SUMMARY

When 25 percent of the crude protein (N x 6.25) of a Puerto Rican modified rural diet was replaced by an equivalent amount of crude protein of either food or brewers' yeasts, no appreciable improvement could be observed in the nutritive value of the former. However, the same amount of either one of these two yeasts corrected an evident deficiency in some of the factors belonging to the B group of vitamins.

So far as the rat requirements were concerned, the rural modified diet was found to be adequate in its thiamine content but deficient in riboflavin. As fed (air-dried), this diet contained 1.24 μg. per gram of thiamine and only 0.75 μg. of riboflavin.

When dried skimmed milk was used in the same proportion in place of the yeasts, a slight but definite improvement in the quality of the protein was noted.

II. Growth and Reproduction Performance of Rats Fed on Yeast as Sole Source of Protein

Dried yeast, as a food, functions in two very important ways. One is by supplying all known B vitamins and the other, by acting as a source of protein when utilized in the proper proportions.

It is the purpose of the writers to discuss the adequacy of yeast protein for the maintenance of normal growth and reproduction in the rat. The literature dealing with the subject has been not only scarce but also somewhat contradictory; for this reason, the authors have thought it well to review it here.

In 1919 Osborne and Mendel[9] fed rats on brewers' yeast as the sole source of protein at levels of 30 and 40 percent. The animals were maintained on these diets for more than a year without exhibiting any gross abnormality, those on the 40 percent level growing at a normal rate. The rats fed the 30 percent level did not do so well. In 1923 Nelson, Heller, and Fulmer[10] utilized diets containing from 25 to 50 percent of dried brewers' yeast and observed normal growth and reproduction in the animals receiving 45 percent level. Three generations of rats were reared on these diets, the growth of the off-

9. T. B. Osborne and L. B. Mendel, The nutritive value of yeast protein. J.Biol.Chem., 38:223–227, 1919.

10. V. E. Nelson, V. G. Heller, and E. I. Fulmer, Studies on yeast. VII. The dietary properties of yeast. J.Biol.Chem., 57-415–424, 1923.

spring being always slightly below normal. At a 50 percent level, the growth curve flattened out after three months. Mangold, Columbus, and Hock[11] reported somewhat similar results in 1939 when feeding a *Torula* yeast grown on sulphite waste.

Recently (1946), Sure[12] fed a brewers' yeast at 30 and 40 percent planes of intake, which furnished 14 and 18 percent protein, respectively, and observed that the growth, reproduction, and lactation performance in three consecutive generations of rats was comparable to that obtained when using a diet containing 15 percent purified casein. The yeast in Sure's ration not only supplied proteins to the diet but also B vitamins, phosphorus, potassium, magnesium, and trace elements as well. The rations were well fortified with an abundance of all the fat-soluble vitamins. Sure asserts that those strains of yeast studied and reported by him "in addition to furnishing all the vitamin B complex, are also excellent tissue builders when fed as the only source of protein for growth, reproduction and lactation."

This same investigator, however, failed to obtain even normal growth in his rats with another type of brewers' yeast studied at a later date.[13] There are other reports in the literature pointing to the inadequacy of some types of yeast in maintaining normal growth and reproduction when utilized as the sole source of protein in the ration.

Again in 1928, Still and Koch[14] made a careful study of bakers' yeast and reported subnormal growth of rats fed a ration containing 30 percent of bakers' yeast as the sole source of protein, concluding that yeast protein is not utilized readily by the rat. In 1931 Kon and Markuze[15] reported that the growth rate on a brewers' yeast diet was improved by replacing part of the yeast by wheat flour. Utilizing a diet containing 9.3 percent of crude protein, in which one sixth of the total protein was provided by a mixture of rye and wheat, Hock,[16] in 1942, observed that when *Torula* or brewers' yeast were added to the ration, the rats grew only half as rapidly as when the remainder

11. E. Mangold, A. Columbus, and H. Hock, Long-time feeding of rats with yeast grown on the sulphite waste from cellulose manufacture. Biedermanns Zentr. B. Tierernähr, **11**: 357–369, 1939.

