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Association Between Maternal Nutrition Status and Birth Weight of Neonates in Selected Hospitals in Mysore City, India

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Abstract: A cross sectional study designed to measure the nutritional status of women in the third trimester of pregnancy and to relate the birth weight of neonate as outcome of pregnancy. Participants comprised of 500 pregnant women in the age group 18-40 years who represented 7.5% of population. Mean height, weight, fundal height and haemoglobin of pregnant women were 155.6 cm, 59.5 kg, 34.0 cm and 10.6 g/dl respectively. Computation of nutrient intakes (based on 24 h recall method) showed that the mean intake of energy was 1785.0 kcal, protein 56.2, calcium 775.6 mg, iron 17.9 mg, zinc 8.5 mg and magnesium 482.3 mg. Percent adequacy of nutrient intake with reference to recommended allowances showed that only magnesium was adequate but other nutrients mentioned above were inadequate. The mean birth weight of neonates was 2.9 kg. Height, head and chest circumferences were 48.5, 33.6 and 32.3 cm respectively. Twenty five percent of neonates considered as low birth weight. Statistical analysis indicated that male neonates were heavier, taller and their head and chest circumferences were higher than female. Maternal height, weight, fundal height and haemoglobin level were significantly correlated with birth weight. Nutrient intakes, namely energy, protein, calcium, magnesium, iron and zinc in the third trimester were significantly correlated with birth weight. Using binary logistic regression analysis weight, fundal height, energy and protein intake of pregnant women could be considered as predictor factors for birth weight. It is suggested that consumption of enough nutrients should be emphasized in the nutrition education component of maternal health programmes.

Key words: Maternal nutrition status, birth weight, neonates, Mysore city, India

INTRODUCTION

It is a universally acknowledged medical truth that adequate nutrition before and during pregnancy has greater potential for a long term health of both mother and the child and it is important during the course of pregnancy (Singh *et al*., 2009). A woman who has been well nourished before conception begins her pregnancy with reserves of several nutrients so that the recurrent needs of the growing foetus can be met without adversely affecting her health. Infants, who have been well nourished in the womb, have an enhanced chance of entering life in very good health. Mother's diet should provide adequate nutrients so that maternal stores do not get depleted (Singh *et al*., 2009). The crucial recommendation to such pregnant women in India is to consume a balanced diet as described by the Indian Council of Medical Research (ICMR) which includes extra nutrients for pregnancy, lactation and childhood. Poor foetal growth has been attributed to widespread maternal under-nutrition. Maternal nutrition is an important factor responsible not only for health of baby, but also for the baby's long term growth (Jackson and Robinson, 2001). Therefore understanding maternal nutrition and foetal growth relationship is critical (Rao *et al*., 2001; Rao *et al*., 2007; Kramer, 2003). Assessment of maternal nutritional status relies on measure of pre-pregnancy weight and weight gain during pregnancy, weight gain at different trimesters, Height and skinfold thickness and limb circumferences. Some measures reflect a women's nutritional status or energy stores as she enters pregnancy (Nahar *et al*., 2007) and literature reviewed showed maternal height, weight and pregnancy weight gain has effect on birth weight. Birth weight is an important determinant of newborn survival, healthy growth and development. As mentioned above birth weight is an index of mother's health and nutritional status during pregnancy and a positive correlation is reported between the quality of maternal diet and birth weight of infant (Mirdula *et al*., 2003).

Birth weight of neonates is also affected by factors such as age, occupation, family income, pregnant experience and morning sickness (Bang and Lee, 2009; Freisling *et al*., 2006; Laria *et al*., 2006). In India, poor foetal growth has been attributed to widespread maternal under nutrition (Rao *et al*., 2001). Presently, about 22% of the babies born in India are low birth weight (less than 2.5 kg) (Kapil, 2009). This figure has remained more or less stationary for the last few decades in spite of striking declines in neonatal and infant mortality. Low birth weight in India has been attributed to widespread

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maternal under nutrition. Therefore understanding maternal nutrition and foetal growth relationship is critical. Various maternal anthropometric criteria (pre-pregnancy weight, height weight gain during pregnancy period have been significantly associated with intrauterine growth and prematurity. These parameters should be viewed as "predictors" of Low Birth Weight (LBW) to be used for risk detection (Sachdev, 2001). Studies on the nutritional status of pregnant women in Mysore are very few and the relationship between maternal nutritional status and birth weight of infants demands further attention. Therefore this study was designed to measure the maternal nutritional status of expectant women in the third trimester of pregnancy and to relate the birth weight as the outcome of nutritional status of pregnancy.

