Further Studies of the Rachitogenic Effect of Dried Yeast in Pig Diets

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In previous papers Braude, Kon & White (1943, 1944) described the development of rickets in fattening pigs on diets containing a large proportion of yeast, its cure or complete prevention with vitamin D, and its partial prevention with additional calcium salts. The yeast was added as a protein supplement to normal fattening rations to the extent of 8–20%. The cause of the rickets remained obscure, though it was unlikely (Braude *et al.* 1944) that it was due to the high phosphorus content of the yeast.

The present paper describes further attempts to determine the nature of the rachitogenic factor, some of them, including a test of stability of the factor to heat, made some time ago, others recent, dealing with the possible connexion between the rachitogenic effect and the phytate phosphorus content of the diet.

Practical rations for pigs, because of their high cereal content, are relatively rich in phytic acid; this is normally hydrolysed in the gut by the phytase present in the majority of cereals (Pedersen, 1940; Møllgaard, 1946). It seemed possible that yeast might in some way inhibit the hydrolysis of phytic acid in the diet and that this would affect the availability to the pig of calcium and phosphorus and lead to rickets. Experiments were therefore planned to test this point. In the meantime Hoff-Jørgensen (1947) published results of experiments in vitro supporting the view that brewer's yeast inhibited phytase activity.

EXPERIMENTAL

Exp. 6.* *Autoclaved yeast, irradiated yeast and yeast residue in relation to yeast rickets in pigs*

Selection of animals

Large White pigs, 10–11 weeks old, from five litters were divided into five blocks of six pigs, and a pig from each block was allocated by toss of coin to one of the six diets described below.

Diets

The composition of the diets is given in Table 1 and their chemical analysis in Table 2. They were given as a wet mash in the way and in the quantities described by Braude *et al.* (1943).

(1) *Rachitogenic diet*. This was the 20% yeast diet of Braude *et al.* (1943, 1944). The dried brewer's yeast was purchased from Messrs A. Guinness, Son and Co. Ltd.

* Exps. 1–5 have already been described by Braude *et al.* (1943, 1944).

(2) *Autoclaved yeast diet.* This was the same as diet 1 with the yeast autoclaved for 6 hr. at 120°. The purpose of this change was to find out whether the factor causing rickets was stable to heat.

Table 1. *Percentage composition of diets used in Exp.* 6

Component	1, 3* and 5†	2	4	6
			Diet no.	
Wheat feed	35	35	35·5	47
Barley meal	20	20	20	20
Ground oats	20	20	20	20
Dried grass	3	3	3	3
Dried yeast	20	—	—	—
Autoclaved dried yeast	—	20	—	—
Dried yeast residue	—	—	20	—
Fish meal	—	—	—	10
Calcium carbonate	1·5	1·5	1·5	—‡
Salt	0·5	0·5	—†	—‡

* Diet 3 was as diet 1 but with 2 g./pig daily of irradiated yeast added directly to the trough.
† Diet 5 was as diet 1 but with 2·5 parts of sodium bicarbonate replacing 1·5 parts of calcium carbonate.
‡ Not added to the diet as sufficient is provided by dried yeast residue or fish meal.

Table 2. *Analytical data for diets used in Exp.* 6 *and for their components*

Diet or component	Moisture (%)	Crude protein (%)	Ether extract (%)	Carbo-hydrate (%)	Fibre (%)	Ash (%)	Ca (%)	P (%)
Wheat feed	11·4	13·4	4·0	53·1	12·7	5·4	0·12	1·02
Barley meal	13·8	11·2	1·6	66·8	4·3	2·3	0·06	0·34
Ground oats	13·0	12·0	3·8	57·2	11·3	2·7	0·10	0·33
Dried grass	11·7	7·2	2·5	48·6	23·4	6·6	0·67	0·18
Dried yeast	9·4	47·4	0·3	32·4	—	10·5	0·27	2·32
Dried yeast residue	16·4	32·3	3·8	35·1	—	12·4	0·04	1·59
Fish meal	10·2	62·3	6·2	—	—	21·8	6·46	3·67
Diets 1, 2, 3 and 5*	11·8	19·1	2·6	51·3	8·3	6·9	0·73	0·96
Diet 4	13·0	16·1	3·3	52·0	8·3	7·3	0·68	0·81
Diet 6	12·1	17·4	3·7	51·2	9·7	5·9	0·74	0·98

* The composition of the diets is calculated from that of the components. The autoclaved yeast used in diet 2 came from the same batch as the dried yeast and was not analysed again after autoclaving. The irradiated yeast added to diet 3 and the sodium bicarbonate added to diet 5 are not included in these figures.

