AFZELIA AFRICANA, A NOVEL NON STARCH POLYSACCHARIDE, RAISED FASTING PLASMA CHOLESTEROL AND TRIGLYCERIDE LEVELS OF RAT

[1]ONYECHI Uchenna Agatha, [2]JUDD Patricia Ann and [2]ELLIS Peter Rory
[1]Department of Home Science, Nutrition and Dietetics, University of Nigeria Nsukka, Enugu State
[2]Division of Life and Health Science, King's College, University of London, London

Corresponding Author: Onyechi, U. A. Department of Home Science, Nutrition and Dietetics, University of Nigeria Nsukka, Enugu State. Email: uche.onyechi@yahoo.com. Phone: 08066794874

ABSTRACT

The effects of vegetable flour prepared from indigenous plant Afzelia africana, a legume, on the fasting plasma cholesterol and triglyceride levels of rats were investigated. Chemical analysis indicated that Afzelia flour contained significant amount of non-starch polysaccharides (NSP). The flour of Afzelia was incorporated into semi-synthetic diet to provide 10g of dietary fibre which is 300g/kg Afzelia flour. This replaced some of the casein, oil and starch in the control diet .The test and control diets were fed to young Sprague-Dawley rats for 14 days ad libitum. Food intake, weight gain, crude digestibility, faecal fat excretion, fasting plasma cholesterol and triglyceride were evaluated. The result showed a statistically significant difference (p > 0.05) between the control diet and the test diet in food intake, weight gain and energy digestibility. Afzelia fed rats had a significant higher fasting plasma cholesterol and triglyceride levels than rats fed the control diet.

Keywords: *Afzelia africana*, Plasma cholesterol, Triglyceride levels, rat

INTRODUCTION

The ability of certain sources of dietary fibre to lower serum cholesterol has been demonstrated in human clinical studies. Water soluble dietary fibre sources (SNSP) tend to be most effective in lowering total cholesterol and LDL cholesterol. Jenkins *et al.* (1975) first reported the hypocholesterol effect of guar gum, a water soluble NSP. Similar reports have also shown reduction in total and low density lipoprotein (LDL) cholesterol in normal subjects (Khan *et al.,* 1981; Smith and Holm, 1982; Penagini *et al.,* 1986).

Different fibres have various effects on rat metabolism. The early studies of Wells (1961) and Judd and Truswell (1985) demonstrated variable effects of supplementing rat diets with different forms of dietary fibre. The authors showed that SNSP such as pectin, guar gum, locust bean gum and carrageen in the diet decreased cholesterol levels, while insoluble dietary fibre does not usually demonstrate hypocholesterolemic effects.

The suggested mechanism involved in the lowering of plasma cholesterol are reduction in bile acid re-absorption, alteration of the metabolism and ratio of bile acid absorbed by changing the intestinal secretion and the hepatic production of lipoproteins or modification of the peripheral disposal of lipoproteins (Chen and Anderson, 1979, 1986). Viscosity is an important factor in the lipid lowering effects of gel-forming polysaccharide such as guar gum (Kay and Truswell, 1977). Furthermore, dietary fibre tends to swell within the aqueous medium of the intestine thus increasing intestinal filling (Eastwood, 1973) which may reduce food intake in rats.

Afzelia africana (AA) is an underexploited and under utilised leguminous crop seed indigenous to the Ibos in the South Eastern part of Nigeria. The fruits of *Afzelia* are very hard and woody, nearly black and busting violently to discharge the seeds (Hutchinson and Dalziel, 1931). This legume is cheap, and is traditionally processed in homes and used on a daily basis for thickening vegetable soups. *Afzelia* is locally known as *'akparata'.* On the basis of these observations, the authors believed that such plant foods could be a useful source of water-soluble dietary fibre and was selected for the study. There is dart of information in the literature on this food and it is largely uncharacterized. The present investigation was designed to determine the effects of *Afzelia* on the plasma cholesterol and triglyceride level of rats. The flour of *Afzelia* was incorporated into semi–purified rat diet to find out if it will lower the plasma cholesterol and triglyceride concentration in rats. Chemical analysis showed that *Afzelia* is rich in non-starch polysaccharides (Onyechi, 1995)

MATERIALS AND METHODS

Preparation and Processing of Plant Food Extracts: The seed samples used in the present study were purchased at the local market in Nsukka, Enugu State and transported to the United Kingdom for processing into flour. *Afzelia* was processed using the traditionally processing method. This involves sorting the seeds to remove spoilt ones. The seeds were then roasted for 10 - 20 minutes in wide aluminium stainless steel pan. The roasted seeds were cracked with the use of wooden pestle to remove the skin. The roasted endosperm was cracked in smaller pieces and ground into fine powder in a coffee grinder (Moulinex blender/mill) and air dried at room temperature. The processing yields a fine yellowish powder with strong aromatic odour (Onyechi, 1995).