12. B. Sure, Biological value of food yeast proteins and their role as supplements to the proteins of the cereal grains. J.Am.Dietet., **22**:114–116, 1946.

13. Personal communication to J. A. G.

14. E. U. Still and F. C. Koch, The biological value of yeast proteins for the rat. Am.J. Physiol., **87**:225–248, 1928.

15. S. K. Kon and Z. Markuze, The biological values of the proteins of breads baked from rye and wheat flours alone or combined with yeast or soya bean flour. Biochem.J., **25**:1476–1484, 1931.

16. A. Hock, Über den biologischen Ergänzungswert verschiedener Nahrungs-proteine; der Ersatz von Tierischem Eiweiss durch Hefeeweiss. Biochem.Ztschr., **311**:385–401, 1942.

of the protein consisted entirely of fish meal. Normal growth was obtained, however, when 50 percent of the fish meal was replaced by yeast.

EXPERIMENTAL METHODS

Two types of yeasts were studied: a brewers' yeast[17] and a *Torula* yeast [18] produced in a Puerto Rican molasses medium at the Pilot Plant of the Puerto Rico Industrial Development Company. The latter was derived from a *Torula utilis* no. 3 strain in the University of Wisconsin collection.

The experimental animals were Wistar albino rats which were separated from their mothers on the twenty-eighth day and reared on the regular laboratory colony stock ration. They were weighed weekly and, once mature, the breeding experiments were commenced. The cages used had raised bottoms.

The composition of the various rations fed the animals is shown in Table 3. Protein was fed on the basis of the crude protein content (total Kjeldahl N x 6.25).

TABLE 3

Composition of the Various Diets Fed

Diet	*Percentage of Ingredients*										
	1	*2*	*3*	*4*	*5*	*6*	*7*[a]	*8*[a]	*9*[a]	*10*[a]	*11*[a]
Dried brewers' yeast	20	30	40		30	40		18			
Dried *Torula* yeast				30					17		
Casein							10				38
Cooked and dried red kidney beans										20	
Cooked and dried soybeans											
Cornstarch	56	46	36	46	46	36	66	58	59	56	38
Butter-fat							10	10	10	10	10
Soybean oil[b]	9	9	9	9							
Mazola oil					9	9					
Cod liver oil	1	1	1	1	1	1					
Sucrose	8	8	8	8	8	8	8	8	8	8	8
Salt mixture[c]	3	3	3	3	3	3	3	3	3	3	3
Cellu flour[d]	3	3	3	3	3	3	3	3	3	3	3

[a] Supplements: 2 mg. thiamine, 2 mg. pyridoxine, and 8 mg. riboflavin per kilo.
[b] Table grade of soybean oil; proved to be practically free of vitamin E.
[c] Modified Osborne and Mendel salt mixture (Hawk and Oser, Science, **74**:369, 1931).
[d] Obtained from Chicago Dietetic Products Co.

17. Fleischmann, Type 2019.
18. Supplied by R. Fernández García, Vice-President in Charge of Research, Puerto Rico Industrial Development Company, San Juan, Puerto Rico.

GROWTH OF RATS ON BREWERS' AND *Torula* YEASTS AS SOLE SOURCE OF PROTEIN

Procedure. Three trials were conducted at 20, 30, and 40 percent levels with brewers' yeast. As the amount of *Torula* available was small, only one single trial was run. A 30 percent level was utilized, previous results with brewers' yeast having indicated that, at this level, its maximum utilization was obtained. In the trials six rats (three males and three females), of an average initial weight of 39.3 g., were placed on each diet, a total of twenty-four animals. A test period of ten weeks was assigned them. The animals were given food and water *ad libitum*.

Another group of 92 rats fed on the laboratory colony stock diet was kept as positive controls,[19] their growth and reproduction performance being taken as the standard of comparison. This was always far superior to that observed in rats receiving the yeast diets, except in the case of growth performance in the first generation animals fed on Diet 2 (30 percent brewers' yeast).

