The relationship between amino acid and protein content of yellow maize

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A study was undertaken to determine the relationship between the amino acid and crude protein content of maize using samples of yellow maize from the four main maize-producing areas of South Africa. After determining moisture, crude protein and amino acid content, the data were subjected to statistical analyses to determine to what extent protein quality changes with crude protein content, and whether this relationship differs between the four areas. All 17 amino acids studied, when expressed as g amino acid/kg dry maize, increased with crude protein content. But when amino acid concentrations were expressed as g amino acid/16 g N only four (aspartic acid, threonine, methionine and histidine) did not change with crude protein content, four others (glycine, lysine, arginine and cysteine) showed a negative relationship, whilst the remaining amino acids increased in concentration as the crude protein content increased. Of economic and nutritional significance to the balanced feed industry are the relationships between isoleucine, leucine, lysine and arginine with crude protein content. Equations to predict the content of these amino acids from the amount of crude protein in maize are given. The remaining amino acids can be estimated without loss of accuracy from their mean value expressed as g/16 g N. Although the relationship between certain amino acids and crude protein differed between certain areas, it is unlikely that these differences are more than empirical statistical observations, so there is little scope in attempting to improve the accuracy of these relationships by considering the areas in which the maize is grown.
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'n Studie is uitgevoer om die verwantskap tussen die aminosuur- en proteïeninhoud van mielies te bepaal. Monsters van geelmielies is van die vier vernaamste mielieproduserende streke van Suid-Afrika verkry. Nadat die vog-, ruproteïen- en aminosuurinhoude van die monsters bepaal is, is statistiese ontledings gedoen om vas te stel tot watter mate die proteïengehalte volgens ruproteïeninhoud verander en of sodanige verwantskappe tussen die vier streke verskil het. Die konsentrasie van elke aminosuur, uitgedruk as g aminosuur/kg droë mielies het toegeneem met toename in ruproteïeninhoud. As aminosuurkonsentrasie egter uitgedruk word as g aminosuur/16 g N, het slegs vier (aspartiensuur, treonien, metionien en histidien) van die 17 aminosure wat ondersoek is, nie met proteïeninhoud verander nie. Vier ander (glisien, lisien, arginien en sisteien) het 'n negatiewe verwantskap getoon, terwyl die oorblywende aminosure met 'n toename in die ruproteïeninhoud van die mielies in konsentrasie toegeneem het. Die verwantskappe van isoleusien, leusien, lisien en arginien met ruproteïeninhoud is vir die gebalanseerde voerindustrie van ekonomiese en voedingkundige belang. Vergelykings om die inhoud van hierdie aminosure volgens die hoeveelheid ruproteïen in mielies te voorspel, word verskaf. Die konsentrasie van die oorblywende aminosure kan sonder 'n verlies aan akkuraatheid vanaf hul gemiddelde waardes in g/16 g N uitgedruk, geskat word. Alhoewel die verwantskap tussen sekere aminosure en ruproteïen tussen streke verskil, is dit onwaarskynlik dat hierdie verskille meer as empiriese statistiese waarnemings is. Dit het dus weinig nut om te poog om die presiesheid van hierdie verwantskappe te verbeter deur die streke waar die mielies gegroei het, in ag te neem.
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Introduction

Provision of adequate levels of the so-called essential amino acids is of major importance in the compilation of mixed animal feeds, especially for monogastric animals. As yellow maize constitutes the main ingredient of South African animal feeds it would be useful if the amino acid content of this ingredient could be readily assessed. Amino acid analysis is too costly and time consuming for routine investigation in the feed industry and it would be helpful if the amino acid content of maize could be deduced from its protein content, which is much more readily determined. Whilst the concentrations of all amino acids in the kernel, when expressed as g/kg maize, increase as the protein content increases, individual amino acids do not all increase to the same extent. The reason for this is that for normal maize, a higher protein content is associated with an increased proportion of the alcohol-soluble fraction zein, which is almost devoid of lysine and tryptophan and has an abnormally high leucine content (Prince, 1954; Sauberlich, Chang & Salmon, 1953; von Fintel & Quicke, 1962). It is therefore necessary to establish the relationships between the concentration of each amino acid and protein content.

The results of such a study undertaken with 80 samples of yellow maize collected at receiving depots in the four main maize-producing areas in South Africa are reported in this article.

