Amino Acid Fortification of Bread Fed at Varying Levels During Gestation and Lactation in Rats[1]

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ABSTRACT The effects of protein quality improvement of white bread fed to female rats during pregnancy and lactation, under varying degrees of adequacy in food energy, on growth and brain development of the offspring have been studied. Weaning weight and the weight, protein and DNA of whole brain and major regions of the brain at weaning were determined. Lysine addition significantly increased all parameters, and threonine addition gave additional significant increments. Restricting energy intake decreased most parameters independent of protein quality. Brain cellularity was significantly higher in offspring of dams fed lysine and threonine fortified bread than in offspring of dams fed casein. All parameters measured in the offspring of dams fed lysine and threonine fortified bread at 70% ad libitum consumption were significantly increased over the values obtained in the offspring of dams fed unfortified bread ad libitum even though they had consumed 13% less protein and dietary energy during the pregnancy and lactation period. The implications of these findings for practical problems in human nutrition are considered. J. Nutr. *107:* 300–309, 1977.

INDEXING KEY WORDS lysine · threonine · protein-energy · early development

There would appear to be general agreement that protein-energy malnutrition of young children, complicated by deficiencies in vitamins and minerals is one of the most serious public health problems in developing countries (1). The relative importance of protein versus energy, however, has become somewhat controversial. Harper et al. (2) suggest that primary protein deficiency in young children is not likely to be the cause of malnutrition if enough food is consumed, even when most of the protein and food energy is supplied by cereal grains. Therefore it is their opinion that such programs as development of protein-rich foods, amino acid fortification, improvement of cereal protein genetically through plant breeding, or efforts to increase legume production and utilization, may not be of real practical value in countries where cereals are the primary staples. On the other hand, the Protein-Calorie Advisory Group (PAG) agree on the importance of food energy but feel that protein-energy malnutrition in most developing countries cannot be reduced by merely providing more of the same food, but that improving the supply of utilizable protein in the diet is also important (3).

An important aspect of this debate is the extent to which increases in the level of utilizable protein in a diet are of benefit under conditions where the supply of food energy is inadequate. In a previous paper (4), this issue has been reviewed with the conclusion that increasing the amount of

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utilizable protein in the diet will increase the efficiency of protein utilization even when the need for dietary energy has not been completely met. Specifically in regard to amino acid fortification, it was demonstrated that the addition of lysine, or lysine and threonine to white bread fed to weanling rats significantly improved growth and the efficiency of nitrogen utilization even under conditions of inadequacy in the supply of dietary energy (4). In the present work, these observations have been extended and the value of amino acid fortification of bread fed to rats through pregnancy and lactation demonstrated in terms of growth of the offspring, including growth and cellular development of the brain, even when dietary energy was consumed at a level below ad libitum intake.

METHODS

Animals. The methods used were, in general, those described previously (5, 6). Virgin female rats[2] weighing approximately 175 to 200 g were caged in groups of three in an air-conditioned room with a temperature of 23° (range 21° to 25°) and relative humidity of 50% (range 30% to 70%). Until breeding, the rats were fed a stock diet.[3] When the females reached a body weight of 210 to 240 g a male of similar strain was placed with each group of females, and the females examined each morning for a vaginal breeding plug. Upon confirmation of breeding, the female was weighed, placed in an individual cage, and started on one of the 15 dietary treatments listed in table 2. Dams and pups were weighed following delivery and the eight heaviest offspring returned to the dam for nursing. Offspring and dam were again weighed on the 22nd postpartum day and the offspring killed by decapitation. Rats were bred until, with one exception, at least four dams on each treatment had produced adequate offspring and nursed eight pups to age 22 days. Dams fed unfortified bread at 70% of ad libitum intake were unsuccessful at raising any pups and this treatment group was abandoned. With this exception, all other treatment groups consisted of four or five dams, as listed in table 2, and all litters consisted of eight pups.

