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EFFECT OF SOYABEAN DIET ON THE SERUM IMMUNOGLOBULIN PROFILE AND CD_4^+ COUNT OF MALE DIABETIC RATS CHALLENGED WITH K.PNEUMONIAE

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ABSTRACT

In this study, the effect of soyabean supplemented diet on the immunoglobulin profile and CD_4^+ count of male diabetic rats challenged with Klebsiella pneumoniae were evaluated in a total of sixty-four mature albino rats, weighing 150-250 grams. The rats were separated into eight groups of eight rats each. The groups were as follows: A- rats fed on rat feed, B-rats fed on rat feed and soyabean meal, C-diabetes-induced rats feed on rat feed, D-diabetes-induced rats fed on rat feed and soyabean meal, E-rats fed on rat feed but challenged with Klebsiella pneumoniae, F- rats fed on rat feed and soyabean meal challenged with Klebsiella pneumoniae, G-diabetes induced rats fed on normal rat feed but challenged with Klebsiella pneumoniae and H-diabetes-induced rats fed on rat feed and soyabean meal but challenged with Klebsiella pneumoniae. The study lasted for 28days. At the end of 28 days, the rats were bled and blood collected, processed and the serum obtained used for assessment of immunoglobulin profile and CD_4^+ T-cell count. Immunoglobulin profile was carried out using immunoturbidimetric assay with Randox(R) kit, while CD_4^+ T-cell count was done using CY-flow machine. Results showed that there were significant increase ($P< 0.05$) in serum lgA, 1gM, 1gG levels and $CD4^+$ T-cell of diabetic rats fed on normal feed and soyabean meal when compared with their counterparts fed with only normal feed. Results of the study suggest that soyabean diet could assist in boosting the serum immunoglobulin levels and CD_4^+ cell of diabetic rats and hence protecting the rats from K. pneumoniae infection.

KEY WORDS

Diabetes mellitus, Immunoglobulins, CD_4^+-T-cell, K. pneumoniae and Soyabeans.

INTRODUCTION

Diabetes mellitus, often referred simply as diabetes, is a syndrome characterized by disordered metabolism and abnormally high fasting blood sugar (hyperglycaemia) resulting from insufficient levels of the hormone, insulin or its insensitivity (Tierney et al., 2002). It is a chronic disease characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism (Sky, 2000, Rother, 2007). The World Health Organization, recognizes three main form of diabetes mellitus type I , type II and gestational diabetes (occurring during pregnancy) (WHO, 1999).

Type I diabetes mellitus formerly called juvenile onset or insulin dependent diabetes mellitus (IDDM) is characterized by loss of the insulin

producing beta cell of the islets of langerhans of the pancreas, leading to a deficiency of insulin. The main cause of this loss of β- cell is a T-cell mediated autoimmune attack (Rother, 2007). There is no known preventive measure which can be taken against type I diabetes. While type 2 diabetes mellitus, also known as non-insulin dependent diabetes mellitus (NIDDM), is due to insulin resistance or reduced insulin sensitivities combined with reduced insulin secretion (Rosenbloom and Silverstain, 2003). Furthermore, gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness (WHO, 1999). In Nigeria, diabetes mellitus has emerged as one of the biggest health problems and its prevalence is increasing at an alarming rate (Unadike, 20120).

Soyabean, is a plant of the family leguminoseae. Legumes are noted for their relatively high protein content when compared with cereals, tubers, fruits and vegetables. Soyabean contains about 40% protein, 18% fat and has a high concentration of minerals such as calcium, iron etc (Anosike et al., 2007).

Infections, no matter how mild, have adverse effects on nutritional status. The significance of these effects depends on the nutritional status of the individual, the nature and duration of the infection and the diet during the recovery period (Scrimshaw and Sangiovanni, 1997). Furthermore, it is not surprising that protein deficiency is so consistently observed to interfere with resistance to infection because most immune mechanisms are dependent on cell replication or the production of active protein compounds. However, because protein cannot be synthesized without a balance of essential amino acids, experimental amino acid deficiencies have the same effect as protein. Essentially all forms of immunity have been shown to be affected by protein-energy malnutrition in young children, depending on the severity of the protein deficiency relative to energy. The effects include impaired antibody formation (Pretorius and De Villiers, 1962, Reddy and Srikantia, 1964 and Reddy et al., 1977), decreased serum immunoglobulin (Aref et al., 1977 and Watson et al., 1987), decreased thymic function (Watts, 1969, Purtilo and Connor, 1975 and Chandra et al., 1982) and splenic lymphocytes, delayed cutaneous hypersensitivity (Simythe et al., 1971 and Chandra, 1974), decreased complement formation (Sythe et al., 1971, Suskind et al., 1976), decreased interferon and effects on nonspecific mechanisms that include anatomic barriers and secretory substances such as lysomes and mucus (Scrimshaw and Sangiovanni, 1997).

