The Use of Methanol-Grown Yeast LI-70 in Feeds for Broilers

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ABSTRACT In 60-day feeding trials, broilers were fed commercial diets in which different amounts of methanol-grown yeast LI-70 replaced fish and soybean meal. In the first trial, all-mash diets containing up to 15% yeast produced growth rates and efficiencies of feed conversion almost equal to those of the soybean meal control and slightly below those of the fish meal control.

In the second trial, pelleted diets containing up to 25% yeast were used. For yeast levels up to 15%, growth rates were faster than for the soybean meal control and slightly slower than for the fish meal control. Diets with more than 15% yeast lacked selenium. Diets containing 25% yeast as the sole source of protein but supplemented with .3 ppm selenium produced growth rates and efficiencies of feed conversion equal to those of the controls.

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INTRODUCTION

In recent years the search for alternative protein sources has led to the development of fermentation processes for the production of single cell proteins (SCP) from methanol, a substrate offering considerable economic and technological advantages (Young, 1973). The microorganisms used are bacteria (McLennan *et al.*, 1973; Faust *et al.*, 1978) or yeasts (Cardini *et al.*, 1975; Kuraishi *et al.*, 1978). Along with the development of these new procedures, studies have been carried out on the use of SCP from methanol as a protein source for animal feeds instead of the traditional fish or soybean meal. The most extensive nutritional studies have been conducted on bacterial SCPs which have been developed commercially (Whitemore *et al.*, 1978).

It has been shown that up to about 10% bacterial SCPs can be effectively used in broiler diets. Waldroup and Payne (1974) obtained growth rates and efficiencies of feed conversion similar to those of the control group using diets containing up to 10% of a bacterial SCP produced by Phillips Petroleum. Vogt *et al.* (1974) substantially confirmed these results for the ICI product, indicating 8% as the optimal amount in the diet. D'Mello and Acamovic (1976) obtained results superior to those of the control group with diets containing 9.6% lyophilized bacterial SCP.

If the diets contain more than 10% SCP, the results are in general unsatisfactory, presumably because the nucleic acid content is too high or the amino acid composition of the bacterial SCPs is unbalanced (Vogt *et al.*, 1974). Instead, yeasts from methanol seem to be well tolerated at higher concentrations, as is the case for yeasts such as those grown on n-paraffins (Shannon and McNab, 1972) or whey (Vogt *et al.*, 1974).

Little has been reported in the literature, however, and research is limited to the results of short-term feeding trials to study single factors such as the Ca/P ratio, methionine concentration, and physical form of the diet. Thus, in 7-day trials Yoshida and Hoshii (1974) found that diets containing 16% yeast from methanol, with a suitably balanced Ca/P ratio, gave growth rates and efficiencies of feed utilization equal to those of the yeast-free control diet. In 21-day trials, White and Balloun (1977) obtained good results with pelleted mixtures containing up to 15% yeast from methanol.

This article describes a series of feeding trials over the entire production cycle in which broilers were fed diets containing different levels of methanol-grown LI-70 yeast in partial or total substitution for the protein meals commonly used in formulating chicken feeds. This product is obtained from a strain of the species *Candida boidinii* (Craveri *et al.,* 1976). Earlier papers (Cardini *et al.,* 1975, 1976a,b) described its production on a pilot-plant scale and its composition and principal nutritional characteristics. According to preliminary studies on the nutritional value, the LI-70 yeast has a biological value of 75% and a protein efficiency ratio determined in the rat by Campbell's method (Campbell, 1961) of 2.06, increasing to 2.43 on addition of .1% methionine to the diet.

MATERIALS AND METHODS

Experiment 1. The LI-70 yeast used after spray drying was a light yellow, very fine powder with a typical yeast odor but free of foreign tastes and odors. Its chemical composition is given in Table 1. Different amounts of the product were included in two typical mixtures for broilers, one containing both fish and soybean meal and the other soybean meal alone. The fish meal was Norwegian herring; 68% crude protein, and the soybean meal was from the United States, defatted, and 45% protein.