MATERIALS AND METHODS

A survey of the hospitals existing in Mysore city was done to identify the hospitals to select the subjects. Four hospitals among 20 hospitals in Mysore city namely, Chaluvamba Hospital (KR. Hospital), Basapa Memorial Hospital (BM. Hospital), Kamakshi Hospital and Apollo Hospital were selected based on their acceptance and willingness to extend support for the study. With the help of a statistician and based on statistics of deliveries per year in each hospitals, 7.5% of pregnant women were selected to represent the target population i.e., pregnant women. Total pregnant women included for the study was as follow; BM. Hospital 60, Apollo Hospital 80, Kamakshi Hospital 110 and KR. Hospital 250 to represent different section of social economic status. All the 500 participants were healthy, between 18-40 years of age and had continuously the selected hospital visited for the three trimesters and registered for delivery and followed up for a week after delivery. The inclusive criteria were age group (18-40 years) and who continuously visited for health care during the three trimesters of pregnancy in selected hospitals. The pregnant women with diabetes mellitus and Cardio Vascular Disease (CVD) and the pregnant women who had parity more than four were excluded from the study. The study was carried out from November 2007 to August 2008.

A written consent to participate in the study was obtained from each subject. The study was approved by the Human Ethical Committee of the University of Mysore. The required information about various aspects proposed to study was obtained by structured questionnaires. Suitable questionnaires were constructed in the Department of Food Science and Nutrition, Manasaganghotri, University of Mysore, India and pre-tested in with small population (pilot study) and suitable modifications were introduced so as to obtain standard questioners.

Anthropometric measurements namely height and weight were carried out by investigator using standard methodology as described by Jelliffee (1966). The measurements were made on the participants wearing a minimum amount of clothing. The weight of pregnant women was recorded at the time of registration by using digital weighing balance to the nearest 100 g (Calibrated after every 10 measurements). Height was measured using a locally made stadiometer. The pregnant women was asked to maintain an upright and erect posture with her feet together and the back of her heels touching the pole anthropometer the horizontal headpiece was lowered onto the women's head and the measurements was taken to the nearest 0.1 cm. Fundal height was measured by a physician as distance between the symphysis pubis and the highest point of the uterine fundus, defined with a gentle pressure on a plain at right angle of the abdominal wall and was marked.

The dietary assessment of pregnant women was done at the end of the third trimester, by investigator and nutrient intake was obtained using 24-h dietary recall method. Probing questions were used to help the subjects to remember all foods and drinks consumed on previous day. Questions were extended to methods of food preparation, portion sizes, as well as to approximate sizes of meals. Standard cups were used to measure the quantity of intake of the cooked food (Thimmayamma and Parvathi, 1996). The information about the quantity of raw material (raw quantity) taken for cooking as well as the cooked food by the subject was recorded in terms of household measures/number/kg to find out the quantum of raw food intake. From the information provided, the cooked and raw amount of foods consumed by each subject was then calculated. The mean intake of different nutrients consumed was then computed for a day the help of ready recknor to calculate nutritive value. The ready recknor was developed by Department of Food Science and Nutrition, Manasa ganghotri, University of Mysore, using nutritive value given in food composition table from Indian Council and Medical Research (ICMR, 1998). Nutrient adequacy was calculated using nutrient intake data and compared with recent ICMR recommendation (National Institute of Nutrition, 2009).

The haemoglobin value of each subject was taken from clinical records, when the subjects were hospitalized for child birth. It was analyzed in laboratory hospital by cyanmetheamoglobin Method (WHO, 1998).

