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Emulsion-Stabilizing Effect of Gum from *Acacia senegal* (L) Willd. The Role of Quality and Grade of Gum, Oil Type, Temperature, Stirring Time and Concentration

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Abstract: Gum Arabic (*Acacia senegal*) from Kordofan (Central Sudan) and Damazin (Blue Nile, Western Sudan) were used in this study. Physicochemical properties of gum samples were studied (moisture, ash, nitrogen, total soluble fiber, specific rotation, relative viscosity, refractive index and pH). Results show significant differences in moisture content, protein content and relative viscosity between Kordofan and Damazin gums. Damazin gum contained higher protein (3%) and characterized by higher viscosity (24.81) compared to Kordofan gum. Stability of Acacia gum emulsion in regard to type of refined oil (sesame, groundnut, cotton seed, sunflower and corn), temperature, stirring time, concentration and gum grade was also investigated. Results revealed that emulsion stability is significantly affected by the type of oil used. Cotton seed oil gave the most stable emulsion while groundnut resulted in the lowest stable emulsion. Increase in the length of the stirring time is significantly increased stability of the emulsion. Also emulsion stability was affected by gum grades. Other factors of concentration and temperature did not significantly influence emulsion stability.

Key words: *Acacia senegal* gum, quality, emulsion stability, emulsification factors

Introduction

Gum Arabic refers to dried exudates obtained from the stems and branches of *Acacia senegal* (L) Willdenow or Acacia seyal. However, the quantity and quality produced varies between and among species and this determines the type and extent of use by man. A study shows that there are about 17 species producing commercial Acacia gum in Sub-Saharan Africa. Most of these gums are edible and some are used in the food and pharmaceutical industries (FAO, 1996).

The gum from *Acacia senegal* is a water soluble polysaccharide of the hydrocolloid group and comprised mostly of arabinogalactan and protein moiety, in addition to some mineral elements (Williams and Phillips, 2000). It has considerable variation in physicochemical, functional and toxicological properties according to different locations, type of soil and age of the tree (Anderson *et al*., 1968). Gum Arabic is known by the worldwide food, beverage and pharmaceutical industry as a versatile additive with polyvalent functions: Protective colloid, film-building and coating agent, encapsulating agent, oxidation inhibitor, stabilizer, emulsifier, texturant, clouding and clarifying agent, food adhesive. More recently, western countries discovered that acacia gum is also a dietary fiber with very interesting nutritional properties (NGARA, 2005).

Many food products in the markets are in the emulsion state such as cheese, milk, salad dressings, sauces, beverages and coconut milk (Gonzalez, 1991; McClements, 1999). An emulsion is a dispersed system that consists of two immiscible liquids (usually oil and water), with one of the liquids dispersed as small droplets in the other called continuous phase (McClements, 1999). The emulsions are thermodynamically unstable systems and have a tendency to break down over time (Dickinson, 1992; Friberg and Larsson, 1997; McClements, 1999). The breakdown of an emulsion may manifest itself through different physicochemical mechanisms such as gravitational separation, coalescence, flocculation, Ostwald ripening and phase inversion (Friberg and Larsson, 1997; McClements, 2000). Therefore, the production of high quality food emulsions that can remain kinetically stable for a certain period of time is necessary. In general, emulsifiers are needed for stabilizing emulsions because they decrease the interfacial tension between the oil and water phases and form a protective coating around the droplets which prevents them from coalescing with each other (McClements, 1999).

Dicknison and Sainsby (1988) distinguished between emulsifier and stabilizer in food system. Emulsifier is defined as a single chemical or mixture of components having the capacity for promotion emulsion formation and short term stabilization by interfacial action while stabilizer is defined as a single chemical or mixture of components which can offer long term stability on emulsion, possibly by mechanism involving adsorption

but not necessarily so. Certain polysaccharides such as gum arabic (Silber and Mizrahi, 1975), Xanthan gum (Prud and Long, 1983) and Tragancanth gum (Dea and Madden, 1986; Bergenstahl and Steinius, 1986) have been noted to display specific surface activities and stabilize dispersed particles of oil droplets in aqueous system. The gum from *Acacia senegal* has a functional ability to act as emulsifier that stabilizes oil-in-water emulsion (Yokoyama *et al.*, 1988; Randall *et al.*, 1988). It is now known that the protein-rich high molecular mass component adsorbs preferentially onto the surface of the oil droplets. It is envisaged that the hydrophobic polypeptide chains adsorb and anchor the molecules to the surface while the carbohydrate blocks inhibit flocculation and coalescence through electrostatic and steric repulsions (NGARA, 2005). Therefore, the protein moiety had effects on emulsifying behavior of gum Arabic and the best emulsion capacity and stability is found in gums with highest nitrogen content (Randall *et al.*, 1988; Dickinson, 1992). The emulsifying properties of gum Arabic, however, are directly influenced by the botanical type, the nature of the growing soils and the climate (NGARA, 2005). In addition to the emulsifying properties of Acacia gum, other factors related to the emulsification process influence the properties of an emulsion (Brosel and Schubert, 1999).

In Sudan, the gum belt lies within the arid/semi-arid zone of mainly 520,000 km^2 in area that extends across Central Sudan between latitudes 10° and 14 N, accounting for one fifth of the country's total area (IIED and IES 1990). This belt covers parts of Kordofan, Darfur, Eastern Sudan and Blue Nile and Upper Nile (Hamza, 1990). The vast production area of gum Arabic in Sudan with its variable climatic and edaphic conditions may produce gums with different physical and chemical properties that reflected in their functional behavior. In line with this concept, the present study is designed to compare effects of factors, such as type of oil, temperature, stirring time and concentration on stability of emulsions prepared from gum Arabic samples collected from two environmentally different locations in Sudan.

Materials and Methods

Sample preparation: Dried gum samples from *Acacia senegal* were obtained from the farms of "Acacia Agricultural Company" in Kordofan (Central Sudan) and Damazin (Blue Nile, Eastern Sudan). Gum samples were sorted out into hand picked select intact nodules with purity of about 98% and cleaned fractured nodules (CFG) with purity less than 98%. Then the samples were ground into fine powder to pass 0.4 mm mesh screen. The prepared samples were kept in tight containers and stored at room temperature until analysis. Hand picked select gum (HPSG) was employed in this study.

Moisture, ash, protein and total soluble fiber: Ash and nitrogen (micro-Kjeldahl) were determined according to AOAC (1990). Moisture content of gum samples was determined by drying samples at 105°C overnight (AOAC, 1990). Total soluble fiber was obtained by subtraction of contents of moisture, ash and protein from 100.

pH: pH of 1% aqueous solution of gum (w/v) was measured using a glass electrode pH-meter (HANNA-pH 210).

Specific rotation: Specific rotation of gum samples was measured in a filtered 1% aqueous solution using a polarimeter (Bellingham and Stanley) equipped with a sodium lamp and a cell of 20 cm path length (Abu Baker, 1996).

Relative viscosity: Relative viscosity of gum Arabic samples was measured in filtered 1% aqueous solution using U-shaped viscometer (AOAC, 1990). A flow time (seconds) of distilled water was measured by filling the viscometer tube (held at 30°C in water bath) with water and then drawn by suction to the upper mark of the viscometer. The distilled water was allowed to fall down passing the lower mark of the viscometer. Initial and final times were recorded using stop watch while the water passing the upper and the lower marks of the U-shaped tube. The flow time of a carbon dioxide free aqueous gum solution (2%) was measured as before.

Calculations:

$$\text{Relative viscosity } (30^{\circ}C) = [T - T_o]/[T_o]$$

Where: T, Flow time of gum Arabic solution (1%) in seconds; T_o, Flow time of distilled water in seconds.

Refractive index: Refractive index of gum samples was measured in a filtered 1% aqueous solution using an Abbe refractometer as described by Karamalla *et al.* (1998).

Measurement of stability of emulsions prepared from gum solution and different types of oils: Five types of refined oil (sesame, groundnut, cotton, sunflower and corn oil) and 20% aqueous gum (HPSG) solution were used to prepare stock emulsions. Emulsions were prepared by blending a measured amount of the gum solution (20%) and the oil (2:1 v/v) for one minute at 1800 rpm using kitchen blender (triplicate preparations were made for each study). Aliquot (1 mL) of the stock emulsion was diluted in distilled water to give final dilution of 1/1000. The absorbance was then read at 520 nm in spectrophotometer (Analgue, S104). Another reading of absorbance was recorded after an hour following the same procedure as before (Karamalla *et al.*, 1998). Emulsion stability was calculated as:

$$\text{Emulsion stability} = \frac{\text{First reading of absorbance}}{\text{Reading of absorbance after an hour}}$$

Tests for stability under influence of emulsification factors: These tests were done with the objective of studying effect of emulsification factors of stirring time, temperature, concentration, gum grade and quality, on emulsion stability. The tests for stability were performed in emulsions prepared by mixing the cotton seed oil (Selected as giving the highest emulsion stability) with 20% aqueous gum solution, as mentioned previously (Karamalla *et al.*, 1998).

Stirring time: This test was done to study effect of the length of stirring on emulsion stability. One mL of the stock emulsion was diluted with distilled water to a concentration of 1/1000 and then stirred for different times (1, 2, 3 and 4 minutes) using magnetic stirrer. Emulsion stability was determined following the previous procedure.

Concentration: Distilled water diluted concentrations of stock emulsion solution of 1, 2, 3 and 4/1000 were prepared and examined for emulsion stability under a fixed stirring time of one minute at room temperature. Emulsion stability was measured as before.

Temperature regime: Emulsions of 1/1000 concentration were subjected to four temperature regimes; 50, 100, 150 and 200°C; for 30 minutes and then emulsion stability was measured as before.

Gum grade: Emulsions were prepared by blending 20% aqueous gum (HPSG and CFG) solutions and cotton seed oil in a ratio of 2:1. Determination of emulsion stability was done following the method described by Karamalla *et al.* (1998) as previously stated.

Statistical analysis: Data are means of three samples. An appropriate statistical analysis of variance (ANOVA) was done (Snedecor and Chochran, 1987).

Results and Discussion

Moisture, protein, ash and total soluble fiber: Table 1 shows the contents of moisture, ash, protein and total soluble fiber of *Acacia senegal* gum (HPSG) collected from Kordofan and Damazin. Results indicated that the moisture content of Damazin gum is significantly ($p \leq 0.05$) lower than that of Kordofan. Moisture in both samples is lower than the value reported by FAO (1998). Ash content of both Kordofan and Damazin gums is similar, which falls within the range reported by FAO (1998). Protein contents of Damazin and Kordofan gums were 3% and 1.7%, respectively. Results revealed that the content of protein in samples studied is significantly ($p \leq 0.05$) different, but both values coincided with those found by Karamalla *et al.* (1998). On the other hand, total soluble fiber of Kordofan gum is significantly ($p \leq 0.05$) higher than that of the Damazin gum. The lower content of protein in Kordofan gum may be responsible for the increase in amount of soluble fiber compared to that in Damazin gum. Considering that total soluble fiber was obtained by subtraction.

Table 1: Physicochemical properties of *Acacia senegal* gum collected from Kordofan and Damazin

	Property*	
Acacia gum	Kordofan	Damazin
Moisture (%)	$10.80^{a} \pm 0.10$	$10.40^{b} \pm 0.20$
Protein (%)	$1.70^{b} \pm 0.20$	$3.00^{a} \pm 0.10$
Ash (%)	$3.40^{a} \pm 0.1.00$	$3.40^{a} \pm 0.10$
Total soluble fiber (%)	$84.10^{a} \pm 1.00$	$82.10^{b} \pm 0.90$
Specific rotation (°)	$-29.00^{a} \pm 2.00$	$-29.00^{a} \pm 1.00$
Relative viscosity	$14.29^{b} \pm 0.03$	$24.81^{a} \pm 0.02$
Refractive index	$1.34^{a} \pm 0.001$	$1.35^{a} \pm 0.001$
pH	$3.90^{a} \pm 0.10$	$3.90^{a} \pm 0.10$

*Means of triplicate samples ± SD. Means having different superscripts within the row are significantly different ($P \leq 0.05$).

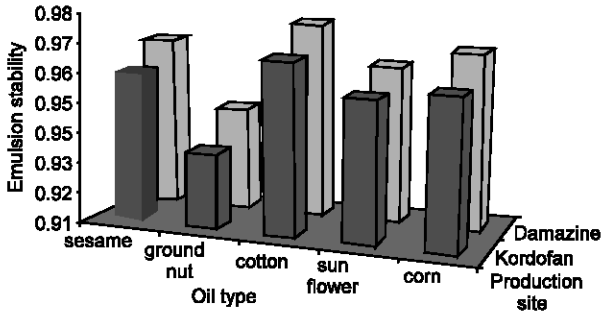

Fig.1: Stability of Kordofan and Damazin gum emulsions as affected by different oil types.

Specific rotation, relative viscosity, refractive index and pH: Results in Table 1 indicated that Kordofan and Damazin gums had similar specific rotation (-29°). This value found within the range reported previously (Anderson *et al.*, 1968; FAO, 1998). Moreover, results illustrated that the relative viscosity (30°C) of Damazin and Kordofan gums were 24.81 and 14.29, respectively (Table 1). These values are significantly ($p \leq 0.05$) different; indicating the more viscous of Damazin gum compared to Kordofan gum and hence reflected the influence of the environmental conditions on some physicochemical properties of the gum produced. Refractive indices and pH of Kordofan and Damazin gums are almost similar, which agreed with those obtained by Karamalla *et al.* (1998).

Effect of emulsification factors on stability of emulsion: Effect of type of oil, temperature, stirring time, concentration and gum grade on emulsion stability is presented in Fig. 1 to 5.

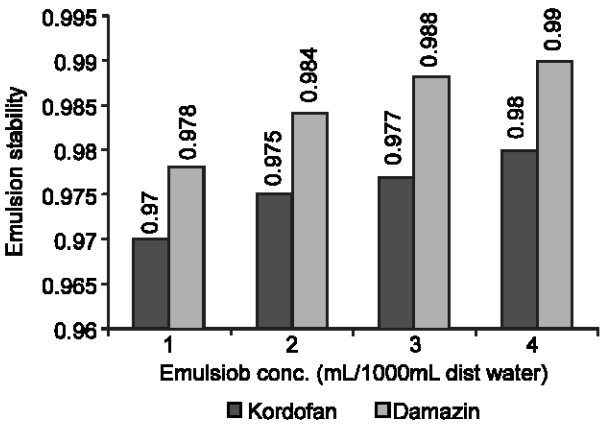

Fig. 2: Effect of varied concentrations on measurement of stability of Kordofan and Damazin gum emulsions

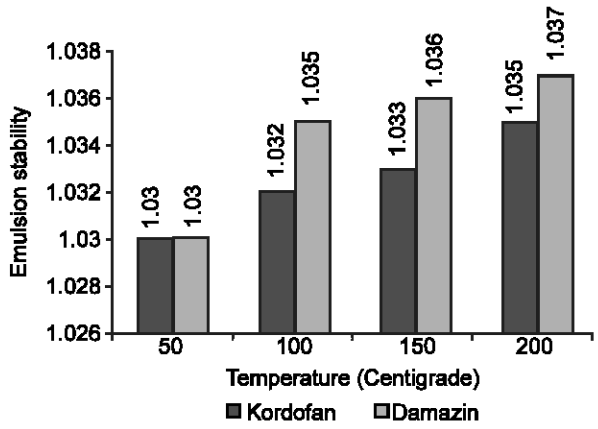

Fig. 3: Stability of Kordofan and Damazin gum emulsion under different heating temperature

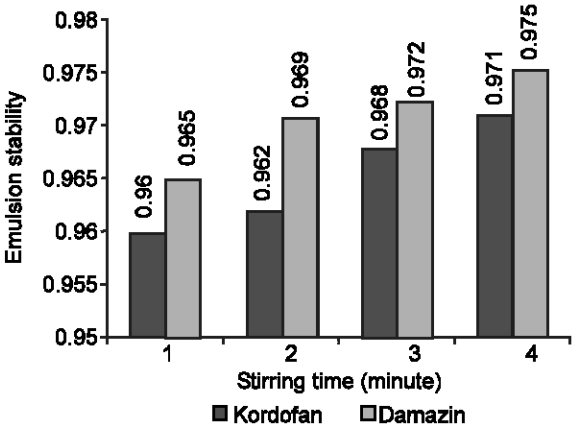

Fig. 4: Stability of Kordofan and Damazin gum emulsions under different stirring times

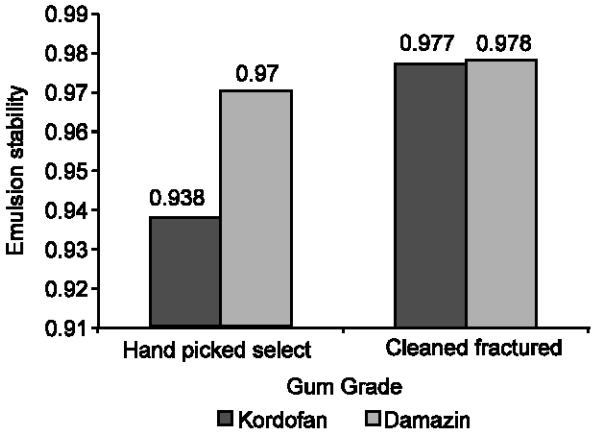

Fig. 5: Effect of gum grade on stability of Kordofan and Damazin gum emulsions.

Oil type: Results show that emulsions prepared by mixing pure oils (sesame, groundnut, cotton seed, sunflower and corn), with aqueous solutions of gums had stabilities varied between 0.935 to 0.967 and 0.944 to 0.974, for Kordofan and Damazin samples, respectively (Fig. 1). However, results indicated that using different types of oils resulted in significant ($p \leq 0.05$) differences in emulsion stability (ES) for both samples of gum. ES when using groundnut oil was the lowest for Kordofan and Damazin gums (0.935 and 0.944, respectively), while that of the cotton seed oil was the highest (0.967 and 0.974, respectively). Damazin gum gave the highest ES compared to Kordofan gum. Differences in ES may be ascribed to difference in protein content (Table 1). Protein is the fraction that provides the functionality of the gum Arabic as emulsion stabilizer. Therefore, the best emulsion capacity and emulsion stability in regard to coalescence and flocculation were recorded in gums with highest nitrogen content (Dickinson *et al.*, 1991). Variability in ES with varied oil type was reported (Pearce and Kinsella, 1978).

Concentration: Fig. 2, illustrates stability of emulsions with concentrations of 1, 2, 3 and 4/1000 for Acacia gum samples. Data revealed an increase, but with insignificant magnitude, in ES with increasing the concentration of the stock emulsion for Kordofan and Damazin gums.

Temperature regime: Emulsion stability at a temperature regime of 50, 100, 150 and 200°C, for both Kordofan and Damazin gums increased, but insignificantly ($p \geq 0.05$), with increasing the heating temperature (Fig. 3). These findings agreed with those reported by Dickinson (1988).

Stirring time: Fig. 4, illustrates that stability of emulsions of Acacia gum, for Kordofan and Damazin, is significantly ($p \leq 0.05$) increased with the increase in stirring time from 1 to 4 minutes. Mechanical blending and homogenization affects droplet-size distribution and hence influences the properties of an emulsion (Brosel and Schubert, 1999). Droplets can be made smaller by

applying more intense emulsification giving more stability (Walstra, 1996).

Hand picked selected gum versus cleaned fractured gum: Stability of oil-in-water emulsion of cleaned fractured gums of Kordofan and Damazin is significantly ($p \leq 0.05$) higher than ES from hand picked selected gum samples studied (Fig. 5).

In conclusion, results of this study indicate that location influence the quality of gum produced, which in turn reflected in emulsification properties of the gum. Factors such as oil type, stirring time and gum grade influence emulsion stability.

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Iron Deficiency and Anaemia in Rural School Children in a Coastal Area of Morocco

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Abstract: Iron deficiency anaemia is the major public health problem encountered in the world. In Children, this trouble has deleterious consequences on the global health and weak cognitive development. This study aims to determine the prevalence of anaemia and iron deficiency and its association with socio-economic and anthropometric parameters of the Schoolchildren in a rural coastal region of Morocco. 295 students between 6 and 16 years old composed the study sample. The level of Haemoglobin was measured in a sub group of 280 school children. The iron status was determined by ferritin level in serum. A questionnaire was developed to collect information on the socio-economic and demographic status of the family such as the size of household, the working status of parents and their level of education. The mean haemoglobin concentration was 12.41 g/dl in boys and 12, 5 g/dl in girls, whereas the mean seric ferritin level was 26, 7 µg/l in boys and 27, 9 µg/l in girls. The overall prevalence of anaemia was 12, 2 % and iron deficiency was found in 20.4 %. Serum ferritin (SF), serum iron concentrations and mean corpuscular volume (MCV) were significantly correlated with Hemoglobin There was an inversely significant relationship between education of the mother and anaemia in children (p=0.01) but not with gender, nor parents' employment. It is concluded that anaemia is relatively less prevalent in this study population. Further studies are needed to explore the dietary determinants of this situation.

Key words: Anaemia, iron deficiency, serum ferritin, schoolchildren and morocco

Introduction

Iron deficiency is the commonest form of malnutrition worldwide and according to the World Health Organization it affects 43% of the world's children (De Maeyer and Adiels-Teasman, 1985). Iron deficiency may be due to inadequate dietary intake of iron, the low level of absorption because of small bowel pathology, increased physiological requirements during rapid growth in infancy and adolescence and chronic blood loss usually from the gastrointestinal or urinary tracts or because of menorrhagia in adolescent girls (World Health Organization/United Nations University/UNICEF, 2001). Iron-deficiency anaemia leads to serious health problems, such as poor cognitive and motor development and behavioural problems, in children (Grantham-McGregor and Ani, 2001). The demographic and Health Surveys in Morocco (Ministry for the Public Health, 2001) reported that 31, 6 % of younger children than 5 years were anaemic. In a previous study in rural and urban Moroccan, we observed a positive correlation between serum ferritin (SF) and haemoglobin (Hb) concentrations, suggesting that a significant proportion of anaemia cases might be related to iron deficiency (Ministry for the Public Health, 2001). Another study showed that higher iron intake is associated with a decreased prevalence of anaemia. However, only one-third of the incidence of anaemia in this study was attributed to iron deficiency suggesting the existence of other causal factors (Aboussaleh *et al*., 2004).

This study was undertaken to estimate the prevalence of anaemia and iron deficiency among rural schoolchildren in the OULAD BERJAL KENITRA region and to determine the various factors associated with anaemia in this population.

Materials and Methods

Study sample: This study was conducted in the district of Menasra, WELAD BERJAL. All the pupils of the unique school of the village are integrated to the study. The sick one are eliminated but treated with the medical staff. The sample was 295 pupils aged between 6 and 16 years old enrolled in a primary school.

Socio demographic data were collected with questionnaire filled out by the parents. The household socio economic status was defined by the following parameters: The income, the parent educational status, the size and the working status of the parents outside the household since women declared not active are working mainly in the house and the garden nearby without any direct cash income. They may however get returns from eggs or poultry sold in the village.

Table 1: Socio-economic and anthropometric characteristics of children

	All children		Children with anaemia				
	N	%	N	%	X^2	P	CI 95%
Sex							
Male	123	41.7	14	11.4			
Female	172	58.3	22	12.8	0.06	0.72	1.53-1.64
Age (years)							
= 12	159	53.9	26	16.3			
> 12	136	46.1	10	7.3	4.22	0.012*	9.70-10.28
Mother's education							
Primary (0 - 5 years)		227	76.9	22	9.7		
Secondary (5-10 years)	68	23.1	14	20.9	6.56	0.010*	1.15-1.26
Father's education							
Primary (0 - 5 years)		186	63	18	9.8		
Secondary (5-10 years)	109	37	17	15.6	4.25	0.104	1.27-1.40
Mother's working status							
Yes	14	4.7	1	7.1			
No	281	95.3	35	12.5	0.61	0.44	0.99-1.04
Father's working status							
Yes	274	92.9	34	12.4			
No	21	7.1	2	9.5	0.42	0.51	2.08-2.28
Household size							
= 5	120	40.7	14	11.7			
> 5	175	59.3	22	12.6	0.78	0.71	5.89-6.40
Wasting < -2Z (Z-W)							
Yes	17	5.8	3	17.6			-0.85- (-0.35)
No	278	94.2	33	12	-0.59	0.43	
Stunting < -2Z (Z-H)							
Yes	16	5.4	2	12.5			-0.82- (-0,61)
No	279	94.6	34	12.1	1.1	0.29	

* P< 0, 05 significantly level, Z-W, weight-for-age Z score, Z-H, height-for-age Z score

Anthropometric data: Body size and growth were assessed through height and weight measurements. Anthropometric index of weight-for-age (WHZ) and height-for-age (HAZ) were calculated as indicators of the growth status of the children. The anthropometric measurements of the study population were determined using Z scores, based upon the World Health Organization International Reference Population (WHO, 1983; UNICEF, 1986) and less than minus 2 z score from the mean was used as the cut-off point for growth retardation.

Blood test: Blood was collected by antecubital venipuncture and drawn into a container with EDTA for red blood cell (RBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) analyses.

The prevalence of anaemia is defined as the percentage of children with hemoglobin values below 2 SD of the reference range.

The lower limit for the haemoglobin (Hb) level in 6-8.9-year-old children was accepted as 11.5 g/dl.

The values for 9-11.9 years were 12 g/dl for Hb. For ages 12-13.9 the lower limits were different according to sex. For girls the lower limits were 12 g/dl for Hb and for boys the limits were 12.5 g/dl for Hb. For ages 14-6, the lower limits for girls were 12 g/dl for Hb, for boys the lower limits were 13 g/dl for Hb.

The severity of anemia was classified as mild (Hb>10, 5 g/dl), moderate (Hb = <10, 5 g/dl) and severe (Hb<7, 5 g/dl) depending on the hemoglobin value of anemic children.

Iron deficiency was defined as plasma ferritin level <15 µg/L. Anaemia, iron depletion, iron deficiency and iron deficiency anaemia were defined according to World Health Organization criteria (World Health Organization, 2002).

Statistical analysis: The chi-squared test and logistic regression analysis were used to investigate the relationship between the prevalence of anaemia and the socio demographic factors. Differences were considered statistically significant at a p level of 0.05.

Results

Initially, 295 students between 6 and 16 years included in the study, 41.7 per cent (123 subjects) were male and 58.2 per cent (172 subjects) were younger than 11 years old. The distribution of risk factors for anaemia between the anaemic and non-anaemic participants is shown in Table 1.

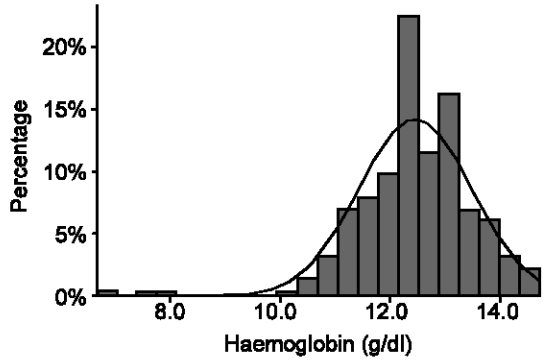

Fig. 1: Distribution of haemoglobin (g/dl) concentration

Socio demographic status

Parent education: Only 23.1 % of the mothers and 37% of fathers went beyond 5 years of formal education but do not go further to get jobs outside the village. The prevalence of anaemia in children is paradox ally inversely associated to parents' education level. Indeed children anaemia prevalence is only about 10% for less educated men or women and increases to 15% and to 20 % in kids whose mothers and fathers completed more than 5 years of instruction.

Most parents who got secondary education are younger than those who got only primary education because the high school is far from the village. These young parents do not complete education and come back to the village to get married and build a family. Their nutrition status is worse may be because they spend money in extra nutritional items (cigarettes, coffee etc.).

Fourteen (4.7 %) mothers and 275 (92.9 %) fathers were active workers. The prevalence of anaemia among children whose mothers and fathers were working found to be 7.1 % and 12.4%, respectively. Comparatively, the anaemia prevalence in children whose mothers and fathers were unemployed was 12.5 % and 9.5 % respectively. Here again the trend is that employment seems not associated or inversely but not significant to the occurrence of anaemia in children. It is worth to note that the majority of active workers are seasonal workers in agriculture or fisheries.

In 59.3 % of families, household size is greater than five. The prevalence of anaemia among children who had five or less family members was 11.7 per cent, while for those with more than five this figure was 12.6 %. No significant difference was observed.

Physical growth: Physical growth was assessed by Weight for age and height for age. Results show that only 5 % of children were stunted and almost the same percentage wasted according to WHO references integrated in Epi info 2000. These results should be carefully taken because WHO is undergoing a renewal in growth charts.

Prevalence of anaemia and Iron deficiency: The mean haemoglobin concentration was 12.41 g/dl in boys and 12, 5 g/dl in girls, whereas the mean ferritin level was 26, 7 µg/l and 27, 9 µg/l respectively. The prevalence of anaemia was 12.2 %. Regarding the severity of anaemia, the mild type is the major one with 98% of cases.

Iron deficiency was found in 20.4 % of the children. It was associated with anaemia in 85% of the subjects.

No gender differences were revealed by these findings according to iron status though a slight difference is noted 12.8% for girl's vs 11.4% for boys.

Backward-stepwise multiple regression analysis (Table 2) was used to identify the factors influencing Hb levels. Serum ferritin (SF), serum iron concentrations and MCV were significantly related to Hb level. The overall F-ratio for all variables was 15.04 (df = 3) and was highly significant (P = 0.000).

Young children (under 12) are more exposed to anaemia than their elders with 16.3% vs 7.3 % respectively. Among the parents education, only the Mother one is significantly related to the prevalence of anaemia. Anthropometric status are found not to be associated statistically to anaemia.

Discussion

The current study reports on the prevalence of anaemia and iron deficiency among schoolchildren in this rural region of Morocco. In contrast in many developing regions of the world, the prevalence of anaemia in 5-12-year's old is estimated of 46 %, with the highest rates found respectively in Africa and in South Asia at 49 % and 50 % (De Maeyer *et al*., 1985).

However Zimmermann *et al*. (2003) suggested lower prevalence's of anaemia among rural school age children in a mountainous region from northern Morocco was 35 per cent. This rate is comparable to the national prevalence reported by The Ministry of Health in 2000 (Ministry for the Public Health, 2001). Surprisingly the figures found here are very low relatively. Only 12.2% of children were anaemic though almost a fifth has iron reserves limited. Theses results are comparable to a 7.4 % prevalence reported by WHO and MDI (2005) in Tunisia among children aged 6-10 years assessed by the same WHO cut off points(Hb <11.5g/dl).

When simple correlation tests were used, we observed a highly signification between Hb and SF, suggesting that iron status was likely to be an important determinant of Hb and hence anaemia.

It seems that the prevalence of anaemia is not uniform through the whole age specific population in Morocco and some pockets of extreme prevalence coexit with some pockets of improved situations such as the area studied.

Our area of study is a rural area with very tiny agricultural areas or gardens. But the species cultivated are mainly

Table 2: Backward-stepwise multiple regression for haemoglobin concentration of schoolaged children in rural Kenitra

Variable	B	SE	ß	T	p	95% CI for B
Serum ferritin (µg/l)	1.520	0.004	0.246	3.88	0.000	(0.007, 0.023)
Serum iron (µg/dl)	1.145	0.007	0.99	1.66	0.097	(-0.002,0.025)
MCV (fl)	3.68	0.010	2.24	3.54	0.000	(0.016, 0.057)

B-Ordinary least-squares regression coefficient; SE B-standard error of B; Beta-standardised ß coefficient; CI-confidence interval.
Model Summary: Multiple R = 0.4; R^2 = 0.16; adjusted R^2 = 0.149; F-ratio = 15.04 (df = 3); P = 0.000.

green vegetables, beans and some cereals. Moreover our study area is a coastal territory where the fish consumption is a habit. This may enhance the bioavailability of iron in the diet. A detailed study on food intake is needed to confirm the findings on iron status deficiencies.

Conclusion: The results suggest that iron deficiency is an important factor of anaemia in this population. However, whole anaemia cannot be solely explained by iron deficiency. Further studies are needed to consider micronutrients status, parasite infestation, hereditary disorders and environmental pollutants. A positive deviation study is needed to investigate the factors involved in improving nutritional status is this rural area.

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The Influence of Glimepiride on the Biochemical and Histomorphological Features of Streptozotocin - Induced Diabetic Rabbits

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Abstract: An experiment was conducted to study the efficacy of glimepiride (a new sulphonylurea) on the biochemical values and histomorphological features of streptozotocin-induced diabetic rabbits. Eighteen New Zealand white male rabbits were selected for the study. The rabbits were randomly divided into three Groups of six each. Group I was kept as normal healthy control (NC), Group II and Group III were made diabetic by single intravenous administration of streptozotocin (@ 65 mg/kg. b.w.). Glimepiride was given to Group III rabbits by gavage @ 2mg/kg. b.w. daily for twenty-one days whereas Group I and Group II rabbits received normal saline in the similar manner. The results indicated a significant decrease in the blood sugar ($P<0.01$), blood urea ($P<0.001$) and serum creatinine ($P<0.001$) in Group III rabbits. Further, the histomorphological study showed an increase in the percentage of beta cells in pancreatic islets and recovery of renal tubules in Group III rabbits. The findings indicate that glimepiride improves the biochemical values and ameliorates the histopathology of diabetic rabbits particularly restoring the morphology of beta cells of islets of Langerhan's and thus, seems to be encouraging.

Key words: Glimepiride, streptozotocin, diabetes, biochemistry and histopathology

Introduction

Streptozotocin induces severe and irreversible hyperglycemia in experimental animals (Mitra *et al.*, 1996). The action of streptozotocin in beta cells is accompanied by characteristic alterations in blood insulin and thereby glucose concentrations (West *et al.*, 1996). It impairs glucose oxidation (Bedoya *et al.*, 1996), decreases insulin biosynthesis and secretion (Nukatsuka *et al.*, 1990) and alters the histomorphology of most of the organs (Mir *et al.*, 2008). The microvascular and macrovascular complications in diabetes are the major causes of morbidity and death in diabetic subjects (Nagappa *et al.*, 2003). The search for more effective and safer hypoglycemic agents therefore, has continued to be an area of search of interest (Krishna *et al.*, 2004).

Sulphonylurea have represented the backbone of NIDDM therapy for more than 30 years (Groop, 1992). The insulinotropihic effect of sulphonylurea is augmented by glucose and they apparently increase beta cell sensitivity to glucose and non-glucose stimuli (Pfeifer *et al.*, 1980).

Glimepiride has been developed for glycemic control in diabetic patients and represents the third generation sulphonylurea. It effectively inhibits the development of oxidative stress in diabetes (Krauss *et al.*, 2004) by possessing a potent extrapancreatic effect on glucose metabolism and may directly stimulate glucose transport activity through phospholipid signalling pathway (Takada *et al.*, 1996).

The present study was designed to investigate the effect of glimepiride on biochemical values and histopathological features of streptozotocin induced diabetic rabbits.

Materials and Methods

The study was conducted in 18 New Zealand white male rabbits of almost uniform age acclimatized to prescribed standard laboratory conditions (Anonymous, 2000). Twelve rabbits were made diabetic by single dose of streptozotocin (@ 65mg/kg. b.w.) dissolved in 1ml of freshly prepared citrate buffer, pH 4.5 and administered intravenously following twelve hours fasting whereas remaining six were kept as control that received equal volume of normal saline.

When diabetes mellitus was well established in rabbits which was confirmed by fasting hyperglycemia, the rabbits were divided into three groups of six each. Group I comprised of normal untreated rabbits (NC), Group II comprised saline treated diabetic rabbits (DC) and Group III comprised diabetic rabbits that received glimepiride (@ 2mg/ kg.b.w.) daily for 21 days. The assessment of treatment was based on blood sugar, blood urea and serum creatinine levels estimated on day 7th, 14th and 21st. At the end of 21st day the rabbits were sacrificed for histological examination of pancreas and kidneys.

Analytical procedure: The blood sugar of rabbits was estimated by Glucometer Gx (Bayer Diagnostic India, Ltd.), blood urea by "DAM Method" and serum creatinine

Table 1: Effect of glimepiride on blood sugar (f), blood urea and serum creatinine of streptozotocin-induced diabetic rabbits

	DAYS											
	Initial Value			7th			14th			21st		
Factor												
Group	BS (F)	BU	SC	BS (F)	BU	SC	BS (F)	BU	SC	BS (F)	BU	SC
NC	94±	19.5±	1.25±	99±	19±	1.05±	105±	21.03±	1.29±	102±	9.75±	1.21±
	6.06	0.64	0.20	5.95	1.46	0.20	4.66	1.58	0.25	3.191	0.84	0.04
DC	292±	53±	3.32±	285±	52.9±	3.12±	210±	46.6±	3.00±	192±	40.1±	2.80±
	10.60	2.11	0.16	9.46	1.80	0.16	8.83	1.25	0.15	9.41	1.16	0.14
Glimepiride	281±	48.25±	3.13±	154±	30.25±	2.46±	119±	24.25±	1.48±	86±	18.75±	0.98±
treated	9.11	1.54	0.08	4.96	1.79	0.05	4.29	0.84	0.17	8.39+	0.84++	0.05+

Values represent mean±SEM; BS(F): Blood Sugar (Fasting); BU: Blood Urea; SC: Serum Creatinine; NC: Non-diabetic control rabbits; DC: Diabetic rabbits treated with normal saline; +p < 0.01, ++p < 0.001 compared with DC.

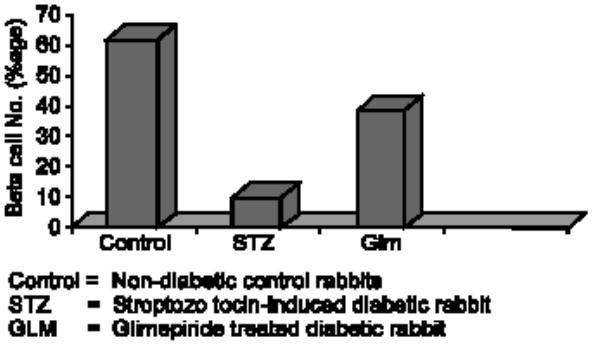

Fig 1: Comparative percentage of beta cells in different groups of Rabbits.

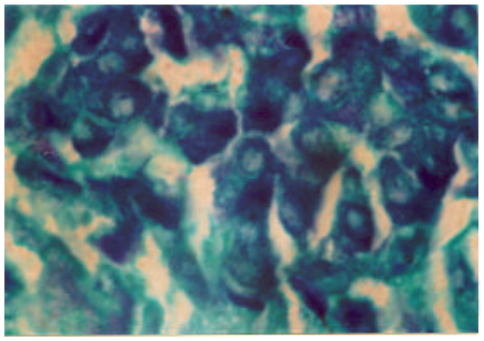

Fig. 2: Pancreatic islet of glimepiride treated diabetic rabbit showing beta cells with deep purple granules [modified Gomori's aldehyde fuchsin (Scott, 1952) x 1000].

by 'Alkaline Picrate Method" using commercially available kits. Histological examination was done by fixing pancreas and kidneys of rabbits in 10% formalin, processed and embedded in paraffin wax. Tissue blocks were sectioned 5 micron thick and stained with Harris Haematoxylin and Eosin (Luna, 1968). However, to demonstrate pancreatic islet cells, a modification of Gomoris staining technique (Scott, 1952) was used.

For quantitative assessment of beta cells, cells of approximately four islets of each tissue and forty islets of each group were counted under trinocular microscope at a magnification of ×1000.

Statistical analysis: All values were expressed as mean and analyzed using students 't' test (Prasad, 2000). 'P' value was obtained from the distribution of 't' probability chart.

Results

Diabetes mellitus in rabbits was induced by single intravenous administration of streptozotocin that was confirmed by elevated levels of fasting blood glucose, blood urea and serum creatinine levels. The subsequent effects of streptozotocin induced diabetes resulted in degenerative and lytic changes in pancreatic islets and kidneys of rabbits.

Glimepiride treatment effectively improved the biochemical levels in diabetic rabbits and the drug was not found to cause hypoglycemia. Table 1 shows the efficacy of glimepiride on blood glucose, blood urea and serum creatinine levels of diabetic rabbits. The histological study of the pancreatic sections by using special stain showed restoration of beta cell morphology in Group III rabbits (Fig. 2) that was also evident by the comparative percentage of beta cells with Group II rabbits (Fig. 1). Further, recovery of renal morphology was observed histologically in Haematoxylin and Eosin stained sections of Group III rabbits.

Discussion

The induction of diabetes mellitus in rabbits was confirmed by elevated levels of fasting blood glucose. Streptozotocin is well known for its selective pancreatic islet beta-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals. It interferes with cellular metabolic oxidative mechanisms (Papaccio *et al.,* 2000) and induces severe and irreversible hyperglycemia (Mitra *et al.*, 1996). Intravenous administration of streptozotocin (@ 65 mg/kg b.w.) in the present study effectively induced diabetes mellitus in rabbits and is in consonance with earlier methods of induction (Tawfeeg and Sherif, 2001; Mir *et al.*, 2008).

A decline of blood sugar level following glimepiride treatment observed in the present study is in total

agreement with earlier workers (Takada *et al.*, 1996; Krauss *et al.*, 2004). Sulphonylurea bind to specific receptors on beta cells resulting in closure of potassium ATP channels and subsequently open calcium channels leading to an increase in cytoplastic calcium that stimulates insulin release (Pilipson and Steiner, 1995). There is much controversy about the mode of action of sulphonylurea and specifically whether they lower blood glucose through extra pancreatic mechanisms other than stimulation of insulin secretion (Groop, 1992). However, studies suggest that glimepiride has a potent extra pancreatic effect on glucose metabolism and may directly stimulate glucose transport activity through phospholipid signaling pathway (Takada *et al.*, 1996). Further studies have shown that glimepiride has more rapid onset than previous sulphonylureas (both glyburide and glipizide) and consequently less risk of hypoglycemia (Geisen, 1988).

The degenerative changes in pancreatic beta cells of streptozotocin-induced diabetic rabbits observed in the present study can be attributed to the production of free radicals (Tesfamariam, 1994) that damage beta cells selectively (Pusztai *et al.*, 1996). However, an increase in the number of beta cells in the islets of Langerhan's in glimepiride-treated diabetic rabbits in comparison to saline treated diabetic rabbits can be attributed to the fact that glimepiride affect the activation of the redox sensitive transcription factor NF(Kappa)B in vitro and in vivo and possesses the free radical quenching properties (Schiekofer *et al.*, 2003). Although the mechanism of beta cell neoformation is not clear but there is strong evidence that islet stem cells may exist in the pancreatic duct and that these ductal epithelial cells may be switched into a proliferative/regenerative phase leading to nesideoblastosis (neogenesis of islets) (Hellerstrom, 1984; Bonner-Weir *et al.*, 1993). Lipsett and Finegood (2002) reported beta cell neoformation from precursor cells in the pancreatic duct of diabetic rats. According to Waguri (1997) the beta cells can regenerate either through differentiation of the precursor cells from the pancreatic duct, or proliferation from existing or surviving mature beta cells.

In the present study the improvement in blood urea, serum creatinine and subsequent amelioration of histomorphological changes in kidneys of glimepiride treated rabbits can be attributed to the recovery of renal function (Tedong *et al.*, 2006), which is explained by the regenerative capability of the renal tubules (Kissane, 1985). Studies have shown that good metabolic control is beneficial in slowing the progression of nephropathy in diabetes and if the duration of diabetes is prolonged before reinstitution of normoglycemia, nephropathy is not easily reversed (Floretto *et al.*, 1998; Renu *et al.*, 2004). Tedong *et al.* (2006) have reported that the normoglycemia in diabetic rats with treatment therapies could ameliorate the glomerular and tubular lesions that characterize diabetic nephropathy and subsequently recover renal morphology and function.

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Management vs Productive and Reproductive Performances of Dairy Farm

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Abstract: The study was conducted at dairy and cattle improvement farm, Hathazari, Chittagong for a period of one year. A total of 82 dairy cows were selected during the period from June 03-July 04 and their information regarding milk production upto 180 days, productive and other reproductive parameters were recorded from farm record book. Average milk production (459.09±138.09 liter), calving interval (634.59±223.92 days), age at puberty (1205.02±313.80 days), service per conception (1.88±1.09), gestation length (273.08±7.48 days) and birth weight of fetuses (18.78±34 kg) were observed. The minimum and maximum value of these results was also observed. The highest milk production (487.54±109.73 liter) was found in the cows with parity number 4-8 and the second highest (456.84±169.58 liter) was found with the parity number 2 and the lowest milk production (445.79±86.64 liter) was found with the parity number 3. The average production of milk was observed (473.44±134.15 liter) in the cows required interval between 370-590 days and 444.74±142.11 liter found in the cows required 591-1365 days. When the range of age at puberty was 1186-2270 days and 665-1185 days the average production of milk was 468.56±161.92 liter and 450.50±113.56 liter was observed, respectively. Comparatively more milk production was found (461.52±151.89 liter) in cows required less number of services per conception was 1-2 than the cows required more number of services per conception was 3-6 and their milk production was 452.84±96.60 liter. There was no any significance difference of production of milk among the different variables.

Key words: Management, productive, reproductive, milk yield

Introduction

In Bangladesh the total cattle population is about 24.4 million of which 11.49 million are female. Among the cattle population 3.53 million are milking cows and 2.61 million are dry cows (cows without milking)). Most of the dairy farms in rural and periurban areas of Bangladesh are small scale and each farm consists of 5-20 lactating cows. There are some non-government and government dairy farm that consists of 50-450 lactating cows (personal communication). In spite of our large number of cattle population, the production of calf and milk are insufficient as per our demand and requirement. Generally crossbred dairy cows produce from 600-800 liter milk per lactation of 210 to 240 days (Islam, 1992). The profit of dairy farm depends on the production of more calf and more milk from the dairy cows with optimum fertility management. Calving interval, age at puberty, service per conception, gestation length and birth weight of fetus are most important parameters to measure the farm economy. A farm with 13-15 months calving interval, 24 months for age at puberty, 1.33 services per conception and 5 kg milk per day per cow could be economically profitable (Azizunnesa, 2002). Milk production depends on the systemic management of dairy farm including proper feeding, breeding, housing and also taking preventive measure against diseases and parasitic infestations (source from internet). Milk production also depends on the parity. Azizunnesa (2002) stated that the milk production of subsistence dairy farm in Mymensingh was 3.2±2.2 kg per cow per day. The reasons for low production of dairy cows could be due to their poor genetic makeup, inadequate feeding, management, disease control programme, hot humid environments (Jabber and Green, 1998; Shamsuddin, 1988; Alam and Ghosh, 1988; Shamsuddin *et al*., 2001). Most of the farmers are illiterate and they are rearing their cattle with so called management system. It is also necessary to mention that more than 80% of the cattle population is being kept by the individual farmers in the rural areas under village management that is why the productivity is very low. On the other hand the demand for milk and milk products is rapidly increase in Bangladesh day by day.

Usually the production performances of cross-bred cows are better than local cows. For why at present the demand of cross bred cows are many times higher due to higher production of milk ranges between 8-15 liter/day and it is also profitable business in our country. But some constrains decline the rate of production ultimately decline the profit of the dairy farms due to lack of technology. So it is important to measure the different productive and reproductive performances of dairy farms for overall productive and reproductive pictures of farms and accordingly putting some recommendation to

Table 1: Measures of productive and reproductive traits of dairy cows at dairy and cattle improvement farm

Parameters	Mean±SD	Minimum	Maximum	No.
Milk production 180 days (liter)	459.09±138.09	144.00	1132.12	82
Calving interval (days)	634.59±223.92	370	1365	82
Age at puberty (days)	1205.02±313.80	665	2280	82
Service per conception	1.88±1.09	1	6	82
Gestation length (days)	273.08±7.48	262	290	82
Birth weight of fetus (kg)	18.78±34	12	34	82

improve the status. Therefore, the present study was carried out to describe the productive and reproductive performances of cross bred dairy cows at dairy and cattle improvement farm and to recommend suggestions for improvement.

Materials and Methods

Description of the farm: The cattle and dairy improvement farm, Hathazary, is one of the government dairy farm located in Hathazary upazilla under Chittagong district which was established in 1995. There were 382 cattle population in this farm during the period of 2003-2004 where in most of the cattle population was Friesian×Local. Very few numbers were Sahiwal×Local and Friesian×Sahiwal cow.

Management practice: Both stall and group feeding are being practiced round the year, where the common feedstuffs are grown in the own cultivable land. The frequency of supply of feed in thrice a day. Heat detection and insemination has been performed by own fixed stuffs. Frozen semen is used for AI. There is a culling system in this farm and heifer replacement for future reproduction. Unproductive cow having 4/5 parities or any other incurable diseases are normally culled and the vacant places are replaced by heifer for future reproduction. Animals are vaccinated against FMD, BQ and HS and also dewormed with broad-spectrum anthelmintics at 4 months interval.

Study design and data collection: A retrospective study was carried out on 82 lactating cows of the farm between June 2003 and May 2004. A structured questionnaire was developed to obtain the necessary information as per objectives targeted. The questionnaire included the following information: genotypes of the animals, average milk production per day per cow up to 180 days, total milk production per lactation up to 180 days, lactation length, calving interval, gestation length, service per conception, age at puberty (latest) and birth weight (last calving) of fetus were recorded in the questionnaire by record reviewing of the farm register with the help of farm manager. The main investigator took this responsibility in person to fill up the questionnaire.

Statistical analysis: The data were sorted and entered into Microsoft Excel 2000 and exported to the STATA 7.0^{R} (Stata corporation college, station) for statistical analyses. Descriptive analyses were done to perform the results in percentage, mean and standard deviation where applicable.

Results and Discussion

Milk production: The measures of different productive and reproductive traits were presented in Table 1.

The average milk production per cow up to 180 days was 459.09±138.09 liter and ranged from 144.00-1132.12 liters which is some extent similar to the result of Azizunnesa (2002). She found the average production of milk of subsistence dairy farm at Mymensingh district was 3.2±2.2 kg per cow per day for a lactation length. However the dissimilar result was reporting by Bhuiyan and Sultan (1994). They found the highest milk yield in Holstein Friesian cow was 10.41±0.17 kg per day for a full lactation period. He also found the average milk production per cow per day from crossbred and indigenous dairy cows was 4.10 and 2.28 kg, respectively. In adequate feed supply, lack of proper management, poor genetic makeup might be the possible causes of low milk production.

Calving interval: The present result showed the calving interval ranged between 370-1365 days. The average calving interval was 634.59±223.92 days. These results are not considered with the previous study of Uddin (2001). He found the highest calving interval of 472.55±169.27 days in indigenous cows and the lowest value of 413.77±53.87 days for Fx cows. For an economic profitable dairy farm, calving interval should be 365 days is considerable (Jainudeen and Hafez, 2001) but this study did not show the expectable calving interval.

Age at first puberty: As the farm economic depends on reproductive lifespan of dairy cows, so it is very important to shows estrous as early as possible for a heifer. By showing estrous as early as possible a female animal can contribute more on the reproductive point of view. In this study the average age at puberty was 1205.02±133.80 days. This result differs from Rahman (1993) and Asraf (1998) who recorded 33×30 = 960 days required for puberty of Fx heifer and 42×30 = 1210 days required for local×Fx heifer in other parts of Bangladesh.