In this study, the effect of soyabean diet on the serum immunoglobulin profile (i.e. lgM, lgG and lgA) and CD_4^+ count of male diabetic rats challenged with Klebsiella pneumoniae (a Gram-negative bacteria) was evaluated.

MATERIALS

***Chemical used**: All the chemical used in this research were of analytical grade.*

***Animal used**: Mature apparently healthy male albino rats weighing 150-250 grams were procured from the Animal House of Faculty of Veterinary Medicine, University of Nigeria, Nsukka campus. The animals were allowed to acclimatize for fourteen days before the commencement of the study.*

***Soyabean meal**: Rats on soyabean meal were given 9.6g/kg body weight of soyabean powder dispersed in water by intubation each morning before the animals were fed for the day.*

Rat feed: *The normal rat feed used in this study is pelleted growers mash of Vital(R) feed. It was produced by Grand Cereals and Oil Mills, Jos, Nigeria.*

Pathogenic Organism: *Klebisella pneumoniae was used as the pathogenic organism in this study. Its stock was procured from the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.*

METHODS

Experimental Design

Sixty-four mature male albino rats, weighing 150-250grams were used in the study. The rats were separated into eight groups of eight rats each and kept in eight different standard cages as follows:

Groups	Treatment
A	Rats fed on rat feed
B	Rats fed on rat feed and soyabean meal
C	Diabetes-induced rats fed on rat feed
D	Diabetes-induced rats fed on rat feed and soyabean meal.
E	Rats fed on rat feed but challenged with Klebsiella pneumoniae
F	Rats fed on rat feed and soyabean meal challenged with Klebsiella pneumoniae
G	Diabetes-induced rats fed on normal rat feed but challenged with Klebsiella pneumoniae
H	Diabetes-induced rats fed on rat feed and soyabean meal but challenged with Klebsiella pneumoniae

All the protocols as approved by Institutional Animal Ethics Committee (IAEC) were observed in the study. At the end of the 28days, the rats were bled from the retro-bulbar plexus of the medical canthus of the eye. The blood samples were collected into EDTA tubes and plain sample bottles. The EDTA acted as anticoagulant. The blood samples in the EDTA bottles were used for CD_4^+ T-cell counts, while the blood sample collected in the plain tubes without EDTA were allowed to stand for 30 minutes and centrifuged at the speed of 3,000 revolutions per minutes for 10minutes. The serum was extracted and used for lgA, lgG and lgM assays using standard biochemical methods.

Induction of Diabetes

Diabetes was induced in the rats by injecting 180mg/kg body weight of alloxan monohydrate intraperitoneally in 0.9% w/v NaCl (normal saline) after fasting the rats for 12hours. Seven days after the alloxan administration, the rats were fasted for 12hours and their blood glucose level was measured. Rats having blood glucose level of 400mg/dl and above were selected and used as diabetic rats in this study. Rats that were described as fasted were deprived of food for at least 12hours but were allowed free access (ad libitum) to drinking water.

Pathogen Challenge

The rats were challenged with Klebsiella pneumoniae by intraperitioneal administration of 0.4ml of the bacterial suspension containing 10^8 c.f.u/ml in 0.9% (w/v) normal saline on the 22^{nd} day of the study.

Proximate Analysis

The proximate composition of soyabean and the normal animal feed used in the study were evaluated. Their protein content were determined by micro khfelldal method as was described by

AOAC (2000) and Changsam (2003), while their ash, moisture and fats contents were determined by gravimetric method as described by AOAC (2000). Crude fiber were determined by gravimetric method as described by James (1995). The total carbohydrate were then estimated using the formula as was given by Bemiller (2003).

Immunoglobulin Profile and CD_4^+ T-cell Determination

The serum lgm, lgA and lgG were determined using immunoturbidimetric assay (Whicher et al., 1983) with Randox(R) kit, while CD_4^+ count was carried out using CY Flow SL Green Machine.

RESULTS

*The result of proximate analysis for the soyabean powder used in this study (**Fig.1**) indicated that its moisture content was 7.40%, protein 48.22%, fibre 3.80%, fat 15.90%, ash 4.60% and carbohydrate content 20.08%. Also, the result of proximate analysis of normal feed (pelleted growers mash of vital(R) feed) used in this study (**Fig. 2**.) showed that the feed's moisture content was 16.75%, fat 5.80%, ash 7.25%, fibre 2.60% and protein 17.95% and crbohydrate content was 49.65%.*

Furthermore, when the different groups of rats were challenged with Klebsiella pneumoniae, it was observed that only the group G with diabetic rats fed with normal feed came down with the infection. This was indicated by the sickly appearance of the rats in the group and mortality of half of the rats in the group G before the end of the study.

