THE USE OF COTTONSEED FLOUR IN VEGETABLE PROTEIN MIXTURES FOR HUMAN FEEDING
I. BIOLOGICAL STUDIES[1]

by

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The numerous surveys carried out in many of the Latin American countries have shown that corn consumed in many different forms is the most important staple food for the rural

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populations of the area. Table I shows the average consumption of corn in several countries in Central America and the average amount of protein and of calories contributed by the cereal grain to the rural diet. It is evident from the figures that corn provides significant amounts of the two nutrients to the daily rural diet. It is also well known that the nutritive value of the proteins of corn is extremely poor so that the actual amount of protein available for growth and maintenance is much less than that indicated by the total amount ingested. It is therefore of great practical importance to enrich corn diets so that they will provide better quality and a larger quantity of protein.

Table I
Daily Quantities of Corn Consumed in Rural Areas in Central America Per Person [1]

	Weight g.	Calories	% of Total Calories	Protein g.	% of Total Protein
Costa Rica	185	635	34	15	32
Nicaragua	300	1030	57	24	40
Honduras	398	1370	69	32	48
El Salvador	374	1286	65	30	58
Guatemala	423	1456	64	34	49

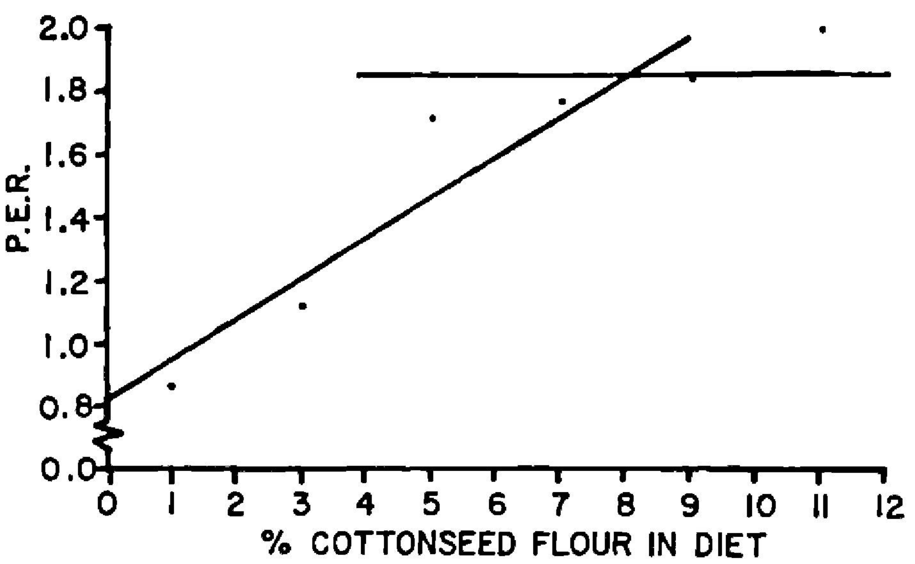
[1] Data Kindly Supplied by Miss Marina Flores, INCAP.

The protein enrichment of corn with cottonseed flour has been approached in two ways. One has been to find the minimum amount of cottonseed flour needed to bring about a maximum improvement in the nutritive value of corn proteins. The second approach, which is one of wider applicability in human nutrition, has been the enrichmant of corn and other cereals with cottonseed flour in the form of vegetables mixtures.

An example of the use of cottonseed flour for the enrichment of lime-treated corn flour is shown in Figure 1. This experiment was carried out with young rats. To a 79% lime-treated corn flour-basal diet, levels of 1 to 11% cottonseed flour were added. The figure shows the PER obtained at each level of cottonseed flour supplement. It is evident that maximum improvement was obtained with about 8% cottonseed flour in the diet.

In Table II the improvement in nutritive value of lime-treated corn with cottonseed flour is compared to the improvement obtained by adding other protein rich foods of both

SUPPLEMENTATION OF LIME-TREATED CORN WITH COTTONSEED FLOUR



animal and vegetable origin. In general, all protein rich foods used increased the nutritive value of the proteins of lime-treated corn. A higher improvement with smaller additions is evident in the animal protein group of supplements, although the vegetable protein supplements, including cottonseed flour, brought about a significant increase in the nutritive value of lime-treated corn proteins as judged by the PER.