Table 2: Measures of milk production up to 180 days in relation to different reproductive parameters

Variables	Category	Mean±SD (liter)	P value
Parity	2	456.84±169.58	0.64
	3	445.79±86.64	
	4-8	487.54±109.73	
Calving interval (days)	370-590	473.44±134.15	0.35
	591-1365	444.74±142.11	
Age at puberty (days)	665-1185	450.50±113.56	0.55
	1186-2270	468.56±161.92	
SPC	1-2	461.52±151.89	0.79
	3-6	452.84±96.60	

Service per conception: The minimum number of service per conception is one of the indicator of economically profitable dairy farm. The average service per conception of studied farm was 1.88±1.09. The number of service per conception in a dairy farm should be maximum 1.33 (de Kruif, 1978). The present result agrees in some extent with the result of Azizunnesa (2002). She observed an average service per conception of subsistence dairy farm was 1.5. In the other experiment by Shamsuddin *et al.* (2001) showed an average service per conception of 2.2 in some selected parts of Bangladesh.

Gestation length: Average gestation length was 273.08±7.48 days which is consistent with the findings of Mondal (1998). He reported an average gestation length for Holstein cross was 275.15±3.95 days. Gestation length of this study might have been varying because of seasonal and manage mental variability of the farm.

Birth weight of calves: The desired level of birth weight of calves is most important for faster growth of heifer to show estrous as early as possible. The Table 1 showed an average birth weight of calves was 18.78±3.57 kg which is almost the same findings of Rahman (1999) and Khan *et al.* (2000). Rahman found an average birth weight of F, Sahiwal×F and local calves were 32.00±5.83, 22.34±3.81 and 29.09±5.70 and 21.03±3.36 kg, respectively. While Khan reported an average birth weight was 17.28±0.76 and 16.00±1.52 kg for farm condition and rural Red Chittagong calves respectively. The reason of low birth weight could be due to nutritional deficiency, any disease condition of mother as well as fetuses or early delivery of fetuses.

The Table 2 showed the effect of some variables on milk production.

The average milk production per cow for 180 days was highest in 487.54±109.73 liter, the cows having 4-8 parity while the average production of milk was in between 456.84±169.58 and 445.79±86.64 liter in cows having 2nd and 3rd parities, respectively. However, the difference of milk production among different parities was not statistically significant. This result agrees with Sarder *et al.* (1997). They reported that parity had significantly effect on milk production and they found more milk in cows with greater parities than those with lesser parities (6.0 vs 7.0 to 7.5 kg for 1st vs 4 to 5th parity). This result also support by Sainz *et al.* (1992). They observed that daily milk yield is maximized when cow aged at 53.90 to 69.67 months. The highest amount of milk (7.7 kg /day) was produced by cows at an age of >84 to 144 months old stated by Sarder *et al.* (1997).

Calving interval: The result showed an average production of milk up to 6 months was 473.44±134.15 and 444.74±142.11 liter by the cows those having calving interval 370 to 590 and 591 to 1365 days, respectively. In this experiment comparatively more milk production was found from cows having minimum interval between calving. However this result is not statistically significant. Longer the calving interval looser the reproductive life, ultimately farm owner looser economically. It is observed that less milk production found when the cows with long calving interval. The author think, the causes behind this less milk production were various reproductive disorders in the post partum period.

Age at puberty: The average milk production/cow was highest in 468.56±161.92 liter, the cows taking long time to shows 1st estrous. On the other hand comparatively less production of milk was found from those cows taking less time to shows 1st estrous. The observed analysis showed that there was no significance difference of milk production among the age at puberty. Islam (2000) reported the average milk production is affected by age at first puberty.

Service per conception: The maximum milk production was found 461.52±151.89 liter from cow's required minimum number of services for conception (1-2) and comparatively lower production of milk was found from cows required higher number of services per conception was 3-6. The differences production of milk among service per conception is not significant statistically. It is observed that cows with less milk production required more number of services for conception and long time for inter calving interval. It indicates that postpartum management was not good for this farm.

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Determination of the Shelf-Life of Trout (*Oncorhynchus mykiss*) Raw Meatball That Packed under Modified Atmosphere

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Abstract: Raw meatball is a traditional food in Turkey which is produced by mixing and kneading of various ingredients. There isn't any thermal inactivation process during the production. Because of high initial microbial load and absence of a pasteurization process and because of its raw consumption, raw meatball has high risk of microbial infection. In this study, it is aimed to detect the shelf-life of trout (*Oncorhynchus mykiss*) raw meatball that was packed under 3 different conditions; Control Group, MAP1 (GroupA) and MAP2 (GroupB). Sensory, pH and microbiological analyses were done during the study. According to the results, it has been determined that the Control (%100 air) samples saved their freshness until 5th day, the MAP1 (5% O_2+ 35% CO_2+ 60% N_2) and the MAP2 (5% O_2+ 25% CO_2+ 70% N_2) groups saved their freshness until 9th. These two groups showed no difference between themselves about storage.

Key words: Trout, raw meatball, MAP, microbiological quality, shelf life

Introduction

Raw Meatball is mostly consumed in East and South East Anatolia which are economically and educationally less developed regions of Turkey. It also recently takes place in the menus of luxury hotels, restaurants and holiday villages as an appetizer which is appreciated. As there is no standard for the ingredients of raw meat ball, its contents may differ according to the region. The main contents are: small, boiled and pounded wheat and minced lean meat. Other ingredients are: onion, garlic, tomato sauce, parsley, red pepper, black pepper, thyme, cumin, allspice, ice or water. All these ingredients are mixed together in an order and are kneaded by hand (traditionally by naked hand). The quality of the raw meatball is directly depends on the quality of meat and spices, hygiene of the personnel and the process. It increases the risk of human health that raw meatball is consumed without any kind of cooking process. Generally, raw meatball is consumed as soon as it is prepared (Sagun *et al*., 2003). Some studies, which have been done on raw meatball, have showed that the microbial load of this food is high and it may create an important risk for the public health (Göktan and Tuncel, 1988; Küplülü *et al*., 2003; Vural *et al*., 2006). Göktan and Tuncel (1988) have notified that raw meatball can be conserved in the fridge for 24 h. Foods may have some changes such as oxidation and microbiological effects during their storage. If food doesn't contact with O_2 from its process step to its arrival to the customer, it can save its freshness for a long time. This may occur by changing the atmosphere conditions in the package (Weber and Laux, 1992). Thus, it has been started to use packing technology with modified atmosphere recently. When Modified Atmosphere Packaging (MAP) combined with refrigeration, it can extend the shelf-life of the seafood according to its hunting operation time and quality (Dhananjaya and Stroud, 1994). The main parameters which affect the shelf-life of the stocked products are the production form, the gas mixture inside the package, features of the package material, storage temperature, packing process and machines that used, etc. In this study, it is aimed to prepare the raw meatball with trout meat and then to detect the shelf life of this product by packaging at cold and by packaging with MAP at different gas mixtures.

Materials and Methods

In this study, totally 40kg cleaned rainbow trout (*Oncorhynchus mykiss*, W.,1792) which were obtained from LIMAN Alabalik Co. Ltd. as 200- 250g pieces was used. The frozen fish which was transported to the laboratory in foam boxes, were holded in hot water (85° C) for two minutes, then head, skin and fish bones were boned and meat was cleaned (As it is possible to be risky to consume much and deficient raw fish meat, it was thought to use cooked fish meat and a sensory panel test had been done about this. After evaluating this sensory test results which had been scored by 10 panellists group, it was decided to use cooked fish meat). These cleaned meat were kneaded by hand with small pounded wheat which had been kept waiting in drinking water for 60 min. The quantity of the small pounded wheat was half of the fish meat quantity. Different kinds of spices (red pepper, black pepper,

cumin, hot pepper, sweet pepper sauce, garlic, onion, salt and garden rocket) were added. Kneading took 3 h and after this, the mixture was shaped as 40-50g flat meatballs. These meatballs were put in foam boxes as 10-12 pieces, 400-500g weight and were taken to the store at cold (+4 ± 1°C) packing with as follows:

The analysis groups

Control group: 100 % air, MAP_1 (Group A): 5 % O_2 + 35 % CO_2 + 60 % N_2, MAP_2 (Group B): 5 % O_2 + 25 % CO_2 + 70 % N_2.

The analyses were run on the first day and then the quality changes were determined by analysing once in two days. In our study, KOROZA PA/PE package (unit weight 37,24 ± 10 %), packing material with thickness 90 micron ± 10 %, packing machine (HENKOVAC E-173 model, The Netherlands) with mix gas mixture were used.

Sensory analyses: The sensorial quality of raw trout meatball was evaluated by 10 experienced panelist according to "the hedonic scale" by using 0-10 scores. According to this evaluation; a score of between 10-8 accepted as "very good", between 8-6 accepted as "good", between 6-5 accepted as "proper to consume" and a score of under 5 accepted as "spoilt" for each sensorial characteristic. Fresh trout was evaluated according to its appearance, odour and colour. After frying in hot oil, it was evaluated according to its taste, odour, appearance and tissue by panellists (Amerina *et al.*, 1965).

pH Measurements: These measurements were done with WTW 537 model micropressor pH meter. While measuring; 10g fish sample had been weighted, diluted 1:1 and the pH probe had been immersed into the solution. Four readings were made on each sample and the mean recorded (Manthey *et al.*,1988).

Microbiological analyses: 10 g samples, that had been taken from each group's each 3 packages under aseptic conditions, have been rarefied with 90 mL peptone water (Merck, Cat No: 107228) and homogenized in stomacher (IUL Instrument, Spain) for 1 min. The dilutions as rarefication solutions have been made from this homogenized sample with 1 mL rarefication and 9 mL peptone. From these dilutions, mesophilic aerobic bacteria, phsycrophilic bacteria, *S. aureus* have been planted with spread plac method and total and fecal coliform have been planted with MPN (Most Possible Number) method. The counting results after the incubation were calculated as log colony forming units (cfu/g and MPN/g).

Total Mesophilic Aerobic Bacteria: To count Total Mesophilic Aerobic Bacteria, Plate Count Agar (PCA) medium (Oxoid CM 463) and spread plac method were used. The results were evaluated by counting after 24 h incubation at 37°C (Anonymous, 1994).

Phsycrophilic Bacteria: The results were evaluated by counting after 10 days incubation at 7°C by using Plate Count Agar (PCA) medium (Oxoid CM 463) and by spread plac method (Anonymous, 1994).

Staphylococcus aureus: The planting was done into medium which is poured into petri dish with Egg yok tellürit (Oxoid) addition into Baird Parker (BP) (Oxoid) medium by spread plac method. The results were evaluated by counting after 2 days incubation at 37°C (Anonymous, 1994).

Total coliform bacteria: To count total coliform bacteria, Lauryl Tryptose Broth (LST) (Oxoid CM 451), Brilliant Gren Bile 2 % Broth (Oxoid CM 31) medium were used and the planting was done by MPN (Most Possible Number) method. The tubes, which were planted into LST, were incubed for 24-48 h at 37°C. One full of transfer loop from each tube which showed positive reaction were planted into BGLB medium, and then were incubed for 24-48 h at 35-37°C. It was confirmed that coliform bacterias exist in the tubes which form gas (Baumgard, 1986).

Fecal coliform bacteria: It was planted from the tubes which were confirmed to have coliform by forming gas in the BGLB medium to EC Broth (Oxoid 10765) medium with transfer loop. The tubes were incubed at 45.5°C for 24 h. It was confirmed that fecal coliform bacterias exist in the tubes which formed gas and they were called as positive (Baumgard, 1986).

***Salmonella* spp.:** 25g sample was taken under aseptic conditions and pre-enhanced in 225 mL phosphate buffer solution (Sigma, Cat No:P-4417) at 37°C for 16-20 h in autoclaved bottles. For selective enhancement, 1 mL pre-enhanced sample from each bottle were taken and planted into the tubes which have 10 mL Selenite Cystine Broth (Merck, Cat No: 1.07709) and Tetrathionate Broth (Merck, Cat No: 1.05285) inside and were waited for 24 h at 37°C. Passing to the selective solid medium was done by smearing method with Bismuth Sulfite Agar (Merck, Cat No: 1.05418) and Xylose Lysine Desoxycholate Agar (Merck, Cat No:1.05287) at 37°C for 24 h incubation. For Salmonella identification, planting was done with transfer loop into the medium that consisted TSI Flat Agar (Merck, Cat No: 1.03915) and LI Flat Agar (Merck, Cat No: 11640). The incubation time was 24 h at 37° C for TSI Agar and 48 h at 37°C for LI Agar. After incubations, Urea Tests were done for the suspicious colonies in the TSI Agar and LI Agar (The colour changes for the suspicious colonies were bottom yellow/top red for TSI Agar and purple for LI Agar). If there were urea negative isolates, biological and serological tests were done (Andrews, 1992).

Measurements of Packing Material Permeability: The O_2 measurements were done by Servomex Oxygen Analyser 574; the CO_2 measurements were done by Servomex Infra-Red Gas Analyzer PA (404 SVS; Servomex, Sussex, UK) Range: 0-100 % CO_2 vehicles in TUBITAK (The Scientific and Technical Research Council of Turkey).

Statistical analyses: Analyses results are reported as Mean ± Standart Deviation (Sd). Differences in mean values were determined using the t-test method (significance was defined at P < 0.5) (Sokal and Rohlf, 1987).

Results and Discussion

The results of the pH and sensory microbial analyses are shown on Table 1 and Fig. 1-4. The values of the sensory and pH analyses of cooked and fresh fish that were used for this study are 6.29±0.01 and 6.33±0.01; 9.80±0.38 and 9.33±0.26. For fresh samples, the microbiological loads are; for mesophilic aerobic bacteria 1 log cfu/g and for phsycrophil, coliform and fecal coliforms, the number was counted lower than the minimum level. For cooked trout, the microbiological loads were counted lower than the minimum level for mesophilic aerobic bacteria, phsycrophil, coliform and fecal coliform microorganisms.

After the raw trout meatball had been prepared, the initial and the 5th day (the day on which the sensory spoilage was seen) pH values of Control group were determined as 6.18±0.01 and 6.03±0.01 ($p<0.05$); sensory analyses values were determined between 9.58±0.38 and 5.33±0.41 points ($p>0.05$). At the end of the 5th day, the microbial values of our Control Group samples were determined respectively; for total mesophilic aerobic bacteria count, as 4.42 and 6.88 log cfu/g ($p<0.05$); for phsycrophil microorganism count, as 3.92 and 5.90 log cfu/g; for total coliform bacteria count, as 2.04 and 1.47 MPN/g ($p<0.05$) and fecal coliform bacteria count, as 0.35 and 1.48 log MPN/g ($p<0.05$).

After raw trout meatball had been prepared, the pH value of MAP_1 group, on the initial day (mixture) and on the 9th day which the sensory spoilage had been seen; were 6.18 ± 0.01 and 6.04±0.01 ($P<0.05$) and sensory analyses values were detected between 9.58±0.38 and 5.42±0.58 ($P>0.05$). On the initial day and at the end of the 9th day, the microbial values of our MAP_1 group samples were determined relatively; for total mesophilic aerobic bacteria count, as 4.42 and 6.61 log cfu/g ($P<0.05$); for psycrophil microorganism count, as 3.92 and 6.77 log cfu/g ($P<0.05$); for coliform count, as 2.04 and 2.30 log MPN/g ($P<0.05$); for fecal coliform count, as 0.35 and 2.18 log MPN/g ($P<0.05$). After raw trout meatball had been prepared, the pH values of MAP_2 group were 6.18±0.01 and 6.02±0.02 ($P<0.05$) and sensory analyse values were detected between 9.58±0.38 and 5.75±0.32 ($P>0.05$), on the initial day (mixture) and on the 9th day which the sensory spoilage had been seen. The microbial values of our MAP_2 group samples on the initial day and on the end of the 9th day were determined relatively; for total aerob mesophilic bacteria count, as 4.42 and 5.0 log cfu/g ($P<0.05$); for psycrophil microorganism count, as 3.92 and 5.87 log cfu/g ($P<0.05$); for total coliform count, as 2.04 and 1.60 log MPN/g ($P<0.05$); for fecal coliform count as <0.35 and 1.60 log MPN/g ($P<0.05$). Also, we have never come across with *Salmonella* spp. and *S. aureus* during our study.

The gas permeability of the package material that was used in this study was determined as 31.5 $cm^3/m^2/d$ bar for O_2 at +4°C; the water steam permeability was determined as 7.27 g/m^2/bar.

Generally, one of the first chemical changes on the fish meat, is the pH changes. pH value of the fish is changeable according to the species. So, this is not an exact criteria for the detection of freshness and quality. This is used as a supporter for other quality control parameters. It is defined that for fresh fish meat, the pH value is 6-6.5 and this can exceed according to the storage time. The limit pH degree for consuming is 6.8-7.0 for fish (Varlik *et al.*, 1993). For the tilapia fillets which have been packed with normal air at 4°C, the pH value was analysed as 6.6 at the 9th day of the storage (Reddy *et al.*, 1994). For the herring vegetable salads which were packed under modified atmosphere conditions and stored, it was seen that the packaging rules and the changes of quality do not effect the product's pH significantly (Ahvenainen *et al.*, 1990). It is defined that; the pH values that analysed at the end of 24 h storage with different gas mixture packages, do not differ from the control group samples significantly. But, at the end of 21 days, it was seen that these samples differ from the control group samples very much (pH 8.03). In our study, the pH value decrease of the raw trout meatball has been caused by the acidity of the spices and boiled and pounded wheat.

If a kind of product is acceptable for its quality parameters but not acceptable for its sensory features, then it is not proper to consume this product (Varlik *et al.*, 1993). It has been determined that, after storing the Morina fillets at MA, the fillets were better than the control group at the 6th and 9th days of the storage. It has been notified that, the fillets in the control packages were spoilt at the 9th day (Woyewoda *et al.*, 1984). In one of the studies of Reddy *et al.* (1994) for the tilapia fillets, at the end of the 9th day of the storage at +4°C with normal air, fillets were spoilt according to the sensory evaluation. Brown *et al.* (1980) have been detected that, for rockfish and salmon fish, after packaging with MA, sensory control samples differ very much from the packed with gas samples at the end of the 7th day. Çetin and Bostan (2002) determined that control group samples of meatballs prepared from red meat spoil at the 4th day of

Table 1: pH and Sensory analyses results of raw trout meatball packed under Modified Atmosphere

Analysis	Analysis Grups	Raw	Cooked	Mixture	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
pH	Control	6.29±0.01	6.33±0.01	6.18±0.01	6.15±0.02	6.14±0.01	6.03±0.01	6.01±0.01	6.06±0.01	5.80±0.01
	MAP_1			6.18±0.01	6.15±0.01	6.14±0.01	6.07±0.01	6.03±0.01	6.04±0.01	5.97±0.01
	MAP_2			6.18±0.01	6.11±0.01	6.14±0.01	6.06±0.01	6.03±0.01	6.02±0.02	6.03±0.01
Sensory	Control	9.80±0.38	9.33±0.26	9.58±0.38	9.42±0.58	7.75±0.27	5.33±0.41	4.83±0.52		
	MAP_1			9.58±0.38	9.25±0.52	7.67±0.27	5.91±0.58	6.50±0.32	5.42±0.58	4.17±0.41
	MAP_2			9.58±0.38	9.50±0.32	7.83±0.26	7.25±0.27	7.17±0.26	5.75±0.32	4.58±0.49

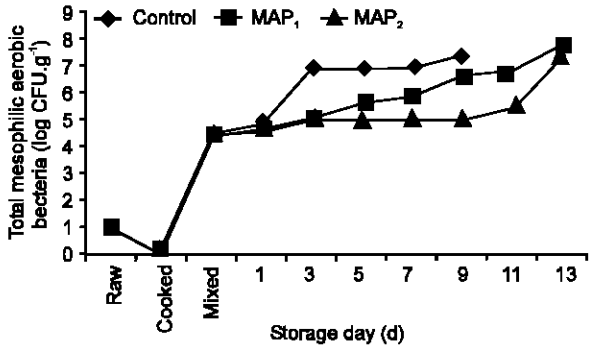

Fig. 1: Total mesophilic aerobic bacteria (log cfu/g) analyses results of raw trout meatball packed under Modified Atmosphere

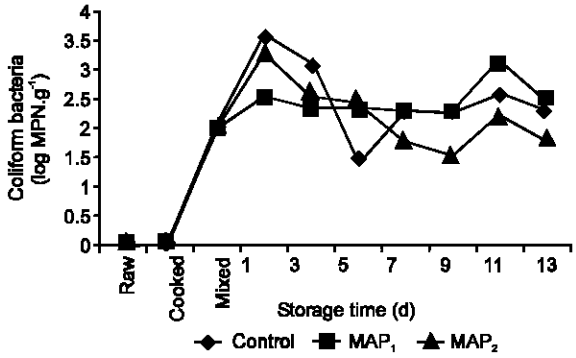

Fig. 3: Coliform bacteria (log MPN/g) analyses results of raw trout meatball packed under Modified Atmosphere

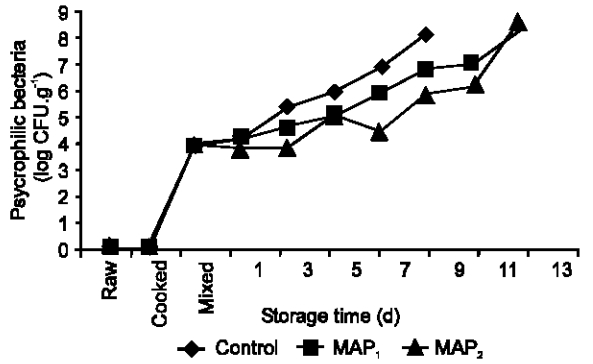

Fig. 2: Psycrophilic bacteria (log cfu/g) analyses results of raw trout meatball packed under Modified Atmosphere

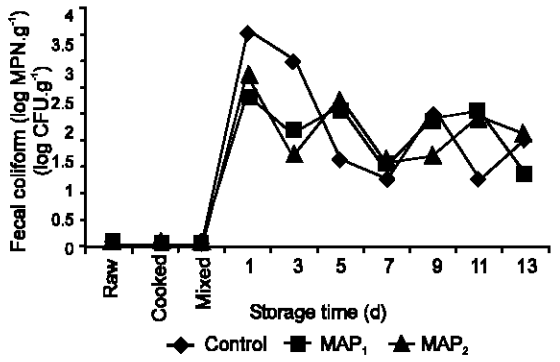

Fig. 4: Fecal coliform (log MPN/g) analyses results of raw trout meatball packed under Modified Atmosphere

the shelf-life. In our study, it was determined that control group samples are appropriate to consume till 5th day and both of the groups that operated by MAP are appropriate to consume till 9th day according to sensory. The limit value of the total aerob mesophilic bacteria is 7-8 log cfu/g. Thus, the standards and regulations suggest much lower values generally (Olafsdottir *et al.*, 1997). According to Anonymous (1992), the limit values for fresh or frozen seafood are 6-7 log cfu/g for total aerob mesophilic bacteria count, 160- 210g for coliforms and 9-12g for *E. coli*. Yildirim *et al.* (2005) defined that, in the study of determinations for the quality changes of the red meat Raw trout meatball; The total aerob mesophilic bacteria count is 4.74 log cfu/g, *S. aureus* number is 4.64 and coliform number is 3.04 log cfu/g in the control group samples. They also did not come across any *Salmonella* spp. Çetin and Bostan (2002), defined that, for the red meat raw trout meatball stored at cold, the initial and 10th day values are 6.15 and 9.59 log cfu/g for total aerob mesophilic bacteria, 4.41-7.91 log cfu/g for coliform bacteria, 3.98-6.91 lof cfu/g for *S. aureus*. In one of the studies related with the hamburger meatballs that packed and stored at 3°C under MA, because of the initial excess of microorganisms, at the 7th day of the storage total aerob mesophilic bacteria count was 6 log cfu/g, at the 13rd day the number was higher than 7 log cfu/g which was the limit value (Çiftçioglu and Gün, 1994). Dondero *et al.* (2004) did not come across with

Salmonella spp. and *S. aureus* in any step of the storage for their study which was about the cold storage of vacuum packages of smoked somon.

Küplülü *et al.* (2003) determined that; for different samples of red meat raw meat balls that were transported from different places of Ankara (Turkey), total aerob mesophilic bacteria count was 6 log cfu/g, coliform bacteria count was 3-5 log cfu/g, average number of *S. aureus* were 4 log cfu/g. They also did not come across any *Salmonella* spp. For the tilapia fillets which were packed with normal air at 4°C and stored for 13 days, the total mesophilic aerobic bacteria count was found as 9.6 log cfu/g and total anaerob bacteria load was found as 8.7 log cfu/g (Reddy *et al.*, 1995). As for the catfish which were packed with normal air at 0°C, as the initial total mesophilic aerobic bacteria count was 5.88 log cfu/g, this was determined as 8.22 log cfu/g at the 8th day. In another study for rainbow trout and herring fillets, after 6 days of storage with vacuum and wrap packaging, total aerob mesophilic bacteria count was 4 log cfu/g and more, for MA groups, it was about 3 log cfu/ g. In the study of hamburger meatballs that stored at 3°C with MA, it was seen that the coliform amount increased logarithmically till the 13rd day and became lower than the initial degree of 5.64 log cfu/g for the rest days (Çiftçioglu *et al.*,1994). Akkus *et al.* (2004), determined that the shelf-life of the anchovy meatballs is 9 days. In our study, there was no significant microbiological load for fresh and cooked fish. Especially, the microbiological content of the product has been increased after adding spicy mixture. Also, it has been thought that the reasons for the increase of colifom amount had been caused by packaging material, by washing water and by different people who kneaded. Elmali and Yaman, (2005) have been detected microbiological quality of raw meat balls produced in Turkey and they have concluded that consumption of raw meat ball poses a risk of food borne infections or toxication due to its raw meat for human health.

Conclusion: In our study, using cooked fish meat affected the shelf-life of the raw trout meatball by extending the duration. Although, it was determined that raw trout meatball had spoilt at the 7th day for microbiological control groups and at the 11th day of storage with MA, they lost their consuming features. Thus, our study is proper to the literature datas. It has been determined that normal vacuum packaged products save their freshness till 5 days and MAP_1 and MAP_2 samples save their freshness till 9 days.

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Lead, Zinc and Cadmium in Root Crops from Mineralized Galena-Sphalerite Mining Areas and Environment

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Abstract: Concentrations of the metals leads, cadmium and zinc were estimated in root crops: Cassava (*Manihot esculenta crantz*), yam (*Dioscorea rotundata*), potato (*Ipomea batatas*) and cocoyam (*Colocosia esculenta*) harvested in some mineralized areas of galena and sphalerite deposits using atomic absorption spectrophotometer. The Investigation showed that the sample analyzed contained lead concentration ranging from 0.030-0.190 mg/kg. Cadmium levels were in the concentration range of 0.136-2.633 mg/kg in all crops, while zinc concentration ranged from 0.340-3.890 mg/kg. There was no significance difference observed between the levels of contamination of the different crops, while high variation in concentration was observed in the level of predominance of heavy metals in different location. These results reflected higher concentration of metals in selected crops from these zone.

Key words: Lead, cadmium, zinc, root crops, mineralized area, galena and sphalerite

Introduction

Uncontrolled mining activities and illegal mining in developing countries has left a lot of environmental hazards and enormous amount of wastes and different types of pollutants are generated.

Adverse environmental and ecological changes as a result of anthropogenic input has become more tangible and menacing (Tomov and Kouzmova, 2005). There exist concerns and question on the state of the soil and quality of food crops, fruits and vegetables cultivated and grown in areas where heavy mining and exploration are carried out.

Some heavy metals such as lead, zinc and cadmium in crops are studied because they are related to environmental problems and also have accumulative properties. Traces of these metals can be found naturally in the environment but industrial activities increase their level so lead to pollution (Adediran *et al.*, 1990). In mineralized areas such as Ishiagu, Nigeria, one of the major sources of lead and associated cadmium to the environment arise from lead and zinc mining activities by industrial and local miners.

Heavy metals such as lead (Pb) is mainly absorbed through leaves, roots and aerial deposition (Flam, 1978). Cadmium accumulation by plants are greatly influenced by the supply of zinc to the plant (Abbdel-Sabour and Mortvedt, 1998; Honma and Hirata, 1978; Haghiri, 1974). However high level of heavy metals in the soil could indicate similar concentration in plant by accumulation at concentration causing serious risk to human health when consumed (Vousta *et al.*, 1996). Moreso, constant exposure to very low levels of elements such as lead (Pb), cadmium (Cd) and mercury (Hg) have been shown to have cumulative effects since there is no homeostatic mechanism which can operate to regulate their toxicity (Carter and Fernando, 1979; Yeast and Brewers, 1983). Recent work has shown the study of heavy metals (Pb, Cd, As and Hg) in edible grains grown and marketed (Osu and Odoemelam, 2007), trace metal (Pd, Fe, Cu and Zn) in crops harvested in some oil prospecting locations (Hart *et al.*, 2005), bio-accumulation of heavy metals in Periwinkle and Oyster (Fubara and Christian, 2006) and the uptake of Cu, Pd, Cd, As and DDT by vegetables grown in urban environments has been reported.

This study therefore, is designed to investigate the concentration of heavy metals lead(Pb), zinc(Zn) and cadmium (Cd) in root crops namely cassava (*Manihot esculenta crantz*), yam (*Dioscorea rotundata*), potato (*Ipomea batatas*) and cocoyam (*Colocosia esculenta*) cultivated and harvested in the galena - sphalerite rich zone of Ishiagu, Nigeria. This will also ascertain the effect of mining activities on these areas in relation to agricultural produce.

Materials and Methods

Study locations: The study areas were, Amita, Amagu, Amaeze, Amaonye, Amaeke and Ihetutu, all in Ishiagu community in Ebonyi State, Nigeria. These sites where chosen because they are rich in lead and zinc minerals and mining activities are carried out in these area. The crops from Federal University of Technology, Owerri, farm was used as the control site for the experiment.

Sample Collection: Food crops used for the study were root tubers; cassava, yam, potato and cocoyam. These crops were harvested at peak of the harvest. By arrangement with the respective farm owners, the

Table 1: Mean values of lead, zinc and cadmium in crops in relation to environment.

Crops	Metal in mg/Kg	Replications					
		Ihetutu	Amita	Amaeke	Amagu	Amaonye	Amaeze
CASSAVA	Lead	0.140	0.030	0.080	0.000	0.080	0.080
	Zinc	1.020	0.340	1.520	1.760	1.890	0.700
	Cadmium	0.993	0.666	1.035	1.285	1.070	0.000
YAM	Lead	0.080	0.000	0.020	0.000	0.100	0.040
	Zinc	1.240	1.700	2.620	1.050	1.460	3.890
	Cadmium	0.136	0.917	1.265	0.017	0.000	0.019
COCOYAM	Lead	0.030	0.000	0.160	0.000	0.000	0.190
	Zinc	0.590	2.950	1.050	2.430	1.890	1.080
	Cadmium	0.258	2.633	0.751	0.804	1.470	2.032
POTATO	Lead	0.050	0.020	0.040	0.030	0.140	0.090
	Zinc	0.820	2.140	1.230	2.840	1.860	2.620
	Cadmium	0.817	0.907	0.000	0.455	1.496	1.910

Table 2: Variance among metals, locations and crops.

	Locations	Crops	Metals	Crop X Metal
$F_{CAL\ 0.05}$	$0.71^{N.S}$	$1.93^{N.S}$	17.120**	$1.330^{N.S}$
	3.03 **	$0.059^{N.S}$	26.970**	$0.970^{N.S}$
$LSD_{0.05}$	N.S	N.S	0.550	0.760
	N.S	N.S	0.492	0.692

various crops were randomly harvested from two or three farms within each location. There was no history of fertilizer and other agro-chemicals used in the farms studied. Samples of each crops collection were wrapped in black calico bags properly labeled and taken to the laboratory for analysis. Each sample removed from calico bags were washed in distilled water to remove soil and dirt, then placed in labeled envelops and were oven dried at 105°C for 2hrs. The dried samples were then milled into powder using Author Thomas Milling Machine and stored in an airtight container until required for analysis.

Determination of metals: 1g of the ground samples was homogenized and was digested with 20ml of 1:1 (v/v) concentrated HNO_3 and HCl (Analar grade) in 100ml beaker. The flask was swirled gently and heated in an electrothemal heater until evolution of white fumes marking the end of the digestion process. The digest was then cooled and filtered through whatman No.1 filter paper into 50ml volumetric flask and diluted to 50ml mark with distilled water. The resulting solutions were subsequently analyzed for lead, zinc and cadmium concentration by an air-acetylene flame atomic absorption spectrophotometry (Apha-4-model) by the standard calibration techniques.

All reagents used in the analysis were of analytical grade. Analysis were done in duplicates. In all determinations, blanks were included.

Results and Discussion

The mean level of lead (Pb), cadmium (Cd) and zinc (Zn) concentration obtained was subjected to statistical analysis. Using analysis of variance (ANOVA). The least significance difference (LSD) was applied to show the mean difference at 5% level of significance.

The mean levels of lead, zinc and cadmium in crops; cassava, yam, cocoyam and potato and with respect to the six locations namely; Ihetutu, Amita, Ameke, Amagu, Amaonye and Amaeze in Ishiagu communities are shown in Table 1. The relatively high concentration of these metals occurring in crops from these areas strongly indicate the presence of heavy metal pollution due to the deposits of lead-zinc minerals and the mining activities in these areas. Previous work on trace metals levels of soils and crop around Ishiagu rock mining site had also shown lead concentration 6mg to 32mg/100g (Ehosum, 2002). The relatively higher level of lead in crops from Ihetutu, Amaeze and Amaeke with values 0.140mg/Kg, 0.0190mg/Kg and 0.160mg/Kg, all were due to the nearness of these villages to the mining sites (1-2 Km) compared to those harvested from villages far away from the mining site. Highest value of zinc concentration was obtained in yam from Amaeze with the values 3.890mg/Kg with associated high concentration of cadmium. This is explained by the reports of Abbdel-Sabour and Mortvedt, 1998.

Table 2 shows the analysis of variance of the crops with respect to the concentration level of heavy metals in different locations. No significant difference was observed between the levels of contamination of the different tubers in question within each environment. No significant difference was observed between the level at which cassava was contaminated with heavy metals and the level at which yam was contaminated. High variation in concentration was observed in the level of predominance of metal in different locations.

Table 2 also shows the least significance difference test on crops with respect to the contamination and levels of metals in different locations. The results obtained were in consonance with that obtained in the analysis of variance except for the interaction of crops with the metals which showed a significant difference between crop contamination with different metals.

Generally, there was high levels of cadmium in crops from these mineral rich lead-zinc zones. This result could be due to the rate with which cadmium is taken up from the soil through the roots and more so, cadmium occurs in association with lead and zinc.

It has been reported that in industrial societies, up to 0.2-0.4mg lead may be ingested in the food daily and 90% of this excreted in the faces. From Table 1, the values of cadmium are above normal range of metal concentration daily intake, which is 0.018mg/kg (Okoronkwo *et al.,* 2005). The values were relatively high compared to the crops from the university farm which has the following values for lead, cadmium and zinc in cassava, yam, cocoyam and potato as 0.000, 0.002, 0.001 and 0.008 all in mg/Kg respectively.

Conclusion: This study has revealed the various concentrations of metal lead (Pb), cadmium (Cd) and zinc (Zn), in cassava, yam, cocoyam and potato tubers harvested at areas of high mining activities in Ishiagu community. These findings are indicative of pollution due to mining activities. The values of these metals are within acceptable range. These crops are stable foods in Ishiagu, consequently people depending on these crops as source of stable food are indirectly ingesting heavy metals into their body system. In view of these findings, there is need to monitor more closely the environment under review and to ensure appropriate mining technology in order to reduce the availability of these metals (Pb, Zn and Cd) in agricultural crops and also preserve the health of communities within the vicinity of the mineralized area, particularly as the effects of heavy metals are bio-accumulative.

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Biochemical Evaluation of Millet Offal as Feeds for Broiler Chickens

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Abstract: Studies were conducted to chemically characterize and biologically evaluate millet offal as a replacement for maize in the diets of broiler chickens. Two types of millet offal were chemically characterized: the one obtained as a by-product of brewing industry and the other a by-product of pap manufacture. Studies were further carried out to further determine the effects of varying levels of millet offal obtained from the brewing industry on the performance of broiler chickens. The results of the studies indicated that millet offal from the brewery contained 14.60% CP, 4.57% CF, 2.25% EE, 2.90% Ash and 2148.0kcal/kg ME while the one from pap manufacture contained 20.65% CP, 3.12% CF, 3.01% EE, 3.36% Ash and 2506.0kcal/kg ME. The results further indicated final body weight values of 602.02, 605.85, 605.83 and 561.72g/bird for starter chicks (5 weeks old) and 2283.7, 2192.2, 2145.9 and 1904.5g/bird for finisher chickens (9 weeks old). Generally, there was an increase in feed intake as dietary millet offal increased. Feed cost per bird generally decreased when millet offal replaced maize in the diets. Therefore, millet offal can be classified as medium energy and protein sources in poultry diets. Moreover, millet offal can replace up to 50% maize in the diet without any adverse performance of broiler chickens and at reduced cost of feed production.

Key words: Millet offal, feed for broiler, maize

Introduction

Livestock consume more than a third of all the world's grains particularly maize (Thompson and Weber, 1981). Maize has remained the chief source of energy in compound feeds and constitutes about 50-60% in most poultry diets. Consequent upon its high demand, maize has been in short supply with resultant price increase at an average of 11% per annum since 1976 (Thompson and Weber, 1981). Several attempts have been made to reduce cost of feed production by replacing part of the maize in the diet with industrial by-products such as maize offal, brewers' dried grains, wheat offal and others (Ademosun, 1973; Dafwang Shwarmen, 1996; Ogbonna *et al*., 2000). Such alternative feedstuffs have the advantage of low cost and possess very low human food preference, thereby reducing competition between man and animals. Millet offal is one of the industrial by-products which could substitute for maize as an energy source in poultry diets. However, two types of millet offal have been identified. One is obtained as a by-product of the brewing industry and the other as a by-product of local manufacture of pap both of which are readily available in Nigeria.

Much of the research work on the use of agro-industrial by-products for non-ruminant animal feeding have concentrated on their utilization in terms of growth and production with limited data available on chemical characterization. An in-depth understanding of the nutro-chemical characteristics of millet offal types vis-à-vis their effects on broiler chicken performance would ensure a more judicious utilization of the ingredient. The aim of this study therefore, is to chemically characterize two types of millet offals in terms of their proximate composition and metabolizable energy values. This study is also designed to investigate the effects of varying levels of millet offal from the brewery on live performances and apparent nutrient retention in the diet of broiler chickens.

Materials and Methods

The millet offal type from the brewing industry was obtained from Guinness Nigeria PLC, while the other type was obtained from a small scale pap manufacturer. Each of the types of millet offal was obtained wet and sun-dried to reduce the moisture content.

Proximate analysis: To determine the proximate composition, representative samples were assayed for moisture, crude protein, crude fibre, fat and ash. Nitrogen free extracted was computed accordingly (A.O.A.C., 1990).

Metabolizable energy study: To determine the metabolizable energy values of the millet offal types, 6 weeks old Anak broiler chickens were used. The birds were managed in standard wire cages equipped with dropping pans. At the beginning of the studies, the birds were divided into 9 similar groups on equal weight basis at 3 birds per group. Three groups were randomly assigned to each of the 3 dietary treatments. Among the treatments, a standard broiler finisher diet served as the basal diet. (Diet 1, Table 1) Diet 2 contained 80% of the basal diet and 20% millet offal from the brewery while Diet 3 contained 80% basal diet and 20% millet offal

Table 1: Percentage composition of Starter and finisher diets

	Diet 1		Diet 2		Diet 3		Diet 4	
Ingredients	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher
Maize	50.00	60.00	37.50	45.00	25.00	30.00	12.50	15.00
Millet offal	00.00	00.00	12.50	15.00	25.00	30.00	37.50	45.00
Soyabean meal	35.00	26.00	35.00	26.00	35.00	26.00	35.00	26.00
Palm kernel meal	10.00	10.30	10.00	10.30	10.00	10.30	10.00	10.30
Bone meal	3.00	2.00	3.00	2.00	3.00	2.00	3.00	2.00
Oyster shell	1.20	1.00	1.20	1.00	1.20	1.00	1.20	1.00
Premix	0.25	0.15	0.25	0.15	0.25	0.15	0.25	0.15
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Methionine	0.10	0.10	0.10	0.10	0.20	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10	0.20	0.10	0.10	0.10
Cost/kg diet N	53.78	54.77	47.91	47.72	42.02	40.37	36.16	33.62
Calculated composition								
Crude protein %	23.51	20.10	23.91	20.60	24.31	21.00	24.71	21.50
Metabolizable energy (kcal/kg)	2866.00	3000.30	2747.00	2859.90	2627.30	2716.60	25080.00	2573.40
Crude fibre (%)	4.24	4.13	4.25	4.47	4.79	4.80	5.07	5.13
Methionine + Cystine (%)	0.71	0.62	0.73	0.65	0.75	0.60	0.77	0.70
Lysine	1.40	1.18	1.46	1.18	1.48	1.19	1.43	1.20
Calcium	1.46	1.07	1.47	1.08	1.48	1.08	1.48	1.10
Total Phosphorus (%)	0.91	0.73	0.93	0.74	0.94	0.76	0.95	0.80

Table 2: Proximate composition and metabolizable energy value of millet offal types (on dry matter basis)

	Millet offal from the brewery	Millet offal from pap manufacture
Crude protein (%)	14.60	20.65
Crude fibre (%)	4.57	3.12
Ether extract (%)	2.25	3.01
Ash (%)	2.90	3.36
Nitrogen free extract (%)	65.39	59.74
Metabolizable energy (Kcal/kg)	2148.00	2506.00

from pap manufacture. To acclimatize the birds to the cages and feed, a 3-day adaptation period was allowed. After this period, total excreta voided were collected quantitatively for 3 days at 24-hourly intervals. Feed and water were provided *ad libitum* during the period and the feed maintained at low levels in the troughs to avoid spillage. The feed for each group was weighed at the start and end of the collection period to determine feed consumption during the trial period. On each collection day, the excreta was blown free of feathers and other debris before collection. The excreta collected were weighed, labeled and oven dried to a constant weight to determine moisture content. The three-day collection for each group was bulked and finely ground to obtain a homogenous mixture. Samples of the diets and dried excreta as well as millet offal types were assayed for Gross Energy (GE) using an adiabatic bomb calorimeter. The Apparent Metabolizable Energy (AME) of the basal diet and substituted diets were calculated as follows.

$$\text{AME (kcal/kg)} = \frac{\text{GE of feed} - \text{GE of excreta}}{\text{Feed intake}}$$

From the metabolizable energy of the basal and substituted diets, the metabolizable energy of the millet offal types were calculated using algebraic equations.

Live performance and nutrient retention studies: Studies were conducted to determine the effects of replacing maize with millet offal from the brewery in broiler diets on live performance and nutrient retention by broiler chickens. The experiment was conducted in two stages: broiler starter and broiler finisher stages. Four diets were tested during the broiler starter stage. Diet 1 which served as the control diet was formulated to meet the nutrient requirements of broiler starter chicks according to the recommendation of Olomu (1995). In Diets 2, 3 and 4, 25%, 50% and 75% by weight respectively of the maize contained in Diet 1 was replaced with millet offal. No attempt was made to make the diets iso-nitrogenous or iso-carlic in order not to underestimate the value of the ingredient. Thus, the levels of other ingredients remained constant. The compositions of the broiler starter diets are shown in Table 1.

One hundred and twenty Anak broiler chickens obtained at day-old were used for the study. The chicks were brooded during the first 4 weeks. During this period, the chicks were vaccinated according to schedule. Coccidiostat and antibiotics were administered at regularly intervals all through the experimental period to prevent coccidiosis and bacterial infections. The birds were reared on deep litter in a standard tropical poultry building divided into 12 pens each measuring about 2.52m^2.

The chicks were placed on commercial broiler starter mash for one week to stabilize them prior to the

Table 3: Effect of partial replacement of maize with millet offal on performance of broiler starter chicks (1-5 weeks old)

Performance parameter	Diet 1 (Control) 50% Maize 0% Millet offal	Diet 2 37.5% Maize 12.5% Millet Offal	Diet 3 25% Maize 25% Millet Offal	Diet 4 12.5% Maize 37.5% millet offal	SEM
Initial body weight (g/bird)	89.53	89.53	86.75	86.54	2.23
Final body weight (g/bird)	602.02	605.85	605.83	561.72	28.23
Weight gain (g/bird)	512.49	516.02	518.90	463.05	28.84
Feed intake (g/bird)	1312.4^{d}	1403.3^{c}	1520.8^{b}	1626.8^{a}	34.45
Feed to gain ratio	2.57^{c}	2.73^{bc}	2.95^{b}	3.55^{a}	0.134
Feed cost per bird (N)	70.58^{a}	66.22^{ab}	63.92^{b}	58.83^{c}	1.79
Feed cost per kg gain (N)	138.21	130.95	127.40	124.13	5.89
Water intake (ml/bird/day)	117.9^{d}	141.4^{c}	173.6^{b}	190.9^{a}	2.71
Water to feed ratio	2.53	2.82	3.11	3.00	0.095
Water to gain ratio	6.47^{c}	7.73^{c}	9.48^{b}	10.96^{a}	0.157

a,b,c,d implies that means within rows followed by same or no superscripts are not significantly ($p>0.05$) different. SEM, standard error of means

commencement of the study. At 1 week of age, the chicks were weighed and randomly allotted to 12 similar groups of 15 birds per group on equi-weight basis. Each group constituted a replicate. Three replicates were allocated to each dietary treatment in a randomized complete block design. Throughout the experiment, feed and water were provided *ad libitum*. The birds were observed daily and a recorded of mortality was kept. Weight gain and feed intake per bird were determined at weekly intervals and feed to gain ratio was computed accordingly. Average daily water intake per bird was also determined for each week. The starter stage lasted from 1-5 weeks of age.

At 5 weeks of age, 3 birds were randomly selected from each group and transferred to metabolism cages to determine nutrient retention. Thus, there were 3 replicates of 3 birds each per treatment. The management and feeding of the birds were as described under metabolizable energy above. Excreta collection and handling were the same as in metabolizable energy study also. The dried faecal samples for each group over the 3 days period were bulked and finely ground to obtained a homogenous mixture. Representative samples of feed and excreta were analyzed for proximate composition using the procedure of A.O.A.C. (1990). From the proximate composition of the feed and excreta, percentage nutrient retentions were determined. At the end of the broiler starter stage, all the birds used for the trial were fed the control diet from 5 to 6 weeks of age.

The broiler finisher stage lasted from 6 to 9 weeks of age. At 6 weeks of age, all the birds were mixed up and randomly divided into 12 similar groups in terms of starting weight. Each group constituted a replicate. Three replicates were assigned to each treatment diet in a randomized complete block design. Four diets were tested as with the broiler starter trial. The replacement regimen was the same as described for the broiler starter diets (Table 1). The parameters studied and methods of data collection were similar to those described for the starter stage.

Data obtained were subjected to analysis of variance in a randomized complete block design using the method described by Steel and Torrie (1980). Duncan multiple range test was used to determine significant differences among means as recommended by Alika (2006).

Results and Discussion

The results of the proximate analyses and metabolizable energy study are presented in Table 2. The results showed that millet offal from local manufacture of pap had higher amount of crude protein (20.65%) than that from the brewing industry (14.60%). Crude fibre contents were 4.57 and 3.12% for millet offal obtained from the brewing industry and pap manufacture respectively. Percentage ether extract and ash were higher with millet offal from pap than with the one from the brewery. Millet offal from the brewery gave a higher value of nitrogen free extract than that obtained from pap manufacture. The results of the metabolizable energy studies (Table 2) showed that millet offal from pap manufacture gave higher apparent metabolizable energy value (2506.0 kcal/kg) than that obtained from the brewing industry which gave a value of 2148.0 kcal/kg.

The results of the trial with broiler starter chicks are presented in Table 3. The results indicated that final body weight and body weight gain were not significantly ($p>0.05$) affected by diet. However, there was a slight depression in final body weight and weight gain on Diet 4, with 75% replacement of maize with millet offal. Feed intake per bird increased significantly ($p<0.05$) with increasing level of millet offal in the diet. There was also increase in feed to gain ratio with increasing levels of millet offal in the diet, significantly so when millet offal replaced 50% or 75% of the maize in the diet. Feed cost per bird decreased with increasing level of millet offal in the diet at the expense of maize. However, the decrease in feed cost per bird on with 25% replacement of maize with millet offal was not significant. Feed cost per kilogram live weight gain was lower on the millet offal diet, but significantly so. Average daily water intake per bird increased significantly as the level of millet offal in the diet increased.

Table 4: Effect of partial replacement of maize with millet offal on performance of broiler finisher chickens (6-9 weeks of age)

Performance parameter	Diet 1 (Control) 100% Maize 0% Millet offal	Diet 2 75% Maize 25% Millet Offal	Diet 3 50% Maize 50% Millet Offal	Diet 4 25% Maize 75% millet offal	SEM
Initial body weight (g/bird)	703.33	701.00	713.33	706.67	6.04
Final body weight (g/bird)	2283.7^{a}	2192.2^{ab}	2145.9^{a}	1904.5^{c}	39.35
Weight gain (g/bird)	1573.6^{a}	1465.6^{b}	1432.6^{b}	1197.8^{c}	40.31
Feed intake (g/bird)	3157.0^{b}	3675.9^{a}	3892.4^{a}	4034.4^{a}	190.08
Feed to gain ratio	2.00^{b}	2.48^{b}	2.72^{b}	3.37^{a}	0.17
Feed cost per bird (N)	172.91^{a}	175.41^{a}	157.12^{a}	135.63^{b}	8.72
Feed cost per kg gain (N)	109.54	118.53	109.80	113.41	5.06
Water intake (ml/bird/day)	460.43^{d}	619.25^{c}	666.44^{b}	747.50^{a}	15.15
Water to feed ratio	3.06^{b}	3.56^{a}	3.70^{a}	3.89^{a}	0.19
Water to gain ratio	6.13^{s}	8.87^{c}	9.77^{b}	13.13	0.36

a,b,c,d implies that means within rows followed by same or no superscripts are not significantly ($p>0.05$) different. SEM, standard error of means

Table 5: Effect of partial replacement of maize with millet offal on percentage nutrient retention by broiler chickens

Response Criteria	Diets 1	2	3	4	SEM
Dry matter	75.87^{c}	76.71^{c}	79.11^{b}	81.63^{a}	0.80
Crude protein	72.41^{b}	72.16^{b}	73.3^{b}	77.31^{a}	1.39
Crude fibre	43.22^{c}	44.81^{c}	61.15^{b}	73.47^{a}	1.51
Fat	70.08	69.86	70.34	69.66	1.55
Ash	64.13^{b}	60.69^{b}	64.22^{b}	68.84^{a}	1.82
Nitrogen free extract	80.86^{d}	83.41^{c}	86.35^{b}	88.15^{a}	0.56

a,b,c,d implies that means within rows followed by same or no superscripts are not significantly ($p>0.05$) different. SEM, standard error of means, Diet 1, 2, 3 and 4 are respectively Diets 1, 2, 3 and 4 shown in Table 3

The results of the trial with broiler finisher chickens are presented in Table 4. The results showed that body weight gain was significantly lower on the millet offal diets. The birds fed Diets 2 and 3 (with 25 and 50% levels of replacement) gave almost similar values of weight gain which were significantly higher than that of Diet 4 (with 75% level of replacement). Feed intake values were significantly higher on the millet offal diets than on the control diet. Feed intake values were not significantly different among the millet offal diets (Diets 2, 3 and 4). Feed to gain ratio increased as the level of millet offal in the diet increased. However, the increase only became significant at 75% level of replacement. Feed cost per bird was lower on Diets 3 and 4 as compared to the control but only significantly so on Diet 4 with 75% level of replacement. Feed cost per kilogram gain was not significantly affected by diets. Water intake significantly increased with increasing levels of millet offal in the diet.

