

## INFLUENCE OF NONFAT DRY MILK SOLIDS ON THE NUTRITIVE VALUE OF BREAD

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Wheat flour is a good food and an excellent source of energy, which is one of the first requirements that must be adequately met in a diet, and since white bread may contribute an appreciable proportion of the required calories to the American dietary, considerable effort has been expended toward improving its nutritive value. Modifications of standard milling processes so as to retain more of the nutritive value of whole wheat (particularly in Canada and the British Isles); the addition of nonfat dry milk solids; the use of yeast products having a high vitamin potency, and more recently enrichment, are all measures which have been suggested to improve the nutritive value of white bread. The use of nonfat dry milk solids has had wide acceptance because in addition to improving the nutritive qualities of white bread it also improves the physical properties.

Fairbanks (1, 2) first demonstrated the value of nonfat dry milk solids in improving the nutritive value of white water bread. His results have recently been confirmed by Mitchell, Hamilton, and Shields (9) and Light and Frey (8). Henry, Houston, Kon, Powell, Carter, and Halton (7) have attributed the better nutritive value of white bread made with nonfat dry milk solids to an increase in quantity and quality of protein and increased amounts of calcium, riboflavin, and possibly other members of the B<sub>2</sub> complex. Harris, Clark, and Lockhart (6) and Volz, Forbes, Nelson, and Loosli (11) found that milk solids and soybean flour and combinations of these two supplements improve the nutritive value of white bread protein.

The studies here reported were undertaken for the purpose of making comparisons of the nutritive values of several types of bread largely used in this country, viz.: water bread, enriched water bread and two whole wheat breads which differed considerably in composition on account of the flour from which each was made. Each of these types of bread was studied with and without the addition of 6 per cent nonfat dry milk solids. Mitchell *et al.* (9) studied white bread enriched according to the standards proposed in 1941 (Federal Register (3)). Since that time new enrichment standards have been promulgated (Federal Register (4, 5)) and it seemed important to conduct studies at the new enrichment levels.

### METHODS

Young rats of the Sprague-Dawley strain weighing 35 to 50 grams were used in these studies. They were housed in individual cages and weighed

Received for publication July 25, 1946.

twice weekly. Weighed portions of air-dried bread crumbs, mixed with water so as to prevent scattering, were fed daily. The bread crumbs, water, and a daily dose of 0.1 cc. USP cod-liver oil constituted the sole articles of diet throughout the 8-week test period. *Ad libitum* feeding was employed in Experiments No. 1 and No. 2. In Experiments No. 3 and No. 4 the animals were pair fed for equal gains.

Hemoglobin determinations were made on a Hellige clinical model hemoglobinometer using a sample of blood taken from the tip of the rat's tail. The values reported represent the average of duplicate determinations. A.O.A.C. (10) analytical methods for bread were used in determining the chemical composition of the diets and recognized chemical and microbiological methods were used in determining the amounts of iron and vitamins. The carcasses were prepared and analyzed in the following manner: At the end of the eight-week growth test the animals were killed, the intestinal contents removed, and the entire carcasses were quickly frozen. The frozen carcasses were ground three times with a food chopper, being adequately mixed and refrozen after each grinding. Appropriate aliquots of the entire ground carcasses were taken for chemical analyses following the methods for meat given in the A.O.A.C. (10).

#### DESCRIPTION OF BREAD SAMPLES

Usually enough bread was baked at one time to last throughout each experiment. The breads were prepared using the following formula which is much like a commercial baker's formula. Sponge: 58 per cent flour, 2 per cent yeast, 0.5 per cent bread improver and 41.6 per cent water. Dough: 42 per cent flour, 4 per cent sugar, 2 per cent salt, 2 per cent malt, 1.5 per cent shortening (hydrogenated vegetable oil) and 27.7 per cent water. The same lot of white flour (0.42 per cent ash) or whole wheat flour was used to prepare all the breads fed in any one experiment; the supplements being added at the time the bread was made.

The following types of bread were studied.