Table II
Amount of Protein Rich Foods, Found Optimum For Supplementing Lime-Treated Corn

Protein Rich Foods	Amount Found % of Diet	Protein Efficiency Ratio
None	------	1.00
Egg Protein	3.0	2.25
Casein (V.F.)	4.0	2.21
Meat Flour	4.0	2.34
Fish Flour	2.5	2.44
Soy-bean protein	5.0	2.30
Soy-bean meal	8.0	2.25
Cottonseed flour	8.0	1.83
Torula yeast	2.5	1.97
Pumpkin seed flour	5.5	1.73

As mentioned previously, another approach to the problem of the improvement in the nutritive value of lime-treated corn has been through the development of protein rich vegetable mixtures (1,5,6).[2] It is now widely accepted that an important and practical approach to supplying the needed dietary protein

[2] Figures in parentheses refer to References at end of this article.

in areas where milk and other products of animal origin are costly or in short supply is the development of suitable combinations of vegetable protein sources to supply both essential and non-essential amino acids in the quantity and proportions required. The first of the vegetable protein mixtures developed by INCAP to receive extensive biological testing is known as Vegetable Mixture No. 8 (1,2,3). It consists of lime-treated corn 50% sesame flour (33% fat) 35%, cottonseed flour 9%, Torula yeast 3% and Kikuyu leaf meal 3%. This mixture contains approximately 25% crude protein with a protein score of 67% based on the FAO amino acid reference pattern (4).

Although in chicks, rats, and children (1,2, 3,9) Vegetable Mixture No. 8 proved to be good protein source, it was not sufficiently economical for the Central American area because of the short supply of sesame seed. A less expensive mixture eliminating sesame flour was developed using cottonseed flour as the vegetable protein concentrate. The relative nutritive quality of cottonseed flour as a substitute for sesame flour was first studied as part of the development of INCAP Vegetable Mixture 9. The results of a representative chick growth experiment are shown in Table III. Cottonseed flour was added to replace sesame flour isoproteically. It can be seen that the growth of the chicks and the feed efficiencies after 35 days on trial improved as

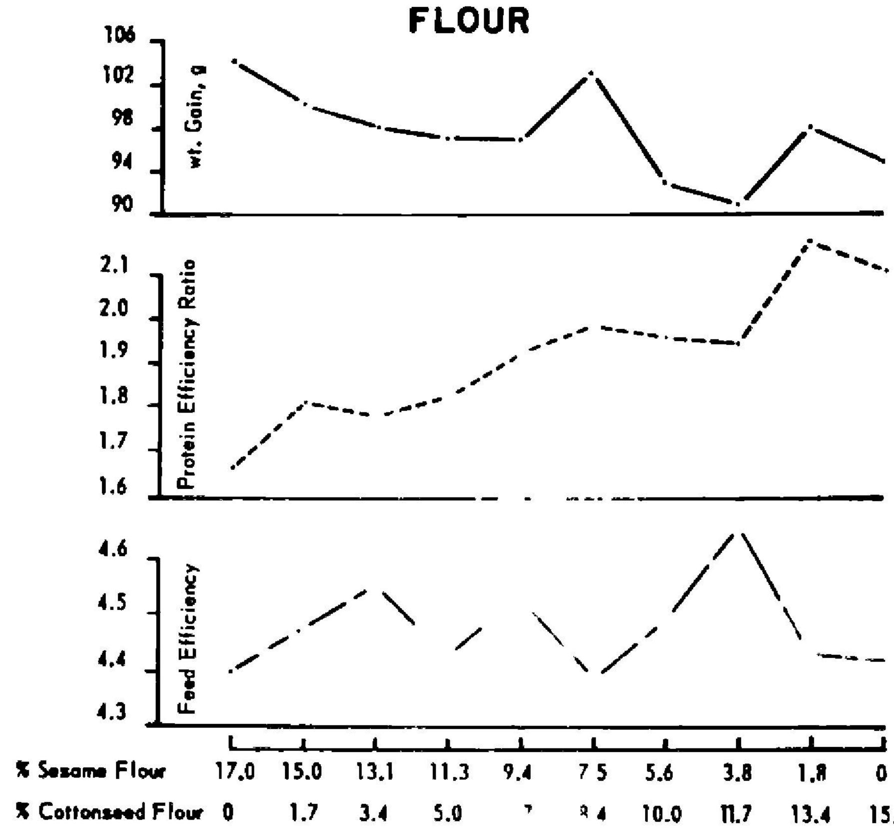
Table III
Substitution of Sesame Flour by Cottonseed Flour [1,2]

