Comparison of the Utilization of Supplemented Wheat Gluten and Other Amino Acid Sources in the Rat

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The utilization of nitrogen from supplemented wheat gluten, supplemented casein–lactalbumin (5 to 1), and pure amino acids by rats was studied. When diets containing six nitrogen levels of supplemented wheat gluten were fed, the highest ratio of g-N retained/g-N consumed (apparent NPU) was found at the 2.4% N level; and the three sources of amino acids were compared at this level of dietary nitrogen. All sources were brought to the same total nitrogen and essential amino acid content by supplementation of the intact proteins with pure amino acids to the FAO provisional pattern. The casein–lactalbumin diet produced larger weight gains from 2 to 6 weeks and higher carcass fat levels after 4 weeks. Greater carcass protein levels and higher ratios of g-N retained/g-N consumed were found after 2 and 4 weeks when the protein consumed was supplemented wheat gluten. Utilization of all three amino acid sources over a period of 8 weeks was found to be similar.

Wheat gluten, the most important flour protein, is a cheap abundant protein concentrate obtained as a byproduct of wheat starch extraction. Unsupplemented wheat gluten is known to be of poor quality (Arnrich *et al.*, 1951; Barnes *et al.*, 1945); however, studies with rats have shown that the protein quality of gluten can be enhanced by supplementation with crystalline amino acids, especially lysine and threonine (Banks *et al.*, 1964; Chang and Chao, 1969; Howe and Dooley, 1963).

Already there is a great need for protein in the underdeveloped nations of the world, many of which are now demanding huge quantities of wheat from the United States, and a relief of the present critical shortage of protein does not appear imminent (Paddock and Paddock, 1967). As the amount of available animal protein per person decreases in the coming years, a greater demand will ensue for plant protein concentrates such as gluten.

Ten experiment stations cooperating in Northeastern Regional Project NE-52 have investigated differences in utilization of proteins equalized in essential amino acid pattern and total nitrogen by supplementation with crystalline amino acids. Casein–lactalbumin (a 5:1 mixture by weight) and wheat gluten were selected for study, representing an animal protein and a plant protein, respectively. A crystalline amino acid control diet was used rather than one with intact protein since many factors affect the availability of amino acids from intact protein. Essential amino acids were fed according to the FAO provisional pattern (FAO Committee on Protein Requirements, 1957) since this pattern represented an estimation of essential amino acid needs for humans on a worldwide basis. Nonessential amino acids were supplied by the test proteins and by crystalline amino acids in the pattern found in milk to provide the remainder of nitrogen needed to bring the diets to their proper nitrogen level. Test subjects included humans, pigs, rats, and protozoa, and standard methodology was used for comparison of results from different species or the same species. Differences in utilization of amino acids for protein formation were found among the three diets. The casein–lactalbumin diet showed a consistent superiority for nitrogen utilization compared to the wheat gluten diet when studied in humans (Morse *et al.*, 1969), pigs (Babcock and Markley, 1967), and rats (Radke *et al.*, 1969).

Since the wheat gluten diet showed a poor utilization of its amino acids at the 1.2 and 1.6% N dietary levels after 2 and 4 weeks when fed to rats, it was considered that another level of dietary nitrogen might be more optimal for gluten nitrogen utilization into carcass protein and that the effects of feeding over a longer period of time should be examined. These aspects were investigated in this study.

MATERIALS AND METHODS

Two experiments were carried out with weanling male rats of the CFN strain (Carworth Farms) with starting weights of approximately 50 g. The rats were housed in individual hanging wire-bottomed cages with access to water at all times in a room held at constant temperature and humidity. For 5 days the rats were fed a 1.2% N supplemented casein diet (Table I) to accustom them to a semisynthetic diet and to bring their weights within the range of 57 to 67 g. Following this preexperimental period, a control group of six rats was killed to determine the initial carcass nitrogen.

In the first experiment six nitrogen levels of supplemented wheat gluten (1.2, 2.4, 3.2, 4.0, 4.8, and 8.0% N) were fed for 2 weeks to determine which level produced the highest ratio of g-N retained/g-N consumed or apparent NPU (McCollum and Simmonds, 1929) in the carcasses. This ratio has also been termed the Productive Protein Value (National Academy of Sciences–National Research Council, 1963).

The second experiment involved feeding wheat gluten, casein–lactalbumin, and pure amino acids at the same nitrogen level (2.4% N) for 2, 4, 6, and 8 weeks with the evaluation of growth and the utilization of dietary nitrogen after each time period.

The NE-52 diet pattern of amino acids has been described by Babcock and Markley (1967). The diets in the present work were based on the same amino acid pattern. The components of the 2.4% N diets are given in Table I. The unaltered FAO amino acid pattern was used in these experiments so that the findings might be compared with those of the other animal and human studies by the Regional group, although it has been stated that the tryptophan content of the FAO amino acid pattern may be needlessly high for rats (Howe *et al.*, 1960).

