



Protein Contents of Maize Varieties (QPM and Normal Maize) as Influenced by Nitrogen and Micronutrients

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ABSTRACT

Field experiments were conducted in 2008 and 2009 in the Guinea savanna ecology of Nigeria to investigate the protein content of maize varieties (QPM and normal varieties) as influenced by nitrogen fertilizer and micronutrients. The treatments were four rates of inorganic fertilizer N (0, 50, 100, 150kgNha⁻¹) and two rates of cocktail micronutrient mixtures. These were tested in a randomized complete block design with three replications and the treatments were factorially combined. The results from the study revealed that micronutrients application increased the lysine and tryptophan content of the QPM varieties since values were increased by it. The result showed that addition of nitrogen fertilizer and micronutrients increased the crude protein content of the maize varieties and so also with micronutrients addition the QPM varieties differed significantly from each other with respect to lysine and tryptophan contents ($p < 0.05$). It can be inferred from this that though normal maize and QPM varieties could be exposed to the same environmental conditions and take up same amounts of micronutrients, the QPM varieties have genetic capacity to synthesize high levels of amino acids and so would have nutritionally higher quality grains. Plant breeders therefore may found this attribute useful in genetic manipulation and cultivar development for enhance protein biochemical components.

INTRODUCTION

Improving nutritional quality of agricultural crops is a noble goal, which is particularly important in cereal crops where meeting protein nutrition needs is one of the greatest challenges because plants tend to be low in protein and the protein is of poor nutritional quality (Vassal, 2006). The nutritional quality of maize is determined by the amino acid make up of its protein. Proteins are linear polymers built of monomer units called amino acids, which contain a wide range of functional groups which include alcohols, carboxamides, carboxylic acid, thioethers and a variety of basic groups (Berg *et al.*, 2001). Protein is essential in human's diet as a source of amino acids that cannot be synthesized from simple sources of nitrogen and energy for growth, reproduction and maintenance (Okai, *et al.*, 2005, Vassal, 2006). It constitutes important sources of carbohydrate, vitamins B, minerals, 15% of all food crop protein and other nutrients such as micronutrients. The predominant protein in maize is the alcohol soluble prolamins protein called Zein. Zein stores N, C, S and other nutrients but is characterized by low levels of lysine and tryptophan (Biston *et al.*, 1996). These deficiencies lead to poor utilization of plant protein in diets so improving the balance of amino acids is an important grain quality objective. Nitrogen (N) is an important plant nutrient and is the most frequently deficient of all nutrients in tropical soil systems. This is because of the relatively large amount required by plants and its high mobility in the soil. Nitrogen is of particular interest because it is usually the most limiting nutrient for crop production while fertilizer N represents a major variable input cost (Gil and Fick, 2001). Micronutrients are absolutely essential and play an active role in gene expression, biosynthesis of protein, nucleic acids, plant metabolism processes starting from cell wall development to respiration, photosynthesis, chlorophyll formation, enzyme activity, nitrogen fixation and reduction (Bishnu *et al.*, 2010). Micronutrients are becoming increasingly important to world agriculture as crop removal of these essential element increases (Adhikary *et al.*, 2010). Maize is assuming the position as the major crop of the sub-humid and semi- arid savanna with respect to economic prospects for the farmers and being a staple food crop in the ecological zone. However it is devoid of some major amino acids, such as lysine and tryptophan (Obi, 1982; Okai *et al.*, 2005; Vassal, 2006).

The development of QPM varieties improved the nutritional properties of maize and has given hope to many as a source of affordable protein for good health. However, the QPM varieties introduced to the Nigerian savanna ecologies still have problems of adaptation when the levels of amino acid content that characterize the varieties are considered. Though breeders may have developed genetically stable materials, environmental factors seem to have great influence on the protein content of the crop. Several studies have been conducted on maize especially the QPM varieties but this work wants to establish the

effect of nitrogen and micronutrient levels on lysine and tryptophan content of maize varieties (conventional and quality protein maize).