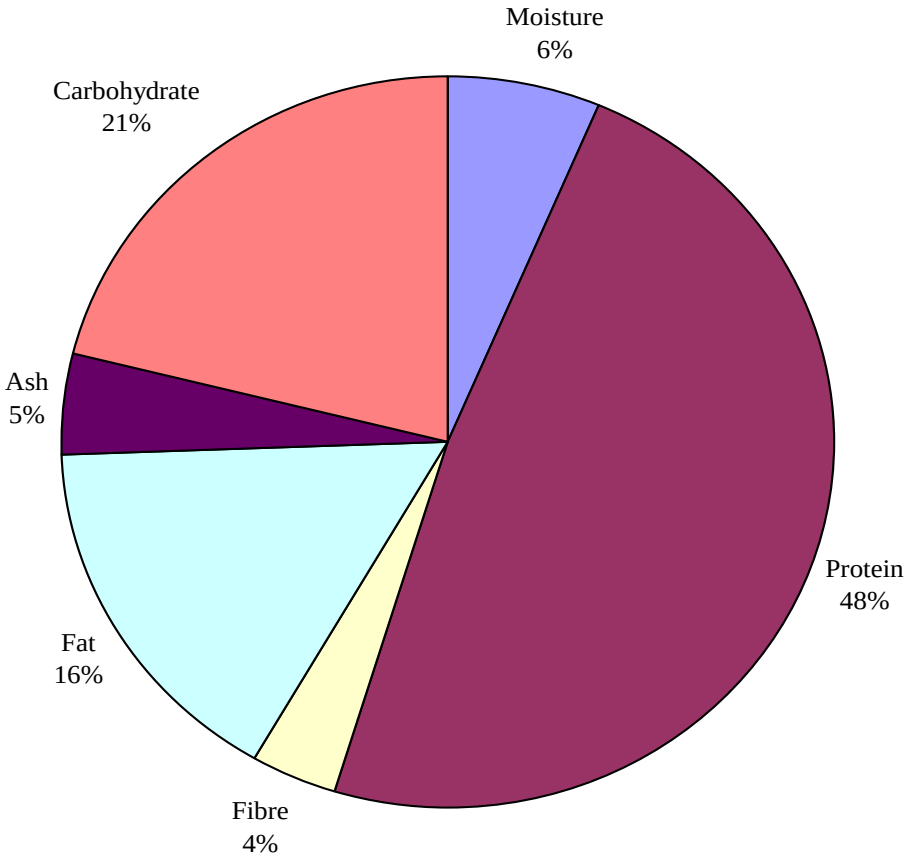

Fig. 1: Soyabean proximate composition

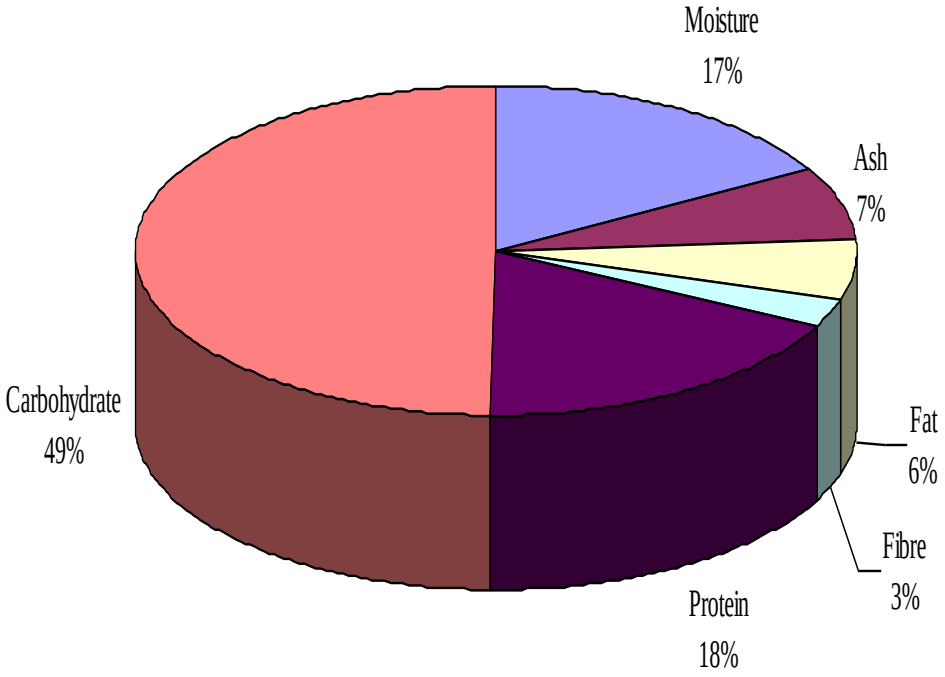

Fig. 2: Normal feed proximate composition

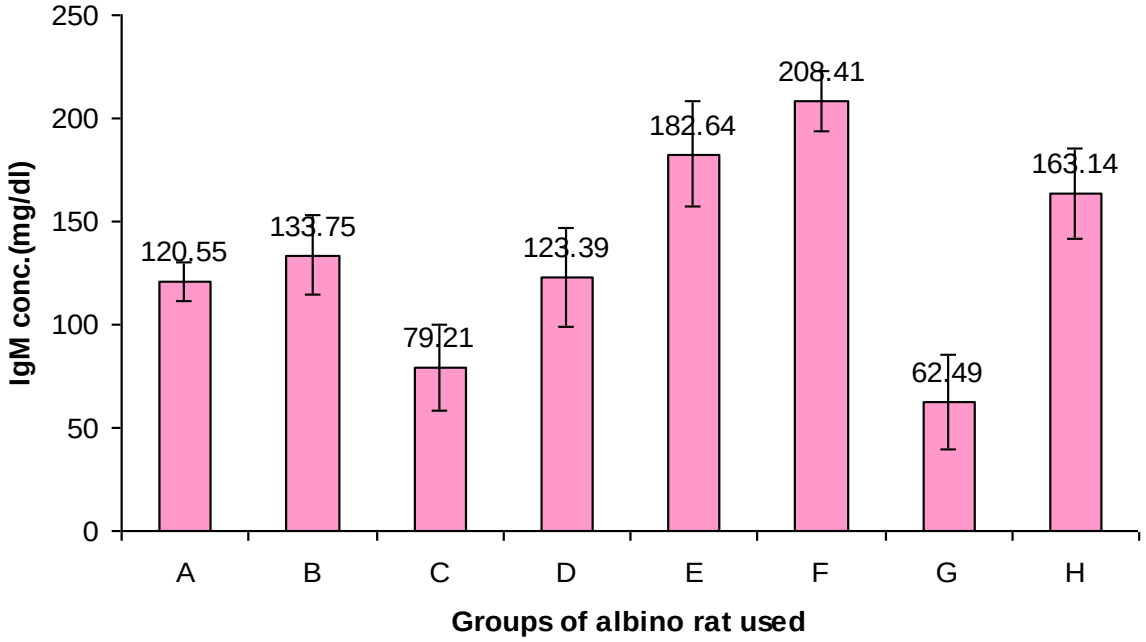

Fig. 3: Mean serum IgM concentration for different groups of rats used

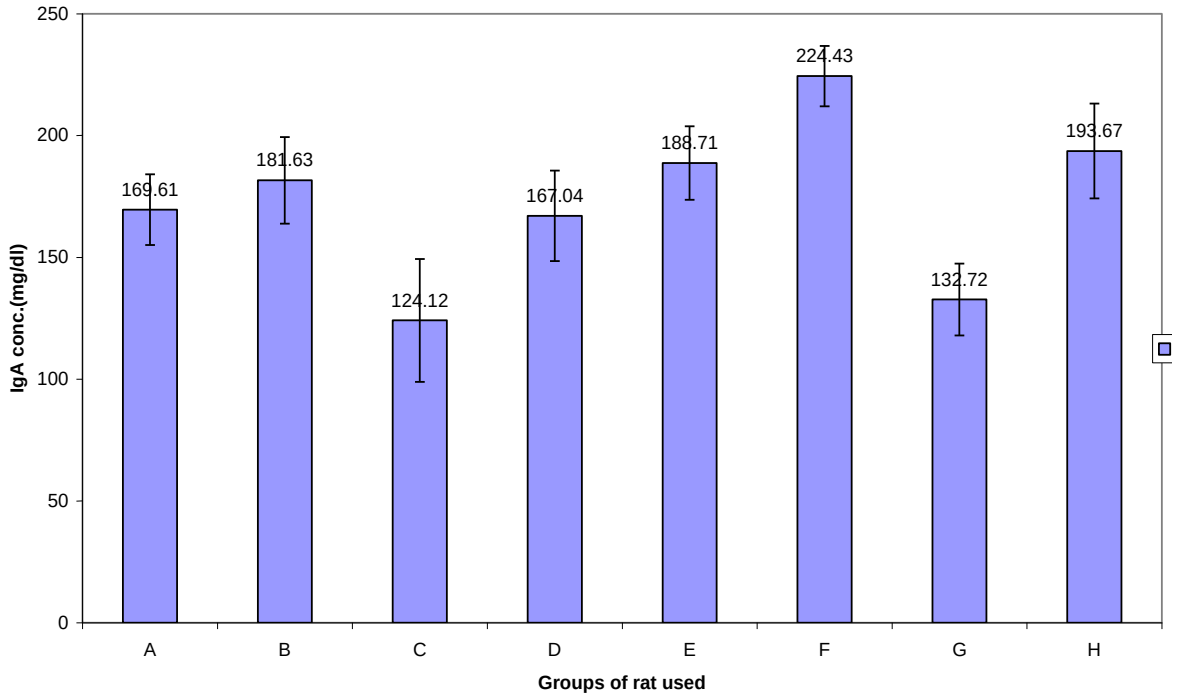

Fig. 4: Mean serum IgA concentration for different groups of rats used

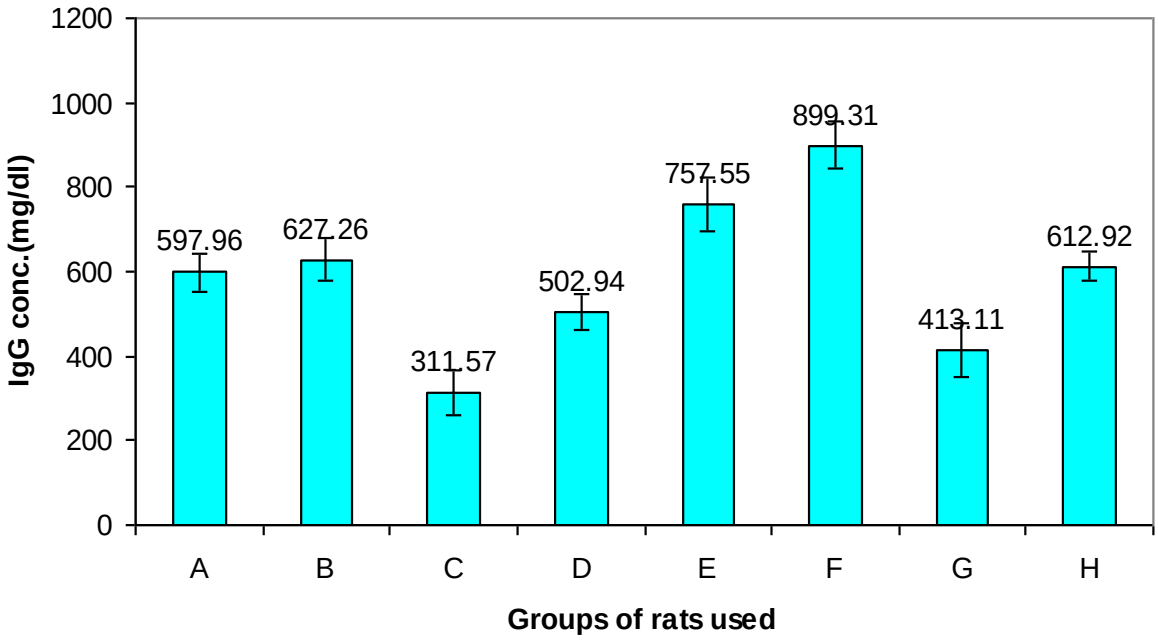

Fig. 5: Mean serum IgG concentration for different groups of rats used

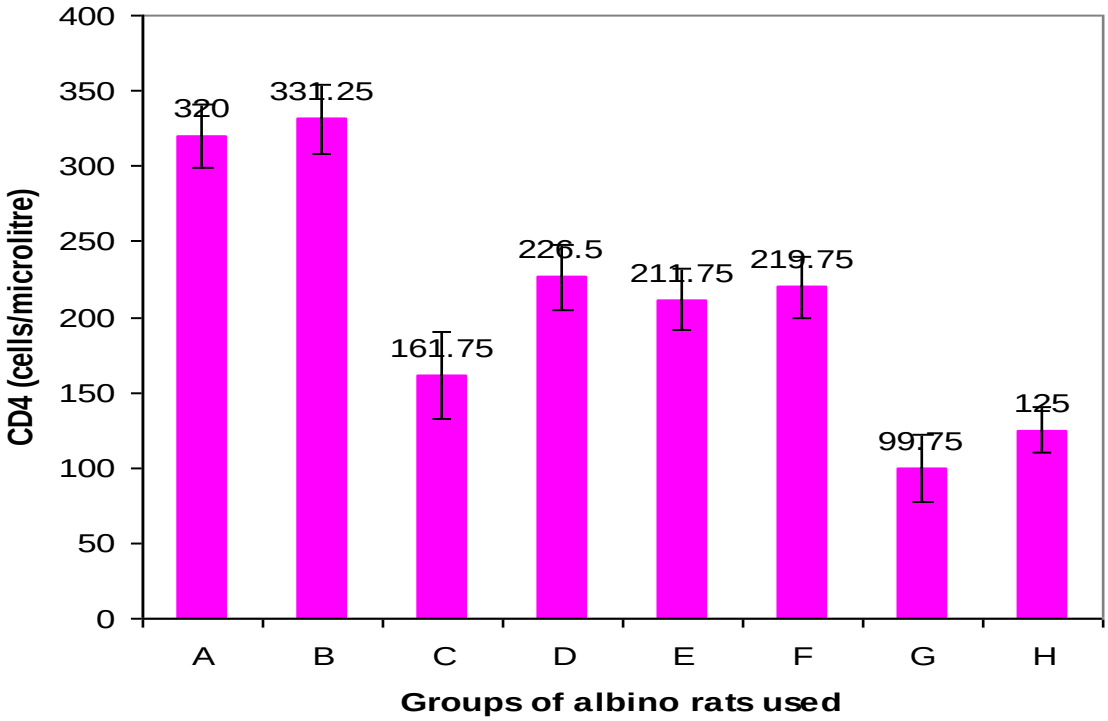

Fig. 6: Mean serum CD_4^+ concentration for different groups of rats used

*The result of the mean serum immunoglobulin M (IgM) for different groups of rats used in the study (**Fig 3**) indicated that the mean serum IgM of groups E, F and H were significantly higher (P<0.05) than that of the control (group A). The result (**Fig. 3**) also showed that there were significant reductions (P<0.05) in the serum IgM concentrations of diabetic rats fed on normal feed only, group C (79.21± 20.73 mg/dl) and diabetic rats fed on normal feed but challenged with