Materials and Methods

Maize (International Feed Code no. 4-02-879)

The maize kernels used in this analysis were collected by the maize board during the 1981/1982 season. A representative sample of kernels was collected (bulk probe) from single consignments of maize arriving at 20 different receiving depots in each of the four main maize-producing regions. Only yellow maize was involved as the emphasis was on maize destined for the animal feed industry.

Analytical procedures

Dry matter was determined as the residue remaining after exposure to infrared light in a Mettler moisture balance.

Protein (g N × 6,25/kg) was determined both by a conventional macro-Kjeldahl procedure using mercuric oxide as catalyst during digestion and trapping the ammonia in saturated boric acid solution, and by means of an auto-analyser technique using a Technicon Auto Analyser II.

Amino acids were determined on 24 h hydrolysates (6N HCL, 110°C in evacuated tubes) using a Beckman Model

119 analyser (single column system); cysteine was determined separately, following reaction with dimethylsulphoxide (DMSO) as outlined by Dennison & Gous (1980).

Results and Discussion

As a preliminary analysis, the mean and standard error of the mean concentration of each amino acid was calculated for the 20 maize samples from each area. These results were expressed as g amino acid/kg dry sample (Table 1) and as g amino acid/16 g N (Table 2). The mean amino acid composition over all areas is also shown in the above tables.

Scatter diagrams of individual amino acid concentrations (both as g/kg and as g/16 g N) versus crude protein content, demonstrated clearly that the concentration of some amino acids in maize differed between areas. Figures 1 – 4, the scatter diagrams for lysine and leucine (expressed as g/kg and g/16 g N) and Figure 5, for phenylalanine (g/16 g N), illustrate the type of results obtained.

Two separate regression analyses were performed on the data, one in which areas were ignored, the other in which the effect of area was quantified by fitting a regression model for grouped data. In both cases the statistical package, Genstat was used.

Analysis over all areas

Assuming that amino acid concentration is a constant proportion of the protein, then, when expressing amino acid concentration as g/kg maize, a linear regression of amino acid

Table 1 Mean amino acid composition (g/kg oven dried sample) and protein content of maize grown in four different areas of South Africa. Values are the means for 20 samples per area

	Area[a]				
Amino acid	A (± *SE*)	B (± *SE*)	C (± *SE*)	D (± *SE*)	Mean (± *SE*)
ASP	6,74(1,18)	7,36(0,71)	7,09(0,49)	6,49(0,61)	6,92(0,85)
THR	3,42(0,35)	3,95(0,28)	3,91(0,26)	3,62(0,34)	3,73(0,37)
SER	4,40(0,45)	5,40(0,43)	5,28(0,38)	4,86(0,51)	4,98(0,59)
GLU	17,98(2,21)	21,48(1,89)	21,42(2,02)	19,96(2,14)	20,21(2,48)
PRO	8,36(1,04)	10,31(0,89)	9,88(0,97)	9,34(1,18)	9,47(1,24)
GLY	3,63(0,25)	4,05(0,26)	4,02(0,23)	3,80(0,31)	3,88(0,31)
ALA	6,97(0,95)	8,70(0,76)	8,45(0,75)	7,66(0,88)	7,95(1,07)
VAL	6,54(1,19)	7,84(0,85)	7,52(0,72)	6,71(0,89)	7,15(1,06)
MET	1,42(0,40)	2,17(0,46)	2,32(0,42)	2,09(0,44)	2,00(0,55)
ILE	3,65(0,45)	4,44(0,34)	4,40(0,41)	3,97(0,47)	4,11(0,53)
LEU	11,77(1,78)	14,68(1,33)	14,35(1,38)	13,08(1,53)	13,47(1,88)
TYR	3,57(0,48)	4,41(0,30)	4,42(0,44)	3,92(0,42)	4,08(0,54)
PHE	4,46(0,69)	5,55(0,49)	5,46(0,53)	5,00(0,52)	5,12(0,70)
HIS	2,68(0,48)	3,08(0,27)	3,06(0,26)	2,86(0,29)	2,92(0,37)
LYS	2,66(0,21)	2,82(0,25)	2,84(0,16)	2,75(0,18)	2,77(0,21)
ARG	4,53(0,53)	4,82(0,33)	4,92(0,32)	4,71(0,38)	4,74(0,42)
CYS	1,27(0,24)	1,22(0,11)	1,28(0,12)	1,18(0,11)	1,24(0,16)
Protein g N × 6,25/kg	94,05(9,62)	106,60(7,37)	106,88(7,84)	99,78(7,09)	101,82(8,03)