Percentage in Diet Sesame Flour	Percentage in Diet Cottonseed Flour	Final Weight g [3]	Feed Efficiency [4]
34.00	0	235	2.97
30.00	3.43	225	3.08
26.25	6.75	253	2.86
22.50	10.07	260	2.84
18.75	13.39	283	2.86
15.00	16.71	244	3.46
11.25	20.03	312	2.86
7.50	23.35	336	2.71
3.75	26.67	342	2.58
0	30.00	337	2.69

No of Chicks/group, 12.
Percentage protein in Diet, 20.4
Experimental period 35 days.
[4] Average feed consumed/Average weight gained.

cottonseed flour replaced sesame flour. From this and other experiments (7,8), it was concluded that the nutritive value of cottonseed flour protein for this purpose is higher than that of sesame flour, presumably due to a higher lysine content in cottonseed flour. Results of a similar experiment carried out with young growing rats are shown in Figure 2. The gain in weight and feed efficiencies were similar in all groups as cottonseed flour protein replaced sesame flour. Protein efficiencies, however, improved from 1.62 when the corn based diet contained sesame flour to 2.10 when it contained only cottonseed flour. Again it was evident that properly processed cottonseed flour is a good protein supplement for corn proteins and somewhat superior to sesame flour.

SUBSTITUTION OF SESAME FLOUR BY COTTONSEED FLOUR



In order to determine the optimum protein combination between corn and cottonseed flour protein, experiments were carried out in chicks and young rats in which the protein of the diets was contributed by various combinations of the two foods. The results of a representative chick trial are shown in Table IV. Gain in weight and feed efficiencies were superior when 15-25% of the protein of the diet was from corn and 85-75% from cottonseed flour In Figure 3, the results in young rats are described. The bars in the lower part of the figure represent the dietary protein distribution. The curves from top to bottom are gain in weight, feed and protein efficiencies, re-

Table IV
Optimum Protein Combination Between Corn and Cottonseed Flour in Chicks

10 Chicks/group

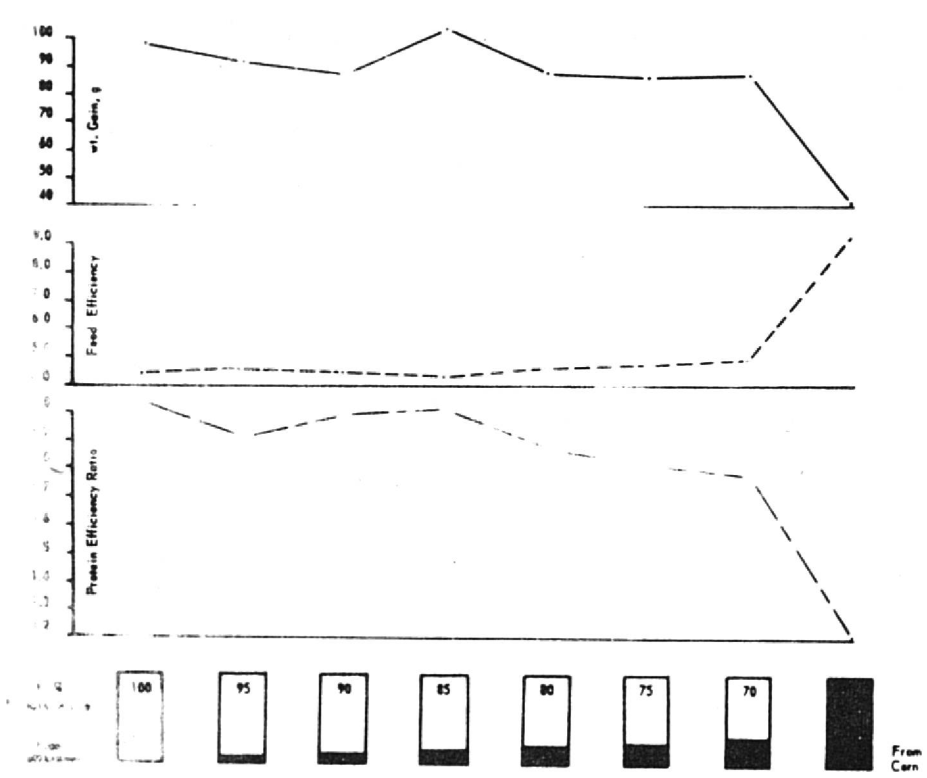
% Protin Distribution in Diet [1]		Weight Gained g. 28 days	Feed Efficiency [2]
Corn	Cottonseed Flour		
10.3	89.7	228	2.19
15.3	84.7	194	2.08
20.3	79.7	284	1.92
25.4	74.6	307	2.00

