FORTIFICATION OF DEFATTED GLANDLESS COTTONSEED FLOUR WITH LYSINE AND THREONINE

A THESIS

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CHAPTER I

INTRODUCTION

As the population of the world increases, the adequacy of the supply of the protein decreases. It is estimated that more than fifty percent of the population of the world is undernourished and some twenty-five percent of the people in the developing countries are severely malnourished (1,2). Since the cost of producing and purchasing animal protein are expensive, it is unlikely that such protein can be used as a means of correcting protein deficiency. Furthermore protein deficiency in both quality and quantity most often occurs in the developing countries where cereal grains are the major source of proteins (3,4). Glandless cottonseed flour has been recognized as being able to contribute significantly to our diet, because of its relatively high protein content (approximately sixty percent), and its availability (5-8). The composition and nutritional quality of cottonseed meals vary with the species of cotton, the time of harvest, and the industrial process by which oil is extracted from the seed (9). One of the disadvantages of using cottonseed as a protein source is its gossypol content, a substance which is harmful to monogastric animals

(10,11). The method of high temperature processing of cottonseed reduces the toxic effects of gossypol but reduces the nutritional quality of the protein by making lysine unavailable (11). Deglanded cottonseed achieved by the method of Liquid Cyclone Process (LCP) and glandless cottonseed achieved by breeding technique are the two newly developed cottonseed varieties which contain a reduced amount of gossypol (12).

Texas Woman's University has been playing an important role in determining and improving the nutritional quality of cottonseed protein. The results of these studies are well summarized in a professional paper by Bhanot (13). The nutritional quality of cottonseed protein determined by the Protein Efficiency Ratio (PER) test ranged from 1.2 to 2.2 in comparison to 2.5 for casein (14-17).

Amino acid composition, especially of essential amino acids in proper ratio, is an important factor in determining the nutritive value of protein foodstuffs (18-20). Cottonseed protein has been shown to be deficient in lysine, methionine, and possibly isoleucine and threonine (6,14). Among these limiting amino acids, lysine is recognized to be the first limiting amino acid (5-6,9,12,14,17). The deficiencies of amino acids in the protein may be corrected by amino acid fortification, protein supplementation, or

protein complementation (21,22).

Very few studies have been reported on the amino acid fortification of cottonseed protein. Yoo (14) recently found that the PER value of defatted glandless cottonseed flour was not significaltly improved when lysine, methionine and/or isoleucine were added. A low plasma threonine level was also found in the rats fed defatted cottonseed flour. These findings suggested that lysine and threonine might be equally limiting in cottonseed protein. In an earlier study by Howe (17), lysine and threonine were suggested to be equally limiting in cottonseed protein.

The purpose of this study was to further investigate the possibility of improving the quality of cottonseed protein by fortifying defatted cottonseed flour with lysine and threonine. The specific objectives of this proposed study were:

1. To determine the amount of lysine and threonine in defatted glandless cottonseed flour (CSF) and casein (C).

2. To fortify defatted glandless CSF with lysine and/or threonine, so that both C and CSF contained the same amount of lysine and/or threonine per 16 grams of nitrogen.

3. To test the quality of lysine and/or threonine fortified CSF by protein efficiency ratio test.

4. To determine the levels of fasting plasma amino

acids, particularly threonine, in rats fed CSF or C diet.

CHAPTER II

REVIEW OF LITERATURE

Cottonseed flour has not yet been widely incorporated into the diet of the people in malnourished sections of the world even though glandless cottonseed has been demonstrated to be suitable for human consumption (3). Clinical studies by Graham et al. (23) showed that cottonseed flour used as sole source of dietary protein did not have any deleterious effect on the growth of convalescent malnourished children. These investigators also demonstrated that at adequate levels of protein intake, properly processed cottonseed flours could be served as the main or sole source of dietary protein for rapidly growing infants and children. Alford et al. (24) showed that cottonseed protein from Liquid Cyclone Process (LCP) was adequate to maintain nitrogen equilibrium in young college women when thirteen percent of the total caloric intake was contributed by the protein. In this study, it was also found that LCP cottonseed protein appeared to have lower essential amino acid (EAA) to non-essential amino acid (NEAA) ratio than the reference protein (RI-5), and the diet containing LCP cottonseed protein as sole protein source contained lesser amounts of lysine, methionine, leucine, and valine than the diet

containing RI-5 as sole protein source. Studies by Sneed et al. (25) showed that fasting plasma threonine, proline, isoleucine, leucine, tyrosine, and lysine concentrations of college women whose diet contained cottonseed flour decreased significantly as the protein intake was decreased from 14.6 g to 6.3 g nitrogen per day. Sutton (26) found that fasting plasma lysine concentrations in college women increased as glandless cottonseed flour was supplemented with seventeen milligrams of Lys-HCl per gram of protein.

Protein deficiency in the developing countries is largely due to quality rather than quantity of the dietary protein (3,19). Several investigators suggested that the protein nutritive value of cereals might be improved by addition of small quantities of the limiting amino acid (3-5,17,19). Results of animal and human studies on amino acid fortification have been extensively reviewed by Jensen (27,28)and Hegsted (29).

The protein in the glandless cottonseed flour has been suggested to be deficient in lysine, methionine (30), and possibly threonine (6). As occurs in most of the vegetable proteins, the first limiting amino acid in cottonseed protein is lysine (5,6,9,12,14,17). Reber et al. (15) demonstrated that the addition of 0.4 percent L-lysine to roasted whole kernel cottonseed would improve its protein

efficiency ratio (PER) to that of the cooked cottonseed kernel.

In addition to the content of amino acids, the quality of cottonseed protein can also be affected by its gossypol content or by the heat treatment process (10,11). Both factors can decrease the availability of lysine in the cottonseed protein.

Recent study by Yoo (14) showed that the quality of cottonseed protein was not significantly improved when it was fortified with lysine either to the same level of total lysine (0.5 percent L-Lys-HCl) or to the same level of available lysine (0.55 percent L-Lys-HCl) in casein. Analysis of amino acid contents of raw defatted glandless cottonseed showed lysine, methionine, and isoleucine to be the three most limiting amino acids (14). Neither the fortification of 0.5 percent L-Lys-HCl, 0.13 percent L-methionine nor the fortification of 0.5 percent L-Lys-HCl, 0.13 percent L-methionine and 0.2 percent L-isoleucine did significantly increase the PER or the biological value (BV) of the defatted glandless cottonseed protein.

