

EFFECT OF QUALITY PROTEIN MAIZE DIET ON LIVER INTEGRITY AND SERUM AMINOTRANSFERASES OF ALBINO WISTAR RATS.

M.A.AGIANG, I.O.WILLIAMS, O.O.EKPE, U.O.EKANEM and M.U. ETENG

(Received 15 July, 2005; Revision Accepted 2 September, 2005)

ABSTRACT

The study was designed to evaluate the effect of quality protein maize (QPM) diet on the histology of the liver and on the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in albino wistar rats. The AST level in rats fed QPM diet was $57.4 \pm 8.92\text{U/L}$ which compared favourably with that in rats fed the reference casein diet ($46.0 \pm 8.48\text{U/L}$) at $p > 0.05$. There was also no significant difference ($p > 0.05$) in the serum AST levels in rats fed the basal diet ($61.2 \pm 4.63\text{U/L}$), common maize diet ($60.2 \pm 8.59\text{U/L}$) and the reference diet. Similarly, there was no significant difference ($p > 0.05$) in the levels of serum ALT in rats fed the basal diet ($33.5 \pm 6.50\text{U/L}$), QPM diet ($21.6 \pm 3.98\text{U/L}$), common maize diet ($27.4 \pm 6.13\text{U/L}$) and the reference casein diet ($21.6 \pm 2.11\text{U/L}$). Histological examination of the livers of rats fed the experimental diets showed mild degeneration of the hepatic vessels and a small central vein in the group fed the basal (protein-free) diet while the livers of the rats fed the QPM diet showed a smaller central vein but generally gave the same picture as that of the group fed the reference casein diet. The results indicate that consumption of QPM diet by rats does not have adverse effect on the liver.

KEY WORDS: Quality protein maize, Liver integrity, Serum aminotransferases.

INTRODUCTION

Though maize is a staple food for the large population, it is low in nutritive value with respect to protein, especially some essential amino acids, which may cause malnutrition and deficiency diseases. Evidence exists of the association between maize consumption and pellagra, a deficiency disease which occurs whenever the intake of nicotinamide or its precursor tryptophan, is insufficient (Vardlaw, 1999). Efforts were made to improve the biological utilization of the nutritive contents of maize through genetic manipulation, processing and fortification which eventually yielded maize lines of high quality protein, called quality protein maize, QPM (CIMMYT, 2000).

Quality protein maize is thus, genetically modified maize whose quality, especially protein, has been improved through the addition of essential amino acids, lysine and tryptophan, through the opaque-2 gene (Villegas, 2000). It looks, grows and tastes like common maize and contains double the lysine and tryptophan, and a more balanced amino acid content that greatly enhances its nutritive value (Ernest, 2000; Watson, 1987). Repeated studies in several countries have shown that pigs or poultry raised on quality protein maize-based feed gain weight faster and produce more than animals raised on normal maize-based feed (Watson, 1987; Bressani and Martz, 1958; Hubell, 1991). In other studies by Bressani and Elias (1970), the protein quality of QPM as a component of the maize/bean diet of 82.8% maize and 10.5% cooked beans was evaluated in young and adult dogs fed at two levels of % protein. Nitrogen balance data showed that nitrogen retention levels for young or adult dogs fed QPM/bean diet were as high as or higher than those in which common maize in the diet was supplemented with lysine and tryptophan and the levels were significantly higher with both diets than with maize and beans alone.

Although molecular probe has it that QPM is genetically modified, toxicity test demonstrated shows that it presents no acceptable health risk. But there are controversies surrounding the use of genetically modified foods which QPM is one several countries are yet to accept its value. This may be due to the fact that genetically modified foodstuffs, like potatoes and peanuts, are seen to be toxic to the liver when eaten in excess. The changes in serum AST and ALT levels have been used as indices of toxicity on the liver (Stoer and Makarora, 1984; Eteng *et al*, 1998; Atangwho, 2000). However, histological changes in the liver and the assessment of the AST and ALT concentrations in the serum of experimental albino Wistar rats fed with quality protein maize may nevertheless show some changes similar to man, as the rat has close metabolic similarity to man, although some species variations may be present. The study therefore evaluates effect of QPM on histology and activities of serum AST and ALT of Wistar rats.

