SUPPLEMENTAL VALUE OF LIQUID CYCLONE PROCESSED COTTONSEED FLOUR ON THE PROTEINS OF SOYBEAN PRODUCTS AND CEREALS

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Studies were conducted to determine whether supplementation with cottonseed flour produced by a liquid cyclone process (LCP) would improve the protein quality of soy concentrate (soy 70/HS, 70/LS), soy isolate (soy 90/HS, 90/LS), triticale, wheat, and rye. When compared with the FAO/WHO suggested pattern, all the soybean products and cereals contained more essential amino acids than the requirements for the adult human, whereas the total sulfur amino acids of soy 70/HS and 90/HS and the lysine of triticale, wheat, and rye do not meet the requirements of either the infant or school child. In Experiment One, young rats were fed an otherwise adequate but protein-free diet, or the same diet supplemented with 10% protein (N × 6.25) from casein, LCP cottonseed flour, soy concentrate, soy isolate, triticale, wheat, or rye. Average weight gain of the rats receiving a 10% LCP cottonseed protein diet was significantly greater than that of the rats receiving any other experimental diet. The protein efficiency ratios (PER) of casein and cottonseed were similar but higher than those for all other diets. In Experiment Two, rats were fed diets containing 5% protein from LCP cottonseed flour, and 5% protein from one of the above soy products or cereals. The PER values for the diets containing 5% protein from cottonseed and 5% protein from soy concentrate, soy isolate, triticale, or wheat were significantly greater than those for the comparable diets containing 10% protein from soy products or cereals alone. The significant improvement in the protein quality of soy concentrate, soy isolate, triticale, and wheat suggests that LCP cottonseed flour is a valuable supplement to these and possibly to other grain products.

Protein malnutrition exists in various areas of the world. In many of these areas, there is a high production of cottonseed (1), offering a potential source of high quality protein. It follows, then, that grain products such as soybean, triticale, wheat, and rye, some of which are native to these areas, may be supplemented with cottonseed flour, resulting in a product with a higher protein quality.

Bressani *et al.* (1) and Squibb *et al.* (2) have supplemented corn, sesame flour, and sorghum with cottonseed flour in formulating a vegetable mixture to alleviate protein malnutrition in Central America. Jones and Divine (3) supplemented white wheat flour with cottonseed flour, and found that the addition of as little as 5 parts of cottonseed flour to 95 parts of wheat flour produced mixtures containing 16 to 19% more protein than the wheat flour alone, and a protein combination that was definitely superior in its growth-promoting value in rats to the same quantity of wheat flour.

Previous investigations were conducted with cottonseed flour produced by older processing methods, such as the screw press technique (1). A more recent technique, the liquid cyclone process, has been developed (4). This process yields a flour of superior quality and high protein content, in which the intrinsic nutritive value of cottonseed is maintained while the total gossypol content is markedly decreased. It was the objective of this investigation to determine whether the supplementation with liquid cyclone processed cottonseed flour would improve the protein quality of soybean products and cereals.

MATERIALS AND METHODS

Protein Samples

Nine protein samples used in this study were: liquid cyclone processed (LCP) cottonseed flour, soy concentrate 70/HS (70% crude protein, high solubility), soy concentrate 70/LS (70% crude protein, low solubility), soy isolate 90/HS (90% crude protein, high solubility), soy isolate 90/LS (90% crude protein, low solubility), ground triticale grain meal, white wheat flour, ground rye grain meal, and Animal Nutrition Research Council (ANRC) casein, which served as the reference protein.

Protein and Amino Acid Analyses

The crude protein content (N $\times$ 6.25) of each sample was determined by a macro-Kjeldahl procedure (5). The amino acid composition of each sample was determined by a column chromatographic method (6). A sample containing 7.0 $\pm$ 0.5 mg of protein was placed in a special Pyrex tube, followed by addition of 1.0 ml of 12*N* HCl and 1.0 ml of deionized water. The sample was inserted in a Dry Ice-trichloroethylene cooling bath. After solidification of the sample, it was vacuumed and then hydrolyzed in an air oven at 110° $\pm$ 1°C for 22 hr. The hydrolysate was filtered through a glass filter and evaporated to dryness with a Buchler rotary evaporator. Deionized water was added and the evaporation repeated twice. The flask containing the dried hydrolysate was made to 5.0 ml with a pH 2.2 citrate buffer and then stored at 4°C. Aliquots of 0.2 ml were introduced into each column of a Beckman Model 116 amino acid analyzer in a standard 4-hr run.