Anthropometric measurements of neonates (weight, height, head and chest circumferences) were taken within 24 h after birth, using standard procedure (Jelliffee, 1966). A beam balance by which accuracy of 50 g was employed for weighing the infants. Infants were weighted, with minimum clothing, when the child was restful. Infantometers were used for measuring the recumbent length of newborn infant. A lightweight fibreglass infantometer was used to measure length up to 100 cms. Infants were laid on the board of the infantometer, which was kept on flat table. The crown of the head was in contact with the top end of the device. The knee was extended, so that the feet were at right

angles to the leg. The mobile device was then brought in contact with the feet of neonate and was allowed to be in contact with the device at the bottom end of the board. The distance between the two right angle devices was measured. The reading was recorded in centimetres with accuracy of 0.1 cms. The infant's head was steadied and the greatest circumference measured, by placing the fibreglass tape firmly round the frontal bones, just superior to the supra-orbital ridges, passing it round the head at the same level, on each side and laying it over the maximum occipital prominence at the back. The infant's chest circumference measurement was made at the nipple line, when the child was calm and breathing normally. The fibreglass tape was used for measuring the chest circumference.

The statistical analysis of data was conducted using SPSS version 11.5 (SPSS Inc., Chicago, Illinois). Descriptive statistic was used for summarization of data. The statistical difference among the groups were analyzed by student's t-test and one-way ANOVA. When the one-way ANOVA results were significant, the Bonferroni test was used to determine whether significant difference exist between different variable means. Binary logistic regression analysis was carried out to find out the 'independent' relationship of variables, which were found significant in the bivariate analysis with birth weight of neonates. Results were considered significant if $p<0.05$.

RESULTS

Details of all selected pregnant women under examination are presented in Table 1. The mean age of pregnant women was 24±4.2 years and the age range was 18-40 years. Majority (29%) of pregnant women were in age group 21-23 years and followed by 18-20 years. Highest percentage of subjects (54%) had married at the age of 18-20 years. Ninety two percent of subjects were not employed. Highest percentage of subjects (62%) were expecting first child. Forty six percent of pregnant women had higher secondary level of education, followed by graduation level. Majority (48%) of subjects had income of Rupees 5000-10000 per month; other details of the subjects are given in Table 1. Anthropometric measurements, fundal height and haemoglobin of pregnant women at the end of the third trimester are presented in Table 2. The mean height, weight, fundal height and haemoglobin were, 155.6 (cm), 59.5 (kg), 34 (cm) and 10.6 (g/dl) respectively.

Energy and nutrient intake per day of pregnant women and percent adequacy of nutrient intake with reference to ICMR recommendation (National Institute of Nutrition, 2009) were computed and are presented in Table 3. As shown in the Table, consumption of energy and protein per day were 69 and 68% respectively and they were not adequate. Among minerals magnesium intake was adequate and others like calcium, iron and zinc were inadequate.

Table 1: General information about pregnant women (n = 500)

Variables	Number
Age (year)[a]	24.0±4.2
Age group (year)[b]	
18-20	140(28)
21-23	144(29)
24-26	111(22)
27 and above	105(21)
Age at marriage	
18-20	272(54)
21-24	212(43)
25-29	16(3)
Occupation	
Non employed	462(92)
Employed	38(8)
Parity (Number of pregnancy)	
1	312(62)
2	157(31)
3-4	31(7)
Education level	
Illiterate	23(5)
Primary	66(13)
Higher and secondary	232(46)
Graduation	179(36)
Total income of family in Rs[†]. (month)	
<5000	127(25)
5000-10000	239(48)
>10000	134(27)

[a]Mean±SD; [b]Number (%); [†]Rupees (Indian currency)
[1 Euroe equal Rs. 56 and 1 USDA equal Rs. 46]

Table 2: Anthropometric, fundal height and haemoglobin measurements of pregnant women (n = 500)

Parameters	Mean±SD
Height (cm)	155.6±5.1
Weight (kg)	59.5±5.9
Fundal height (cm)	34.0±2.2
Haemoglobin (g/dl)	10.6±1.4

Table 3: Energy and nutrients intake (per day) of pregnant women with reference to RDA (ICMR, 2009) (n = 500)

Energy and nutrients	Mean±SD	RDA	Percent adequacy
Energy (kcal)	1785.0±374.0	2590	69
Protein (g)	56.2±15.5	82	68
Calcium (mg)	775.6±297.9	1200	65
Magnesium (mg)	482.3±169.4	320	151
Iron (mg)	17.9±5.5	38	47
Zinc (mg)	8.5±2.3	12	71

Table 4: Neonate information (n = 500)

Birth weight	% (N)
Normal birth weight (2.5 kg-3.5 kg)	75(377)
Male	44(221)
Female	31(156)
Low birth weight (less than 2.5 kg)	25(123)
Male	12(58)
Female	13(65)