The results of the nutrient retention study are presented in Table 5. The result showed that percentage dry matter retention significantly decreased as the level of maize in the diet decreased. Percentage crude protein retention was almost similar for the control diet and Diets 2 and 3 with 25% and 50% replacement respectively of maize with millet offal. Diet 4 with 75% replacement level gave a significantly higher crude protein retention as compared to the other diets. Crude fibre retention increased with increasing level of millet offal in the diet. However, the increase was not significant at 25% level of replacement. Percentage fat retention was almost similar for all the diets. Ash retention was significantly higher on Diet 4 with 75% replacement of maize with millet offal compared to other diets which gave almost similar values. Nitrogen free extract retention increased linearly as the level of millet offal in the diet increased. ($p<0.05$) From the results of the proximate chemical analysis, millet offal from pap manufacture gave higher crude protein value than millet offal from the brewery. The difference in percentage crude protein may be related to difference in processing method adopted. The higher degree of extraction resulting from mechanical processing and the level of heat treatment of the raw material in the brewery may have affected the crude protein level of the material. Only slight differences existed between the two millet offal types in terms of crude fibre, ether extract and ash contents suggesting that processing method may not have had any effect on the nutrients.

The metabolizable energy of the millet offal from pap production was higher than that from the brewery. The reason for this is not immediately apparent. It is likely that in the brewery, more of the energy yielding materials is extracted than in the local manufacture of pap since the process in the brewery is mechanical. It is however, pertinent to note that the metabolizable energy value of the millet by-products are much higher than those of other by-products like wheat offal, maize gluten feed and brewers' dried grains with metabolizable energy values of 1145 kcal/kg, 1700 kcal/kg and 1900 kcal/kg respectively (Olomu, 1995). The metabolizable energy values of 2148 and 2506 kcal/kg for millet offals from beer and pap manufacture respectively place the millet offals in the category of medium energy ingredients. The metabolizable energy value of millet offal from pap manufacture (2506 kcal/kg) compares very well with the value of 2500 kcal/kg reported for palm kernel cake

($p<0.05$) (Olomu, 1995). This clearly implies that millet offal may be effective if used as an energy source in poultry diets.

The comparable performance of the birds on millet offal diets inspite of the lower metabolizable energy levels of the diets compared to that of the control diet may be related to the increase in feed intake by the birds as the dietary millet offal increased. The increase in feed intake may be due to the attempt by the birds to eat to meet their energy requirement (Olomu, 1995). The increased feed intake by the birds is associated with increased protein intake at a time when protein requirement was critical. The increased feed intake could also be an attempt by the birds to satisfy energy needs as fibre serves as an energy diluent (Thompson and Weber, 1981; Uko *et al*., 1991). The increase in feed to gain ratio observed on the millet offal diets can be attributed to the increase in feed intake on the diets since weight gain was apparently similar for all the groups. The decrease in feed cost per bird as millet offal in the diet increased is due to the lower market price of the millet as compared to that of maize. The linear increase in average daily water intakes of the birds with increasing levels of millet offal in the diet may be related to the increase in feed intake of the birds which was accompanied with increase in dietary crude fibre. This observation in agreement with the findings of Ezieshi and Olomu (2003; 2004). It has been reported that more water is needed to soften the fibrous tissues in the gastro-intestinal tract preparatory to digestion (Neumann, 1977). The depression in body weight at the highest level (75%) of replacement may be related to the low M.E. level of the diet coupled with the high crude fibre intake. Results of the retention study showed that the inclusion of millet offal in the diet did not adversely affect nutrient retention. The range of values for nutrient retention is in agreement with the findings of Ezieshi and Olomu (2003; 2004).

Conclusion: Information on proximate chemical composition and metabolizable energy values of the two types of millet by-products presented here should form important reference data for feed millers, researchers and farmers who may wish to use millet offals as feed stuffs in poultry diets. From the result of this study, millet by-products can be classified as medium energy and protein sources in poultry diets. Millet offal can replace up to 50% maize in the diet of broiler chickens without any significant effect on performance of the birds and at reduced cost of feed production.

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Accuracy Analysis of the Food Intake Variety Questionnaire (FIVeQ). Reproducibility Assessment among Older People

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Abstract: The study included 131 people aged 65 + (62 men and 69 women). Food intake variety questionnaire (FIVeQ) included questions about eating (yes/no) during last 7 days the named amounts of 65 subgroups of products. Interviews were made using the face-to-face situation, twice with a two-week interval (test and retest). For the further analysis products were aggregated into 9 main groups: cereal products, dairy products, meat products, vegetables, fruit, fats, sugar and sweets, beverages, spices. The questionnaires' accuracy measures were sensitivity index (%) and specificity index (%) and the test power. High reproducibility of the results obtained by the FIVeQ questionnaire was stated. It shows a good accuracy of the questionnaire as a tool for studying food intake variety and allows to recommend its usage among older people.

Key words: Food intake variety questionnaire, reproducibility, accuracy, test power

Introduction

Questionnaires for analyzing food intake are widely used in epidemiologic studies. They are measure tools that usually are elaborated for a specific project. Before applying a questionnaire in scientific studies it is necessary to check earlier its quality in a pilot study and examine its validity using the referential method in order to determine its accuracy and reliability (Jedrychowski, 1982). A good standard of proceeding is to check a new food questionnaire in a pilot study on the same or very similar sample to the one being in the question of the study, correct the questionnaire and retest it (Jedrychowski, 1982; Pisani *et al*., 1997). Next the questionnaire should be validated using the referential method (evaluation of the method's reliability), for example nutritional status evaluation method (validation) (Bathalon *et al*., 2000; Bohlscheid-Thomas *et al*., 1997; Jedrychowski, 1982; Johansson *et al*., 2002; Katsouyanni *et al*., 1997; Kroke *et al*., 1999; Lee *et al*., 2002; Ocke *et al*., 1997; Pisani *et al*., 1997; Shu *et al*., 2004; Yee *et al*., 2001) and/or food intake evaluation method (calibration) (Jedrychowski, 1982; Yee *et al*., 2001) and/or by repeating nutritional interview (results reproducibility evaluation) (Bohlscheid-Thomas *et al*., 1997; Boucher *et al*., 2006; Frankenfeld *et al*., 2003; Hu *et al*., 1999; Jedrychowski, 1982; Katsouyanni *et al*., 1997; Ozsoy *et al*., 2007; Wang *et al*., 2007). The obtained results referring to the pilot study, precise construction of the questionnaire and its modification and the applied validation method are popularized. In Poland only a few food questionnaires are checked and validated and results of such practices published (Czarnocinska and Wadolowska, 2006; Gawecki *et al*., 2002; Ilow *et al*., 2005; Szymelfejnik *et al*., 2006; Wadolowska, 2005). It creates a risk that the results of studies carried out using a not validated questionnaire have a unknown precision and may be questioned (Czarnocinska and Wadolowska, 2006; Wadolowska, 2005). That makes a food questionnaire's validation very important, especially that, as every research method, it should have a high reproducibility of results and be reliable, that is have a known validity and accuracy (Jedrychowski, 1982).

Test's validity describes the ability of the questionnaire to measure a precise feature in such a way that the value obtained in the measurement was correspondent with the real value (Jedrychowski, 1982). Validity measures are i.a. correlation coefficient and sensitivity and specificity indices. Reproducibility describes a degree in which the questionnaire applied to the same person, used by the same or other researcher, gives the same results (Jedrychowski, 1982).

Diet diversifying is an important feature of nutrition that favours an adequate intake many different nutrients and their proper balancing with the body needs. It is the dietary recommendation that appears the most often in proper nutrition rules and national dietary prevention recommendations in different countries and WHO (2003). The recommended diet diversification is particularly forwarded to older people (Roszkowski *et al*., 1995; Roszkowski, 2003). Taking into consideration proceeding of the aging processes, connected to changes in the alimentary canal, decrease in muscles vigour, inner organs working and physical activity, seniors have bigger problems in fulfilling body needs than younger people (Roszkowski, 2003). Often there are even more problems. For example because of lacks in dentition seniors avoid eating some products, especially hard and malleable foods. Those problems may deepen because of excessive usage of diuretic,

purging or neutralizing medicines or antibiotics (Roszkowski, 2003). A separate issue is the economic limitations of older people's households and many problems connected to foods, i.a. shopping and/or preparing dishes (Niedzwiedzka *et al.*, 2004; 2005a; 2005b). These and other factors significantly influence older people's nutrition manner and diversification of their daily diets and, as follows, diet-related diseases occurrence. Thus studying diet diversification is a frequent subject of studies on correlations between nutrition and diet-related diseases occurrence risk (Drewnowski and Specter, 2004; Elmadfa and Freisling, 2005; Gerhard *et al.*, 2004; Ledikwe *et al.*, 2004; Lichtenstein *et al.*, 2006; Matthiessen *et al.*, 2003; McCrory *et al.*, 1999; Norat and Riboli, 2003; Psaltopoulou *et al.*, 2004; Roberts *et al.*, 2005).

The aim of the work was the analysis of the validity of the created half-quantitive questionnaire of food intake diversification with acronym FIVeQ and testing its reproducibility among older people.

Materials and Methods

The study included 131 people (62 men and 69 women) aged above 65 years from people stating a so called basic sample. The basic sample was chosen by the quota method, granting that the total sample size amounts to 400 people, 50 persons each of 8 subgroups. The selection criteria of the basic sample were: sex, age (65-74 years old and 75+ years old) and family status (living alone and with other people). The basic study was carried out in five chosen provinces: Warminsko-Mazurskie, Slaskie, Mazowieckie, Podkarpackie and Wielkopolskie. During the recruitment we tried to reach older people with different education level, incomes, place of living etc., so as to have the studied sample similar to national differentiation. Finally the basic studies included 422 people. In the calibration study all people included in the basic studies and living in two provinces, Warminsko-Mazurskie and Slaskie (131 people) took part in the calibration study. Choosing sample for the basic study was realized according to the criteria of the project with the acronym SENIOR FOOD QOL (www.foodinlaterlife.org) and the calibration study was carried out within the status studies of the Department of Human Nutrition UWM in Olsztyn and the obtained financial aid from European Social Fund and national budged within the Integrated Operational Regional Development Program 2004-2006.

The created questionnaire of food intake variety with acronym FIVeQ (Food Intake Variety Questionnaire) consisted of two parts. The first part included questions about sex, age, place of living, total monthly income of the household and the average amount of money spent on food and beverages during a week. Second, the essential part of the FIVeQ questionnaire, concerned intake frequency (yes/no) during last 7 days of 65 subgroups of food products, being yet after thermal treatment (in the form "ready to eat"), in the amount usually bigger than 2 table spoons or 7 bread slices or 7 glasses (Appendix A). In this way information was gathered if a product was consumed in the amount bigger than insignificant. The division of products into 65 subgroups was created on the basis of their content and nutritive value (Kunachowicz *et al.*, 1998), our experience (Czarnocinska and Wadolowska, 2006; Szymelfejnik *et al.*, 2006; Wadolowska, 2005) and other authors results (Hu *et al.*, 1999; Lee *et al.*, 2002; Pisani *et al.*, 1997; Shu *et al.*, 2004) and erspecially studies of Horwath *et al.* (1999).

Before the basic research in the Warmia area, pilot studies were carried out twice with older people meeting the agreed criteria of the sample choosing (age, sex, family status). In the pilot study it was examined if the questions included in the FIVeQ questionnaire are understandable and properly formulated. After finishing the pilot studies the basic studies were carried out twice with a two-week break (test and retest) in the time from August till September 2005. The pilot and basic studies were carried out face-to-face. Public Opinion Research Center in Warsaw (CBOS) was responsible for collecting data. The CBOS pollsters were instructed by a person responsible for the study and all questions concerning the questionnaire were in detail described and explained. Each pollster, before starting an interview with a respondent, in detail described the aim of the study and ways of answering. Both studies (test and retest) among all subjects were carried by the same pollster.

For further analysis 65 subgroups were aggregated in 9 main groups: cereal products, dairy products, meat products, vegetables, fruit, fats, sugar and sweets, beverages, spices (Appendix A). During aggregating products we mainly took their content, origin and nutritive value into consideration (Kunachowicz *et al.*, 1998) and also features facilitating further separating of nutrition habit (Hu *et al.*, 1999).

The measures of questionnaire validity were indices of sensitivity (%) and specificity (%) (Jedrychowski, 1982) and the test power (in the range from 0 to 1) (Hall, 1983; Nelson *et al.*, 2004). Questionnaire's sensitivity describes its ability to proper classifying people answering yes-yes in both studies (test-retest) and determined the percentage of people (%) consuming specific groups and products. Questionnaire's specificity is its ability to reveal people answering no-no in both studies (test-retest) and describes the percentage of people (%) not consuming the mentioned groups of products. The test power was determined as a probability (in the range from 0 to 1) of taking the right decision connected to rejecting a false hypothesis and defining it as 1-β, where β is the probability of making a type II error. Accepting a true diagnostic hypothesis and

Appendix A. Applied products grouping

Food groups	Food subgroups	Food items	Amount of food items consumed during the last 7 days
1.Cereal products	Wholemeal cereal products	Wheat, rye, wholemeal bread, bread with grains, pumpernickel, grahams	7 slices of whole bread or roll
	Refined cereal products	White wheat bread, rye, wheat-rye, toast bread, normal rolls, butter rolls and bagels, French bagels, raised rolls	7 slices of whole bread or roll
	Large grains groats	Buckwheat groats, peeled barley, brown rice	2 table spoons
	Small grains groats	Manna, crushed barley	2 table spoons
	Ready-to-eat breakfast cereal products	Not cooked milk supplement, for example muesli, corn flakes	2 table spoons
	Potatoes, potato pancakes, French fries	Potatoes, potato pancakes, French fries	2 table spoons
2. Fruit	Stone	Apricots, avocado, cherries, nectarines, peaches, plums, wine grapes etc.	2 table spoons
	Kiwi and citrus	Kiwi, oranges, mandarins, citrons, grapefruit	2 table spoons
	Tropical fruit	Pineapples, melons, dactyls etc.	2 table spoons
	Berry fruit	Raspberries, blackberries, blueberries, strawberries, etc.	2 table spoons
	Bananas	Bananas	2 table spoons
	Apples and pears	Apples, pears	2 table spoons
	Dried fruit	Raisins, apricots, figs, apples, plums, etc..	2 table spoons
	Sweet fruit preserves	Stewed fruit, jam, plum jam, candied fruit, dactyls	2 table spoons
3.Vegetables	Flowers	Broccoli, brussels sprouts, fresh cabbage, cauliflower	2 table spoons
	Yellow-orange vegetables	Carrot, paprika	2 table spoons
	Leafy green vegetables	Different kinds of lettuce, pores, celery, spinach	2 table spoons
	Tomatoes	Tomatoes	2 table spoons
	Root and other vegetables	Red beets, parsnip, onion, garlic, celeriac, radish, turnip, mixed vegetables	2 table spoons
	Marrows	Fresh cucumber, eggplant, marrow, pumpkin, zucchini	2 table spoons
	Fresh and canned beans	Corn, green peas, French bean, green bean	2 table spoons
	Dry beans	Bean, lupine, pea, lentil, broad bean, soy	2 table spoons
	Sauerkraut and cucumber	Sauerkraut and cucumber	2 table spoons
	All other fermented products	Fermented rye soup, fermented beetroot soup etc.	2 table spoons
	All kinds of mushrooms	Champignons, dried, marinated and fried mushrooms etc.	2 table spoons
	Almonds, hazelnuts etc.	Almond, chestnut, cashew, coconut, hazelnut, pistachio, walnut, peanut, peanut butter, chocolate-nut cream	2 table spoons
	Pumpkin seed, sesame seed, sunflower seed	Pumpkin seed, sesame seed, sunflower seed	2 table spoons
	Olives	Olives	2 table spoons
4.Dairy products	Milk and dairy products	Milk, milk soup, milk drinks, yoghurt, kefir, buttermilk	7 glasses
	Cottage cheese	Different cottage cheese, natural and flavoured cheese, mozzarella	2 table spoons
	Cheese	Hard and processed cheese, spread cheese	2 table spoons
	Ice-creams and pudding	Ice-creams and pudding	2 table spoons
5.Meat products	Sausages	Different kinds of sausages, mingles meat sausages, frank-furters	Amount for 1 slice of bread, well covered
	Good quality cold meats	Poultry and pork-beef good quality cold meats	Amount for 1 slice of bread, well covered
	Organ meat and cold meat products	Liver, black pudding, brains, brawn, meat pies, bacon	2 table spoons
	Red meat	Pork, beef, veal, beef cold meat, for example beef ham	2 table spoons
	Poultry	Poultry meat from hens, chickens, ducks, turkey	2 table spoons
	Game	Ouail, wild duck, rabbit	2 table spoons
	Lean fish	Pollock, cod, hake, carp to 1 kg etc.	2 table spoons
	Fatty fish	Tuna, salmon, sardines, herring, mackerel, smoked herring, big carp etc.	2 table spoons
	Mussels and oysters	Mussels, squids, oysters etc.	2 table spoons
	Shellfish	Lobsters, crabs, shrimps etc.	2 table spoons
	Hard roe	Caviar	2 table spoons
	All kinds of eggs	From hens, ducks, quails etc.	2 items
6.Fats	Oil	Oil	2 table spoons
	Butter	Butter	2 table spoons
	Margarine in cubes or cups	Margarine in cubes or cups (for spreading)	2 table spoons
	Cream	Cream	2 table spoons
	Other animal fats	Other animal fats, for example lard, fat	2 table spoons
	Mayonnaise and dressings	Mayonnaise and dressings (salad sauces)	2 table spoons
7.Sugar and sweets	Sugar, honey, fruit candies, hard caramels	Sugar, honey, fruit candies, hard caramels	2 table spoons
	Biscuits, cakes with cream, short,	Biscuits, cakes with cream, short, semi-short cakes,	2 table spoons

Appendix (A) continued

	semi-short cakes etc.	fruit, yeast, cheese cakes, donuts, poppy-seed cake	
	Different kinds of chocolate and chocolate sweets	Different kinds of chocolate and chocolate sweets	10 cubes of chocolate
	All kinds of salty snacks	Chips, salty crunchies, crackers, fingers etc.	2 table spoons
8.Beverages	Non-alcohol	Fizzy drinks such as coca-cola, fruit fizzy drinks, herbs and fruit infusions, fruit and herbs "teas"	7 glasses
	Tea	Black, green, red	7 glasses
	Coffee	Coffee	7 glasses
	Beer	Beer	7 glasses
	Wine	Wine, drinks	1 "wine" glass (100 ml)
	Vodka	Vodka and other strong alcohols	1 "vodka" glass (50 ml)
	Fruit juices	Apple, orange, grapefruit, currant, multifruit and other juices	7 glasses
	Vegetable and vegetable-fruit juices	Tomato, carrot and other juices	7 glasses
	Water	Water	7 glasses
9.Spices	Herbs and spices	Herbs and spices	2 table spoons
	Soya sauce	Soya sauce	2 table spoons

Table 1: Comparison of the test power values in sex and age groups

	In total N = 131		Men N = 62		Women N = 69		65-74 years old N = 68		75+ years old N = 63	
Food groups	x	95% CI	x	95% CI	x	95% CI	x	95% CI	x	95% CI
1.Cereal products	0.48	0.17 ÷ 0.79	0.45	0.08 ÷ 0.82	0.53	0.25 ÷ 0.81	0.58	0.30 ÷ 0.86	0.39	0.05 ÷ 0.74
2.Fruit	0.70	0.50 ÷ 0.90	0.68	0.43 ÷ 0.93	0.70	0.51 ÷ 0.89	0.72	0.53 ÷ 0.91	0.69	0.48 ÷ 0.89
3.Vegetables	0.70	0.58 ÷ 0.83	0.64	0.47 ÷ 0.81	0.75	0.64 ÷ 0.86	0.73	0.59 ÷ 0.87	0.68	0.55 ÷ 0.81
4.Dairy products	0.67	0.44 ÷ 0.89	0.70	0.58 ÷ 0.82	0.62	0.26 ÷ 0.97	0.66	0.42 ÷ 0.91	0.67	0.43 ÷ 0.90
5.Meat products	0.73	0.58 ÷ 0.87	0.68	0.51 ÷ 0.86	0.75	0.61 ÷ 0.90	0.76	0.63 ÷ 0.89	0.68	0.49 ÷ 0.86
6.Fats	0.64	0.46 ÷ 0.82	0.59	0.36 ÷ 0.82	0.71	0.57 ÷ 0.84	0.67	0.50 ÷ 0.84	0.61	0.38 ÷ 0.84
7.Sugar and sweets	0.61	0.35 ÷ 0.86	0.63	0.37 ÷ 0.89	0.58	0.33 ÷ 0.82	0.69	0.46 ÷ 0.91	0.52	0.15 ÷ 0.89
8.Beverages	0.73	0.54 ÷ 0.91	0.67	0.42 ÷ 0.92	0.74	0.57 ÷ 0.92	0.70	0.48 ÷ 0.93	0.75	0.60 ÷ 0.90
9.Spices	0.66	-3.47 ÷ 4.78	0.66	-3.47 ÷ 4.78	0.66	-3.47 ÷ 4.78	0.71	-2.79 ÷ 4.20	0.64	-3.75 ÷ 5.02

x-mean value; 95% CI-95% confidence interval

rejecting a false hypothesis is connected to taking a right decision based on an interview. However there was chance of making a type I error (α), that is the incorrect rejection of a true null hypothesis and there is the chance of making a type II error (β), that is the incorrect acceptance of a false null hypothesis. The greater the power in a study, the less likelihood of making a type II error (Jedrychowski, 1982; Nelson *et al.*, 2004).

Indices and the test power were determined for all 65 subgroups of products (Appendix B) and then for 9 main groups the mean values and 95% confidence interval were calculated (95% CI; Table 1-3). The statistical analysis was held using the computer programme STATISTICA PL v.7.1.

Results

The mean test power for the total sample amounted from 0.48 for cereal products to 0.73 for meat products and beverages (Table 1). The lowest mean values of the test power were stated for cereal products both for sex and age groups (from 0.45 to 0.58).

The mean sensitivity index amounted for the total sample from 55.3% for spices to 77.9% for fats, while the mean value of the specificity index from 47.8% for cereal products to 72.7% for meat products (Table 2-3). The lowest mean values of the specificity index in sex and age groups were for cereal products (from 39.4% to 57.8%) and the sensitivity index for spices (from 43.5% to 59.2%), excluding people aged 75+ years.

In the group analysis the low values of the test power of the cereal products (0.48) were influenced by the low test power value for small grains groats (0.19) and potatoes and/or potato pancakes and/or French fries (0.20; Table 1, Appendix B). At the same time in the cereal products group the highest test power was stated for ready-to-eat breakfast cereal products (0.88) and large grains groats (0.79). High mean test power for meat products (0.73) in the total sample was influenced by high test power for mussels and/or molluscs (1.00), shellfish (1.00), hard roe (0.99) and game (0.99), while in beverages group high value for beer (0.96), vodka (0.95) and wine (0.93). At the same time in the meat products group the lowest test power values were stated for poultry (0.46) and all kinds of eggs (0.47), while in beverages group for tea (0.29) and water (0.44).

In the group analysis low values of the sensitivity index for spices (55.3%) depended on low value of that index for Soya sauce (25.0%; Table 2, Appendix B). In the spice group high index was obtained for herbs and spices (85.5%). High mean sensitivity index in the total sample for fats (77.9%) was influenced by high values of that index for oil (94.4%) and butter (93.7%). At the same time in fats group the lowest sensitivity was stated for mayonnaise and/or dressings (57.1%).

In the group analysis low mean specificity index values for cereal products (47.8%), similarly like for the test power, were influenced by low values of that index for small grains groats (18.9%) and for potatoes and/or

Table 2: Comparison of the sensitivity index values (%) in sex and age groups

	In total N = 131		Men N = 62		Women N = 69		65-74 years old N = 68		75+ years old N = 63	
Food groups	x	95% CI	x	95% CI	x	95% CI	x	95% CI	x	95% CI
Cereal products	73.3	51.2÷95.3	78.0	62.2÷93.9	69.5	41.6÷97.5	69.2	41.3÷97.1	77.7	58.7÷96.7
Fruit	61.0	44.8÷77.1	64.9	50.0÷79.7	60.1	40.5÷79.8	52.2	26.7÷77.7	68.5	56.4÷80.7
Vegetables	63.6	49.1÷78.2	68.6	56.1÷81.0	59.4	41.4÷77.4	58.2	40.4÷76.1	70.4	58.8÷81.9
Dairy products	69.1	36.7÷101.5	63.4	25.2÷101.5	73.0	40.9÷105.0	68.1	37.4÷98.7	70.0	35.1÷105.0
Meat products	75.7	63.3÷88.1	76.8	65.6÷87.9	74.1	59.6÷88.7	75.5	63.5÷87.4	75.8	61.0÷90.6
Fats	77.9	61.1÷94.8	76.1	59.7÷92.5	79.3	61.7÷97.0	77.1	56.3÷97.8	79.2	65.8÷92.7
Sugar and sweets	68.8	30.9÷106.6	68.9	39.4÷98.3	69.3	25.2÷113.4	65.6	25.3÷106.0	72.0	30.6÷113.3
Beverages	65.5	49.2÷81.8	62.4	46.3÷78.6	72.6	52.9÷ 92.3	73.1	59.6÷86.6	58.4	37.0÷79.8
Spices	55.3	-329.1÷439.6	59.2	-269.9÷388.2	52.9	-364.6÷ 470.3	43.5	-508.6÷595.5	61.9	-215.8÷339.5

x-mean value; 95% CI-95% confidence interval

Table 3: Comparison of the specificity index values (%) in sex and age groups

	In total N = 131		Men N = 62		Women N = 69		65-74 years old N = 68		75+ years old N = 63	
Food groups	x	95% CI	x	95% CI	x	95% CI	x	95% CI	x	95% CI
Cereal products	47.8	16.4÷79.1	44.7	7.6÷81.9	53.0	24.6÷81.4	57.8	29.9÷85.8	39.4	4.9÷73.9
Fruit	70.1	50.4÷89.7	68.5	43.3÷93.6	70.0	51.3÷88.7	71.7	52.7÷90.6	68.4	47.8÷89.0
Vegetables	70.2	58.0÷82.5	64.2	47.5÷81.0	73.1	61.5÷84.6	70.7	56.5÷84.9	68.0	54.5÷81.4
Dairy products	66.6	44.4÷88.7	69.9	57.2÷82.5	61.5	25.8÷97.1	66.3	42.2÷90.4	66.6	43.4÷89.7
Meat products	72.7	58.3÷87.2	57.9	39.8÷76.1	67.3	52.3÷82.3	64.0	53.3÷74.7	61.2	41.3÷81.2
Fats	63.9	46.3÷81.4	58.6	935.6÷81.7	70.4	57.0÷83.8	67.1	50.3÷83.8	61.1	38.4÷83.7
Sugar and sweets	60.5	34.8÷86.1	63.2	36.9÷89.5	57.8	32.7÷82.9	68.6	45.9÷91.3	52.3	15.2÷89.3
Beverages	72.5	54.0÷91.1	67.0	41.7÷92.3	71.4	52.4÷90.3	70.2	48.1÷92.4	75.4	60.2÷90.6
Spices	65.9	-347.7÷479.4	65.8	-347.2÷478.8	65.9	-347.7÷479.4	70.7	-282.5÷423.9	63.5	-379.4÷506.3

x-mean value; 95% CI-95% confidence interval

potato pancakes and/or French fries (20.0%; Table 3, Appendix B). In the group of cereal products the highest values of that index were obtained for ready-to-eat breakfast cereal products (87.9%) and large grains groats (79.3%). High mean specificity for meat products (72.7%), similarly like in the case of the test power, was influenced by high values of that index for mussels and/or molluscs (100.0%), shellfish (100.0%), hard roe (99.2%) and game (99.2%). At the same time in that group of products the lowest specificity was stated for poultry (46.2%) and all kinds of eggs (46.7%).

For groups of products with the highest values of the test power, sensitivity and specificity indices, a narrow range of the confidence interval was stated (95% CI). In the group analysis the highest test power values and as well a narrow range of confidence interval were stated for meat products (0.73; 95% CI = 0.58÷0.87) and beverages (0.73; 95% CI = 0.54÷0.91; Table 1). Narrow range of the confidence interval and high value of the sensitivity index was stated for fats (77.9%; 95% CI = 61.1÷94.8%; Table 2) and narrow range of the confidence interval and high value of the specificity index for meat products (72.7%; 95% CI = 58.3÷87.2%; Table 3).

For the spices group a very wide range of the confidence interval was stated (95% CI). For example in the total sample 95% confidence interval for the test power amounted from -3.47 to 4.78, for the sensitivity index from -329.1% to 439.6%, while for the specificity index from -347.7% to 479.4% (Table 1-3).

Discussion

The created FIVeQ questionnaire turned out to be a good measure tool to determining groups of products both consumed and not consumed by older people. On the basis of the high values of the specificity index ($\geq$ 80%) we showed products subgroups that were not consumed by the respondents or consumed very rarely (>once a week). They included: ready-to-eat breakfast cereal products; tropical fruit, dried fruit, bananas, berries; olives, almonds and/or hazelnuts etc., pumpkin and/or sesame and/or sunflower seeds, all kinds of mushrooms, dry beans; ice-creams and/or pudding; mussels and/or molluscs, shellfish, hard roe, game, fatty fish; all kinds of salty snacks; beer, vodka, wine, vegetable juices and/or vegetable-fruit juices and Soya sauce. One of the reasons for such a low intake of the mentioned products could be their quite high price and/or small knowledge of (Gronowska-Segner, 2002; Gutkowska, 2002; Niedzwiedzka *et al.*, 2005a,b). At the same time the highest values of the sensitivity index ($\geq$ 80%) in the group analysis enabled for showing subgroups of products often consumed by seniors, stating the base of their diets. That were: potatoes and/or potato pancakes and/or French fries, refined cereal products, small grains groats; apples and/or pears; tomatoes, root vegetables and/or other vegetables, yellow-orange vegetables, flowers; milk and/or dairy products, cottage cheese; poultry, all kinds of eggs, good quality cold meats, sausages; oil, butter, cream; sugar and/or honey and/or fruit candies and/or

Appendix B. Detailed comparison of the test power, the specificity (%) and the sensitivity index values (%) in age and sex groups

		Test power					Sensitivity index (%)					Specificity index (%)				
Food groups	Food subgroups	O	M	K	65-74	75+	O	M	K	65-74	75+	O	M	K	65-74	75+
1.Cereal products	Wholemeal cereal products	0.49	0.62	0.35	0.61	0.33	77.8	85.4	71.4	84.4	71.1	48.8	61.9	35.0	60.9	33.3
	Refined cereal products	0.32	0.23	0.44	0.31	0.33	85.3	79.6	90.0	83.6	87.0	31.8	23.1	44.4	30.8	33.3
	Large grains groats	0.79	0.78	0.80	0.84	0.74	49.0	66.7	35.7	29.2	68.0	79.3	78.0	80.5	84.1	73.7
	Small grains groats	0.19	0.19	0.19	0.29	0.13	84.0	85.4	83.0	79.6	90.0	18.9	19.0	18.8	28.6	13.0
	Ready-to-eat breakfast cereal products	0.88	0.86	0.89	0.93	0.83	45.8	54.5	38.5	42.9	50.0	87.9	86.3	89.3	92.6	83.0
	Potatoes, potato pancakes,French fries	0.20	0.00	0.50	0.50	0.00	97.6	96.6	98.5	95.5	100.0	20.0	0.0	50.0	50.0	0.0
2.Fruit	Stone	0.59	0.55	0.63	0.54	0.64	72.9	70.0	75.6	75.0	70.7	58.7	54.5	62.5	54.2	63.6
	Kiwi and citrus	0.74	0.72	0.75	0.76	0.71	67.8	65.4	69.7	66.7	69.0	73.6	72.2	75.0	76.3	70.6
	Tropical fruit	0.94	0.93	0.95	0.97	0.92	33.3	50.0	25.0	0.0	66.7	94.4	93.3	95.4	96.9	91.7
	Berry fruit	0.80	0.80	0.80	0.85	0.74	41.5	33.3	50.0	42.9	40.0	80.0	80.5	79.6	85.1	74.4
	Bananas	0.82	0.82	0.82	0.84	0.79	66.7	61.1	70.0	60.9	72.0	81.9	81.8	82.1	84.4	78.9
	Apples and pears	0.20	0.00	0.27	0.29	0.13	89.7	91.4	87.9	90.2	89.1	20.0	0.0	27.3	28.6	12.5
	Dried fruit	0.89	0.89	0.88	0.89	0.89	43.8	80.0	27.3	16.7	60.0	88.7	89.5	87.9	88.7	88.7
	Sweet fruit preserves	0.63	0.76	0.50	0.59	0.67	72.0	67.6	75.6	65.2	80.6	63.3	76.0	50.0	59.1	66.7
3.Vegetables	Flowers	0.50	0.38	0.58	0.47	0.60	80.2	79.6	80.7	86.8	74.1	50.0	37.5	58.3	46.7	60.0
	Yellow-orange vegetables	0.67	0.50	1.00	1.00	0.50	95.3	96.7	94.1	94.0	96.7	66.7	50.0	*	*	50.0
	Leafy green vegetables	0.71	0.83	0.61	0.65	0.78	74.4	68.2	80.4	71.1	77.8	70.7	83.3	60.9	65.2	77.8
	Tomatoes	0.50	0.25	0.50	0.50	0.25	98.4	98.3	98.5	95.5	98.3	50.0	25.0	50.0	50.0	25.0
	Root and other vegetables	0.17	0.00	0.29	0.14	0.20	95.8	94.7	96.8	96.7	94.8	16.7	0.0	28.6	14.3	20.0
	Marrows	0.64	0.50	0.75	0.70	0.61	60.2	54.0	66.0	53.4	68.9	64.3	50.0	75.0	70.0	61.1
	Fresh and canned beans	0.77	0.73	0.82	0.81	0.73	53.8	66.7	45.2	50.0	57.7	77.2	73.2	81.6	81.0	73.0
	Dry beans	0.82	0.80	0.83	0.81	0.83	29.6	33.3	26.7	18.8	45.5	81.7	80.0	83.3	80.8	82.7
	Sauerkraut and cucumber	0.64	0.52	0.75	0.59	0.68	73.8	79.5	68.9	65.2	84.2	63.8	52.2	75.0	59.1	68.0
	All other fermented products	0.79	0.84	0.75	0.88	0.71	75.6	75.7	75.6	76.7	74.3	79.2	84.0	75.0	88.0	71.4
	All kinds of mushrooms	0.83	0.84	0.82	0.81	0.85	45.5	52.0	40.0	46.2	44.8	82.9	83.8	82.1	81.0	85.3
	Almonds, hazelnuts etc.	0.93	0.91	0.95	0.93	0.93	54.5	77.8	38.5	38.5	77.8	92.7	90.6	94.6	92.7	92.6
	Pumpkin seed, sesame seed, sunflower seed	0.90	0.91	0.88	0.92	0.88	28.6	50.0	20.0	22.2	40.0	89.7	91.4	88.1	91.5	87.9
	Olives	0.98	0.98	0.97	0.98	0.97	25.0	33.3	0.0	0.0	50.0	97.6	98.3	97.1	98.5	96.7
4.Dairy products	Milk and dairy products	0.55	0.65	0.48	0.52	0.59	87.1	80.0	93.8	87.2	87.0	55.3	64.7	47.6	52.4	58.8
	Cottage cheese	0.54	0.63	0.38	0.56	0.50	83.2	80.4	85.2	76.9	89.1	54.2	62.5	37.5	56.3	50.0
	Cheese	0.75	0.72	0.77	0.71	0.80	63.2	63.6	62.8	65.9	60.5	75.0	72.2	76.9	70.8	80.0
	Ice-creams and pudding	0.82	0.80	0.84	0.86	0.78	42.9	29.4	50.0	42.3	43.5	81.7	80.0	83.8	85.7	77.5
5.Meat products	Sausages	0.50	0.22	0.63	0.56	0.42	82.5	83.0	82.0	86.5	78.4	50.0	22.2	63.2	56.3	41.7
	Good quality cold meats	0.60	0.53	0.65	0.75	0.42	83.0	84.4	81.4	77.3	88.6	60.5	52.9	65.4	75.0	42.1
	Organ meat and cold meat products	0.65	0.57	0.69	0.55	0.79	68.9	73.2	63.6	57.1	79.5	64.9	57.1	69.4	54.5	79.2
	Red meat	0.50	0.33	0.63	0.58	0.39	70.1	65.8	74.4	70.3	70.0	50.0	33.3	63.3	58.1	39.1
	Poultry	0.46	0.43	0.50	0.56	0.25	95.8	94.5	96.8	94.9	96.6	46.2	42.9	50.0	55.6	25.0
	Game	0.99	1.00	0.99	1.00	0.98	*	*	*	*	*	99.2	*	98.6	*	98.4
	Lean fish	0.70	0.67	0.72	0.67	0.73	59.6	62.1	56.5	61.5	57.7	69.6	66.7	71.7	66.7	73.0
	Fatty fish	0.86	0.81	0.91	0.90	0.82	54.3	60.0	46.7	64.7	44.4	86.5	81.0	90.7	90.2	82.2
	Mussels and oysters	1.00	1.00	1.00	1.00	1.00	*	*	*	*	*	100.0	*	*	*	*
	Shellfish	1.00	1.00	1.00	1.00	1.00	*	*	*	*	*	100.0	*	*	*	*
	Hard roe	0.99	0.98	1.00	1.00	0.98	*	*	*	*	*	99.2	98.4	*	*	98.4
	All kinds of eggs	0.47	0.67	0.33	0.56	0.33	91.4	91.1	91.7	91.5	91.2	46.7	66.7	33.3	55.6	33.3
6.Fats	Oil	0.43	0.25	0.67	0.40	0.50	94.4	91.4	97.0	96.8	91.8	42.9	25.0	66.7	40.0	50.0
	Butter	0.45	0.40	0.50	0.58	0.25	93.7	90.4	96.6	96.4	90.9	45.0	40.0	50.0	58.3	25.0
	Margarine in cubes or cups	0.75	0.70	0.81	0.70	0.81	73.6	74.4	72.9	77.8	69.0	75.0	69.6	81.0	69.6	81.0
	Cream	0.62	0.60	0.64	0.71	0.57	86.4	84.6	87.9	83.6	89.8	61.9	60.0	63.6	71.4	57.1
	Other animal fats	0.79	0.81	0.77	0.79	0.78	62.3	62.9	61.5	60.0	65.4	78.6	81.5	76.7	78.8	78.4
	Mayonnaise and dressings	0.80	0.76	0.84	0.84	0.75	57.1	52.9	60.0	47.8	68.4	79.8	75.6	84.1	84.4	75.0
7.Sugar and Sweets	Sugar. honey. fruit candies. hard caramels	0.48	0.50	0.47	0.73	0.25	88.9	83.3	94.4	89.5	88.2	47.8	50.0	46.7	72.7	25.0
	Biscuits. cakes with cream. short. semi-short cakes etc.	0.47	0.50	0.43	0.50	0.42	85.9	84.1	87.3	85.4	86.3	46.9	50.0	42.9	50.0	41.7
	Different kinds of chocolate and chocolate sweets	0.67	0.68	0.65	0.68	0.66	62.3	62.5	62.1	46.4	80.0	66.7	68.4	65.0	67.5	65.8
	All kinds of salty snacks	0.80	0.84	0.76	0.84	0.76	37.9	45.5	33.3	41.2	33.3	80.4	84.3	76.5	84.3	76.5
8.Beverages	Non-alcohol	0.61	0.67	0.56	0.65	0.57	66.1	48.3	81.8	61.8	71.4	60.9	66.7	55.6	64.7	57.1

Appendix (B) continued

	Tea	0.29	0.00	0.31	0.13	0.50	95.7	93.4	98.2	95.0	96.5	28.6	0.0	30.8	12.5	50.0
	Coffee	0.72	0.77	0.68	0.61	0.82	90.6	88.9	92.9	97.1	82.8	71.6	76.9	68.3	60.6	82.4
	Beer	0.96	0.91	1.00	0.95	0.98	58.8	53.3	100.0	80.0	28.6	96.5	91.5	*	94.8	98.2
	Wine	0.93	0.94	0.92	0.92	0.94	46.7	50.0	40.0	66.7	33.3	93.1	94.2	92.2	91.9	94.4
	Vodka	0.95	0.89	0.98	0.96	0.93	60.0	56.0	70.0	66.7	55.0	94.8	89.2	98.3	96.2	93.0
	Fruit juices	0.73	0.71	0.74	0.80	0.65	52.2	54.2	50.0	47.8	56.5	72.9	71.1	74.5	80.0	65.0
	Vegetable and vegetable-fruit juices	0.90	0.89	0.91	0.92	0.88	33.3	33.3	33.3	55.6	16.7	90.0	88.7	91.2	91.5	88.2
	Water	0.44	0.25	0.60	0.40	0.50	86.1	84.5	87.5	87.3	84.7	44.4	25.0	60.0	40.0	50.0
9.Spices	Herbs and spices	0.33	0.33	0.33	0.43	0.29	85.5	85.1	85.7	86.9	83.7	33.3	33.3	33.3	42.9	28.6
	Soya sauce	0.98	0.98	0.98	0.98	0.98	25.0	33.3	20.0	0.0	40.0	98.4	98.3	98.4	98.5	98.3

*no possible statistical analysis; O-total sample; M-men; K-women; 65-74-people aged 65-74 years; 75+-people aged 75 years and more

hard caramel, biscuits and/or cakes with cream and/or short and/or semi-short etc.; tea, coffee, water; herbs and spices. Those were products traditionally consumed by Polish and in reasonable price (Gutkowska, 2002; Laskowski, 2005; Slowinska and Wadolowska, 2000; Wierzbicka *et al*., 1997).

The carried out evaluation of the FIVeQ questionnaire showed that it can be recommended as an accurate and reliable measure tool in epidemiologic studies among older people. For the majority of products (about 2/3 items) we obtained good results reproducibility (test and retest). High test power values (over 0.8) were stated for 31% subgroups of products, while moderately high test power (from 0.6 to 0.8) for 35% items. Similarly for the sensitivity and specificity indices their high values (over 80%) were revealed for 38% and 32% subgroups of products, respectively and moderately high sensitivity and specificity (from 60% to 80%) for 28% and 34% items, respectively.

References show that in order to examine questionnaire's reproducibility it is necessary to make an interview twice with the same person, the same pollster and to compare obtained results of test and retest (Bohlscheid-Thomas *et al*., 1997; Boucher *et al*., 2006; Frankenfeld *et al*., 2003; Hu *et al*., 1999; Jedrychowski, 1982; Katsouyanni *et al*., 1997; Ozsoy *et al*., 2007; Wang *et al*., 2007). The time gap between two interview should be respectively short, so that the change in nutrition habit is minimal, but on the other hand long enough, in order not to have the respondent remember his answers. The reason for small reproducibility of results may be: (i) respondent (not meeting the recruitment requirements, not stable emotionally, etc.) (ii) non-adequate questionnaire (not understandable and long questions, lack of explanations, etc.) (iii) pollster (his attitude, behaviour, expectations concerning answers, age, sex, intellectual level, etc.) (Jedrychowski, 1982). During carrying out the study we made an effort to eliminate outer reasons interfering with the interview results, i.e. connected to respondent, pollster and questionnaire. Thus it should be expected that the stated for some products smaller results reproducibility had different reasons.

During the second food interview (retest) it is assumed that people's nutrition did not change and the results are to be repeated. However that assumption is not always true. The first interview may change nutritional behaviour of respondents, for example encourage them to eat products earlier not known. Another reason may be individual changeability of nutrition, so called day-to-day, which especially concerns products consumed rarely (Gibson, 1990; Wadolowska *et al*., 2004). For those reasons in the retest results may be reliable, but different than those obtained in test (Gibson, 1990). In this way the calibration method shown small results reproducibility, but it is not a proper conclusion with reference to nutritional behaviour. Unfortunately, each calibration proceeding is burdened by some error and real precision may never be determined, as repeating the nutritional observation of people is impossible (Block, 1982 according to Gibson, 1990; Wadolowska *et al*., 2004). Repeating a nutritional interview gives new information on consumption in next day or week, since day-to-day changeability (of one person) is stated by short-term consumption of different products by the same person (Gibson, 1990). This conviction does not entitle to absolute ceasing working on evaluation of food questionnaire's accuracy, but forces a critical approach and indicates on purposefulness of regular improving food intake evaluation methods (Wadolowska *et al*., 2004).

An important conclusion arising from the study is fact that sex and age of older respondents (65-74 or 75+ years old) did not influence significantly the sensitivity and accuracy indices and the test power values. It enables to acknowledge the FIVeQ questionnaire as a tool properly classifying the examined older people regardless of their sex and age. The advantage of the questionnaire is its simple construction and the ability to give dichotomic answers (yes/no). Thus the questionnaire, after a short description of the study's aim and essence, may be filled during an interview by a pollster or individually by older people and probably by younger adults.

The results obtained for meat products, beverages and fats should be especially considered as they had the

highest mean test power and the sensitivity and specificity indices and at the same time narrow ranges of the confidence interval for those groups of products. It shows that products were aggregated into groups properly and that questionnaire had similar high accuracy for assortment subgroups constituting main groups. On the other hand spices group were found to have a very wide range of the confidence interval. Thus in the future it can be predicted that spices group and its assortment subgroups will be excluded from the studies on seniors nutrition differentiation.

The noted high values of the sensitivity indices indicate on a good ability of the questionnaire to proper classifying the examined to a group of people consuming specified products, while high values of the specificity indices indicate on a good ability of the questionnaire to proper classifying the examined to a group of people not consuming specified products. That questionnaire's feature is extremely useful in epidemiologic studies because of possible detecting by the FIVeQ questionnaire both negative and positive features of people's nutrition. It means that the created questionnaire may be applied in studying relations between consuming by seniors different foods and diet-related diseases occurrence. Nutritional conditionings of diseases, i.a. obesity, cardiovascular disease, cancer, hypertension, diabetes, are often analyzed with regards to eating or not pro-healthy or unhealthy and in the context of diet diversity (Drewnowski and Specter, 2004; Elmadfa and Freisling, 2005; Lichtenstein *et al*., 2006; McCrory *et al*., 1999; Norat and Riboli, 2003; Psaltopoulou *et al*., 2004). Realization of such studies needs applying proper research tools: sensitive and accurate and at the same time simple. The carried out analysis gives the basis for stating that the FIVeQ questionnaire meets these requirements.

Conclusion: High values of the sensitivity, specificity and test power indices were stated for the food intake variety questionnaire FIVeQ and proper classification of the subjects to the group of people eating or not specific products, regardless of their sex and age. That questionnaire's feature is extremely useful in epidemiologic studies because of possible detecting by the FIVeQ questionnaire both negative and positive features of people's nutrition. The obtained results prove good accuracy of the FIVeQ questionnaire as a tool for studying food intake variety and enable us to recommend its applying among older people.

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Magnesium, Zinc and Copper Intake by Polish University Students

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Abstract: The aim of the work was to analyze the consumption of three minerals: magnesium, zinc and copper among Polish university students. The study included 708 university students aged 18-26 years. Nutritional value of students' diet was assessed using the 24-hour recall method. The minerals intake among students was compared to Polish Recommended Dietary Intake (RDI). Male students consumed more magnesium, zinc and copper than female students in comparison to the RDI (on average, male: Mg-91%, Zn-99%, Cu-67%; female: Mg-73%, Zn-80%, Cu-42%). The average male students' diets were compatible with the recommended dietary intake of magnesium and zinc. The copper intake connected with high deficiency risk was observed among over 50% of male students. The average female students' diets had too low content of all three minerals. The intake indicating high deficiency risk was revealed among 40-50% of female students for magnesium and zinc and about 90% of them for copper.

Key words: Copper, intake, magnesium, minerals, students, zinc

Introduction

Incorrect nutrition in terms of amount and quality causes unprofitable health consequences. Over consumption of energy, animal fats and sugar is usually accompanied by small consumption of fibre (Borowska and Socha, 2005; Kaluza and Brzozowska, 2005; Szymelfejnik *et al*., 2003; WHO, 2003; Wadolowska *et al*., 2000; Przyslawski *et al*., 1999; Stopnicka *et al*., 1999; Alvarez-Pineiro *et al*., 1998; Ziemlanski, 1998; Burke *et al*., 1997; Przyslawski and Gertig, 1997, Ilow and Regulska-Ilow, 1996ab). Too small intake of fibre, observed among children, teenagers as well as adults comes from Over consumption of highly refined products, which supply large amounts of energy without enough amount of vitamins and minerals, especially micronutrients (Szponar *et al*., 2003; Przyslawski *et al*., 1999; Alvarez-Pineiro *et al*., 1998; Ziemlanski, 1998; Burke *et al*., 1997; Ilow and Regulska-Ilow, 1996a,b; Montana and Lopez, 1996).

The aim of the work was to analyze the consumption of three minerals: magnesium, zinc and copper among Polish university students. These components play vital functions in human body. Magnesium and zinc, next to calcium, phosphorus and fluorine, are the constructive material of skeleton, teeth, skin and hair (WHO, 2003; Ziemlanski, 1998). Magnesium with natrium, potassium and calcium play the main role in electrolytic-water balance. In turn, copper is a component of the copper-dependent enzymes and with iron and cobalt is essential in the process of red blood cells production. The human organism can only stay healthy if it is supplied with food products in proper quantity and value proportions.

Table 1: Sample characteristics

	Females		Males	
Parameter	x	SD	x	SD
Age (year)	20.6	1.62	20.8	1.79
Height (cm)	166.5	5.74	179.3	6.98
Weight (kg)	59.0	9.01	74.3	11.37
BMI (kg/m^2)	21.3	2.73	23.1	3.06

x - mean value, SD - standard deviation

Materials and Methods

Sample: The study included university students aged 18-26, studying in years 1996-1998 at the Faculty of Food Sciences of University of Warmia and Mazury in Olsztyn, Poland, on the 1st and 4th year of study. In total diets nutritional value of 708 people, including 488 women (1st year of study: 267 subjects; 4th year of study: 221 subjects) and 220 men (1st year of study: 123 subjects; 4th year of study: 97 subjects) was assessed. Height and weight of students were measured by well-trained researches. Then the Body Mass Index (BMI) of students was calculated (Table 1). This data are a part of longitudinal study carried out since 1989 in the Department of Human Nutrition of the University of Warmia and Mazury by our team.