In a study by Grau (31), lysine and methionine were shown to be the first and second limiting amino acids for growing chicks fed 20 percent cottonseed meal. Howe et al. (17) subsequently demonstrated that fortification of 0.1

percent L-Lys-HCl and 0.2 percent DL-threonine to cottonseed protein produced the best supplementary effect using PER test and suggested that lysine and threonine might be equally limiting in cottonseed protein for growing rats. Fisher (32) also suggested that no single amino acid (one of which should be threonine) was first-limiting in cottonseed protein for growing chicks. It was possible that more than one amino acid might be first limiting in cottonseed protein.

Plasma amino acid levels are affected by the various dietary, physiological, and pathological factors (33). Plasma amino acid concentrations are influenced by the balance between the influx of amino acids into the blood from digested food protein and the breakdown of tissue protein, and the efflux of amino acids from the blood to the tissues for protein synthesis (34). Therefore, amino acids in the plasma reflect a portion of the metabolic pool of free amino acids in the body (35). In 1959, Longenecker and Hause (36) used the fasting plasma amino acid pattern as a reference in the evaluation of the quality of dietary protein. Swendseid and Kopple (37) suggested that the plasma amino acid pool might be a sensitive indicator of depletion and repletion condition since it reflected rapid flux of extravascular and intracellular pools. Graham et

al. (38) demonstrated that the fasting plasma amino acid levels could serve as a good indicator for protein status of an individual and for identification of a first limiting amino acid in dietary protein after prolonged consumption. It was found in the study by Yoo (14) that fasting plasma threonine concentration of rats fed defatted glandless cottonseed flour for 28 days was significantly lower than those fed casein diet even though the cottonseed flour was fortified with lysine, methionine and/or isoleucine.

In animal and human studies, it was found that plasma threonine declined markedly whereas plasma phenylalanine and lysine remained relatively constant when the study subjects were fed a diet lacking both phenylalanine and lysine (37). Free threonine value in the plasma of rats fed diets containing excessive amounts of lysine and threonine was elevated markedly, whereas the content of lysine in the plasma remained constant (42). These results indicated that threonine might be a sensitive amino acid and its level in the plasma of test subject would increase or decrease markedly according to the amount of such amino acid in the diet.

CHAPTER III

METHODS AND PROCEDURE

This proposed study includes: 1) the determination of lysine and threonine contents of test proteins (casein and defatted glandless cottonseed flour); 2) the application of protein efficiency ratio test to test the protein quality of lysine and/or threonine fortified cottonseed flour; and 3) the quantitative determination of amino acids, particularly lysine and threonine in the plasma of rats fed the test diets.

Determination of lysine and threonine concentration in the test proteins (casein and cottonseed flour)

Amino acid compositions of acid hydrolyzed ANRC (Animal Nutrition Research Council) casein and defatted glandless cottonseed flour (CSF) were determined on a Beckman 121M Microcolumn Amino Acid Analyzer according to the procedure provided by Beckman Instruments, Inc. (43). The detailed procedure is presented in Appendix A. The amounts of lysine and threonine in both ANRC casein and CSF were identified.

Fortification of lysine and threonine to defatted glandless cottonseed flour

The contents of lysine and threonine in ANRC casein and CSF were calculated from the results obtained from the previous section. L-Lysine-HCl and L-threonine were added to the CSF so that the contents of the total lysine and total threonine in CSF and ANRC casein (per 16 grams of nitrogen) were equal.

Diet

Five diets were prepared according to the formula prescribed by the Association of Official Analytical Chemists (AOAC) for Protein Efficiency Ratio (PER) Test (44). All diets contained protein, 10%; oil, 8%; mineral, 5%; vitamin, 1%; fiber, 1%; water, 5%; and sucrose, to make 100%. The dietary composition of the test diets is shown in Table 1. Diet 1 contained ANRC casein as protein source and was the control diet. Diet 2 contained CSF as protein source. Diet 3 contained CSF fortified with 0.484% L-lysine-HCl. Diet 4 contained CSF fortified with 0.08% L-threonine. Diet 5 contained CSF fortified with 0.484%

L-lysine-HCl and 0.08% L-threonine. A summary of the dietary groups is presented in Table 2.

Animal

Twenty-two day old male Holtzman rats were purchased from Holtzman Co. in Madison, Wisconsin. Upon arrival, animals were individually housed in suspended wire mesh cages in a temperature (20-23°C) and light (12 hr/12 hrs - light/dark) controlled animal room. All animals were fed a diet containing 20% protein from casein and water ad libitum for three days.

Protein efficiency ratio test

After the 3-day acclimation period, sixty-six male rats within a body weight range of fifteen grams were randomly divided into six groups. The first group of sixteen rats were sacrificed for determinatin of fasting plasma amino acid concentrations before PER Test was initiated. The remaining five groups of ten rats each were used for PER Test according to the method of AOAC (44). Each group of rats was fed one of the five test diets listed in Table 1. Food consumption and body weight of the rats were measured

Table 1

DIETARY COMPOSITION (%)

Ingredient	Diet				
	1	2	3	4	5
Casein (ANRC)[a]	11.66	-	-	-	-
Cottonseed flour[b]	-	17.39	17.39	17.39	17.39
L-Lys-HCl[c]	-	-	0.484	-	0.484
L-Threonine[d]	-	-	-	0.08	0.08
Sucrose	68.97	64.20	63.72	64.12	63.64
Corn oil[e]	7.99	7.54	7.54	7.54	7.54
Salt Mixture (USP XVII)	5.00	5.00	5.00	5.00	5.00
Vitamin mix (AOAC)	1.00	1.00	1.00	1.00	1.00
Non-nutritive fiber[f]	1.00	1.00	1.00	1.00	1.00
Water	4.38	3.87	3.87	3.87	3.87

[a]Protein (N x 6.25), 85.75%;lipid, 0.1%; ash, 2.05%; moisture, 5.3%; and carbohydrate, 0.25%
[b]Protein (N x 6.25), 57.50%;lipid, 2.62%; ash, 6.37%; moisture, 6.5%; fiber, 4%; and carbohydrate, 24.62%.
[c]L-(+)- Lys-HCl, Fisher Scientific Co., Fair Lawn, New Jersey.
[d]L-Threonine, Sigma Chemical Co., St. Louis, MO.
[e]Mazola Corn oil, Englewood Cliff, New Jersey.
[f]Cellulose type, Teklad Test Diet, Madison, Wisconsin.