[a]Area: A eastern Transvaal; B western Transvaal; C Orange Free State; D Natal

Table 2 Mean amino acid composition (g/16 g N) of maize grown in four different areas of South Africa. Values are the means of 20 samples per area

	Area[a]				
Amino acid	A (± *SE*)	B (± *SE*)	C (± *SE*)	D (± *SE*)	Mean (± *SE*)
ASP	7,15(0,94)	6,91(0,53)	6,64(0,27)	6,49(0,28)	6,80(0,62)
THR	3,65(0,32)	3,70(0,14)	3,67(0,16)	3,63(0,15)	3,66(0,21)
SER	4,68(0,24)	5,06(0,22)	4,94(0,21)	4,86(0,25)	4,89(0,26)
GLU	19,12(1,10)	20,15(1,02)	20,02(0,98)	19,99(1,24)	19,82(1,14)
PRO	8,89(0,61)	9,67(0,52)	9,24(0,57)	9,34(0,77)	9,29(0,67)
GLY	3,88(0,25)	3,80(0,19)	3,76(0,18)	3,81(0,12)	3,82(0,19)
ALA	7,39(0,41)	8,16(0,33)	7,91(0,39)	7,67(0,47)	7,78(0,49)
VAL	6,93(0,76)	7,35(0,58)	7,04(0,49)	6,71(0,55)	7,01(0,64)
MET	1,51(0,42)	2,04(0,43)	2,17(0,37)	2,09(0,41)	1,95(0,48)
ILE	3,88(0,22)	4,16(0,16)	4,12(0,22)	3,97(0,27)	4,03(0,25)
LEU	12,47(0,69)	13,76(0,58)	13,41(0,63)	13,09(0,79)	13,18(0,82)
TYR	3,79(0,21)	4,14(0,23)	4,13(0,28)	3,93(0,22)	4,00(0,28)
PHE	4,72(0,28)	5,20(0,24)	5,11(0,23)	5,01(0,27)	5,01(0,31)
HIS	2,84(0,31)	2,89(0,24)	2,86(0,15)	2,87(0,15)	2,87(0,22)
LYS	2,84(0,18)	2,65(0,21)	2,66(0,13)	2,76(0,09)	2,73(0,18)
ARG	4,82(0,42)	4,53(0,26)	4,61(0,23)	4,72(0,22)	4,67(0,31)
CYS	1,35(0,23)	1,15(0,08)	1,20(0,08)	1,18(0,08)	1,22(0,15)

