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Effect of Amino Acid(s) and Pulse Supplementation on Nutritional Quality of Normal and Modified *Opaque-2* Maize (*Zea mays* L.)

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Chemical analysis as well as rat feeding experiments indicate superior nutritive quality of modified *opaque-2* (*o2*) as compared with normal maize. Supplementation with lysine alone in modified *o2* and lysine + tryptophan in normal maize substantially improved the nutritive quality. Addition of Bengal gram (*Cicer arietinum*) to normal and modified *o2* maize in the ratio of 3:7 on protein basis improved their nutritional quality substantially and brought it at par to diet supplemented with amino acids. Diet protein quality affected growth, protein, and nucleic acids in rats.

Maize occupies an important place next to wheat and rice in the world food supply map. However, because of a deficiency of lysine and an excess of leucine, its nutritional quality is very low. The discovery of association of high lysine and tryptophan and low leucine with *o2* maize by Mertz et al. (1964) gave a major breakthrough and realization that maize proteins could be improved in quality by genetic manipulation. Although many new *o2* varieties have been developed, these have not found acceptability with the farmer and consumer mainly due to low yield, softness of the kernel, and susceptibility to insect attack during storage. Therefore, in the past few years breeders have developed modified *o2* maize with hard endosperm to overcome the agronomic and acceptability problems of original chalky *o2* maize. Chemical analysis of modified *o2* maize has shown improvement in nutritional quality as compared to normal maize.

However, the biological value of even modified *o2* is slightly lower than that of chalky *o2* (Gupta et al., 1979). Therefore, in the present study the effect of amino acid supplementation with lysine and tryptophan on nutritional quality has been studied. In addition the synergistic effects of addition of pulse to maize diet on nutritional quality has been studied because of the complementary nature of essential amino acid composition of pulses and cereals.

EXPERIMENTAL PROCEDURES

Sample Preparation. The maize varieties SO/SN composite (a modified *o2* hard endosperm composite) and Vijay (normal) were tested. The BG-206 variety of Bengal gram (*Cicer arietinum*) was used for pulse. For endosperm separation, kernels were soaked in distilled water at 4 °C for 3 h, then the pericarp was removed, and the endosperm and embryo were separated with scalpel, and the endosperm was collected. Dried endosperm ground to 100 mesh flour was defatted by Soxhlet extraction method.

Protein Fractionation. Protein fractionation of the defatted endosperm samples was done according to the method of Nagy et al. (1941). At each stage completeness of extraction was checked by measuring the absorbance of the last extract at 280 nm. Nitrogen was determined by micro-Kjeldahl method (AOAC, 1965).

Preparation of Isonitrogenous Diets. Normal, modified *o2*, and pulse isonitrogenous (1.5% N) diets were

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Table I. Basal Diet Composition for Normal Maize, Modified *Opaque-2* Maize, and Pulse (on moisture-free basis)[a,b]

	basal diet composition (g/kg of diet)		
diet constituents	normal maize diet	modified *opaque-2* maize diet	pulse diet
normal maize	781.2		
modified *opaque-2* maize		823.7	
Bengal gram			491.8
mineral mixture[c]	40	40	40
vitamin mixture[d]	16	16	16
N-free mixture[e]	162.8	120.3	452.2
total diet weight	1000	1000	1000

[a] Each rat received 10 g of diet containing 150 mg of N daily. [b] Protein (percent of dry matter): normal maize, 12.0; modified *opaque-2* maize, 11.38; and pulse, 19.06. [c] Forty grams of mineral mixture added per kilogram of diet consisted of: $CaCO_3$, 2.74 g; calcium citrate, 12.33 g; $CaHPO_4 \cdot 2H_2O$, 4.5 g; K_2HPO_4, 8.75 g; KCl, 4.99 g; NaCl, 3.08 g; $MgSO_4$, 1.53 g; $MgCO_3$, 1.41 g; ammonium ferric citrate (brown, 20.5–22.5% Fe), 0.61 g; $MnSO_4 \cdot H_2O$, 8.0 mg; $CuSO_4 \cdot 5H_2O$, 3.1 mg; KI, 1.6 mg; NaF, 20.3 mg; $AlNH_4(SO_4)_2 \cdot 12H_2O$, 3.6 mg. [d] Sixteen grams of vitamin mixture added to 1 kg of diet comprised of the following: vitamin A, 16 mg (~3200 IU); vitamin D_3, 128 μg (~240 IU); thiamin, 640 μg; riboflavin, 1.6 mg; nicotinamide, 6.4 mg; pantothenic acid, 1.6 mg; α-tocopherol, 320 μg; pyridoxine, 160 μg; potato starch (autoclaved), 15.97 g. [e] The composition of the N-free mixture is given in Table II.