Cysteine content was determined by the cysteic acid procedure of Moore (7). A sample containing approximately 0.1 mg of cysteine was mixed with 2 ml of cold performic acid for oxidizing cysteine to cysteic acid. After the sample was allowed to stand overnight, 0.30 ml of 48% hydrobromic acid was added to the reaction tube in an ice bath to prevent overoxidation. The sample was dried under vacuum and prepared for hydrolysis with 2.0 ml of 6*N* HCl according to the procedures described above. For calculating the cysteine content, aspartic acid was used as the internal reference. As tryptophan was destroyed by acid hydrolysis, the tryptophan content of the sample was not determined.

Animal Feeding Experiments

For biological evaluation of protein qualities, 24-day-old male albino rats of the Sprague-Dawley strain weighing from 52 to 68 g were used. In Experiment One, 54 rats were allotted randomly to nine experimental groups of 6 rats each, whereas in Experiment Two, 70 rats were distributed among 7 experimental groups of 10 rats each. The animals were housed individually in raised, wire-bottomed cages. Water and food were offered *ad libitum* during the 28-day experimental period.

At the end of the feeding period, feces of each rat for the last 7 days were placed in a 125-ml Erlenmeyer flask containing 50 ml of 6*N* HCl. The fecal samples were stored at room temperature, and then autoclaved for 4 hr. After being cooled to room temperature, the fecal suspension was forced through a sieve and made to 200 ml with water. Duplicate aliquots of 10 ml were used for nitrogen determination.

In Experiment One, diets containing 10% crude protein (N × 6.25) from cottonseed, soy 70/HS, soy 70/LS, soy 90/HS, soy 90/LS, triticale, wheat, or rye were used, whereas in Experiment Two, the protein in the diets remained constant at 10%, with 5% being furnished by cottonseed and 5% by soy products or cereals. An otherwise adequate but protein-free basal diet and the same basal diet supplemented with 10% ANRC casein protein were used as the control diets in both experiments. The basal diet used in the present study was patterned after that of the AOAC Official Methods (5) which contained the following in %: corn starch, 85; Wesson oil, 8; salt mixture, 5; vitamin mixture, 1; and cellulose, 1. The dietary protein was added to the basal diet at the expense of corn starch.

Statistical Analysis

A one-way analysis of variance (8) was used to test the nutritional status of the rats fed the experimental diets in each growth experiment. When significant differences ($P<0.01$) were found among treatments, Duncan's multiple range test (8) was applied to determine where the differences occurred. Student's "t" test (9) was used to determine the differences, if any, of means of nutritional values between the cottonseed flour supplemented diets and the unsupplemented soy products or cereal diets.

RESULTS AND DISCUSSION

Protein Content and Amino Acid Composition

Casein, LCP cottonseed flour, soy 70/HS, soy 70/LS, soy 90/HS, soy 90/LS, triticale, wheat, and rye contained 97.3, 67.4, 69.2, 65.5, 90.2, 89.6, 16.4, 12.1, and 11.9% protein (N × 6.25), respectively.

The essential amino acid composition of the samples and the FAO/WHO suggested pattern of amino acid requirements of infant, school child, and adult (10) are shown in Table I. All the samples contained adequate quantities of essential amino acids to meet the requirements of the adult. The lysine and leucine contents of triticale, wheat, and rye; leucine content of LCP cottonseed flour; total sulfur amino acids of soy 70/HS and 90/HS; threonine of triticale and wheat; and valine of soy 90/HS, triticale, and wheat, however, were lower than the quantities required for supporting the normal growth of the infant. Cottonseed, soy 90/HS, soy 90/LS, triticale, wheat, and rye were deficient in lysine, while all the soy products were low in total sulfur amino acids, and triticale and wheat were deficient in threonine for meeting the growth requirements of the school child.

The low lysine values found in triticale and wheat are in agreement with the observations reported by Kies and Fox (11) that lysine was the first limiting amino acid of both grains. In a rat feeding experiment, Kihlberg and Ericson (12) demonstrated that lysine was the first and threonine the second limiting amino acid of rye. Grau (13) found that lysine was the first and methionine the second limiting amino acid in the proteins of cottonseed meal for supporting the growth in chicks. Fisher (14) indicated that not only lysine and methionine but also

TABLE I
Essential Amino Acid Content of Samples and FAO/WHO Suggested Patterns of Amino Acid Requirements[a]

