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Effect of Aqueous Extract of *Tridax procumbens* Linn on Plasma Electrolytes of Salt-Loaded Rats

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Abstract: The effect of aqueous extract of *Tridax procumbens* Linn on the weight, packed cell volume and plasma electrolyte profiles of salt-loaded rats were investigated. The test and test-control groups received a diet consisting 8% salt and 92% commercial feed, while the control group received a diet consisting 100% of the commercial feed. The test group also received daily by intra-gastric gavages, 50 mg/100 g body weight of the extract, while the test-control and control groups received appropriate volumes of water by the same route. The mean daily weight gain and Packed Cell Volume (PCV) of the test rats were significantly higher ($p<0.05$) than those of the test-control and control. The treated animals had significantly lower ($p<0.05$) plasma sodium and chloride levels, compared to the test-control. There was no difference in the plasma calcium and potassium levels of the test and test-control animals. This result suggests that the antihypertensive action of *T. procumbens* may be mediated via reduction of weight and alteration of plasma sodium and potassium levels and in addition suggest its use in the management of obesity and diabetes mellitus.

Key words: *Tridax procumbens*, hypertension, plasma electrolytes, salt-loading

INTRODUCTION

The observation that high salt intake is associated with hypertension is not new (Blaustein *et al*., 2006). In addition to raising the blood pressure, dietary salt is responsible for several other harmful effects, some of which though independent of the arterial pressure, also harm the cardiovascular system. It increases the mass of the left ventricle, thickens and stiffens conduit arteries and thickens and narrows resistance arteries, including the coronary and renal arteries. It also increases the number of strokes, the severity of cardiac failure and the tendency for platelets to aggregate (De Wardener and MacGregor, 2002; Meneton *et al*., 2005). In renal disease, a high salt intake accelerates the rate of renal functional deterioration (Bakris and Smith, 1996). Apart from its effect on the cardiovascular system it also affects calcium and bone metabolism, which underlies the finding that in postmenopausal women salt intake controls bone density of the upper femur and pelvis. It controls the incidence of carcinoma of the stomach and is associated with the severity of asthma in male asthmatic subjects (Carey *et al*., 1993; De Wardener and MacGregor, 2002).

In normotensive as well as hypertensive subjects, blood pressure can be judged to be "salt-sensitive" when observed to vary directly and substantially with the net intake of sodium chloride. From both a clinical and public health perspective, the phenomenon of normotensive salt sensitivity may be important. Not only is normotensive salt sensitivity a likely and possibly common precursor of hypertension, but the phenomenon might be susceptible to dietary suppression, which could prevent or delay its progression to hypertension (*vide infra*) (Morris *et al*., 1999; O'Shaughnessy and Karet, 2004).

Tridax procumbens Linn belongs to the family Compositae (alt Asteraceae). Native to Central America and tropical South America, it has spread throughout the tropical and subtropical parts of the world. Its common names are coat buttons, tridax daisy, erva-de-touro, cadillo chisaca, tridax (http://www.lucidcentral.org/keys/FNW/FNW seeds/html/fact sheets/*Tridax procumbens*.htm; http://www.ars-grin.gov/cgi-bin/npgs/html/family.pl). The Ibo people of South Eastern Nigeria call it "mbuli". Traditionally, it is used for the treatment of bronchial catarrh, dysentery, malaria, stomachache, diarrhoea, high blood pressure and to check haemorrhage from cuts, bruises and wounds and to prevent falling of hair. It possesses antiseptic, insecticidal, parasiticidal and hepatoprotective properties and has marked depressant action on respiration (Salahdeen *et al*., 2004; Edeoga *et al*., 2005; Ravikumar *et al*., 2005; Saxena and Albert, 2005; Hemalatha, 2008). In the present study, we investigated the effect of aqueous extract of *T. procumbens* Linn on the weight, Packed Cell Volume (PCV) and plasma electrolytes of salt-loaded rats, with a view to finding any clue to the mechanism of its antihypertensive property and/or any protective role of the plant against the pathogenesis of salt sensitive hypertension.

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MATERIALS AND METHODS

Collection of animals and preparation of the leaf extract: Albino rats were collected from the animal house of the Department of Biochemistry, University of Port Harcourt, Port Harcourt, Nigeria. The plants were collected from behind the Ofrima Hall Complex of University of Port Harcourt, Port Harcourt, Nigeria. After due identification at the Herbarium of the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria, their leaves were collected, rid of dirt, oven dried at 55°C and ground into powder. The resultant powder was soaked in boiled distilled water for 12 h, after which the resultant mixture was filtered. A known volume of the resultant filtrate was evaporated to dryness and the weight of the residue used to determine the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract to the test animals.

Experimental design and composition of diet: The rats were randomly sorted into three groups of five animals each, so that the average weight difference was ±1.8 g. The animals were individually housed in plastic cages. After a one-week acclimatization period on guinea growers mash (Bendel Feed and Flour Mills Ltd., Ewu, Nigeria), the treatment commenced and lasted for a week. The control group received a diet consisting 100% of the commercial feed, while the test-control and test received a diet consisting 8% salt and 92% commercial feed. The 8% dietary salt-loading was adapted from Obiefuna *et al.* (1991). The test received daily by intra-gastric gavages, 50 mg/100 g body weight of the extract while the test-control and control group received appropriate volumes of water by the same route. The animals were allowed food and water *ad libitum*. At the end of the treatment period the rats were weighed and anaesthetized by intra-peritoneal injection of 5 mg/kg body weight of 25% Urethane saline solution. While under anesthesia, blood was collected from each rat via heart puncture and transferred into heparin sample bottles after which they were painlessly sacrificed.

Determination of PCV and plasma electrolyte profile: PCV was measured with micro haematocrit, with 75 x 16 mm capillary tubes filled with blood and centrifuged at 3000 rpm for 5 min. The electrolytes were analyzed with an auto-analyzer. Sodium and potassium were determined by using a flame photometer (Model, 405, Corning, UK) using NaCl and KCl to prepare the standards. All other metals were determined by Atomic Absorption Spectrophotometer (Perkin-Elmer Model 403, Norwalk CT, USA).

Statistical analysis of data: All values are quoted as the mean ± SD. The values of the various parameters for the test, test-control and control groups were analyzed for statistical significant differences using the student's t-test. $P<0.05$ was assumed to be significant.

RESULTS AND DISCUSSION

The effect of the extract on the PCV and mean daily weight gain of salt-loaded rats is shown in Table 1. The PCV of the test rats was significantly higher ($p<0.05$) than those of the test-control and control. This means that the extract had positive effect on the haemopoietic system of the test rats and could protect the animals against the salt-loading induced lowering of PCV. The raised haematocrit is an indication of haemoconcentration which may be due to increased RBC mass. The mean daily weight gain of the test group is significantly lower than those of the test-control and control. Weight loss helps improve and control coronary risk incidence, diabetes mellitus, dyslipidemia, hypertension, obesity and physical functioning (Reisin *et al.*, 1978; Shah, 1991; Trussell *et al.*, 2005; Bantle *et al.*, 2006; Krauss *et al.*, 2000, 2006) and is one of the strategies for increasing low HDL-C levels (Assmann and Gotto, 2004), as well as improving the insulin resistance (Krauss *et al.*, 2006). Therefore, the implication of the significantly low mean daily weight gain produced by the extract in test animals is that it may be useful in the management of hypertension, obesity and dyslipidemia. This corroborates the use of the plant in traditional medicine for the management of hypertension.

Table 1: Effect of aqueous extract of *Tridax procumbens* on the weight and PCV of salt-loaded rats

Parameter	Control	Test-control	Test
Mean daily weight gain (g/day)	4.048±0.673[a]	3.238±1.019[b]	2.524±1.258[c]
PCV (%)	35.926±5.762[a]	31.111±1.571[b]	40.476±3.367[c]

Values are means±SD, n = 5 per group. Entries with different superscripts are significantly different at $p<0.05$

Table 2: Effect of aqueous extract of *Tridax procumbens* on plasma electrolyte concentrations of salt-loaded rats

	Concentration (mmol/L)		
Parameter	Control	Test-control	Test
Sodium	40.667±5.133[a]	42.367±5.187[b]	41.000±2.646[a]
Potassium	0.747±0.098[a]	0.807±0.202[a]	0.807±0.264[a]
Calcium	4.250±0.250[a]	3.250±0.250[b]	3.250±0.000[b]
Chloride	25.067±1.793[a]	26.600±2.163[b]	25.800±1.058[c]

Values are means±SD, n = 5 per group. Entries with different superscripts are significantly different at $p<0.05$

The effect of the extract on plasma electrolytes of salt-loaded rats is shown in Table 2. The treated animals had significantly lower ($p<0.05$) plasma sodium and chloride levels, compared to the test-control. Reduction in plasma sodium and chloride concentrations is one of the mechanisms of action of antihypertensive drugs,

especially the diuretics (Burton and Theodore, 2007). According to them, diuretics act by diminishing sodium chloride reabsorption at different sites in the nephrons, thereby increasing urinary sodium chloride and water losses, consequently leading to decreased plasma levels of these electrolytes. This again corroborates the use of this plant as an antihypertensive, in traditional medicine practice. There was no difference in the plasma calcium and potassium levels of the test and test-control animals. This probably means that the plant has no effect on calcium and potassium metabolism.

Conclusion: In conclusion, our result suggests that the antihypertensive action of *T. procumbens* may be mediated via reduction of weight and alteration of plasma sodium and potassium levels.

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Impact of Breakfast Eating Pattern on Nutritional Status, Glucose Level, Iron Status in Blood and Test Grades among Upper Primary School Girls in Riyadh City, Saudi Arabia

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Abstract: The aim of the study was to examine the effect of breakfast eating pattern (between breakfast consumers and non consumers), on nutritional status (body mass index), glucose level, iron status and test grades (school performance) among Saudi school children (girls). A total of 120 female students aged 9-13.9 years from Riyadh city, Saudi Arabia Participated in this cross-sectional study. A pre-designed questioner was used to collect information on their breakfast eating habits. Body weight and height of the girls were measured and Body Mass Index (BMI) was calculated. Tests for blood Glucose (GLU), Hemoglobin (HG), Hematocrit (HT), Serum Iron (SI), serum ferritin were performed and Total Iron Binding Capacity (TIBC) was calculated. School marks of the previous semester were also collected (used). The results shows that 23.33% of girls ate breakfast only once a week or less often, whereas (40.83%) of the girls ate breakfast daily. Skipping breakfast increased with age. Breakfast skipping was significantly noticed among over weight-obese students compared to lean students, the mean level of HB, HCT, SI, TIBC and ferritin of the girls who ate breakfast regularly was the highest with no significant difference. Regular habit of eating breakfast had beneficial impact on nutritional status.

Key words: Breakfast, school children, nutritional status, performance

INTRODUCTION

Children require an adequate supply of nutrients for growth, energy and to maintenance of body functions. Their brains rely on a constant supply of nutrients in order to function properly. Eating breakfast provides children with energy for their brains as it improves their learning skills. However, without breakfast, body energy reserves become depleted over night. A gap of about to 12 h between dinner and breakfast causes a decline in blood glucose levels, which may lead to glucose deprivation. If this happens in sufficient degree, it can result in a rapid disturbance in cerebral function (Wurtman *et al*., 1977). Habitually missing breakfast can adversely affect cognitive performance. The gradual decline of insulin and glucose levels could cause a stress response, which interferes with different aspects of cognitive function, such as attention and working memory. It is plausible that the decline in cerebral iron level, likely to result from diet that is deficient in heme, intensifies the stress associated with overnight and morning fast (Center on Hunger, Poverty and Nutrition Policy, 1995). This is why breakfast is commonly referred to as the most important meal of the day. The consumption of breakfast has many benefits for children, such as its positive effects on daily nutritional intake (Peter Wiliams, 2007; Uma Chitra and Radha Reddy, 2007; Noela *et al*., 2006; Theresa *et al*., 1993), body weight (Fabritius and Rasmussen, 2008; Henríquez Sánchez *et al*., 2008) and macro and micro-nutrients (Ruxton and Kirk, 1997; Cueto, 2001). Breakfast consumption may improve cognitive function reflected on memory, better marks and school attendance (Rampersaud *et al*., 2005; Kleinmana *et al*., 2002). Children who do not eat breakfast have reduced memory function, poorer attention spans and reduced performance in tasks requiring concentration when compared with those who consume an adequate breakfast. Therefore, consuming a healthy breakfast improves cognitive function and learning outcome (Mahoney *et al*., 2005). Habitually consuming an inadequate breakfast in quality and quantity is associated with poorer test scores (Lo ´pez-Sobaler *et al*., 2003). Effects of macro-nutrients on cognitive performance may be dependent on their effects on glucose metabolism, metabolic activation, or serotonin. Other factors that modify effects include time of day, habitual diet and vulnerability of the population (Louise Dye *et al*., 2000). Yet, young people are more likely to skip breakfast than any other meal. Those who skip breakfast are more likely to be overweight than those who don't, skipping breakfast means probably feeling very hungry by morning tea-time. Studies have found that children who skip breakfast are more likely to have a higher intake of sugar and fat in their diets. They are also more likely to be lacking essential vitamins and minerals in their diet, such as calcium, iron and zinc (Williams, 2007; Friedman and Hurd-Crixell, 1999). Food behaviors and food choices established in childhood

may track into adulthood (Mikkila *et al*., 2004). In Saudi Arabia a previous study has shown that failure to eat breakfast or lack of nutritious food for breakfast is common in childhood (Abalkhail and Shawky, 2002). A study carried in Jeddah city (western province) shows that the increase in BMI among Saudi Arabian children and female adolescents aged 10-20 years is apparent in all age groups (Abalkhail, 2002). Another study in Al-khobar city (Eastern province) showed that the prevalence of overweight and obesity among female school-aged children and adolescents was very high (Al-Saeed *et al*., 2006). The limited number of studies on this field triggered us to perform this study to recognize the relationship between eating/skipping breakfasts and the nutritional status, glucose levels, iron status in blood and test grades, among upper primary school girls in Riyadh city.

MATERIALS AND METHODS

Riyadh city is divided into four educational districts (north, south, east and west). One local governmental school was chosen randomly from each district. A total of 120 female students aged 9-13, 9 years (studying in 4th, 5th and 6th grades) participated in this cross-sectional study, by completed a questionnaire. A pre-designed questioner was used to collect the information about breakfast eating habits. Breakfast consumption is categorized as usual/always, often and rarely/never (5-7, 2-4 and 0-1 times/week, respectively). Anthropometric measurements including height and weight has been used to assess the nutritional status by calculation Body Mass Index (BMI) using the formula: BMI = Weight (in kg)/Height (in m^2). And an early-morning blood sample of 5 ml has been taken by trained physicians. Blood Glucose (GLU), Hemoglobin (HG), Hemotocrit (HT), Serum Iron (SI), serum ferritin were performed and Total Iron Binding Capacity (TIBC) has been calculated. Blood Glucose level has been tested using BM-Test 1-44 blood glucose test strips, following the manufacturer's procedure and then measured with a Prestige Medical Healthcare Ltd. HCI digital Blood Glucometer. Hemoglobin and hemotocrit has been measured by using Culter S+4 according to Dacie and Lewis (1975), serum iron by the method of Williams and Conrad (1966) and Ferritin has been measured according to Addison *et al*. (1972). According to the following formulae: (TIBC = Transferrin *24). Transferrin has been measured first in order to calculate the TIBC; the assay of transferrin has been carried out using the Cobas Mira plus Analyzer. A school mark of the first semester has been collected, and the questioner was filled at the first week of the second semester. Data analysis was done using Statistical Package for Social Science (SPSS) for means and (MNOVA) test was employed to analyze the data and study the association of the eating pattern of breakfast and other variables. Statistical inferences were made at sig. $\leq$0.05.

RESULTS

Table 1 shows the breakfast eating frequencies of the study sample. Although the majority of girls think that eating breakfast at home is very important, Breakfast skipping was relatively common: 23.33% of girls ate breakfast only once a week or less often, whereas (40.83%) of the girls ate breakfast daily. 7.14% subjects had completed 9 years of age, 30.0% had completed 10 years of age, 35.83% subjects had completed 11 years of age, 23.33% had completed 12 years of age and 6.67% had completed 13 years of age. Skipping breakfast increased with age. Mean age of the study sample who ate breakfast daily was 10.82±1.05 which is less than the mean age of girls who ate breakfast Some times or rarely.

The mean height of the girls who habitually consume breakfast was 141.26±9.28 cm, while the mean weight was 37.89±12.94 kg. Their mean BMI was 18.63±4.28 which is less than the mean weight and the mean BMI of the girls who did not consume breakfast but the difference does not reach statistical significance (Table 2).

Table 3 shows nutritional status according to BMI. Of the study sample, 66.67% were normal (lean), 13.33% were overweight while 12.5% were found to be obese and 7.5% were under weight. Most of the girls who ate breakfast daily (71.4%) were normal while 20.4% were overweight or obese and 8.3% were found to be under weight, the highest percentage of obesity and under weight were found among the girls skipping breakfast 25.0%, 10.7% consecutively. There was a significant association between eating breakfast and nutritional status for the benefit of normal students.

The mean level of glucose and iron in blood are shown in Table 4, level of glucose was proximate for the three groups. The mean level of HB, HCT, SI, TIBC and ferritin of the girls who ate breakfast regularly was the highest and no significant difference was noticed.

The mean grade tests for girls consuming breakfast regularly was the highest between the study sample groups in all subjects but the difference was not significant (Table 5).

DISCUSSION

Girls in Saudi Arabia do not involve in any physical activity at schools (sport is not included in curriculum) or at outside (due to the hot weather), yet food habits and nutritional status is a very important factors for growth and health maintenance. This study shows that about 40.83% eat breakfast daily while 23.33% do not eat it at all or once a week. These results are much higher than the results of previous studies that were conducted in Saudi Arabia. In Riyadh city, a study shows that 16, 5% of the girls age 7-14 years do not eat breakfast (AL-Othaimeen *et al*., 1999) And in Jeddah city skipping breakfast is reported by 14.9% of school students

Table 1: Distribution of study sample according to breakfast consumption and age

Age group (years)	Daily		Sometimes		Rarely or never		Total	
	No	%	No	%	No	%	No	%
9-9.9	3	2.5	1	0.83	1	0.83	5	7.14
10-10.9	19	15.83	11	9.17	6	5.0	36	30.0
11-11.9	15	12.5	14	11.67	14	11.67	43	35.83
12-12.9	8	6.67	13	10.83	7	5.83	28	23.33
13+	4	3.33	4	3.33	-	-	8	6.67
Total	49	40.83	43	35.83	28	23.33	120	100.0
Mean age	10.82±1.05		11.19±1.01		10.96±0.79		10.98±0.99	

Table 2: Distribution of study sample according to breakfast consumption and anthropometric measurements

	Daily n = 49	Sometimes n = 43	Rarely or never n = 28		
	Mean±SD			F	Sig.
Height (cm)	141.26±9.28	142.05±8.16	141.13±8.24	3.382	0.918
Weight (kg)	37.89±12.94	40.29±12.02	41.01±14.37	0.432	0.166
BMI	18.63±4.28	19.68±4.35	20.18±5.31	0.379	0.144

Table 3: Distribution of study sample according to breakfast consumption and nutritional status

Nutritional status	Daily		Sometimes		Rarely or never		Total		F	Sig.
	No	%	No	%	No	%	No	%		
Normal	35	71.4	31	72.1	14	50.0	80	66.67	3.153	0.046*
Overweight	7	14.3	5	11.6	4	14.3	16	13.33		
Obese	3	6.1	5	11.6	7	25.0	15	12.5		
Under weight	4	8.2	2	4.7	3	10.7	9	7.5		
Total	49	100.0	43	100.0	28	100.0	120	100.0		

*Significant differences between groups (Sig. <0.05)

Table 4: Distribution of study sample according to breakfast consumption and glucose level, iron status in blood

	Daily	Sometimes	Rarely or never		
	Mean±SD			F	Sig.
GLU (mmol/L)	100.46±15.90	101.81±20.56	98.45±19.19	1.620	0.202
HB (g/dl)	13.18±0.91	12.91±1.67	12.89±0.96	1.379	0.256
HCT (%)	39.00±2.16	38.09±2.44	38.32±3.58	0.597	0.674
SI (mmol/L)	90.98±26.54	84.53±32.83	90.85±27.92	0.298	0.743
TIBC (mmol/L)	346.59±74.64	335.06±68.82	330.10±75.97	0.382	0.683
Ferritin (µg/L)	17.54±12.44	17.09±12.52	16.53±9.64	0.219	0.804

Table 5: Distribution of study sample according to breakfast consumption and subjects test grades

	Daily n = 49	Sometimes n = 43	Rarely or never n = 28	F	Sig.
Math (% of the marks)	≥90	> 80 to < 90	≥70	0.355	0.132
Science (% of the marks)	≥90	> 80 to < 90	≥70	0.313	0.401
Reading (% of the marks)	≥90	> 80 to < 90	≤80	0.290	0.264

(Abalkhail and Shawky, 2002). But these findings are lower than those reported in Abha, Southwestern region of Saudi Arabia, where breakfast is a regular meal for 72% of primary school students (Nadia *et al*., 2007). Another study involving 124 girls, age 9-10 years in Qatar (Gulf Region) has found that 89.5% eat breakfast daily (Abdelmonem and Al-Dosari, 2008). In another study in India 62.3% of the children age 11-13 years habitually consume breakfast, 33.8% consume it irregularly, skipping it 2 or 3 times a week and 3.9% do not consume breakfast at all (Gajre *et al*., 2008). In Hong Kong 30.5% of the student age 10-14 years old reported skipping breakfast for at least 1 day in a school week (Tereza Cheng *et al*., 2008). Skipping breakfast increase with age, these findings are consistent with other studies (Pearson *et al*., 2009; Williams *et al*., 2008; Abalkhail and Shawky, 2002). Girls who eat breakfast regularly have the lesser mean body mass index; whereas girls who do not eat breakfast have the highest mean weight and body mass. These findings are similar to some other studies (Fabritius and Rasmussen, 2008; Albertson *et al*., 2007; Lo ´pez-Sobaler *et al*., 2003). In this study breakfast consumption has an important impact on nutritional status; obese girls are more likely to skip breakfast than their normal peers and are at higher risk for growth deficits and health problems. These results are not different from other studies (Tereza Cheng *et al*., 2008; Amin *et al*., 2008; Arash Rashidi *et al*., 2007). Levels of glucose in blood are convergent either due to the quality and/or quantity of

breakfast eaten or due to time of testing. Eating breakfast before 1.5-2 h prior to testing registers as not eating breakfast. That may explain the findings, since there appears to be no significant difference between groups. Healthy breakfast should contain whole grain cereals, fruit and/or vegetables, milk or fresh juice, whole grain foods are high in fiber which helps in feeling full and helps to slow the release of energy into the blood. Energy, therefore, lasts longer throughout the day. However, little research has been published on the types of foods and beverages consumed by Saudi school children for breakfast. One study shows that 23.9, 22.8, 18.2 and 18.8% of the anemic school children do not eat green vegetables daily, do not eat fruits at school as snack between meals, do not eat junk food between meals as snack and do not eat junk food at school respectively (Abalkhail and Shawky, 2002). In the USA, African-American children still had mean intakes of most nutrients below recommended levels (Williams *et al*., 2008). Although the mean biochemical tests for iron status in blood were within tolerable levels for all the groups but the highest level was for girls consuming breakfast regularly. The lack of information about using supplements and about the content of other meals consumed throughout the day by the study samples may explain the significant differences between groups in level of HG, HCT and TIBC. Results of a study in Peru (Jacoby *et al*., 1996) shows that children in the school breakfast programme improved their average daily intake of energy, protein and iron by 15, 16 and 60%, respectively. Similar results have been reported in developing countries (Mathews, 1996). The mean level of HB in this study is higher than that reported in a study in Peru (Cueto and Chinen, 2008) where they have found that the average hemoglobin level in a group of children having school breakfast is 12 g dl^{-1}, while in the comparison group it is 11 g dl^{-1}. That's probably due to the high consumption of meat, poultry and eggs by the population in Saudi Arabia. Breakfast quality is an important component in the complex interaction between lifestyle factors and mental health (O'Sullivan *et al*., 2008). Habitually consuming an inadequate breakfast in quality and quantity is associated with poorer test scores (Barbara Radcllffe *et al*., 2004; Lo ´pez-Sobaler *et al*., 2003). In Rural Peru, a school breakfast programme shows a significant and positive effect on short-term memory, arithmetic and reading comprehension (Cueto and Chinen, 2008). Indian students who regularly consume breakfast achieve higher scores on the immediate recall memory test and perform significantly better on the letter cancellation test (attention-concentration) (Gajre *et al*., 2008). In the present study, eating breakfast has no effect on tests grades. The design of this study does not distinguish whether children who habitually have better breakfasts have better test grades. Breakfast quality and quantity need to be taken into account when designing future studies. Further research is needed to investigate the relationships between consuming breakfast (including quality and quantity) and mental function in Saudi schoolchildren.

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Karyological and Chromosomal Study of Catfish (Clariidae, *Clarias gariepinus*, Burchell, 1822) from Anambra River, Anambra State, Nigeria

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Abstract: Karyological and Chromosome analysis of *Clarias gariepinus* (Burchell, 1822) inhabiting Ananmbra River, Anambra State of Nigeria was carried out using Modified Air-drying technique. *Clarias gariepinus* was found to have standard karyotype and diploid chromosome number of 2n = 56. The study further revealed that the males and females catfish consist of 8 metacentric, 24 and 25 submetacentric and 24 and 23 acrocentric chromosomes, respectively. The work documented the karyotypic polymorphism of *Clarias gariepinus* resident in the River.

Key words: Karyotype, chromosome, *Clarias gariepinus*

INTRODUCTION

Clarias gariepinus is widely considered as the most tropical catfish species ideal for aquaculture in West Africa (Clay, 1979). It has a pan African distribution, being preponderance from Nile to West Africa and from Algeria to South Africa as the Orange system and Umtamvuna (east coast), going by Teugels (1986) report. The species is also preponderance in Asia Minor and Potamodromous (Teugels, 1986). In Nigeria, *Clarias gariepinus* is known by different names among various ethnic groups; Tarwada (Hausa), Imunu (Ijaw), Ejengi (Nupe), Aro (Yoruba) and Arira (Igbo).

According to Ozouf-Costaz *et al.* (1990), in *Clarias gariepinus*, the karyotype and chromatin materials are very stable. They observed no detectable karyotypic differences among the *Clarias gariepinus* populations from three different geographical locations. Working on the chromosomal structure, Levan *et al.* (1964) maintained that fish chromosomes with centromere index less than 35 were classified as acrocentric, while those between 35 and 45 were regarded as submetacentric and those within 45-50 were the metacentric chromosomes.

Data on chromosomal constitution, architecture and by extension, the photomicrograph of the chromosome morphology of catfish is rather limited compared to other vertebrates because of the small size and limitations of techniques employed (Klinkhardt, 1991; Ergene *et al.*, 1999). Air-drying technique which was originally developed for the study of mammalians' chromosomes (Serap and Tolga, 2004) can now be applied to chromosome studies in other species. According to the authors, while the basic steps are the same, few modifications have been applied to this technique for different species (Foresti *et al.*, 1993; Cucchi and Barufaldi, 1990). Doussau and Ozouf-Costaz (1985) has described a technique for studying fish chromosomes using anterior kidney and in some cases, stimulation of fish sample with yeast suspension is advocated for induction of mitosis (Lee and Elder, 1980). However, the use of Air-drying technique appears to be simpler, cheaper and more reliable when compared to the existing techniques. The study was therefore, designed to determine the karyotype, chromosomal architecture, the proportion of acrocentric, submetacentric and metacentric chromosomes and number of chromosomes in *Clarias gariepinus*, Burchell, 1822 (Pisces, Clariidae) in Anambra River, Anambra State, Nigeria using Air Drying Technique.

MATERIALS AND METHODS

Live *Clarias gariepinus* specimens were collected from Anambra River, Anambra State, Nigeria; brought to the laboratory in air-tight containers and put into aquariums where they were kept for several days. They were fed twice a day and 0.5% Colchicine was injected intra-peritoneally into the fish sample to disrupt spindle formation at mitosis and prevent the replicated chromosomes from migrating to their respective poles (Hartwell *et al.*, 2000). Four hours later, the fish sample was sacrificed by decapitation and dissected. Gill arches and kidney tissues were extracted and crushed to obtain an epithelial cell suspension. The suspension was aspirated into centrifuge tubes and after hypotonic treatment with 0.75% KCL solution for 30 min (at room temperature); they were centrifuged at 2000 rpm for 10 min.

The supernatants were recovered and the cell buttons were broken up with the tip of pipette. Cells were fixed in Glacial Acetic Acid (GAA) and Methanol at the ratio of 1:3. The solution was allowed to stand for 30 sec and the fixative changed by three successive centrifugations for

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10 min. Cells from the fixative were transferred to clean and cold slides, air-dried and stained with 10% giemsa solution in 6.8 phosphate buffer. Finally, the slides were rinsed with distilled water and air-dried. Slides were covered, microscopic studies were performed and well-separated metaphase chromosomes photographed. A total of sixty slides were prepared at the rate of five slides from a fish sample. A karyogram was prepared by high-contrast chromosome photographs (Fig. 1) and the individual chromosomes were cut out of the photographs. Classification and karyogram of the chromosomes were performed according to the techniques described by Levan *et al.* (1964) and Ergene *et al.* (1998a,b). The final karyogram was scanned and printed.

RESULTS AND DISCUSSION

The diploid chromosome number of *C. gariepinus* in Anambra River was found to be twenty-eight pairs (2n = 56) and autosomes basic arm number (NF) is 88 for males and 89 for females. It was also found that the male *Clarias gariepinus* has 8 metacentric, 24 submetacentric and 24 acrocentric chromosomes, while the female recorded 8 metacentric, 25 submetacentric and 23 acrocentric chromosomes. Figure 1 presents diploid metaphase chromosomes from gill epithelial cells of *C. gariepinus*, while Fig. 2 and 3 display the male and female Karyotypes, respectively of gill epithelial cells of *C. gariepinus*. The percentage occurrences of metacentric, submetacentric and acrocentric chromosomes in sixty-four samples examined is shown Table 1.

Karyotypes of *Clarias gariepinus* living in Anambra River, Anambra were 2n = 56. The most recent and complete data summarizing siluroid karyotypes have been produced by Rab (1981) and Vasiliev (1985). In siluroid families, chromosome and/or chromosome arm numbers exhibit a great variability and we can assume that the karyotype is specific and that this criterion can be used for the species characterization.

It is a well known phenomenon that acrocentric chromosomes have a tendency to stick to each other by their centromeres and in this way they form metacentric chromosomes (Dogramaci *et al.*, 1994). If we consider the same condition for *C. lazera*, the metacentric number will increase but the acrocentric number will decrease; but since the acrocentric number would decrease and this would result in a decrease in chromosome number, this is considered to be a weak probability (Ergene *et al.*, 1999).

The variation seen in the karyotype of *C. gariepinus* in the Anambra River can be viewed as a small part of the main population and variations in chromosome number and chromosome morphology were equally determined for *C. gariepinus* inhabiting the River (Table 1). Variations such as these show that various karyotypic forms exist in this species, since the highest value in the count is accepted as the number of chromosomes and portray karyotypical polymorphism of the resident *C. gariepinus* species in Anambra River.

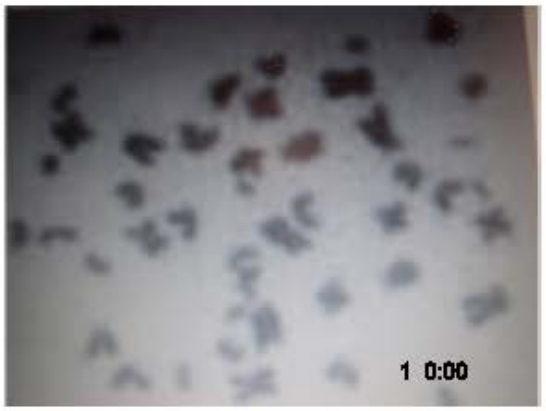

Fig. 1: Diploid metaphase chromosomes from gill epithelial cells of *C. gariepinus*, giemsa staining, x 1500

Fig. 2: Karyotype from gill epithelial cells of a male of *C. gariepinus*, giemsa staining, x 1500

Fig. 3: Karyotype from gill epithelial cells of a female of *C. gariepinus*, giemsa staining, x 1500

Table 1: Frequency distribution of diploid chromsome number of *Clarias gariepinus* in Anambra River, Anambra

Examined Specimen	Evaluated Metaphase Number	Diploid Chromosome Number (2n)	Males			Females			Occurrence (%)
			M	SM	A	M	SM	A	
-	2	49	8	12	14	10	23	20	3.10
-	3	51	12	25	12	10	23	20	4.70
-	6	52	9	23	12	12	22	19	9.40
-	2	53	13	23	12	6	18	17	3.10
-	5	54	12	18	25	12	17	18	7.80
-	3	55	12	22	24	8	18	24	4.70
-	17	55	8	23	24	8	12	22	26.60
-	24	56	8	24	24	8	25	23	37.50
-	2	58	5	24	24	8	25	20	3.10
10	64								100

M: Metacentric, SM: Submetacentric, A: Acrocentric

Ozouf-Costaz *et al*. (1990) supporting Teugels (1982) study, stated that *C. lazera* and *C. mosambicus* are synonymous with *C. gariepinus*. In order for *C. lazera* to be assumed synonymous to *C. gariepinus*, it must be determined whether they can breed and produce fertile individuals. However, taking only karyological and morphological characteristics into account in defining species has its shortcomings. Karyotypical studies on *C. gariepinus* living in other parts of Nigeria are necessary for more detailed knowledge about karyotypical forms.

The first successful hybridization between the two species of Clariidae, *Clarias gariepinus* and *Heterobrunchus longifilis*, was done in 1985 by Hecht and Lublinkhof. But, karyotypes of the parental species and of their hybrids remained unknown. It is of interest to know the karyotype of the species used for this hybridization in order to estimate their genetic purity (Ozouf-Costaz *et al*., 1990). A study of the phylogenetic relationships between these species by karyological and biochemical methods would allow a prediction of the possible genomes obtainable in the hybrids. Finally, the analysis of their karyotypes would clarify the mechanism involved in karyogamy, as well as why these hybrids are viable and relatively fertile (Ozouf-Costaz *et al*., 1990). This study is the first in a series to characterize the chromosomes of clariid catfishes used for fish culture from Anambra River and will be followed by definition of the genetic structure of hybrids. Although studying chromosomes at meiosis are particularly difficult to undertake in the field, the marker chromosomes detected in the standard karyotypes of *Clarias gariepinus* appear to be useful in comparing different species. Furthermore, these markers may also be useful in identifying parental genetic input in hybrid karyotypes.

In addition to chromosomal architecture of *clarias gariepinus*, gene characterization and mapping and DNA analysis should be embarked upon to determine various species, additional information on the fish cytogenetic database and provide clues for improvement of the economic species.

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Replacement of Feed Concentrate with Graded Levels of Cassava Leaf Meal in the Diet of Growing Rabbits: Effect on Feed and Growth Parameters

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Abstract: Forty growing rabbits with mean live-weight of 0.774kg+0.04 were randomly fed on five diets containing graded levels of Cassava Leaf Meal (CLM). The general objective of the work was to determine the replacement value of concentrate with CLM in the diet of growing rabbits by assessing the feed quality and growth parameters. Specifically, the Voluntary Feed Intake (VFI), Average Daily Gain (ADG), Feed Conversion Ratio (FCR) and digestibility of various nutrients were estimated. The Dry Matter (DM) intake of the rabbits ranged from 44.24-66.85 g/day per rabbit whereas the Crude Protein (CP) and energy intakes per day per rabbit ranged from 9.95-13.16 g and 204.39-291.47 kcal, respectively. Digestibility coefficients ranging from 67.06-81.09; 54.13-77.50; 24.95-44.43; 48.99-75.65 and 65.40-79.16 were obtained for DM, CP, CF, EE and energy, respectively. The FCR and ADG ranges of 3.13-5.27 and 8.43-21.36 g/d/rabbit were obtained. Rabbits performed satisfactory at 15% and 30% inclusion levels of CLM. It was observed that higher levels of CLM inclusion resulted in a significant decrease in the values of all the parameters examined and a forage free diet is not ideal for rabbit production, as it could induce diarrhoea.

Key words: CLM, concentrate, digestibility and rabbits

INTRODUCTION

The constant increase in the cost of protein and energy concentrates coupled with the scarcity of most feed ingredients make research into replacement of concentrate with forage imperative in third world countries. Reducing the levels of concentrate used in compounding rabbit feed will invariably entail reduction in the cost of rabbit production. The rabbit (*Oryctolagus cuniculus*) is a monogastric animal with a distinct preference for high fibre diets (Carregal, 1977). This may be accredited to its functional caecum and the practice of caecotrophy. Cheap feedstuffs are digested through the action of microorganisms inhabiting the caecum of the rabbit. So, rabbits being unique from other monogastric animals can utilize roughages more efficiently. Several research reports have shown that rabbits can neither be raised on roughages alone nor on roughage free diets (Iyeghe-Erakpotobor *et al*., 2002; Olorunsanya *et al*., 2007; Omole *et al*., 2005 and Iyeghe-Erakpotobor *et al*., 2006). Olorunsanya *et al*. (2007) and Omole *et al*. (2005) observed that more than 45% inclusion level of forage in the diet of rabbits bring remarkable reduction in performance while Davidson and Spreadbury (1975), Carregal (1977) and Oluremi and Nwosu (2002) reported incidence of diarrhoea in rabbits raised under forage free diets.

Generally, cassava is predominant in the humid tropic and its cultivation is amiable to wide range of environment. Though, several aspects of cassava leaf have been used in formulation of rabbit diets, there is dearth of information on the inclusion of the Cassava Leaf Meal (CLM) in rabbit diet. The CLM contains more crude protein and are highly digestible when compared to cassava peel, cassava tuber and cassava stem. This study was therefore designed to evaluate the replacement value of concentrate with Cassava Leaf Meal (CLM) on the diet of growing rabbits. It was also intended to establish the optimal inclusion level of cassava leaf meal for excellent rabbit production.

MATERIALS AND METHODS

The study was carried out at the University of Nigeria, Nsukka, Rabbit Unit. The rabbit house and cages measuring 61 x 45 x 40 cm were thoroughly washed and disinfected before the commencement of the experiment. The feeding and watering troughs were also washed, disinfected and fixed on the cages. The cages were randomized into five treatments and eight replicates, and numbered accordingly. Forty growing rabbits (25 females and 15 males) were used. Both the males and the females were randomly distributed independently into five treatment pens of eight rabbits per treatment. There were a total of three males and five females per treatment. The average weights of each animal per treatment were 0.588, 0.581, 0.577, 0.576 and 0.573 kg, respectively. Each treatment has eight replicates (rabbits), which were housed individually in hutches.

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Fresh cassava leaves (*Manihot esculenta* var *otu-pam*) were collected from the University of Nigeria, Nsukka farm. The leaves were sun-dried for five days after which they were pulverized and used to compound the experimental diets. The concentrate diet was formulated using Maize, Soyabean, Brewers Dry Grain (BDG), Fish Dust, Bone Meal, Salt and Molasses to Contain 18.81% Crude protein and 4.30 kcal/g energy. Five experimental diets were prepared with graded levels of CLM inclusion in the concentrate diet. The levels were 0% (Diet 1), 15% (Diet 2), 30% (Diet 3), 45% (Diet 4) and 60% (Diet 5). The proximate compositions of the diets are given in Table 1.

Table 1: Proximate compositions of the experimental diets

	Diets				
Nutrients	0% (Diet 1)	15% (Diet 2)	30% (Diet 3)	45% (Diet 4)	60% (Diet 5)
CP %	18.81	19.69	20.66	21.50	22.48
EE %	7.40	7.48	7.60	7.76	7.90
CF %	5.80	6.65	8.13	9.60	11.07
Energy (Kcal/g)	4.30	4.36	4.45	4.52	4.62

The animals were re-weighed at the beginning of data collection. Known quantities of feed were provided daily *ad libitum* for a period of three weeks. The remnants of feed were measured to determine the feed intake. After three weeks, the rabbits were weighed again and the weight gains were calculated. Faeces voided were collected in labeled polythene bags, bulked, oven-dried for 48 h at 80°C and weighed. The feeds and faeces were milled separately to pass through a 1 mm sieve. After this, the samples were subjected to proximate analysis. The crude protein, crude fibre and ether extract components of the feeds and faces were determined using the AOAC (1990) methods. The gross energy contents were determined using the Parr Adiabatic Bomb Calorimeter.

The data obtained were subjected to statistical analysis under a completely randomized design with five treatments and eight replicates. The SPSS (2004) statistical programme was used and differences between treatment mean values were separated using Duncan's New Multiple Range Test (DNMRT).

RESULTS

The dry matter intake, average daily gain, crude protein intake and feed conversion ratio of the rabbit are presented in Table 2. The dry matter intake was highest at 15% inclusion of cassava leaf meal and poorest at 60% level of inclusion. Diets 1, 3 and 4 with 0, 30 and 45% levels of inclusion were not significantly different from one another ($p>0.05$). Diets 2 and 3 gave the highest Average Daily Weight Gain (ADWG) values compared to diets 1, 4 and 5 which gave lower values. No significant ADWG differences were obtained between diets 2 and 3; similarly none existed among diets 1, 4 and 5. Diets 1, 2 and 3 had no significant differences in Feed Conversion Ratio (FCR), but there was a meaningful difference between diets 2 and diets 4 and 5.

The nutrient digestibility coefficients of the various diets by the rabbits are shown in Table 3. The digestibility coefficients of dry matter for 0% and 15% inclusions of cassava leaf meal were the same. No differences were obtained between 15, 30 and 45% inclusion. But there existed significant differences between 30, 45 and 60% inclusions. There were no significant difference between CF digestibility coefficients of diet 2, 3 and 4, but difference existed between 15% and 60% inclusion levels. The same was true for ether extract digestibility. Crude protein and energy digestibility followed the same pattern. There were no observable differences for 15, 30, 45 and 60% inclusion levels of cassava leaf meal ($p>0.05$). The values obtained were inversely proportional to the cassava leaf meal inclusion. That is, generally the coefficients decreased in value with increasing levels of the CLM inclusion in the diets for each nutrient.