(3) *Irradiated yeast diet.* This was diet 1 supplemented with 2 g. irradiated yeast/pig daily. A sample of the dried brewer's yeast was finely ground so that 70% of it passed through a 100 mesh sieve and 2 kg. of it were spread as evenly as possible over a circle 6¾ ft. in diameter marked out on a large sheet of glazed paper. Over the centre of this circle, and 30 in. from it, a quartz mercury-vapour lamp was suspended. After 20 min. irradiation the powder was collected and the operation repeated twice, i.e. the yeast was irradiated in all for 1 hr. The vitamin D potency of the irradiated yeast was not determined, but from results obtained under similar conditions by Booth (1934) it can be gauged that it was not less than 500 i.u./g. As the addition of a small quantity of irradiated yeast would be convenient under practical conditions, the mixture was

tested to find out whether it would make safe the feeding of large quantities of dried yeast to pigs.

(4) *Yeast-residue diet.* The yeast in diet 1 was replaced by 20% of the residue remaining in the preparation of a proprietary yeast extract. As special fractionation of yeast in quantities required in experiments with pigs was impossible for practical reasons, the giving of this residue, which was made available to us by Phillips Yeast Products Ltd., London, was undertaken in an endeavour to locate the factor in yeast responsible for rickets.

(5) *Yeast diet with sodium bicarbonate.* This was diet 1 with 1·5% calcium carbonate replaced by 2·5% sodium bicarbonate. Earlier work had shown the beneficial effect of calcium carbonate in preventing yeast rickets. This was most probably due to calcium as such, but it was thought desirable to check whether the effect was in any way connected with the alkaline nature of the supplement.

(6) *Fish-meal diet.* This was a normal fattening ration, with fish meal as a protein supplement, used for purposes of comparison with the yeast diets.

Experimental details

The main experiment lasted 24 weeks and records of growth and food intake were kept. With a few animals the feeding was extended beyond 24 weeks for reasons given below. Blood samples were taken at the beginning of the experiment, after 12 weeks and after 24 weeks, and the serum calcium was determined by the Clark & Collip (1925) modification of the Kramer & Tisdall (1921) method. The pH of the intestinal contents of two blocks of pigs was determined at slaughter by means of a glass electrode.

Results

The pigs in the first three blocks completed the experiment without any disturbance. In blocks 4 and 5 five pigs died rather suddenly during a fortnight in February in which abnormally cold weather was experienced. At the time of death some of them were already affected by the experimental treatments, but it is most unlikely that these were directly responsible for the sudden deaths. Poisoning was suspected but could not be proved. Post-mortem examinations did not provide any clue besides showing that the deaths were due to non-specific causes, pneumonia and enteritis. All surviving pigs in block 4, which were nearly of bacon weight, were slaughtered as a precautionary measure, and only three pigs remained in block 5. It was, therefore, decided not to include the incomplete records of these animals in the present paper. Thus the results are based on the first three blocks only, i.e. each of the treatments was replicated three times.

Table 3 gives details of growth and food consumption of the pigs in these three blocks. The pigs which received the normal fish-meal diet and the yeast diet supplemented with irradiated yeast (diets 3 and 6) appeared normal. They consumed their food readily; their daily live-weight gain and food utilization were as good as could be expected from the wartime quality of the feeding-stuffs which made up their rations.

On the other hand, the behaviour of some of the pigs which received the other four diets was obviously abnormal. In block 1 the pigs receiving the yeast diet (diet 1), the