TABLE 1. *Chemical composition of LI-70 yeast*

Ingredient	(%)
Moisture	3.8
Protein (N × 6.25)	59.3
Lipids	4.8
Fiber	1.0
Ash	6.9
Carbohydrates	24.2
Aspartic acid	6.32
Threonine	2.86
Serine	2.92
Glutamic acid	7.50
Proline	2.14
Glycine	3.15
Alanine	3.69
Valine	3.51
Cystine	1.19
Methionine	.89
Isoleucine	3.33
Leucine	4.34
Tyrosine	1.96
Phenylalanine	2.86
Lysine	4.58
Histidine	1.49
Arginine	3.15
Tryptophan	1.01
NH_3	.60

The compositions and chemical analyses of the experimental diets are given in Table 2. Diets were formulated in such a way that replacing soybean or fish meal with yeast did not significantly change the protein content, which was about 23% of the dry matter, or the methionine, calcium, phosphorus, potassium, and sodium contents in the final feed. Fats were not added to the diets so that any effects due to fats in the LI-70 yeast would be evident. The calculated ME values of the diets were 2945 (± 1.2%) kcal/kg, based on the assumption that the yeast has an ME of 2640 kcal/kg (D'Mello and Acamovic, 1976).

One-day old Hubbard chicks, started in heated 34 C cages and then transferred at 21 days to approximately 22 C cages were used. Each diet was offered *ad libitum* in all-mash form to three groups of chicks, each containing 5 males and 5 females, for 60 days. The individual body weights and feed consumptions for each group were ascertained periodically and on the last morning. All chicks were slaughtered and the degree of pigmentation and dressing percentage determined with blood, viscera, and feathers removed. The weights of liver and kidneys were determined for 10 (5 males and 5 females) of the 30 animals receiving each diet. They were expressed as the percentage live weight. Finally, the fatty acid content of the perianal fat of some of the chicks of groups A (fish meal control), D (soya meal control), and F (10% yeast) was determined according to a method described in detail by Landone *et al.* (1976).

All data were subjected to analysis of variance according to the method of Snedecor and Cochran (1967) and significant differences between the means were by the multiple range test of Duncan (1955).

Experiment 2. In view of the results of the first experiment, a second experiment was conducted with the same LI-70 yeast from methanol 1) to study the effect of replacing higher percentages of soybean meal, up to complete replacement; 2) to eliminate the problems due to the powdery nature of the yeast by using pelleted diets; 3) to determine the advantages of using a yeast-free starter diet rich in fish protein for the first 10 days before giving the yeast diets; and 4) to ascertain any effects due to selenium deficiencies in the yeast for diets in which the yeast completely replaced soybean meal.

This last point must be discussed more fully. Effects of selenium deficiencies in diets have been reported by a number of authors. Nesheim

TABLE 2. *Composition of the experimental, all-mash diets (Experiment 1)*

	Diet						
	Fish + soybean meal control A	B	C	Soybean meal control D	E	F	G
Composition, % w/w							
Corn meal	64.0	65.0	66.0	62.5	63.5	64.5	65.0
Soybean meal (45%)	26.0	16.0	13.0	32.0	26.0	18.5	12.5
Alfalfa meal (17%)	3.0	3.7	3.8	2.0	2.6	3.5	4.0
Herring meal	4.0	2.0	4.0	...	...	...	...
LI-70 yeast	...	10.0	10.0	...	4.5	10.0	15.0
Dicalcium phosphate	1.5	1.6	1.6	1.9	1.7	1.9	1.85
Calcium carbonate	1.0	1.0	1.0	.8	.9	.8	.8
Sodium chloride	.2	.3	.2	.4	.4	.4	.4
Vitamins and trace elements[1]	.3	.3	.3	.3	.3	.3	.3
DL-methionine	...	.1	.1	.1	.1	.1	.15
Chemical analysis							
Moisture content (%)	11.20	11.50	10.50	10.30	10.50	10.80	10.80
Crude protein, % of dry weight	22.90	23.00	23.10	22.70	22.80	22.80	23.10
Crude lipids, % of dry weight	4.70	4.50	4.80	5.10	4.70	4.90	4.90
Crude fiber, % of dry weight	4.90	5.00	4.80	5.10	4.90	4.90	4.90
Ash, % of dry weight	6.60	7.10	7.10	7.20	6.80	6.90	6.80
Carbohydrates, % of dry weight	60.90	60.40	60.60	60.70	60.80	61.30	60.80
Calcium, % of dry weight	1.25	1.32	1.30	1.32	1.35	1.35	1.31
Total phosphorus, % of dry weight	.78	.74	.80	.78	.82	.77	.81
Sulfur amino acids, % of dry weight	.91	.92	.93	.86	.87	.89	.96
ME, kcal/kg	2950	2955	2980	2905	2925	2940	2950