MATERIALS AND METHODS

The study was carried out at Samaru, Zaria for two years (2008/2009 and 2009/2010) at the Institute for Agricultural Research (IAR) Experimental farm in Samaru, Zaria, Nigeria (Longitude 11° 11'N and Latitude 7° 38' E, at an elevation of 686m above Sea Level). The soil is classified as Alfisol in the USDA Soil Classification system and it is developed in deeply weathered pre-Cambrian, basement complex rock overlain by Aeolian drift materials of varying thickness (Moberg and Esu, 1989; Ogunwole, 2000). The main soil subgroup is typicHalplustalf (USDA Soil Survey staff, 2006).

The site was divided into three blocks each consisting of 32 plots, giving a total of 96 plots and each plot measuring 12m². There were 4 ridges in a plot, 5m long at 0.75m x 0.25m spacing on a row. The experiment was laid out in a randomized complete block design with three replications and treatments were factorially combined. Two maize seeds were sown per stand and thinned to one per stand at two weeks after germination. Four rates of nitrogen fertilizer (0, 50, 100 and 150 kg/ha) was applied as Urea in 2 split doses at 2WAP and 4WAP. The micronutrients treatments (Fe, Zn, B, Mo, and Cu) were applied as cocktail of the mixture to half the number of plots at the rate of 22.85g/ha. Basal application of phosphorus and potassium were done at 60kgP₂O₅ha⁻¹ as single super phosphate (SSP), and 60kgK₂Oha⁻¹potash (MOP), (60%) respectively. All the fertilizers were applied at planting. In addition to the initial herbicide application to control weed, plots were manually weeded with hand hoe.

Before 50% silking stage, leaf sampling was done. The index plant samples were oven-dried at 65°C for 48 hours and then ground in a mill and stored for tissue analysis. N, P, K and micronutrients (Cu, Fe, Zn, B and Mo) were analyzed. Analysis of the maize grain was carried out on the endosperm of the maize seed (open pollinated and the quality protein maize varieties) as follows:

Random sample of 30 seeds were soaked in distilled water for 30 minutes. The pericarp was then peeled off and the germs were removed with scalpel and tweezers. The remaining endosperm was thereafter air-dried overnight and ground in a mill to fine powder and this was used for the determination of grain N, crude protein and the amino acids. The protein content of the leaves and grain sample was determined from total nitrogen and multiplied by a factor of 6.25.

Data collected were subjected to statistical analysis using SAS statistical computer software (SAS, 2005). Duncan Multiple Range Test (DMRT) was used to compare treatments means. Analysis of variance

was employed to determine significant differences between means.

RESULTS AND DISCUSSION

The table below shows properties of the soil used for the experiment.

Table 1: Physico –chemical properties of the soil used for the study

Parameters	Field Study	
	0-15 (cm)	15-30 (cm)
Sand (gkg^{-1})	540	525
Silt (gkg^{-1})	330	350
Clay (gkg^{-1})	130	125
Textural class	Sandy-loam	
$\text{pH}_{\text{H}_2\text{O}}$	5.70	5.60
$\text{pH}_{\text{CaCl}_2}$	5.40	5.20
Organic carbon (g kg^{-1})	5.20	5.00
Total nitrogen (%)	0.06	0.07
Available P (mgkg^{-1})	7.58	6.80
Exchangeable acidity (cmolkg^{-1})	0.60	0.62
Exchangeable bases (cmolkg^{-1})		
Calcium	3.98	4.50
Magnesium	1.36	1.59
Sodium	0.40	0.30
Potassium	0.70	0.58
Effective CEC (cmolkg^{-1})	2.60	3.45
Micronutrients (mgkg^{-1})		
Extractable Zinc	16.75	18.40
Extractable Iron	52.00	45.50
Extractable Copper	0.58	0.55
Extractable Molybdenum	11.00	11.08
Extractable Boron	0.10	0.11

The soil is sandy –loam in texture and very low in N and available P. Micronutrient contents indicated low to moderate. It is expected that the maize varieties would benefit from the added fertilizers.