Diets. The experimental design was to feed unfortified and amino acid fortified white bread and casein diets at 100%, 85% and 70% of ad libitum intake. The composition of the diets is given in table 1. The basal bread diet contained 90% air dried white bread, the highest level that could be fed and still enable a vitamin mixture, salt mixture, and corn oil to be added to the diet. The bread averaged 2.3% N, dry weight, as determined by the microKjeldahl method (7) and the basal bread diets (groups 1 to 3) contained 2.1% N. The lysine, or lysine and threonine diets were made isonitrogenous with the basal bread diet by slightly reducing the amount of

TABLE 1

Composition of experimental diets[1]

Ingredient	Group number				
	1–3	4–6	7–9	10–12	13–15
Dried bread[2]	900	881	870	850	—
L-lysine HCl[3]	—	2.6	4.4	4.2	—
DL-threonine[3]	—	—	—	2.6	—
Salts USP XIV[4]	40	40	40	40	40
Vitamin mix[5]	20	20	20	20	20
Corn oil[6]	40	40	40	40	40
Cornstarch	—	16	26	43	759
Casein[7]	—	—	—	—	141

[1] Expressed as g/Kg diet. [2] Freshly baked standard white bread (Wonder Bread) was obtained from the Continental Baking Company in Denver, air dried and ground in a Wiley Mill. Weights listed are for the dry weight of the bread and cornstarch. [3] L-lysine monohydrochloride (food grade) and DL-threonine (allo-free) were obtained as a gift from Dr. Eugene Howe, Merck and Co., Rahway, New Jersey. [4] Salt mixture USP XIV (Nutritional Biochemicals Company) contained (in %): Al $NH_4(SO_4)_2 \cdot 12\ H_2O$, 0.009; $CaCO_3$, 6.86; $Ca_3(C_6H_5O_7)_2 \cdot 4\ H_2O$, 30.83; $Ca(H_2PO_4)_2 \cdot H_2O$, 11.28; $CuSO_4 \cdot 5\ H_2O$, 0.008; $FeNH_4\ C_6H_5O_7$, 1.53; $MgCO_3$, 3.52; $MgSO_4$, 3.83; $MnSO_4 \cdot H_2O$, 0.020; KCl, 12.47; KI, 0.004; K_2HPO_4, 21.88; NaCl, 7.71; NaF, 0.050. [5] Vitamin Diet Fortification Mixture (Nutritional Biochemicals Company) supplied the following nutrients/kg diet; (in I.U.) retinyl acetate, 18,000; cholecalciferol, 2,000; α-tocopherol, 110; (in mg) ascorbic acid, 900; inositol, 100; choline chloride, 1,500; menadione, 45.0; *p*-aminobenzoic acid, 100; niacin, 90.0; riboflavin, 20.0; pyridoxine-HCl, 20.0; thiamin-HCl, 20.0; calcium pantothenate, 60.0; folic acid, 1.80; biotin, 0.40; vitamin B_{12}, 0.03. [6] Mazola corn oil, CPC International Inc., Englewood Cliffs, New Jersey. [7] Animal Nutrition Research Council (ANRC) casein obtained from the Sheffield Chemical Co., Norwich, New York.

[2] Carworth CFE rats obtained from Carworth Farms, Portage, Michigan.
[3] Purina Laboratory Chow. Ralston Purina Company, Checkerboard Square, St. Louis, Missouri.

bread and adding cornstarch. The casein was analyzed for nitrogen as above, and the casein diet made isonitrogenous with the bread diets.

Food consumption was measured daily for all ad libitum fed rats and averaged for each diet. The restricted fed rats were fed approximately 85% or 70% of the ad libitum value for each particular diet each day for the duration of pregnancy and lactation.

Analytical Methods. At autopsy, fresh brains were immediately removed from the cranium, dissected free of meninges, weighed, and the cerebellum and brain stem separated from the cerebrum and weighed, individually. DNA was determined by the method of Zamenhof et al. (8) and protein was determined on the perchloric acid precipitated pellets by the method of Lowry et al. (9) as modified by Chase et al. (10). DNA and protein analyses were done on each of the three brain areas with the total brain values representing the sum of the three determinations. Statistical comparisons were made using Duncans Multiple F test (11) and all results are expressed as means ± SE. In the tables, the least significant differences (LSD) for each parameter are indicated for two levels of significance.

RESULTS

Lysine fortification level. Two levels of lysine fortification were studied. In no parameter did 0.5% L-lysine monohydrochloride increase the response over that obtained with 0.3% L- lysine monohydrochloride unless threonine was also added. These results are consistent with results obtained in growth trials with weanling rats (12). The higher level of lysine addition alone was used in this study to exclude the possibility that any responses in the lysine and threonine group could be due to the higher level of lysine rather than the threonine. This possibility is excluded by the data and the results obtained with the higher level of lysine added alone will not be further described in this paper.

