

Biosafety Baseline for African *Biofortified sorghum* (ABS188 and ABS203) Through Feeding Bioassay

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ABSTRACT

A number of biofortified crops have been generated through transgenic technologies including sorghum. A key step towards the release of genetically modified (GM) biofortified sorghum is its nutritional evaluation and risk assessment study. In this work, two genetically modified sorghum ABS188 and ABS203 were administered on mice to evaluate the effect of their consumption on liver and spleen. Following the molecular analysis of the two GM sorghum, the transgenes *Zeamays* Phytoene synthase gene (*Zm-PSY1*) and *Pantoea ananatis* Carotenoid Biosynthesis gene (*PaCrT1*) were confirmed in ABS 188 and ABS 203. There was a loss in weight of mice fed with ABS 188 and ABS 203 while weight gain was recorded in mice fed with local sorghum. In conclusion ABS 188 and ABS 203 are considered to be as safe and nutritious as local sorghum, with the advantage that the GM sorghum are biofortified with Vitamin A, Zinc and Iron.

Keywords- Genetically Modified Sorghum, Biofortification, Nutrition and Transgene.

I. INTRODUCTION

Micronutrient deficiency or hidden hunger, which is characterized by chronic deficiency of essential vitamins and minerals such as vitamin A, iron, zinc and iodine, affects millions of people, especially the rural poor and other vulnerable populations. In Sub-Saharan Africa, where the highest prevalence of hidden hunger is recorded, vitamin A deficiency alone affects 48% of children under five years while iron deficiency is responsible for many cases of anaemia affects 63% of children under 5 years, contributing to 20% of all maternal deaths. Furthermore, about one-third of the world's population suffer from zinc deficiency while 26% of Africa's population is at risk of becoming zinc deficient. The most significant and common clinically micronutrient deficiencies in children and women of childbearing age include deficiencies of iron, iodine, zinc, and vitamin A and are estimated to affect as many as two

billion people (Luchuo *et al*, 2013).

Sorghum [*Sorghum bicolor* (L.) Moench], a tropical plant belonging to the family of Poaceae is one of the most important crops in Africa, Asia and Latin America (Anglani, 1998). In terms of total cereal consumption, sorghum represents about 30% (USAID, 2010) Nigeria is the largest cereal producer in West Africa accounting for about 71% of the regional sorghum production in 2006 (RECA Niger, 2010). Therefore, sorghum plays a crucial role in contributing to house-hold food security in many of the world's poorest and most food-insecure regions, that cannot afford imported rice and wheat-based food (ICRISAT/FAO, 1997). Sorghum grain has a nutritional profile similar to corn and other cereals as it shares the typical nutritional deficiencies of cereal grains, a low content of several essential amino acids, a low vitamin A and E content and a low bio-availability of iron and zinc (Shewry and Halford, 2003). Therefore, a diet, based mostly on sorghum, is not adequate to meet the nutritional growth or maintenance requirements for children and adults and needs to be supplemented with essential amino acids and micronutrients. Advances in genetics and molecular biology have enabled the development and commercial release of genetically modified organisms (GMOs) such as sorghum, with traits that transcend the species barriers. The development of biofortified sorghum offers the potential for increased agricultural productivity or improved nutritional values that can contribute directly to enhancing human health and development.

Biofortification is a process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or genetic modification. Howarth and Amy (2017) reported that biofortified crops can improve human nutrition and address micronutrient deficiencies by increasing the daily adequacy of micronutrient intakes among individuals throughout the lifecycle Biofortified crops are also a feasible means of reaching rural populations who may have limited access to diverse diets or other micronutrient interventions (Howarth *et al.*, 2011).

Genetically modified plant has been one of the most rapidly adopted technologies in the history of agriculture. (Harlander, 2002). Since the introduction of the first genetically modified plant in the 1983, genetic engineering techniques and their applications have developed rapidly. The development of the nutritionally enhanced sorghum lines will rely on transgenes and technologies that have shown high efficacy in transgenic maize and that resulted in a significantly improved nutritional quality of maize grain. As a proof of concept, a first-generation transgenic sorghum line (ABS #1) that possesses grain with a 50% increase in lysine has already been developed. The goal of the ABS project is to develop transgenic sorghum varieties that will overcome most of the described nutritional deficiencies by substantially improving grain digestibility, by delivering vitamins, the essential amino acids lysine, threonine and tryptophan, and by improving the bioavailability of iron and zinc. (Zhao *et al.*, 2003)

The main objective of this study was to assess the safety of genetically modified sorghum by determining the histopathological effects of genetically modified sorghum (ABS 188 and ABS203) on the liver and spleen of experimental mice.