Table 4 shows the body weight of males and females belonging to the first, second, third, and fourth generations receiving diets containing different levels of brewers' and *Torula* yeasts. Growth and reproduction in the animals receiving a 30 percent level of *Torula* yeast was significantly inferior to that of the animals on the brewers' yeast, at the same level. Table 4 also demonstrates the growth of

TABLE 4

Body Weight of First, Second, Third, and Fourth Generation Males and Females Receiving Various Diets during a Ten-Week Period

Generation	*Sex*	*Body Weight at the End of Experimental Period*				
		(Stock) Colony Diet	*Brewers' Yeast*			*Torula Yeast*
			20 Percent[a] *(Diet 1)*	*30 Percent*[a] *(Diet 2)*	*40 Percent*[a] *(Diet 3)*	*30 Percent*[a] *(Diet 4)*
		G.	*G.*	*G.*	*G.*	*G.*
First	Male	240	114	238	196	146
	Female	170	89	172	130	138
Second	Male	240	No second	120 (2)[b]	108 (2)	No second
	Female	170	generation	88 (3)	95 (4)	generation
			obtained			obtained
Third	Male	240		No third	163 (6)	
	Female	170		generation	117 (14)	
				obtained		
Fourth	Male	240			103 (13)	
	Female	170			90 (13)	

[a]The diet of these animals was supplemented by an adequate amount of vitamin E.

[b]Figures in parentheses indicate number of animals used in the second, third, and fourth generation growth-trials.

19. M. Goettsch, Growth and reproduction in the rat on diets of rice and beans. Puerto Rico J.Pub.Health & Trop.Med., **21**:239–255, 1946.

first generation animals receiving the colony stock diet, which showed perfect reproduction performance during successive generations and maintained the same rate of growth.

Two groups of fourth generation animals (Table 5) reared on Diet 3 (40 percent of brewers' yeast), after weaning, were given Diet 3 plus 1 percent methionine and the regular colony stock diet, respectively. The group receiving the methionine exhibited better growth than the animals on Diet 3, unsupplemented (Table 4); their general health appeared to have also improved.

While the animals on Diet 3 had a 38 percent mortality during the ten weeks of the experiment, the one with the methionine supplement had a 100 percent survival. The effect of the colony stock diet on the fourth generation was still more dramatic, as these animals had a growth performance during the ten weeks similar to normal fourth generation animals on the colony stock diet; they also survived 100 percent. Fig. 1 illustrates the condition of the rats in each of the groups six weeks after the experiment commenced.

TABLE 5

Fourth Generation Animals on Diet 3, Supplemented by Methionine, and on Stock Colony Diet

Ration	*No. of Rats*	*Average Initial Weight at 28 Days* G.	*Average Final Weight* G.	*Gain in Weight 10 Weeks* G.	*Percentage Survival*
Diet 3 + 0.1 percent methionine	6 ♂	35	132	97	100
	6 ♀	35	95	60	100
Stock colony diet (No. SB VIII)	5 ♂	33	261	228	100
	6 ♀	33	167	134	100

PAIR-FEEDING EXPERIMENTS

In order to compare the growth-promoting value of brewers' and *Torula* yeast with that of other protein foods, a pair-feeding experiment was conducted during a period of five weeks with diets containing 8 percent protein derived exclusively from one of the following: brewers' yeast, *Torula* yeast, casein, soybeans, or red kidney beans (Diets, 7, 8, 9, 10, and 11). These same diets had been pre-

viously utilized in the determination of the biological values of each of the protein foods now studied, the results of which have already been reported.[20]

Procedure. In each of the pair-feeding trials conducted, six rats (three males and three females) were used. The food given each animal was determined by the amount consumed by the rat with the smallest appetite. Chart II shows the weight gain made by the rats on the diets containing different protein foods.

