

cans of frozen egg yolk given to this laboratory for this study. The assistance in the analytical work performed by Sander M. Goodman of this laboratory is appreciated. The authors are also grateful for the critical reviews of the manuscript made by C. W. Schroeder and James J. Winston.

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Corn Proteins Improved with Amino Acids

PROTEIN EFFICIENCY

Improvement in Whole Yellow Corn with Lysine, Tryptophan, and Threonine

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THAT THE PROTEINS IN CORN are deficient in lysine and tryptophan and that the proteins in wheat are deficient in lysine was found in 1932 by Mitchell and Smuts (2). The recent finding of Pecora and Hundley (3) that the proteins in white polished rice can be improved by the addition of threonine, and of Sure (4) that the proteins in whole wheat and in patent wheat flour can be further improved by threonine in pres-

ence of lysine and tryptophan, stimulated the study of the influence of threonine supplementation to the proteins in whole yellow corn. As a typical United States variety, the Staley Manufacturing Co., Decatur, Ill., supplied representative samples from 10 different cars, 95% of which was hybrid whole yellow corn.

Two typical Guatemala varieties of whole yellow corn, marked TGY and 142-48, were supplied by the Institute

of Nutrition of Central America and Panama. Because in Guatemala 70% of the total proteins are derived from whole yellow corn, which are deficient biologically, it was of interest to determine whether they could be improved by addition of threonine in the presence of lysine and tryptophan.

This study was carried out on the Wistar strain albino rat; in all groups, which contained 12 animals in each

As threonine was found to improve the proteins in polished rice and in milled and whole wheat, its effect on the proteins in whole yellow corn was investigated. Marked improvement in the efficiency of the proteins in two types of Guatemala whole yellow corn was secured by the addition of 0.5% threonine in the rations in the presence of lysine and tryptophan. However, a supplementary effect of threonine to the proteins in U. S. hybrid whole yellow corn was obtained only in the presence of lysine, tryptophan, and methionine. Threonine produced a marked specific effect on economy of food utilization. As corn supplies about 70% of the proteins in Guatemala and the greater proportion of the proteins among African natives and in many other countries of the world, and is used to a large extent in the southern United States in quick breads, enrichment of corn meal with lysine, tryptophan, and threonine would be very desirable when these amino acids reach a stage of economic commercial production.

group, the sexes were equally divided. The animals were about 28 days old when started on the experiments and weighed 50 to 55 grams each. The composition of the rations is given in Table I.

The following components of the vitamin B complex were administered separately from the ration to each animal 6 times weekly and a double allowance was given on Saturdays: 25 γ each of thiamine, riboflavin, pyridoxine, and niacin, 150 γ of calcium pantothenate, 3 mg. of *p*-aminobenzoic acid, 1 mg. of inositol, and 6 mg. of choline chloride. The fat-soluble vitamins A, D, and E were furnished by the cod liver oil and wheat germ oil in the rations.

The protein content ($N \times 6.25$) of U. S. whole yellow corn was 8.94%, and the protein contents of the Guatemala varieties were 8.3% for TGY and 9.1% for 142-48. The animals were weighed once weekly and accurate records of food consumption were kept. The yardstick used for protein efficiency was gain in body weight per gram of protein intake, expressed as protein efficiency ratio (PER).

The amino acids, DL-lysine, DL-

tryptophan, and L-lysine, were generously supplied by the chemical manufacturers. The authors also had a small amount of L-tryptophan on hand; hence, DL-lysine and L-tryptophan were used in some experiments and L-lysine and DL-tryptophan in others.