Table 3. *The growth of pigs and their food consumption in Exp. 6*

No. and description of diet	Block and sex	Initial weight (lb.)	Final weight (lb.)	Mean daily gain (lb.)	Amount of food offered (lb.)	No. of occasions on which the animal refused food	Approx. amount of food refused (lb.)	Food refused expressed as a percentage of that offered, mean for the group	Average food consumption/lb. live weight gain (corrected for refusals) (lb.)
1. Rachitogenic	1 ♂	51	154	0·613	736·4	69	103		
	2 ♀	40	194	0·917	742·0	37	55		
	3 ♂	45	224	1·065	793·1	13	17		
	Mean	45·3	190·7	0·865	757·2	39·7	58·3	7·7	4·81
2. Autoclaved yeast	1 ♂	57	153	0·572	756·0	84	98		
	2 ♂	38	190	0·904	707·7	9	3		
	3 ♂	47	213	0·988	762·3	0	0		
	Mean	47·3	185·3	0·821	742·0	31·0	33·7	4·6	5·13
3. Irradiated yeast	1 ♂	59	232	1·030	845·6	0	0		
	2 ♀	43	216	1·030	760·9	8	4		
	3 ♂	39	226	1·113	784·7	0	0		
	Mean	47·0	224·7	1·058	797·1	2·7	1·3	0·2	4·47
4. Yeast residue	1 ♂	51	209	0·940	787·5	4	1		
	2 ♀	36	131	0·565	618·8	76	80		
	3 ♂	45	152	0·637	722·4	68	99		
	Mean	44·0	164·0	0·714	709·6	49·3	60·0	8·5	5·41
5. Yeast with sodium bicarbonate	1 ♂	46	152	0·631	718·9	54	68		
	2 ♂	45	198	0·911	764·4	6	5		
	3 ♀	42	150	0·643	727·3	27	58		
	Mean	44·3	166·7	0·728	736·9	29·0	43·7	5·9	5·67
6. Fish meal	1 ♀	43	204	0·958	763·0	5	2		
	2 ♀	44	215	1·018	777·0	4	1		
	3 ♂	46	233	1·113	809·2	0	0		
	Mean	44·3	217·3	1·030	783·1	3·0	1·0	0·1	4·52

autoclaved yeast diet (diet 2) and the diet with sodium bicarbonate (diet 5) developed symptoms of rickets similar to those observed in previous experiments. Their live-weight gain and food utilization were subnormal and they refused food on many occasions. The pig receiving the yeast-residue diet (diet 4) grew at a normal rate.

Table 4. pH *of intestinal contents of two blocks of pigs in Exp.* 6

Diet	Block 1	Block 2
1	6·80	7·50
2	7·13	7·45
3	6·86	7·60
4	6·88	7·60
5	6·97	7·42
6	6·91	7·68

Table 5. *Calcium content of the blood serum of pigs in Exp.* 6

No. and description of diet	Block and sex	Initial value (mg./100 ml.)	Value after 12 weeks (mg./100 ml.)	Value after 24 weeks (mg./100 ml.)
1. Rachitogenic	1 ♂	10·56	7·05	6·04
	2 ♀	11·49	10·57	7·43
	3 ♀	10·64	9·98	9·25
	Mean	10·90	9·20	7·57
2. Autoclaved yeast	1 ♂	11·32	7·54	7·65
	2 ♂	10·25	10·09	10·55
	3 ♂	10·43	10·28	11·69
	Mean	10·67	9·30	9·96
3. Irradiated yeast	1 ♂	10·87	10·52	10·91
	2 ♀	11·10	10·30	10·98
	3 ♀	10·55	10·68	11·75
	Mean	10·84	10·50	11·21
4. Yeast residue	1 ♂	11·45	9·95	10·17
	2 ♀	10·77	8·00	7·49
	3 ♀	10·78	5·87	6·60
	Mean	11·00	7·94	8·09
5. Yeast with sodium bicarbonate	1 ♂	10·20	7·16	7·26
	2 ♂	11·14	10·81	9·85
	3 ♀	11·25	10·48	10·29
	Mean	10·86	9·48	9·13
6. Fish meal	1 ♀	11·21	11·08	11·26
	2 ♀	11·37	11·12	11·84
	3 ♂	10·89	10·00	11·55
	Mean	11·16	10·73	11·55

In block 2 none of the pigs showed definite symptoms of rickets. The pigs receiving diets 1 and 5 refused food in the later stages of the experiment, while the pig receiving diet 4 refused food throughout the experimental period and grew at a slow rate, but showed no other abnormal symptoms. The pig receiving diet 2 grew at an irregular rate but otherwise appeared normal.

In block 3 only pigs receiving diets 4 and 5 definitely deviated from normal. They grew slowly and frequently refused food, but no other symptoms were observed.

The pH of the intestinal contents of the pigs in the two blocks tested was not affected by the diet (Table 4). Pigs on diets 3 and 6 maintained their serum-calcium level throughout and even showed a slight increase in the final estimation (Table 5).

On the other hand, the values for all the other pigs declined, especially for the three pigs in block 1 which showed symptoms of rickets. At the conclusion of the main experiment each of these three animals was given 30 g. daily of controlled cod-liver oil mixture for a period of 9 weeks and recovered completely.