TABLE 2

AMINO ACID FORTIFICATION OF DEFATTED GLANDLESS COTTONSEED PROTEIN (CSF)

Diet group	Protein source	Amino acid fortified (%)	
		L-Lys-HCl	L-Thr
1	ANRC casein (control)[a]	-	-
2	CSF[b]	-	-
3	CSF[b]	0.484	-
4	CSF[b]	-	0.08
5	CSF[b]	0.484	0.08

[a]ANRC (Animal Nutrition Research Council) casein, Sheffield Chemical Co., Lynhurst, New Jersey.

[b]Raw defatted glandless cottonseed protein obtained from Roger Delinted Cottonseed Co., Waco, Texas. Free gossypol content, 200 ppm. (analyzed by POPE Testing Laboratories, INC., Dallas, Texas).

weekly. At the end of four weeks, the protein efficiency ratio was calculated according to the following formulae (44,45):

$$\text{PER} = \frac{\text{Weight gain (gm)}}{\text{Protein consumed (gm)}}$$

$$\text{Adjusted PER} = \frac{\text{Mean PER of test protein}}{\text{Mean PER of reference ANRC protein}} \times 2.5$$

Plasma amino acid concentration

Plasma amino acid concentrations of the rats were determined from overnight fasting (15 hrs) blood samples obtained from the inferior vena cava. Blood samples were centrifuged at 4,000 rpm for 30 minutes (Clini-cool, Damon/IEC Division, Needham heights, MA.) for separation of the plasma from the red blood cells. Plasma samples were centrifuged at 10,000 rpm for 15 minutes (Sorvall RC-5B Refrigerated Superspread Centrifuge, Du Pont Instruments, Newton, Conn.) after the addition of sulfosalicylic acid (45 mg/ml plasma). The deproteinized plasma samples were diluted at 1:1 with 1% lithium citrate (pH 2.2) and filtered with a 0.45 um pore size filter. Twenty ul of the filtrate

filtrate was applied to the Beckman 121 M microcolumn amino acid analyzer for amino acid analysis.

Statistical analysis

One-way analysis of variance and Newman-Keuls multiple range test were applied to test for the statistical differences in overall and between groups analyses. A probability of $p \leq 0.05$ was considered as significant.

CHAPTER IV

RESULTS AND DISCUSSION

Lysine and threonine concentrations of casein and defatted glandless cottonseed flour

The amino acid composition of raw defatted glandless cottonseed flour (CSF) and ANRC (Animal Nutrition Research Council) casein are shown in Table 3. The results indicate lysine, methionine, and isoleucine as the first, second and third limiting amino acids, respectively in the CSF, in reference to ANRC casein. Threonine in CSF was deficient to a lesser degree and was about 80 percent of that of the casein. These results were in agreement with the findings of Yoo (14) and other published reports (46,47). Yoo (14) found that (a) fortifying CSF with 0.50 percent or 0.55 percent L-lysine-HCl had similar effect on the PER results, (b) the fortification of lysine, methionine and isoleucine to CSF did not significantly improve the PER of CSF, and (c) the plasma threonine concentrations of rats fed CSF diet was significantly lower than that of the rats fed ANRC casein. Howe et al. (17) showed that the most complementary effect of fortifying degossypolized CSF could be achieved at the levels of 0.1 percent L-lysine-HCl and 0.2 percent

TABLE 3

ESSENTIAL AMINO ACID (g/16g N) COMPOSITIONS OF DEFATTED GLANDLESS COTTONSEED FLOUR (CSF) AND ANRC CASEIN

Amino acid	ANRC Casein	CSF	$\frac{\text{CSF}}{\text{Casein}} \times 100$
Lys	7.98	4.11	51.50
His	2.81	2.48	88.26
Arg	4.06	11.81	290.89
Thr	4.07	3.27	80.34
Val	6.04	3.56	58.94
Met (+Cys)	2.91 (4.15)	1.53 (3.05)	52.58 (73.49)
Ile	4.14	2.21	53.38
Leu	9.23	5.35	57.96
Phe	5.39	5.09	94.43
Total	39.76	25.12	

DL-threonine.

This investigator elected to fortify defatted glandless CSF with lysine and/or threonine and tested the PER of the lysine and/or threonine fortified CSF. In addition, the plasma amino acid concentrations, especially threonine, of rats fed CSF diet with or without fortification were determined. To equalize the content of lysine and threonine (per 16 grams of nitrogen) in CSF and ANRC casein, 0.484 grams of L-lysine-HCl and 0.08 grams of L-threonine were added to each 100 grams of CSF protein. The diets listed in Table 1 were accordingly formulated.

Protein Efficiency Ratio Test (PER Test)

Table 4 presents the initial and final body weights of the rats fed ANRC casein or defatted glandless cottonseed flour (CSF) with or without lysine and/or threonine fortification. The initial body weights of the five groups of rats were similar. The mean final body weight of the rats consuming casein was significantly higher than those consuming CSF with or without lysine and/or threonine fortification. It was apparent that the body weight gain of the casein group was significantly greater than the CSF

groups (Table 5). Since the protein consumption (Table 5) was not different among the five groups of rats, a significantly higher PER (Table 6) was found in the control protein (casein) than that of the test protein (CSF with or without fortification).

Neither the body weight gain, nor the protein consumption was significantly different among the four test protein groups (Groups 2,3,4 and 5). Cottonseed flour fortified with 0.484 percent L-Lys-HCl and 0.08 percent L-threonine (Group 5) showed significantly higher PER than the CSF without any amino acid fortification (Group 2) or with fortification of threonine alone (Group 4). The PER of CSF fortified with lysine alone (Group 3) was very similar to that of CSF fortified with threonine alone (Group 4), but Group 3 was not statistically different from Group 5. The large value of standard deviation in Group 3 or dietary treatment may be responsible for these insignificant findings. These data indicated that significant improvement in the PER of CSF was achieved when both lysine and threonine were fortified to CSF; however, such improvement was not sufficient to reach the adjusted PER value (2.5) of the ANRC casein. Other investigators (14,17) had also reported that fortification of lysine or threonine alone did not improve the quality of cottonseed protein. The results

TABLE 4

BODY WEIGHT OF RATS FED ANRC CASEIN AND DEFATTED GLANDLESS COTTONSEED FLOUR (CSF) WITH OR WITHOUT AMINO ACID(S) FORTIFICATION FOR 28 DAYS[1].