Amino Acid	Casein	Cottonseed	Soy 70/HS	Soy 70/LS	Soy 90/HS	Soy 90/LS	Triticale	Wheat	Rye	Suggested Patterns		
										Infant	School child	Adult
Histidine	3.6	3.4	3.1	2.9	2.4	2.8	2.2	1.9	2.4	1.4		
Isoleucine	5.5	3.9	5.8	5.7	5.4	5.5	3.7	3.6	3.8	3.5	3.7	1.8
Leucine	10.7	7.0	9.9	9.7	9.3	9.4	7.6	7.6	7.4	8.0	5.6	2.5
Lysine	10.1	5.5	9.4	8.5	6.9	7.3	3.9	2.2	4.3	5.2	7.5	2.2
Methionine	4.8	2.5	1.5	2.1	1.9	2.0	2.6	2.2	2.3			
Cysteine	0.3	1.6	1.0	1.0	1.0	1.1	1.8	1.6	1.9			
Total sulfur amino acids	5.1	4.1	2.5	3.1	2.7	3.1	4.4	3.8	4.2	2.9	3.4	2.4
Phenylalanine	5.7	6.5	6.4	6.0	5.9	6.0	5.5	3.7	5.5			
Tyrosine	6.7	4.2	5.0	4.4	4.1	4.8	3.4	3.2	2.4			
Total aromatic amino acids	12.4	10.7	11.4	10.4	10.0	10.8	8.9	6.9	7.9	6.3	3.4	2.5
Threonine	4.7	4.4	5.6	5.2	5.0	5.1	4.0	3.6	4.4	4.4	4.4	1.3
Valine	6.2	4.9	5.4	5.0	4.5	4.9	4.3	4.4	4.7	4.7	4.1	1.8

[a]g/100 g protein.

leucine, threonine, and isoleucine were limiting for optimum growth of chicks receiving cottonseed meal as the sole source of dietary protein.

According to Almquist (15), methionine was the first limiting amino acid of soybean. For supporting the normal growth of rats and pigs, threonine was the second and lysine the third limiting amino acid of soybean (16), while in chicks, threonine was also the second limiting amino acid but followed by valine (17). As shown in Table I, all the soy products were deficient in sulfur amino acids for meeting the requirement of the child, and soy 90/HS did not contain a sufficient amount of valine for the infant. The variations in amino acid composition of soy products, as shown in Table I, may be explained in part by the studies of Liener (18) which show that lysine and a number of other amino acids were partially destroyed during the processing of soybean meal.

Animal Feeding Experiments

The growth data of young rats fed an otherwise adequate but protein-free diet, or the same diet supplemented with 10% protein (N × 6.25) from various sources (Experiment One), are shown in Table II. For the 28-day experimental period, the average weight gain ranged from −19 g for the rats receiving the protein-free diet to 122 g for the rats consuming 10% protein from cottonseed. Significant differences ($P<0.01$) in weight gains existed among various dietary groups. Weight gained (13 g) by rats consuming a wheat diet was significantly lower than the weight gained by rats on all other protein diets. Weight gains of the rats on diets in which the protein was supplied by casein, soy 70/LS, rye, or triticale were similar but significantly higher than the weight gains of those consuming the protein from soy 70/HS, soy 90/HS, or soy 90/LS.

Such considerable differences among weight gains were thought to be

TABLE II
Effect of Feeding Young Rats for 28 Days an Otherwise Adequate but Protein-Free Basal Diet, or the Same Basal Diet with 10% Protein from Different Sources

Protein Source	Weight Gain g	Food Intake g	Adjusted PER[a]	Fecal Nitrogen[b] mg	Nitrogen Digestibility[c] %
Protein-free	−19	134		41	
Casein	76	271	2.50	146	97.7
LCP Cottonseed	122	410	2.34	299	96.8
Soy 70/HS	47	285	1.39	177	97.4
Soy 70/LS	57	286	1.67	188	97.2
Soy 90/HS	44	292	1.31	137	98.1
Soy 90/LS	43	306	1.19	134	98.3
Triticale	60	328	1.49	375	94.6
Wheat	13	233	0.48	124	97.7
Rye	64	317	1.96	492	90.3

[a]Adjusted protein efficiency ratio $= \frac{\text{weight gain g}}{\text{protein intake g}} \times \frac{2.50}{2.61}$

[b]Last 7 days only.

[c]$\frac{\text{N intake} - (\text{fecal N} - \text{fecal N of protein-free group})}{\text{N intake}} \times 100$

attributable to differences in food intakes. Food intake for rats fed cottonseed was significantly higher than for all other protein sources. The food intake of the rats fed the rye diet was significantly higher than that of the rats fed the wheat diet, but similar ($P>0.01$) to that of the rats fed the diets containing soy 70/HS, soy 70/LS, soy 90/HS, soy 90/LS, triticale, or casein. Food intake of the rats on diets containing triticale, soy 70/HS, soy 70/LS, soy 90/HS, soy 90/LS, wheat, or casein was very similar.