The rats fed a casein diet gained 33 g., those on the brewers' yeast, 29 g., and the ones receiving soybeans, 22 g. The animals which received *Torula* yeast and red kidney beans showed practically no gain in weight during the five weeks of the experiment. It is interesting to note (Table 6) that the growth-promoting value of the different proteins studied, that is, the gain in weight in grams per gram of protein consumed, could be arranged in the same order as the net protein values of the respective foods containing them. In a way, this outcome should not be surprising, as the growth-promoting value of a protein, when fed as a foodstuff, is influenced by the coefficient of digestibility of the protein, as well as by the amount of protein present per unit weight of food. These two factors are also functions of the net protein value of a food.

On the other hand, the biological value is represented by a figure totally independent of the coefficient of digestibility and of the percentage of protein in the foodstuff. It represents the percentage of absorbed nitrogen that is retained in the body. Generally speaking, it is considered that a protein with a high growth-promoting value and a low biological value is more suitable for growth than for maintenance, while one with these values reversed is more suitable for maintenance than for growth. The biological values of the casein, the brewers' yeast, and the soybeans did not differ greatly from each other and were far above those of the red kidney beans and *Torula* yeast. The same happened with the growth-promoting values of their respective proteins. Red kidney beans seemed to be better utilized for maintenance than for growth when compared with *Torula* yeast, which appeared to be more suited for growth promotion than for maintenance. Casein and brewers' yeast rated approximately the same for both functions, while soybeans seemed to be slightly better for maintenance than for growth when compared to casein or brewers' yeast.

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20. J. A. Goyco and C. F. Asenjo, Estudios sobre el valor nutritivo de las levaduras comestibles y otros alimentos protéicos. El Crisol, **1**:2–9, 1947.

TABLE 6

Growth-promoting, Biological, and Net Protein Values of the Various Proteins and Foods Studied, When Fed at an 8 Percent Level of Protein in the Diet

Diet and Source of Protein	*Growth-promoting Value of Protein*	*Biological Value of Protein*	*Net Protein Value of Food*
Diet 7 (casein)	1.81	71.7	57.1
Diet 8 (brewers' yeast)	1.76	69.3	30.1
Diet 10 (soybeans)	1.39	73.1	25.6
Diet 9 (*Torula* yeast 3)	0.57	45.3	20.0
Diet 11 (red kidney beans)	0.11	57.1	9.3

VITAMIN E IN YEAST

When mature, males and females reared exclusively on Diets 1, 2, 3, and 4 from weaning were mated with proved fertile females and males from the breeding colony. The right time for mating was determined by following the estrus cycle, using a vaginal smear technic very similar to that of Long and Evans.[21] Briefly, it is as follows: A small portion of the vaginal content is obtained with a micro-spatula moistened in normal saline. The animal is held loosely in the left hand, while the vaginal margins are separated and the spatula is introduced. For accurate diagnosis, large quantities of the vaginal content are not necessary, the material at the lips being as diagnostic as that near the uterine os. The vaginal content so obtained is then smeared on a slide with a drop of water and examined under the low-power objective. The stage of the estrus cycle is determined by the type of epithelium present.[22] When the animal is found in estrus, it is then paired overnight with a normal male in a separate cage. To determine whether or not insemination has occurred, the female is examined the next morning for the presence of the vaginal plug and the occurrence of spermatozoa in the vaginal contents.

In almost every case, the RBC sign of implantation was observed in females inseminated by normal males from the breeding colony on the fourteenth day after mating. However, only in very few instances were young cast. By following the body weight of the preg-

21. J. A. Long and H. M. Evans, The estrus cycle in the rat and its associated phenomena. Experimental studies in the physiological anatomy of reproduction. Memoirs U.California, Berkeley, Vol. 6, 1922.

22. *Ibid.*

nant females, evidence was obtained that resorption was taking place. Two females were killed, two or three days after the date on which parturition was expected, and in both cases resorption sites were found. It was then decided to feed the females 11 mg. of alpha-tocopherol (vitamin E), the day after mating, to carry them through pregnancy. Females that in a previous pregnancy had shown resorption were used for this test. In all cases litters were cast on the expected day. The results of the experiments using first generation animals fed the different vitamin E-free yeast diets are presented in Table 7.