Maternal weight. Changes in maternal weight are shown in table 2. Differences as a function of protein quality and level of food intake were small. Maternal weight loss during pregnancy was significantly less in lysine, and lysine and threonine ad libitum fed groups than in the unfortified bread group. Weight changes during lactation were not affected by protein quality when the diets were fed ad libitum. Food restriction during pregnancy or lactation caused very slight losses in body weight that did not vary significantly as a function of protein quality.

Maternal food consumption. Ad libitum food consumption during pregnancy was significantly increased by lysine, or lysine and threonine addition. The increases in food consumption during lactation associated with amino acid fortification were significant, and were slightly greater than the increases during pregnancy but still were not large. During the entire gestation and lactation period, dams fed lysine and threonine fortified bread consumed 20% more food than dams fed unfortified bread. The effect of protein quality on food consumption was, as expected, much less than that observed during postweaning growth (4).

Pup weights. The number of pups per litter, birth weights, and total litter weights for all dietary treatments are shown in table 3. In ad libitum fed dams, protein quality did not significantly affect the number of pups per litter or total litter weight. Birth weights were significantly higher in the offspring of dams fed lysine and threonine fortified bread ad libitum as compared to unfortified bread. Birth weights of pups from dams fed casein ad libitum were significantly greater than those of pups from dams fed bread, or bread plus lysine, but not bread plus lysine plus threonine. Food restriction reduced the number of pups per litter, birth weights, and total litter weights for all diet groups with no differences discernible as a function of protein quality. The size of the reductions appeared to be independent of the degree of food restriction, suggesting that the phenomenon may have been in part a result of the pair feeding procedure per se rather than being solely related to the extent to which food intake was reduced. This is speculation, however, and the reason for the observation that the reductions in these parameters were approximately as large in 85% as 70% ad libitum fed dams is not understood.

TABLE 2

Maternal body weight and food consumption

Group	Diet	Number of mothers	Food intake-% of ad libitum	Initial body weight	Post-partum body wt.	Post-weaning body wt.	Food consumption gestation[1]	Food consumption lactation[2]	Δ Wt.[3] pregnancy	Δ Wt.[4] lactation
				g	*g*	*g*	*g/day*	*g/day*	*g*	*g*
1	White bread	4	100	224±5[7]	214±6	221±4	16.8±1.3	31.4±1.4	−9.8±1.6	9.0±4.0
2	White bread	5	85	224±5	215±4	216±4	13.9±0.2	27.0±0.1	−8.8±0.9	1.0±3.2
3	White bread	5[6]	70							
4	Bread + 0.3% L-lysine HCl[5]	5	100	223±3	220±3	229±1	20.4±0.5	36.5±1.7	−4.2±2.3	7.1±3.1
5	Bread + 0.3% L-lysine HCl[5]	4	85	225±3	219±2	218±3	17.7±0.7	32.3±1.0	−6.5±2.2	−0.5±7.5
6	Bread + 0.3% L-lysine HCl[5]	4	70	218±5	207±5	198±5	14.5±0.5	26.3±0.9	−11.8±1.8	−9.0±2.0
7	Bread + 0.5% L-lysine HCl[5]	5	100	224±6	225±4	229±7	18.7±1.0	37.6±1.1	1.0±2.1	3.2±5.0
8	Bread + 0.5% L-lysine HCl[5]	4	85	219±2	216±2	217±1	15.5±0.8	31.2±0.8	−3.0±2.1	0.5±1.8
9	Bread + 0.5% L-lysine HCl[5]	4	70	222±5	207±3	210±3	12.3±0.8	25.6±0.7	−14.5±4.5	3.3±2.3
10	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[5]	4	100	224±6	222±6	228±8	19.9±0.5	38.5±0.9	−1.5±2.1	5.8±2.3
11	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[5]	4	85	225±5	221±6	220±2	16.6±0.0	33.1±0.1	−4.2±4.4	−0.8±5.7
12	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[5]	4	70	220±5	212±6	213±5	14.6±0.8	27.2±0.1	−7.8±2.5	1.2±5.4
13	Casein	4	100	218±3	223±3	229±5	16.4±0.3	40.8±1.9	4.8±2.9	8.6±9.6
14	Casein	4	85	217±5	218±4	218±4	13.4±0.2	33.3±1.2	3.5±1.8	4.2±2.1
15	Casein	4	70	218±5	216±5	225±5	11.2±0.1	28.4±0.9	−2.8±2.7	13.0±1.5