Group	Diet	Body Weight (grams)	
		Initial	Final
1	Casein	72.71 ± 2.57	209.25 ± 12.59
2	CSF	73.79 ± 1.95	175.84 ± 25.87*
3	CSF+Lys	73.06 ± 2.01	182.78 ± 18.10*
4	CSF+Thr	73.04 ± 2.96	181.50 ± 17.99*
5	CSF+Lys+Thr	73.02 ± 2.61	186.15 ± 15.19*

[1]Values are mean ± SD of 10 rats, except Group 3 which consisted of 9 rats.

*Significant at $p < 0.05$ compared to Group 1 (Control).

TABLE 5

BODY WEIGHT CHANGE AND PROTEIN CONSUMPTION OF RATS FED ANRC CASEIN AND DEFATTED GLANDLESS COTTONSEED FLOUR (CSF) WITH OR WITHOUT AMINO ACID(S) FORTIFICATION FOR 28 DAYS[1].

Group	Diet	Body Weight Gain	Protein Consumed
		gm	gm
1	Casein	136.23 ± 13.12	44.33 ± 4.00
2	CSF	102.05 ± 25.81*	44.28 ± 6.08
3	CSF+Lys	109.63 ± 18.28*	45.46 ± 5.28
4	CSF+Thr	108.83 ± 18.06*	45.45 ± 5.13
5	CSF+Lys+Thr	113.08 ± 12.91*	43.44 ± 4.62

[1]Values are mean ± S.D. of 10 rats except Group 3 which consisted of 9 rats.

*Significant at $p < 0.05$ in comparison to Group 1 (Control).

TABLE 6

PROTEIN EFFICIENCY RATIO (PER) OF CASEIN AND DEFATTED GLANDLESS COTTONSEED FLOUR (CSF) WITH OR WITHOUT AMINO ACID(S) FORTIFICATION

Group	Diet	PER	Adjusted PER
1	Casein	3.07 ± 0.17	2.5
2	CSF	2.27 ± 0.33*,**	1.87
3	CSF+Lys	2.41 ± 0.25*	1.96
4	CSF+Thr	2.39 ± 0.17*,**	1.95
5	CSF+Lys+Thr	2.60 ± 0.16*	2.12

[1]Values are mean ± S.D. of 10 rats except Group 3 which consisted of 9 rats

*Significant at $p < 0.05$ compared to Group 1 (Control).

**Significant at $p < 0.05$ compared to Group 5.

of the present study and the reported findings of these investigators on the fortification of lysine or threonine alone to cottonseed protein are in agreement. Furthermore, the addition of lysine at 0.484 percent and threonine at 0.08 percent to CSF was not sufficient to improve the quality of CSF to the same degree as casein.

Fasting plasma amino acid concentrations

Fasting plasma amino acid concentrations of rats fed ANRC casein or CSF with or without lysine and/or threonine fortification are shown in Tables 7 and 8. Group B represents the fasting plasma amino acid composition of weanling rats fed a diet containing 20 % protein from casein for three days. The effect of fortification of dietary amino acid on the levels of plasma free amino acids can be very complex. Under the present experimental condition, the discussion will be mostly limited to the concentrations of plasma lysine, thretoine and arginine.

Lysine concentration in the fasting plasma of rats fed CSF without fortification (Group 2) was not significantly lower than that of the casein group (Group 1), although the content of lysine in CSF was approximately 50 percent of that of the casein.

TABLE 7

FASTING PLASMA ESSENTIAL AMINO ACID CONCENTRATIONS (μ mole/l) OF RATS FED ANRC CASEIN (C) OR DEFATTED GLANDLESS COTTONSEED FLOUR (CSF) WITH OR WITHOUT AMINO ACID(S) FORTIFICATION FOR 28 DAYS[1].

Amino acid	Group					
	B[2]	1(C)	2(CSF)	3(CSF+Lys)	4(CSF+Thr)	5(CSF+Lys+Thr)
Lys	860.4[a] (79.8)	530.8 (53.4)	507.3 (105.5)	627.0[b] (90.1)	692.2[b] (158.6)	566.0 (67.7)
His	59.8 (18.9)	62.0 (28.2)	37.8[c,d] (5.6)	37.8[c,d] (14.7)	50.7 (12.8)	53.1 (7.7)
Arg	237.0 (25.9)	131.5[c] (51.6)	144.4[c] (34.3)	227.0[b,d] (47.4)	174.7 (61.2)	168.6 (34.4)
Thr	356.1 (46.9)	602.4[a] (93.0)	192.0[c] (59.8)	230.4[c] (118.9)	303.3[b] (51.5)	286.0 (130.8)
Val	252.7[a] (79.3)	143.6 (50.6)	91.8 (45.6)	146.1 (37.2)	130.8 (47.9)	83.9[d,e] (24.7)
Met	76.8[a] (5.8)	45.2 (5.4)	34.4[d] (5.0)	43.3[b] (14.1)	38.0 (5.3)	36.7[d] (4.0)

[1]Values are mean (SD) of 7 samples.
[a]Significant at $p<0.05$ compared to all groups.
[b]Significant at $p<0.05$ compared to Group 2.
[c]Significant at $p<0.05$ compared to Group B.
[d]Significant at $p<0.05$ compared to Group 1 (Casein).
[e]Significant at $p<0.05$ compared to Group 3.

TABLE 7-CONTINUED

FASTING PLASMA ESSENTIAL AMINO ACID CONCENTRATIONS (μ mole/l) OF RATS FED ANRC CASEIN (C) OR DEFATTED GLANDLESS COTTONSEED FLOUR (CSF) WITH OR WITHOUT AMINO ACID(S) FORTIFICATION FOR 28 DAYS[1].