Exp. 7. *The effect of yeast on the hydrolysis of phytic acid in the intestine of the pig*

Analytical methods

The phytate phosphorus of the diets and faeces was determined by the method of McCance & Widdowson (1935) as modified by Cruickshank, Duckworth, Kosterlitz & Warnock (1945). The final phosphorus determinations were done by the strychnine-molybdate method described by Peters & Van Slyke (1932).

Table 6. *Percentage composition and phytate phosphorus content of the diets used in Exp. 7*

Component	Normal fattening diet	Control meat meal diet	Yeast diet
Wheat feed	48	46	40
Barley meal	20	20	18
Maize meal	20	20	18
Meat meal	—	10	—
Fish meal	10	—	—
Dried grass	2	2	2
Calcium carbonate	—	1·5	1·5
Salt	—	0·5	0·5
Dried brewer's yeast	—	—	20
% phytate P	0·38	0·38	0·32

Experimental details

Four pairs of litter-mate Large White pigs, 12 weeks old, were divided into two groups of four pigs each. Both groups were given a normal fattening ration for 5 weeks when a composite sample of faeces was taken from each group and analysed for phytate phosphorus. Subsequently one group received a control meat-and-bone meal diet similar to that used previously in this series of experiments (Braude *et al.* 1943), while the other group was given the 20% yeast diet. The composition of the diets and their phytate phosphorus contents are given in Table 6.

When the pigs had been 1 week on these diets, and again 2 weeks later, samples of faeces were taken from each group and analysed for phytate phosphorus. The diets were given for another month and were then followed for a period of 5 weeks by diets in which the phytase had been destroyed. The diets were fed as a wet mash (Braude *et al.* 1943) and to destroy the phytase, instead of cold water, boiling water was used for mixing according to the method of Pedersen (1940); the mash was allowed to cool before being given. The phytate phosphorus content of the faeces was determined after 1, 4 and 5 weeks on these diets.

Results

The results of the experiment are given in Table 7. They do not suggest any inhibitory effect of yeast on the intestinal hydrolysis of phytate. On the contrary, pigs receiving yeast excreted no measurable amounts of phytate phosphorus though some was present in their faeces when they were eating the control fattening ration. With the meat-and-bone meal ration, however, more phytate passed through the gut unchanged and it is remarkable that the replacement of 10% fish meal by 10% meat-and-bone

Table 7. *Phytate phosphorus excretion by groups of four pigs in Exp. 7*

Diet	Length of time on diet (weeks)	Meat-meal diet group Phytate P/g. faeces (mg.)	Yeast diet group Phytate P/g. faeces (mg.)
Normal fattening	5	4·4	3·2
Experimental	1	14·8	0
	3	16·5	0
Experimental with phytase destroyed	1	10·4	4·9
	4	78·7	10·6
	5	82·2	13·0

meal should have had such a definite effect. In fact the result could be interpreted as indicating inhibition of phytase activity by the meat-and-bone meal. When the phytase in the diet was destroyed, the excretion of phytate phosphorus by both groups of pigs increased, but again much more phytate was found in the faeces of the pigs which received the meat-and-bone meal.

It is clear that these findings neither support our original tentative explanation of the rachitogenic effect of yeast nor agree with the results of Hoff-Jørgensen's (1947) tests in vitro. A repetition of his tests with the yeast used in the pig experiment seemed, therefore, desirable.

Exp. 8. *The effect of fresh and dried brewer's yeast and of fresh baker's yeast on the hydrolysis, in vitro, of sodium phytate by wheat phytase*

Method of measurement

The effect of yeast on the hydrolysis of solutions of pure sodium phytate was determined by a method essentially that of Hoff-Jørgensen (1947). The phytase solution was prepared by shaking wheat bran for 2 hr. with ten times its weight of water; it was then filtered through muslin and toluene was added as a preservative.

To a suitable amount of sodium phytate in a 250 ml. centrifuge tube, 50 ml. of the phytase solution were added, followed by 0·2 M-acetate buffer to give the desired volume (about 100 ml.). In experiments with yeast it was shaken for 1 hr. with 50 ml. of the acetate buffer and the sodium phytate and the phytase solution were then added. In all cases the pH of the final system was adjusted to 5·20. The tubes were incubated for 2 hr. at 30°. After incubation 10 ml. of solution were removed from each tube and

were replaced by 10 ml. of 5N-HCl to stop the hydrolysis. After centrifuging, the phytate phosphorus was measured in the supernatant liquid.