DISCUSSION

The dry matter intake of the rabbit ranged from 44.24 g to 66.85 g/day/rabbit and it is within the range of 40-80 g per day per rabbit reported by Joyce *et al.* (1971), Iyeghe-Erakpotobor *et al.* (2002) and Olorunsanya *et al.* (2007) and close to the ranges obtained by Omole *et al.* (2005) and Iyeghe-Erakpotobor *et al.* (2006). As the percentage incorporation of the cassava leaf meal increased from 0-15%, the DM intake increased from 53.00 g to 66.85 g/d/rabbit and also diarrhoea, which was observed in treatment I (0% inclusion level), disappeared. This agreed with the finding of Davidson and Spreadbury (1975), Carregal (1977) and Omole *et al.* (2005) who claimed that the dietary level of crude fibre less than 6% will promote diarrhoea in the rabbit. As the cassava leaf meal inclusion exceeded 15%, the Voluntary Feed Intake (VFI) started to decline from 56.76 g in 30% inclusion to 44.24 g in 60% inclusion. This result is in agreement with that of Maff (1978) and Omole *et al.* (2005) who recommended 12-14% inclusion level of forage in the diet of rabbits. The decline in DM intake may be as a result of physical barrier to feed intake due to the coarseness of the feed or as a result of change in satiety level at the satiety control centre in the hypothalamus. Again, as the cassava leaf meal inclusion increased from 30-60%, the crude protein and energy contents of the diet increased but the VFI and energy intake decreased. The rate of decrease in VFI was so high that the total CP and energy consumptions per day were also decreased.

Table 2: Dry matter intakes, average daily gain, energy intake, feed conversion ratio of the rabbits fed graded levels of cassava leaf meal

	Diets				
Parameters	1	2	3	4	5
DM intake					
- g/day/rabbit	53.00^{b}	66.85^{a}	56.76^{b}	55.61^{b}	44.24^{c}
- g/day/kg live-weight	73.22	84.39	74.20	70.08	54.60
Daily gain: g/d/rabbit	11.63^{b}	21.36^{a}	17.05^{a}	10.55^{b}	8.43^{b}
CP Intake g/day/rabbit	9.97	13.16	11.73	11.96	9.95
Energy Intake Kcal/day	227.90	291.47	252.58	251.36	204.39
Feed Conversion Ratio (FCR)	4.56^{ab}	3.13^{a}	3.33^{ab}	5.27^{b}	5.25^{b}

*^{a,b,c}Indicates values with different superscripts within row differ significantly ($p<0.05$)

Table 3: Mean nutrient digestibility coefficients of the diets by rabbits

	Diets						
Nutrients (%)	1	2	3	4	5	Mean	SE
DM	81.09^{a}	76.17^{ab}	72.12^{bc}	73.13^{bc}	67.06^{c}	73.91	±2.59
CP	77.50^{a}	67.67^{b}	65.71^{b}	65.11^{b}	54.13^{b}	68.02	±2.60
CF	44.43^{a}	36.79^{b}	30.20^{bc}	29.99^{bc}	24.95^{c}	33.27	±3.76
EE	75.65^{a}	66.46^{b}	59.93^{b}	60.73^{b}	48.99^{c}	62.35	±4.88
Energy	79.16^{a}	70.79^{b}	71.14^{b}	70.40^{b}	65.40^{b}	71.38	±2.47

*^{a,b,c}Indicates values with different superscripts within row differ significantly ($p<0.05$)

The Average Daily Weight Gain (ADWG) decreased with increasing level of cassava leaf meal except that 15% inclusion was better than 0% inclusion. 15% inclusion level proved best with ADWG of 21.36 g. Also at 30% level of CLM, the ADWG of 17.05 g/day/rabbit was obtained which was reasonably higher than the ranges reported by Olorunsanya *et al.* (2007), Omole *et al.* (2005) and Oluremi and Nwosu (2002) when 30% of other aspects of cassava were fed. The difference between these results could be attributed to more proteins and digestible nutrients contained in CLM than other aspects of cassava meal (Oyenuga, 1968). Olorunsanya *et al.* (2007), Omole *et al.* (2005) and Oluremi and Nwosu (2002) also obtained similar ADWG for the rabbits when only concentrate diets were fed.

The FCR range of 3.13-5.29 obtained in this work showed a very good performance of the rabbits. The values obtained in this study are comparable with 3.9-4.1 range reported by Cheeke (1971) when arginine, lysine and methionine were added to rabbit diet. Olorunsanya *et al.* (2007), Omole *et al.* (2005) and Oluremi and Nwosu (2002) reported poorer FCR for the rabbits. The digestibility coefficients obtained in this study are within the range reported elsewhere (Omole *et al.* 2005; Oluremi and Nwosu, 2002). In general the digestibility coefficients declined with increased level of CLM and this agreed with the reports of Bassendina (1969), Omole *et al.* (2005) and Olorunsanya *et al.* (2007) who observed decrease in nutrient digestibility with increased level of crude fibre in the diet. This may be as result of the masking effect of crude fibre on the bacteriostatic activity in the caecum. It reduces the microbial protein synthesis and fermentative ability of the caecum thereby resulting in decreased caecum microbial population.

Conclusion: Forage free diet is not ideal for optimal performance of rabbits. It induces diarrhoea, reduction in FCR, ADG and DM intakes. Again the level of crude fibre in the diet affects the digestibility of nutrients by the rabbits. Furthermore profitable rabbit production cannot be achieved by feeding more than 30% CLM. Since 15% inclusion of CLM was not superior to 30% inclusion, rabbits can be profitably maintained on a diet with up to 30% of CLM.

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Effects of Breed, Sex and Source Within Breed on the Blood Bilirubin, Cholesterol and Glucose Concentrations of Nigerian Goats

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Abstract: Effects of breed, sex and source within breed on the blood bilirubin, cholesterol and glucose concentrations of the Nigerian goats were studied using eighty-one yearly goats; comprising equal numbers of the Sahelian Goat (SG), Red Sokoto Goat (RSG) and West African Dwarf goat (WADG) breeds. The studies indicate that there were no significant breed differences in total serum bilirubin concentrations and the range of 0.63-0.65 mg/100 ml was observed. However, the conjugated (direct) bilirubin is generally lower in the male goats than in the female goats, while the unconjugated (indirect) bilirubin is higher in the male goats than in their female counterparts. Source within each breed exhibited no pronounced effects on the Sahelian goats; but had effects on the RSG and WADG breeds. The study further revealed that the Sahelian goat breed has the highest concentration of serum glucose and the lowest level of serum cholesterol; the reverse is the case for the West African Dwarf goats, while the Red Sokoto goats recorded moderate concentrations of both cholesterol and glucose in the serum. The serum cholesterol level in goat is inversely proportional to the glucose concentration.

Key words: Goat breeds, sex, source within breed, serum bilirubin, cholesterol and glucose

INTRODUCTION

Bilirubin, a brownish yellow substance, is one of the end products of haemoglobin catabolism. After the protein and iron are broken away from haemoglobin, a green pigment, biliverdin, remains, which becomes reduced to bilirubin (Harper *et al*., 1977; Frandson, 1981; Singh, 2004). Bilirubin tests measure the amount of the bilirubin in the blood sample and it is considered the true test for the liver function, as it reflects the ability of the liver to take up, process and secrete bilirubin into the bile (Frandson, 1981; Singh, 2004). Total bilirubin in the blood stream may be partitioned into two forms namely, direct (unconjugated/water soluble) and indirect (conjugated/non-water soluble). Usually, the conjugated form travels through the blood to the liver where it is converted to the soluble form (Harper *et al*., 1977; Frandson, 1981; Singh, 2004). The serum total bilirubin ranges of 0.00-0.9 mg/100 ml of serum have been reported for both man and livestock (Harper *et al*., 1977; Frandson, 1981; Singh, 2004). High bilirubin concentration in the body causes jaundice in man, a condition in which the skin and the white part of the eyes appear yellowish. Singh (2004) maintained that the high blood bilirubin is caused by liver disease, blood disorder and or blockage of the tube.

The blood sugar level in man and livestock is maintained at a constant range through the action of several hormones (Scott, 1999; Singh, 2004; Randox, 2006). Scott (1999), Zubcic (2001), Lazzaro (2001) and Tambuwal *et al*. (2002) have reported the range of 60-100 mg of glucose per 100 ml of blood for different breeds of goat. Similar values have been documented for man (Harper *et al*., 1977; Singh, 2004; Randox, 2006), for cat (Nottidge *et al*., 1999), for Sheep (Hunter, 1996; Kelly, 1974) and for poultry (Schalm *et al*., 1975). Insulin and glycogen are the principal hormones involved in the regulation of blood sugar. While insulin is hypoglycemic, glycogen is hyperglycemic. Generally when the sugar in the blood reaches the range of 160-180 mg, it appears in the urine indicating abnormality caused by excess sugar in the blood, which could lead to health hazard (Harper *et al*., 1977; Singh, 2004; Randox, 2006). Very low level of glucose in the blood is typified in livestock by gross reduction in weight gain, milk yield and alteration in the fatty acid composition of the milk (Scott, 1999; Zubcic, 2001; Lazzaro, 2001; Tambuwal *et al*., 2002).

Cholesterol is a combination of sterol and lipids, which plays a key role in formation of cell membrane, production of hormones, cortisone and bile and metabolism of fat soluble vitamins, as well as acting as antioxidant (Macdonald and Low, 1995; Ritter, 1996). They noted that excess cholesterol in the serum blocks blood vessels thereby leading to angina and or heart attack in man and very low level is equally dangerous. The work therefore was designed to determine the levels of bilirubin, cholesterol and sugar in the blood of Nigerian goats and to study the effects of breed, sex and source within breed on these blood biochemical indices.

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MATERIALS AND METHODS

The study was conducted at the Goat Research Unit of the Department of Animal Science, Faculty of Agriculture, Delta State University, Asaba Campus, Asaba, Delta State. Delta State falls within the humid tropic. Each breed was sourced from three different sources based on areas its predominance in the country. A total of nine goats (comprising three males and six females) were selected from each location.

Design of the experiment: The experiment was conducted under a 2 x 3 factorial in CRD to test the effects of sex, breed and their interactions on the blood bilirubin, cholesterol and glucose concentrations of the Nigerian indigenous goats. Sex was tested on two levels with unequal replicates of twenty-seven bucks and fifty-four does; while there were three breeds with twenty-seven goats for each breed. In addition, effect of source of goat within each breed on these parameters was tested using one-way classification.

The statistical model used:

$$Y_{ijk} = \mu + B_i + S_j + (BS)_{ij} + e_{ijkl}$$

Where:

- Y_{ijk} = is the observed blood bilirubin, cholesterol or glucose
- μ = The population mean
- B_i = The effect of the breed, $_i$ = 1, --, 3
- S_j = The effect of $_j$th sex of the animal, $_j$ = 1, 2
- $(BS)_{ij}$ = is the interaction between breed and sex and
- e_{ijk} = is the error term associated with the observations

Assumptions; error term is independently, identically and normally distributed, with zero mean and constant variance, that is, iind (0, δ^2).

Analytical procedure: 10 ml of blood was collected from each goat via the jugular vein; properly identified and centrifuged at 4000 revolutions per minute for 15 min. Then, the parameters were determined as follow:

Determination of total bilirubin: The number of test tubes to represent all the samples was set up on a test tube stand. All were properly labeled against each sample. One tube was labeled blank. To the blank, 0.2 ml of sulphanilic acid, 1 ml of caffeine and 0.2 ml of sample were added. In each sample tube, 0.2 ml sulphanilic acid, 1 ml caffeine, 1 drop sodium nitrate and 0.2 ml respective samples were added. They were thoroughly mixed and allowed to stand for 10 min at 25°C. Thereafter, 1 ml of titrate was added to both the sample blank and rest of the tubes. They were again mixed and allowed to stand for 15 min at 25°C. The absorbance values ($A.V_b$) of the samples against the sample blank were read in a colorimeter at 540 mn. Then, the total bilirubin value was derived from the equation:

$$\text{Total bilirubin (mg/dl)} = 10.8 \times A.V_b\,(540\text{ mn})$$

Determination of direct bilirubin: The test tubes were set as in total bilirubin determined in 1.1 above. To the sample blank, 0.2 ml of sulphanilic acid, 2 ml of normal saline acid and 0.2 ml of the sample were added. Then, in each sample 0.2 ml of sulphanilic acid, 1 drop of sodium nitrate, 2 ml of normal saline and 0.2 ml of required sample were added. They were all mixed and allowed to stand for exactly 5 min at 25°C. At the end of this period the absorbance values ($A.V_d$) of the samples were read against sample blank at 546 mn. Then,

$$\text{Direct bilirubin} = 14.4 \times A.V_d\,(546\text{ mn})$$

Estimation of indirect bilirubin: Indirect bilirubin was calculated as a difference between the values of total and direct bilirubin. That is:

$$\text{Indirect bilirubin} = \text{Total bilirubin-Direct bilirubin}$$

Determination of cholesterol: Test tubes were prepared and labeled as blank, standard and sample specimen. 10 ml of distilled water and 1000 ml of working reagent (4-Aminoantipyrine 0.30 mmol/l, phenol 6 mmol/l, peroxidase = 0.5 μ/ml, cholesterol esterase = 0.15 μ/ml, cholesterol oxidase = 0.1 μ/ml and pipe buffer 80 mmol/l all mixed together) were pipetted into the test tube labeled blank. 10 ml of standard solution and 1000 ml of the working reagent were added to the test tube labeled standard. Again, 1000 ml of the working reagent and 10 ml of the required samples were pipetted into the appropriate test tubes. Each tube was thoroughly mixed and incubated for 5 min at 37°C. The absorbencies of the samples were measured against the blank. From this, the cholesterol value was determined from the equation:

$$\text{Cholesterol conc.} = \frac{\Delta\ \text{Sample x conc. of standard}}{\Delta\ \text{Standard}}$$

Determination of glucose: Tubes were prepared and labeled as blank, standard and sample specimen. Then, 1.5 ml of working reagent (glucose oxidase 15 μ/ml, peroxidase 1.2 μ/ml, mutarotase 40 μ/ml, 4-aminoantipyrime 0.38 mM and phydrobenzensulfonate 10 mM) was added to all the tubes. They were incubated in water bath at 37°C for 5 min. Thereafter, 0.01 ml of the sample was pipetted to the respective tubes, thoroughly mixed and incubated at 37°C for exactly 10 min. Colorimeter was zeroed with the reagent blank and the absorbencies of the sample tubes were read and recorded at 540 mn. Glucose concentration was determined from the equation given below:

$$\text{Glucose conc.} = \frac{\text{Abs x conc. of standard}}{\text{Ab standard}}$$

Data analysis: All the data collected were subjected to Analysis of Variance (ANOVA) appropriate for a 2 x 3 factorial in a CRD to test the effects of breed, sex and their interactions on the measured parameter. However, percentage values were transformed into Arcsine angles prior to analysis. The differences between means were separated using Duncan's New Multiple Range Test (DNMRT). SPSS (2004) Statistical Package was used for data analysis.

RESULTS

The breed effects on the blood bilirubin, glucose and cholesterol levels of the Nigerian Goats studied are given in Table 1.

Total bilirubin were similar ($p>0.05$) in all the Nigerian goat breeds. However, the SG recorded high direct bilirubin value but a low indirect bilirubin level. The reverse is true for the RSG, that is, the RSG scored high indirect bilirubin and least ($p<0.05$) direct bilirubin. The WADG on the other hand contained almost equal levels of direct bilirubin and indirect bilirubin. The level of glucose was significantly higher ($p<0.05$) in the RSG and SG than in the WADG. Significantly low ($p<0.05$) level of cholesterol was recorded in the SG compared to the levels in other breeds.

Table 2 presents sex effect on the blood bilirubin fractions, glucose and cholesterol levels of the Nigerian goat breeds. The total bilirubin fraction did not differ ($p>0.05$) between the male and female goats. The buck recorded higher levels of indirect bilirubin than did the doe, while the reverse was the case for direct bilirubin ($p<0.05$). The glucose level is significantly higher ($p<0.05$) in the doe than in the buck while cholesterol is higher in the buck than in the doe.

The mean levels of total bilirubin, direct bilirubin, indirect bilirubin, glucose and cholesterol as affected by sex x breed interaction are in Table 3. Except for the RSG, the total bilirubin is significantly the same ($p>0.05$) in the males and females goats studied. However, males recorded consistently higher numerical values in indirect (conjugated) bilirubin than their females' counterparts in all the goat breeds, but were significant ($p<0.05$) only in the RSG breed. Similarly, the Does persistently outscored the Bucks' counterparts in direct (unconjugated) bilirubin, but were significant ($p<0.05$) in the RSG and WADG breeds. There was no significant sex x breed interaction effects on the glucose level of the goats no matter the breed. It is only in the RSG group that the Bucks gave higher values of cholesterol ($p<0.05$) than did the Does. No other sex x breed interaction effect was exhibited on both glucose and cholesterol fractions of the goats, breeds and gender not withstanding.

Table 1: The mean levels of bilirubin, glucose and cholesterol in SG, RSG and WADG breeds

Parameters	SG	RSG	WADG
Total bilirubin (mg/dl)	0.63±0.05	0.61±0.17	0.65±0.15
Direct bilirubin (mg/dl)	0.37±0.04^{b}	0.28±0.11^{a}	0.34±0.07^{b}
Indirect bilirubin (mg/dl)	0.26±0.04^{a}	0.33±0.14^{b}	0.31±0.11^{b}
Glucose (g/litre)	72.63±1.74^{b}	73.78±3.36^{b}	66.78±3.89^{a}
Cholesterol (g/litre)	53.86±1.92^{a}	68.41±3.87^{b}	77.21±2.7^{b}

^{a,b}Means bearing different superscripts in the same row are significantly different ($p<0.05$). Where; SG = Sahel Goat, RSG = Red Sokoto Goat and WADG = West African Dwarf Goat

Table 2: The effect of sex on the total bilirubin, direct and indirect bilirubin, glucose and cholesterol levels across breed and source

	Sex	
Index	Male	Female
Total bilirubin (mg/dl)	0.64±0.03	0.62±0.04
Direct bilirubin (mg/dl)	0.29±0.08^{a}	0.35±0.09^{b}
Indirect bilirubin (mg/dl)	0.35±0.12^{b}	0.27±0.09^{a}
Glucose (g/litre)	67.92±3.35^{a}	72.64±3.35^{b}
Cholesterol (g/litre)	71.90±3.72^{b}	63.79±3.59^{a}

^{a,b}Means bearing different superscripts in the same row are significantly different ($p<0.05$)

The mean values of total bilirubin, direct and indirect bilirubin, glucose and cholesterol levels of the experimental breeds of goat obtained from different sources for each breed are presented in Table 4. The Sahel goats have statistically the same levels ($p>0.05$) of unconjugated bilirubin, glucose and cholesterol, irrespective of source of the breed. Except for the Sahelian goat ecotype from Gumel, the same is true for total and conjugated bilirubin.

The RSG derived from Sokoto were statistically low in total bilirubin, direct and indirect bilirubin, and cholesterol level compared to ecotypes from Katsina and Gusau. RSG from Gusau were remarkably high in cholesterol, indirect bilirubin and total bilirubin, but low ($p<0.05$) in glucose level.

The WADG ecotype from Akure statistically gave lower ($p<0.05$) blood concentrations of total bilirubin, direct bilirubin and cholesterol, while they are comparable to the ecotype from Ughelli in glucose and indirect bilirubin levels. WADGS from Umuahia were exceptionally high ($p<0.05$) in cholesterol, total bilirubin and indirect bilirubin. However, they were statistically lower ($p<0.05$) in glucose level when compared to the ecotypes from Ughelli and Akure. The WAD goats from Ughelli were remarkable high ($p<0.01$) in conjugated bilirubin. It appears that the higher the glucose level, the lower the cholesterol concentration and vice versa.

DISCUSSION

The concentrations of cholesterol obtained for the SGs are numerically lower than the range of 70-116 mg/100 ml documented by researchers (Jovanovic *et al*., 1989; Macdonald and Low, 1995; Ritter, 1996; Scott, 1999; Zubcic, 2001; Aikhuomobhogbe and Orheruata, 2006), but those of the RSGs and WADGs fall within the range.

Table 3: Effect of sex x breed interaction on bilirubin, glucose and cholesterol in bucks and does

Breed	Sex	Total bilirubin Mg/dl	Direct bilirubin Mg/dl	Indirect bilirubin Mg/dl	Glucose g/litre	Cholesterol g/litre
SG	Male	0.61±0.05[a]	0.35±0.03[a]	0.26±0.03[a]	70.38±0.98	58.00±1.05[a]
	Female	0.64±0.06[a]	0.38±0.03[a]	0.26±0.04[a]	73.51±3.35	51.78±2.63[a]
RSG	Male	0.70±0.11[b]	0.23±0.06[a]	0.47±0.10[b]	68.42±14.63	76.29±15.52[b]
	Female	0.57±0.10[a]	0.30±0.12[b]	0.27±0.10[a]	76.46±7.95	64.47±13.04[a]
WADG	Male	0.62±0.06[a]	0.30±0.07[a]	0.32±0.07[a]	64.44±11.92	81.41±5.26[a]
	Female	0.66±0.06[a]	0.37±0.07[b]	0.29±0.12[a]	67.94±16.53	75.11±7.24[a]

[a,b]Means bearing different superscripts in the same column are significantly different ($p<0.05$). Where; SG = Sahel Goat, RSG = Red Sokoto Goat and WADG = West African Dwarf Goat

Table 4: The mean values of the total bilirubin, direct and indirect bilirubin and glucose and cholesterol levels according to source for each breed

Breed	Location of origin	Total bilirubin Mg/dl	Direct bilirubin Mg/dl	Indirect bilirubin Mg/dl	Glucose level g/litre	Cholesterol g/litre
SG	Mad	0.65±0.06[b]	0.39±0.02[b]	0.26±0.02[a]	70.22±1.08[a]	53.00±4.29[a]
	Pot	0.65±0.01[b]	0.39±0.02[b]	0.26±0.02[a]	73.14±2.77[a]	54.61±3.64[a]
	Gum	0.58±0.01[a]	0.33±0.05[a]	0.25±0.05[a]	74.52±3.09[a]	53.96±2.77[a]
RSG	Sok	0.41±0.11[a]	0.17±0.02[a]	0.24±0.02[a]	75.37±7.44[b]	55.90±5.03[a]
	Kat	0.69±0.03[b]	0.40±0.10[c]	0.30±0.10[b]	77.19±9.04[b]	63.60±8.81[b]
	Gus	0.75±0.01[c]	0.29±0.01[b]	0.46±0.01[c]	68.79±14.37[a]	85.73±9.57[c]
WADG	Umu	0.79±0.13[c]	0.34±0.07[b]	0.45±0.07[b]	50.11±13.09[a]	84.49±2.47[c]
	Ugh	0.62±0.09[b]	0.40±0.06[c]	0.22±0.06[a]	77.00±4.19[b]	76.63±6.16[b]
	Aku	0.54±0.03[a]	0.28±09.04[a]	0.26±0.04[a]	73.22±8.51[b]	70.51±4.15[a]

[a,b,c]Means bearing different superscripts in the same column within the same breed are significantly different ($p<0.05$). Where; SG = Sahel Goat, RSG = Red Sokoto Goat and WADG = West African Dwarf Goat, Mad-Maiduguri, Pot-Potiskum, Gum-Gumel, Sok-Sokoto, Kat-Katsina, Gus-Gusau, Umu-Umuahia, Ugh-Ughelli and Aku-Akure

The low cholesterol concentration observed in the SGs indicates poor cell membrane and vitamin utilization, which might be responsible for the poor meat yield and quality often reported in the breed. In addition, the roughness and lightness of their skins could be attributed to the extreme low level of cholesterol in the breed. Higher levels of cholesterol recorded by bucks compared to the does could be physiological. The sahelian goats recorded similar values in cholesterol concentrations, irrespective of source. It could be that the sahelian goat breed is more homogenous compared to other breeds and extreme high levels of cholesterol recorded by the RSG goats from Gusau and WAD goats from Umuahia might be responsible for neonatal deaths often reported by farmers in those areas. However, there is lack of literature reports to buttress these facts.

The concentrations of glucose recorded for the Nigerian goats are within the literature values. Scott (1999) reported the range of 60-100 mg/100 ml; Jovanovic *et al.* (1989) gave the range of 68-82 mg/100 ml, while Grabkowski and Rutkowiak (1989) documented the range of 60-78 mg/100 ml for different breeds of goat. Blood glucose level is of great value in determining the efficiency of sugar absorption in the body. The level of glucose in the WADG is lower than the referral ranges, and indicates high glucagon and low insulin concentrations in the blood. Schalm *et al.* (1975), Harper *et al.* (1977), Kaneko *et al.* (1997), Mohammed *et al.* (1991) and Zapata *et al.* (2003) reported that very low level of glucose in the blood will lead to reductions in weight gain and milk yield and to a change in the fatty acid composition of the milk. Consequently, the low weight gain and poor milk yield in the WADGs compared to other breeds might be as a result of low level of glucose in their blood. The males have lower glucose levels than the females, which could be due to catabolism as the bucks frequently engage in exercise. The exceptional low level of glucose recorded by the WAD goats from Umuahia (South-East) accounts for the miniature size of the goats from the area. The study further revealed that the higher the blood glucose, the lower the cholesterol concentration in the blood and vice-verse.

The total serum bilirubin is similar in the three breeds of goats studied and lies within the range of 0.00-0.90 mg/100 ml reported by notable researchers for goats and other classes of livestock (Harper *et al.*, 1977; Frandson, 1981; Singh, 2004). However, the SG breed has the highest serum concentration of unconjugated (direct) bilirubin, followed by the WADG and the least being the RSG. This implies that the Sahelian goats have the greatest inherent tendency to suffer from liver disease and or more of blocked blood vessels. Consequently, the SG breed kids are prune to neonatal jaundice. Significantly higher levels of unconjugated bilirubin recorded by does in this study could be as a result of the fact that bucks engage in more exercise than the does and as such, the conjugated bilirubins are broken down, though there is scarcity of information to buttress these findings.

Conclusion: The cholesterol level in Nigerian goats is inversely proportional to the glucose concentration. WADG should be the meat of choice for people suffering from diabetes or family with chronological records of diabetes, while the Sahelian chevron is preferable for individuals with high levels of cholesterol (cholesterol arum). Nigerian goat breeds have similar concentrations of total bilirubin, but sex plays key role in the fraction of the conjugated (direct) and unconjugated (indirect) bilirubin. The Sahelian goats are more homogeneous, irrespective of source. Again, poor weight gain and milk yield often reported in WADGs are as a result of low level of glucose in their blood, and miniature size of the WADG ecotype from South-East is a function of their blood glucose.

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Preliminary Survey on Nutritional Status among University Students at Malaysia

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Abstract: The objective of this survey was to measure the Nutritional status through Body Mass Index (BMI) profile among Universiti Sains Malaysia (USM) main campus students. The data was randomly collected from students around USM lecture theaters during day time. A number of 624 students (male 264 and female 360) were involved in the survey. The result showed that the mean age, weight and height among the students were 21.42±1.38 years, 55.65±12.21 kg and 163.43±8.89 cm, respectively. The mean BMI of all the samples was 20.81±3.61 kg/m^2, with male students showing slightly higher BMI (21.84±4.13 kg/m^2) compared to female students (20.05±2.96 kg/m^2). Malay students showed the highest BMI, followed by Indian and Chinese students. The BMI's for the Malay, Indian and Chinese students were 21.01±3.67 kg/m^2, 20.80±3.72 kg/m^2 and 20.43±3.19 kg/m^2, respectively. Based on the BMI result, about 61% of all the samples were in the normal range, 27% were underweight and about 12% were overweight or more. A higher percentage of the female students were in the underweight category (33%) compared to the male students (20%). Based on race, about 63% of the Malay students were normal weight, 25% were underweight and 12% in the overweight or more category. For the Chinese students, about 60% were normal weight, 30% were underweight and 10% in were in the overweight or more category. For the Indian students, about 57% were normal weight, 28% were underweight and 15% were in the overweight or more category. This preliminary data showed that there is a high percentage of underweight students among USM students. Further assessments need to be carried out to survey if these underweight students show symptoms of clinical problems related to nutritional deficiencies.

Key words: Nutritional status, BMI, anthropometry, university students

INTRODUCTION

Evaluation of the nutritional status of individuals and population groups is a tool of vital importance in public health and a feasible indicator of standards of living. There are many measures to assess the nutritional status of a population. Body Mass Index (BMI) is one of them. Assessment of nutritional status of individuals and population has attracted the attention of not only nutritionist and other biological scientist, but also economists and other social scientists with a view to understanding the health and socioeconomic status of population (Herrera *et al*., 2003). BMI is expressed as the ratio of weight to height squared and can be a good parameter to grade Chronic Deficiency (CED) in adults. However, BMI should be used cautiously when classifying fatness or body composition. Using BMI for measuring amount of fat in a person's body may not be as accurate as originally thought (Pivarnik *et al*., 2007).

Universiti Sains Malaysia (USM) is one of the competitive universities in Malaysia. Ballard (2006) reported competitive universities foster eating disorder, especially among female students. Female university student typically desire to lose weight and are more likely than male students to diet or try other weight-loss practices. Report on BMI status among university students in Malaysia is limited. Hence the objective of this survey is to measure the BMI profile among USM main campus students in Minden, Penang.

MATERIALS AND METHODS

The survey sample is based on basic anthropometry data (height and weight) among USM main campus students aged 18-26 years old. USM is one of the competitive Universities in Malaysia located at the Northern part of Malaysia. The data was collected randomly among students around USM lecture hall during lecture hours. A number of 624 students (male 264 and female 360) from three main race at Malaysia (Malay, Chinese and India) some number of International students were involved in the survey. The weight of the students without shoes were taken using a bathroom scale, while heights were measured using a commercial tape. The BMI was computed by dividing the weight (kg) by the square of the height (m). BMI (kg/m^2) of the students was classified as follows: underweight (BMI < 18.5); normal (BMI 18.5-24.9); overweight (BMI 25.0-29.9) and obese (BMI > 30.0) (WHO, 1990).

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Table 1: BMI profile among female and male USM students

	Female	Male	Overall
n	300	264	624
Age (years)	21.32±2.52	21.58±2.45	21.42±2.52
Weight (kg)	50.33±8.35	62.82±13.12	55.65±12.21
Height (cm)	158.41±5.99	169.74±8.29	163.43±8.98
BMI (kg/m^2)	20.05±2.96	21.84±4.13	20.81±3.61
Underweight (%)	32.78	20.07	27.40
Normal (%)	60.56	62.12	61.22
Overweight (%)	6.11	14.39	9.61
Obese (%)	0.56	3.41	1.77

Table 2: BMI profile among USM students sample according to race

	Chinese	Indian	Malay	Other (International students)
n	204	93	319	8
Age (years)	21.48±1.93	21.60±1.74	21.31±3.04	22.37±1.59
Weight (kg)	55.56±10.21	58.39±13.94	54.93±12.70	55.50±8.67
Height (cm)	164.70±7.89	166.96±9.84	161.07±8.98	166.25±5.92
BMI kg/m^2	20.43±3.19	20.80±3.72	21.01±3.67	20.00±2.19
Underweight (%)	29.81	27.96	25.33	25.00
Normal (%)	60.38	56.99	63.38	75.00
Overweight (%)	9.31	15.05	8.15	0.00
Obese (%)	0.49	0.00	3.13	0.00

RESULTS AND DISCUSSION

The overall mean age, weight, height and BMI of the students' samples were 21.42±2.52 years, 55.65±12.21 kg, 1.63±0.08 m and 20.81±3.61 kg/m^2, respectively. The mean BMI of male students were slightly higher (21.84±4.13 kg/m^2) compared to female students (20.05±2.96 kg/m^2) (Table 1). The mean BMI of USM students were slightly lower than the mean BMI among Universiti Putra Malaysia (UPM) students which were about 22.17±3.41 kg/m^2 (Quah and Zaitun, 2005). UPM is another Malaysian competitive University located at the central part of Malaysia. Higher BMI of male students compared to female students was also reported among Venezuelan university students (Herrera *et al*., 2003) and among groups of college students at a large Midwestern University, US (Davy *et al*., 2006). According to the BMI classification, most of the USM students were in the normal category (61.22%), followed by about 27% in the underweight category and about 12% in the overweight or more category. Davy *et al*. (2006) also reported that the number of underweight students were higher for females compared to males. Ming *et al*. (2005) reported that more female students than male students in a public university in Kuala Lumpur skipped lunch and dinner. It is commonly known that young women including female students typically desire to lose weight and young men typically want to gain weight. Women are more likely than male to diet or try other weight-loss practices. If men do attempt weight loss, they typically try exercise rather than dieting.

Table 2 shows the mean age, weight, height and BMI of the students' samples according to race. Malaysia is a unique country where multiple ethnic groups (race) exist in this country. The Malays race and other Bumiputera groups make up 65% of the population, Chinese race around 26%, Indians race around 8% and other unlisted race around 1%. The BMI of Malay students (21.01±3.67 kg/m^2) was slightly higher than Indian, Chinese and others (international students, mainly Indonesian) which was 21.01±3.67, 20.80±3.72, 20.43±3.19 and 20.00±2.19 kg/m^2, respectively. Higher BMI among Malay students compared to Chinese students was also reported in UPM (Yaw *et al*., 2005). They found that the mean BMI for female Malay students was 21.33±3.28 kg/m^2 and for female Chinese students was 20.30±2.37 kg/m^2.

According to the BMI classification, 63% of Malay students fall into the normal category, followed by 25% in the underweight category and 12% in the overweight or more categories. For the Chinese students, about 60% were in the normal category, followed by 30% in the underweight category and 10% in the overweight or more categories. For the Indian students, about 56% were in the normal category, followed by 28% in the underweight category and 13% in the overweight or more categories. The number of underweight students among all races in Malaysia is higher than Venezuelan students which was only about 11% (Herrera *et al*., 2003). This percentage is also higher than the number of underweight young adults in United Stated which was only about 7% (Davy *et al*., 2006).

Conclusion: This preliminary data showed that the prevalence of underweight among USM students was alarmingly high and this problem necessitates further nutritional status survey to determine if these underweight groups were at risk of nutrient deficiencies.

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Assessment of Protein Needs of Nigeria Adult Males Using Short-Term Nitrogen Balance Technique

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Abstract: Protein requirement of Nigerian adult males aged 21-27 years from two different geographical locations in Nigeria was determined as a basis of comparison with previous studies. Eighteen young men from Northern and Southern Nigeria were recruited into the study and fed on a customary Nigerian mixed diet. The subjects were divided into dietary regimen groups of four protein levels (0.4, 0.7, 0.8 and 0.9 g protein/kg/day) at ordinary level of energy intake of 0.18 MJ/kg/day. The groups were assigned either an ascending or descending sequence of dietary changes. From regression analysis, the Nitrogen requirement for Nitrogen equilibrium was estimated to be 108.0±9.45 mg N/kg/day (0.68 g protein/kg/day) and 110.8±14.53 mg N/kg/day (0.69 g protein/kg/day) for the Northern and Southern groups respectively. Estimates for allowances to cover 97.5% of the population were 126.9 mg N/kg/day (0.79 g protein/kg/day) and 139.9 mg N/kg/day (0.87 g protein/kg/day) for the groups respectively. The mean Net Protein Utilization (NPU) for the diet was 64±1.29 with a true digestibility of 95.1±1.82. The requirement compared favorably and difference was not significant from previous studies. Thus, there is no need to set different requirements for any part of the country.

Key words: Protein requirement, N-equilibrium, Nigerian mixed diet

INTRODUCTION

The requirements of individuals and populations for protein and energy have been discussed extensively by experts and committees over the past few decades (Yarez *et al*., 1982; Morse *et al*., 2001; Elango *et al*., 2009). Although a rigorous definition for either protein or energy needs has not been unanimously agreed upon, various operational definitions have been formulated, and these have varied with time.

The current agreement indicates that the Recommended Dietary Allowance (RDA) for protein of 0.8 g of protein/kg/day safely and adequately meets the dietary needs of virtually all healthy people at or older than the age of 19 years (Council, 1989). There is also a general consensus that the protein requirement and suggested safe and adequate protein intake (i.e., protein allowance) of adults should be established primarily from shorter-term (2- to 3-week) nitrogen balance studies (Rand *et al*., 1977).

These recommendations are based on a meta-analysis of nitrogen balance studies (Rand *et al*., 2003), in which protein requirements are estimated by fitting a linear regression analysis model to the data and measuring zero nitrogen balance as the criterion of nutritional adequacy. However, the physiological response relationship between nitrogen intake and balance is not linear due to a decreased efficiency of protein utilization as zero balance is approached (Young *et al*., 1973; Rand and Young, 1999).

Conclusions from a very limited number of shorter-term nitrogen balance studies are conflicting; some support (Cheng *et al*., 1978; Zanni *et al*., 1979) and some question (Gersovitz *et al*., 1982; Bunker *et al*., 1987) the adequacy of 0.8 g of protein/kg/day for elderly people. Support for the conclusion that the RDA for protein may not adequately meet the dietary needs of many older people is found in a retrospective reanalysis of these shorter-term nitrogen balance data, based on calculations recommended by the 1985 Joint Food and Agriculture Organization, World Health Organization, and the United Nations University Expert Consultation (Council, 1989).

The question is 'if this estimate will be adequate for all young adult males in Nigeria, irrespective of population groups of different ethnic backgrounds and environments'. It is a known fact that variation in protein requirements is the result of both growth potential and the modifying effect of the environment on the expression of such potential. It is therefore necessary to know the appropriate estimates for protein and energy requirement among different population groups in the country with different geographical environment, cultural background and consuming diets in physical characteristics. Also the capacity of the diets to meet

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protein requirements necessary to maintain adequate protein nutritional status under their prevailing environmental conditions should be determined.

This present study is aimed at determining the protein requirements of young adult Nigerian males living in different parts of the country with different geographical locations, dietary habits and culture.

MATERIALS AND METHODS

Subjects: Eighteen young men aged 21-27 years were recruited into the study. Seven of them were students of University of Maiduguri, Maiduguri (Northern Nigeria) designated M and the other eleven were from the University of Nigeria, Nsukka (South Eastern Nigeria) designated N. Characteristics of these are shown in Table 1.

The subjects were studied in their various campuses and subjected to their respective environmental conditions. Medical history, physical examination, as well as biochemical analysis of blood were obtained from each subject. Subjects certified as healthy were allowed to participate in the study. They maintained normal activity which included their full academic schedule and were free living but without hard physical exercise. Each student maintained a diary of all his activities through the study period.

To determine the energy intake needed to maintain the body weight under ordinary conditions, subjects were questioned in detail about their dietary intakes and physical activities prior to the Nitrogen balance studies. Each morning the subjects were weighed under the standardized conditions of preprandial, post-voiding and light indoor clothing.

Diet and experimental design: Food ingredients of the ordinary Nigerian mixed diets were carefully selected based on a combination of root tubers, cereals, vegetables and animal products which are common to the two groups studied, but cooked to the specification unique to respective areas. The basic ingredients and nutrient composition of the diet is shown in Table 2.

The average energy intake of all subjects was 0.18 MJ/kg/day. Complete mineral and vitamin supplements were added to prevent any deficiencies.

Subjects in each zone were divided into two groups; one group was assigned an ascending sequence of dietary changes while the other group has a descending sequence of dietary changes. Each dietary level was proceeded by one day on a practically protein free diet of less than 0.1 g protein/kg/day (Atinmo *et al*., 1985) to enhance adaptation, followed by 10 days on the test protein level. A break of three days between the different protein levels was allowed. Meals were consumed in patterns the subjects were accustomed to, i.e. 7-8 am, 1-2 pm and 7-8 pm under supervision.

Samples and analysis: Urine specimens were collected everyday on a 24 h basis in 2 litre plastic bottles containing 10 ml of 1N HCl. After measuring, an aliquot of each urine sample was frozen for analysis of total nitrogen. Faecal samples were collected with aid of a feal marker (carmine) as 5 day pools in pre-weighed plastic containers. Collections were held frozen prior to homogenization with a blender. The nitrogen content of the diets, urine and feaces were determined by the modified microkjedahl method of Munro and Fleck (1969).

For the last 5 days of each experimental period, N-balance was calculated from intake, fecal, urine and miscellaneous losses. Skin N loss was taken as 10.14 mg N/kg/day (Atinmo *et al*., 1988a).

Estimates of individual N requirements were by linear regression equation relating N intake to balance. The protein intake at which N balance was equilibrium was estimated as the requirement (Rand *et al*., 1977). Estimates of allowances for individual variations to cover the 97.5% population were taken as (+2 SD) of the mean (Rand *et al*., 1977).

The Biological Value (BV), Net Protein Utilization (NPU) and the true and apparent digestibilities for the mixed diet at different levels of N-intake in the young men were calculated by the conventional procedures of UNU (1979). Obligatory urinary, fecal and integumental Nitrogen losses were assumed to be 43.45, 18.32 and 7.46 mg.

Two way analysis of variance was used to test the level of significance between treatments, taking individual subjects as blocks. The student t-test was used to compare results between population groups.

RESULTS

Subjects: Changes in body weights and mid arm circumference of the subjects are presented in Table 1. The changes were not significantly ($p<0.05$) affected throughout the study period, among and within the two groups studied.

Nitrogen balance and requirements: Details of Nitrogen balance data are summarized in Table 3. An increase in urinary nitrogen was observed with increase in protein intake in both groups studied. All groups showed negative N-balance at 0.4 g protein/kg/day intake. More subjects reached N-balance with increase in dietary protein.

