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UTILIZATION OF PROTEIN FROM OPAQUE –2 MAIZE BY CHICKEN AND RAT

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OPSOMMING: BENUTTING VAN PROTEIN VAN OPAQUE –2 MIELIES DEUR KUIKENS EN ROTTE

Proewe met kuikens en rotte toon dat die netto benutting van protein (NPB) van Opaque –2 (0) mielies betekenisvol hoër is as die NPB waardes van normale (N) mielies. Altesaam sewe mielie variëteite is aan die toetse onderwerp en die drie variëteite wat die O geen besit, het die drie hoogste posisies van NPB waardes beklee. In die kuikenproewe is mieliemeel van heel pitte van die N variëteit met 0,013% 1-triptofaan en 0,04% 1-lisien aangevul en hierdie rantsoen was nie betekenisvol swakker as twee van die O variëteite nie. 'n Kommersiële N tipe geelmieliemeel wat in die proef ook met bogemelde peile van dieselfde aminosure aangevul is het ook nie betekenisvol swakker NPB waardes as twee van die O variëteite getoon nie, dog met verdubbeling na die aanvulling van beide aminosure het die geelmieliemeel nie betekenisvol van die beste O variëteit verskil nie.

SUMMARY:

Opaque –2 maize gave significantly better nett protein utilisation (NPU) values than normal maize varieties in trials performed with rats and chickens. Altogether seven varieties of maize were tested of which three carried the opaque –2 gene and these proved to rank first, second and third in NPU values. In the chicken trial normal white maize supplemented with 0,013% 1-tryptophan and 0,04% 1-lysine was not significantly different to two of the Opaque –2 maize samples. A commercial normal yellow maize fortified with similar levels of these amino acids held a similar position as the fortified N variety, however, supplemented with higher levels of these amino acids (double the former levels), yellow maize was not significantly different to the top Opaque –2 maize variety in the trial.

The Opaque –2 gene causes a marked alteration of the amino acid composition of maize endosperm protein. Mertz, Veron, Bates & Nelson (1965) were the first to demonstrate that the nutritional value of Opaque –2 maize is superior to normal maize in rat feeding trials. Subsequently Opaque –2 maize was shown to have higher nutritional value than normal maize for pigs (Cromwell, Pickett and Beeson, 1967), for the chicken (Cromwell, Rogler, Featherstone and Pickett, 1967; Cromwell, Rogler, Featherstone and Cline, 1968) and for laying hens (Fonseca, Featherstone, Rogler and Cline, 1970).

This gene was incorporated into South African lines (Gevers, 1970) and preliminary biological tests of two opaque –2 composites showed the whole grain to be decidedly superior in terms of rat growth and nett protein utilization to the grain of their normal counterparts (Quicke & Gevers, 1972).

The subsequent production, in larger quantities, of fresh strains of locally-developed whole-kernelled Opaque –2 maize prompted further biological evaluation of this type of maize. This paper reports the nett protein utilization of the new material as determined in preliminary experiments with rats and in more extensive tests with young chickens. Whole kernel meal of two opaque –2 top-cross hybrids and an Opaque –2 variety were compared with whole-kernel meals of 3 white strains, and a commercial yellow mealie meal. The effect of additions of lysine and tryptophan to the meal of one of the normal white varieties and to the yellow maize meal was also studied.

Procedure

Maize samples and diets

All maize meal were ground to pass a 1 mm sieve and, with the exception of the commercial yellow mealie meal, were prepared as whole-grain meals.

Rat diets Maize diets, made up to contain approximately 9% protein and N-free diet were prepared as previously described (Quicke & Gevers, 1972).

Chicken diets The general composition of the chicken diets which contained 92,5% maize meal is given in Table 1.