First generation males raised on Diets 1, 2, 3, and 4, when tested with proved fertile females from the breeding colony, were also found to be functionally sterile after repeated matings. When these animals were killed and smears made from the *vas deferens*, in Locke's solution, motile spermatozoa were found in the testes of only one male. All other males showed either complete absence of spermatozoa or the presence of nonmotile tails. The average weight of the testes of these animals was 0.5 g., while those of normal males of the same age from the breeding colony weighed 1.15 g. Pathological examination performed on all male rats fed yeast diets, including the one in which motile spermatozoa were found, showed a more or less advanced degenerative change of the germinal epithelium in every case.

The sterility of rats fed yeast as the sole source of protein was previously reported by Funk and Douglas[23] in 1913 and by Drummond,[24] in 1918. However, at the time these reports were made, vitamin E had not yet been identified as the dietary factor particularly concerned with reproduction in the rat. The absence of vitamin E in yeast was probably one of the factors that helped the identification of this vitamin, for at the time yeast was the product generally utilized to furnish the B vitamins to synthetic diets. If yeast had contained appreciable amounts of vitamin E, it would have been impossible to prepare diets free of this factor. Both the brewers' and *Torula* yeast used in these experiments were almost devoid of vitamin E as is indicated by the results obtained.

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23. C. Funk and M. Douglas, Studies on beri-beri. VIII. The relationship of beri-beri to glands of internal secretion. J.Physiol., London, **47**:475–478, 1914.

24. J. C. Drummond, I. A study of the water-soluble accessory growth-promoting substance II. Its influence upon the nutrition, and nitrogen metabolism of the rat. Biochem.J., **12**:25-41, 1918.

TABLE 7

Vitamin E-Deficiency Exhibited by Some of the Yeast Diets

Rat No.	*Diet No.*	*Supplement*	*R.B.C.*	*Weight of Female*			*No. of Animals per Litter*	*Average Weight of*		*Percentage of Mortality before 28 Days*	*Remarks*
				At Time of Mating G.	*At Time of R.B.C. G.*	*On 20th Day After Mating G.*		*Litter at Birth G.*	*Individual at Birth G.*		
20 ♀	1	None	+	157	176	164	No litter cast				Resorption; sacrificed and four implantation sites found
21 ♀	1	None	+	146	160	158	No litter cast				Resorption
21 ♀	1	11 mg. alpha-tocopherol day after mating	+	156	162	178	7	31	4.4	100	Young lived only three days
31 ♀	2	None	+	214	225	219	No litter cast				Resorption
31 ♀	2	11 mg. alpha-tocopherol day after mating	+	200	221	230	Number of animals not recorded			100	Litter born dead; parturition trouble
41 ♀	3	None	+	172	188	173	No litter cast				Resorption; sacrificed; five implantation sites in right horn and seven in left
37 ♀	4	None	+	152	160	158	No litter cast				Resorption
37 ♀	4	11 mg. alpha-tocopherol day after mating	+	140	157	187	7	31	4.4	100	Young killed by mother

FERTILITY, REPRODUCTION, AND LACTATION ON SYNTHETIC DIETS SUPPLEMENTED WITH VITAMIN E AND CONTAINING DIFFERENT LEVELS OF YEAST AS SOLE SOURCE OF PROTEIN

To gain some knowledge of the influence that the level of yeast protein in the diet could have on the fertility, reproduction, and lactation of the rat, several groups of these animals were fed rations containing different levels of yeast as the only source of protein.

Procedure. The rats receiving Diet 8 (Table 8) were ten normally mature female animals raised on the regular colony stock diet. Half of them commenced receiving the experimental diet one month previous to mating, while the balance were fed the same diet from the day after mating. As both groups responded in the same manner, the results were combined and reported as from one group. Diet 8 contained 18 percent brewers' yeast, that is, about 9 percent of protein. All these animals received 7.6 mg. of alpha-tocopherol per week.

