Chapter III

EXPERIMENTS

1. Preparation of Dry Brewery Yeast

Brewery yeast used in this study was a waste yeast solution from the bottom part of fermentation tank of the Thai Amarit Brewery Plant. The species of the yeast was identified as Saccharomyces carlbergenesis 286 (Amatavivat, personal communication). The yeast solution was brown in color with strong odor of beer. Hence, various processing steps as shown in Fig. 1 were carried out of the yeast solution before use in broiler's feed.

The yeast was first washed with water to remove beer and foreign materials that came with yeast. From preliminary investigations on washing the yeast with water, it was found that the ratio of yeast solution to water of 1:5 (by volume) was desirable. After washing, the yeast was separated by using filter press as shown in Fig. 2. The frame size of the filter press was 14 x 14 $in.^2$, the number of plate was 18, and the pressure was controlled at 0.5 $kg/cm.^2$ Following filtering, yeast paste was dried in compartment tray dryer as shown in Fig. 3. The moisture content of yeast was reduced to 5-6 per cent. The dryer was controlled at 60-65 $^{\circ}$c. The tray size was 16 x 32 $in.^2$ and the thickness of yeast paste on each tray was about 0.25 in. The drying time was about 3 hrs. It was necessary to dry at this low temperatures (60-65 $^{\circ}$c.) because essential amino acids expecially lysine would bind with reducing sugars to form Maillard reaction especially at higher temperatures (Braverman, 1963). The effect could cause the reduction of nutritive value of the yeast.

The dry brewery yeast was pale brown in color, with rather mild in odor. It was weighed and expressed in Kg. dry yeast per 10 litres of yeast

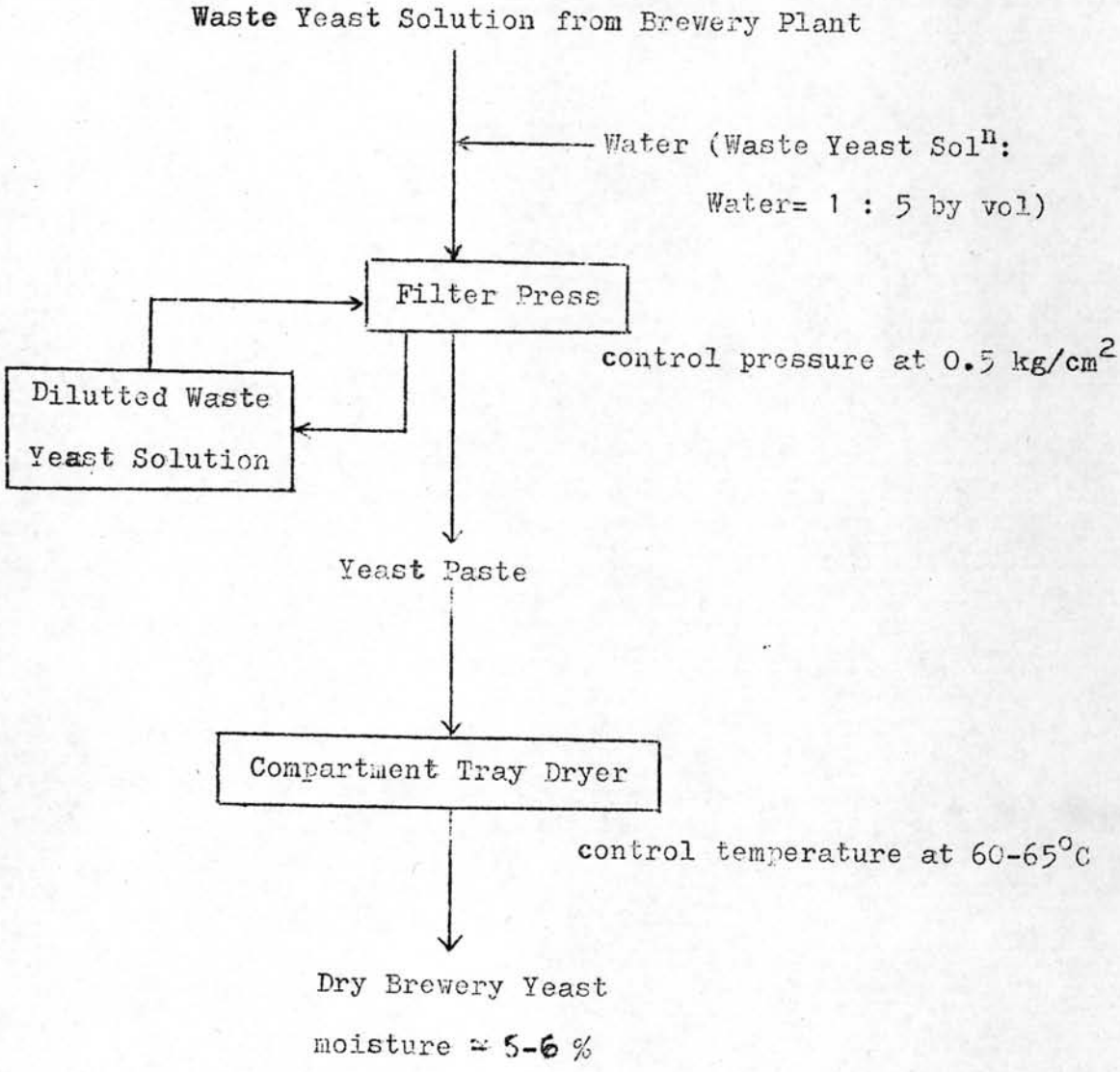
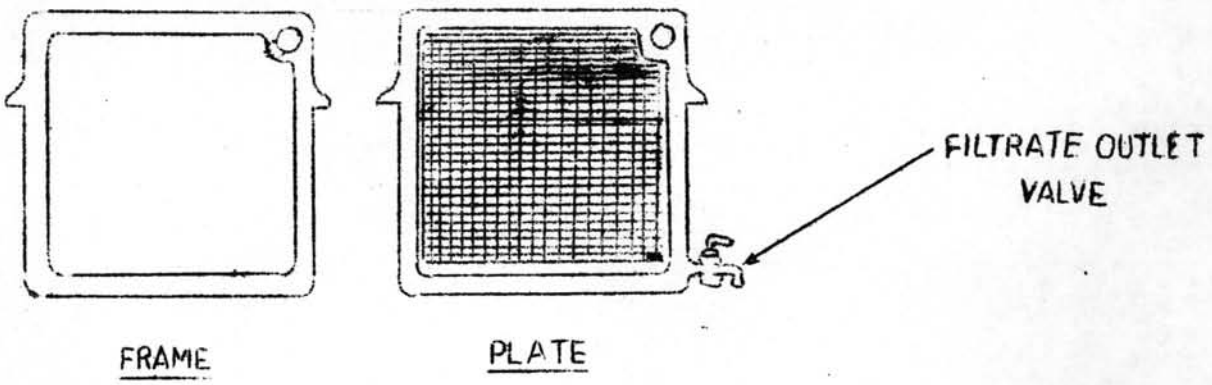


