

*Full Length Research Paper*

## **Effect of soya bean (*Glycine max*) on coagulation profile of New Zealand white rabbits**

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The effect of differently cooked soya bean (*Glycine max.*) on some blood coagulation parameters was studied using forty (40) New Zealand white rabbits. The animals were grouped into four (A-D) groups of ten (10) rabbits each and fed 100% growers mash and water ad libitum for three (3) weeks. Group A (control) animals were maintained on the standard pelletized feed while group B, C and D Rabbits had 25% of their feed substituted with 'fermented and boiled', 'roasted and boiled' and 'roasted' soya bean respectively for two weeks. The percentage of diet substituted with soya was increased to 50% and 75% each for a period of two weeks, after which the rabbits were returned to 100% growers mash for three weeks to observe recovery form effects. Blood was collected from the marginal ear vein at the end of each stage of the research to determine prothrombin time, activated partial thrombin time, serum calcium concentration and platelet counts. The result show that soya bean prolonged prothrombin time and activated partial thrombin time but decreased serum calcium and blood platelet concentration. Soya bean intake prolonged blood coagulation. These effects were dependent on the method of processing and percentage composition of soya bean in the feed. Soya bean is rich in protein but contain numerous antinutritional factors which are not completely eliminated in traditional methods of cooking.

**Keywords:** Soya bean, prothrombin, cooking, platelets, coagulation.

### **INTRODUCTION**

Soya bean is the yellow skin seed of *G. max*, a plant belonging to the Fabaceae family. The protein in soya bean is of high quality, consisting of most of the essential amino acids. Soya bean is also rich in minerals and vitamins such as iron, zinc, copper, thiamine, riboflavin, niacin and panthothenic acid. Most of these are well-known haematinics and are essential in the formation of red cells (McArthur et al., 1988). High protein intake affects normal hemostasis, fluid balance and organ growth (Kung-Chi et al., 1993).

Since the beginning of 1970, there has been a great breakthrough in the popularization of soya bean based

food in Nigeria and in many parts of the developing world. As a result, soya bean products have been incorporated into many traditional Nigerian foods (Theodore, 1998). High protein foods of animal origin such as meat, fish, milk and eggs are very expensive especially to the low income earners who are the majority in the population of West African sub region (Belloque et al., 2002). Poverty has therefore aggravated the incidence of protein energy malnutrition in the West African sub region and particularly in Nigeria. In an attempt to combat the ill-effects of protein deficiency, soya bean consumption has been promoted rapidly as a cheap alternative source of protein for low income earners.

Chemical or physical methods are employed in the processing of soya bean. The applications vary in industrial processing or kitchens for human or animal

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consumption. They include roasting, cooking, sprouting, fermentation, use of proteolytic enzymes, infra red heating, alkali and or acid treatment. Steaming at 100°C inactivates the anti-nutritional factors in raw soya flour, thus rendering a maximum protein efficiency ratio. The objective of processing soya bean is to detoxify haemagglutinin and other harmful substances present in the bean while preserving the nutrients (Norman, 2000). According to Nwokolo (1996) the more the degree of processing of soya bean the higher the digestibility.

Consumption of soya beans has been suggested to be beneficial in different health aspects such as cancers, osteoporosis and menopausal symptoms, diabetes, besides exerting a cholesterol lowering and anti-viral activity. It is weakly diaphoretic and stomachic. It is also used in the treatment of colds, fevers and headaches, insomnia, irritability and stuffy sensation in the chest. These health effects are mainly attributable to the presence of saponins and isoflavones in soya beans (Vincken et al., 2008). Nevertheless, despite the good properties associated to soya bean proteins, there are many countries in which the addition of these proteins is forbidden or in which the addition of soya bean proteins is allowed up to a certain extent (Belloque et al., 2002).

Raw soya bean contains numerous anti-nutrients. Although processing can reduce them, it does not completely eliminate them (Perez, 2006). The anti-nutrients in soya bean include phytates, protease inhibitors, trypsin inhibitors, lectins, oxalates and goitrogens. Soya bean consumption has also been associated with cases of infantile leukaemia, increased osmotic fragility, rashes (allergy) and has carcinogenic/mutagenic activity. It is also rich in haemagglutinin, which promotes the clumping of blood cells (Kyaala, 2005).

