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TECHNO-ECONOMIC ASPECTS OF OUR NEWLY DEVELOPED N-PARAFFIN YEAST[1/]

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For many years we have produced baker's yeasts and an RNA-rich yeast from molasses on a commercial basis. We have successfully developed a unique process of economically producing a n-Paraffin Yeast (KANEPRON) in large quantities by combining most up-to-date chemical engineering technology with a new fermentation technology developed on the basis of such an abundant experience in yeast production. This process has many features like continuous fermentation using a unique air-lift fermentor originally developed by us, efficient supply of oxygen, fine dispersion of n-paraffin, an excellent drying system, etc.

Results of feeding tests where KANEPRON produced was fed to poultry, pigs and cultured fishes at the formulation ratio of 5-15%, 5-10% and 30-60% respectively have shown the superiority of KANEPRON to conventional protein sources such as soybean and fish meals with respect to growth rate and feed conversion. This superiority appears to consist in good composition of KANEPRON.

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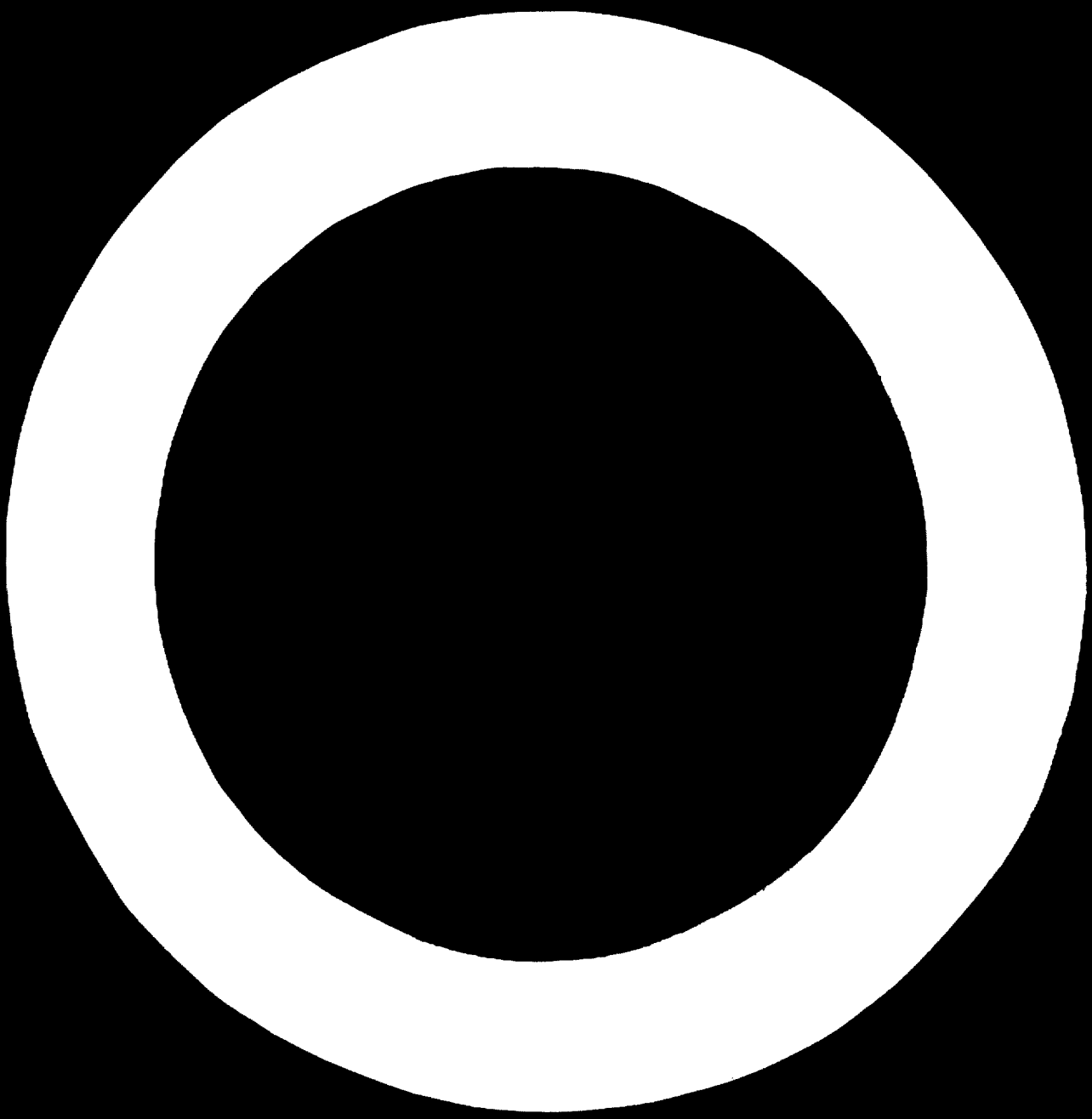
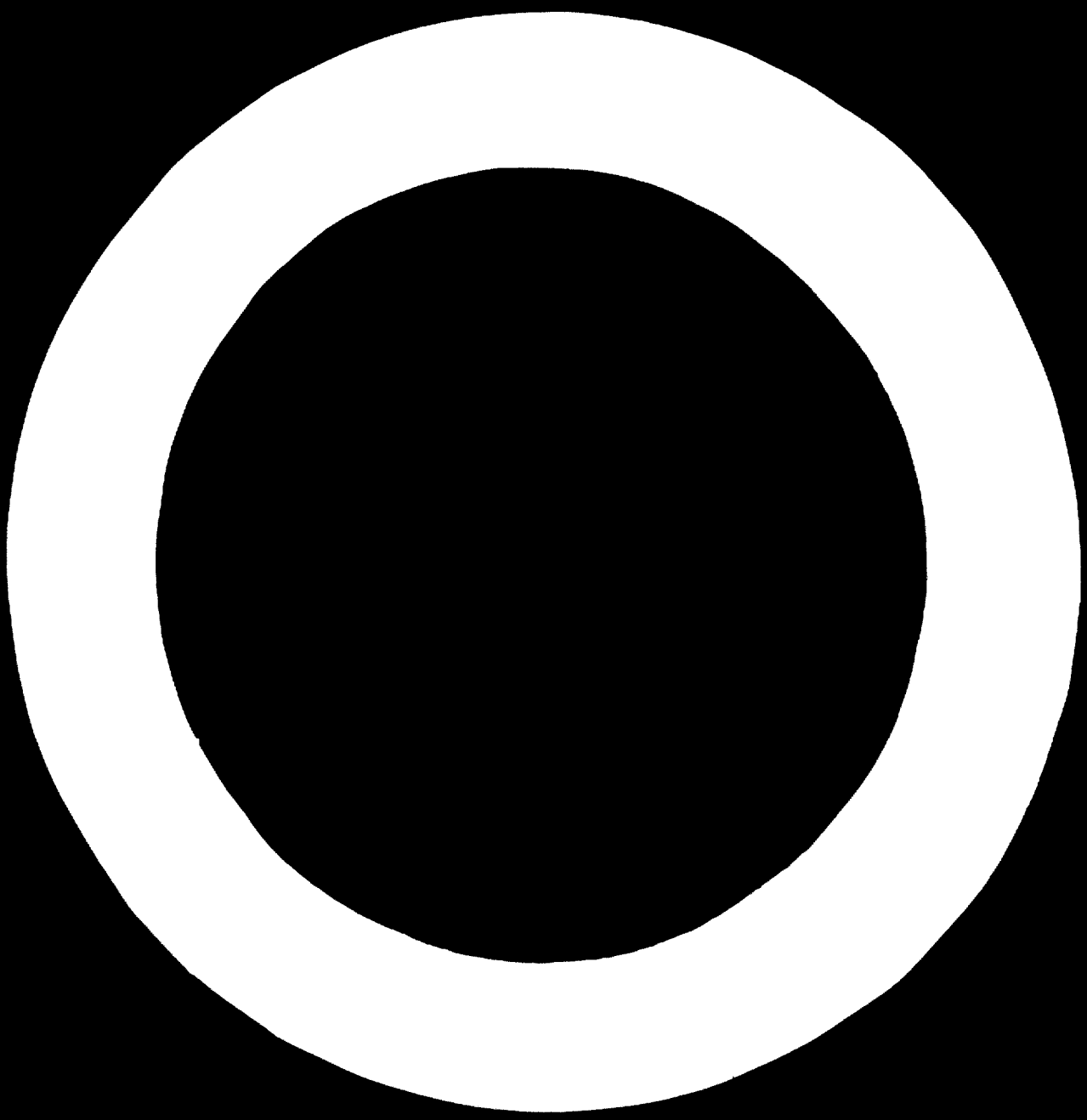