II. MATERIALS AND METHODS

Source of Sorghum Bicolor

The Genetically Modified sorghum were obtained from The National Biosafety Management Agency Abuja, through institute for Agricultural Research Zaria (IAR). The local sorghum was obtained from the Central market Kaduna and was identified in National research institute for chemical technology (NARICT) Zaria with voucher number 0/2018.

Detection of Trance Genes in the Sorghum Varieties

DNA Extraction

DNA of the GM sorghum (ABS 188 and ABS 203) and local sorghum were extracted and amplified using the methods described by Shaista *et al.*, (2009). PCR primer was synthesized by Bioneer Company, USA.

Polymerase Chain Reaction

DNA amplification was carried out using specific primers for the phytoene synthase gene: ZmPSY1 For 5'GTGTAGGAGGACAGATGAGCTTGT3'; and ZmPSY1 Rev 5'CATCTGCTAGCCTGTGAGAGCTCA3' and carotenoid reductase gene: PaCRT1 For 5'TGGAGAAGCGTTTACAGTAAGGT3' and PaCRT1 Rev 5'GCGTGACAGATAAAGTGAGAAGTC 3' (Shaista *et al.*, 2009).

Amplification was carried out in a DNA thermal cycler PTC-100. Two microlitres of extracted DNA was added in to a 0.2ml tube containing PCR component from Bioneer (1U of taq polymerase, 1.5mM of MgCl₂, 250µM of each dNTPs, 2X PCR Buffer) 1 µl of each primer forward and reverse were added and the reaction mixture

was made up to 20ul with PCR grade water. The PCR was conducted with the following amplification steps in thermal cycler PTC 100 by MJ Research: Initial Denaturation of 94°C for 5min; 35 cycles of denaturation at 94°C for 30seconds, annealing at 55°C for 30 seconds, Extension at 72° C for 1 minute and final extension at 72°C of 5minute.

Gel Electrophoresis

Amplified PCR products were separated by agarose gel electrophoresis using 1.5% agarose gel at 100Volt for 40minutes. The result was analyzed using a gel documentation system (Universal hood II, Biorad laboratories Segrate (Milan) italy). Automated DNA sequencing was performed at the DNA Labs located in kinkino road, Kaduna State, Nigeria. The samples were sequenced to confirm the nucleotide arrangement of the genes.

Sequencing

The amplified PCR products were purified using QIAGEN® PCR Purification Kit. Sequencing reaction was carried out using the same PCR primers (ZmPSY1 and PaCRT1) using the BigDye termination sequencing. The reaction was purified and Electrophoresed using ABI Prism, 310 genetic analyzer Applied biosystem at the DNA Laboratory, Kaduna.

The sequence obtained was align with other sequence on gene bank using The National Centre for Bioinformatics (NCBI) BLAST option and the percentage identity match was obtain.

III. EXPERIMENTAL ANIMAL STUDIES

Source of Experimental Mice

Twenty mice with body weight ranging from 15-36g were obtained from Department of physiology Ahmadu Bello University Zaria, Kaduna.

Animal Housing and Feeding

The animals were housed in cages and were kept in DNA labs Kaduna. Feed and water ad libitum were provided. The animals were allowed to acclimatize to the new environment for two weeks before they were introduced to the experimental diets.

Grouping of Experimental Animals

The mice were separated into four groups, five mice per each group. The animals were fed with diet each containing 20% of either GM Sorghum or Local Sorghum. Feeding was for a period of three months. Proper sanitation of the cages was kept throughout the duration of the study period.

Determination of Growth of Experimental Animals

The growth of animals in each of the groups was measured through changes in body weight. This was done by weighing each individual mice in each group, at the beginning of the experiment, every week and at the end of the experiment. The increase or decrease in weight was recorded against weeks. The feed conversion ratio was

determined by measuring the amount of feed consumed per unit weight gained.

The percentage mortality rate is used to assess the death recorded in the experiment from the beginning to the end.

$$\text{Percentage mortality} = \frac{\text{no of dead experimental animals}}{\text{total no experimental animals in a group}} \times 100$$

Histopathology of the liver and Spleen

Experimental animals were sacrificed after 90days of feeding with experimental diet. Employing the method of Aysun and Turan (2008), the liver and spleen were collected and fixed in fixative (10% neutral buffered formalin) and were stored until required. All specimens were dehydrated in ascending grades of isopropyl alcohol (70%, 85%, 95% and 100%), cleaned in xylene, and

embedded in paraffin wax. Serial sections were cut to a thickness of 6 μm and stained with hematoxylin and eosin for histopathological investigation.