Amino acid	Group					
	B	1(C)	2(CSF)	3(CSF+Lys)	4(CSF+Thr)	5(CSF+Lys+Thr)
Ile	129.6[a] (29.2)	89.2 (10.1)	73.1 (8.6)	87.7 (23.1)	84.1 (16.8)	86.7 (13.9)
Leu	188.2[a] (24.7)	125.9 (8.7)	95.5[d] (12.7)	110.0 (30.4)	104.6 (30.2)	108.1 (19.8)
Phe	72.1[a] (9.4)	54.4 (8.1)	42.6[d] (7.3)	43.1[d] (9.7)	46.9 (10.1)	44.0[d] (7.0)
Total	2232.8[a]	1828.5	1219.0[d]	1552.4[b,d]	1518.4[b,d]	1433.0[d]

TABLE 8

FASTING PLASMA NON-ESSENTIAL AMINO ACID CONCENTRATIONS (μ mole/l) OF RATS FED ANRC CASEIN(C) OR DEFATTED GLANDLESS COTTONSEED FLOUR(CSF) WITH OR WITHOUT AMINO ACID(S) FORTIFICATION FOR 28 DAYS[1].

Amino acid	Group					
	B	1(C)	2(CSF)	3(CSF+Lys)	4(CSF+Thr)	5(CSF+Lys+Thr)
Asp	26.0 (9.5)	29.3 (6.5)	29.1 (4.8)	31.0 (4.6)	30.1 (4.2)	29.9 (6.4)
Ser	341.4[a] (40.3)	544.3 (75.0)	527.4 (112.0)	604.5 (146.2)	622.6 (39.6)	634.1[b] (90.3)
Gln	462.2[a] (32.7)	259.0 (43.8)	216.4[c] (39.0)	164.8[b,c] (39.3)	199.7[c] (20.9)	244.7[d,e] (18.4)
Glu	138.1 (20.7)	160.9 (28.2)	193.9[f] (48.6)	227.5[c,f] (67.7)	167.7[d] (30.1)	178.4[d] (16.7)
Gly	290.8[a] (54.5)	429.6 (83.5)	390.4 (48.1)	379.0 (108.8)	510.1 (44.4)	441.9 (68.0)
Ala	309.2 (44.8)	397.9[f] (95.5)	227.1[c,f] (63.6)	217.6[c,f] (87.0)	276.8[c] (69.0)	287.6 (37.4)

[1]Values are mean (SD) of 7 samples.
[a]Significant at $p<0.05$ compared to all groups.
[b]Significant at $p<0.05$ compared to Group 2.
[c]Significant at $p<0.05$ compared to Group 1.
[d]Significant at $p<0.05$ compared to Group 3.
[e]Significant at $p<0.05$ compared to Group 4.
[f]Significant at $p<0.05$ compared to Group B.

TABLE 8-CONTINUED

FASTING PLASMA NON-ESSENTIAL AMINO ACID CONCENTRATIONS(μ mole/l) OF RATS FED ANRC CASEIN(C) OR DEFATTED GLANDLESS COTTONSEED FLOUR(CSF) WITH OR WITHOUT AMINO ACID(S) FORTIFICATION FOR 28 DAYS[1].

Amino	Group					
acid	B	1(C)	2(CSF)	3(CSF+Lys)	4(CSF+Thr)	5(CSF+Lys+Thr)
Cys	36.4[a] (7.0)	21.2 (13.9)	16.9 (14.4)	15.8 (7.5)	24.5 (15.8)	32.7 (12.3)
Tyr	80.4 (11.9)	91.3 (7.9)	78.6 (11.7)	84.9 (24.3)	76.0 (8.5)	76.9 (10.6)
Tau	313.5[a] (81.4)	63.2 (27.0)	124.5 (63.3)	180.2[c] (66.3)	98.8[d] (31.4)	69.0[d] (17.8)
Cit	93.7[a] (11.4)	50.3 (17.8)	44.1 (19.3)	76.0[b,c] (9.4)	64.5[b] (11.3)	38.5[e,d] (16.2)
Orn	58.2 (10.5)	73.2 (11.6)	93.2[f] (17.8)	87.9[f] (25.6)	90.2[f] (21.9)	112.8[c,f] (53.8)
Total	2150.0 (130.1)	2153.7 (289.9)	1918.1 (103.6)	2069.1 (442.6)	2158.0 (128.7)	2137.6 (183.4)

When CSF was fortified with either lysine (Group 3) or threonine (Group 4) and was fed to the rats, the fasting plasma concentrations of lysine of these two groups were significantly higher than those of the rats fed CSF without fortification (Group 2). When both lysine and threonine were added to CSF, no statistically significant difference was found between these two groups (Group 2 vs Group 5). These data indicated that the fortification of L-Lys-HCl or L-threonine alone to CSF at the level of 0.484 percent and 0.08 percent, respectively had an influence on the fasting plasma lysine concentration.

Said et al. (42) using synthetic amino acid mixture had demonstrated that plasma lysine concentration of rats increased with increased intake of lysine alone. When threonine intake was increased along with lysine intake, the increase in the plasma lysine concentration observed earlier was depressed. The present study shows similar results.

In the amino acid imbalance studies by Ip and Harper (47), it was suggested that an amino acid imbalanced diet would stimulate protein synthesis and increase the efficiency of the utilization of the limiting amino acid. A reduction of the limiting amino acid in the plasma of rats fed the amino acid imbalanced diet was observed. It is possible that when both threonine and lysine were fortified

to CSF, an imbalance in the amino acid content of the CSF was produced which in turn accelerated the uptake of lysine for protein synthesis. The increased utilization of lysine contributed to the lower fasting plasma concentration of lysine. The reason for the high level of plasma lysine with threonine alone (Group 4) is unknown.

Fasting plasma concentrations of threonine of rats fed CSF with or without lysine and/or threonine fortification (Groups 2,3,4 and 5) were significantly lower than those of the rats fed casein diet (Group 1). In an earlier study by Yoo (14), it was also found that the mean serum threonine concentration of rats fed CSF with or without fortification of lysine, methionine and/or isoleucine was significantly lower than that of the casein fed rats. Yoo suggested that serum free threonine level was possibly affected most sensitively when the diet was slightly deficient in threonine (80 % of the casein). In the present study although the CSF diet was fortified with threonine (Groups 4 and 5), the plasma threonine concentrations of these rats were low. The rats fed CSF diet fortified with threonine alone (Group 4) had significantly increased level of plasma threonine in comparison to the rats fed CSF diet without fortification (Group 2). The plasma threonine of rats fed 10% protein from ANRC casein (Group 1) was also

significantly higher than that of the rats fed a 20 % protein from the casein (Group B). The same result was observed by Yoo (14). One would expect that when almost twice as much of the protein was fed to the rats, the amount of each essential amino acid in the plasma of these rats would be either similar or higher than that of the rats fed lower amount of protein. This was true in the comparison of all the plasma essential amino acids except threonine between Group B and Group 1. This unexpected finding in plasma threonine is unexplanable.