Phytic acid blocks the uptake of essential minerals – calcium, magnesium, iron and zinc in the intestinal tract (Katz, 1987). These minerals play an essential role in blood coagulation and the maintenance of haemostasis. Hence, a deficiency in these factors will create disequilibrium in the blood coagulation process. Tagnon and Soulier (1946) reported that crystallized soya bean trypsin inhibitor prevents the formation of thrombin from a mixture of prothrombin and blood thrombokinase. The crystallized soya bean trypsin inhibitor had marked anticoagulant activity on whole blood.

Crystallized soya bean trypsin inhibitor, at a concentration of 100 mg/mL, suppresses the production of thrombin from a mixture of prothrombin and blood thrombokinase (Tagnon and Soulier, 1946). Reduced thrombin formation will result in prolongation of blood clotting time.

However, Kung-Chi et al. (1993) opined that a high protein intake caused rapid coagulation of blood in rats without affecting the activity of clotting factors, the diet sensitized rats to factors that initiate clotting *in vivo*.

The broad objective of this work is to assess the

effect(s) of *G. max* dietary preparations on some blood coagulation components of New Zealand White Rabbits. Measures of prothrombin time (PT), activated partial thromboplastin time (APTT), platelet and serum calcium levels in tests and control animals were compared.

## MATERIALS AND METHODS

### Animals

Forty (40) New Zealand White (*Oryctolagus cuniculus*) rabbits were selected for use in this study. The rabbits were housed in collective cages (2 rabbits per cage) measuring 37.0 x 31.0 x 16.0 cm, under controlled temperature conditions (22°C) and with a 12 h light-dark cycle (lights on at 6:00 AM). All experiments involving animals were approved by the Committee on Animal House / Ethics at Igbinedion University, Okada. Edo state.

### Experimental design and groups

Soya bean was weighed dried, cooked according to the method of processing indicated and mixed with commercial feed. 5 g/100 g body weight/day of feed and water *ad libitum* was given to all the animals.

### Preparation of soya bean diet

1. Roasted soya bean: Soya bean was handpicked to remove chaff. Aluminium plate was placed on a red- hot electric heater. Soya bean was poured into the plate and stirred intermittently. Roasting continued until the coat appeared golden brown with visible cracking. This was blend into fine particles using a dry electric grinding machine.
2. Boiled soya bean: Soya bean was handpicked to remove chaff. Aluminium plate was placed on a red- hot electric heater. Soya bean was poured into the plate and stirred intermittently. Roasting continued until the coat appeared golden brown with visible cracking. This was blend into fine particles using a dry electric grinding machine. Water was boiled to 120°C. Roasted, grated soya bean was added and stirred while heating for 5 min to obtain a smooth paste.
3. Fermented and boiled soya bean: Soya bean was handpicked to remove chaff. This was blend into fine particles using a dry electric grinding machine. Soya powder was soaked in the dark for 2 days. This was heated to 120°C for 5 min.

### Sample collection and analysis

Two mL of blood were collected via the marginal ear vein into sodium citrate and lithium heparin anticoagulant tubes, first after three weeks and subsequently at two-week intervals throughout the study. PT, APTT, platelets counts and serum calcium estimation were carried out on the samples following the manual methods described by Dacie and Lewis (1991).