1. *Water bread.* White flour without supplement.
2. *Enriched water bread.* White flour enriched to 10 per cent above the minimum levels for thiamine, riboflavin, niacin, and iron as specified in the new standards for enriched flour (Federal Register (4)). Analyses of the bread crumbs (table 1) show that these amounts of enrichment ingredients produced bread enriched well above the new minimum requirements of 1.1 mg. thiamine, 0.7 mg. riboflavin, 10 mg. niacin and 8 mg. of iron per pound of enriched bread (Federal Register (5)).
3. *Nonfat milk bread.* 6 per cent nonfat dry milk solids based upon weight of white flour.
4. *Enriched nonfat milk bread.* A combination of the supplements given under 2 and 3.

5. *Whole wheat bread.* 100 per cent whole wheat flour.

6. *Whole wheat nonfat milk bread.* 6 per cent nonfat dry milk solids based upon weight of whole wheat flour.

Two different lots of whole wheat flour were used and the breads made from them showed differences in chemical composition as shown in table 1. For example, whole wheat bread #1 contained 17.13 per cent protein whereas whole wheat bread #2 contained 15.14 per cent protein.

Complete chemical and vitamin analyses were not made on all the bread samples fed. However, the same formula was followed wherever confirmatory tests were made and the analyses of each type of bread studied is shown in table 1. Every lot of bread was tested for moisture and protein. The analyses of the bread crumbs have been calculated to the basis of fresh bread containing 38 per cent moisture and these values are also shown in table 1.

#### RESULTS OF FEEDING EXPERIMENTS

The results from the feeding experiments are summarized in table 2. The relative nutritive values of these several types of bread can be fairly accurately evaluated on the basis of the grams of solids required to produce one gram of gain and this has been done as shown in the last column of table 2. When evaluated on this basis the smaller the weight of food required to produce a gram of gain, the higher the nutritive value of the food. The nutritive values calculated in this manner were subjected to statistical analysis in order to determine the significance of differences referred to in this report.

*Experiments No. 1 and No. 2 (ad libitum feeding).* The small differences between the nutritive values of water bread and enriched water bread are not significant when the data are analyzed by Student's "t" test for unpaired differences. (Experiment No. 1,  $P > 0.9$ ; Experiment No. 2,  $P = 0.4$ ).<sup>1</sup>

The addition of 6 per cent nonfat dry milk solids greatly improved the nutritive value of enriched water bread in both experiments, and the results from Experiment No. 2 indicate that water bread containing 6 per cent nonfat dry milk solids is equal in nutritive value to enriched water bread containing 6 per cent nonfat dry milk solids. Mitchell *et al.* (9) obtained similar results; however, they observed a slight but significant increase in hemoglobin levels when nonfat milk bread was enriched according to the 1941 standards. Hemoglobin determinations were not made on the animals used for this comparison in these studies.

<sup>1</sup> The numerical value of  $P$  (Probability) indicates whether the difference between observed values are due to chance or to the imposed experimental conditions. When  $P \leq 0.05$  (odds  $\geq 19:1$ ) we consider the difference between two means to be statistically significant. When  $P \leq 0.01$  (odds  $\geq 99:1$ ) we consider the difference to be highly significant.