IV. RESULTS

Molecular Analysis of Sorghum Samples

Molecular detection of the Trans genes is shown in Plate 1. Bands at 550bp in lane 1 and 2 shows the present of the phytoene synthase gene (Zeamays) in ABS 188 and ABS 203 but absent in the local sorghum (lane 3). Also 500bp bands lane 7 and 8 shows the present of Carotenoid reductase gene (CRT) in both ABS 188 and 203 but absent in local sorghum (lane 9). Lane 5 and 10 which shows no bands are the negative controls.

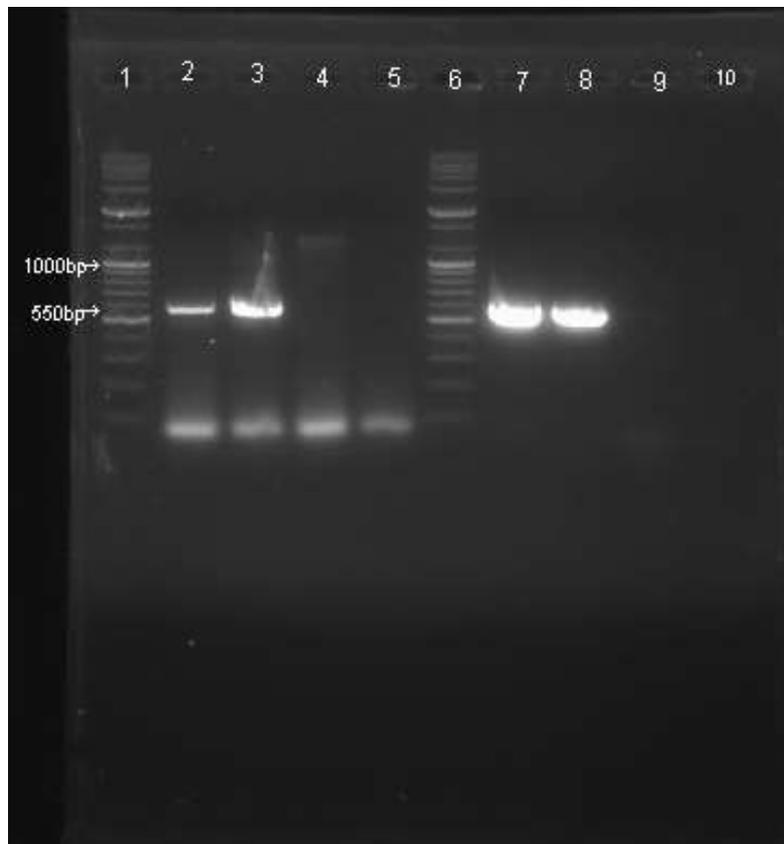


Plate I: Agarose gel electrophoresis showing the transgenes ZmPsy in ABS 188 lane 2 and ABS 203 lane 3 and CrT1genes in the two transgenic sorghum lane 7 and 8 (ABS 188 and ABS 203 respectively), lane 4 and 9 are the local sorghum. Lane 1 and 6 are 100basepair plus molecular ladder and lane 5 and 10 are the negative controls.

Weight of the Experimental Animals

There was a noticeable decline in the mean weight of mice fed with ABS 188 and ABS 203 (Table 4). Mice fed with ABS 188 loss a weight of about 3.1 g while the mice fed with ABS 203 loss a weight of about

0.6 g. On the other hand, there was a gain in weight of the mice fed with local sorghum (3.8 g). The feed intake of 25 g/day was recorded for all the mice fed with both genetically modified sorghum and local sorghum. Negative feed conversion ratio was observed in mice fed with ABS 188 and ABS 203 whereas the feed conversion ratio of mice fed with local sorghum was positive (6.6g). Statistical analysis reveals that there is a significant difference ($p= 0.0039$) in the initial weight of all the 4 groups while there is no significant difference ($P= 0.4499$) in the final mean weight of the experimental animals, (Table 4).