MATERIALS AND METHODS

Quality protein maize was obtained from the Institute of International and Tropical Agriculture (IITA) through the Ministry of Agriculture, Calabar while the common maize (white) was obtained from Watt market, Calabar. The protein contents of the two maize samples were analyzed by the Kjeldal distillation method (AOAC, 1980) before administration to the animals.

Feeding trial

Twenty adult albino rats aged thirty-one days and weighing 100g-200g were obtained from the animal house of the department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar. The animals were randomly

M.A.AGIANG, Department of Biochemistry, University of Calabar, Calabar Nigeria
I.O.WILLIAMS, Department of Biochemistry, University of Calabar, Calabar Nigeria
O.O.EKPE, Ministry of Agriculture, Calabar, Cross River State Nigeria
U.O.EKANEM, Department of Biochemistry, University of Calabar, Calabar Nigeria
M.U. ETENG, Department of Biochemistry, University of Calabar, Calabar Nigeria

assigned to four study groups (A, B, C, and D) of five rats each. The four groups (A, B, C, D) of rats were fed a basal (nitrogen free) diet, a standard casein diet, a test diet (TDI) of QPM and a test diet (TDII) of common maize respectively. The diets were compounded to supply about 10% protein on dry matter basis. Diets and drinking water were given *ad libitum* for 28 days of which 7 days were for acclimatization. The weights of the animals were taken at the beginning and at the end of the study period. Food intake was also recorded. At the end of the feeding period, the rats were anaesthetized in chloroform vapour and dissected; blood was collected through cardiac puncture into plain sample tubes, allowed to clot and serum separated by centrifugation at 800 revolutions per minute for 15 minutes. The liver tissue was also extracted for histological analysis.

Enzyme assay

The serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using the Randox diagnostic kit whose principle is based on the Reitman and Frankel method (1957), revised 1995.

Histological studies (Bradbury, 1973)

The liver was removed and washed in 0.9% physiological (normal) saline before it was transferred into 10% buffered formalin for 48 hours. It was later transferred to 70% alcohol, two changes for two hours each and to 90% alcohol, two changes for two hours each. These were then transferred to absolute alcohol of two changes for two hours each. The liver tissues were then removed to a mixture of equal volumes of absolute alcohol and xylol, and then transferred to two changes of xylol for two hours each to produce "clear" tissues. The "clear" tissues were then transferred to melted paraffin wax of melting point 52°C. The wax is kept at this temperature in a thermostatically controlled bath with two changes of bath at one hour each. The liver tissues were later embedded in a fresh molten paraffin wax and allowed to solidify. The embedded blocks were trimmed and sections were made from blocks with the microtome at 6 microns each. The tissues were then floated on a water bath and mounted in clean water albumenized slides. They were allowed to dry in an incubator for 24 hours at 37°C and were later stained with haematoxylin and eosin.

Statistical Analysis

Data were presented as mean \pm SEM and the significant differences in the effects of basal, reference and test diets were measured by ANOVA and the student t-test.

RESULTS AND DISCUSSIONS

Pictomicrographs of the histological changes in the liver of rats fed with experimental diets are shown in plates 1, 2, 3, and 4 respectively. The histology of the liver of rats fed the basal diet show a small central vein with narrow hepatic vessels which appear degenerated (plate 1). This could have been as a result of malnutrition since this diet was protein-free. The liver of the rats fed the reference (casein) diet had larger sinusoids that were irregularly displaced, larger hepatocytes, distinct portal triad almost filled with blood (plate 2). The histological picture of the liver of rats fed the QPM diet (plate 3) is similar to that seen in plate 2. Plate 4 shows the liver of rats fed the common maize diet that indicates a central vein not too large compared to that seen in plate 2.