### Statistical analysis

The data obtained was expressed as mean  $\pm$  SEM (Standard Error of Means of ten observations) and statistically by application of the Statistical Package for Social Science (SPSS) version 11. P-values < 0.05 were considered to be significant. All experimental results were first evaluated to establish the necessity for using parametric

**Table 1.** Coagulation profiles of rabbit fed soya bean with respect to processing methods.

Coagulation parameters	Group A (Control)	Group B (Roasted)	Group C (Roasted and Boiled)	Group D (Fermented and Boiled)	Concentration
PT	14.8±2.1	13.9±2.1	14.8±1.6	13.7±1.1	Baseline data
APTT	22.6±1.9	22.7±1.9	22.8±1.8	22.8±1.6	
Platelets	4.4±0.4	4.5±0.4	4.6±0.5	4.8±0.6	
Calcium	2.8±0.3	2.9±0.1	3.0±0.1	3.0±0.2	
PT	13.8±1.3	20.2±1.5	21.3±3.5	28.7±3.9	25% Soya bean
APTT	22.8±1.9	29.8±2.6	26.6±1.2	27.8±1.9	
Platelets	4.5±0.4	4.2±0.3	4.1±0.3	4.1±0.4	
Calcium	2.7±0.2	1.8±0.6	1.6±1.3	1.8±0.9	
PT	14.5±1.4	43.1±1.7	31.3±3.7	37.8±5.7	50% Soya bean
APTT	23.0±1.9	30.8±3.1	26.0±2.0	28.6±1.9	
Platelets	4.5±0.4	3.9±0.2	3.8±0.3	3.4±0.4	
Calcium	2.6±0.3	2.0±0.1	2.1±0.2	2.0±0.3	
PT	14.5±2.3	46.8±4.5	38.1±3.8	44.8±6.2	75% Soya bean
APTT	22.8±1.5	29.6±3.7	29.0±1.7	29.8±2.9	
Platelets	4.6±0.4	3.6±0.3	3.0±0.3	2.9±0.6	
Calcium	2.5±0.3	2.1±0.2	2.3±0.1	2.4±0.1	
PT	15.7±2.9	19.5±2.7	17.4±3.1	19.7±2.1	Recovery
APTT	25.5±2.1	25.5±2.1	25.5±2.1	25.5±2.0	
Platelets	4.5±0.4	3.2±0.2	3.2±0.3	3.7±0.4	
Calcium	2.7±0.2	2.7±0.1	3.3±0.2	2.5±0.3	

statistics. The data were determined to have a normal distribution

pattern was similar for all the methods of cooking employed.

## RESULTS

### PTT and APTT

There were significant increases in PTT and APTT of all the groups compared to control with few exceptions (Table 1). Increase in blood clotting times (PTT and APTT) was dose related (Table 1, Figures 1 and 2). The values obtained at 75% soya bean concentration were higher than those at 50% which were also higher than values for 25% soya bean concentration i.e. 75% (PTT and APTT) > 50% (PTT and APTT) > 25% (PTT and APTT) > baseline (PTT and APTT). F&B soya bean prolonged blood clotting time than R&B soya bean, which recorded higher values than RST soya bean.

### Serum Calcium and Platelets

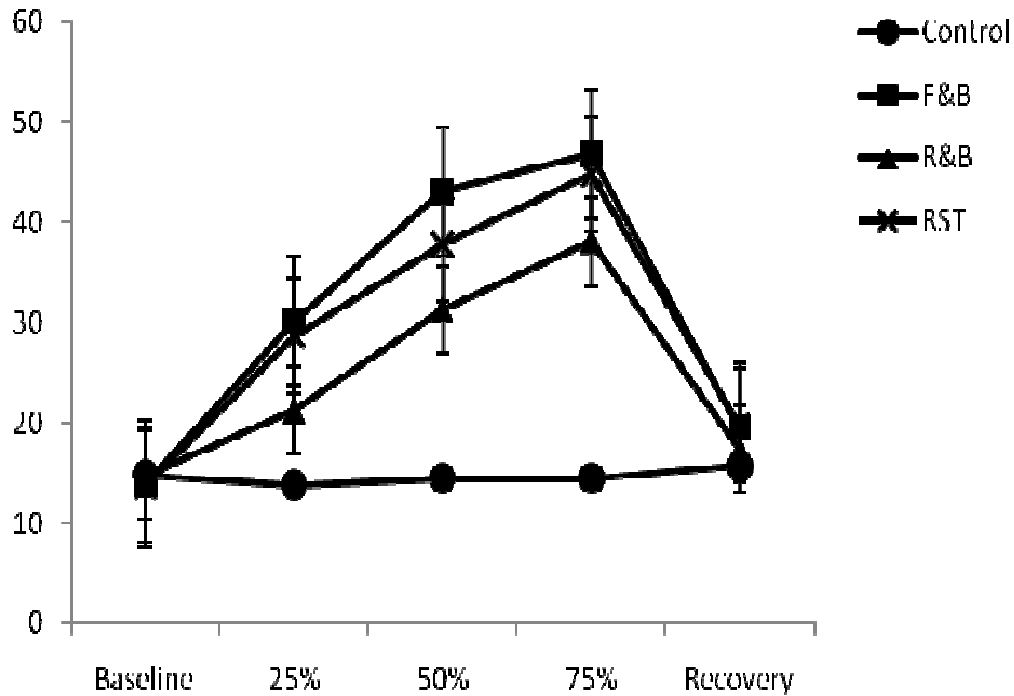
Ca<sup>+</sup> and Platelet counts recorded were lower in the treatment group (Table 1; Figure 3-4). The effects of soya bean on these variables were also dose related. The