All rats and their food were weighed to the nearest tenth of a gram daily. Each group of rats in the first experiment

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Table I. Composition of the Preexperimental and 2.4% N Diets

	Preexperimental (*ad libitum*)	2.4% N Test Diets (restricted intake)		
		Wheat gluten (WGAA)	Casein–lactalbumin (CLAA)	Amino acid (AA)
	g/100 g of Diet			
Casein	6.52	...	...	...
Casein–lactalbumin	...	...	9.08	...
Wheat gluten (NBC)	...	8.48	...	...
Amino acids	2.69	10.38	9.38	18.91
Cellulose	2.00	2.00	2.00	2.00
Mineral mix (NBC)	4.00	4.00	4.00	4.00
Vitamin mix (NBC)	2.20	2.20	2.20	2.20
Corn oil	5.00	5.00	5.00	5.00
Corn starch	38.80	33.97	34.17	33.94
Sucrose	38.80	33.97	34.17	33.94

was fed its respective diet *ad libitum*. In the second experiment restricted food intake was used by feeding all groups 1 g more of food each day than the mean food consumption of the lowest consuming group for the preceding day. Thus, a method similar to pair-feeding was used based on whole groups of rats rather than individual rats. This method allowed differences in food consumption to be held to less than a gram per day per group.

The rats were sacrificed by ether treatment and their digestive tracts immediately removed and washed free of food and fecal material. The carcasses were weighed and frozen individually in plastic bags. After thawing, the carcasses were cut into small pieces and dried to constant weight in a vacuum oven at 80° to 90° C. The dried carcasses were ground in a Wiley mill and lipids removed with ether in a Soxhlet extractor. The extracted rat tissue was ground with a mortar and pestle and triplicate samples were used for nitrogen determinations by a microKjeldahl method (A.O.A.C., 1955). Student's t-distributions were used in the evaluation of data.

RESULTS

Weight gains and nitrogen retention data of the groups fed six nitrogen levels of supplemented wheat gluten for 2 weeks are summarized in Table II. A major difference in growth occurred between the group fed 1.2% N and the other groups fed higher nitrogen levels, with the 1.2% N level producing a much lower weight gain ($P < 0.01$). This effect was probably due to the 1.2% N level being marginal for growth. The difference in nitrogen levels of the wheat gluten diet had no significant effect on the protein levels of the rat carcasses. There were, however, significant differences due to changes in the dietary nitrogen level when the ratios of g-N retained/g-N consumed were compared. The highest ratio was obtained at the 2.4% N level and was significantly higher than the ratio obtained at the 1.2% N level ($P < 0.05$) and all other nitrogen levels ($P < 0.01$). Ratios obtained from consumption of diets at the 1.2% N and 3.2% N levels were similar. In the range of 3.2 to 8.0% dietary nitrogen, each diet with a lower level of nitrogen produced a higher ratio ($P < 0.01$) than any of the diets with more nitrogen in them. Because of the superior ratio at the 2.4% N level, this level was selected to compare the wheat gluten diet (WGAA) with the casein–lactalbumin diet (CLAA) and the pure amino acid diet (AA) in the second experiment.

The results of the second experiment are summarized in Table III. Significantly better growth occurred in rats fed CLAA as compared to those fed either WGAA ($P < 0.01$) or AA ($P < 0.05$) for 2 weeks. CLAA produced greater

Table II. Effect of the Level of Wheat Gluten on Growth and the Utilization of Dietary Nitrogen by Rats after 2 Weeks

(mean of six rats ± SEM)

% N in Diet	Weight Gain, g	Total Food Consumption, g	Wet Weight Carcass Protein, % (N × 6.25)	g-N Retained/g-N Consumed
1.2	19.2 ± 0.7	139.4 ± 2.7	17.1 ± 0.20	0.378 ± 0.016
2.4	61.7 ± 1.3	167.7 ± 2.4	17.7 ± 0.60	0.440 ± 0.015
3.2	68.6 ± 3.2	164.8 ± 2.5	17.3 ± 0.58	0.366 ± 0.013
4.0	60.1 ± 2.0	152.9 ± 3.0	17.4 ± 0.12	0.286 ± 0.007
4.8	73.5 ± 1.6	166.5 ± 2.2	16.9 ± 0.19	0.246 ± 0.009
8.0	69.3 ± 1.4	142.8 ± 3.0	16.8 ± 0.41	0.153 ± 0.007

Table III. Effect of Amino Acid Source on Growth and the Utilization of Dietary Nitrogen by Rats after 2, 4, 6, and 8 Weeks

(mean of six rats ± SEM)