The Effect of Nitrogen Fertilization on Nitrogen Concentration of the Maize Grain, Crude Protein, Lysine and Tryptophan Content

Increase in nitrogen rates increased the concentration of the grain from the control to the highest level of nitrogen applied (150kgNha^{-1}). The combined analysis also showed that grain N increased as the nitrogen rates increased from the control to 150kgNha^{-1} in the QPM varieties - SAMMAZ 14 (2.78%) and SUSUMA (2.80%) while for the normal maize varieties (SAMMAZ 12 and SAMMAZ 11) though N increased to the highest rate of N applied (150kgNha^{-1}) the N content was lower than it was for quality protein maize varieties. SAMMAZ 12 and SAMMAZ 11 produced (2.52%) and (2.64%) respectively. The nitrogen concentration in the grain increased as amount of nitrogen applied (150kgNha^{-1}). This was supported by Thomson *et al.* (2004) who reported that grain protein concentration showed more consistence response to increasing nitrogen rates than did yield.

The application of N at the rate of 150kgNha^{-1} gave maximum crude protein contents of 17.45% for SAMMAZ 14, (17.36%) for SUSUMA, (15.72%) for SAMMAZ 12 and (14.94%) for SAMMAZ 11 (Table 2).

The protein content of SAMMAZ 14 (QPM) was 19.31% > SAMMAZ 12 and 24.47% > SAMMAZ 11. SUSUMA had protein content of 24.59% > SAMMAZ 12 and 24.08% > SAMMAZ 11.

When the lysine and tryptophan contents of the maize varieties were combined for the two years, SUSUMA recorded 3.14% and tryptophan contents 0.72% at (100kgN) while SAMMAZ 14 had maximum lysine content of 3.06% and tryptophan content of 0.55% at (150kgN). SAMMAZ 11 had lysine content of 2.96% and tryptophan contents 0.48% at (150kgN). The grain crude protein significantly increased with increase in nitrogen rates. Since N is a major constituent of protein, applying N fertilizer would enhance protein synthesis or build up in cereal grains.

The grain crude protein significantly increased with increase in nitrogen rates. Since N is a major constituent of protein, applying N fertilizer would enhance protein synthesis or build up in cereal grains. The highest crude protein recorded in this study was 17.45% for SUSUMA (QPM) and 15.72% for SAMMAZ 11 (normal maize) these were high compared to 9.11% recorded by Osei *et al.* (1999) and 14.94% recorded by Prasanna *et al.* (2001) for QPM. Aduku (2005) reported a value of 8.0% crude protein for normal maize and QPM. The variation in the quantity and quality of the crude protein in the grain maize could be attributed to the level of nitrogen in the soil since the level of nitrogen fertilizer influences the quantity and quality of protein in maize (Deosthale and Visweswar, 1972).

[illegible]

Effects of Micronutrients on Nitrogen, Crude protein, Lysine and Tryptophan Contents of the Maize Grain

SUSUMA (QPM) had N content of 2.74% with micronutrients application while SAMMAZ 14 (QPM) produced combined N content of 2.67% in the grain. The normal maize, SAMMAZ 12 recorded N content of 2.40% without micronutrients application while SAMMAZ 11 (normal maize) had 2.55% N in the grain with micronutrients application.

The application of micronutrients increased the crude protein of the QPM varieties in that SUSUMA had the highest crude protein of 17.09% followed by SAMMAZ 14 with 16.67% and SAMMAZ 11 had 15.93% all with micronutrients application while SAMMAZ 12 produced crude protein of 14.97% without micronutrients application.

QPM varieties SUSUMA had the lysine and tryptophan contents of 3.20% and 0.53%, SAMMAZ 14 had lysine and tryptophan contents of 3.01% and 0.49% and SAMMAZ 11 produced lysine and tryptophan contents of 2.93% and 0.47% all with

micronutrients application. SAMMAZ 12 had lysine content of 2.84% and tryptophan content of 0.44% without micronutrients application as presented in Table 3.

Micronutrients application increased the lysine and tryptophan content of the QPM varieties since values were increased by the application of micronutrients. This suggests in clear terms that QPM varieties had higher capacity to utilize applied micronutrients for the synthesis of the relevant amino acids. It can be inferred from this that though normal maize and QPM varieties could be exposed to the same environmental conditions and take up same amounts of micronutrients, the QPM varieties have genetic capacity to synthesize high levels of amino acids and so would have nutritionally higher quality grains.

SAMMAZ 11 though, a normal maize responded to micronutrients application although at the highest application of N fertilizer which subsequently increased the protein content. This infers that the micronutrients content of the QPM varieties are similar to the normal maize.