General information about neonates is given in Table 4. Majority (75%) of them had normal birth weight and considerable percentage (25%) belonged to Low Birth Weight (LBW). Fifty six percent of neonates were male and forty four percent of them were females. Other details like weight, height, head and chest circumferences of neonates were 2.9 (kg), 48.5 (cm),

Table 5: Anthropometric measurements of neonates (n = 500)

Parameters	Mean±SD	Male (Mean±SD)	Female (Mean±SD)	t-test results	p-value
Weight (kg)	2.9±0.5	3.0±0.5	2.8±0.5	2.7	0.015
Height (cm)	48.5±2.0	48.8±2.0	48.2±2.0	3.6	0.001
Head circumference (cm)	33.6±1.1	33.8±1.0	33.4±1.1	4.0	0.001
Chest circumference (cm)	32.3±1.8	32.5±1.9	32.0±1.6	2.9	0.004

Table 6: LBW and NBW of neonates: Nutritional status of pregnant women (Third trimester, n = 500)

Parameter	LBW (kg)	NBW (kg)	t-test results	p-value
Weight (kg)	54.4±4.3	61.2±5.3	14.1	0.001
Height (cm)	154.0±4.4	156.2±5.2	12.8	0.001
Fundal height (cm)	32.7±2.4	34.5±1.9	7.4	0.001
Haemoglobin (g/dl)	10.0±1.4	10.8±1.3	6.3	0.001
Energy (kcal/day)	1363.0±201.3	1922.0±309.8	23.1	0.001
Protein (g/day)	42.0±6.9	60.8±14.7	19.1	0.001
Calcium (mg/day)	642.4±251.0	819.1±299.5	6.4	0.001
Magnesium (mg/day)	337.6±122.3	529.5±155.3	12.5	0.001
Iron (mg/day)	13.8±4.39	19.3±5.3	11.5	0.001
Zinc (mg/day)	6.6±1.7	9.2±2.1	13.4	0.001

33.6 (cm) and 32.3 (cm) respectively (Table 5). Details about difference between anthropometric measurements based on gender of neonates are given in Table 5. Mean anthropometric measurements of neonates according gender groups indicated that, the mean weight of male neonates was 3.2 kg vs. 2.9 kg in female neonates. Mean height, head and chest circumferences in male neonates versus female were 51.3 cm vs. 49.7 cm, 34.7 cm vs. 33.9 cm and 33.0 cm vs. 32.6 cm respectively. As clear in the Table 5 male neonates were heavier, taller and their head and chest circumferences were higher than female.

DISCUSSION

As clear in the Table 3 consumption of nutrient intake showed inadequate percentage intake of energy, protein, calcium, zinc and iron with reference to ICMR recommendation (National Institute of Nutrition, 2009). The findings of the present study was similar with other study that were conducted in different socioeconomic status of urban and rural areas in India (Shihareni and Lakshmi, 2001; Kharade and Antony, 2002; Pathak *et al*., 2003; Chaturvedi *et al*.,1994; Panwar and Punia, 1998; Kapil *et al*., 1999). The results of their study showed that the mean energy and nutrients intake (protein, calcium, iron, zinc) were inadequately consumed by the subjects when compared with RDA recommendation.

Birth weight is the most sensitive and reliable indicator of health of neonates. It is strongly associated with the health and survival of infant. Mean birth weight of neonates was 2.9 kg. LBW is an indicator of poor health of the neonates. Considerable percentage of neonates (25%) was classified as LBW and 75% showed NBW (Normal Birth Weight). Similar results in Mysore hospitals were reported by Raman *et al*. (2001) and Shobiri and Nazari (2006) which revealed that 25% and 21% of neonates were LBW babies respectively. Percentage LBW in female neonates was slightly higher than males neonates (12% vs 13% respectively, Table 4). Comparison of the two groups (male and female neonates, Table 5) indicated that male neonates showed significantly higher height, weight, head and chest circumferences than females. Similar findings were also reported by Som *et al*. (2004) from India, Kato, (2004) from Japan and Moore *et al*., (2004) from Australia, Their finding indicated that male neonates were heavier than female neonates.