[1] LSD, $P < 0.05$; 1.7 for closest, 2.0 for most distant means. $P < 0.01$; 2.3 for closest, 2.7 for most distant means. [2] LSD, $P < 0.05$; 2.6 for closest, 3.1 for most distant means. $P < 0.01$; 3.4 for closest, 4.0 for most distant means. [3] LSD, $P < 0.05$; 6.9 for closest, 8.2 for most distant means. $P < 0.01$; 9.2 for closest, 10.8 for most distant means. [4] LSD, $P < 0.05$; 11.4 for closest, 13.6 for most distant means. $P < 0.01$; 15.3 for closest, 18.0 for most distant means. [5] Amino acid percentages based on dry weight of the bread. [6] No offspring of dams fed bread at 70% of ad libitum consumption survived. [7] Mean ± SE.

TABLE 3

Birth weight, litter size, and total litter weight

Group	Diet	Food intake % of ad libitum	No. of pups	Mean birth wt.[1]	No. of pups per litter[2]	Total litter weight[3]
				g		*g/litter*
1	White bread	100	37	6.61±0.10[6]	9.2±0.8	61.2±3.7
2	White bread	85	42	6.20±0.09	8.4±0.6	52.0±3.2
3	White bread	70	0[5]			
4	Bread + 0.3% L-lysine HCl[4]	100	54	6.62±0.08	10.8±0.9	70.9±5.5
5	Bread + 0.3% L-lysine HCl	85	34	6.14±0.08	8.5±0.3	52.2±1.7
6	Bread + 0.3% L-lysine HCl	70	35	6.13±0.06	8.8±0.5	52.9±2.8
7	Bread + 0.5% L-lysine HCl	100	53	6.79±0.09	10.6±0.7	72.6±3.8
8	Bread + 0.5% L-lysine HCl	85	34	5.85±0.10	8.5±0.6	49.7±2.1
9	Bread + 0.5% L-lysine HCl	70	32	6.87±0.09	8.0±0.7	54.9±4.2
10	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine	100	38	7.14±0.10	9.5±1.2	68.6±7.0
11	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine	85	35	5.78±0.10	8.8±0.6	50.2±1.3
12	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine	70	33	6.41±0.11	8.2±0.2	53.0±0.7
13	Casein	100	40	7.37±0.11	10.0±0.9	73.7±4.4
14	Casein	85	34	5.93±0.07	8.5±0.3	50.4±1.2
15	Casein	70	32	6.47±0.11	8.0±0.8	51.7±3.7

[1] LSD, $P < 0.05$; 0.25 for closest, 0.30 for most distant means. $P < 0.01$; 0.33 for closest, 0.39 for most distant means. [2] LSD, $P < 0.05$; 1.7 for closest, 2.0 for most distant means. 2.3 for closest, 2.7 for most distant means. [3] LSD, $P < 0.05$; 9.2 for closest, 10.9 for most distant means. $P < 0.01$; 12.2 for closest, 14.4 for most distant means. [4] Amino acid percentages based on dry weight of the bread. [5] No offspring survived. [6] Mean±SE.

As shown in table 4, weaning weights progressively increased as the protein quality of the bread was increased through amino acid fortification. Weaning weights in offspring of dams fed lysine and threonine fortified bread were not significantly different from those found for casein. Maternal food restriction reduced weaning weights significantly for all diet groups. However, the beneficial effects of protein quality improvement were clearly apparent, even under conditions of maternal dietary energy inadequacy. For example, offspring of dams fed lysine and threonine fortified bread at 70% of ad libitum intake weighed 28.8 ± 0.3 g at weaning (group 12) compared to only 16.8 ± 0.4 g in the offspring of dams fed bread ad libitum (group 1) in spite of the fact that the former dams consumed 13% less food from conception to weaning than did the latter.

Brain weights. Offspring whole brain, cerebrum, cerebellum, and brain stem weights at weaning for all dietary treatments are listed in table 4. Whole brain weight and the weight of all major regions of the brain were progressively increased by lysine and then lysine plus threonine addition to the bread when fed ad libitum. The only exception was that lysine addition alone did not significantly increase brain stem weight. In all cases, weights for the offspring of dams fed lysine and threonine fortified bread were comparable to the values obtained with casein. Food restriction during pregnancy and lactation significantly reduced whole brain weight and cerebrum weight of the pups in all diet groups. Brain stem and cerebellum weights were significantly reduced by food restriction in the offspring of dams fed bread, bread plus lysine plus threonine, or casein. Surprisingly, these weights were not affected significantly by food restriction in the offspring of dams fed bread fortified with either level of lysine alone. As was the case for weaning weight, the beneficial effects of protein quality improvement on brain weight were apparent even when food intake was reduced. The brain weight in offspring of dams fed lysine and threonine fortified bread at 70% of ad libitum intake (group 12) was 1.344 ± 0.012 g