Table 3: The effect of micronutrients on nitrogen in grains, crude proteins, lysine and tryptophan content of the maize varieties

	Micronutrients (g ha ⁻¹)	Nitrogen in grains		Crude protein in Grains		Tryptophan (%)		Lysine (%)	
		2008	2009	2008	2009	2008	2009	2008	2009
SAMMAZ 14	-M	2.03	2.88	12.67	18.03	0.47	0.36	2.94	2.62
	+M	2.31	3.02	14.45	18.89	0.53	0.45	3.13	2.89
SUSUMA	-M	1.91	2.66	11.94	16.77	0.43	0.59	2.83	3.16
	+M	2.39	3.08	14.95	19.23	0.51	0.54	3.07	3.33
SAMMAZ 12	-M	2.04	2.75	14.08	17.77	0.39	0.48	2.69	2.99
	+M	2.00	2.71	12.53	16.96	0.35	0.46	2.59	2.92
SAMMAZ 11	-M	2.11	1.99	13.00	16.18	0.42	0.47	2.79	2.96
	+M	2.25	2.85	12.76	17.18	0.47	0.46	2.94	2.92
Mean		2.15	2.87	13.42	17.88	0.44	0.48	2.87	2.97
SE±		0.28	0.31	1.77	1.89	0.03	0.02	0.27	0.26
CV(%)		22.81	18.74	22.82	18.37	11.63	8.50	16.51	15.35
V*N		NS	NS	NS	NS	**	**	**	**

The Effect of Treatments on Nitrogen, Crude Proteins, Lysine and Tryptophan Contents of the Maize Grain

The effect of treatments on the grain parameters as presented in Table 4a showed grain N generally seemed to increase with N rates in the QPM varieties and decrease in the normal maize varieties, with or without the application of micronutrients. SAMMAZ 14 had 2.54kgNha^{-1} and 2.53kgNha^{-1} nitrogen content with or without micronutrients application. SUSUMA had a nitrogen content of 2.90kgNha^{-1} and 2.77kgNha^{-1} with added micronutrients or without micronutrients application. The normal maize SAMMAZ 12 and 11 recorded 2.85 kgNha^{-1} , 2.74 kgNha^{-1} , 2.83 kgNha^{-1} and 2.68kgNha^{-1} with or without the application of micronutrients respectively.

The results also showed that the crude protein content varied from 16.78% to 19.60% among the varieties. SAMMAZ 14 had crude protein content of 16.78% at 150kgNha^{-1} with micronutrient addition and had 14.27% at 150kgNha^{-1} without micronutrients. Also SUSUMA had the highest crude protein of 17.91% at 50kgNha^{-1} with no micronutrient addition, but had 19.60% at 50kgNha^{-1} with addition of micronutrients. SAMMAZ 12 had crude protein content of 18.32% with micronutrients and 17.23% without micronutrient at 150kgNha^{-1} . For SAMMAZ 11, (normal maize) recorded the crude protein of 17.24% and 16.41% with and without micronutrients at 150kgNha^{-1} respectively. The mean crude proteins content among the varieties were 14.45% - 18.90% for both years respectively as presented on Table 4a.

The lysine and tryptophan contents of the different varieties were presented in Table 4b. The lysine content was 3.19% and 2.82% for SAMMAZ 14 with or without micronutrients. SUSUMA had highest lysine content of 3.30% and 3.24% with or without micronutrients with 50kgNha^{-1} . SAMMAZ 12 recorded a lysine content of 3.02% and 2.89% with or without micronutrients while SAMMAZ 11 had lysine content of 3.20% and 2.93% with or without micronutrients respectively.

The mean values for tryptophan in the two years were highly significant, $p < 0.05$ with the highest value of 0.59% (SUSUMA) and the lowest value of 0.39% (SAMMAZ 14). The tryptophan content of 0.55% (100kgNha^{-1}) and 0.43% (0kgNha^{-1}) were recorded with or without micronutrients application by SAMMAZ 14 while SUSUMA variety had tryptophan content of 0.59% and 0.52% at 50kgNha^{-1} with or without micronutrient application. SAMMAZ 12 had tryptophan content of 0.46% (0kgNha^{-1}) without micronutrients and 0.45% (150kgNha^{-1}) with micronutrient applications. SAMMAZ 11 recorded 0.50% and 0.50% (50kgNha^{-1}) with or without micronutrients. In the study, SUSUMA (QPM) had highest crude protein, lysine and tryptophan contents with nitrogen fertilizer and micronutrients while SAMMAZ 14 performed better at no micronutrients but with optimal level of nitrogen. The conventional maize had protein, lysine and tryptophan contents at the highest application of nitrogen. SUSUMA, quality protein maize had just lysine content of 1.23% and 11.21% with or without micronutrient better and tryptophan content of 3.85% and

