A STUDY OF THE AMINO ACID PROFILE OF COTTONSEED PROTEIN DIETS FED YOUNG WOMEN

A DISSERTATION

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CHAPTER I

INTRODUCTION

Plant foodstuffs will play a major role in supplying the protein needs of an ever-increasing world population. Possible protein shortages may be a future food issue facing the United States of America, as it is now a pressing problem in certain other countries. Animal protein foods have already been pushed to a higher cost plateau and will not likely decline until economical plant proteins attain a greater degree of importance.

Despite its importance as a staple food in developing countries, little research concerning cottonseed flour has been conducted with human consumption. Researchers in human nutrition at Texas Woman's University (TWU) have been studying the nutritional adequacy and the quality of cottonseed protein for human consumption and in small laborabory animals in hopes that the knowledge obtained will add further developments toward solving the protein problem (30, 53).

The Natural Fibers and Food Protein Committee (NFFPC) of Texas granted funds to the TWU Research Institute for the development of food containing cottonseed protein and evaluation of the nutritional properties of cottonseed. A later grant by the United States Department of Agriculture (USDA) supported a study of the effects of diets (7, 59) containing Liquid Cyclone Process (LCP) cottonseed protein concentrate on growing children. A study with elderly adults (72) consuming glandless cottonseed flour was supported by NFFPC.

Purpose of the Study

Differences in protein quality due to the composition of amino acid are thought to be most easily detected when diets have suboptimal amounts of total protein-nitrogen. Nitrogen balance cannot be achieved, however, unless requirements for both the essential amino acids and total nitrogen are met. The purpose of the study was to evaluate the effect of the amino acid patterns in cottonseed protein on the nitrogen balance of young women when fed liquid formula diets. The following topics were therefore investigated:

Part A. The pattern of amino acids in the diet, the level of essential amino acids in the diet, the level of total nitrogen in the diet, and the effect of these factors on nitrogen balance in human subjects.

Part B. The pattern of amino acids in the diet, the pattern of amino acids in the serum of the subjects, the effects of the diets on blood urea nitrogen, the serum total protein and albumin.

CHAPTER II

REVIEW OF LITERATURE

Influence of Amino Acid Intake on Nitrogen Balance

Protein Quality and Amino Acid Availability

Since protein utilization is related to the amino acids present, the amino acid composition of protein plays an important role in determining the nutritive value of plant protein foodstuffs (24). Plant proteins, in general, are deficient in one or more amino acids. Cereals are mainly deficient in lysine while legumes and leaf proteins are deficient in methionine (24, 73). The primary deficiency of an amino acid, namely lysine or methionine, in many instances is further intensified by a secondary deficiency of other amino acids, threonine and/or tryptophan for example. The most important oilseeds are soybean, peanut, cottonseed, coconut, sesame seed, and sunflower seed. Soybean proteins are rich in lysine and threonine, but deficient in methionine. Cottonseed and coconut proteins are deficient in lysine and methionine. Peanut proteins are deficient in lysine, methionine, and threonine. On the other hand, sesame seed and sunflower seed proteins are rich in sulphur amino acids and tryptophan, but deficient in lysine (73).

Although supplementation of amino acid deficiency in plant proteins generally results in an increased protein utilization, there still seem to exist some differences in protein quality of various plant sources. This is particularly true with cereals and oilseeds where the ratio of total essential amino acids to the total amino acids or nitrogen is low (27). Thus, this ratio recognizes the importance of adequate amounts of all essential amino acids in protein synthesis. Various investigators, therefore, have studied the following aspects of plant proteins: factors affecting their nutritive value, mutual supplementation, and amino acid supplementation (73).

The availability of amino acid depends upon the digestibility coefficient of a protein, and the rate of release of amino acids during digestion. There is evidence that some of the essential amino acids present in proteins may not be fully released after digestion, and the rate of release of different amino acids varies from protein to protein during digestion (47). For instance, ten amino acids were all highly available from peanut flour and wheat flour (92-100 per cent), whereas in cottonseed meal the availability ranged from 64.5 to 93.4 per cent. The utilization of lysine from nineteen food proteins ranged widely from 49 per cent to 98 per cent, and of methionine from 48 per cent to 83 per cent (18, 21, 47). Raw soybean meal gave the lowest values for the availability of both lysine and methionine.

Amino Acids Requirement in Dietary Intake

The results of numerous animal studies indicate that amino acids from certain foods may be utilized less efficiently than purified amino acids (21, 27). Since the ultimate goal of amino acid requirement studies is to make possible the translation of these requirements into quantitative terms relative to particular foods, the desirability of determining availability to man of individual amino acids from specific foods is apparent.

The extensive investigation by Rose and co-workers (63 - 66) identified the eight amino acids essential for nitrogen balance in human adult male subjects and were the first studies that attempted to measure dietary needs for specific amino acids. About the same time Linkswiler, *et al*. (42 - 45) reported the determination of availability of individual amino acids from specific foods. The replacement of a portion of the twelve amino acids in purified diets by those in wheat or in corn resulted in a slight improvement in nitrogen balance in young women subjects.

Hundley and co-workers (29) reported evidence that the amino acid balance in human diets is indicated as an important factor. In the study with four men, there was a highly significant decrease in nitrogen retention in one subject when the diet, containing 350 grams of rice (total nitrogen intake was 5.07g/day) and 3000 Kcal, was supplemented with lysine and threonine. Similarly, another subject responded with a highly significant shift towards negative balance when his diet was supplemented with high levels of the essential amino acids. On the other hand, Truswell and Brock (78) observed a positive nitrogen balance with seven men eating a corn diet when lysine, tryptophan, and isoleucine were added to the diet.

Under the conditions of both high and low total nitrogen intake, lysine has been shown to be the first limiting amino acid in ordinary corn for apparent nitrogen retention in human adults (34, 39, 78). Results of these studies and others (11, 33, 77) suggested that minimum requirements for human adults for both protein and lysine as provided by corn can be substantially reduced by additions of nonspecific nitrogen, for example, nitrogen from any metabolically usable, nontoxic source.

Effect of Dietary Amino Acid Content on Serum Amino Acid Levels

For many years, published information has indicated the concentration of free amino acids in portal blood increases after a protein meal. Denton and Elvehjem (8) measured free plasma amino acid (PAA) levels in portal and in systemic blood at intervals after feeding dogs with beef, casein, or zein. Concentration of most amino acids and of most essential amino acids decreased after a meal of zein. When a nonprotein diet containing sucrose was given, PAA levels fell markedly. After a meal containing a low or moderate amount of protein, systemic PAA changes may be insignificant.

Guggenheim, et al. (20) demonstrated that the relation between PAA levels and the availability of the limiting amino acids in foods in portal blood was found to depend not only upon the amino acid composition of food protein but also on the digestive release of amino acids after a protein meal. The type of carbohydrate fed with the protein markedly affected PAA levels. Peak plasma concentrations of lysine were much lower when glucose rather than starch or lactose was given simultaneously with soya protein.