Time in Weeks	2.4% N Diet (see text)	Weight Gain, g	Total Food Consumption, g	g Gained/g of Food Consumed	Wet Weight Carcass Fat, %	Wet Weight Carcass Protein, % (N × 6.25)	g-N Retained/g-N Consumed
2	WGAA	67.2 ± 0.6	169.2 ± 1.5	0.397 ± 0.005	13.0 ± 0.69	16.1 ± 0.31	0.414 ± 0.012
2	CLAA	71.8 ± 1.0	172.5 ± 2.1	0.416 ± 0.003	12.7 ± 0.94	14.2 ± 0.39	0.360 ± 0.020
2	AA	67.2 ± 1.1	167.6 ± 1.3	0.401 ± 0.007	12.1 ± 0.57	15.2 ± 0.20	0.399 ± 0.011
4	WGAA	129.8 ± 2.9	393.0 ± 4.2	0.330 ± 0.004	13.7 ± 0.86	18.4 ± 0.08	0.428 ± 0.006
4	CLAA	152.4 ± 1.3	411.6 ± 0.3	0.370 ± 0.003	18.1 ± 0.57	14.9 ± 0.44	0.342 ± 0.015
4	AA	131.6 ± 3.4	382.4 ± 7.5	0.344 ± 0.003	16.3 ± 1.10	15.4 ± 0.42	0.356 ± 0.013
6	WGAA	192.7 ± 3.0	642.3 ± 11.0	0.300 ± 0.004	17.4 ± 0.83	18.3 ± 0.20	0.382 ± 0.007
6	CLAA	211.6 ± 1.5	660.6 ± 2.8	0.320 ± 0.002	16.6 ± 0.94	18.0 ± 0.33	0.390 ± 0.010
6	AA	191.5 ± 3.3	627.0 ± 9.6	0.305 ± 0.002	16.2 ± 1.50	18.0 ± 0.38	0.375 ± 0.008
8	WGAA	247.1 ± 6.7	898.4 ± 11.2	0.275 ± 0.005	17.5 ± 1.60	18.0 ± 0.34	0.335 ± 0.012
8	CLAA	252.6 ± 9.0	898.5 ± 11.0	0.281 ± 0.007	17.1 ± 0.93	18.1 ± 0.25	0.339 ± 0.008
8	AA	244.3 ± 7.8	903.8 ± 12.2	0.270 ± 0.008	15.6 ± 0.38	18.7 ± 0.21	0.346 ± 0.011

growth than the other two diets after 4 and 6 weeks ($P < 0.01$), but not after 8 weeks, when growth resulting from the three diets was not significantly different. Food efficiency ratios corroborated the weight gain relationships, except that after 2 weeks the ratios obtained for the rats fed CLAA and AA were not statistically different. In only one instance was the level of carcass fat different due to dietary treatment. After 4 weeks the feeding of CLAA resulted in a higher fat content than the feeding of WGAA ($P < 0.01$). Carcass protein levels were found to be affected by the type of diet used. After 2 weeks the rats fed WGAA had a higher carcass protein level than those fed CLAA ($P < 0.01$) or AA ($P < 0.05$). Also during this time period the consumption of AA resulted in a higher carcass protein level than the consumption of CLAA ($P < 0.05$). After 4 weeks, feeding WGAA still resulted in a higher carcass protein level than feeding both CLAA or AA ($P < 0.01$). When the three diets were fed for periods of 6 and 8 weeks, all the carcass protein levels were similar. Of the three diets studied, only WGAA produced a significantly greater ratio of g-N retained/g-N consumed/time period. After 2 weeks rats fed WGAA had a higher ratio than those fed CLAA ($P < 0.05$). The greatest ratio for the rats fed WGAA was obtained after 4 weeks, and this ratio was greater than those produced from feeding both CLAA and AA ($P < 0.01$). After 6 and 8 weeks the ratios from all three protein diets were not significantly different.

DISCUSSION

By determining carcass nitrogen at six levels of dietary nitrogen, a new level of dietary nitrogen (2.4%) different from those studied before was found that resulted in a greater utilization of amino acids from WGAA. The gluten diet at this nitrogen level compared more favorably than in previous experiments (Radke *et al.*, 1969) with the other diets (CLAA and AA), showing a greater or similar efficiency of nitrogen utilization depending on the length of dietary treatment.

After 2 weeks the wheat gluten diet allowed a greater carcass protein level and apparent NPU than the casein–lactalbumin diet. The CLAA diet allowed greater overall growth, but the carcass fat levels of the rats fed the two diets were similar, indicating utilization of the dietary amino acids only for maintenance and growth, not for calorie storage, and more efficient use of the amino acids from WGAA for protein formation. The wheat gluten and control diets produced similar ratios of g-N retained/g-N consumed; however, there were higher carcass protein levels in the WGAA-fed animals. Thus, after this time period at this dietary nitrogen level, CLAA but not the control FAO diet appeared to be inferior to WGAA in efficiency of nitrogen utilization for protein formation.