Table 4: Growth performance parameters of mice fed experimental diet

	ABS 188	ABS 203	Local Sorghum	Standard diet
Initial mean weight (g)	29.4±2.1	29.0±3.9	27.2±5.7	19.4±3.6
Final mean weight (g)	26.3±3.1	28.4±3.3	31.0±6.3	25.5±6.2
Feed intake (g/day)	25	25	25	25
Feed Conversion ratio	-8.1	-41.7	6.6	4.1
Weight gain	-3.1	-0.6	3.8	6.1
Mortality	2	0	1	1
N	5	5	5	

N: number of mice in each group

There is significant difference in the initial weight of all the four groups, $P = 0.0039$

Histopathology of Liver and Spleen of Experimental Mice

Histopathological examination of the spleen (plate 11) shows an unremarkable white pulp shown by the straight arrow, and a red pulp shown by step arrow in the Microscopic cross sections of the mice fed with local

sorghum (group B). Group A, C, and D did not show any inflammation.

Plate 111 shows the photomicrographs of liver of mice fed with the experimental diet with no inflammation in group A, B and D, while group C which was fed with ABS 188 shows a periportal inflammation in the liver.

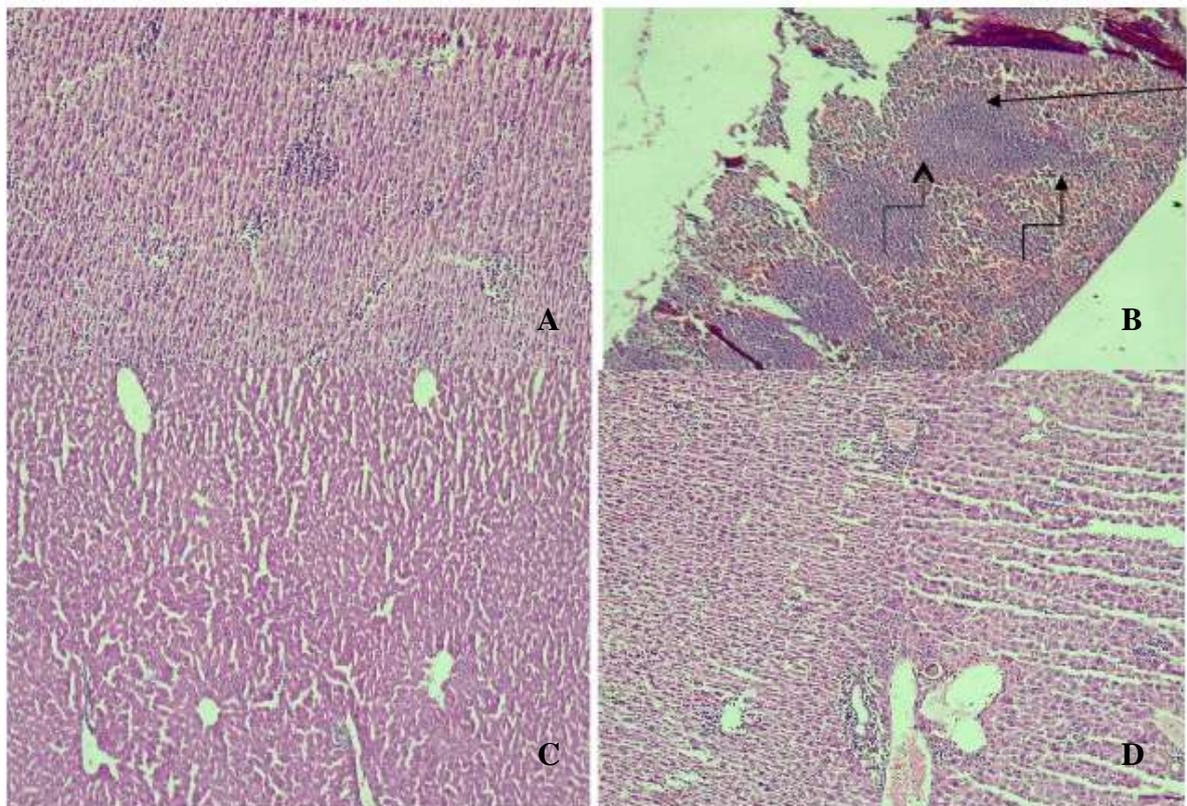


Plate II: Photomicrographs of Spleen tissues (A–D) of mice stained with H&E. A is the control group; B (fed

with local sorghum), C (fed with ABS 188, and D (fed with ABS 203). (Magnification: x200).