TABLE 1  
*Typical analyses of breads used in feeding trials*

Identification	Moisture	Protein (N x 6.25)	Ash	Calcium	Phos- phorus	Iron per lb.	Thiamine per lb.	Ribo- flavin per lb.	Niacin per lb.
Composition of dry bread crumbs									
	%	%	%	%	%	mg.	mg.	mg.	mg.
Enriched water bread	6.78	12.95	2.60	0.060	0.098	12.20	1.86	1.36	15.62
Enriched nonfat milk bread	7.15	14.25	2.87	0.120	0.139	12.00	1.80	1.86	15.57
Water bread	7.57	12.96	2.54	0.055	0.096	5.45	0.50	0.38	4.31
Nonfat milk bread	9.22	13.38	3.00	0.114	0.136	11.32	0.43	1.23	4.80
Whole wheat bread #1	10.45	17.13	3.39	0.065	0.234	22.60	1.36	1.27	18.06
Whole wheat nonfat milk bread #1	10.42	17.45	3.74	0.132	0.247	24.00	1.09	2.50	17.45
Whole wheat bread #2	10.75	15.14	3.34	0.070	0.415	17.60	1.75	0.80	17.80
Whole wheat nonfat milk bread #2	11.01	16.24	3.53	0.140	0.475	15.70	1.20	2.47	15.30
Composition of bread (38% moisture) calculated from crumb analyses									
	%	%	%	%	%	mg.	mg.	mg.	mg.
Enriched water bread	38.0	8.61	1.73	0.040	0.065	8.11	1.24	0.90	10.39
Enriched nonfat milk bread	38.0	9.52	1.92	0.080	0.093	8.01	1.20	1.24	10.40
Water bread	38.0	8.69	1.70	0.037	0.064	3.66	0.34	0.25	2.89
Nonfat milk bread	38.0	9.14	2.05	0.078	0.093	7.73	0.29	0.84	3.27
Whole wheat bread #1	38.0	11.86	2.35	0.045	0.162	15.64	0.94	0.88	12.50
Whole wheat nonfat milk bread #1	38.0	12.08	2.59	0.091	0.171	16.61	0.75	1.73	12.08
Whole wheat bread #2	38.0	10.52	2.32	0.049	0.288	12.22	1.22	0.56	12.36
Whole wheat nonfat milk bread #2	38.0	11.31	2.46	0.098	0.331	10.95	0.84	1.72	10.66

Nonfat milk bread was not equal in nutritive value to whole wheat bread #1. Also the addition of 6 per cent nonfat dry milk solids did not produce a significant improvement ( $P = 0.12$ ) in the nutritive value of whole wheat

TABLE 2  
Summarized data from feeding tests

Diet	No. of rats	Av. initial weight	Av. wt. at end of 8 weeks	Av. gain	Av. wt. bread solids consumed	Bread solids required for one gram gain
Experiment No. 1 ( <i>ad libitum</i> feeding)						
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Enriched water bread ...	8	42.4	85.5	43.1	312.9	7.34
Water bread .....	8	43.0	90.6	47.6	340.7	7.25
Enriched nonfat milk bread .....	8	40.9	132.0	91.1	442.4	4.90
Experiment No. 2 ( <i>ad libitum</i> feeding)						
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Enriched water bread ...	8	41.0	79.2	38.4	296.8	7.85
Water bread .....	8	39.6	73.6	34.0	275.4	8.28
Enriched nonfat milk bread .....	8	40.5	100.5	60.2	367.6	6.15
Nonfat milk bread .....	8	39.0	98.0	59.0	355.6	6.10
Whole wheat bread #1	8	40.0	154.1	114.2	527.0	4.66
Whole wheat nonfat milk bread #1 .....	8	41.5	192.6	151.1	638.8	4.30
Experiment No. 3 (paired feeding for equal gain)						
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Enriched water bread ...	12	40.5	65.3	27.4	261.1	10.75
Water bread .....	12	40.4	63.8	22.6	269.1	12.10
Nonfat milk bread .....	12	38.3	80.7	42.3	279.8	6.61
Enriched water bread ...	12	38.8	76.5	37.7	333.9	8.86
Whole wheat nonfat milk bread #1 .....	12	39.4	135.1	95.6	434.6	4.57
Whole wheat bread #1	12	39.0	130.6	91.6	486.6	5.35
Nonfat milk bread .....	12	38.3	101.4	63.2	380.3	6.13
Whole wheat bread #1	12	37.4	103.7	66.3	348.8	5.28
Experiment No. 4 (paired feeding for equal gain)						
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Enriched nonfat milk bread .....	8	39.6	97.9	58.3	335.9	5.85
Whole wheat bread #2	8	40.5	100.6	60.1	334.3	5.62
Whole wheat bread #2	8	37.9	103.0	65.1	360.7	5.57
Whole wheat nonfat milk bread #2 .....	8	38.5	104.8	66.3	332.1	5.02

bread #1. It should be noted that whole wheat bread #2 which was fed in Experiment No. 4 had a lower nutritive value.