An attempt was made to investigate the relationship between anthropometric measurements, fundal height and haemoglobin status of pregnant women and birth weight of neonates. As a first exercise to find out association with birth weight and nutritional status of pregnant women, birth weight of neonates was classified into two categories LBW and NBW and subjected to student's t-test and results are presented in Table 6. As shown in the Table pregnant women who gave birth to LBW babies were significantly lower in height, weight, fundal height and haemoglobin level than women who gave birth to neonates with normal birth weight. It was also observed that pregnant women who gave birth to normal neonates had significantly higher consumption of energy and nutrients than pregnant women who gave birth to neonates with LBW babies. Similar results are reported by Rao *et al*. (2007) from India and Al-Shosan (2007) from Saudi Arabia.

It was interesting to analyze the results of different categories of height, weight, fundal height, haemoglobin level, energy and nutrient intake of pregnant women with reference to variations in birth weight of neonates. The data was subjected to one-way ANOVA and the findings are presented in Table 7. It is clear from Table that the taller pregnant women (more than 160 cm) gave birth to significantly heavier babies (3.1 kg) than shorter women. Pregnant women with weight less than 50 kg gave birth

Table 7: Nutritional status (level) of pregnant women vs. Birth weight of neonates (n = 500)

Parameters	Mean birth weight	F-value	p-value
Height (cm)			
<150.0	2.8[a]		
150.0-160.0	2.9[a]	13.6	0.001
>160.0	3.1[b]		
Weight (kg)			
<50.0	2.3[a]		
50.0-54.9	2.5[b]	61.6	0.001
55.0-59.9	2.8[c]		
60.0-64.9	3.0[d]		
Fundal height (cm)			
28.0-30.0	2.5[a]		
31.0-33.0	2.8[b]	24.3	0.001
34.0-36.0	3.0[c]		
Hemoglobin (g/dl)			
<9.0	2.7[a]		
9.0-9.9	2.7[a]		
10.0-10.9	2.8[a]	17.8	0.001
≥11.0	3.1[b]		
Energy intake (kcal)			
>1500.0	2.3[a]		
1500.0-1999.9	3.0[b]		
2000.0-2499.9	3.2[c]	177.1	0.001
≥2500.0	3.5[d]		
Protein intake (g)			
<40.0	2.2[a]		
40.0-49.9	2.5[b]		
50.0-59.0	3.0[c]	154.5	0.001
≥60.0	3.0[c]		
Calcium intake (mg)			
<800.0	2.8[a]		
800.0-1200.0	3.1[b]	26.0	0.001
>1200.0	3.2[b]		
Magnesium intake (mg)			
<280.0	2.7[a]		
280.0-320.0	2.8[a]	78.2	0.001
>320.0	3.0[b]		
Iron intake (mg)			
<19.0	2.8[a]		
19.0-29.0	3.1[b]	37.6	0.001
>29.0	3.2[b]		
Zinc intake (mg)			
<9.0	2.7[a]		
9.0-12.0	3.2[b]	47.6	0.001
>12.0	3.2[b]		

Note: Different superscript indicate significant difference at 5% level as shown by post hoc Bonferroni

to neonates with 2.3 kg, while subjects with more than 65 kg gave birth to heavier neonates (3.3 kg). Studies conducted by Fawzi *et al.* (1997) from North Africa and Parvathi and Khyrunnisa Begum from India (2007) reported maternal height and weight at 6 and 9 month of pregnancy was positively associated with mean birth weight and length of neonates ($p<0.001$) and their finding supported present findings. Higher level of fundal height in pregnant women (28-36 cm) at the end of the third trimester, showed significant increase in birth weight of neonates born to them. Shobiri and Nazari (2006), Parvathi and Khyrunnisa Begum (2007) reported similar results. It is revealed from the Table 7 pregnant women with haemoglobin more than 11 g/dl, which is considered as normal level gave birth to neonates with normal weight, while pregnant women with lower haemoglobin level (<11 g/dl), who were considered as anaemic gave birth to low birth weight babies. Shoberi *et al.* (2006) in their study also indicated the importance of normal haemoglobin level on pregnancy outcome and their results was agree with current findings. Their finding showed normal concentration of haemoglobin have a significant influence on birth weight. Effect of different level of haemoglobin also reported by Rosenberg *et al.* (2004) from China, their finding also showed that both mild and moderate anaemia (95 = Hb<120 g/L and Hb <95 g/L respectively) were significantly associated with lower birth weight.