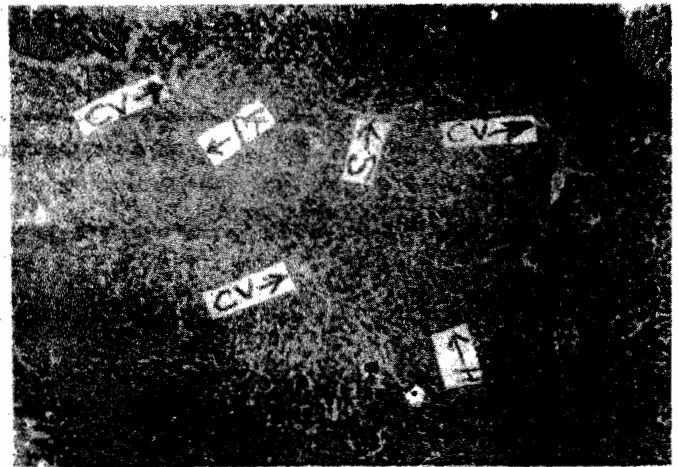


Plate 1: Histology of the liver of rats fed basal diet

CV=Central vein
PT=Portal triad
1. Portal vein
2. Hepatic artery
3. Bile duct
S=Sinusoid
H=Hepatocytes

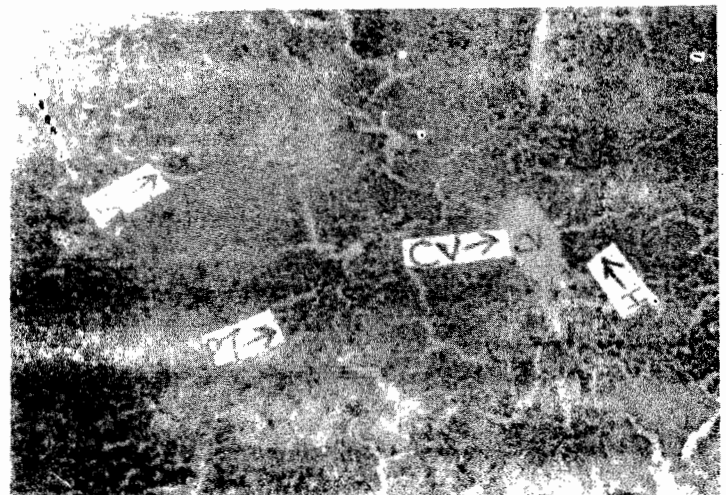


Plate 2: Histology of liver of rats fed reference diet

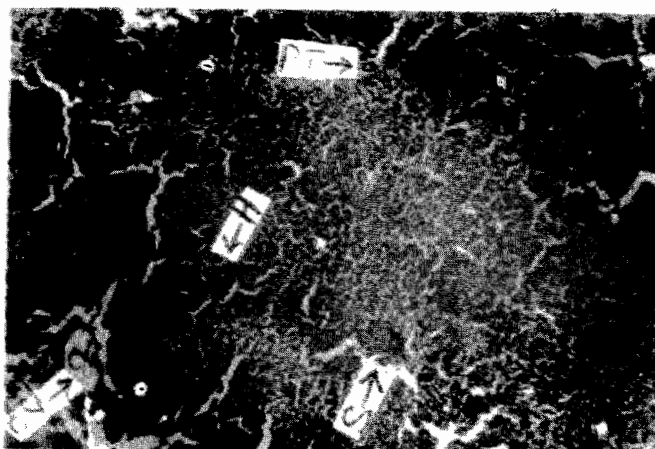


Plate 3: Histology of liver of rats fed quality protein maize diet



Plate 4: Histology of liver of rats fed common maize diet

Table 1: The activities (U/L) of serum AST and ALT in rats fed the experimental diets.

Groups	Aspartate aminotransferase	Alanine aminotransferase
A:		
Basal diet	61.2 ± 4.63	33.5 ± 6.50
B:		
Reference	46.0 ± 4.48	21.6 ± 2.11
C:		
Test diet 1	57.4 ± 8.92	21.6 ± 3.98
D:		
Test diet 11	60.2 ± 8.59	27.4 ± 6.13

Each value represents mean ± SEM of 5 Determinations.