The regression equation obtained from individual data of the subjects who participated in the study is shown in Table 4. The protein requirements obtained for the Nigerian mixed diet for Maiduguri and Nsukka groups were 108.01±9.45 and 110.82±14.53 mg N/kg/day or 0.675 and 0.693g protein/kg/day respectively. The overall mean was calculated at 109.725±2.56 mg N/kg/day (0.686 g protein/kg/day) with an allowance of 0.843 g protein/kg/day.

Table 1: Characteristics of the young adult Nigerian male studied

Subject	Age (Years)	Weight (kg) Initial	Weight (kg) Final	Height (cm)	BMR* (MJ/day)	Mid arm circumference Initial	Mid arm circumference Final
Maiduguri							
M1	23	61.0	61.3	170.1	6.74	28.4	28.4
M2	26	58.0	58.6	171.0	6.55	29.0	29.0
M3	24	59.5	60.4	173.5	6.64	28.6	28.7
M4	23	52.3	52.3	170.6	6.18	24.7	24.5
M5	23	52.4	53.4	170.6	6.19	25.5	25.0
M6	22	49.7	49.6	166.3	6.02	26.1	25.4
M7	21	54.4	54.9	163.3	6.32	27.0	28.0
Nsukka							
N1	23	60.0	60.3	171.1	6.68	28.4	29.5
N2	22	56.3	54.9	167.6	6.44	26.0	26.5
N3	22	59.7	57.4	179.6	6.66	27.0	26.0
N4	25	68.3	68.3	170.6	7.21	29.6	30.0
N5	24	72.6	72.5	177.8	7.48	28.1	28.5
N6	23	59.4	59.4	168.5	6.64	27.1	27.0
N7	23	54.5	54.7	151.6	6.32	28.2	28.5
N8	27	61.9	61.6	171.6	6.80	28.0	28.0
N9	23	62.6	62.6	172.4	6.84	29.4	30.0
N10	24	58.2	57.8	162.1	6.56	27.8	26.5
N11	22	58.0	57.9	165.0	6.55	27.5	27.5
Mean	23.3	58.82	58.77+	169.37	6.60	27.58	27.61+
SD	1.50	5.55	5.52	6.02	0.36	1.35	1.61

*BMR is the Basal Metabolic Rate calculated from the equation BMR = 0.064 + 2.84 (FAO/UNU, 1985) where W = Weight; +No significant difference between final and initial values

Table 2: Ingredients and nutrient composition of the Nigerian mixed diet used in the study

Ingredients	Levels of protein intake (g/day) 0.4	0.7	0.8	0.9	Protein free
Custard flour	-	-	-	-	100
Corn meal	170	-	-	-	-
Bean cake	130	-	-	-	-
Refined sugar	25	100	100	50	20
Granted cassava	500	560	560	560	560
Onion	20	20	20	20	20
Pepper	2	2	2	2	2
Tomatoes	20	20	20	20	20
Red Palm oil	40	40	40	40	40
Okra	28	28	28	28	28
Boiled egg	-	56	56	56	-
Bread	-	100	100	125	-
Butter	-	28	28	28	-
Tea	Nil	Milk	-	-	-
Meat	-	40	60	80	-
Yam	-	400	400	400	-
Caloreen (glucose polymer)	100	-	-	-	200
Orange drink (0.5 MJ/bottle)	Varied among individuals				
Vitamin and Mineral supplements*				1 cap	
Nutrients					
Protein (g)	25.79	39.0	45.0	53.5	4.9
Energy (MJoule)	10.74	10.94	10.96	10.96	-

*The amount is for an average 59 kg subject. Vitamin and mineral was in the form of a capsule prepared by Ricker Laboratories. Langhborogh, England, UK

Protein utilization and digestibility: The Biological Value (BV), Net Protein Utilization (NPU) and true and apparent digestibilities for the Maiduguri and Nsukka groups are presented in Table 5. There were no significant differences ($p<0.005$) in the BV, NPU and true digestibility at different levels of protein intake in the same set of group. Also the differences observed in the mean parameter of 67.28 versus 67.23 for BV and 64.54

Table 3: Daily nitrogen data with diet (Mg N/kg/day)

Nitrogen intake	Urinary nitrogen	Feacal nitrogen	Total nitrogen loss*	Nitrogen balance
Maidugri!				
74.85±5.71	63.55±5.81	21.96±2.29	95.90±6.74	-21.04±7.07
112.84±9.14	73.83±7.44	24.16±3.54	108.39±6.69	2.85±8.54
130.03±9.66	82.14±7.44	22.80±1.99	115.13±8.94	14.90±10.22
156.23±12.17	92.45±7.83^{ff}	22.80±2.07^{ee}	125.66±7.56	30.57±14.02^{ff}
Nsukka§				
63.92±5.39	57.87±11.04	23.98±1.95	92.25±12.05	-28.33±11.02
92.71±7.90	70.13±12.13	23.09±3.22	104.36±11.81	-11.64±9.86
116.79±9.78	75.55±13.31	23.79±2.60	109.76±12.31	7.04±11.34
147.69±12.07	90.73±14.15^{ff}	24.36±1.94^{ee}	125.50±12.41	22.20±13.01^{ff}

*Total nitrogen loss include 10.41 mg N/kg/day for sweat Nitrogen (ref. 9). ! = Values are mean±SD (n = 7); § = Values are mean±SD (n = 11); ff = Significant difference from other levels of protein intake ($p<0.05$); ee = No significant difference from other levels of protein intake ($p<0.05$)

Table 4: Linear regression equations relating N-balance to N-intake for subjects

Subject	Regression equation*	Maintenance requirement	r	p
Maiduguri				
M1	Y = 0.527x-59.53	112.86	0.990	<0.05
M2	Y = 0.758x-79.02	104.24	0.992	<0.05
M3	Y = 0.553x-62.23	112.58	0.998	<0.05
M4	Y = 0.604x-71.61	118.63	0.989	<0.05
M5	Y = 0.677x-61.09	90.19	0.996	<0.05
M6	Y = 0.763x-86.61	113.56	0.995	<0.05
M7	Y = 0.563x-55.77	103.98	0.999	<0.05
Mean		108.01		
SD		9.45		
Nsukka				
N1	Y = 0.657x-85.48	130.04	0.991	<0.05
N2	Y = 0.740x-69.73	94.25	0.994	<0.05
N3	Y = 0.533x-68.48	128.61	0.986	<0.05
N4	Y = 0.322x-32.19	99.94	0.962	<0.05
N5	Y = 0.734x-72.41	98.68	0.938	<0.05
N6	Y = 0.946x-99.90	105.66	0.977	<0.05
N7	Y = 0.959x-70.00	106.28	0.976	<0.05
N8	Y = 0.586x-59.41	101.37	0.991	<0.05
N9	Y = 0.546x-53.65	98.19	0.949	<0.05
N10	Y = 0.563x-71.89	127.74	0.986	<0.05
N11	Y = 0.480x-61.56	128.23	0.975	<0.05
Mean		110.82		
SD		14.53		
Overall mean		109.73		
SD		12.56		

*Y = N-balance (mg/kg/day). x = N intake (mg/kg/day)

versus 63.39 for NPU of Maiduguri and Nsukka group respectively were not significant. The same applied to the 95.94 versus 94.32 for true digestibility.

Thus the overall values for the diet protein were 67.25±1.83 for BV and 63.96±1.29 for NPU. True digestibility was quite high at 95.13±1.82.

Values of apparent digestibility were somewhat lower at low protein intakes than at higher protein intakes ($p<0.05$) (Table 5) in both groups, although true digestibility did not differ.

DISCUSSION

Nicol and Phillips (1976) suggested that it may not be possible to establish a single safe level of protein intake based on body weight and assuming an adequate energy intake, which would apply to all men of different ethnic, socio-economic and nutritional background.

Thus the requirements in terms of protein for man are estimated to depend on a large extent on his ecological as well as socio-economic background. The customary diet plays an important role in terms of composition. Thus, our interest to investigate the extent of influence of ecological factors and customary diets on protein requirements in this particular study.

The experimental design was basically the recommendation of the United Nations University workshop (UNU Hunger program, 1979). Our subjects were from different geographical locations in the country but actually had a similar pattern as they were all resident university students. Thus, forming a good basis

Table 5: Biological Value (BV), Net Protein Utilization (NPU) and true and apparent digestibilities of the mixed Nigerian diet at different levels of protein intake

Level of protein intake (g/kg/day)	BV	NPU	Digestibilities	
			Apparent	True
Maiduguri*				
0.4	67.65±7.43^{ee}	64.39±8.04^{ee}	0.50±3.93^{ee}	95.10±3.19^{ee}
0.7	68.96±5.28^{ee}	65.34±4.88^{ee}	78.49±3.60^{ee}	94.81±3.28^{ee}
0.8	66.83±6.02^{ee}	64.70±6.09^{ee}	82.63±0.68^{ee}	96.79±1.29^{ee}
0.9	65.62±5.36ff^{ee}	63.71±5.45ff^{ee}	85.28±2.22§^{ee}	97.07±1.50ff^{ee}
Nsukka!				
0.4	69.54±19.72^{ee}	63.92±19.04^{ee}	62.32±3.68^{ee}	91.19±2.89^{ee}
0.7	65.99±11.99^{ee}	62.58±11.49^{ee}	74.95±3.88^{ee}	94.84±3.43^{ee}
0.8	68.92±10.60^{ee}	65.51±9.48^{ee}	79.40±3.51^{ee}	95.20±2.50^{ee}
0.9	64.46±8.42ff^{ee}	61.84±7.46ff^{ee}	83.32±2.36§^{ee}	96.07±1.47ff^{ee}

*Values are mean±SD (n = 7). ! = Values are mean±SD (n = 11); § = Significant difference from other levels of protein intake ($p<0.05$); ff = No significant difference from other levels of protein intake ($p>0.05$); ee = No significant difference between groups at same level ($p>0.05$)

of comparison with our previous studies at the University of Ibadan, Ibadan located in the south western part of Nigeria (Atinmo *et al*., 1985;1988a,b).

Though it is extremely difficult to know if changes in the body weight as well as body composition occurred from day to day, yet maintenance of steady body weight is the accepted criterion for adequacy of energy intake (Rand *et al*., 2003; Elango *et al*., 2009).

Energy intake for weight maintenance varies with the protein intake. Therefore, the non significant changes observed in body weight and mid arm circumference of the subjects suggests that energy intake was adequate.

The nitrogen loss in urine increased linearly with increase in protein intake as previously observed (Atinmo *et al*., 1988b; Calloway and Margen, 1971; Young and Scrimshaw, 1968; Gersovitz *et al*., 1982), while the fecal nitrogen was observed to be essentially the same irrespective of the level of protein intake. Thus, all subjects absorbed more nitrogen with increase in nitrogen intake. Therefore, the proportion of the intake absorbed (apparent digestibility) increased with increase in nitrogen intake.

In this study N-balance was taken up within the region of zero balance from our previous estimates (Atinmo *et al*., 1988b) so as to evaluate critically the efficiency of utilization of the protein of the diet as opposed to over estimation with very low intakes.

Nevertheless, the paradox of a large nitrogen retention in normal subjects which is not reflected in the change in body weight has also posed a problem to this study. The assumption to this is the probability of an overestimation of nitrogen retention which could be attributed to many factors like the underestimation of total N-losses from actual skin and other miscellaneous losses. An estimated value based on similar totally different from the hotter drier Northern parts was used for the study (Atinmo *et al*., 1988b). This value might not be adequate. Moreover, N-balance is dependent on other factors such as energy intake, physical activity, stress of any kind and the habitual intake (Milward *et al*., 1989).

Net Protein Utilization (NPU) remained constant over the entire range of protein intake. The average value over the range was 64. This value was comparable to that in our previous studies (Atinmo *et al*., 1988a;b) and also that of Huang and Lin (1982) on Chinese young men on a mixed diet.

Mean protein requirement of all individual subjects had a rather large coefficient of variation though they were exposed to minimal experimental variations. However the mean value of 108 mg N/kg/day obtained for the Maiduguri group was not significantly different ($p<0.05$) from that of the Nsukka group which was 110.82 mg N/kg/day. The safe level of N-intake taken as the mean requirements (+ 2 SD) which was expected to cover the needs of nearly 97.5% of the population was calculated to be 126.9 and 139.9 mg N/kg/day respectively for the Maiduguri and Nsukka group respectively. The mean requirement was taken as 134.85 mg N/kg/day (0.84 g protein/kg/day).

Conclusion: Though higher in value than our previous estimate, the requirements compare favorably and the difference was not significant. The lower estimate obtained in our previous study can be attributed to a higher energy intake.

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Determination of Cyanide in *Amanitia muscaria* Samples Using Alkaline Picrate Method

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Abstract: Analytical methods were developed for the detection of cyanide in *Amanitia muscaria* samples using the alkaline picrate method. Dried mushroom samples contained in glass bottles were treated with acid solution and maintained at 80°C for 10 min. Hydrogen cyanide vapours librated from the samples reacted with alkaline picrate solution on Whatman filter paper strips to form red-coloured complex on the test strips. The red coloured compound on the test strips were extracted with 50% ethanol solution and the extract absorbance read at 510 nm. A linear relationship was obtained between the ranges of 0-200 µg CN. Cyanide was detected in mushroom samples at the levels of 84-712 µg/10 g sample. Recovery of cyanide from the samples demonstrated that cyanide poisoning could occur in populations due to prolonged intake of toxigenic mushroom samples. Association between cyanide intake from cyanogenic mushroom and the manifestation of severe human diseases were discussed.

Key words: *Amanitia muscaria*, picrate method, cyanide

INTRODUCTION

Mushrooms are a general term applied to the fruiting bodies of saprophytic fungi. Mushrooms have for so many years been used as human food. With the realization of the nutritional value of mushrooms as protein sources, people in several parts of the world now rely on mushroom as an important source of protein. Mushrooms have the potential to convert nutritionally valueless substances into high protein food (Lintzel, 1941; Chang and Hayes, 1978).

Many food sources are highly cyanogenic (Lasch and El-Shawa, 1981; Hall and Rumack, 1986; Akiatonwa *et al*., 1994; Suchard *et al*., 1998). Cyanogenic food sources have caused numerous cases of acute cyanide poisoning. Cyanide is a potent toxin which exerts its toxicity by inhibiting cytochrome oxidase causing a cytotoxic hypoxia. It is toxic to a number of enzyme systems. The main target enzyme is cytochrome C oxidase, the terminal oxidase of the respiratory chain and involves the interaction with ferric ion of cytochrome a_3 (Cummings, 2006).

Amanitia muscaria is a mushroom which occurs singly or in groups and it is found in the forest growing on decaying tress (Alexopoulos, 1962). It is variously classified as edible, as hallucinogenic, as poisonous and as deadly (Tsunoda *et al*., 1989). Mushroom poisoning is caused by the consumption of poisonous mushrooms. Consumption of wild mushroom is wide spread in Africa and the choice of the species to be consumed depends on the tradition. Food poisoning by wild mushrooms including *Amanitia muscaria* occurs very often (Benjamin, 1995; Diaz, 2005). Toxic mushrooms cannot be made nontoxic by cooking, freezing, drying or any other means of processing (Nieminen *et al*., 2006). The only way to avoid food poisoning by toxic mushrooms is to avoid consumption of toxic species. The aim of this work was to develop a rapid and simple spectrophotometric method to screen *Amanitia muscaria* samples collected from different locations in Nsukka for their cyanide levels.

MATERIALS AND METHODS

Sample collection: *Amanitia muscaria* samples were collected from different locations in Nsukka. The mushroom samples were collected into polythene bags and were identified based on the taxonomic descriptions given by Alexopoulos (1962). The samples were dried in an oven at 60°C and ground using a sterile Corona grinder (Medellin, Colombia). The samples were used immediately for cyanide determination.

Determination of cyanide: Alkaline picrate reagent was prepared by a modification of the method described by Williams and Edwards (1980) as follows: Test tubes with 2 ml of 2% KOH and 1 ml of picric acid: Na_2CO_3:H_2O (1:5:200 v/w/v) were prepared. Standard absorbance curves were made with 3 Whatman No 1 papers each with a dimension of 8 x 1 cm. The papers were dipped into the alkaline picrate solution for 15 min. The picrate impregnated papers were removed from the solution and used immediately for cyanide determination. Cyanide solutions containing (50-200 µgKCN/mL) were each prepared in glass bottles. The cyanide was acidified with 20% HCl solution and immediately sealed with 3 picrate impregnated papers. The system was maintained at 80°C in a thermostatic

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water bath (Kotterman, Germany) for 10 min. The bottles were removed from the incubator and kept on the laboratory bench at room temperature (28± 2°C) for 24 h. The red-coloured complex formed on the test strips was eluted with 50% ethanol solution for 30 min and the eluate absorbance was measured at 510 nm using a Spectrumlab 23A spectrophotometer.

Preparation of Samples and cyanide analysis: Whatman number 1 filter papers (8 x 1 cm) were dipped into the alkaline picrate solution and drained free of excess liquid just before use. The filter paper strips were prepared under identical conditions. The samples (10 g per sample) were loaded into glass bottles and acidified with 20% HCl solution. The bottles were sealed with 3 picrate impregnated strips suspended above the acidified samples as the bottles were sealed. The system was maintained at 80°C in a thermostatic water bath (Kotterman, Germany) for 10 min. The bottles were removed from the incubator and left at room temperature (28±2°C) for 24 h. The red-coloured picrate paper strips were removed from the bottles and rinsed in 50% ethanol solution for 30 min and the absorbance of the solution measured at 510 nm using a Spectrumlab 23A spectrophotometer. Cyanide levels of the samples were extrapolated from the standard curve.

RESULTS AND DISCUSSION

Samples of edible mushroom, *Amanita muscaria* were analyzed for their cyanide content using the alkaline picrate method. Data in Table 1 show the cyanide levels of the mushroom samples. The cyanide levels of the samples ranged from 84-712 µg/10 g sample. The formation of red coloured complex by the reactiion of cyanide and alkaline picrate has become a standard agronomic technique used for quantitative estimation of cyanide (Williams and Edwards, 1980). Some procedures used for quantitative estimation of mushroom toxins are elaborate and time consuming and the patient might have recovered by the time the analysis is completed. Therefore development of a simple method for cyanide determination is important for routine analysis of mushroom samples.

A linear relationship was obtained between cyanide concentrations of 0-200 µg HCN equivalents/mL. The values obtained were reproducible and cyanide as low as 1 µg could be detected. The cyanide in the test samples evaporated as HCN with the addition of 20% HCl solution as the sample was maintained at 80°C. Tompsett (1959) reported the volatitization of HCN from cyanogenic biological substances with the addition of mineral acid followed by heating of the sample. The alkaline picrate papers acted as a trapping agent of the liberated HCN (Williams and Edwards, 1980). The HCN liberated slowly changed the colour of the picrate paper strips from orange to red at 28±2°C. The colour was fully developed after 20 h.

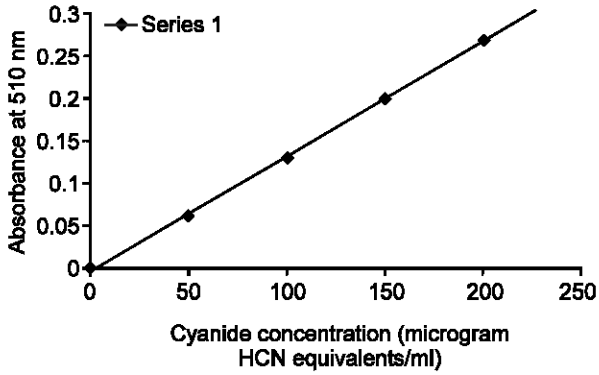

Fig. 1: Calibration curve for cyanide determination by the alkaline picrate method

Table 1: Cyanide contents of *Amanitia muscaria* samples

Sample number	Place of collection	Cyanide content (µg/10 g)
1	Opi	285
2	Ibagwa	522
3	Uzouwani	97
4	Obollo Afor	216
5	Edem	115
6	Aku	586
7	Ukehe	628
8	Ekwegbe	185
9	Ichi	712
10	Orba	669
11	Ikem	84
12	Enugu-Ezike	480
13	Imilike	177

The widespread occurrence of cyanide in many edible mushrooms has been reported (Akiyama *et al*., 2006). The role of cyanogenic foods as etiology of human diseases such as goiter, cretinism, konzo and tropical ataxic neuropathy has been demonstrated from both clinical and epidemiological studies (Tylleskar *et al*., 1991; Banea-Mayambu *et al*., 2000). All humans are susceptible to cyanogenic mushroom poisoning. The poisonous species are ubiguitous. Species of poisonous mushrooms are characterized by their variations in toxin content based on their genetic and growing conditions. Severity of intoxication depends on the amount and type of toxin consumed. From our study, if an individual consumes 1 kg of the mushroom samples, the maximum dose of cyanide intake will be 71.2 mg. The lethal dose of cyanide intoxication of humans is 200-300 mg for an adult human (Akiyama *et al*., 2006). Although the cyanide levels of our samples are low when compared with the lethal dose for humans, epidemiological studies have shown that small doses of cyanide given over a long period of time produced histological changes in the central nervous system (Smith, 1964). The toxicity of cyanide given over

a period of time caused cerebral damage primarily to the basal ganglia (Rachinger *et al*., 2002). Other effects of cyanide poisoning include transcient hyperpnea, headache, dyspnea, central nervous system excitement and progressive histotoxic tissue hypoxia (Hall and Rumack, 1986); konzo, an upper motor neuron disease (Howlett *et al*., 1990; Banea-Mayambu *et al*., 1997; Ernesto *et al*., 2002); cyanide induced parkinsonism (Uitti *et al*., 1985) and chronic neuropathy (Osuntokun 1972; Osuntokun, 1994). Therefore to minimize these health disorders due to cyanide intoxication, development of a simple and rapid analytical procedure to assay cyanogenic mushroom samples becomes necessary especially in developing countries where mushroom has become an important food source.

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Physio-Chemical Characteristics and Dietary Metal Levels of Oil from *Elaeis guineensis* Species

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Abstract: Three Species of local palm oil (*Elaeis guineensis*) were harvested and some of their physio-chemical and dietary metal components were analyzed using standard test methods such as Spectrophotometry, Titrametry and Gravimetry. The three species of *E. guineensis* studied were *Elaeis pisifera*, *Elaeis dura* and *Elaeis tenera*. Determination of the antioxidants as total carotenoids (Vitamin A) and Vitamin E showed that Vitamin A content in *E. pisifera*, *E. dura* and *E. tenera* were 8677.17, 8927.65 and 880.80 ug/g respectively, while Vitamin E obtained for the species were 476.88, 443.52 and 181.92 mg/100 g for Pisifera, Dura and Tenera species respectively. The protein content in the three species (with proximate) also gave 2.21, 2.30 and 2.70% for Pisifera, Dura and Tenera respectively while the iodine values which were determined using Wijis method gave results to be 44.38, 46.93 and 44.05 for Pisifera, Dura and Tenera in that order. The acid value and the saponification values were determined (by titrametric method) in the three species which gave acid value results to be 0.65, 0.67 and 0.57 mgkOH/g for Pisifera, Dura and Tenera respectively while the saponification values where 191.45, 185.77 and 188.48 mgKoH/g for Pisifera, Dura and Tenera in that order. Also the percentage moisture content gave 0.05, 0.04 and 0.05 for the three species in same order above. The dietary magnesium for the Pisifera, Dura and Tenera in that order were 0.95, 1.13 and 0.37 mg/dm^3 and 0.08, 0.24 and 0.05 mg/dm^3 for dietary zinc. The Dietary calcium level were 0.46, 0.41 and 0.34 mg/dm^3 and the potassium 0.46, 0.39 and 0.48 mg/dm^3 for Pisifera, Dura and Tenera respectively. The iron concentration levels were 38.30, 67.70 and 78.30 mg/dm^3 for the three in the same order above.

Key words: *Elaeis guineensis*, physio-chemical, dietary metal, oil,

INTRODUCTION

Palm oil (*Elaeis guineensis*) is a form of edible vegetable oil obtained from the mesocarp of the oil palm fruits. Previously the second most widely produced vegetable oil after Soya bean oil, it may have now surpassed Soya bean oil as the most widely produced vegetable oil in the world today.

Palm oil itself is reddish in colour because of its rich in beta carotene (a provitamin responsible for pigmentation of most fruits and vegetables and the precusor of vitamin A also an antioxidant that destroys singlet oxygen and free radicals in the body). (Ellan Johannesen, 2005).

It is used as cooking oil and also in the processing of other foods.

Red palm oil, besides providing calorie density to the diets is also the largest natural source of tocotrienol and tocopherol (vitamin E family). (Manorama and Rukmini, 1991).

Chemical analysis of the fatty acids composition of the red palm oil indicates that it has about 50% saturated, 40% mono unsaturated and 10% poly unsaturated fatty acids. (Manorama and Rukmini, 1991).

Previously, (Njoku, 2006; Njoku and Ejele, 2004) have done some work on the isolation characterization and a physiochemical property survey of *Elaeis guineensis* at varying temperature regimes just like other works in *Elaeis guineensis* compositional checks but emphasis on their antioxidative potentials and dietary metal levels were not captured, so that formed the basis and scope of this work. Obetta (2000) also worked on palm kernel physio-chemistry.

A study by a group of researchers in China comparing palm, soyabean, peanut oils and lard showed that palm oil actually increased the levels of good cholesterol and reduced the levels of bad cholesterols (LDL) in the blood (Zhang *et al*., 1995; 1997; cited by Koh, 2006).

A study by Hornstra in 1990 also showed similar results.

The composition of palm oil: Palm oil consists mainly of glycerides made up of a range of fatty acids. Triglycerides constitute the major components, with small proportion of diglycerides and monoglyceride. (Tan and FCH, 1981).

The 2 most predominant fatty acids in palm oil are C16:O (saturated) palmitic acid and C18:1 (Unsaturated) Oleic acid. Typical fatty acid composition of palm oil is given as: ie Malaysian palm oil (Tan and FCH, 1981).

Other major constituents of palm oil are mono and diglycerides. Free fatty acids, moisture, diet and minor component of non oil fatty matter referred to collectively an unsaponifiable matter (Goh *et al*., 1985).

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Table 1: Fatty acid composition of Malaysian oil

ACID	Name	% Range	% Mean
C12: 0	Lauric	0.1-1.0	0.2
C14:0	Myrstic	0.9-1.5	1.1
C16:0	Palmitic	41.8-46.8	44.0
C16:1		0.1-0.3	0.1
C18:0	Stearic	4.2-5. 1	4.5
C18:1	Oleic	37.3-40.8	39.2
C18:2	Linoleic	9.1-11.0	10.1
Others	Others	0.0-1.8	0.9

Minor components: These are classified into one category because they are fatty in nature but are not really oils. They are referred to as unsaponifiable matter and they include the following:
(1) Carotenoids (2) Tocopherols and tocotrienols (3) Sterols (4) Phospholipids (5) Triterpene alcohol (6) Methyl Sterols (7) Squalenes etc. (Goh *et al*., 1985).

Carotenoids: The carotenoids, whose name is derived from the fact that they constitute the major pigment in the carrot root, *Daucus carota*, are undoubtedly among the most widespread and important pigments in living organisms. Carotenoids are a group of more than 700 compounds (eg alpha-carotene, beta-carotene). The human body uses carotenoids as Vitamin A which enhances eye health. Carotenoids also play an important potential role by acting as biological antioxidants protecting cells and tissues from the damaging effects of free radicals, which could also cause cancer (Koh, 2006).
Studies also suggest that carotenoids enhance immune function by a variety of mechanisms and improve cardiovascular health. Carotenoids are present in numerous vegetable oils, including yellow maize (corn) oil, groundnut oil, soya-bean oil, rape seed oil, linseed oil, olive oil, barley oil, sunflower oil and cotton seed oil. The concentration of carotenoids in these vegetable oils is generally low, less than 100 ppm (Ong and Tee, 1992).
Of the vegetable oils that are widely consumed palm oil contains the highest known concentration of agriculturally derived carotenoids. In fact, crude palm oil is the world's richest natural plant source of carotenes in terms of retinal (pro vitamin A). Palm oil contains about 15-300 times as many retinal equivalent as carrot, leafy green vegetables and tomatoes (Tan, 1989).

MATERIALS AND METHODS

The physiochemical parameters used in the evaluation of the three palm oil species composition were determined using standard analytical method such as spectrophoto-metric, titimetric and gravimetric etc.

- Metallic measurements-Unicam solar 32 AAS
- Total Carotenoid-Genesys 10 uv spectrophotometer
- Vitamin E Content-Genesys 10 uv spectrophotometer
- Protein content-Titration
- Iodine value-Titration
- Acid value-Titration
- Saponification value-Titration
- Moisture content-Gravimetric

The three palm oil samples were collected from their respective bunches and processed by heating (boiling) the nuts, pounding and pressing the pounded fibre to obtain the palm oil while the nuts were removed. The palm oil samples were collected in plastic containers and later stored in smaller glass containers with good cork.

Sample collection: The three palm oil species were extracted by the traditional method of boiling the palm nuts, pounding them in motar and pressing the pounded mesocarp while the palm kernels were isolated.
These samples were collected in Amaawom-Oboro village near Umudike Umuahia in Abia State, Nigeria. The 3 species, which has their characteristic physical properties, were identified and isolated based on those properties. Examples of their physical properties:

- Okpuruka (*E. dura*): This specie is usually red when ripe and has a thick-hard kernel which cannot be broken with ease with the teeth. Preferable for PKO yield.
- Osukwu (*E. pisifera*): This specie is very much like the Elaeis Dura but differs in its yield and thicker endosperm. Pisifera has higher palm oil yield with little or no kernel. The kernels are usually soft and can be broken with teeth.
- Obia (*E. tenera*): This specie usually has a characteristic green seed when unripe but equally red when ripe. It has a mixture of both small and large kernels. It's palm oil yield is higher than that of Dura but less than Pisifera.

Determination of metals: 2.0 g of the homogenized palm oil sample was weighed in a beaker on a mettler balance and 2 cm^3 of concentrated sulphuric acid was added to it.
The beaker was heated on the hot plate for 10 min to digest the oil sample in the acid. The digested sample was then transferred to an electric furnace and ashed at 550°C for 30 min. After the ashing and cooling of the sample, 6 cm^3 of HCL was used to wash the sample into another beaker. The beaker was further washed with 25 cm^3 of distilled water and resultant solution filtered with a 125 mm whatman filter paper.
The filtered sample solution was then assayed using the Atomic Absortion Spectrophotometer (AAS) combusting and atomizing the sample using air-acetylene oxide [Unicam Solar 32 AAS].

Standard solutions of Calcium, Magnesium, Zinc, Iron and potassium were prepared according to ASTM standard: Carotenoid content was determined by the screening method developed by Kimura Meiko (2004)

Table 2: Composition of minor component in Malaysian crude palm oil

Names	ppm Concentration
Carotenoids	500-700
Tocopherel and Tocotrienels	600-1,000
Sterols	326-527
Phospholipids	5-130
Triterpene alcohol	40-80
Methyl Sterol	40-80
Squalene	200-500
Aliphatic alcohols	100-200
Aliphatic hydrocarbon	50

Courtesy of Malaysian food and nutrition bulletin, volume 15 (1993/1994)

Table 3: Retinol equivalent (RE) of red palm oil compared with other foods

	RE
Fish liver oils (preformed retinol)	
Halibut	900,000
Shark	180,000
Cod	18,000
Fruits and vegetables (carotene derived)	
Red palm oil	30,000
Carrot	2,000
Leafy vegetables	685
Apricots	250
Tomatoes	100
Bananas	30
Orange juice	8

Courtesy of American oil chemical society (Tan, 1989)

following the guidelines of Rodriguez-Amaya (1999) while the Vitamin E content was determined by the method described by Pearson (1976) and the protein by Kjeldahl method described by James (1995). The Iodine value was also determined by the Wijis method while the, Acid value and saponification number were determined by titrametry.

The moisture content was determined by the gravimetric method (James, 1995). 5.0 g of samples were weighed into clean, dry crucibles of known weight. The crucibles containing the samples were then transferred into an oven and heated to dryness at 105°C for 3 h.

The crucibles were removed, cooled in a desiccator and reweighed repeatedly until constant weights were obtained.

The % moisture content were determined by the calculation below:

$$\%\text{Moisture} = \frac{w_2 - w_3 \, x100}{w_2 - w_1}$$

Where:

W_1 = Weight of empty crucible
W_2 = Weight of crucible + sample before drying
W_3 = Weight of crucible + sample after drying to a constant weight

RESULTS AND DISCUSSION

Metal components: The analysis results obtained from the three palm oil species showed that *Elaeis tenera* (obia) which is a cross of the *Dura* and *Pisifera* species had highest concentration of dietary metal composition for iron and potassium. For example its iron and potassium concentrations were 78.30 mg/dm^3 and 0.48 mg/dm^3 while, *E. dura* and *E. pisifera* had 67.70 and 38.30 for iron and 0.39 and 0.46 for potassium respectively.

Tenera had lower concentration in Zinc (0.05 mg/dm^3), Magnesium (0.37 mg/dm^3) and Calcium (0.34 mg/dm^3) compared to *Pisifera* and *Dura* which had 0.08 mg/dm^3 Zinc, 0.95 mg/dm^3 Magnesium, 0.46 mg/dm^3 Calcium and 0.24 mg/dm^3 zinc, 1.13 mg/dm^3 Magnesium, 0.41 mg/dm^3 Calcium respectively.

Summarily, the *Elaeis tenera* has the highest concentration of dietary metal components analyzed.

Carotenoid: The results showed that the cross breed specie (*Elaeis tenera*) had the least concentration of vitamin A (880.80 mg/g). The other species analyzed for example, *E. dura* and *E. pisifera* had 8927.65 ug/g and 8677.17 ug/g respectively.

The *E. tenera* showed a general lower concentration of vitamins than the other species.

Vitamin E: The analysis results obtained from the three palm oil species also indicated that the *E. tenera* which is the cross of *E. dura* and *E. pisifera* had the least concentration of Vitamin E., which was 181.92 mg/100 g. While *E. dura* and *E. pisifera* had 443.52 and 476.88 mg/100 g respectively. The results above depicts a steady lower concentration of vitamins in the *Elaeis tenera* specie.

Protein: The three species of palm oil analyzed indicated a close and almost equal concentration of dietary protein content. The *E. tenera* was 2.75% while the *E. dura* and *E. pisifera* had 2.30% and 2.21% respectively. The result also showed that the cross (*E. tenera*) had the highest concentration of protein among the 3 species.

Iodine value: The result obtained on the iodine value of the palm oil samples also indicated a very close value in their iodine values includes the values which gave 44.05 for *E. tenera* while the *E. dura* and *E. pisifera* had 46.93 and 44.38 respectively. The result depicts the degree of unsaturation of the fatty acids in the species hence, *E. tenera* has the least contents of the unsaturated fatty acids among the 3 species.

Acid value: There is equally a close relationship in the acid value obtained from the three palm oil species. The *E. tenera* also had the least value of 0.57 mg kOH/g while the *E. dura* and *E. pisifera* had (mgkOH/g) 0.67 and 0.65 respectively. Though the values were close but the *E. tenera* still had the least acid value.

Table 4: Analysis result summary

Metal Content		Mg/dm^3		
Parameter	Method	Obia (*E. tenera*)	Okpuru (*E. dura*)	Osukwu (*E. pisifera*)
Zinc (Zn)	ASTM D1691	0.05	0.24	0.08
Magnesium (Mg)	ASTM D511	0.37	1.13	0.95
Calcium (Ca)	ASTM D511	0.34	0.41	0.46
Potassium (K)	ASTM D3561	0.48	0.39	0.46
Iron (Fe)	ASTM D1058	78.30	67.70	38.30
Caroteniods (mg/g)				
Carotenoids	E/E	888.80	8927.65	8677.17
Vitamin E (mg/100 g)				
Vitamin E	E/E	181.92	443.52	476.88
Protein (%)				
Protein	E/E	2.75	2.30	2.21
Iodine value				
Iodine Value	E/E	44.05	46.93	44.38
Acid value (mgKOH/g)				
Acid Value	E/E	0.57	0.67	0.65
Saponification value (mgKOH/g)				
Saponification value	E/E	188.48	185.77	191.45
Moisture content (%)				
Moisture Content	E/E	0.05	0.04	0.05

E/E = Explained Earlier

Since the acid value measures the extent to which the glycerides in the oil have been decomposed by lipase action and depicts the tendency of rancidity, it means then that the *E. tenera* would be the most stable on the shelf (Manorama and Rukmini, 1991).

Saponification value: The analysis results of the three palm oil species indicated that the Elaeis Dura had the least saponification value (185.77 mg/KOH/g) while the *E. pisifera* had the highest value among the three (191.45 mgKOH/g). The cross (*E. tenera*) had 188.48 mgKOH/g.

Since S.V. measures the volume of KOH required to neutralize the fatty acids produced by hydrolysis of 1 g of sample, the results above therefore depicts that the *E. dura* has the least value of both free and combined fatty acids.

Moisture content: The three (3) species had almost an equal moisture content as indicated in the results of samples analyzed.

The *E. tenera* was 0.05%, *E. pisifera* was 0.05% while the *E. dura* gave a slightly lower value of 0.04%.

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Nutritive Value of Oak Leaves in Sheep

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Abstract: Oak (*Quercus* sp.) leaves and branches is an important source of forage in the north-west of Iran, during winter season, when the pasture herbages are not available, but the nutritive value of this forage is not well known. In this study nutritive value of three species of oak tree leaves: *Quercus persica, Q. infectoria* and *Q. libani* were assessed by chemical analysis and *in situ* method. The chemical composition (g/kg DM basis) of the above species, respectively were as follow; 951, 927, 946, Organic Matter (OM); 115, 92, 123, Crude Protein (CP); 532, 540, 512, Neutral Detergent Fiber (NDF); 317, 300, 331, Acid Detergent Fiber (ADF); 98, 103, 95, lignin (ADL); 78, 115, 104, total phenols; 73, 109, 100, Total Tannins (TT); 14, 15, 12, Condensed Tannin (CT) and 46, 87, 62, Hydrolysable Tannin (HT). Protein Precipitable Phenolics (PPP) were respectively 160, 190 and 230 (g/kg total phenols). Rumen liquor taken from three male Ghezel sheep was used to measure the *in situ* degradability characteristics of oak leaf. The soluble component (a), insoluble but fermentable fraction (b), the potential degradability (a+b) and the Effective Degradability (ED) were higher in *Q. persica* ($p<0.01$). There was a strong negative correlation between TT, HT and ED in sheep. The rank order of nutritive value, in terms of chemical composition and *in situ* degradability were as follows: *Q. persica* > *Q. libani* > *Q. infectoria*.

Key words: Oak leaves, chemical composition, *in situ* degradability, sheep

INTRODUCTION

Oak leaves and twigs are often grazed by ruminants or harvested for use as livestock feed during feed shortages (Singh *et al*., 1996). Approximately 3 million ha of forest are covered by various oak species, mainly dominated by *Quercus persica*, *Quercus infectoria* and *Quercus libani*, in the north-west of Iran (Fatahi, 1995). In this region, oak leaves are an important source of forage for small ruminant during periods of the year when quality and quantity of pasture herbages is limited. However, *Quercus* species have been reported to contain high levels of tannins in both hydrolysable (Makkar, 2003) and condensed (Makkar *et al*., 1991) forms. Therefore, the value of these leaves as feeds for ruminants is offset by their potentially negative effects on protein utilization, and the risk of toxicity when intake is high (Garg *et al*., 1992). *In situ* rumen disappearance technique is useful for rapid screening of feeds to assess their potential as feed energy sources for ruminants (Preston, 1995). There is little information available on the nutritive value of *Quercus* Spp. in Iran. The present study was, therefore, carried out to determine the chemical composition, phenolic composition and degradation of *Quercus* leaves.

MATERIALS AND METHODS

***Quercus* species:** Samples consisted of three indigenous *Quercus* species, being *Q. persica*, *Q. infectoria* and *Q. libani*. Samples were harvested by hand during the summer at several locations in the NW of Iran. Branches were randomly sampled from at least 10 plants per species. leaves were removed from branches, pooled to five samples per species and air dried in the shade to minimize changes in tannin content and activity (Makkar and Singh, 1991b). Water Soluble Carbohydrate (WSC) was Measured using the anthrone method (MAFF, 1982).

Chemical analysis: Standard methods as described in AOAC (1990) were used for determination of Dry Matter (DM), ash and CP (N x 6.25). Ash-free Neutral Detergent Fiber (NDF) was determined using sodium sulfate according to the method of Van Soest *et al*. (1991) and ash-free Acid Detergent Fiber (ADF) was determined based on AOAC (1990). Lignin (ADL) was determined by solubilization of cellulose with sulphuric acid as described by Robertson and Van Soest (1981).
Samples were analyzed for Total Phenols (TPH), Total Extractable Tannin (TT), Condensed Tannins (CT), Hydrolysable Tannins (HT) and Protein Precipitible Phenolics (PPP), as described below.

Total phenols: Dried plant material (200 mg) was extracted with acetone: water (10 ml; 70:30, v/v) in ultrasonic bath for 20 min. Contents were centrifuged (4°C, 10 min, 3000 g) and the supernatant was kept on ice until analysis. Total phenols were determined with the Folin-Ciocalteau reangent and detected at 725 nm (Makkar, 2000). A calibration curve was prepared using tannic acid (Merck Gmbh, Darmstadt, Germany). Total phenols were calculated as tannic acid equivalents and expressed as equiv. g/kg DM.

Tannins: Condensed tannins were measured by the HCL-butanol method (Makkar, 2000). An aliquot from the above acetone: water extract (0.5 ml; although this extract occasionally needed diluting with the extractant, acetone: water, if final absorbance at 550 nm exceeded 5.6 absorbance units) plus HCL-butanol (3 ml) and ferric ammonium sulphate (0.1 ml) regents were heated in a boiling water bath for 60 min. Absorbance was read at 550 nm. The colorimetric data (in absorbance units) were converted to leucocyanidin equivalents based on the assumption that the color yield of condensed tannins, E 1%, 550 nm, is 460 (Porter *et al*., 1986).