Table 1

General composition of experimental chick-diets

Item	Maize diets
Maize meal	92,50
Starch	0,01
Vitamin mixture *	0,44
Mineral mixture **	1,01
KH_2PO_4	0,60
$CaHPO_4$	1,44
$CaCO_3$	1,00
Sunflower oil	3,00
	100,00

* Provides

mg per kg ration: choline chloride 2000; niacin 50; thiamine HCL 25; riboflavin 16; Ca pantothenate 20; pyridoxin 6; folic acid 4; biotin 0,6; B_{12} 0,02; menadion 5; vitamin C 250; i.u. per kg ration: A 5000; D 800; E 12.

** Provides

g per kg ration; NaCl 6; $MgSO_4$ 3; Fe Citrate 0,5; $MnSO_4.H_2O$ 0,35; $ZnCO_3$ 0,2; $CuSO_4.5H_2O$ 0,3; KI 0,01; $Na_2MoO_4.H_2O$ 0,01; Na_2SeO_4 0,002.

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Table 2

Description of individual diets. All maize meals comprised 92,5% of diet as shown in Table 1 (T = top cross hybrid; V = variety)

Code	Type maize meal added	Amino acid supplement mg per 100 g diet		N-content of diet g/100 g	Lysine content of diet g/100 g
		L-tryptophan*	L-lysine**		
MPF–5	Normal white T	–	–	1,58	0,281
MPF–6	Opaque –2 white T	–	–	1,42	0,362
MPF–7	Opaque –2 white T	–	–	1,39	0,403
MPF–8	Normal white T (S.A. 15)			1,54	0,304
MPF–9	Opaque –2 white V	–	–	1,50	0,391
MPF–10	Normal white V	–	–	1,47	0,246
MPF–10–T_1L_1	"	13,84	51,79	1,47	0,288
MPF–10–T_2L_2	"	27,72	103,6	1,49	0,332
YM	Commercial yellow maize meal	–	–	1,46	0,228
YM–T_1L_1	" " " "	13,84	51,79	1,42	–
YM–T_1L_2	" " " "	13,84	103,6	1,41	–
YM–T_2L_1	" " " "	27,72	51,79	1,43	–
YM–T_2L_2	" " " "	27,72	103,6	1,44	0,320

* L-Tryptophan — Additions of 13,84 and 27,72 mg/100 g correspond to 0,013 and 0,026% of complete diet

** L-Lysine monohydrochloride — Additions of 51,79 and 103,6 mg/100 g correspond to 0,04 and 0,08% of complete diet

The N-free diet was prepared in the same way except that maize meal was replaced by starch giving a total starch content of 92,51 per cent. Table 2 provides a brief description of the maize samples tested, the amino acid supplements and the N- and lysine content of the complete diets.

Rat trial

Diets containing whole kernel meals MPF-5 to 9 and a corresponding N-free diet were each fed to a separate group of 4 albino rats in a single 8-day trial. Weight gains and daily feed intakes were recorded and the data used to evaluate protein efficiency ratio (PER) and net protein utilization (NPU) by the method of Bender & Doell (1957).

Chicken trial

Cornish Game x Plymouth Rock broiler chickens were raised to one week of age on a chicken starter diet. After discarding very heavy and very light birds, the remaining 280 chickens were split up into four weight classes. Each of the 13 experimental diets (Table 2) and the N-free control diet were fed in a randomised block design to 4 groups of 5 chickens, each of these groups being selected to represent one of the 4 weight classes, thus affording 4 replications per treatment. NPU values were determined in a 8 day feeding trial according to the method of Bender & Doell (1957) as recommended for chickens by de Muelenaere, Quicke and Wessels (1960), the N-content of each carcass being estimated from the moisture content using the regression equation of Wessels & Bundock (1968). The weight gains were also used to evaluate nett protein ratio (NPR) as described by Render & Doell (1957).

Chemical analyses

Nitrogen and protein (N x 6.25) were determined by the Kjeldahl prosedure and lysine was determined using a Beckman 120B analyser as described by Quicke & Gevers (1972).