Amino acids are known to be conserved when the supply is low and to be oxidized rapidly when the supply exceeds the requirement (48). The body of an animal has the ability to conserve various essential amino acids in varying degrees by modifying the rates of catabolism when the diet is deficient in any of the essential amino acids (42). Yamashita et al. (41) have demonstrated that the catabolism of lysine was depressed in rats fed a lysine-free diet, whereas the catabolism of threonine was not significantly affected in rats fed a threonine-free diet. Harper and Kang-Lee (49) showed only when a mixture of amino acids was devoid of threonine, the NPU (Net Protein Utilization) became zero. Several investigators have found that there was minimal adaptation of threonine in rats when the supply

of this amino acid was either in excess or in depletion. The rate of catabolism or the ability of conservation of threonine were not modified (20,41,49-51). It was suggested that threonine might not be conserved as well as the other essential amino acids when the intake was low.

Harper and Kang-Lee (49) also showed that plasma threonine would increase sharply only after the level of threonine in the diet exceeded 0.3 percent. This phenomenon was not observed in the present study. No consistent increase was found in plasma threonine concentration when the level of threonine in the CSF diet was increased to 0.407 percent after fortification.

In the paper by Yoo (14), it was found that the concentration of serum free arginine of rats fed CSF diet was significatly higher than that of the casein fed rats. Such difference in the plasma argininep concentration was not found in the present study between the rats consuming the CSF diet (Group 2) which contained approximately three times the amount of arginine as in casein and the rats consuming casein diet (Group 1). This result was unexpected. A significant increase in the plasma arginine was found in the rats fed CSF fortified with lysine. alone (Group 3).

Weanling rats that were fed a 20 % protein diet for 3

days (Group B) showed higher fasting plasma concentrations of all the essential amino acids, except histidine than those in Group 1. Rats in Group 1 were sacrificed after being fed the 10 % casein protein diet for 28 days. The difference in the plasma essential amino acid concentrations between Group B and Group 1 was probably due to the different level of protein in the two diets.

The total essential amino acid (TEAA) in the plasma of rats fed CSF diet with or without lysine and/or threonine fortification (Groups 2,3,4 and 5) was significantly lower than that of the rats fed the casein diet (Group 1). Since almost all the individual essential amino acid content was less in the CSF than in the csein (Table 3), the low TEAA found in the plasma of rats after consuming CSF diet for 28 days was reasonably expected. Histidine, threonine, methionine, leucine, and phenylalanine levels of fasting plasma of rats fed CSF diet (Group 2) were found to be significantly lower than those fed the casein diet (Group 1). These amino acids were also shown to be in lower amount in CSF in comparison to ANRC casein (ranging from 53 % to 94 % of the content of ANRC casein). The plasma threonine and phenylalanine concentrations of the rats fed CSF fortified with threonine and lysine remained significantly lower than those in Group 1. The fortification of threonine alone to

CSF diet had also increased the plasma amino acid concentrations of lysine and arginine comparing to those of the rats fed 10 % casein (Group 1). The addition of lysine and threonine (Group 5), only improved the plasma levels of histidine and leucine.

Table 8 shows fasting plasma non-essential amino acid (NEAA) concentrations of rats fed defatted CSF with or without amino acid(s) fortification for 28 days. Since NEAA can be synthesized in the body, it would not be meaningful to relate the plasma NEAA concentrations to the dietary NEAA concentrations. Group B, again was composed of rats fed 20 % casein protein diet for three days and were sacrificed before PER experiment was initiated. Plasma glutamine, cystine, taurine, and citrulline concentrations were significantly higher in Group B rats than those of the rats in Groups 1 through 5 which were fed the 10 % protein diet. Plasma serine and plasma glycine were lower in Group B rats than those in groups 1-5. No significant differences were found in the concentrations of plasma aspartic acid, glycine,cystine and tyrosine among the groups of rats fed CSF with or without lysine and/or threonine fortification (Groups 2,3 and 4) or between the CSF fed groups and the casein fed group (Group 1). When both lysine and threonine were added to the CSF diet, rats consuming such diet (Group

5) showed significantly higher content of plasma serine than those consuming CSF diet without any fortification (Group 2). There was a gradual increase in the plasma serine concentration from Group 2 to Group 5.

Plasma concentration of glutamine was lower in rats fed CSF (Group 2), CSF fortified with lysine (Group 3), or CSF fortified with threonine (Group 4) in comparison to that of the rats fed the casein diet (Group 1). Fortified CSF with both lysine and threonine (Group 5) increased plasma glutamine concentration significantly in comparison to CSF fortified with lysine or threonine alone (Group 3 or Group 4). Fortified CSF with lysine alone (Group 3) showed a significant depression in plasma glutamine in comparison to CSF without any fortification (Group 2).

Mean glutamic acid concentrations in the plasma of rats fed CSF (Group 2) or CSF fortified with lysine alone (Group 3) was higher than that of rats fed 20 % casein protein diet (Group B). When CSF was fortified with threonine alone (Group 4), or lysine and threonine (Group 5), there was a significant decrease in the plasma concentration of glutamic acid (vs Group 3). Mean plasma alanine concentration of the rats fed CSF (Group 2), CSF with lysine (Group 3) or threonine (Group 4) was significantly lower than that of the rats fed casein (Group 1). When lysine and threonine were

both added to the CSF diet (Group 5), this difference disappeared (Group 5 vs Group 1). The concentration of plasma alanine in rats fed CSF (Group 2) or CSF with lysine (Group 3) was significantly lower than that of the rats in Group B. Plasma alanine concentration of the rats in Group 1 was significant higher than that of the rats in Group B. Fernstrom and others (52) found that in human, plasma glycine and alanine (the smaller molecular weight neutral amino acids) levels varied inversely with dietary protein content. In this study, it was also observed that the plasma glycine and alanine concentrations of rats fed 10 % casein protein diet were higher than those of the rats fed the 20 % casein protein diet (Group 1 vs Group B). When the 10 % casein protein was replaced by 10 % CSF protein (Groups 2-5), this relationship was not found. The inverse relationship between plasma smaller molecular weight neutral amino acids and the quantity of dietary protein may only limit to comparison using the same type of dietary protein.