Klebsiella pneumoniae, group G (62.49±22.96mg/dl) when compared to control, group A (120.55 ± 9.29mg/dl). **Fig.3** indicated that the mean serum IgM of diabetic rats fed on soyabean diet (group D) was significantly higher (P<0.05) than that of diabetic rats fed on normal feed. Result (**Fig.3**) also showed that the mean serum IgM of diabetic rats fed on soyabean diet challenged with klebsiella pneumoniae (group H) was significantly higher (P< 0.05) than that of diabetic rats fed on normal feed but challenged with Klebsiella pneumoniae (group G). **Fig.3** also indicated that the mean serum IgM of normal rats fed on soyabean diet (group B) and normal rats fed on soyabean diet but challenged with Klebsiella pneumoniae (group F) were higher than their counterparts fed on normal feed (groups A and E respectively). The increases were statistically not significant (P >0.05).*

*The result of the mean serum IgA concentrations for the different groups of rats used in this study (**Fig. 4**) revealed that the IgA levels of normal rats fed on normal rat feed and soyabean meal but challenged with klebsiella pneumoniae, group E (188.71 ± 15.09mg/dl) and diabetic rats fed on normal rat feed and soyabean meal but challenged with Klebsiella pneumoniae, group H (193.67 ± 19.43 mg/dl) were significantly higher (P<0.05) than that of the control, group A (169.61 ± 17.53mg/dl). **Fig.4** further indicated that the mean serum IgA concentration of diabetic rats fed on normal feed, group C (124.12± 25.21 mg/dl) was significantly lower (P<0.05) than those of the*

*control, group A (169.61 ± 17.53mg/dl) and those of the diabetic rats fed on normal feed and soyabean meal, group D (167.04 ± 18.61mg/dl). **Fig.4** also indicated that the mean serum lgA of rats fed on soyabean but challenged with Klebsiella pneumoniae (group F) and diabetic rats fed on soyabean but challenged with Klebsiella pneumoniae (group H) were significantly higher (P<0.05) than their counterparts fed on normal feed (group E and G respectively).*

*The result of the mean serum lgG concentrations for the different groups of rats used in this study (**Fig.5**) showed that the mean serum lgG concentrations of normal rats fed on normal feed but challenged with Klebsiella pneumoniae, group E (757.55 ± 63.22mg/dl) and normal rats but also challenged with Klebsiella pneumoniae group F (899.31 ± 56.23mg/dl) were significantly higher (P< 0.05) than that of the control, group A (599.96 ±45.06mg/dl). **Fig.5** also revealed that the serum mean lgG concentration of diabetic rats fed on normal feed, group C (311.57± 53.36mg/dl) was significantly reduced (P<0.05) when compared to the control, group A (597.96 ± 45.06mg/dl) and to that of the diabetic rats fed on normal rat feed and soyabean meal, group D (502.94± 41.54mg/dl). Figure 5 further indicated that the mean serum lgG of rats fed on soyabean diet challenged with Klebsiella pneumoniae (group F) and diabetic rats fed on soyabean diet challenged with Klebsiella pneumoniae (group H) were significantly higher (P< 0.05) than their counterparts fed on normal feed only (groups E and G respectively). The result also revealed that there was no significant difference (P< 0.05) between the mean serum lgG of rats fed on soyabean diet (group B) and that of rats fed on normal feed only (group A).*