[a]Areas: A eastern Transvaal; B western Transvaal; C Orange Free State; D Natal

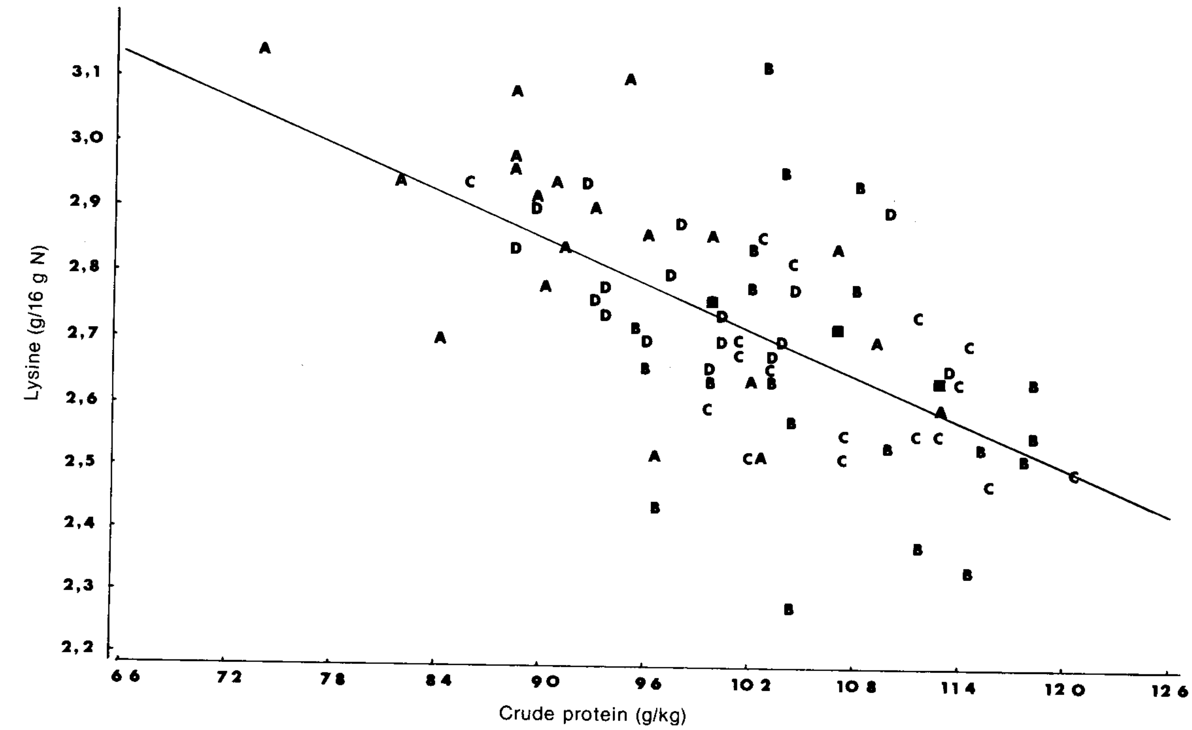

Figure 1 Scatter diagram and least squares regression of lysine (g/16 g N) versus crude protein content (g/kg of dry sample). A eastern Transvaal; B western Transvaal; C Orange Free State; D Natal. ■ Observations coincident between areas.

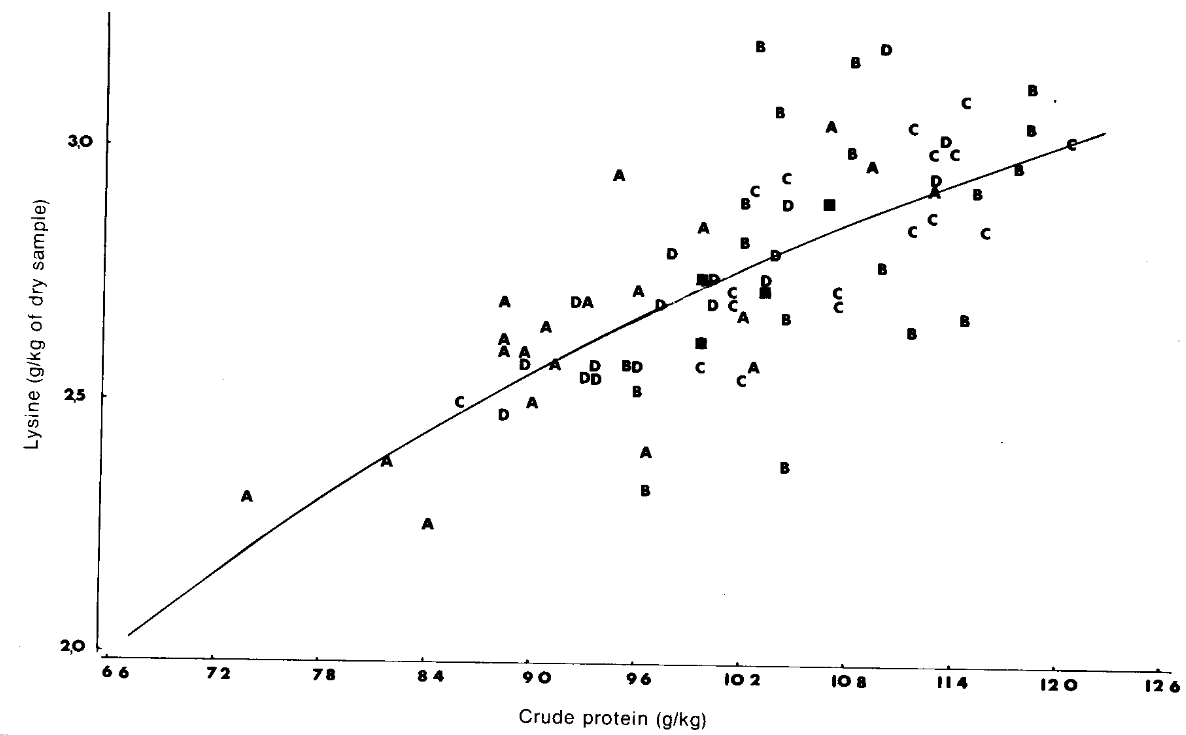

Figure 2 Scatter diagram and least squares regression of lysine (g/kg of dry sample) versus crude protein (g/kg dry sample). A eastern Transvaal; B western Transvaal; C Orange Free State; D Natal. ■ Observations coincident between areas.

concentration on protein concentration, with zero intercept, would be expected. Similarly, where amino acid concentration is transformed and expressed as g/16 g N the regression slope would be expected to be zero but with a positive (non-zero) intercept.