Consequently, McLaughlan and associates (48) reported that the PAA levels and the amount of the amino acids in the dietary protein exhibited a rough correlation. In the study with adult human subjects, several test foods (fish, egg, fish plus butter, protein cereal, protein cereal plus milk) were employed. The increase of each of the PAA lysine, methionine, threonine, and tryptophan measured was approximately proportional to their concentrations in the test diets.

Fish, which is high in lysine, produced elevated plasma lysine levels, whereas egg, which contains a large amount of tryptophan, gave high plasma tryptophan levels. The protein cereal had a low lysine content, which was reflected by very low PAA ratio for lysine.

Weller (79) also found a low concentration of plasma valine in adult subjects fed a nonprotein diet for only one day and an only slightly lower level after two weeks. Inter- and intra-subject variation in PAA levels was wide, but the authors concluded that fasting PAA patterns were more characteristic of the diet than of short term nutritional status. Although PAA methods are not fully satisfactory in diagnosis of protein malnutrition, such studies appear to be a valuable adjunct to such other methods as the determination of plasma proteins.

Several investigators (14, 46, 50) have demonstrated that the measurement of PAA concentration can provide a guide to identifying the amino acid that is limiting. The studies cited indicated clearly that the plasma concentration of the limiting amino acid in two adults fell to unusually low levels when the dietary protein (test meal: containing wheat gluten, sugar, and corn oil slurried in orange juice) was grossly deficient in one amino acid. From this report, lysine was indicated to be limiting in wheat gluten.

CHAPTER III

PROCEDURE

The present study investigated the effect of cottonseed protein in comparison to a non-cottonseed protein diet, RI-5, on young college women. The defatted glanded cottonseed flour, Liquid Cyclone Process (LCP), used in this study was produced by the Southern Marketing and Nutrition Research Division, United States Department of Agriculture in New Orleans, Louisiana. The reference protein, RI-5, was obtained from Ross Laboratories (Columbus, Ohio). RI-5 was developed to be a complete protein composition similar to egg white. The chemical analysis of LCP and RI-5 is shown in Table 1. The relative abundance of amino acids in LCP and RI-5 is also shown in Table 2.

Subjects

Twelve young college women aged 19 to 24 years participated in the 35-day experiment. The carefully selected subjects were of normal weight and in good health as determined by medical history and examination. The subjects continued their normal daily activities and were housed together in one section of a dormitory on the campus at TWU throughout the experimental period. Pertinent characteristic information concerning the subjects is given in Table 3.

Table 1

CHEMICAL ANALYSIS OF LCP AND RI-5

Approximate chemical analysis on LCP[a]		Approximate chemical analysis on RI-5[b]	
	per cent by weight		per cent by weight
moisture	2.80	moisture	6.920
lipid	1.24	lipid	0.780
free gossypol	0.02	carbohydrate	39.200
total gossypol	0.06	protein	50.200
nitrogen	10.61	Ca	0.580
protein	68.93	P	0.580
Lysine (gm/16 gm N)	3.96	Na	0.008
fiber	2.30	K	1.250
ash	8.86	Cl	0.470
N. solubility	99.62	Mg	0.012

[a] Chemical analysis from the Southern Utilization Research Branch, USDA in New Orleans.

[b] Chemical analysis from Ross Laboratories, Columbus, Ohio.

Table 2

RELATIVE ABUNDANCE OF AMINO ACIDS IN PROTEIN PRODUCTS

Amino Acid	LCP (g/ 16 g N)		RI-5		Egg white protein[d/]
	TWU[a/]	A&M[b/]	TWU[a/]	Ross[c/]	
essential amino acid					
lysine	3.78	3.60	6.06	7.50	4.10
threonine	3.75[e/]	3.10	3.80	3.70	3.10
valine	5.70[f/]	4.40	6.29	6.00	5.40
methionine	1.48	1.50	2.76	2.30	2.70
isoleucine	4.20[f/]	3.00	4.96	4.90	4.50
leucine	6.40	5.70	8.53	8.40	6.20
phenylalanine	5.89	5.70	4.30	4.40	4.40
tryptophan	1.09[g/]	1.40	1.00	1.00	1.00
non-essential amino acid					
histidine	2.40	2.70	2.11	--	--
arginine	10.35	12.70	2.52	--	--
aspartic acid	9.91	9.10	6.51	--	--
serine	5.40[e/]	4.20	4.81	--	--
glutamic acid	23.34	21.10	19.35	--	--
proline	3.83	3.70	8.91	--	--
glycine	4.62	4.00	1.69	--	--
alanine	4.19	3.70	2.98	--	--
tyrosine	3.55	3.10	4.48	4.40	3.00
eystine + cysteine	1.68	--	0.52	0.43	1.70

[a/]Analysis of LCP and RI-5 from Texas Woman's University.

[b/]Values from Texas A & M University.

[c/]From Advances in Metabolic Disorders (1). Values were converted to tryptophas as 1.0.

[d/]*Ibid*.

[e/]Values extrapolation to zero hydrolysis times.

[f/]Values after 72 hours of hydrolysis.

[g/]Values after alkaline hydrolysis.

Table 3

CHARACTERISTICS OF YOUNG WOMEN SUBJECTS

Subject case number	Age Yrs	Weight Kg	Height Cm	Surface area m^2	Calculated caloric intake Kcal/day
2977	20	55.9	156.9	1.57	2,000
2978	22	54.5	152.5	1.52	1,900
2979	20	57.7	165.0	1.68	2,000
2980	20	52.2	147.5	1.43	1,900
2981	21	54.5	152.5	1.52	1,900
2982	22	62.0	160.6	1.67	2,000
2983	20	86.8	170.0	2.03	2,000
2984	22	59.1	155.6	1.59	2,100
2985	20	56.4	153.1	1.54	1,900
2986	20	66.1	158.1	1.68	2,100
2987	24	66.1	156.3	1.68	2,250
2988	19	56.8	150.5	1.52	1,900

Diets

Liquid formula diets were prepared using four different levels of cottonseed flour, 0, 30, 60, and 100 per cent, with the remainder of the protein being supplied by RI-5. The composition of the diets is shown in Table 4.

Design of Experiment

The 35-day study period was divided as follows. Initially seven days were allowed for equilibration to the dietary treatments. During the equilibration period, all subjects consumed diet A, 100 per cent RI-5 reference protein formula diet. Following equilibration, the four experimental diets were administered for seven days each in a randomized pattern with the subjects divided into four groups, each group receiving a different diet (Table 5). Total dietary nitrogen was constant throughout the study. The first three days of each period were used to equilibrate the subjects. The nitrogen balance was determined during the last four days. The liquid formula diet was given under supervision as four equal meals daily at approximately 8:00 A.M., noon, 3:00 P.M., and 7:00 P.M. Caloric intake was adjusted to allow subjects to maintain approximately the same weight throughout the study.