Food and minerals intake assessment: Nutritional value of diets was assessed using the 24-hour recall method (Charzewska *et al*., 1997). The interviews were carried out during the autumn in years 1996-1998, every day, keeping the proportions between interviews in weekdays and weekends. Every person was interviewed

Table 2: Description of consumption classes

Class number	Consumption classes
1	< 66.7% of the safe intake level
2	66.7-89.9% of the intake safe level
3	90.0-109.9% of the safe intake level
4	110.0% of safe the intake level-100.0% of the recommended intake level
4A*	> 110.0% of the safe intake level
5	> 100.0% of the recommended intake level

*for Mg only because of the value of 110% of the safe level is higher than the value of recommended level

Table 3: Ranges of minerals consumption classes'

	Mg (mg/day)		Zn (mg/day)		Cu (mg/day)	
Class No.	Females	Males	Females	Males	Females	Males
1	<187.0	<233.0	<6.7	<9.3	<1.30	<1.30
2	187.0-251.9	233.0-314.9	6.7-8.9	9.3-12.5	1.30-1.79	1.30-1.79
3	252.0-308.0	315-384.9	9.0-10.9	12.6-15.3	1.80-2.19	1.80-2.19
4	-	-	11.0-13.0	15.4-16.0	2.20-2.50	2.20-2.50
4A	>308.0	>385.0	-	-	-	-
5	-	-	>13.0	>16.0	>2.50	>2.50

for copper recommended safe level operaters, in order to indicate classes the value of 2,0 mg was accepted as the safe intake level, 2.5 mg as recommended intake level

once. The information about the consumption of food products, dishes and drinks was collected by proper questionnaires. The amount of consumed food products, dishes and drinks was estimated on the basis of "Album of photographs of food products and dishes" (Szczyglowa *et al.*, 1991). The results were analysed using the software "Dietetyk v. 2.0" containing the database from the tables of content and nutritional value of food products (Nadolna *et al.*, 1994).

The content of energy, protein, fat, carbohydrates and magnesium, zinc and copper, after including losses (10%), was calculated. The nutrients intake among students was compared to the Polish recommended dietary intake on the safe level (RDI) created by Ziemlanski *et al.* (1994). For the valuation the RDI was accepted for women and men in the age of 19-25, with moderate physical activity and body weight for women 60 kg, for men 75 kg.

Statistical analysis: Nutritional value of diets was also termed as nutritive density, calculating the amount of nutrients for 1000 kcal. The intake of magnesium, zinc and copper is presented in determined consumption classes (Table 2). For determining ranges of 5 consumption classes the following limits were supposed: (I) 66.7% of the safe intake level (ii) 90% of the safe intake level (iii) 110% of the safe intake level (iv) 100% of the recommended intake level (Ziemlanski *et al.*, 1994). For zinc and copper 5 consumption classes were determined, for magnesium 4 consumption classes (4A class for magnesium only) because of higher value of 110% of the safe intake level than recommended intake level. The values of these nutrients ranges are collected in Table 3.

The comparison of the average values and the differentiation of collocation of women and men in consumption classes were verified on the basis of one-factor variance analysis (ANOVA) and chi^2 test. The statistical analysis was executed with the software Statistica 5.5 PL, at the significance level $p \leq 0.05$.

Results

Diets of male students had proper content of energy (2967 kcal, *i.e.* about 99% of the RDI), lower than proper content of carbohydrates (about 75% of the RDI) and higher than proper content of protein (about 178% of the RDI) as well as fat (about 118% of the RDI). The intake of energy, fat and carbohydrates by female students was lower than the recommended dietary intake and amounted about 72%, 83%, 58% of the RDI, respectively. Total women intake of protein exceeded the RDI in safe level by 14 percent points (Table 4).

Male students consumed on average more magnesium, zinc and copper than female students (Table 4). Male students consumed adequate to the RDI amounts of magnesium (on average 319 mg, i.e. about 91% of the RDI) and zinc (on average 13.8 mg, i.e. about 99% of the RDI), however lower than recommended amounts of copper (on average 1.34 mg, *i.e.* about 67% of the RDI). The analysis of the minerals after computing for 1000 kcal stated recommended amount of zinc in male students' diets (4.69 mg/1000 kcal vs. 4.67 mg/1000 kcal), however not enough magnesium (111 mg/1000 kcal vs. 117 mg/1000 kcal) and copper (0.46 mg/1000 kcal vs. 0.67 mg/1000 kcal, Table 4) in comparison to the RDI.

About 50% of men consumed magnesium and zinc in proper or higher amount (over 90% of the RDI; Table 5-6,

Table 4: The average nutrients intake by university students

	Females			Males			
Nutrient	N	x	SD	N	x	SD	p
Energy (kcal)	488	1696	689.8	220	2967	1130.0	<0.001
Energy (kJ)		404	164.2		706	269.0	
Energy (% RDI)	488	72.2	29.35	220	98.9	37.67	<0.001
Protein (g)	488	54.7	24.78	220	99.8	46.06	<0.001
Protein (g/1000 kcal)	488	32.4	8.00	220	33.5	7.97	0.096
Protein (% RDI)	488	114.0	51.63	220	178.2	82.24	<0.001
Fat (g)	488	64.8	32.70	220	118	56.6	<0.001
Fat (g/1000 kcal)	488	37.6	10.3	220	39.30	9.23	0.034
Fat (% RDI)	488	83.1	41.92	220	118.2	56.65	<0.001
Carbohydrates (g)	488	221	98.1	220	366	150.4	<0.001
Carbohydrates (g/1000 kcal)	488	132	26.01	220	124	22.7	<0.001
Carbohydrates (% RDI)	488	58.1	25.67	220	75.1	30.86	<0.001
Mg (mg)	488	204	91.2	217	319	132.4	<0.001
Mg (mg/1000 kcal)	488	127	50.0	217	111	28.3	<0.001
Mg (% RDI)	488	73.0	32.56	217	91.2	37.83	<0.001
Zn (mg)	488	7.98	3.957	220	13.8	6.10	<0.001
Zn (mg/1000 kcal)	488	4.78	1.419	220	4.69	1.120	0.404
Zn (% RDI)	488	79.9	39.57	220	98.9	43.61	<0.001
Cu (mg)	485	0.83	0.400	219	1.34	0.556	<0.001
Cu (mg/1000 kcal)	485	0.51	0.205	219	0.46	0.132	0.001
Cu (% RDI)	485	41.8	19.98	219	67.1	27.82	<0.001
Zn/Cu	485	9.95	3.089	219	10.8	4.12	0.003

N-sample size; x-mean value; SD-standard deviation; p-level of significance

Table 5: The average magnesium intake by university students' in consumption classes

	Females N = 488				Males N = 217			
Class number	N	x (mg/d)	SD (mg/d)	% RDI (%)	N	x (mg/d)	SD (mg/d)%	RDI (%)
1	239	134.7[a]	34.81	48.1	61	183.9[a]	44.20	52.5
2	129	218.9[b]	19.44	78.2	54	266.9[b]	24.24	76.2
3	63	276.4[c]	16.86	98.7	50	349.9[c]	19.10	110.0
4A	57	385.0[d]	75.80	137.5	52	503.4[d]	103.31	143.8
ANOVA	p<0.001				p<0.001			

N-sample size; x-mean value; SD-standard deviation; p-level of significance for one-factor variance analysis; a, b, c, d, e-using different letters marked significance difference at $p \leq 0.005$ in columns

8). Among over 20% of men the intake of magnesium and zinc was around 50% of the RDI and it was connected with high deficiencies risk. Very low intake of copper (about 47% of the RDI) was observed among over the half of men (Table 7). The intake of copper at the level of 76% of the RDI was stated among 30% of men, while proper or higher intake was stated among merely 16% of the men sample (Table 7-8).

Among women the average intake of every analysed mineral was lower than the RDI (Table 4). The average intake of magnesium (204 mg *i.e.* 73% of the RDI) and zinc (7.98 mg, *i.e.* about 80% of the RDI) did not exceed 80% of the RDI and the intake of copper (0.83 mg) fulfilled the recommendations in 42%. The women's diets had, after computing for 1000 kcal, higher than recommended intake of magnesium (127 mg/1000 kcal *vs.* 119 mg/1000 kcal) and zinc (4.78 mg/1000 kcal *vs.* 4.26 mg/1000 kcal) and lower than recommended intake of copper (0.51 mg/1000 kcal *vs.* 0.85 mg/1000 kcal). The consequence of very low intake of copper, both among women and men and additionally among women lower than recommended intake of zinc was higher than recommended value of zinc to copper ratio (Zn/Cu; Table 4).

Proper intake of magnesium and zinc was stated among 13-14% of women (Table 5-6, 8). Almost all of female students (about 97% of the sample) had lower than recommended intake of copper (below 90% of the RDI; Table 7). The intake of copper below 66.7% of the RDI, connected with high deficiencies risk, was stated among about 90% of women (Table 7-8).

Discussion

Our results revealed an agreement with an average magnesium intake among male students and lower than recommended average magnesium intake among female students (about 80% of the RDI). Insufficient intake of magnesium, conducive to high deficiencies risk, referred to almost a half of women and one third of men. Proper amount of magnesium in men's and

Table 6: The average zinc intake by university students' in consumption classes

Class number	Females N=488				Males N=220			
	N	x (mg/d)	SD (mg/d)	% RDI (%)	N	x (mg/d)	SD (mg/d)%	RDI (%)
1	203	4.84[a]	1.425	48.4	49	7.25[a]	1.834	51.8
2	129	7.78[b]	0.653	77.8	59	11.10[b]	0.947	79.3
3	69	9.94[c]	0.560	99.4	39	13.87[c]	0.808	99.0
4	47	11.70[d]	0.543	117.0	11	15.73[cd]	0.161	112.4
5	40	16.90[d]	5.104	169.1	62	21.33[e]	5.490	152.3
ANOVA	p<0.001				p<0.001			

N-sample size; x-mean value; SD-standard deviation; p-level of significance for one-factor variance analysis; a, b, c, d, e-using different letters marked significance difference at p≤0.005 in columns; * the statistic analysis was not executed

Table 7: The average copper intake by university students' in consumption classes

Class number	Females N=484				Males N=219			
	N	x (mg/d)	SD (mg/d)	% RDI (%)	N	x (mg/d)	SD (mg/d)%	RDI (%)
1	435	0.73[a]	0.255	36.7	115	0.95[a]	0.246	47.3
2	33	1.53[b]	0.127	76.4	68	1.52[b]	0.134	75.8
3	13	1.94[c]	0.133	96.8	23	2.02[c]	0.130	101.2
4	1	2.32*	0.000	116.1	6	2.36[cd]	0.094	117.8
5	2	2.74*	0.280	136.8	7	3.01[e]	0.664	150.4
ANOVA	p<0.001				p<0.001			

N-sample size; x-mean value; SD-standard deviation; p-level of significance for one-factor variance analysis; a, b, c, d, e-using different letters marked significance difference at p≤0.005 in columns; * the statistic analysis was not executed

women's diets was stated among university students from Lublin (Marzec *et al.*, 2004). Proper amount of magnesium in men's diets and on the level of 66-67% of the RDI for women were stated among university students from Bialystok (Stopnicka *et al.*, 1999). Low intake of magnesium (85% of the RDI) was stated among young Polish women from Bialystok (Borowska and Socha, 2005). Low intake of magnesium was also recorded among school children aged 13-15 from Poznan (girls 72-75% of the RDI, boys 82-84% of the RDI, Przyslawski *et al.*, 1998). Proper intake of magnesium (91-107% of the RDI) was revealed among adults (20-59 years old) from Warsaw (Kaluza and Brzozowska, 2005) and low intake of magnesium was recorded in seniors` diets from Warsaw (Dybkowska *et al.*, 2004). In turn in diets, recreated on the basis of family budget, 60% realization of the recommended intake was found (Gawecki and Hryniewiecki, 1998). Insufficient intake of this mineral in Poland was confirmed by the analysis of hair in all age groups of people, revealed a deficiency of magnesium. Its particularly low level was shown among children (Oleszkiewicz, 1996). On the other hand the analysis of the amount of magnesium in hair of university students of Medical Academy in Gdansk revealed no necessity for supplementing of the tested group with magnesium, however individual supplementation with magnesium is recommended, according to the authors of that project (Lukasiak *et al.*, 1998).

These facts are worrisome because of the data indicating on the influence of magnesium deficiency in etiopathogenesis of cancer and heart coronary disease (Leone *et al.*, 2006; Singh, 1997; Oleszkiewicz, 1996). Moreover it was stated that the magnesium deficiency among young women may cause delay of the first menstruation, varying periods length, painful menstruations. During pregnancy hyper contraction of the womb muscle may occur, what can be in many cases a cause of miscarriage and premature birth. Children born by mothers with the magnesium deficiency are weaker, eat worse, are less disease-resistant, sleep badly and may react with convulsions to high temperature (Oleszkiewicz, 1996).

Proper magnesium intake was stated among Spanish university students (Quiles *et al.*, 1996; Alvarez-Pineiro *et al.*, 1998). These authors noticed that the magnesium intake below 80% of recommended intake was stated among 40% of women and men (Quiles *et al.*, 1996). Lower than recommended intake of magnesium was stated among young people aged 16-20 from Valencia (Farre-Rovira *et al.*, 1999) and among university students from Luisiana, however more women than men consumed magnesium below 66% of the RDA (Zive *et al.*, 1996).

Our study revealed in university male students' diets proper average zinc content, however less than a half of the men consumed zinc in proper or higher quantity. On the other hand among female students an average zinc intake oscillated around 80% of the RDI and as much as 42% of the female students consumed it below 66% of the RDI. These findings are corresponding with the results of the research of diets, recreated on the basis of family budgets (Gawecki and Hryniewiecki, 1998). It was revealed that girls' and women's diets are poor in zinc. Also, other authors stated low zinc intake among 10-year old girls (below 70% of the RDA; Champagne *et al.*,

Table 8: Sample distribution in magnesium, zinc and copper consumption classes

	Sample percentage in minerals intake classes (%)								
	Mg			Zn			Cu		
Class number	Total N=705	Females N=488	Males N=217	Total N=708	Females N=488	Males N=220	Total N=703	Females N=484	Males N=219
1	42.5	49.0	28.1	35.6	41.6	22.3	78.2	89.9	52.5
2	26.0	26.4	24.9	26.6	26.4	26.8	14.4	6.8	31.1
3	16.0	12.9	23.0	15.2	14.2	17.7	5.1	2.7	10.5
4	–	–	–	8.2	9.6	5.0	1.0	0.2	2.7
4A	15.5	11.7	24.0	–	–	–	–	–	–
5	–	–	–	14.4	8.2	28.2	1.3	0.4	3.2
chi^2 test*	chi^2 = 39.78 p<0.001			chi^2 = 63.21 p<0.001			chi^2 = 125.36 p<0.001		

*comparison of females and males distribution in minerals consumption classes

1998), young people aged 16-20 in Valencia (Farre-Rovira *et al.*, 1999), among Spanish female students (80% of the RDA) and male students (70% of the RDA), university students from Luisiana (Zive *et al.*, 1996) and Korean female students (Chung-Ja-Sung and Young-Hwa-Yoon, 2000). In the Spanish university students population zinc intake below the border value pertained 45% of men and 72% of women (Quiles *et al.*, 1996). In diets of Polish female students and male students very low zinc intake were stated too (60% and 76% of the RDI, respectively; Bialas *et al.*, 2005). Zinc amount in primary school students' diets from Poznan did not overspread the recommended dietary intake and furthermore in the 1990s was a bit lower than in the 1980s, however no statistically important differences were stated (Przyslawski *et al.*, 1998).

It is believed that moderate zinc deficiencies are quite common, however it is difficult to confirm it unequivocally, because of the lack of simple nutritional status evaluation methods. The risk groups of this element deficiency occurrence are children, pregnant and lactating women, the elderly, people eating mainly vegetable food and alcoholics (Gawecki and Hryniewiecki, 1998). Low zinc deficiency may occur among children with its low intake, because of the lack of appetite and taste disorder (Ziemlanski, 1998). The deficiency of this mineral may result in roughness of the skin, hair loss, changes in the nail structure and difficulties in healing wounds. The zinc deficiency usually occur along with other nutrients deficiency especially protein and energy, what also makes their diagnosis difficult (Gawecki and Hryniewiecki, 1998). Zinc deficiency may cause insulin level reduction and glucose tolerance malfunction, may carry weight on the inefficiency of immune system and cancer. It was stated that mental illnesses occurrence binds with the zinc deficiency. In turn the zinc deficiency among pregnant women may be a risk element of pregnancy disorders, a cause of a bad foetus growth, high ratio of prebirths, relatively frequent growth defects (Oleszkiewicz, 1996). The differentiation in zinc intake was stated to be connected to the amount of consumed energy and with the race of pregnant women (Neggers *et al.*, 1998). The analysis of the amount of zinc in Medical Academy students hair in Gdansk did not reveal any individual, substantial deficiencies of this mineral (Lukasiak *et al.*, 1998).

The Olsztyn university students' diets had very low copper content (women: 42% of the RDI on average, men: 67% of the RDI on average). It was revealed that as many as over 50% of male students and about 90% of female students was consuming copper below 66% of the RDI, what means in the insufficient amount, connected with high deficiencies risk. In diets of university students from Lublin and Poznan very low intake of copper was stated (Lublin: male 70% of the RDI, female 50% of the RDI; Poznan: male 56% of the RDI, female 60% of the RDI; Bialas *et al.*, 2005; Marzec *et al.*, 2004). Diets of many population groups from the region of Wielkopolska in Poland had improper copper content and men consumed much more copper than women (Szajkowski, 1996; Przyslawski *et al.*, 1998). In Poland the amount of copper in diets, recreated on the basis of family budgets, was very low in comparison to the recommended dietary intake (Gawecki and Hryniewiecki, 1998). The average copper intake below 70% of the RDA was revealed among children aged 10 (Champagne *et al.*, 1998).

According to Ziemlanski (1998) the copper deficiency is rare among people. However, hipocupremium states may occur, what was stated in several disease units, for instance kwashiorkor and to a very large extent malnutrition. The copper deficiency may also occur among people consuming mainly dairy products and in lingering illnesses of alimentary canal. The symptoms of the copper deficiency are the anaemia, which is caused by hampered iron transport to the haemoglobin synthesizing tissues and the curtailment of the red blood cells life expectancy, as well as snapping of blood vessels, increased bone fragility, heart dysfunction,

increase of the cholesterol level, reduction of the humoral and cellular immunity and the lack of pigment in the skin (Gawecki and Hryniewiecki, 1998).

Our results revealed in students' diets too high Zn/Cu ratio, which was caused mainly by very low copper intake. The data show that the stated Zn/Cu ratio is unprofitable because its increase is conductive to and at the same time its decrease prevent, the development of ischemic heart disease (Szajkowski, 1996; Lukasiak *et al.*, 1998). Szajkowski (1996) proved that diets having high amount of animal fat and sugar and low amount of fibre have high zinc to copper ratio. It was also revealed that the diets, which had too much protein and saturated fatty acids and insufficient amount of carbohydrates and fibre had too low amount of magnesium and zinc (Farre-Rovira *et al.*, 1999). In turn high fibre intake (>20 g/day) was connected with higher proper magnesium and zinc intake probability (Nicklas *et al.*, 2000) and the main source of the analysed nutrients were cereals, vegetables and fruit as well as milk and meat (Zive *et al.*, 1996).

In many studies it was stated that diets of different populations, including university students, had too high saturated fats and animal protein intake and too low complex carbohydrates content (Switoniak *et al.*, 1995; Koszewski and Kuo, 1996; Montana and Lopez, 1996; Quiles *et al.*, 1996; Alvarez-Pineiro *et al.*, 1998; Hendricks and Herbold, 1998; Wadolowska *et al.*, 1998). This state was a result of high fats as well as meat and its products intake and of insufficient intake of cereals, fruit and vegetables (Ilow and Regulska-Ilow, 1996a,b). Whereas it is taken into consideration that cancer occurs twice less frequently among people consuming vegetables and fruit in large amount, in comparison to those who eat only a few of them and among whom micronutrients deficiency occurres, including zinc deficiency (Kruk, 2006; Ames, 1998; Ziemlanski, 1998). The analysis of changes in minerals consumption in diets of school children from Poznan in the 1990s in comparison to the 1980s showed changes in the food intake structure, however they did not have any vital effect on the observed too low level of their consumption. According to the authors even similar intake cannot lead to rush conclusions, because it is essential to consider different assimilation of minerals of various food, as well as their interaction. In the 1990s, in comparison to the 1980s the role of animal products (meat, pork-butcher's meat, milk, eggs) as the source of magnesium has decreased, however the role of cereals, vegetables and fruit has increased. A regularity in zinc and copper intake structure was observed. In diets of the 1990s the share of animal products as well as carotene rich vegetables and fruit (boys' diets), eggs and beans (girl's diets) as the source of zinc and copper has decreased, while the share of cereals, vegetables and fruit has increased (Przyslawski *et al.*, 1998).

Observed insufficient cooper intake among university students from Olsztyn, as well as the occurrence of high low-Mg-and-Zn-intake population percentage seems to confirm other authors' data about unprofitable dietary habits, finding expression in improper food selection-low cereals intake, especially wholemeal (Sekula, 1997), dairy products, vegetables and fruit as well as beans intake (Przyslawski *et al.*, 1999; Stopnicka *et al.*, 1999). Attention should be drawn to education of young people from the point of view of proper intake structure, the frequency of valuable food consumption, leading to the change of improper dietary habits. Not varied, monotonous diet is the factor of the development of illnesses caused by improper dieting such as: blood circulation system diseases, cancer, insulin-not-dependent diabetes, obesity, the iron-deficiency anaemia and osteoporosis.

Conclusions: Male students consumed more magnesium, zinc and copper than female students. The average male students' diet were compatible with the recommended dietary intake of magnesium and zinc. The copper intake connected with high deficiency risk was observed among over 50% of the male students. The average female students' diets had too low content of all three minerals. The intake indicating high deficiency risk was revealed among 40-50% of female students for magnesium and zinc and about 90% of them for copper.

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Potentials of Sweet Potato (*Ipomoea batatas*) Leaf Meal as Dietary Ingredient for *Tilapia zilli* Fingerlings

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Abstract: An 8-week feeding trial was conducted to evaluate the potential of sweet potato (*Ipomoea batatas*) leaf meal as dietary protein source in the diet of *Tilapia zilli* fingerlings. Five isonitrogenous diets of 30% crude protein were formulated to contain 0, 5, 10, 15 and 20% sweet potato leaf meal (Diets 1-5) to partially replace other protein ingredients in the tilapia diet. The diet containing 0% leaf meal served as the control. *Tilapia zilli* fingerlings were reared in 50L circular plastic tanks maintained in a flow-through system. Each dietary treatment was tested in triplicate groups of 10 fingerlings per tank. The results of the growth and nutrient utilization responses show that there were no significant ($p>0.05$) differences among the fish fed diets 1-4 (0-15% sweet potato leaf meal) but were significantly ($p<0.05$) different from fish fed on diet 5 (20% sweet potato leaf meal) which had lower growth and feed utilization values. There were no significant ($p>0.05$) differences in the carcass composition of *Tilapia zilli* fingerlings fed on experimental diets. The present findings show that sweet potato leaf meal has good potential for use as one of the protein sources in *Tilapia zilli* diet up to 15% level without compromising growth.

Key words: Sweet potato leaf meal, plant protein, feed utilization, growth, tilapia

Introduction

One of the problems facing the aquaculture industry today is the high cost of fish feed. Nutritionist all over the world are constantly searching for the dietary protein sources in which fish will maximize growth and increase production within the shortest possible time and at lowest cost. Leaf meals are one of the cheapest sources of proteins that may reduce the high cost of fish feed. Many studies have been conducted using various sources of leaf meal proteins (Ng and Wee 1989, on cassava leaf meal, Yousif *et al*., 1994 on Alfalfa, Reyes and Fermin, 2003 on *Carica papaya* and other leaf meal, Bairagi *et al*., 2004 on *Leucaena leucocephala*).

The sweet potato, *Ipomoea batatas* L. (Lam) belongs to the morning-glory family Convolvulaceae. It is cultivated in over 100 nations and ranks fifth among the most important food crops in the tropical areas (An, 2004). The leaves of this plant have been used in the tropics as a cheap protein sources in ruminant feeds.

Studies have been conducted to determine the nutritive value of sweet potato leaf meal. According to Woolfe (1992), Ali *et al*. (1999), Ishida *et al*. (2000), An (2004), Ekenyem and Madubuike (2006), the leaf meal has a high protein content of between 26 to 33%, with high amino acid score. It has good mineral profile and vitamins such as A, B_2, C and E. Aside from its nutritive values, sweet potato leaves can be harvested many times throughout the year (Hong *et al*., 2003) thereby making the leaf meal to be abundant. One major factor limiting the use of this leaf meal in fish feed is the presence of anti-nutritional factors (Tacon, 1993). The antinutritional substances present in the sweet potato leaves, according to Oyenuga (1968), are the invertase and protease inhibitors. These substances can be inactivated by various processing methods such as oven or sun-drying, boiling or steaming and grinding prior to inclusion in fish feeds.

Although, various leaf meals have been tested as potential fish feed ingredient to decrease diet cost, the use of sweet potato leaf meal has not been tested. It is against this background that the present study was designed, to evaluate the potentials of incorporating sweet potato leaf meal into the pelleted feed of *Tilapia zilli*, a widely culturable fish species in Africa. The objective of this work, therefore, was to determine the growth performance, feed utilization and carcass composition of *Tilapia zilli* fingerlings fed on graded levels of sweet potato leaf meal.

Materials and Methods

Collection and preparation of sweet potato leaf meal: Fresh leaves of sweet potatoes were collected from evergreen farms Idiroko road, Ogun State, Nigeria. The collected samples were washed thoroughly with tap water to remove dirt and debris, drained properly and later sun dried to a constant weight. The dried leaves were milled using a laboratory miller, packed in the freezer at-20°C until use.

Diet formulation and preparation: Five isonitrogenous and isocaloric diets were formulated to contain 30% crude protein. The sweet potato leaf meal was incorporated into each of these diets at 0, 5, 10, 15 and 20% to replace other protein ingredients in the diets. The

diet containing 0% leaf meal serves as the control. Feed ingredients were weighed according to the gross composition table in Table 1. The ingredients were mixed together using a kitchen mixer before the addition of vitamin premix. Oil was later added to the dry ingredient and mixed thoroughly. Warm water was added to the premixed ingredients and homogenized until a dough-like paste was formed. The dough was passed through an improvised pelleting machine. The moist pellets were oven dried at 60°C to a constant weight and kept in air tight containers.

Experimental design and feeding trials: Fingerlings of *Tilapia zilli* were purchased from Animashaun fish farms, Badagry, Lagos, Nigeria. The fish were allowed to acclimatize for 10days, during this period, they were fed on commercial diet. Prior to the commencement of the experiment, all fish were starved for 24 hours. This practice was to eliminate variation in weight due to residue food in the gut and also to prepare the gastro intestinal tract for the experimental diets, while at the same time to increase the appetite of the fish.

The feeding trial was conducted in flow-through plastic aquaria each with 50L capacity of water. 150 fingerlings of initial mean weight of 2.13±0.85g were randomly allotted at the rate of 10 fingerlings per aquarium into five dietary groups designated Diet 1, Diet 2, Diet 3, Diet 4 and Diet 5 and each group was fed on 0, 5, 10, 15 and 20% sweet potato leaf meal respectively. Fish were fed on allotted experimental diets at 3% of their total body weight per day. Feedings were generally done in the mornings at 09:00 and 16:00 h. Except on weighting days when they were fed after weighing. All fish were reweighed every fortnight and feed weight was adjusted accordingly to accommodate for weight changes. For statistical reasons, each of the dietary group was triplicate. The experiment lasted for 56 days.

Digestibility study: The digestibility trial was conducted separately in static aquaria. Removal of the uneaten feed was done by siphoning. Faecal samples were then collected from the three replicates of each dietary treatment. The faeces were easily detected and immediately removed from the water with a glass canula and dried to a constant weight in an oven at 60°C (Ramachandran and Ray, 2007). Apparent digestibility coefficient for protein (ADC_P) was calculated according to Zhao *et al.* (2006).

$$\% \text{ ADCp} = \frac{100 - (100 \times \% \text{ Cr2 O3 in diet} \times \% \text{ protein in diet}}{\%\text{Cr2 O3 in faeces} \times \% \text{ protein in faeces.}}$$

Chemical analyses: Samples of sweet potato leaf meal, the experimental diets, faecal samples and experimental fish at the beginning and at the end of the feeding trial were subjected to proximate analyses. Moisture was obtained by drying the sample at 105°C in an oven until constant weight was achieved. Crude protein was determined by using the microkjeldah digestion method (N×6.25). Crude lipid content was done by soxhlet-extraction. Ash content was done by combustion in muffle furnace to constant weight at 550°C. Crude fiber was determined using the acid/base digestion process. Nitrogen Free Extract (NFE) was computed by taking the sum of values for crude protein, crude lipid, crude fiber and moisture and subtracting this from 100 (Maynard *et al.*, 1979). All analyses followed the procedures of A.O.A.C (1990).

Water analyses: Water quality was monitored every week throughout the feeding trials. Temperature ranged between 26-28°C, dissolved oxygen 5.0-6.5mg/l and pH 6.5-7.3. These water parameters were within the recommended range for tilapia culture (Balarin and Halton, 1979).

Evaluation of growth and nutrient utilization parameters: Growth and nutrient utilization parameters were assessed in terms of body weight gain (WTG), Percentage Weight Gain (PWG), Specific Growth Rate (SGR), Food Conversion Ratio (FCR) Gross Food Conversion Efficiency (GFCE) and Protein Efficiency Ratio (PER). The following formulas were used.

WTG = Mean final body weight-mean initial body weight

$$\text{PWG} = \frac{\text{Mean weight gain}}{\text{Mean initial weight}} \times 100$$

$$\text{SGR} = \frac{\text{Log}_e W_2 - \text{Log}_e W_1}{T_2 - T_1} \times 100$$

Where W_2 = Weight of fish at time T_2 in days
W_1 = Weight of fish at time T_1 in days
Log_e = Natural log to base e

$$\text{FCR} = \frac{\text{Weight of dry feed fed (g)}}{\text{Live weight gain of fish (g)}}$$

$$\text{PER} = \frac{\text{Gain in weight of fish (g)}}{\text{Protein consumed (g)}}$$

Statistical analysis of data: All experimental data were subjected to the analysis of variance test (ANOVA) using Microsoft software STATISTICA followed by Duncan's multiple range test (Duncan, 1955).

Results

The result of the proximate analysis of the sweet potato leaf meal is presented in Table 2. Sweet potato leaf meal had a crude protein level of 23.57% crude fat 3.07, crude fiber 8.28%, total ash, 11.01% and 49.05% for nitrogen free extract.

Table 1: Gross composition of the experimental diets (%)

Ingredient	Diet 1 0%SPL	Diet 2 5%SPL	Diet 3 10%SPL	Diet 4 15%SPL	Diet 5 20%SPL
Yellow Maize	31.85	25.18	19.15	13.73	8.82
Groundnut cake	30.20	28.56	27.07	25.75	24.15
Fish meal	12.08	11.44	10.84	10.30	9.82
Sweet potato leaf meal	-	9.99	18.97	27.30	34.37
Soybean meal	18.12	17.13	16.26	15.43	14.73
Corn oil	5.00	5.00	5.00	5.00	5.00
Bone meal	1.00	1.00	1.00	1.00	1.00
Chromic Oxide	1.00	1.00	1.00	1.00	1.00
Vitamin premix	0.50	0.50	0.50	0.50	0.50
Sodium chloride	0.25	0.25	0.25	0.25	0.25

SPL = Sweet potato leaf meal

Table 2: Proximate composition of sweet potato (*Ipomoea batatas*) leaf meal

Nutrient	Percentage composition
Moisture	4.02
Crude protein	23.57
Crude fat	3.07
Crude fiber	8.28
Total Ash	11.01
Nitrogen Free Extract (NFE)	49.05

The growth performance and feed utilization efficiencies of *Tilapia zilli* fingerlings in terms of weight gain, specific growth rate feed conversion ratio and protein efficiency ratio are presented in Table 3. The mean final weight of the fish increased from the initial value in all the dietary treatments. *Tilapia zilli* fingerlings fed on Diet 1 had the highest weight gain while Diet 5 had the poorest weight gain. The general trend was that decreasing growth rate was observed with increasing inclusion level of sweet potato leaf meal in the experimental diets. However, there were no significant differences ($p>0.05$) in the weight gain of fingerlings fed Diet 1 with those fed on Diets 2, 3 and 4. Fingerlings fed on Diet 5 had significantly ($p<0.05$) lower weight gain than the other diets.

The FCR was lowest 1.53±0.20 in fish fed on Diet 1 and highest 3.8±1.76 in fish fed on Diet 5, however, FCR values were not significantly ($p>0.05$) different in all the diets except Diet 5 which was significantly ($p<0.05$) different from other diets.

The protein efficiency ratio were not significantly ($p>0.05$) different in Diets 1, 2, 3 and 4 but were all significantly ($p<0.05$) different from Diet 5. The apparent protein digestibility of the experimental diets by the tilapia fingerlings range from 75.39% in Diet 5 to 79.79% in Diet 1.

The results of carcass composition of *Tilapia zilli* fingerlings before the commencement of the feeding trials and at the end of the experiment are presented in Table 4. There were no significant ($p>0.05$) differences in the carcass composition of *Tilapia zilli* fingerlings fed on diets containing different levels of sweet potato leaf meal. However, the initial carcass composition of fish before the feeding trials had lower moisture, crude protein and crude lipid content than the final body composition of the fish.

Discussion

When alternative sources of feedstuff such as plant protein are used in fish diets, one of the common problems is the acceptability by fish and this has to do with the palatability of the diet (Rodriguez *et al.*, 1996). In the present investigation, all the experimental diets were accepted by *Tilapia zilli* fingerlings, indicating that the levels of incorporation of sweet potato leaf meal did not affect the palatability of the diets. This might be due to the processing technique employed in this study. These drying and the grinding techniques might have reduced the antinutrient in the sweet potato leaf meal thereby increasing its palatability in *Tilapia Zilli*. This observation is in support of the work of Siddhuraju and Becker (2003), Francis *et al.* (2001) and Fagbenro (1999). These workers reported that reduction in antinutrient by different processing techniques resulted in better palatability and growth in fish.

The potentials of a feedstuff such as leaf meal in fish diets can be evaluated on the basis of its proximate chemical composition, which comprises the moisture content, crude protein, crude fibre, crude lipid, total ash and nitrogen- free extract. The proximate composition of sweet potato leaf meal in the present investigation revealed that the crude protein content was 23.57%, crude fibre 8.2% and ash 11.01%. These values were lower than the values reported by Woolfe (1992) and An (2004) for sweet potato leafmeal. These differences might be due to different environmental conditions such as soil type, harvesting time, local varieties and processing methods.

On the protein digestibility, the overall efficiency of *Tilapia zilli* fingerlings to digest protein in the experimental diets decreased as the level of sweet potato leafmeal increased in the diet. These values were in a close range (75.39-79.79%) and were not likely to be significant. This decreasing trend have been reported in diets containing black gram seedmeal (Ramachandran

Table 3: Mean growth and nutrient utilization parameters of *Tilapia zilli* fingerlings fed on experimental diets

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Initial weight (g)	2.34±0.28[a]	2.23±0.31[a]	2.09±0.18[a]	2.17±0.33[a]	2.14±0.29[a]
Final weight (g)	4.93±0.43[a]	4.67±0.48[a]	4.31±0.31[a]	4.36±0.64[a]	3.19±0.38[b]
Weight gain (g)	2.58±0.29[a]	2.45±0.46[a]	2.22±0.19[a]	2.19±0.34[a]	1.05±0.29[b]
Feed intake (g)	3.93±0.47[a]	3.74±0.52[a]	3.51±0.31[a]	3.64±0.56[a]	3.59±0.49[a]
(SGR)% day	1.69±0.19[a]	1.56±0.37[a]	1.42±0.15[a]	1.38±0.27[a]	0.01±0.57[b]
FCR	1.53±0.20[a]	1.60±0.47[a]	1.58±0.15[a]	1.67±0.15[a]	3.80±1.76[b]
PER	2.22±0.33[a]	2.23±0.58[a]	2.12±0.23[a]	2.00±0.19[a]	0.99±0.31[b]
[1]APD	79.79	77.62	77.02	75.63	75.34

Figures in the same row having similar superscript are not significantly different at p>0.05; [1]Statistical analysis not determined, as samples were pooled

Table 4: Proximate carcass composition of *Tilapia zilli* fingerlings at the start and end of the feeding trials

Carcass composition%	Initial fish	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moisture	3.27±0.02[a]	5.26±0.20[a]	5.15±0.02[a]	5.10±0.20[a]	5.00±0.02[a]	5.20±0.01[a]
Crude protein	59.0±0.20[a]	62.13±0.20[a]	63.50±0.15[a]	63.0±0.13[a]	62.80±0.10[a]	63.00±0.10[a]
Crude lipid	5.13±0.15[a]	8.50±0.50[a]	8.45±0.50[a]	8.10±0.05[a]	8.00±0.40[a]	7.85±0.20[a]
Total ash	11.14±0.60[a]	11.28±0.40[a]	11.50±0.50[a]	10.85±0.40[a]	11.00±1.30[a]	11.20±0.25[a]

Figures in the same row having similar superscript are not significantly (p>0.05) different

and Ray, 2007) diets with leaf meal (Ray and Das, 1994; Bairagi *et al*., 2004) and diets with grass pea seed meal Ramachandran and Ray, 2004; Ramachandran *et al*., 2005). The reasons for this present observation might be due to high fibre content of leafmeal and the presence of protease inhibitor in sweet potato leaf meal (Oyenuga, 1968); decreased in proteolytic enzyme activity (Falge *et al*., 1978; Eusebio *et al*., 2004) and nutrient absorption (Shiau, 1997; Olivera-Novoa *et al*., 2002). Although the nutritional quality of sweet potato leaf meal as determined by Tilapia body weight gain, specific growth rate, food conversion ratio and protein efficiency ratio was higher in fish fed the control diet (0% leaf meal) no significant (p>0.05) differences were observed in other experimental diets containing leaf meal up to 15% level. To date, there is no published information on the incorporation of sweet potato leaf meal in fish diets, available information on other leaf meals revealed that 1pil-1pil, *Leucaema leucocephala* leaf meal in the diets of *Oreochromis niloticus* at 12.5% inclusion did not affect growth, however, at high levels of inclusion, 25% or more, the growth of *O. niloticus* was adversely affected (Santiago *et al*., 1988). In the present investigation, inclusion of sweet potato leaf meal at 20% level reduced the growth rate and feed utilization of *Tilapia zilli* fingerlings.

Fasakin *et al*. (1999) reported that 30% inclusion of duckweed, *Spirodela polyrrhiza* in the diet of *O. niloticus* supported growth. Ritcher *el al*. (2003) reported that *Moringa oleifera* leaf meal could replace 10% fish meal based dietary protein for Tilapia without causing any adverse effect on fish growth.

However, Afuang *et al*. (2003) reported that solvent-extracted moringa leafmeal could replace 30% of fish meal from *O. niloticus* diets. These various workers have shown that leaf meal protein at low levels of inclusion (less than 50%) in fish diets were able to support growth, therefore, supporting the results of this study.

The proximate carcass composition data of *Tilapia zilli* fingerlings showed insignificant (p>0.05) differences in fish fed diets containing different levels of sweet potato leaf meal, these amount however increased more than the initial values. This observation is in accordance with the report of Ramachandran and Ray (2004). The body moisture and crude protein content were similar in all the experimental groups, but there were reductions in the body lipid of fish fed on sweet potato leaf meal. The reason here might be due to the reduction of the level of fishmeal lipid as the level of sweet potato leafmeal increased in the diets. This is in agreement with the results of Siddhuraju and Becker (2001) and Afuang *et al*. (2003) who observed similar reductions in body lipid of fish fed on diets containing plant-based proteins.

In conclusion, the results of this study show that sweet potato leaf meal could be included up to 15% level in Tilapia zilli diets without any negative effects on the growth and feed efficiency. Furthermore, sweet potato leaves are locally available in the tropics and can be obtained throughout the year.

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Survey of Foetal Wastages: A Case Study of Makurdi Abattoir in Benue State from 1997 to 2002

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Abstract: This study was conducted to evaluate the causes and effects of foetal wastage in the Nigeria livestock industry, following indiscriminate slaughter of pregnant cows in Makurdi Abattoir, Benue State. A total of 45, 742 were slaughtered in the abattoir, with a total of 1,508 (3.9%) fetuses recovered from 1997 to 2002. There was no significant difference (P>0.05) between fetuses and years.

Key words: Foetal wastage, protein, malnutrition

Introduction

The need for adequate human nutrition cannot be overemphasized; yet, acute protein malnutrition is endemic in most developing countries (FAO/WHO, 1983). In Nigeria, this situation has largely been due to in adequate development of the livestock sub sector of the economy. However, it is noteworthy that other factors including adequate meat inspection practices have also been contributory. An undesirable effect of this lapse in veterinary public heath duties is the indiscriminate slaughter of pregnant animals (Garba *et al*., 1992).

Today, there exist a wide disparity between the food Agricultural Organization quantity of animal protein intake (3sg/person/day) and this is subject to several factors that account for inadequate supply of meat in Nigeria, bringing less consumption of meat compared to plant sources of protein which are relatively cheaper. The animal product in the diet of an average Nigerian has been diminishing year after year due to marginal improvement in animal population and productivity (Oyenuga, 1987).

The common slaughtered animals for meat in Nigeria are Cattle goat, sheep, pig and poultry others include camel, buffaloes, donkey, horses, rabbit and others games and forest animals that are edible (Alabi, 1993). A decrease in annual growth rate of livestock population in Nigeria (CBN, 1983) annual report of 1997 showed that there was a decline in the percentage contribution of the livestock sector to the gross domestic product (GDP) between 1991 and 1995. However, marginal increase was recorded in the absolute worth of the sub sector between 1993 and 1995. if the situation is compared with the rapid growth in human population of 21% per annum and the diminishing disposable income, an average growth rate of 1.6% per annum in the livestock production index holds a grim prospect for animal protein supply and this situation is tragic.

The economic recession that has been witnessed in Nigeria since the 1980s has brought in its wake a deterioration in the quality and quantity of animal protein in the diet of Nigerians. This has also dictated new trends in ameliorating the situation. This has entailed the slaughtering of not only prime breeding males but also pregnant animals resulting in foetal wastages, as reported by different workers with respect to camels (Ataja and Uko, 1994) small ruminants (Ogwuegbu, *et al*., 1987) and cattle (Oyekunle *et al*., 1992). Ataja and Uko (1994) found that 24.06% of female camels slaughtered for meat in Sokoto abattoir in 1992 were pregnant. Oyekunle *et al*., 1992) reported that between 14% and 20% of cows slaughtered in Abeokuta and ijebu-igbo abattoirs from 1984 to 1989 were pregnant.

The slaughter of pregnant domestic animals vies-avis cattle, goat and sheep will no doubt worsen the already precarious supply of animal protein to the populace (Abdullahi, 1985). It is most uneconomical to continue the practice of slaughtering pregnant animals, a situation that greatly threatens the Nigeria livestock industry. One possible actor contributing to the high rate of slaughter of pregnant cows is the season of the year. In analyzing the effect of draught on the effect of draught on livestock in sub Saharan Africa, Toulmm (1984) observed that at the extreme dry periods, herders increased their sales of aged cows and less productive females in order to meet house hold cash needs. As the dry season progressed and the stress on cattle increased, herders were compelled to liquidate pregnant females before they die naturally. Most livestock farmers sell off their animals without considering the fertility of the stock before selling off due to illiteracy and poverty and or diseases condition of the animal. It therefore becomes necessary of study the pattern of fetal wastages with Benue State as a case study.

Benue State is said to be the food basket of the Nation. The links before the North and Southern States of Nigeria and as such form a good case study.

Materials and Methods

The study was carried out in Makurdi Metropolis. The primary data used for the study were obtained from the livestock division, Benue State Ministry of Agriculture, Makurdi. The data which covered a period of six years (January, 1997 to December, 2002) were collected and analyzed from Makurdi.

The completed meat inspection forms at the Makurdi abattoir. The number of cattle slaughtered annually was calculated and the sex incidence was obtained with the number of fetuses recovered at the time of slaughter though not classified according to the trimester of pregnancy.

A total of 20 farmers (Fulani herds men) and 20 butchers were orally interviewed on the reasons for premature slaughter of pregnant females.

Results were analyzed using a chi-square test. Analysis of variance (ANOVA) was also employed to test the significance difference between the numbers of fetuses with the number of years.

Graphs were plotted using micro soft excel window XP.

Results and Discussion

Figure 1 revealed yearly trend of fetal wastages at Makurdi, abattoir, 1998 had the highest peak of wastage and there was also significant rise within the years determined by different seasons of the year.

While Fig. 2 shows the monthly increase of fetal wastage at the abattoir, there was a rise in the months of May to July with the highest peak in June. There was also a significant rise in September.

The rise in fetal wastages from 1997 having the highest peak may have been as a result of the economic hardship that prevailed around that period. Perhaps farmers need money to send children to school and meet some other domestic needs.

The season of the years under review also shows that the rains are just about to begin in the months of May-June.

These periods are characterized by drought, hunger which expose animals to poor nutrition, diseases and as such to forestall losses due to natural death or diseases farmers prefer to sell their animals. Also the problem of anthelheminthes occurs at unset of rains and the ends of rains and cost of treating the animals may also be another reason why farmers sold their animals.

The reports of Beckm *et al.* (1974) showed that 70% of the cattle slaughtered during the extreme dry periods were females, compared to 30% during the normal periods of the year. Germen (1975) also observed a similar phenomenon, that most of the cattle sold for slaughter during the dry season were females. The rise in wastage in September of most of the years May be as result of emergence of festivals and ceremonies, this period farmers need money for marriages, Christmas and Moslem Festivals.

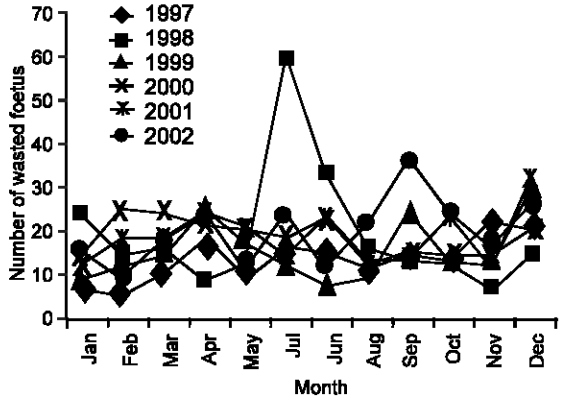

Fig.1: Monthly incidence of fetal wastage at Makurdi abattoir

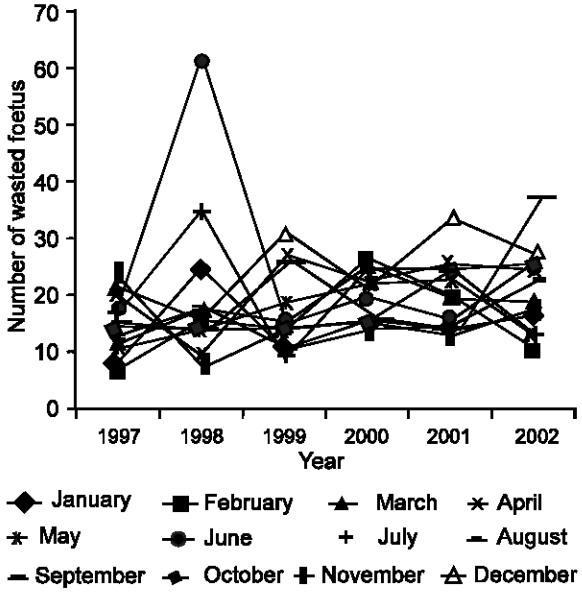

Fig. 2: Yearly incidence of fetal wastage at Makurdi abattoir

Looking at the relatively high rate of calf wastage in Makurdi abattoir, two important questions come to mind. First, what are the effects of this on the supply of beef in a Country where meat production lags behind Consumption? And secondly what are the economic implications of this for both total and per caput meat production and consumer and the policy implications for adequate planning of the livestock sector in Nigeria? The net effect of the continuous calf wastage would mean a reduction in both the consumer and the producer welfare, through meat shortages and reduced farmer increase. Nigeria (through Makurdi abattoir, Benue State alone) loses about 3.9 percent of its future productive herd as a result of the indiscriminate slaughtering of pregnant cows. An important factor contributing to the increased slaughter of pregnant cows

in Nigeria is the poor enforcement of existing livestock legislation. Three important policy objectives are emphasized in Nigerians livestock sector to increase domestic animal protein production so as to attain national self sufficiency in meat production; to increase farmer's income in order to raise their standard of living and to cut down on meat imports thereby conserving scarce foreign exchange. Although government regulations discourage the slaughter of pregnant cows, the structure of the formal cattle marketing and slaughter system in Nigeria has flaws limiting their application. As a result of the lax enforcement of existing regulations, trade cattle markets for abattoirs without routine veterinary checks.

Attempts to reduce meat deficits in Nigeria must focus on ways of reducing calf wastage during slaughter. Government intervention in cattle marketing remains essential, particularly in the enforcement of policies relating to the sale of pregnant cows for slaughter. Policy efforts must concentrate on instituting routine veterinary checks at cattle control posts and abattoirs. In addition, producers of cows in order to avoid disposing of them during calving season. If meat supplies are to be maintained or increased to meet future domestic demand, the incidence of slaughtering pregnant cows must be reduced or halted completely.

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Effects of Salt Concentrations on the Functional Properties of Some Legume Flours

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Abstract: The effect of various salt concentrations on the functional properties of gourd seed, white melon, yellow melon and bulma cotton seed was investigated. The studies on the effect of salts commonly used in food industries such as NaCl, KCl, Na_2SO_4, K_2SO_4 and CH_3CO_2Na on the functional properties of the sample suggested that water absorptivity, emulsification, foamability and gelation were affected by the salts and that these effects were functions of the types of salt considered and their various concentrations. The data generated showed that salts may be used at appropriate concentrations to enhance or inhibit the functionalities of the flours.

Key words: Salt concentrations, legume flours, gourd seed, white melon, yellow melon, bulma cotton seed

Introduction

Plant sources of protein are the major ways of protein intake in many developing nations (Oshodi, 1992). Some of these nations are in dearth food supply especially that of protein. Legumes are increasingly used by thickly populated regions of the world to alleviate the impasse of low protein diet. However, some of the functionality of these legumes have not been well articulated and are termed underutilized. Some of these under utilized seeds such as gourd seed, white melon, yellow melon and bulma seed are reported in this present paper.

Gourd seed (*Legarania vulgaris*) is an annual herb with a climbing or trailing habit. The leaves are about 10-40cm wide. Sometimes with 3-7 lobes and hairy on the lower surface. It belongs to the family of cucubitaceae which is found predominantly in tropical Africa. The leaves are edible when cooked and seeds can either be roasted or used as soup condiment.

White melon (*Cucumeropsis edulis*) is an annual climbing vine with lobed flowers 13-20cm in diameter. The fruit is about 18cm in length with shinning and white flesh. The mature fruit may be ready for harvesting within 200 days from the planting period and normally 2-5 fruits weighing 0.8-1.5kg are produced per plant.