## DISCUSSION

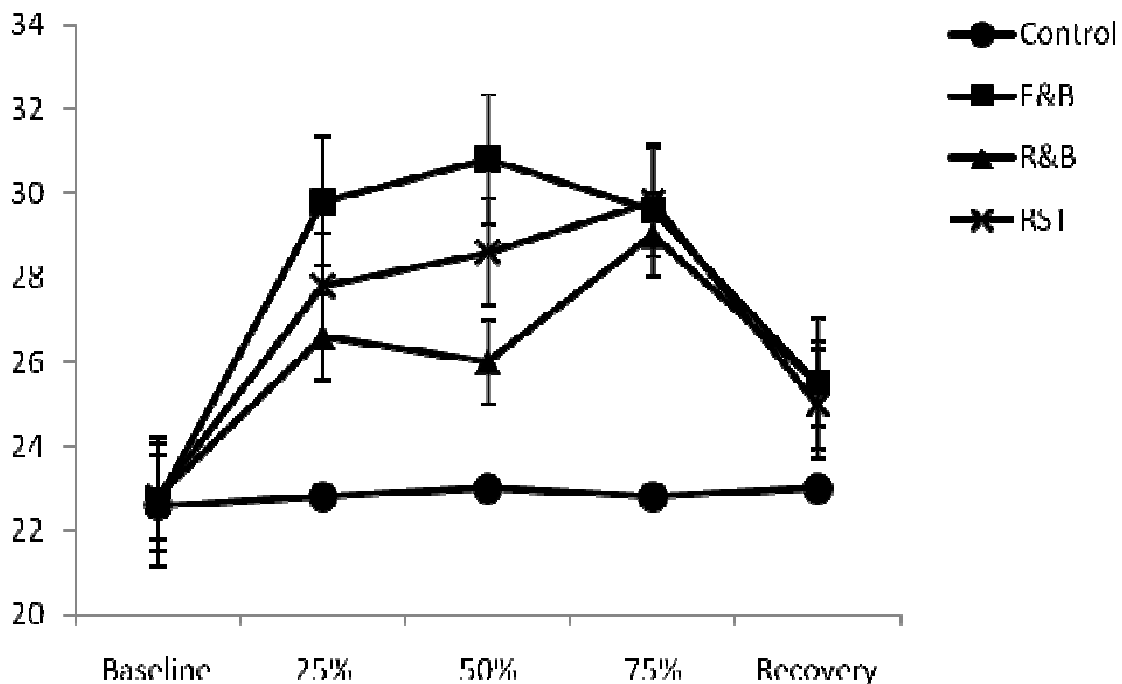
Soya bean is a widely used inexpensive source of protein both for human and animal consumption. It also has other applications in health and industries. There have been growing concerns on the dietary utilization of soya bean because of the anti-nutritional factors highly present in it. In the present study, it is confirmed that the components of soya bean elicit variable effect on blood coagulation parameters.