[1] Percentage protein in the Diet 22.5.
[2] Average Feed Consumed/Average Weight Gained.

spectively. It can be noticed that better gains in weight and higher protein efficiencies are obtained when corn and cottonseed flour provide 15 and 85% of the protein of the diet. It is also apparent that when cottonseed flour contributes less than 70% of the protein of the diet, gain in weight, protein efficiency and feed efficiency decrease (7).

The essential amino acid pattern of the 15 to 85% protein corn-cottonseed flour mixture is shown in Table V. The supplementary value of cottonseed flour in the proportions found experimentally are evident. Comparison with the FAO pattern (4) reveals deficiencies in isoleucine, lysine, total sulphur, amino acids and tryptophane. Addition of cottonseed flour to corn improves but does not wholly correct the lysine and tryptophane deficiency in the cereal grain according to the FAO pattern. The amino acid proportions in the 15 and 85% protein mixture, using tryptophane as the base, are similar to those of the FAO pattern.

COMBINATIONS BETWEEN COTTONSEED FLOUR AND CORN PROTEINS



From experimental results of this type and corn and cottonseed flour, INCAP Vegetable Mixture 9 was formulated. The corn-cottonseed protein combination chosen for Vegetable Mixture 9 was the one in which corn provides 20% of the protein and cottonseed flour 80%. This meant that the maximum protein content in the final mixture would be from 26 to 28%. Vegetable Mixture 9 was then formulated as follows in percent: 56 whole ground corn, 38 cottonseed flour, 3 Torula yeast and 3 dehydrated leaf meal. Experimental results also indicated that sorghum grain could replace all or part of the corn (3,8), and to lower its cost, the experimental formula was made of 28% whole ground corn, 28% ground sorghum grain, 38% cottonseed flour, 3% Torula yeast and 3% dehydrated leaf meal.

This formula was then subjected to extensive testing in chicks, rats, dogs, and pigs (10,11,12). A representative chick trial is shown in Table VI. Chick growth and feed efficiencies are similar when the mixture is made with 28% corn and 28% sorghum, with 56% corn or with 56% sorghum. The response is 82% of that obtained with a commercial chick feed containing animal protein. As shown in the lower part of Table VI, supplementations with lysine significantly improved

Table V
Essential Amino Acid Patterns

Amino Acid	Corn	Cottonseed Flour	15-85 Protein Combination Corn-Cottonseed	FAO Ref. Prot.
		mg/g N		
Arginine	262	719	647	......
Histidine	231	113	132	......
Isoleucine	213 [1]	231 [1]	228 [1]	270
Leucine	572	413	437	306
Lysine	126 [1]	256 [1]	235 [1]	270
Methionine / Cystine	189 [1]	169 [1]	172 [1]	270
Phenylalanine	276	294	291	180
Threonine	214	294	281	180
Tryptophan	32 [1]	75 [1]	68 [1]	90
Valine	281	331	323	270

[1] Limiting Amino Acids.

growth of the chicks fed Vegetable Mixture 9 and methionine addition had a small effect of doubtful significance.