As regards the safety of KANEPRON, Food Sanitation Investigation Council of Ministry of Health & Welfare of Japan made the formal announcement that KANEPRON was safe enough to be used as an animal feed.

Further, our tentative production cost has shown that KANEPRON has more economic advantages than comparable products.

These techno-economic properties enable us to draw the conclusion that KANEPRON is a promising feed protein source of superior quality and safety, therefore the emergence of KANEPRON could be a powerful means of a solution to the worldwide problem of protein deficiency.



CONTENTS

Introduction

We have for many years produced baker's yeasts and an RNA-rich yeast, a raw material for chemical seasonings, from molasses on a commercial basis. By combining most up-to-date chemical engineering technology with a new fermentation technology developed on the basis of such abundant experience in yeast production, we have successfully developed a unique, large-scale process for producing a n-Paraffin-Yeast —— KANEPRON. This new process was examined from a viewpoint of its commercial practicability. In addition, the product produced by the process was subjected to comparative feeding tests on pigs, poultry, cultured fishes, etc., and safety and nutritional quality tests using various test animals and chemical analyses.

Some techno-economic aspects of our new process and product are discussed hereunder.

I. n-Paraffin

On Dec. 19, 1970, the Food Sanitation Investigation Council of the Ministry of Health & Welfare of Japan announced a report entitled "On the safety of n-paraffin-yeast as animal feed", which provides that n-paraffins as raw material must:

(a) be higher than 98 WT% in purity;

(b) not contain more than 1.0 ppb of 3,4-benzpyrene, 1,2;5,6-dibenzanthracene and 20-methylcholanthrene respectively;

(c) pass the test of FDA 121,1156 for food additives.

There are molecular sieve processes and urea processes available for the production of n-paraffins. Representative methods of the former are Isosiv process and Molex process, whereas those of the latter are Nurex process and Edeleanu process. n-Paraffins produced by the Isosiv process is suitable for SCP production —— particularly with respect to the purity of n-paraffins and therefore the safety of n-Paraffin-Yeast.

II. Process

A. Features of the process

As mentioned above, we have developed a new process for mass-production of yeast from n-paraffins. Features of the process are summarized as follows:

(a) Continuous production —— production is carried out in a continuous system throughout the process except the step of seed culture, thereby ensuring high productivity and uniform properties of the product;

(b) Efficient aeration —— since hydrocarbon fermentation involves high oxygen demands, effective supply of oxygen is one of the critical parts of hydrocarbon fermentation.

This problem has been overcome by developing an efficient aeration apparatus;

(c) Economical heat removal —— large amounts of heat evolved by propagation and metabolism is economically removed to maintain an optimum temperature of the broth in a fermentor;

(d) Efficient dispersion of n-paraffins —— an efficient system has been established by which n-paraffins are broken up finely into microdroplets to thoroughly disperse in an acqueous medium, thus accelerating the growth rate of yeast;

(e) Economical drying —— a large-scale, continuous drying system has been developed.

In addition to the technical features above, various bio-engineering problems have been solved, and it was confirmed that economical, large-scale production of yeast from n-paraffins is quite feasible with due consideration of satisfactory n-paraffin based yield, productivity, product quality, plant cost, etc.

B. Outline of the process

The process outline is given in Figure I. The process consists of raw material and additives supply, seed culture, fermentation, separation and dehydration, drying and product handling, utilities supply, and wastewater treatment.

The raw material is n-paraffin, and the additives are nitrogen source, various types of inorganic salts, trace elements, and an antifoam agent.

n-Paraffin and the additives are prepared in accordance with their respective properties, and then supplied to the seed vessel and fermentor. Features of this step consist in continuous dissolution and sterilization of the nutrients.

Seed cultivation is carried out to obtain the necessary amount of inoculum.

On completion of seed cultivation, the nutrients are fed to the fermentor along with the required amount of inoculum from the seed vessel. Continuous fermentation starts when the desired biomass concentration is attained. The feed inputs and outputs are controlled for smooth and continuous operation. The yeast biomass produced by continuous fermentation passes to separators, and the acqueous medium is recycled to the fermentor. This fermentation step is characterized by the employment of a unique air-lift fermentor which gives a satisfactory gas-liquid dispersion, a fine dispersion of paraffin-droplets; and efficient heat removal; continuous fermentation which ensures high yield factor and productivity; and the recycling of the acqueous medium for reuse.

The yeast milk obtained by the above separation is water-washed and condensed in another separation step and dehydrated. The dehydrated yeast is sent to a drying step to give the product. The drying system is continuous, high capacity and easy to operate and maintain. The product is stored in silos to be ready for shipment as the end product.

The utilities supply includes supply of fermentation air, cooling water, process water, steam and electric power. Features are the sterilization of air by adiabatic compression, and deodorization of vented fermentation air by combustion.

Wastewater is treated by a combination of coagulation-precipitation method and activated sludge method to attain a reduced COD, oils and suspended solids. Impurities eliminated as sludge are dehydrated and then incinerated.

C. Air-lift fermentor

As compared with the conventional carbohydrate fermentation, the hydrocarbon fermentation has such factors as sparing solubility of the substrate, high oxygen demands, large heat evolution, etc., which therefore present special bio-engineering problems.

In view of these factors, the design of fermentor for hydrocarbon fermentation must meet such requirements as to allow efficient supply of large amount of oxygen to the fermentor and sufficient dispersion of the oil-droplets into the acqueous medium for attaining good growth rate of the yeast.