*Experiment No. 3 (paired feeding for equal gain).* Since Experiment

No. 1 and Experiment No. 2 were conducted by the *ad libitum* feeding method, differences in the palatability of the several types of bread may have influenced the results. Also, some of the animals attained greater body weights than others and thus required a larger amount of energy for maintenance. Therefore, a number of these breads were fed in Experiment No. 3 wherein comparisons between two types of bread were made by feeding them to 12 pairs of rats for equal gains. As is indicated by the grouping of the summarized feeding test data from this experiment in table 2 the following comparisons were made:

1. Enriched water bread versus water bread.
2. Nonfat milk bread versus enriched water bread.
3. Whole wheat nonfat milk bread #1 versus whole wheat bread #1.
4. Nonfat milk bread versus whole wheat bread #1.

When compared by this method and the data analyzed by Student's "t" test for paired differences, enriched water bread exhibited a nutritive value which was significantly higher than that of water bread ( $P < 0.01$ ). Whereas we have been able to demonstrate a slight improvement in the nutritive value of water bread by enrichment to the new levels it should be noted that neither of these two types of bread has as high nutritive value as are exhibited by the other types of bread fed in these experiments.

The results on nonfat milk bread and enriched water bread are in agreement with those obtained in Experiments No. 1 and No. 2, and again show the superior nutritive value of water bread made with 6 per cent nonfat dry milk solids.

When fed for equal gains the addition of 6 per cent nonfat dry milk solids produced a significant improvement in the nutritive value of whole wheat bread #1 ( $P < 0.01$ ). However, this lot of whole wheat bread still exhibited a higher nutritive value than nonfat milk bread ( $P < 0.01$ ).

*Experiment No. 4 (paired feeding for equal gain).* It appeared that we had been using a whole wheat flour of unusually high nutritive value in Experiments No. 2 and No. 3. The whole wheat bread crumbs had a very high protein content (17.13%) and the results from the feeding tests did not agree with similar experiments reported in the literature (Mitchell *et al.* (9) and Light and Frey (8)). Therefore we repeated the experiments involving whole wheat bread using a whole wheat flour of lower protein content or one which more closely approached the average protein content of whole wheat flour. The breads made from this flour are referred to as whole wheat bread #2.

The results of this feeding test are also shown in table 2. The difference between the nutritive value of enriched nonfat milk bread and whole wheat bread #2 was not significant ( $P > 0.1$ ) and the nutritive value of whole wheat bread #2 was improved by the addition of 6 per cent nonfat dry milk solids ( $P < 0.01$ ).

## CARCASS ANALYSES

Rate of growth on a diet and the nutritive value of the diet as measured by the grams of solids required to produce a gram of grain are generally recognized methods of evaluating a diet. However, further information about the nutritive value of a diet can be obtained from the chemical composition of the deposited tissue since this can vary in percentage of solids, fat, ash and protein. In order to evaluate some of the bread diets from this standpoint a number of the carcasses from experiments No. 3 and No. 4 were analyzed. Since the animals were pair fed for equal gain the analytical data were analyzed statistically by Student's "t" test for paired differences.

*Experiment No. 3.* The 12 pairs of rats which had been fed for equal gains on nonfat milk bread and enriched water bread were continued on the same dietary regime for two weeks following the 8 week growth test during which time hemoglobin determinations were made. The hemoglobin values, weights of the cleaned carcasses and results of the chemical analyses of the carcasses are summarized in table 3.