Results and Discussion

Rat trial

The results of the rat trial (Table 3) clearly indicate the superiority of Opaque –2 white maize over normal white maize. The PER values of opaque kernels compared well with those (1,73 and 2,32) reported previously (Quicke & Gevers, 1972) as do the NPR-values although the latter are slightly higher for the opaque kernels than the earlier values (3,40 and 3,88). The PER values have been used in similar comparisons by a number of authors, but as pointed out by Quicke & Gevers, 1972, the poor intake

Table 3

Protein efficiency ratio (PER) and nett protein ratio (NPR) values of opaque –2 (O) and normal (N) white kernelled maize (whole grain) for growing rats

Maize sample		PER	NPR	Estimate NPU*
MPF–5	(N)	n.v.**	2,89	46,2
MPF–6	(O)	1,78	4,38	70,1
MPF–7	(O)	1,60	4,25	68,0
MPF–8	(N)	n.v.	2,67	42,7
MPF–9	(O)	1,78	4,18	66,8

* Estimated NPU = NPR x 16

** n.v. Negative values as rats did not gain weight

on normal maize diets is a distinct disadvantage and may result in an under evaluation of normal relative to Opaque –2 maize. This problem is emphasized in the present study in which the performance of the rats on the normal maize was so poor that it was not possible to evaluate the PER.

Notwithstanding the shortcoming of the Bender & Doell procedure NPR values offer a useful criterion for comparing the nutritive value of normal and opaque –2 maize. Following the suggestion of Bender & Doell (1957) NPU's were estimated from the NPR values by multiplying by the factor 16. These calculated values agree well with the NPU's of Opaque –2 and normal maize obtained by the carcass analysis procedure in the previous study (Quicke & Gevers, 1972).

Chick trial

The results of this trial are presented in Table 4, each value being the mean of 4 replications. The diets are arranged in descending order according to their respective NPU

Table 4

Nett protein utilization (NPU) and nett protein ratio (NPR) values for chicks, of opaque –2 and normal white kernelled maize (whole grain) and a commercial yellow mealie meal with and without lysine and tryptophan supplementation. Diets ranked according to NPU values. All values are means of 4 replicates

Diet code	NPU	NPR	Ranking NPU	Ranking NPR
MPF–6	74,59 (a)*	4,94	1	1
MPF–7	70,62 (ab)	4,76	2	3
MPF–9	68,69 (abc)	4,63	3	5
YM–T_2L_2	68,63 (abcd)	4,79	4	2
YM–T_1L_2	66,98 (bcde)	4,74	5	4
MPF–10–T_1L_1	64,67 (bcdef)	4,35	6	7
YM–T_1L_1	64,26 (bcdef)	4,40	7	6
MPF–8	63,79 (cdef)	4,00	8	11
YM–T_2L_1	62,02 (ef)	4,28	9	8
MPF–10–T_2L_2	61,71 (ef)	4,22	10	9
MPF–10	60,89 (ef)	3,93	11	12
MPF–5	59,18 (f)	3,77	12	13
YM	58,75 (f)	4,07	13	10

* Averages not followed by the same alphabetic character differ significantly from one another ($P \leq 0{,}05$).

values. MPF–6 a top-cross opaque –2 white hybrid had the highest mean NPU value while the commercial yellow maize meal had the lowest. Direct comparison of the white maize meals with the yellow maize meal is not strictly valid as the former include the germ with its more favourable amino acid balance, whereas the germ is excluded from the commercial meal. However, the three normal white maize meals (MPF –5,8 and 10) serve as valid controls and the NPU values of the Opaque –2 samples are significantly higher ($P \leq 0{,}05$) than those of these normal whole-kernel controls.

The results reported here are in agreement with findings of other workers such as Cromwell *et al.* (1967), who applied growth methods to show that the protein of Opaque –2 maize is of higher nutritional value than normal maize for chickens. The N.P.U. method, which is a modified nitrogen balance method and a test of greater sensitivity than growth studies, has to our knowledge, not been applied hitherto in nutritional comparisons of Opaque –2 and normal maize with chickens although it has been used, with similar results, in tests on rats (Quicke & Gevers, 1972). The present results also support the work of Young, Osalp, Cholakos and Scrimshaw (1971), who assessed the NPU value of Opaque –2 maize with human subjects. They concluded that, for human nutrition, Opaque –2 maize protein is comparable to some proteins from animal origin.

