*

	0–8 weeks		0–17 weeks	
Group	Absolute	% of control	Absolute	% of control
Control 1	2·63	100	3·17	100
7·5% BP protein	2·60	98·9	3·14	99·1
Control	2·60	100	3·15	100
15% BP protein	2·50	96·2	3·07*	97·5

*Significant at $P=0.05$.

Table 6. *Mean daily live-weight gain of pigs (litter F1b) over 8- and 17-week feeding periods*

	0–8 weeks		0–17 weeks	
Group	g/day	% of control	g/day	% of control
Control 1	589	100	646	100
7·5% BP protein	562	95·4	641	99·2
Control 1	612	100	666	100
15% BP protein	584	95·4	657	98·6
Control 2	621	100	674*	100
20% BP protein	627	101·0	691*	102·5

*After 14 weeks.

Table 7. *Feed conversion efficiency of pigs (litter F1b) over 8- and 17-week feeding periods*

	0–8 weeks		0–17 weeks	
Group	Absolute	% of control	Absolute	% of control
Control 1	2·46	100	3·07	100
7·5% BP protein	2·41	98·0	2·98	97·1
Control	2·35	100	2·99	100
15% BP protein	2·33	99·1	2·94	98·3
Control 2	2·28	100	2·73	100
20% BP protein	2·27	99·6	2·68*	98·2

*At 14 weeks.

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were 101·2, 94·7 and 94·0% of the controls (van der Wal & Shacklady, 1968).

Both the F1a and F1b litters were taken up to slaughter weight—approximately 100 kg live—with the results shown in Tables. The existence of a 20% yeast group in the F1b litters was because we had enough pigs to make up a third treatment group and were anxious to see what would be the effect of such a high level (Shacklady & van der Wal, 1968).

Commercial grading of the carcasses failed to differentiate between any of the treatments.

Liver, kidney, muscle and fat from these pigs and eggs from the laying birds have been fed to rats for periods varying from 2 to 12 weeks with no indication of the presence of toxic materials.

Regarding palatability, we have fed a ration with 65% of gas oil grown yeast to a pig for 11 weeks without any trouble. After slaughter we submitted hams from this pig and those from a control pig to tasting by 250 people. Both were equally acceptable.

We have now farrowed gilts from this F1 generation and the F2a litters will soon be weaned. So far the performance of the control gilts in farrowing 10·35 pigs weighing 1398 g seems better than the experimental ones with 9·0 pigs weighing 1296 g but beyond merely quoting these figures there are no observations that can be made at present. We intend to go up to the F3a generation which we would expect late in 1969.

No doubt this exposition suffers from trying to present some years of work in 30 min and from describing experiments which are, as yet, incomplete. All that can be claimed for the results so far is that they give us reasonable grounds for expecting that our object of producing a safe and useful ingredient of animal feeds will be achieved in the relatively near future.

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Economic and production problems in the development of new protein sources

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While it is true that there is an overall world shortage of protein, it is equally true that there is protein available now which is not used in improving the nutrition of those in desperate need. Previous papers have described the problems and potential of fish protein, leaf protein, and single-cell proteins derived from yeasts