COMPOSITION OF EXPERIMENTAL DIET

Ingredient	Experimental Dietary a/			
	Diet A	Diet B	Diet C	Diet D
	-- 100 % RI-5* (g/day)	30% LCP 70% RI-5* (g/day)	60% LCP 40% RI-5* (g/day)	100% LCP -- (g/day)
LCP	--	31.4± 2.5	72.4± 4.5	104.8± 5.5
RI-5	129.5± 6.5	90.7± 1.0	54.8± 5.5	--
Corn oil	78.8± 4.0	76.5± 5.3	77.5± 4.5	78.5± 4.5
Dextrose	174.5±10.5	180.0±15.5	186.0±16.3	195.5±16.3
NaCl	2.0±	2.0±	2.0±	2.0±
Water	820	820	820	820

Glycerolomonleate (1.0 gm each) used as an emulsifying agent

Addition to liquid formula diet:

Apples, celery	
Lemon, salt, low calorie soft drink	Variable
methylcellulose wafers	Allowed up to 36 Cal.
Vitamin supplements b/	
Mineral Supplements c/	

* Expressed as percentage of total protein provided.
a/ Ranges according to body weight of individual subjects.
b/ Unicaps-M by Upjohn. Each pill contains: vitamin A 5,000 USP units, vitamin D 400 USP units, riboflavin 2.5 mg, thiamine mononitrate 2.5 mg, ascorbic acid 50 mg, niacinamide 20 mg, vitamin B_6 0.5 mg, B_{12} 2g, calcium pantothenate 5 mg, vitamin E 10 units, folic acid 1 mg every other day.
c/ Total mineral supplements were in milligrams: Iron, 10, iodine 0.15, copper 1.0, manganese 1.0, magnesium 6, potassium 5.0, calcium 800.

Table 5

DIET CONSUMED BY SUBJECTS DURING EACH WEEK OF THE STUDY

Subjects (Case number)	Experimental period (weeks of study)				
	1	2	3	4	5
2977	A	B	C	A	D
2978	A	D	A	B	C
2979	A	C	B	A	D
2980	A	C	D	A	B
2981	A	B	C	D	A
2982	A	B	D	C	A
2983	A	D	A	B	C
2984	A	A	D	C	B
2985	A	A	C	D	B
2986	A	C	B	D	A
2987	A	D	A	B	C
2988	A	A	B	C	D

Collection and Analysis of Samples

Food samples: Daily formula diets were made and an aliquot of each composite was frozen for analysis.

Blood samples: Immediately prior to the onset of the study and at the end of each seven-day experimental period, overnight fasting blood samples were collected from the cubital vein through venipuncture by a trained medical technician. Serum samples were stored in a freezer and maintained at -10^{o}C until analyzed.

Urine samples: Complete 24-hour urine collections were made throughout the experiment and an aliquot was taken from the individual 24-hour collections and then frozen.

Fecal samples: Brilliant blue, FCF, capsules were given to the subjects as fecal markers to indicate the beginning of the four-day balance period and carmine markers on the last day of each seven-day period. The fecal samples were frozen after delivery to the laboratory. Fecal samples were pooled for the four-day period, blended in a blender with deionized water, and aliquots were weighed for nitrogen analysis.

No allowances were made for menstrual nitrogen losses although total nitrogen loss varies between 300 and 800 mg per period (58). This results in slightly more than 100 mg nitrogen for an average day of menstruation. Integumentary nitrogen loss of men and women was computed by Sirbu (71).

Allowances for hair and nail growth was 25 mg nitrogen per day and loss from insensible perspiration was taken to be 100 mg nitrogen with about 7 g nitrogen per day intake. Thus the sum of calculated experimental errors in balance technique was approximately 125-150 mg nitrogen per day.

Analysis of Samples

Dietary nitrogen, urinary nitrogen, and urinary creatinine excretion were determinated daily, and the mean daily fecal nitrogen output was determined from pooled four-day samples. Urinary nitrogen was determined by the conventional micro-Kjeldahl technique with slight modification as suggested by Henry (28), replacing mercuric oxide with cupric sulfate as the digestion catalyst. Dietary and fecal nitrogen were determined by the macro-Kjeldahl procedure. Determination of creatinine in the urine was done by the Biggs and Cooper (3) modification of the Folin-Wu procedure.

The amino acids in LCP, RI-5, and each of the formula diets were analyzed by ion exchange chromatography on a Beckman 121 M microcolumn automatic amino acid analyzer (35). Hydrolysis of samples was performed with 6 N HCl at 110^{o}C for 24 hours in a forced-draft oven to obtain hydrolysates suitable for analysis of all amino acids except cystine plus cysteine and tryptophan. After hydrolysis, the HCl was removed from the hydrolysate mixture by rotary evaporation.

To correct for the destruction of serine, threonine, and the branched-chain amino acids, three hydrolysis times (24, 48, and 72 hours) were used and extrapolated to zero and infinite time according to a procedure by Tkachuk (76).

For the measurement of cystine plus cysteine as cysteic acid and of methionine as the sulfone, a sample was oxidized with performic acid by the modified procedure of Moore (54). For tryptophan, samples were subjected to alkaline ($Ba(OH)_2 \cdot 8 H_2O$) hydrolysis and measured colorimetrically using p-dimethylamino-benzaldehyde according to the method described by Miller (52).

For the determination of free amino acids in serum, the samples were deproteinated by the use of a 10 per cent sulfosalicylic acid solution (69). The samples were filtered through a 0.45 milli micron pore size filter and an aliquot was applied to the ion exchange column. The amino acids were separated by a dual column, sodium citrate buffer procedure developed by the Beckman Company.

Total serum protein, albumin, and globulin were measured colorimetrically by the method of Saifer and Zymaris (68) with a modification of the biuret reagent as outlined by Gornal (16). Sodium sulfite and ether-span were used to separate the protein fractions.

Blood urea nitrogen (BUN) was determined by the colorimetric method developed by the Sigma Chemical Company (70).

Hemoglobin, hematocrit, cholesterol, and cell body mass were measured by routine procedures (17) at the beginning and the end of the study. Because these quantities did not change during the experimental period, the data have not been indicated.

CHAPTER IV

RESULTS

Amino Acid Composition of the Diets

The quantities of essential and non-essential amino acids in the experimental diets are presented in Table 6. The major differences in essential amino acid content between the diets were in the content of lysine, methionine, leucine, and valine. Since the RI-5 protein contained more of each of these amino acids than did the cottonseed protein, diet A contained more of each than diet D. Diets B and C were intermediate between the others. Among the non-essential amino acids, arginine, proline, and glycine were the only ones where major differences were observed. Diet D (the cottonseed diet) contained more arginine and glycine while the RI-5 diet (A) contained the most proline.