Although amino acid concentrations can be expressed in terms of both g/kg and g/16 g N, it is the latter that is of greater interest in this study — samples with a higher crude protein content would be expected to contain more amino acids per kilogram; and changes in the composition of the protein are more readily apparent when the amino acids are expressed as g/16 g N; also, the former units can be calculated from the latter by appropriate transformation (see footnote, Table 3). So to avoid duplication, all subsequent analyses concern amino acid concentrations expressed as g/16 g N. In order to verify whether the amino acid composition of maize protein changes as the protein content of maize is increased, linear regression models based on all 80 sample means were analysed.

The possibility of a quadratic term was also considered but,

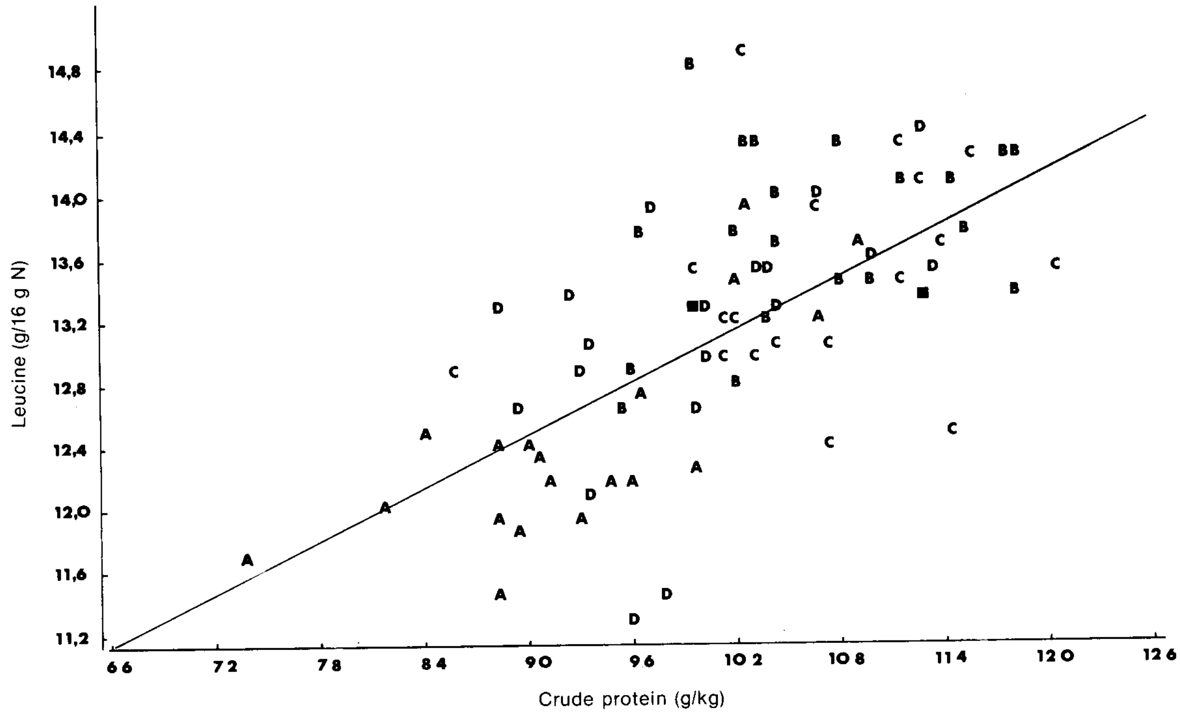

Figure 3 Scatter diagram and least squares regression of leucine (g/16 g N) versus crude protein (g/kg of dry sample). A eastern Transvaal; B western Transvaal; C Orange Free State; D Natal. ▪ Observations coincident between areas.