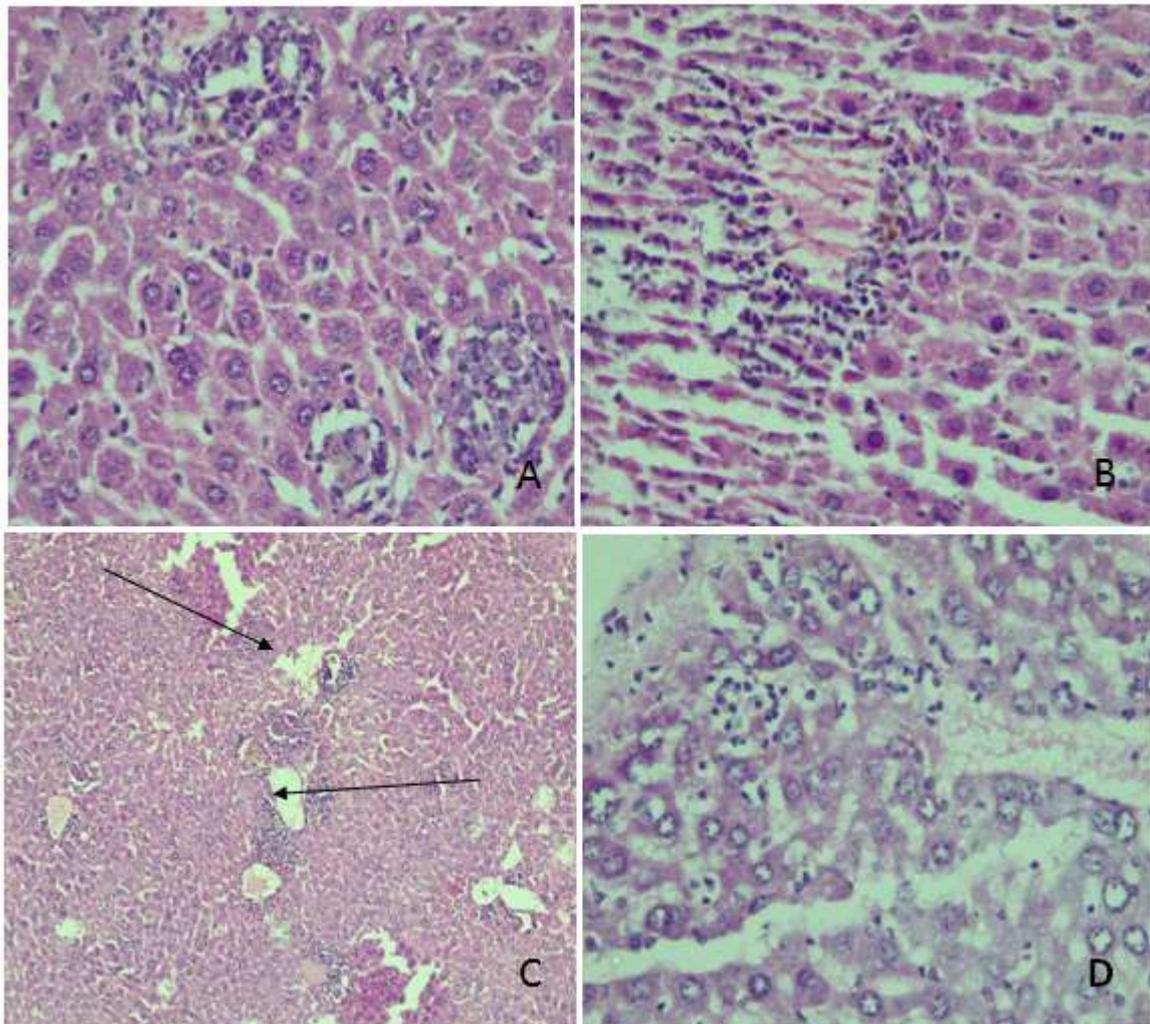


Plate III: Photomicrographs of liver tissues (A–D) of mice stained with H&E. A is the control group; B (fed with local sorghum), C (fed with ABS 188, and D (fed with ABS 203). (Magnification: C, x100 ; A,B and D, x 200).

V. DISCUSSION

Genetic modification technology ensures improvement in productivity and quality of crops (Hashimoto *et al.*, 1999). Guidelines for the safety or risk assessment process to demonstrate that GM crops are as safe as the conventional non-GM crops, requires proximate analysis of key nutrients in GM crops and Feeding studies, to compare the nutritional performance of GM crops to non-GM crops (WHO, 1995).

Results of this studies confirms the presence of transgenes (ZmPsy and CrT1genes) in the genetically modified sorghum but absence in the local sorghum. Which agrees with the work of Shaista *et al.*, (2009) who biofortified and detect the presence of ZmPsy and CrT1 gene in maize. When align with sequence already submitted to gene bank the sequence of the genes for

ABS 188 and ABS 203 shows a 99% identity which shows that the similarity of the query and subject sequence those not occur by chance.