Increases in content of cottonseed protein in the diet decreased the total content of essential amino acids while the content of non-essential amino acids increased only very slightly. The ratio of essential to non-essential amino acids was lowest in diet D and the highest in diet A with diets B and C being intermediate. The total essential amino acids in the daily intake of diets A, B, C, and D constitute approximately 40 per cent, 39 per cent, 37 per cent, and 34

Table 6

AMINO ACID COMPOSITION OF FORMULA DIETS

Amino Acid	Experimental diets (g/day)			
	A	B	C	D
Essential amino acid (EAA)				
lysine	3.48	3.09	2.96	2.34
threonine	3.03	2.61	2.50	2.38
valine	4.91	4.90	4.23	3.61
methionine	1.57	1.10	1.02	0.82
isoleucine	3.86	3.76	3.68	3.02
leucine	5.25	5.07	3.98	3.21
phenylalanine	2.63	2.63	2.41	2.92
tryptophan	0.72	0.72	0.70	0.69
Total	24.45	23.88	21.48	18.99
Mean	3.18	2.98	2.69	2.37
Non-essential amino acid (NEAA)				
histidine	1.21	1.24	1.33	1.43
arginine	1.95	3.11	4.12	5.53
aspartic acid	3.99	4.28	4.65	4.91
serine	3.78	3.70	3.42	3.42
glutamic acid	12.22	11.72	11.32	11.24
proline	5.37	4.39	3.76	2.42
glycine	1.06	1.71	2.13	2.24
alanine	1.91	1.93	1.99	2.16
cystine+cysteine	2.49	2.30	2.11	2.04
tyrosine	2.48	2.30	2.26	2.24
Total	36.46	36.68	36.92	37.63
Mean	3.65	3.67	3.69	3.76
Ratio EAA to NEAA	0.87	0.81	0.73	0.63
EAA/TN (%)	40.1	39.4	36.8	33.5

Each is the mean value of three separate hydrolysate preparation
TN:total nitrogen

per cent of total nitrogen respectively. The proportion of essential to non-essential amino acids in cottonseed protein is lower or slightly imbalanced when compared to an animal reference protein.

Nitrogen Balance

The mean nitrogen balances of subjects receiving the four different diets during the seven-day experimental periods are shown in Table 7. Mean daily nitrogen balances resulting from the four diets (A, B, C, and D) furnishing approximately 10 grams of nitrogen were -0.05, +0.76, +0.35, and +1.42 grams of nitrogen respectively.

Data of randomized subjects, one way analyses of variance with repeated measures, indicated mean differences at smaller than the 5 per cent level of significance. Duncan's multiple range test indicated that the only significant difference was between diets A and D ($P<0.05$).

A mean nitrogen intake of 10.16 g per day maintained positive balance in most of the subjects and in most cases considering an allowance to cover integumental losses (71). Although diet A resulted in a slightly negative balance with a loss of 0.05 g nitrogen, it would still be considered within the equilibrium zone by definition of retention of 0 ± 5 per cent of nitrogen intake (29, 31, 61, 74). All diets containing LCP produced slightly positive nitrogen balances

Table 7

NITROGEN BALANCE[b] OF YOUNG WOMEN ON DIFFERENT LEVELS OF COTTONSEED FLOUR DIETS

Experimental diet	Experiment days	Nitrogen intake[a] (g/day)		Urinary excretion[c] (g/day)		Fecal excretion (g/day)		Nitrogen balance (g/day)		Creatinine excretion (g/day)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A	7	10.13	(0.95)	9.59	(2.03)	0.59	(0.25)	-0.05	(2.18)	0.79	(0.12)
B	7	9.95	(0.73)	8.58	(1.91)	0.61	(0.18)	+0.76	(1.62)	0.72	(0.20)
C	7	10.41	(0.57)	9.38	(2.48)	0.68	(0.21)	+0.35	(2.46)	0.75	(0.18)
D	7	10.14	(0.70)	7.98	(1.35)	0.74	(0.25)	+1.42	(1.88)	0.77	(0.16)

a/ In grams per day based on nitrogen intakes of 9.95-10.41 (13% of the total calories as protein.

b/ Values (g N/day) for 12 subjects based on final 4 days of experimental periods.

c/ Total integumental loss such as skin, hair, nail, sweat was counted as 0.150 g/day and was computed in urinary excretion.

with the 100 per cent LCP diet (D) giving the most positive results. Thus cottonseed protein appears at least as effective as RI-5 protein.

Since amino acids in the diet were supplied in the form of the bound protein, it was assumed that the nitrogen was not totally absorbed. Fecal nitrogen could be influenced by variations in endogenous factors and diet. The differences in fecal nitrogen for the dietary levels were not an important factor influencing the ability to maintain nitrogen balance in this study.

It has been observed that creatinine excretion for adults is related to the dietary nitrogen intake (12, 34). There were no significant differences in the urinary creatinine excretion with any diet. Overall creatinine excretion was approximately one-half of the values reported when meat diets are fed (13, 25).

Serum Amino Acids

The comparison of serum free amino acid concentration of subjects receiving the four different levels of cottonseed flour during the seven day experimental period is shown in Table 8. With the exception of lysine all of the essential amino acids in the serum were at approximately the same concentration for all diets. Diet A gave a considerably higher concentration of lysine than other diets. The branched-chain

Table 8

SERUM FREE AMINO ACID CONCENTRATION OF SUBJECTS ON LCP DIETS

Amino Acid	Experimental diet (micro moles/100 ml serum)			
	A	B	C	D
Essential amino Acid (EEA)				
lysine	18.0	12.1	12.4	11.7
threonine	14.5	13.7	12.2	13.7
valine	18.5	14.3	18.3	16.6
methionine	2.3	2.4	2.6	2.8
isoleucine	5.4	4.2	5.9	5.4
leucine	10.3	8.3	11.0	9.6
phenylalanine	4.1	3.3	3.6	4.4
Total	73.1	58.3	65.9	54.2
Mean	10.4	8.3	9.4	7.7
Non-essential amino acid (NEAA)				
histidine	8.6	7.7	7.5	8.4
arginine	9.7	8.8	9.7	13.4
aspartic acid	1.4	0.9	0.9	1.3
asparagine plus glutamine	55.5	45.1	62.2	66.7
serine	11.7	10.0	9.7	14.7
proline	20.1	15.2	15.0	10.9
glutamic acid	28.3	16.9	16.1	21.5
alanine	27.9	24.1	31.3	30.3
tyrosine	4.4	4.6	4.5	4.9
ornithine	6.8	5.1	6.6	7.3
glycine	21.3	18.4	18.1	29.1
Total	195.7	156.8	163.5	208.5
Mean	17.8	14.2	14.8	18.9
Ratio EAA to NEAA	0.6	0.6	0.6	0.4
EAA to TN (%)	27.2	27.1	28.7	20.6

Each value is the mean of the serum concentrations from four subjects.

amino acids leucine and valine were found to have substantially higher levels in serum than isoleucine in all diets.

Diet D produced the lowest total essential amino acid level and the highest total non-essential amino acid. The ratio of essential to non-essential amino acids was lowest in the serum of subjects fed the 100 per cent LCP diet while all of the other diets produced the same ratio. Overall the proportion of essential and non-essential amino acids in the serum of subjects fed a diet in which the total dietary protein was cottonseed flour protein was slightly imbalanced when compared to a mixed diet with combination of LCP and RI-5 proteins.

Results of the blood analyses indicated no measurable changes in most blood constituents which could be related to different levels of cottonseed protein in the diets. As shown in Table 9, the concentrations of serum total protein, albumin, and globulin were not different from dietary treatments; and the mean for each value was within the normal range suggested for healthy young adult women. It is, therefore, suggested that the protein values obtained were not influenced by the different levels of cottonseed diet treatments.