The conventional agitate' fermentor could be used for a small working volume of hydrocarbon fermentation, but with a large amount of working volume this type of fermentor would not be suitable for the hydrocarbon fermentation on account of economic disadvantages of the bulkiness of the agitator and big power requirement for mechanical agitation, in addition to mechanical and complex structural problems associated with mechanical strength of the fermentor and agitator, installation of agitator, etc. For these reasons, the necessity of development of a new type of fermentor has been stressed which would be different from the conventional patterns and could meet the requirements of large-scale hydrocarbon fermentation.

Since over a decade ago, we have been involved in intensive studies on air-lift fermentors, and successfully developed a new air-lift design which is suitable for large-scale production of biomass from hydrocabons. A simplified schematic representation of the fermentor is given in Figure II.

Air is blown dispersedly into the fermentor from an air-dispersion apparatus equipped at the lower end of the primary body, thereby increasing oxygen transfer rate. The broth containing micro air-bubbles is forced to move upwardly at a high velocity in the primary body by the air-lift effect, and the broth is deaerated in a separating chamber located at the upper end of the fermentor, and then the broth is allowed to flow back to the bottom of the fermentor through the circulatory tube, thus causing a more thorough breakup of air-bubbles and paraffin droplets. Thus, almost thorough homogenization of the broth is attained thanks to the circulation of the broth between the primary body and the circulatory tube.

As regards the oxygen supply, the fermentor is designed in such a manner that gas-liquid interfacial area can be increased by the fine dispersion of air into the medium and, at the same time, the partial pressure of oxygen be also increased by making the liquid depth sufficiently high, thus raising the oxygen transfer rate.

III. End product

A. Quality

Table 1. shows an example of analysis of KANEPRON on main components, minerals composition, amino acids composition and vitamins. Table 2. shows an example of the result of nutritional tests on KANEPRON.

B. Safety

In Japan, the Committee on n-Paraffin-Yeast under the Food Sanitaion Investigation Council of the Ministry of Health & Welfare demonstrated the viewpoints and methods for confirmation of safety of the n-Paraffin-Yeast in the Committee's report entitled "On the safety of n-Paraffin-Yeast as an animal feed", in which the following subjects came into question:

i) Carcinogenic substances of polycyclic aromatic hydrocarbon compounds and heavy metals contained both in n-paraffins as the raw material and in constituents of culture medium;

ii) Unknown toxic substances which might be contained in n-paraffins as the raw material;

iii) Mycotoxins which might be produced by microorganisms;

The Committee arrived at a consensus of opinion on these subjects and established methods for confirmation of safety on the basis of the following considerations:

i) Chemical tests and toxicity tests themselves may, in theory, be authentic as means to examine the safety of meat, milk and the others of animals fed with feeds containing n-Paraffin-Yeast. Apart from chemical tests, however, it is technically very difficult to carry out toxicity tests on such meat, milk and the others.

Furthermore, data to be obtained from detailed tests on the strain used, living cells, broth filtrated and final product may be more useful than those to be obtained from toxicity tests on such animal products.

ii) It is possible to determine the presence of any known carcinogenic substances of polycyclic aromatic hydrocarbon compounds and heavy metals in terms of chemical means.

iii) It is possible to determine the presence of any known mycotoxins by chemical and biological means.

iv) There is no effective method at present other than toxicity tests by biological means to determine the presence of any unknown toxic substances which might be contained in n-paraffins or unknown mycotoxins.

The test series entailed by the Committee in the report are classified into 6 chapters with 22 items as given in Appendix.

We conducted various safety tests in accordance with the methods entailed by the Committee, and submitted the Committee a report, "On the safety of KANEPRON" consisting of over 1200 pages.

The Committee conducted very careful deliberation and examination on the report and materials submitted by us, in accordance with the above-mentioned viewpoints and methods, and obtained the following viewpoints;

i) The strain to be used has neither pathogenicity nor infectivity, and no main known mycotoxins are detected both in living cells and in broth filtrate by chemical and biological means.

ii) n-Paraffins used as the raw material have a purity of more than 98 per cent, and do not contain more than 1.0 ppb of 3,4-benzpyrene, 1,2;5,6-dibenzanthracene, or 20-methylcholanthrene.

iii) No recongnizable toxicity is detected in the acute and subacute toxicity tests on living cells, broth filtrate, and their extracts, and in chronic toxicity tests including carcinogenecity and multiple generation tests on the product.

iv) In chemical tests it is recognized that any harmful substances of polycyclic aromatic hydrocarbons and heavy metals are not contained, in a concentrated form, in the meat and milk, egg and others of livestock, poultry, fishes fed on the product as a feed.

Finally, on Dec. 15, 1972, the Food Sanitation Investigation Council under the Ministry of Health & Welfare made an official announcement that KANEPRON is safe enough to be used as animal feed.

C. Feeding tests

Comparative feeding tests were carried out on pigs, poultry, cultured fishes, etc. using KANEPRON and the conventionally available protein sources like fish meal and soybean meal. The results of these tests have shown that KANEPRON is better than or equal to the comparable conventional products with respect to growth rate and feed conversion.

Some examples of the test procedures and results are given below.

(a) Broiler

Procedure:

In order to compare the effect on growth rate and feed conversion of KANEPRON as a protein source for feed with that of fish meal and soybean meal, KANEPRON was administered to two major groups of broilers in the incorporation of 5% and 20% in the feed as shown in Table 3. The effect of the feed form -powder or granular- was also studied.

One group was fed on powder KANEPRON during the whole test-period, while the other was fed on powder KANEPRON during the first half period, and on granular KANEPRON during the second half.

Result:

Table 5. shows that the KANEPRON groups were better than the control in weight gain and feed conversion, and that the granular form was more efficient than the powder form.

(b) Layer

Procedure:

Five groups consisting of 30 Hi-Line layers each were adminstered with different types of feeds for 22 weeks, The incorporation of KANEPRON in the feed was in the range of 5 - 13% (Table 6). The number of eggs produced and the egg weights were recorded every day, while the feed consumption was recorded every 4 weeks

Results:

i) Though the number of eggs produced per 100 hen-day of the KANEPRON groups during the first four weeks was less than that of the control, from the 5th week on, the KANEPRON groups showed better results (Table 6).

ii) Egg weights and feed conversions of the KANEPRON groups were much better than those of the control group (Table 8).

iii) The 6% group (group 3) showed the most outstanding results with respect to egg production per 100 hen-day, egg weight, and feed conversion.

iv) Mortality during the 22 weeks was about the same (Table 7).

v) Eggs of the KANEPRON groups were similar to the commonly marketed eggs in taste, color and smell.

(c) Pig

Procedure:

In order to test the nutritional effect and safety of KANEPRON, fish meal and soybean meal in feeds for pigs were replaced by maximum possible amount of KANEPRON (23% in the first stage and 17% in the second stage) as shown in Table 9. The feeding tests were conducted on 6-week old pigs until they reached 90 kg at marketing time.

Result:

weight gain and feed conversion of the test group were better than those of the control group during the whole test period (Fig. III & Table 10).