TABLE 3  
*Average hemoglobin values and carcass analyses—Experiment No. 3*

Diet	Av. weight of cleaned carcass	Av. hemo- globin	Carcass analyses		
			Ash	Calcium	Nitrogen
	<i>gm.</i>	<i>gm./100 cc.</i>	%	%	%
Enriched water bread .....	88.7	13.27	2.79	0.68	3.070
Nonfat milk bread .....	92.2	12.96	3.35	0.88	3.172

The carcasses of the nonfat milk bread rats contained a significantly higher percentage of nitrogen ( $P = 0.02$ ) and a significantly higher percentage of calcium ( $P < 0.01$ ). The difference between the concentration of hemoglobin in the blood of the two groups of rats was not significant ( $P = 0.3$ ). Fairbanks (2) showed that the addition of 6 per cent nonfat dry milk solids to white water bread increased the calcium content but not the nitrogen content of the carcass.

*Experiment No. 4.* The four groups of 8 rats each used in this paired feeding experiment were killed immediately following the 8-week growth test and the prepared carcasses were analyzed for total solids, fat, protein, and ash. The weights of the cleaned carcasses and their average chemical composition calculated to the dry basis are summarized in table 4.

The carcasses of the animals which had been fed enriched nonfat milk bread contained a significantly higher percentage of ash than the carcasses of the animals which had been fed whole wheat bread #2 ( $P < 0.01$ ). Other

TABLE 4  
Average carcass analyses—Experiment No. 4

Diet	Av. weight of cleaned carcass	Solids	Fat dry basis	Ash dry basis	Crude protein (N × 6.25) dry basis
	gm.	%	%	%	%
Enriched nonfat milk bread	94.0	34.10	32.94	9.79	56.96
Whole wheat bread #2 .....	96.4	33.43	32.08	7.96	59.93
Whole wheat bread #2 .....	99.5	33.78	33.72	7.65	57.96
Whole wheat nonfat milk bread #2 .....	101.5	33.04	30.04	9.33	59.96

differences in the chemical composition of the carcasses of these two groups of experimental animals were not statistically significant. Therefore, it appears that enriched nonfat milk bread is equivalent in nutritive value to whole wheat bread of average composition both from the standpoint of weight gained per unit of bread solids consumed and from the chemical composition of the carcass produced. Judged on the basis of per cent ash in the carcasses, enriched nonfat milk bread seems to be nutritionally superior to whole wheat bread. Mitchell *et al.* (9) found that white bread made with 6 per cent nonfat milk solids was superior to whole wheat bread in the promotion of bone calcification.

The carcasses of the animals which had been fed whole wheat nonfat milk bread #2 contained a significantly higher percentage of ash than the carcasses of the animals which received whole wheat bread #2 ( $P < 0.02$ ). Otherwise the differences in chemical composition between these two lots of carcasses were not statistically significant. These results confirm those of the growth experiment wherein whole wheat nonfat milk bread was found to have a higher nutritive value than whole wheat bread. It required less of the whole wheat nonfat milk bread to produce a one-gram gain, yet the carcass composition was equivalent to that of those fed the whole wheat bread in solids, fat and protein, and superior to those of the rats fed whole wheat bread in ash.

#### CONCLUSIONS

1. The addition of 6 per cent nonfat dry milk solids improves the nutritive value of water bread, enriched water bread and whole wheat bread.
2. Water bread supplemented with 6 per cent nonfat dry milk solids is superior in nutritive value to enriched water bread as measured by the grams of solids required to produce a gram of gain and the per cent nitrogen and calcium in the carcasses of the experimental animals. There was no significant difference between hemoglobin levels in the rats on which these comparisons were made.
3. Water bread supplemented with 6 per cent nonfat dry milk solids and enriched to the levels of the new standards is equivalent in nutritive

value to whole wheat bread of average composition as measured by grams of solids required to produce a one-gram gain and the chemical composition of the carcasses of the experimental animals.

4. Enrichment at the new levels causes a slight improvement in the nutritive value of water bread which can be observed by paired feeding for equal gain.

5. The improvement in the nutritive value of whole wheat bread brought about by the addition of 6 per cent nonfat dry milk solids has been confirmed by carcass analyses.

6. Whole wheat breads vary in nutritive value depending upon the type of whole wheat flour used.

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