Of the other measurements made during this experiment, the only noticeable changes were the slightly elevated

Table 9

COMPARISON OF BLOOD ANALYSES

Experimental diet	Serum			Blood urea nitrogen mg/100 ml
	Protein	Albumin g/100 ml	Globulin	
A	7.18	4.61	2.72	21.2
B	7.42	4.71	2.70	22.0
C	7.34	4.62	2.72	22.0
D	7.48	4.63	2.86	23.7

Each is the mean value of serum concentration from four subjects.

serum-urea nitrogen on diet D while the diets B and C were at an intermediate level. These levels were within or slightly higher than the normal limits for the young adults.

CHAPTER V

DISCUSSION

Since protein utilization is related to the amino acids present, the amino acid composition of protein plays an important role in determining the nutritive value of plant protein food stuffs. In determining the adequacy of the amino acid composition of LCP cottonseed protein to maintain nitrogen equilibrium in adult women, it was observed that diets in which all or part of the protein was supplied by cottonseed flour maintained equilibrium, or slightly positive nitrogen balance, when the subjects consumed approximately 10 g nitrogen daily.

The effectiveness of the diets containing LCP can not be explained in terms of the concentration of a single amino acid. Diet D which contained 100 per cent LCP as its protein source contained the lowest level of lysine and methionine, but it was as effective as diets A, B, and C which supplied 2.96 to 3.48 g lysine and 1.02 to 1.57 g methionine. The cystine plus cysteine value in the 100 per cent LCP diet was almost the same as other diets. The 100 per cent LCP diet was as useful in meeting protein needs of these young women as the two diets in which RI-5 provided either 70 per cent or 40 per cent of the nitrogen or the RI-5 diet alone.

All diets that permitted a mean positive nitrogen balance contained at least three times the minimal requirements of crystalline essential amino acids for women (39) except for methionine (Figure 1). Total sulfur-containing amino acids, however, were considerably higher than the amount of methionine required in the absence of cystine. This comparison suggests that sulfur containing amino acids were disproportionately low in the 100 per cent LCP diet.

The interesting observation from the values in Table 7 is that the daily intake of essential amino acids relative to the total nitrogen decreases as the proportion of cottonseed flour increases. According to these estimates the total essential amino acids in diets A, B, C, and D constitute approximately 40 per cent, 39 per cent, 37 per cent, and 34 per cent of the total nitrogen. The first value is close to that found in high quality proteins; the value of 34 is below that of any commonly available meat protein, but is typical for plant protein. Although the total essential amino acids supplied by diet containing only cottonseed protein is slightly lower than the other diets, this amount is twice as high as the reported adult requirements (60).

Improvement in nitrogen retention was indicative of improvement in protein adequacy according to the interpretation of nitrogen balance in the classical studies on total protein and specific amino acid requirement of human diet (66). As

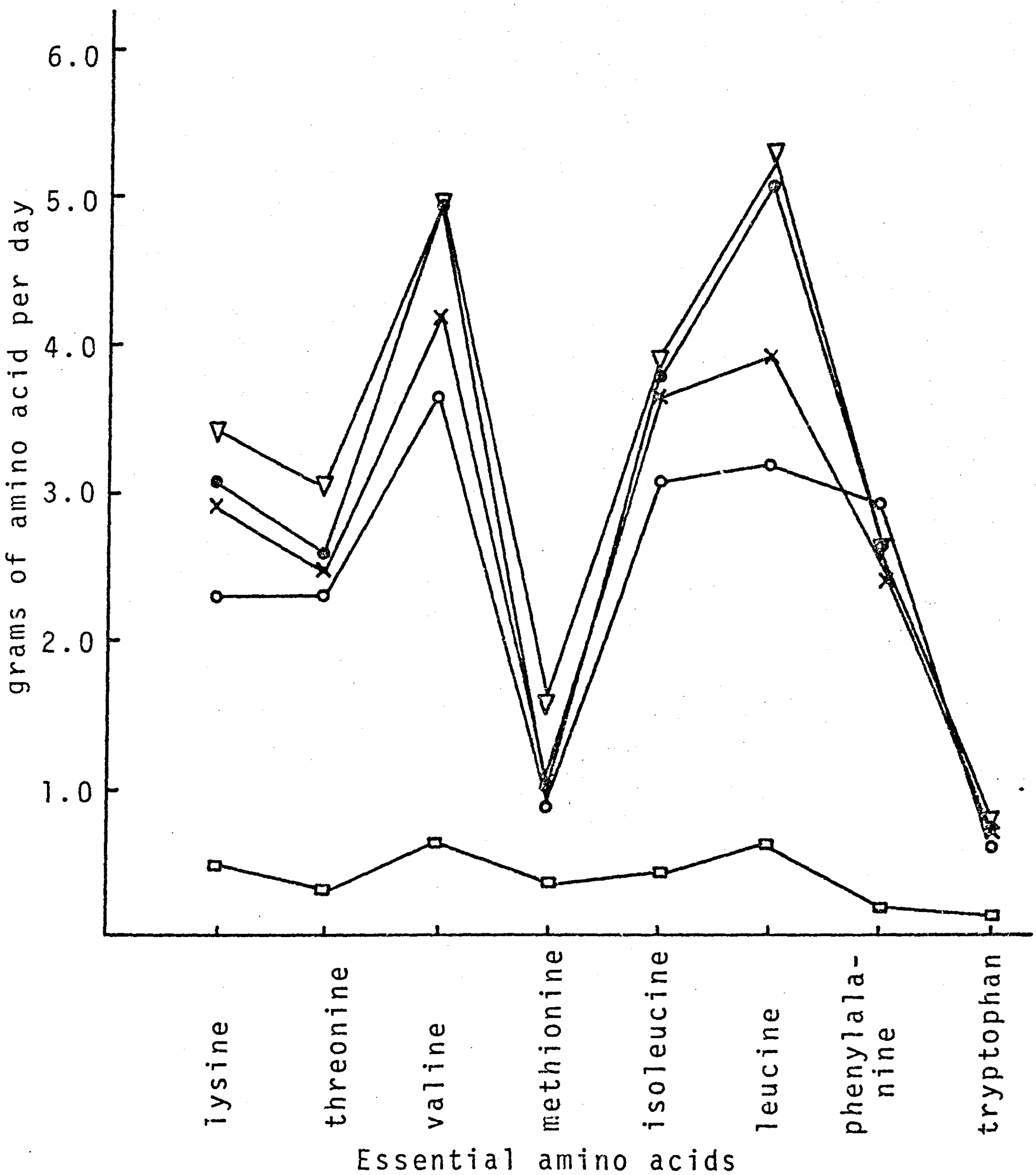

Figure 1. Relationship between minimum daily requirement of essential amino acids for young women and amounts of amino acids supplied by study diets.

□ requirement, △ diet A, • diet B,

x diet C, ○ diet D.

total dietary nitrogen is increased, nitrogen concentration of urine and feces also tends to increase; thus, collection errors might tend to favor a progressively greater apparent nitrogen retention under the circumstances. In the present study, changes and the low level of nitrogen retention in spite of a higher level of nitrogen intake might be the result of cumulative errors rather than of true changes in nitrogen retention.