Non-Tannin Phenols (NTP) were determined using absorption to insoluble polyvinylpyrrolidone. The insoluble Polyvinylpyrrolidone (PVPP; 100 mg) was weighted into 100 x 12 mm test tubes. Distilled water, 1 ml and then 1 ml tannin containing extract were added and vortexed. The tube was kept at 4°C for 15 min, vortexed again, then centrifuged (3000x g) for 10 min and supernatant collected. The phenolic content of the supernatant was measured by the Folin-Ciocalteau reaction and this was accepted as the NTP (Makkar, 2000).

Total tannins were calculated as the difference between TPH and NTP. Hydrolysable tannins were analyzed using Rhodanin assay according to Makkar (2000). The results were expressed as gallotannin. Protein precipitable phenolics were determined according to Makkar (2000) and results were expressed as tannic acid equivalent.

***In situ* degradability:** Three remun-fistulated Ghezel sheep (live weight 64±2.5 kg) were used to determine the rate of degradability of DM (AFRC, 1992) from oak leaves. Sheep were fed a ration consisting of Lucerne hay, oak leaves (mix of three oak spices), wheat bran and barley grain (50:50) with a ratio of forage to concentrate of 60:40 (DM basis), which was calculated to ME at their maintenance level. Sheep were adapted to the diet for 10 days.

In situ bags were made from a Dacron material (21 x 10 cm) with a pore size of 45 μm (AFRC, 1992). All samples of feeds were dried and milled through a 4.0 mm sieve. Then 5 g of each sample was put in the *in situ* bags and incubated at the same time in the rumen for 3, 6, 12, 24, 48, 72 and 96 h. In each sheep, one bag was used for time interval. Bags were attached on semi-rigid stalks to ensure immediate insetion within the liquid of the rumen contents while allowing free movement. After withdrawing the bags from the rumen, they were washed in a washing machine for 1 h using cold water and dried for 48 h at 50°C. The degradability value at t = 0 was obtained by washing two bags in a washing machine for 1 h using cold water. For each bag, the residue was analyzed for DM. Degradability at each incubation time was calculated by taking the values obtained from the three bags (i.e., n = 3). The ruminal degradability (Y) of DM at time (t) was obtained from an exponential curve of the type:

$$Y = a + b\,(1-e^{(-ct)})$$

This was fitted to the experimental data by iterative regression analysis (Ørskov and McDonald, 1979). In this equation, the constant a represents the soluble and very rapidly degradable component and b represents the insoluble but potentially degradable component which degrades at a constant fractional rate (c) per unit time. The effective degradability of DM in each species was then estimated (Ørskov and McDonald, 1979) by the equation: effective degradability (g/kg DM) = a + bc/c+ k. In this equation, k refers to the fractional outflow rate of small particles from the rumen. A value of 0.05 fraction/h was used for k.

Statistical analysis: Data on chemical and tannin composition and *in situ* degradability were subjected to analysis using the General Linear Model (GLM) procedure of SAS (2001), based on the statistical model:

$$Y_{ij} = \mu + S_i + e_{ij}$$

Where Y_{ij} is the general observation on chemical composition and tannin composition and *in situ* degradability, μ the general mean, S_i the ith effect of oak species on the observed parameters and e_{ij} the standard error term. Means were tested using Duncan test.

RESULTS AND DISCUSSION

Chemical composition and phenolics: *Quercus infectoria* leaves had the lowest OM, CP and ADF (Table 1), but contained the highest NDF and ADL versus other species. In all *Quercus* spp., the level of HT was high (Table 2). Among the oak leaves, *Q. infectoria* had the highest TPH, TT, CT and HT content ($p<0.05$) in *Quercus libani* versus other species.

The variation in chemical composition among these species of *Quercus* may be partly due to the genotypic factors that control accumulation of forage nutrients (Minson, 1990). In our oak leaves, the CP content was more than 80 g/kg DM (range: 92-123 g/kg DM) which, according to Norton (1998), should provide ruminal ammonia levels above the minimum required by rumen microorganism to support optimum growth. It seem to be likely the *Quercus* leaves studied in this experiment will meet the CP requirements of small ruminants for maintenance. Makkar and Singh (1991b) reported a similar range of CP content in mature *Q. incana*, *Q. semecarpifolia*, *Q. serraata*, *Q. ilex* and *Q. glauca*. However, Kamalak *et al*. (2004) obtained lower CP content in *Q. branti*, *Q. coccifera*, *Q. ceris*, *Q. libari* and *Q. infectoria* than our *Qercus* sp. The ADF and ADL content

Table 1: Chemical composition (g/kg DM) of oak species

	Q. persica	*Q. infectoria*	*Q. libani*	SEM
DM	939b	943a	946a	0.4
OM	951a	927c	946b	0.9
CP	115b	92c	123a	0.1
ADF	317ab	300b	331a	0.7
NDF	532a	540a	512b	0.4
ADL	98b	103a	95b	1.3
WSC	8.9c	11.7b	19.2a	0.51

Means in the same row with different letters differ (p<0.05)

Table 2: Phenolic composition (g/kg DM) of oak species

	Q. persica	*Q. infectoria*	*Q. libani*	SEM
TPH	78c	115a	104b	0. 2
TT	73c	109a	100b	0.2
CT	14b	15a	12c	0.05
HT	46c	87a	62b	0.1
PPP	160c	190b	230a	0.4

Means in the same row with different letters differ (p<0.05)

of our oak species were lower than in *Q. incana*, *Q. semecarpifolia*, *Q. serraata*, *Q. ilex* and *Q. glauca* (Makkar and Singh, 1991b), but higher than in *Q. libari* (Kamalak *et al*., 2004). The level of NDF in our experiment was lower than in *Q. incana*, *Q. semecarpifolia* and *Q. serraata* than *Q. libari* (Kamalak *et al*., 2004). There was a relationship between TPH and TT which is similar to findings of Makkar *et al*. (1993) who noted a high positive correlation between content of total tannins and total phenolics. Levels of TPH and total tannins in the experimental species were higher than in *Q. incana* (Singh *et al*., 2005), *Q. hartwissiana* (Yildiz *et al*., 2005), *Q. coccifera* (Ben Salem *et al*., 2003;2005), similar to *Q. rotundifolia* (Khazaal *et al*., 1994), but lower than *Q. coccifera* (Khazaal *et al*., 1994). Condensed tannins in our oak leaves were similar to *Q. hartwissiana* (Yildiz *et al*., 2005), *Q. coccifera* (Khazaal *et al*., 1994) and *Q. suber* (Gasmi-Boubaker *et al*., 2005), but lower than *Q. coccifera* (Ben Salem *et al*., 2003;2005); *Q. incana* (Singh *et al*., 2005), *Q. cercis* (Canbolat *et al*., 2005). The level of HT is high in *Quercus* sp. Similarly, some groups have reported that oak leaves are rich in HT (Garg *et al*., 1992; Makkar, 2003).

However, others noted low levels of HT in oak leaves (Yildiz *et al*., 2005; Singh *et al*., 2005). The variations between our oak leaves and other oak species in the chemical composition and phenolics contents is probably due to any or all of the vegetative stage (Makkar and Singh, 1993), method of storage (Makkar and Singh, 1993), drying conditions (Makkar and Singh, 1991a), species (Makkar and Singh, 1991b; Makkar *et al*., 1991) and habitat (Goncalves-Alvim *et al*., 2004).

***In situ* DM disappearance and estimated parameters:** The *in situ* dry matter constants are given in Table 3. The soluble component (a), insoluble but fermentable component (b), the degradation rate of b (c), the potential degradability (a+b) and the Effective Degradability (ED) of the oak leaves were influenced (p<0.01) by species. In the samples, the DM disappearance characteristics (a, b, a+b, ED) of *Q. persica* were higher (p<0.01) than those in other species.

Table 3: *In situ* dry matter disappearance in oak species

	Q. persica	*Q. infectoria*	*Q. libani*	SEM
a	20.66a	18.14b	20.14a	0.33
b	16.50a	13.16c	15.37b	0.15
a+b	37.17a	31.29c	35.52b	0.32
c	0.026a	0.028a	0.018b	0.001
ED	30.00a	25.73c	27.47b	0.16

a: Water-soluble fraction (g/kg DM); b: Insoluble but fermentable fraction (g/kg DM); c: The degradation rate of b (/h); a+b: The potential degradability (g/kg DM); ED: The effective degradability of dry matter calculated for an outflow rate of 0.05/h (g/kg DM). Means with different letters within species differ (p<0.01)

These variable values of *in situ* degradability among our *Quercus* sp. could be due to variations in tannin activity (PPP, Table 1) between *Quercus* spp. Values obtained using a protein precipitation assay better relate to the nutritional values of tannin-rich feeds (Hagerman and Butler, 1989; Makkar, 1989). Tannin activity has been reported to vary among forage species due to the in functionality of tannin and chemical structure (Dalzell and Kerven, 1998), degree of polymerization (Schofield *et al*., 2001), biochemical processes (Wong, 1973) and to the tannin structure-biological activity relationship (Haslam, 1998).

Conclusion: This study has shown that the *in situ* rumen degradability measurement appear to be suitable for assessing the potential nutritive value of *Quercus* leaves. On the basis of TT, TPH, HT, PPP and DM degradation parameters, oak leaves of *Q. persica* species were judged to be nutritionally best. However, further research is needed to assess their impacts on animal performance.

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Comparison of Different Wheat Varieties Grown in Punjab for Leavened Flat Bread (Naan) Production

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Abstract: Seven wheat varieties i.e. Inqulab 91, Bhakkar 2002, AS 2002, Shafaq 2006, Sehar 2006, Auqab 2000 and GA 2002 collected from different locations of Punjab were subjected to physicochemical, rheological, minerals (copper, manganese, iron, zinc) and sensory analyses to determine their suitability for leavened flat bread (naan) preparation. Naans were prepared from different wheat flours and evaluated for colour, taste, flavor, texture, chewing ability, folding ability and overall acceptability. It was observed that Shafaq 2006 had the highest test weight (81.50 kg/hl), thousand kernel weight (41.20 g), zinc (8.50 mg kg^{-1}) and lowest broken/shrunken grains (0.79%), insect damaged grains (0.45%), moisture (12.92%) and dough stability (4.11%), Bhakkar 2002 had the maximum broken / shrunken grains (1.61%), ash (0.61%), falling number (432) and minimum water absorption (54.05%), dough development time (2.21 min) and manganese (5.29 mg kg^{-1}), whereas Sehar-2006 had the highest protein (10.84%), wet gluten (27.56%), dry gluten (9.35%), dough development time (4.12 min), copper (4.68 mg kg^{-1}) and lowest edible foreign matter (0.42%), tolerance index (35.00 BU) and iron (14.80 mg kg^{-1}). Naans prepared from Auqab 2000 were ranked highest and more acceptable than others.

Key words: Punjab, wheat varieties, physicochemical, farinographic properties, sensory evaluation, naan

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the largest grain crop and staple food of Pakistan. It occupies a central position in agriculture and economy (Shuaib *et al*., 2007). It contributes 13.1% to the agricultural value addition and 2.8% to GDP. Total wheat production during 2008-09 touched 23.4 million tons (GOP, 2009). Besides being a rich source of carbohydrates, wheat contains protein, essential amino acids except lysine, minerals such as phosphorus, magnesium, iron, copper and zinc and vitamins like thiamin, riboflavin, niacin and vitamin E (Khan and Zeb, 2007).

The term wheat quality is a complex of many factors depending on milling, chemical, baking and rheological dough properties. Wheat quality reflects suitability for a particular purpose or intended use. The major factors influencing wheat quality are cultivar, climatic conditions, cropping year, process of harvest and storage conditions (Pasha, 2006).

Wheat is unique among cereals since its milled product '' flour'' is capable of forming the dough due to its gluten content. The unique characteristics of wheat can be attributed to the ability of its proteins gliadin and glutenin, which upon hydration form viscoelastic network gluten: the actual substance that imparts gas retention property to dough (Shah *et al*., 2008). In Pakistan, the most commonly consumed and least expensive product of wheat flour is flat bread like chapattis, rotis and naan. Furthermore, wheat is used for various other bakery products like bread, cookies, cakes, buns, pastries etc. In Punjab and Sindh provinces chapatti and roti doughs are unleavened while in Baluchistan and NWFP provinces fermented rotis are prepared. Naan is flat leavened bread prepared from flour, water, salt and yeast (Aidoo *et al*., 2006). Fermented dough is used for making naan. Therefore, naan is made from finer granulation flour than that used for chapattis because finer the granulation, the more rapid is the process of fermentation (Qarooni, 1996). It has better digestibility and greater storage life than chapattis. It is mostly consumed at breakfast, while it is also available at lunch and dinner with specific dishes (Farooq *et al*., 2001). The present study was designed to evaluate wheat varieties grown in Punjab for various physico-chemical and rheological characteristics in order to assess their suitability for the production of leavened flat bread (naan).

MATERIALS AND METHODS

Collection of raw materials: Seventy eight wheat samples were collected randomly from nine regions of Northern, Central and Southern Punjab during 2008. Samples were drawn directly from farmers field. Wheat samples were packed airtight in polyethylene bags and

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taken to Food Quality and Nutrition Program (FQNP) Lab. National Agricultural Research Centre (NARC). Representative samples of each variety were prepared for physico-chemical analysis, farinographic studies and naan preparation by combining individual variety samples from different regions. Physico-chemical, farinographic analysis of composite samples and sensory evaluation of prepared naan were done in triplicate.

Physical characteristics of wheat: Wheat samples were uniformly divided through Boerner Divider and analyzed for physical quality characteristics such as thousand kernel weight, test weight, foreign matter, broken/shrunken grains and damaged grains according to standard procedures as described in AACC (2000).

Milling of wheat: Wheat grains were tempered and then milling was done using Quadrumate Senior Mill according to standard method (AACC, 2000). Four products were obtained i.e. reduction flour, break flour, shorts and bran. Reduction flour and break flour were mixed to get straight grade flour for further studies.

Chemical/general characteristics of wheat flour: Wheat flour of different varieties was subjected to determine its chemical/general characteristics such as moisture, ash, crude protein (N x 5.7), wet and dry gluten and falling number according to standard procedures of AACC (2000). Perten Glutomatic was used to determine wet and dry gluten whereas Falling Number system (Perten 1500) was used for the determination of alpha amylase activity in wheat flour. Trace elements (copper, manganese, iron, zinc) were analyzed using a Varian SpectrAA 220FS Atomic Absorption Spectrometer. The samples were prepared according to the standard methods of AOAC (2005).

Farinographic studies: Rheological behaviour of different wheat varieties flour was evaluated by running flour samples through Brabender Farinograph equipped with a bowl of 50 g capacity. The dough characteristics such as water absorption, dough development time, dough stability, tolerance index and softening of dough were determined according to standard procedure of AACC (2000).

Preparation of leavened flat bread (naans): Leavened Flat Bread (Naans) were prepared by taking 250 g straight grade flour, mixed with 50 g yoghurt and water for 10 min, kept in an incubator at 35°C overnight covered with wet cloth. It was then mixed with 750 g flour, 15 g sugar, 5 g salt, 5 g sodium bicarbonate and water (quantity as determined by farinograph water absorption) for dough preparation. Dough balls of 100 g each were made and sheeted into a disk of 7 inch diameter with rolling pins, pressed with fingertips in the centre and allowed to proof for 30 min. Then naans were prepared by baking in an oven at 315°C for 3 min.

Sensory evaluation: Sensory evaluation of naans was carried out by a panel of judges for colour, taste, flavour, texture, chewing ability and folding ability. Samples were presented in succession and panelists were asked to rate evaluation variables according to 9- point Hedonic scale as described by Land and Shepherd (1988).

Statistical analysis: The data obtained for each parameter was subjected to statistical analysis using Statistica 6.0 software according to methods described by Steel *et al.* (1996).

RESULTS AND DISCUSSION

Seven wheat varieties collected from different regions of Punjab were evaluated for physicochemical, rheological and sensory characteristics for leavened flat bread preparation.

Physical characteristics: Data regarding physical parameters of wheat grains reveals that Shafaq-2006 variety had the highest test weight (81.50 kg/hl) whereas AS 2002 samples possessed the lowest test weight (76.80 kg/hl) (Table 1). Test weight is considered as one of the important tool in wheat grading system (Pasha, 2006). It is imperative in the grain trade because most grains are sold at a certain test weight. Highest thousand kernel weight (41.20 g) was observed in Shafaq 2006 wheat and lowest value was recorded in Inqulab 91 (36.60 g). Thousand kernel weight as well as test weight is useful index for potential milling yield. The differences observed in test weight and thousand kernel weight among wheat varieties may partly be due to the differences in the genetic make up of the varieties and partly attributed due to different growing and environmental conditions prevailed during growing periods (Randhawa *et al.*, 2002).

In case of foreign matter, wheat grains of GA 2002 had the highest (1.19%) and Auqab 2000 had the lowest (0.25%) non-edible foreign matter. AS 2002 possessed the highest (1.29%) and Sehar-2006 had the lowest (0.42%) edible foreign matter. The differences in foreign matter may be due to varied climatic conditions of different locations, harvesting and threshing operations as well as planting time (Anjum *et al.*, 2003). Maximum broken/shrunken grains was observed in Bhakkar 2002 (1.61%) followed by Inqulab 91 (1.38%) samples whereas, Shafaq 2006 had the minimum (0.70%) broken/shrunken grains. As regards damaged grains, AS 2002 had the lowest (0.45%) and Inqulab-91 had the highest (0.92%) insect damaged grains, whereas GA 2002 had the lowest (0.54%) and Auqab-2000 had the highest (0.87%) other damaged grains (fungus/black tipped, heat damaged, immature grains etc).

Table 1: Physical characteristics of wheat grains

Varieties	Test weight (kg/hl)	Thousand kernel weight (g)	Foreign matter (%)		Broken/ shrunken grains (%)	Insect damaged grains (%)	Other damaged grains (%)
			Non-edible	Edible			
Inqulab 91	77.50±1.03	36.60±0.86	0.79±0.21	1.01±0.19	1.38±0.43	0.92±0.25	0.61±0.12
Bhakkar 2002	78.20±1.12	38.50±0.67	0.88±0.29	0.73±0.15	1.61±0.32	0.79±0.14	0.73±0.15
AS 2002	76.80±0.95	37.10±0.74	0.46±0.17	1.29±0.32	1.07±0.25	0.57±0.09	0.78±0.17
Shafaq 2006	81.50±0.73	41.20±0.57	0.37±0.13	0.64±0.17	0.79±0.15	0.45±0.11	0.64±0.08
Sehar 2006	79.30±0.86	39.00±0.78	0.54±0.16	0.42±0.09	0.94±0.27	0.66±0.14	0.58±0.13
Auqab 2000	78.60±0.67	38.00±0.51	0.25±0.10	0.87±0.21	1.16±0.28	0.52±0.10	0.87±0.19
GA 2002	80.00±0.54	40.60±0.65	1.19±0.24	1.13±0.28	1.32±0.19	0.73±0.17	0.54±0.10

*All values are means of three replications

Table 2: Chemical/general characteristics of wheat flour

Varieties	Moisture (%)	Ash (%)	Protein (%)	Wet gluten (%)	Dry gluten (%)	Falling No.
Inqulab 91	13.19±0.14	0.58±0.09	10.57±0.12	26.98±0.94	9.12±0.53	371±13.53
Bhakkar 2002	13.28±0.19	0.61±0.07	9.75±0.14	24.63±1.16	8.28±0.44	432±18.56
AS 2002	13.25±0.11	0.52±0.06	10.45±0.11	26.29±0.90	9.04±0.48	388±11.01
Shafaq 2006	12.92±0.15	0.57±0.03	9.98±0.16	25.55±0.61	8.67±0.39	364±8.82
Sehar 2006	13.07±0.08	0.56±0.05	10.84±0.10	27.56±0.67	9.35±0.50	406±10.44
Auqab 2000	13.16±0.17	0.54±0.04	10.36±0.18	26.64±0.72	9.21±0.32	352±9.49
GA 2002	13.32±0.10	0.53±0.02	9.32±0.23	24.17±0.55	7.84±0.21	417±9.88

*All values are means of three replications

Chemical/general characteristics: It is evident from the data on chemical characteristics of wheat flour that mean moisture content of different varieties were quite close to each other due to tempering of wheat grains before milling. Highest moisture content was observed in GA 2002 (13.32%) while Shafaq 2006 had the lowest moisture (12.92%) (Table 2). Moisture content is dependent on genetic makeup of wheat varieties and is largely influenced by agronomic and climatic conditions (Mahmood, 2004). In case of ash, highest value was observed in Bhakkar 2002 (0.61%) while lowest ash was recorded in AS 2002 (0.52%) samples. The ash content of flour is related to the amount of bran in the flour and therefore to flour yield.

As regards protein content, Sehar-2006 had the highest protein (10.84%) followed by Inqulab-91 (10.57%), while GA 2002 (9.32%) wheat had the lowest protein content. The protein content is an important criterion while considering the wheat quality. Protein content is an inherent characteristic but the quantity of protein depends on the growing conditions (Kent and Evers, 1994). Variation in protein content among wheat varieties is due to differences in their genetic makeup as well as differences in environmental and production conditions prevailed during growth stages (Randhawa, 2001).

Highest wet and dry gluten content was observed in Sehar 2006 (27.56% and 9.35%) whereas lowest value was observed in GA 2002 (24.17% and 7.84%). The differences in gluten content among different samples may be ascribed to the variation in genetic makeup of wheat varieties, climatic conditions and differences in cultural practices and growth locations (Randhawa *et al*., 2002).

In case of falling number, Bhakkar 2002 had the highest falling number (432) and conversely lower alpha amylase activity while Auqab 2000 had lowest falling number (352) and therefore higher amylase activity. Alpha amylase activity depends on weather conditions, especially precipitation and mineral fertilizer (Gyiri and Sipos, 2006). Similar results were reported by Pasha (2006) during his study on fifty different wheat varieties during 2004-05.

Data regarding mineral composition of wheat reveals that the concentration of copper ranged from 2.61-4.68 mg kg^{-1} (Table 3). Sehar-2006 had the highest value (4.68 mg $kg^{-1)}$ whereas, GA 2002 possessed the lowest copper content (2.61 mg kg^{-1}). Highest iron content was observed in Inqulab 91 (21.37 mg kg^{-1}) and lowest value was observed in Sehar 2006 (14.80 mg kg^{-1}). The concentration of manganese and zinc among different varieties varied from 5.29-9.16 and 4.67-8.50 mg kg^{-1} respectively. Highest manganese and zinc were detected in GA 2002 (9.16 mg kg^{-1}) and Shafaq 2006 (8.50 mg kg^{-1}) respectively. Similar findings were reported by Araujo *et al*. (2008) in their studies on mineral composition of wheat flour consumed in Brazil.

Farinographic studies: Farinographic studies were conducted to determine the rheological properties of wheat flour (Table 4). Highest water absorption (57.38%) was observed in Inqulab 91 followed by Sehar 2006 (57.02%) while Bhakkar 2002 had the lowest water absorption (54.05%). DMR test for water absorption reveals that all wheat flour samples were significantly different from each other except Sehar 2006 and Auqab 2000 which had non-significant differences. Water absorption is considered to be an important characteristic of wheat. Stronger wheat flours have the ability to absorb and retain more water as compared to weak flours. Higher water absorption is required for good flat bread characteristics which remain soft for a longer time (Simon, 1987).

Table 3: Mineral composition of wheat flour

Varieties	Copper (mg kg^{-1})	Iron (mg kg^{-1})	Manganese (mg kg^{-1})	Zinc (mg kg^{-1})
Inqulab 91	3.43±0.25	21.37±0.22	8.20±0.24	6.34±0.08
Bhakkar 2002	4.12±0.13	20.42±0.26	5.29±0.18	7.06±0.28
AS 2002	2.81±0.11	17.61±0.30	5.44±0.26	5.35±0.11
Shafaq 2006	3.26±0.16	17.30±0.34	5.88±0.22	8.50±0.15
Sehar 2006	4.68±0.19	14.80±0.20	7.18±0.13	7.60±0.19
Auqab 2000	3.75±0.13	20.40±0.20	6.72±0.24	7.17±0.04
GA 2002	2.61±0.26	18.79±0.32	9.16±0.20	4.67±0.33

*All values are means of three replications

Table 4: Farinographic characteristics of different wheat varieties

Varieties	WA (%)	DDT (min)	DS (min)	TI (BU)	SD (BU)
Inqulab 91	57.38a	3.63b	8.14c	46.67cd	70.00c
Bhakkar 2002	54.05f	2.21e	5.83e	86.67b	93.33b
AS 2002	55.87c	2.57de	9.17a	53.33c	60.00cd
Shafaq 2006	55.19d	3.08c	4.11g	60.00c	76.67bc
Sehar 2006	57.02b	4.12a	7.66d	35.00d	53.33d
Auqab 2000	56.78b	3.24bc	8.55b	43.33cd	46.67d
GA 2002	54.56e	2.70d	5.22f	110.00a	116.67a

*All values are means of three replication. *Means followed by same letters do not differ significantly ($p<0.05$).
WA = Water Absorption, DDT = Dough Development Time, DS = Dough Stability, TI = Tolerance Index, SD = Softening of Dough

Table 5: Sensory attributes of chapattis prepared from different wheat varieties

Varieties	Colour	Taste	Flavour	Texture	Chewing ability	Folding ability	Overall score
Inqulab 91	6.53e	7.00c	7.27bc	6.47c	6.13c	5.80c	6.53d
Bhakkar 2002	7.07d	6.27d	7.00c	5.60e	5.87cd	5.40d	6.21e
AS 2002	8.07a	7.47ab	7.40b	6.67bc	6.80b	6.73a	7.19b
Shafaq 2006	7.47c	7.33b	7.53b	7.00ab	6.07c	6.13b	6.92c
Sehar 2006	7.60b	7.60a	6.87cd	7.33a	6.93b	6.20b	7.09bc
Auqab 2000	7.87ab	7.80a	7.93a	6.80b	7.60a	6.27b	7.38a
GA 2000	7.20d	7.07c	6.60d	6.13d	5.67d	5.93bc	6.43d

*All values are means of three replication. *Means followed by same letters do not differ significantly ($p<0.05$)

In case of Dough Development Time (DDT), Sehar 2006 had the highest value (4.12 min) whereas Bhakkar 2002 had the lowest time (2.21 min). Higher dough development time reflects strong flour while its lower value is an indication of weak flour. Dough stability of different wheat varieties flour varied from 4.11 min (Shafaq, 2006) to 9.17 min (AS, 2002). DMR test for dough stability shows that wheat flours of all varieties were significantly different from each other. Dough stability is an indicator of flour strength. For Tolerance Index (TI), highest value was observed in GA 2002 (110 BU) whereas Sehar 2006 had the least tolerance index (35.00 BU). Generally, higher the tolerance index value, weaker is the flour. As regards Softening of Dough (SD), Auqab 2000 had the lowest value (46.67 BU) followed by Sehar 2006 (53.33 BU). Flours that have lower SD are stronger and the ones having higher SD values are weaker. Differences in farinographic characteristics among different wheat varieties may be due to variations in protein quantity and quality (Rehman *et al*., 2001).

Sensory evaluation: Naans prepared from different wheat varieties flour were subjected to sensory evaluation for colour, taste, flavour, texture, chewing ability and folding ability in triplicate and their scores were calculated (Table 5). Highest mean score for colour (8.07) was obtained by AS 2002 whereas Inqulab 91 got the lowest score (6.53). As regards taste, Auqab 2000 was at the top (7.80) followed by Sehar 2006 (7.60) and found to be least (6.27) for Bhakkar 2002. Maximum flavor score (7.93) was attained by Auqab 2000 whereas GA 2002 received the minimum score (6.60). The differences in colour, taste and flavour scores may be attributed to the differences in hardness/softness of wheat grains and other factors like wheat varieties and milling characteristics of wheat (Farooq, 2001). For texture, highest mean score (7.33) was obtained by Sehar 2006 and lowest (5.60) by Bhakkar 2002. In case of chewing ability, Auqab 2000 got the maximum score (7.60) and GA 2002 obtained the minimum score (5.67). For folding ability, Bhakkar 2002 obtained the least score (5.40) whereas AS 2002 received the highest score (6.73). With respect to overall acceptability of naans, highest score (7.38) was obtained by Auqab 2000 and thus regarded as more acceptable than other flour naans while lowest score (6.21) was obtained by Bhakkar 2002 thus considered least acceptable.

Conclusion: It was concluded that physic-chemical and rheological characteristics of wheat varieties affect the quality of the end product. Overall, quality of wheat varieties was good and comparable to International

standards. Wheat variety Auqab 2000 was ranked highest and most suitable for leavened flat bread (naan) preparation.

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Reduction in Hypertension and Related Lipid Profile Parameters after Exercise in Females

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Abstract: The effect of exercise on BMI and plasma lipid profile has been investigated in hypertensive females of four ethnic groups of Baloch (B), Pathan (P), Hazara (H) and Punjabi (PU). A total of 32 females, i.e. 8 from each group as control and another batch of 32 females' in similar distribution followed prescribed exercise protocol for ten weeks. After the trial fasting blood samples were collected from controlled group and exercised subjects. Exercise manifested significant decrement in Systolic Blood Pressure (SBP) in P, H and PU groups and of Diastolic Blood Pressure (DBP) in all the ethnic sub-populations. Marked and significant decrease of total cholesterol in all the ethnic groups was observed and the lowering of LDL cholesterol was also found in most except Pathan sub-population. Striking and significant rise of HDL of the exercising females was observed in all ethnic sub-populations. Triglyceride levels were considerably and significantly lowered in B, P, and H subjects. The beneficial effects of exercise on blood pressure and plasma lipids in female hypertensive subjects have been clearly demonstrated.

Key words: Exercise, blood pressure, cholesterol, high density lipoprotein, low density lipoprotein

INTRODUCTION

Hypertension is one of the most frequent chronic conditions in medical consultation (Grundy *et al*., 1998; Moleiro and Perez, 2003) and leading cause of death worldwide (Kearney *et al*., 2005) being major risk factor for stroke and Coronary Heart Disease (CHD) in adults. Blood pressure above optimal (<120 <80 mm Hg) is established as a major cardiovascular disease risk factor (Chiriac *et al*., 2002; Stamler *et al*., 2003).

High blood pressure is associated with overweight tendencies and obesity (Marc *et al*., 2001). According to Guimaraes (2002) elevation of blood pressure is associated with body mass index, life style and family history of hypertension, cardiovascular diseases and diabetes. Saito *et al*. (2003) reported the presence of positive correlation of blood pressure with Body Mass Index (BMI). BMI index has been reported to be the most important predictor of both types of blood pressure independent of life style and family history (Fu *et al*., 2003). Hypertension has been shown to be particularly important in women because of it being modifiable risk factor and is extremely prevalent in older women (August and Oparil, 1999).

Vigorous exercise has been proven the best way to help lower blood pressure (Diya, 2009). An immediate (acute) reduction in BP following exercise has been termed 'post-exercise hypotension' and is agreed to be caused by reductions in vascular resistance (the resistance to flow that must be overcome to push blood through the circulatory system) (Hamer, 2006). The beneficial effect of physical activity has been demonstrated by many trials. Data from more than 40 observational studies showed clear evidence of an inverse relationship between physical activity and all cause mortality (Kahn *et al*., 2002). Regular physical activity of about forty five minutes daily is associated with a significant 20-30% reduction in risk of all cause mortality. Contrarily it is also suggested that exercise has little effect on reducing resting systolic and diastolic blood pressures in children and adolescents (Alpert and Wilmore, 1994).

Regular participation in physical activity as well as a single exercise session can positively alter cholesterol metabolism (Durstine and Haskell, 1994). Exercise is involved in increasing the production and action of several enzymes that function to enhance the reverse cholesterol transport system (Durstine and Haskell 1994). Exercise has been shown to improve blood pressure, lower the risk of cardiovascular heart disease, improve lipid profiles (i.e. raise HDL, lower LDL and total cholesterol) and enhance insulin sensitivity (Diya, 2009). Many studies have shown that regular exercise is beneficial and increases the HDL level and decreases total cholesterol and LDL levels (Khan *et al*., 1987; Thompson *et al*., 1997; Leon and Sanchez, 2001). These responses are, however, affected several interacting factors such as age, sex, ethnicity, and steroid hormones, although the strongest predictors are exercise-induced plasma volume changes, dietary habits and initial fitness levels (Krummel *et al*., 1993; Leon, 1991).

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There are reports of no effect of exercise on lipid profile as of Rowland *et al.* (1996) and Welsman *et al.* (1997) did not observe any significant changes in blood lipid levels after aerobic training. Aellen *et al.* (1993) reported that exercise failed to induce beneficial alterations in the lipoprotein profiles, especially in the anti-atherogenics (reduces LDL and increases HDL). The study of Tsekouras *et al.* (2008) has reported the partial beneficial effects. These contrary results expound the need of further investigation in assessing the effect of exercise on lipid profile from different aspects.

Hypertension is becoming an increasing common disease in the developing countries (WHO, 1978) including Pakistan and because of traditional sedentary lifestyle women population is more vulnerable to increase in BMI and hypertension (N.H.S of Pakistan, 1997). The present study is carried out for a trial of evaluating the effect of exercise in the women particularly to promote healthy lifestyle and reduce health risks.

MATERIALS AND METHODS

The work was carried out on hypertensive subjects of different ethnic groups including Baloch (B), Pathan (P), Hazara (H) and Punjabi (PU) inhabitants of Quetta region of Balochistan. Participant volunteers were recruited from the local community, primarily through newspaper advertisements and through pasting posters in all departments of University of Balochistan, Bolan Medical College, hospitals, telephonic messages, emails and by counseling in different communities and also in various medical camps.

The subjects smoking, with history of CAD, renal impairment or proteinuria, hepatic impairment, gout or hyperuricemia, diabetic neuropathy or retinopathy was excluded from the trial. It was ascertained that participating subjects did not participate in an exercise program for at least 6 months prior to study and followed only the exercise protocol of the trial.

Assortment of hypertensive subjects were according to the WHO (1978) standard, where hypertension in adults is arbitrarily defined as systolic blood pressure to or greater than 160 mm Hg or/or diastolic pressure equal to or greater than 95 mm Hg. Resting systolic and diastolic blood pressure was measured indirectly using a standard sphygmomanometer (Wenzhou, China) after the subjects sat for 10 min rest. Measures were taken 3 times at 2 min interval and an average of the last two measures was calculated (Khan *et al.*, 1993).

Body mass and body height were measured in control as well as exercised group and body mass index was calculated following the procedure of Santos *et al.* (2001).

Sixty four sedentary hypertensive women participated in the study. All participants were free living and consumed self selected foods. Following 12 h fasting blood was drawn from 64 participating volunteer women with 16 from each ethnic group. Of these 32 (8 from each ethnic group) were prescribed exercise for 10 weeks. Motorized treadmill (Green master, china) was used for exercise. Exercise consisted of walking on the treadmill 4 times per week. Each session lasted about 50-60 min including a warm up time (5-10 min), fat burning period (10 min), aerobic exercise (15 min) the main performance with treadmill exercise (15 min) and a cool down period (10 min). After completion of 10 week exercise session the exercising along with non exercising participants were again sampled blood with 12 h fast and serum was stored at -20°C.

Body mass index, blood pressure (systolic and diastolic) and lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride) were estimated with commercial kits (Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany) under the similar laboratory conditions for all the control and exercise performers.

Statistical analysis was undertaken with statistical program of Sigma Stat 3.5. Student t test was used for comparison between normal and obese subject groups and $p<0.05$ was considered as statistically significant.

RESULTS

The blood pressure comprised Diastolic Blood Pressure (DBP) and Systolic Blood Pressure (SBP), while lipid profile comprised the monitoring of total Cholesterol (CHO), Low Density Lipoprotein Cholesterol (LDL), High Density Lipoprotein Cholesterol (HDL) and Triglycerides (TG) concentrations.

Age and BMI: In non exercising control subjects the mean age was 43.125±6.0, 44.3±5.4, 47.6±5.2 and 44.9±4.2 years in Baloch, Pathan, Hazara and Punjabi sub-populations respectively. In exercising volunteers average age was 48.9±6.6, 40.1±5.0, 43.4±4.9 and 38.9±5.2 years in Baloch, Pathan, Hazara and Punjabi sub-populations respectively (Table 1).

The average value of BMI in control female volunteers was 30.7±2.3, 31.0±1.6, 28.3±1.2 and 32.0±1.7 kg/m^2 and in exercising subjects was 28.9±2.3; 29.5±1.1; 29.0±1.2 and 28.0±0.8 kg/m^2 (Table 1).

Table 1: Age (years) and BMI (kg/m^2) of hypertensive control and exercise performing group

	Age (Control)	Age (Exercise)	BMI kg/m^2 (Control)	BMI kg/m^2 (Exercise)
Baloch	43.1±6.0	48.9±6.6	30.7±2.0	28.9±2.3
Pathan	44.3±5.4	40.1±5.0	31.0±1.6	29.5±1.1
Hazara	47.6±5.2	43.4±4.9	28.3±1.2	29.0±1.2
Punjabi	44.9±4.2	38.9±5.2	32±1.7	28±0.8

Blood pressure

Systolic: Exercise showed appreciable fall of systolic blood pressure in all ethnic subjects except in Baloch where slight reduction has been noticed. The statistically significant ($p<0.051$), ($p<0.023$) and ($p<0.040$) decrease levels of 9.7, 9.8 and 11% were noticed in P, H and PU subjects respectively in exercised group (Fig. 1).

Diastolic: Exercise of ten weeks manifested tremendous and significant ($p<0.001$), ($p<0.001$), ($p<0.015$), ($p<0.020$) reduction of 18.1, 12.8, 12.1 and 9.2% in DBP in B, P, H and PU ethnic groups, respectively (Fig. 2).

Cholesterol: Fraction of total cholesterol was significantly ($p<0.002$), ($p<0.007$), ($p<0.001$), ($p<0.018$) reduced in all ethnic groups with noticeable change of **14.3, 9.7, 9.8 and 10%** in B, P, H and PU subjects respectively. However, the level ranged between 261.1±6.6 to 263±6.6 mg/dl and 226±7.3 to 236 mg/dl in control and exercised hypertensive group correspondingly (Fig. 3).

LDL cholesterol: Exercise trained hypertensive subjects manifest marked and significant ($p<0.006$), ($p<0.002$), and ($p>0.001$) reduction in LDL-cholesterol levels in B, H and PU volunteers respectively. Nevertheless the levels ranged 164.5±7.6 to 173±1.8 mg/dl in controlled hypertensive and 47.8±4.4 to 156±3.4 mg/dl in exercised hypertensive subjects respectively.

HDL cholesterol: The HDL cholesterol levels in control hypertensive subjects ranged between 33.5±0.9 to 36±0.8 mg/dl and in exercised volunteers it varied between 43.5±2.5 to 47.8±1.7 mg/dl. Exercise exhibited tremendous and significant ($p<0.01$), ($p<0.001$), ($p<0.001$) and ($p<0.001$) rise with 26, 33, 42 and 25% of HDL-cholesterol levels in B, P, H and PU sub ethnic populations respectively.

Triglyceride: Exercise explicit evident and statistically significant ($p<0.001$), ($p>0.001$) and ($p<0.001$) fall in triglycerides levels with 14.2, 6.9 and 8.9% in B, P and H subjects, respectively. However, the levels ranged between 197±1.8 to 201.5±2.8 mg/dl in controls and 170.1±5.8 to 183.7±3.6 mg/dl in exercise followers.

DISCUSSION

It is a sign of modern times that increasing rates of urbanization and associated behavioral changes have led to a higher prevalence of a sedentary lifestyle and less exercise. For example, it is estimated that children today spend 600 kcal/day less on physical activity than their counterparts 50 years ago (Boreham and Riddoch, 2001). Unsurprisingly, we are facing an epidemic of hypertension, obesity, metabolic syndrome and diabetes mellitus-which, unless tackled proactively, will result in

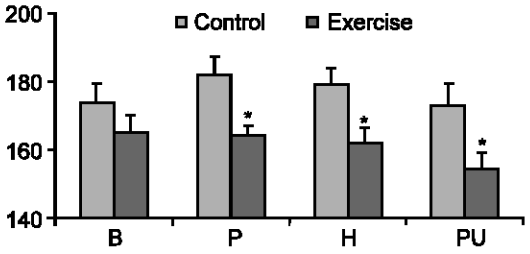

Fig. 1: Systolic blood pressure (mm Hg) in hypertensive female subjects of control and exercise trainers in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. ***p<0.001

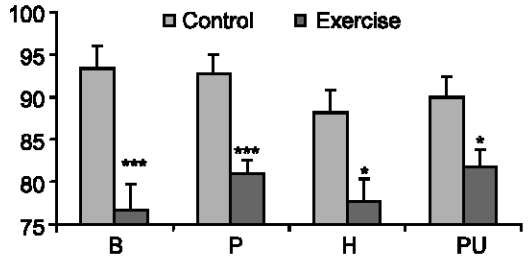

Fig. 2: Diastolic blood pressure (mm Hg) in hypertensive female subjects of control and exercise trainers in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. *p<0.05, ***p<0.001

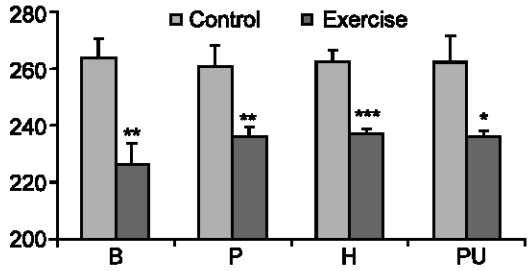

Fig. 3: Serum cholesterol mg/dl in hypertensive female subjects of control and exercise trainees in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. *p<0.05, **p<0.01, ***p<0.001

an increase in cardiovascular diseases, especially in the young and middle aged. Lifestyle changes-especially exercise- and relationships to hypertension may need greater attention, especially at a population level.