Plasma cystine and taurine concentrations of rats in Group B were significantly higher than those in Groups 1-5. Since cystine and taurine are synthesized from methionine, higher levels of plasma cystine and taurine may be expected in the group of rats fed a diet composed of higher level of casein (Group B) which contained greater amount of

methionine than the groups of rats fed either lower level of casein (Group 1) or lower level of CSF (Groups 2-5). When dietary protein level was reduced to 10 %, rats consuming CSF diet (Groups 2-5) had similar or higher level of plasma taurine than those consuming diet (Group 1), especially when CSF was fortified with lysine alone (Group 3).

No significant difference was found in the plasma concentration of citrulline between the rats fed the casein diet (Group 1) and the CSF diet without fortification (Group 2). Adding lysine (Group 3) or threonine alone (Group 4) increased the level of plasma citrulline of the rats significantly in comparison to Group 1 or Group 2. But the addition of both lysine and threonine (Group 5) to CSF diet exhibited a suppressing effect on the plasma citrulline level of the rats. Citrulline concentration of plasma of rats fed 20 % casein diet (Group B) was significantly higher than those fed the 10 % protein diet whether its source was casein or CSF (Groups 1-5).

Ornithine concentration in the plasma of rats fed CSF diet with or without fortification (Groups 2-5) was higher than that of the rats fed the 20 % casein protein diet (Group B). Rats fed the CSF diet fortified with lysine and threonine (Group 5) had the highest plasma ornithine level among the groups of rats fed the CSF diet and this level was

significantly higher than that of the rats fed the 10 % casein protein diet (Group 1). No significant difference was found in the total NEAA concentrations among the six dietary groups.

CHAPTER V

SUMMARY AND CONCLUSION

The present study was designed to follow up the previous study conducted by Yoo (14) on the possibility of improving the quality of cottonseed protein. Defatted glandless cottonseed flour (CSF) fortified with 0.484 % L-lysine-HCl and/or 0.08 % L-threonine was tested by protein efficiency ratio test (PER) for quality. The plasma amino acid patterns of rats tested by PER were determined before and after the PER test. The levels of lysine and threonine supplementation were determined to satisfy the criterion that both ANRC (Animal Nutrition Research Council) casein (as control), and CSF would contain the same amount of these two amino acids per 16 g of nitrogen after the fortification of CSF.

Analysis of CSF showed that lysine was the first-limiting amino acid using ANRC casein as a reference protein. Threonine was not shown to be a major limiting amino acid in CSF and it was approximately eighty percent of that in the casein. Methionine, isoleucine in CSF were shown to be the second and third limiting amino acid, respectively.

The PER value of CSF diet before fortification

was significantly lower than that of the casein. When CSF was individually fortified with 0.484 % L-Lys-HCl or 0.08 % L-threonine, there was no significant increase of the PER value. Addition of both lysine and threonine to CSF produced a significant increase of PER value in comparison to CSF without any amino acid fortification or with threonine fortification alone. This improvement was not sufficient to reach the PER value of the casein.

Fasting plasma level of lysine of rats fed CSF fortified with lysine or threonine alone was significantly increased in comparison to those of the rats fed the CSF diet without fortification. Fortification of CSF with both lysine and threonine led to the disappearance of the significant increase in the plasma lysine seen in the individual fortification of lysine or threonine. Fortification of threonine alone to CSF brought a significant increase in the plasma threonine of rats in comparison to the rats fed CSF without any fortification. Rats consuming CSF diet with or without lysine and/or threonine fortification showed lower levels of plasma threonine than those fed the casein diet.

Total essential amino acid (TEAA) concentration of the plasma was significantly higher in rats fed 20 % casein protein diet than those fed the 10 % protein diet whether

the source of protein was casein or CSF. Rats consuming 10 % CSF protein diet whether it was fortified or not showed significantly lower concentrations of TEAA than those consuming 10 % casein protein diet. Fortified CSF with lysine or threonine alone increased plasma TEAA in comparison to CSF without fortification. Fortified CSF with both lysine and threonine did not bring significant increase in the plasma TEAA concentration. No consistent increase or decrease in the concentration of plasma non-essential amino acid was found when CSF was fortified with lysine and/or threonine. The total non-essential amino acid concentrations in the rats fed 20 % casein protein diet, 10 % casein protein diet and 10 % CSF protein diet with or without lysine and/or threonine fortification were similar.

In conclusion, fortifying defatted cottonseed flour with 0.484 percent L-Lys-HCl and 0.08 percent L-threonine brought significant improvement of the protein quality of CSF tested by the PER assay. This improvement in the quality did not reach to the same level as that of the casein. Fortification of lysine or threonine alone to CSF brought significant increase in the fasting plasma lysine concentration. Fortification of threonine alone to CSF significantly increased the fasting plasma threonine concentration in comparison to that of rats fed CSF without

any fortification. The plasma concentration of threonine of rats fed CSF with threonine fortification remained significantly lower than that of the rats fed the casein diet. The fortification of lysine and/or threonine to CSF did not significantly improve the concentration of plasma threonine of rats fed the fortified diet.

APPENDIX A

PREPARATION OF HYDROLYSATE OF DIETARY PROTEIN

1. Approximately 50 mg of the samples were placed in hydrolysate tubes.

2. Add 2 ml of 6 N HCl into the samples.

3. Evacuate the samples for 4-5 hours under ice/ methanol.

4. Hydrolyze the samples at 100 C for 24 hours.

5. Evaporate HCl in a vacuum desiccator with NaOH in it.

6. Add 2 ml of lithium citrate buffer (pH 2.2) into the dried samples, and mix.

7. Dilute the samples to have proper concentrations (about 1:50 dilution).

8. Filter the diluted samples through 0.45 μm pore size filters.

9. The filtered samples are ready to be run by the Beckman 121M Microcolumn Amino Acid Analyzer.

10. Amount of lysine obtained by this procedure is the total lysine.

APPENDIX B

SHEFFIELD PRODUCTS
CASEIN: A.N.R.C. REFERENCE PROTEIN

CASEIN: A.N.R.C. REFERENCE PROTEIN

DESCRIPTION

Casein has been approved by the Animal Nutrition Research Council (A.N.R.C.) as a reference protein for use in biological assays for determining the nutritive value of protein-containing materials. This grade of casein is a consistent, high quality product designed for use in such assays.