It appears likely that the variability in the protein quality among some of the subjects may be due to the variation in the utilization of amino acids (4). Individual factors could reduce the rate of protein hydrolysis, which in turn affects the protein digestibility as well as an amino acid availability. Interactions between tryptophan and isoleucine in human diets have been reported (5, 56) even though these amino acids are not related metabolically.

Ganapathy, et al. (15) reported that excessive arginine in relation to lysine is a factor contributing to the poor utilization of millet protein. In this connection, it should be pointed out that the two diets containing the most cottonseed protein contained a considerably higher ratio of arginine compared to lysine than the diet containing only RI-5.

The factor which is most likely to affect the amino acid availability is the protein digestibility. It is well known that trypsin exhibits a strict specificity for arginyl and lysyl peptide bonds; therefore, the total number of peptide bonds split in the protein molecule should equal the sum of arginine and lysine residues (51). As can be seen in Table 10 the sum of arginine and lysine content in diets C and D are almost 1.5 times as much as that of 0 per cent LCP diet. Moreover, it was found that lysylprolyl and arginylprolyl linkages are completely resistent to trypsin (2). It is quite conceivable that the higher proline couples with the low arginine, and lysine content of many plant proteins may favor the formation of lysylprolyl and arginylprolyl bonds in their proteins. Hence, the ratio of arginine plus lysine to proline could be an important factor in determining the protein utilization of plant foods. Although proline is not an essential amino acid, its level in proportion to lysine and arginine content could be a factor contributing to poor digestibility of plant proteins (2). It is interesting to observe that 100 per cent LCP was found to be lower in proline, 1.5-2.5 times lower than the other diets, but the proportion to lysine was almost a 1:1 ratio. Thus in the diet D, the ratio is nearly equal to 3.0 while the ratio of each diet C, B, and A were 1.8, 1.4 and 1.0 respectively. This finding would appear to be related to the good amino acid availability of cottonseed protein.

Beside the influence of linear structure, the rate of protein hydrolysis is considerably affected by its tertiary

Table 10

DISPROPORTIONATE AMINO ACIDS IN FOUR COTTONSEED DIETS

Experimental diet	arginine	leucine	lysine	methionine	proline	glycine
	(G/day)					
A	1.95	5.25	3.48	1.57	5.37	1.06
B	3.11	5.07	3.09	1.10	4.39	1.71
C	4.12	3.98	2.96	1.02	3.76	2.13
D	5.53	3.21	2.34	0.82	2.42	2.24

structure. The susceptibility to proteolytic digestion, dependant upon the availability of amino acid residues which are compatible bonds, will result in an increased rate of protein hydrolysis (23, 38). This would explain, at least in part, the improvement in protein quality usually observed with heat-treated plant food stuffs (41). From data presented it can be postulated that the overall nitrogen retention from the subjects was slightly low, although the nitrogen intake from the diets was slightly higher than recommended daily intake for women (60). Not having heat treated liquid formula diets available may have influenced the results.

An interesting factor, and possibly a significant aspect of pancreatic enzymes in response to undigested proteineous material, was reported by Green, et al. (19). Since pancreatic enzymes are rich in sulfur-containing amino acids, the preferential synthesis of pancreatic enzymes would create an increased requirement for methionine and/or cystine for synthesis of other tissue proteins, thus emphasizing the deficiency of sulfur-containing amino acids which already exist in many plant proteins.

There appears to be no difference in the digestibility of the two proteins fed to the subjects. If it is assumed that much of the nitrogen excreted in feces is that which is not digested and therefore not absorbed, it was observed that both proteins were highly digestible. Values for fecal nitrogen were similar for all diets and were relatively low. The digestibility for the proteins was 92.7 per cent and 94.2 per cent for diets D and A.

Although the relative proportions among essential amino acids must be important, quantitative relations have not been established, partly because of differences in requirements of individual subjects for particular amino acids (29, 39, 40, 56). Therefore, quantities of all essential amino acids in experimental diets containing ordinary foods must be considered; and data obtained in the present experiment indicate that it is difficult to predict the outcome of a particular combination. Combinations of oilseed proteins with or without egg protein or animal protein evidently provide amounts of amino acids considerably above minimal requirements established for synthetic amino acids and are able to maintain nitrogen equilibrium in adults.

Serum amino acid concentrations possibly reflect the balance between the entry of amino acids into the blood from food that is eaten and digested, and the tissue protein breakdown as well as the exit of amino acids from blood to the tissue where the amino acids are used for tissue protein synthesis and removed by degenerative reactions (23, 24). The regulation of serum amino acid concentration includes adjustments in food intake, in the rate of gastric emptying, in the rate of absorption from the intestine, in the rate of reabsorption in the kidney, in the rate of synthesis of tissue proteins, and in the rate of amino acid degradation (22).

This relationship has been confirmed by several researchers who have compared the levels of amino acids in portal blood during absorption with amino acid content of the diets (8, 20, 48, 49). On the other hand, some investigators have failed to establish a relationship between the relative abundance of dietary amino acids and their increase in serum during the absorptive period. The differences in availability of the individual amino acids within the same protein may be attributed to differences in the rate of absorption of the individual amino acids.

In Figure 2, the dietary intake of the amino acids is compared with the serum levels. In order that more direct comparison can be made, both values have been converted to weight units instead of micromoles as the serum values are reported in Table 8. It is noteworthy that for most of the amino acids (especially the essential ones) there is a relationship between dietary and serum levels. In most cases the amino acids that were present in the larger amounts in the diet are also present in the larger amounts in the serum. The few exceptions to this are histidine, alanine, and glycine. Of course, alanine and glycine are non-essential and are synthesized by transamination of relatively simple and readily available substrates. Tyrosine is present at lower levels