Blood coagulation profile of rabbits fed soya bean deviated from the values obtained at baseline and recovery period. The differences between the values were dependent on the method of cooking employed and the percentage composition of soya bean in the diet (Figures 1 to 4).

Fermentation of soya bean resulted in a marked extension of prothrombin time and activated partial thrombin time compared to values obtained from roasted and boiled soya or roasted soya bean (Figures 1 and 2). Isolated trypsin inhibitor from soya bean has been proven



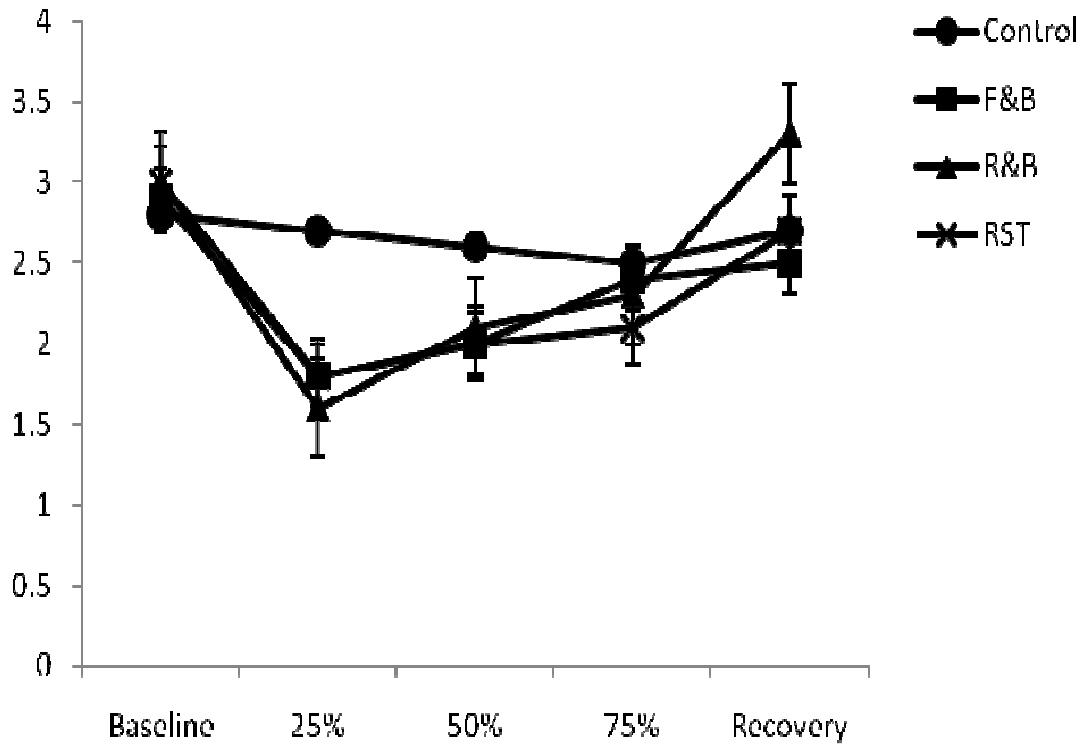
**Figure 1.** PT of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B- fermented and boiled soya bean; R&B – roasted and boiled soya bean; RST – roasted soya bean.