Table II. Composition of Nitrogen-Free Mixture

sucrose	9.0%
cellulose powder	5.2%
groundnut oil	5.2%
potato starch (autoclaved)	80.6%

Table III. Protein, Lysine, and Tryptophan Content of Normal and Modified *Opaque-2* Maize

amino acid, g/16 g of N	normal	modified *opaque-2*	FAO/WHO, 1973, ref. amino acid pattern
lysine	2.42	3.87	5.50
tryptophan	0.48	0.71	1.00
protein (%)	11.57	11.00	
chemical score[a]	44.0	70.4	

[a] Chemical score is based on lysine as the first limiting amino acid and 5.5 g of lysine/16 g of N in 1973 reference amino acid pattern. Values given are averages of duplicate analyses which agreed closely.

Table IV. Protein Distribution in Endosperm of Normal (Vijay) and Modified *Opaque-2* (SO/SN Composite) Maize

protein fractions[a]	normal		modified *opaque-2*	
	%	mg/end.	%	mg/end.
water soluble	3.56	0.58	14.58	1.66
5% NaCl soluble	4.75	0.77	6.95	0.79
70% alcohol soluble	43.33	7.04	18.06	2.06
0.1% NaOH soluble	43.04	6.99	52.79	6.02
residue	5.32	0.86	7.62	0.87
total protein	9.19	18.89	7.91	13.37
total protein recov.	85.9		85.3	

[a] Percent of the total protein recovered. Values given are averages of duplicate extractions which agreed closely.

prepared according to the procedure described by Eggum (1973). In the pulse supplementation experiment, the diets were prepared using maize and Bengal gram flour in a ratio of 7:3 on protein basis. Deficient amino acid(s), individually or in combination, were added to the basal diets so as to meet the amino acid requirements of preschool children given by FAO/WHO (1973): lysine 5.5 g and tryptophan, 1.0 g/16 g of N. The composition of basal diet and nitrogen-free mixture is given in Tables I and II, respectively. The formulas for calculating true digestibility biological value, and other quality characters are

$$\text{t.d.} = \{[\text{N intake} - (\text{fecal N} - \text{metabolic N})] \times 100/\text{N intake}\}$$

$$\text{b.v.} = \{[\text{N intake} - (\text{fecal N} - \text{metabolic N}) - (\text{urinary N} - \text{endogenous N})] \times 100/[\text{N intake} - (\text{fecal N} - \text{metabolic N})]\}$$

$$\text{n.p.u.} = (\text{t.d.} \times \text{b.v.})/100$$

$$\text{u.p.} = [\text{n.p.u.} \times \text{N(in \% of dry matter)} \times 6.25]/100$$

Nitrogen Balance Study. Nitrogen balance of each sample was determined in four Wistar male growing rats by the method of Mitchell and Carman (1926) in a room maintained at 24 ± 1 °C as described by Lodha et al. (1976). Each rat received 10 g of dry matter containing 150 mg of N daily. The rats were arranged in groups of four in such a way that group means did not differ much. Each rat was housed in a perspex cage. The total experimental period of 9 days consisted of 4 days preliminary feeding, followed by a 5 days balance period in which pooled feces and pooled urine for each rat were analyzed for nitrogen.

To study the growth pattern of rats on different diets, the experiment was continued up to 12 days and weight of each rat was recorded on days 3, 6, 9, and 12. At the end of the experiment, the rats were sacrificed. Two hours before killing, their food was restricted. Rats were anesthetized by giving chloroform and quickly cut open; their livers and muscles were removed, weighed, and stored in liquid nitrogen until further analysis.

Determination of Protein, Lysine, and Tryptophan. Protein (N × 6.25) in maize samples, diets, liver, muscles, feces, and urine was estimated by the micro-Kjeldahl method.

Lysine and tryptophan in defatted samples were estimated by the colorimetric methods as described by Villegas and Mertz (1971) and Hernandez and Bates (1969), respectively.

Determination of Nucleic Acids in Liver. Total nucleic acids of liver were estimated from absorbance at 260 nm of the combined perchloric acid extracts assuming that 1 mg/mL of nucleic acid had $E_{260\text{nm}}^{1\text{cm}}$ of 30 units. DNA was estimated colorimetrically by the method of Burton (1967). RNA was estimated by subtracting DNA from total nucleic acids.