Fig. 1 Preparation Scheme of Dry Brewery Yeast



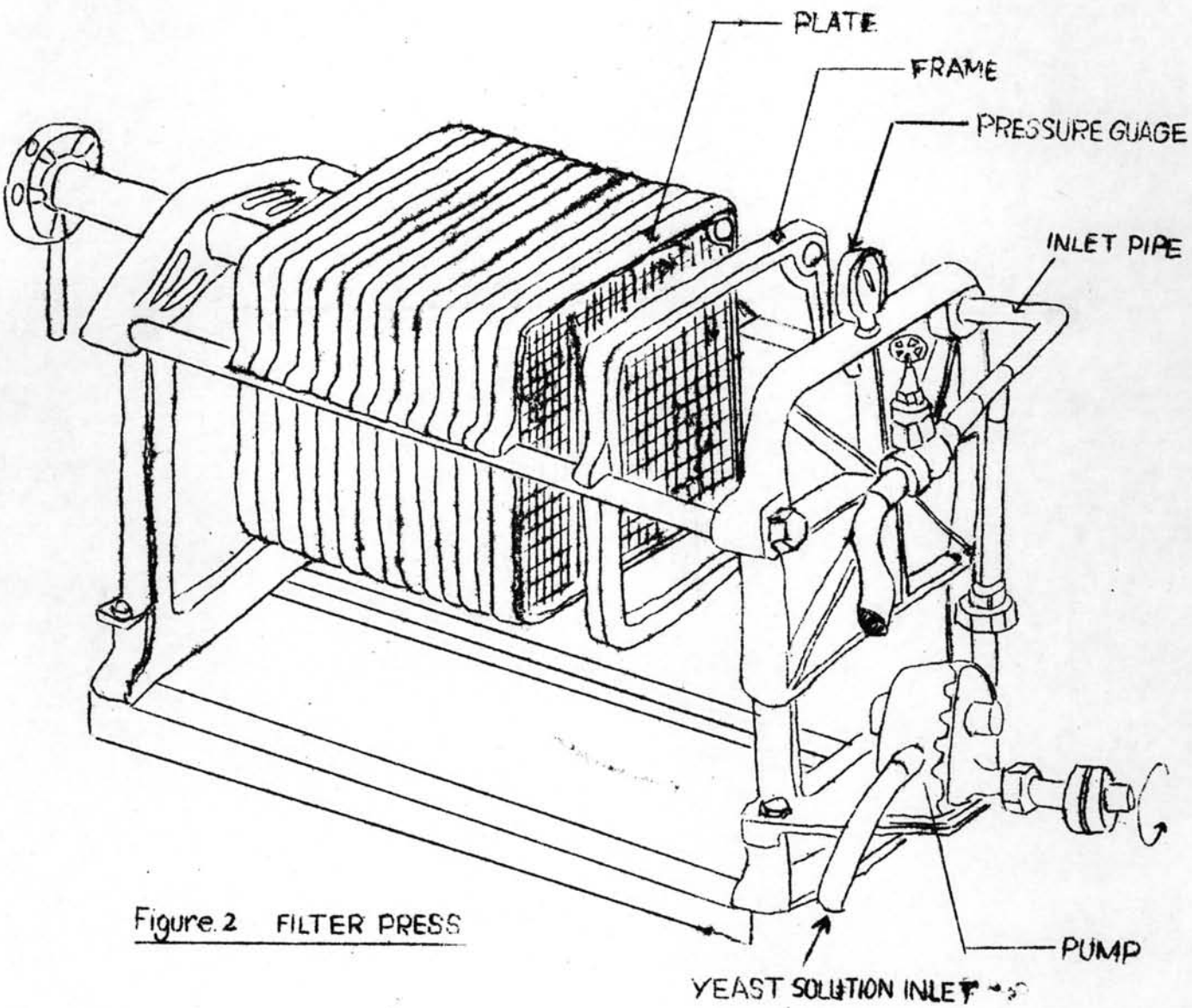


Figure. 2 FILTER PRESS

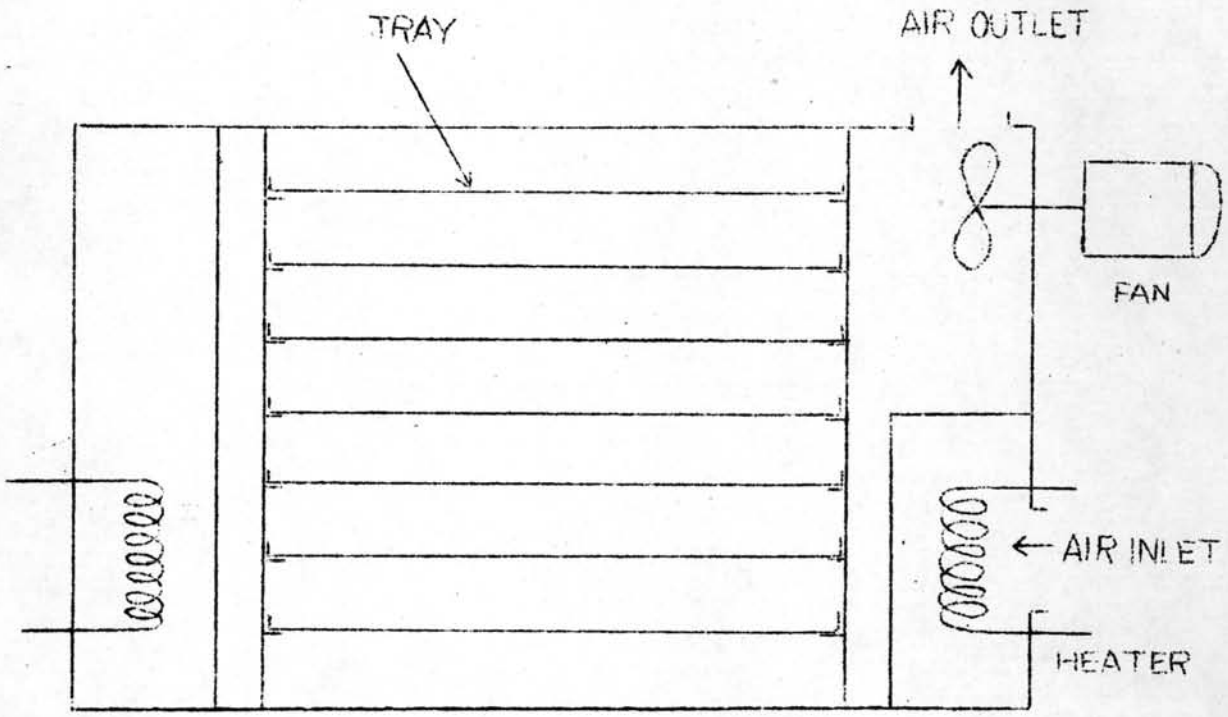


Fig. 3 Compartment Tray Dryer

solution. The concentration of yeast in the waste yeast solution before the preparation step was also determined by drying a known volume of yeast solution (about 50 ml.) in the tray dryer at 60-65 °c. until dry. The dry yeast was expressed in Kg. per 10 litres.

2. Chemical Analysis

As the protein requirement for broiler is critical, hence Kjeldahl protein, nucleic acids and amino acids were analysed in samples of yeast and various feed meals. Fat, calcium, phosphorus, fiber and ask content were also determined for comparison. The analytical methods were described as follows:

Kjeldahl protein (K-protein)

The Kjeldahl nitrogen was determined by method of AOAC (Lepper, 1945). Protein was calculated from the amount of nitrogen multiplied by 6.25. A sample size of 2.5 to 3.0 g. was used for digestion, 0.85 g. of catalysts (0.7 g HgO + 0.15 g Cu SO .5 H_2O) were used and the digestion was done in macro-Kjeldhl digestion flask until a clear solution was obtained (about 2.5 - 3.0 hrs). The clear solution was diluted with 200 ml of water and distilled with a mixture of 25ml, 45% of sodium-hydroxxide and 50 ml of 8% sodium thiosulfate solution. This was carried out in distillation apparatus until the distillate received in 50 ml of 0.5 N hydrochloric acid attained a total volume of about 200 ml. The quantity of nitrogen was determined by titration the distillate with 0.2 N sodium hydroxide. Four drops of methyl red (1 g in 200 ml alcohol) was used as an indicator, giving a yellow end point.