(d) Carp

Procedure:

As a protein source for carp's feed, KANEPRON was tested comparatively with fishmeal. One year old carp were fed in net crawls for 115 days on different kinds of feeds. The incorporation of KANEPRON in the feed ranged from 15 to 75% as shown in Table 11. Calculated analysis of each feed is shown in Table 12. All the feeds were administered in a pellet form every day. The body weights and feed consumptions were recorded every two weeks.

Results:

i) The 30% KANEPRON group showed the most outstanding result. Body weights and feed efficiency of the groups fed on less than 60% KANEPRON-based feeds, were better than those of the control as shown in Table 13.

ii) Protein efficiency of the KANEPRON groups was higher than that of the control as shown in Table 13.

iii) The KANEPRON groups showed better feed consumption; indicating that KANEPRON is of better palatability.

iv) Carp fed on KANEPRON based feed were of better taste, tenderness and colour; this indicates that KANEPRON is a desirsble protein source for carp.

(e) Eels

Procedure:

Twenty gram weighing yearling eels were fed in an outdoor concreat pool (water temparature. 23 - 24°C, pH: 7.81) for 77 days with feeds based on KANEPRON, fish meal, sulfite waste liquor and beer yeasts. Incorporation of KANEPRON in the feeds was 35% as shown in Table 14. Calculated analysis of each feed is shown in Table 15. The body weights were recorded every 2 - 3 weeks. Amount of the daily ration was about 2% of the total eels weight. Health conditions of the eels were indicated by the Hematocrite value.

Result:

i) No noticeable difference in growth rate and feed efficiency was observed among all the groups (Table 16.).

ii) With respect to Hematocrite value, the KANEPRON group was better than the other groups (Table 16.).

iii) This test showed that KANEPRON is a suitable protein source for eels.

(f) Rainbow trout

As a protein source for rainbow trout, KANEPRON was compared with fish meal. At the same time, effect of methionine added to the feed was also studied. 150 g weighing trout were bred for a period of 280 days. The incorporation of KANEPRON in the feed was 52% for both group 2 and group 3 as shown in Table 17. The feed for group 3 was added methionine in the incorporation of 0.8%. Each group consisted of about 100 trout. Feed was administered twice a day. The body weights were measured every two weeks.

Result:

i) The KANEPRON groups showed better weight gain and feed conversion in the latter half period as shown in Table 20.

ii) No noticeable effect of methionine on growth rate was observed as far as this test was concerned.

iii) No recognizable difference was noted between trout fed on KANEPRON based feeds and the commonly marked one with regard to color, taste and smell.

IV. Plant investment cost and production cost

Estimated plant investment cost and production cost of KANEPRON based on 100,000 tons per annum are given in Table 21.

V. Conclusion

The techno-economic aspects discussed in the preceding chapters regarding our newly-developed n-Paraffin-Yeast -KANEPRON- are summarized as follows:

(a) The process is a large-scale and continuous one which is characterized by efficient system of oxygen supply, efficient dispersion of n-paraffins, economical heat removal system and excellent drying system.

(b) The product is of uniform quality, and can be supplied steadily in large quantities at a stable price.

(c) The results of feeding tests have shown that KANEPRON is better than or equal to the comparable products such as fish meal and soybean meal with respect to growth rate and feed conversion.

(d) As regards the safety of KANEPRON, the Food Sanitation Investigation Council of the Ministry of Health & Welfare of Japan made an official announcement that KANEPRON is safe enough to be used as animal feed.

Table 1. Analysis of the product

Main components (Percentage, dry base)	
Moisture	4.5
Crude protein	61.0
Crude fat	3.2
Crude fiber	4.2
Crude ash	9.8

Minerals composition (Percentage, dry base)		
Phosphorus	(P_2O_5)	5.3
Potassium	(K)	1.9
Magnesium	(Mg)	0.25
Calsium	(Ca)	0.05
Zinc	(Zn)	0.06
Iron	(Fe)	0.04

(to be continued)

Table 1. Analysis of the product
(continued)

Amino acids composition
(Percentage, dry base)

Aspartic acid	6.13	Methionine	0.60
Threonine	3.28	Isoleucine	2.89
Serine	2.84	Leucine	4.46
Glutamic acid	8.54	Tyrosine	1.49
Proline	2.54	Phenylalanine	2.46
Glycine	2.81	Tryptophan	0.90
Alanine	3.64	Lysine	4.57
Cystine	0.87	Histidine	1.01
Valine	3.36	Arginine	2.92

Vitamins
(Dry base)

Vitamin E	147	mg/kg
Thiamine	15	mg/kg
Riboflavin	77	mg/kg
Pyridoxine	11	mg/kg
Pantothenic acid	335	mg/kg
Niacin	534	mg/kg
Choline	0.50	%
Inositol	0.59	%
Vitamin B_{12}	0.41	mg/kg
Biotin	1.1	mg/kg
Folic acid	3.9	mg/kg

Table 2. Nutritional value

Protein digestion rate (Percentage)	
Poultry	84.8 - 88.0
Pig	88.3 - 89.3
Carp	85.4
Eel	81.6

Metabolizable energy (Calorie per gram)	
Chick	3.15 - 3.38
Layer	3.48 - 4.08

Digestible energy (Calorie per gram)	
Piglet	4.16
Swine	4.55

Table 3. Composition of the feeds
(Percentage)

Period	0-3rd Week			4-9th Week		
Group	1,2	3,4	5,6	1,2	3,4	5,6
Corn	43	43	43	40	40	40
Milo	15	15	15	23	23	23
Soybean meal	26	22	10	22	18	6
Fishmeal	8	7	4	7	6	3
Kanepron	-	5	20	-	5	20
Soybean oil	1.5	1.5	1.5	3	3	3
Alfalfa	3	3	3	2	2	2
$CaCO_3$	1.66	1.66	1.66	1.25	1.25	1.25
$CaHPO_4$	0.70	0.70	0.70	0.65	0.65	0.65
NaCl	0.22	0.22	0.22	0.20	0.22	0.22
Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Vitamins A, D & E	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin B group	0.25	0.25	0.25	0.25	0.25	0.25
Choline Chloride	0.05	0.05	0.05	0.05	0.05	0.05
Minerals	0.05	0.05	0.05	0.05	0.05	0.05
Furazolidone	0.10	0.10	0.10	0.10	0.10	0.10
Amprolium	0.12	0.12	0.12	0.10	0.10	0.10
Antibiotics	0.05	0.05	0.05	0.05	0.05	0.05

Note: Poder --- group 1, 3, 5
Granular --- group 2, 4, 6

Table 4. Chemical analysis of the feeds
(Percentage)

Group	1,2		3,4		5,6	
Week	0-3	4-9	0-3	4-9	0-3	4-9
Moisture	12.59	13.11	12.43	12.84	12.14	12.31
Crude protein	20.09	20.92	23.19	20.93	21.67	21.50
Crude fat	4.97	5.96	5.61	6.25	5.04	6.63
Crude fiber	2.74	2.64	2.42	2.49	2.85	2.44
Crude ash	6.55	5.53	6.48	5.61	6.24	5.51
NFE	51.06	51.84	49.87	51.88	52.06	51.61