RESULTS

Protein, Lysine, and Tryptophan Contents. The results presented in Table III, indicate protein, lysine, and tryptophan contents in kernels as well as chemical score values based on lysine as the first limiting amino acid for normal and modified *o2*. Lysine and tryptophan levels are higher in modified *o2* compared to normal maize. The protein content in modified *o2* was slightly lower. As a result of increase in lysine in modified *o2*, the chemical score value showed 50% increase as compared to normal.

Protein Fractionation Study. Protein fractionation data of normal and modified *o2* maize endosperm (Table IV) indicate major difference in alcohol-soluble prolamine (zein) fraction and water-soluble albumin fraction. Modified *o2* had considerably less prolamine and higher albumin compared to normal maize. On comparing the absolute level of different protein fractions per endosperm, it is seen that the change in salt-soluble and alkali-soluble fraction on percent basis is mainly due to reduction in prolamine level.

Effect of Supplementation with Lysine, Tryptophan, and Pulse on Protein Quality. The results of nitrogen balance study are presented in Table V. True digestibility (t.d.) was not affected much by amino acid supplementation in normal maize. However, with modified *o2*, t.d. increased significantly on supplementation with pulse. The biological value (b.v.) of normal maize was increased by 5.7 and 35.5%, respectively, on supplementation with lysine and lysine + tryptophan. On supplementation of normal maize with pulse b.v. increased significantly and was comparable to that when lysine + tryptophan were added. The b.v. of modified *o2* was significantly higher compared to normal maize. Supplementation with lysine and lysine + tryptophan increased b.v. respectively by 21.1 and 22.8% of modified *o2* diet. Addition of pulse increased b.v. substantially and was similar to the diet supplemented with amino acids. One of the differences between normal and modified *o2* was in the increase in biological value on lysine supplementation alone. Lysine supplementation in normal increased b.v. only slightly while in the case of modified *o2* the increase was significant. Addition of tryptophan along with lysine to normal maize diet increased the b.v. significantly. These results indicate that in normal maize diet both lysine and tryptophan were limiting while in the case of modified *o2*, lysine is the only limiting amino acid. This is also reflected by the chemical analysis data given in Table III for normal maize.

The net protein utilization (n.p.u.) values also showed a trend similar to b.v. on supplementation with lysine,

Table V. Effect of Amino Acid(s) Supplementation on Normal and Modified *Opaque-2* Maize Protein Quality in Rats[a]

amino acid(s) supplement	true digestibility	b.v.	net protein utilization	utilizable protein
normal				
none	86.47 ± 4.26	58.83 ± 3.51	50.87 ± 7.97	6.13 ± 0.84
+ 0.45% L-Lys·HCl	86.53 ± 1.38	62.18 ± 2.96	53.83 ± 3.42	6.50 ± 0.28
+ 0.45% L-Lys·HCl + 0.06% DL-Trp	85.59 ± 1.66	79.71 ± 3.52	68.28 ± 3.68	8.19 ± 0.44
+ pulse[b]	89.32 ± 1.43	81.92 ± 2.57	73.23 ± 3.14	8.81 ± 0.37
modified *opaque-2*				
none	84.92 ± 3.44	68.71 ± 4.12	58.15 ± 1.81	6.63 ± 0.18
+ 0.22% L-Lys·HCl	87.42 ± 2.45	83.12 ± 3.08	72.77 ± 3.63	8.25 ± 0.41
+ 0.22% L-Lys·HCl + 0.03% DL-Trp	88.53 ± 0.47	84.37 ± 2.18	74.58 ± 4.37	8.50 ± 0.43
+ pulse[b]	93.38 ± 0.76	82.66 ± 2.59	77.19 ± 2.59	8.81 ± 0.26
pulse (alone)	90.96 ± 2.42	82.80 ± 1.26	75.30 ± 2.42	14.38 ± 0.46

[a] LSD for t.d. at 1%, 2.88; at 5%, 2.18; LSD for b.v. at 1%, 5.30; at 5%, 4.00. [b] These diets in this and subsequent tables were prepared using maize and Bengal gram (pulse) flour in the ratio of 7:3 on protein basis.