Table VI
Representative Chick Growth Trial With INCAP Vegetable Mixture 9'

(35 DAYS—20 CHICKS PER GROUP)

Diet	Protein in Diet %	Final Weight g.	Feed Efficiency
V.M.9'	23.5	479 [1]	2.31
V.M.9' with 56% Corn	23.8	460 [1]	2.25
V.M.9' with 56% Sorghum	24.1	479 [1]	2.27
"Ace-Hi"	23.9	587 [1]	2.01
V.M.9'	23.0	310 [2]	2.45
" + 0.3% DL-Met	23.0	361 [2]	2.26
" + 0.2% L-Lys HC1	23.0	472 [2]	2.14
" + both A.A.	23.0	490 [2]	2.04

[1] 55 g. initial weight. [2] 45 g. initial weight

Since other cereal grains are more important staple foods in other regions of the world than corn and sorghum, the results of a chick experiment fed Vegetable Mixture 9 in which the corn-sorghum combination was substituted for several other cereal grains, are shown in Table VII. In general, both the growth of the chicks and the feed efficiencies in 35 days were very satisfactory with these other cereals and rice gave the best results. It can be concluded that other cereals can be used in the formula of Vegetable Mixture 9 without lowering the nutritive value of the mixture.

A representative rat growth experiment is shown in Table VIII where Vegetable Mixture 9 was fed at five levels of protein in the diet and the response compared with that obtained by feeding five levels of protein from casein.

Table VII
Substitution of Yellow Ground Corn In Vegetable Mixture 9 By Other Cereal Grains

Cereal Grain in V.M.9'	% Protein in Diet	Final Weight [2] g.	Feed Efficiency [3]
Yellow Corn	24.5	399	2.17
Wheat Flour	25.3	357	2.32
Barley	25.9	420	2.27
White Rice	24.7	426	2.07
Oats	24.7	383	2.30
Whole Wheat	25.8	380	2.41

[1] Chicks per group, 17.
[2] Experimental period, 35 days.
[3] Average feed consumed/Average weight gained.

Table VIII
Representative Rat Growth Experiment

(21 DAYS—6 RATS/GROUP)

Protein in Diet %	Average Weight Gain g. V.M.9	Average Weight Gain g. Casein	Protein Efficiency [1] V.M.9	Protein Efficiency [1] Casein
5	22	22	1.88	2.14
10	66	67	2.30	2.38
15	104	105	2.11	2.31
20	114	117	1.75	2.00
25	115	121	1.47	1.65

[1] Ave. Wt. Gain/Ave. Protein Consumed.

Weight gains in 21 days were similar for both proteins, however, the protein efficiency ration of casein was higher than the PER of the vegetable mixture, particularly at low protein levels in the diet. A representative rat protein-repletion study is shown in Table IX. Good repletion weights were observed in the rats fed Vegetable Mixture 9 at the 10% protein level in the diet and whether the mixture was made with 56% corn, 56% sorghum or 28% corn plus 28% sorghum grain. The response from all diets was similar to that obtained with skim milk fed at iso-proteic levels. The results presented in Table X show the effect of supplementing Vegetable Mixture 9 at the 10% level of protein in the diet, with lysine and methionine added alone and measured in the protein-depleted rat. It is evident that addition of 0.20% L-lysine HC1 improved repletion weight gains while higher lysine levels did not. Methionine addition at any level tested did not improve the nutritive quality of Vegetable Mixture 9.

Representative tests with young growing dogs are given in Table XI. This table shows

Table IX
Representative Rat Repletion Trial With INCAP Vegetable Mixture 9'

(10% PROTEIN IN DIET, 6 RATS PER GROUP)

Mixture	Ave. Wt. Gain in 15 days g.
V.M. 9' (56% Corn)	63
V.M. 9' (28% Corn, 28% Sorghum)	61
V.M. 9' (56% Sorghum)	69
Skim milk	66

Table X
Representative Rat Repletion Study
Amino Acid Supplementation of
INCAP Vegetable Mixture 9
(10% PROTEIN IN DIET, 6 RATS PER GROUP)

A.A. Added	g%	Repletion Gain in 14 days
		g.
None	0.0	51
L-Lysine HC1	.1	60
L-Lysine HC1	.2	71
L-Lysine HC1	.3	62
DL-Methionine	.1	53
DL-Methionine	.2	51
DL-Methionine	.3	53

the biological value of Vegetable Mixture 9 as compared with casein. The study was carried out by feeding the dogs from 3.0 to 4.5 gm. of protein per K. of body weight per day of either casein or Vegetable Mixture 9. A nitrogen-free feeding period was also included to obtain data on endogenous urinary and metabolic fecal nitrogen to be applied in the formula of Thomas and Mitchell (13,14). The average biological value for casein was 78% while that for Vegetable Mixture 9 was 74%.