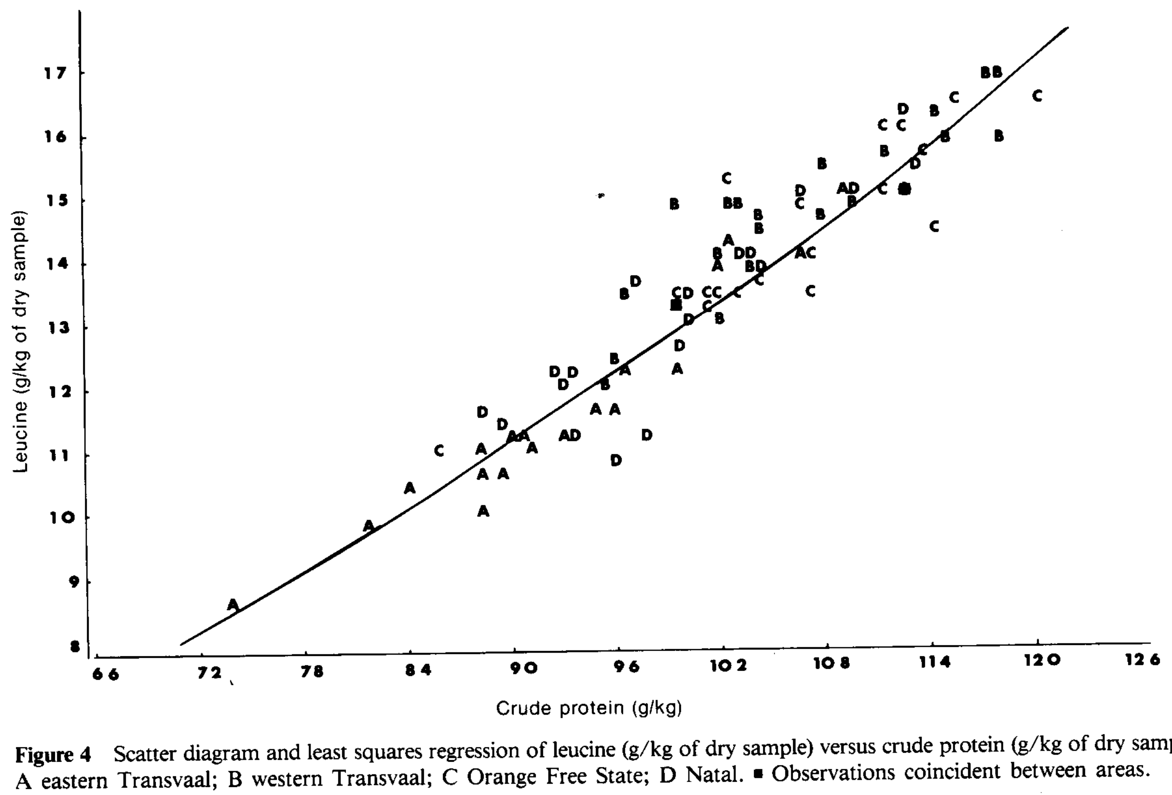

Figure 4 Scatter diagram and least squares regression of leucine (g/kg of dry sample) versus crude protein (g/kg of dry sample). A eastern Transvaal; B western Transvaal; C Orange Free State; D Natal. ▪ Observations coincident between areas.

in one case only, i.e. phenylalanine, was the additional term marginally significant. In only four cases of the 17 amino acids studied (aspartic acid, threonine, methionine and histidine) was the slope not significantly different from zero (Table 3). Of the 13 amino acids whose composition within the protein changed with the protein content of maize, four amino acids (glycine, lysine, arginine and cysteine) showed a negative relationship with protein content, i.e. as the protein content of maize increased, the concentration of these amino acids fell. As an example, the lysine content of maize with a protein content of 80 g N × 6,25/kg is estimated to be 2,97 g/16 g N (2,38 g/kg) whilst at a protein concentration of 120 g N × 6,25/kg the lysine content is 2,52 g/16 kg N (3,02 g/kg). The other amino acids whose regression coefficients differed significantly from zero showed increasing concentrations of amino acid as the protein content of maize increased.

These results are consistent with those quoted above (Prince, 1954; von Fintel & Quicke, 1962) in which it was shown that the alcohol-soluble fraction of maize protein, zein, which is almost devoid of lysine and tryptophan but has an abnormally