Table VI. Synergistic Effect of Pulse and Amino Acid(s) Supplementation to Normal and Modified *Opaque-2* on Weight Gain in Rats[a]

amino acid(s) supplement	total av wt gain (12 days)	wt gain/day	wt gain/g of feed consumed
normal			
none	12.09 ± 1.14	1.01 ± 0.09	0.15 ± 0.02
+ 0.45% L-Lys·HCl	12.25 ± 0.09	1.02 ± 0.02	0.15 ± 0.01
+ 0.45% L-Lys·HCl + 0.06% DL-Trp	23.87 ± 4.19	1.99 ± 0.35	0.24 ± 0.03
+ pulse	17.00 ± 3.87	1.42 ± 0.30	0.21 ± 0.03
modified *opaque-2*			
none	21.33 ± 2.78	1.78 ± 0.23	0.20 ± 0.06
+ 0.22% L-Lys·HCl	22.75 ± 1.36	1.90 ± 0.12	0.24 ± 0.02
+ 0.22% L-Lys·HCl + 0.03% DL-Trp	26.88 ± 4.64	2.24 ± 0.39	0.26 ± 0.03
+ pulse	24.67 ± 2.77	2.06 ± 0.20	0.24 ± 0.02
pulse (alone)	22.87 ± 2.51	1.91 ± 0.21	0.22 ± 0.01

[a] LSD for total average weight gain: at 1%, 4.70; at 5%, 3.55. Values given are averages of four experimental rats in each group.

Table VII. Average Fresh Liver Weight and Protein Content of Liver and Muscles in Rats Fed on Normal and Modified *Opaque-2* Diet with Amino Acid(s) Supplementation[a]

additions to basal diet[b]	fresh liver wt, g	protein in liver, %	protein in liver, mg/liver	protein in muscles, mg/g
normal				
none	3.73 ± 0.52	12.97	493	164.6
+ 0.45% L-Lys·HCl	3.83 ± 1.22	14.06	538	173.1
+ 0.45% L-Lys·HCl + 0.06% DL-Trp	3.85 ± 0.09	14.63	564	174.6
+ pulse	3.50 ± 0.24	15.06	527	261.0
modified *opaque-2*				
none	4.0 ± 0.07	13.84	554	158.4
+ 0.22% L-Lys·HCl	4.09 ± 0.35	14.59	591	169.6
+ 0.22% L-Lys·HCl + 0.03% DL-Trp	4.17 ± 0.71	14.66	611	172.3
+ pulse	3.54 ± 0.12	17.88	633	231.0

[a] LSD for fresh liver weight: at 1%, 0.53; at 5%, 0.40. [b] Basal diet as given in the Methods section. The values for liver weight are averages of four experimental rats in each group. Livers and muscles from all the rats of individual group were pooled and protein estimated by independent duplicate analyses, and the values from duplicates agreed closely. Values given for protein in liver and muscles are averages of the duplicate values.

lysine + tryptophan, and pulse. The increase in n.p.u. by addition of pulse was slightly higher to that obtained by supplementation with lysine + tryptophan in both normal and modified *o2* maize diet. The utilizable protein value, which takes into account both protein quantity as well as protein quality, also showed substantial improvement with amino acid supplementation as well as by addition of pulse to normal and modified *o2* diet. The utilizable protein value for modified *o2* was slightly higher than normal maize. The increase in utilizable protein as a result of supplementation either with amino acids or pulse was comparable in both normal and modified *o2*. The b.v. of pulse alone was higher compared to normal and modified *o2* diet. On comparing the b.v. after amino acid supplementation and supplementation with pulse in the ratio of 7:3 (maize:pulse on protein basis), the b.vs. were nearly comparable for normal and modified *o2*. These results indicate very clearly the synergistic effect of addition of pulse to normal and modified *o2* maize diet on nutritional quality.

Synergistic Effect of Pulse and Amino Acid Supplementation on Growth. The weight gain in rats on isonitrogenous normal and modified *o2* diets supplemented with amino acid(s) and pulse is shown in Table VI. The weight gain with modified *o2* was significantly higher compared to normal maize diet. Addition of lysine alone did not affect weight gain while addition of lysine + tryptophan resulted in 100 and 25% increase in weight gain with normal and modified *o2*, respectively. Addition of pulse alone to normal and modified *o2* diet also increased weight gain. However, the weight gain obtained with pulse addition was considerably lower compared to the weight gain with lysine + tryptophan supplementation. Feed conversion efficiency (g of weight gain/g of feed consumed) of modified *o2* and normal maize diets on supplementation with lysine + tryptophan was also higher.