Table 5. Overall results of the feeding experiment

Week	Group	1 (Control)	2	3	4	5	6
0	Average weight (g)	38.5	38.5	38.5	38.5	38.5	38.5
3	Average weight (g)	402.4	394.4	415.5	418.0	412.0	404.4
	Average weight gain (g)	363.9	355.9	377.0	379.5	373.5	365.9
	As % of control	100		105.1		102.7	
	Feed conversion	2.04	2.10	1.98	1.97	2.00	1.99
7	Average weight (g)	1,466.2	1,549.0	1,510.7	1,599.7	1,438.2	1,575.3
	Average weight gain (g)	1,427.7	1,510.5	1,472.2	1,561.2	1,399.7	1,536.8
	As % of 1st group	100	105.8	103.1	109.4	98.0	107.6
	As % of 2nd group	-	100	-	103.4	-	101.7
	Feed conversion	2.02	2.06	2.04	2.03	2.03	1.98
9	Average weight (g)	1,903.9	2,166.5	1,997.4	2,225.4	1,947.8	2,181.8
	Average weight gain (g)	1,865.4	2,128.0	1,958.9	2,184.9	1,909.3	2,143.3
	As % of 1st group	100	114.1	105.0	117.1	102.4	114.9
	As % of 2nd Group	-	100	-	102.7	-	100.7
	Feed conversion	2.32	2.24	2.37	2.14	2.29	2.21

Table 6. Composition of the feeds
(Percentage)

Group	1	2	3	4	5
Soybean meal	15	0	7	15	16
Fish meal	6	6	6	0	3
Kanepron	0	13	6	8	5

Table 7. Mortality during 22 weeks
(Percentage)

Group	1	2	3	4	5
Mortality	3.3	3.3	6.6	0	3.3

Table 8. Eggs produced per 100 hen-day
egg weight and feed conversion

Weeks	Group	Eggs per 100 hen-days	Egg weight (grams)	Feed per hen-day (grams)	Feed conversion
1-4	1	73.1	58.9	106.2	2.47
	2	71.7	58.7	103.0	2.45
	3	71.5	61.2	102.9	2.36
	4	72.7	60.3	109.1	2.49
	5	63.8	61.4	104.4	2.67
5-8	1	66.7	60.3	110.8	2.76
	2	66.9	59.9	102.8	2.56
	3	68.7	62.0	108.4	2.51
	4	69.8	61.8	112.5	2.61
	5	63.4	62.0	105.1	2.63
9-12	1	62.0	61.1	98.5	2.60
	2	64.8	60.5	96.5	2.46
	3	71.3	62.6	98.4	2.21
	4	67.3	61.6	100.6	2.43
	5	64.4	63.3	96.8	2.38
13-16	1	57.0	61.0	96.7	2.78
	2	60.3	60.9	97.2	2.59
	3	60.6	62.7	96.9	2.55
	4	57.4	61.7	95.6	2.70
	5	59.4	62.8	96.2	2.58
17-20	1	49.6	61.5	100.9	3.31
	2	54.6	6 .6	92.5	2.75
	3	58.2	6 .4	97.6	2.61
	4	60.1	64.0	101.9	2.63
	5	58.5	64.4	98.4	2.61
21-22	1	52.0	62.0	97.0	3.01
	2	52.7	62.8	89.7	2.71
	3	57.7	64.8	105.0	2.81
	4	57.9	65.2	109.5	2.90
	5	53.9	66.2	102.2	2.86
0-22	1	60.9	60.6	102.2	2.77
	2	62.7	60.4	97.3	2.57
	3	65.4	62.8	101.3	2.47
	4	64.8	62.1	104.3	2.59
	5	61.2	63.2	100.4	2.60

Table 9. Composition and chemical analysis of the feeds (Percentage)

Ingredients	1st period (a)		2nd period (b)	
	Control	Experimental	Control	Experimental
Corn	63.7	63.4	67.7	67.5
Dried skim milk	4	4	-	-
Defatted rice bran	-	-	8	8
Glucose	7	7	5	5
Soybean meal	17	-	13	-
White fishmeal	(	-	4	-
Kanepron	-	23	-	17
$CaCO_3$	0.2	1.5	0.5	1.4
$CaHPO_4$	1.1	-	0.8	-
Salt	0.5	0.5	0.5	0.5
Vitamins & Minerals	0.5	0.5	0.5	0.5
Methionine	-	0.1	-	0.1
Moisture	12.43	11.35	11.24	11.33
Crude protein	21.06	19.39	16.34	16.48
Crude fat	3.30	3.74	3.45	3.90
Crude fiber	1.52	2.44	3.32	3.38
Crude ash	5.38	4.94	4.88	4.73

(a) 7-11 weeks of age
(b) 12-31 weeks of age

Table 10. Daily gain and feed conversion

	Initial body weight(kg)		Final body weight(kg)		Daily gain(kg)		Total feed intake(kg)		Daily feed intake(kg)		Feed conversion(kg)	
	A	B	A	B	A	B	A	B	A	B	A	B
Weeks of age												
6 - 11	7.3	6.8	22.1	21.2	0.42	0.41	29.4	27.4	0.84	0.78	2.0	1.9
12 - 19	22.1	21.2	50.4	50.7	0.51	0.53	78	78	1.39	1.39	2.7	2.6
20 - 31	50.4	50.7	102.2	96.2	0.62	0.54	168	168	2.0	2.0	3.2	3.7
Total	7.3	6.8	102.2	96.2	0.54	0.51	275.4	273.4	1.57	1.56	2.9	3.1

Note: A --- Experimental
B --- Control

Table 11. Composition of the feeds
(Percentage)

Group	1	2	3	4	5	6
Fish meal	50	40	30	20	10	0
Kanepron	0	15	30	45	60	75
Wheat flour	44	39	34	29	24	19
Vitamins	1	1	1	1	1	1
Feed oil	5	5	5	5	5	5
Total	100	100	100	100	100	100

Table 12. Calculated analysis of the feeds
(Percentage)

Group	1	2	3	4	5	6
Moisture	12.39	11.80	11.65	10.71	10.55	10.85
Crude protein	44.05	42.91	43.24	42.97	43.08	47.77
Crude fat	5.35	5.04	5.10	5.30	5.52	5.89
Crude ash	10.02	9.19	8.62	7.68	7.32	6.96
Crude fiber	1.86	2.51	3.20	3.86	2.71	4.52

Table 13. Weight of carp and feed efficiency

	Days	1	2	3	4	5	6
Number of carp	0	383	384	395	391	400	409
	30	381	374	390	389	399	407
	57	374	364	383	381	389	404
	86	356	356	374	366	380	399
	115	346	349	363	358	371	395
Number of dead carp		37	35	32	33	29	14
Body weight of dead carp(kg)		8.5	8.1	8.1	6.7	6.7	2.9
Total body weight(kg)	0	38.0	38.0	38.0	38.0	38.0	38.0
	30	62.6	65.8	67.5	69.6	65.0	58.5
	57	92.7	106.4	113.2	114.5	98.0	88.5
	86	114.8	141.7	155.9	150.4	127.7	112.2
	115	122.2	157.0	174.1	168.0	142.5	125.7
Total weight gain(kg)		92.7	127.1	144.2	8.6	111.2	90.6
Average body weight(g)	0	99.2	99.0	96.2	97.2	95.0	93.0
	30	164.5	176.0	173.0	179.0	163.0	143.9
	57	248.0	292.0	296.0	300.5	252.0	219.0
	86	322.5	398.0	416.5	411.0	336.0	281.5
	115	354.0	449.5	480.0	469.5	384.0	318.0
Feed consumption(kg)		135.7	153.6	161.6	161.6	146.9	135.1
Feed efficiency(%)		68.3	82.7	89.2	85.8	75.7	67.0
Protein efficiency(%)		163.7	203.7	219.7	212.2	187.2	166.7

