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BETTER QUALITY MAIZE PROTEIN

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The poor nutritional quality of cereal proteins has been a concern of nutritionists ever since Osborne and Mendel (1914a, b) demonstrated that the gliadin fraction of wheat is deficient in lysine and that zein, the alcohol soluble protein of maize, is deficient in both lysine and tryptophan. Nevertheless, as shown in Table 1, cereals continue to play a dominant role in the provision of protein both for human and animal consumption.

Table 1

World protein production (1963–4)
(Adapted from Mossberg, 1970)

Source	Protein, Million metric tons	
	Total production	Human consumption
Cereals	100,5	60,3
Oilseeds	24,4	8,0
Legumes	7,0	6,0
Other plant sources	14,5	9,0
Animal sources	31,3	23,7
TOTAL	177,7	107,0

It is not surprising therefore that the announcement, ten years ago, by Mertz, Bates & Belson (1964) that the lysine and tryptophan content of maize endosperm can be increased by the introduction of the opaque-2 gene, engendered great interest amongst nutritionists. In particular, those concerned with improving the nutritional status of underprivileged population groups for whom maize is the dietary staple, appreciated the opportunity afforded by this genetic breakthrough and breeding programmes aimed at the development of opaque-2 maize were initiated in several countries. The possibility of achieving the same result with other cereals, notably wheat and barley has also received active attention.

The problem of the poor quality of maize protein, and the improvement in nutritive value achieved by the introduction of the opaque-2 gene, have been the subjects of earlier reviews by the present author (Quicke, 1972, 1974; Quicke & Gevers, 1972), while the progress made in the Natal breeding programme for the introduction of the opaque-2 gene into local lines and hybrids has also been previously reported (Gevers, 1972, 1974). The present paper emphasizes the possible role of opaque-2 maize in animal feeding but some of the earlier material is presented by way of introduction in order to provide as complete a picture as possible of the status of opaque-2 maize at the end of the first decade of its development.

Improved protein quality in opaque-2 maize

The primary effect of the opaque-2 gene is a marked reduction (almost 50%) in the level of zein relative to the non-zein endosperm proteins, especially the glutelins (Table 2). The altered distribution of endosperm proteins results in a change in the balance of essential amino acids the most important effect being a virtual doubling of the levels of lysine and tryptophan in the maize kernel (Table 3).

Table 2

Effect of the opaque-2 gene on the major nitrogenous fractions in defatted maize endosperm. (Compiled from data reported by Jimenez, 1966; taken from Quicke, 1974)

Nitrogenous fraction	Percentage of total N	
	Normal maize	Opaque-2
5% NaCl and water-soluble fractions	4,7	17,1
0,2% NaOH-soluble (glutelins)	35,1	50,0
70% ethanol-soluble (zein)	47,2	22,8
Insoluble fraction	4,3	6,2
N not recovered	8,7	3,9

Table 3

Lysine and tryptophan content of normal and opaque-2 maize (Bauman & Mertz, 1972)

Amino acid	g/100 Protein	
	Normal	Opaque-2
Endosperm		
Lysine	1,5–2,5	3,0–4,0
Tryptophan	0,3–0,5	0,7–1,2
Whole kernel		
Lysine	2,0–3,0	4,0–5,0
Tryptophan	0,4–0,6	0,8–1,2

A further important change in amino acid composition is the reduction in leucine content of the kernel. This amino acid, which is abnormally high in maize endosperms, antagonizes the utilization of the niacin reserves in maize (Kies & Fox, 1972) and the lower leucine content of opaque-2 maize is considered to be an important factor in the improved availability of niacin observed with this type of maize (Bressani, Elias & Gomez-Brenes, 1969).

In view of the increasing emphasis being placed on the provision of sulphur-containing amino acids in the diet, it must be mentioned that in an early report by Nelson, Mertz & Bates (1965) the cystine content of endosperm proteins of opaque-2 maize was shown to be only half that of normal maize. It was pointed out however, that cystine values equal to or greater than those of normal maize had been obtained in other comparisons and examination of several analyses now available in the literature permits the conclusion that no marked loss of sulphur amino acids is incurred by the introduction of the opaque-2 gene. This point requires further careful evaluation.