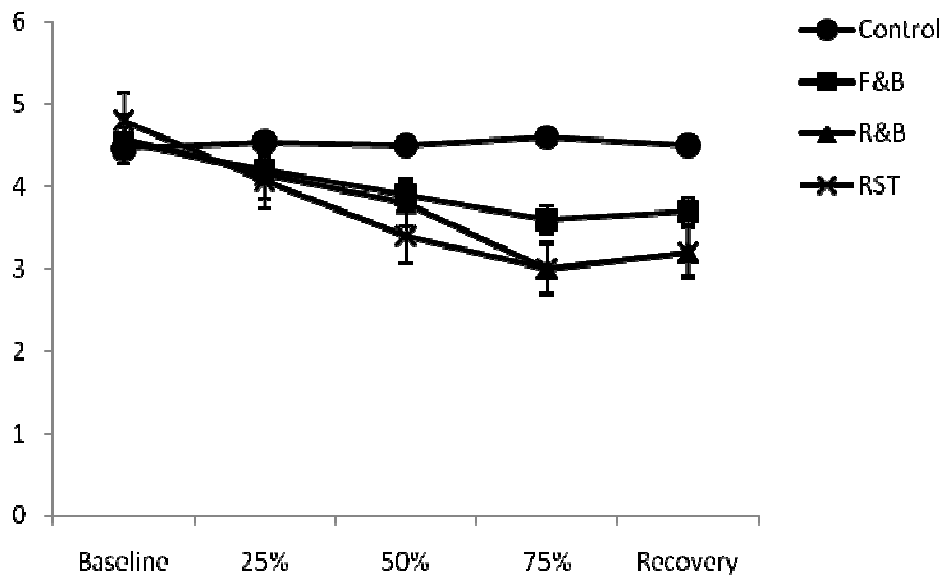
**Figure 2.** APTT of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B- fermented and boiled soya bean; R&B – roasted and boiled soya bean; RST – roasted soya bean

to be capable of delaying the coagulation of blood. Previous studies by six investigators have shown that it

inhibits the first phase of coagulation and has no effect on the activity of thrombin (Tagnon and Soulier, 1946;



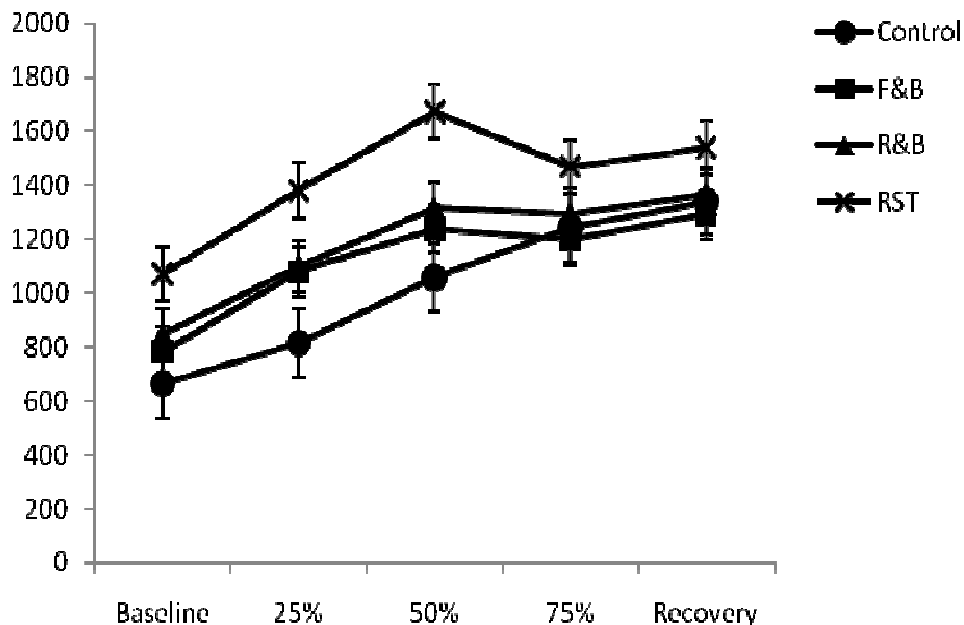
**Figure 3.** Serum calcium of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B- fermented and boiled soya bean; R&B – roasted and boiled soya bean; RST – roasted soya bean.



**Figure 4.** Platelet count of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B- fermented and boiled soya bean; R&B – roasted and boiled soya bean; RST – roasted soya bean.

Macfarlane et al., 1946; Croxatto, 1946; Glazko, 1947; Macfarlane, 1947; Guest and Nelson, 1949; Glendening and Page, 1951a).