Chemical and Physical Methods of Analysis of the Plant Food: Afzelia flour was analysed using standard methods (Kirk and Sawyer, 1991) for moisture (104 ^{o}C for 16h), ash (total minerals; 525 ^{o}C for 12h), fat (Soxhlet; light petroleum-diethyl ether extraction) and protein (micro-Kjeldahl method; N x 5.7). The starch content of the flour was determined by an enzymatic method (Englyst *et al.,* 1992a.). The Englyst method (Englyst *et al.,* 1992b) was used to determine total NSP and the water-insoluble fraction of the NSP; the water-soluble fraction of the NSP was determined as the difference. The procedure involves acid hydrolysis of the NSP followed by gas chromatography of the alditol acetate derivatives of the neutral sugars. The test food sample was boiled with 80 % ethanol for 1 hour under reflux. The residue obtained by filtration was washed with 95 % ethanol and air dried at room temperature. The dried residue was extracted with 7 volume of distilled water then followed by centrifugation. The supernatant was collected, pH adjusted and centrifuged. The SNSP in the supernatant was precipitated by addition of absolute ethanol. The precipitate was collected by filtration and stored at 4 ^{o}C. The particle size distributions of the test foods were determined by a standard laboratory mechanical sieve analysis method (Lauer, 1966); the viscosity of 1% aqueous dispersion of the test foods was obtained by the U tube capillary viscometer.

Rats: Ten litters each containing two male Sprague-Dawley rats, supplied by A. Jack and sons, London, were used for the experiment. On arrival, each rat weighed between 71 - 87g. Each litter of rats was placed in a cage and fed a stock diet (CRM Labsure, Christopher Hill, London)

Formulation of the Control Diet: The batch size of diet prepared was 5 kg. The calculated quantities of casein (New Zealand Milk Products UK Ltd), vitamin mix and mineral mix (King's College, London mix), sucrose (Booker Fitch Food Services), solka floc (Jordensen and Wettre Limited) and corn starch (Cerestar, Manchester HHIPA), were each weighed and transferred to Hobart mixer and blended for 15 minutes. Sufficient corn oil was heated in beaker to approximately 80 ^{o}C and calculated amount of cholesterol (BDH Chemicals Limited) was weighed and stirred into the corn oil and mixed well to dissolve. This mixture of cholesterol and corn was added to the dry ingredients and blending continued for another 30 min until well distributed. The mixture was passed through a 1/8 inch mesh size. Homogenization of the total mixture was ensured by mixing for a further 30 min in the Hobart mixer. The diet was stored at -20^{0}C in self- sealed freezer bags. Composition of the diets is shown in Table 1.

Formulation of the Test Diet: The test diet was prepared to contain afzelia flour and was formulated to provide approximately 10g total dietary fibre per 100g diet while maintaining similar protein and fat levels. This was determined from the result of preliminary analysis (Onyechi, 1995). The amount of casein and corn oil was adjusted to maintain similar protein and fat contents with the control diet. The test food replaced some of the protein, fat and starch in the diet as shown in Table 1. Enough corn starch was used to make up a batch size of 5 kg diet. Proximate composition of the diets is shown in Table 1.

Table 1: Quantity and proximate composition of the Control and test diet containing Afzelia flour

Ingredients	**Quantity of ingredient (g/1000g) diet**	
	CD	**AAD**
Casein	150	99
Fat (corn oil)	100	100
Vitamins	20	20
Minerals	40	40
Sucrose	100	100
Cholesterol	10	10
Solka floc	50	50
Food sample	-	300
Corn starch	530	281
Proximate Composition		
Moisture %	6.11	5.61
Fat %	10.00	10.4
Protein %	15	15
Ash %	4.8	5.0
Available CHO	58.0	42.7
Fibre difference %	6.1	21.4
Total CHO	64.1	64.0
Kcal by bombing	406.0	409.0

Note: CD = Control diet, AAD = Afzelia Africana diet

Feeding of the Rats: On arrival the rats were fed stock diet for 2 days and placed on ground stock diet for a further 5 days to acclimatise them to eating a ground diet. After one week the rats were assigned into two groups with mean weight of 115 – 116 g, so that one rat from a litter went into each experimental group. The groups were therefore assumed to be genetically similar and fed for 14 days *ad libitum.* The rats were individually housed in stainless steel cages with suspended trays containing filter paper linings for collection of spill and faeces. The rats were weighed daily for the first two days and then on alternate days. Weight was determined by difference from week to week. Food intake, faecal out put, weight gain, energy digestibility, plasma cholesterol and triglyceride were the parameters assessed in the rats.