As anticipated, the more favourable balance of essential amino acids in opaque-2 maize promotes better protein utilization. Most workers have used whole kernel meal for biological evaluation with the rat as test animal. Protein efficiency ratio (P.E.R.) is the criterion most generally employed. Two such studies on opaque-2 and normal maize have been carried out in this laboratory. In view of the lower food intake and, consequently, lower protein intake of the rats on the normal maize diets, and the adverse effect this may have on P.E.R.-values, nett protein ratios (NPR-values, Bender & Doell, 1957b) were also determined. The first study was also extended to include the determination of nett protein utilization (N.P.U.) by the carcass analysis method (Bender, & Doell, 1957a). The results are summarized in Table 4.

Table 4

Protein quality of opaque-2 (O) and normal (N) maize as determined in 8-day rat feeding trials. Diets consisted of whole kernel meal plus supplementary vitamins and minerals, and maize starch to adjust protein levels to approximately 9% (first trial) or 8% (second trial). (For experimental detail see Quicke & Gevers, 1972)

Protein source	PER	NPR	NPR x 16[a]	NPR x 17,5[b]	NPU by carcass analysis
First trial (1970/71 season)					%
MPF–1 (O)	2,32	3,68	61,1	67,9	29,5
MPF–2 (N)	0,27	2,30	36,8	40,3	44,6
MPF–3 (O)	1,75	3,40	54,4	59,5	61,2
MPF–4 (N)	0,73	2,68	42,9	47,0	51,5
Casein	1,84	3,62	57,9	63,4	68,7
Second trial (1971/72 season)					
MPF–5 (N)	n.v.[c]	2,89	46,2	50,6	–
MPF–6 (O)	1,78	4,38	70,1	76,7	–
MPF–7 (O)	1,60	4,25	68,1	74,4	–
MPF–8 (N)	n.v.	2,67	42,7	46,6	–
MPF–9 (O)	1,78	4,18	66,9	73,2	–

a Bender & Doell (1957b)

b Morrison (1964)

c n.v. = no value as test animals experienced a slight loss in weight

The superior quality of opaque-2 maize, when this cereal provides the only source of dietary protein is clearly evident. The data serve incidentally, to underline the difficulty of using PER-values in evaluating maize proteins. Although NPR-values are also subject to criticism (Morrison, 1964) they appear to give a fairer evaluation of normal maize relative to opaque-2 maize than do the PER-values. Following the suggestions of Bender & Doell (1957b) and of Morrison (1964) respectively, theoretical estimates of NPU were made by multiplying NPR-values by the factors 16 and 17,5. Comparison of the estimated NPU-values with those determined experimentally in the first trial shows that, as found by Morrison, the factor 17,5 gave the better estimate.

The super quality of opaque-2 maize for man has also been demonstrated in N-balance studies with children (Bressani, 1966) and young adults (Clark, Allen, Meyers, Tuckett & Yamamura, 1967; Young, Ozalp, Cholakos & Scrimshaw, 1971).

Opaque-2 maize in pig rations

Almost half of the maize crop in the United States of America is used in the feeding of pigs and the evaluation of opaque-2 maize in pig nutrition has received major emphasis particularly in the Mid-Western States. Most of the work has been done by the Purdue group (Pickett, 1966; Cromwell, Pickett & Beeson, 1967; Cromwell, Pickett, Cline & Beeson, 1969; Klein, Beeson, Cline & Mertz, 1971; Gipp & Cline, 1972). In all these studies, involving growing and/or finishing pigs, opaque-2 maize promoted better weight gains (generally a three-fold increase) and feed efficiency than normal maize when maize was fed as the only protein source in the diet. It is unfortunate that in most of the studies the protein content of the normal maize (8%) was considerably lower than that of the opaque-2 maize (11%), thereby complicating direct comparisons in terms of protein quality. In attempts to overcome this problem maize diets were made isonitrogenous either by adding a non-essential nitrogen source (Cromwell, Pickett & Beeson, 1967) or by diluting opaque-2 diets by the addition of glucose monohydrate (Klein *et al.*, 1971). The former procedure did not improve performance on the normal maize diet, but dilution of opaque-2 maize by the addition of glucose resulted in a reduction in weight gains. However, the diluted opaque-2 diet was still superior to the undiluted normal maize diet with 50% higher gains when tested at a level of 8% protein in the diet.