TABLE 4

Body and brain weight at weaning

Group	Diet	Food intake % of ad libitum	No. of pups	Total food consumed[1]	Weaning wt.[2]	Brain wt.[3]	Cerebrum wt.[4]	Cerebellum wt.[5]	Brainstem wt.[6]
				g	*g*	*g*	*g*	*g*	*g*
1	White Bread	100	32	1,012	16.8±0.4[8]	1.235±0.010	0.847±0.012	0.148±0.003	0.202±0.004
2	White Bread	85	40	859	12.8±0.2	1.166±0.007	0.771±0.006	0.140±0.002	0.181±0.002
3	White Bread	70	0[8]						
4	Bread + 0.3% L-lysine HCL	100	40	1,195	26.4±0.2	1.322±0.008	0.894±0.008	0.155±0.001	0.208±0.004
5	Bread + 0.3% L-lysine HCl[7]	85	32	1,050	23.3±0.3	1.248±0.013	0.858±0.007	0.145±0.001	0.211±0.002
6	Bread + 0.3% L-lysine HCl[7]	70	32	857	21.4±0.2	1.256±0.007	0.843±0.007	0.154±0.001	0.218±0.005
7	Bread + 0.5% L-lysine HCl[7]	100	40	1,182	25.6±0.3	1.291±0.009	0.877±0.008	0.148±0.001	0.204±0.002
8	Bread + 0.5% L-lysine HCl[7]	85	32	981	22.5±0.4	1.247±0.005	0.841±0.004	0.151±0.001	0.206±0.002
9	Bread + 0.5% L-lysine HCl[7]	70	32	796	20.3±0.3	1.242±0.006	0.827±0.007	0.152±0.001	0.209±0.002
10	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[7]	100	32	1,226	38.8±0.4	1.491±0.008	0.966±0.010	0.177±0.001	0.235±0.002
11	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[7]	85	32	1,044	34.4±0.3	1.407±0.009	0.936±0.005	0.161±0.001	0.234±0.005
12	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine	70	32	878	28.8±0.3	1.344±0.012	0.882±0.008	0.159±0.001	0.213±0.002
13	Casein	100	32	1,201	39.1±0.6	1.482±0.010	0.953±0.008	0.175±0.001	0.232±0.004
14	Casein	85	32	981	30.4±0.3	1.317±0.008	0.865±0.009	0.158±0.001	0.211±0.004
15	Casein	70	32	832	27.2±0.2	1.288±0.011	0.853±0.009	0.157±0.001	0.217±0.001

1 Total food consumed per dam during pregnancy and lactation. 2 LSD, $P < 0.05$; 0.8 for closest, 1.0 for most distant means. $P < 0.01$; 1.1 for closest, 1.3 for most distant means. 3 LSD, $P < 0.05$; 0.025 for closest, 0.030 for most distant means. $P < 0.01$; 0.033 for closest, 0.039 for most distant means. 4 LSD, $P < 0.05$; 0.022 for closest, 0.027 for most distant means. $P < 0.01$; 0.029 for closest, 0.034 for most distant means. 5 LSD, $P < 0.05$; 0.003. $P < 0.01$; 0.004. 6 LSD, $P < 0.05$; 0.008 for closest, 0.010 for most distant means. $P < 0.01$; 0.011 for closest, 0.013 for most distant means. 7 Amino acid percentages based on dry weight of the bread. 8 Mean±SE.

compared to the value of 1.235 ± 0.010 g for ad libitum fed bread (group 1).

Brain protein. Values for protein in whole brain, cerebrum, cerebellum, and brain stem of the offspring at weaning are listed in table 5. With one exception, total brain protein and the amounts of total protein in all major regions of the brain were all progressively increased by the addition of lysine and then lysine and threonine to the bread fed to the dams. The one exception was that brain stem protein was not increased by lysine and threonine over that found for lysine alone. Brain protein levels in the lysine and threonine group in all cases were fully comparable to values obtained in the casein group. Maternal food restriction significantly reduced brain protein levels in all brain regions and in whole brain for all diets.