Lowry protein (L-protein)

The Lowry protein of whole yeast cells was determined according

to the method described by Herbert et al (Herbert, Phipps and Strange, 1971). Five g of sample was transfered in 500 ml of 0.1 N sodium hydroxide. The yeast suspension was blended at high speed for 10 min. After blending, the protein solution was filtered, 3 ml of the filtrate was diluted to 100 ml with water. One ml of the diluted protein solution was transfered into a test tube and 4 ml of a mixture of 1 ml of 1% copper sulfate, 1 ml of 2% sodium - potassium tartrate, and 100 ml of 2% sodium carbonate in 0.1 N sodium hydroxide was added and allowed to stand for 15 min. A quantity of 0.4 ml of 1 N Folin solution was added rapidly to the above solution. A blank containing 1 ml of distilled water instead of diluted protein solution was treated in the same way. After standing for 45 min., the absorbance was measured against the solution blank at 750 nm using Spectrophoto-meter (Baush and Lomb, Inc. Spectronic 20). Albumin was used to prepare a standard curve (See Appendix 1).

Nucleic acid

It was assumed that nitrogen content in brewery yeast represents protein nitrogen and non - protein nitrogen which comprises largely of nucleic acid (Vananuvat, 1975). Nucleic acid content in dry brewery yeast was therefore the difference between the values of K-protein and L-protein.

Fat

Fat in dry brewery yeast and feed meal was determined by method of AOAC (Lepper, 1945). Thimble containing 3 g of sample was placed in Soxhlet apparatus. A quantity of 100 ml of petroleum ether of boiling point 30 - 60 °c was poured into the apparatus and refluxed for 30 min. The fat - containing flask was dried at 60 °c to constant weight, cooled and weighed. Fat content was calculated as percentage.

Calcium

Calcium content was determined by method of AOAC (Lepper, 1945). Two grams of sample was ignited in muffle to carbon - free ash for 4 hrs. A few drops of nitric acid was added and it was ignited again at 550 °c for 1.5 hr. The residue was boiled in 10 ml of 50 % hydrochloric acid and then washed with 100 ml water. The residue suspension was neutralized with 1:1000 (by vol.) ammonium hydroxide using methyl red as indicator. Then 1.5 ml of 6 N hydrochloric acid, 5 g of urea and 5 ml of 4% of ammonium oxalate were added. The solution was brought to boil until the color was slightly orange, cooled and filtered through Whatman filter paper no 40. The paper and residue were washed with water, added 2.5 ml of sulfuric acid and heated to 85 °c. Calcium content was determined by titrated the solution with 0.05 N potassium permanganate.

Phosphorus

Phosphorus content was determined by method of AOAC (Lepper, 1945). Two grams of sample was ignited in muffle at 600 °c for 4 hrs. The ash was cooled, added 40 ml of 3.25 N hydrochloric acid and several drops of nitric acid and brought to boil. It was diluted to 100 ml with water and 6 ml of the diluted solution was added with 20 ml of molybdovanadate reagent (40 g ammonium molybdate H_2O in 400 ml hot water + 2 g ammonium metavanadate in 250 ml hot water + 250 ml of 70% hyperchloric acid and diluted to 2 lit with water), and diluted to 100 ml with water. After standing for 10 min, the transmittance was measured against a reagent blank at 400 n m using the Spectronic 20 spectrophotometer. Phosphorus standard solutions were used to prepare a standard curve. (See Appendix 2).

Fiber

Fiber content was determined by a modified method (ผลิตภัณฑ์อุตสาหกรรม ผลิตภัณฑ์มันสำปะหลัง, 2516.) A sample of 2.5 g was mixed with 0.255 N sulfuric acid and digested for 30 min. The solution was filtered through filter cloth, washed the residue with 200 - 300 ml hot water. The residue was added 200 ml of 0.313 N sodium hydroxide. It was then brought to digest for 30 min. The solution was filtered immediately through Gooch Crucible and washed with hot water. The residue was washed again with 10 ml ethyl alcohol. Then it was dried at 105-110 °c for 3 hrs, weighed, ignited in muffle to fibre-free at 600 °c for 30 min and reweighed. The fibre content was the difference between the weight of sample before and after ignition steps.

Amino Acid

A defated sample was hydrolyzed with 12 ml. 6 M hydrochloric acid at 110 °c for 24 hrs. This was done in an evacuated prescored, 10 ml. ampoule. The hydrolyzate was filtered through a milipore filter and 3 ml. of the filtrate was transfered into a small vial and dried under sodium hydroxide in a vacuum desiccator. The dried sample was dissolved in 3.8 ml. of 13% sucrose solution. A portion of 0.2 ml. norleucine was added in the sample as internal standard.

Amino acid analyses were conducted on a Technicon Auto Analyzer Colorimeter, 0.8 ml. of the sample containing about 0.4 mg. protein was applied to each of the columns. Amino acids were quantitatively determined by comparison with chromatograms of amino acid standards (the height-width method of measuring peak area was used). Norleucine appeared in the acidic and neutral amino acids' chromatogram immediately after leucine. Results were expressed as gm. of amino acid per 16 gm. of nitrogen. However, the hydrochloric acid treatment caused the full destruction of tryptophan

and half destruction of cystine (More and Stein, 1954). The amino acid analysis was performed at the Department of Chemistry, Kasetsart University.