That the superiority of opaque-2 maize is primarily due to its higher lysine and tryptophan content was clearly shown by adding these amino acids to the normal maize diets. It is of interest that there was no response to the inclusion of synthetic lysine as the only amino acid supplement, but tryptophan alone gave a better response indicating that for pigs, tryptophan is the more limiting amino acid in normal maize. As expected, addition of both lysine and tryptophan to give dietary levels equal to those of the opaque-2 diets gave much better weight-gains although performance was still slightly below that of opaque-2 maize (Cromwell, Pickett & Beeson, 1967). In one experiment (Klein *et al.*, 1971) there was some evidence that lysine was still limiting in an opaque-2 maize sample with a protein content of 11,7%, confirming a similar report by Gallo, Jimenez & Maner, (1968). The best growth gains (0,53 kg/day) and feed efficiency (feed/gain 2,6) reported to date for amino acid supplemented maize diets appear to be those obtained with opaque-2 maize (11% protein) supplemented with ten essential amino acids to meet N.R.C. standards and made up to 12% crude protein by the further addition of glutamic acid (Klein *et al.*, 1971). Higher lysine and tryptophan levels may not be the only factor responsible for the improved performance of pigs on opaque-2 maize. As pointed out by Klein *et al.* (1971) the amino acids of opaque-2 maize are likely to be more available than those of normal maize as glutelin has been shown to be more digestible than zein while the zein/glutelin ratio is much reduced in opaque-2 relative to that in normal maize. Food intake is also considerably higher when opaque-2 replaces normal maize.

Cromwell, Pickett & Beeson (1967) studied the effect of replacing normal maize with opaque-2 maize in maize-soya bean meal diets. They concluded that opaque-2 maize is superior to normal maize when substituted on an equal weight basis at suboptimal levels of dietary protein. When diets were made isonitrogenous by adjustment of the levels of maize and soya bean meal comparable gains and feed conversions were obtained with normal and opaque-2 maize. A fuller investigation of maize-soya bean meal diets was carried out by Drews, Moody, Hays, Speer & Ewan (1969) who found that replacement of normal maize by opaque-2 maize effected a saving of approximately 5% soya bean meal, an important consideration as in the final analysis, economic factors will determine the acceptability of opaque-2 maize in pig feeding. In this connection it is to be noted that in the USA where much of the maize is grown for direct feeding to pigs on the farm it is accepted that savings in feed costs can only be achieved if yields of opaque-2 maize approach those of normal hybrids.

While most of the work has been concerned with growing and finishing pigs, Baker, Becker, Jensen & Harmon (1970) evaluated opaque-2 maize as sole source of dietary protein in gestation diets. These workers concluded that opaque-2 maize is a satisfactory source of protein for the gestating gilt. The opaque-2 diet contained only 9,7% crude protein however, and maize-soya bean meal diets with a higher protein content (12 to 20%) promoted better gains during gestation.

Possibly the most interesting application of opaque-2 maize in pig feeding is that carried out in Colombia and reported by Harpstead (1971). Although weight gains on diets containing opaque-2 maize as sole protein source are substantially less than maize-soya bean meal diets with 16% protein, it was realised that optimal diets are beyond the means of the small Colombian farmer because of the relatively high cost and limited availability in that country of high-protein supplements such as fish meal and soya bean meal. A feeding regimen based on opaque-2 maize was therefore sought which would promote as high a level of meat production as possible with a minimum of protein supplementation. It was found that an opaque-2 maize diet containing 10% protein without any supplementary protein could be used throughout gestation, as well as over the 156-day period from weaning to marketing. Supplementation with soya bean meal, to provide an additional 2% of protein to the diet, was introduced only during the 56-day lactation.

Opaque-2 maize in the feeding of poultry

Because of the higher protein levels (15 to 20%) required in poultry rations, most studies with chicks have involved the use of maize-soya bean meal diets. An important feature of such diets is that methionine and not lysine, is the first limiting amino acid for chicks and comparison of opaque-2 and normal maize in diets containing soya bean meal without methionine supplementation reflected adversely on the opaque-2 maize particularly at suboptimal levels of dietary protein (Cromwell, Rogler, Featherstone & Pickett, 1967).

When the diets were supplemented with methionine opaque-2 maize proved superior to normal maize at the lower protein levels (suboptimal). Disappearance of this effect at higher protein levels is ascribed to the increased amounts of lysine and tryptophan derived from the additional soya bean meal added to the diets. An amino acid suppementation experiment revealed that for normal maize-soya bean diets supplemented with methionine, lysine is the most limiting amino acid and, in contrast to the results obtained with pigs, addition of tryptophan alone elicited no response (Cromwell, Rogler *et al.* 1967).