Yellow melon (*Colocynthis citrullus*) is a creeping annual crop which belongs to the gourd (*cucubitaceae*) family. It possesses hairy stems, triangular leaves and small yellow flowers. It is widely cultivated plant in Nigeria (Aoyenuga, 1978; Akobundu *et al*., 1982). It is very popular condiment in local soup when cooked and mixed with pepper, onions, palm oil, fish/meat and salt. It forms well known "egunsi soup" which can compliment carbohydrate foods.

Bulma cotton seed (*Cochlospermus reliogiosum*) is a native of tropical Asia and it is cultivated in several parts of West Africa especially Nigeria. The fruits are often hairy obriod capsule endocarp and the seeds are covered with long cotton hairs or silk. The long cotton hairs are closely or loosely attached to membranous outer testa. The seeds can serve as condiment, sources of foods and soup ingredient when toasted. Oshodi and Ekperigin (1989) reported that in order to successfully introduce a new supplementation into any food item, it is important to find out whether the supplementation possesses appropriate functional properties for food application and consumer acceptability.

The addition of salt may increase the total water content of the protein system at specific water activity value, although it may decrease the preferential binding of water to the protein. These effects are marked by dependent on anion and cation components (Sathe and Salunkhe, 1981; Altchul and Wilke, 1985; Oshodi and Ojokan, 1997; Ogungbenle *et al*., 2002).

The effect of salt is significant because, in many foods, salt concentrations are approximately 0.2-0.3M (Altchul and Wilke, 1985). This work will provide information on the selective use of salt for the enhancement of food properties.

Materials and Methods

The gourd seed, white melon, yellow melon where purchased from Erekesan market in Akure while bulma cotton seed was harvested from University of Ado Ekiti, Nigeria. The whole seeds were thoroughly washed in distilled water and air dried, then made into flours using blender. The flours were packaged in polythene bags and stored in freezer until used. The salts used for the study were: NaCl, KCl, Na_2SO_4, K_2SO_4 and CH_3CO_2Na. The concentrations of salt solutions used were: 0.5 1.0, 2.0, 5.0 and 10.0% w/v respectively. The procedure of Inklaar and Fortuin (1969) was used for the emulsion capacity and stability. Two grams of protein flour was made into a slurry in $40cm^3$ of water in a conical flask by

Table 1: Least gelation concentration of the samples in various salt concentrations

Samples	Salt/%	0.0	0.5	1.0	2.0	5.0	10.0
A		18	14	10	12	12	12
B		6	6	4	4	4	4
C	KCl	12	10	8	6	6	4
D		16	16	14	14	12	12
A		18	16	16	14	14	14
B		6	6	4	4	4	4
C	NaCl	12	10	10	8	8	6
D		16	16	16	14	12	12
A		18	14	14	14	12	12
B		6	6	6	6	4	4
C	K_2SO_4	12	10	10	10	8	6
D		16	14	14	12	12	12
A		18	14	14	14	12	12
B		6	6	6	4	4	4
C	Na_2SO_4	12	10	10	8	8	6
D		16	14	14	14	12	12
A		18	16	16	16	14	14
B		6	4	4	4	4	4
C	CH_3COONa	12	10	10	10	8	8
D		16	16	16	14	12	12

A = Gourd seed, B = White melon, C = Yellow melon, D = Bulma cotton seed

stirring at 1000 rpm for 15 min. After 10cm^3 of vegetable oil was added over a period of 5min with stirring at 1000 rpm, stirring was continued for some minutes. The system was transferred to a centrifuge tube, heated in a bath maintained at 85°C for 15min with occasional stirring and then cooled for 15min in a water-bath maintained at 25°C. The tube was finally centrifuge at 3000 rpm until the volume of oil separated from the emulsion was constant. Results were expressed as percentage of oil that separated from emulsion layer.

Foaming capacity and foaming stability were determined by method (Coffman and Garcia, 1977). 2% w/v salt solution was homogenized for 1 min in a food blender at maximum speed and the contents were immediately poured into a 100cm^3 graduated cylinder. Foaming stability was the foam volume increase immediately after mixing. Foaming stability was measured as the foam volume after 2h.

Water absorption capacity was determined by method (Beuchat, 1977). One gram of the flour was mixed with 10cm^3 of the salt solutions. The sample was allowed to stand at 25°C for 30min. centrifuged at 5500rpm for 30min and the volume of the supernatant noted in a 10cm^3 graduated cylinder. Density of water assumed to be 1.000g/cm^3. The volume of water absorbed was converted to gram.

The method of Sathe *et al.* (1982) was used for lowest gelation concentration with slight modification. Sample suspensions of 2-20% w/v were prepared in salt solution. From each suspension, 10cm^3 was put in a test tube and heated for 1h in a boiling water bath followed by a rapid cooling in a cold water bath. The test tubes were further cooled for 4°C for 2h. The lowest gelation was determined as that concentration which did not fall when the test tube was inverted.

The experimental procedures were slightly modified by exchanging distilled water with appropriate salt solutions.

Results and Discussion

Table 1 indicates the variation of least gelation concentration in the various concentration of salts. The least gelation concentrations for gourd seed, white melon, yellow melon, bulma cotton seed are 18.0, 16.0, 12.0 and 16.0% respectively. The addition of salts resulted in a general decrease in least gelation concentration which depended on the concentration and nature of salts under consideration and values obtained in the presence of salts ranged 12-16%, 14-16%, 6-10% and 12-16% for gourd seeds, white melon, yellow melon and bulma cotton seed respectively. It was observed that the addition of salts at relatively low concentration (0.5%) improved the gel forming properties of the samples studied and this effect was well pronounced with the use of KCl and Na_2SO_4. The results obtained in the presence of salts especially for bulma cotton seed are higher than those of benniseed reported (Oshodi and Ojokan, 1997) and bovine plasma protein concentration (2% to 4%) reported (Ogungbenle *et al.*, 2002). At high concentration of salts (10.0%), the gelling capacities of the samples are improved better than at low salt concentrations. The ability of protein to form gels and provide a structural matrix for holding water, flavours, sugars and food ingredients is useful in food applications and in new products development. The values obtained for emulsion capacity and stability for the samples are presented in Table 2 and 3. These results showed that the samples have good emulsion capacity and stability depended on the salt concentrations and type of salts under consideration. There was steady and progressive

Table 2: Emulsion Capacity of the Samples in Various Salt Concentrations

Samples	Salt/%	0.0	0.5	1.0	2.0	5.0	10.0
A		18.5±0.02	17.1±0.2	17,6±0.1	17.8±0.4	18.3±0.1	16±0.5
B		175±0.3	95±0.3	94±0.4	92.5±0.1	91±1.0	90.5±0.2
C	KCl	61±0.9	59±0.2	56±0.3	57±0.1	56±0.4	55.5±0.1
D		29±0.4	26±0.2	25±0.3	25.8±0.6	26±0.5	24±0.3
A		18.5±0.01	17.8±0.4	17.5±0.1	17±0.3	16±0.2	15±0.1
B		175±1.0	94.1±0.9	93±0.2	92±0.1	91±0.7	90±0.3
C	NaCl	61±0.8	58±0.2	57±0.1	56±0.3	55±0.1	54±0.4
D		29±0.3	25±0.4	24.8±0.6	24.5±0.1	25±0.2	23±0.5
A		18.5±0.03	17.5±0.01	16±0.1	15.5±0.3	15±0.1	14±0.3
B		175±0.8	91±0.6	88±0.3	91±0.2	93±0.4	92.5±0.3
C	K_2SO_4	61±0.3	62±0.2	61.5±0.1	61±0.4	62±0.5	59±0.9
D		29±0.5	28±0.2	27.5±0.1	27.8±0.3	28±0.9	24.5±0.5
A		18.5±0.3	17±0.04	15±0.3	14±0.2	13.5±0.1	13±0.3
B		175±0.9	96.5±1.0	96.0±1.5	95±0.7	94±0.8	93±0.9
C	Na_2SO_4	61±0.2	62±0.2	61.5±0.1	61±0.4	62±0.5	59±0.9
D		29.0±0.6	24±0.3	23±1.0	23.5±0.3	24±0.1	19±0.4
A		18.5±0.1	17.9±0.3	17.8±0.2	17.5±0.4	17±0.1	16±0.2
B		175±2.0	97±1.0	96±0.8	95±0.6	94±0.5	93.5±0.4
C	CH_3COONa	61±0.6	58.5±0.3	58±0.1	57±0.2	56±0.6	55±0.5
D		29±0.4	27±0.1	27.2±0.3	27.5±0.7	25.5±0.3	24±0.4

Table 3: Emulsion Stability of the samples in various salt concentrations after 24hr

Samples	Salt/%	0.0	0.5	1.0	2.0	5.0	10.0
A		0	9.2±0.3	9.3±0.1	8.5±0.5	8.0±0.2	8.3±0.1
B		0	83 V 0.4	84.9±0.1	82.8±0.3	85±0.2	86±0.4
C	KCl	0	26±0.3	24. 5±0.6	25.6±0.1	25.6±0.3	24±0.5
D		0	12.5±0.4	11.6±0.1	13.5±0.3	14±0.1	13.3±0.4
A		0	9±0.01	9.5±0.03	8.0±0.04	7.5±0.05	7.9±0.02
B		0	82.5±0.1	84.8±0.3	82.5±0.4	87.1±0.2	85±0.1
C	NaCl	0	25±1.0	23.5±0.3	24±0.2	22±0.1	23±0.5
D		0	12±0.1	10±0.3	13±0.2	13.5±0.1	12.5±0.4
A		0	7.0±0.1	8.0±0.2	8.5±0.3	7.5±0.1	7.6±0.2
B		0	80.2±1.0	81.2±0.9	81.5±0.8	82.5±0.3	83±0.2
C	K_2SO_4	0	26±0.9	28±0.7	26±0.1	25±0.2	25.5±0.3
A		0	6±0.3	8±0.5	7±0.1	6.0±0.3	6.5±0.4
B		0	81.2±0.3	84.0±0.5	85.0±0.1	87.0±0.6	83.5±0.3
C	Na_2SO_4	0	27 V 0.3	28±0.5	30±0.3	25±0.1	23±0.4
D		0	13±0.4	12±0.2	14±0.4	12.5±0.3	11±0.5
A		0	8±0.02	9±0.01	8.5±0.03	8.0±0.02	8.2±0.03
B		0	82.7±0.3	84.9±0.2	85±0.1	85.9±0.4	84.0±0.4
C	CH_3COONa	0	24±0.5	26±0.4	23±0.3	22.5±0.1	23.5±0.6
D		0	15±0.6	14±0.4	16±0.3	17±0.1	13.5±0.5

A= Gourd Seed, B = White melon, C= Yellow melon, D= Bulma cotton seed

increase in emulsion capacity with salt concentrations up to 5.0% salt. There was a general decrease in emulsion capacity as the salt concentration was increased for other samples studied up to 10% salt solutions. Table 3 indicates the emulsion stability and it was measured by the volume of water separated after some time. Table 3 further shows that no water was separated after 24h in the absence of salts and there was also a steady increase in the quality of water separated in the presence of salts used. The degree of water separated varies from salt to salt. In the presence of CH_3COONa, Na_2SO_4 and NaCl, the value of water separated from the emulsion of the samples was almost consistent up to 5% of these salts while the presence of KCl and K_2SO_4 the amount of water separated increases progressively as the salt concentration increased. Three separate mechanisms that appear to be involved in the formation of a stable emulsion may be (i) reduction of interfacial tension, (ii) electrical charge and (iii) formation of a rigid interfacial film (Mcwatters and Cherry, 1981). The surfactancy of proteins is related to their ability to lower the interfacial tension between water and oil in the emulsion. The surface activity is a function of the ease with which protein can migrate to absorb at, unfold and rearrange at an interface and presumably salts reduce the surface activity of gourd, white melon and bulma cotton seed and thereby increase interfacial tension which leads to a decrease in emulsion capacity. Salts may also reduce charge repulsion between the proteins and enhance hydrophilic association at the interface (Kinsella, 1979).

Table 4: Foaming capacity of the samples in various salt concentrations at zero hour

Samples	Salt/%	0.0	0.5	1.0	2.0	5.0	10.0
A		18.7±0.05	19.2±0.1	21±0.3	19±0.5	23±0.4	24.5±0.2
B		6±0.02	4.3±0.01	6.1±0.03	8.3±0.2	9±0.04	12.5±0.1
C	KCl	24.7±0.2	26.5±0.5	26±0.1	28±0.8	29.8±0.1	30.5±0.3
D		14.7±0.2	19±0.1	19.5±0.5	21±0.3	23±0.1	24±0.3
A		18.7±1.0	19±0.3	20.5±0.2	18±0.1	22±0.3	23±0.4
B		6±0.01	4.2±0.03	6.0±0.06	8±0.01	8.5±0.03	13±0.0.4
C	NaCl	24.7±0.1	26±0.2	25±0.1	28±0.6	29.5±0.4	30±0.3
D		14.7±0.1	18±0.3	19±0.3	20±0.5	22±0.3	23±0.2
A		18.7±0.2	20±0.1	22±0.3	23±0.3	25±0.1	26±0.4
B		6±0.03	3.5±0..4	4±0.01	4.5±0.02	6±0.04	8±0.06
C	K_2SO_4	24.7±0.8	28±0.4	26±0.1	30±0.3	32±0.8	34±0.3
D		14.7±0.6	20±0.9	19±0.5	20±0.8	21±0.2	22±0.3
A		18.7±0.3	24±0.1	25±0.2	20±0.3	23±0.2	24±0.1
B		6±0.06	5±0.01	4±0.02	3.5±0.04	4.2±0.05	6±0.1
C	Na_2SO_4	24.7±0.6	28.5±0.5	.275±0.4	31±0.2	33±0.1	35±0.9
D		14.7±0.6	21±0.2	20.5±0.1	21.9±0.4	22±0.3	24±0.1
A		18.7±0.1	19.8±0.3	21.0±0.4	22±0.5	22.5±0.4	24.0±0.1
B		6±0.01	6.9±0.04	8.0±0.0.6	10±0.01	12±0.02	2.5±0.01
C	CH_3COONa	24.7±0.6	27±0.1	26.0±0.3	29±0.2	30±0.3	32±0.4
D		14.7±0.8	18.5±0.2	18.8±0.4	21±0.5	23±0.1	24±0.6

Table 5: Foaming stability of the samples in various salt concentrations at zero hour

Samples	Salt/%	0.0	0.5	1.0	2.0	5.0	10.0
A		9.0±0.2	10.5±0.1	15±0.1	16±0.3	20±0.5	22±0.1
B		5.5±0.02	5.6±0.3	6.5±0.03	7±0.1	7.2±0.6	8±0.4
C	KCl	12.5±0.1	13.8±0.5	13.9±0.4	14.1±0.1	14.8±0.1	15±0.2
D		7.0±1.0	8.5±0.8	9.0±0.1	9.5±0.3	11±0.4	12±0.5
A		9.0±0.1	10±0.7	14±0.4	15.5±0.2	19.5±0.5	20±0.8
B		5.5±0.9	5.8±0.3	6.0±0.4	6.5±0.3	7.0±0.4	7.8±0.6
C	NaCl	12.5±0.2	26±0.6	23.6±0.1	14±0.3	14.2±0.2	14.5±0.1
D		7.0±0.9	8.2±1.5	0.5±0.8	9.0±0.4	9.5±0.5	10.5±0.1
A		9.0±0.6	11±0.5	15±0.1	17±0.2	21±0.2	24±0.8
B		5.5±0.1	5.9±0..3	7.3±0.05	7.5±0.4	8±0.1	9.3±0.2
C	K_2SO_4	12.5±0.1	14.1±0.4	14.9±0.5	15±0.6	15.2±0.8	15.4±0.3
D		7.0±0.5	10.5±0.8	12±0.4	12.5±0.2	13±0.3	16±0.8
A		9.0±0.3	12±0.8	16.5±0.6	17±0.3	20±0.2	23.5±0.5
B		5.5±0.2	6.5±0.3	7.0±0.4	9±0.01	9.2±0.3	10±0.2
C	Na_2SO_4	12.5±0.6	14±0.2	14.5±1.0	14.9±0.6	15±0.9	15.8±0.6
D		7.0±0.8	10±0.5	11.5±0.8	12±0.4	12.8±0.3	15±0.1
A		9±0.9	13±0.2	16.5±0.1	18±0.9	22±0.3	25.0±0.9
B		5.5±0.01	6.0±0.3	7.5±0.2	10±0.4	10.3±0.1	11±0.5
C	CH_3COONa	12.5.±0.5	14±1.0	15.1±0.3	15.8±0.2	15.9±0.3	16.2±0.6
D		7.0±0.6	9.5±1.0	10±0.9	11.5±0.9	13±0.1	15±0.1

A = Gourd Seed, B = White melon, C = Yellow melon, D = Bulma cotton seed, Error as standard deviation

The observed decrease in emulsion stability (Table 3) may be due to increased contact leading to coalescence which thereby reduces stability (Parker, 1987).

The effect of salts on the foaming capacity is presented in Table 4. The foaming capacity depends on the type of salt under consideration. For most of the salts used, there was an increase in foaming capacity of the samples with increase in concentration of salts from 0.5% to 10%. This may be due to the fact that salts usually reduce surface viscosity and rigidity of protein films but increase spreading rate, thereby weakening interpeptide attractions and increasing foam volume for certain protein (Altchul and Wilke, 1985). In the case of bulma cotton seed, salts at appropriate concentrations aid foaming, presumably by aiding diffusion and spreading at the interface, but high levels of salts will depress foaming (Altchul and Wilke, 1985). The improved foaming capacity of these samples studied in the presence of salts may consequently improve their functionalities to be useful for the production of cakes (Johnson *et al.*, 1979; Lee and Love, 1993) and whipped topping where foaming is an important property (Kinsella, 1979). Foaming stabilities after 24hrs are shown in Table 5 which, imply that all salts used at various concentrations had significantly improved the foaming stabilities of the samples studied.

The results for water absorption capacities of gourd seeds, white melon, yellow melon and bulma cotton seed in different salt solutions are presented in Table 6.

Table 6: Water Absorption capacity of the samples in various salt concentrations

Samples	Salt/%	0.0	0.5	1.0	2.0	5.0	10.0
A		100±0.3	65.0±0.3	48.6±0.1	45.0±0.2	35.0±0.1	27.5±0.3
B		200±0.8	160±0.5	140±0.1	130±0.7	126±1.0	120±0.2
C	KCl	117±1.2	80±0.6	70±0.3	65±0.1	33±0.5	30±0.1
D		175±1.8	90±0.1	78±0.3	75±0.4	62±0.2	50.6±0.1
A		100.0±0.9	60±0.1	45.5±0.2	40±0.4	30±0.1	25±0.3
B		200±0.9	155±0.1	135±0.3	125±0.9	122±0.1	109±0.6
C	NaCl	117±1.0	58±0.8	50±0.1	40±0.2	35.5±0.4	30±0.3
D		175±1.5	88±0.6	75±0.1	70±0.2	50±0.3	40.5±0.5
A		100±0.1	80.5±0.2	80±0.3	70±0.1	60.5±0.2	50±0.1
B		200±0.5	195±1.0	190±0.8	175±0.9	170±1.5	160±0.6
C	K_2SO_4	117±0.9	94±0.6	80±0.3	70±0.4	55±0.3	50±0.5
D		175±1.0	160±0.8	150±0.5	145±0.2	140±0.1	130±0.9
A		100±0.2	80±0.1	70.5±0.3	60±0.2	50±0.5	40.5±0.2
B		200±2.0	155±0.3	135±1.0	125±1.5	122±0.7	109±0.5
C	Na_2SO_4	117±1.3	95±0.1	85±0.2	75±0.1	60.5±0.7	60.0±0.6
D		175±1.3	150±0.6	149±0.8	140±1.5	130±1.0	120±0.8
A		100±0.8	59±0.7	40±0.1	30.5±0.3	30±0.2	25±0.1
B		200±1.0	150±0.3	140±0.4	135.5±0.3	129±0.1	120±0.5
C	CH_3COONa	117±1.5	58.5±0.6	54±0.3	40.5±0.1	35±0.2	30±0.4
D		175±0.9	87.5±0.6	73±0.8	65.5±0.5	45.5±0.6	40±0.1

A = Gourd Seed, B = White melon, C = Yellow melon, D = Bulma cotton seed, Error as standard deviation

The water absorption capacities in distilled water were found to be 100% for gourd seed, 200% for white melon, 117% for yellow melon and 175% for bulma cotton seed. These values are lower than that of protein concentrate of Adenous breviflorus benth flour (201%) reported (Oshodi, 1992). But this value is also lower than quinoa (147%) and pearl millet (115%) reported (Oshodi *et al*., 1999), sun flower protein concentrates (107% and 137%) reported (Lin, 1974) and some varieties of lima bean (*Phaseolus lunatus*) reported (Oshodi and Adeladun, 1993) (130. 1-142.2%). The present values reported for white melon and bulma cotton seed were comparable and fall within the range of some seeds reported (Lin, 1974). This implies that high water absorption of these samples may make them more susceptible to heat denaturation (Kinsella, 1979). Table 6 further indicated a progressive decrease in water absorptivities with increase in salt concentration generally up to 10%. The decrease or increase in water capacity varies with type of salt. This may be due to the fact that the effect of salt vary with the cation and anion species involved (Kinsella, 1979). The observed trend at low salt concentrations may be due to masking of charges which may reduce electrostatic interaction and hydration but increase hydrophobic interaction. At high concentrations of salts, the macro molecules preferentially bind the ions, that is, the proportion of water to salt in the vicinity of macro molecules is greater than the ratio in bulk phase solvent (Kinsella, 1979). Salts bound to protein may depend on the ions and the ability of the ions to enhance hydration, when protein attracts such ions (electrostatic effect). The moderately high water absorptivity in the presence of Na_2SO_4 compared to other salts used may be due to the high hydration potential of Na_2SO_4 (Kinsella, 1979). At high salt concentration (10%), electrostatic effects are apparently of little importance with regards to the amount of water bound to protein because of competitions between the ions and proteins for water become predominant (22, Kinsella, 1979). Furthermore, at low concentrations of salts (ions), macro molecules preferentially bind the ions, that is more ions are associated with the macromolecule than in the case of bulk solution. Therefore, water absorptivity at relatively low salt concentrations obtained may be an advantage in the production of meat analogues where the capacity of the matrix to imbibe and hold water and to stimulate the juiciness and texture of the product is critical (Ogungbenle *et al*., 2002). However, it is pertinent to note that water binding capacity caused by addition of salt may cause the protein to imbibe disproportionate amount of water and dehydrate other components in the food system or vice versa for example, in bread baking the water binding capacity of added protein extender must be compensated to ensure proper hydration of flour protein (Kinsella, 1979).

Conclusion: It can be concluded that the functionality and potentiality of these flours can be improved/ inhibited by selective application of salts at appropriate concentrations.

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Effect of Non Forage High Fibrous Feedstuffs as Fiber Sources in Total Mixed Ration on Gas Production Characteristics and *in vitro* Fermentation

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Abstract: The objective of this study was to determine the effect of non forage high fibrous feedstuffs as fiber sources in a total mixed ration on gas production characteristics and *in vitro* fermentation using an *in vitro* gas production technique. The experiment was designed in CRD with five replicates per treatment. The fiber sources in the total mixed ration were rice straw (rt-TMR), tomato pomace (tp-TMR), palm meal (pm-TMR), dried brewer gain (db-TMR) and soybean hulls (sh-TMR). The results showed that kinetic gas production, *in vitro* dry matter digestibility and *in vitro* organic matter digestibility, were significantly different among treatments ($p<0.05$). The soybean hulls as a fiber source in the total mixed ration gave the highest IVDMD, IVOMD and gas production parameter. *In vitro* fermentation end-products consisting of NH_3-N, TVFA and pH were significantly different among the treatments ($p<0.05$); however, the pH values were relatively stable at 7.01-7.16. All treatment means were within the normal range. NH_3-N concentration was in the optimal range for rumen ecology microbial activity. Future research should investigate the impact of the ability of non forage high fibrous feed to replace forage in intact animal.

Key words: Non forage high fibrous feedstuffs, total mixed ration, fermentation and *In vitro*

Introduction

In recent years, feeding cattle a total mixed ration (TMR) has become widely accepted. The benefits of a TMR include increased milk production, enhanced use of low cost alternative feed ingredients, ability to control the forage concentrate ratio, lower incidence of metabolic and digestive disorders and reduced labor input for feeding. Silage, forage and hay are conventional roughages found in TMR. Long hay, however, when added to the TMR, becomes a problem for mixing machines, as such, it is recommended to reduce the particle size of long hay prior to adding it to the machine. Chopping long hay to reduce particle size is expensive and time consuming. Non forage high fibrous feed is an alternative fibrous feed for ruminant. In tropical zones, there are many varieties of non forage high fibrous feedstuffs: tomato pomace, soybean hull, palm meal, leucaena meal, coconut meal, mung bean meal and dried brewer grain are abundant. The degradation characteristics of non forage high fibrous feedstuffs are the same as forage (Chumpawadee *et al*., 2005, 2006). The fiber source of TMR is very important because it can affect feed intake, chewing activity, digestibility and production. Soybean hulls appeared to be mixed in a total mixed ration of about 20-25% and they did not affect dry matter intake and production (Sukulthanasorn *et al*., 2007; Grant, 1997). Additionally, tomato pomace can be fed at 100 % as replacement forage for dairy cows and beef cattle (Sanitwongnaayutaya, 2005).

However, the non forage high fibrous feeds are small in particle size and the effective NDF is much lower. The NRC (1989) recommends a minimum of 25 % NDF in total dietary DM, when used as traditional forage and concentrate combinations. Grant (1997) recommends, when using non forage high fibrous feeds as a fiber source, it should be up to 7-10 % from NRC recommendations.

With respect to non forage high fibrous feeds in tropical zones, limited information is available on its use as a fiber source of TMR. The aim of this study was to investigate the *in vitro* fermentation using TMR from different fiber sources.

Materials and Methods

Preparation of TMRs: Non-forage high fibrous feedstuffs and others were collected from various feed mills and organizations in the northeast of Thailand. All feed samples (Table 1) were ground to pass through a 1 mm screen for chemical analysis. The feedstuff samples were analyzed for dry matter (DM), crude protein (CP) and Ash (AOAC, 1990), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (Van Soest *et al*., 1991).

Five TMRs were formulated, to have similar total digestible nutrient (TDN), CP, NDF, ADF, but differ in fiber source (Table 2). The experiment was designed in CRD with five replicates per treatment. The fiber sources of the total mixed ration were rice straw (control), tomato

pomace, palm meal, dried brewer gain and soybean hulls. Five TMRs for the gas production test were ground to pass through a 1 mm screen in a hammer mill.

***In vitro* gas production test:** Strict anaerobic techniques were used in all steps during the rumen fluid transfer and incubation period. Rumen fluid inoculum was removed before the morning feeding under vacuum pressure via the rumen fistula into a 2 liter glass flask and transferred into two pre-warmed 1 liter thermos flasks which were then transported to the laboratory. The medium preparation was as described by Makkar *et al.* (1995). Mixed rumen fluid inoculums were obtained from two fistulated Brahman-Thai native crossbred steers (weighing about 250±15 kg). The animals were offered rice straw on *ad libitum* and fed 0.5 % body weight of concentrate (concentrate mixture: 49.80% cassava chip, 17.5% rice bran, 14.60% palm meal, 7.0% soybean meal, 1.40% urea, 0.4% salt, 1.0 % mineral mix and 8.30% sugarcane molasses). The animals were fed twice daily; water and a mineral lick were available *ad libitum* for 14 days.

The feed sample of approximately 500 mg on a fresh weight basis was transferred into a 50 mL serum bottle (Sommart *et al.*, 2000). The bottles were pre-warmed in a hot air oven at 39°C for about 1 hour prior to injection of 40 mL of rumen fluid medium (using a 60 mL syringe) to each bottle. The bottles were stoppered with rubber stoppers, crimp sealed and incubated in a hot air oven set at 39°C.

The rate of gas production was measured by reading and recording the amount of gas volume after incubation using a 20 mL glass syringe connected to the incubation bottle with a 23 gauge, 1.5 inch needle. Readings of gas production were recorded from 1 to 72 h (hourly from 1-12 h, every 3 h from 13-24 h, every 6 h from 25-48 h and every 12 h from 49-72 h) after incubation periods. Amounts of cumulative gas volume at 2, 4, 6, 12, 24, 48 and 72 after incubations were fitted using the equation $y = a+b [(1-Exp(-ct)]$ (Orskov and McDonald, 1979), where a = the intercept, which ideally reflects the fermentation of the soluble fraction, b = the fermentation of the insoluble fraction, c = rate of gas production (a+b) = potential extent of gas production, y = gas production at time 't'.

In vitro digestibility of dry matter and organic matter was measured at 72 h after incubation. The residues of the TMRs were removed by filtering through a glass filtering crucible, residue was washed with 250 mL boiled distilled water and the amount of DM and OM in the residue was estimated. Calculation of *in vitro* DM and OM digestibility as a percent of total DM and OM followed the equation: % *In vitro* DM or OM digestibility = [(DM or OM initial - DM or OM after incubate) / DM or OM initial] x 100.

***In vitro* fermentation measurement:** The bottles were sampling at 0, 3, 6, 9 and 12 h after incubation. Rumen fluid medium pH was measured immediately after sampling using a portable pH meter. The rumen fluid medium was acidified with 5 mL 6 N HCl and centrifuged at 3000 rpm for 15 minutes and the clear supernatant was stored in plastic tubes at -20°C until analyzed for ammonia nitrogen (Bremner and Keeney, 1965) and total volatile fatty acid concentration (Briggs *et al.*, 1957).

Statistical analyses: All data obtained from the trials were subjected to the analysis of variance procedure of statistical analysis system (SAS, 1996) according to a completely randomized design. Means were separated by Duncan New's Multiple Range Test. The level of significance was determined at $P<0.05$.

Results and Discussion

Chemical composition of feedstuffs and TMRs: The chemical compositions of feed ingredients used in the experiment are shown in Table 1. The feed ingredients varied widely in terms of composition. The non-forage high fibrous feedstuffs have high NDF content, more than 49.5 %. A chemical composition analyses of the five TMRs are presented in Table 2. All five TMRs had a similar chemical composition. The ration CP, ash and NDF content were approximately 12.84%, 11.02% and 36.47%, respectively.

Gas production characteristics of TMRs: Amounts of cumulative gas volume at 2, 4, 6, 12, 24, 48 and 72 after incubations were fitted using the equation $y = a+b [(1-Exp (-ct)]$ (Orskov and McDonald, 1979). Although there are other models available to describe the kinetics of gas production, the Orskov and McDonald (1979) model was chosen because of the compatibility of its parameters with intake, digestibility and degradation characteristics and concentrate feedstuffs has been documented (Blummel and Orskov, 1993; Khazaal *et al.*, 1993; Sommart *et al.*, 2000; Nitipot and Sommart, 2003). Gas production characteristics are presented in Table 3. The a intercept value for all TRMs ranged from -1.22 to -10.41 mL. The values for a, intercept, were negative in the incubations of all TMRs in this study. These data suggested that a lag phase due to a delay in microbial colonization of the substrate may occur in the early state of incubation. Several authors (Khazaal *et al.*, 1993; Blummel and Becker, 1997) have also reported negative values with various substrates when using mathematical models to fit gas production kinetics. This is due to either a deviation from the exponential cause of fermentation or delays in the onset of fermentation due to the microbial colonization. It is well known that the

Table 1: Chemical analysis of feedstuffs used for feed formulation in the experiments

	%DM basis				
Feedstuffs	DM (%)	CP	Ash	NDF	ADF	ADL
Rice straw	91.6	3.4	11.0	72.2	53.2	3.5
Tomato pomace	87.3	20.2	8.4	49.5	44.4	17.4
Palm meal	92.9	7.8	5.1	58.3	47.1	8.5
Dried brewer's gain	91.1	21.0	4.6	49.7	35.1	5.8
Soybean hulls	90.6	10.0	5.0	58.5	50.0	1.8
Leucaena meal	90.9	10.3	9.7	58.0	50.8	17.0
Cassava chip	93.4	1.9	2.0	6.9	6.4	1.9
Sugar cane molasses	72.4	2.2	8.5	0.0	0.0	0.0
Rice bran	91.7	14.3	6.3	20.3	8.1	2.6

Table 2: Feed formulation and chemical composition of dietary treatments

	Dietary treatments[1]				
Ingredients	rt-TMR	tp-TMR	pm-TMR	db-TMR	sh-TMR
Rice straw	40.0	-	-	-	-
Tomato pomace	-	40.0	-	-	-
Palm meal	-	-	40.0	-	-
Dried brewer gain	-	-	-	40.0	-
Soybean hulls	-	-	-	-	40.0
Leuceana meal	3.0	21.6	18.3	24.0	12.0
Cassava chip	25.0	25.0	25.0	25.0	25.0
Sugar cane molasses	3.0	4.3	8.9	9.3	6.5
Rice bran	25.0	8.0	4.6	0.5	13.6
Salt (NaCl)	0.5	0.5	0.5	0.5	0.5
Shell flour	0.4	0.1	0.1	0.1	0.2
Urea	2.6	0.05	2.1	0.1	1.7
Mineral mixed	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0
Chemical composition					
DM, %	93.68	93.86	92.28	92.39	92.38
Ash, %	12.98	12.20	10.54	9.70	9.70
CP, %	12.06	13.51	12.19	13.36	13.08
NDF, %	36.32	35.90	37.36	38.00	34.77
ADF, %	26.29	22.67	21.15	25.42	22.62
ADL, %	7.90	10.60	8.08	7.01	5.01
Total digestible nutrient* (TDN),%	60.48	60.09	63.70	62.42	62.87
Calcium (Ca)*, %	0.40	0.43	0.54	0.54	0.49
Phosphorus (P)*, %	0.57	0.40	0.27	0.26	0.34

*Calculated value. [1]rt = Rice straw as fiber source, tp = Tomato pomace as fiber source, pf = Palm meal as fiber source, db = Dried brewer gain as fiber source, sh = Soybean hulls as fiber source

value for absolute |a|, described ideally, reflects the fermentation of the soluble fraction. In this study the |a| was highest for sh-TMR and significance difference (P<0.05) with rs-TMR, tp-TMR, pm-TMR and db-TMR. It is indicated that the soluble fraction in sh-TMR was also highest. The soluble fraction makes it easily attachable by ruminal microorganisms and leads to much gas production (Table 3). Boyle (2007) also reported the fiber in soybean hull is rapidly fermented.

The gas volume at asymptote (b) described the fermentation of the insoluble fraction. The gas volume at asymptote was significantly higher in sh-TMR than that rt-TMR, tp-TMR, pm-TMR and db-TMR (P<0.05). The gas volumes at asymptote have the advantage of predicting feed intake. Blummel and Orskov (1993) found that the gas volume at asymptote could account for 88% of variance in intake. Sommart *et al.* (2000) suggested that gas volume is a good parameter from which to predict digestibility, fermentation end-product and microbial protein synthesis of the substrate by rumen microbes in the *in vitro* system. Additionally, *in vitro* dry matter and organic matter digestibility were shown to have high correlation with gas volume (Sommart *et al.*, 2000; Nitipot and Sommart, 2003). In this study, sh-TMR showed the highest gas volume which was also the highest *in vitro* digestibility (Table 3). However, when using soybean hull as a fiber source in TMR for intact animals, the user should be aware that fiber in soybean hull ferments rapidly and has a small particle size. Soybean hulls can be used and mixed in total mixed ration about 20-25% (Sukulthanasorn *et al.*, 2007; Grant, 1997). Increasing soybean hull in the TMR has a negative affect on digestibility and growth rate (Ludden *et al.*, 1995).

Rate of gas production (c) expressed in %/h as ranked from the fastest to the slowest were; pm-TMR, tp-TMR, db-TMR, rt-TMR and sh-TMR. Fast rates of gas production were observed in pm-TMR and tp-TMR. This result might have been influenced by the carbohydrate fraction that was readily available to the microbial population.

The potential extent of gas production (|a|+b) of sh-TMR was the highest and significantly different (p<0.05) with rt-TMR, tp-TMR, pm-TMR and db-TMR. This implies that sh-TMR was highly fermentable in the rumen. However, the potential extent of gas production of pm-TMR and db-TMR are the same with rt-TMR (control). Remarkably, the potential of gas production for rt-TMR, tp-TMR, pm-TMR and db-TMR was slightly lower compared with sh-TM, possibly due to the influence of carbohydrate fraction in the TMR. Fibrous constituents had negatively influenced *in vitro* gas production (Melagu *et al.*, 2003). The fiber content of the soybean hull rapidly ferments compared to most fiber sourced (Grant, 1997). Therefore, much gas production was shown in the sh-TMR.

***In vitro* digestibility of dry matter and organic matter:** The *in vitro* dry mater (IVDMD) and organic matter digestibility (IVOMD) at 72 h after incubation are given in Table 3. It can be seen that IVDMD and IVOMD are similar. The IVDMD and IVODM were significantly different (p<0.05) among treatment. The sh-TMR gave the highest IVDMD and IVOMD. This result implies that the microbe in the rumen and animal have high nutrient uptake. The result agrees with Loest *et al.* (2001) who found that soybean hulls have high digestibility in the rumen. In addition, the fiber in soybean hull ferments rapidly (Boyle, 2007) leading to high gas volume (Table 3). Sommart *et al.* (2000) reported that gas volume is a good parameter to predict digestibility, fermentation end product and microbial protein synthesis of substrate by rumen microbes in the *in vitro* system. The IVDMD and

Table 3: Effect of non forage high fibrous feedstuffs as fiber source in TMRs on Gas production characteristics and in vitro digestibility

	Dietary treatments[1]					
Parameters	rt-TMR	tp-TMR	pm-TMR	db-TMR	sh-TMR	SEM
Gas production characteristics[2]						
a, mL	-2.99^{a}	-4.62^{a}	-4.37^{a}	-1.22^{a}	-10.41^{b}	0.94
b, mL	109.23^{b}	90.79^{c}	98.14^{c}	98.14^{c}	161.25^{a}	5.39
c, %/h	0.034^{c}	0.056^{a}	0.057^{a}	0.044^{b}	0.030^{c}	0.00
|a|+b, mL	112.23^{b}	95.42^{c}	102.51^{bc}	100.99^{bc}	171.66^{a}	5.97
In vitro digestibility of dry matter and organic matter at 72 h (%)						
IVDMD	77.58^{c}	76.61^{c}	80.93^{b}	80.07^{b}	93.37^{a}	1.19
IVOMD	79.15^{c}	78.27^{c}	82.27^{b}	81.36^{b}	93.27^{a}	1.14

^{a,b,c,d} Means within a row different superscripts differ ($p<0.05$), [1]rt = Rice straw as fiber source, tp = Tomato pomace as fiber source, pf = Palm meal as fiber source, db = Dried brewer gain as fiber source, sh = Soybean hulls as fiber source, [2]a = the intercept (mL), which ideally reflects the fermentation of the soluble fraction, b = the fermentation of the insoluble fraction (asymptote) (mL), c = rate of gas production (%/h), |a|+b = potential extent of gas production (mL)

Table 4: Effect of non forage high fibrous feedstuffs as fiber source in TMRs on pH, ammonia nitrogen (NH_3N) and total volatile fatty acid (TVFA) in *in vitro*

	Dietary treatments[1]					
Parameters	rt-TMR	tp-TMR	pm-TMR	db-TMR	sh-TMR	SEM
pH						
0 h	7.07^{b}	7.11^{ab}	7.14^{a}	7.08^{b}	7.16^{a}	0.01
3 h	7.09	7.08	7.09	7.07	7.09	0.00
6 h	7.07^{ab}	7.07^{ab}	7.06^{ab}	7.04^{b}	7.09^{a}	0.01
9 h	7.08^{a}	7.06^{b}	7.05^{b}	7.07^{ab}	7.07^{ab}	0.00
12 h	7.07^{a}	7.02^{b}	7.03^{b}	7.01^{b}	7.02^{b}	0.01
			---------- Ammonia nitrogen (NH_3N), mg% ----------			
0 h	19.70^{b}	14.97^{c}	21.84^{a}	18.83^{b}	21.81^{a}	0.55
3 h	24.74^{b}	17.20^{c}	29.34^{a}	18.05^{c}	22.69^{b}	0.98
6 h	20.77^{a}	14.09^{b}	19.19^{a}	13.42^{b}	21.70^{a}	0.78
9 h	22.45^{a}	14.86^{c}	20.72^{b}	13.47^{d}	18.22^{c}	0.71
12 h	24.82^{a}	15.18^{c}	20.99^{b}	14.86^{c}	21.36^{b}	0.84
			---------- Total volatile fatty acids, mM --------			
0 h	36.80^{b}	38.40^{ab}	36.18^{b}	47.26^{a}	30.28^{b}	1.70
3 h	43.08	47.75	39.88	43.57	39.75	1.38
6 h	57.85^{c}	60.31^{c}	71.38^{a}	64.25^{b}	45.79^{d}	1.80
9 h	78.03^{a}	80.74^{a}	52.18^{c}	74.10^{b}	78.77^{a}	2.25
12 h	79.26^{a}	76.31^{a}	58.46^{ab}	48.49^{b}	71.39^{a}	3.68

^{a,b,c,d} Means within a row different superscripts differ ($p<0.05$). [1]rt = Rice straw as fiber source, tp = Tomato pomace as fiber source, pf = Palm meal as fiber source, db = Dried brewer gain as fiber source, sh = Soybean hulls as fiber source.

IVOMD of rt-TMR and tp-TMR lower than pt-TMR, db-TMR and sh-TMR. The reason for that is possibly that the fiber fractions of rt-TMR and tp-TMR have a large proportion of lignified cell walls leading to attachment difficulty by microorganism, with low fermentation rates, low digestibility rate and limited intake (Ibrahim *et al.,* 1995; Hindrichsen *et al.,* 2001). The higher fiber content (Table 1) of rice straw and tomato pomace probably resulted in lower *in vitro* dry matter and organic matter digestibility since high NDF and ADL content in feedstuffs result in lower fiber degradation (Van Soest, 1988).

***In vitro* fermentation pattern:** Concentrations of NH_3-N, TVFA and pH in the *in vitro* fluid were used to monitor the *in vitro* fermentation pattern (Table 4). The pH was significantly affected by fiber source in TMRs. The pm-TMR and sh-TMR had higher pH (7.14 and 7.16) than those rt-TMR, tp-TMR and db-TMR. When monitoring pH pattern at 0, 3, 6, 9 and 12 hr after incubation, the pH values were relatively stable at 7.01-7.16 and all treatment means were within the normal range that has been reported as optimal pH (6.0-7.0) for microbial digestion. The buffer in the rumen fluid medium is the reason for pH remaining stable at all times of fermentation. The buffer is a factor that should be considered when using the gas production technique. The exhaustion of the buffer would lead to a lowering of the pH (Getachew *et al.,* 1998). At a lower pH, the celluolytic bacteria becomes less active (Russell and Dombrowski, 1980). In this study the buffer was not exhausted. Therefore, this condition was optimal for microbial activity. However, non forage fiber sources should be considered when feeding intact animals because the physical form of the fiber affects their chewing activity. Less mastication, may reduce their saliva excretion leading to less buffering capacity in the

rumen (Grant, 1997). Beauchemin *et al.* (2003) found that a reduction of mean particle size of alfalfa hay from 5.7 to 2.2 mm decreased ruminal pH. Generally, non forage high fibrous feedstuffs have small particle size. It was expected that they negatively affect chewing activity, rumen condition and digestion.

Ammonia nitrogen concentration was significantly different ($p<0.05$) among treatments at each hour of sampling. The difference in NH_3-N concentrations among treatments may have been related directly to urea and degradability of protein in the TMRs. Although, nitrogen recycling in the rumen and *in vitro* is different, NH_3-N concentration was in the optimal range for rumen ecology, microbial activity (Perdok and Leng, 1990; Wanapat and Pimpa, 1999). At 0 to 3 hours after incubation pm-TMR had the highest NH_3-N, when compared with other TMRs. When ammonium nitrogen is high it indicates that the soluble fraction of protein is also high. Remarkably, NH_3-N concentration of tp-TMR and db-TMR were low at all time of sampling. It may have been that the urea level in both TMRs was lower than others. In addition, the protein in tomato pomace and dried brewer grain had low degradability (Chumpawadee *et al.*, 2006). Although, NH_3-N concentration of all TMRs was different with rt-TMR (control), it was in the normal range. Therefore, it can be used as non forage high fibrous replacement dietary forage. Future research should investigate the impact of the ability of non forage high fibrous feed to replace forage in intact animals.

Total volatile fatty acid concentrations were significantly different ($p<0.05$) among treatments at all times of sampling, accepting 3 hours after incubation. Remarkably, TVFA concentrations in the *in vitro* medium, from 0 to 12 hours after incubation, tend to be increased. The reason for that is possibly VFAs accumulated in the medium. The VFA can not absorb via the *in vitro*, but most of the VFA can be absorbed into rumen wall. Although, VFA increased in the medium, pH did not change because the buffer in the medium was not exhausted. This is the advantages of the gas production technique. The VFA production of tp-TMR and sh-TMR are the same with rt-TMR and difference from pm-TMR and db-TMR, this result might have been influenced by carbohydrate fraction in TMRs. The rate and extent of carbohydrates degradation are influenced by the condition of rumen fermentation and rate and extent of VFAs production (Cheng *et al.*, 1991). Keadly and Mayne (2001) also suggest that VFAs concentration is similar when the animal fed diets contained a similar carbohydrate composition. In this study, have difference source of fiber in TMRs, thus VFA concentration was also different.

Conclusion: In *in vitro* study, non forage high fibrous feed stuffs had an affect on gas production characteristic, *in vitro* digestibility and *in vitro* fermentation. The soybean hull as a fiber source in TMR gave the highest parameters of gas production characteristic and *in vitro* digestibility. Concentration of NH_3-N, TVFA and pH were different when TMRs contained a different non forage fiber. Future research should investigate the impact of a non forage high fibrous feed replacement of forage for intact animal.

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Effect of Carbohydrate Drink Intake Patterns on Exercise in Heat

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Abstract: Carbohydrate drink can increase work capacity by maintaining power output or speed or prolonging the time to fatigue at a fixed workload. Literature is available on fluid intake content and exercise performance. No study has been undertaken till now on fluid intake patterns during high intensity exercise in heat. Therefore the present study is devised to fill this gap and to design an appropriate fluid volume intake pattern during high intensity exercise in hot environmental condition. 15 moderately trained men participated in the study. Each subject was given two different intake patterns of carbohydrate drink in two exercise sessions conducted on different days. A gap of seven days was kept between two exercise sessions. Total amount of fluid intake by the subjects in each session is 1000ml. During the experiment ambient temperature was kept constant at 35°C. Parameters evaluated were blood glucose concentration during and after the high intensity exercise, time to exhaustion and mental concentration. Findings of the present study revealed that when carbohydrate ingestion was done in large quantities before exercise, the rate of glucose disappearance was significantly ($t = 10.34$, $p<0.05$) earlier as compared to carbohydrate ingestion intermittently during exercise in hot environmental condition. Time of exhaustion was significantly ($t = 13.73$, $p<0.05$) higher in intermittent drink intake pattern. Performance in mental concentration was significantly ($t = 4.98$, $p<0.05$) better in intermittent drink intake pattern.

Key words: Blood glucose, high intensity exercise, dehydration

Introduction

Dehydration resulting from physical exercise in the heat, followed by a brief period of rehydration and the continuation of activity or competition is a common scenario for athletes, labourers, military personnel and recreational exercises. Thirst sensation is an appropriate measure for rehydration. But thirst does not provide a good index of body water requirements; because thirst is probably not perceived until an individual has incurred a water deficit of approximately 2% of body weight.

The onset of fatigue during prolonged high intensity exercise is associated with reduction in blood glucose concentration and dehydration (Cheuvront *et al*., 2001). Fortunately, there are a number of strategies that athletes can use to prevent and/or to reduce the dangers that are associated with exercise in heat such as muscle cramps, stomach upset, stomach cramps, nausea, flatulence, diarrhea, headaches, dizziness, unusual fatigue and delirium, coma and even death, can be blamed to extreme heat stress. Nutritional intervention seems to be the most effective to prevent athlete from heat stress and this also includes the optimization of hydration status by the use of fluid replacement beverages (Wendt *et al*., 2007).

Ingestion of carbohydrate drink during high intensity exercise helps alleviate heat dissipation problems (Melin *et al*., 1994). Carbohydrates, especially in the heat are able to supply quick energy.

Researches have been completed in the area of fluid intake content and exercise performance. But till now there is no study available on appropriate fluid intake patterns before and during high intensity exercise in the heat and its effect on mental concentration and performance.

In this regard Wright *et al*. (1991) had previously demonstrated that a combination of carbohydrate drink when given both before and during exercise has an additive effect on performance. They did not explain that what would be the effect of carbohydrate ingestion on performance when it is given either prior to exercise or during exercise separately, therefore the relative importance of each practice could not be elucidated. In addition glucose concentration was not determined in the study, so it was impossible to determine the underlying mechanism behind their observed performance enhancement.

In order to obtain glucose concentration during different fluid intake pattern Febbraio *et al*. (2000) had conducted a comprehensive study by obtaining glucose concentration throughout exercise. But this study was conducted in the thermo neutral environmental conditions. As much of the athletic event occur in hot environmental condition, this does not present practical scenario.

For long endurance and high intensity sports performed in hot environmental condition it is well known that ingestion of carbohydrate drink is essential to delay the

Table 1: Comparison of blood glucose concentration values at different time duration during exercise between two different carbohydrate drink intake patterns.

	Bolus pattern			Intermittent pattern			
Time duration	Mean	SD	SE	Mean	SD	SE	t
30 min before exercise	86.370	4.35	1.09	84.94	2.84	0.71	1.61^{NS}
At the onset of exercise	102.200	5.59	1.39	86.37	3.11	0.78	12.65**
At 30 min during exercise	70.060	5.48	1.37	96.25	4.59	1.15	13.83**
At 1 hour during exercise	88.370	4.31	1.08	108.69	6.85	1.71	10.25**
At time to exhaustion	75.630	4.81	1.21	91.64	4.33	1.08	10.34**

NS-Non Significant *p<0.05 - Significant **p<0.01 - Highly Significant

onset of fatigue. But the exact pattern of carbohydrate drink ingestion in hot environmental condition remains controversial. Hargveaves *et al.* (1996) suggested that during exercise in the heat there is an increase in liver glucose output without any change in the whole body glucose utilization. Due to this fact coaches and sports physician normally prescribe ingestion of large amount of glucose prior to exercise. This raises a question is this pattern appropriate to delay fatigue and enhance mental concentration during the late stage of game by maintaining blood glucose at euglycemic level. Therefore the aim of the present study is to design an appropriate fluid intake pattern so the athletes had a better maintenance of blood glucose concentration during the late stage of exercise in hot environmental condition.

Materials and Methods

Subjects: Sixteen recreational athletes (age 23±1.13 years, weight 67±3.02kgs, maximal oxygen uptake 52.08±3.44 ml/kg/min) were recruited as subjects for this study. Informed written consent was obtained from each participant prior to the data collection and each participant was debriefed about the procedure. All subjects had a competitive athletic background but none were currently training for endurance or sporting activities. The study was approved by the Institutional ethics committee of the Guru Nanak Dev University, Amritsar.