The site of inhibition of blood clotting by soya bean trypsin inhibitor was first identified by Glendening and Page (1951b). They opined that soya bean trypsin



**Figure 5.** Weight of rabbits fed increasing proportion (25%-50%-75%) of soya bean compared to initial data (Baseline) and recovery values. F&B – fermented and boiled; R&B – roasted and boiled; RST - roasted

inhibitor delays the coagulation of blood by a mechanism unique among other biological inhibitions. It apparently forms a dissociable complex with the substrate, prothrombin or a derivative of the substrate. The inhibitor does not exert its effect upon the enzyme of the first phase (thromboplastin), nor upon the cofactor (accelerator globulin) nor upon the product (thrombin). It would interfere with the conversion of prothrombin to thrombin in 25% sodium citrate when all other factors are absent.

Soya bean trypsin has anti-thrombin properties (Tagnon and Soulier, 1946). Since thrombin is required for the formation of blood clot, it is not surprising that dietary soya bean prolonged blood clotting (Figures 1 and 2) in this study. Heat treatment which is employed in most traditional cooking was applied, but does not completely destroy trypsin inhibitors present in Soya bean. The role of trypsin inhibitor in the coagulation process was confirmed in the report of Tagnon and Soulier (1948) in which soya bean preparation containing a trypsin inhibitor was injected intravenously into 2 dogs and 3 rabbits producing the following effects: prolongation of the clotting time and of the prothrombin time, and increase in the antiproteolytic activity of the blood plasma or serum.

Serum calcium concentration was lower (Figure 3) in all the rabbits fed soya bean at 25, 50 and 75% concentration. Phytic acid inhibits the absorption of calcium in the gastrointestinal tract (Katz, 1987). Both calcium and phytic acid are highly present in separate fractions of soya bean. Calcium is required in the initial and final stages of blood clotting. Therefore, a reduction

in plasma calcium will further prolong blood coagulation *in vivo* in addition to the effect of trypsin inhibitors on thrombin.

Platelet count reduced (Figure 4) in all the rabbits administered soya bean diet irrespective of the processing method employed.

Heat-labile trypsin inhibitors are not the only toxic factors in the beans. Other factors identified include haemagglutinin activity, cyanide production, amylase inhibition, and urease activity (Ekpenyong and Borchers, 1981) which essentially affects growth or weight gain rather than coagulation. Weight gain pattern of control and treatment groups was similar from the onset to the period the rabbits were fed 50% soya bean (Figure 5). The rabbits began to record significant weight loss when the proportion of soya in the diet was increased to 75% and recovered slowly when returned to 100% CF. Kiers et al. (2003) reported similar effects of soya bean on weight in weaned piglets.

Weight gain was observed at lower concentration of soya bean. Gupta (2009) obtained similar results after addition of soya bean oil to standard diet of rabbits. The reduction in weight gain as the soya bean concentration increased may result from the presence of genistein in soya bean which promotes weight loss (Naaz, 2003).

In summary, Soya bean diet affects coagulation profiles in rabbits. The effects observed were dependent on the method of processing and the proportion of soya bean in the diet. Soya bean diet had a significant reduction effect on serum calcium concentration and platelet counts ( $P < 0.05$ ). The anti-nutritional factors present in soya bean are not completely eliminated by the methods of

processing employed and are responsible for these effects.

When the consumption of soya bean is inevitable because of its cost effectiveness as a source of protein, fermentation or pre-soaking of whole soya bean is recommended. Soya rich diet should be supplemented with dietary calcium. The consumption of soya bean should be with caution in people who have abnormal tendency to bleed, thrombocytopenia or other blood clotting problems. There is need for further research into soya bean and health in infants, women and experimental models.