3. Nutritional Assessment with Broilers

3.1 Broiler's feed meal

In feeding trial with poultry, broilers fed diet containing brewery yeast was compared with broilers fed control diet. The control rations were based on commercial formulation using fish meal and soy bean meal as protein sources and contained nutrients equal to our formulation which brewery yeast partly replaced the fish or soy bean meal. Protein content in initial feeding period (1-35 days) of the diet was 23%, whereas in final feeding period (35-56 days) the protein content of the diet was 20%.

The composition of enrichment and ingredients used in feed meals were shown in Appendice 3 and 4. All diet formulations were presented in Table 11 to 20.

3.2 Replacement of fish meal and soybean meal with brewery yeast

In the first trial, our feed meal was formulated using dry brewery yeast replaced 12.5 and 25 per cent of fish meal (see Appendix 5). The selection of 25% replacement was on arbitrary basis. Even though yeast contains protein comparable to fish meal(Appendix 4) but fish meal has characteristic fishy flavor which is very desirable to broilers. Hence, it was speculated that the use of more than 25 per cent yeast replaced fish meal would not be accepted by broiler in this preliminary study on the use of brewery yeast replacing fish meal. Furthermore, the study of 12.5 per cent replacement of fish meal with yeast was also carried out to check an indication of any harmful effect that might be observed in this study. This feeding trial was started on November 6, 1974 and finished on January 1, 1975.

Table 11

Formulation of feed meal containing brewery yeast replaced fish meal at 12.5 and 25 %. Values are expressed in percentage.

	Initial Feeding Period			Final Feeding Period		
	Control	12.5% Yeast	25% Yeast	Control	12.5% Yeast	25%Yeast
Corn	62.00	62.00	62.00	70.00	70.00	70.00
Fish meal #2	12.00	10.50	9.00	10.00	8.50	7.00
Soy bean meal	23.00	23.00	23.00	17.00	17.00	17.00
Brewery yeast	-	1.50	3.00	-	1.50	3.00
Oyster shell	0.75	0.75	0.75	0.75	0.75	0.75
Calcium diphosphate	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Enrichment	1.00	1.00	1.00	1.00	1.00	1.00
	100.00	100.00	100.00	100.00	100.00	100.00

Table 12

Formulation of feedmeal containing brewery yeast replaced soy bean meal at 24%. Values are expressed in percentage

	Initial Feeding Period		Final Feeding Period	
	Control	24% Yeast	Control	24% Yeast
Corn	62.0	62.00	70.00	70.00
Fish meal # 2	12.0	12.00	10.00	10.00
Soy bean meal	23.0	17.50	17.00	11.50
Brewery yeast	-	5.50	-	5.50
Oyster shell	0.75	0.75	0.75	0.75
Calcium diphosphate	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50
Enrichment	1.00	1.00	1.00	1.00
	100.00	100.00	100.00	100.00

Table 13

Chemical composition of the initial feeding period for control feed meal.

	Crude Protein	Crude Fat	Total Calcium	Total Phosphorus	Crude Fiber
	(Values are expressed in percentage)				
Corn	5.57	2.59	0.04	0.17	1.52
Fish meal #2	6.60	0.66	0.88	0.37	0.17
Soy bean meal	10.40	0.86	0.09	0.17	1.67
Brewery yeast	-	-	-	-	-
Oyster shell	-	-	0.26	-	-
Calcium diphosphate	-	-	0.18	0.13	-
Salt	-	-	-	-	-
Enrichment	-	-	-	-	-
	22.57	4.11	1.45	0.84	3.36

Table 14

Chemical composition of the initial feeding period feed meal containing brewery yeast replaced fish meal at 12.5%. Values are expressed in percentage.

	Crude ptotein	Crude Fat	Total Calcium	Total Phophorus	Crude Fiber
Corn	5.57	2.59	0.04	0.17	1.52
Fish meal # 2	5.78	0.59	0.77	0.32	0.15
Soy bean meal	10.40	0.86	0.09	0.17	1.67
Brewery yeast	0.81	0.038	0.003	0.016	0.086
Oyster shell	-	-	0.26	-	-
Calcium diphosphate	-	-	0.18	0.13	-
Salt	-	-	-	-	-
Enrichment	-	-	-	-	-
	22.56	4.058	1 343	0.806	3.426

Table 15

Chemical composition of initial feeding period feed meal containing brewery yeast replaced fish meal at 25 %

	Crude Protein	Crude Fat	Total Calcium	Total Phosphorus	Crude Fiber
	(Values are expressed in percentage)				
Corn	5.57	2.59	0.04	0.17	1.52
Fish meal # 2	4.95	0.49	0.66	0.27	0.13
Soy bean meal	10.40	0.86	0.09	0.17	1.67
Brewery yeast	1.62	0.076	0.006	0.032	0.172
Oyster shell	-	-	0.26	-	-
Calcium diphosphate	-	-	0.18	0.13	-
Salt	-	-	-	-	-
Enrichment	-	-	-	-	-
	22.54	4.016	1.236	0.772	3.492

Table 16

Chemical compossition of the final feeding period for control feed meal. Values are expressed in percentage.