The overall results regarding blood pressure of present study suggested that exercise has beneficial and encouraging effect on reducing systolic and diastolic blood pressures in almost all studied pub-populations of hypertensive females. Blood pressure was significantly decreased in all ethnic groups except SBP in Baloch individuals. The SBP was markedly reduced in

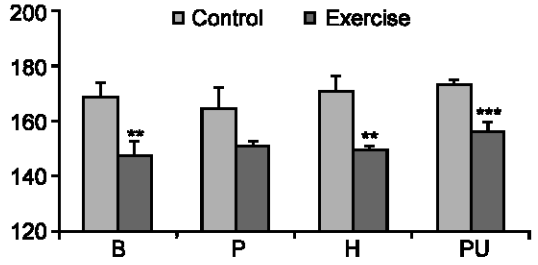

Fig. 4: Serum LDL cholesterol mg/dl in hypertensive female subjects of control and exercise trainers in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. **p<0.01, ***p<0.001

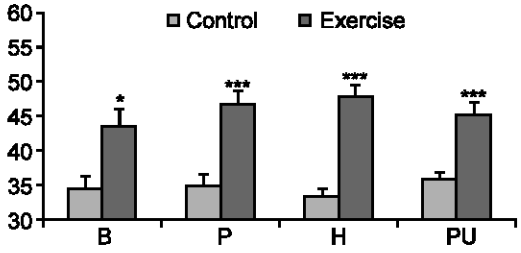

Fig. 5: Serum HDL cholesterol mg/dl in hypertensive female subjects of control and exercise trainers in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. *p<0.05, ***p<0.001

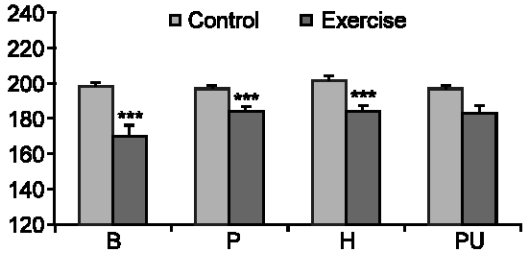

Fig. 6: Serum triglycerides mg/dl in hypertensive female subjects of control and exercise trainers in B (Baloch), P (Pathan), H (Hazara) and PU (Punjabi) ethnic groups. ***p<0.001

P (p<0.05), H (p<0.05) and PU (p<0.05), where as remarkable decrement [B (p<0.001), P (p<0.001), H (p<0.05) and PU (p<0.05)] was noticed in DBP in all ethnic groups.

Similar results have been reported by Kelley *et al.* (2003) and Tsai *et al.* (2002) who in 12 week exercise trials observed that the mean maximal reductions in clinic BP were 11 mm Hg for systolic and 5 mm Hg for diastolic pressure. Hagberg *et al.* (2000) and Chiriac *et al.* (2002) indicated that exercise training decreases Blood Pressure (BP) in approximately 75% of individuals with hypertension, with systolic and diastolic BP reductions averaging approximately 11 and 8mm Hg, respectively. Recently Kovacs *et al.* (2009) demonstrated that blood pressure decreased without any change in BMI or body fat seemed to confirm the independent effect of exercise. Women may reduce BP more with exercise training than men and middle-aged people with hypertension may obtain greater benefits than young or older people.

The study of Alpert and Wilmore, 1994 also supported these results that exercise reduced resting blood pressure in normotensive adolescents, but that aerobic exercise consistently reduced resting blood pressure in hypertensive adolescents. However, the study suggested that exercise has little effect on reducing resting systolic and diastolic blood pressures in children and adolescents.

The mechanisms associated with the chronic adaptations to blood pressure are more complex. A recent meta-analysis supports this chronic role being partially explained by a decreased systemic vascular resistance in which the autonomic nervous system and renin-angiotensin system (a hormone system that helps normalize long-term blood pressure and blood volume in the body) are most likely the underlying regulatory mechanisms (Cornelissen and Fagard, 2005). Another factor contributing to this decrease in vascular resistance is the increase of nitric oxide production (from different sites in the body) causing a vasodilation (increase in the internal diameter of a blood vessel that results from relaxation of smooth muscle within the wall of the vessel) in response to regular aerobic exercise.

Patients with hypertension also improve plasma lipoprotein-lipid profiles and improve insulin sensitivity to the same degree as normotensive individuals with exercise training. These results continue to support the recommendation that exercise training is an important initial or adjunctive step that is highly efficacious in the treatment of individuals with mild to moderate elevations in BP. A large number of studies from American college of sports (1994) indicated that endurance exercise training will elicit a 10 mm Hg average reduction in both systolic and diastolic blood pressures in individuals with mild essential hypertension (blood pressures 140-180/90-105 mm Hg).

The present study explores the beneficial effects of exercise on lipid profile also. In all ethnic groups total cholesterol was markedly decreased with 14.3, 9.7, 9.8 and 10% in B, P, H and PU groups respectively. Exercises manifested pronounce effect on LDL cholesterol with significant reduction in all groups except Pathan. Greater and pronounced outputs of HDL levels were noticed in exercised group with statistically significant elevation. Ten week of exercise training period explicit the positive response on triglycerides, marked and highly significant reduction has been observed in all ethnic groups except PU where non significant lowering was estimated.

Numerous studies and reviews have concluded that moderate exercise training has vital role on blood lipid

profile. Daniels (1999) reported that physical activity appears to have beneficial impact on lipid profile and lipoproteins. While significant improvements had been detected by different authors (Chang *et al*., 2008; Ferguson *et al*., 1999; Huang *et al*., 2007) and this could also explain our results. Studies of Gandapur *et al*. (2001) find out that prolonged aerobic exercise is capable of decreasing total cholesterol, LDL levels and particularly Apoprotein B levels and increasing HDL levels. A greater effect on HDL cholesterol has been indicated by Ferguson *et al*. (1998). Park and Ransone (2003) reported the existence of the threshold intensity of acute aerobic exercise necessary to promote a significant increase in HDL cholesterol (HDL-C).

Our finding are in confirmation of the most of the studies as it demonstrates that after exercise on treadmill for ten weeks, HDL cholesterol levels were statistically significant higher in all hypertensive female volunteers. The subject of the study are middle aged and almost on the final phases of the reproductive life. The earlier studies have reported of marked increase in HDL-C in women of this age. Lindheim *et al*. (1994) reported increased HDL-C levels in postmenopausal women that exercised. Generally, physically active women exhibit higher levels of HDL-C when compared to their sedentary counterparts (Kikkinos and Fernhall, 1999).

However reviewing the literature of Rowland *et al*. (1996) and Welsman *et al*. (1997), they could not observe any significant changes in blood lipid levels after aerobic training. Aellen *et al*. (1993) reported that exercise intensity acts as an important modulator of the beneficial effects of exercise on lipoprotein profile, since high intensity training (above the anaerobic threshold in cycling) failed to induce beneficial alterations in the lipoprotein profiles, especially in the anti-atherogenics (reduces LDL and increases HDL).Thus the results of the present study are unlike these reports. Tsekouras *et al*. (2008) examined the effect of high intensity intervals of aerobic training on VLDL-TG secretion in men. They observed that subjects trained on treadmill for 8 weeks had reduced VLDL-TG, our findings are consistent with these results whereby LDL significantly reduced in all hypertensive female ethnic groups except in Pathan sub-population.

Cauza *et al*. (2006) assessed that a 4 month training period, proved highly beneficial with reductions in fasting blood glucose, HbA1C, total cholesterol, LDL-cholesterol, triglyceride and an elevation in HDL-cholesterol concentrations in diabetes mellitus type 2 patients, thus resulting in a reduced atherogenic lipid profile. While in our findings cholesterol was significantly decreased in all female ethnic participants and triglycerides levels also shows marked reduction in all pub-populations, however in Punjabi hypertensive female's exhibit fall at low levels. Tsai *et al*. (2002) elaborated in his study that significant reductions were found in plasma total cholesterol (-6.1%), Low-Density Lipoprotein Cholesterol (LDL-C) (-14.1%) and triglyceride (-11.4%). Elevation of High-Density Lipoprotein Cholesterol (HDL-C) (+11.2%) was also noted.

On the reports of beneficial effect of exercise many national guidelines for the prevention and treatment of hypertension recommend lifestyle modifications in the form of 'regular aerobic exercise', as well as a reduction of dietary sodium intake, weight loss and moderation of alcohol intake (Appel *et al*., 2006).

It is also apprehended, although regular exercise training has beneficial effects on blood lipid profiles, a period of detraining as little as three months can offset all the advantages gained during training and reverse the beneficial effects of regular exercise training, thus underscoring the need for uninterrupted regular exercise throughout life.

Thus further studies from different aspects to determine precise effects of exercise on blood pressure and associated lipid profile remain in demand. Nevertheless exercise has evident beneficial effects on blood pressure and lipid profile of middle age female populations of different ethnic groups in study.

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Comparative Protection of Cowpea, *Vigna unguiculata* (L.) Walpers Against Field Insect Pests Using Cashew Nut Shell Liquid and Cypermethrin (Cymbush)

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Abstract: The efficacy of ethanolic extract of Cashew Nut Shell Liquid (CNSL) in protecting Cowpea, *Vigna unguiculata* L. against field insect pests was ascertained through two field trials at two different planting seasons by comparing with a suitable insecticide, Cymbush 10 EC containing 100 g Cypermethrin per litre of water under natural infestation at Akungba-Akoko, Nigeria. The liquid which is viscous and contains Phenolic compounds was first standardized in the screen house using concentrations of 0.01, 0.1, 0.25, 0.5 and 1.0% to determine a marginal position of high protective capability and non-toxicity to the leaves. About 1.0% proved most effective and it was subsequently transferred to the field for comparison with Cymbush. CNSL was evaluated through the assessment of Insect Pest Number and Percentage Flower Infestation. From these, results were obtained on pod characteristics, yield and yield components, which showed that the 1.0% formulation of CNSL selected was as effective as Cymbush showing a comparatively higher protective ability.

Key words: Cowpea, cashew nut shell liquid, protection, akungba-akoko, cypermethrin

INTRODUCTION

Due to the devastating effect of insect pests of Cowpea at almost every stage of its development, several approaches have been adopted in its control. Research into the control of these insect pests has centred primarily on the use of synthetic insecticides (Echendu, 1991). Amongst the insecticides are Azodrin, Thiodan, DDT, Dursban and Dimecron, which have been found to be effective against the leafhoppers. The use of leafhopper resistant cowpea varieties was also adopted at the International Institute of Tropical Agriculture (IITA, 1974). The varieties include Tvu59, Tvu123, VITA -1, VITA -3 that do not need insecticide protection against leafhoppers.

Research in recent years has been turning more towards selective biorational pesticides, generally perceived to be safer than the synthetic (Arnason *et al*., 1989), while, extensive works on the use of plant extracts in pest control were also documented (Mordue and Blackwell, 1993) and the use of inexperience and safe protectants of plant origins was extensively reviewed (Lale, 1995).

The use of CNSL has been gaining more attention due to its possession of the active Phenolic compounds, Anacardic acid and Cardol, which also have corrosive and abrasive properties, JOF Ideal Farm, Unpublished. It was demonstrated that low concentration of CNSL could be effective in controlling *Callosobruchus maculatus* (Echendu, 1991). Similar work was also reported in preventing oviposition in *C. maculatus* (Ofuya and Fayape, 1999).

This study reports the results of fieldwork at Akungba-Akoko on the comparative control of Cowpea using a protectant of plant origin, CNSL and a recognized synthetic insecticide, Cymbush 10 EC, which contains 100 g Cypermethrin per litre.

MATERIALS AND METHODS

The study was first conducted at Adekunle Ajasin University, Akungba-Akoko in 2005 but later reviewed at the same site between 2006 and 2007. The first field trial was conducted during the late planting season (August – November) of 2006 while, the second trial took place during the early planting season (April-June) of 2007.

The Cashew Nut Shell Liquid was obtained from a cashew processing factory in Owo, Ondo State, Nigeria. Different formulations of 0.01, 0.1, 0.25, 0.5 and 1.0% were made for screening in the screen house. The most effective concentration (1.0%) was subsequently transferred to the field for evaluation with cypermethrin in controlling cowpea against insect pests. The field experiments consisted of five blocks each measuring 2.1 m wide by 11 m long and consisting of three main plots corresponding to treatments. Planting distances were 80 cm between rows and 25 cm within rows. The plots were planted in randomized block design. The spray of Cymbush 10 EC, which contains 100 g

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Cypermethrin per litre was done at a recommended reduced dosage of 25 mL in 20 L of water in a full load of a conventional knapsack CP 20 sprayer. Spray treatments in the field studies resumed 2 Weeks after Planting (WAP), progressed forth nightly and terminated at eight WAP. Natural infestation of experimental plots by the insects was not monitored.

Evaluation of CNSL for the control of pest

Assessment of insect pest number: The number of insects present in each plot was determined through count forth nightly. Both sides of all the leaves on each plot were examined for the insects. Sampling consisted of counting in situ *Ootheca mutabilis*, *Aphis craccivora* and *Maruca testulalis*. Total insect count was computed for each plot.

Percentage flower infestation: Percentage flower infestation was measured by randomly collecting 20 flowers from the two outer rows of each plot (i.e. 10 flowers row^{-1}). The flowers were examined for any sign of insect presence or injury. Insect flowers were expressed as a percentage of the total number of flowers collected from each plot.

Effect of CNSL on yield and yield components

Pod analysis: Ten pods were randomly harvested at crop maturity from each plot. Each pod was examined for characteristic insect entry / exit holes. Pod damage was computed for each plot. Scores were thus, taken on Pod Load (PL), which measures the degree of successful pod production and Pod Damage (PD) as represented by entry holes and the presence of frass, both using a 1-9 scale (one the inverse of the other) developed at IITA (IITA, 1987, 1988; Jackai and Singh, 1988) as shown in Table 1.

From these scores, using [PL x (9-PD)], a Pod evaluation index (Ipe) was calculated for the determination of the extent of damage/the quality of cowpea as shown in Table 2. The performance of concentration was expressed on the pods using this index.

Evaluation of trial: The evaluation of trial was based on the determination of efficacy using Henderson-Tilton's formula (Puntener, 1981).

$$\% \text{ Efficacy} = \left(1 - \frac{Ta}{Ca} \times \frac{Cb}{Tb}\right) \times 100$$

Tb = Infestation in the treated plot before application (1.0% Extract or Cymbush)
Ta = Infestation in the treated plot after application (1.0% Extract or Cymbush)
Cb = Infestation in the check plot before application
Ca = Infestation in the check plot after application

Data collection and analysis: The data collected up to the date of termination of experiment included the flea beetle and aphid infestation count, *Maruca* flower infestation, pod-sucking bug damage and yield. All data were subjected to Analysis of Variance (ANOVA) and means compared for significance differences using Least Significance Difference (LSD) values at the 5% level of probability of Tukey's Honestly Significance Test. Counts were normalized by square root and arc sin transformations $(x + 0.5)^{1/2}$.

RESULTS

The selection of 1.0% from the screen house experiment and its transfer to the field was based on its higher mortality rate compared to other lower concentrations.

Table 1: Scores for Pod Load (PL) and Pod Damage (PD) and selection criteria for field evaluation of cowpea resistance to selected insect pests

PL score	PD score
1	9
2	8
3	7
4	6
5	5}
6	4}
7	3} Selection range
8	2}
9	1}

Table 2: Matrix of pod evaluation index (Ipe) used for the screening of the extent of damage done by pod insects

	Pod Damage (PD)								
	Undamaged							Heavily damaged	
PL	1	2	3	4	5	6	7	8	9
Poor 1	8	7	6	5	4	3	2	1	0
2	16	14	12	10	8	6	4	2	0
3	24	21	18	15	12	9	6	3	0
4	32	28	24	20	16	12	8	4	0
5	(40)	(35)	(30)	(25)	(20)	15	10	5	0
6	(48)	(42)	(36)	(30)	(24)	18	12	6	0
7	(56)	(49)	(42)	(35)	(28)	21	14	7	0
8	(64)	(56)	(48)	(40)	(32)	24	16	8	0
Heavy 9	(72)	(63)	(54)	(45)	(36)	27	18	9	0

Equation: Ipe = PL x (9 - PD), Values in parenthesis ( ) represent the selection range derived from Table 1. Underlined '--' numbers are outliers, which are within the selection range

Table 3: Cumulative means of parameters of cowpea in the two field trials

Treatment (%)	Mean insect count	Flower infestation (%)	Pod load	Pod damage	Pod evaluation index (Ipe)	Mean seed count	Converted (Ipe) values
1.0	14.18±3.6a	0.1±0.0a	7.5±0.5b	1.7±0.1a	54.47±2.5b	1598.8±14.4b	6.8b
Cymbush	7.55±3.7a	0.06±0.0a	8.0±0.1b	1.5±0.1a	60.11±3.6b	1624.0±12.5b	7.5b
Control	47.15±2.5b	0.28±0.1b	5.0±0.0a	2.4±0.0b	32.64±2.1a	488.8±6.8a	4.1a

Means in each column bearing the same letter are not significantly different at the 5% level of probability by Tukey's test

Table 4: Correlation matrix showing the relationships of the cumulative of the various parameters measured in the use of cymbush in the two field trials

	(%) Flower infestation	Ipe value	Seed count
Insect count			
Flower infestation (%)	+0.99771		
Ipe value	-0.90869	-0.84216	
Seed count	-0.99206	-0.97209	+0.93964

Table 5: Correlation matrix showing the relationships of the cumulative of the various parameters measured in the use of 1% concentration in the two field trials

	(%) Flower infestation	Ipe value	Seed count
Insect count			
Flower infestation (%)	+0.95665		
Ipe value	-0.99926	-0.95389	
Seed count	-0.99842	-0.95434	+0.99551

Table 3 shows the cumulative of the means of yield parameters of cowpea obtained in the two field trials. This result shows that a direct relationship exists between insect count and other parameters (Ofuya, 1989; Annan, 1992) that pest density threshold is an important factor in influencing aphid damage to plant growth and yield. The highest infestation was observed in the control. The parameters observed in Cymbush and 1.0% concentration showed no significance difference. Since, infestation has the tendency of influencing yield (Ofuya, 1989; Annan, 1992) and severe infestation could greatly reduce pod production (Jackai, 1995), the different levels of infestation in Cymbush and extract protected plants have resulted in no significant difference $p<0.05$ in the other yield parameters. The converted Ipe Values in Table 3 were derived from the Ipe Equation where, heavy pod is assigned the value of 9 to which, other values were related. Table 4 and 5 show the Correlation Matrix of the various parameters measured in the use of Cymbush and 1.0% Ethanol extract of Cashew Nut Shell Liquid.

The insect count was positively correlated to Flower infestation and the seed count was also positively correlated to the Pod Evaluation Index while, other relationships were negatively correlated. From the determination of %. Efficacy using Henderson-Tilton, Cymbush and 1.0% Extract of CNSL have 84.00 and 70.00%, respectively.

DISCUSSION

Considerable efforts have been made world-wide to find safer biodegradable substitutes for synthetic insecticides (Crombie, 1990). Among these efforts is the use of Cashew Nut Shell Liquid (CNSL), whose efficacy as seed protectant against *C. maculatus* L. on cowpea seeds in storage was reported (Echendu, 1991; Ofuya and Fayape, 1999.). Similar work on the protection of Okra, *Abelmoschus esculentus* L. against *Podagrica* beetles was also documented (Olotuah, 2003). CNSL is a viscous liquid, although it has been confirmed to contain the phenolic compound, which is active in pest control and has been tested and found successful as bactericide (Unpublished). Since the use of plant products in pest control had been documented and had been found reliable, the use and economic of use of CNSL is as well desirable. Moreover, the hazards often associated with the use of some synthetic insecticides have not been reported from the use of CNSL. Due to the viscosity of CNSL, it became pertinent to standardize this liquid using a suitable solvent and also to determine its optimal level of protective capability in the screen house to obtain a concentration that will be non-toxic to the plant but will effectively protect it against insect pests.

The observed Yield vigour results from efficacy of insecticide in pest control while, the Low Ipe value indicates that there is no resistance to flower damage and that severe damage at the flowering stage could result in poor production, with concomitant low Ipe values (Jackai, 1983).

It must be stressed that the more effective the insecticide the more its protective capacity and consequently the greater the yield.

The determination of efficacy of CNSL against the use cypermethrin in this study had revealed that severe damage due to high pest densities could result in poor yield and the application of 1.0% CNSL, a natural plant extract in ethanol could be an effective protectant and a substitute to the synthetic insecticides.

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Gender Differences of Body Mass Index in Adults of Pakistan: A Case Study of Multan City

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Abstract: Obesity is an epidemic health problem worldwide that can result in many serious and sometimes, fatal diseases. It is very important to study such prevalence in developing countries, like Pakistan where people cannot afford the medical tolls, additionally, added to their non-meeting budgets, due to problems of obesity. The present study addresses the same issue by taking into account of 2000 adults from Multan city as case study of Pakistan. Although many of similar studies have also been carried out in the region but the present study evaluates the obesity prevalence according to recommendations of World Health Organization that in Asia Pacific Region, a person is considered to be overweight if BMI $\geq$ 23 rather BMI $\geq$ 25 and to be obese if BMI $\geq$ 25 rather than if BMI $\geq$ 30. According to this new definition, we report that more than 46% people are overweight (18.95%) and obese (27.85%). The percentage of normal people is just 28.65 while 24.55% are underweight. We report the mean MBI to be 22.87±0.086 (S.E), males have 13 kg more weight as compared to that of females have and males are more than 5 inches taller as compared to females, on the average. It is noted that mean BMI of males (23.51±0.11) and mean BMI of females (22.05±0.133) are different significantly. We find that 55.12% among males and 36.15% among females are either overweight or obese. The percentile plot of the data also displays the similar picture. We further report that married people are three times obese as compared to unmarried ones.

Key words: Obesity, BMI of females, BMI of males

INTRODUCTION

Obesity is used to describe body weight that is much greater than what is considered healthy. It is considered a worldwide challenge to public health. As it has been related to numerous health risks, both physical and psychological therefore, its prevalence has led the World Health Organization (WHO) to declare it a "global epidemic" (WHO, 1998). On the behalf of International Diabetes Federation (2004), Jawad (2005) reports that more than 1.1 billion people in the world are estimated to be overweight and 320 million are calculated to be obese. More than 2.5 million deaths each year are attributed to obesity, a figure expected to double by 2030. Many of recent studies show that obesity should be taken scarily as it can result in many serious, and potentially deadly, health problems. These problems include hypertension, Type II diabetes mellitus, coronary diseases, infertility, kidney diseases and a higher risk for certain forms of cancer, such as those that affect the colon, prostate, endometrium and possibly breasts (Bray *et al*., 1998; Marion and Jacobson, 2000; Ferris, 2007).

In developed countries, obesity is an intensifying public health problem. For example, Mokdad *et al*. (2002) report that 19.8% of U.S. adults in 2000 (20.2% for males and 19.4% for females) are obese and its prevalence rate is rising alarmingly. Zohoori *et al*. (1998) show that the obesity rate among men aged 18-60 has increased considerably from 1992-1996 in Russia. Peytremann-Bridevaux (2007) reports the increase in prevalence of obesity in different European countries. Similarly, as a notion of obesity to be world wide health issue, El-Hazmi and Warsy (1997) estimate that 13.05% of Saudi males and 20.26% females also being obese.

Such irksome conditions of obesity prevalence are not much different in Pakistan. Bharmal (2000), foucing in Pakistan, reports that obesity in childhood and adolescent is increasing in developing countries. On the basis of National Health Survey of Pakistan (1998,) Pappas *et al*. (2001) report on the health status of the Pakistani population that the overweight prevalence rates for adults aged 25-64 years are 13.2% for men and 22.6% for women. Nanan (2002) also gives the similar comments about Pakistani population w.r.t. obesity. Afridi and Khan (2004) also reported similar details.

There are many ways to determine if a person is obese, but a person's Body Mass Index (BMI), defined as the ratio of weight (kg) to squared height (m^2), has been popularly used as a measure of overweight and obesity. According to WHO's standards, a person is overweight if BMI $\geq$ 25 and is obese if BMI $\geq$ 30.

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According to Kan and Tsai (1993), the possession of knowledge on obesity's health risks prevents an individual from being overweight. So by the analysis of BMI, we can, implicitly, assume it to contribute to lessen the said hazards of obesity for public. The growth of obesity usually, results in high medical expenditures and is elevated risk of mortality and morbidity likely to accompany it. Obesity accounts for 2-6% of total health care costs in several developed countries (Khan *et al*., 2008). Thus, there is a need to study such prevalence in developing country like Pakistan where people cannot afford to spend more on medical tolls due to their financial conditions. But most of the developing, transitional and newly industrialized countries are still not alert from the above-mentioned hazards of obesity. Pakistan is one amongst such countries.

In available literature, many studies can be found about the prevalence of obesity in Pakistan. But majority of them follow the same BMI cut-offs as defined by WHO, internationally and have already defined above. Such cut-offs are used by many of the researchers, in their studies, namely, Kiyani *et al*. (2002), Rehman *et al*. (2003), Shah *et al*. (2004), Khurram *et al*. (2006) and Khan *et al*. (2008) among many others, addition to them already mentioned above. But Nanan (2002), in a study, concludes, "In South Asia, including Pakistan, social and environmental changes are occurring rapidly, with increasing urbanization, changing lifestyles, higher energy density of diets and reduced physical activity. The coexistence of underweight in early life with obesity in adults may presage both a higher prevalence and incidence for Noncommunicable Diseases (NCDs) such as hypertension and diabetes". Thus, she favours the provisional recommendations for Asia Pacific Region published in February 2000 by the WHO Regional Office for the Western Pacific. These recommendations state to use of BMI $\geq$ 23 for overweight and BMI $\geq$ 25 for obesity, for the countries of Asia Pacific Region. So according to Nanan (2002), these new definitions may provide a more accurate determination of the health of Pakistanis, especially in those with more than one risk factor for NCDs. Recently, a large study in Chinese population has been published, with same reference (Leung *et al*., 2008). Such details can also be found by Jaleel (2009).

The main objective of the present study is two-fold; first to study the obesity prevalence in adults in Pakistan by taking Multan city as a case study, according to new bounds of BMI and the second to study the differences in BMI among male and female adults. The reason for the study to be focused on adults is that according to Guo *et al*. (1994), the risk of being overweight in adulthood is greater with higher degrees of being overweight in childhood (Bovet *et al*., 2004; Padula, 2008).

MATERIALS AND METHODS

A cross-sectional data comprising 2000 adult (aged 14 years or more) individuals, both males and females were taken from Multan city from January 1, 2007 to December 31, 2008. The sample was taken by convenient sampling, from Bahauddin Zakariya University, Multan, different colleges and different public places, including parks and markets etc. For the present study, we take data on the variables, gender (1 = male and 2 female), marital status (0 = unmarried and 1= married), age (in years, rounded to next year), weight (in kg) and height (in inches).

BMI of the individuals are calculated as weight in kilograms divided by height in meters squared, using measurements obtained from medical examinations employing standardized procedures and equipments:

$$\text{BMI} = \frac{\text{Weight in kg}}{(\text{Height in meters})^2}$$

According to the recommendations of WHO (2000) for Asia Pacific Region, a person will be underweight (if BMI $\leq$ 19), normal (if 20$\leq$ BMI < 23), overweight (if BMI 23$\leq$ BMI < 25) and obese (BMI $\geq$ 25).

RESULTS AND DISCUSSION

In our data set of 2000 individuals, 1123 are males (56.2%) and 877 are females (43.8%). Moreover, among these 2000 respondents, 1501(75.05%) are unmarried and 499 are married (24.95%). The mean age of the respondents is 24.35±0.16 (SE) years. These figures are 25.92±0.23 and 22.33±0.18 for males and females, respectively.

The summary of weight (in kg) and height (in inches) are given in Table 1 along with mean comparisons of males and females, using t-test. The table shows that mean weight of males is significantly greater than that of females and similar findings for mean height. We note that males have about 13 kg more weight, on the average, as those of females have. Similarly, males are about 5 inches taller, on the average, as compared to females.

We compute BMI as mentioned above. We report the mean MBI to be 22.87±0.086 (SE) that reflects that the respondents are normal in regard of obesity, on the average. But the mode value of 23.46 shows that majority of the respondents are overweight, according to new WHO's standards for the regions in which Pakistan lies. Furthermore, the value of median shows that more than 50% of the respondents have BMI greater than 22.72. It is a clear notion that people have notable tendency to become overweight and stepping towards obese. It can be noticed that mean, median and mode of BMI are almost equal and the same can be depicted by Fig. 1 that shows the symmetric histogram of BMI.

Table 1: Summary of weight (kg) and height (inches)

Variable	Gender	N	Mean	SE	t-statistic	d.f	p-value
Weight	Male	1123	68.11	0.318	27.92	1998	0.00
	Female	877	55.23	0.327			
	Total	2000	62.46	0.269			
Height	Male	1123	67.06	0.090	36.42	1998	0.00
	Female	877	62.37	0.088			
	Total	2000	65.01	0.082			

Table 2: Obesity status (Comparison among males and females)

	Obesity status				
Gender	Underweight	Normal	Overweight	Obese	Total
Male	199 (17.72)	305 (27.16)	240 (21.37)	379 (33.75)	1123
Female	292 (33.30)	268 (30.56)	139 (15.85)	178 (20.30)	877
Total	491 (24.55)	573 (28.65)	379 (18.95)	557 (27.85)	2000

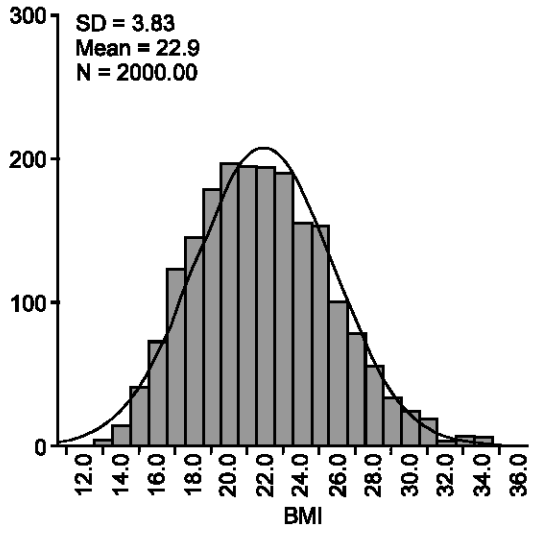

Fig. 1: BMI histogram

Table 2 presents the frequencies and percentages (shown in parentheses) of the underweight, normal, overweight and obese persons dividing in 1123 males and 877 females and also the same measures for the overall sample of 2000. From the table, we note that more than 46% people are overweight and obese. Percentage of normal people is just 28.65 while 24.55% are underweight. When we want to note the gender differences so far, we see that percentage of female underweight is double than that of males while 55.12% among males and 36.15% among females are either overweight or obese. We also compute Chi-square test for the association between gender and obesity status showing the similar findings that obesity status is highly associated with gender (Chi-square statistic = 87.266, d.f = 3 and p-value = 0.00). So, generally, in Multan city, males have higher tendency to become obese as compared to females. The same is also true when we compare mean BMI of males (23.51±0.11) and mean BMI of females (22.05±0.133), we note that mean BMI of males is significantly (p-value = 0.00) larger than that of females. These estimated means of BMI for males and females are quite closer as reported by Shah *et al.* (2004) in their study and according to them the mean BMI was 22.4 (95% CI; 21.9, 22.9) for men and 22.6 (95% CI; 21.9, 23.2) for women. But they further use the previously available definitions of BMI as discussed earlier.

Table 3 gives a comparison between married and single adults. It presents the frequencies and percentages (shown in parentheses) of the underweight, normal, overweight and obese persons dividing in 1501 unmarried and 499 married people. We note that married people are about three times obese as when compared with unmarried ones. The percentage of married people who are successful in controlling their weights is almost half to that for unmarried ones. The one chief reason for these figures is that married people are indulged in many activities regarding economical, their children, social commitments etc. so they are not able to take good care of their health. The percentage of unmarried underweight is about five times as compared to that of married people. When we compare the mean weights of married and unmarried people, it is found that mean weight married people is about 10 kg more than that of unmarried people (p-value = 0.00).

Fig. 2 portrays a percentile plot where 10^{th}, 20^{th}, … , 90^{th} percentiles have been drawn against the BMI for males and females in order to indicate gender differences and for the whole sample as well. According to new WHO standards, we take BMI cut-offs at 19, 23 and 25. The same interpretation can be derived as Table 3 does. The overweight of males starts above 40^{th} percentile while the same starts above 60^{th} percentile for females. The obesity for males exits between 60^{th} and 70^{th} percentile while the same begins near 80^{th} percentile for females. The plot also shoes that about one-quarter number of females are underweight.

Table 3: Obesity status (Comparison among married and unmarried people)

	Obesity status				
Marital status	Underweight	Normal	Overweight	Obese	Total
Unmarried	457 (30.45)	492 (32.78)	267 (17.79)	285 (18.99)	1501
Married	34 (6.81)	81 (16.23)	112 (22.44)	272 (54.51)	499
Total	491	952	479	78	2000

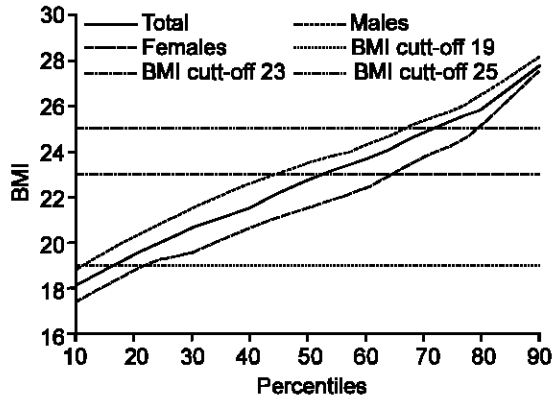

Fig. 2: Percentile plot

Conclusion: The present study about BMI of 2000 adults including 1123 males (56.2%) and 877 females (43.8%) conducted in Multan city, concludes that males have about 13 kg more weight, on the average, as that of females have. According to new recommendation of WHO regarding obesity definition in Asia Pacific Region, a considerable number (more than 46%) of the respondents is found to be overweight and obese. It is reported that the percentage of female underweight percentage of female underweight is double than that of males and males are about 20% more overweight and obese as compared to females. When we compare obesity of married people as compared to unmarried ones, we report that married people are about three times obese as compared to unmarried ones. These figures show an alarming situation that needs serious attention for policy makers and the people of health concern.

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Haematological Parameters of Savanna Brown Does Fed Varying Dietary Levels of Flamboyant Tree Seed Meal

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Abstract: Fifteen (15) nuliparous Savanna Brown does aged 6-8 months with a mean live weight of 9.55 kg were randomly allotted into five dietary treatments comprising of three animals per treatment. Five different diets with varying levels of flamboyant tree seed meal were fed as supplement at the rate of 0.50 kg/head/day. T_1 which had no flamboyant tree seed served as control while diet T_2, T_3, T_4 and T_5 which served as the treatment diet had 25, 50, 75 and 100% flamboyant tree seed meal, respectively. The animals were managed semi-intensively. 5 mls of blood samples were collected via jugular vein puncture into well labeled EDTA bottles and were immediately placed in an ice-chest containing ice cubes and analyzed within 2 h of collection for blood glucose, protein and plasma urea. The average total blood glucose and protein were significantly ($p<0.05$) different with diets T_1 and T_2 recording higher values while plasma urea level did not differ significantly ($p<0.05$) among the treatment groups. All the values obtained were within the recommended normal range. It is therefore concluded that up to 100% level of inclusion of flamboyant tree seed meal in the diets of Savanna Brown does was not deleterious on the blood parameters.

Key words: Haematology, savanna brown does, flamboyant tree seed meal

INTRODUCTION

Goats constitute a very important component of the livestock sub-sector of the Nigerian agricultural economy. The potential of goat production in alleviating the low level of consumption of animal protein by human in developing nations, Nigeria inclusion needs no emphasis (Animashaun *et al*., 2006). The high cost of formulating concentrate and pelletized feed has been a major constraint militating against the increased production of valuable sources of animal protein (Animashaun *et al*., 2006). Hence, the urgent need to incorporate non-conventional feedstuffs into goat diet. One of such feedstuffs is the flamboyant tree (*Delonix regia*) seed which is widely grown as an ornamental plant.

Animashaun *et al*. (2006), observed that nutritional studies should not be limited to performance, carcass quality and nitrogen alone, but the effect on blood constituent is also very relevant. Laboratory tests on the blood are very vital tools that help to detect any deviation from normal in the animal or human body. Haematology aid the clinician to arrive at a definitive diagnosis of a disease, enables him/her to make a prognosis and also to assess the efficiency of therapy and toxicity of drugs and chemical substances (Ihedioha and Ibeachu, 2005). Currently in veterinary practices, a diagnosis is considered incomplete or not definitive if information obtained from history and chemical examination is not combined with laboratory test results including the result of haematology (Ihedioha and Ibeachu, 2005).

Consequently, this study aims to determine the effect of replacing groundnut cake with varying levels of flamboyant tree seed meal on some blood parameters of Savanna Brown does.

MATERIALS AND METHODS

The study was conducted at the Ruminant Animal Production Unit of the Teaching and Research Farm, Federal University of Technology, Minna, Niger State. The study area lies within the Southern Guinea Savanna ecological zone of Nigeria. It has a mean annual rainfall of 1102.6-1361.7 mm and an annual temperature range of between 26.66°C and 27.77°C.

Fifteen (15) nulliparous Savanna Brown does aged 6-8 months with average body weight of 9.55 kg were used for the study which lasted 12 weeks. The animals were allowed a pre-treatment periods of two weeks prior to study in order to enable them acclimatize. They were given prophylactic treatment against helminthes and other parasites and were randomly allotted into five treatment groups with three animals per treatment. Flamboyant tree seed were collected during the dry season, dried properly and roasted using open flame for 15 min in an open pot with little sand to prevent friction and burning. The roasted seed was sieved, ground and incorporated at different levels into the experimental diets.

The five treatment diets were designated T_1, T_2, T_3, T_4 and T_5. The feeds were formulated to be isonitrogenous and isocaloric. T_1 served as the control with 0% inclusion of flamboyant tree seed and 100% groundnut cake while T_2, T_3, T_4 and T_5 had the groundnut cake component substituted for flamboyant tree seed meal at 25, 50, 75 and 100%, respectively (Table 1). The

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Table 1: Composition of experimental diets fed to Savanna Brown does during the experimental period

	Treatments				
Ingredients	T_1	T_2	T_3	T_4	T_5
Maize grain	65.35	55.10	43.50	31.90	20.57
Rice waste	29.91	37.18	48.78	60.38	71.71
Groundnut cake	6.22	4.66	3.11	1.56	0.00
Flamboyant tree seed meal	0.00	1.56	3.11	4.66	6.22
Bone meal	0.75	0.75	0.75	0.75	0.75
Salt	0.75	0.75	0.75	0.75	0.75
Total (kg)	100.00	100.00	100.00	100.00	100.00
Crude protein (%)	12.05	11.99	11.99	12.00	11.99
Energy (Kcal/kg)	3220.75	3197.13	3167.82	3138.52	3110.13

T_1 = 0% Flamboyant tree seed meal T_2 = 25% Flamboyant tree seed meal T_3 = 50% Flamboyant tree seed meal
T_4 = 75% Flamboyant tree seed meal T_5 = 100% Flamboyant tree seed meal

Table 2: Proximate composition of the experimental diets raw and roasted Flamboyant Tree Seed Meal (FTSM)

Nutrients (%)	T_1	T_2	T_3	T_4	T_5	Raw FTSM	Roasted FTSM
Dry matter	91.00	92.01	91.00	93.00	90.00	81.80	89.40
Moisture	9.00	7.99	9.00	7.00	10.00	12.20	10.60
Crude protein	12.07	12.01	11.99	12.03	12.01	18.10	18.92
Crude fibre	12.25	18.32	22.86	24.69	29.35	7.50	11.00
Ether extract	13.36	17.38	10.74	18.16	13.96	7.50	9.00
Ash	5.01	7.00	7.00	9.01	11.00	3.60	3.40
Nitrogen Free Extract	57.31	45.29	47.41	36.11	33.68	63.30	57.70
Energy (Kcal/kg)	3977.60	3856.20	3342.60	3560.00	3084.00	3931.00	3874.70

T_1 = 0% Flamboyant tree seed meal T_2 = 25% Flamboyant tree seed meal T_3 = 50% Flamboyant tree seed meal
T_4 = 75% Flamboyant tree seed meal T_5 = 100% Flamboyant tree seed meal

animals were supplied clean water and salt lick *ad-libitum* and were allowed adequate grazing time from 10.00 am to 4.00 pm. 5 mls of blood were collected via the jugular vein of each animal into bottles containing disodium salts of Ethylene Diamine Tetra-acetic Acid (EDTA) as anti-coagulant. The EDTA bottles containing blood samples were transferred into an ice chest containing ice cubes and taken to the laboratory for analysis. The parameter analyzed were blood glucose, protein and urea.