Food intake was recorded by providing each rat with an individual weighed pot of food. These food pots and food were weighed on alternate days before topping up the food supply and reweighing. At the end of each week of the two experimental periods, the spillage was collected by sifting the faeces from the spilt food. The faeces samples were collected separately from each animal and stored in self-sealed bags at -20 ^{o}C until analysis. Dry weight (DW) of spilt was determined by drying this in an oven at 105 ^{o}C for 48 hours, together with the cage lining paper and food adhering to it. The dry weight of the paper was subtracted from the total to give dry weight of spillage. Dry weight of the remaining food

in the pot was similarly determined after drying for 48 hours at 105 ^{o}C. Food intake was calculated.

Bleeding of the Rats and Plasma Cholesterol and Triglyceride Analysis: At the end of 14 days of experimental feeding, the rats fed the diets were anaesthetized and bled from the heart using a heparinized needle and syringe to prevent the blood from clotting. The blood was collected in a centrifuge tube and centrifuged at 2,500 rpm for 15 minutes. The plasma was separated from the cells and stored in LP tubes at a temperature of -20 ^{o}C until analysis. The fasting plasma cholesterol and triglyceride levels were determined by enzymatic method (Roschkau *et al.,* 1975; Sidel *et al.*, 1981) and (Tiffany *et al.,* 1974) respectively using Boehringer-Mannheim kit method.

Statistical Analysis: The data collected were analysed using descriptive statistics for their central tendencies and analysis of variance with Fischer least significant level at 0.05 probability.

RESULTS

Chemical and Physical Analysis of *Afzelia:* Chemical analysis showed that A*fzelia* flour contained 3.3g/100g of fat; 17.7g of protein; 1.4g starch; 3.3g ash. The total NSP was 35.8g/100g; 29.3g was the value for the water soluble NSP; 6.5g was the insoluble NSP content per 100g afzelia flour. The sugar composition of the SNSP fraction showed that afzelia contained a proportion of xylose, glucose and galactose. The mean particle size of afzelia flour was 272 µm and the viscosity of a 1% aqueous dispersion was 1,000-15,000 cps (Onyechi, 1995).

Food Intake: Food intake of rats at 1 - 7 days, 8 – 14 days and cumulative are shown in Table 2. The mean dry food intake for rats fed the control diet was significantly (P<0.05) higher than the food intake of rats fed afzelia diet.

Table 2: Dry food intake (g) of rats fed control and test diets containing *Afzelia* over 1 - 7, 8 - 14 and 14 cumulative days

Diet	Days 1-7	Days 8-14	Cumulative
Control	192 ± 25.60^{a}	138 ± 25.60^{b}	332 ± 23.42^{c}
Afzelia	72 ± 8.91^{b}	74 ± -5.79^{c}	147 ± 12.23^{d}

Column values with unsimilar superscripts are significantly different from each other

Faecal Fat Excretion: There was no significant difference in the faecal fat excretion of the rats fed the two diets at 1 - 7, 8 – 14 and 14 cumulative days (Table 3).

Table 3: Fecal fat excretion (g) of rats fed control diet and test diets containing *Afzelia* for days 1 - 7, 8 - 14 and 14 cumulative days

Diet days	Days 1-7	Days 8-14	Cumulative
Control	1.75 ± 0.08	1.85 ± 0.21	3.60 ± 0.19
Afzelia	1.149 ± 0.21	1.95 ± 0.13	3.44 ± 0.38

Weight Gain: The rats fed the control diet had more significant weight gain (P < 0.05) than the rats fed the test diet through out the feeding periods (1 – 7 and 1 – 7, 8 – 14 and cumulative days) (Table 4).

Table 4: Weight gain (g) of groups of rats fed control diet and test diet containing *Afzelia* during days 1 – 7, 8 – 14 and 14 cumulative days

Diet	Days 1 – 7	Days 8 – 14	Cumulative
Control	44 ± 3.11^{a}	67 ± -25.39^{b}	111± -539^{c}
Afzelia	17 ± -1.02^{b}	21 ± -2.57^{c}	38 ± 2.98^{d}

Column values with the unsimilar superscripts are significantly different from each other p < 0.05.

Energy Digestibility: Energy digestibility was determined at 14 days of the feeding period. There was a higher significant difference (P < 0.05) in the energy digestibility of rats fed the control diet compared to the ones fed the test diet containing afzelia diet (Table 5).