*The result of CD_4^+ cell count for the different groups of albino rats used in this study as shown in **Fig.6** indicated that the mean CD_4^+ cell count of normal rats fed on normal feed and soyabean meal, group B (331-25 ± 22.50 cells/µl) and normal rats fed on soyabean diet but challenged with Klesibiella pneumoniae (group F) were significantly not different (P>0.05) from their counterparts fed on normal feed only (groups A and E respectively). However, with the exception of group B, the mean CD_4^+ count of all the other groups were siginificantly lower (P< 0.05) than that of the control, group A. Moreover, the results shown in **Fig.6** indicated that there were significant reduction (P<0.05) in CD_4^+ count of all the groups challenged with Klebsiella pneumoniae when compared with their counterparts not challenged with the pathogen. The results also showed that the mean CD_4^+ counts of diabetic rats fed on normal rat feed and soyabean meal, group D (226.50± 21.41 cells/µl) was significantly higher than that of the diabetic rats fed on only normal feed, group C (161.75± 28.74 cell/µl).*

DISCUSSION

Over the years, most investigations on diabetes have concentrated on the effect of various diets or herbal extracts on glucose levels without adequate consideration of their effect on the immune response. However, the effect of soyabean diet on some immune markers (lgG, lgA, lgM and CD_4^+cells) of diabetic rats were evaluated in this study.

*The results of the immunoglobulin profile indicated significant increase (P< 0.050 in the lgM concentrations (**Fig.3**) of normal rats fed on normal rat feed but challenged with Klebsiella pneumoniae (group E), normal rats fed on normal feed and soyabean meal challenged with Klebsiella pneumoniae (group F) and diabetic rats fed on normal feed and soyabean meal but challenged with Klebsiella pneumoniae when compared to the control group (group A). This*

suggested that the presence of Klebsiella pneumoniae (antigen), the state of health of the rats and the nutritive value of the soyabean diet used in the study could have likely been the reason for the increased lgM antibodies. This observation corroborated Edelman (1973) observation that in active immunity, the effective contact with antigens causes the host to produce antibodies in response to the antigens. When the lgM concentration of diabetic rats fed on normal rat feed (group C) was compared to the control, a significant reduction (P<0.05) was observed in group C, suggesting that diabetes also causes depletion of the lgM level, thereby reducing immune competence in the rats. However, when the lgM concentration of diabetic rats fed on normal feed (group C) was compared with those of their counterparts fed on normal feed and soyabean meal (Group D), it was observed that the reduction in lgM concentration in group C was restored to normalcy with the incorporation of soyabean diet in group D, an observation that could be attributed to soyabean's high protein composition with its high content of essential amino acids. These amino acids could have helped in boosting the plasma proteins which could have led to increase in the IgM concentration, since immunoglobulins are synthesized by plasma cells. This observation is in accord with McGee and McMurray (1977) report that soyabean (protein) could help in boosting immunoglobulin concentration in the intestine of mice. Immunoglobulins are proteins. So the increase in IgM concentration observed in the diabetic rats fed on soyabean diet (group D) when compared to that of the diabetic rats fed on normal feed (group C) could be as a result of high essential amino acids present in the soyabean used.

*Also, the results of IgA and IgG concentrations for normal rats fed on normal feed challenged with Klebsiella pneumoniae (group E), normal rats fed on normal feed and soyabean meal challenged with Klebsiella pneumoniae (group F) and diabetic rats fed on normal feed and soyabean meal but challenged with Klebsiella pneumoniae (group H) indicated significant increases (P<0.05) in IgA and IgG concentrations when compared with control group A (**Fig. 4 and 5**). This also suggested that the production of more antibodies (IgA and IgG) in both diabetic and non-diabetic rats (except in group G) as soon as they were challenged with Klebsiella pneumoniae in order to protect the rats from invading antigen could have been affected by the presence of Klebsiella pneumoniae, the state of health of the rats (i.e. whether diabetic or non-diabetic) and the nutritive value of the diet (soyabean) used. This observation is also in consonance with the report that the presence of antigen could trigger off the production of antibodies as protective measure of the host against invading antigen (Edelman, 1973).*