The remaining animals in the experiment were twenty-eight-day-old female rats fed the experimental diets from the time of weaning. On attaining maturity, they were mated with proved fertile males. At this time, each female on Diets 1, 2, and 4 received 11 mg. of alpha-tocopherol, since these diets, like Diet 8, were deficient in vitamin E. The twelve animals on Diet 6 (40 percent brewers' yeast) were not fed alpha-tocopherol, since this diet contained 9 percent of Mazola oil, which is rich in vitamin E.

The results obtained showed that fertility, reproduction, and lactation were greatly impaired by levels of yeast protein below 18 percent. The only group in which nearly normal fertility and reproduction was attained was that on Diet 6, containing 40 percent of brewers' yeast, equivalent to 18 percent of protein. Although the animals receiving Diet 6 were weaned at twenty-eight days, their average weight at this age was always below that of the young of the same age raised on the colony stock diet. Nevertheless, second generation females were fertile and reared third generation animals.

Oviduct infection was observed in some females on Diets 1, 2, and 6. The infection usually arose after they had given birth to more than one litter, when they generally became sterile and gave no RBC signs. Autopsies showed severe infection of one or both oviducts in every instance

TABLE 8

Reproduction Performance of Rats Receiving Colony Stock Diet (SB VIII) and Synthetic Diets with Yeast as Only Source of Protein

Item	*SB VIII*	*Diet 8*	*Diet 1*	*Diet 2*	*Diet 6*	*Diet 4*
1. Age of animals when placed on diet	28 days old	Mature animals	28 days old	28 days old	28 days old	28 days old
2. Number of females in group	12.0	10.0	2.0	5.0	12.0	3.0
3. Number of positive matings	15.0	35.0	7.0	5.0	30.0	4.0
4. Number of litters born	15.0	10.0	3.0	4.0	26.0	2.0
5. Percentage of fertility $\frac{(4)}{(3)}$	100.0	28.6	42.9	80.0	86.7	50.0
6. Average number of young born per litter	9.1	3.8	4.7	4.1	7.6	4.5
7. Total number of animals weaned at 28 days	120.0	5.0		1.0	1.78	
8. Percentage of young weaned $\frac{(4 \times 6)}{7}$	87.5	13.2		6.1	89.9	
9. Average weight of animals at 28 days of age	58.0	15.0		18.3	33.7	
10. Number of cases of oviduct infection			2.0	1.0	3.0	

SUMMARY

A study has been conducted of the growth, reproduction, and lactation of rats fed different levels of brewers' and *Torula* yeasts as the sole source of protein. The performance of the animals receiving the yeast diets was compared with that of normal female rats on the colony stock diet. All the yeast diets proved to be inferior to the latter, as indicated by the following results:

1. Growth performance of normal rats, fed from their twenty-eighth day on the different experimental yeast diets, was inferior to that of rats raised throughout the experiment on the colony stock ration, except in the case of the first generation animals receiving Diet 2 (30 percent brewers' yeast), which exhibited a growth response similar to that of the colony animals.

2. Both the brewers' and *Torula* yeasts were found to be practically free of vitamin E.

3. The reproduction performance of rats receiving brewers' yeast diets, supplemented with vitamin E, was always inferior to that of the colony rats. Second generation animals were obtained only from the groups receiving Diet 2 (30 percent brewers' yeast), 3, and 6 (40 percent brewers' yeast). Third and fourth generations were only obtained in the groups feeding on Diets 3 and 6. Only 6.1 percent of second generation animals from mothers on Diet 2 attained twenty-eight days of age. On the other hand, the second, third, and fourth generations raised on Diets 3 and 6 had normal reproduction, but lactation was poor; the twenty-eight-day-old rats weighed almost half as much as rats of the same age from mothers on the colony stock diet.

4. *Torula* yeast fed at a 30 percent level proved to be a very poor source of protein for growth, reproduction, and lactation.

5. The growth-promoting values of casein, brewers' yeast, soybeans, *Torula* yeast, and red kidney beans were compared in a pair-feeding experiment. Casein, brewers' yeast, and soybeans gave, respectively, values of higher order, while the value for *Torula* yeast was only about one third as much. Red kidney beans had the lowest value of all the foods tested.