Brain DNA. Values for total brain DNA and the amount of DNA found in the various regions of the brain are listed in table 6 for the offspring at weaning of dams in all dietary treatments. In all cases, addition of lysine alone resulted in significant increases in DNA over values obtained with the unfortified bread. Addition of threonine with the lysine gave additional significant increases in DNA in all major regions of the brain and in whole brain. Whole brain DNA values in the lysine and threonine group were significantly higher than the values observed for the casein group. Food restriction during pregnancy and lactation significantly reduced DNA in the whole brain and all regions of the brain in the offspring of dams fed all diets except bread. The maximum maternal food restriction possible in the unfortified bread group, 15%, did not reduce cerebrum DNA and caused only slight, although significant, reductions in cerebellum and brain stem DNA.

Amino acid fortification at reduced energy intake. A major objective of the present study was to examine the effects on the offspring of amino acid fortification of bread fed to female rats at food intake levels less than energy need during pregnancy and lactation. Dams fed the lysine and threonine fortified bread diet at 70%

TABLE 5

Protein levels in whole brain and in various regions

Group	Diet	Food intake % of ad libitum	No. of pups	Whole brain protein[1]	Cerebrum protein[2]	Cerebellum protein[3]	Brainstem protein[4]
				mg	*mg*	*mg*	*mg*
1	White Bread	100	32	99.29±1.39[7]	71.16±0.99	11.90±0.20	16.23±0.33
2	White Bread	85	40	95.46±0.72	70.67±0.62	10.11±0.16	14.68±0.16
3	White Bread	70	0[6]				
4	Bread + 0.3% L-lysine HCl[5]	100	40	111.72±0.87	78.13±0.72	14.38±0.13	19.20±0.36
5	Bread + 0.3% L-lysine HCl[5]	85	32	103.91±0.71	71.93±0.59	13.53±0.11	18.45±0.16
6	Bread + 0.3% L-lysine HCl[5]	70	32	100.29±0.72	70.31±0.56	12.70±0.10	17.28±0.39
7	Bread + 0.5% L-lysine HCl[5]	100	40	110.27±0.85	76.95±0.72	14.22±0.09	19.10±0.20
8	Bread + 0.5% L-lysine HCl[5]	85	32	104.67±0.41	73.10±0.35	13.42±0.08	18.15±0.17
9	Bread + 0.5% L-lysine HCl[5]	70	32	98.81±0.63	69.19±0.58	13.16±0.08	16.46±0.13
10	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[5]	100	32	116.96±0.94	81.12±0.86	16.34±0.10	19.49±0.13
11	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[5]	85	32	111.91±0.59	78.22±0.41	15.13±0.10	18.56±0.39
12	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[5]	70	32	109.61±0.77	77.14±0.70	14.26±0.08	18.22±0.14
13	Casein	100	32	115.09±0.87	79.81±0.69	16.56±0.10	18.72±0.30
14	Casein	85	32	109.06±0.87	75.56±0.87	15.24±0.10	18.26±0.18
15	Casein	70	32	107.85±0.84	75.44±0.78	14.39±0.10	18.01±0.12

[1] LSD, $P < 0.05$; 2.22 for closest, 2.70 for most distant means. $P < 0.01$; 2.91 for closest, 3.45 for most distant means. [2] LSD, $P < 0.05$; 1.86 for closest, 2.26 for most distant means. $P < 0.01$; 2.44 for closest, 2.89 for most distant means. [3] LSD, $P < 0.05$; 0.30 for closest, 0.37 for most distant means. $P < 0.01$; 0.40 for closest, 0.47 for most distant means. [4] LSD, $P < 0.05$; 0.64 for closest, 0.78 for most distant means. $P < 0.01$; 0.84 for closest, 0.99 for most distant means. [5] Amino acid percentages based on dry weight of the bread. [6] No offspring survived. [7] Mean ± SE.