	Crude Protein	Crude Fat	Total Calcium	Total Phosphorus	Crude Fiber
Corn	6.29	2.93	0.05	0.19	1.72
Fish meal # 2	5.50	0.55	0.59	0.24	0.14
Soy bean meal	7.69	0.64	0.07	0.14	1.24
Brewery yeast	-	-	-	-	-
Oyster shell	-	-	0.26	-	-
Calcium diphosphate	-	-	0.18	0.13	-
Salt	-	-	-	-	-
Enrichment	-	-	-	-	-
	19.48	4.12	1.15	0.70	3.10

Table 17

Chemical compossition of the final feeding period feed meal containing brewery yeast replaced fish meal at12.5 % Values are expressed in percentage.

	Crude Protein	Crude Fat	Total Calcium	Total Phosphorus	Crude Fiber
Corn	6.29	2.93	0.05	0.19	1.72
Fish meal # 2	4.68	0.46	0.48	0.20	0.12
Soy bean meal	7.69	0.64	0.07	0.14	1.24
Brewery yeast	0.83	0.038	0.003	0.016	0.086
Oyster shell	-	-	0.26	-	-
Calcium diphosphate	-	-	0.18	0.13	-
Salt	-	-	-	-	-
Enrichment	-	-	-	-	-
	19.49	4.038	1.043	0.676	3.166

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Table 18

Chemical composition of the final feeding period feed meal containing brewery yeast replaced fish meal at 25 %. Values are expressed in percentage.

	Crude Protein	Crude Fat	Total Calcium	Total Phosphorus	Crude Fiber
Corn	6.29	2.93	0.05	0.19	1.72
Fish meal # 2	3.85	0.38	0.37	0.15	0.10
Soy bean meal	7.69	0.64	0.07	0.14	1.24
Brewery yeast	1.65	0.076	0.006	0.032	0.172
Oyster shell	-	-	0.26	-	-
Calcium diphosphate	-	-	0.18	0.13	-
Salt	-	-	-	-	-
Enrichment	-	-	-	-	-
	19.48	4.026	0.936	0.642	3.232

Table 19

Chemical composition of the initial feeding period feed meal containing brewery yeast replaced soy bean meal at 24 %. Values are expressed in percentage.

	Crude Protein	Crude Fat	Total Calcium	Total Phosphorus	Crude Fiber
Corn	5.57	2.59	0.04	0.17	1.52
Fish meal # 2	6.60	0.66	0.88	0.37	6.17
Soy bean meal	7.91	0.66	0.06	0.13	1.27
Brewery yeast	2.97	0.14	0.02	0.06	0.32
Oyster shell	-	-	0.26	-	-
Calcium diphosphate	-	-	0.18	0.13	-
Salt	-	-	-	-	-
Enrichment	-	-	-	-	-
	23.05	4.00	1.44	0.86	3.28

Table 20

Chemical composition of the final feeding period feed meal containing brewery yeast replaced soy bean meal at 24%. Values are expressed in percentage.

	Crude Protein	Crude Fat	Total Calcium	Total Phosphorus	Crude Fiber
Corn	6.29	2.93	0.05	0.19	1.72
Fish meal # 2	5.50	0.55	0.59	0.24	0.14
Soy bean meal	5.20	0.43	0.04	0.08	0.84
Brewery yeast	2.97	0.14	0.02	0.06	0.32
Oyster shell	-	-	0.26	-	-
Calcium diphosphate	-	-	0.18	0.13	-
Salt	-	-	-	-	-
Enrichment	-	-	-	-	-
	19.96	4.05	1.14	0.70	3.02

As both fish meal and soybean meal are the important sources of protein in broiler's feed, hence it was decided to study on the effect of replacement of soybean meal with yeast at similar percentage as those in the first trial. In the second trial, brewery yeast was used to replace soybean meal at 24 per cent. The result of the first trial appeared that there was somewhat in difference in body weight and overall protein efficiency of broilers fed with feed meals containing 12.5 and 25 per cent yeast replaced fish meal, (See next chapter), therefore only 24 per cent replacement was carried out in this trial. The later trial was began on Jan 8, 1975 and term inated on March 5, 1975. The birds used in all experiments were "Arbor Acres". The diet was fed in powder form. These feeding trials were intended to simulate the commercial practice. Hence, they were performed at Research farm Station of Charoen Pokphan Feedmill Co., Ltd., at kilometer 13, Bangka Bangkok.