Table 14. Composition of the feeds
(Percentage)

Group	1	2	3	4
Fish meal	70	35	35	35
α-starch	29	29	29	29
Vitamin mix	1	1	1	1
Sulfite waste liq. yeat		35		
Beer yeast			35	
Kanepron				35

Table 15. Calculated analysis of the feeds
(Percentage)

Group	1	2	3	4
Moisture	10.3	8.8	7.5	7.4
Crude protein	46.2	43.4	41.6	41.8
Crude fat	5.3	3.8	4.5	4.4
Crude fiber	0.3	0.8	0.4	1.6
Crude ash	11.2	8.3	8.4	8.6

Table 16. Average weight of eels and feed efficiency

Experimental period	Group	1	2	3	4
1 - 26	Initial Number	129	135	121	129
	Final	129	135	121	129
	Number of dead eels	0	0	0	0
	Av. weight (g) Initial	23.3	22.2	24.8	23.3
	Final	31.6	29.7	30.6	31.4
	Av. weight gain	8.3	7.5	5.8	8.1
	Feed efficiency (%)	96	90	62	94
27 - 43	Initial Number	75	73	77	74
	Final	75	73	76	74
	Number of dead eels	0	0	0	0
	Av. weight (g) Initial	40.0	41.1	39.0	40.5
	Final	54.0	54.9	51.7	54.7
	Av. weight gain	14.0	13.8	12.7	14.2
	Feed efficiency (%)	102	98	96	102
44 - 77	Initial Number	74	72	73	72
	Final	74	72	73	71
	Number of dead eels	0	0	0	1
	Av. weight (g) Initial	60.6	61.6	59.6	61.8
	Final	74.6	78.2	74.6	79.1
	Av. weight gain	14.0	16.6	15.0	17.3
	Feed efficiency (%)	60	67	63	65
	Hematocrit value (%)	25.8	11.4	19.8	27.8
	Serum protein (%)	9.7	7.3	9.8	8.8

Table 17. Composition of the feeds
(Percentage)

Group	1	2	3
Fish meal	64.0	26.0	26.0
Kanepron	0	52.0	52.0
α -starch	15.0	15.0	14.2
β -starch	14.0	0	0
Vitamin mixture	2.0	2.0	2.0
Methionine	0	0	0.8
Feed oil	3.0	3.0	3.0

Table 18. Calculated analysis of the feeds
(Percentage)

Group	1	2	3
Moisture	10.19	7.21	7.19
Crude protein	44.73	46.12	46.87
Crude fat	3.53	3.78	4.12
Crude fiber	0.26	2.68	2.61
Crude ash	7.14	8.60	8.59
N.F.E.	34.15	31.61	30.62

Table 19. Average body weight (Gram)

Group \ Days	0	42	84	126	154
1	151.5	194.4	237.5	283.2	318.6
2	153.1	192.6	232.2	284.0	320.0
3	150.0	189.9	228.8	276.0	311.6

Selection

Group \ Days	154	196	238	280
1	354.8	430.6	519.4	632.1
2	379.3	468.8	579.8	715.5
3	360.7	446.7	550.8	686.1

Table 20. Average weight gain and feed conversion

Days	Group	Feed consumption (g)	Weight gain (g)	Feed conversion
0	1	69.22	43.1	1.60
|	2	63.39	39.5	1.60
42	3	65.54	39.9	1.64
43	1	66.99	43.1	1.55
|	2	66.18	39.6	1.67
84	3	65.57	38.9	1.69
85	1	81.22	45.7	1.78
|	2	90.72	51.8	1.75
126	3	84.96	47.2	1.80
127	1	65.65	35.4	1.85
|	2	66.31	36.0	1.84
154	3	64.87	35.6	1.82
		Selection		
155	1	136.77	75.8	1.80
|	2	147.51	89.5	1.65
196	3	155.68	86.0	1.81
197	1	178.54	88.8	2.01
|	2	190.39	111.0	1.72
238	3	202.04	104.1	1.94
239	1	197.12	112.7	1.75
|	2	210.85	135.7	1.55
280	3	207.53	135.3	1.53
0	1	795.52	444.4	1.79
|	2	834.99	503.1	1.66
280	3	846.19	487.0	1.74

Table 21. Estimated plant investment cost and production cost
(Yen, Japan base, 1973)

(a) Battery-limit plant investment cost[1] for a 100,000 tpa plant:

7,500 million Yen 1/

(b) Production cost (Yen per ton):

n-Paraffins	17,300
Additives	11,000
Utilities	14,000
Packaging	1,000
Taxes & insurance	1,500
Labor	700
Plant overhead	700
Maintenance & plant supplies	1,500
Depreciation	6,800
Total production cost	54,500

1/ The following items are excluded from the cost: water-treatment facilities, boiler, power station, waste-water-treatment facilities, fire-fighting facilities, maintenance room, office, ware house, temporary facilities, etc.

