



Effect of Genetically Modified *Glycine max* and *Zea mays* on Cell - Mediated Immunity of Albino Rabbits

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Abstract

Genetic modification is an aspect of biotechnological methods involve in the intentional alteration of the DNA of plants, animals and microorganisms with the aim of transferring desired genetic traits across related or unrelated species. This study seeks to assess the effects to genetically modified *Glycine max* (Soybeans) and *Zea mays* (Maize) on some cell-mediated immune parameters (CD₄⁺ T and CD₈⁺ T cells) of rabbits. The feeds; were given in different proportions (10%, 20%, 30% and 100%) of GM diet incorporated in standard growers mash to thirty-five rabbits divided into different groups A-E. Physical, behavioural and anatomical features were monitored and blood samples were collected from the marginal ear vein of the rabbits fortnightly for a period of 14 weeks. The blood samples were assessed for CD₄⁺ T and CD₈⁺ T cells using flow cytometric method. Values obtained from the CD₄⁺ T cell count ranged from 3.33cells/ μ l - 5.00 cells/ μ l while the CD₈⁺ T cell count ranged from 2.05cells/ μ l - 6.00cells/ μ l across the different groups. No negative effect on the health status and cellular immune response indices analysed on consumption of GM diet in the rabbits hence could be considered safe for consumption.

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Introduction

Immunity involves the capability of multicellular organisms to resist harmful microorganisms from entering it by possessing sufficient biotic protection to avert infectious diseases or opportunistic biological takeover. The immune system serves the essential function of protecting a host from numerous pathogenic organisms or cancer cells, and as a result plays a critical function in the preservation of health (Alan,2006). The equilibrium between the combination of antigen driven triggers of the immune component with the host response determines the extent/gravity of the resolution of immune response. Protein nutritional reputation is dynamically related to the function of B-cells, T-cells and the outcome of immunity (Alan,2006). Human immune response is mitigated, balanced and stabilized in immune related physical disorders due to the interaction of many food ingredients and functioning of the immune system. Several studies have shown that there has been evidence of an apparent decline in contagious diseases like tuberculosis, mumps and measles; and a collateral rise in immune associated defects such as multiple sclerosis, allergic diseases, and cardiovascular disorders over the past decade (Bach, 2002).

Proposing from this observation, a connection with non-genetic influence which could include life style metamorphosis, medical and wellness programme practices and dietary habits such as consumption of refined food with reduced micronutrients could be attributable to the cause of these disorders Berg and Forman (2006).

The immune system is complex. It involves a variety of biological activities such as cell division and proliferation, energy metabolism and production of proteins. This complexity supports the idea that nutrition plays a pivotal role in modulating immune function. Modulating immune response is one of the most important functions of food because it contains various substances that can control the physiological functions of the body. Food -derived substances can modulate either innate or acquired immunity. The defence of the body against attack by pathogens or cancer cells and the maintenance of health is the exclusive role of the immune system (Bednarek *et al.*,2013). Malnutrition, aging, physical and mental stress or undesirable lifestyle can disturb immune functions. Vitamins A, C, E and B6, folate, iron, zinc and selenium are macro nutrients most often cited as being important to immune function. Beta-carotene (a

precursor to Vitamin A), Vitamin B₁₂ and Vitamin D are some of the nutrients essential in immune function. Vitamins, minerals and fatty acids enhances DTH (delayed hypersensitivity) vitamins and minerals and thereby enforcing antigen – specific antibody production, oligosaccharides increase and the argumentation of T cells proliferative response. Lactic acid bacteria, vitamins and minerals promote phagocytic and NK cell activity. Therefore, an efficient way to prevent immune function from declining and to reduce the risk of infection or cancer is by the ingestion of foods with immune modulating properties (Buzoianu *et al.*,2012). The immune system attacks the food protein by developing its own protein to attack the allergen. The activation of effector cells (Mast cell and basophils) through food specific immunoglobulin E (IgE) antibodies, IgE-mediated food allergy and cell-mediated reactions result in sub-acute or chronic inflammation. IgE-mediated reactions are rapid and occur within seconds to minutes of the ingestion of the offending food (Ebo and Stevens, 2016). Such manifestations include: acute urticaria and angioedema, rhinoconjunctivitis, asthma, nausea, vomiting, abdominal cramps and diarrhoea (Bednarek *et al.*,2013).