3.3 Housing and feeding preparation .

Housing of broilers was arranged according to Fig 4 and lay-out in each room was shown in Fig 5. The feeding house was divided into 6 rooms, each room was built for 150 broilers. The floor was covered with saw dust which was rather rough for birds, but better than husk because saw dust could absorb water much more than husk. The dimension of each room about 6×3.5 m^2., generally one square metre area was used for 10 birds. In this study each room was occupied with 100 birds. One or two days before the broilers was brought to the research farm, the house was cleaned, a protector and brooder was prepared. The protector, 10 ft. in diameter and $1\frac{1}{2}$ ft. high, was used to keep birds inside of it. In the middle of the protector there was a brooder, 6 ft. in diameter, which supply heat using an electrical lamp. The temperature of the brooder was control at 95 F on the first day that one-day old broilers were brought. During the

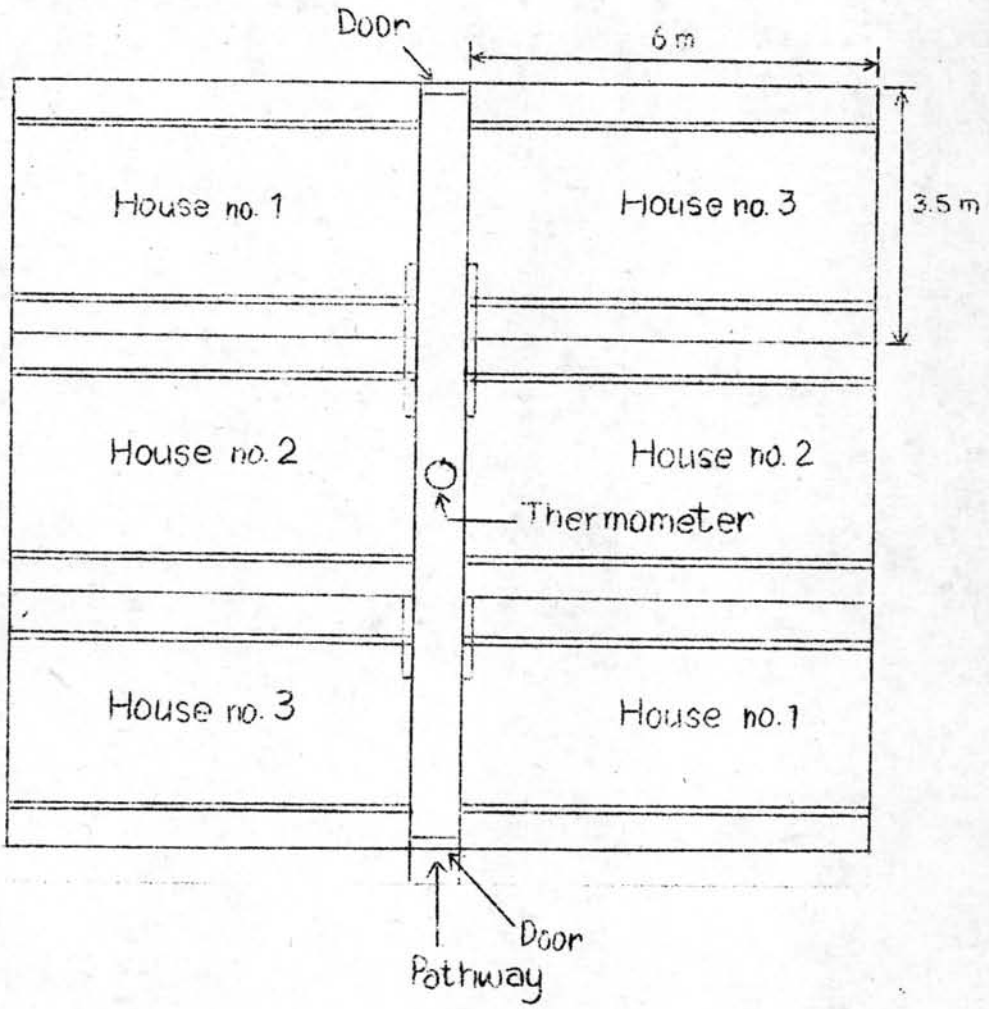

Fig. 4 Lay out of chicken feeding House

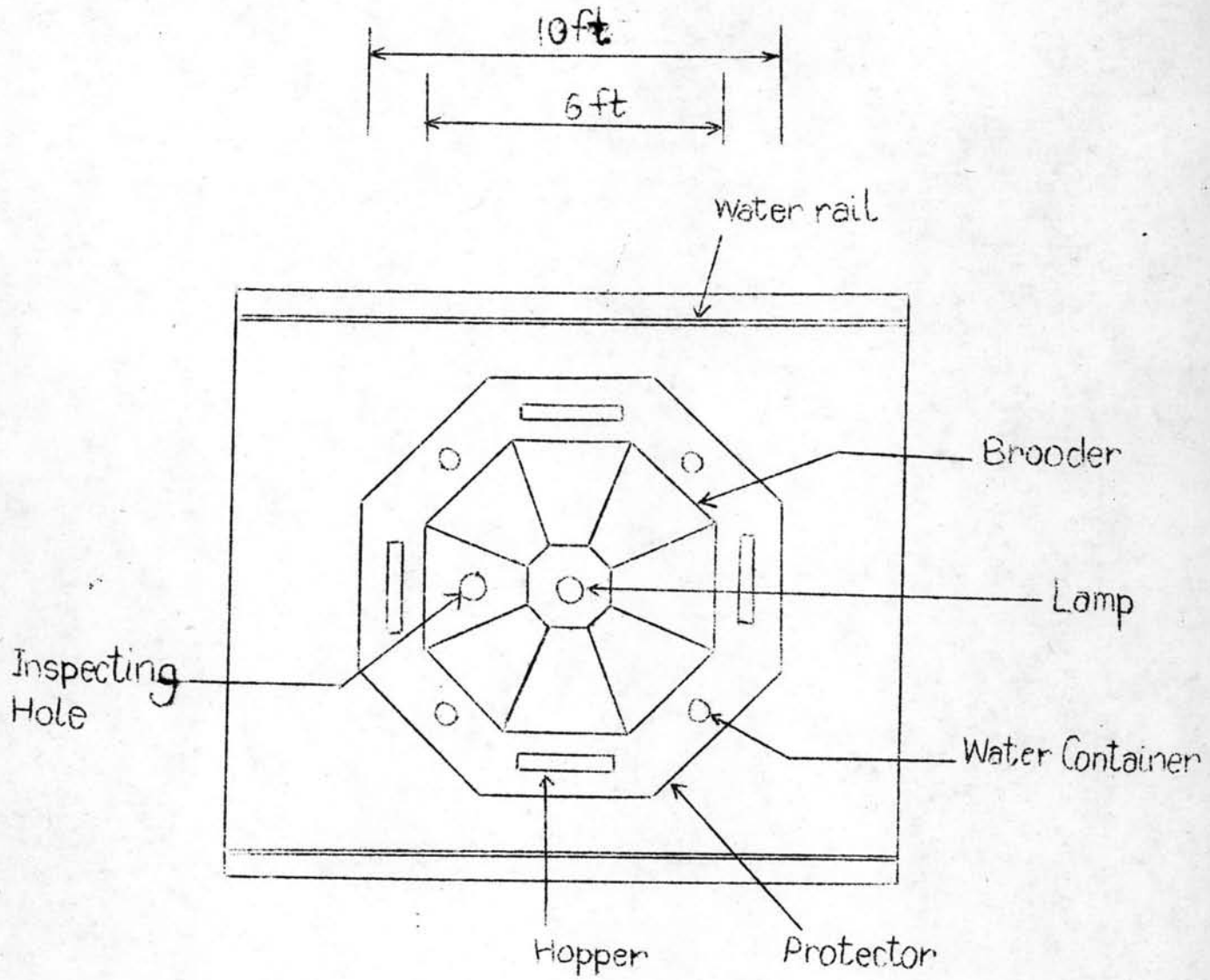

Fig. 5 Lay out of each room in feeding House

first week it was controlled at about 90 F (Both of the brooder and the protector were removed when the birds passed one week old.) Around the brooder, 4 bowls containing water and 4 hoppers were arranged in the manner that shown in Fig. 5. In this way broilers could drink water after eating. One bowl contained about one gallon of water, the bowls were removed when the broilers was two-week old and water rails were used in place of them.

3.4 Feeding trials with broilers

Feeding trials with broilers in commercial research farm are performed with not less than 100 birds per group with equal number of males and females. However, in this study it was carried out at 100 birds per group; 50 males and 50 females. A summary of experimental design in both the first and the second trials was shown in Table 21. For the diet containing brewery yeast replaced 24% soybean meal, it was not done in duplicates due to some errors in preparation of the meal.

During feeding trials, there are two feeding periods, i.e., initial feeding and final feeding periods as explained below.

3.4 a. Initial feeding period (1-35 days)