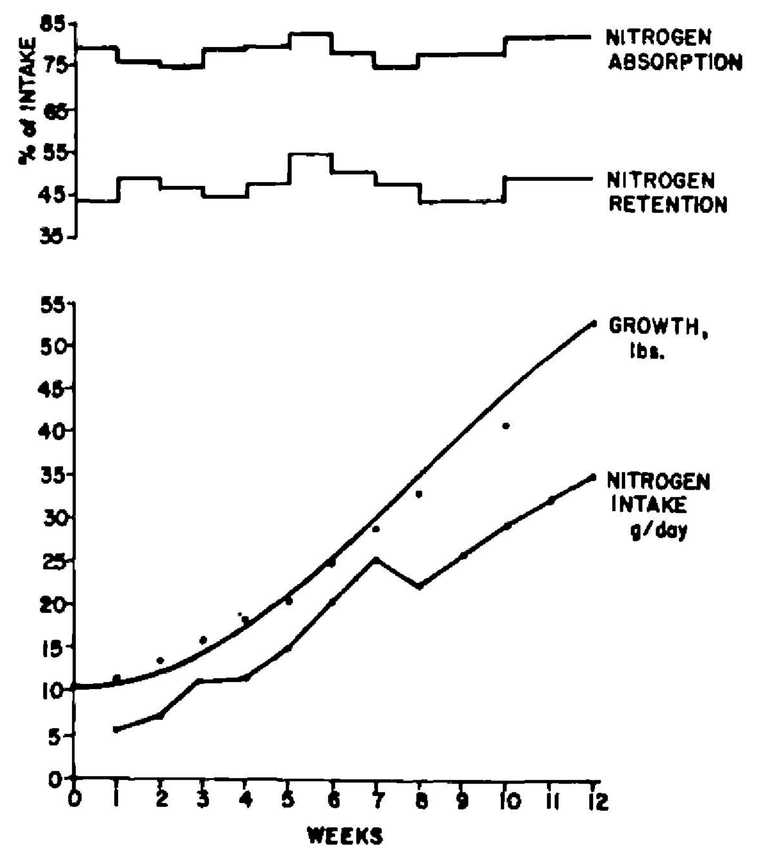
Table XI
Biological Value of Vegetable Mixture 9 and Casein

Dog No.	V.M.9' Protein Intake g/k/day	B.V.[1]	Casein Protein Intake g/k/day	B.V.[1]
7	3.52	76.3	3.81	78.1
8	3.29	71.0	3.92	73.4
9	4.04	76.0	4.02	78.4
11	4.48	70.7	4.43	82.1
Ave.	------	73.5	------	78.0

[1] $$B.V. = \frac{NI\text{-}(FN\text{-}MN) - (UN - EN)}{NI\text{-}(FN\text{-}MN)} \times 100$$

The mixture was tested further in young growing pigs. The results are shown in Figure 4. The pigs were fed Vegetable Mixture 9 cooked in water for half an hour in the proportions of one part solid to ten parts liquid. After cooking the vegetable mixture, minerals and vitamins were added to reduce moisture content and when cool it was fed to the pigs. The upper part of the figure shows the weekly percentage of nitrogen absorption and retention of nitrogen intake over a 12-week period. The lower part of the figure indicates the nitrogen intake in grams per day and the growth curve in pounds, respectively, over the 12-week period. Nitrogen absorption and retention were very consistent and high during the whole experimental period and the growth of the pigs was satisfactory. No signs of ferocity were noticed at any time.