The sinusoids are smaller compared to those shown in plates 2 and 3. The portal triads are distinct and almost completely filled with highly eosinophilic tissues, probably red blood cells.

The serum AST levels in rats fed basal, reference, QPM and common maize diet were 61.2 ± 4.63U/L, 46.0 ±

4.48U/L, 57.4 ± 8.92U/L, and 60.2 ± 8.59U/L respectively. The serum ALT levels were 33.5 ± 6.50U/L, 21.6 ± 2.11U/L, 21.6 ± 3.98U/L and 27.4 ± 6.13U/L respectively (Table 1). Enzymes are essential factors which enable the many biochemical reactions that constitute life to proceed in the cells of the body. Changes in enzyme concentration in

tissues should reflect the state of health. Aminotransferase activity in serum has been of use in diagnostic enzymology when heart or liver damage has occurred. A rise in serum ALT points more to pathology of the liver rather than the heart. Although changes in the activities of some enzymes in the serum or plasma have been associated with tissue damage and as a consequent leakage of the enzyme from the (Alkyne *et al.*, 1979), the activities of the enzymes (AST and ALT) were generally not significantly different between the different dietary groups ($P > 0.05$). In particular, ALT value of animals fed QPM diet is the same with that fed the standard Reference (casein) diet, hence no toxicity on the hepatic tissue.

CONCLUSION

This study has demonstrated that despite the controversy surrounding the use of quality protein maize, this improved protein maize can be considered free from having any toxic effect on the liver of animals.

REFERENCES

- Alkyne, GAO; Hay, R.W; Picou, D.I; Standfield, J.P; Whitehead, R. G. 1979. The Pathology of protein-energy malnutrition. In Protein-Energy Malnutrition. ELBS.London.
- Atangwho, I. J. 2005. Assessment of the protective effect of aqueous extract of *Venoma Amydalina* on pancreatic and hepatic tissues and its impact on serum biochemical and selected heamatological indices of alloxanized wistar rats.M.Sc thesis.
- Washington DC. 1980. University of Calabar. Calabar, Nigeria. Association of Official Analytical Chemists. Official Methods of Analysis. (13th ed) .
- Bradbury, S., 1973. Hewer's Textbook of Histology for Medical Students (Rev.) ELBS and William Heinemann Medical Books Ltd. London. 431-438.
- Bressani, S. and Martz, T. 1958. Mutant gene that changes protein composition and increase Lysine content of maize endosperm. Nutritionally enhanced corn. 11: 220-223.
- Bressani, S. and Elias, W. 1970. Inheritance of modified endosperm structure. Cereals Research Communication. 4: 401-410
- CIMMYT., 2000. Economics working papers on Quality protein maize. Centre for Quality protein maize study. Mexico 13: 258-858
- Ernest, S. 2000. Issues regarding target and adoption of quality protein maize. Purdue University, Indiana 10: 120-135.
- Eteng, M. U., Ebong, P. E., Ettarh, R. R., and Umoh, I. B. 1998. Aminotransferase activity in serum, liver and heart tissue of rats exposed to theobromine. Indian J. Pharm.30:339-342.
- Hubell, H. 1991. Feed stuff analysis table. Feeding Studies. Purdue Univ. Indiana 11: 40-42.
- Reitmann, S. and Frankel, S., 1957. Determination of Aminotransferases in serum. Amer, J.Clin Pathol 28:56
- Struer, E. A., Makarora, U. G., 1984. Laboratory manual in Biochemistry. Miv. Publishers Moscow. 162-164.
- Villegas, E... 2000. Researchers for quality protein maize. Mexico. 13:139-143.
- Wardlaw, G. M., 1999. Perspectives in Nutrition (4th Ed). WCB/Mc Graw-Hill. 423
- Watson, F., 1987. Physical and chemical kernel characteristics of normal and Opaque-2 endosperm maize hybrids. Crop Science. 17: 362-366.