Maternal nutrition has been recognized for its important in the course and outcome of pregnancy. Young (1976) has suggested that nutritional factors may account for 60% of the observed variations in birth weight. Variations in the energy intake of pregnant women showed significant influence on birth weight of babies. It is evident from the Table 7, pregnant women who consumed <1500 kcal per day (<59%RDA) gave birth to neonates with LBW (2.3 kg), while pregnant women with ≥2500 kcal per day (≥96% RDA) of energy per day gave birth to neonates with NBW (3.5 kg). Pregnant women with protein intake <40 g per day (<49% RDA) gave birth weight to neonates LBW (2.2 kg) while pregnant women with higher intake of protein (≥60 g/day or 73%RDA) gave birth to neonates with NBW (3.3 kg). Similar results with regard to protein and energy intake were reported by Al-Shosan (2007) and Rao *et al.* (2007). The finding showed high intake of calcium, magnesium, iron and zinc significantly influenced the birth weight of babies. As it clear from the Table 7 pregnant women with higher intake of minerals gave birth to neonates with normal weight, while pregnant women with lower intake gave birth to neonates low birth weight. It may be stated from this finding that different levels of nutritional status of pregnant women significantly influenced the birth weight of neonates.

It was interesting to find out which nutritional factors were very important in influencing birth weight of neonates. The binary logistic regression was carried out to find out the possible factors associated with birth weight (results are presented in Table 8). Weight, fundal height of pregnant women along with energy and protein intake could be considered as primary predictor factors for birth weight. In a study conducted by Rao *et al.* (2007) in the rural areas of district Ambala, Haryana, energy intake and mother's weight were best predicting factors for birth weight where as in Saudi Arabia Al-Shosan (2007) reported that caloric intake was the best indicator for birth weight. Moore *et al.* (2004) in South Australia reported caloric deficit in the third trimester and low maternal anthropometric measurements are associated

Table 8: Summary of results of binary logistic regression

Variables (pregnant women)	B	SE	Wald	Sig	95.0% C.I. for EXP(B) Lower	Upper
Weight (kg)	0.079	0.035	4.937	0.026	1.009	1.159
Fundal height (cm)	0.079	0.075	10.008	0.002	1.095	1.472
Energy (kcal/day)	0.005	0.001	29.104	0.001	1.003	1.007
Protein (g/day)	0.058	0.027	4.655	0.031	1.005	1.118

a Variable(s) entered on step 1: Height, Weight, Fundal height, Hemoglobin, Energy intake, Protein intake, Calcium intake, Magnesium intake, Iron intake, Zinc intake

with high percentage of LBW. The findings reported by Rao *et al.* (2007); Al-Shosan (2007) and Moore *et al.* (2004) are similar with present findings.

To conclude, maternal nutritional status influenced birth weight of neonates. Adequate nutrient intake especially dietary energy, protein, calcium intake are important for pregnancy outcome. Weight, fundal height and energy and protein intake of pregnant women could be considered as predictor factors for birth weight. The findings of the study indicate that male neonates were heavier, taller and their head and chest circumferences were higher than female neonates. Further studies with large sample size may have to be conducted to throw light on various aspects of nutritional factors and their association with birth weight of neonates.

The limitation of the present study was that haemoglobin measurements could have been done by investigator and sample size at higher number (i.e., 658 pregnant women) could have been taken to cover 10% of the respective population.

It may be recommended from the present study that the government and non government agencies should focus on the effective implementation of program to improve the dietary intake of pregnant women to optimize their health and that of to improve the health condition of neonates.

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Effects of Aqueous Extract of Spices Mixture Containing Curry, Garlic and Ginger on Plasma Glucose and Lipids in Alloxan-induced Diabetic Rats