Protein in Liver and Muscles. The results presented in Table VII show fresh liver weight and protein in liver and muscles in rats fed on normal and modified *o2* diet with and without amino acid(s) and pulse supplementation. Amino acid supplementation resulted in an increase in protein per liver. Liver weight and protein in muscle were also affected. Supplementation with pulse, however, resulted in a slight decrease in liver weight but on the other hand it increased protein levels both in muscles and liver. It is important to note that the maximum protein was

Table VIII. DNA and RNA Content of Liver in Rats Fed on Normal and Modified *Opaque-2* Diet with Amino Acid(s) and Pulse Supplementation[a]

additions to basal diet	total nucleic acid, mg/g	DNA, mg/g	RNA, mg/g	RNA, mg/liver
normal				
none	8.5	1.3	7.2	26.8
+ 0.45% L-Lys·HCl	9.4	1.3	8.1	30.9
+ 0.45% L-Lys·HCl + 0.06% DL-Trp	11.2	1.3	9.9	38.3
+ pulse	12.0	1.8	10.2	35.8
modified *opaque-2*				
none	8.7	0.9	7.8	31.1
+ 0.22% L-Lys·HCl	9.8	1.1	8.7	35.1
+ 0.22% L-Lys·HCl + 0.03% DL-Trp	12.2	0.9	11.3	47.2
+ pulse	12.6	1.7	10.9	38.5

[a] Livers from all the rats under each group were pooled and DNA and RNA content estimated by independent duplicate analyses. The values given are averages of duplicates which agreed closely.

obtained in muscles in rats fed on either normal and modified *o2* diet which was supplemented with pulse, whereas maximum protein in liver was obtained on supplementation with lysine + tryptophan in normal and pulse in modified *o2*. It is, therefore, quite evident that the quality of diet is of paramount importance in determining the liver protein as well as protein in muscles.

RNA and DNA Content in Liver. RNA/gram of liver as well as per liver and total nucleic acid per gram of liver were found to have a positive correlation with protein quality of diet, while DNA content was not affected by the improvement in protein quality with amino acid(s) supplementation (Table VIII). DNA content showed an increase when normal and modified *o2* diets were supplemented with pulse. Total nucleic acids and RNA per gram of liver did not differ much in rats fed on normal and modified *o2* diets.

DISCUSSION

Comparison of nutritional quality of normal and modified *o2* maize based on chemical analysis as well as rat feeding experiment in the present study indicated superiority of modified *o2*. Amino acid(s) supplementation as well as addition of pulse apparently improved the nutritional quality of both normal and modified *o2* substantially. The nutritional quality of normal and modified *o2* after supplementation with lysine + tryptophan was nearly comparable to each other. Addition of lysine alone improved markedly the nutritional quality of modified *o2*, while in the case of normal, the nutritional quality improved substantially only on addition of tryptophan along with lysine. These results indicate the limitation of both lysine and tryptophan in normal maize, while only lysine limitation in modified *o2*. Significant improvement in PER has been obtained by Bressani et al. (1969) on supplementation with lysine + tryptophan. The high response to lysine and tryptophan added together to normal maize obtained during present investigation compared to when added to modified *o2* maize could probably be due to more availability of niacin from modified *o2* compared to normal maize (Bressani et al., 1969). The synergistic effect of addition of pulse on improvement in nutritional quality of normal and modified *o2* has been clearly established. Improvement in nutritional quality of other cereal grains has been observed by several workers (Yang et al., 1961; Rosenberg and Culik, 1957; Bressani et al., 1969). With the improvement in protein quality of normal and modified *o2* maize due to amino acid(s) and pulse supplementation, growth rate as well as protein content of liver and muscle in rats increased apparently. Highly significant and significant positive correlation between b.v. and other characters like weight gain per day ($r = 0.827$) and protein content per liver ($r = 0.742$), respectively, has been observed (Table IX). This could be due to faster rate of protein synthesis. In vitro amino acid incorporation study carried out by Chinchalkar and Mehta (1978) showed that polysomes from *o2* maize fed rats liver were more efficient in protein synthesis than those from normal maize fed rat liver. RNA on per gram of liver basis was found to have highly significant positive correlation with b.v. ($r = 0.828$), while DNA content was not affected by the improvement in protein quality with amino acid(s) supplementation. The DNA content of an organ reflects the cell number (Winick, 1968) and does not change under normal conditions. However, an increased incorporation of labeled precursor into RNA in rats fed with an adequate protein diet has been reported by Girija et al. (1965). It is therefore apparent that protein quality of diet has marked influence on animal metabolism.

Table IX. Correlations for Some Protein Quality Characters of Modified *Opaque-2* and Normal Maize Diet

characters	correlation coeff.[a]
b.v. and wt gain/day	0.827**
b.v. and fresh liver wt	0.080
b.v. and protein content in liver, %	0.644*
b.v. and protein content in liver, mg/liver	0.742*
b.v. and RNA/g of liver	0.828**
b.v. and protein content in muscles, mg/g	0.460

[a] Significant (*), highly significant (**).

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