The growth data obtained with the chicks in this experiment were also used to estimate NPR values which are included in Table 4. These values are somewhat higher than the corresponding values obtained in the rat trial, this trend being noticeably more marked for the normal maize (MPF –5 and MPF –8) than for the three Opaque –2 meals (MPF –6, 7 and 9). It is noteworthy however that the order of ranking of these 5 meals is exactly the same irrespective of the criterion used, i.e. rat or chick NPR values, estimated rat NPU values, or chick NPU values by the carcass analysis procedure. Although the data are limited, there is a strong indication that growing chicks make better use of the protein of normal maize than do rats, so that differences in the nutritive value of Opaque –2 and normal maize are less marked, although still significant, when the chick is used as test animal.

The nutritional superiority of the Opaque –2 samples is largely attributable to the beneficial effect of the higher lysine and tryptophan levels in these samples. This is clearly substantiated by the results obtained with the diets in which the amino acids were added to yellow maize meal and to the whole-kernel normal white variety (MPF–10–T_1–L_1). The poor response to the addition of higher levels of these amino acids to this meal (diet MPF–10–T_2–L_2) is surprising however, and not readily explained. This diet contained 0.331 per cent lysine by analysis which is close to the lysine level in MPF–6 and higher than that of the most heavily supplemented yellow maize meal (YM–T_2L_2). In the absence of further data no definite conclusion can be reached but it seems that in some instances the beneficial effects of amino acid supplementation of maize meals may be subject to interference by complicating factors.

That part of the experiment in which the yellow maize meal was supplemented with two levels of lysine and tryptophan constituted a 2 x 2 factorial design with each treatment replicated four times. These results could therefore be subjected to further statistical analysis as summarized in Table 5. This analysis shows that while a doubling of the level of lysine supplementation from 0,04 to 0,08 (corresponding respectively to 2,8% and 5,6% of the dietary N) resulted in an improvement of nett protein utilization no such response was effected by increasing the level of tryptophan supplementation from 0,013 to 0,026% (corresponding respectively to 0,918% and 1,81% of the dietary N). It appears therefore that in future work of the amino acid supplementation of maize diets tryptophan additions amounting to more than 0,013% need not be considered although additional tryptophan may be required if lysine levels are increased above the levels used here.

An important feature of the data obtained in the chick trial was the demonstration of a highly significant ($P \leq 0{,}01$) effect of the initial mass of the chicks on NPU values, as revealed in the analysis of variance of the randomised block design. This finding raises the possibility that the breed of chicken used may influence the resulting NPU values especially when breeds differ as widely as the modern specialized breeds as for example broilers and layers. This aspect requires further investigation.

The shortcomings of the methods used in these studies are well recognised as is the difficulty associated with the assessment of protein quality of foods with such a low protein content. These difficulties notwithstanding, the three Opaque maize samples tested in this study were clearly superior to the normal maize meals investigated and these findings are in line with the results of most other workers in the field. It may be concluded therefore that Opaque –2 maize can make a useful contribution to the now acute problem of protein supplies for animal feeding. This contribution will be all the more valuable if the incorporation of the Opaque –2 gene goes hand-in-hand with the development of lines which also have an increased protein content.

Table 5

Effect of higher levels of lysine and tryptophan supplementation on the NPU value of yellow maize meal. Each value is a mean of 4 replicates, each replicate consisting of 5 chickens

		L-lysine		
		0,04%	0,08%	Means
L-tryptophan	0,013%	64,2599	66,9749	65,6174
	0,026%	62,0224	68,6299	65,3262
Means		63,1412	67,8024	65,4718

	Standard error	Least significant difference	
		$P \leq 0{,}05$	$P \leq 0{,}01$
For a mean in the body of the table	1,9570	4,4269	6,3605
For a marginal mean	1,3838	3,1303	4,4975

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