The above findings were confirmed in a second series of experiments in which opaque-2 and normal maize were compared with a floury-2 hybrid (Cromwell, Rogler, Featherstone & Cline, 1968). An interesting aspect of this study was the demonstration that in the absence of added methionine floury-2 maize produced faster and more efficient gains than either the normal or the opaque-2 maize. This finding was attributed to the higher methionine content of floury-2 maize. When methionine was added to the maize-soya bean diets however, opaque-2 maize promoted the best response while the floury-2 diets were only slightly better than the normal maize controls. This was ascribed to the lower lysine content of floury-2 compared with opaque-2 maize.

A third study by the Purdue group (Fonesca, Featherstone, Rogler & Cline, 1970) compared the nutritional value of opaque-2 and normal maize in diets for laying hens. Egg production over an 8-month period was higher on the diets containing opaque-2 maize although eggs were smaller than those from hens fed the normal maize-soya bean meal diets. In one of the experiments, hens fed a 12,5% protein diet containing only high-protein (13,6%) opaque-2 maize, vitamins and minerals, produced as well as hens fed a normal maize-soya bean meal diet containing 15% protein.

In an attempt to obtain a more quantitative evaluation of the nutritional value of the proteins of opaque-2 maize for chicks, NPU-values were determined on locally bred material (du Preez, Gevers, Quicke & Gous, 1974). Three white-kernelled opaque-2 composites were compared with three normal white-kernelled lines and a commercial yellow maize meal. The results are summarized in Table 5 which also includes the corresponding estimated NPU values for rats obtained as NPR x 17,5. The superiority of opaque-2 maize is again apparent but the difference between the NPU of this type of maize and of normal maize is much smaller in the case of chickens than with rats. If the estimated NPU-values for rats are accepted, it is evident that this smaller difference is due to a much better utilization of the protein of normal maize by the chick than by the rat.

It must be emphasized that in all the work reviewed above, whole kernel maize meals served as the protein source for the various diets tested. Such studies may be expected to minimize differences between opaque-2 and normal maize owing to the inclusion of the good quality proteins of the embryo which are unaffected by the opaque-2 gene.

Table 5

Nett protein utilization (N.P.U.) values for chicks, of opaque-2 and normal white maize (whole kernel meal) and a commercial yellow maize meal. Estimated N.P.U.-values (NPR x 17,5) for rats (cf. Table 4) included for comparative purposes. (For experimental detail see du Preez et al., *1974)*

Protein source	Chicks	Rats
	N.P.U.	N.P.R. x 17,5
Opaque-2 maize		
MPF-6	74,6	76,7
MPF-7	70,6	74,4
MPF-9	68,7	73,2
Normal maize		
MPF-5	59,2	50,6
MPF-8	63,8	46,6
MPF-10	61,7	n.d.*
Commercial yellow meal	58,8	n.d.

* n.d. = not determined

The only available data on the nutritive value of maize endosperm appear to be those of Wichser (1966) who obtained NPR-values of 4,20 and 2,91 respectively for whole kernel meals prepared from opaque-2 and normal maize compared with values of 4,09 and 2,53 for the relevant endosperms. These results suggest that the nutritional advantages of feeding opaque-2 rather than normal maize will be greater when diets are based on commercially milled meals. On the other hand the decrease in total protein content associated with the removal of the germ is likely to be greater in the case of opaque-2 maize as the germ/endosperm ratio of this type of kernel is generally higher than in normal maize. The practical significance of these differences between whole kernel and degermed meals requires further investigation.

Conclusion

The beneficial effect of the opaque-2 gene on the nutritive value of maize is clearly established but its full exploitation awaits the solution of several related problems. In particular, short-comings in agronomic features, yield, resistance to disease and, in the case of maize for human consumption, acceptability to the consumer all require attention.

On the other hand, full exploitation of the advantages of opaque-2 maize in animal feeding can best be achieved by a concomitant increase in the protein content. This aspect is also receiving the attention of the plant breeders and the objective here is to produce an opaque-2 strain with 12% protein. The higher the protein content of the maize used, the greater the saving in terms of high-cost high-protein feeds.

The past decade has seen considerable advance in the breeding of better-quality maize and it is anticipated that the first S. African adapted lines may be released to breeders and growers within 3 to 4 years. While it cannot be claimed that opaque-2 maize will resolve all our problems in respect of provision of adequate dietary proteins for human and animal consumption, the progress made is encouraging and, in the face of continued effort by overseas workers at an increasing tempo, it is essential that a lively breeding and evaluation programme be maintained in this country.

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