Yeasts tested

Dried brewer's yeast, fresh brewer's yeast (from Messrs H. and G. Simonds, Reading) as received from the brewery, washed brewer's yeast, i.e. fresh brewer's yeast filtered on a Buchner funnel and washed with water in order to remove the beer residues, and fresh baker's yeast were used.

Table 8. *The hydrolysis of sodium phytate by wheat phytase in the presence or absence of yeast*

Unless otherwise stated sodium phytate was incubated with phytase for 2 hr. at 30° and pH 5·2.

		Phytate phosphorus			
		Originally present (mg.)	Split (mg.)	Hydrolysis (%)	
Exp.	Addition				Inhibition
1	None	51·4	26·0	50·6	—
	1 g. dried brewer's yeast	51·4	28·9	56·2	0
	5 g. fresh brewer's yeast*	51·4	29·5	57·4	0
2	None	51·4	28·2	54·9	—
	5 g. fresh brewer's yeast*	51·4	31·1	60·5	0
	5 g. fresh brewer's yeast, washed†	51·4	29·2	56·8	0
	4·5 g. fresh baker's yeast	51·4	32·3	62·8	0
3	None	46·9	25·2	53·7	—
	5 g. dried brewer's yeast	46·9	28·4	60·5	0
	10 g. fresh baker's yeast	46·9	26·4	56·3	0
4	None	43·5	19·1	43·9	—
	No enzyme	43·5	6·6	15·2	—
5	None	47·3	23·3	49·3	—
	No enzyme	47·3	7·4	15·6	—

* As obtained from the brewery.
† Filtered on a Buchner funnel and washed with water.

Results

The results of this experiment are given in Table 8. They show that yeast, whether fresh or dried, does not inhibit, in vitro, the hydrolysis of sodium phytate by a preparation of wheat phytase. If anything, the breakdown was rather more pronounced in the presence of the yeast.

DISCUSSION

The outcome of these experiments is essentially negative in that they give little clue to the nature of the rachitogenic factor in yeast. Its presence in autoclaved yeast indicates that it is stable to heat. Whatever the mode of action of yeast it is clear that the attractive suggestion that it acts by inhibiting the breakdown of phytic acid (cf. p. 66) is fallacious. It seems clear that most of the phosphorus present in the yeast diet must be available to pigs. As a matter of fact the phytic acid was more completely hydrolysed in the gut with the yeast diet than with the meat-and-bone meal diet. The difference

became more marked after treatment of the diet with boiling water suggesting, perhaps, greater heat stability of the phytase in the presence of yeast. Previous work (Braude *et al.* 1944) showed that the replacement in our pig diets of yeast phosphorus by phosphorus as inorganic phosphate in quantities in excess of those supplied by yeast, did not lead to rickets. A study of the effect of yeast on the metabolism of calcium and phosphorus is clearly needed, but facilities for this type of work are, for the present, not available here.

The addition of sodium bicarbonate to the yeast diets did not prevent the appearance of rickets, thus showing that the protective value of calcium carbonate (Braude *et al.* 1943, 1944) lies in the calcium moiety. The observation that the inclusion of a small proportion of irradiated yeast makes large amounts of yeast innocuous to the pig may be of practical value, but merely confirms the previous knowledge about the protective action of vitamin D.

From a practical point of view the use of yeast in pig diets presents no problem (Braude *et al.* 1943), but the theoretical aspect of its rachitogenic effect is, to us, still obscure.

SUMMARY

1. Three further experiments on the nature of the factor in yeast rachitogenic to pigs (Braude *et al.* 1943, 1944) have yielded essentially negative results.

2. The factor is heat stable, as it is present in autoclaved yeast.

3. The addition to the rachitogenic diet of 2 g./pig daily of irradiated yeast prevented the occurrence of rickets.

4. The addition to the rachitogenic diet of 2·5% sodium bicarbonate failed to prevent rickets, showing that the protective value of calcium carbonate (Braude *et al.* 1943, 1944) was due to calcium *per se* and not to the alkaline nature of the addition.

5. The feeding of the residue from the preparation of a yeast extract gave inconclusive results.

6. Experiments with pigs disproved our tentative assumption that dried yeast caused rickets by inhibiting the intestinal breakdown of the phytic acid of the diet. In agreement with this, but contrary to the finding of Hoff-Jørgensen (1947), in vitro tests did not show any inhibition of phytase activity by dried yeast.

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