TABLE 6

DNA levels in whole brain and in various regions

Group	Diet	Food intake % of ad libitum	No. of pups	Whole brain DNA[1]	Cerebrum DNA[2]	Cerebellum DNA[3]	Brainstem DNA[4]
				(mg)	(mg)	(mg)	(mg)
1	White Bread	100	32	2.176 ± 0.036[7]	0.922 ± 0.016	1.023 ± 0.019	0.231 ± 0.005
2	White Bread	85	40	2.146 ± 0.016	0.929 ± 0.008	0.993 ± 0.016	0.221 ± 0.002
3	White Bread	70	0[6]				
4	Bread + 0.3% L-lysine HCl[5]	100	40	2.374 ± 0.018	0.975 ± 0.011	1.136 ± 0.012	0.264 ± 0.005
5	Bread + 0.3% L-lysine HCl[5]	85	32	2.284 ± 0.016	0.937 ± 0.010	1.089 ± 0.010	0.257 ± 0.002
6	Bread + 0.3% L-lysine HCl[5]	70	32	2.254 ± 0.017	0.927 ± 0.012	1.080 ± 0.009	0.247 ± 0.006
7	Bread + 0.5% L-lysine HCl[5]	100	40	2.328 ± 0.016	0.962 ± 0.010	1.114 ± 0.011	0.252 ± 0.003
8	Bread + 0.5% L-lysine HCl[5]	85	32	2.296 ± 0.014	0.942 ± 0.006	1.104 ± 0.012	0.250 ± 0.003
9	Bread + 0.5% L-lysine HCl[5]	70	32	2.214 ± 0.015	0.919 ± 0.008	1.057 ± 0.011	0.238 ± 0.002
10	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[5]	100	32	2.837 ± 0.019	1.088 ± 0.014	1.467 ± 0.010	0.281 ± 0.002
11	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[5]	85	32	2.418 ± 0.019	0.998 ± 0.011	1.154 ± 0.011	0.266 ± 0.006
12	Bread + 0.5% L-lysine HCl + 0.3% DL-threonine[5]	70	32	2.375 ± 0.016	0.973 ± 0.013	1.144 ± 0.007	0.258 ± 0.002
13	Casein	100	32	2.535 ± 0.027	1.036 ± 0.013	1.228 ± 0.021	0.271 ± 0.004
14	Casein	85	32	2.351 ± 0.017	0.966 ± 0.010	1.129 ± 0.010	0.255 ± 0.002
15	Casein	70	32	2.321 ± 0.014	0.954 ± 0.011	1.126 ± 0.011	0.242 ± 0.002

[1] LSD, $P < 0.05$; 0.053 for closest, 0.064 for most distant means. $P < 0.01$; 0.069 for closest, 0.082 for most distant means. [2] LSD, $P < 0.05$; 0.030 for closest, 0.037 for most distant means. $P < 0.01$; 0.040 for closest, 0.047 for most distant means. [3] LSD, $P < 0.05$; 0.033 for closest, 0.041 for most distant means. $P < 0.01$; 0.044 for closest, 0.052 for most distant means. [4] LSD, $P < 0.05$; 0.008 for closest, 0.010 for most distant means. $P < 0.01$; 0.011 for closest, 0.013 for most distant means. [5] Amino acid percentages based on dry weight of the bread. [6] No offspring survived. [7] Mean ± SE.

of ad libitum consumption (group 12) consumed 13% less food from conception until weaning than did dams fed the basal bread diet ad libitum (group 1). This reduction in food intake was significant during both pregnancy and lactation. In the rat, most of the cellular development of the brain takes place during lactation (13). In spite of this reduced level of food intake, the increases in weaning weight and all brain parameters in the lysine and threonine group were significant. It is notable that protein quality improvement was able to cause these increases at a time when there was a 13% decrease in both protein and energy intake. Dams fed lysine fortified bread at 70% of ad libitum consumption (group 6) consumed 15% less food than did dams fed the basal bread diet ad libitum (group 1). The increases in the offspring of these lysine fed dams in weaning weight, cerebellum weight, brain stem weight, cerebellum protein, brain stem protein, whole brain DNA, cerebellum DNA and brain stem DNA were all significant as compared to the offspring of dams fed bread ad libitum. Increases in cerebrum and whole brain parameters with the exception of whole brain DNA, were not significant, however. The lesser degree of quality improvement resulting from lysine addition was apparently not sufficient to overcome the effects on whole brain and cerebrum of a 15% reduction in both protein and energy intake.