REPRESENTATIVE NITROGEN BALANCE AND GROWTH OF PIGS FED VEGETABLE MIXTURE 9'



One of the most important factors limiting the use of cottonseed meals as a food for humans has been the presence of gossypol pigments and their effect on the quality of cottonseed protein. Since Vegetable Mixture 9 contains 38% cottonseed flour and has a gossypol content of .016%, it was of interest to find what effect cooking had on the pigment. Representative results of two experiments are shown in Table XII. In both experiments, samples were analyzed for free and total gossypol after cooking for 5,10,15,20, and 25 minutes. In the second and third column the gossypol content is given on samples that were cooked in the laboratory with constant stirring, using 20 gm. of mixture and 200 ml. of water. After cooking, the material was lyophilyzed and analyzed. Columns 4 and 5 show the results of cooking Vegetable Mixture 9 using the same ration of solids to liquids as before by determining the gossypol in the wet material immediately after cooking and cooling. Moisture determinations were then carried out and the values presented are on a moisture-free basis.

Table XII
Changes in Free and Total Gossypol of Vegetable Mixture 9 During Cooking

Cooking Time Minutes	V.M.9'[1] Gossypol Free mg/100g	Total g%	V.M.9B[2] Gossypol Free mg/100g	Total g%
0 dry	15.8	0.45	15.8	0.42
0 wet	17.3	.46	6.8	.43
5	15.0	.49	7.4	.35
10	12.9	.47	6.4	.46
15	11.4	.45	8.7	.42
20	8.8	.44	5.1	.38
25	10.2	.44	3.7	.42

[1] Analysis on freeze-dryed cooked sample.
[2] Analysis on cooked wet sample.

The results in columns 2 and 4 show that free gossypol decreases as cooking time increases, the drop being more marked in the samples analyzed in the wet state. It was noticed repeatedly that a drop in free gossypol occurred immediately after wetting the sample. Total gossypol did not change in either group of samples during cooking. The drop in free gossypol could be explained as the binding of the pigment to epsilon amino-lysine groups in the protein of the material. Moreover, it is also known that in many vegetable proteins as much as 20% of the total nitrogen is nonprotein in nature. It is thus theoretically possible that some of the lysine binding occurs with free amino acids or other nonprotein nitrogenous constituents.

One of the most important requirements in the development and use of low cost vegetable protein mixtures for human feeding in technically underdeveloped areas is that they can be formulated from materials grown and processed in the area where they are to be used. Cottonseed cake samples from mills in several of the Central American countries were analyzed to find one suitable for use in the Mixture 9 formula. A cottonseed meal produced in El Salvador by prepress solvent extraction operations met WHO-FAO-UNICEF Protein Advisory Group specifications in all essential respects. The screened flour incorporated in the formula of Vegetable Mixture 9 as the sole source of protein was tested biologically in chicks. Its performance was compared to that obtained from "Pro-flo" cottonseed flour fed in the mixture and alone. The results of several such experiments are shown in Table XIII. It is evident that the "Borgonovo" cottonseed flour from El Salvador, as judged from weight gains and feed efficiencies after an experimental period of 28 days, is nutritionally superior to "Pro-flo" flour when fed either in the vegetable mixture or as the only source of protein. The chemical analysis of the two flours given in Table XIV shows them to be similar in composition although the "Borgonovo" flour is slightly higher in both lysine (g./16g. N) and total gossypol. In the lower part of the table PER and FE values obtained from rat studies with the two flours are shown; they again indicate a higher nutritive value for the Salvadorenean cottonseed flour.

Table XIII
Evaluation of Borgonovo Cottonseed Flour Alone and in Vegetable Mixture 9

Trial No.	Cottonseed Flour	No. of Chicks Initial	No. of Chicks Final	Average Wt. g. Initial	Average Wt. g. Final[3]	F.E.[4]
1	Borgonovo[1]	10	10	55	246	2.73
	Pro-flo[1]	10	10	55	212	3.16
2	Borgonovo[1]	10	9	46	277	2.09
	Pro-flo[1]	10	10	46	222	2.49
3	Borgonovo[1]	10	10	46	258	2.05
	Pro-flo[1]	10	10	46	200	2.85
1	Borgonovo[2]	10	10	52	301	2.30
	Pro-flo[2]	10	10	53	233	2.68
2	Borgonovo[2]	10	9	46	317	2.00
	Pro-flo[2]	10	9	46	237	2.20

[1] In Vegetable Mixture 9'
[2] Tested Alone
[3] Experimental period 28 days.
[4] Average feed consumed/ Average Wt. gained.