[1] The following were added per kilogram of diet: vitamin A, 7500 IU; vitamin D_3, 2400 IU; vitamin E, 3 mg; choline, 600 mg; d-pantothenic acid, 6 mg; vitamin B_{12}, .015 mg; vitamin B_2, 3.4 mg; vitamin PP, 38.6 mg; vitamin K, 1.5 mg; vitamin C, 15 mg; vitamin B_1, 1.5 mg; vitamin B_6, 1.5 mg; folic acid, .4 mg; vitamin H_1, 1.5 mg. Trace elements: Mn, 82.5 mg; Fe, 37.5 mg; Co, 3 mg; Cu, 9 mg; Zn, 30 mg; I, 85 mg.

and Scott (1958) observed exudative diathesis in chickens fed diets containing Torula yeast as the protein source and also deficient in selenium and vitamin E. Thompson and Scott (1969) observed pancreatic dysfunctions in chickens fed diets lacking selenium. Ikumo *et al.*, (1978) noted similar symptoms in chickens fed diets containing a methanol-grown yeast as the sole protein source. They showed that the addition of .5 ppm selenium to such diets completely eliminated the exudative diathesis and pancreatic dysfunctions.

Since the LI-70 yeast is produced in a pilot plant in which the culture medium is prepared from very pure raw materials and the equipment is made of corrosion-resistant materials, the absence even of traces of selenium was expected. The selenium content determined on the dry yeast with the fluorescence method of Wilkie and Young (1970) was less than .01 ppm. Moreover, the LI-70 yeast, although containing a large amount of water-soluble vitamins, contained less than 10 ppm vitamin E. However, adequate vitamin E was present in the supplementary vitamin mixture.

Diets for Experiment 2 (Table 3) were formulated with the protein, methionine, calcium, phosphorus, potassium, and sodium contents maintained constant, as in Experiment 1. Again, fats were not added to the diets. Calculated ME values were 2960 (± 1.3%) kcal/kg.

Each diet, as .1 in pellets, was offered *ad libitum* for 60 days to 30 male Hubbard chicks divided into three groups of 10. The chicks were assigned to the groups at random. Housing and management of the chicks and determination of growth rates and feed consumption for each group were as described in the first experiment. The individual dressing percentage and degree of pigmentation were noted when the chicks were killed. Statistical treatment of the data was the same as in the first experiment.

RESULTS AND DISCUSSION

Experiment 1. For the entire experimental period, a mean mortality was 3.8%, with no significant differences between groups. Table 4 shows the results of the experiment, including the growth rate for males and females, total efficiency of feed conversion, and the findings on slaughtering.

For diets A, B, and C containing fish meal it was observed that males fed diets in which only soybean meal had been replaced showed the same growth rate as the control group; those fed diets in which half the fish meal also had been replaced had a growth rate significantly poorer than the controls ($P<.01$). Females fed the control diet and diets in which only soybean meal had been replaced showed similar growth rates; those receiving diets with replacement of half the fish meal in addition to soybean meal replacement showed slightly better rates. For the diets containing only soybean meal (DEFG) it was noted that for males, groups receiving diets with 5 and 10% yeast had slightly slower growth rates than controls, while those receiving the 15% diet had about the same growth rates as the controls. For females, groups receiving 5 and 10% yeast had slightly slower growth rates than controls, whereas those receiving 15% yeast had superior growth rates. Differences, however, were not statistically significant.