The results of this investigation are summarized in Tables II to V. The addition of 1% DL-lysine to basal ration 1 resulted in 81% increased growth but in only 19.2% increase in protein efficiency ratio (Table II), as there was a 51.3% increase in food consumption. However, the addition of 0.5% DL-tryptophan in the presence of lysine produced not only 137.7% increase in growth but a 65.8% increase in protein efficiency ratio, because food intake was less than on ration 2 supplemented with lysine. In other words, DL-tryptophan produced a marked increase in economy of food utilization, which is also expressed in the protein efficiency ratio. While addition of 0.5% DL-methionine or 0.5% DL-threonine resulted in negative responses, the incorporation in ration 6 of 0.5% DL-threonine in the presence of methionine, lysine, and tryptophan resulted in over 50% increased growth, but an insignificant increase in protein

efficiency ratio because of greater food consumption. In supplementing the proteins of whole yellow corn with amino acids, the latter contributed additional nitrogen; therefore, in addition to data on protein efficiency ratios, figures are also presented on gains in body weight per gram of nitrogen intake. However, the relative differences between these results and those on protein efficiency ratio are very slight.

In Table III further evidence is presented on the improvement of the proteins in U. S. hybrid whole yellow corn with threonine in the presence of lysine, tryptophan, and methionine, using the technique of controlled food intake. In this series of experiments each animal was given for 15 days 7 grams of food, which was consumed without any waste. The addition of 0.5% DL-threonine to basal ration 7, containing 0.4% L-lysine and 0.5% DL-tryptophan, produced insignificant gains in body weight, and the addition of 0.5% DL-methionine replacing the DL-threonine, on controlled food intake, was accompanied by an inhibiting effect on growth. However, the addition of 0.5% DL-threonine in the presence of methionine, lysine, and tryptophan was followed by about 33%

Table I. Composition of Rations

	Type of Ration													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
U. S. whole yellow corn	86.0	86.0	86.0	86.0	86.0	86.0	86.0	86.0	86.0	86.0
Guatemala whole yellow corn (TGY)	86.9	86.9
Guatemala whole yellow corn (142-48)	79.3	79.3
DL-Lysine	..	1.0	1.0	1.0	1.0	1.0
L-Lysine	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
DL-Tryptophan	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
L-Tryptophan	0.2	0.2	0.2	0.2
DL-Methionine	0.5	..	0.5	0.5	0.5
DL-Threonine	0.5	0.5	..	0.5	..	0.5	..	0.5	..	0.5
Sure's salts No. 1 (5)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vegetable shortening	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Cod liver oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Wheat germ oil	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cerelose	4.0	3.0	2.5	2.0	2.0	1.5	3.1	2.6	2.6	2.1	2.5	2.0	10.1	9.6

Table II. Influence of Lysine, Tryptophan, Methionine, and Threonine on Efficiency of Proteins in U. S. Whole Yellow Corn

7.7% protein in rations. Experimental period 6 weeks. Average results per animal. 6 males and 6 females in each group

Ration	Type of Ration	Gains in Body Weight		Total Food Intake, G.	Protein Intake, G.	Protein Efficiency Ratio ^a	Increase, %	Nitrogen Intake, G.	Gains in Body Weight per G. of Nitrogen Intake	
		Weight, G.	Increase, %						Intake, G.	Increase, %
1	US WYC	24.7	...	267.9	20.6	1.20 ± 0.05 ^b	..	3.2960	7.50	..
2	US WYC +1% DL-lysine	44.7	81.0	405.3	31.2	1.43 ± 0.06	19.2	5.0031	8.88	18.4
3	US WYC +1% DL-lysine +0.5% DL-tryptophan	58.7	137.7	383.5	29.5	1.99 ± 0.08	65.8	4.7242	12.42	65.6
4	US WYC +1% DL-lysine +0.5% DL-tryptophan +0.5% DL-methionine	59.7	141.3	375.8	28.9	2.07 ± 0.07	72.5	4.6283	12.89	71.8
5	US WYC +1% DL-lysine +0.5% DL-tryptophan +0.5% DL-threonine	57.3	132.0	390.1	30.0	1.91 ± 0.06	59.2	4.8054	11.93	59.0
6	US WYC +1% DL-lysine +0.5% DL-tryptophan +0.5% DL-methionine +0.5% DL-threonine	72.9	195.1	439.0	33.8	2.15 ± 0.08	79.2	5.4157	13.34	77.8