It was also observed that more IgM, IgA, and IgG antibodies were synthesized in the groups in which soyabean meal was incorporated into their diet when compared with their counterparts fed on only normal feed. This suggests that soyabean diets could have helped in boosting their immunoglobulin levels, due to the presence of essential amino acids in the soyabean. These essential amino acids could have helped in boosting the immunoglobulin level. This observation also corroborates McMurray (1977) which stated that protein (essential amino acids) helps in boosting immunoglobulin concentration in the intestine of mice. Furthermore, the IgM, IgA and IgG concentrations of diabetic rats fed on normal feed (group C) were significantly reduced (P<0.05) when compared to the control, an indication that diabetes causes depletion of immunoglobulin levels. This could be due to the diabetics' inability to utilize blood glucose for energy generation. Muscle proteins are therefore

metabolized for energy generation, leading to loss of muscle amino acids which could result to the shrinking of the muscles. The depletion of the muscle's amino acids could have caused the decrease in the immunoglobulin levels, since immunoglobulins are synthesized from amino acids. However, it was observed that the reduced immunoglobulin concentrations in group C was restored almost to normal level in diabetic rats fed on Soyabean diet (group D). The suspicion is that the essential amino acids in soyabean could have been replenished the lost amino acids for energy generation. The replenished essential amino acids from Soyabean Diet could have assisted in boosting the immunoglobulin concentration in the rats fed with Soyabean supplemented diet, since immunoglobulins are proteins.

*Moreover, **Fig.6** indicated that with the exception of normal rats fed on normal rat feed and soyabean meal (group B), that the mean CD_4^+ count of all other groups (groups C, D, E, F, G and H) were significantly lower ($P< 0.05$) than the control (group A). This suggests that diabetes and the presence of pathogen (Klebsiella pneumoniae) could have caused reduction in CD_4^+ cells. The utilization of muscle protein for energy generation could have brought about the depletion of essential amino acids in the diabetic rats. This in turn could have caused the decreased CD_4^+ cell count in diabetic rats as observed, since CD_4^+ cells are produced from essential amino acids. Also results in **Fig.6** showed that both diabetic and non-diabetic rats challenged with Klebsiella pneumoniea but fed on soyabean incorporated diet had higher CD_4^+ count than their counterparts fed on only normal rat feed. This is because soyabean is rich in essential amino acids. These essential amino acids could have helped in replenishing the lost muscles and tissues amino acids during energy generation in diabetic rats fed in soyabean diet. The replenished essential amino acids in diabetic rats fed on Soyabean supplemented diet could have boosted their CD_4^+ cell count when compared to that of the diabetic rats fed on normal feed. This should also be expected considering the high nutritive value of Soaybean (**Fig.1**) with high percentage of protein, minerals and vitamins including antioxidants (Regel et al., 2000; Anosike et al., 2007; and Villegas and Gao, 2008). The above observation also corroborates the report that decreased dietary intake of antioxidants could lead to a concomitant decrease in CD_4^+ T-cells (Oguntibeju et al., 2005 and 2006). Since the CD_4^+ T-cells, perform a central and coordinating role in the immune response (Hughes et al., 1997; Vajpayee et al., 2009), and Soyabean diet helped in raising the CD_4^+ cells in diabetic rats as observed in this study, Soyabean diet therefore could help in the general boosting of the immune system of diabetics.*

CONCLUSION

This study revealed significant increases ($P<0.05$) in serum IgA, IgM and IgG levels and CD_4^+ T-cells concentration of diabetic rats fed with normal feed supplemented with soyabean meal (group D) and the rats infected with K. pnemoniae and fed with normal feed and supplemented with soyabean meal (group H) when compared with their counterparts fed with only normal feed (groups C and F). Moreover, it was observed that when various groups of rats were challenged intraperitoneally with 0.4ml of Klebsiella pneumonia suspension containing 10^8 c.f.u/ml, establishment of infection and high mortality rate of the infected rats were observed in diabetic rats fed with normal feed only (group G), whereas no mortality was observed in diabetic rats fed with normal rats feed supplemented with soyabean meal (group H). These results suggest that soyabean diet could be useful in the general

boosting of the immune system and in the general management of diabetes mellitus.

SUGGESTIONS FOR FURTHER STUDIES

It is suggested that further studies be carried out as follows:

- *Inducing diabetes that could last for more than 3 months in order to appreciate the effect of soya protein more, in chronic complications of diabetes.*
- *Feeding the animals with soyabean diet for more than 3 months (i.e. longer period), since the longer the animal is exposed to the diet, the more the effect of the diet could be discovered.*
- *Using more than two different pathogenic organisms that are infectious to albino rats in subsequent study, to confirm these results.*

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