Body weights in these trials with all-mash diets were somewhat variable, because the yeast is much finer than the other components of the diet and the mixtures in the feeding trays may not have been homogeneous. Also, the chicks had some difficulty in eating the yeast, as it adhered to their beaks; this was particularly true for groups F and G. White and Balloun (1977) also have made this observation for all-mash diets containing SCP.

There were no significant differences in the means of the efficiencies of feed conversion, dressing percentages, and weights of liver and kidneys for the various diets tested. The experimental diets had no effect on the degree of pigmentation of the skin of the broilers. No odd-numbered fatty acids were found in the perianal fat of the two control groups, A and D, or of group F, receiving 10% yeast. The fatty acids ranged from C_{14} to C_{18}, the distributions varying slightly for the three diets; the unsaturated fatty acid content was 80% for the fish-soybean meal control group and 70% for the other two diets. (Table 5).

Experiment 2. As shown in Table 6, the growth rates of groups fed 10 and 15% yeast were slightly better than for those of chicks fed the soybean meal control and slightly poorer than that for the fish meal control. Differences, however, were not statistically significant. Conversion efficiencies and the yields on killing were not significantly different.

The group receiving 20% yeast showed a growth rate that was significantly slower ($P<.01$) than that of groups receiving less yeast

TABLE 3. *Composition of the experimental, pelleted diets (Experiment 2)*

	Diet								
	Soybean meal control A	B	C	D	E	F	G	Fish + soybean meal control H	Starter diet I
Composition % w/w									
Corn meal	63.5	63.5	65.5	65.7	66.0	67.55	67.55	64.0	61.0
Soybean meal (45%)	31.0	26.0	18.0	12.0	6.75	...	...	26.0	26.0
Alfalfa meal (17%)	2.2	2.2	3.2	4.0	4.0	4.0	4.0	3.0	3.0
Herring meal	...	...	...	...	...	...	...	4.0	7.0
LI-70 yeast	...	5.0	10.0	15.0	20.0	25.0	25.0	...	...
Dicalcium phosphate	1.2	1.5	1.5	1.3	1.1	1.0	1.0	1.5	1.5
Calcium carbonate	1.0	.9	.9	1.0	1.1	1.3	1.3	.8	.9
Sodium chloride	.5	.4	.4	.45	.45	.3	.5	.3	.2
Vitamins and trace elements[1]	.4	.4	.4	.4	.4	.4	.4	.4	.4
DL-methionine	.2	.1	.1	.15	.20	.25	.25	...	...
Selenium (ppm)	...	...	...	...	...	...	.3	...	...
Chemical analysis									
Moisture content (%)	10.50	10.70	10.80	10.20	10.10	10.20	10.20	11.00	10.20
Crude protein, % of dry weight	22.90	23.20	22.90	23.30	23.40	23.50	23.50	23.70	24.80
Crude lipids, % of dry weight	4.70	3.90	4.10	4.30	4.40	4.50	4.50	4.10	5.70
Crude fiber, % of dry weight	4.30	4.30	4.50	4.40	4.70	4.60	4.60	4.80	4.90
Ash, % of dry weight	6.60	6.30	5.90	6.50	6.40	6.30	6.30	6.60	7.20
Carbohydrates, % of dry weight	61.50	62.30	62.60	61.50	61.10	61.10	61.10	61.40	57.90
Calcium, % of dry weight	.93	.90	.93	.97	.99	.89	.89	.88	.98
Total phosphorus, % of dry weight	.77	.76	.78	.80	.78	.87	.87	.72	.75
Sulfur amino acids, % of dry weight	.96	.89	.88	.95	1.04	1.10	1.10	.80	.88
ME, kcal/kg	2920	2950	2955	2960	2980	3000	3000	2950	2940

[1] The following were added per kilogram of diet. Vitamin A, 10000 IU; vitamin D_3, 3200 IU; vitamin E, 4 mg; choline, 800 mg; d-pantothenic acid, 8 mg; vitamin B_{12}, .02 mg; vitamin B_2, 4.5 mg; vitamin PP, 51.5 mg; vitamin K, 2 mg; vitamin C, 20 mg; vitamin B_1, 2 mg; vitamin B_6, 2 mg; folic acid, .5 mg; vitamin H_1, 2 mg; Trace elements: Mn, 110 mg; Fe, 50 mg; Co, 4 mg; Cu, 12 mg; Zn, 40 mg; I, 1.14 mg.