After 4 weeks WGAA produced its highest ratio of g-N retained/g-N consumed, greater than the ratios from the other diets, and also a higher carcass protein level than was found from CLAA or AA. Although the carcass protein level was higher from WGAA-fed animals than CLAA-fed animals, the carcass fat level of the rats fed CLAA was higher than that of the rats fed WGAA. These results might indicate that the CLAA-fed rats were exceeding their requirements of amino acids for maintenance and growth, with the excess amino acids becoming deaminated and synthesized into fatty acids for adipose tissue formation. If such were the case, this condition would represent a less desirable use of amino acids from CLAA-protein at this stage of feeding. The wheat gluten diet, on the other hand, appears to express maximal usage of its amino acids for protein formation after 4 weeks. With respect to the pure amino acid diet, WGAA seemed to allow a true superiority for protein formation since both groups of animals were of similar weights and had similar levels of carcass fat.

After 6 weeks the rats fed CLAA were heavier than those fed the other two diets; however, carcass fat and protein levels were similar from all three diets as well as were ratios of g-N retained/g-N consumed. After 8 weeks all parameters were similar from feeding the three diets. Thus, over an extended feeding period at the 2.4% N level, economic aspects rather than the efficiency of utilization would be paramount in selection of the source of dietary amino acids.

Since a difference in response to the three diets was found, the cause must lie in the properties of the intact proteins, since the total nitrogen content, total content of essential amino acids, and pattern of added nonessential amino acids was the same. Digestibilities of the nitrogen portions of the three diets were the same for pigs (Babcock and Markley, 1967); therefore, timing of the release of amino acids from the protein and in their movement through the intestinal wall was likely to have had a greater effect on nitrogen utilization during the initial 6 weeks. After 8 weeks, enzyme activity changes in the intestinal wall or liver could have been responsible for similar utilization of nitrogen from the three diets.

The lower food consumption in some instances of rats fed the pure amino acid diet might have resulted from osmotic effects in the gastrointestinal tract due to the crystalline amino acids as described by Rogers and Harper (1965). This effect also might have been a factor in consumption of WGAA compared to CLAA; for 55.2% of the nitrogen of the wheat gluten diet came from crystalline amino acids, whereas 50.2% of the nitrogen portion of the casein–lactalbumin diet was made up of crystalline amino acids.

This study showed that both dietary nitrogen level and length of feeding period must be considered when evaluating the utilization of amino acids for protein formation from diets such as those described here. Previous experiments had tentatively ascribed WGAA as inferior to CLAA in this respect (Babcock and Markley, 1967; Morse *et al.*, 1969; Radke *et al.*, 1969). The experiments in this study showed that such was not the case if the dietary nitrogen level was increased from 1.6% N to 2.4% N. Instead, the CLAA diet appeared to show a less efficient use of its amino acids for protein formation; however, this function was time dependent. This study only showed comparisons in utilization at the apparent optimal level of utilization for the wheat gluten diet. Since in one instance the amino acids from the casein–lactalbumin diet appeared to be partially utilized for calories, perhaps a lower level of dietary nitrogen would show a more optimal use of the amino acids from CLAA for protein formation. These experiments did not resolve this question. What the results suggest, however, is that a plant protein concentrate such as gluten might be utilized as well for protein formation as an animal protein concentrate such as casein–lactalbumin if fed under the dietary conditions described here.

Although pure amino diets are at present impractical for protein distribution due to the high cost involved (as well as acceptability problems), this study also showed the crystalline amino acid diet to be of no advantage over consumption of the diets containing intact protein.

Gluten should not be overlooked as a protein source for human consumption in the future. It is a cheap abundant by-product that can be important nutritionally. Wheat will

continue to hold its prominence as a cereal crop in the years ahead, and extensive research will continue to be carried out on the physical and chemical characteristics of its major protein gluten. The present high cost of amino acids shouldn't be a drawback in consideration of their future use for protein supplementation. Already the price of lysine reflects the effect that anticipated consumer demand has on lowering the price, and there is no reason to expect that the same situation will not apply to other amino acids if demanded in large enough quantities. This study has investigated the effects from the use of wheat gluten as the sole source of intact protein in the diet. Further studies by this laboratory will investigate the utilization of gluten with a higher dietary ratio of intact protein to crystalline amino acids and also its value in mixtures with other proteins. In whatever proportion it is used in the diet, gluten can make a favorable contribution to protein nutrition and help alleviate the strong demand on animal sources, especially in the years ahead when there will be less animal protein available for worldwide use.

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Received for review April 13, 1970. Accepted March 5, 1971. This study was part of Northeastern Regional Research Project NE-52, Utilization of amino acids from protein, and was supported in part by Regional Research Funds.