Preliminary Testing: Subjects attended pre-participation screening phase 2 days prior to the actual experiment in which they were asked to fill the screening questionnaire. VO_2 max was determined by Queen's College step test. Subjects with VO_2 max 45-55 ml/kg/min had participated in the study.

Experimental Trials: Subjects reported to the laboratory on two occasions after an overnight fast, having abstained from alcohol, caffeine, tobacco and strenuous exercise for the previous 24 hours. To minimize differences in resting muscle and liver glycogen concentration, subjects were provided with prepared food packages for 24 hours before each trial. Such a dietary exercise regimen has previously been shown to minimize differences in pre-experimental metabolism and substrate availability (Angus *et al.*, 2000). Each experiment was separated by a period of 7 days.

A complete cross over design was used. During each trial subject continued cycling till exhaustion at an intensity 60-70 % age predicted maximum heart rate derived from Karvonen's (1957) equation in a climatic chamber at 35°C till exhaustion.

Subjects exercised after ingesting 1000ml of an isotonic carbohydrate drink, 30 min before exercise in single bolus drink intake pattern. In the second exercise session, 1000 ml of fluid was divided into 4 equal parts and given at the start of exercise, 15 minutes, 30 minutes and 45 minutes. Drink temperature was maintained at 10°C.

On arrival in the laboratory, the subjects voided and blood sample was collected for the analysis of baseline blood glucose concentration with the help of glucometer (Elegance CT X - 10). Subjects were asked to complete mental concentration test - Stroop test (Ridley and Mulcahy, 1930). This test is a psychological test of mental (attentional) vitality and flexibility. The test takes advantages of ability to read words more quickly and automatically than naming colours. The word is printed or displayed in a colour different from the colour it actually represents. After a basal blood glucose concentration was derived, subjects undergoing cycle ergometry were made to wear the polar short-range telemetry strap on the chest and monitoring watch on their wrists. Leger and Thirierge (1988) suggested that portable light weight telemetric heart rate monitors were valid and accurate tools for measuring heart rate.

Further fingertip blood samples were obtained at 30 minutes, I hour and at the time of exhaustion. Post experimental reading of mental concentration test was also recorded.

Statistical analysis: A statistical computer software program [SPSS 14.0] was used for statistical analysis. The metabolic data from the two trials was compared with related t-test.

Results

The purpose of this investigation was to determine that which of the two intake patterns of carbohydrate drink

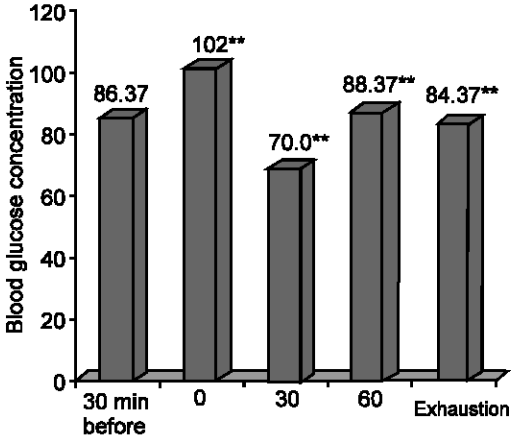

Fig. 1: Comparison of Blood Glucose Concentration at Different Time Duration in Single Bolus Drink Intake Pattern.

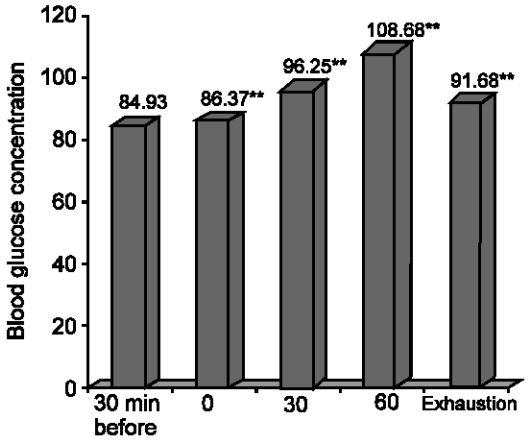

Fig. 2: Comparison of Blood Glucose Concentration at Different Time Duration in Intermittent Drink Intake Pattern.

Table 2: Comparison of time of exhaustion (in minutes) in two different drink intake pattern

	Time of exhaustion			
	Mean	SD	SE	t
Bolus pattern	77.06	4.09	1.02	-13.73**
Intermittent pattern	90.56	5.85	1.46	-

NS-Non Significant *p<0.05 - Significant **p<0.01 - Highly Significant.

Table 3: Comparison of pre value and post values of mental concentration test in two different carbohydrate drink intake pattern.

	Bolus Pattern			Intermittent Pattern			
	Mean	SD	SE	Mean	SD	SE	t
Pre exercise	13.31	1.30	0.33	12.81	1.11	0.28	1.73^{NS}
Post exercise	16.68	2.41	0.66	14.56	1.93	0.48	4.98**

NS-Non Significant *p<0.05 - Significant **p<0.01 - Highly Significant

i.e. bolus or intermittent is more effective to maintain blood glucose concentration at euglycemic level and enhance mental performance.

Comparison of blood glucose concentration values were made with related t test between two different drink intake pattern, there was significant difference ($p<0.05$) at all time durations except the basal values ($t = 1.61$) ($p>0.05$), (Table 1). At the time of exhaustion blood glucose concentration was significantly ($t = 10.34$) ($p<0.05$) higher in intermittent drink intake pattern.

Blood glucose concentration after ingestion of carbohydrate drink 30 minutes before exercise in single bolus drink intake pattern, was significantly ($t=33.64$, $p<0.05$) raised at the start of exercise. After 30 minutes of exercise it declined significantly ($t = 18.63$, $p<0.05$) below the level of fasting blood glucose. At 1 hour of exercise blood glucose concentration was raised significantly ($t = 11.25$, $p<0.05$). At the time of exhaustion it declined to a greater extent ($t = 9.62$, $p<0.05$) (Fig. 1).

Blood glucose concentration after ingestion of carbohydrate drink intermittently during exercise, was significantly ($t = 5.96$, $p<0.05$) raised at the start of exercise and it significantly raised at all time durations during exercise i.e., at 30 minutes ($t = 11.47$, $p<0.05$) and at 1 hour ($t = 14.44$, $p<0.05$) of exercise. After 1 hour, at the time of exhaustion it was significantly ($t = 17.67$, $p<0.05$) declined (Fig. 2).

Time to exhaustion was compared with related t test between the two different drink patterns, it was significantly ($t = 13.73$) ($p<0.05$) higher in intermittent pattern in comparison to single bolus drink intake pattern (Table 2).

The time taken for the completion of mental concentration test was significantly ($t = 4.98$) ($p<0.05$) towards lower side in intermittent drink intake pattern (Table 3).

Discussion

The purpose of the study was to design an appropriate fluid intake pattern for athletes participating in high intensity sports in hot environmental condition and to enhance mental concentration during the late stage of a game. Specific parameters included were blood glucose concentration at regular intervals, time to exhaustion and mental concentration test.

Blood glucose concentration: In the present study it was observed that there was a decline in blood glucose concentration in both the groups post experiment but the extent of decrease in blood glucose concentration was more in the single bolus pattern ($p<0.05$). This indicates that ingestion of carbohydrate drink intermittently during exercise in heat would help to maintain blood glucose concentration at normoglycemic level after 1 hour of strenuous exercise. It was previously suggested that fall in blood sugar leads to several performance related problems including lack of energy, headache, dizziness and the treated 'bonking' or 'hitting the wall' (McArdle *et*

al., 2001). Hargreaves (1996) had suggested that in heat there is an increase in liver glucose output which results in hyperglycemia. To blunt this increase in liver glucose output, Marmy *et al.* (1996) had revealed that pre exercise glucose ingestion results in rapid fall in plasma glucose due to higher muscle glucose uptake with the onset of exercise. They also suggested that with pre exercise glucose ingestion liver glucose output can be reduced up to 62%.

The decrease in blood glucose concentration when fluid was ingested in a single bolus pattern was observed from the start of exercise. Previous studies suggest that when pre exercise glucose ingestion was done in large amounts 30 minutes before exercise it causes blood glucose to rise rapidly which may trigger insulin release in large quantity (Brand-Miller and Factor, 1996). This will led to rebound hypoglycemia, which can be observed after 30 minutes of strenuous exercise (McArdle *et al.*, 2001). The results of the present study observed that blood glucose concentration rapidly fell after 30 minutes of exercise. This decrease in blood glucose concentration after 30 minutes of exercise was not observed in the intermittent drink intake pattern, this could be due to the fact that the amount of pre exercise carbohydrate glucose ingestion did not match with the single bolus pattern session. Thus, pre exercise glucose ingestion had completely suppressed endogenous glucose production (Jeukendrup *et al.*, 1999) in single bolus drink intake pattern.

Significant fall in blood glucose concentration in single bolus pattern can also be observed due to decrease in liver glucose output with large amount of pre exercise glucose ingestion (Fontvieille, 1992). The amount of intermittent drink intake pattern was not sufficient to blunt the increase in liver glucose output. Furthermore, it partially blunted the rise in blood glucose concentration. After 1 hour of strenuous exercise, blood glucose concentration in single bolus pattern, returned to the basal values due to increase in the endogenous glucose production i.e. liver glucose output. But this increase did not match with the blood glucose concentration attained with intermittent drink intake pattern after 1 hour of exercise. This difference in blood glucose concentration was found to be statistically significant. After 1 hour of exercise blood glucose concentration was significantly higher in intermittent drink intake pattern. This could be due to the fact that a supply of exogenous glucose was there in latter pattern. In single bolus pattern the rise in blood glucose concentration after rebound hypoglycemic values could be due to the release of stress hormones such as glucogan (McArdle *et al.*, 2001).

After the completion of exercise blood glucose concentrations declined in both the patterns from the blood glucose concentration values at 1 hour of exercise. The result also suggest that the decrease relatively more with single bolus pattern. Even though the time taken in completion for exercise was towards higher side in intermittent pattern, blood glucose was maintained at euglycemic levels. The results thus suggest that intermittent drink intake pattern can partially suppress the endogenous glucose production by liver, which allows the athlete to continue exercise for a longer period of time in comparison to single bolus pattern. This stored endogenous glucose will then allow the athlete to utilize this stored glucose during later stage of exercise without reaching glycogen depletion state. This could also be related to the fall in blood glucose concentration but this factor alone could not be responsible for fatigue in high intensity exercise.

In the present study, percentage of glucose rate of appearance came from exogenous glucose was not determined. Nonetheless, even if hepatic glucose production was completely suppressed as is possible when large amounts of glucose are ingested during exercise (Wagenmakers *et al.*, 1999), we cannot account for all the ingested glucose appearing in the blood and/or being taken up by the tissue. Therefore our data is in agreement with the previous suggestion that the rate of exogenous glucose oxidation may be limited by digestion, absorption and glucose rate of appearance into the blood stream.

It must be noted that, in the present study, it was assumed that glucose rate of disappearance matched with the glucose oxidation as it was not directly measured using primed continuous infusion (6, 6-2H). However, this assumption of blood glucose measurement is valid because it has been previously demonstrated that 98% of glucose rate of disappearance is oxidized during exercise (Jeukendrup *et al.*, 1997).

Time of exhaustion: There was significant difference in time to exhaustion between two different carbohydrate drink intake patterns. Time to exhaustion was considerably towards higher side in intermittent drink intake pattern in comparison to bolus drink intake pattern (Table 2).

Previous findings of Coyle *et al.* (1986) suggested that when carbohydrate drink ingestion was done during exercise it postpones fatigue by 15-30 minutes. They had compared carbohydrate drink with a placebo drink. In the present study, although carbohydrate drink was ingested in both the sessions but the pattern of intake was different. From the findings of the present study it can be suggested that carbohydrate drink can post pones fatigue if consumed intermittently throughout exercise.

Mental concentration: The present study revealed that there was a significant decrease in mental concentration ability in single bolus drink intake pattern in comparison to intermittent drink intake pattern ($P<0.05$) after the completion of high intensity exercise in heat (Table 3).

Gopinathan *et al.* (1988) had suggested that even 2% of body mass loss impair mental functioning. Mental functioning is important during the late stage of many athletic events or team games. As the game reaches towards the end there is a need to have a good mental concentration ability to succeed in achieving the task with tactical skills. Reilly (1996) had suggested that reduction in work rate, mental fatigue, technical errors and deterioration in skills were observed as the game reaches towards the end.

Blood glucose is the main substrate for energy metabolism within the central nervous system. It is not surprising; therefore, that hypoglycemia has been suggested as a possible reason for deterioration in performance observed in sports, which require both tactical thought and co-operative intervention between players.

In the present study differences were found in blood glucose concentration in two different drink intake patterns. Blood glucose concentration was significantly higher in the intermittent drink intake pattern in comparison to the single bolus drink intake pattern. Therefore the decrease in the blood glucose concentration in the single bolus intake pattern might be the cause for decrement in mental concentration ability in comparison to intermittent drink intake pattern.

Conclusion: From the results it is concluded that even when carbohydrate is ingested in large quantities before exercise the glucose rate of disappearance is earlier, as compared to ingestion of carbohydrate drink intermittently during exercise in hot environmental conditions. The time of exhaustion might be linked to this decrease in blood glucose concentration. Mental concentration will be better when carbohydrate drink was ingested intermittently during exercise.

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The Effects of Germination of Low - Tannin Sorghum Grains on its Nutrient Contents and Broiler Chicks Performance

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Abstract: Sorghum (Fetrita) was soaked in water for 12 hours, germinated for 3, 5 and 7 days in a shadow under cover and dried by sunlight for 2 - 3 days. Crude protein and tannin content of germinated sorghum were determined and it's metabolizable energy value was calculated. Four isonitrogenous and isocaloric diets were formulated using Fetrita germinated for zero (control), 3, 5 and 7 days each in a starter and a finisher diet and were fed to unsexed commercial broiler chicks (Hypeco) for 42 days. The results showed that by seventh day of germination crude protein increased by 31%, tannin content increased by 100% and reached toxic level and metabolizable energy was decreased by 6%. Germination of low - tannin sorghum for 3 days had no effect on feed intake, body weight gain and feed conversion ratio of broiler chicks throughout the whole period. Increase in days of germination decreased growth of broiler chicks. It seems that germination of low - tannin sorghum grains has little beneficial effects on broiler chicks' performance and it increased tannin concentration.

Key words: Sorghum, germination, tannin and broiler

Introduction

Sorghum is deficient in lysine content. Hulse *et al.* (1980) noticed that first limiting amino acid in sorghum is lysine as in most cereals. Also Wall and Blessin (1970) reported that the amino acids composition of the total protein in sorghum is the same to that of corn and other cereals, where lysine is the most limiting amino acid. These studies showed that lysine content is of a crucial importance in diets composed mainly of cereals (Jansen, 1972). In addition to the insufficient amount of lysine, sorghum grains contain an antinutritive factor called tannin. Tannins are polyphenols that occur widely in plant - based food. They are considered as to be part of the plant defense system against environmental stressors. Herstad (1980); Barry and Manley (1984) found that high - tannin sorghum decreased feed consumption of broiler chicks. Elkin *et al.* (1978) and Luis and Sullivan (1982) found frequent use of high - tannin sorghum diet decreased growth rate and led to poor feed conversion ratio of broiler chicks. Musharaf and Latshaw (1991) reported that intermediate - tannin sorghum (1.35% CE) increased incidence of leg abnormalities when compared with low - tannin sorghum.

Bennick (2002) noticed that tannins have a number of effects on animals, including growth - rate depression and inhibition of digestive enzymes. Hassan *et al.* (2003) reported that high - tannin sorghum caused a highly significant reduction in the weight gain and feed intake of broiler chicks compared to low - tannin sorghum and increased the feed conversion ratio.

Various processing methods have been employed to improve the nutritive value of cereal grains, such as tannin extraction (Musharaf and Latshaw, 1991) germination or malting (Shayo *et al.*, 2001) who concluded that germination was superior to the other processing methods in improving the nutritional and functional qualities of sorghum. Malting increased the protein, lysine and reduced tannin contents of sorghum (Wu and Wall, 1980; Okoh *et al.*, 1989 and FAO, 1995). Idris *et al.* (2005) reported that malting of low - tannin sorghum reduced tannin content of seeds. Hamid (2001) found that germination of low - tannin sorghum increased protein content of grains. Falfiolu *et al.* (2006) noticed that average weight of growing hens increased with increasing levels of malted sorghum sprout.

This study was performed to evaluate the efficiency of germination method in improving the nutritive value of low - tannin sorghum for broiler chicks.

Materials and Methods

Low - tannin sorghum (Fetrita) was soaked in water for 12 hours, germinated for 3, 5 and 7 days in a shadow under cover and dried by sunlight for 2 - 3 days. Germination process was performed as commonly practiced at home to prepare traditional local beverage (Hulu - mur) used during the Holy month of Ramadan. Crude protein of germinated and ungerminated sorghum was determined by micro - Kjeldhal method. Tannin content was determined by the modified vanillin - HCl method of Price *et al.* (1978). Metabolizable energy

Table 1: Composition, calculated and determined analysis of experimental diets (dry matter)

	Ungerminated Fetrita		3-days germination		5-days germination		7-days germination	
Ingredients	Starter %	Finisher %	Starter %	Finisher %	Starter %	Finisher %	Starter %	Finisher %
Sorghum (Fetrita)	54.00	57.10	54.00	57.10	54.00	57.10	54.00	57.10
Groundnut cake	24.00	23.96	16.50	18.00	11.00	17.13	-	-
Sesame cake	14.20	05.20	20.25	09.22	19.50	05.98	24.50	15.08
Wheat bran	02.66	09.00	04.23	11.00	10.40	15.00	16.43	23.13
Oil	01.50	01.50	01.50	01.50	01.50	01.50	01.50	01.50
Bone meal	01.55	00.86	01.38	00.73	01.27	00.71	01.04	00.35
Oyster shell	00.97	01.37	01.06	01.45	01.17	01.50	01.33	01.74
Lysine – HCl	00.51	00.38	00.53	00.41	00.59	00.45	00.66	00.54
DL – Methionine	00.11	00.13	00.05	00.09	00.07	00.13	00.04	00.06
Vitamin (Premix) *	00.25	00.25	00.25	00.25	00.25	00.25	00.25	00.25
Salt	00.25	00.25	00.25	00.25	00.25	00.25	00.25	00.25
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Determined								
Crude protein (%)	23.55	20.55	23.78	20.46	23.70	20.66	23.86	20.85
Lysine (%)	01.20	01.00	01.20	01.00	01.20	01.00	01.20	01.00
Methionine (%)	00.50	00.41	00.50	00.41	00.50	00.41	00.50	00.41
Calcium (%)	01.00	00.90	01.00	00.90	01.00	00.90	01.00	00.90
Available phosphorous (%)	00.45	00.35	00.45	00.35	00.45	00.35	00.45	00.35
Calculated metabolizable energy (MJ/Kg)	13.41	13.33	13.25	13.12	13.36	13.18	13.08	12.97

*Supplied per Kg of diet: vitamin A, 3500 IU; vitamin D3, 1300 IU; vitamin E, 11 IU; vitamin B12, 0.009 mg; riboflavin, 2.2 mg; niacin, 66 mg; panthothenic acid, 7 mg; choline, 1700 mg; thiamine, 2 mg; pyridoxine, 3 mg; biotin, 0.4 mg; folic acid, 0.4 mg.

Table 2: Changes in protein content (%), metabolizable energy (MJ/Kg) and tannin content (% as catechin equivalent) of sorghum seeds as a result of germination for 3, 5 and 7 days

	Ungerminated Fetrita	3-days germination	5-days germination	7-days germination
Crude protein (%)	13.10	12.95	15.42	17.15
Metabolizable energy (MJ/Kg)	14.34	13.82	14.06	13.37
Tannin (%)	00.34	00.59	00.54	0.68
% Changes in tannin contents	00.00	74.00	59.00	100.00

values of sorghum were calculated by the modified equation of Ellis (1981):

ME = 1.549 + 0.0102 CP + 0.0275 oil + 0.0148 NFE - 0.0034 fibre.

ME: Metabolizable energy (MJ/Kg).

CP: Crude protein (g/Kg).

NFE: Nitrogen free extracts (g/Kg).

The experiment was carried out in the premises of poultry unit which belongs to Elnazil Company, Soba south east to Khartoum. It lasted six weeks. Minimum and maximum temperature were 10°C and 34°C, respectively. One hundred and forty four, one - day - old, unsexed commercial broiler chicks (Hypeco) were assigned into 24 pens in groups of 6 chicks in a pen. Each experimental diet was fed to 6 replicates, in a completely randomized design. Broiler chicks were kept on a deep litter floor system. Each of the germinated Fetrita was incorporated into broiler chicks' diet forming three experimental diets. The fourth diet was the control diet containing ungerminated Fetrita. Experimental diets were formulated to meet or exceed the (NRC, 1994) requirements of broiler chicks. The diets were isonitrogenous and isoenergetic. Feed and water were provided *ad ibitum*. Feed consumption, weight gain and feed conversion ratio were recorded weekly for the individual replicate of each dietary treatment. Mortality was recorded as it occurred. Routine and occasional management, vaccination and medication were carried out as and when due.

Proximate analysis of the diets was carried out according to official method of analysis of AOAC (1980). Crude protein was determined and metabolizable energy was calculated as mentioned for Fetrita. Table 1 shows calculated composition and determined analysis of experimental diets.

The data generated from the experiment was subjected to analysis of variance. Least Significant Difference (LSD) test was used to assess significance of difference between means as described by Little and Hills (1978).

Results and Discussion

Table 2 shows changes in protein content, metabolizable energy and tannin of Fetrita seeds as affected by germination process. By seventh day of germination protein content (%) of Fetrita increased by 31%. These results agreed with Wu and Wall (1980); Okoh *et al.* (1989); Elkhalifa (1993); Elmaky (1994); Hamid (2001) who reported that germination of low -

Table 3: Effects of germination of Fetrita grains for different days, when fed to broiler chicks during the starter period (0 – 21 days)

	Ungerminated Fetrita	3-days germination	5-days germination	7-days germination	SE ±	L.S.D 5%	L.S.D 1%
Number of birds	36	36	36	36			
Feed intake (g/bird)	904^{a}	901^{ab}	810^{ab}	803^{b}	33	101	148
Body weight gain (g/bird)	443^{a}	432^{ab}	383^{bc}	341^{c}	18	54.21	79.46**
Feed conversion ratio (g feed/g gain)	2.04^{a}	2.10^{a}	2.14^{a}	2.43^{b}	0.08	00.24	0.35**

^{a,b}: Row means followed by the different letter are significantly different. ** : (P< 0.01). SE: Standard error

Table 4: Effects of germination of Fetrita grains for different days, when fed to broiler chicks during the finisher period (22 - 42 days)

	Ungerminated Fetrita	3-days germination	5-days germination	7-days germination	SE ±	L.S.D 5 %	L.S.D 1%
Number of birds	35	36	36	33			
Feed intake (g/bird)	2271^{a}	2014^{ab}	1751^{b}	1888^{b}	128	382	561
Body weight gain (g/bird)	1005^{a}	1015^{a}	878^{ab}	831^{b}	57	170	249
Feed conversion ratio (g feed/g gain)	2.25^{a}	2.09^{a}	2.02^{a}	2.30^{a}	0.11	0.32	0.66

^{a,b}: Row means followed by the different letter are significantly different. **: (P<0.01). SE: Standard error.

Table 5: Effects of germination of Fetrita grains for different days, when fed to broiler chicks during the whole period (0 – 42 days)

	Ungerminated Fetrita	3-days germination	5-days germination	7-days germination	SE ±	L.S.D 5%	L.S.D 1%
Number of birds	36	36	36	36			
Feed intake (g/bird)	3175^{a}	3004^{ab}	2560^{b}	2960^{b}	151	453	656
Body weight gain (g/bird)	1447^{a}	1447^{a}	1261^{ab}	1172^{b}	67	200	293**
Feed conversion ratio (g feed/g gain)	2.18^{ab}	2.09^{ab}	2.05^{a}	2.31^{b}	0.08	0.25	0.37

^{a,b}: Row means followed by the different letter are significantly different. ** : (P<0.01). SE: Standard error

tannin sorghum increased protein content and most important protein fractions that rich in lysine (albumin - globulin fraction). Also by seventh day of germination metabolizable energy (MJ/Kg) decreased by 6%. The same results were obtained by Dalvi (1974) and Elmaky (1994). They noticed that decrease in metabolizable energy of sorghum as the result of germination process is due to the degradation of the starch to soluble sugars to meet the seedling requirements. Tannins content (as % catechin equivalent) of Fetrita increased as the days of germination increased, however after 3 days it started to decrease then increased again, but it reached toxic effect (0.68% as reported by Fuller *et al.,* 1966) only by seventh day of germination. The same findings were reported by Elkhalifa (1993); Elmaky (1994); Hamid (2001). Ahmed (1988) suggested that this increase in tannin content could be due to solubilization occurring as the grain took up water, or to synthesis of polyphenols during germination.

Tables (3, 4 and 5) show effect of different germination days of Fetrita when fed to broiler chicks during the starter, finisher and the whole period, respectively. During starter, finisher and the whole periods, body weight gain, feed consumption and feed conversion ratio of broiler chicks offered 3 - days germinated Fetrita diet were insignificantly different (P>0.05) from those offered ungerminated Fetrita diet. This agreed with the results obtained by Hamid (2001) who found better broiler performance have been observed if germination process did not exceed 3 - days. Broiler chicks fed 5 - days germinated Fetrita diet showed significant (P<0.05) poor body weight gain than those fed ungerminated Fetrita diet during starter period, although tannin content did not reach the toxic level (0.64 - 0.84 %) as suggested by Fuller *et al.* (1966). It has been reported that sprouting of sorghum reduced protein and energy utilization in rats and caused inadequate performance in growing pigs (Shem *et al.,* 1990). Hamid (2001) mentioned that germination of low - tannin sorghum for more than 3 days tends to reduce feed consumption and body weight gain.

During starter, finisher and the entire rearing periods, 7 - days germinated Fetrita diet resulted in significant (P<0.01) poor weight gain and significant (P<0.05) decrease in feed consumption. Also it resulted in significant (P<0.01) poor feed conversion ratio except during finisher period. These effects are mainly due to increase in tannin content which reached toxic level (0.64 - 0.84 %) by the seventh day of germination. Some studies reported that lysine content of sorghum grains increased as a result of germination process (Wu and Wall, 1980; Okoh *et al.*, 1989; Elkhalifa, 1993). Therefore lysine content of the diets in this study that contained germinated Fetrita was expected to increase, particularly protein content of the germinated Fetrita has increased by 31%. However, this increase in lysine was not demonstrated on the performance of broiler chicks. This is probably due to the high increase in tannin content which reached 100% by seventh day of germination. Si *et al.* (2001) reported that at starter period addition of 0.2% lysine to broiler chicks diet containing NRC (1994) recommended level of lysine resulted in insignificant

increase in body weight when compared to those fed diet supplemented with 0.1% lysine. Also they found addition of 0.3% lysine resulted in a significant reduction in body weight when compared to broiler chicks fed diet supplemented with 0.2% lysine. Furthermore, they reported that there were insignificant differences in body weight of birds fed diet supplemented with various levels of lysine at finisher period. Corzo *et al.* (2006) found that supplementation the diet of female broilers with lysine at 42 day of age had no effect on feed conversion ratio and body weight. Okoh *et al.* (1989) reported insignificant differences ($P>0.05$) between feed consumption, body weight gain and feed conversion ratio of supplemented malted sorghum with lysine and that of supplemented unmalted sorghum with lysine. All these reports may explain why increase of protein and lysine in the 7 - days germinated Fetrita diet failed to support optimum broiler performance in this diet.

Some studies have reported that tannin decreases the growth of broiler chicks. Hassan *et al.* (2003) reported that high - tannin sorghum caused a highly significant ($P< 0.05$) reduction in the weight gain and feed intake of broiler chicks compared to low - tannin sorghum and increased the feed conversion ratio ($P<0.01$). Herstad (1980); Mitaru *et al.* (1983); Barry and Manley (1984); Ibrahim *et al.* (1988) found significant decrease in feed intake of broiler chicks fed diets containing tannin. Banda-Nyirenda and Vohra (1990) and Douglas *et al.* (1991b) found poor feed efficiency when they fed high - tannin sorghum to broiler chicks. Douglas *et al.* (1990b) and Elkin *et al.* (1995) attributed this reduction in growth of broiler chicks to that tannins decrease utilization of energy, protein and specific amino acids.

It can be concluded that under the conditions of this study, germination of low - tannin sorghum increased protein and tannin contents and decreased metabolizable energy. When the germination period exceeded 3 days poor performance of broiler chicks took place.

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Protein, Mineral Content and Amino Acid Profile of Sorghum Flour as Influenced by Soybean Protein Concentrate Supplementation

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Abstract: This study was conducted to investigate the affect of supplementation of sorghum flour with soy protein concentrate (SPC) on ash, protein, mineral composition (Cu, Ca, Fe, Na and K) amino acids profile and electrophoretic patterns of their blends. The protein contents were found to be 14, 68, 18, 22 and 26% for sorghum flour, SPC, meal one, meal two and meal three, respectively. However, their ash contents were 2.29, 6.51, 2.82, 3.01 and 3.67%, respectively. No significant difference ($P \leq 0.05$) in terms of the ash content was observed between meal one and two, while meal three was significantly different from the remaining meals. Mineral composition of the tested samples significantly differs between the meals ($P \leq 0.05$) Supplementation of sorghum flour with SPC showed a significant increase in lysine and threonine contents, with a slight increase in methionine level in meal one and two. Electrophoretic patterns of samples indicated the appearance of new bands in all blends as well as intensification of protein bands due to the interaction between the two native proteins.

Key words: Chemical composition, electrophoretic patterns, sorghum flour, soy-protein concentrate

Introduction

Cereals are important sources of energy and protein in human diets. Although carbohydrates are their main dietary contribution, they provide protein and smaller amount of lipid, fibre and vitamins. It is commonly known that the main nutritional drawback to cereals, particularly sorghum (*Sorghum bicolor*), is their low protein content and the limited biological quality of their protein (highly deficient in lysine and tryptophan) (Mertz, 1970; Ortega *et al*., 1986 and Waliszewski *et al*., 2000) compared to animal protein. Nevertheless, the protein quality cereals can be improved by combining it with other rich sources of protein. Soybean (*Glycine max* L, Merril) proteins are used in human food in a variety of forms, including infant formula, flours, protein isolate and concentrates and textured fibres. Soy foods include cheese, drinks, miso, temph, tofu, salami and vegetarian meat substitutes. New soy foods are continually being developed. (Liu, 2000 and Singh *et al*., 2000). Effort to increase the availability of protein in human diet from plant materials, particularly soybean concentrate and isolate have partially replaced wheat flour in some baked products (Tsen *et al*., 1982). Soybean blend have been used as an excellent source of protein for corn tortilla fortification (Figueroa *et al*., 2003) with improved essential amino acid balance, specially lysine and tryptophan which are low in cereals (Serna-Saldivar *et al*., 1988 and Waliszewski *et al*., 2000). Fortification of wheat flour with soy proteins improved the protein quality reflected in amino acid profile (Stark *et al*., 1975). The present investigation was undertaken to study the nutritive value of sorghum as affected by supplementation with soybean protein concentrate and electrophretic assay of native proteins.

Materials and Methods

Commercial soy protein concentrate (SPC) was obtained from Loli Meat Manufacturing Company, Khartoum and Sudan. Sorghum (*Feterita*) was obtained from the local market. The seeds were cleaned and freed from foreign material and broken seeds. The clean seeds were milled into flour to pass a 0.4 mm screen. The flour was stored in polyethylene bags at 4°C for further analysis. Unless otherwise stated, all reagents used in this study are of analytical - grade.

Sample preparation

Cooking: Cooked samples were prepared by suspending the flour of each sample in distilled water in the ratio of 1:10 (W/V) flour to water and stirring to avoid lumps, while boiling in a water-bath for 20min. Then the samples were freeze-dried and kept in polyethylene bags at 4°C for further analysis according to Arbab and El Tinay (1997).

Soybean protein concentrate (SPC) supplementation: The supplementation of sorghum with soybean protein concentrate was elevated by 4, 8 and 12%.

Protein and ash content: Crude protein (N x 6.25), ash

Table 1: Protein and ash content (%) of sorghum flour, soy protein concentrate (SPC) and their meals.

	Treatments				
Parameter	Sorghum flour	SPC	Meal 1	Meal 2	Meal 3
Protein	14.00^{e} (± 0.00)	68.25^{a} (±0.00)	18.00^{d} (±0.00)	22.00^{c} (±0.00)	26.00^{b} (±0.00)
Ash	2.29^{d} (±0.01)	6.51^{a} (±0.03)	2.82 ^{c} (±0.05)	3.01^{c} (±0.45)	3.67^{b} (±0.05)

Values are means (±SD), Values not sharing a common superscript in a column are significantly ($p \leq 0.05$) different.

Table 2: Mineral Contents (mg/100g) of Sorghum Flour, Soy Protein Concentrates (SPC) and their Meals.

	Treatments				
Parameter	Sorghum flour	SPC	Meal 1	Meal 2	Meal 3
Cu	0.41^{e} (±0.01)	0.85^{a} (±0.05)	0.76^{b} (±0.01)	0.62^{d} (±0.00)	0.68^{c} (±0.00)
Ca	2.43^{e} (±0.02)	27.79^{a} (±0.15)	6.17^{d} (±0.01)	9.23^{c} (±0.01)	11.38^{b} (±0.01)
Fe	15.54^{d} (±0.01)	7.15^{d} (±0.01)	18.10^{b} (±0.06)	16.64^{c} (±0.07)	19.41^{a} (±0.30)
P	263.30^{e} (±0.10)	469.63^{a} (±0.42)	282.11^{d} (±0.19)	331.40^{c} (±0.10)	342.08^{b} (±0.07)
Na	6.18^{e} (±0.16)	82.50^{a} (±0.10)	7.23^{d} (±0.06)	17.68^{b} (±0.05)	15.61^{c} (±0.01)
K	225.23^{e} (± 0.16)	1020.30^{a} (±0.10)	310.00^{d} (±0.06)	314.79^{c} (±0.05)	389.99^{b} (±0.01)

Values are means (±SD), values not sharing a common superscript in a column are significantly ($p \leq 0.05$) different.

content of the raw material (sorghum and SPC) and cooked samples were determined according to AOAC (1984).

Determination of mineral content: Minerals were extracted from the samples by dry ashing method as described by Chapman and Pratt (1982) the amount of iron, Ca and Cu were determined using atomic absorption spectroscopy (Perkin-Elmer 2380). Phosphorus was determined according to Chapman and Pratt (1982). Sodium and potassium were determined by flame photometer (CORNIGEEL) according to AOAC (1984).

Determination of amino acids: The amino acid content was determined according to official method of analysis AACC (2000) using LKB Biochrom 4150 (Alpha) Automatic Amino acid Analyzer based on ion-exchange Chromatography.

SDS-polyacrylamide gel electrophoresis: Electrophoretic pattern of sorghum flour and soy protein concentrate and their blends was conducted as follow. Deffated soybean and sorghum blends were extracted with 0.03 M Tris-HCl (pH 8.0) buffer at room temperature to yield whole buffer extract as described by Iwabuchi and Yamauchi (1987), then centrifuged at 500 rpm for 30min at 15^{0}C. Soluble protein was estimated using the method of Comassie Brillent Blue G-250 according to Bradford (1976). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (1970).

Statistical Analysis: Each determination was carried out on three separate samples and analyzed in triplicate and figures were then averaged. Data was assessed by the analysis of variance (ANOVA) (Snedecor and Cochran, 1987). Duncan Multiple Range Test (DMRT, 1955) was used to separate means. Significance was accepted at $P \leq 0.05$.

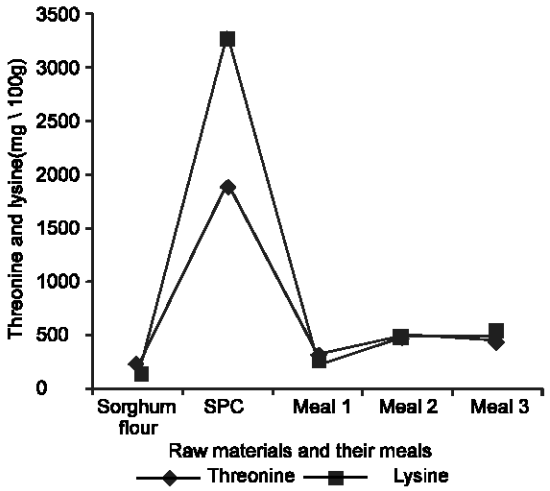

Fig. 1: Effect of supplementation of sorghum flour with SPC on Lysine and Threonine.

Results and Discussion

Chemical composition of sorghum flour, soy protein concentrates (SPC) and their meals: Table 1 shows the protein and ash content of sorghum flour, SPC and soy-sorghum flour meals. The soy-sorghum flour meals have increased protein content (18-26%) and ash (2.82-3.67%). In all meals there were significant improvements in the quality of soy-enriched sorghum. The protein content of sorghum increased significantly with the addition of soy protein concentrate. There were significant differences ($P < 0.05$) in ash content. The protein and ash contents were higher in meal three. This work confirms earlier report by Fashakin (1994) on the beneficial effect of vegetable protein. Supplementation of sorghum flour with the soy protein concentrate (SPC)

Table 3: Amino acids profile (mg/100g) of sorghum flour, soy protein concentrate (SPC) and their meals.

Amino acids	Sorghum flour	SPC	Meal 1	Meal 2	Meal 3
Aspartic	517.81	5304.30	831.12	1281.73	1202.75
Thrionine	204.72	1910.50	324.81	503.32	456.80
Serine	231.55	1778.10	352.40	515.82	473.16
Glutamic	995.86	6520.40	1457.65	2130.27	2020.15
Glycine	72.22	2017.16	144.61	286.16	323.03
Alanine	984.00	2392.30	1108.98	1227.42	1090.40
Valine	504.85	2728.20	597.50	774.85	697.78
Methionine	134.95	608.50	139.11	176.72	150.66
Isoleucine	411.73	2402.70	546.41	738.17	685.75
Leucine	1230.76	3896.10	1473.27	1721.78	1508.85
Tyrosine	147.33	1398.50	164.06	224.18	96.51
Phenylalanine	443.47	2620.90	601.21	807.66	688.74
Histidine	219.23	1546.66	313.91	441.15	448.01
Lysine	105.75	3306.50	252.40	510.73	506.58
Ammonia	877.22	2460.57	1071.11	1393.46	1440.69

Means are of duplicate samples, Meal 1: 18% on protein base. Meal 2: 22% on protein base. Meal 3 :24% on protein base.

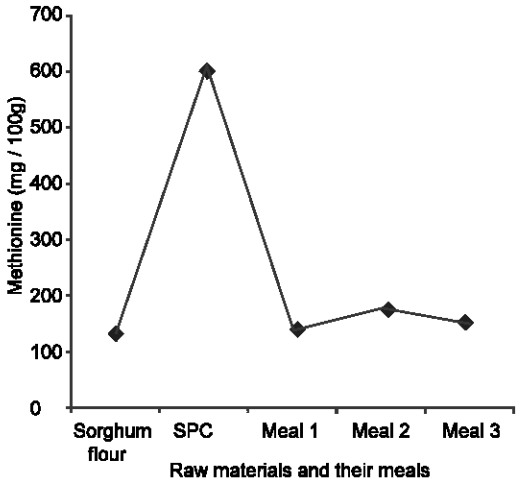

Fig. 2: Effect of supplementation of sorghum with SPC on methionine level.

reaching 18, 22 and 26% protein which meets the RDI of infant fed solely on sorghum-soy meals. About 285 - 310g to meat the daily protein requirements of 55g proteins (FAO/WHO, 1974). The increase in protein values of food that was supplemented with soy protein concentrate had been reported earlier by many investigators (Shuey and Tipples, 1982).

Mineral content of sorghum flour, soy protein concentrates (SPC) and their meals: Table 2 shows the results of mineral composition of sorghum flour, soy protein concentrate (SPC) and soy-sorghum flour blends. Sodium and potassium contents of the meal samples varied from 7.23 to 15.61 mg/100g and 310 to 389.99 mg/100g, respectively. The 12% SPC supplementation had significantly ($P \leq 0.05$) higher sodium and potassium contents. All meals were significantly different in sodium and potassium contents. Phosphorus was found to be varying from 263.30 to 469.63 mg/100g. The values of phosphorus in all the meal samples were significantly different ($P \leq 0.05$) from each other. Iron, calcium and copper contents of meal samples varied from 7.15 to 19.41 mg/100g, 2.43 to 27.79 mg/100g and .41 to .85 mg/100g, respectively. The copper and calcium contents of SPC were significantly higher than others while the iron content was significantly higher in sorghum flour compared to SPC and the three meals. The samples differ significantly among themselves with regard to all cations tested. The results obtained are in full agreement with Ijarotimi and Ashipa (2005) who studied the mineral content of weaning foods made of sweet potato supplemented with soybean flours.

Amino acid profile of sorghum flour, soy protein concentrates (SPC) and their meals: Table 3 shows the essential amino acids composition of sorghum flour, soy protein concentrate (SPC) and their meals. The amino acids content of sorghum flour was in agreement with Dendy (1995) who studied the amino acid composition of sorghums (regular and brown cultivars). The results revealed that lysine and threonine have the lowest values among all meals. Amino acids content of SPC was higher than sorghum flour. Methionine content was the lowest one. Result agreed with the those reported by Friedman *et al.* (1991). Supplementation of sorghum by soy protein concentrate (SPC) elevated protein content as well as protein quality (essential amino acid profile). The results obtained are in agreement with Stark *et al.* (1975) who reported that fortification of wheat flour with soy proteins increased protein quality by improving amino acids profile. Lysine and threonine contents of sorghum flour and soy protein concentrate (SPC) and soy-sorghum flour meals are shown in Fig. 1. Sorghum proteins have been reported to be limited in lysine and threonine (Shelton *et al.*, 1951). The presence of relatively high concentration of leucine in sorghum has been suggested as possible

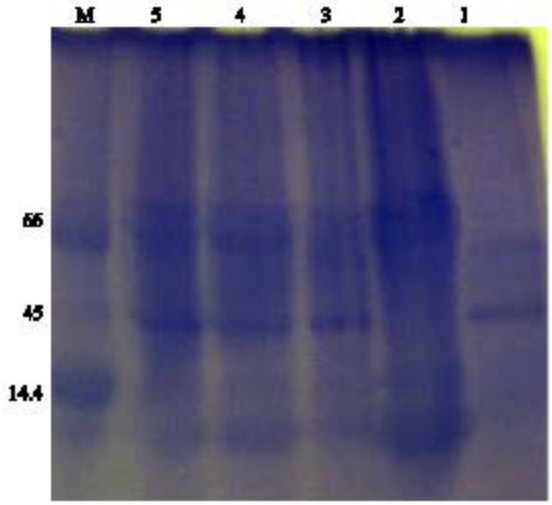

Fig. 1: Electrophoretic separation of protein subunits in Tris - glycine buffer pH 8.3 of sorghum and soybean samples in SDS - PAGE 5 and 15 % With 0.1% SDS.
1 = Sorghum, 2 = SPC, 3 = Blend 1, 4 = Blend 2, 5 = Blend 3. M = Molecular weight protein markers; Albumin bovine 66.0, Ovalbumin 45.0, Lysozyme 14.4 K Da.

factor in the development of pellagra in a population group subsisting principally on this crop (Belavady *et al.,* 1967). The correlation between protein and lysine is significantly negative while that between the protein and leucine is positive. Lysine content of soy proteins falls between that of cereal proteins such as wheat gluten and that of animal proteins such as casein (Betschart, 1978 and Friedman and Finot, 1990).Supplementation of sorghum flour with soy protein concentrate (SPC) increased the lysine content from 105.7 mg/100g in sorghum flour to 252.40 mg/100g in meal one (based on 18% proteins content) and this increment was doubled (510.73 mg/100g) in meal two (22% protein content) while no change was observed in meal three (26% protein content). Threonine contents were 204.72mg/100g in sorghum flour and 813.12, 1281.73 and 1202.75 mg/100g in supplemented meals 1, 2 and 3, respectively. Fig. 2 shows the methionine levels of sorghum flour, soy protein concentrate and soy-sorghum flour meals. The low contents of the essential acid L-methionine in soy protein limits its nutritive value. Methionine content of soy protein concentrate was 608.50 mg/100g which agrees with Brandon *et al.* (1991) who reported methionine content of soy flour as only 650mg/100g. This value is much lower than that of other cereals and meat (Friedman, 1996 and Sarwar *et al.*, 1993). Although cysteine has a sparing effect on methionine, it does not make up for the low methionine levels. The problem is further compounded for two reasons, first, during food processing and storage L-methionine and other amino acids are chemically modified, further reducing nutritional quality in case of methionine, such modification included oxidation to methionine sulfoxide and methionine sulfone, racemization to D-methionine and degradation to compounds with undesirable flavors, second, protein bound methionine in some plant foods is poorly utilized, presumably because of poor digestibility (Begbie and Pusztai, 1989 and Gumbmann *et al.,* 1983). The methionine content of soy-sorghum flours meals were 139.11, 176.72 and 150.66mg/100g, respectively. Methionine content was low in sorghum flour and SPC but higher in meal one and two (18 and 22% protein content and respectively) while no increase was observed in meal three (based on 26% protein content) this may be due to SPC being subjected to excessive heat during cooking.

Electrophoretic patterns of soy-sorghum blends: Results of electrophoretic patterns of soy protein concentrate (SPC), sorghum flour and soy-sorghum blends are shows in Fig. 3. The SDS-PAGE revealed that for sorghum protein (lane 1) the MW of the protein bands confined between 14.4 to 45KD, while those of SPC were extended in the range of 14.4 to 66 KD, considering that other bands had molecular weight lower than 14.4 KD. In composite flour samples bands of molecular weight of 45 to 66 KD were observed .Bearing in mind that the detectable bands in blends of higher intensity, suggesting the effect of addition of SPC proteins to the sorghum flour native protein. However, in composite flour samples with SPC have higher protein quantity and quality (Stark *et al.,* 1975) and the intensification of bands in soy blends indicate protein aggregation, suggesting interaction between two native proteins in the blends.

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Poor Dietary Intake of Energy and Retinol among Pregnant Women: Implications for Pregnancy Outcome in Southwest Nigeria

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Abstract: This paper examines the impact of adequate intake energy and retinol on pregnancy outcome among selected pregnant women in Osun State, Southwest Nigeria. Eight hundred and forty (840) pregnant and 250 non-pregnant women were involved in the survey conducted in 2006 which used a 24-hour diet recall to assess their dietary intake. Food models were used to assist memory and portion size of foods taken. The results indicate that dietary energy intake in this study was inadequate in about 75% of the pregnant women and 65% of the non-pregnant women. With the exception of protein intakes in Ife North, inadequate intake was less than 15% in all LGA. The structure of the menu, however, point to a preponderance of protein of plant origin. Compared with protein intake, the dietary intake of Vitamin A (Retinol) was inadequate in all the LGA and among pregnant and non-pregnant women. The paucity of animal protein in the diet may be responsible for this. Almost 70% of the pregnant women also had inadequate Vitamin C intakes. The study therefore, reiterates that emphasis on dietary sources of energy, iron, vitamin A and folic acid during pregnancy should be the key discussion during antenatal visits. Food demonstration during the antenatal visits offers the best strategies.

Key words: Malnutrition, pregnancy, antenatal, outcome, miscarriage, dietary, prevalence

Introduction

Nigeria is one of the countries with the highest maternal mortality rate in the world (Harrison, 1997; Orji *et al*., 2002) and malnutrition during pregnancy has been recognized as one of the major factors for this high maternal mortality in developing countries (Rush, 2000; Keen, 2003). This is because maternal malnutrition worsens the other causes of maternal morbidity such as anaemia, postpartum haemorrhage, obstructed labour and infections among others. Several studies have shown that poor dietary pattern before and during pregnancy is one of the major causes of malnutrition (Sanusi and Oredipe, 2002; Ojofeitimi *et al*., 1982).

Although, the demand for energy and other nutrients increase during pregnancy, some pregnant women deliberately reduce their dietary intake in an attempt to have smaller babies (Ojofeitimi *et al*., 1982). This is because of the fear that uncontrolled dietary intake during pregnancy results in big baby which in turn leads to increased risk for caesarean section (Ojofeitimi *et al*., 1982; Ojofeitimi and Tanimowo, 1980). The consequence of this faulty dietary habits is maternal malnutrition leading to increase in the incidence of anemia in pregnancy, low birth weight, poor maternal weight gain and increase risk for neural tube defects, cleft lip, cleft palate and maternal mortality and morbidity (Keen, 2003; Sanusi and Oredipe, 2002; Sanusi and Omoni, 2000; Sanusi and Akinyele, 1999; Fall, 2003; Wald *et al*., 1998; Langley-Evans and Langley-Evans, 2002; Barker, 1997).

Adequate nutrition, especially for women and children remains an important concern in Nigeria. A comparison of the National Demographic and Health Survey (NDHS) 1990 and 1999 showed that stunting and wasting seem to have increased among children aged 0-36 months (from 36 to 46 percent and from 11 to 12 percent respectively). Early nutritional intervention at the onset of pregnancy will certainly reduce the prevalence of stuntingness and wasting among these children, hence the need for this study.

Materials and Methoods

The study was conducted in Osun state, Nigeria. The state which came into existence on 27th August 1991 has 30 Local Government Areas with a total population of 2,158,143 (1,043,126 males and 1,115,017 females). According to the 2002 Nigeria Reproductive Health service provision survey, the state is the fourth primary level health facility providers by number in Nigeria with 15 Dispensaries, 65 Health posts, 10 Maternity Centers and 408 Primary Health Centers with a ratio of 1 PHC to 10,000 people. There are three Teaching Hospitals, two Federal Hospitals, six States General Hospitals located

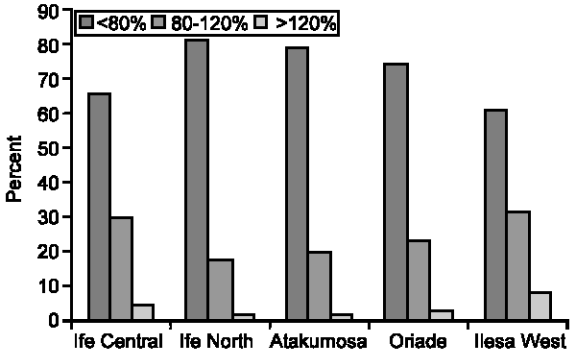

Fig. 1: Adequacy of Energy Intakes in the Selected LGAs

in urban and rural communities in the state. The major occupation of the people is farming, both for export and food crops.

For data generation, a descriptive and cross-sectional design was employed. The first stage of sampling was the selection of six Local Government Area (LGA) (3 urban and 3 rural) from the 30 LGA in the state using urban and rural status. The urban LGA are: Ife Central, Ife East and Ilesa West, while the rural LGA include Atakumosa East and West and Oriade.