The protein efficiency ratio (PER) of the animals receiving the 10% casein protein diet for 4 weeks was 2.61. However, it was adjusted to 2.50 (19), and the PER values of all the other dietary groups were adjusted accordingly by multiplying the values obtained experimentally with the factor of 2.50/2.61. The PER values for casein (2.50) and cottonseed (2.34) were not significantly different, but were higher than the values of other dietary groups. PER for the wheat diet (0.48) was the lowest among all the treatments. The PER for the rye diet (1.96) was significantly higher than those for the diets containing soy 70/HS, soy 70/LS, soy 90/HS, soy 90/LS, triticale, and wheat. Soy 70/LS had a higher PER than soy 70/HS, soy 90/HS, soy 90/LS, and wheat. The PER for triticale was similar to that of soy 70/HS, soy 70/LS, and soy 90/HS, but higher than the values for soy 90/LS and wheat. For soy 70/HS, soy 90/HS, and soy 90/LS, the PER values were similar.

The relatively low PER values for the diets containing soy concentrates (70/HS, LS) and isolates (90/HS, LS) were consistent with the results of Uri *et al.* (20), who found that PER values of an extraction mixture, a filtered extract, and isolated soybean proteins were inferior to those of the original soybean oil meal. The authors suggested that the inferiority of the extraction mixture and filtered extract as a food was due, not to a loss of methionine or available lysine, but perhaps to antitryptic activity. De Groot and Slump (21) found that the protein isolated from soybean meal had a much lower net protein utilization value than the original meal. However, they attributed this decrease in nutritive value to the destruction of cystine and the formation of lysinoalanine, which is produced by the interaction of dehydroalanine, a decomposition product of

TABLE III
Effect of Feeding Young Rats for 28 Days an Otherwise Adequate but Protein-Free Diet or the Same Basal Diet Supplemented with 10% Protein from Casein or 5% Protein from Soy Products or Cereals and 5% Protein from LCP Cottonseed Flour

Protein Source	Weight Gain g	Food Intake g	Adjusted PER[a]	Fecal Nitrogen[b] mg	Nitrogen Digestibility[c] %
Protein-free	−19	129		42	
10% Casein	79	287	2.50	167	97.4
5% Soy 70/HS + 5% cottonseed	75	336	2.20	287	95.2
5% Soy 90/HS + 5% cottonseed	62	283	2.00	194	96.1
5% Triticale + 5% cottonseed	72	345	1.98	342	94.6
5% Wheat + 5% cottonseed	38	248	1.49	178	96.4
5% Rye + 5% cottonseed	68	307	2.16	408	92.3

[a,b,c]See footnotes of Table II.

cystine and serine and the ε-amino group of lysine.

As shown in Table II, fecal nitrogen values during a 7-day period ranged from 124 mg for rats consuming 10% protein from wheat to 492 mg for those receiving 10% protein from rye. The nitrogen digestibility values for rats fed diets with 10% protein from casein, soy 70/HS, soy 70/LS, soy 90/HS, soy 90/LS, and wheat were similar but higher than for the cottonseed, triticale, and rye diets. The rats consuming the cottonseed, triticale, and rye diets had the lowest digestibility values and the greatest amount of nitrogen in the feces, which probably related to the high fiber content of whole triticale and rye grains used in the experimental diets.

The growth data for young rats fed an otherwise adequate but protein-free diet or the same diet supplemented with 10% protein from casein or 5% protein from cottonseed and 5% protein from other grains (Experiment Two) are shown in Table III. When 5% cottonseed protein was added to soy 70/HS, triticale, or rye, the average weight gains were very similar to that obtained with a 10% casein protein diet. The weight gains of the animals receiving 5% protein from cottonseed and 5% protein from soy 70/HS, soy 90/HS, or wheat were significantly greater than the gains of animals fed comparable diets without cottonseed flour. Harden and Yang (22) found that the weight gains and net protein utilization values of weanling rats fed LCP or glandless cottonseed bread diets at the 10% protein level were significantly higher than those of the rats which were fed a white wheat flour bread diet.

The adjusted PER values of casein, soy 70/HS plus cottonseed, and rye plus cottonseed flour were not significantly different. The PER values of soy 70/HS, soy 90/HS, triticale, or wheat supplemented with 5% cottonseed protein were significantly higher than those of the nonsupplemented diets (Tables II and III). For triticale and wheat, the significant improvement with cottonseed supplementation may be attributable to the cottonseed protein's higher content of lysine, which was the first limiting amino acid in triticale and wheat for human nutrition (11). The improvement in protein quality of soy 70/HS and soy 90/HS with cottonseed flour may be due to the cottonseed's higher content of methionine which was the first limiting amino acid of soybean (15). The supplementation of rye with cottonseed flour improved the PER, but not significantly so. The rats fed diets containing 5% protein from triticale or rye had markedly higher fecal nitrogen and lower digestibility values than other dietary groups, which is similar to the results obtained in Experiment One (Table II).

Data from the present study indicated that when various protein sources were supplemented with 5% LCP cottonseed flour protein, PER values were significantly increased. LCP cottonseed flour improved the PER values of the diets containing soy concentrate, soy isolate, triticale, wheat, or rye.

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