The data obtained were subjected to one way analysis of variance (ANOVA) while means were separated using the Duncan's (1955) multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Proximate composition of the raw and roasted flamboyant tree seeds and the experimental diets is presented in Table 2. Dry matter, crude protein, crude fibre and ether extract were higher in the roasted than raw seeds while Moisture, Ash, Nitrogen Free Extract and Energy were higher in the raw seeds. The anti-nutritional factors in the raw and roasted seeds are presented in Table 3. The anti-nutritional factors in the raw seeds were greatly reduced by roasting. This is in agreement with the findings of Grant *et al.* (1991) that traditional processing methods can effectively reduce anti-nutritional factors in legume seeds. Table 4, 5 and 6 show the weekly record of haematological parameters

Table 3: Anti-nutritional composition of raw and roasted flamboyant tree seed

Factors	Raw flamboyant tree seed	Roasted flamboyant tree seed
Tannin (mg/100 g)	93.10	11.20
Phytate (mg/100 g)	2.13	0.58
Saponin (%)	12.23	2.22
Typsin inhibitor (mg/g)	273.00	62.00

observed in the does. With the exception of the 1st and 4th week of study, the blood glucose level differed significantly ($p<0.05$) among the different groups. Remarkable decline was observed for animals in T_3-T_5 in the 7th and 8th week of study. Weekly protein level did not differ significantly ($p<0.05$) among treatments in the 1st six weeks of study. However, in the 7th and 8th week, a significant ($p<0.05$) decline in T_3-T_5 was recorded. Except in the 2nd week of study, the plasma urea level showed significant differences among all the treatment group. The overall summary of the value obtained for blood glucose, protein and plasma urea is presented in Table 7. Blood glucose and protein differed significantly among the treatments. It was observed that the weekly readings and cumulative reading obtained fell within the normal range prescribed by Fasae *et al.* (2005). The increase in total blood protein reflects the ability of the animals to store reserve protein when animals have reached the maximum capacity for less liable protein intake (Fasae *et al.*, 2005). Plasma urea levels

Table 4: Average weekly blood glucose level of savanna brown does fed varying levels of flamboyant tree seed meal

	Diets							
Weeks	T_1	T_2	T_3	T_4	T_5	SEM	LS	N/R
1	1.40[a]	1.36[a]	2.16[a]	1.40[a]	2.10[a]	0.61	NS	3.5-6.0
2	3.67[ab]	3.43[ab]	4.23[a]	3.80[ab]	4.30[a]	0.97	*	3.5-6.0
3	3.73[ab]	3.60[ab]	4.37[a]	3.93[ab]	4.45[a]	0.87	*	3.5-6.0
4	4.63[a]	3.60[a]	4.37[a]	4.30[a]	4.25[a]	0.63	N S	3.5-6.0
5	4.77[a]	4.83[a]	3.17[ab]	2.80[b]	4.55[a]	1.48	*	3.5-6.0
6	5.10[a]	5.13[a]	3.50[b]	3.03[b]	4.90[a]	1.55	*	3.5-6.0
7	5.17[a]	5.50[a]	3.50[ab]	3.23[ab]	2.45[b]	2.30	*	3.5-6.0
8	5.13[a]	5.53[a]	3.53[ab]	3.55[ab]	2.55[b]	2.42	*	3.5-6.0

[a,b]Means along the same row with the same superscript are not significantly (p>0.05) different.
SEM = Standard Error of Means LS = Level of Significance NS = Not Significantly Different (p>0.05) N/R = Normal Range.
T_1 = 0% Flamboyant tree seed meal T_2 = 25% Flamboyant tree seed meal T_3 = 50% Flamboyant tree seed meal
T_4 = 75% Flamboyant tree seed meal T_5 = 100% Flamboyant tree seed meal

Table 5: Average weekly total protein level of savanna brown does (mg/l) fed varying level flamboyant tree seed meal

	Diets							
Weeks	T_1	T_2	T_3	T_4	T_5	SEM	LS	N/R
1	6.57[a]	7.83[a]	6.80[a]	6.47[a]	6.85[a]	0.76	NS	6.4-7.0
2	6.97[ab]	7.43[a]	7.50[a]	7.60[a]	7.60[a]	0.59	NS	6.4-7.0
3	7.03[a]	7.40[a]	6.60[a]	7.57[a]	7.65[a]	0.59[a]	NS	6.4-7.0
4	6.27[a]	6.60[a]	7.67[a]	7.63[a]	7.75	0.1.07	N S	6.4-7.0
5	6.43[a]	8.43[a]	5.83[a]	5.47[a]	8.45[a]	2.85	NS	6.4-7.0
6	6.79[a]	7.03[a]	6.00[a]	5.57[a]	8.30[a]	2.46	NS	6.4-7.0
7	7.70[a]	8.37[a]	5.03[ab]	5.30[ab]	4.35[b]	3.75	*	6.4-7.0
8	7.67[a]	8.20[a]	5.03[ab]	5.23[ab]	4.40[b]	3.75	*	6.4-7.0

[a,b]-Means along the same row with same superscript are not significantly (p>0.05) different
SEM = Standard Error of Means LS = Level of Significance N/R = Normal Range
T_1 = 0% Flamboyant tree seed meal T_2 = 25 % Flamboyant tree seed meal T_3 = 50% Flamboyant tree seed meal
T_4 = 75 % Flamboyant tree seed meal T_5 = 100% Flamboyant tree seed meal

Table 6: Average weekly urea level in the blood of savanna brown does (m/mol) fed varying level of flamboyant tree seed meal

	Diets							
Weeks	T_1	T_2	T_3	T_4	T_5	SEM	LS	N/R
1	6.20[ab]	4.36[b]	5.00[a]	6.33[ab]	6.95[a]	1.12	*	4.5-8.5
2	5.07[a]	5.33[a]	6.20[a]	6.23[a]	5.80[a]	0.96	NS	4.5-8.5
3	5.37b	5.60[ab]	6.27[ab]	6.87[a]	5.95[ab]	0.67	*	4.5-8.5
4	5.90[ab]	6.13[a]	6.03[a]	6.40[a]	4.70[b]	1.67	*	4.5-8.5
5	6.10[a]	6.20[a]	3.83[b]	4.37[b]	4.80[b]	2.34	*	4.5-8.5
6	6.63[a]	6.43[a]	4.07[b]	4.57[b]	5.45[a]	2.24	*	4.5-8.5
7	6.47[a]	6.70[a]	4.47[b]	4.73[b]	3.10[b]	3.08	*	4.5-8.5
8	6.50[a]	6.63[a]	4.27[b]	4.77[b]	3.50[b]	3.08	*	4.5-8.5

[a,b]Means along the same row with same superscript are not significantly (p>0.05) different.
SEM = Standard Error of Means LS = Level of Significance N/R = Normal Range
T_1 = 0% Flamboyant tree seed meal T_2 = 25% Flamboyant tree seed meal T_3 = 50% Flamboyant tree seed meal
T_4 = 75% Flamboyant tree seed meal T_5 = 100% Flamboyant tree seed meal

Table 7: Mean contents of some blood parameters in savanna brown does fed varying levels of flamboyant tree meal

	Diets							
Parameters	T_1	T_2	T_3	T_4	T_5	SEM	LS	N/R
Blood glucose (mmoi/l)	4.20[a]	4.28[a]	3.60[ab]	3.23[b]	3.69[ab]	0.29	*	3.5-6.0
Protein(mg\l)	6.95[ab]	7.79[a]	6.43[ab]	6.09[b]	6.92[ab]	0.48	*	6.4-7.0
Urea (mmol\l)	6.03[a]	5.59[a]	5.01[a]	5.53[a]	5.03[a]	0.41	NS	4.5-8.5

[a,b]Means along the same row with same superscript are not significantly (p>0.05) different.
SEM = Standard Error of Means LS = Level of Significance N/R = Normal Range
T_1 = 0% Flamboyant tree seed meal inclusion T_2 = 25% Flamboyant tree seed meal inclusion
T_3 = 50% Flamboyant Tree seed meal inclusion T_4 = 75% Flamboyant tree seed meal inclusion
T_5 = 100% Flamboyant tree seed meal inclusion

increased throughout the period of study. The amount of urea is dependent on the protein content of the daily diet. When within normal range, it is an indication of proper functioning of the kidneys. The mean total value for blood parameters so measured fell within the normal range specified by Fasae *et al*. (2005). The implication of these findings is that flamboyant tree seed meal did not have any detrimental effect on the haematological performance of the animals.

Conclusion and recommendations: The result obtained from this study indicate that flamboyant tree seed meal can be used to substitute groundnut cake up to 100% without any deleterious effects on the blood parameters. Also, farmers should be encouraged to protect and plant flamboyant trees to serve as plant protein sources in addition to performing ornamental functions.

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Characterization of Ready-to-Eat Composite Porridge Flours Made by Soy-Maize-Sorghum-Wheat Extrusion Cooking Process

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Abstract: The materials used included sorghum, maize, wheat and soybean and two composite flours were formulated Sorghum-Maize-Soy 1 (SMS1) and Sorghum-Maize-Soy 2 (SMS2). Nutritional and functional characteristics of the two products were determined after High Temperature Short Time (HTST) extrusion; SMS2 had higher content ($p \leq 0.05$) of zinc, magnesium and phosphorus than SMS2. SMS1 and SMS2 had protein content of 23.87 and 17.95% wt (percent weight) respectively with energy value of 1 694.89 and 1 540.88 Kilojoule/100g (KJ/100g) respectively while *In vitro* Protein Digestibility (IVPD) was found at 72.32 and 68.85% wt respectively. Linoleic acid, linolenic acid and all the essential amino acids were found in both extrudates and lysine was well retained during HTST extrusion. In general, changes in proximate composition, minerals and amino acids content during HTST extrusion were significant in both flours while fatty acids content did not change significantly. SMS1 was characterized by a decrease in viscosity on cooling as indicated by setback viscosity value, 12.00 Brabender Unit (BU), a pointer towards low retrogradation property of the flour. SMS1 had the lowest ($p \leq 0.05$) peak viscosity of 15.00 BU but showed the highest ($p \leq 0.05$) paste stability with 2.0 BU of breakdown viscosity. Results showed that SMS1 flour would have better gelation characteristics than SMS2 at similar concentrations with a least gelation concentration of 16.66% (w/v).

Key words: Composite flour, ready-to-eat, extrusion, nutritional composition, functional characteristic

INTRODUCTION

Maternal and child undernutrition remain pervasive and damaging conditions in low income and middle-income countries (Black *et al*., 2008) although the development of an adequate and accessible food product to a vast majority of the population is one cure for this scourge.

Sorghum is a staple food in many African countries and contains reasonable amount of protein, ash, oil and fibre (Drich and Pran, 1987), however, is deficient in essential amino acid content, particularly with respect to lysine. Maize is the world's most widely grown cereal, cultivated across a range of latitudes, altitudes, moisture regimes, slopes and soil types (Smale and Jayne, 2003). In many African and Middle Eastern countries, corn is used for various food preparations. These foods are major sources of calories and nutrients (Akinrele, 1970). Wheat's adaptability to various climates and soils is evident from its wide distribution throughout the world. Wheat is grown to some extent on every continent except Antarctica (Matz, 1991). According to De Ruiter (1974), the use of soy flour in composite flours is emphasized and is quite understandable with regard to the worldwide cultivation of soybean, its protein content and nutritional protein quality. The addition of soy flour to that of sorghum, maize and wheat will overcome their deficiency of some nutritional composite like the essential amino acid lysine.

Pelembe *et al*. (2002) reported that, in Africa, due to deforestation by utilization of wood for fuel, there is a great need for pre-cooked foods. High-Temperature, Short-Time (HTST) extrusion cooking could be used to produce sorghum-based foods of high nutritional quality and in a ready-to-eat form. According to Brennan (2006), there are many benefits to using extruders to process food materials. Extrusion systems are able to process highly viscous materials that are difficult or impossible to handle using conventional methods. The ability of extrusion systems to carry out a series of unit operations simultaneously and continuously gives rise to savings in labour costs, floor space costs and energy costs whilst increasing productivity. Besides processing advantages, extrusion cooking also can induce some beneficial nutritional and chemical changes in foods (Camire, 2002). Previous important research work on the extrusion cooking of cereals and legumes has been reported (Ding *et al*., 2006; Bredie *et al*., 1998; Iwe *et al*., 2001; Pelembe *et al*., 2002) but nothing has been reported on HTST extrusion cooking of sorghum-maize-soy-wheat composite flour.

The main objectives of this study were to determine the chemical and functional properties of the developed composite flours and the effect of HTST extrusion on some nutritional characteristics of the flours. In addition, an eventual incorporation of the extrudates in others

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foods could be considered according to their functional characteristics.

MATERIALS AND METHODS

Material procurement: Sorghum, soybean, wheat and maize were purchased from Wuxi (Jiangsu province) local markets.

Sample preparation: Sorghum, wheat and maize grains were sorted, cleaned, dried in an oven at 40°C for 48 h and finally milled into flour to pass a 1.0 mm screen. Soybean grains were sorted, cleaned, dried in an oven for 12-18 h, roasted at 60°C for 25 min and finally milled into flour to pass a 1.0 mm screen. Two composite flours were formulated; Sorghum-Maize-Soy 1 (SMS1) and Sorghum-Maize-Soy 2 (SMS2). *SMS1* was a mixture of 45% of soybean, 25% of maize, 25% of sorghum and 5% of wheat flour and *SMS2* was a mixture of 25% of soybean, 35% of maize, 35% of sorghum and 5% of wheat flour.

Extrusion process: The blend was extruded using a twin screw co-rotating extruder; model A DS-32-II (Jinan Food Machinery China) with a smooth barrel. The extruder had four heating independent zones and the effective cooking zone temperatures were set at 60, 100, 120 and 150°C, respectively. The Length to Diameter (L/D) ratio for extruder was 20:1. The diameter of the hole in the die was 6 mm with a die length of 27 mm. The ingredients were fed into the extruder in the form of flour (particle size: 1.0 mm), after adjusting the moisture content to 10%. The rotation speed was 120 rpm at an average pressure of 8-11 Pascal (Pa). The extrudates were collected and dried in an air oven at 40°C for 10 min. The product were then cooled and milled into flour to pass 0.4 mm screen. Finally, the flour was stored in polyethylene bags at 4°C for further analysis.

Determination of the functional properties of the product

Nutritional composition: Moisture, fat and ash contents were determined according to the procedures specified by the AOAC (1970). Crude protein (N x 6.25) content was determined by the Kjeldahl nitrogen method of the AOAC (1980). Carbohydrate content was determined by the Alkaline 3, 5-dinitrosalicylic acid (DNS) colorimetric method James, 1995). The calorie values were calculated by the Atwater formula (FAO, 1973) using values of 9, 4 and 3.75 Kilocalories for fat, protein and carbohydrate respectively. The conversion from kilocalorie (Kcal) to Kilojoule (KJ) was done using the formula 1 Kcal = 4.184 KJ.

Water-binding capacity (WBC): The water-binding capacity was determined by the modified method of Lin and Humbert (1974). A 2 g (10% wt moisture content) sample was added to 20 ml of distilled water in a test tube, stirred briefly with magnetic stirrer (78HW-1, China) and allowed to stand for 1 h at room temperature (28°C) before being centrifuged at 2460 rpm (Anke TDL-5, China) for 25 min. Supernatant water was decanted by inverting the tubes over filter paper placed in a volumetric flask. The samples were allowed to drain for about 35 min and the weight of bound water was determined by the difference between initial and final weights of the sample.

The least gelation concentration (LGC): The least gelation concentration was determined by the method of Coffmann and Garcia (1977).

Bulk density (BD): A 50 g flour (8% wt moisture content) samples was put into a 100 ml measuring cylinder. The cylinder was tapped continuously until a constant volume was obtained. The bulk density was calculated as weight of flour (g) divided by flour volume (cm^3) (Okaka and Potter, 1979).

Pasting properties: The pasting properties were evaluated using a Micro Visco-amilograph (Brabender, Type: 803201, Germany). Flour slurry containing 10% solids (w/v, dry basis) was stirred at 160 rpm and heated from 30-95°C at a rate of 5.0°C/min, held at 95°C for 15 min then cooled at the same rate to 50°C. The following parameters were obtained from plotted graphs: Peak viscosity (the maximum hot paste viscosity), final viscosity (viscosity at the end of the test after cooling at 50°C and holding at this temperature), setback viscosity (final viscosity-holding strength), breakdown viscosity (peak viscosity-holding strength or trough) and beginning of gelatinization.

Nitrogen solubility index: Nitrogen solubility of the proteins was determined using the method of American Association of Cereals Chemists (Mirmoghtadaie *et al.*, 2009).

***In vitro* protein digestibility (IVPD):** *In vitro* protein digestibility was determined according to the method of Saunders *et al.* (1973).

Amino acid analysis: Amino acid analysis was performed using ion exchange chromatography following the release of amino acids from the extrudates. A 60 mg sample was mixed with 8 ml of 6 $molL^{-1}$ HCl. The hydrolysation was done under vacuum at 110°C for 24 h. After cooling, the hydrolysate was washed, filtered and dried, also under a vacuum, in a water bath at 60°C. The amino acids in the hydrolysate were separated and quantified by injecting 50 µL of the hydrolysate into a Hitachi (Tokyo, Japan) 835-50 amino acid analyzer equipped with a 2.6 mm x 150 mm ion exchange column coated with resin (Hitachi, Tokyo, Japan) 2619. The column temperature was 53°C.

Minerals analysis: Samples for minerals analysis were prepared according to the method of James (1995). Minerals were determined using an atomic absorption spectrophotometer (Varian AAS model 220Z).

Fatty acids analysis: Fat was extracted from flour with ether absolute. 0.1 g plant oil and 2 ml of 0.5 M NaOH in methanol solution were added to 20 ml tube. The mixture was incubated at 60°C water bath for 30 min until the oil was solubilized. 2 ml of 25% BF3 in methanol solution were added to the cooled mixture. Then the mixture was incubated at 60°C water bath for 20 min for esterification. Subsequently, 2 ml hexane was added to the cooled mixture and shaken. The solution was centrifuged and the upper no aqueous layers were placed in a tube and dried with anhydrous Na_2SO_4. The solution obtained was used for analysis. Fatty acids were identified and quantified by injecting the prepared sample into GC/MS system (model GC-2010 SHIMADZU).

Statistical analysis: Each determination consisted of two separate samples, which were analyzed, in triplicate and the figures were then averaged. Data was assessed by the Analysis of Variance (ANOVA) (Snedecor and Cochran, 1987) and by the Duncan's multiple range test with a probability $p \leq 0.05$ (Duncan, 1955).

RESULTS AND DISCUSSION

Proximate composition: Proximate composition is shown in Table 1. The moisture content of SMS1 and SMS2 after HTST extrusion was 9.63 and 13.88% wt respectively. This can be attributed to starch gelatinization during extrusion cooking as during the process the intermolecular bonds of starch molecules in the presence of water and heat break down, allowing the hydrogen bonding sites (the hydroxyl hydrogen and oxygen) to engage more water. SMS2 contained more sorghum (35%) and more maize (35%) than SMS1, which contained only 25 and 25% of sorghum and maize respectively. More sorghum and maize refer to more starch in the flour thus more water absorption during starch gelatinization. As expected, the protein content of the extrudates increased proportionally with the amount of soybeans in the formulation. The content was 21.51% wt in SMS1 and 17.11% wt in SMS2 before HTST extrusion. Both extrudates showed a significant ($p \leq 0.05$) decrease in crude protein content after HTST extrusion that was 23.87 and 17.95% wt for SMS1 and SMS2 respectively. Camire (2002) reported that the total protein change was not significant during most extrusion operations. The Codex Alimentarius Commission at its 19th session (1991) reported that protein content of supplementary foods for older infants and young children should be in the order of 15 g per 100 g of the food on dry matter basis, we concluded that SMS1 would cover the requirement. SMS1 had a higher ($p \leq 0.05$) calorie value of 1 694.89 Kilojoule/100g compare to 1 540.88 Kilojoule/100g for SMS2 after HTST extrusion.

Water-binding capacity (WBC): Water binding capacity measures the amount of water absorbed by starch and can be used as an index of gelatinization (Anderson *et al*., 1969). It also depends on the availability of hydrophilic groups that bind water molecules and on the gel-forming capacity of macromolecules (Onyeka and Dibia, 2002). The ability to absorb water is particularly important during reconstitution into the blend before consumption. SMS1 and SMS2 had water-binding capacities of 2.27 and 4.10 g/g respectively, as shown in Table 2. Lower absorption capacity is desirable for making thinner gruels. WBC increased as the proportion of maize increased in SMS2, this can be attributed to the higher amylose/amylopectin ratio in maize. Mercier and Feillet (1975) observed that higher amylose ratio results in a higher WBC.

The least gelation concentration (LGC): Least Gelation Concentration (LGC), an index of gelling tendency of samples, is very important with respect to porridges (Onyeka and Dibia, 2002). Kinsella (1979) reported that protein gels are composed of three dimensional matrices or networks of interwoven and partially associated polypeptides in which the water is trapped. Gels are characterized by a relatively high viscosity, plasticity and elasticity. The ability of protein to form gel and provide a structural matrix for holding water, flavours, sugars and food ingredients is useful in food applications (cited in Yemisi and Kayode, 2007). Protein gel formation usually requires prior heating of a protein to cause at least partial denaturation or unfolding of the polypeptide chains (Coffman and Garcia, 1977).

Elofsson *et al*. (1997) noted that gel formation of proteins is the result of a two-step process involving, first

Table 1: Proximate composition of SMS1 and SMS2 before and after HTST extrusion

Constituents	SMS1x	SMS2x	SMS1	SMS2
Moisture (% wt)[1]	8.38±0.14[d]	8.88±0.12[c]	9.63±0.11[b]	13.88±0.12[a]
Fat (% wt)	14.34±0.0.12[a]	10.92±0.15[c]	13.36±0.12[b]	9.23±0.16[d]
Protein (% wt)	21.51±0.14[b]	17.11±0.12[d]	23.87±0.16[a]	17.95±0.16[c]
Ash (% wt)	2.60±0.14[a]	2.15±0.15[b]	2.62±0.12[a]	2.02±0.11[b]
Carbohydrate(%wt)	53.17±0.15[c]	60.94±0.12[a]	50.5±0.11[d]	56.91±0.12[b]
Calorie Value(J/Kg)[2]	1734.18±0.12[a]	1653.68±0.18[c]	1694.89±0.12[b]	1540.88±0.15[d]

[1]Percent weight. [2]Kilojoule per 100 gram. [a,b,c,d]Values with different letters in the same raw are significantly different ($p \leq 0.05$).
SMS1x and SMS2x, products before HTST extrusion; SMS1 and SMS2, products after HTST extrusion

Table 2: Functional properties of SMS1 and SMS2 after HTST extrusion

Property	SMS1	SMS2
Water binding capacity (g/g)	2.27±0.21[b]	4.10±0.19[a]
LGC[1] (% w/v)	16.99±0.19[b]	22.55±0.17[a]
Bulk density (g/ml)	0.69±0.18[a]	0.68±0.19[a]
IVPD[2] (% wt)	72.32±0.29[a]	68.85±0.25[b]

[1]Least gelation concentration. [2]*In Vitro* Protein Digestibility.
[a,b]Values with different letters in the same raw are significantly different ($p \leq 0.05$). SMS1 and SMS2, products after HTST extrusion

the partial denaturation of individual proteins to allow more access to the reactive side groups within the protein molecules and second the aggregation of these proteins by means of reactive side groups into a continuous three dimensional network structure capable of retaining significant amount of water and also exhibiting some structural rigidity. This phenomenon is of importance in foods since it contributes significantly to the textural and rheological properties of various foods (cited in Yemisi and Kayode, 2007).

LGC was taken as a measure of the gelation capacity and the lower the LGC the better the gelation characteristics of the flour. SMS1 had a lower LGC ($p \leq 0.05$) of 16.99% (w/v) than 22.55% (w/v) of SMS2 at pH 6.3 as shown in Table 2, a consequence of higher protein concentration in SMS1. This demonstrated that SMS1 porridge would have better gelation characteristics than SMS2 at similar concentrations. The least gelation concentration reported for legume flours was 14% for lupin seed proteins (Sathe *et al*., 1982).

Bulk density (BD): Bulk density is a measure of heaviness of flour (Oladele and Aina, 2007). SMS1 had a bulk density of 0.69 g/ml, which was not significantly different of that of SMS2 (0.68 g/ml) as shown in Table 2. Their bulk densities were lower than 0.71 g/ml reported for wheat flour (Akubor and Badifu, 2004) and higher than 0.62 g/ml reported for cowpeas (Okaka and Iseih, 1990). Increase in bulk density is desirable in that it offers greater packaging advantage as greater quantity may be packed within constant volume (Molina *et al*., 1983). However, low bulk density is desirable in preparation of infant and weaning foods. Stojceska *et al*. (2009) reported that Bulk density is highly correlated to the moisture content of the product during extrusion.

Pasting properties: The onset of starch gelatinization in SMS2 was found to occur at temperature 22.1°C lower ($p \leq 0.05$) than SMS1. The presence of more starch in SMS2, may contribute, to some extent to its faster gelatinization and lower gelatinization temperature as SMS2 was composed of 35% of maize and 35 % of sorghum compare to 25% of sorghum and 25% maize for SMS1. SMS1 required a longer time (13.21 min) to reach maximum viscosity; this might be due to the lower rate of absorption and swelling of starch granules, as can be seen in Fig. 1.

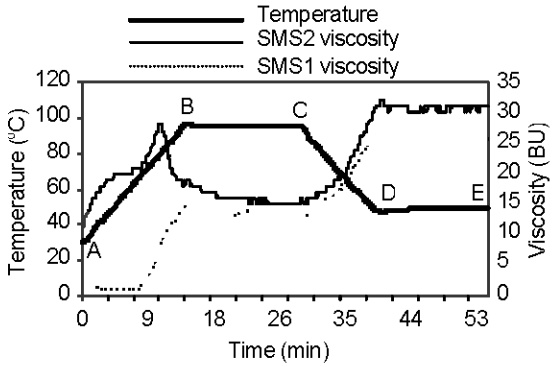

Fig. 1: Amylograph pasting characteristics of SMS1 and SMS2 after HTST extrusion. A-B: Heating period, B-C: Holding period, C-D: Cooling period, D-E: Final holding period

Ragaee and El-Sayed (2006) reported that during the holding period of the viscosity test, the material slurries are subjected to high temperature and mechanical shear stress, which further disrupt starch granules in the grains, resulting in amylose leaching out and alignment. This period is commonly associated with a breakdown in viscosity. The ability of starches to withstand heating at high temperature and shear stress is an important factor in many processes. During cooling, re-association between starch molecules, especially amylose, will result in the formation of a gel structure and therefore, viscosity will increase to a final viscosity. This phase is commonly described as the setback region and is related to retrogradation and reordering of starch molecules. The low setback values indicate low rate of starch retrogradation and syneresis. The peak viscosity often correlates with quality of end product and also provides an indication of the viscous load likely to be encountered by a mixing cooker.

As shown in Table 3, the maximum viscosity reached upon starch gelatinization in SMS1 was much lower ($p \leq 0.05$) than that measured on SMS2, their maximum viscosities were respectively 28.0 and 15.0 BU, likely as a consequence of the fat content of the two samples. As fat content increases, the amount of air en-trapped in the structure of the dispersion during mixing increases which causes a decrease in viscosity. SMS1 had the lowest ($p \leq 0.05$) maximum viscosity but it showed the highest ($p \leq 0.05$) paste stability, as indicated by its lowest ($p \leq 0.05$) breakdown viscosity (2.0 BU). This indicates that SMS1 may have good potential as a food ingredient for food exposed to heat treatment at high temperature and mechanical stirring (Ragaee and El-Sayed, 2006).

SMS1 was characterized by little decrease in viscosity on cooling as indicated by setback viscosity value, 12.00 BU, a pointer towards low retrogradation property of the flour.

Table 3: Pasting properties of SMS1 and SMS2 after HTST extrusion

Property	SMS1	SMS2
Beginning of gelatinization		
Time (min)[1]	11.05±0.04[a]	6.41±0.05[b]
Viscosity (BU)[2]	11.00±0.05[b]	21.00±0.06[a]
Temperature (°C)[3]	85.00±0.05[a]	62.90±0.06[b]
Maximum viscosity		
Time (min)	13.21±0.03[a]	9.51±0.05[b]
Viscosity (BU)	15.00±0.05[b]	28.00±0.06[a]
Temperature (°C)	96.10±0.04[a]	78.70±0.04[b]
Final viscosity		
Time (min)	52.31±0.06[a]	52.31±0.05[a]
Viscosity (BU)	26.00±0.05[b]	31.00±0.04[a]
Temperature (°C)	50.00±0.06[a]	50.00±0.06[a]
Breakdown viscosity (BU)	2.00±0.05[b]	13.00±0.04[a]
Setback viscosity (BU)	12.00±0.06[b]	13.00±0.05[a]

[1]Minutes. [2]Brabender Unit. [3]Degree Celicius. [a,b]Values with different letters in the same raw are significantly different ($p \leq 0.05$).
SMS1 and SMS2, products after HTST extrusion

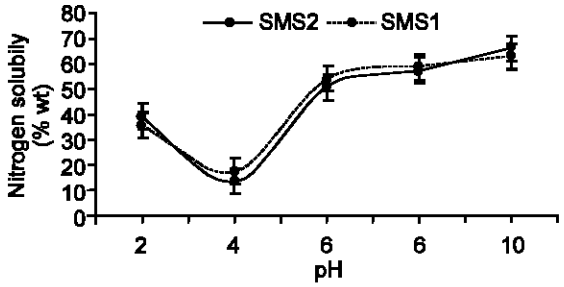

Fig. 2: Nitrogen solubility index of SMS1 and SMS2 after HTST extrusion

Nitrogen solubility index: The pH-dependent protein solubility profile for the extrudates is presented in Fig. 2. Both extrudates protein isoelectric point was found between 4.5 and 5.5 and the solubility reduced as the pH increased until it reached the isoelectric point; this was followed by progressive increase in solubility with further increase in pH. Maximum nitrogen solubility was observed at pH 10, 64.52 and 66.02 % wt for SMS1 and SMS2 respectively. Similar observations was reported for winged bean and Chickpea (Sathe *et al*., 1982) and (Sanchez-Vioque *et al*., 1999) and coincide with curves reported by Mensa-Wilmot *et al*. (2001).

The characteristics described above can be understood on the basis of the overall ionic charge of the protein with the pH. At low pH values, most of the carboxyl and amino groups from the lateral amino acid chains are protonated in the -COOH and $-NH_3^+$ forms respectively, and the overall charge of most protein molecules is positive. As the pH increases some of the carboxyl groups are dissociated into $-COO^-$ and $-H^+$, according to their dissociation constants and the positive charges associated with the proteins diminish up to the isoelectric point, where these are neutralized (Yemisi and Kayode, 2007).

At this point, the protein cannot be hydrated by water molecules, due to the modification of its tertiary and quaternary structures and its solubility reaches a minimum value (Sathe *et al*., 1982). As the pH increases even more, the amino groups dissociate into $-NH_2$ and $-H^+$ and the overall protein charge becomes negative due to the presence of $-COO^-$ groups and can consequently be hydrated and dissolved in water.

Onyeka and Dibia (2002) noted that during HTST extrusion cooking process, the quaternary structure of proteins opens in the hot moist conditions, to produce a viscous plasticized mass. The proteins are then polymerized, cross-linked and reoriented to form a new fibrous structure. HTST extrusion cooking reduces protein solubility as a function of temperature, probably as a result of thermally induced cross-links among subunits of proteins by heat.

***In vitro* protein digestibility (IVPD):** The mean values of the *in vitro* Protein Digestibilities (IVPD) for the extrudates are presented in Table 2. They were 72.32 and 68.85% wt for SMS1 and SMS2 respectively and the presence of more sorghum in SMS2, contributed, to the lower digestibility. Previous works showed that the sorghum has low protein digestibility. Tannin content and high levels of disulphide bonds in sorghum protein are mainly responsible for the reduction in digestibility of sorghum protein. Tannins interact with proteins causing their precipitation. Hamaker *et al*. (1987) attributed this to the formation of disulphide bonds, which results in toughening at the surface and interior of the protein bodies. Camire (2002) reported that extrusion may improve protein digestibility by denaturating proteins, exposing enzyme-accessible sites. Enzymes and enzyme inhibitors generally lose activity due to denaturation. Reductions in protease inhibitors can contribute to better plant protein utilization. 49.25 and 55.85% IVPD were reported for Sudanese and Indian sorghum cultivars respectively (Awadelkareen *et al*., 2009).

Amino acid: The amino acid composition of both SMS1 and SMS2 before and after HTST extrusion is presented in Table 4. Both SMS1 and SMS2 contained all the essential amino acid before and after HTST extrusion. After HTST extrusion, Threonine, Valine, Methionine, Isoleucine, Leucine and tryptophan were higher ($p \leq 0.05$) in SMS1 than SMS2. However, Phenylalanine and Lysine presented a highest score ($p \leq 0.05$) in SMS2. Lysine content of SMS2 was 7.74 g/100 g; higher than 2.706 g/100 g reported for soybeans and 0.265 g/100 g reported for maize (Matz, 1991).

Table 4: Amino acids content of the SMS1 and SMS2 before and after HTST extrusion

Amino acid (g/100 g)	SMS1x	SMS2x	SMS1	SMS2
Aspartic acid	2.27±0.02^{a}	1.74±0.03^{b}	2.35±0.04^{a}	1.72±0.03^{b}
Threonine*	8.59±0.03^{b}	6.77±0.02^{d}	9.05±0.03^{a}	6.87±0.03^{c}
Serine	1.18±0.04^{c}	9.41±0.02^{a}	1.19±0.04^{c}	9.15±0.01^{b}
Glutamic acid	4.41±0.04^{b}	3.63±0.03^{c}	4.66±0.01^{a}	3.63±0.04^{c}
Glycine	8.93±0.04^{b}	6.67±0.07^{c}	9.27±0.03^{a}	6.75±0.04^{c}
Alanine	1.16±0.03^{a}	1.03±0.03^{a}	1.20±0.06^{a}	1.02±0.02^{a}
Cysteine	2.17±0.04^{b}	1.75±0.02^{c}	1.18±0.02^{d}	8.37±0.04^{a}
Valine*	8.97±0.06^{b}	7.27±0.04^{d}	9.75±0.02^{a}	7.50±0.01^{c}
Methionine*	2.69±0.06^{a}	2.54±0.04^{b}	2.33±0.04^{c}	1.98±0.01^{d}
Isoleucine*	7.86±0.04^{b}	6.20±0.03^{c}	8.43±0.03^{a}	6.25±0.06^{c}
Leucine*	1.87±0.03^{b}	1.66±0.04^{c}	1.99±0.04^{a}	1.65±0.03^{c}
Tyrosine	6.43±0.03^{b}	5.06±0.03^{d}	6.95±0.01^{a}	5.23±0.04^{c}
Phenylalanine*	1.04±0.07^{d}	8.65±0.06^{b}	1.24±0.03^{c}	9.56±0.03^{a}
Lysine*	1.14±0.04^{b}	8.30±0.03^{a}	1.10±0.03^{b}	7.74±0.06^{ab}
Histidine	5.52±0.06^{b}	4.45±0.07^{c}	6.07±0.03^{a}	4.59±0.07^{c}
Arginine	1.38±0.04^{a}	1.01±0.05^{b}	1.48±0.04^{a}	1.02±0.06^{b}
Tryptophan*	1.80±0.02^{b}	1.51±0.04^{d}	2.31±0.01^{a}	1.64±0.04^{c}
Proline	1.88±0.03^{a}	1.25±0.03^{d}	1.71±0.02^{b}	1.49±0.03^{c}

*Essential amino acid. ^{a,b,c,d}Values with different letters in the same raw are significantly different ($p \leq 0.05$).
SMS1x and SMS2x, products before HTST extrusion; SMS1 and SMS2, products after HTST extrusion

Table 5: Minerals content of SMS1 and SMS2 before and after HTST extrusion

Minerals (ug/g)	SMS1x	SMS2x	SMS1	SMS2
Zinc	29.47±0.16^{a}	27.65±0.17^{b}	23.96±0.16^{d}	24.18±0.17^{c}
Iron	53.03±0.15^{c}	47.70±0.18^{d}	96.03±0.18^{a}	64.43±0.16^{b}
Magnesium	84.16±0.18^{a}	83.77±0.17^{b}	75.56±0.19^{d}	81.87±0.15^{c}
Calcium	1720.75±0.19^{a}	1675.50±0.19^{b}	966.04±0.21^{c}	890.82±0.18^{d}
Phosphorus	534.01±0.18^{d}	585.11±0.18^{c}	602.52±0.18^{b}	618.41±0.19^{a}

^{a,b,c,d}Values with different letters in the same raw are significantly different ($p \leq 0.05$).
SMS1x and SMS2x, products before HTST extrusion; SMS1 and SMS2, products after HTST extrusion

De la Gueriviere *et al.* (1985) reported that excessive Maillard browning during extrusion cooking could result in losses of lysine up to approximately 50% (cited by Camire, 2002). However, changes in Lysine content for both SMS1 and SMS2 was not significant, this coincide with the observations from Konstance *et al.* (1998), Corn-soy blends extruded for reconstitution as porridge or gruel had good lysine retention.

Minerals: The Minerals content of both SMS1 and SMS2 before and after HTST extrusion are presented in Table 5. Zinc, iron, magnesium, calcium and phosphorus content have been determined. After HTST extrusion SMS1 showed the highest score ($p \leq 0.05$) in iron (96.03 ug/g) and calcium (966.04 ug/g) content and SMS2 showed the highest score ($p \leq 0.05$) in zinc (24.18 ug/g), magnesium (81.87 ug/g) and phosphorus (618.41 ug/g) content. Minerals content before and after HTST extrusion was significantly different for both SMS1 and SMS2, this did not coincide with what was reported by Camire (2002), mineral content and bioavailability are generally retained well during extrusion. However, Iron and phosphorus content increased significantly in both SMS1 and SMS2 after HTST extrusion. Camire (2002) noted that total iron increased by as much as 38% due to extrusion.

On the other hand, Cisse *et al.* (1998) reported that weaning food blends of pearl millet, cowpea and peanut had greater iron availability and protein digestibility compared to similar foods processed by roasting (cited by Camire, 2002). None of the processed blends provided adequate iron to meet infant needs, however.

Fatty acids: Table 6 presents the fatty acid composition of SMS1 and SMS2 before and after HTST extrusion. After HTST extrusion, both extrudates contained 52% wt of linoleic acid and linolenic acid content was 6.54 and 5.94% wt for SMS1 and SMS2 respectively. In general, HTST extrusion did not promote significant changes in fatty acids content in both SMS1 and SMS2. However, myristic acid, which was not present in both SMS1 and SMS2 before HTST extrusion, was found in both extrudate, in the range of 0.05-0.06% wt. Linoleic and linolenic acid average content reported for soybean oil were 50.8 and 6.8% respectively (Erickson, 1995). Unlike other processing methods, extrusion cooking does not promote significant *cis-trans* isomerisation of unsaturated lipids. Maga (1978 reported that corn and soy blends had 1.5% more *trans*-fatty acids after extrusion (cited in Camire, 2002).

Table 6: Fatty acids composition of SMS1 and SMS2 before and after HTST extrusion

Fatty acids (% wt)		SMS1x	SMS2x	SMS1	SMS2
Capric acid	(C10:0)	-	-	-	-
Lauric acid	(C12:0)	-	-	-	-
Myristic acid	(C14:0)	-	-	0.06±0.04^{a}	0.05±0.04^{a}
Palmitic acid	(C16:0)	11.52±0.04^{b}	11.71±0.04^{a}	11.62±0.04^{ab}	11.61±0.05^{ab}
Stearic acid	(C18:0)	4.00±0.04^{a}	3.67±0.05^{b}	4.10±0.07^{a}	3.79±0.07^{b}
Arachidic acid	(C20:0)	0.30±0.02^{a}	0.32±0.02^{a}	0.34±0.07^{a}	0.34±0.05^{a}
Behanic acid	(C22:0)	-	-	-	-
Palmitoleic acid	(C16:1)	0.09±0.02^{a}	0.11±0.05^{a}	0.10±0.04^{a}	0.11±0.07^{a}
Oleic acid	(C18:1)	23.55±0.04^{d}	24.61±0.03^{b}	23.91±0.05^{c}	24.79±0.04^{a}
Arachidinic acid	(C20:1)	0.18±0.07^{a}	0.17±0.07^{a}	0.17±0.07^{a}	0.17±0.05^{a}
Linoleic acid	(C18:2)	53.47±0.05^{a}	53.22±0.04^{b}	52.84±0.05^{c}	52.85±0.05^{c}
Linolenic acid	(C18:3)	6.53±0.04^{a}	5.87±0.05^{b}	6.54±0.04^{a}	5.94±0.04^{b}

^{a,b,c,d}Values with different letters in the same raw are significantly different ($p \leq 0.05$).
SMS1x and SMS2x, products before HTST extrusion; SMS1 and SMS2, products after HTST extrusion

Conclusion: This study revealed that sorghum, maize, soybean and wheat could be used to produce nutritious and ready-to-eat composite flours. The blends were extruded to provide pre-cooked foods that could be reconstituted at 60°C to a porridge or gruel, eliminating prolonged cooking or degradation of heat labile nutrients. The use of these locally grown cereals and legumes could make a great contribution to food security in sub-Saharan region and other developing countries. However, certain aspects like the digestibility and bio-availability of the macronutrients in these composite flours need further investigation. On the other hand, the composite flours did not meet the recommended micronutrient (minerals) requirements for infants, children and adults therefore, fortification with appropriate micronutrients or micronutrient-dense foodstuffs will be necessary.

Finally, the functional properties such as bulk density, water binding capacity, least gelation concentration and pasting properties analysis helped to consider an eventual incorporation of the extrudates in others foods formulation.

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Antinutritional Assessment of *D. alata* Varieties

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Abstract: The antinutritional components of five hybrid varieties of water yam (*Dioscorea alata*) and two land races were evaluated. The antinutritional factors evaluated were phenol ranging from 0.16 to 0.27%; hydrogen cyanide, 9.62 to 12.00 mg/kg; alkaloids, 0.12 to 0.55%; tanmins, 46.50 to 180.25%; phytate, 0.22 to 0.28%; heamagglutinin, 1.22 to 5.75 Hu/g and trypsin inhibitor, 24.02 to 49.51. TI unit/mg. There were significant differences ($P < 0.05$) in some of the antinutritional factors among the water yam varieties investigated. Generally most of antinutritional factors are low to cause health hazard. The overall results are suggestive of high nutritional quality of the water yam varieties due to low presence of antinutritional factors compared to other tropical root crops.