Eight hundred and forty-four women of reproductive age (15-49) were randomly selected for interview with probability of selection proportional to the population size and availability of health facility in each of the LGA. Based on these criteria, two hundred and seventy-five (275) were from Ife Central LGA, one hundred and nineteen (119) from Ife North LGA, seventy-six (76) from Atakumosa east and west LGAs, one hundred and four (104) from Oriade LGA and one hundred and twenty-five (125) from Ilesa West LGA. However, to qualify for inclusion in the sample, certain conditions must be met. Among these are: the woman must be willing to participate, be under 35 years, should be at least 3 months pregnant with adequate evidence and should be willing to deliver at the maternity centers, her height should be above 1.5 metres to obviate cephalo-pelvic disproportion, should not have had history of miscarriage, still birth or obstructed labour and not suffering from oedema or any other related medical problems that increase weight or accumulates fluids related to pregnancy and should be of singleton pregnancy. Dietary intake of these pregnant women was assessed using the 24 hour dietary recall. The pregnant women were interviewed at the health facilities during the antenatal care while the non-pregnant women of reproductive age in the same communities were interviewed in their homes to serve as control. Socio-demographic data such as age, occupation, family income and parity were collected.

To assess the dietary intake of the respondents, a 24-hour diet recall was done for each subject to assess the usual dietary intake. Food models were used to assist memory and portion size of foods taken. However, for comparison, dietary intakes of two hundred and fifty (250) randomly selected non-pregnant women were assessed using the same instrument. The interviewers were trained and techniques standardized.

The instrument for data harvesting was validated and pre-tested. Interviewers were trained and procedures standardized. Food models were used to aid correctness of portion sizes. The dietary supplement was analysed to know the nutrient context/100g. Weighing scales were procured for each of the health facilities used for the study.

The 24-hour dietary recall was analysed using an electronic software Total-Diet Assessment (TDA)® to obtain the nutrient content of the foods. Prior to this, the amount of food consumed was converted to weight. Adequacy of nutrient intake was assessed using the WHO Recommended Dietary intake (RDA) for international use (Passmore and Nicol, 1974).

Descriptive Statistics that includes means, standard deviation, percentages and proportion were derived. The chi-squared was used to compare frequency between nominal values. The Statistical Package for Social Sciences (SPSS) version 11 was used for all analyses.

The protocol for this study was reviewed and approved by the Ethical Board of the Obafemi Awolowo University Teaching Hospital Comlex Ile-Ife. The participants in this study also gave informed consent after the details of the study were explained to each of them and voluntary participation requested.

Results and Discussion

The background characteristics of respondents showed a youthful population with more than 40% of the population in age group 20-29. The major ethnic group is Yoruba; though other ethnic groups are still found in the study locations. More than 70% had received formal education, the highest being secondary education. More than 80 percent professed to be Christian as against 16.4 percent Muslims. Examination of occupational distribution revealed that the dominant occupation among the respondents was trading (41.2%).

Fig. 1 shows that in each of the LGA, more than sixty percent had inadequate dietary energy intake (see Table 1). As presented in Fig. 2, dietary protein intake was adequate in more than 65% of the women. This however, is predominantly plant proteins, which is limited in essential amino acids. As shown in Table 1, dietary intake of vitamin A in all the LGA was inadequate, but dietary intake of vitamin C was far better in Ife North, Atakumosa and Ilesa West than in Ife Central and Oriade.

Table 1 further shows further that dietary foliate intake was adequate in all the LGA. Intake of calcium was

Table 1: Adequacy of Dietary Energy and Micronutrient Intakes by Local Government Area

%RDA Energy	Ife Central (N = 275)	Ife North (N = 119)	Atakumosa (N = 76)	Oriade (N = 104)	Ilesa West (N = 125)
<80%	65.5	80.7	78.9	74.0	60.8
80-120%	29.8	17.6	19.7	23.1	31.2
>120%	4.7	1.7	1.7	2.9	8.0
%RDA Protein					
<80%	13.1	21.0	9.2	13.4	8.8
80-120%	18.2	12.6	13.2	18.3	15.2
>120%	68.7	66.4	77.6	68.3	76.0
%RDA Vitamin A					
<80%	100.0	100.0	100.0	100.0	100.0
80-120%	-	-	-	-	-
>120%	-	-	-	-	-
%RDA Vitamin C					
<80%	85.4	36.1	25.0	64.4	29.6
80-120%	5.8	16.8	26.3	10.6	16.8
>120%	8.7	47.1	48.7	25.0	53.6
%RDA Folate					
<80%	25.5	22.7	15.8	12.5	11.2
80-120%	14.3	13.4	11.8	2.5	4.8
>120%	60.2	63.9	72.4	85.0	84.0
%RDA Calcium					
<80%	96.0	94.1	89.5	95.0	88.7
80-120%	3.3	4.2	10.5	5.0	8.9
>120%	0.7	1.7	-	-	2.4
%RDA Zinc					
<80%	94.2	82.4	86.8	88.3	80.8
80-120%	4.4	12.6	10.5	9.7	11.2
>120%	1.4	5.0	2.6	1.9	8.0
%RDA Iron					
<80%	84.6	89.0	94.7	83.6	82.2
80-120%	9.3	8.5	2.6	13.5	11.4
>120%	6.1	2.5	2.6	2.9	6.5
Total	100.0	100.0	100.0	100.0	100.0

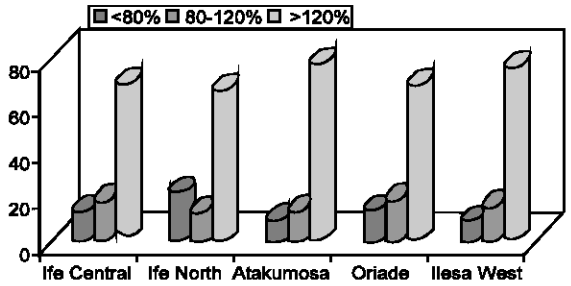

Fig. 2: Adequacy of Protein Intakes in the Selected LGAs

generally poor among the respondents. In addition, intake of dietary iron and zinc were extremely poor in all the LGA, particularly among the pregnant women.

Dietary energy intake in this study was inadequate in about 75% of the pregnant women and 65% of the non-pregnant women (Table 2). The Recommended Dietary Allowance (RDA) was 2200Cal and 2550Cal for non-pregnant and pregnant women respectively (WHO, 1997). This inadequacy of dietary energy intake is similar in the five LGA assessed. Furthermore, the difference in intakes of dietary energy between the pregnant and non-pregnant was not significant ($p = 0.05$). Earlier investigators had reported diet restrictions and nutritional aversion during pregnancy based on health, religion and tradition in these locations (Oboro *et al.*, 2003; Ojofeitimi *et al.*, 1982). This high degree of inadequate dietary energy is consistent with household food insecurity (Abudu and Akinkugbe, 1982). Sanus *et al.* (2006) had found a 70% prevalence of household food insecurity in their study in Lagos and Ibadan. Similarly, Maziya-Dixon *et al.* (2004) had reported a high prevalence of about 60% of severe household food insecurity in the moist savannah in which the present study locations situate.

Dietary protein intakes were more than adequate in all LGA, averaging 65%. With the exception of protein intakes in Ife North, inadequate intake was less than 15% in all LGA. The structure of the menu, however, point to a preponderance of protein of plant origin. Compared with protein intake, the dietary intake of Vitamin A (Retinol) in the LGAs was inadequate in all the LGA and among pregnant and non-pregnant women. The paucity of animal protein in the diet may be responsible for this. Almost 70% of the pregnant women had inadequate Vitamin C intakes (Table 1).

Table 2: Adequacy of Dietary Energy and Micronutrient Intakes

Variable	Not Pregnant (N = 250)	Pregnant (N = 844)
%RDA Energy		
<80%	65.4	75.5
80-120%	28.9	21.9
>120%	5.7	2.6
%RDA Protein		
<80%	12.0	16.4
80-120%	10.9	20.3
>120%	77.1	62.8
%RDA Vitamin A		
<80%	100.0	100.0
80-120%	-	-
>120%	-	-
%RDA Vitamin C		
<80%	46.6	68.0
80-120%	11.1	15.9
>120%	42.8	16.1
%RDA Folate		
<80%	11.6	28.2
80-120%	7.3	16.1
>120%	81.0	55.7
%RDA Calcium		
<80%	88.2	99.4
80-120%	10.3	-
>120%	1.5	0.6
%RDA Zinc		
<80%	81.4	93.9
80-120%	12.9	4.9
>120%	5.7	1.2
%RDA Iron		
<80%	79.9	78.3
80-120%	16.6	16.2
>120%	3.4	5.5

Intake of foliate is high in all the LGA with over 60% having intakes of over 120% RDA. Over 71% of the pregnant women had adequate dietary intake of folate indicating that as high as 28% of the pregnant women did not have adequate intake of foliate. This however does not pose a problem since pregnant women usually use supplementary folic acid. The inadequacy of dietary iron in all the LGA is an issue which is surprising in this study, although iron supplements are given during pregnancy.

As shown in Table 2 a third of the pregnant women and about 30% of non-pregnant women had adequate dietary energy intakes. But dietary protein was adequate in both pregnant and non-pregnant women. Both pregnant and non-pregnant women's intake of vitamin A was inadequate (Table 2). There was a higher intake of vitamin C among the non-pregnant than the pregnant women. However, more of the pregnant than non-pregnant women had inadequate intake of foliate.

Conclusion: Studies have established that women who have better nutritional status at the time they become pregnant are better able to meet the demands imposed by the pregnancy and tend to have more successful outcomes (Ojofeitimi *et al.*, 1982). There is no doubt that malnutrition is pervasive and even if it does not result in death, the burden it implies in terms of retarded development and vulnerability to diseases is great.

Though there are other social and behavioural factors that clearly impair foetal development such as malaria, HIV/AIDS, maternal smoking, but maternal nutritional status is an important determinant of the outcome of a pregnancy. The ability of a woman to carry a healthy pregnancy and successfully suckle her infant during its first year of life requires that she has the capability to provide all the energy and nutrients that are required. Woman with good nutritional status at the time they become pregnant are better able to meet the demands imposed by the pregnancy and have more successful outcomes. This study had shown that nutritional intervention during pregnancy would overcome poor maternal weight gain and reduce the incidence of low birth weight and premature deliveries using locally available and affordable foodstuff.

In spite of iron supplementation during pregnancy, inadequacy of dietary iron intake could still exist, more so when the major sources of proteins are from plant origin. More importantly, iron deficiency anaemia has been linked with adverse pregnancy outcomes; low birth weight, preterm, stillbirth, neonatal infection, maternal morbidity and mortality. These findings indicate that specific policies should be developed by appropriate governmental agencies and ministries to control and prevent both micronutrients and energy deficiencies during pregnancy in order to reduce the high maternal morbidity and mortality in Nigeria. Emphasis on dietary sources of energy, iron, vitamin A and folic acid during pregnancy should be the key discussion during antenatal visits. Food demonstration during the antenatal visits offers the best strategies.

The role of health workers in improving maternal health is quite instructive; if health workers are given the necessary orientation, they could be a very useful outlet for enlightening the women on discarding old-fashioned practices of food avoidance and achieve a better maternal nutrition status. This could help in reduction of maternal malnutrition, morbidity and mortality. Policy makers and programme officers could thus use health workers as instruments to achieve necessary social change as regards food prohibitions.

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Diet and Nutrition Status of Children in Four Tribal Blocks of Thane District of Maharashtra, India (Nutrition Status of Children)

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Abstract: India accounts for about 40% of undernourished children in the world, which contribute to high morbidity and mortality in the country. Recently reports on deaths due to malnutrition among Tribal communities of Thane district in the State of Maharashtra were widely published in the newspapers and also communicated via other media. A rapid survey was undertaken to investigate the extent of the problem in selected villages of Thane district where the malnutrition deaths were reported. Information was collected from a total of 118 households in 4 villages on nutritional status through clinical examination, anthropometry, dietary intake and general socio-economic status. The results revealed that majority of the population belonged to Scheduled tribe community (95.8%). Data on food and nutrient intake indicated that rice is consumed as the major dietary staple in the region followed by ragi (*Eleusine coracana*), wheat (*Triticum aestivum*) and bajra (*Pennisetum typhoideum*). The average calorie intake per Consumption Unit (CU) was observed to be 1857 kcal which was less than the RDA by 23% and protein 30% (42g) less than the RDA. The percent distribution of children from 0-6 years of age according to nutritional status indicated that the overall prevalence of underweight (<Median-2SD) among 0-6 year old children was 68.7% while that of severe underweight (<Median-3SD) was 28.6%. The overall prevalence of stunting (<Median-2SD) in the children of the 0-6 year age group was 60.4% while that of severe stunting (<Median-3SD) was 38.5%. The index of current nutritional status and measure of wasting as indicated by weight for height showed overall prevalence as 30.2% and severe wasting as 4.4%. The results of the rapid survey indicated that various factors including health status of mothers, dietary and socio-economic factors have been contributory to the malnutrition of the children and that malnutrition alone may not be the direct cause of the deaths that are reported in the media.

Key words: Nutritional status, tribal community, malnutrition

Introduction

Many countries that are lower than India on GDP ladder have taken better care of their children. India accounts for about 40% of undernourished children in the world, which contribute to high morbidity and mortality in the country (James Levinson, 1998). Health and nutrition status of Indian women and children is in vast and systemic crisis. About 46% of children below the age of 3 years in India, i.e. about 45 million little boys and girls are underweight or malnourished in terms of the standard weight for age criterion (NFHS III Report, 2007). There are of course wide variations across states in both levels and trends in indicators of health and malnutrition. Punjab and Kerala report the lowest proportion of underweight children (27% and 28.8% respectively) while Jharkhand and Madhya Pradesh, more than 59% of children below the age of 3 were underweight. Child malnutrition has actually risen in seven states, most rapidly in Madhya Pradesh and Haryana. The National Plan of Action on Nutrition (National Nutrition Policy ,1993 and 1995) aims to bring down the prevalence of existing under nutrition among children by half and lower birth weight to less than 10%.

The State of Maharashtra is situated at a North latitude 18°42' to 20°20' and East longitude of 72°42' to 73°48' having a total population of 96,878,627 of which 9,881656 belong to Scheduled Caste and 8,577,276 to Scheduled Tribe (Census of India, 2001). The State has a tribal population of 8.9% as against the National average 8%. However the tribal population in Thane district represents 1.96% of the population that is concentrated in some pockets of which Jawahar, Mokhada and Vikramghad blocks which consist of 90, 90.6, 52.67 and 90.35% respectively.

Scheduled tribe is a proxi of socio-economic backwardness in India and the recent malnutrition deaths reported in 4 regions of Thane district namely, Mokhada, Jawahar, Wada and Vikramgadh blocks. The total population in these regions is 70,000 and a total of 718 deaths that were attributed to malnutrition were reported from these regions.

During 2004-2005 reports on malnutrition deaths in Tribal communities in Thane district in the State of Maharashtra occurred that were widely published in the newspapers (The Hindu 21.4.2006) and also communicated via other media. Regions in the district

Table 1: Coverage particulars of the study villages of Thane district

Taluk	Village	No. of HHS	Total population surveyed
Jawahar	Sarsun	31	163
Mokhada	Gomghar	31	233
Mokhada	Suryamal	28	177
Vikramgadh	Jambha	28	149
Total		118	722

Table 2: Average household consumption of foodstuffs (g/CU/day)

n	25
Cereals and Millets (gm)	505±236.58
Roots and Tubers (gm)	73.2±37.74
Other vegetables (gm)	67.7±42.07
Milk and milk products (ml)	27.2±55.09
Flesh food (gm)	10.4±10.55

Table 3: Average household intake of nutrients (CU/day) as compared to RDA

Name of nutrient	Actual intake*	RDA**	% RDA (+/-) to actual intake
Protein	42.0	60 gm	-30.0
Fat	8.0	20gm	-
Energy (Kcal)	1857.0	2425 Kcal	-23.0
Calcium	881.0	400mg	+55.0
Vitamin A (µg/day)	75.0	600 µg	-87.0
Thiamine	1.3	1.2 mg	+8.3
Riboflavin	0.7	1.4 mg	-50.0
Niacin	10.8	16 mg	-33.0
Vitamin C	27.2	40 mg	-32.0
Iron	14.6	28 mg	-48.0
Folic acid (µg/day)	34.4	100µg	-66.0

*N = 25, **% RDA±calculated based on RDA for sedentary worker. Source: Nutritive value of Indian Foods National Institute of Nutrition, Hyderabad, India

Table 4: Distribution (%) of 0-6 year children according to SD classification (<Median-2SD)

N	40
Under weight (weight for age)	68.7
Wasting (weight for height)	30.2
Stunted (Height for age)	60.4

reported to be affected were Jawahar, Mokhada, Wada and Vikramghad Blocks. In order to evaluate the extent of the problem and also identify the associated factors responsible for malnutrition deaths was the aim of the present study.

Materials and Methods

A rapid survey was undertaken in Mokhada, Jawahar and Vikramgadh talukas in the villages where the deaths were reported. These villages were Gomghar (Mokhada taluk), Jamba (Vikramgadh taluk), Sarsun (Jawahar taluk) and Suryamal (Mokhada taluk). A house-to-house survey was carried out using a specially prepared questionnaire in all available households numbering 118. The coverage particulars are given in Table 1. Data was collected on demographic features and socio-economic status. Anthropometric measurements and clinical examination for nutrition deficiency signs was performed for all the available children between 0-6 years of age and lactating mothers in all four villages. A total of 42 children below the age of 6 years (represented by 15.6%) and 40 lactating mothers were covered. Food and nutrient intake was carried out with the help of a food frequency questionnaire in 20% of the households. The nutrient intake was calculated using Nutritive value of Indian foods (Gopalan *et al.*, 2004; Expert Group ICMR, 1990).

Results and Discussion

Demographic and socio-economic profile: About 96% of HHS belonged to Scheduled Tribe (ST) and 4.2% to Scheduled Caste (SC) community. Within the ST community 44.1% belonged to *Madhavakoli,* 25.4% *Worli* and 42% *K.Thakur* caste groups. Some of the following poverty indicators reveal the severity of the economic backwardness of the villages surveyed. About 42% lived in *Kutcha* houses (mud/thatched wall and roof) while 33.9% lived in houses with tiled/asbestos roof and 22% had their houses made of brick/stonewall with thatched roof. A negligible percentage (0.8%) had concrete houses. Electricity was available in 33.9% of the HHS. The source of drinking water was mostly open wells (94%), which were situated at far distances from some villages. Firewood was the major source of fuel for cooking (95.8%).

Disguised Agriculture was the major occupation (46.6%) and major crops were *ragi* and *warai* (*Panicum miliaceum)*. All the land was rain fed. The land used for agriculture was distributed by the State government, which gives 5 hectares per family as *patta*. About 19% were landless agricultural labourers. Migration was about 34% in the villages and was mostly in search of daily wages during the lean season by the families.

Even though illiteracy rate was very high (67.8%) about 11% only completed their schooling. About 67% comprised nuclear families and 30.5% joint families. Sanitation was observed to be very poor. Animals like cattle were also kept in the living area of the family. No latrine facilities were available in these villages.

Food and nutrient intake: Rice was the staple food in surveyed population followed by ragi, wheat and bajra. The government supplies 10 kg of rice and wheat to each of the family as monthly ration at a subsidized price. In addition the household procure rest of the rice and wheat under the food-for-work programme of the government.

The nutrient intake was observed to be below the RDA with respect to all nutrients except for calcium and thiamin (Table 2 and 3). Protein and vitamin A intake were observed to be 30-87% below the RDA (Table 2).

Anthropometry: Data on the percent distribution of pre school children according to NCHS standards for weight for age, height for age and weight for height are given in

Table 5: Anthropometrical measurements (mean±SD) of lactating mothers, age at marriage number of children and prevalence of anemia

Weight (Kg)	Height (cm)	% Mothers with <20 BMI	Age at marriage (year)	Number of live children	Prevalence of anemia (%)
43.14±4.66	150.74±6.766	67.5%	17.27±1.372	2.97±1.233	35%
N=40	N=40	N=40	N=51	N=98	N=40

Fig. 1: A case of marasmus

Table 3. It was observed that the overall prevalence of underweight was about 69% that of stunting was 60% in the same age group. The weight for height, which gives the prevalence of wasting and used as index of current nutritional status was observed to be 60% in the 0-6 year age group (Table 4). A scrutiny of the records from one of the major hospitals in Jawahar indicated that 104 children (<6 yr) died during 2005 to 2006 due to various reasons like premature for death (24), premature for weight (27), infectious asepectia (8), septicemia (16), meningitis (3), pneumonia (16), TB (3), congenital heart disease (1). Out of 104, 5 were in malnutrition Grade III and 5 from Grade IV. In the same year 384 children were in Grade III and 266 in Grade IV malnutrition indicates severity of nutrition problem in the area. During the survey we found case of Marasmus (Fig. 1).

Anthropometric measurements of lactating mothers as shown in Table 5 indicated the intake of protective foods such as green leafy vegetables and protein was grossly inadequate as compared to RDA. In the present survey we found that 67.5% of lactating mother had BMI less than 20. (which complicates by giving birth to low weight babies). Our report correlates with NNMB 2001 survey where the percentage of adult females with chronic energy deficiency in rural Maharashtra was 45.1% and 5.6% adult females were obese (BMI>25).

The state of Maharashtra is progressive state with an average per capita income of Rs. 32,170 per annum (Economic Survey of Maharashtra, 2005-2006). The major occupation is farming. However, the surveyed Talukas in Thane District are tribal taluks constituting 90% tribal population having no match with State per capita income. Due to various reasons their nutritional status is very poor. These villages are located in the hills with no irrigation facility, only ragi and millet grows, no cash crop except warai, more than 4 children, marriage at early age. These are the probably contributory factors for malnutrition in the area. Unemployment in the area force people to migrate and take their children along. Height can be used as a basis for advising women about their level at risk and appropriate choice of place of delivery. Twenty percent of surveyed lactating women had height below 145 cms. As per NFHS-2 survey percent of married women with height below 145 cms in Maharashtra was 11.9%. Short stature may be associated with small pelvis, which can complicate delivery. Majority of the lactating mothers in the study had inadequate nutrients intake than RDA expect calcium, which could be the probable reason for very high rates of malnutrition in children.

Nutrition status is a major determinant of the health and well being of children. Inadequate diets and infections are associated with poor nutrition (NFHS II, 1999).

Children are the first call on agenda of development. Not only because young children are the most vulnerable but because the foundation for life long learning and human development is led in the crucial early years. It is now globally acknowledged that investment in human resources development is a pre-requisite for economic development of any nation. Early childhood (first 6 years) constitutes the most crucial period in life, when the foundations are led for cognitive, social and emotional language, physical/motor development and cumulative life long learning. By the end of the second year of life, most of the growth of the human brain is already complete and critical brain structure is in place. The young child under three years is more vulnerable to the vicious cycle of malnutrition, disease/infections and resultant disability, all of which constitute risks, development opportunities determine both the present of every child and family as well as the future human resource development of the nation.

The results of the rapid survey indicated that various factors including health status of mothers, dietary and

socio-economic factors have been contributory to the malnutrition status of the children and malnutrition alone may not be the direct cause of the deaths as reported in the media.

Limitations of the study: The survey was carried out during a period of 8 days where more number of households could not be covered due to time constraints. Added to this was the problem of high rate of migration during the survey period, which also limited our survey to only available households.

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Performance of Starter Broilers Fed Anaerobically Fermented and Lyle Treated *Delonix regia* Seed Meal

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Abstract: One hundred and twenty day-old Hubbard broiler birds were used to study the effects of anaerobic fermentation and lyle treatment of *Delonix* seed meal on the performance of starter broilers. The experimental period spanned 5 weeks. The birds were divided into four treatment groups of three replicates each using complete randomized design. The anaerobically fermented and lyle treated *Delonix* seed meal (AFLTDSM) was used to replace groundnut cake (GNC) at 0%, 5% and 7.5% level. Untreated raw *Delonix* seed meal (URDSM) was also used at 5% level in order to evaluate its effects on starter broiler performance. The four treatments were designated as T_1, T_2, T_3 and T_4 respectively. Parameters evaluated were mean body weight, daily body weight gain, mean feed intake, mean feed conversion ratio as well as apparent nutrient digestibility. The results showed that mean body weight, daily body weight gain, mean feed intake and mean feed conversion ratio were not significantly affected ($p>0.05$) by the substitution of GNC with AFLTDSM in the diets. However, apparent nutrient digestibility of dry matter (DM), crude protein (CP), ash, ether extract (EE) and Nitrogen free extract (NFE) were significantly affected ($p<0.05$) by the treatment diets. Crude fibre (CF) digestibility was however not affected ($p>0.05$) by the inclusion of *Delonix* seed meal in the diets. It was concluded that AFLTDSM could be used as a substitute for GNC in starter broilers diet without any significant effect on the performance of the birds.

Key words: Performance, starter broilers, anaerobic fermentation, *delonix* and lyle treated

Introduction

Food security has become a major concern in Africa and most of the developing countries due to rapid population growth. According to Gueye (2007), the total mid-year African human population in 2004 was estimated to be 871 million people. This rapid growth is characterized by increasing pressure on biological resources used for the production of food. A fall out of this increase in population is competition between man, the industry and livestock reared to serve as a source of protein needed by the teeming population. In order to be able to provide protein in quantity and quality, there is the need to accelerate the production of livestock generally and poultry in particular.

The rearing of poultry birds either locally which represents more than 80% of the total poultry production in Africa (Gueye, 2000) or commercially appears to be a way out of meeting the need occasioned by the rapid growth in population. With a total poultry population of 1, 356 million chickens, 16 million ducks, 12 million geese and guinea fowls and 9 million turkeys, producing 2, 180, 125 metric tones (MT) of hen eggs, 7, 143 MT of other poultry eggs, 3, 257, 292 MT of chicken meat, 56, 619 MT of duck meat, 55, 340 MT of goose and guinea fowl meat and 66, 252 MT of turkey meat (FAO Aide News, 2007) a solution seems to be around the corner for overcoming the protein deficit apparent in African human population. These birds however need to eat in order to grow, maintain themselves and produce the needed eggs and meat of biological value.

This is where the problem is as most feed ingredients, particularly those of protein and energy origins, are quite expensive and also scarce due to competition for them. The recent drive for alternative energy (ethanol from maize and cassava) should therefore be seen as a great threat to the livestock industry and poultry production in particular. The beacon is therefore now on alternative sources of feed otherwise known as non-conventional feedstuff. These non-conventional feedstuffs are mostly not competed for by man and animals and hence represent a great potential as cheaper and readily available feed resources. There abound in Africa a lot of tree legumes whose seeds can be harvested and incorporated into poultry and other monogastric animal feed.

This study was undertaken therefore to evaluate one of such non-conventional protein source (*Delonix* seed meal) and its effect on the performance of starter broiler birds. The tree plant grows extensively in most African countries where it is used for beautification because of its aesthetic flowers when in season. The seeds are not used as food by man and can easily be collected from the tree during the dry season. Hence it is cheap.

Materials and Methods

Location of study: The study was carried out at the poultry unit of the Department of Animal Production, School of Agriculture and Agricultural Technology of the Federal University of Technology, Minna, Niger state,

Nigeria. Minna is situated on longitude 6° 33' east and latitude 9° 45' north of the equator. Minna experiences two distinct seasons (dry and wet) with an annual precipitation varying from 1,100-1,600mm and a mean temperature of 36.5°C between March and June and 21°C between December and January.

Processing of *delonix regia* and experimental diets: *Delonix* seeds were collected during the dry season within and around Minna environment. Some portion of the collected seeds was anaerobically fermented and then treated with lyle solution using an adaptation of the method described by Annongu *et al.* (2004). It involved milling the seeds using a hammer mill with sieve size of 3mm. The milled seeds were then soaked in a given quantity of tap water for 7 days after which the dough was removed and packed in double layered polythene bags and tied to exclude air. It was then placed in a drum, covered to make it air-tight and left to ferment for another 7 days. After this, the dough was soaked in lyle solution (constituted by dissolving 20Kg of ash in 100 liters of water) for 2 days. It was then strained, sun-dried and stored until further use as anaerobically fermented and lyle treated *Delonix* seed meal (AFLTDSM). Some other portion of the collected seeds was left untreated. This was sun-dried and milled using the same hammer mill as above and stored until further use as untreated raw *Delonix* seed meal (URDSM).

Four different diets were formulated using *Delonix* seed meal (Table 1) and fed to the birds. The formulated diets were:

T_1 = 0% inclusion of *Delonix* seed meal (control).
T_2 = 5% inclusion of URDSM.
T_3 = 5% inclusion of AFLTDSM.
T_4 = 7.5% inclusion of AFLTDSM.

The diets were formulated to be isonitrogenous and isocaloric to meet the protein and energy requirements of starter broilers.

Experimental birds and their management: A total of 120 day-old Hubbard broiler birds were acquired and used for the experiment. The birds on arrival were randomly distributed into four treatment groups of three replicates representing thirty birds/treatment and ten birds/replicate. The poultry house was washed and disinfected before the arrival of the birds. The birds were housed in pens covered with wood shavings as litter material and heated electrically throughout the experimental period with 100watt bulbs. The birds were fed and watered *ad libitum*. They were also vaccinated against Newcastle and Gumboro diseases, the common poultry diseases in the area. Vitalyte® was given as anti-stress whenever operations such as vaccination and weighing were carried out. Coccidiostat was also administered weekly through water as a preventive measure. Other routine management practices were also observed.

Table 1: Composition of experimental diets

	Diets			
Ingredients	T_1	T_2	T_3	T_4
Maize	54.30	54.30	54.30	54.30
GNC	32.90	27.90	27.90	25.40
URDSM	0.00	5.00	0.00	0.00
AFLTDSM	0.00	0.00	5.00	7.50
Maize bran	4.45	2.45	2.45	2.45
Fish meal	3.00	5.00	5.00	5.00
Bone meal	0.80	0.80	0.80	0.80
Limestone	1.00	1.00	1.00	1.00
Palm oil	3.00	3.00	3.00	3.00
Salt	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Methionine 0.10	0.10	0.10	0.10	
Premix*	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
%CP	22.00	22.00	22.00	22.00
ME (Kcal/Kg)	3138.00	3049.00	3047.00	3014.00
Chemical composition (%)				
DM	77.20	80.00	81.00	85.80
CP	23.00	22.75	22.75	22.75
CF	3.30	4.60	3.61	5.60
EE	20.00	17.50	17.50	19.00
Ash	5.40	4.80	5.40	4.80
NFE	25.50	30.35	32.74	33.65

*2.5Kg of premix contains: Vitamin A (10000000iu), Vitamin D_3 (2000000iu), Vitamin E (12000iu), Vitamin K (2iu), Thiamine B (1.5g), Riboflavin B_2 (5g), Pyriboflavin B_6 (1.5g), Vitamin B_{12} (10mg), Biotin (20mg), Niacin (15g), Pantothenic acid (5g), Folic acid (0.6g), Manganese (75g), Zinc (50g), Iron (25g), Copper, Iodine (1g), Selenium (100mg), Cobalt (300mg), BHT (125g), Choline chloride (150g). T_1 = 0% inclusion of *Delonix* seed meal (control). T_2 = 5% inclusion of URDSM. T_3 = 5% inclusion of AFLTDSM. T_4 = 7.5% inclusion of AFLTDSM.

Chemical analysis: The nutrient content of the diets, proximate composition of the raw untreated and processed *Delonix* seed meals were all analyzed according to the procedures of AOAC (1990). Cynogenic content, tannins and trypsin inhibitor activity (TIA) were analyzed by modifying the procedures of AOAC (1984). Phytic acid was determined by the method of Latta and Eskin (1980).

Digestibility trial: A digestibility trial was conducted in order to ascertain the level of nutrient usage by the birds. The parameters determined were DM, CP, CF, EE, ash and NFE. This was done by the method of AOAC (1990).

Statistic: All data collected were subjected to one-way analyses of variance (ANOVA) using SPSS (2001). Where statistical differences were observed, means were separated using the method of Duncan (1955).

Results and Discussion

Table 2 represents the proximate composition of *Delonix* seed meal both in its untreated raw and processed forms. The processing slightly led to a decrease in DM and CP of the test material. However, the CF, EE and ash content were greatly reduced as a result of the anaerobic fermentation and lyle treatment to which the *Delonix* seeds were exposed. The decreases might be due to leaching out of nutrients as a result of

Table 2: Proximate composition of Delonix seed meal (%)

Parameters	URDSM	AFLTDSM
DM	87.90	87.13
CP	18.40	18.10
CF	17.00	11.00
EE	9.50	7.50
Ash	8.60	3.60
NFE	34.40	46.93

Table 3: Effect of anaerobic fermentation and lyle treatment on anti-nutritional factors in Delonix seed meal

Parameters	URDSM	AFLTDSM
Phytic acid (mg/100g)	503.10	238.50
(% decrease in phytic acid)	-	52.59
Cyanide (mg/100g)	18.07	14.75
(% decrease in cyanide)	-	18.37
TIA (mg/g)	36.85	19.42
(% decrease in TIA)	-	47.30
Tannin (g/Kg)	22.64	28.11
(% increase in tannin)	-	24.16

prolonged soaking during processing. Akinmutimi (2006) reported similar decrease in CP content in *Mucuna pruriens* following cooking for 90 minutes.

Table 3 shows the effect of anaerobic fermentation and lyle treatment on anti-nutritional factors present in *Delonix* seed meal. The processing greatly led to a reduction in phytic acid, cyanide and TIA. This is a positive occurrence as these anti-nutritional factors have been reported to limit the utilization of seeds containing them (Wu and Inglett, 1974) and particularly in broilers, where they have been reported to reduce growth rate due to reduced protein and specific amino acid utilization (Douglas *et al*., 1992; Elkin *et al*., 1995). The tannin content was however increased following treatment. Tannins have been reported to be resistant to treatment such as cooking (Akinmutimi, 2004). It is possible that due to the removal of moisture during processing, the tannin content became more concentrated in the seed meal hence the increase in tannin content.

The performance of broilers fed AFLTDSM is shown in Table 4. The inclusion of AFLTDSM in the diet resulted in marked growth differences with broilers on the raw untreated *Delonix* seed meal having the least average body weight compared to those on the treated diets. The daily weight gain of the birds fed the raw untreated *Delonix* seed meal was however higher than for the birds fed the treated diets and the control although not significantly ($p>0.05$). This disagrees with the findings of Olupona *et al*. (1999) and Amaefule and Obioha (2001) who observed poorer weight gains in monogastric animals fed raw legume seeds. Perhaps this is because *Delonix* seed meal is well utilized by poultry birds. This is in consonant with the findings of Grant *et al*. (1991) that *Delonix* seeds are among those fringe legume seeds well utilized by animals.

Feed intake was better in birds fed the *Delonix* seed meal based diets compared to those fed the control diet.

Table 4: Performance of starter broilers fed Delonix seed meal based diets

	Diets				
Parameters	T_1	T_2	T_3	T_4	SEM
Initial body weight (g)	60.30	60.36	61.20	60.00	
Av. Body weight (g)	282.89	292.38	312.92	300.16	20.93^{ns}
Av. Feed intake (g)	285.45	386.45	381.47	394.05	18.96^{ns}
Daily body weight gain(g)	13.45	14.93	13.78	13.77	5.35^{ns}
Feed conversion ratio	4.34	4.31	3.34	3.70	0.18^{ns}
Protein efficiency ratio	1.41	4.40	4.34	4.48	
Energy efficiency	7.84	9.58	10.36	10.65	

ns = not significant ($p>0.05$). T_1 = 0% inclusion of *Delonix* seed meal (control). T_2 = 5% inclusion of URDSM. T_3 = 5% inclusion of AFLTDSM. T_4 = 7.5% inclusion of AFLTDSM.

This might be due to the increasing level of fibre in the diet (Table 1) particularly in diets 2 and 4. High fibre in the diet meant that in order for the birds fed these diets to meet their requirements for energy, protein and other dietary components, the birds had to eat more. This agrees with the report of Savory and Gentle (1976) and Abdelsamie *et al*. (1983).

Feed conversion ratio was better in birds fed AFLTDSM. This might be due to the effect of fermentation which has been reported to improve nutrient density, digestibility as well as control anti-nutrients and toxins (Hamad and Field, 1978).

Protein efficiency ratio increased with increasing level of AFLTDSM in the diet. This might not be too surprising as fermentation improves nutrient digestibility as reported above. The implication is that the efficiency of protein utilization will be enhanced. Besides, the reduction in TIA in the treated meal means protein digestion will be improved in diets containing them. Norton *et al*. (1985) reported that trypsin inhibitor delay protein digestion. This could be through the inhibition of trypsin and chymotrypsin, enzymes necessary for protein digestion in the gastro-intestinal tract of the birds.

The estimates of apparent nutrient utilization are shown in Table 5. It revealed that the inclusion of AFLTDSM in the diets significantly ($p<0.05$) affected DM, CP, EE, ash and NFE digestibility by the birds. With the exception of DM and CP, all the other nutrients were poorly digested. This might not be unconnected with the age of the birds and the length of time it took the birds to get acclimatized to the test material. The high digestibility values observed for DM and CP in this study is in agreement with the report of Jaffe (1975) which stated that processing of legume seeds leads to improvement in DM and protein quality.

The high fibre content of *Delonix* seed meal (Table 2) could be faulted for the low digestibility values observed for CF, EE, ash and NFE. This is because it decreased the exposure time of these nutrients to digestive enzymes due to increased rate of passage through the gastro-intestinal tract induced by fibre. High fibre diet is known to aid quick bowel movement. This according to Trait and Wright (1990), decreases the availability of

Table 5: Apparent nutrient digestibility by starter broilers fed *Delonix* seed meal based diets (%)

	Diets				
Parameters	T_1	T_2	T_3	T_4	SEM
DM	78.95^a	78.90^a	78.35	75.95^b	0.76*
CP	69.60^c	76.47^b	81.45^a	79.62^b	1.84*
CF	40.91	44.52	44.95	43.65	0.73^{ns}
EE	33.30^c	48.12^a	47.29^a	44.76^b	2.28*
Ash	37.38^c	48.95^b	51.28^a	45.78^b	2.61*
NFE	43.26^c	48.86^b	54.29^a	47.76^b	1.80*

ns = not significant ($p>0.05$). Means denoted by different superscripts are significantly different ($p<0.05$). T_1 = 0% inclusion of *Delonix* seed meal (control). T_2 = 5% inclusion of URDSM. T_3 = 5% inclusion of AFLTDSM. T_4 = 7.5% inclusion of AFLTDSM.

nutrients. This eventually will affect growth in birds as most of the nutrients will be lost via the faeces. Onifade and Babatunde (1997) reported the interference of high fibre with nutrient availability at the tissue level. According to them, this reduces nutrient available for growth and maintenance. This probably affected the average daily body weight gain and by implication, the average body weight of the birds at five weeks.

Conclusion: The result of this study indicates that AFLTDSM could be used as a substitute for GNC in starter broilers diet without any serious effect on the performance of the birds. Effort should be geared therefore on finding other means of processing the seeds in order to render them more useful and useable as a protein source for poultry and other monogastric animals.

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Effect of Processing Treatments on Quality of Cereal Based Soyabean Fortified Instant Weaning Food

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Abstract: The study reports on processing of weaning food using different proportions of wheat flour and Soybean flour, with whole milk powder and sugar. The raw materials and processed weaning food samples were analyzed for their moisture, protein, fat, ash and total carbohydrate content. Six samples of weaning food were prepared using 5% and 10% Soya flour and control sample were processed without Soya flour. The moisture content of the processed weaning food was found lower than that of control. The protein ash and fat of the samples with Soya flour were higher and carbohydrate contents were lower than that of control sample. No remarkable change in moisture content, peroxide value, fatty acid value and flavor were observed up to 4 months of storage in ambient condition indicating that the products were shelf stable up to 4 months of storage. The bacterial count increased with the increase of storage time.

Key words: Soya bean, wheat, milk powder, sugar, weaning food

Introduction

Weaning is a gradual process the infant becomes accustomed to the adult diet. Weaning foods should be given to the baby at about the age of four to six months. At four months most babies start to need extra food in addition to breast milk; because they grow fast and breast milk is no longer enough to support their growth (Srivastava, 2002). The weaning period, from around 4-6 months until 2 years of age, is a critical period of a child's life when it is mostly at risk from malnutrition and disease. Protein energy malnutrition is the one of serious problem in Bangladesh. One main reason is scarcity and high price of foods of animal origin. Among the plant protein sources pulses and soybean have immediate potential for alleviating malnutrition because of their relatively high protein content. The children between the age of 4 months and 2-3 years are suffering from malnutrition because they are neither getting mothers milk nor the supplementary foods. To combat this situation soybean based weaning food may be an alternative. Major problems are generally low incomes, poor environmental conditions and lack of education. The need to educate families to exploit locally produced foods to produce nutritionally adequate products is stressed (Cameron and Hofvender, 1983). Another studies reported semi-solid infant foods using α-Rice flour and Modified Potato starch is effective for retro gradation prevention and physicochemical stability (Choi and Sohn, 2000). A normally growing child doubles its birth weight by the time it is six months old and triples its birth weight by the time it is one year. A rapid growth and tissue build up is very necessary during this period and unless proper type of protein rich foods are provided during this period, protein malnutrition and under nutrition will develop, which when unattended will lead to kwashiorkor (Jon and Webb, 1964). This study was undertaken to achieve the objectives as follows: To analyze the composition of raw materials, to determine the effect of processing parameters on composition of the weaning food, to evaluate the sensory attributes of weaning food an to evaluate the shelf life of the processed weaning food at ambient condition.

Materials and Methods

Soybean was collected from Bangladesh Seed Foundation, Department of Plant Breeding and Genetics, Bangladesh Agricultural University, Mymensingh. Whole milk, powder and sugar were collected from the local market. The Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh supplied the other relevant materials for chemical analysis.

Preparations of soya flour: Soya flour was processed from the straw yellow variety of soybean free from immature, field damaged and black soybeans. Using grain cleaners, the foreign materials were removed. Heavy aspiration removed loose hulls, weed seeds and other light foreign matter. The clean and fresh soybean seeds were then soaked in water [water contained 0.25-0.5% sodium bicarbonate ($NaHCO_3$)] for over night and applied steam 10 min, 15 min and 20 min for measuring the acceptability and quality of the product.

Table 1: Formulation of weaning food

		Ingredients			
Samples No.	Heat treatment (Min)	Wheat flour (%)	Soya flour (%)	Milk powder (gm)	Sugar (gm)
A	10, 15, 20	100	0	3	5
S_1	10	95	5	3	5
S_2		90	10	3	5
S_3	15	95	5	3	5
S_4		90	10	3	5
S_5	20	95	5	3	5
S_6		90	10	3	5

A: control without Soya flour. S_1-S_6: Different Sample used different ratio Soya flour and heat treatment

Table 2: Composition of raw materials used for weaning food samples

Ingredients	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Total carbohydrate (%, By difference)
Wheat flour	12.80	11.80	1.50	1.50	72.40
Soybean flour	10.54	39.78	19.67	4.57	25.44
Milk powder	2.70	27.40	28.20	4.70	37.00

The main purpose of using $NaHCO_3$ was to remove the bitterness and anti-nutritional factors. The hulls were then removed and dried the dehulled soybean and grinded in a huller mill. The Soya flour was packed in a high-density polyethylene bags, sealed and stored.

Processing of weaning foods: The prepared soybean flour were mixed with milk powder, sugar and wheat flour at different percentage as shown in Table 1. The mixed sample was blended for uniform mixing. After mixing the finished products were packed in polyethylene bag, sealed and stored at ambient temperature.

Serving process: At first clean drinking water was boiled for 5 minutes and allowed to cool. Then 75 mL of water was added to 25 g of weaning food in a bowl and stirred until the weaning food was smooth.

Microbiological studies: The bacterial count was done according to the "Recommended Method for the Microbiological Examination of Food" published by American Public Health Association (APHA, 1967).

Sensory evaluation: Weaning foods were tasted by a panel of judges. The panelists were selected from the teachers, students and staff of the Department of Food Technology and Rural Industries, Bangladesh Agricultural University, Mymensingh. All the judges consisted the panel were conversant with the factors governing the quality of the products. The products were served to each judge who independently examined the following characteristics: a) Color b) Flavor c) Texture and d) Overall acceptability.

The hedonic rating test Rangana (Ranganna, 1994) was used to measure the consumer acceptability of the product. The relative importance of each factor was compared numerically on a scale of 9 (9 = like extremely, 1 = dislike extremely). Each judge gave a score. The average score of each sample was then calculated. To ascertain uniformity of judgments among the total score assigned by each of them for the sample product was calculated by adding up the scores for the various individual characteristics. ANOVA (Analysis of variance) and DMRT were adopted to select the best samples (Gomez and Gomez, 1984).

Storage studies: The samples were stored in the laboratory for 6 months in double polyethylene bags at ambient temperature (27-32°C). Properly sealed plastic bags are good moisture barriers (Goddard, 1980). The stored weaning food was analyzed initially at an interval of 15 days up to one month, then at an interval of 30 days for the rest period. During storage studies the change in moisture content, peroxide and fatty acid values and flavor were observed and the shelf life of the processed weaning foods were assessed.

Results and Discussion

Composition of raw materials: The raw materials used for the weaning food samples were analyzed for moisture, protein, fat, ash and total carbohydrate. The results are presented in Table 2. Moisture, protein, fat, ash, total carbohydrate of wheat flour were found 12.8%, 11.8%, 1.5%, 1.5% and 72.4% respectively whereas the same components in soybean were also found 10.54%, 39.78%, 19.67%, 4.57% and 25.44% respectively. Moisture, protein, fat, ash and total carbohydrate of whole milk powder were found 2.7%, 27.4%, 28.2%, 4.7% and 37.0% respectively. Protein, fat, ash content in soybean flour was maximum but moisture content was lower when compared with control. The moisture content of the raw materials ranged from 2.7% to 12.8%, protein content ranged from 11.8% to 39.78%, fat content ranged from 1.5% to 28.2% and total carbohydrate ranged from 25.44% to 72.4%.

Chemical analysis of weaning food: The weaning food was analyzed for moisture content, protein, ash, fat and total carbohydrate. All the determinations were done in triplicate and the results were expressed as average value. The results are presented in Table 3.

Sensory evaluation a panel of 10 judges evaluated the color, flavor, texture and overall acceptability of the weaning food prepared with water. The judges gave the score for preference of color, flavor, texture and overall acceptability. The mean scores for color, flavor, texture and overall acceptability of different samples with different heat treatment are presented in Table 4.

Storage studies of weaning foods: The shelf life of the processed weaning foods was studied for a period of 6

Table 3: Composition of prepared weaning food

Sample	Heat treatment (min.)	Composition (%)				
		Moisture	Protein	Fat	Ash	Carbohydrate (By difference)
A (control)	10, 15, 20	8.67	11.54	3.52	1.43	74.69
S_1	10	7.73	12.52	4.58	1.47	73.70
S_2		7.70	13.29	4.76	1.52	72.73
S_3	15	7.54	12.60	4.66	1.51	73.69
S_4		7.49	13.43	4.83	1.54	72.71
S_5	20	7.30	12.72	4.72	1.54	73.72
S_6		7.23	13.63	4.88	1.57	72.69

A = Control with 100% wheat flour; S_1 = 95% wheat + 5% soybean + 3 gm milk + 5 gm sugar; S_2 = 90% wheat + 10% soybean + 3 gm milk + 5 gm sugar; S_3 = 95% wheat + 5% soybean + 3 gm milk + 5 gm sugar; S_4 = 90% wheat + 10% soybean + 3 gm milk + 5 gm sugar; S_5 = 95% wheat + 5% soybean + 3 gm milk + 5 gm sugar; S_6 = 90% wheat + 10% soybean + 3 gm milk + 5 gm sugar

Table 4: Mean Scores of sensory evaluation for weaning food

Samples	Sensory attributes			
	Colour	Flavour	Texture	Overall acceptability
A (control)	7.9^{a}	7.6^{ab}	7.9^{a}	7.8^{a}
S_1	7.6^{a}	8.0^{a}	7.7^{ab}	7.1^{abcd}
S_2	6.1^{bc}	6.7^{bcd}	7.0^{bc}	6.6^{cd}
S_3	7.8^{a}	7.3^{abc}	7.6^{ab}	7.7^{ab}
S_4	6.9^{ab}	5.8^{dc}	6.8^{c}	6.8^{bcd}
S_5	5.5^{c}	6.5^{cde}	6.7^{c}	7.5^{abc}
S_6	5.1^{c}	5.5^{e}	6.6^{c}	6.4^{d}
LSD ($p<0.01$)	1.132	1.005	0.7263	0.9708

Mean with same superscripts within a column are not significant at $p<0.01$; A = Control with 100% wheat flour; S_1 = 95% wheat + 5% soybean + 3 gm milk + 5 gm sugar; S_2 = 90% wheat + 10% soybean + 3 gm milk + 5 gm sugar; S_3 = 95% wheat + 5% soybean + 3 gm milk + 5 gm sugar; S_4 = 90% wheat + 10% soybean + 3 gm milk + 5 gm sugar; S_5 = 95% wheat + 5% soybean + 3 gm milk + 5 gm sugar; S_6 = 90% wheat + 10% soybean + 3 gm milk + 5 gm sugar

Table 5: Storage studies of weaning foods with heat treatment

Period of storage (days)	Sample code	Observations					
		Moisture content (%)	Peroxide value mL. eq kg^{-1}	Free fatty acid value mg of KoH/kg of fat	Flavour	Microbial load cfu mL^{-1}	Remarks
0	A	8.67	8.47	2.88	Good	4.59	Good
	S_1	7.73	8.67	2.93		4.51	
	S_2	7.70				4.49	
	S_3	7.54				4.46	
	S_4	7.49				4.45	
	S_5	7.30				4.40	
	S_6	7.23				4.38	
15	A	8.62	8.40	2.89	Good	4.68	Good
	S_1	7.74	8.62	2.93		4.61	
	S_2	7.71				4.60	
	S_3	7.56				4.57	
	S_4	7.50				4.55	
	S_5	7.30				4.51	
	S_6	7.24				4.49	
30	A	8.60	8.34	2.89	Good	4.77	Good
	S_1	7.75	8.55	2.95		4.68	
	S_2	7.72				4.66	
	S_3	7.56				4.63	
	S_4	7.51				4.61	
	S_5	7.33				4.58	
	S_6	7.27				4.57	
60	A	8.65	8.27	2.92	Good	4.86	Good
	S_1	7.77	8.48	2.96		4.76	
	S_2	7.74				4.73	
	S_3	7.57				4.71	
	S_4	7.53				4.69	
	S_5	7.35				4.66	

Table continued

Period of storage (days)	Sample code	Observations: Moisture content (%)	Peroxide value mL. eq kg^{-1}	Free fatty acid value mg of KoH/kg of fat	Flavour	Microbial load cfu mL^{-1}	Remarks
	S_6	7.28				4.65	
90	A	7.72	8.22	2.94	Good	4.93	Good
	S_1	7.79	8.41	2.98		4.82	
	S_2	7.75				4.80	
	S_3	7.59				4.77	
	S_4	7.54				4.76	
	S_5	7.36				4.74	
	S_6	7.31				4.72	
120	A	7.77	8.22	2.94	Good	5.00	Good
	S_1	7.85	8.34	3.01		4.89	
	S_2	7.80				4.87	
	S_3	7.64				4.85	
	S_4	7.60				4.83	
	S_5	7.38				4.80	
	S_6	7.34				4.78	
150	A	7.89	8.18	3.01	Slightly	5.05	Fresh-ness
	S_1	7.94	8.35	3.06	rancid	4.96	declined
	S_2	7.89				4.96	
	S_3	7.73				4.93	
	S_4	7.71				4.91	
	S_5	7.45				4.87	
	S_6	7.30				4.85	
180	A	8.02	8.20	3.03	Rancid	5.11	Not
	S_1	8.06	8.37	3.14		5.06	acceptable
	S_2	8.02				5.03	
	S_3	7.92				5.01	
	S_4	7.83				5.00	
	S_5	7.54				4.98	
	S_6	7.41				4.97	

A = Control with 100% wheat flour; S_1 = 95% wheat + 5% soybean + 3 gm milk + 5 gm sugar; S_2 = 90% wheat + 10% soybean + 3 gm milk + 5 gm sugar; S_3 = 95% wheat + 5% soybean + 3 gm milk + 5 gm sugar; S_4 = 90% wheat + 10% soybean + 3 gm milk + 5 gm sugar; S_5 = 95% wheat + 5% soybean + 3 gm milk + 5 gm sugar

months at ambient condition. No remarkable change in moisture content, peroxide and fatty acid values and flavor were observed up to 4 months. After 4 months of storage greater increase in moisture content peroxide and fatty acid values were noticed and also rancid flavor was developed. These results are presented in Table 5. Observing the moisture, fat and ash contents, results of organoleptic evaluation and shelf life of the weaning food, it may be concluded that weaning foods may be processed substituting the wheat flour by soy flour up to 10%. Further investigation such as feeding program, effect on health is in progress for recommending the food for infants.