15.25% better than SAMMAZ 11 a normal maize variety. This implies that giving the normal maize variety same environment by exposing them to same management practices and soil factors can help the normal maize pick up more essential amino acids.

The QPM and the normal maize differed significantly from each other with respect to lysine and percentage tryptophan ($P < 0.05$). This probably suggests a high variability that exists in maize genotypes with respect to these biochemical components. Plant breeders may therefore find this attribute useful in genetic manipulation and cultivar development for enhance protein biochemical components. Forages and some cereals other than maize support the view that nitrogen fertilization up to and beyond the point of maximum yield increases the concentration of nitrogen in the tissue. Lysine and tryptophan values in this study were comparable with Vassal *et al.* (1979) who reported range of lysine content of 1.8- 2.0% and tryptophan content of 0.9-1.06%. Sema and Rooney (1994) reported higher lysine content of 4.08 g/100g protein and tryptophan content of 0.75 g/100g protein in QPM. They reported lysine contents of 3.04 g/100g protein and tryptophan contents of 0.59 g/100g protein in the normal maize.

SUSUMA had higher marginal protein, lysine and tryptophan contents with micronutrients application than the normal maize which infers that QPM cultivars had greater lysine and tryptophan contents than the normal cultivars and that lysine and tryptophan contents increased in both the QPM and normal varieties as the N level in the soil increased. This shows that given the set of conditions that influenced quality in QPM, the quality of the normal maize may be improved. This is consistent with the results of Pixley and Bjamason (1993) and Bhatnagar *et al.* (2003) who reported the superiority of QPM cultivars over non- QPM cultivars for protein quality. The contrast analysis showed no significant difference in all the parameters however this may indicate there is genotypic variation in grain protein content in both the QPM and the normal cultivars.

Santayehu (2008) reported that the protein content of the kernels of corn increased with increasing nitrogen supply in the soil. Potassium increases grain protein content of wheat thus improving quality while it increases the oil content of maize grain (Rejado, 1979). Whitehouse (1971) also reported that the cause of high protein content in maize is a restriction on growth which is due to a shortage of water or some adverse condition during the later stages of grain-filling.

Anonymous (2004) showed that there was a direct relationship between the soil and the nitrogen applied to the soil and the contents of crude protein, zein and leucine in maize grain. He concluded that variation in content of the amino acids suggest that nitrogen-fertilization in relation to plant population as well as variety has an important effect on protein composition. The contrast analysis showed no significant difference in all the parameters however this may indicate there is genotypic variation in grain protein content in both the QPM and the normal cultivars.

[illegible]

Table 4b: The interactive treatments on grain lysine and tryptophan contents of the maize varieties

[illegible]

CONCLUSION

The addition of nitrogen fertilizer and micronutrients increased the nitrogen and protein contents of the quality protein maize. The crude protein recorded was higher in this study than that recorded in literature. The crude protein was higher compared to 9.01% recorded by Osei *et al.* (1999) and Prasanna *et al.* (2001) for QPM. Aduku (2005) reported a value of 8.0% crude protein for normal maize and QPM.SUSUMA had higher marginal protein, lysine and tryptophan contents with micronutrients application than the normal maize which infers that QPM cultivars had greater lysine and tryptophan contents than the normal cultivars and that lysine and tryptophan contents increased in both the QPM and normal varieties as the N level in the soil increased. SUSUMA, the better of the QPM had just lysine content of 3.19% and 5.08% tryptophan better than SAMMAZ 11 a conventional maize variety which implies that giving the normal maize variety the same environment and same management factors can help the normal maize to pick up more essential amino acids. This is consistent with the results of Pixley and Bjarnason (1993) and Bhatnagar *et al.* (2003) who reported the superiority of QPM cultivars over non-QPM cultivars for protein quality.

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