^a Expressed as gains in body weight per gram of protein intake.
^b Standard deviation of means.

increased growth and increase in protein efficiency ratio, because of greater economy in food utilization produced by threonine addition. The same animals were then fed *ad libitum* for 30 days rations 9 and 10; because of 15 days' previous caloric restriction on the low food intake of only 7 grams daily, the threonine supplementation resulted in about 33% increase in growth and 22% increase in protein efficiency ratio.

In Table IV is shown the influence of threonine addition to the proteins in U. S. whole yellow corn, using the Mitchell method (7) of nitrogen retention. The addition of 0.5% DL-threonine (ration 10) to ration 9 resulted in an increase of 7.4 in biological value and an increase of 6.0 in net utilization.

The role of threonine in economy of food utilization is further demonstrated in its supplementary value to the proteins of the two types of Guatemala whole yellow corn (Table V). However, contrary to experience with the U. S. hybrid corn, the effect of threonine on increased growth was obtained in the absence of methionine. In this series of experiments the animals were placed on 7.2% proteins rations furnished by the Guatemala types of corn, and for 30 days were given 7 grams daily. On the 142-48 variety there was 27.1% increase in protein efficiency ratio as a result of the threonine addition, and on the TGY variety there was 72.1% increase in protein efficiency ratio. Such results indicate the significant role threonine plays

Table III. Improvement in the Protein Efficiency of United States Hybrid Whole Yellow Corn with Threonine

Controlled feeding, 7 grams fed daily to each animal. Experimental period 15 days. Average results per animal. Protein in ration, 7.7%. 12 animals in each group

Ration	Type of Ration	Gains in Body Weight		Protein Efficiency Ratio ^a	
		G.	%	Increase, %	
7	US WYC +0.4% L-lysine +0.5% DL-tryptophan	17.0	..	2.10	..
8	US WYC +0.4% L-lysine +0.5% DL-tryptophan +0.5% DL-threonine	17.5	2.9	2.16	2.9
9	US WYC +0.4% L-lysine +0.5% DL-tryptophan +0.5% DL-methionine	14.6	..	1.80	..
10	US WYC +0.4% L-lysine +0.5% DL-tryptophan +0.5% DL-methionine +0.5% DL-threonine	19.4	32.8	2.40	33.3
Same Animals on <i>ad libitum</i> Feeding Subsequent 30 Days					
9	US WYC +0.4% L-lysine +0.5% DL-tryptophan +0.5% DL-methionine	40.9	..	1.97	..
10	US WYC +0.4% L-lysine +0.5% DL-tryptophan +0.5% DL-methionine +0.5% DL-threonine	54.3	32.8	2.39	21.7

^a Gains in body weight per gram of protein intake.
Food consumption per animal in ration 9 during this 30-day period was 268.7 grams, or 20.7 grams of protein.
Food consumption per animal in ration 10 during this period was 296.7 grams, or 22.8 grams of protein.

in economy of food utilization, which, of course, also influenced the protein utilization. During the subsequent 30 days the same groups of animals were given the same rations but were fed *ad libitum*. From Table V it is evident that the threonine addition was followed by 54% increase in growth and 31.1% increase in protein efficiency ratio in the 142-48 variety, and by 43.6% increase in growth and 19.0% increase in protein efficiency ratio in the TGY variety.