TABLE 4. *Experiment 1 (All-mash diets)—Body weight gains, efficiencies of feed conversion, and findings on slaughtering with all-mash diets (Experiment 1)*

	Group with diet											
	Fish + soya meal Control A			B			C			Soya meal Control D		
	M	F	M+F	M	F	M+F	M	F	M+F	M	F	M+F
Body weights (g)												
21 days	460	428	444	458	440	449	472	438	455	470	440	455
34 days	1088	928	1008	1054	966	1010	1058	951	1004	1043	936	989
49 days	1961^{A}	1611^{A}	1786^{A}	1847^{B}	1671^{A}	1759^{A}	1911^{ABa}	1625^{A}	1768^{A}	1850^{B}	1589^{AB}	1719^{AB}
60 days	2478^{A}	2007^{AB}	2242^{A}	2262^{B}	2061^{A}	2161^{AB}	2467^{A}	1974^{AB}	2207^{A}	2338^{B}	1939^{AB}	2138^{B}
Feed conversion (+)[1] (Means, M+F) +												
21 days			1.42			1.42			1.41			1.60
34 days			1.82			1.90			1.89			1.82
49 days			2.04			2.16			2.14			2.03
60 days			2.34			2.46			2.39			2.43
Findings on slaughtering												
Dressing percentage			85.3			85.7			85.7			85.2
Weight liver, % of live weight			1.95			2.01			1.88			2.07
Weight kidneys, % of live weight			.38			.38			.41			.33

	E			F			G		
	M	F	M+F	M	F	M+F	M	F	M+F
Body weights (g)									
21 days	427	400	413	440	420	430	460	430	445
34 days	1015	900	975	1003	935	969	1025	925	975
49 days	1824^{Bb}	1566^{B}	1695^{B}	1805^{Bb}	1545^{B}	1675^{B}	1831^{Bb}	1617^{A}	1724^{AB}
60 days	2283^{B}	1931^{B}	2107^{B}	2299^{B}	1897^{B}	2098^{Ba}	2333^{B}	2030^{A}	2181^{ABb}

Feed conversion (+)[1] (Means, M+F) +			
21 days	1.54	1.67	1.51
34 days	1.91	1.90	1.86
49 days	2.14	2.16	2.11
60 days	2.43	2.44	2.36
Findings on slaughtering			
Dressing percentage	85.3	85.8	86.2
Weight liver, % of live weight	1.95	2.03	1.94
Weight kidneys, % of live weight	.38	.34	.38

A,B,a,b Means having different superscripts in the same line differ significantly for P<.05 if a small letter is used and for P<.01 if a capital letter is used.

[1] (+), Feed consumption/body weight (%).

TABLE 5. *Fatty acid content of the perianal fat, % w/w for all-mash diets (Experiment 1)*

Fatty acid	Group A	Group D	Group F
C_{14}	0.1	...	...
C_{16}	18.1	21.8	26.5
$C_{\bar{16}}$	11.1	7.2	2.1
C_{18}	.8	7.4	2.4
$C_{\bar{18}}$	51.7	42.0	48.6
$C_{\bar{\bar{18}}}$	17.5	21.3	20.4
$C_{\bar{\bar{\bar{18}}}}$	.7	.2	...

and controls. This difference, already evident on the 21st day, was attributed to a partial lack of selenium. This explanation was upheld by the results from selenium supplementation. Defatted soybean meal contains about .6 ppm selenium (Kellor, 1974), whereas LI-70 contains none. Thus, the 6.75% of soybean meal provided at least minimal amounts of selenium.