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Abstract: With increasing use of spices worldwide in the face of increasing burden of Diabetes Mellitus (DM), especially type 2, coupled with claims about the favourable effects of spices on some health conditions, the present study investigated the effect of aqueous extract of spices mixture containing curry, garlic and ginger on plasma glucose and lipids in alloxan induced diabetic rats, a type 2 DM model. The animals were assigned into six groups (I-VI) of six animals per group. Group I-III animals were made diabetic by intraperitoneal administration of alloxan while group IV-VI were non diabetic. Groups I & II and V & VI were administered 300 mg and 600 mg/Kg body weight respectively, of the extract by oral compulsion for four weeks while groups III and IV acted as diabetic and non-diabetic control respectively. Plasma glucose and lipid profile were analyzed by standard laboratory methods. The extract had no significant effect on body weights of the animals irrespective of their diabetic status. However, the extract had significant ($p<0.05$) hypoglycaemic effect on both diabetic and non-diabetic rats, with plasma glucose lower in the groups (diabetic and non-diabetic) treated with 600 mg/kg body weight of the extract in comparison to those treated with 300 mg/kg body weight (4.43±0.56 vs. 5.03±0.50 and 4.08±0.13 vs. 4.41±0.22 mmol/l respectively). While plasma High Density Lipoprotein-Cholesterol (HDL-C) was comparable among the animal groups, plasma Total Cholesterol (TC), Triglyceride (Tg) and Low Density Lipoprotein-Cholesterol (LDL-C) were significantly ($p<0.05$) lower in the groups (diabetic and non-diabetic alike) treated with the extract when compared with those untreated. This effect however appeared to be abolished at higher concentration of the extract as evidenced by lower decreases in the lipid fractions at the concentration of 600 mg/kg body weight against that at 300 mg/kg body weight. In conclusion, intakes of curry, garlic and ginger concurrently at culinary dose exerts beneficial effects on plasma glucose and lipids in health and disease. It also reaffirms the safety of spices combinations as practiced currently.

Key words: Spices mixture, type 2 diabetes model, hypoglycaemia, hypolipidaemia

INTRODUCTION

The burden of diabetes mellitus type 2, characterized by insulin resistance and hyperglycaemia is on the increase worldwide (Razieh *et al*., 2007). The disease has been recognized as an important public health problem in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive and unaffordable (Djrolo *et al*., 1998). Again, the incidence of cardiovascular diseases has been found to increase two-to-fourfold in people with type 2 diabetes mellitus (Raza and Movahed, 2003). The inability of the modern therapy to control all the pathophysiological aspects of diabetes and its complications coupled with the enormous costs it poses on the economy of the developing nations of the World, underscore the alternative strategies urgently sought (WHO, 2002). Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the management of this disease. Amongst such plants reported to have beneficial effects in the treatment of diabetes are spices such as cinnamon, cloves, bay leaves, ginger, turmeric, garlic amongst others (Khan *et al*., 2003; Broadhurst *et al*., 2000; Liu *et al*., 2007; Srinivassan, 2005), although both experimental and epidemiological studies have been consistently equivocal on their antidiabetic effects. While some studies have reported beneficial effects of these plants on some biomarkers of the disease, such as plasma glucose, lipid profile and antioxidant capacity (Ojiako and Nwanjo, 2009; Ugwuja *et al*., 2008; Al-Amin *et al*., 2006), others did not (Islam and Choi, 2008). Also most studies on medicinal/culinary plants have focused on individual plant. However, spices are seldom consumed singly, but rather as mixtures of two or more spices used to improve flavour of foods. In the light of previous findings of beneficial effects of dietary supplement containing

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mixture of raw curry, garlic and ginger on plasma glucose and lipids on normal rats, this study is aimed at investigating the effects of aqueous extract of raw spices mixture containing curry, garlic and ginger on alloxan-induced diabetic rats with specific interest on plasma glucose and lipid profile. Since the use of spices is on the increase, the result from this study would provide scientific evidence of the safety or otherwise of their uses by individuals with or at risk of diabetics mellitus.

MATERIALS AND METHODS

Animals: Male Wister albino rats (n = 36), weighing 108-162 g purchased from animal house of the Department of Pharmacy, University of Nigeria, Nsukka were randomly assigned into six (6) groups (I-VI) of six (6) rats per group. The animals were allowed free access to feed and water *ad libitu* for a period of one week to allow them acclimatise. All the rats received human care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals.

Induction of diabetes: Diabetes mellitus was induced in rats in groups I-III by intraperitoneal injection of 200 mg/Kg body weight of alloxan dissolved in distilled water while groups IV-VI were not induced. Fasting blood glucose levels were determined after three days of alloxan injection with a glucometer (ACCUTREND GC, Boerhinger, Mannheim, Germany), using blood from tail tips and diabetes mellitus was confirmed by elevated fasting plasma glucose > 7.8 mmol/l.