Figure I. Block flow diagram of the process

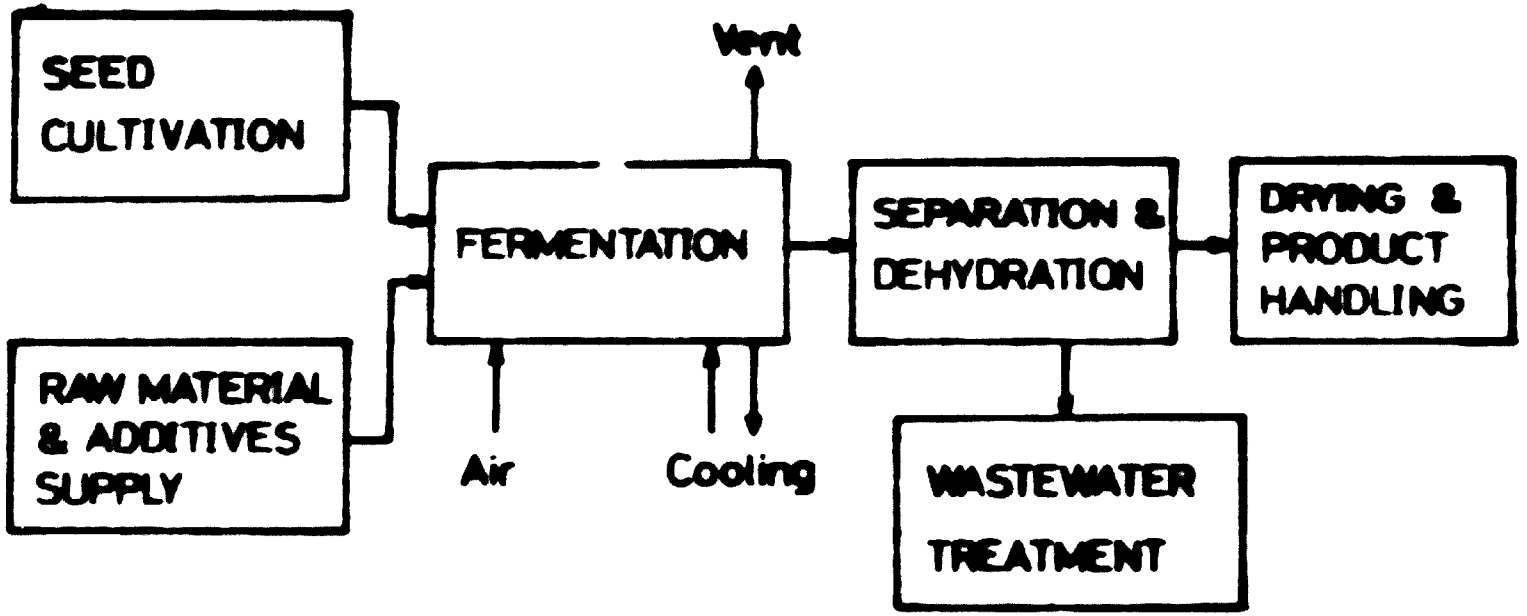


Figure II. Simplified schematic representation of air-lift fermentor

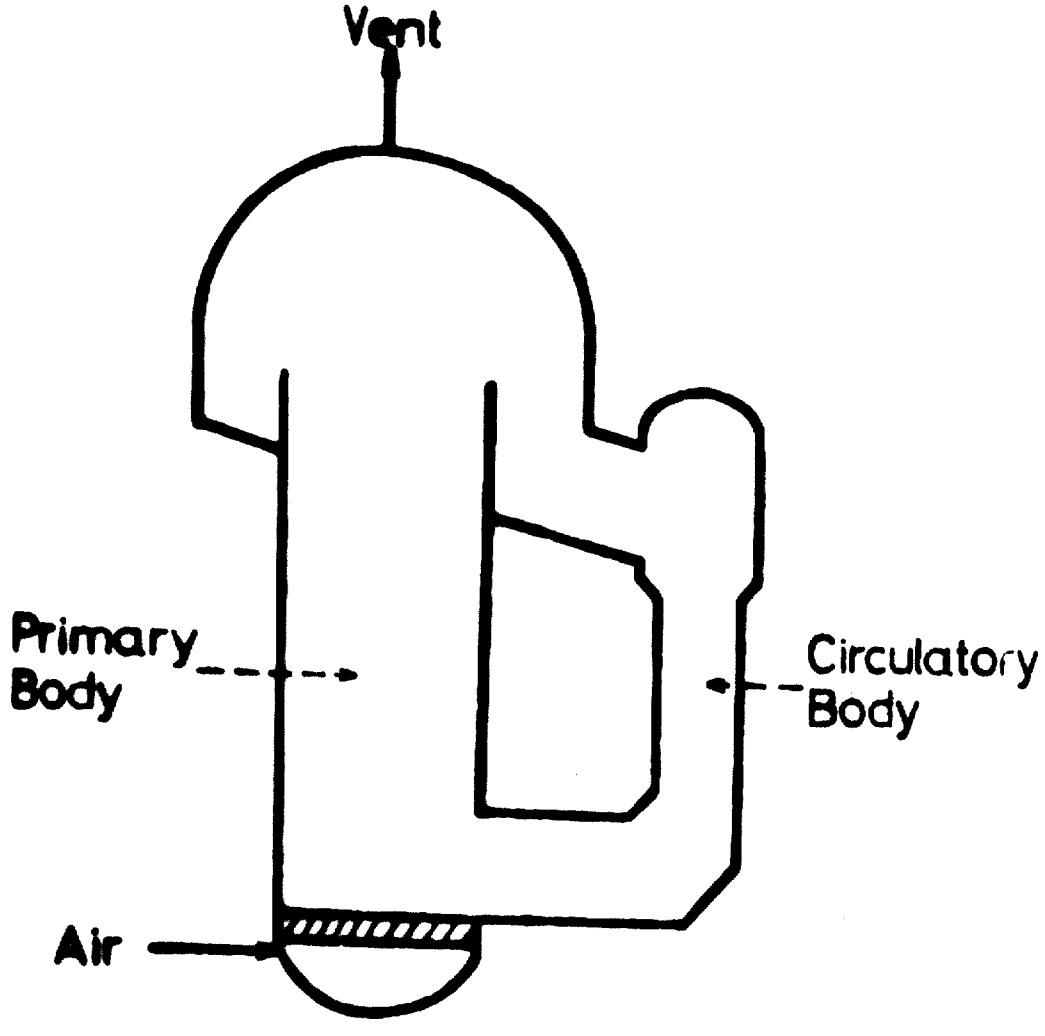
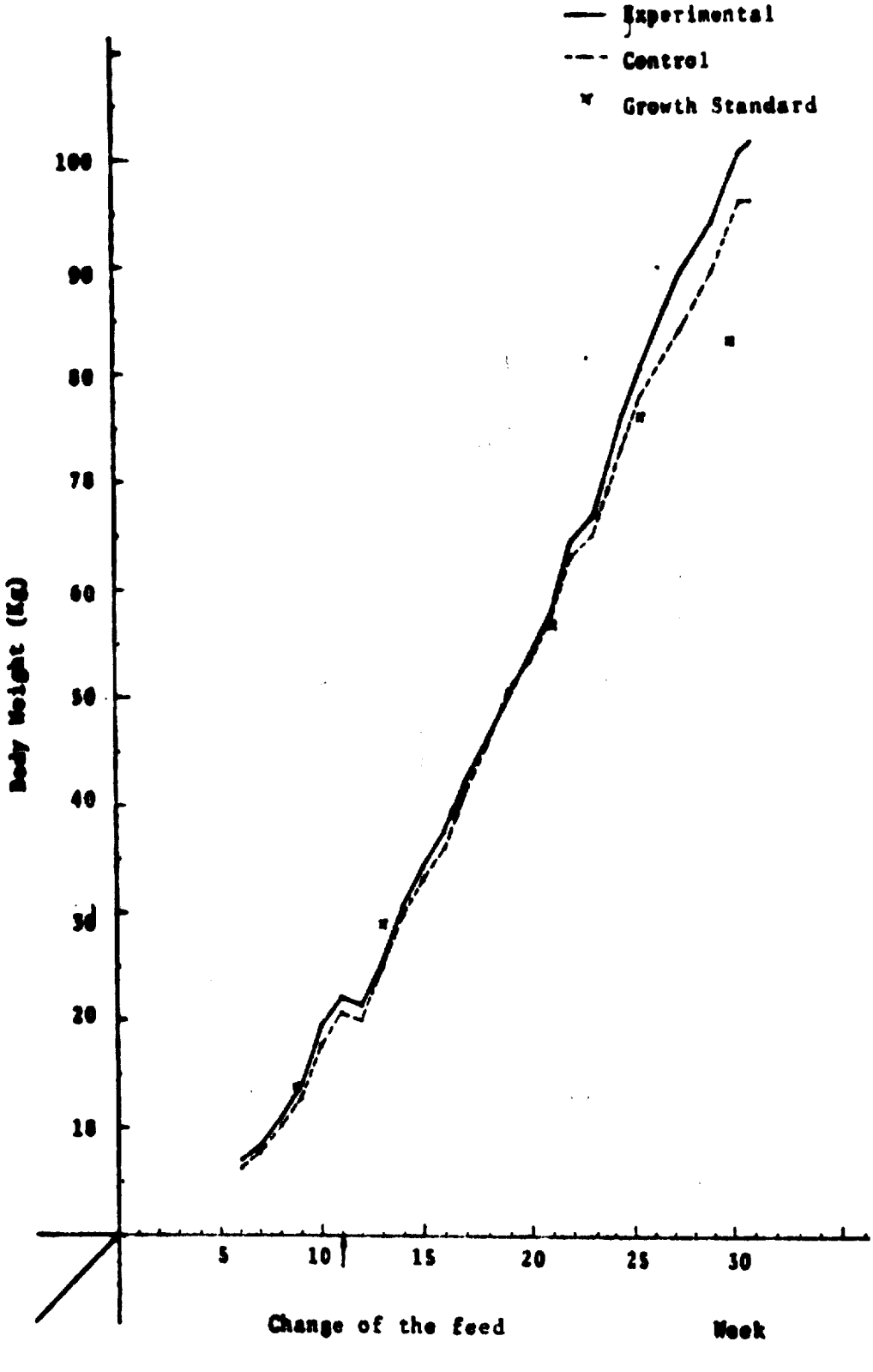