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Effect of Compensatory Growth on the Performance and Carcass Characteristics of the Broiler Chicks

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Abstract: An experiment was conducted to examine the effect of compensatory growth on the performance and carcass characteristics of broiler chicks. The chicks were divided into three experimental groups (A, B and C). Group A of chicks was offered the control diet which was formulated to meet the nutrient requirement of the broiler chicks for 45 days. Group B was subjected to physical feed restriction in the term (8-21 days), these chicks were offered 35% of their nutrient requirement in this period then they were refed with the control diet to the end of the experiment. While group C was restricted by given a low protein and high fibre diet in the term (8-21 days), then they were refed with the control diet to the end of the experiment. Results revealed that there was no significant difference in feed intake, weight gain, carcass weight, liver weight, abdominal fat weight and gizzard weight. Also results revealed that there was significant difference ($p < 0.05$) in feed conversion ratio and alimentary tract weight. Dressing percentage was 74.13%, 74.93% and 75.72% for group A, B and C respectively.

Key words: Compensatory growth, performance, carcass characteristics and broiler chicks

Introduction

Compensatory growth has been shown to occur in most farm animals, even the broiler chicken, which has a very short grow-out cycle. This catch-up growth follows a period of feed nutrient restriction imposed usually by either physical feed restriction or the feeding of diets very low in nutrient density. To be of economic interest, such animals must achieve normal weight-for-age prior to market and/or show improved efficiency of growth and/or exhibit superior carcass characteristics.

Both the beef cow and the Turkey have feeding periods extending into months, as compared to the chicken broiler which has only a relatively short growing period. Therefore, it would be expected that broilers on a compensatory growth program might have problems achieving similar weights at market age as compared to birds reared on a regular feeding program. Several metabolic disorders such as various leg problems, sudden death syndrome and ascites, were thought to be enhanced by the rapid early growth of the modern day broiler. Thus, studies were done to look at the possibility of slowing down growth at an early age, to see what effect this would have on these problems. A number of studies were reported from several areas around the world, all indicating that slowing weight gain of broilers down during the second week of life often resulted in a significant reduction in mortality from the above mentioned conditions. However, in most but not all cases, a slight reduction in body weight and enhanced fat deposition was noted at market age.

So the objective of this study is to examine the effect of compensatory growth on the performance and carcass characteristics of broiler chicks.

Materials and Methods

An experiment was conducted in the premises of Poultry Research Unit in the Faculty of Animal Production, University of Khartoum, at Khartoum North (Shambat). Seventy tow unsexed day old broiler chicks (Ross) were used in this experiment to examine the effect of compensatory growth on the performance and carcass characteristics of broiler chicks. Tow experimental diets were prepared which were approximately isocaloric, but they contained different levels of protein and crude fibre. The first diet was prepared according to the nutrient content of the broiler chicks as outlined by NRC (1984) (Table 1) .

Seventy-two chicks were selected and allocated randomly in nine experimental pens, of eight chicks each (three pens per treatment). The initial body weights of chicks in each pen were adjusted to be approximately the same. Group A was given the control diet throughout the experimental period, feed and water were provided *ad libitum* and 24 hours light was maintained. Group B was subjected to feed restriction in the term (8-21 days) of age, these chicks were offered 35% of their nutrient requirement in this period then they are refed with the control diet to the end of the experiment. While group C was restricted by given a low protein and high fibre diet in the same term (8-21 days) of age, then they are refed with the control diet to the end of the experiment.

Table 1: Nutrient Composition of the experimental diets

Ingredient	A*	B
Sorghum	58.00	61.0
Groundnut meal	19.00	0.0
Super concentrate**	5.00	5.0
Wheat bran	15.00	31.0
Oyster shell	2.70	2.7
Salt	0.30	0.3
Total	100.00	100.0
ME (Kcal/ Kg)	3057.00	2939.9
Crude protein	21.20	15.8

*Chicks of the second group (B) were given restricted amounts of this feed (35% of their nutrient requirement) in the term (8-21 days). **Super concentrate composition: protein 45%, fibre 3%, calcium 12%, phosphorus 6%, methionine + cystine 4.75%, lysine 11%, NaCl 2.8-3% and M.E. KCl/Kg 2000.

Feed intake, body weight and weight gain were recorded weekly for the individual replicates of each treatment and mortality was recorded. At the end of the experiment (at day 45), birds were starved overnight and one bird from each replicate was randomly selected, wing banded and individually weighed. Then, it was slaughtered by jugular severing. The bird thereafter was feathered and the carcass was weighed. The alimentary tract, abdominal fat, gizzard and liver were weighed. The layout of the experiment was statistically analyzed according to the analysis of variance applicable to complete randomized design as described by Snedecor and Cochran (1980).

Results and Discussion

Performance data

Feed intake and body weight gain: There was no significant difference in feed intake and weight gain between the experimental groups (Table 2). Group B and C ate more feed than group A in the compensating period, this could be due to the fact that group B ate more to compensate for the previous hunger while group C ate more for their protein needs. In the 80's Israeli workers, (Plavnik and Hurwitz 1985, 1988) investigated the possibility of utilizing a compensatory growth program to improve the feed utilization of market weight broilers. While the program did achieve enhanced feed utilization, in some cases, body weight of the compensatory birds did not equal to that of the control group at market age.

Auckland *et al.* (1969) and Auckland and Morris (1971a, b), showed that reduced body weight of turkeys up to 6 weeks of age, from the feeding of low protein diets resulted in weight gain equal to the controls at market age, but with a significant improvement in feed: Gain ratio and an overall reduction in protein intake. Lesson and Summers (1978), with investigating compensatory growth, also looked at diet self selection for turkeys. They found that birds given the choice to select a high energy low protein diet, or a high protein low energy diet, had similar body weights to that of control birds at market age. Plavnik and Hurwitz (1991) looked at restricting feed with turkeys, starting at 7 days of age for a 10 day period. At 20 weeks of age they noted better body weight, feed utilization and meat yield for the early feed restricted birds as compared to the controls.

Table 2: Performance of broiler chicks subjected to compensatory growth

	Chick groups				
Item	A	B	C	L.S	S.E
Number of birds	24	24	24	-	-
Number of days	45	45	45	-	-
Initial body weight (g)	64	64	64	N.S	-
Final body weight gain (g)	1430	1210	1400	N.S	0.60
Body weight gain (g)	1366	1146	1336	N.S	0.18
Feed intake (g/ bird)	4090	3160	3830	N.S	0.75
Feed conversion ratio	3.0	2.8	2.9	*	1.28

In this and subsequent table values are means of 3 replicates of 8 birds each. L.S = Least significant. N.S. = Not significant. * = P < 0.05. S.E = Standard error.

Feed conversion ratio: There was significant difference ($P < 0.05$) in the feed conversion ratio. Group (B) and (C) has better feed conversion ratio than the control group (A). Many works in poultry and turkey support this fact. In the early 90's Dr. Lesson's lab at Guelph, also began looking at the possibility of improving feed: Gain ratio of broilers by restricting nutrient intake at an early age, so that the growth curve of the bird was more concave in nature rather than linear. As pointed out by Zubair and Lesson (1994), the improvement in feed efficiency noted with compensatory growth was due to the smaller body mass of the bird up to the point of growth compensation. Thus a lower nutrient requirement for maintenance. Also the results stated herein were in line with those of Lesson and Summers (1978).

Carcass data

Carcass weight: As seen in Table 3 there was no significant difference in the carcass weight between the tested groups. Carcass weight is the reflection of the body weight, so these results were in line with those of Auckland *et al.* (1969) and Auckland and Morris (1971a, b). Leeson and Summers (1978), with investigating compensatory growth, also looked at diet self selection for turkeys. They found that birds given the choice to select a high energy low protein diet, or a high protein low energy diet, had similar body weights to that of control birds at market age but with an improved feed: Gain ratio and more carcass protein. Also Zubair and Leeson (1994) observed little or no difference was noted in carcass characteristics for the compensatory versus birds grown on a normal feeding program.

Alimentary tract weight: There was significant difference ($P < 0.05$) in the weight of the Alimentary tract (Table 3). Group C which was fed a high fibrous diet (Table 1) has the heaviest Alimentary tract weight

Table 3: Carcass characteristics of broiler chicks subjected to compensatory growth

	Chick groups				
Item	A	B	C	L.S	S.E
Live wt (g/bird)	1430	1210	1400	N.S	0.6000
Carcass wt (g/bird)	1070	840	1060	N.S	2.7500
Abdominal fat wt (g)	30	10	30	N.S	0.0010
Alimentary Tract wt (g)	10	60	70	*	6.9000
Gizzard wt (g)	30	20	20	N.S	0.0030
Liver wt (g)	20	21	20	N.S	0.0003
Dressing Percentage	74.13%	74.93%	75.72%	N.S	0.0320

followed by group B which was subjected to period of feed restriction, so it ate more to compensate for the past hunger. Group A which was finished in a control diet has the light Alimentary tract weight.

Liver weight: As seen in Table 3 there was no significant difference in the liver weight between the tested groups and this may be due to the compensatory activity of the liver when birds of group B and C were refed with the control diet.

Gizzard weight: Gizzard is an early maturing organ and according to its muscular nature slight or no change in its volume and weight will be expected by the progress of the age of the bird, so this may explain the insignificant difference in the gizzard weight between the tested groups (Table 3).

Abdominal fat weight: Compensatory growth had no significant effect on the abdominal fat weight (Table 3) this may be due to scientific fact that fat is the last forming tissue. In broilers major fat deposition occurs at the last tow weeks and upon this time the three experimental groups were fed the same diet.

Dressing Percentage: Dressing out percentage is the proportion of the carcass weight from the slaughter weight as seen in Table 3, there was no significant difference in the dressing percentage of the experimental groups.

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Levels of Serum Iron, Total Iron Binding Capacity, Transferrin Saturation Fraction and Packed Cell Volume of Blood Donors in Calabar, Cross River State, Nigeria

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Abstract: The purpose of this study was to determine the serum iron and total iron binding capacity, transferrin saturation fraction and packed cell volume of blood donors in University of Calabar Teaching Hospital, Calabar. Serum iron concentration (SI), Total Iron Binding Capacity (TIBC), Transferrin Saturation Fraction (TSF) and Packed Cell Volume (PCV) showed mean values of 16.60±2.93umol/l, 62.20±4.02umol/l, 27.20±3.00% and 0.398±.030l/l respectively for the blood donors. These values were not significantly (p>05) different from that reported for the control subjects (Serum Iron, 17.50±1.80umol/l. Total Iron Binding Capacity, 61.70±3.43umol/l, Transferrin Saturation Fraction, 28.60±3.37% and Pack Cell Volume 0.410±0.37 l/l respectively). However, the SI, 16.60±2.93umol/l, TSF, 27.20±3.00% and PCV 0.398+0.30l/l respectively of blood donors from Calabar in South-Eastern part of Nigeria were significantly (p>0.50) lower than values obtained from Ibadan (SI, 19.06±1.00umol/l, TSF, 35.10±1.90%, PCV, 0.451±.068l/l) in South-Western part of Nigeria. Age did not significantly (p>0.05) influence the levels of SI, TIBC, TSF and PCV of the donors and controls in this study. From this study it is observed that there may be regional differences in the iron parameters suggesting regular nutritional education for the donors on the importance of improving their nutritional status.

Key words: Blood donors, serum iron, total iron binding capacity, transferrin saturation fraction, packed cell volume

Introduction

The voluntary unpaid blood donation is a humanitarian act towards the sick by the healthy (Ali *et al*., 2001). No blood transfusion service can survive without blood donors. The demand for blood is soaring all over the world and on any given day, approximately 32,000 units of red blood cells are needed for accident victims, people undergoing surgery and patients receiving treatment for leukemia, cancer and other diseases, such as sickle cell disease and thalassaemia (Ranney and Rapaport, 1997). More than 23 million units of blood components have been reported to be transfused every year (Huestis and Busch, 1991). In most countries, strict regulations have been established for the selection of blood donors that incorporate criteria that serve to protect both the donor and recipients (Huestis and Busch, 1991).

A donor donates approximately 450mls of blood at the time of donation (Ranney and Rapaport, 1997) which contains approximately 225mg of iron which will be lost by the donor. Reports have shown that some blood banks in some countries do not care for standards and hence the donors health is jeopardized (Jacobs *et al*., 1998). Currently the American Association of blood banks has a standard of minimum haemoglobin of 13.5g\dl for men and 12.5\dl for women donors (Dacie and Lewis, 2001) that are currently in use worldwide. Iron is a universal cofactor for mitochondrial energy generation and supports the growth and differentiation of all cell types. The regulation of systemic iron is through the proteins 'Transferrin' (iron mobilization) and 'Ferritin' (iron sequestration) (Roskams and Connors, 1994). The physiologic importance of the storage iron is that it provides a rapidly available supply in the event of blood loss (Lipschitz *et al*., 1995). The iron content of the body is kept constant by maintaining a balance between the amount absorbed and amount lost and this amount also depends upon the interaction of foods, drugs and abnormal components of diet (Jawad, 1996). Iron requirements depend on age, sex, race, pregnancy lactation and attitude (Molla *et al*., 1992). This work is aimed at assessing the iron status (in terms of Serum iron, Total iron binding capacity, Transferrin saturation fraction and packed cell volume) of male subjects presenting themselves as blood donors at University of Calabar Teaching Hospital, Calabar, Nigeria.

Materials and Methods

A total of eighty five (85) subjects were used for the study. The first group was made up of 52 blood donors attending UCTH blood transfusion bleeding bay. Of this number a few claimed to be relatives of patients while a greater number claimed they were paid to donate blood for their patients in the hospital who required blood transfusion for various reasons. The ages of the donors ranged from 19-40 years. The second group was made

Table 1: Mean Serum Iron, TIBC, PCV and Transferrin Saturation fraction of Blood Donors Compared with Control

Groups	SI (umoll/l)	TIBC (umol/l)	TSF (%)	PCV (l/l)
Blood Donors n = (52)	16.60±2.93	62.20±4.02	27.20±3.00	0.398±030
Control Subjects n = (33)	17.50±1.80	61.70±3.34	28.60±3.37	0.410±037
P Value	>0.05	>0.05	>0.05	>0.05

Result expressed as mean±SD, n= Number of subjects studied

Table 2: Serum Iron, TIBC, PCV and Transferring Saturation fraction of Blood Donors and Controls based on age

	Blood Donors			Control Subjects		
Parameters	15-25 (n = 14)	26-36 (n = 18)	>37 (n = 20)	20-30 (n = 21)	31-40 (n = 7)	>41 (n = 5)
SI (umol/l	17.40±1.80	17.0±2.40	16.0±3.30	16.40±2.30	16.40±2.0	16.0±2.10
TIBC (umol/l	68.60±2.40	63.50±2.40	58.30±3.0	64.90±2.0	57.20±1.90	54.10±1.90
TSF (%)	27.30±2.30	26.90±3.0	26.0±2.0	25.50±3.0	28.0±3.0	26.40±2.0
PCV L/L	0.360±.019	0.370±.020	0.360±.030	0.445±.025	0.420±.020	0.410±.025

ANOVA analysis (p>0.05); Result expressed as mean±SD, n = Number of subjects studied

Table 3: Mean Serum Iron, TIBC, PCV and Transferrin Saturation fraction of Blood Donors from Calabar Compared with donors from Ibadan

	Blood Donors		Control Subjects	
Parameters work	Present (n = 52)	Ibadan work (n = 31) (Usanga, 1990)	Present Work n = 52)	Ibadan work n = 31) (Usanga, 1990)
SI (umol/l)	16.60+2.93	19.06+1.00	17.50+1.80	21.27+2.00
TSF (%)	27.20+3.00	35.10+1.90	28.60+3.37	35.5+2.31
PCV L/L	0.398+.030	0.451+.068	0.410+.037	0.459+.068

ANOVA analysis (p < 0.05); Result expressed as mean±SD, n = Number of subjects studied

up of thirty-three (33) apparently healthy male adults who served as control. They were all healthy individuals who gave no history of medication at least 3 months preceding the study. The consisted of volunteers who were staff in the hospital and also Medical Laboratory Science Students of University of Calabar Teaching Hospital, Calabar, Nigeria. They were aged 18-41 years. All blood donors fulfilled the criteria for suitability as donors (including packed cell volume concentration of >0.390 l/l). Venous blood samples were collected from the subjects between 9.00am-12noon into sequestrene and dry iron-free bottles. The following investigations were performed on the samples haematocrit, Serum Iron (SI), Total Iron Binding Capacity (TIBC) and Transferring Saturation Fraction (TSF). Standard methods of Dacie and Lewis, 2001 were used for haematocrit determination while the serum iron, TIBC and TSF were determined by the method of the international committee for standardization in haematology (ICSH, 1978). The data were analyzed by student's "t" test and ANOVA. Unless otherwise stated the data were expressed as means±standard deviation. P<0.05 was considered significant in all statistical comparisons.

Results

The results of SI, TIBC, TSF and PCV concentrations for the blood donors and controls are presented in Table 1. The SI, TIBC, TSF and PCV concentrations of male blood donors were observed to fall within the reference range of Dacie and Lewis, 2001 and reports of Usanga, 1990; Ukaejiofor *et al.*, 1979. Again there were no significant differences when blood donors were compared with controls. The blood donors and controls subjects were compared based on age (Table 2). The numerical differences within and among the age groups shows no significant (p>0.05) change with increase in age. The SI, TIBC, TSF and PCV of the control and blood donors from Calabar were compared with that observed for the same age group from Ibadan (Usanga, 1990) (Table 3). For all the parameters investigated donor's from Calabar recorded significantly (p<0.05) lower values than their counterparts from Ibadan.

Discussion

Haemoglobin level assessed either by packed cell volume or copper sulphate method is one of the criteria used for selection of donors in UCTH. The parameter indicates whether a donor is fit in terms of haemoglobin content (an iron containing protein) or not. A Pack Cell Volume of >0.390L/L was used as inclusion criteria for the participants in the study. The Serum Iron (SI) Total Iron Binding Capacity (TIBC) Transferring Saturation Fraction (TSF) and Packed Cell Volume (PCV) of the male donors were similar to that reported from other parts of Nigeria and other normal population (Usanga, 1990; Ukaejiofor *et al.*, 1979; Dacie and Lewis, 2001; Jacob's *et al.*, 1972). Our present work has shown that SI, TIBC, TSF and PCV of male blood donors in this locality did not differ significantly (p>0.05) from healthy

male non-donors (control) adults. This finding is similar to the earlier reports (Usanga, 1990; Ukaejiofor *et al.*, 1979) that blood donors had similar values of SI, TIBC, TSF and PCV with non-blood donors.

The values of SI, TSF and PVC of our donors were observed to be significantly lower than that reported for donors in Ibadan (Usanga, 1990). Nutritional and economic differences may account for the differences observed. Two decades ago blood donation was largely done by patient's relation and voluntary non-remunerated donors, however, recent trends show that commercially paid donors who donate blood merely for the financial gains to meet up their needs are the bulk providers of blood used for transfusion services in UCTH and perhaps in other centers in Nigeria. These commercial donors who do the donation just for the monetary benefit may not see the essence of proper feeding hence the lower values observed. Furthermore, Usanga, 1990 used Williams and Conrad, 1966 method for the estimation of serum iron, total iron binding capacity and percent saturation of transferrin with iron while in the present study, kit method of International Committee for Standardization in Haematology (ICSH, 1978) was used. The differences in the sample size may also have contributed to the observed significant differences in the parameters of donors in the present studies.

This study has shown regional differences in the iron parameters of blood donors in Nigeria. From this study, it is suggested that there should be increased awareness campaigns through regular education to commercial donors on the need for them to improve their nutritional status as well as reduce the frequency of donation so as to prevent a depletion of their iron stores. It is believed that by so doing maintenance of donors' good health as well as quality safe blood for transfusion will be achieved.

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Prevalence of Underweight: A Matter of Concern among Adolescents in Osun State, Nigeria

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Abstract: Prevalence of undernutrition among adolescents in developing world has shown a decline during the last decade. Adolescents make up approximately 20% of the world's population. There is a dearth of research on adolescent nutrition in developing countries. The aim of this cross sectional study was to determine the prevalence of undernutrition, overweight and obesity as measured by Body Mass Index (BMI) in a representative sample of adolescents aged 10-19 years in Osun State of Nigeria. Adolescents (n = 401) from 32 schools in urban and rural districts of the state responded to a sociodemographic questionnaire. Body mass index for age was calculated and the prevalence of underweight, overweight and obesity was determined based on WHO/NCHS value of <5th, 85th and 95th percentiles respectively. The results consisted of 182 boys and 219 girls. The prevalence of underweight was 20.1% in the study area, which was higher among the rural adolescents (22.4%) than urban (18.7%) and 25.8% and 15.1% among boys and girls respectively. The prevalence of overweight was 3.2% with 4.1% from urban and 1.5% from rural, while 1.1% were boys and 5.0% were girls. Only 0.5% urban girls were obese. Prevalence of underweight was significantly higher in boys at mid adolescence (24.2%, $p<0.02$), boys who were involved in jobs after school hours (13.7%, $p<0.06$) and who do not travel regularly (22.5%, $p<0.12$). While among girls who reside with extended family member (11.9%, $p<0.05$). In conclusion, adolescents living in Osun state, Nigeria are at high risk of underweight.

Key words: Adolescents, underweight, overweight, obesity, prevalence

Introduction

Adolescence is characterized by rapid physical growth and sexual development, accompanied by changes in the percentage of body fat. Adolescent underweight has been identified as a risk factor for underweight in adulthood and it increases adult and child morbidity and mortality by leading to a variety of adverse health outcomes and low birth weight in babies (Cole *et al*., 2000; Oner *et al*., 2004). Undernutrition is still prevalent in developing countries and continues to be a primary cause of poor health (Sawaya *et al*., 2004; Nandy *et al*., 2005). In Asian countries, the incidence of low birth weight predicts the prevalence of underweight during pre-school and subsequent years. Childhood and adolescent stunting adversely affects the health of adults (Mason *et al*., 1999).

The prevalence of malnutrition is much higher in South Asia than in developing countries in other regions. The risk factors for cardiovascular disease originate in youth and early adulthood (Berenson *et al*., 1992). Numerous studies have reported that cardiovascular disease risk factors are associated with adiposity in children, childhood overweight and obesity are associated with an increased prevalence of cardiovascular disease risk factors (Teixeira *et al*., 2001) and persistent obesity is associated with the development of adverse adult cardiovascular disease risk profile (Srinivasan *et al*., 1996). Childhood obesity has become a severe health problem in some developing countries, especially during the last few decades (Ribeiro *et al*., 2003).

In contrast to children and adults, relatively little information is available about gender differences in weight status among adolescents (Wang *et al*., 2002; Ribeiro *et al*., 2003). However, it has recently been estimated that the prevalence of adolescent obesity is increasing not only in developed countries but also in some developing countries in which malnutrition used to be the major nutritional disorder. During adolescence, the Body Mass Index (BMI) is the preferred method of expressing the body fat percentiles of groups. It is widely accepted that a BMI between the 85th and 95th percentiles is defined as overweight and a BMI greater than the 95th percentile as obesity (Chu, 2001; Barlow and Dietz, 1998; Dietz and Bellizzi, 1999) while BMI that is less than 5th percentile is regarded as underweight.

The aim of this study was to determine prevalence of underweight, overweight and obesity in a representative sample of adolescent aged 10-19 years living in Osun State, Nigeria and to compare the prevalence among boys and girls.

Materials and Methods

Osun State, with a land area of 925,100 hectares or 9,251 km^2 and an estimated projected population of

Table 1: Mean±SD height, weight and Body Mass Index (BMI) of adolescents according to age and sex

	No. of subject		Height (m)		Weight (kg)		BMI (kg/m^2)	
Age (yr)	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
10.00	-	2	--	1.59 ±0.01	--	53.00±5.66	--	20.95 ±1.86
11.00	-	1	--	1.44 -	--	48.00-	--	23.15-
12.00	3	5	1.47±0.07	1.46±0.09	37.33±6.80	44.20±10.32	17.17±1.66	20.40±2.19
13.00	8	17	1.50±0.14	1.48±0.76	43.50±9.10	42.40±7.43	19.10±1.54	19.13±2.05
14.00	30	38	1.47±0.99	1.53±0.06	41.07±6.70	48.16±6.79	18.74±1.69	20.62±2.64
15.00	37	54	1.54±0.86	1.53±0.08	47.16±10.14	48.30±5.76	19.60±2.73	20.34±3.18
16.00	29	39	1.57±0.09	1.54±0.05	49.41±7.70	49.48±6.59	20.06±2.05	20.48±2.28
17.00	28	24	1.61±0.09	1.52±0.05	52.92±8.59	52.45±5.17	20.19±1.80	22.65±2.55
18.00	29	21	1.62±0.07	1.55±0.05	54.07±6.83	53.61±7.74	20.511.69	22.252.46
19.00	18	18	1.63±0.06	1.56±0.62	57.90±4.04	52.47±6.07	21.932.17	21.371.37

2,184,569 in 1998 is divided into 30 Local Government Areas (LGAs) with urban and rural districts. During the study period between September and November 2004, 239,829 adolescents aged 10-19 years according to WHO definition were attending government schools (Osun State Ministry of Education).

The adolescents were the subjects of the cross sectional population study conducted in both urban and rural areas of the state with the use of a multistage stratified, random sampling technique. The original objective was to collect a sample size which is one fifth of the adolescents aged 10-19 years who registered for the school year 2004/2005. A list of names of government schools was obtained from the Department of Planning, Research and Statistics, Ministry of Education. Thirty two schools were selected randomly of these, 25 were proportionally selected from urban areas and 7 from rural areas.

The final sample size was determined by the following steps. First, the number of subject living in urban and rural was estimated at 80% and 20% respectively since some of the subjects in rural area attend schools in urban area. The number of students selected from each school was determined according to the total number of students at each school. Finally, the classrooms were chosen on a systematic random basis and each adolescent was selected randomly from a classroom. Available statistics shows that Osun State has the highest number of children attending school in the nation (FOS, 1996).

The survey instrument which is a structured questionnaire was conducted on 450 students from various schools of these, 401 were included for analysis; 49 were excluded because of incomplete survey. Experts were trained in the correct methods for taking anthropometric measurements of the subjects. To minimize errors in measurement, scales were checked for accuracy by weighing an object of known weight. Height was measured by a portable stadiometer attached to the scales. The necessary posture was taken for the measurement. The portable scale and stadiometer were calibrated daily. Weight was measured in kilograms and height in meters. The BMI was calculated as the ratio of the body weight in kilograms to the square of the height in meters.

The data were entered and processed on an IBM computer by SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA. Descriptive Statistics on BMI, weight and height were calculated. Estimates of the prevalence of underweight, overweight and obesity were based on the NCHS/WHO cut off points values, which defined underweight as a BMI below the 5th percentile, overweight as a BMI equal to or greater than the 85th percentile and less than the 95th percentile for sex and age. Obesity as a BMI equal to or greater than 95th percentile for age and sex.

Correlation analysis was done to test the relationship of some demographic characteristics of the subjects to BMI at $p<0.05$ value for significance.

Results

The final sample of adolescents aged between 10 and 19 years consisted of 182 boys and 219 girls. The final sample in this study consisted of 401 adolescents after removal of all outliners. The mean values (±SD) of weight, height and calculated BMI in relation to age are shown in Table 1. The prevalence of underweight, overweight and obesity for adolescents of different ages according to age and sex are shown in Table 2. The prevalence of underweight, overweight and obesity for adolescents according to location and sex are shown in Table 3.

The prevalence of underweight, overweight and obesity was 25.8%, 1.1% and none respectively among boys and 20.0%, 3.2%, 0.5% among girls. The prevalence of underweight was highest at 15 years of age among boys (8.2%) and at 14 years among girls (4.1%). The prevalence of overweight was observed at age 15 among boys (1.1%) and between 14 and 19 years among girls (5.0%). The incidence of obesity was noticed among female at age 15 and 18 years (0.5%) and none among male. While, the prevalence of underweight, overweight and obesity was 18.7%, 4.1%, 0.7% respectively among urban and 22.4%, 1.5% and

Table 2: Prevalence (%) of underweight, overweight and obesity among adolescents according to age and sex

	Boys			Girls		
Age (yr)	Under weight	Over weight	obesity	Under weight	Over weight	obesity
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	66.7	-	-	20.0	-	-
13	37.5	-	-	47.1	-	-
14	43.3	-	-	23.7	5.3	-
15	40.5	5.4	-	11.1	3.7	1.9
16	20.7	-	-	15.4	2.6	-
17	14.3	-	-	4.2	8.3	-
18	10.3	-	-	9.5	9.5	4.8
19	14.3	-	-	-	66.7	-

Table 3: Prevalence (%) of underweight, overweight and obesity among adolescents according to location and sex

	Boys			Girls		
Location	Under weight	Over weight	obesity	Under weight	Over weight	obesity
Urban	25.0	1.7	-	12.8	6.0	1.3
Rural	26.3	-	-	20.0	2.9	-

Table 4: Prevalence (%) of underweight, overweight and Obesity among adolescents according to sex and personal and household characteristics

	Boys			Girls		
Measure	Under weight	Over weight	Obesity	Under weight	Over weight	Obesity
Age						
Early adolescence (10-12)	1.1	-	-	0.5	-	-
Mid adolescence (13-15)	24.2	1.1	-	14.6	4.1	0.9
Late adolescence (16-19)	0.5	9.3	-	-	0.9	-
P value	0.02			NS		
Household type						
Monogamy	-	1.6	-	2.3	0.5	0.5
Polygamy	7.7	-	-	3.2	1.4	-
Extended	18.1	1.1	-	11.9	3.2	0.5
P value	NS			0.05		
Household size						
≤ 6	12.6	-	-	8.2	3.6	0.5
7-11	8.8	0.5	-	4.6	1.4	0.5
≥ 12	4.4	0.5	-	2.3	-	-
P value	NS			NS		
No of hours spent outside the home						
≤ 8	17.0	0.5	-	10.5	3.7	0.5
≥ 9	6.6	0.5	-	4.6	1.4	0.5
P value	0.05			NS		
Job involvement after school hours						
Yes	13.7	-	-	9.6	1.8	0.5
No	12.1	1.1	-	5.0	3.2	0.5
P value	0.061			NS		
Frequency of traveling						
Regular	3.3	-	-	0.9	0.5	-
Non regular	22.5	1.1	-	14.2	4.6	0.9
P value	0.120			NS		

none among rural adolescents. The prevalence of underweight, overweight and obesity was highest at 14 years (6.0%), 15 years (1.5%) and 15 and 18 years (0.7%) respectively among urban. While, the prevalence of underweight and overweight was highest at ages 14-16 years (13.4%) and 18 years (1.5%) among rural.

The prevalence of underweight, overweight and obesity according to the adolescents' personal and household indicators are shown in Table 4. The results of the correlation analysis showed that the prevalence of underweight and overweight was significantly higher in boys at mid adolescents (24.2% and 9.3% respectively

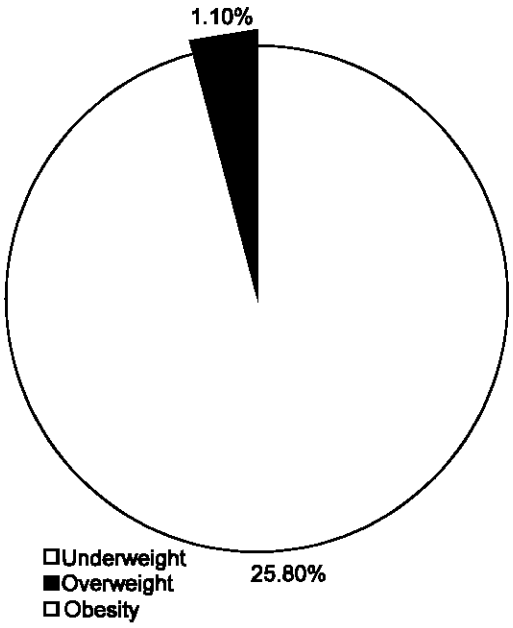

Fig. 1: Distribution of boys according to underweight and overweight

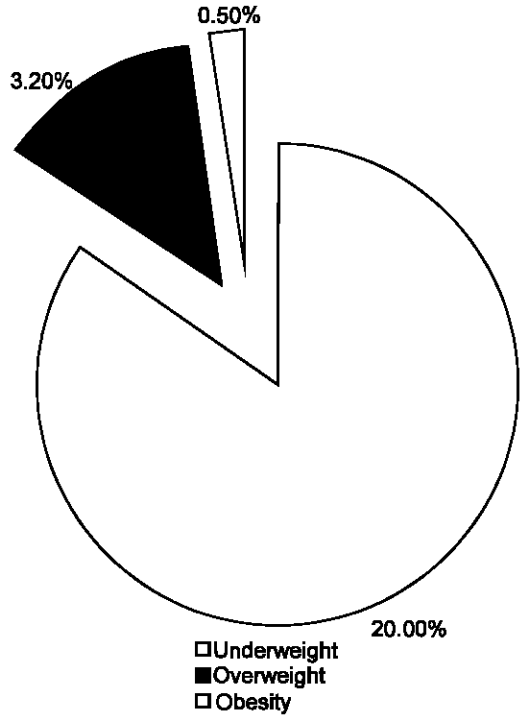

Fig. 2: Distribution of girls according to underweight, overweight and obesity

at p<0.02). While the prevalence of underweight was significantly higher in girls who lived with extended family members (11.9%, p<0.05), boys who were involved in jobs after school hours (13.7%, p<0.061), boys who do not travel out of their place of abode regularly (22.5%, p<0.120).

The prevalence of underweight, overweight and obesity in boys and girls are shown in Fig. 1 and 2.

Discussion

Adolescent malnutrition in developing countries is beginning to receive the attention it deserves. Although data on adolescent nutritional status in Asia and Africa is sparse, that which exists suggests a generally higher prevalence of adolescent undernutrition in South Asia than in South-East Asia or sub Saharan Africa and a higher prevalence in rural than in urban areas (Laquatra, 2000; Carderio *et al*., 2006).

In South Asia, a high prevalence of undernutrition (50% or move with BMI <5th percentile WHO/NCHS references) among adolescents have been recorded. Undernutrition and overweight is a global problem, especially overweight and obesity spreading even to developing world, where it is an increasing threat to health. One third of all deaths globally already stem from ailments linked to excess weight and low consumption of food. The prevalence of overweight and obesity in children has recently been investigated in several countries. The overall prevalence of underweight, overweight and obesity in this study was 20.0%, 3.2% and 0.5% respectively.

Underweight, overweight and obesity are increasing worldwide and are emerging as major risk factors for several chronic diseases. Hence, it is important that countries monitor the weight status of children and adolescents. WHO/NCHS recommends the use of BMI percentiles. This study found a high prevalence of underweight and low prevalence of overweight among adolescent boys and high prevalence of underweight, overweight and low prevalence of Obesity among girls in the study area. Many studies show that overweight and obesity in adolescence are powerful indicators of adulthood overweight and related disease (Al-Sendi *et al*., 2003). A similar study conducted in the Gulf Region reported that the overall prevalence of obesity among Bahraini boys and girls between 12 and 17 years of age was high, especially in girls (21% in boys, 35% in girls (Gargari *et al*., 2004) which is in collaboration with the girls in this study.

Although the prevalence of overweight and obesity was high in both Bahrain and Qatar, a striking finding of this study was that the prevalence was higher among girls than boys. A study of high school girls in Iran found that the prevalence of overweight and obesity were 10.1% and 3.9% respectively (Livingstone, 2000).

A similar study of adolescents in Istanbul found that the prevalence of overweight and obesity were 11.3% and 1.6%, respectively, among boys and 10.6% and 2.1% among girls (Oner *et al*., 2004). Some studies found

significant sex differences in the prevalence of overweight and obesity. Most studies from Asia and Europe e.g, Taiwan (Chu, 2001), Hungary (Elmadfa *et al*., 1993) and Austria (Sibai *et al*., 2003) found a higher prevalence among adolescent boys and girls which is contrary to the pattern in this study. In Qatar, the prevalence of underweight was 8.6% in boys and 5.5% in girls (Bener, 2006). These figures are lower than the 14.4% for boys and 11.1% for girls reported from Istanbul (Oner *et al*., 2004) which is similar to the pattern in this study.

In this study, prevalence of underweight was higher among boys than girls while the prevalence of overweight was higher among girls than boys. None of the boys in the study area was obese while 0.5% of girls was obese, which implied that girls were more at risk of overweight and obesity than boys, while boys were more at risk of underweight than girls. Though the prevalence of underweight in both sexes was high (25.1%) in boys and (15.1%) in girls in the study area.

Health professionals may play a key role in promoting good dietary behaviour among adolescents. Other interventions may involve health education through mass media to influence nutritional norms and practices. Such interventions, aimed at better health awareness and more physical activity, should be monitored for their effectiveness.

Limitation of the study: A limitation of the study is that the measurements of the subjects could be performed only once, so that some measurement errors might not be accounted for.

Also, the study did not provide direct indications of the natural history of overweight and obesity in this population. Despite these limitations, the data presented in this study provide a valuable profile of the physical characteristics of a major segment of the adolescents in Osun State, Nigeria.

Conclusions: This study found a high prevalence of underweight and overweight in the adolescent population in the study area, especially among boys (underweight) and girls (overweight). The prevalence of obesity was found in girls and none in boys.

There is a need to establish a national control programme for the prevention and treatment of malnutrition and related complications. All age groups and segments of society should be targeted.

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Nutritional Comparison of Cow and Buffalo Milk Cheddar Cheese

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Abstract: Cheddar cheese was manufactured from cow and buffalo milk using commercially available starter cultures (*Lactococcus lactis* ssp. *Cremoris* and *Lactococcus lactis* ssp. *Lactis*). Cheese was analyzed for proximate composition, pH, acidity, lactose and mineral contents and sensory perception (flavor, aroma and texture) after 2 and 4 months of ripening. All the chemical composition was significantly influenced by cow and buffalo milk. Buffalo milk cheese was found considerably superior in nutritional profile. Lactose content and pH decreased and acidity increased significantly during ripening of 120 days. On sensory evaluation, buffalo milk cheese was ranked appreciably higher for all the sensory parameters as compared to that of cow milk.

Key words: Buffalo milk, cow milk, cheddar cheese, chemical composition and sensory evaluation

Introduction

Cheese is a form of milk preservation and is highly nutritious, convenient and versatile and offers a diversity of flavors, textures and forms (Fox *et al*., 2000; Singh *et al*., 2003; Farkye, 2004). The diversity is due to an increasing knowledge of the technology of cheese making and the biochemistry and microbiology of cheese ripening (Farkye, 2004).

Cheddar is a hard ripened cheese produced by acidification and concentration of milk following gel formation with rennet (Banks, 2002). It forms a significant proportion of international trade in cheese and as a result of new world trade agreements, the volume of cheese traded is likely to increase (Muir *et al*., 1997).

The Cheddar cheese is a complex mixture, consisting of protein, fat, carbohydrates, vitamins and minerals. The component balance theory states that a mixture of the right chemicals at the appropriate levels would produce a Cheddar aroma and texture. However, when these components are out of balance, it is impossible to predict the sensory responses (House and Acree, 2002). Moreover, it has high nutritional value due to its high concentration of casein which contains various levels of all essential amino acids (Hughes and Willenberg, 1993). It also contains fat and small amount of other nutrients such as vitamin A, B_2, B_6 and B_{12}. Because of its high protein and calcium contents, cheese in moderation is an important component of balanced diet (Considine, 1982).

Quality of Cheddar cheese depends on manufacturing technology, starter cultures and composition of milk (Banks, 2002). A mixture of moisture, fat, salt, peptides, amino acids, micro flora, minerals and other minor constituents, occluded within a casein matrix combine to make Cheddar cheese a complex food (Maarse *et al*., 1994).

Buffalo's milk is ranked second in the world after cow's milk, being more than 12% of the world's milk production (CNIEL, 2002). In India and Pakistan (both producing about 80% of the world's production of buffalo milk), this milk is used for making different dairy products including soft and hard cheeses (Ahmad *et al*., 2008).

As compared to cow milk, buffalo milk is richer in fat, lactose, protein, total solids, vitamins and minerals, such as calcium, magnesium and inorganic phosphate (Fundora *et al*., 2001; Ahmad *et al*., 2008).

In most of the world, Cheddar cheese is manufactured from cow milk but keeping in view the production of buffalo milk in Pakistan and its composition, the present study was conducted to manufacture cow and buffalo milk Cheddar cheeses and their nutritional comparison.

Materials and Methods

Milk procurement: The buffalo and cow milk was procured from farm house, Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan.

Cheese manufacturing and ripening: Commercially available freeze dried cultures of Cheddar cheese (*Lactococcus lactis* ssp. *Cremoris* and *Lactococcus lactis* ssp. *Lactis*) were used to manufacture cheese from cow and buffalo milk. Both of the milk was standardized at 3.5% fat level and cheese was prepared following the standard protocol, with some modifications, as described by Scott (1981). Ripening was done at 4°C for a period of 4 months.

Quality evaluation of cheese

Chemical analysis: Proximate composition, pH, acidity, lactose and mineral contents were determined in fresh cheese and after 2 and 4 months during ripening.

Table 1: Effect of ripening on chemical composition of cow and buffalo milk Cheddar cheeses

Chemical analysis	Cow milk Cheese				Buffalo milk cheese			
	0 day	60 days	120 days	Means	0 day	60 days	120 days	Means
Moisture	37.60	36.58	36.12	36.77[a]	36.38	35.83	35.10	35.77[b]
Fat	30.58	30.23	29.83	30.21[b]	32.62	32.31	32.06	32.33[a]
Protein	25.51	25.20	24.92	25.21[b]	26.60	26.40	26.12	26.37[a]
Ash	3.85	3.84	3.85	3.85[b]	4.01	4.08	4.11	4.07[a]
Lactose	0.13[a]	0.09[b]	0.04[c]	0.09[b]	0.16[a]	0.12[b]	0.08[c]	0.12[a]
Acidity	0.89[b]	0.93[ab]	0.96[a]	0.93	0.90[b]	0.93[ab]	0.96[a]	0.93
pH	5.34[a]	5.25[b]	5.18[c]	5.26	5.33[a]	5.25b	5.19[c]	5.26

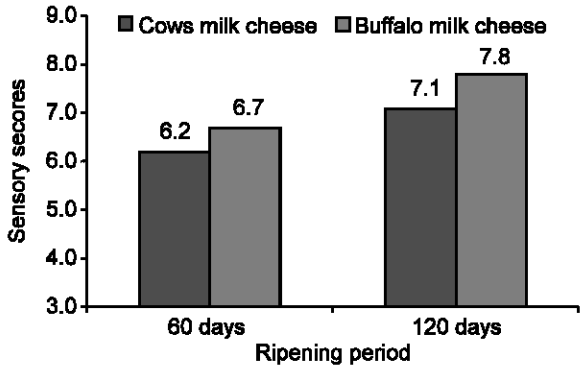

Fig. 1: Effect of ripening on flavor (scores) of Cheddar cheese

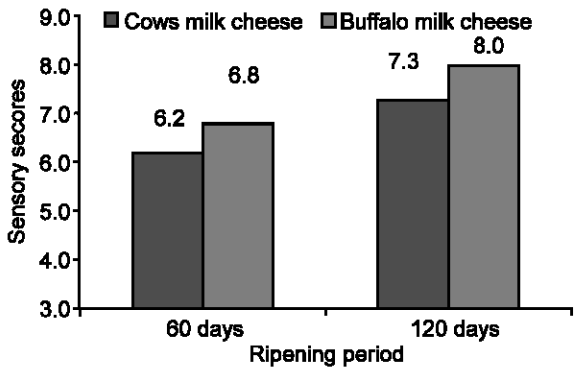

Fig. 2: Effect of ripening on aroma (scores) of Cheddar cheese.

Moisture content was determined by oven drying, fat content using Babcock method, total protein by Kjeldahl method, ash content by igniting the cheese sample, acidity by titrimetric method (AOAC, 1990) and lactose contents as described by Lees (1971). The pH of cheese slurry prepared by blending 20 g of grated and shredded cheese sample with 12 ml of water, was measured with a pH meter (inoLab WTW Series 720) after calibrating with fresh pH 4.0 and 7.0 standard buffers (Onga *et al.*, 2007). Sodium, calcium and potassium contents were determined using flame photometer as described by Kirk and Sawyer (1991).

Sensory evaluation of cheese: Cheese was evaluated for sensory characteristics after 60 and 120 days of ripening by a panel of assessors on 9-point hedonic scale (Land and Shepher, 1988).

Results and Discussion

Chemical composition: Significant differences in chemical composition of cow and buffalo milk Cheddar cheese were found. Moisture content of cow milk cheese (36.77%) was significantly ($p<0.05$) higher than buffalo milk cheese (35.77%). Fat, protein, ash and lactose content (32.33%, 26.37%, 4.07% and 0.12% respectively) were significantly ($p<0.05$) higher in buffalo milk cheese than that of cow milk (30.21%, 25.21%, 3.85% and 0.09% respectively). Non-significant ($p>0.05$) effect of milk was found on acidity and pH values of the cheese (Table 1).

The significant differences in cheese composition were because of the reason that as compared to cow milk, buffalo milk is richer in fat, lactose and protein, especially casein (Ahmad *et al.*, 2008) which is the major constituent that influences the cheese yield and composition. Fundora *et al.* (2001) also reported that the buffalo milk has a higher content of fat, crude protein, lactose, total solids, vitamins and minerals as compared to cow milk.

The Cheddar cheese composition obtained in present study is in accordance with the findings of Hughes and Willenberg (1993) who proposed the average chemical composition of Cheddar cheese as moisture 37%, protein 25%, fat 33% and ash 4%.

During ripening, lactose content significantly ($p<0.05$) decreased resulting in significant increase in acidity and decrease in pH value of buffalo as well as cow milk cheese while ripening had non-significant ($p>0.05$) effect on all other compositional parameters (Table 1).

The significant effects are due to the cheese being a biochemically dynamic product that undergoes significant changes during ripening (McSweeney and Sousa, 2000) and these changes are as a result of several microbiological, biochemical and metabolic processes. (Singh *et al.*, 2003; Farkye, 2004). The most significant one is the metabolism of lactose to lactate and other metabolites by lactic acid bacteria that influence the rate and extent of acidification (McSweeney and Fox, 2004). Onga *et al.* (2007) also reported that

Table 2: Effect of ripening on mineral contents of cow and buffalo milk Cheddar cheeses

	Cow milk Cheese				Buffalo milk cheese			
Minerals	0 day	60 days	120 days	Means	0 day	60 days	120 days	Means
Sodium	662.00	668.50	671.67	667.39[b]	680.33	682.25	685.92	682.83[a]
Calcium	721.08	723.58	726.83	723.83[b]	751.25	753.58	759.75	754.86[a]
Potassium	78.50	83.33	86.92	82.92[b]	82.83	87.33	89.83	86.66[a]

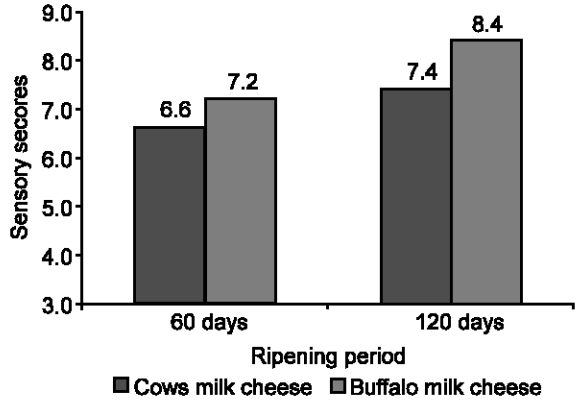

Fig. 3: Effect of ripening on texture (scores) of Cheddar cheese.

during ripening, the residual lactose predominantly metabolized to L-lactate. Moreover, the capacity of milk to be acidified is higher for buffalo milk than for cow milk (Ahmad *et al.*, 2008).

Mineral contents: During ripening, non-significant ($p>0.05$) changes were recorded in mineral contents of buffalo as well as cow milk cheese. However, all the three minerals; sodium, calcium and potassium were found highly significant ($p<0.01$) in buffalo milk cheese as compared to that prepared from cow milk (Table 2). It is for the reason that as compared to cow milk, buffalo milk is higher in mineral content especially calcium (Fundora *et al.*, 2001; Ahmad *et al.*, 2008). On acidification, similar solubilization of calcium and other minerals was observed by Ahmad *et al.* (2008) in both cow and buffalo milk that validate the non-significant changes in mineral content during ripening of cheese.

Sensory evaluation: Significantly higher ($p<0.05$) scores were awarded to the cheese prepared from buffalo milk as compared to that of cow milk for all the sensory parameters (flavor, aroma and texture) as illustrated in Fig. 1, 2 and 3. Fundora *et al.* (2001) described that compared with cow milk; the composition of buffalo milk imparts a rich flavour and taste and makes it a highly suitable ingredient for the manufacture of a wide variety of milk products particularly cheese.

Moreover, ripening had highly significant ($p<0.01$) effect on sensory perception of cheese as the scores for flavor, aroma and texture of cheese obtained after 120 days of ripening are much higher than that awarded after 60 days (Fig. 1, 2 and 3) because during cheese ripening, the biochemical and metabolic processes are responsible for the basic flavour and textural changes (Collins *et al.*, 2003; Lucey and Singh, 2003; Smit *et al.*, 2005). Singh *et al.* (2003) illustrated that the characteristic flavour, aroma, texture and appearance of cheese develop during ripening and these changes are predetermined by the composition of milk and starter culture.

Conclusion: It was concluded that the nutritional value and acceptability of Cheddar cheese manufactured from buffalo milk is much superior to that of cow milk. So, the buffalo milk because of its chemical composition, offers excellent opportunities for the development of different dairy products.

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