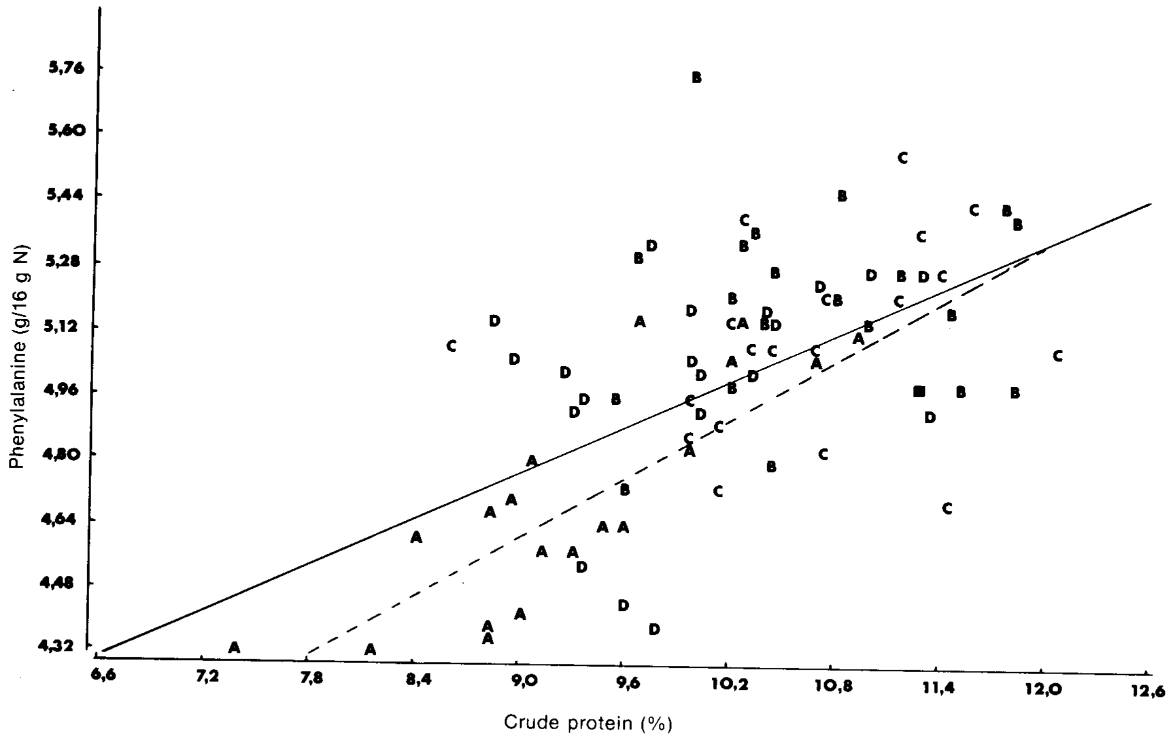

Figure 5 Scatter diagram of phenylalanine (g/16 g N) versus crude protein content (percentage of dry sample). Least squares regression over all areas (– – – –) and for area A (——). A eastern Transvaal; B western Transvaal; C Orange Free State; D Natal. ▪ Observations coincident between areas.

Table 3 Relationship[a] between amino acid concentration (g/16 g N) and crude protein (g N × 6,25/kg) content of 80 samples of maize grown in South Africa

Amino acid	Constant term (± *SE*)	Regression coefficient *b*(± *SE*)
ASP	6,795(0,620)	
THR[b]	3,662(0,207)	
SER[c]	4,248(0,312)	0,0629(0,031)
GLU[c]	16,270(1,331)	0,3479(0,130)
PRO	7,534(0,791)	0,1721(0,077)
GLY	4,932(0,191)	−0,1097(0,019)
ALA	5,159(0,509)	0,2575(0,050)
VAL	4,965(0,734)	0,2005(0,072)
MET	1,953(0,482)	
ILE	3,125(0,279)	0,0891(0,027)
LEU	7,587(0,760)	0,5494(0,074)
TYR[d]	2,953(0,311)	0,1026(0,030)
PHE[b,e]	3,001(0,302)	0,1972(0,030)
HIS[d]	2,865(0,217)	
LYS	3,882(0,169)	−0,1134(0,017)
ARG	6,121(0,334)	−0,1424(0,033)
CYS	1,734(0,177)	−0,0506(0,017)

[a]Equations are of the form *a.a.*(g/16 g N) = constant + *b*. (crude protein). Where the regression is not significant ($P \leqslant 0,05$) prediction from the mean value is indicated. To calculate *a.a.*(g/kg maize) the following equation should be used: *a.a.*(g/kg) = 10^{-1}.(constant.protein + *b*.(crude protein)^2)

[b]A more accurate estimate of this amino acid for maize grown in the eastern Transvaal is given in Table 4

[c]See Table 4 for maize grown in Natal

[d]See Table 4 for maize grown in western Transvaal

[e]A slightly more accurate estimate is obtained by fitting a quadratic term for this amino acid; viz: PHE = − 1,885 + 1,178 × protein − 0,0488 × protein^2

high content of leucine, is the protein fraction that is increased as the protein content of maize is raised. The results of this study quantify these changes, allowing a more accurate adjustment to be made to the assumed amino acid content of maize of varying protein concentrations. These equations are particularly useful to formulators of feeds for poultry and pigs where the amino acid concentrations of the mixed feeds are of paramount importance. As maize usually makes up the bulk of such feeds in South Africa a more accurate assessment of the amino acid concentrations could make a considerable difference to the cost of the feed and to productivity on the farm.