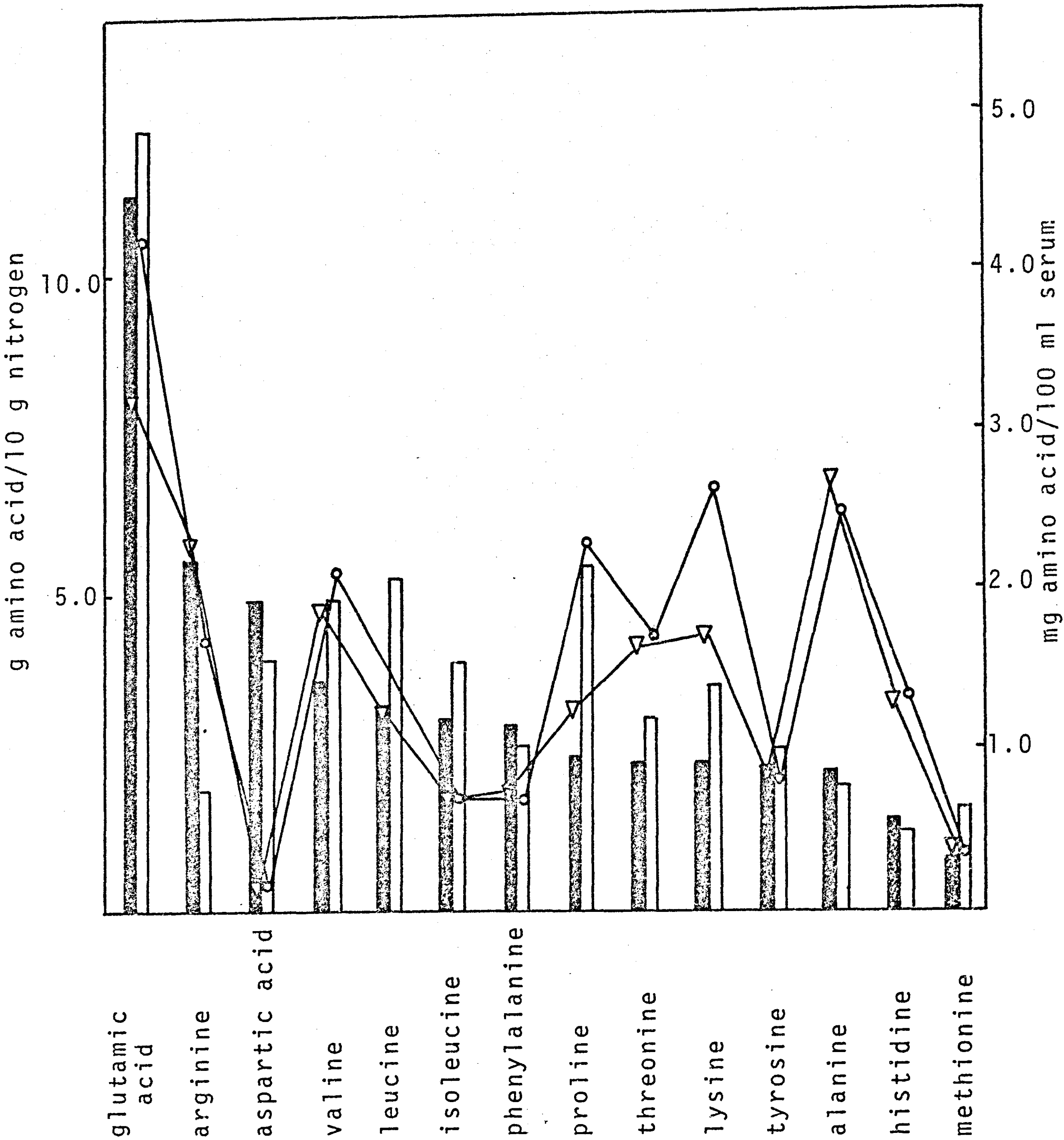

Figure 2. Comparison of amino acids in diet (g/10 g N with free amino acids in serum (mg/100 ml)

▭	0 % LCP diet	o	0 %	Serum
▦	100 % LCP diet	△	100 %	

than might be expected which suggests that much of it is metabolized to other compounds which are required by the body. The very low values of aspartic acid reflect its importance in transamination.

It is also observed that in most cases the diet which contained the highest level of each amino acid produced the higher serum amino acid levels in the subjects consuming that diet. The most obvious exception to this is in the case of methionine where, although the RI-5 diet (diet A) contained the most, the serum levels were higher in the subjects fed diet D. Although Eggum (9) reported no relationship between biological value and the amino acid composition of the diets, in the present experiment, with higher serum levels of proline and lysine in subjects fed the diets A and B, serum lysine and proline concentration also increased in serum one-half to twice that of diets C and D. However, isoleucine and phenylalanine were not appreciably affected, but leucine, valine, and threonine were found to have substantially high levels in serum than in all diets.

It was of interest that considerably lower content of lysine in the diet D and lower content of arginine in the diet A showed markedly increased serum threonine concentration in both diets. According to the report by Zimmerman and Scott (81), this increased serum concentration was not to be considered significant for threonine since several of the amino acids showed the same characteristics but to a lesser degree.

The observed amounts of ornithine in the serum of subjects fed all diets, may be explained in that the arginine and aspartic acid were converted in appreciable amounts to ornithine (10). The largest concentration in serum was the amide nitrogen of aspartic and glutamic acids (asparagine and glutamine). As pointed out by isotopic study (23), after the feeding of isotopic nitrogen it was always noted that asparagine and glutamine have the largest concentration of the isotopes.

CHAPTER VI

SUMMARY AND RECOMMENDATIONS

Influence of Amino Acid Intake on Nitrogen Balance

The major purpose of the study was to investigate the influence of amino acid patterns and protein value in the different levels of defatted cottonseed flour (LCP diet). Twelve young college women served as experimental subjects in a series of four experiments in which nitrogen balance was studied. The experimental liquid formula diet which provided 10 g nitrogen (13 per cent protein) per subject per day was composed of 0 per cent, 30 per cent, 60 per cent, and 100 per cent LCP with the balance of total nitrogen as a reference protein, RI-5 which was very close to egg white protein in amino acid composition.

Mean daily nitrogen balances of the subject fed 0 per cent, 30 per cent, 60 per cent, and 100 per cent LCP diets was -0.05, +0.76, +0.35, and +1.42 grams nitrogen respectively. Generally, the branched-chain amino acids, lysine and methionine, were lower in the diet D and higher in the diet A, but these amino acids were well compensated in the diets B and C. Non-essential amino acid levels in the 100 per cent LCP diet was very similar to or slightly higher than that of the three other diets.

The ratio of essential amino acids to non-essential amino acids in the 0 per cent, 30 per cent, 60 per cent, and 100 per cent LCP diets were 0.87, 0.81, and 0.73, and 0.63, and the mean essential amino acids provided were 3.18, 2.98, 2.69, and 2.37. The total essential amino acids in the daily intake of diets were 0 per cent, 30 per cent, 60 per cent, and 100 per cent while LCP diets constitute nearly 40 per cent, 39 per cent, 37 per cent, and 34 per cent of the total nitrogen respectively. The 100 per cent LCP diet contained high amounts of arginine and low amounts of proline while the 0 per cent LCP diet was high in proline, lysine, methionine, but very low in arginine. On the other hand, 30 per cent and 60 per cent were well proportioned in amino acid content.

Although on the basis of amino acid composition, the LCP protein appears to be of lower biological quality than RI-5 reference protein, the results of this study indicated that at the level fed to these subjects, the quality of the cottonseed protein is adequate to maintain nitrogen equilibrium in adult women. The findings suggest that the nutritive value of the LCP in four levels of diets was similar and is related to the amino acid composition. The nutritive value of LCP protein is reasonably well balanced, almost comparable to that of protein of animal origin.

The evidence presented in this report represents preliminary findings of a continuing study to develop and improve the quality of protein from defatted glanded cottonseed flour. Recommendations for further investigations would include the following:

1. A refinement of the technique which can be used for assessing dietary protein quality in young women subjects to detect even relatively small differences in protein quality. On healthy young adults the dietary protein is used essentially for maintenance purposes.

2. An investigation of the amino acid requirement in a lower level of nitrogen intake when supplied with the same types of diets to young women for a relatively long duration.

3. An investigation of the limiting amino acids in cottonseed flour for young women when fed different levels of LCP.

4. An investigation of the amino acid content of cottonseed flour and availability for young women when fed heat treated formula diet. More research efforts are needed to measure and improve the protein digestibility of LCP.