Table XIV
Comparison of Pro-flo and Borgonovo Cottonseed Flour

Measurement	Pro-flo	Borgonovo
	Percent	
Moisture	3.17	11.19
Total Nitrogen	8.21	8.00
Protein (N x 6.25)	51.34	50.00
Ether Extract	5.21	4.25
Ash	6.61	--------
Crude Fiber	3.68	3.63
Soluble Nitrogen	57.4	73.8
Lysine (g/lb g N)	3.8	4.5
Free Gossypol	0.045	0.044
Total Gossypol	0.92	1.15
P. E. R.[1]	1.82	2.14
F. E.[1]	4.65	4.19

[1] As determined in INCAP

SUMMARY

As a partial solution to the protein malnutrition problem existing in Latin America and many other parts of the world, INCAP initiated in 1951 work on the development of all-vegetable protein mixtures intended primarily for the supplementary feeding of young children. Mixture 8, the first formula to be extensively tested, contained lime-treated corn 50, sesame flour 35, cottonseed flour 9, Kikuyu leaf meal 3 and Torula yeast 3%. This formula was found to have a good protein quality in tests with chicks, rats and children, but the price of the sesame in Central America was too high for practical use.

Experiments were then carried out to determine the optimum protein combination between corn and cottonseed flour. Studies in chicks and rats indicated that cottonseed flour could replace sesame flour in Mixture 8. The best combinations were those in which corn provided 15-20% and cottonseed from 80-85% of the protein of the mixture. Because of the relative abundance and low cost of cottonseed oil meal in Central America, a formula containing a larger amount of cottonseed flour was developed in 1958. It contained cottonseed flour 38, corn 28, sorghum 28, dehydrated leaf meal 3 and Torula yeast 3%.

This formula, identified as Mixture 9, was subjected to extensive biological testing. In chicks, the mixture produced good growth and feed efficiencies and no toxic effects were detected. Other cereal grains can replace totally or partially the corn and sorghum, but Torula yeast contributes toward the protein quality of the mixture, whose first limiting amino acid is lysine. Experimental results with rats also indicated the mixture to be of a quality comparable to milk, casein and meat flour. When fed at moderate protein levels, a deficiency is apparent which can be corrected by addition of 0.1% lysine and 3% of protein concentrates rich in this amino acid, added in place of the leaf meal. Two generations of rats were maintained without any apparent toxic effects.

Dogs were fed the mixture from 4 weeks of age up to 4 months. Biological value determinations in dogs average 74% for Mixture 9 and 78% for casein. Pigs were also fed the mixture from 5 weeks to 4 months of age with no apparent toxic effects. Free and total gossypol determinations on the cooked mixture indicated a decrease in free gossypol with cooking.

It is concluded that the mixture is of good protein quality, free of toxic effects, and suitable for human feeding. In all experiments reported, "Pro-flo" cottonseed flour was used. Recent experiments with a cottonseed flour produced by Borgonovo Hnos., El Salvador, gave slightly better results.

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DISCUSSION

Question: Did you measure available lysine in the cottonseed flours that you used?

Bressani: We did not measure available lysine by determining the free epsilon amine nitrogen. We did measure available lysine *in vivo* in chicks and concluded that for the cottonseed protein samples that we have, about 80% of the total lysine (as measured microbiologically) is available.

Aines: How was the Borgonova meal produced?

Bressani: It was produced by prepress solvent extraction.

Question Is the Borgonova cottonseed flour being used in your present mixtures?

Bressani: Yes. Dr. Scrimshaw is going to talk about this.

Question: With what experimental animals have you tested the Borgonova flour?

Bressani: We have tested it with dogs, rats, and chicks.

Question: What is the capacity of the Borgonova plant?

Paggi: The capacity of the Borgonova plant in Salvador is about 80 tons per day.