Key words: Antinutrients, assessment, *D. alata* varieties, water yam

INTRODUCTION

Water yam (*Dioscorea alata*) is the most economically important yam species, which serve as a staple food for millions of people in tropical and sub-tropical countries (Hahn, 1995; Coursey, 1967). *D. alata* is a climbing plant with glabrous leaves and twining stems which coil readily around a stake (Udensi *et al*., 2008). *D. alata* is a crop with potential for increased consumer demand due to its low sugar content necessary for diabetic patients. In Nigeria five high yielding and disease resistant water yam (*D. alata*) varieties have been developed by International Institute of Tropical Agriculture (Oselebe and Okorie, 2005). The nutritional and functional properties of these seven varieties have been reported (Udensi *et al*., 2008). The report of their work indicated the possibility of selecting good varieties for intensive cultivation in Nigeria and other *D. alata* growing regions according to their chemical composition only. So far the antinutrient composition of *D. alata* varieties have not been widely reported. Antinutritional factors when present in a food system lower the bioavailability of protein and minerals. The present study aims at providing information on the antinutritional factors of five hybrid varieties of water yam and two land races. The objectives of this work were,

a) To evaluate the antinutrient composition of five *D. alata* varieties.
b) To assess the possibility of selecting good varieties according to their nutritional and antinutrient composition.

MATERIALS AND METHODS

Seven *D. alata* varieties: TDa 98/01166, TDa 98/01168, TDa 98/01178, TDa 99/00169, TDa 99/00240, TDa 297 (the institutional check) and a land race genotype "Okwalenkata" (the best local variety) were collected from the Faculty of Agriculture, Ebonyi State University Abakaliki, Nigeria. The varieties were cultivated in the same environment and all were given the same treatment. In this study, the yam tubers were harvested mature at the same time.

Sample preparation: The yam tubers were peeled, washed, sliced into cubes and dried in hot air oven at a temperature of 60°C to a moisture content of about 10%. The dried yam chips were then milled using locally fabricated attrition mill to obtain yam flour. The flour was sieved through 1 mm sieve and packaged in plastic containers for analysis.

Antinutritional factor studies: Alkaloid was estimated using the alkaline precipitation gravimetric method described by Harbone (1973). Trypsim inhibitor and heamagglutinin were determined according to the methods described by Arntifield *et al*. (1985). Phytic acid content was measured by the method of Davis and Reld (1979) while tannins were determined by the Folin-Denis Spectrophotometric method as described by Pearson (1976). Hydrogen cyanide content was determined by the method of Balagophalan *et al*. (1985). Total phenol content of the yam samples were determined by the Colometric method of (AOAC, 1990).

RESULTS AND DISCUSSION

The antinutrients of the *D. alata* varieties studied are presented in Table 1. The phenol content of the water yam varieties (0.16±0029 - 0.27±0.0058 %) is lower than the values obtained for *D. rotundata* as reported in literature. The low content of phenol in *D. alata* is responsible for the slow browning reaction during

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Table 1: The anti-nutritional factors of yam samples (*Dioscorea alata*)

Varieties	Phenol (%)	HCN (mg/kg)	Alkaloid (%)	Tannins (mg/100g)	Phytate (%)	Heamagglu-tinin (Hu/g)	Trypsin inhibitor (Tiunit/mg)
Tda 98/01166	0.22±0.0018^{e}	11.40±0.0058^{e}	0.24±0.0017^{c}	46.50±0.029^{g}	0.28±0.029^{a}	5.75±0.0033^{a}	49.51±0.0058^{a}
TDa 98/01168	0.25±0.20^{b}	11.51±0.013^{b}	0.18±0.0033^{e}	176.09±0.0082^{b}	0.22±0.0033^{d}	4.85±0.003^{b}	39.00±0.0067^{e}
TDa 98/01169	0.16±0.0029^{d}	11.00±0.004^{d}	0.36±0.0033^{b}	54.75±0.0033^{f}	0.25±0.0029^{b}	5.75±0.0033^{a}	48.02±0.015^{b}
TDa 99/00176	0.27±0.0058^{a}	12.00±0.004^{a}	0.55±0.0033^{a}	148.55±0.029^{c}	0.22±0.058^{d}	1.62±0.0017^{e}	41.03±0.017^{c}
TDa 98/00240	0.23±0.0058^{c}	9.62±0.012^{f}	0.23±0.0033^{d}	92.55±0.029^{d}	0.27±0.0029^{a}	3.56±0.01^{c}	24.02±0.02^{g}
TDa 297	0.25±0.0047^{b}	10.92±0.017^{e}	0.12±0.0033^{f}	65.57±0.35^{e}	0.26±0.01^{a}	1.22±0.01^{e}	39.50±0.0033^{d}
Local best	0.24±0.0029^{a}	11.04±0.036^{d}	0.12±0.00296^{f}	180.25±0.0033^{a}	0.24±0.0067^{c}	2.02±0.38^{d}	29.51±0.0033^{f}

Means bearing different superscripts in the same vertical row are significantly different (P < 0.05)

Table 2: Proximate composition of *D. alata* varieties

Varieties	Moisture Content %	Ash %	Ether extract %	Crude protein %	Crude fibre %	Carbohy-drate %	Energy Kcal/100g
Tda 98/01166	6.52^{b}	3.08^{a}	1.10^{a}	6.78^{bc}	1.13^{a}	87.64^{a}	385.33^{a}
TDa 98/01168	6.00^{bc}	2.25^{b}	0.90^{b}	6.34^{cd}	1.05^{ab}	83.46^{a}	367.30^{a}
TDa 98/01176	7.50^{a}	2.30^{b}	0.78^{c}	7.00^{b}	0.80^{bc}	81.62^{a}	361.58^{a}
TDa 99/00169	5.77^{cd}	2.38^{b}	0.75^{c}	7.18^{b}	0.88^{bc}	83.04^{a}	367.63^{a}
TDa 99/00240	5.26^{d}	3.15^{a}	0.85^{c}	8.31^{a}	0.90^{bc}	81.53^{a}	367.01^{a}
TDa 297	7.57^{a}	2.65^{ab}	1.03^{ab}	5.69^{ef}	0.75^{c}	82.31^{a}	361.27^{a}
Local best	6.05^{bc}	2.25^{b}	0.75^{c}	5.78^{de}	0.83^{bc}	84.34^{a}	367.23^{a}

Means with the same superscripts in the same column are not significantly different (P < 0.05). Culled from Udensi *et al.* (2008). The Investigation of Chemical Composition and Functional Properties of water yam (*Dioscorea alata*): Effect of varietal differences. Pakistan Journal of Nutrition. 7 (2): 342-344.

Table 3: Mineral contents of *D. alata* varieties (mg/100g)

Varieties	K	Na	P	Ca	Mg	Vitamin C
TDA98/01168	400.00^{a}	200.00^{b}	120.00^{d}	60.12^{b}	85.08^{b}	18.48^{cd}
TDA98/01178	380.00^{a}	380.00^{a}	140.00^{cd}	60.12^{b}	85.08^{b}	20.22^{cd}
TDA99/00240	380.00^{a}	250.00^{b}	340.00^{a}	80.16^{a}	24.31^{d}	17.60^{d}
TDA 98/01166	240.00^{b}	190.00^{b}	180.00^{c}	40.08^{c}	97.24^{a}	16.72^{d}
TDA 99/00169	320.00^{ab}	200.00^{b}	300.00^{ab}	20.16^{d}	60.77^{c}	35.20^{a}
TDA297	310.00^{ab}	220.00^{b}	260.00^{b}	40.08^{c}	97.24^{a}	28.45^{b}
Local best (LC)	260.00^{b}	360.00^{a}	100.00^{d}	20.04^{d}	60.77^{c}	22.88^{c}

Means with the same superscripts in the same column are not significantly different (P < 0.05). Culled from Udensi *et al.* (2008). The Investigation of Chemical Composition and Functional Properties of water yam (*Dioscorea alata*): Effect of varietal differences. Pakistan Journal of Nutrition. 7 (2): 342-344.

processing which is nutritionally important. Hydrogen cyanide content (Table 1) ranged from 9.62±0.017 - 12.00±0.004 mg/kg. These values are lower than the results for *D. cayenensis* (260 mg/kg) and *D. rotundata* (90 mg/kg) (Ozo *et al.*, 1984). Generally, hydrogen cyanide is known to be toxic, but the levels obtained in the hybrid varieties *D. alata* are quite below the toxic level of 50 mg/kg. The low levels of alkaloids presented in (Table 1) underscored the safety of the *D. alata* varieties studied when consumed, since most alkaloids are known to be toxic and can course a wide range of physiological changes in the body when consumed (Harbone, 1973). However, simple processing such as boiling removes the alkaloids present in most cultivated species of yams (Osagie and Opoku, 1992). The tannins (Table 1) ranged from 46.50±0.29 - 180±0.0033 mg/100g. The values are higher than that reported for *D. rotundata* (20 mg/100g) by Uka (1985), which implies that less protein may be available in the *D. alata* varieties than in *D. rotundata* due to protein-tannin complex formation. However, it is important to note that heat treatment which is normally given to D-alata before consumption will eliminate or reduce the level of tannin in the food system thereby making the protein available. The phytate contents of the seven *D. alata* varieties are relatively lower than that reported for *D. rotundata* and *D. esculenta* (Uka, 1985). The implication of the low values of phytate in these *D. alata* varieties is that the tubers will contain available minerals for absorption in the body. The heamaggluatinin level was low (Table 1) at the range of 1.22±0.01 - 5.75±0.003 (Hu/g) of the test samples. The low level of heamaggluatinin content of the yam varieties will be further reduced/eliminated during processing or cooking (Udensi *et al.*, 2005; Khokhar and Chauhan, 1986) to prevent red blood agglutination commonly caused by heamagglutinin. Table 1 shows the trypsin inhibitor contents of the test samples. The values are very high compared to those obtained for *Mucuna cochinchinensis* (7.47 TI unit/mg) and *Mucuna utilis* (13.00 TI unit/mg) by Ukachukwu and Obioha (1997) and Udensi *et al.*, 2004), respectively. The presence of large quantity of trypsin inhibitor in the body disrupts the digestive process and may lead to undesirable physiological reactions (Booth *et al.*, 1960). The processing method normally applied in the processing of *D. alata* will enhance the nutritional quality

of the yam by reducing or eliminating the toxic substance. The varieties of *D. alata* investigated contain low levels of antinutritional factors, which ensure safety for both man and animal in food and feed composition. The protein and mineral contents of all the varieties indicate also product of good nutritional quality for the consumers. Farmers should therefore be encouraged to select varieties of high protein content for cultivation to prevent the problem of protein malnutrition and hunger. In terms of nutrient components, Udensi *et al.* (2008) reported average crude protein of 6.8 % (Table 2) for the *D. alata* varieties. Thus, *D. alata* should not be considered protein poor, as has been the case. Udensi *et al.* (2008) also reported the seven varieties as good sources of minerals (Table 3), which are nutritionally important.

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The Role of Humic Acid in Palm Kernel Cake Fermented by *Aspergillus niger* for Poultry Ration

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Abstract: An experiment was conducted to improve the nutrient content of palm kernel cake through fermentation by *Aspergillus niger* with combination humid acid dosage and fermented time. The experiment used Complete Randomize Design (CRD) with 3 x 3 factorial and twice replication. The first factor was humic acid dosage: (1) 0 ppm, (2) 100 ppm and (3) 200 ppm. The second factor was fermented time: (1) 5 day, (2) 7 day and (3) 9 day. The parameters were dry matter, crude protein and crude fiber. The result of study showed that there was no significantly ($p>0.05$) interaction between humic acid dosage and fermented time to dry matter, but against crude protein and crude fiber, there were highly significant interaction ($p<0.01$). Humic acid dosage were not ($p>0.05$) effected to dry matter, but against crude protein and crude fiber, there were significant ($p<0.01$) effect. Fermented time showed that there were highly significant ($p<0.01$) effect to dry matter, crude protein and crude fiber. The conclusion was palm kernel cake which was fermented by *Aspergilus niger* showed that humic acid 100 ppm and fermented time 7 day had a better content. This condition can be seen in crude protein 23,20%, crude fiber 10,59% and dry matter 42.38%.

Key words: Fermentation, *Aspergilus niger*, palm kernel cake and humic acid

INTRODUCTION

One of the potential wastes to be used is waste of oil palm processing such as Palm Kernel Cake (PKC). PKC is the side result of oil palm production that can be used as feedstuff for poultry. Palm Kernel Cake (PKC), the major agroindustrial by product of the palm oil industry in Malaysia, Indonesia and Thailand is another good source of energy and protein for ruminants (Setthapukdee *et al*., 1991) and poultry diets (Ezhieshi and Olomu, 2008).

Even though the crude protein contain of PKC is quite high but the usage of it is still low in poultry ration. It is around 10% in duck ration (Yeong *et al*., 1981). It is caused by the low quality (Garcia *et al*., 1999; Perez *et al*., 2000; Odunsei *et al*., 2002). To increase the use value of PKC, there has been done a research by using the fermentation method to use the microorganism of cellulose characteristic such *Aspergillus niger*. Supriyati *et al*. (1998) got the result of the research about the processing of PKC by *Aspergillus niger* gave the improvement of protein that high enough as much as 52,04% and the decreasing crude fiber around 42,03%, but still limited to use in poultry ration.

So in this research, it will introduce the role of humic acid in processing of PKC, in order to get the optimum condition to improve the quality of PKC. Humic acid is one of compound which consisted in "Humic Substance" as the result of decomposition organic substance, especially concerning plants which located young coal, peat moss oil, compost (Senn and Kingman, 1973). Humic acid is also effective in fasten micro substance, such as Cu, Zn and Mn (Tan, 1998, but in animal husbandry especially in processing and woof material biotechnology are still rare to use. The manufacturing through the soaking with humic acid will give the best result and new thing. Besides, humic acid also provides the component such as N, P and S into land and energy for microorganism activity (Stevonson, 1994). By adding of Kucukersan *et al*. (2005) that the usage of humic acid in livestock feed gives some advantages for health and livestock growth, for example, humic acid has ability in carbohydrate metabolism and protein through out catalytic. This theory can be used in PKC fermentation process because the fermentation process also activated the microorganism growth which is expected, so the processing PKC has better quality and expected to replace the soybean meal (Dairo and Fasuyi, 2008). So that the ration cost can be pressed and the breeder income increase.

Therefore, the objective of this study was to determine the effect of humid acid dosage and fermented time to improve the nutrient content of palm kernel cake through fermentation by *Aspergillus niger*.

MATERIALS AND METHODS

This research was conducted to determine various of concentration of humic acid and fermentation time to raise the quality of Palm Kernel Cake (PKC). It will be

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fermented with *Aspergilllus niger*. Materials that are used on this research are: 1). The oil palm tree seeds from PT. Incasi Raya Jl. Baypass Padang, 2). *Aspergilllus niger* from the center of applied research LIPI. 3). Humic acid from the soil. 4). The Media: PDA/Potato Dextrose Agar from Diffo-Becton Dickinson. 5). Aquades and mineral brooks which consist of $MgSO_4$ $7H_2O$, $FeSO_4$ $7H_2O$, $ZnSO_4$ $7H_2O$, $MnSO_4$ $7H_2O$, KH_2PO_4 and Thiamin hydrochloride. 6). Substrate is the mix of PKC and Chicken fesses with the comparison 80% BIS and + 20% chicken fesses.

This research was using a completely randomized design with 3 x 3 factorial and twice repetition. The first factor was three kinds of humic acid dosage: (1) 0 ppm, (2) 100 ppm and (3) 200 ppm. The second factor was fermented time: (1) 5 day, (2) 7 day and (3) 9 day. The data is analyzed by using variation of investigation. If there is an effect of the treatment, so the differences on treatments are determined by Duncan's Double test (Steel and Torrie, 1991).

The parameters were dry matter, crude protein and crude fibe of Palm kernel cake fermentation by *Aspergilllus niger*.

RESULTS

The aim of the research was to determine the condition of fermentation which is suitable to combine the Humic Acid dosage and the fermentation time. So it produces a high quality product Palm Kernel Cake Fermentation (PKC) can be seen in dry matter, crude protein and crude fiber.

Dry matter (DM): The average value of dry matter content of fermented Palm Kernel Cake (PKC) by *Aspergillus niger* at the interaction between humic acid dosage and fermented time is shown in Table 1. At Table 1 can be showed that the average dry matter fermented of PKC about 37.26-44.36% (DM).

The result of statistic analysis shows that there was no interaction ($p>0.05$) among A and B. The factor A were no significant ($p>0.05$) but the factor B were highly significant ($p<0.01$) effect to dry matter content of PKC.

Crude protein (CP): The average value of crude protein content of fermented Palm Kernel Cake (PKC) by *Aspergillus niger* at the interaction between humic acid dosage and fermented time is shown in Table 2. At Table 2 can be showed that the average crude protein fermented of PKC about 16.40-23.20% (CP).

The result of statistic analysis shows that there is interaction ($p<0.01$) effect among A and B than the factor A and B every were highly significant ($p<0.01$) effect to crude protein content of PKC by *Aspergillus niger*.

Crude fiber (CF): The average value of crude fiber content of fermented Palm Kernel Cake (PKC) by *Aspergillus niger* at the interaction between humic acid dosage and fermented time is shown in Table 3. At Table 3 can be showed that the average crude protein fermented of PKC about 10.59-12,50% (CF).

The result of statistic analysis shows that there is interaction ($p<0.01$) effect among A and B than the factor A and B every were highly significant ($p<0.01$) effect to crude fiber content of PKC by *Aspergillus niger*.

Table 1: The average value of dry matter content of fermented PKC by *Aspergillus niger* at the interaction between humic acid dosage and fermented time

	Fermented time			
Humic acid dosage	B1 (5 days)	B2 (7 days)	B3 (9 days)	Average
A1 (0 ppm)	43.38	41.89	39.28	41.52
A2 (100 ppm)	44.63	42.38	37.26	41.42
A3 (200 ppm)	44.34	41.84	39.25	41.81
Rataan	44.12^{A}	42.0491^{B}	38.60^{A}	

Keterangan: Different capital letter on the same row indicated highly significant ($p<0.01$)

Table 2: The average value of crude protein of fermented PKC by *Aspergillus niger* at the interaction between humic acid dosage and fermented time

	Fermented time			
Humic acid dosage	B1 (5 days)	B2 (7 days)	B3 (9 days)	Average
A1 (0 ppm)	19.76^{d}	19.90^{d}	21.32^{c}	20.33
A2 (100 ppm)	19.90^{d}	23.20^{a}	19.09^{e}	20.73
A3 (200 ppm)	21.00^{c}	22.32^{e}	16.40^{f}	19.91
Rataan	20.22	21.81	18.97	

Keterangan: Capital and small letter are different on the same row and column indicated highly significant ($p<0.01$)

Table 3: The average value of crude fiber content of fermented PKC by *Aspergillus niger* at the interaction between humic acid dosage and fermented time

	Fermented time			
Dosis asam humat	B1 (5 hari)	B2 (7 hari)	B3 (9 hari)	Rataan
A1 (0 ppm)	12.51^{a}	12.02^{cd}	12.30^{b}	12.28^{A}
A2 (100 ppm)	11.70^{e}	10.59^{g}	12.22^{bc}	11.50^{C}
A3 (200 ppm)	12.21^{bc}	10.81^{f}	11.93^{d}	15.60^{B}
Rataan	12.14^{A}	11.14^{B}	12.15^{A}	

Keterangan: Capital and small letter are different on the same row and column indicated highly significant ($p<0.01$)

DISCUSSION

Dry matter (DM): Lack of interaction between humic acid dosage and fermented time caused by the low range of humic acid dosage and range of fermented time made it still not yet to show the real difference of interaction.

Based on the test of DMRT towards fermented time (factor B) shows that the treatment of B1 is real difference ($p<0.01$) rather than B2 but the same with B3. The treatment of dry matter B2 was decrease than B1 and B3. It is caused by the degree of water. The higher

of water degree for treatment of B2 relates to microorganism growth activity. Because the growth of mold is more active than the other. A lot of mold which stimulated a mount of food essence are changed to be dry matter. The growth and development of mold so in the last fermentation of water degree increase which caused the decrease of dry material degree.

The increasing of development mold is caused by humic acid given. It is based on Kucukersan *et al.* (2005) stated that Humat Acid is able to do metabolism carbohydrate and protein by catalytic. Added by Kompiang (2006) that Humic acid can stimulate the microorganism growth in intestines. So this fermentation actives the growth of mold It could be seen for decrease of dry matter after adding Humic acid 100 ppm. But in adding until 200 ppm doesn't show the difference result, because pH not suitable to growth of mold.

While the metabolism process was on, the energy retained from carbohydrate (glucose) will produce energy, water (H_2O) and CO_2. The water retained will increase the water content of product which make the dry matter content of product decrease. Fardias (1988) explained that the microorganism used carbohydrate as energy source which proceed from glucose. The degradation of glucose was done through the glycolisis stripe until the energy, water (H_2O) and CO_2 retained. The water obtained will increase the water content of product which made the dry matter content of product decrease after fermentation.

At B3 treatment there was an improvement of dry matter than B2 treatment. This caused by the growth and propagation of mold experienced a reduction because the nutrient available in substrate was decreasing, so the water retained in metabolism process was less than B2 treatment. Because of the low water retained, the water content of product decrease and increase the dry matter of product.

Crude protein (CP): The test of DMRT for interaction between A and B shows that treatment combination A2B2, was highly significant (p<0,01) with other. Then the higher of crude protein for A2B2 compared by other are caused by *Aspergillus niger* growth activity was better than other. A lot of mold will contribute more protein because the body of mold consists of single cell protein. Based on Saono (1974) that around 31-50% mold contains of protein and fermentation produces enzym in which the enzym is also a protein.

The height of crude protein at A2B2 treatment caused by humic acid dosage given 100 ppm which was able to reach the rught condition for the growth of mold, while humic acid available with constituent and energy which really needed in mold growth At the 7 day of fermentation, mold reach the optimum growth and grow more than usual, so the protein from substrate increase. According to Stevonson (1994) that humic acid supplied constituent and energy for the growth of microorganism in soil. Sukara and Atmowidjojo (1980) have a notion that the better growth and development of mold will change more media component composer into a mass cell, so the protein of mold formed and will increase crude protein of material. The low improvement of crude protein content at A3B3 treatment than other treatment caused by 2 possibilities. The first is because humic acid dosage was too high that made the atmosphere become too sour in which this situation is not suitable for the growth of mold. It relates to addition of humic acid with 100 ppm will give growth of mold which is better than adding 200 ppm humic acid. It has relation with pH of fermentation process. A good pH is a round 4-5 and Humic Acid pH is around 4-5. The second is because the nutrient content in substrate was decreasing. Because of these 2 things above, the growth of mold experienced the decreasing. The lower of mold, the lower of crude protein content. Appropriate with the opinion from Moeljoharjo (1979) that the growth of microbe was affected by the sufficiency of food source.

Crude fiber (CF): The test of DMRT for interaction between A and B shows that treatment combination A2B2, was highly significant (p<0,01) with other. The low of crude fiber for A2B2 compared by other are caused by *Aspergillus niger* growth activity was better than other.

From the DMRT experiment against the interaction between factor A and B turn out that the combination A2B2 treatment was real difference (p<0.01) than other combination treatment. From the result above we can see the possibility of reduction along with the improvement of Humic Acid content and fermented time. The best treatment is the combination of A2B2 treatment with humic acid dosage 100 ppm and fermented time 7 days which give the lowest crude fiber content of palm kernel cake fermented with *Aspergillus niger*.

The low content of crude fiber at A2B2 treatment is caused by the addition of humic acid 100 ppm and fermented time 7 days. At 7 days fermented time, mold grows better than other fermented time. The more mold grow, the more cellulose enzyme will retained to tear down cellulose, so at the end of fermentation, the crude fiber decrease. Appropriate with the opinion of Sulaiman (1988) that the longer fermented time given, the longer time to tear down food material, so at the end of fermentation the decreasing of crude fiber.

The low content of crude fiber at A2B2 treatment is caused by the addition of humic acid 100 ppm because humic acid can activate microorganism growth wanted. Appropriate with Kucukersan *et al.* (2005) that the function of humic acid in ration gave an amount of profit for health and growth of livestock, for example humic acid had an ability to metabolic carbohydrate and protein

through catalytic. The higher microorganism activity, the higher retained enzym produced by mold to tear down cellulose, so at the end of fermentation, the crude fiber decrease.

Conclusion: The conclusion was palm kernel cake which was fermented by *Aspergillus niger* showed that humic acid 100 ppm and fermented time 7 day had a better content. This condition can be seen in crude protein 23,20%, crude fiber 10,59% and dry matter 42.38%.

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Impact Analysis of Knowledge Practice for Food Safety in Urban Area of Varanasi

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Abstract: The present study was planned with main objective of identifying buying practices of homemakers and their awareness in food practices in Varanasi (urban area). For this objective, questionnaire was prepared and distributed among selected people depends on their age group, sex and educational background. Statistical test were carried out on the basis of frequency of male and female respondents obtained in total respondents (n = 300). Chi square test were carried out and the calculated value were compared with value of t test (0.05) and on this basis, conclusion were drawn. Correlation between different variables was determined for their impact. Study revealed that educated people of both sexes follow slightly good buying practices while homemakers are not following food practices and also they differ significantly in following the practices in term of use of quality water which affects the family health. Study also revealed that age and awareness are not interlinked while education is interlinked with good practices.

Key words: Food safety behavior, buying practices, education

INTRODUCTION

India is the world's 2nd largest producer of food next to China. With India's food production likely to increase significantly during the next decade. There has been a long debate in India and abroad regarding food quality and safety issues. Varanasi is situated at 5'N, located in the middle of Ganga valley of North India and its population is 1.90 of total population of UP (16,6052,859) according to 2001 census government of India UP. The urban agglomeration is stretched between 82°56'E-83°03'E and 25°14'N-25°23. Generally food security for the urban people is closely related to many factors like their purchasing power, food items bought from retailer, stockiest, local market, or from supermarket. Other factor like scarcity of clean water for processing, cooking, drinking, washing lack hygienic aspects, due to lack of awareness and improper sanitation in food preparation has great impact on health. Most of the above practices directly affect the health of urban food consumers. Beside this, threats to consumer health are due to various factors like dust and airborne pollutants, poor hygiene, improper storage, deteriorating urban environments and finally, the threat of communicable diseases being spread via the food system.

Buying practices involves the determination by market agencies of kind, qualities and quantities of goods desired by consumer. Buyer has to find out the desired qualities of goods sold at satisfactory prices. Effective buying requires a specialized knowledge of content of goods, their resources and their use (Kotler, 1990). The studies conducted by Nimkar (1976) reveal that homemakers were the actual buyers for the food in the family. "Availability of money resource and availability of the product" in the market were the most important factors, whereas "food habit" and "nutritional requirements" were the least important factors while buying a product. Among home makers retail shops were more used than wholesale shops for purchasing grains, monthly purchasing was most common among the employed and unemployed homemakers for grains and grocery.

According to Howes *et al.* (1996), Attitudes, is an important factor besides knowledge which ensures trend of food borne illnesses. A number of studies (Howes *et al.*, 1996; Powell *et al.*, 1997) have indicated that although training may bring about an increased knowledge of food safety this does not always result in a positive change in food handling behavior. It has been suggested that this disparity between knowledge and practice occurs because much of the existing training, particularly formal certificated training, is designed using the KAP model (Rennie, 1995). This approach assumes that an individual's behavior or Practice (P) is dependent on their Knowledge (K) and suggests that the mere provision of information will lead directly to a change in Attitude (A) and consequently a change in behaviour. It has been suggested that this model is flawed in its assumption that knowledge is the main precursor to behavioral change (Ehiri *et al.*, 1997). According to Nidhi and Priti (2009), education, family income and occupation are major factor that effect extent of awareness but overall education has highest impact. The present study was planned with main objective to find about homemakers and their awareness in food practices in Varanasi (urban area) and in aim to know the factors affecting purchasing decisions of food products and for their perception of food quality.

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MATERIALS AND METHODS

Based on literature survey, a list of relevant variables was prepared. A questionnaire was prepared to capture the relevant variables, which was initially pre-tested at urban area of Varanasi. After its finalization; primary data was collected from 300 respondents in Varanasi, Uttar Pradesh. The data were then tabulated processed and analyzed by chi square test, student t-test, f test and link between various factors were determined by correlation. Age group selected for study were 18-25, 26-35, 36-45 and >46 including both M and F respondents while education background were selected from below matric to graduate. For buying practices tendency data were collected for food items selection from retailer, stockiest, local market, or from supermarket. In buying practices various factor such as type of packaging used, branded verses local were studied. While for kitchen practices application and use of available knowledge on water quality and their impact on health were studied. On the basis of the response, the observed and expected frequency were calculated for chi-square value and on the basis of degree of freedom in row and column t 0.05 value was compared for decision of null hypothesis. F test and correlation was perform to know the significance and impact of age and education on buying practices, water quality practices and their impact on health.

Objective of the study:

Effect of education, and age group on

- Buying practice
- Application of knowledge in kitchen (kitchen behavior practice) and
- Their impact on health

RESULTS AND DISCUSSION

Demographic profile of respondents: Age group selected for study were 18-25 (22.8%), 26-35 (28.9%), 36-45 (23.6%) and > 46 (24.5%) of total M and F respondents studied. Education background were selected from junior high school to graduate.

The demographic profile of respondents in terms of age, and their education level, has been presented in Table 1 and educational distribution has been depicted in pie chart Fig. 1. From the demographic Table 1 it can be seen (out of total 38% M and 62% F), 52% of the M and 36% of F respondent were up to intermediate level and up to graduate level the ratio of respondent were just half 32% M and 16% F, at matric level F (36%) respondent were more than male (10%) and almost same pattern was found in below matric education (F, 25% and M 6%).

Buying practices: Data and percentages of respondent are shown in Table 2 and 3, Fig. 2 and 3. It can be observed that 50.8% M purchased from the local market, while 36% M purchased from stockiest in Varanasi and only 3.5% purchased from supermarket. Out of total respondent 69.8% F purchased these items from local market and 19.5% purchased from stockiest, while 9.6% from other market and only 1% from supermarket.

Table 1: Demographic characteristics (n = 300)

Age category	Male	Male (%)	Female	Female (%)
18-25	26	8.6	42	14
26-35	33	28.9	65	21
36-45	27	23.6	40	13.3
>46	28	9.3	39	13
Educational level				
Below matric	7	6.1	47	6.1
Matric	11	9.6	68	36.5
Intermediate	59	51.7	42	22.5

Table 2: Number and percentage of respondent for market practices

Market practices	Male	Male (%)	Female	Female (%)
Local	58	50.8	130	69.8
Stockiest	42	36.8	36	19.35
Supermarket	4	3.5	2	1
Other	10	8.7	18	9.6

Table 3: Number and percentage of respondent for buying practices

Buying practices	Male	Male (%)	Female	Female (%)
Open	33	28.9	35	18.8
Local seal	22	19.2	60	32.2
Brand seal	32	28	49	26.3
Any	27	23.6	42	22.5

Statistical analysis shows that calculated χ^2 value of was greater than t 0.05 at 3 df (15.15>7.815). Therefore, it can be concluded that difference is significant and thus hypothesis is rejected. In other word it can be concluded that both male and female together following a slightly good buying practice.

At individual level male [χ^2 9.37>t 0.05 (3) 7.815] are more aware and follow slightly good buying practices as compare to female [χ^2 5.817>t 0.05 (3) 7.815], it means in such household where buying of kitchen item are based on male this practices are followed at significant level.

Table 3 and Fig. 3 shows in Table 4, Statistical analysis of buying practices show that calculated χ^2 is slightly more than the t 0.05 at 3df (7.9>7.81) which rejects the null hypothesis that respondent are concerned about the buying practices in buying kitchen items. From Table 3 it is clear that male are little concern with the quality of kitchen item but equal percentage of respondent are buying kitchen items from branded sources (local 28.9%, and branded 28%). This means that either male are aware of these practices and not following or they are not well aware of the practices, but data suggests that those who are well aware are following the practices. This statement is strengthened by the correlation data presented in Table 4 which shows that education has positive correlation of 0.759 ($p<0.007$) in buying practices.

In Table 3 female respondent are buying local but sealed items in higher percentage than the branded items (local seal, 32%; branded item 26.3%) equally. Impact of age and education on market practice were

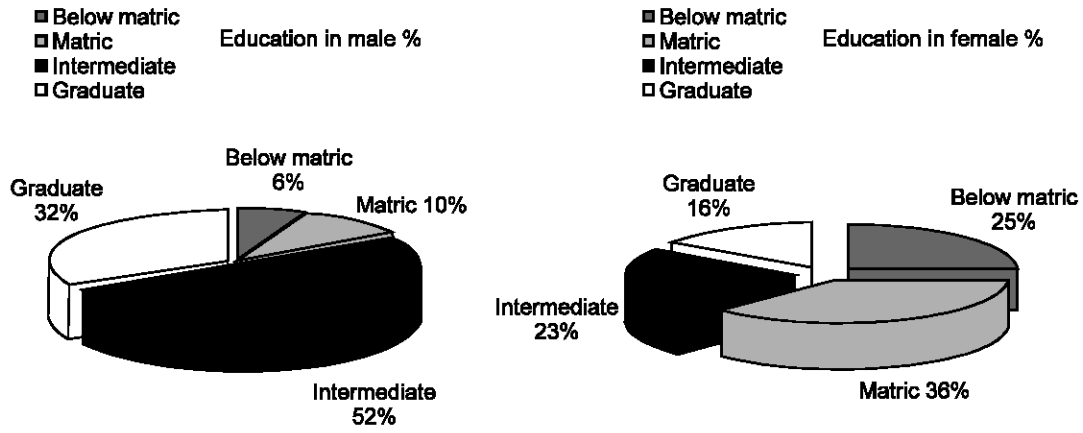

Fig. 1: Pie chart of educational distribution of respondents

Table 4: Comparative correlation of age, education and water on all the variables studied

Correlation (r)	Age	Education	Impact on health	Buying practices	Market practices	Water
Age (r)	0	-0.023	-0.032	-0.024	0.016	0.013
P<0.05		(0.333)	(0.002)	(0.136)	(0.010)	(0.043)
Education (r)	0		-0.6861	0.759	-0.464	-0.0351
P<0.05			(0.004)	(0.007)	(0.023)	(0.088)
Water (r)			0.042			0
P<0.05			(0.057)			
df	3	3	3	3	3	3
χ^2	1.321	56.599	9.37	7.869	15.157	8.9

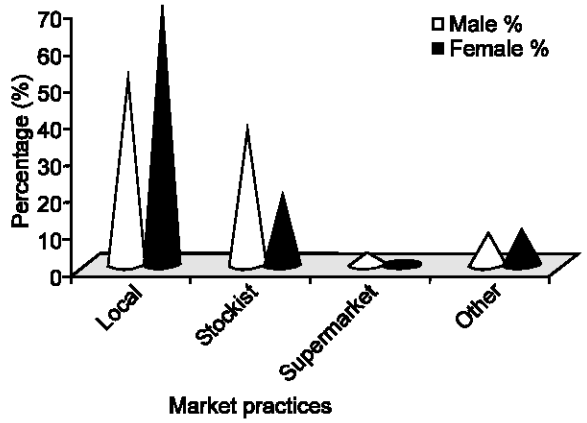

Fig. 2: Percentage of respondent for market practices

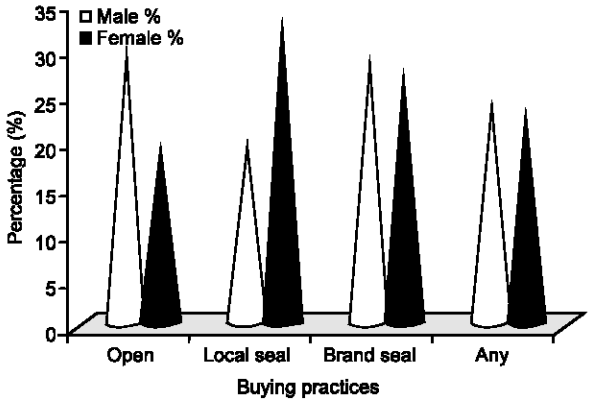

Fig. 3: Percentage of respondent for buying practices

compared by correlation calculation which is shown in Table 4. A correlation value of 0.016 ($p<0.010$) was found with respect to age on market practices which shows that impact of age has slight impact on market practices, while education has negative correlation of -0.464 ($p<0.023$) on market practices. Therefore, the previous conclusion is also supported by the correlation data.

Similar conclusion has been made by Rimal (2001) that educating consumers about preventive methods to reduce food safety threats will lead to reduced concerns and changes in food consumption habits. Kathy Hamilton (2009) concluded that there is connections between the poverty narrative and the family decision making individual control in purchasing and budgeting decisions. Therefore, it may be one of the causes for not following good buying practices.

Food and water practices: The summaries of result are given in Table 5 and 6 and Fig. 4 and 5. It is most commonly observed that water mostly effects health and proper educational background and availability of water both effects whole family health.

Table 5 shows that about 36.8% male (n = 114) uses supply water, while 31.5% uses filtered water while other 29% uses water from Hand pump (ground water) while

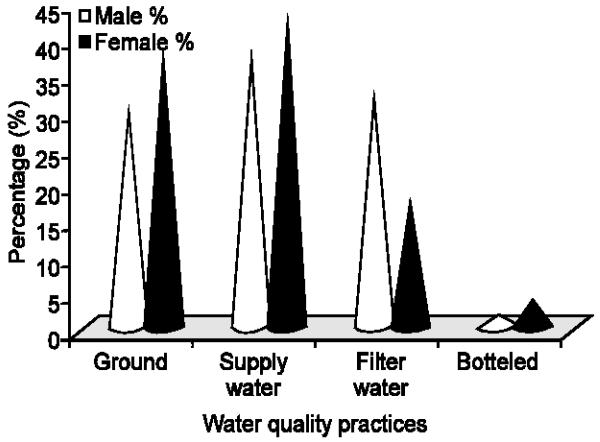

Fig. 4: Percentage of respondent following different water quality practices

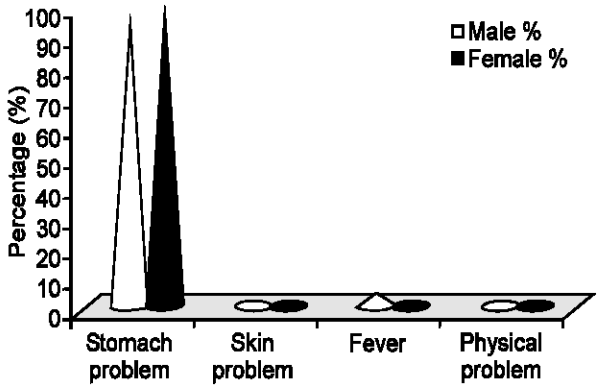

Fig. 5: Percentage of respondent affected by using bad food practices

Table 5: Number and percentage of respondent for Water quality practices

Water quality practices	Male	Male (%)	Female	Female (%)
Ground	34	29.8	69	37
Supply water	42	36.8	78	41.9
Filter water	36	31.5	32	17.2
Bottled	2	1.7	7	3.7

in female (n = 186) 41.9% prefer to use supply water perhaps because of availability, while 37% uses hand pump water and only 17.2% uses filtered water. This means that male are also not well aware of hygienic aspects of water quality and they are relying on supply water but still some respondent are there that are following good hygienic health practices (using filtered water = 31%). In females, similar observation was made in which 41.9% are still using supply water while 37% are using ground water and only 17.2% are using filtered water. In both male and female there is low percentage for using bottled water. Therefore, it can be concluded that both male and female respondent are not following good quality water practices.

Table 6: Number and percentage of respondent for Health problems due to bad food practices

Health problems due to bad food practices	Male	Male (%)	Female	Female (%)
Stomach problem	107	93.85	180	96.77
Skin problem	2	1.75	3	1.61
Fever	5	4.38	3	1.61
Physical problem	0	0	0	0

Calculated χ^2 was slightly more (8.9>7.82) than t-test at 0.05 a 3 degree of freedom. Therefore, it can be concluded that difference is significant and hypothesis is rejected. Knowledge is less followed and use of water is more dependent on availability of water. It was seen that still some frequency are there which follows good water practices by using filtered water which decide overall family health. Therefore, some data were also collected regarding the impact on the health. Table 4 correlation data shows that age has slightly positive correlation with use of quality water 0.013 ($p<0.043$) while education has slightly negative correlation with use of quality water -0.0351 ($p<0.088$).

Table 5 health problem shows that 93% male and 96% female were having problem related to stomach and other were minor problem. Calculated (χ^2) was less than t 0.05 (3) value (2.21<7.81). Therefore, difference is insignificant and hypothesis is valid that due to bad food practices respondents are not healthy and effected by some problems. Water quality has significant impact over health of both male and female especially stomach related problem. Water is shown to have high positive correlation with health 0.042 ($p<0.057$) as shown in Table 4.

Conclusion and suggestions: From the present study, it could be concluded that age has no impact in following buying practices of homemakers and their awareness in food practices in Varanasi (urban area). Study also revealed that educated people of both sexes follow slightly good buying practices while homemakers are not following both buying as well as food practices and also they differ significantly in following the practices in term of use of quality water which affects the family health. Study also revealed that age and awareness are not interlinked while education is interlinked with good practices. This may be due to various reasons like income, awareness and less education of impacts on health. Now a day's various private ("Jago Re") and government agencies ("Jago Grahak Jago") are making good effort for making the consumer well aware of these running various slides on TV and radio but still people are not following good buying and food and water practices.

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Effect of Feeding Canola Oil and Vitamin A on the Fatty Acid Profile of Egg Yolks in Laying Hens

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Abstract: Two hundred and forty White Leghorn laying hens at 48th week of age were randomly divided into 24 experimental units. These experimental units were allotted to eight treatment groups which were fed diets with 0, 2, 3 and 4% canola oil with 3000 or 10000 IU vitamin A/kg of diet (4 x 2 factorial design), for a period of 12 weeks in order to observe the effects of feeding canola oil and vitamin A on the fatty acid profile of egg yolks. Two eggs per replicate were collected at the end of trial and analyzed for fatty acid contents of egg yolks. The increase in dietary canola oil levels increased ($p<0.001$) both n-6 and n-3 Polyunsaturated Fatty Acids (PUFA) but the increase in n-3 PUFA was more pronounced. The n-6/n-3 ratio decreased with the increase in dietary canola oil levels. However, the increase in dietary vitamin A level did not influence ($p>0.05$) the egg yolk fatty acid composition.