Effect of Dietary Amino Acid Content on Serum Amino Acid Levels

With the exception of lysine, most of the essential amino acid concentrations in serum were approximately the same for all diets. The 0 per cent LCP diet gave considerably higher concentration of lysine than other diets. The branched-chain amino acids leucine and valine were at a substantially higher level in serum than isoleucine in all diets. The ratio of essential to non-essential amino acids was the lowest in the serum of subjects fed the 100 per cent LCP diet while all of the other diets produced the same ratio. The proportion of essential to non-essential amino acid in 100 per cent LCP diet was slightly imbalanced when compared to mixed diets with combinations of LCP and RI-5 proteins.

The results of the blood analyses related to this study indicated no significant changes in most blood constituents measured which could be related to different levels of cottonseed flour, the only significant differences were the slightly elevated serum urea nitrogen on a 100 per cent LCP diet intake in comparison with the 0 per cent LCP diet. The findings suggest that the nutritive value of LCP in flour levels of diet was similar and is related to amino acid composition. The observed changes in serum levels are not readily explained as the result of competition transport system or for enzymes involved in their metabolic pathways.

Although the serum amino acid method may provide valuable information regarding the nutritional status, limiting amino acids, and amino acid requirements, no one has yet presented convincing evidence that there are unique advantages in using blood amino acid levels for ranking the relative quality of various foods. Furthermore, several other factors that might obscure the results have not been studied, for example, the influence of energy-yielding nutrients, the effect of dietary carbohydrates, hormones, amino acid antagonism, and toxicity. Therefore, it is not surprising that little success has been achieved in pursuing correlations between intestinal release of amino acids and changes in these amino acids in portal blood.

The evidence presented in this report represents preliminary findings of a continuing study to develop and improve the quality of protein from defatted glanded cottonseed flour. Recommendations for further investigations would include the following:

1. An investigation of the plasma concentration of the amino acids after a high protein meal.

2. An investigation of several factors influencing the changes in plasma concentration of amino acid after a protein meal: level of dietary protien, extent of dietary deficiency of amino acid, duration of feeding test, and the animal's growth rate.

3. An investigation of more extensive evaluation of various yardsticks, such as plasma amino acid ratio and plasma amino acid score, for rating plasma amino acid changes.

4. An investigation of the most suitable initial reference pattern of plasma amino acids, for example, fasting plasma amino acid pattern in young adults.

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APPENDIX

NITROGEN BALANCE OF YOUNG WOMEN ON

0 PER CENT LEP DIET

(DIET A)

Subject Case Number	Nitrogen intake	Urinary excretion	Fecal excretion	Nitrogen balance	Creatinine excretion
	g/day				
2977	11.97	8.99	0.68	+2.30	0.87
2978	9.03	9.52	0.61	-1.10	0.66
2979	10.51	6.93	0.30	+2.98	0.53
2980	10.06	14.03	0.41	-4.38	0.79
2981	10.53	10.50	0.78	-0.75	0.74
2982	9.89	12.18	0.94	-3.23	0.98
2983	9.44	9.27	0.18	-0.01	0.84
2984	9.53	9.95	0.40	-0.82	0.84
2985	9.20	9.09	0.63	-0.52	0.92
2986	11.13	9.62	0.57	+0.94	0.78
2987	11.23	8.52	1.01	+1.70	0.84
2988	9.08	6.56	0.36	+2.16	0.65
Mean	10.13	9.59	0.59	-0.05	0.79
Standard deviation	0.95	2.03	0.25	2.18	0.12

Total integumental loss of nitrogen was counted as 0.13 g/day and computed in urinary excretion.

NITROGEN BALANCE OF YOUNG WOMEN ON

30 PER CENT LCP DIET

(DIET B)

Subject Case Number	Nitrogen intake	Urinary excretion	Fecal excretion	Nitrogen balance	Creatinine excretion
	g/day				
2977	10.03	10.29	0.61	-0.88	0.94
2978	8.79	10.18	0.58	-1.97	0.72
2979	10.00	6.89	0.45	+2.66	0.29
2980	10.26	10.48	0.44	-0.66	0.67
2981	9.15	5.63	0.62	+2.90	0.51
2982	10.23	8.94	1.07	+0.22	0.99
2983	9.51	6.78	0.61	+2.12	0.73
2984	11.00	10.51	0.52	-0.03	0.83
2985	10.27	9.31	0.84	+0.12	0.87
2986	10.37	10.08	0.50	-0.21	0.88
2987	10.95	8.48	0.71	+0.76	0.71
2988	8.87	5.45	0.44	+2.98	0.47
Mean	9.95	8.58	0.61	+0.76	0.72
Standard deviation	0.73	1.91	0.18	1.62	0.20

Total integumental loss of nitrogen was counted as 0.13 g/day and computed in urinary excretion.

NITROGEN BALANCE OF YOUNG WOMEN ON

60 PER CENT LEP DIET

(DIET C)

Subject Case Number	Nitrogen intake	Urinary excretion	Fecal excretion	Nitrogen balance	Creatinine excretion
	g/day				
2977	10.62	8.27	0.79	+1.56	0.77
2978	9.00	9.39	0.62	-1.01	0.60
2979	10.88	5.32	0.42	+5.14	0.45
2980	10.29	10.68	0.64	-1.03	0.81
2981	10.75	8.18	0.62	+1.95	0.62
2982	10.48	14.02	1.13	-4.67	0.97
2983	10.01	11.41	0.41	-1.81	0.94
2984	11.23	8.15	0.80	+2.28	0.72
2985	9.99	11.46	0.76	-2.23	0.92
2986	10.25	9.83	0.67	-0.25	0.89
2987	10.90	10.36	0.93	-0.39	0.86
2988	10.60	5.59	0.48	+4.53	0.45
Mean	10.41	9.38	0.68	+0.35	0.75
Standard deviation	0.57	2.48	0.21	2.46	0.18

Total integumental loss of nitrogen was counted as 0.13 g/day and computed in urinary excretion.

NITROGEN BALANCE OF YOUNG WOMEN ON

100 PER CENT LCP DIET

(DIET D)

Subject Case Number	Nitrogen intake	Urinary excretion	Fecal excretion	Nitrogen balance	Creatinine excretion
	g/day				
2977	11.12	6.91	0.63	+3.58	0.73
2978	9.00	8.21	0.69	+0.10	0.66
2979	10.45	6.71	0.53	+3.21	0.57
2980	10.07	8.39	0.49	+1.19	0.70
2981	10.16	8.93	0.89	+0.34	0.63
2982	10.09	8.38	0.62	+1.09	0.94
2983	9.60	10.20	1.23	-1.83	1.16
2984	10.13	6.92	0.52	+2.69	0.81
2985	9.47	8.54	0.68	+0.25	0.81
2986	10.69	8.36	1.20	+1.13	0.71
2987	11.40	9.13	0.84	+1.43	0.91
2988	9.48	5.12	0.60	+3.76	0.65
Mean	10.14	7.98	0.74	+1.42	0.77
Standard deviation	0.70	1.35	0.25	1.88	0.16

Total integumental loss of nitrogen was counted as 0.13 g/day and computed in urinary excretion.