When the birds were brought to the farm, they were put in the house under the protector. After allowing sufficient time for baby chicks to drink water containing antibiotic, "Pharmocin"[1], the birds were then given diet containing 23% protein. The diet was given in a quantity that it was emptied in 2-3 hrs. Then the diet was given again 3 times a

[1] Pharmocin was a soluble antibiotic, containing chlotetracycline hydrochloric acid 25 g/lb. It was used to promote growth and prevent desease caused by bacteria. Dosage used was 7.2 g dissolving in one gallone water.

Table 21

Experimental design for feeding trials

The 1st Feeding Trial				The 2nd Feeding Trial	
	% brewery yeast used to replace fish meal			% brewery yeast used to replace soy bean meal	
	0	12.5	25	0	24
Total groups	2	2	2	2	1
No. of birds/gr.	100	100	100	100	100
Total birds	200	200	200	200	100

day as well as water. It was necessary to take good care of the broilers because in this period their resistance to environmental conditions was quite low. At 6-9 days old, broilers' mouths were cut at the tips. This is necessary in order to prevent picking each other. The baby chicks could eat more diet, grow regularly and have beautiful skin. After cutting their mouths, the broilers were given concentrated soluvit[1] to relieve stress and tire-ness. It would help the birds to eat more feed meal. When the birds were 12 - 16 days old, they were given vaccines to prevent New-castle desease and inflamative trachea. One or two drops of vaccines were delivered in the birds' noses or eyes to produce antibody in the birds.

3.4 b. Final feeding period. (35 - 56 days)

In this period, the birds were fed 20% protein formulation diet. The broilers were taken care the same as in initial feeding period.

During the initial and final feeding periods various data were determined:

a) Number of alive birds;

b) Broilers' activity and characteristics of growth.

c) Broiler's body weight and the consumption of feed meal. Here, a group of broilers was weighed and averaged. The consumption of feed meal was the difference between the weight of the feed introduced and left over during a week time and averaged.

[1] Soluvit was a water soluble complex vitamins, it was given by dissolving 1 g in 1 gallon of drinking water