CD₄⁺T and CD₈⁺T cells are recruited to bind to different regions of the MHC II making up the majority of T-lymphocyte (Gerard and Bryan, 2007). CD₄⁺T cells activate innate immune system, B-lymphocyte, cytotoxic T-cells as well as non-immune cells. CD₈⁺T cells play a major role in adaptive immune response activation and differentiation into distinct effector subtypes through the secretion of specific cytokines (Galbas *et al.*, 2011). They also play a critical role in the suppression of immune reaction. Hence, CD₄⁺T cells and CD₄ count are used to assess the immune status of a patient and to determine treatment efficacy (Ashwini *et al.*,2010).

The fear and controversies surrounding the safety of biotechnological altered crops for man and the environment have been on the increase. In this part of the world, researches which tend to address the benefit/effects of the consumption of genetically modified foods on immunological parameters of consumers is still at its infancy especially as these foods are gradually gaining entrance into Nigeria. This study seeks to establish scientifically the consequences or otherwise of the consumption of genetically modified *Glycine max* (Soybeans) and *Zea mays* (Maize) on some cell-mediated immune parameters (CD₄⁺ T and CD₈⁺ T cells) of rabbits.

Material and Methods

Collection and Preparation of Experimental Feeds

25g each of genetically modified soybean (TX197-10F) and maize (PVA 5N) obtained from the

International Institute for Tropical Agriculture (IITA) Ibadan were used for this investigation. The grains were milled with 30ml of water using a local streamer pelletizer (Model 42) instrument.

Experimental animals

Thirty-five non- gestational albino rabbits were used for this study. They were 12 weeks old and weighed between 900 - 1100g. The rabbits were purchased from a local market in Benin City, Edo State, Nigeria. The animals were housed in a well-ventilated cage made up of iron gauze and stainless steel at the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. They were equilibrated on standard grower's mash feeds and water was provided *adlibitum*, for two (2) weeks under standard housing conditions of ventilation, temperature and relative humidity and natural 12hr light/dark regimen. The rabbits were divided into five groups namely A- E to make seven (7) rabbits per group while they were fed with the milled GM crop (soybean (TX 197-10F)) and maize (PVA 5N). Group A received 10%, Group B 20% and Group C 30% dietary incorporation of GM diet and standard growers mash respectively while Group D was fed with 100% of GM diet, while Group E(control group) was fed with 100% standard growers mash(without GM food) for a period of 14 weeks.

Blood collection

At the end of two weeks equilibration period, the animals were weighed and 6ml of blood samples was collected from the marginal ear vein of the rabbits and dispensed into EDTA and plain containers. Blood samples were subsequently collected every two weeks and used to carry out appropriate laboratory analysis for a period of 14 weeks (Okojie and Eghafona, 2016).

Estimation of CD₄⁺ T and CD₈⁺ T Cells Counts.

Blood CD₄⁺ T and CD₈⁺ T Cells levels were assessed by adding 20ml whole blood into a test tube with (EDTA) as anticoagulant. 20µl of CD₈ mAbPE and CD₄ mAbPE was added, mixed gently and incubated for 15mins at room temperature. The procedure was conducted in a darkroom. Eight hundred microlitre (800µl) of no lyse buffer was added, shaken to properly mix and then analysed using a PARTEC flow cytometer with an excitation light source of 488nm (Alan, 2006).

Statistical Analysis

The data were recorded as mean ± standard deviation. The independent t-test and one way analysis of variance (ANOVA) was used to analyze the mean value of the treatment and control group for CD₄⁺ T and CD₈⁺ T Counts. Any differences of 5% (*p*<0.05) were considered significant (Ogbeibu, 2014).