TYPICAL ANALYSIS

Moisture	7.0%
Ash (phosphorous fixed)	2.05%
Acidity (as lactic acid)	0.10%
Protein (N x 6.38)(dry basis)	95.0%
Ether extractables (Mojonnier)	1.5%
Carbohydrate (as lactose)	0.25%
Particle size	98% through 30 mesh

MINERAL CONTENT (Estimated)

Magnesium	0.05%
Manganese	<0.2 ppm
Iron	20 ppm
Copper	7 ppm
Zinc	30 ppm
Mercury	<1.0 ppm
Sodium	<0.02%
Potassium	<0.02%
Cobalt	<0.1 ppm
Phosphorous	0.8%
Calcium	0.06%
Chloride	0.5%

VITAMIN CONTENT (Estimated)

Biotin	2 mμg/g
Folic acid	31 mμg/g
Niacin	0.5 μg/g
Pantothenic acid	0.4 μg/g
Pyridoxine	0.4 μg/g
Riboflavin	75 μg/g
Thiamin	0.1 μg/g

P. O. Box 630, Norwich, New York 13815
(607) 334-9951, TELEX 646056

AMINO ACID CONTENT
(Typical)

Alanine	2.8%
Ammonia	1.2%
Arginine	3.6%
Aspartic acid	6.5%
Cystine	0.3%
Glycine	1.7%
Glutamic acid	20.8%
Histidine	2.5%
Isoleucine	4.8%
Leucine	8.8%
Lysine	7.4%
Methionine	2.5%
Phenylalanine	4.8%
Proline	12.1%
Serine	5.6%
Tryptophan	1.1%
Threonine	3.9%
Tyrosine	5.1%
Valine	5.6%

Data presented are typical. Slight variation may occur from lot to lot.

CAS:503
Revised October 1977 and September 1978

APPENDIX C

PROXIMATE COMPOSITION OF DEFATTED COTTONSEED FLOUR ANALYZED BY POPE TESTING LABORATORIES

POPE TESTING LABORATORIES, INC.

CONSULTING ANALYTICAL CHEMISTS
AND TESTING ENGINEERS

FOODS, FEEDS, DAIRY PROD.
WATER, MISCL. ANALYSES
COTTON SEED PRODUCTS
PACKING HOUSE PRODUCTS
SEED GERMINATION
FERTILIZERS

P. O. BOX 903
DALLAS, TEXAS
75221

OFFICIAL CHEMISTS
WEIGHERS AND INSPECTORS
NATL. COTTONSEED PRODUCTS ASS'N.
NATL. SOYBEAN PROCESSOR'S ASS'N.
REFEREE CHEMISTS
AMERICAN OIL CHEMISTS SOCIETY

TO Texas Woman's University
Denton, Texas

File No.

Date Rec'd 3-31-82

Report of Tests on Defatted Cottonseed Flour

Received from Dr. Andie Hsueh

Identification Marks Sample #1

Moisture	6.5%
Protein	55.63
Fat	2.62
Fiber	3.8
Ash	6.37
Carbohydrates	25.08
Free Gossypol	0.02

Remarks

LAB NO. 30688

POPE TESTING LABORATORIES, INC.

By Flora Hunter

F-50—40M

POPE TESTING LABORATORIES, INC.

CONSULTING ANALYTICAL CHEMISTS
AND TESTING ENGINEERS

FOODS, FEEDS, DAIRY PROD.
WATER, MISCL. ANALYSES
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TO Texas Woman's University
Denton, Texas

File No.

Date Rec'd 3-31-82

Report of Tests on Defatted Cottonseed Flour

Received from Dr. Andie Hsueh

Identification Marks Sample #2

Moisture	6.7%
Protein	55.72
Fat	2.62
Fiber	4.1
Ash	6.45
Carbohydrates	24.41
Free Gossypol	0.02

Remarks

LAB NO. 30689

POPE TESTING LABORATORIES, INC.

By Flora Hunter

F-50-40M

POPE TESTING LABORATORIES, INC.

CONSULTING ANALYTICAL CHEMISTS
AND TESTING ENGINEERS

FOODS, FEEDS, DAIRY PROD.
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OFFICIAL CHEMISTS
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NATL. COTTONSEED PRODUCTS ASS'N.
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REFEREE CHEMISTS
AMERICAN OIL CHEMISTS SOCIETY

TO Texas Woman's University
Denton, Texas

File No.

Date Rec'd 3-31-82

Report of Tests on Defatted Cottonseed Flour

Received from Dr. Andie Hsueh

Identification Marks Sample #3

Moisture	6.6%
Protein	55.50
Fat	2.70
Fiber	4.1
Ash	6.72
Carbohydrates	24.38
Free Gossypol	0.02

Remarks

LAB NO. 30690

POPE TESTING LABORATORIES, INC.

By Flora Hunter

F-50—40M

AVERAGE PROXIMATE COMPOSITION OF DEFATTED GLANDLESS COTTONSEED FLOUR

Ingredient	Average of three samples (%)
Moisture	6.50
Protein	55.63
Fat	2.62
Fiber	4.00
Ash	6.37
Carbohydrate	24.62
Free gossypol	0.02

APPENDIX C

NON-ESSENTIAL AMINO ACID (g/16g N) COMPOSITION OF DEFATTED GLANDLESS COTTONSEED FLOUR (CSF) AND ANRC CASEIN

Amino acid	ANRC Casein	CSF
Aspartic acid	7.19	8.59
Serine	5.94	4.11
Glutamic acid	21.81	17.67
Proline	11.13	4.20
Glycine	1.62	3.89
Alanine	3.45	4.08
Cystine	1.24	1.52
Tyrosine	5.74	2.77

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