Key words: Canola oil, n-3 PUFA, laying hens

INTRODUCTION

Omega-3 polyunsaturated fatty acids (n-3 PUFA) play an important role in reducing blood viscosity and pressure, platelet aggregation, cardiac arrhythmia and plasma triglycerides (Simopoulos, 2000) in humans. Dietary intake of n-3 PUFA decreases risk of heart disease (Tample, 1996), provide an inhibitory effect on the growth of prostate and breast cancer (Rose, 1997), delays the loss of immunological functions (Fernandes, 1995) and is required for normal fetal brain and visual development (Neuringer *et al*., 1998). Considering the potential health benefits of n-3 PUFA, these should be increased in the human diet. In this regard, eggs are potential source of n-3 fatty acids because they can be easily enriched with n-3 PUFA by dietary modifications of the laying hens. The enrichment of laying-hen rations with vegetable oils, such as canola oil, readily promotes the deposition of n-3 PUFA into egg yolk (Van Elswyk, 1997). Canola oil may clearly increase the n-3 PUFA contents in the form of Linolenic Acid (LNA), the precursor of whole n-3 family of fatty acids (Lopez-Ferrer *et al*., 2001). Canola oil has what is now considered to be an almost perfect balance of n-6 to n-3 PUFA; the n-6 to n-3 ratio in canola oil is 2:1 which perfectly matches with human requirements. In PUFA-enriched eggs, the control of lipid per-oxidation is required to prevent the loss of nutritional values (Chow, 1992). Supplementation of hens' diet with vitamin A may increase the content of this vitamin in eggs (Mendonca *et al*., 2002) and can prevent any possible lipid per-oxidation in eggs and egg products. Moreover, considering the potential health benefits described for vitamin A (Food and Nutrition Board, 2000), enriched poultry eggs can be a useful source of this vitamin in human diet.

Most of the trials conducted in the past used linseed or marine oils for enrichment of eggs with n-3 PUFA. However, A little information is available on use of canola oil to enhance the n-3 PUFA contents of egg. So, the present trial was conducted to evaluate the supplemental effects of canola oil, in combination of vitamin A, on egg yolk fatty acid composition in laying hens.

MATERIALS AND METHODS

Two hundred and forty White Leghorn laying hens at 48th week of age were randomly divided into 24 basic experimental units; each comprising of 10 laying hens and was designated as a replicate. These replicates were allotted to eight treatment groups (three replicates/treatment) which were fed diets with 0, 2, 3 and 4% canola oil with 3000 or 10000 IU vitamin A /kg of diet (4 x 2 factorial design). The hens were kept in cages (2 birds/cage) providing 0.093 m^2 floor space area to each, throughout the experimental period of 12 weeks. All the diets were isocaloric and isoproteinous formulated according to the recommendations of National Research Council (1994). Hens had ad libitum access to feed and water throughout the experimental period. The light regime was 16L:8D for all treatment groups. Two eggs per replicate were collected at the end of trial and analyzed for fatty acid contents of egg yolks.

Analysis: Chemical analysis of the layer ration was run using international procedures of AOAC (1990). Ingredients and chemical composition of layer ration are shown in Table 1. The fatty acid contents of egg yolks

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Table 1: Ingredient and nutrient composition of layer diets

Diets	T_1^a &T_2^b	T_3^a &T_4^b	T_5^a&T_6^b	T_7^a &T_8^b
Ingredients	---------- % ----------			
Corn	65.00	53.00	50.00	48.00
Rice broken	4.40	10.85	11.60	11.45
Soybean meal	13.00	19.80	23.40	27.10
Fish meal 52%	5.50	0.00	0.00	0.00
Corn gluten	4.00	4.90	2.50	0.00
Canola oil	0.00	2.00	3.00	4.00
Limestone	6.92	7.25	7.35	7.40
DCP	0.72	1.65	1.65	1.60
L-lysine	0.08	0.14	0.06	0.00
Dl-methyonine	0.03	0.07	0.85	0.10
Vit./min. premix[1]	0.35	0.35	0.35	0.35
Total	100	100	100	100
Nutrients				
CP (%)	17.00	17.00	17.00	17.00
ME (Kcal/Kg)	2900	2900	2900	2900
EE (%)	3.22	4.30	5.14	6.00
CF (%)	3.88	3.70	3.91	4.07
Ca (%)	3.24	3.27	3.30	3.26
Av.P (%)	0.41	0.43	0.42	0.44
Lysine (%)	0.90	0.92	0.91	0.90
Methionine (%)	0.38	0.37	0.36	0.4
Threonine (%)	0.64	0.66	0.65	0.63
LA[2] (%)	1.50	1.66	1.81	1.98
LNA[3] (%)	0.07	0.26	0.36	0.46

[1]Provided per kilogram of diet: Cholecalciferol, 1,250 IU; Vitamin E (dl-alpha-tocopheryl acetate), 12 IU; menadione, 2.5 mg; riboflavin, 6 mg; calcium pantothenate, 8 mg; niacin, 15 mg; pyridoxine 2 mg; folic acid, 1 mg; vitamin B_{12}, 7 µg; Mn, 50 mg; Zn, 55 mg; Fe 40 mg; Cu, 4 mg; I, 2 mg; Co, 0.3 mg; ethoxiquin, 150 mg. [a]rations containing 3000 IU/kg of diet vitamin A; [b]rations containing 10000 IU kg of diet vitamin A; [2]LA = Linoleic Acid;[3]LNA = Linolenic Acid

were determined by gas chromatography. The egg yolks were separated by breaking the eggs. Yolk lipids were extracted according to AOAC (1995) by using chloroform and absolute alcohol (1:1). Fatty acids were converted into Fatty Acid Methyl Esters (FAME) according to the method described by Chin *et al*. (1992) by using 4 ml HCl in 100 ml methanol. FAME were Separated and quantified by gas Chromatograph (Varian 3900) using a fused silica capillary column (30 M, 0.25 mm diameter).

Statistical analysis: The statistical analysis was performed using the two-way ANOVA by GLM and Tukey's honestly significant difference test was used to compare means (Minitab 13.1, Minitab Inc., State College, PA).

RESULTS

The amount of oleic acid (OA; an n-9 fatty acid) was increased ($p<0.001$) by feeding canola oil (Table 2). The total n-6 and n-3 PUFA in egg yolks of laying hens increased ($p<0.001$) with the increase in dietary canola oil level (Table 2 and 3). The supplementation of 4% canola oil produced best results with highest n-6 and n-3 PUFA level in the yolks. The ratio of n-6 to n-3 PUFA decreased ($p<0.001$) with the increase in canola oil level

Table 2: Effect of canola oil and vitamin A on egg yolk Oleic Acid (OA), Linoleic Acid (LA), Linolenic Acid (LNA) and Arachidonic Acid (AA) contents (% of total fatty acids) in laying hens

Diet	OA (%)	LA (%)	LNA (%)	AA (%)
Canola oil (%)				
0	37.90[d]	13.70[c]	0.89[b]	1.86
2	41.98[c]	14.76[b]	1.99[a]	1.82
3	43.39[b]	14.79[b]	1.92[a]	1.89
4	44.28[a]	15.64[a]	1.98[a]	1.97
SEM	0.193	0.079	0.035	0.044
Vit. A (IU/kg diet)				
3000	41.79	14.75	1.69	1.88
10,000	41.99	14.70	1.69	1.89
SEM	0.137	0.056	0.025	0.031
ANOVA	---------- Probabilities ----------			
Oil	0.000	0.000	0.000	0.196
Vit. A	0.329	0.587	0.917	0.913
Oil x Vit. A	0.366	0.665	0.637	0.981

[a-d]Means within a column with different superscripts differ significantly ($p<0.001$)

of the diet in laying hens (Table 2). The lowest ratio was observed at the level of 4% canola oil in the diet. However, vitamin A level higher than NRC recommendations did not produce any significant ($p>0.05$) affect on egg yolk n-6 PUFA, n-3 PUFA or n-6/n-3 PUFA ratio in laying hens. No dietary interaction ($p>0.05$) was observed between canola oil and vitamin A for egg yolk n-6 PUFA, n-3 PUFA or n-6/n-3 PUFA ratio in laying hens.

DISCUSSION

The n-6 PUFA: Linoleic Acid (LA) and total n-6 PUFA of egg yolks followed an increasing trend with the increase in canola oil in the diet of hens. Canola oil contains almost 20% LA (Rowghani *et al*., 2007) (Which is double than LNA in canola oil), so the dietetic increase in canola oil resulted into an increase in LA content of egg yolks. The Arachidonic Acid (AA) content in yolks was not increased with the increase in canola oil of the diet. It could be suggested that LNA have more preference than LA for desaturation and elongation in the liver metabolism of hens (Bean and Leeson, 2003). That's why AA level could not be increased instead of increased level of LA in the diet. On overall basis, total n-6 PUFA increased in egg yolks with the increase in canola oil in the diet, mainly because of increased LA contents of yolks. The n-6 PUFA contents remained similar for both dietary vitamin A levels. Similar to the present results, the addition of canola oil to the hens' diet increased the concentration of LA in the egg yolks (Da Silva Filardi *et al*., 2005). Canola oil supplementation resulted into an increase in LA content of thigh and breast muscles of broilers (Nobar *et al*., 2007). Shafey *et al*. (2003) had reported an increase in LA contents in yolks of hens fed on sunflower oil in the diet. The results of current study are not in agreement with Aydin (2005) who fed various

Table 3: Effect of canola oil and vitamin A on Eicosapantanoic Acid (EPA), Docosapantanoic Acid (DPA), Docosahexanoic Acid (DHA), total n-3 and n-6 PUFA (% of total fatty acids) and n-6:n-3 in laying hens

Diet	EPA	DPA	DHA	n-3 PUFA	n-6 PUFA	n-6:n-3
	(%)					
Canola oil (%)						
0	0.07[b]	0.23[c]	1.24[d]	2.44[d]	15.57[c]	6.39[a]
2	0.13[a]	0.26[b]	1.70[c]	4.08[c]	16.59[b]	4.07[b]
3	0.15[a]	0.25[bc]	2.30[b]	4.63[b]	16.69[b]	3.60[c]
4	0.13[a]	0.30[a]	2.97[a]	5.39[a]	17.61[a]	3.27[d]
SEM	0.005	0.006	0.025	0.027	0.094	0.036
Vit. A (IU/kg diet)						
3000	0.12	0.26	2.04	4.12	16.63	4.31
10,000	0.11	0.26	2.06	4.14	16.59	4.35
SEM	0.003	0.004	0.017	0.019	0.066	0.026
ANOVA	Probabilities					
Oil	0.000	0.000	0.000	0.000	0.000	0.000
Vit. A	0.195	1.000	0.342	0.628	0.681	0.414
Oil x **V**it. A	0.352	0.539	0.670	0.119	0.787	0.224

[a-d]Means within a column with different superscripts differ significantly ($p<0.001$)

canola oil levels (0.5-10%) in the diet of laying hens and concluded that oil supplementation did not produce any change in n-6 PUFA of egg yolk. On other hand, Nobar *et al*. (2007) reported a decrease in total n-6 PUFA in the muscles of broilers fed diets with canola oil. The increase in OA contents of egg yolks is due to the fact that canola oil has more than 50% OA in it which favoured the deposition of this fatty acid in the egg yolks.

The n-3 PUFA: Canola oil has a handsome quantity of LNA (more than 10%) in it (Da Silva Filardi *et al*., 2005). The increase in dietary canola oil caused a significant increase in LNA and its derivatives Eicosapantanoic Acid (EPA), Docosapantanoic Acid (DPA), Docosahexanoic Acid (DHA) and total n-3 PUFA in egg yolks of hens, as depicted in the present results. Although canola oil have no EPA, DPA and DHA in it, the increase in these fatty acids with the increase in canola oil in the diet confirmed the ability of laying hens to convert LNA into its long chain metabolites as reported earlier (Schumann *et al*., 2000). With the increase in dietary canola oil levels, the total n-3 PUFA increased at a higher magnitude than total n-6 PUFA in the egg yolks. The present results are in line with Rowghani *et al*. (2007) who observed an increase in LNA, EPA, DPA and total n-3 PUFA contents of egg yolk by the supplementation of 3% and 5% canola oil to the laying hens. The addition of canola oil to the hens' diet promoted the enrichment of egg with LNA, DHA and n-3 PUFA as a whole, to some extant in the egg yolk (Da Silva Filardi *et al*., 2005). Hens fed diets supplemented with combination of fish oil (4%) and rape seed oil (2%) laid eggs with higher content of n-3 PUFA (LNA, EPA, DPA and DHA) in yolk lipids (Skrtic *et al*., 2007). Aydin (2005) fed various canola oil levels (0.5-10%) in the diet of laying hens and concluded that as the proportion of canola oil increased in the diet of layers, the concentrations of LNA and total n-3 PUFA in the yolk increased. Cherian and Sim (1991) reported increased LNA and DHA content of egg yolks with feeding 16% canola seed in the diet. Canola oil supplementation dramatically increased the LNA, EPA and DHA content of thigh and breast muscles of broilers (Nobar *et al*., 2007). As for as vitamin A; its higher level in the diets did not affect the n-3 fatty acid composition of egg yolks.

n-6/n-3 PUFA ratio: Canola oil has an ideal LA/LNA ratio of 2:1 in it (Simopoulos, 2000). Laying hens in this study showed the ability to deposit fatty acids in accordance to what is fed in the ration. The LA/LNA ratio in yolks decreased with the increase in dietary canola oil level. The increase in n-3 PUFA contents at a high magnitude than n-6 PUFA resulted into decreased n-6/n-3 PUFA ratio in egg yolks of hens fed on canola oil in the diet. According to the previous studies also, the addition of canola oil to the hens' diet can lower the n-6:n-3 ratio in the egg yolk (Da Silva Filardi *et al*., 2005). Similarly, Aydin (2005) offered various canola oil levels (0.5-10%) to the laying hens and concluded that n-6:n-3 ratio in the egg yolk decreased in a linear fashion with the increase in canola oil level. Pita *et al*. (2006) found that 6% canola oil in the diet of hens resulted n-6/n-3 ratio of 6.48 in the egg yolks. However, in the present study, vitamin did not produce any favorable effect on n-6/n-3 fatty Acid ratio of egg yolks.

Conclusion: The inclusion of canola oil in the diet of laying hens promoted the deposition of n-6 and n-3 FUPA in egg yolks. The increase in n-3 PUFA was more pronounced. So, the inclusion of canola oil in the diet of laying hens resulted into the eggs with better proportion of n-3 PUFA. As n-3 PUFA are more beneficial for human health than other type of fatty acids, n-3 enriched eggs produced by canola oil feeding to laying hens are more valuable for human beings than ordinary commercial eggs.

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Economics of Using Cocoa Bean Shell as Feed Supplement for Rabbits

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Abstract: The study analyzed the economics of using cocoa bean shell (cbs) as feed supplement in rabbit production. Data used for this study was collected from an experimental study of performance of rabbits fed graded levels of various treatments of CBS as feed supplement. Gross margin and dominance analysis were used to analyze the data. The study showed that untreated CBS can be used economically at 100g/kg inclusion in rabbit feed while hot Water treated CBS (WCBS) can be included up to 200 g/kg in rabbit feed. The study recommends the use of hot water treatment of CBS at 200 g/kg inclusion for optimum profitability of rabbit production.

Key words: Cocoa bean shell rabbit, gross margin, dominance analysis

INTRODUCTION

In Nigeria, the high cost and scarcity of conventional food and feed sources for human and livestock has caused men and livestock to compete with each other for some food supply. Hence, livestock for use should be those that are less dependent and are capable of converting crop waste into meat. Lebas *et al.* (1986) found that rabbits with short generational interval fulfils such criteria in utilizing plant materials and crop wastes more efficiently and are very suitable as panacea to protein deficiency and low meat supply in Nigeria (Joseph *et al.*, 1997).

There is therefore, the need to search for by products and crop wastes with a view to finding feed supplements which can maintain physiological balance and enhance live-stock productivity.

Cocoa bean shell, a by-product of the emerging cocoa is the testa immediately surrounding the cocoa nib and constitute about 10% of the cocoa bean. It is estimated that about 10,500 tonnes of cocoa bean shell is produced annually in Nigeria (Aina, 1998), which constitute a disposal problem in the cocoa milling and chocolate industry. The cocoa bean shell has a proximate composition that is comparable to other agro-allied by-products such as maize bran and wheat offal. Hence, it is useful as a supplement to feed animals especially, rabbits. Cocoa bean shell also has an intermediate buffer value between the protein and cereal sources of feed (Carolien, 2001). This suggests that animals consuming cocoa bean shell might not have difficulty in lowering the gastric pH, thus, improving protein digestibility and utilization.

However, the theobromine content of cocoa bean shell limits its use as feedstuff for monogastric animals due to the imbalance caused by theobromine on growth performance of rabbits (Muhammed *et al.*, 2000). Earlier reports (Odunsi and Longe, 1998; Odunsi *et al.*, 1999) advocate the reduction or neutralization of theobromine as a means of improving the food value of the cocoa cake. Adeyina and Ademoroti's (2003) findings indicate some effectiveness of boiling treatment of cocoa bean shell in reducing the theobromine level for improved performance of broiler finisher. This effectiveness may actually be based on the physical measurement; hence, the study is aimed at analyzing the economic of using cocoa bean shell as feed supplement for rabbit. The study specifically analyzes the economical treatment in the use of cocoa bean shell the profitability and the best level of inclusive of cocoa bean shell as feed supplement in rabbit.

MATERIALS AND METHODS

Data used for this study were mainly of the secondary form. The major data was obtained from an experimental study of rabbits fed various treatments of cocoa bean shell. Data were collected on the body weight gain resulting from different level of cocoa bean shell fed. Information were also sourced from journals, annual reports and internal Network information.

Two treatments were used to investigate the effect of cocoa bean shell on growth performance and economic returns to the farmer. Treatment one evaluated untreated Cocoa Bean Shell (CBS) at 0, 50, 100, 150, 200, 250 or 300 g CBS 1kg diet. Treatment two involved hot Water treated Cocoa Bean Shell (WCBS) a 0, 100, 200, 300 and 400 g/kg (WCBS) diet. In treatment 1, forty-two weaner rabbits (mean body weight 0.37 kg) were randomly allocated to the 7 dietary treatment levels. Each rabbit was treated as a replicate thus, there were six replicates per treatment level.

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Treatment two involved the use of 30 weaner rabbit (mean body weight 0.30 kg) randomly allocated to the five dietary treatment levels. Each rabbit was treated as a replicate thus, giving 6 replicates per treatment level.

The feeding trial lasted 70 days, feed and water were given *ad-libitum* and body weight gain was determined on a body basis. Total estimate of cost on labour feed stuffs and value of foundation stocks were compared with total value production to determine the profit or loss to rabbit production.

Gross margin and Dominance analysis were used to analyze in the study. Gross margin analysis determined and compared the cost and return of rabbit production using different levels of cocoa bean shell as feed supplements and is expressed thus:

$$\text{Gross margin (GM)} = \text{TVP} - \text{TVC}$$

where:

TVP = Total value production(ie. Price at which each rabbit was sold

TVC = Total variable cost (sum total of labour, feedstuff cost and value (VFS) of foundation stock)

Dominance Analysis examined the cost and benefit of each treatment and served in eliminating some of the treatment levels from further consideration.

RESULTS AND DISCUSSION

Table 1 shows the result of the gross margin analysis for treatment one. The weight gain compared with the control diet (0 g/kg) was the same (1.2 kg) for rabbits fed up to 100 g/kg inclusion of untreated CBS. However, with further increase in CBS level, weight gain reduced to the least in 300 g/kg inclusion of CBS. This can be attributed to the presence of theobromine (an anti-nutritional factor) which is capable of suppressing appetite thereby reducing feed intake and hence, the reduction in growth performance (Muhammed *et al*., 2000). Also, the Total Variable Cost (TVC) showed the control diet (0 g/kg) with no CBS inclusion to be highest (969.42 Naira) while, the least TVC (743.28 Naira) was at 300 g/kg inclusion. This confirms the report that the use of non conventional feed ingredient can help in reducing the cost of feeding livestock. Gross margin was shown to increase up to 100 g/kg inclusion level of untreated CBS after which, it started declining. Thus, revealing the superiority of 100 g/kg inclusion level of untreated CBS.

The gross margin analysis of rabbits fed hot Water treated CBS (WCBS) is shown in Table 2. Weight gain up to 200 g/kg inclusion level was the same as the control (1.2 kg). TVC was highest in the control diet (670.58 Naira) and lowest in 400 g/kg WCBS (568.90 Naira). Gross margin however was highest at 200 g/kg inclusion level while, it was lowest at 400 g/kg WCBS inclusion level.

To further ascertain the best economical level of inclusion of the untreated and the hot water treated CBS, the result of the dominance analysis for untreated CBS (Table 3) indicate 100 g/kg inclusion to have the highest return with lower cost. Therefore dominating the other levels of inclusion. In treatment two the dominance analysis (Table 4) showed 200 g/kg inclusion of WCBS to have the highest return with lowest cost compared to other levels of WCBS inclusion.

Table 5 shows the comparison of the dominance analysis of the two treatments. The result showed treatment two with hot water treated CBS dominating treatment one with untreated CBS. This confirms the effectiveness of hot water treatment in reducing the theobromine content of CBS for better performance of livestock (Adeyina and Ademoroti, 2003).

Table 1: Gross margin analysis of rabbits fed graded levels of untreated cocoa bean shell

CBS level (g/kg)	Body weight gain (kg)	VFS (N)	Feed cost (N)	Labour cost (N)	TVC (N)	TVP (N)	GM (N)
0	1.2	600	4.82	364.6	969.42	1000	30.52
50	1.2	550	4.65	364.6	919.25	1000	80.75
100	1.2	500	4.43	364.6	869.03	1000	130.97
150	1.1	450	4.22	364.6	818.82	900	81.18
200	0.9	400	4.02	364.6	768.62	800	31.38
250	0.9	390	3.82	364.6	758.42	800	41.58
300	0.8	380	3.68	364.6	743.38	750	1.72

Table 2: Gross margin analysis of rabbits fed graded levels of hot water treated cocoa bean shell

CBS Level (g/kg)	Body weight gain (kg)	VFS (N)	Feed cost (N)	Labour cost (N)	TVC (N)	TVP (N)	GM (N)
0	1.20	300	5.98	364.6	670.58	1000	329.42
100	1.20	250	5.55	364.6	620.15	1000	379.85
200	1.20	230	5.10	364.6	599.70	1000	400.30
300	1.00	220	4.72	364.6	589.32	850	260.68
400	0.95	200	4.30	364.6	568.90	800	231.10

Table 3: Dominance analysis of rabbits fed untreated cocoa bean shell

CBS level(g/kg)	0	50	100	150	200	250	300
0	-	+50.17	+100.39	+50.60	+0.8	+11	-28.86
50	NA	-	+50.23	+0.43	-4937	-39.17	- 79.03
100	NA	NA	-	-49.79	-99.59	-89.39	-129.25
150	NA	NA	NA	-	-49.80	-39.6	-79.46
200	NA	NA	NA	NA	-	+10.2	-29.66
250	NA	NA	NA	NA	NA	-	-39.86
300	NA	NA	NA	NA	NA	NA	-

Table 4: Dominance analysis of rabbits fed graded level of hot water treated cocoa bean shell

WCBS level (g 1 kg)	0	100	200	300	400
0	-	+50.43	+70.88	-68.75	-98.32
100	NA	-	+20.45	-119.17	-14875
200	NA	NA	-	-139.62	-169.2
300	NA	NA	-	-	-29.58
400	NA	NA	NA	NA	-

NA = Not Applicable, +ve value = Dominant Treatment, -ve value = Non dominant treatment

Table 5: Dominance analysis between of rabbits fed untreated and hot water cocoa bean shell

Treatments	1	2
1	-	+269.33
2	NA	-

Conclusion and recommendation: From the findings of this study, it can be concluded that CBS is economical as a non-conventional feed supplement and can be included in rabbit feed at 100 g/kg inclusion for the untreated CBS and 200 g/kg inclusion for the WCBS in rabbit production for optimum growth performance and highest cost benefit ratio.

It is however recommended that preferably, hot water treated CBS should be used. This treatment has shown to be capable of reducing the theobromine content, reducing feed cost and makes it possible for higher inclusion rate of CBS in rabbit diet.

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Assessment of the Food Habits and School Feeding Programme of Pupils in a Rural Community in Odogbolu Local Government Area of Ogun State, Nigeria

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Abstract: The food habits and school feeding programme of pupils in a rural community in Odogbolu local government area of Ogun State, Nigeria was assessed in this study. A total of 68 pupils from primaries I to III in both public and private primary schools were involved in the study. It was found that majority of the school children had three meals daily. Majority of the pupils do not bring food to school from home. The amount of money brought to school to purchase mid-day meal foods was higher with pupils from private school than those from public schools. However, generally the quantity and quality of the mid-day meal purchased on both schools were poor and therefore did not have significant contribution of their nutritional status.

Key words: Assessment, food, habits, school, feeding

INTRODUCTION

Malnutrition has continued to be a public health problem in developing countries where the poor socio economic condition has continued to work in synergy with malnutrition. The most recent Food Consumption and Nutrition Survey in Nigeria (FCSN 2001-2003) cited by Adebambo (2006) reveals that the nutritional status in Nigerian children is very poor. The data showed that 42% of Nigerian children were stunted, 25% were underweight and 9% were wasted. 29.5% of the children under five years of age suffer from vitamin A deficiency while over 27% were at different stages of iron and iodine deficiency.

Although the importance of education had been internationally acknowledged, it is estimated that in developing countries as many as 26% of boys and 30% girls of primary school age are not attending school (UNDP, 2003). A further 11% of children attending school do not reach grade 5 (Wachs, 2000). Malnutrition has been identified to affect the cognitive development of children (Pollitt, 1995; Grantham-McGregor and Ani, 2001). Apart from the adverse effect of malnutrition on the cognitive achievement of school children, malnutrition is also likely to result in poor attendance at school, low health status which will invariably lead to high withdrawal rate. If the millennium goal of education for all by 2015 is to be achieved, there is the need to put in place measures that will increase children enrolment, retention and improved academic performance. Poverty and poor eating habit have been linked to poor child growth and low school age enrolment among Nigeria children (Adebambo, 2006). Although the Universal Basic Education (UBE) programme had been embarked upon in Nigeria, measures to boost school enrolment, attendance at school and completion as well as improve the learning achievement of the school children must also be put in place. A veritable tool for achieving this is the introduction of good and effective school feeding programme. Although school feeding programme is currently being practiced in Nigeria, there is the need to assess its effectiveness especially in achieving the goals earlier on identified.

The purposes of this study therefore are to assess the food consumption pattern and practice of school feeding programme among school children in a rural community in Ogun State, Nigeria, with view to determining their effect on the nutritional status of the pupils and their attendance in school.

MATERIALS AND METHODS

The study was carried out in Ijagun, a rural village in Odogbolu local government area of Ogun State Nigeria between January and April, 2008. The Tai Solarin University of Education is located in this village. There are two primary schools in the village, one private (government approved) and the government public primary school. Both schools were involved in the study. All the pupils in primaries I to III in both schools were involved in the study. There were thirty nine (39) and twenty nine (29) respondents from the public and private schools respectively. Thus the total sample population was sixty-eight (68).

Information about the food habits of pupils were obtained through a questionnaire designed in line with that used in the assessment of food vending schemes of primary schools in Nigeria (FME/FBI/FAO, 1997). The class teachers were tutored on the objective of the study and use of the questionnaire and thereafter employed in the administration of the questionnaire on the pupils.

The ages of the children and attendance at school were obtained from the school register. Height was measured

using a specifically constructed wooden stadiometer and measurement was to the nearest centimeter. Weight was determined using a Salter bathroom scale. The scale was calibrated periodically with standard weight to ensure its reliability. The pupils heights were determined bare footed and weights determined in their underwares only. Thereafter, the weight for age, height for age and weight for height were converted to the corresponding Z-scores and were them compared with the standard reference measurement by the National Centre for Health and Statistics (NCHS, 1986).

RESULTS

The general characteristics of the school children are show in Table 1. In the private school, 46.2 and 53.8 percent were male and female respectively while in the public school, there were 48.3 and 51.7% male and female respectively. With respect to the father's occupations, majority, 51.3% of pupils' fathers in the private school were engaged in paid employment and 43.6% were traders. In the public school, majority (48.3%) of the fathers were farmers; 34.5% traders and 13.8% engaged in paid employment. With respect to the mothers, majority (59.0%) of mothers of pupils in the private school were traders. The same trend was observed with mothers of pupils in the public school with 51.7% involved in trading. While none of the mothers of pupils in private school was involved in farming, 31.0% mothers of pupils in public schools were engaged in farming.

The food consumption pattern of pupils at home are presented in Table 2. Majority of the pupils, 92.3 and 89.7% for private and public schools respectively, had their breakfast at home. Generally the breakfast, lunch and dinner were prepared at home. All pupils in both schools claimed to have lunch everyday. However, while 92.3% of pupils in private school had lunch at home, all the pupils in the private school (100.0%) had lunch at home. Eating snacks between lunch and dinner was not a common practice among the pupils in both schools as 74.4 and 89.7% of, pupils in the private and public schools respectively, claimed not to be involved in the practice. All the pupils in both schools had dinner in their respective homes.

The description of the staple foods commonly eaten at various meals at home by the pupils is presented in Table 3. For the breakfast, the most popular food is rice among the pupils of both the private and public schools, 43.6 and 30.5% respectively. However, while 10.3% of pupils in private school consumed cassava based food at breakfast, 31.0% of the pupils consumed cassava-based diet among the public schools. Among the pupils of both private and public schools, the most common staple foods for lunch and dinner was cassava based, 48.7 and 72.4% respectively for lunch and 56.3 and 62.1% respectively for dinner. This was followed by rice in both groups, 23.1 and 20.7% in the private and public schools respectively. Majority of the foods for lunch and dinner were consumed along with soup or stew with animal of fish products. However, more pupils in the private school consumed animal and fish products along with the staples than pupils in public schools.

The food consumption pattern of the pupils outside the home are presented in Table 4. This involved bringing food to school from home, buying and eating foods before the beginning of school, during break and after school hours before reaching home. Majority of pupils in both schools were not involved in the practice of bringing food to school from home. Specifically, 74.4 and 100% of pupils in the private and public schools respectively did not bring food to school from home. Buying food during the break within the school premises constituted the major food eaten outside the school by the pupils for both schools. Among the pupils in the private school, 20.5, 64.1 and 41.0% bought food before the commencement of school, during the break time within the school and after the school hours from vendors outside the school respectively. The corresponding values for the pupils in the public school are 10.3, 58.6 and 27.6%.

The frequency of bringing money and the amount brought to school are presented in Table 5. Among the pupils in the private and public schools, 79.5 and 62.1% respectively brought money to school. Among those that brought money to school majority, 41.9%, of pupils in private school brought ten naira (₦ 10.00) while majority, 72.2% of those in public school brought five naira (₦ 5.00). The major type of food purchased after school hours by pupils in both the private and public schools are fruits, 48.7 and 82.8% respectively. With respect to the type of food sold in the school during the break time, cooked rice with fish or meat stew was dominant in both schools.

The nutritional status of the pupils from both schools are presented in Table 6. Majority of the pupils were underweight from primaries I to III in both the public and private schools. In the public schools the prevalence of the underweight pupils ranged from 49.3% in primary I to 51.3% in primary III while that of private school ranged from 40.3-43.6%. With respect to stunting, the prevalence for the public school ranged from 21.6% for primary I pupils to 27.7% for the primary III pupils. The range for the private school is 15.4-20.3% from primary I to primary III. For wasting, 14.2-16.3% were found to be wasted with the highest occurring in among the primary II pupils in the public school. This is at variance with the usual trend where the highest incidence of malnutrition was found among the primary III pupils. But for the private school, the range of the prevalence of wasting was from 7.7% in primary I to 10.5% in primary III. Inspite of the high degree of malnutrition observed, some level of overweight was observed. This ranged from 0.8%

Table 1: General characteristics of school children by school type

	Schools			
	Private		Public	
	Frequency	%	Frequency	%
Sex of Pupils				
Male	18	46.2	14	48.3
Female	21	53.8	15	51.7
Total	39	100	29	100
Occupation of Father				
Farming	02	5.1	15	51.7
Trading/self employed	17	43.6	10	43.5
Gainfully employed	20	51.3	04	13.8
Unemployed	-	-	-	-
Total	39	100	29	100
Occupation of Mother				
Farming	-	-	09	31.0
Trading/self employed	23	59.0	15	51.7
Paid employments	14	35.9	03	10.3
Unemployed	02	5.1	02	7.0
Total	39	100	29	100

Table 2: Food consumption, pattern and practice of pupils at home

	Schools			
	Private		Public	
Characteristics	Frequency	%	Frequency	%
Have breakfast at home				
Yes	36	92.3	26	89.7
No	03	7.7	03	10.3
Total	39	100	29	100
Frequency of breakfast at home (per week)				
Less than four times	05	12.8	09	31.0
More than four times	34	87.2	20	69.0
Total	39	100	29	100
Source of break fast				
Prepare at home	35	89.7	21	72.4
Bought from food vendor	04	10.2	08	27.6
Total	39	100	29	100
Home lunch everyday				
Yes	39	100	29	100
No	-	-	-	-
Place of lunch				
At home	36	92.3	29	100
From food vendor	03	7.7	-	-
Total	39	100	29	100
Meal between lunch and Dinner (snacks) per week				
None	29	74.4	26	89.7
Less none than four times	06	15.4	03	10.3
Four or more times	04	10.2	-	-
Total	39	100	29	100
Dinner everyday				
Yes	39	100	29	100
No	-	-	-	-
Total	39	100	29	100
Place of Dinner				
At home	39	100	29	100
From Food Vendor	-	-	-	-
Total	39	100	29	100

among the primary III pupils in the public school to 3.6% among the primary I pupils in the private school. Also the incidence of pupils of normal nutritional status ranged from 5.0% among pupils in primary III in the public school to 32.8% among primary I pupils in the private school.

Attendances of the pupils in both schools are presented Table 7. Generally, attendance of pupils in the private

Table 3: The daily consumption of common staple foods of school children at home

	Schools			
	Private		Public	
Characteristics	Frequency	%	Frequency	%
Breakfast staple food				
Food based on				
Rice	17	43.6	10	34.5
Yam	02	5.1	02	6.9
Plantain	00	0.0	00	0.0
Maize e.g. Pap	03	7.7	05	17.2
Beans	01	2.5	00	0.0
Wheat e.g. bread	12	30.38	03	0.2
Cassava	04	10.3	09	31.0
Total	39	100.0	29	100.0
Presence of animal products in their soup or stew for breakfast				
Yes	29	74.4	17	53.6
No	10	25.6	12	41.4
Total	39	100.0	29	100.0
Lunch staple foods				
Food based on				
Rice	15	38.5	08	27.6
Yam	00	00.0	00	00.0
Plantain	00	00.0	00	00.0
Maize	00	00.0	00	00.0
Beans	02	5.1	00	00.0
Wheat	03	7.7	00	00.0
Cassava	19	48.7	21	72.4
Total	39	100.0	29	100.0
Presence of animal products In their soup or stew for lunch				
Yes	31	79.5	13	44.8
No	08	20.5	16	55.2
Total	39	100.0	29	100.0
Dinner staple foods				
Food based on				
Rice	09	23.1	06	20.7
Yam	01	2.6	00	00.0
Plantain	02	5.1	02	6.9
Maize	00	0.0	00	0.0
Beans	04	10.3	03	10.3
Wheat	01	2.6	00	0.0
Cassava	22	56.3	18	62.1
Total	39	100.0	29	100.0
Presence of animal/fish products in their soup or stew for dinner				
Yes	34	87.2	19	65.5
No	05	12.8	10	34.5
Total	39	100	29	100.0

school was higher than in the public school. In both schools the highest attendance was recorded on Monday while the least was recorded on Friday.

DISCUSSION

Food habit is a major determinant of nutritional status. The study revealed that majority of the school children had three meals daily. This is highly commendable and should be encouraged. In particular, having breakfast will help in holding the attention of the pupils in school, especially in the morning lessons. In fact it has been reported that missing break fast had detrimental effect on cognition (Pollitt *et al*., 1982; Simeon and Grantham-McGregor, 1989; Pollitt *et al*., 1996; Wyon *et al*., 1997; Benton and Parkner, 1998).

That cassava based foods were consumed by majority of pupils in the public school is probably due to the fact that majority of their fathers are engaged in farming, with cassava as the major food cultivated. This type of positive correlation between the staple foods commonly eaten by school children and the major agricultural produce of the community has been reported in previous study (FME/FBI/FAO, 1997). Surprisingly however, rice is the food commonly eaten by the school children for breakfast. This is most probably due to the ease of preparation. It also saves time. Hence most families found it convenient to prepare and serve for breakfast.

Although majority of the pupils brought money to school, the amount brought by majority of the pupils (₦ 5.00) is so small that the food purchased with it was not likely to

Table 4: Food consumption pattern and practice of school children outside the home

	Schools			
	Private		Public	
Characteristics	Frequency	%	Frequency	%
Frequency of bringing food to school from home per week				
Do not bring food from	29	74.4	29	100.0
Less than three times	07	17.9	00	0.0
Three or more times	03	7.7	00	0.0
Total	39	100.0	29	100.0
Buy food before the beginning of school				
Yes	08	20.5	03	10.3
No	31	79.4	26	89.3
Total	39	100.0	29	100.0
Buy food during the school break within the school				
Yes	25	64.1	17	58.6
No	14	39.9	12	41.4
Total	39	100.0	29	100.0
Buy food after school from food vendors outside the school premises				
Yes	16	41.0	08	27.6
No	23	59.0	21	72.4
Total	39	100.0	29	100.0

Table 5: Amount of money brought daily to school by the pupils

	Schools			
	Private		Public	
	Frequency	%	Frequency	%
Brought money to School daily				
Yes	31	79.5	18	62.1
No	08	20.5	11	37.9
Total	39	100.0	29	100.0
Amount of money Brought to school				
₦ 5.00	10	25.8	21	72.2
₦ 10.00	16	41.9	06	22.2
Above ₦ 10.00	13	32.3	02	5.6
Total	39	100.0	29	100.0

Table 6: Classification of nutritional status based on Z-Scores

		Pry I		Pry II		Pry III	
		Public School	Private School	Public School	Private School	Public School	Private School
Nutritional classification	Z-Scores	(%)					
Under weight (weight for age)	<-2SD	49.3	40.3	49.8	42.3	51.3	43.6
Stunting (height for age)	<-2SD	21.6	15.4	26.2	18.7	27.7	20.3
Wasting (weight for height)	<-2SD	14.2	7.7	16.3	8.4	15.2	10.5
Over weight	>+2SD	1.4	3.6	1.2	3.4	0.8	3.0
Normal	Between -1 and +1SD	13.5	32.8	6.5	27.2	5.0	22.6

have any significant effect on their nutritional status. It is not surprising, therefore, that the incidence of malnutrition is high among the pupils studied especially among pupils in the public school. The socio-economic status of the parents might be responsible for this. Previous study (Olusanya, 1997) has shown that the food purchased by school children during break time is poor in both quantity and quality. The poor socio-economic status of the parents of majority of pupils, especially in the public schools is partly responsible for the meagre amount brought to the school by the pupils.

The high prevalence of malnutrition reported in this study further confirms the high degree of malnutrition among school children in developing countries (Oni and Blossner, 1997). In all the anthropometric indices measured, the pupils from the public school were more malnourished than their counterpart from the private school. This was not surprising as the pupils from the public primary school were from the rural community, with very low socio-economic status. The prevalence of stunting, in particular, in the public school was almost double that of private school. Similar finding was

Table 7: Mean (±SD) of pupils attendance at school during the week for the second term of the school year

	% Attendance at school					
	Pry I		Pry II		Pry III	
Days of the week	Public School	Private School	Public School	Private School	Public School	Private School
Monday	81.4±3.1	87.2± 5.1	83.1±2.1	90.4±3.2	84.9±2.7	89.6±2.6
Tuesday	73.6±2.8	81.7±3.7	76.3±2.1	88.7±4.1	77.4±3.6	91.7±4.1
Wednesday	63.1±4.2	84.3±2.8	70.2±3.6	85.4±3.6	71.1±2.8	86.2±3.1
Thursday	70.2±3.3	90.2±4.1	73.4±3.6	92.1±4.5	74.6±3.1	91.8±3.7
Friday	57.4±2.9	87.3±2.1	60.1±2.1	90.2±2.6	63.5±3.2	91.6±3.3

reported by Tee *et al.* (2002). Stunting, which occurs mostly in the first three years of life, reflects long-term under nutrition and poor health. Similarly, the higher prevalence of overweight among pupils in private school could be associated with the higher socio-economic status of the parents. Such high prevalence of overweight and obesity among children of higher socio economic portion has been reported in some developing countries (Tee *et al.*, 2002; Soekirman *et al.*, 2002; Florentino *et al.*, 2002).

The relief of hunger is most likely to improve a child's ability to concentrate which should facilitate learning. Children's memory may also improve so they are more likely to learn. In fact some previous studies have shown the benefits to cognition especially memory, from early morning glucose drinks or breakfast in elderly and young adults (Korol and Gold, 1998).

Conclusion: Provision of school meal will definitely impart positively on both the attendance and cognition of the pupils. Although provision of school meal is in practice in Nigeria, it is still at a very low ebb and optional. If the huge amount being invested on the Universal Basic Education is to yield the desired results urgent and appropriate action should be placed on the provision of government subsidized school meals. Also, the school meals should be well planned to ensure good nutrient quality. In this respect, the Home Economics units of both the schools and ministry of education should be involved in the planning, formulation and preparation. If school meals are of good nutrient quantity and quality and the supply is efficient and continues for some time, the children's underlying nutrition status such as wasting should improve. However, it is more difficult but possible, to improve stunting (Powell *et al.*, 1998).

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