There is a growing concern that introducing foreign genes into food plants may have an unexpected and negative impact on human health, hence animal experiment is employed in this study. Animal feeding experiment gives valuable and reliable information regarding the safety of a genetically modified plants for both livestock and human consumption (Alexander *et al.*, 2007). Currently, genetically modified varieties of yellow maize, soybean and sorghum are produced for animal feed (Nowicki *et al.*, 2010). The results of feeding studies on mice showed no histopathological effects but caused some minor changes. Malatesta *et al.* (1998) suggested that the minor changes in the liver are as a result of alterations in the metabolic processes. Contrary to the result of this study, Malatesta *et al.* (2008) demonstrated that genetically modified cereal and soybean intake could influence some liver features and cause significant modifications of some nuclear features in the hepatocytes of GM-fed mice.

Histopathological examination of the spleen of the mice fed genetically modified sorghum and local sorghum showed no any inflammation. The GM sorghum had no any effect in the spleen of the experimental mice, indicating that the GM sorghum is as safe as the local sorghum. A 105-day feeding study supporting this finding was with mice which were fed with GM soybean and no histopathological abnormalities in the spleen and mucosa of small intestine were detected (Teshima *et al.*, 2000). Results obtained by Jaszczak *et al.* (2008) did not reveal any difference in DNA damage between the control and experimental groups of mice fed a GM diet over 5 generations. In contrast to this study, another feeding study in rats with MON 863 Bt-corn demonstrated no histopathological damage in spleen and liver (Smith, 2005). No apparent adverse effect was also reported by Sakamoto *et al.* (2008) in rats fed on GM soy at a level of 30% in the diet for 104 weeks. Some of the studies that reported no adverse effects in animals receiving the GM cereal diet were done not only at a low concentration (0.1%) in the diet but also by feeding rats for a very short duration (10 days) (Guimaraes *et al.*, 2010).

Monitoring body weight and food consumption in feeding studies with rats can be a sensitive indicator of overall animal health (Borzelleca, 1996). The full growth performance of animals fed on experimental diet (ABS) and control diet at the start of the experimental study were homogenous. At the end of the feeding trial period, mice fed with local sorghum diet increased in body weight, which on the contrary, there was decrease in the body weight of mice fed with experimental diet. The observation in body weight gain of animals in this studies is a reflection of feed intake; because the mice fed with control diet consumed high amount of feed and thus gained the highest weight when compared to that of other groups fed with experimental diets, while ABS 188 has decrease in weight. A 90-day feeding study on rats carried out by (Schroder *et al.*, 2007) indicated that no statistical difference was observed in body weights of rats fed with *Bacillus thuringiensis* corn (Cry1Ab protein).

VI. CONCLUSION AND RECOMMENDATION

Molecular analysis showed that there is presence of transgenes (ZmPsy and CrT1genes) in the genetically modified sorghum (ABS 188 and ABS 203) but absent in the local sorghum. There is a decrease in the final body weight of mice fed with genetically modified sorghum (ABS 188 and ABS 203) as against mice fed with local sorghum which recorded a noticed increase in body weight. This is a direct consequence and reflection of feeding rate and feed conversion ratio. Histopathological examination revealed that the genetically modified sorghum (ABS 188 and ABS 203) had no toxic effect in the liver and spleen of the experimental mice.

Finally, consistent with agronomic, compositional and animal feeding studies, the nutritional

and safety assessment of genetically modified sorghum did not cause any damage in the organs of the experimental mice. In view of this, ABS 188 and ABS 203 are considered to be substantially equivalent to, and as safe as local sorghum.

Lack of dietary diversity and low intake of many macro and micro-nutrients have led to serious malnutrition; hence the need for biofortified and genetically modified food. Because the consumption of genetically modified food has been on the increase and gradually being accepted by diverse people, it is paramount for risk assessment to be performed on other GM crop species in order to ascertain their potential impact in near future. Taking cognizance, the results of this study, further risk assessment studies should be conducted on GM sorghum so as to tackle any issue arising from conflicting interests` and concerns against GM sorghum. Improved technologies should be employed to further assess the acute, medium-term and longer-term effects of GM crops in order to assure their safety. Finally, holistic approach (histopathological, biochemical, neuropharmacological effects etc.) should be taken in the risk assessment study and effect of GM crops in all the organs.

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