Figure III. Growth Cu ve of Pigs



Appendix

A. Strain (microorganisms used for the production of n-Paraffin-Yeast)

(a) Microbiological studies on morphological and biological properties

(b) Mutability

(c) Infectivity

B. Raw material (culture medium including n-paraffin)

(a) Polycyclic aromatic hydrocarbons

(b) Heavy metals

C. Living cells (living cells at the stage of maximum growth in one cycle of the process of production)

(a) Polycyclic aromatic hydrocarbons

(b) Heavy metals

(c) Mycotoxins

(d) Toxicity tests

D. Broth filtrate (broth filtrate collected at the stage of maximum growth of microorganisms in one cycle of the process of production)

(a) Polycyclic aromatic hydrocarbons

(b) Heavy metals

(c) Mycotoxins

(d) Toxicity tests

E. The product (the product to be used as animal feed after the process of production)

(a) Living cells in the product

(b) Polycyclic aromatic hydrocarbons

(c) Heavy metals

(d) Mycotoxins (This test is not needed if no mycotoxin is detected both in the living cells and in the broth filtrate.)

(e) Toxicity tests

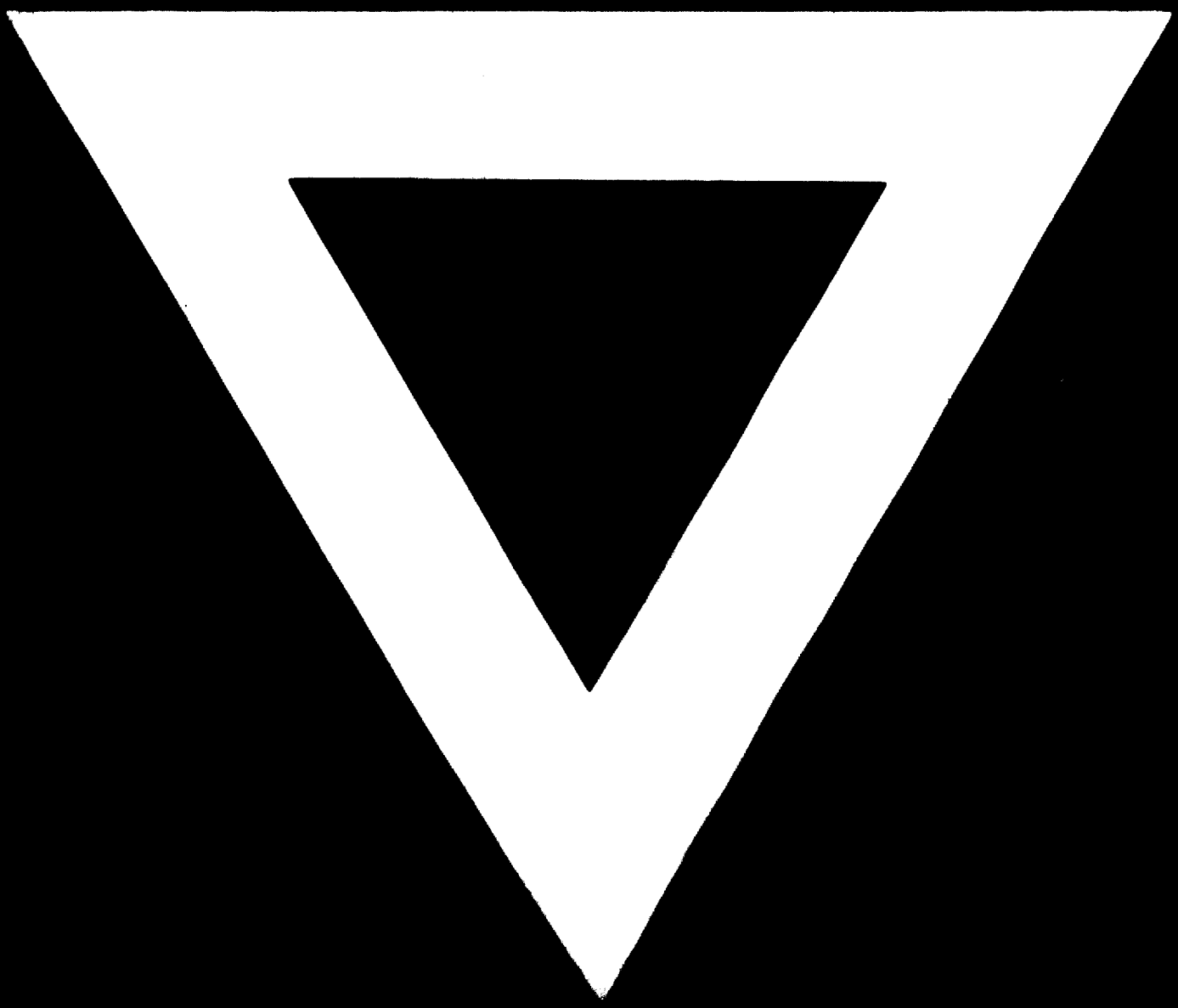
(f) Multiple generation test

F. Meat and milk and the others (meat, eggs, milk, and other edibles from domestic animals, poultry and fishes fed with the product as feed.)

(a) Polycyclic aromatic hydrocarbons

(b) Heavy metals

(c) Mycotoxins (This test is not needed if no mycotoxin is detected both in the living cells and in the broth filtrate.)



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