Results

The responses of different treatments is shown in figure 1. Concentrations 3.33 ± 0.57 cells/ μ l for Group A which received 10% GM supplemented diet, 5.00 ± 1.41 cells/ μ l for Group B (20% GM supplemented diet), 4.00 ± 0.86 cells/ μ l for Group C (30%) GM supplemented diet, 4.00 ± 0.86 cells/ μ l for Group D (100%)GM supplemented diet) and 4.00 ± 0.89 cells/ μ l for Group E (control group). CD_4^+ T cells counts recorded a non-significant difference at ($p < 0.05$) when compared with the control across the various treatments

GM supplement diet treatment group when compared with the control group (Figure 2). Group A which received 10% GM supplemented diet recorded 4.33 ± 2.08 cells/ μ l, 4.50 ± 0.70 cells/ μ l was recorded for Group B (20% GM supplemented diet), 2.50 ± 0.70 cells/ μ l for Group C (30% GM supplemented diet), 4.44 ± 1.42 cells/ μ l for Group D (100% GM supplemented diet) and 6.00 ± 1.09 cells/ μ l for Group E (control group). A non-significant ($p < 0.05$) decrease was witness in CD_8^+ T cells count analyzed across the various.

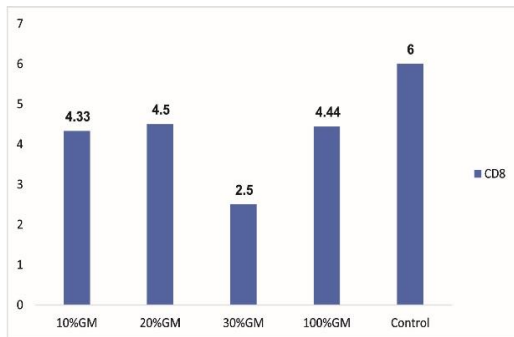


Figure 1: CD_4^+ T Cell Count of rabbit fed with different concentrations of genetically modified feed

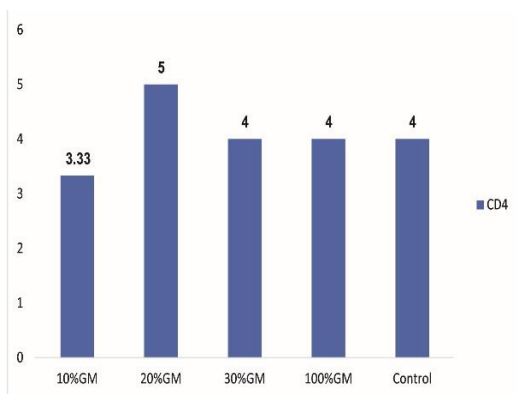


Figure 2: CD_8^+ T Cell Count of rabbit fed with different concentrations of genetically modified feed

Discussion

CD_4^+ T and CD_8^+ T cells are cellular immunological markers whose values indicate the immune state of individuals with regards to its ability to mount appropriate responses against foreign agents (Matthias and Martin, 2010). These immunological markers are grossly affected by food substances (Sivan *et al.*, 2017). Findings from this study revealed an inconsequential contrast in the result obtained in the control group as compared to the GM diet supplemented groups respectively. CD_4^+ T cells are a conjugated protein found on the exterior of T helper cells, monocyte, histiocyte and dendritic cells. They are white blood cells, essential in the immune system which help send signal to other immune cells including CD_8^+ T cells in the advent of infection. Increased number of CD_4^+ T cells in the blood could be suggestive of an allergy (Janeway *et al.*, 2001). Classical symptom of food allergy which may be due to immune response include itchiness, difficulty in breathing, diarrhoea, vomiting swelling of the tongue etc (Helm *et al.*, 2000). These were not noticed in the rabbits in the cause of this study as they appeared normal with bright eyes, normal fur and stools and active motility; which correspond with previous findings by Buzoianu *et al.*, 2012. Observations from this study corresponded with polish cellular immune response study where inconsequential changes were seen in peripheral leukogram of leukocyte subpopulation including Leukocyte, Polymorphonuclear leukocytes or lymphocyte immunophenotype with explicit classification of CD_3^+ T, CD_4^+ T and CD_8^+ T in the blood of animals (Sow and fattener) given genetically modified soybean and BT maize (Bednarek *et al.*, 2013). This confirms that genetically modified feed component did not affect cellular immunity in experimental animals. (Galbas *et al.*, 2011) also found out that antigenic properties of globulin obtained from genetically modified soybean are homogeneous to those of classical soybeans protein.

Conclusion

Findings from this study have revealed that cell-mediated immune parameters (CD_4^+ T and CD_8^+ T cells) of rabbits were not negatively affected upon consumption of GM food; the rabbits appeared normal and stable physically and were also active throughout the course of the study. Hence, the doubts in relation to the health and safety suitability of genetically modified food should be reconsidered.

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