## REFERENCES

- Belloque J, Garcia MC, Torre M, Marina ML (2002). Analysis of soya bean proteins in meat products: A review. *Crit. Rev. Food Sci. Nutr.* 42(5):507–532.
- Croxatto H (1946). Anticoagulant action of crystalline antitrypsin from soya beans. *Rev. Soc. Argent Biol.* 22:477.
- Ekpenyong TE, Borchers RL (1981). In: Benito O D and Pilar S R (2006). Chemical composition studies on winged bean (*Psophocarpus tetragonolobus*). *Tubers* 47(3):821–824.
- Glazko AJ (1947). Effect of blood protease and trypsin inhibitor on the clotting mechanism. *J. Clin. Invest.* 26(3):364–369.
- Glendening MB, Page EW (1951a). Site of action of soya bean trypsin inhibitor on the coagulation. *Fed. Proc.* 5:128-1304.
- Glendening MB, Page EW (1951b). The site of inhibition of blood clotting by soya bean trypsin inhibitor. *J. Clin. Invest.* 30:1298.
- Guest MM, Nelson TE (1949). Action of antitrypsin and antifibrinolysin on the first phase of the blood clotting reaction. *Fed. Proc.* 8:64.
- Gupta V (2009). Comparison of the effects of coconut oil and soya bean oil on TSH level and weight gain in rabbits. *J. Health Allied Sci.* 8(1):7.
- Katz SH (1987). Food and biocultural evolution: A model for the investigation of modern nutritional problems. *Nutritional Anthropology*, Alan R Liss Inc. p. 50.
- Kiers JL, Meijer JC, Nout MJ, Rombouts FM, Nabuurs MJ, Van Der A (2003). Effect of fermented soya beans on diarrhoea and feed efficiency in weaned piglets. *Meulen J.* 95(3):545-552.
- Kung-Chi C, Ping-Ping L, James LH (1993). High casein-lactalbumin diet accelerates blood coagulation in rats. *J. Nutr.* 3:0602.
- Kyaala D (2005). Soy – Not so healthy food. *Scheffneys monthly.* pp. 15-17.
- Macfarlane RG (1947). The action of soya-bean trypsin inhibitor as an anti- thromboplastin in blood coagulation. *J. Physiol.* 106:104.
- Macfarlane RG, Pilling J (1946). Anticoagulant action of soya-bean trypsin inhibitor. *Lancet* 2:888.
- Mc Arthur DS, Murphy PA, Lan SM (1988). Phytoestrogen content of processed soya bean foods. *Food Technol.* 41:60-64.
- Norman R (2000). *Food Science* 3<sup>rd</sup> Ed., The Avi Publishing Company Inc USA pp. 533-534.
- Nwokolo E (1996). Soya bean (*Glycine max* (L.) Merr). In: Legume and Oilseeds in Nutrition SE Nwokolo and J Smert (eds). Chapman and Hull pp. 90-102.
- Perez J (2006). Use of soy in edible vegetable oil production. United States Department of Agricultural Statistics pp. 31-51.
- Tagnon HJ, Soulier JP (1946). Anticoagulant activity of the trypsin inhibitor from soya bean flour. *Proc. Soc. Exp. Biol. Med.* 61:440.
- Tagnon HJ, Soulier JP (1948). The effect of intravenous injection of trypsin inhibitor on the coagulations of blood. *Blood* 3(10):1161-1166.
- Theodore K (1998). The preparation of soya bean foods for use in rural communities of the developing world. *J. Trop. Pediatr.* 44(4):251-252.
- Vincken JP, Petra R, Nout MJ, Judith W (2008). Bio-active compounds in the soya bean foods tofu and tempe. MSc thesis projects. *Food Microbiology, Fermentation and Fungi* p. 3.

**Appendix.** Experimental design and groups.

<b>Duration (weeks)</b>	<b>Group A (Ctrl) n=10</b>	<b>Group B (F&amp;B) n=10</b>	<b>Group C (R&amp;B) n=10</b>	<b>Group D (RST) n=10</b>
3 WK	100% CF	100% CF	100% CF	100% CF
2 WK	100% CF	75% CF; 25% SOY	75% CF; 25% SOY	75% CF; 25% SOY
2 WK	100% CF	50% CF; 50% SOY	50% CF; 50% SOY	50% CF; 50% SOY
2 WK	100% CF	25% CF; 75% SOY	25% CF; 75% SOY	25% CF; 75% SOY
3 WK	100% CF	100% CF	100% CF	100% CF

\* CTRL – control, F&B – fermented and boiled, R&B- roasted and boiled, RST – roasted, CF-commercial feed, SOY- soya bean.