Table 5: Mean energy digestibility (%) of control and test diet containing *Afzelia* fed to rats at 1 – 7 and 8 – 14 days

Diet	Days 1 – 7	Days 8 – 14
Control	0.93 ± -0.02^{a}	0.85 ± -0.004^{b}
Afzelia	0.82 ± 0.01^{b}	0.74 ± -0.02^{c}

Value in column with unsimilar superscripts are significantly different from each other at P < 0.05

The fasting plasma cholesterol and triglyceride levels: The fasting plasma cholesterol and triglyceride levels of groups of rats fed *Afzelia* diets was significantly (P < 0.05) higher than the rats fed on the control diet (Table 6).

Table 6: Mean fasting plasma cholesterol and triglyceride levels (mmol/L) of rats fed control diet and the test diet containing Afzelia four for 14 days

Diets	Plasma cholesterol level (mmol/L)	Plasma triglyceride level (mmol/L)
Control	3.97 ± -0.17^{a}	0.80 ± -0.07^{b}
Afzelia	566. ± -0.31	2.10 ± -0.28^{c}

Column values with unsimilar superscripts are significantly different from each other p<0.05.

DISCUSSION

The result of the study showed a lower mean body weight of *Afzelia* fed rats compared to the rats fed the control diet. This is in line with Fleming and Lee, 1983; Judd and Trustwell, 1985 studies that reported consistent lower weight gain of rats fed pectin. Studies have shown that fibre swells within the aqueous medium of the intestine (Eastwood, 1973). This property of dietary fibre has a tendency to increase intestinal filling. Judd (1980) showed that gut content of rats fed 10% pectin diet for 21 days were heavier than the content of the control group despite a lower food intake of the pectin fed animals. Therefore the rats in the current study may have eaten less food due to the intestinal filling caused by

the water trapped in the GIT and the slower gastric emptying giving the rats a sense of fullness.

Previous works (Riccardi *et al.,* 1967; Chen and Anderson, 1979; Judd, 1980; Judd and Truswell, 1985) have shown cholesterol lowering effect of diets containing SNSP. The different effects of dietary fibre on blood lipids have been reported in previous studies. The addition of cellulose (insoluble NSP) to a diet containing 1% cholesterol increased serum cholesterol levels (Tsai *et al.,* 1976). However viscous soluble NSP have consistently been shown to lower plasma cholesterol levels in rats fed hypercholesterolaemic diets (Judd *et al.,* 1977; Chen and Anderson 1979; Asp 1981). The suggested mechanism by which NSP affect plasma lipid level include, reduction in total energy intake, bile acid excretion/ sequestering, changes in fat absorption, changes in endocrine secretion, resistance to diffusion and effects of short-chain fatty acids (Story and Kritchevsky, 1976). The effectiveness of dietary fibre in reducing cholesterol levels probably lies in its ability to reduce availability of fatty acids and cholesterol for absorption in the upper intestine. This maybe seen as increased faecal fat; increased cholesterol and its bacterial metabolites, and reduced bile acid re-absorption, which affects cholesterol esterification (Dreher, 1987).

The increased level of plasma cholesterol and triglyceride levels seen in rats fed *Afzelia* was contrary to expectation. The result of the chemical composition of the plant seeds showed that Afzelia flour is high in SNSP which has been shown to lower plasma lipid levels in hypercholesterolemic rats. The authors postulated that the negative effect of *Afzelia* to the plasma lipid levels of the rats could be attributed to possible toxic substances that may be contained in *Afzelia* seeds. Hutchinson and Dalziel (1931) noted that the processing method of *Afzelia* seeds include roasting and soaking for several days to rid the seeds of the poisonous substances they may contain. However, the traditional processing method which was used was different, as the seeds of *Afzelia* were not soaked after roasting. This could account for the negative effects of *Afzelia* on the rats like poor weight gain, loss of hair and poor condition at the end of the experimental period.

Furthermore, this confounding result of increased plasma lipid levels maybe due to the fact that in these rats, fat absorption may have been continuing even during the fasting period as food remained in the guts of these animals. It has been suggested that the presence of fibre in the gut generates an increased unstirred water layer in the intestine (Johnson and Gee, 1981). Consequently there is delay in fat absorption in the GIT.

In conclusion *Afzelia* which is consumed in most households as thickening agents in the South Eastern Nigeria among the Ibos may contain undesirable substances that maybe toxic. These toxic substances may require that the seeds to be roasted and soaked for days to rid the seeds of the poison (Hutchinson and Dalziel, 1931). It is important to reconsider the traditional processing method and adopt the new method of roasting and soaking of the seeds.

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