In a study on the acquisition and assessment of data on the amino acid composition of feedingstuffs, Fisher (1984) found that five amino acids (methionine, cysteine, lysine, tryptophan and isoleucine) were significantly correlated with the crude protein content of maize, and that there was very little improvement in the accuracy of prediction of any amino acids when the regression coefficient was included in the prediction equation. When the criterion used by Fisher (1984) was applied in this study to assess the improvement in the accuracy of prediction by including the linear effect of crude protein, the average *SE* for the 13 amino acids with regression coefficients in Table 3 was 6,91% of the mean before regression and 6,36% after the linear effect of protein content was removed. Worthwhile improvements were made only in the following cases: isoleucine (*SE* changed from 6,20 to 5,76% of the mean), leucine (from 6,22 to 4,80%), lysine (from 6,59 to 5,13%) and arginine (from 6,64 to 5,95%).

Because there is an underlying chemical basis for the change in lysine and leucine concentrations in maize with an increase in the crude protein content (von Fintel & Quicke, 1962), the changes observed in the present study are not purely of an empirical nature and should therefore be exploited by the feed industry. Lysine is of importance in that it is the first-limiting amino acid in cereal sources used in poultry and pig feeding, whilst the concentration of leucine in a feed, because of its interaction with isoleucine and valine, will influence the requirement by the animal for these two amino acids (D'Mello & Lewis, 1971). It is therefore recommended that the concentrations of lysine, leucine, arginine and isoleucine be adjusted according to the crude protein content of maize.

Effect of area

Variation in the protein content of maize can be influenced by agronomic, genetic and environmental factors, including fertilizer treatment, soil type, maturity at harvest, rainfall, and temperature during growth. No account of these factors was taken in this study except to separate the samples into the four main geographical areas in which maize is grown in South Africa.

The mean protein content of oven-dried maize in each of the four areas varied from 94,05 g/kg in the eastern Transvaal to 106,88 g/kg in the Orange Free State (Table 1). This is illustrated in Figures 1 – 5 where observations from area A (eastern Transvaal) lie predominantly on the left of the graphs, and those from area C (Orange Free State) on the right.

These differences in crude protein content between areas contributed to differences in the relationship between certain amino acids and crude protein between areas. The relationship between the two amino acids threonine and phenylalanine, and crude protein differed significantly in area A (eastern Transvaal) from those in the other three areas. This difference in areas is illustrated in Figure 5 for phenylalanine, and this is used here to show how such empirical differences can arise. The protein content of a few of the samples contributing to the regression of phenylalanine on crude protein in area A is considerably lower than that for other areas, but in these cases the phenylalanine content is not correspondingly lower. This has had the effect of 'bending' the graph upward for samples with a low protein content, leading to an apparently curvilinear regression of phenylalanine on crude protein content in area A. More accurate estimates of the threonine and phenylalanine content of maize sampled from area A could be obtained by applying the equations given in Table 4.

Similarly, the mean values of serine and glycine in maize from Natal, and tyrosine and histidine in maize grown in the western Transvaal, differed significantly from the corresponding values of maize in the three other areas (Table 4). Without the relevant information regarding agronomic and other differences between areas there is little justification for regarding these as permanent differences in amino acid concentrations between areas. Consequently these empirical statistical observations should not be applied in practice; rather in the absence of further information, the relationships given in Table 3 and determined over all samples would allow a more accurate estimation of the amino acid concentration of maize from its crude protein content.

Table 4 Relationship between amino acid concentration (g/16 g N) and crude protein content (g N × 6,25/kg) in cases where this differs between areas

Area[a]	Amino acid	Constant term (± *SE*)	Regression coefficient (± *SE*)
A	THR	5,003(0,439)	– 0,144(0,046)
D	SER	3,536(0,720)	
D	GLY	3,755(0,477)	
B	TYR	5,589(0,764)	
A	PHE	2,434(0,518)	– 0,243(0,055)
B	HIS	4,053(0,742)	

[a]Area: A eastern Transvaal; B western Transvaal; C Orange Free State; D Natal

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