DISCUSSION

The improvement in growth and brain development in offspring of dams fed amino acid fortified bread throughout the pregnancy and lactation period was reported previously (5, 6). In this previous study bread, bread plus lysine, and bread plus lysine and threonine were compared with isonitrogenous casein (13% in diet), 26% casein and stock diet. Values obtained for the lysine and threonine groups were generally comparable or superior to the values obtained with the 13% casein or stock diets although generally less than the values obtained with 26% casein. In the present study, comparison was made only with the isonitrogenous casein control. The results obtained are similar to those reported previously (5, 6) and confirm that addition of lysine, or lysine and threonine to bread fed to rats during pregnancy and lactation significantly increases growth and brain development of the offspring at the time of weaning. These results are consistent with the earlier work of Culik and Rosenberg (14) who reported that lysine fortification of white bread fed to rats for seven generations improved reproductive and lactation performance including an increase in birth weights and weaning weights of the offspring. The present and previously reported work (5, 6) demonstrates that in spite of its relatively low protein level, white bread fortified with lysine and threonine is an adequate protein source for rats during pregnancy and lactation.

As reported previously (5) the diets used in these studies contained 18 to 33 mg zinc/kg diet, amounts above the requirement (15) but with little margin of safety. The weaning weights of 39.1 g with a casein level of only 13% would strongly suggest that the diet was adequate in zinc and other micronutrients.

Birth weights of pups whose dams were fed unfortified bread were significantly less than those of pups whose dams had been fed lysine and threonine fortified bread or casein, but were not less than the pups born of dams fed lysine fortified bread. The effects of feeding a diet even completely devoid of lysine during pregnancy in the rat are not clear; Niiyama et al. (16) reported no effect on litter size or birth weight, but Zamenhof et al. (17) observed significantly reduced birth weights. The adverse effects on birth weight of feeding unfortified bread would be expected to be considerably less than feeding a diet completely devoid of lysine. It is of interest that dams fed unfortified white bread at 70% of ad libitum intake were not able to deliver and raise viable young, in contrast to the results with lysine fortified bread at only a slightly higher level of food intake.

Results in this paper demonstrate clearly that amino acid fortification of bread improved growth and brain development in the offspring of dams fed diets through pregnancy and lactation in which the bread was the sole protein source, even when total protein and energy intake were 13% to 15% less than the amounts consumed by dams fed unfortified bread. These results are consistent with results obtained with postweaning rats in which growth and nitrogen utilization were significantly improved by amino acid fortification of bread fed substantially below the energy need (4). These improvements, which have been demonstrated to occur both during pregnancy and lactation, and in the postweaning period suggest that protein quality improvement of cereals is of definite value even if food intake is marginally deficient. This conclusion would appear to have relevance for quality improvement through plant breeding programs and possibly other protein improvement programs as well as for quality improvement through amino acid fortification.

In interpreting rat growth experiments in terms of their implications in human nutrition, a major factor to consider is that the human infant grows at a slower rate than does the weanling rat. Graham et al. (18) demonstrated that, for the human infant, lysine fortified wheat is comparable in protein quality to casein, while for the rat it is necessary to add threonine with the lysine (12).

In comparing early development in the human with that in the rat, it is important to consider that rat growth occurs more in the post-natal period than is the case for the human (13). However, Dobbing and Sands (19) have recently concluded that cellular development in the human brain continues well into the second year and that the rat may resemble the human infant more closely in postnatal brain development than was initially recognized. The present experiments included both prenatal and postnatal periods and thus, the entire period of cellular development of the brain was included (13).

In this rat model, threonine addition along with the lysine was of considerable importance and much more so than for growth of the weanling rat. It would appear desirable to study further the metabolism of threonine in the pregnant or lactating rat, especially in relation to brain development of the offspring. It is noteworthy that brain cellularity, as determined by DNA content, was significantly higher in the offspring of dams fed lysine and threonine fortified bread than dams fed casein ad libitum. No data are presently available dealing with the effects of lysine and threonine fortification of wheat fed to women during pregnancy.

There are two important issues to be resolved concerning the practical value of protein quality improvement of cereals. The first is one considered in this and the previous paper (4); namely, the extent to which the beneficial effects of protein quality improvement are maintained under conditions of marginal inadequacy in the supply of dietary energy. The present results demonstrate the value, for the rat, of protein quality improvement under these conditions. It seems likely that such improvement would also be of value for the slower growing infant human even if dietary energy were not completely adequate, but this point remains to be demonstrated. The fact that energy need can be reduced by decreasing the level of bodily activity whereas the protein requirement cannot, would lend support to this suggestion.

Another very important aspect of the practical value of protein quality improvement, by either fortification or plant breeding, is whether lysine, lysine and threonine, or lysine and tryptophan are limiting the protein value of any significant number of human diets. This question has been discussed extensively elsewhere (20–22). The issue has not yet been resolved but is under active investigation.

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