Discussion

A biological assay of the protein efficiency of three types of whole yellow corn used in this study, during an experimental period of 6 weeks, disclosed the following protein efficiency ratios: U. S. hybrid corn, 1.13; Guatemala corn, TGY, 1.09; and Guatemala 142-48, 1.09. In the absence of amino acid supplementation, the protein efficiency of these three corns was about the same. However, the fact that increased growth was obtained following addition of methionine to the two types of Guatemala corn in the presence of lysine and tryptophan, and that a supplementary effect to threonine addition to the proteins in the U. S. variety was obtained only in the presence of lysine, tryptophan, and methionine, necessitated determination of the methionine content of these three types of corn. M. C. Kik of this department determined this amino acid in the three corns microbiologically and found 0.22% in the U. S. hybrid corn, 0.22% in the Guatemala TGY variety, and 0.23% in the Guatemala 142-48 variety. It would appear then that, as there was practically no difference in the methionine content of the U. S. and Guatemala varieties, the methionine in the U. S. variety is not readily available during digestion; hence, its addition was necessary before a response to threonine could be obtained.

The fact that threonine plays a significant role in economy of food utilization, when added as a supplement to the proteins in whole yellow corn, is in agreement with results recently obtained when this amino acid was used as a supplement to the proteins in patent wheat flour (4).

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Table IV. Influence of Threonine Additions on Biological Value of United States Whole Yellow Corn as Determined by Nitrogen Retention Method

Ad libitum feeding. 12 animals in each group.		Protein in ration 7.7%.	Average results per animal.	
Ration	Type of Ration	Biological ^a Value	True Digestibility ^b	Net Utilization ^c
9	US WYC	84.2	90.5	76.2
	+0.4% L-lysine			
	+0.5% DL-tryptophan			
	+0.5% DL-methionine			
10	US WYC	91.6	89.7	82.2
	+0.4% L-lysine			
	+0.5% DL-tryptophan			
	+0.5% DL-methionine			
	+0.5% DL-threonine			

^a Expressed as per cent of absorbed nitrogen retained in animal body.

^b True coefficient of digestibility obtained by subtracting nitrogen lost in feces from total nitrogen intake and dividing by 100.

^c Obtained by multiplying true coefficient of digestibility by biological value and dividing by 100.

Table V. Improvement in Protein Efficiency of Guatemala Whole Yellow Corn with Threonine

Controlled food intake, 7 grams fed daily to each animal. Experimental period, 30 days. Average results per animal. Protein in rations 7.2%. 12 animals in each group.

Ration	Type of Ration	Gains in Body Weight		Protein Efficiency Ratio ^a	
		G.	%	Increase, %	
11	GWYC, 142-48	31.4	..	2.09	..
	+0.4% L-lysine				
	+0.2% L-tryptophan				
12	GWYC, 142-48	39.9	27.1	2.64	26.3
	+0.4% L-lysine				
	+0.2% L-tryptophan				
	+0.5% DL-threonine				
13	GWYC, TGY	21.2	..	1.40	..
	+0.4% L-lysine				
	+0.2% L-tryptophan				
14	GWYC, TGY	36.5	72.1	2.41	72.1
	+0.4% L-lysine				
	+0.2% L-tryptophan				
	+0.5% DL-threonine				
Same Animals on <i>ad libitum</i> Feeding Subsequent 30 Days					
11	GWYC, 142-48	34.1	..	1.77	..
	+0.4% L-lysine				
	+0.2% L-tryptophan				
12	GWYC, 142-48	52.5	54.0	2.32	31.1
	+0.4% L-lysine				
	+0.2% L-tryptophan				
	+0.5% DL-threonine				
13	GWYC, TGY	62.2	..	2.48	..
	+0.4% L-lysine				
	+0.2% L-tryptophan				
14	GWYC, TGY	89.3	43.6	2.95	19.0
	+0.4% L-lysine				
	+0.2% L-tryptophan				
	+0.5% DL-threonine				

^a Gains in body weight per gram of protein intake.

Food consumption per animal during this 30-day period:

Ration 11. 267.7 grams or 19.3 grams protein.

Ration 12. 313.3 grams or 22.6 grams protein.

Ration 13. 348.3 grams or 25.1 grams protein.

Ration 14. 420.5 grams or 30.3 grams protein.

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