With the exception of the selenium deficient group, all groups of this trial showed a mean mortality of 3.7%, with no significant differences between the diets.

The group receiving the diet in which the soybean meal had been completely replaced, with no selenium supplement, showed very retarded growth rate even on the 21st day. On the 28th day, there were clear symptoms of distress that developed into a mortality of 72% by the 40th day, at which time the remaining chicks were slaughtered. As those chicks were necropsied, we observed fragility of the capillaries with extensive hemorrhaging and edemas consisting of a greenish gelatinous fluid, especially in the thorax and groin, characteristic of the exudative diathesis appearing in chickens lacking selenium. Similar mortality rates and anatomico-pathological findings were reported for this kind of trial by Ikumo *et al.* (1978).

The group also receiving a diet containing 25% yeast, but supplemented with .3 ppm of selenium, showed growth rate, conversion efficiency, and dressing percentage similar to that of groups receiving up to 15% yeast, with no statistical differences. Moreover, this group showed no excessive mortality. On the 40th day, two chicks from this group were sacrificed and carcass and viscera were normal. The selenium deficiency described here for the chicken has also been observed in the trout, and it probably occurs in other domestic animals as

TABLE 6. *Body weight gains, efficiencies of feed conversion, and dressing percentages for pelleted diets (Experiment 2)*

	Group with diet										
	Soybean meal control A	B	C	D	E	F	G	Fish + soybean meal control H	I+H	I+B	I+C
Body weights (g)											
21 days	444^{a}	492^{a}	469^{a}	435^{a}	350^{b}	335	472^{a}	517^{a}	494^{a}	526^{a}	482^{a}
34 days	1068^{A}	1087^{A}	1067^{A}	1068^{A}	906^{B}	. . .	1078^{A}	1095^{A}	1085^{A}	1088^{A}	1100^{A}
49 days	1827^{A}	1853^{A}	1864^{A}	1830^{A}	1711^{B}	. . .	1840^{A}	1893^{A}	1798^{A}	1800^{A}	1752^{A}
60 days	2345^{A}	2390^{A}	2405^{A}	2375^{A}	2185^{B}	. . .	2395^{A}	2430^{A}	2350^{A}	2305^{A}	2330^{A}
Feed conversion (+)[1]											
21 days	1.58	1.55	1.54	1.68	1.90	2.03	1.56	1.43	1.58	1.54	1.52
34 days	1.90	1.84	1.82	1.97	1.96	. . .	1.84	1.86	1.84	1.86	2.01
49 days	2.16	2.11	2.13	2.24	2.25	. . .	2.16	2.03	2.00	2.21	2.09
60 days	2.36	2.31	2.45	2.43	2.51	. . .	2.45	2.24	2.25	2.39	2.31
Dressing percentage	86.3	86.1	86.8	86.9	85.3	. . .	85.9	86.3	86.2	86.0	86.3

A,B,a,b Means having different superscripts in the same line differ significantly for $P<.05$ if a small letter is used and for $P<.01$ if a capital letter is used.

[1] (+), Feed consumption/body weight (%).

well. In experiments now in progress, we find that trout grow quite well on diets containing 50% LI-70 yeast, whereas with 65% diets, corresponding to complete replacement of the fish meal, mortality was high and growth irregular. The addition of .3 ppm selenium eliminated the deaths and produced normal growth (Grimaldi and Succi, 1979, unpublished data).

For the groups which received a starter diet the first 10 days and treatment diets thereafter no differences were observed in the results either between treatments or in comparison with the groups which received equivalent diets but no starter.

No adherence of feed to the beaks of the chicks was observed in any of the groups of the second experiment; thus, the findings were more consistent than those of the first experiment in which all-mash diets were used with somewhat better growth.

It can, therefore, be concluded that pelleted diets containing up to 15% LI-70 yeast can be successfully given from the first day of life to growing broilers under practical conditions. Diets containing more than 15% yeast can be used with suitable additions of selenium, as shown by Experiment 2.

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