Preparation of spices' mixture: Raw spices powder of curry, garlic and ginger were purchased from Abakpa main market in Abakaliki metropolis. The spices were mixed together in equal ratio to form a uniform powder. This was soaked in 400 ml of distilled water overnight with occasional shaking after which it was filtered. The filtrate was dried on a rotary evaporator to give a semi-solid residue from which appropriate weight was measured and dissolved in 2.0 ml of physiological saline to obtain the desired concentration of 300 mg/ml of the extract.

Treatment of animals: Animals in groups I & II and groups V & VI received 300 mg and 600 mg/kg body weight of the extract respectively while animals in groups III and IV were given 2.0 ml of physiological saline daily and served as diabetic and non diabetic controls respectively. Throughout the duration of the study, which lasted for four (4) weeks, all the animal were maintained on normal rat feed. At the end of the experiment, the animals were fasted for 12 h after which they were sacrificed and blood (6.0 ml) collected and dispensed into fluoride oxalate bottle (2.0 ml) and EDTA bottles (4.0 ml) for the estimation of plasma glucose and lipids respectively.

Biochemical analyses: Plasma was isolated from the blood samples by centrifugation at 2000 g for 5 min in a laboratory centrifuge. Plasma glucose was determined by glucose oxidase method as described by Barham and Trinder (1972), plasma total cholesterol was estimated using the method described by Lopez-Vitrella *et al.* (1977), HDL-cholesterol and LDL-cholesterol were determined as described by Lopez-Vitrella *et al.* (1977) and Assmann *et al.* (1984) respectively.

Statistical analysis: All the data were analyzed for mean and standard deviation. Comparison of variables among groups was done using one way Analysis of Variance (ANOVA) and value is considered statistically different when p value is less than 0.05.

RESULTS

From Table 1, all the animals showed increments in their body weights irrespective of their diabetic status, although there was no significant difference in their final body weights.

Table 2 shows that aqueous extract of spices mixture of curry, garlic and ginger had significant ($p<0.05$) hypoglycaemic effect on both diabetic and non-diabetic rats, with the effect higher at higher concentration of the extract, as the plasma glucose was lower in the animal groups (diabetic and non-diabetic) treated with 600 mg/kg body weight of the extract in comparison to those treated with 300 mg/kg body weight of the extract (4.43±0.56 vs. 5.03±0.50 and 4.08±0.13 vs. 4.41±0.22 mmol/l respectively). However, while plasma High Density Lipoprotein-Cholesterol (HDL-C) was comparable among the animal groups, plasma Total Cholesterol (TC), Triglyceride (Tg) and Low Density

Table 1: Effect of aqueous extract of spices mixture containing curry, garlic and ginger on body weight of alloxan-induced diabetic rats[1,2]

	Diabetic case		Non-diabetic case		Controls	
Duration/day	Group I (300 mg/kg body weight)	Group II (600 mg/kg body weight)	Group V (300 mg/kg body weight)	Group VI (600 mg/kg body weight)	Group III (Diabetic)	Group IV (Non-diabetic)
0	79.6±5.6	81.2±6.1	81.6±6.7	81.1±4.5	77.9±2.8	80.8±3.3
7	84.6±4.2	82.6±5.1	83.7±4.9	83.7±7.4	79.1±3.2	83.7±3.6
14	85.3±3.0	85.7±2.9	89.3±7.4	87.6±5.7	82.3±2.9	88.9±4.2
21	87.3±3.8	86.6±4.1	90.7±7.4	93.9±4.8	88.6±4.5	92.7±4.4
28	95.4±4.6	94.3±3.9	99.4±6.8	96.6±4.8	94.6±3.7	97.4±2.9

[1]Values are expressed as mean ± standard deviation. [2]Weight of the animals were expressed in grams (g)

Table 2: Effect of aqueous extract of spices mixture containing curry, garlic and ginger on plasma glucose and lipids of alloxan-induced diabetic rats[1,2]

	Diabetic case		Non-diabetic case		Controls	
Plasma parameters	Group I (300 mg/kg body weight)	Group II (600 mg/kg body weight)	Group V (300 mg/kg body weight)	Group VI (600 mg/kg body weight)	Group III (Diabetic)	Group IV (Non-diabetic)
Glucose (mmol/l)	5.03±0.50[a]	4.43±0.56[a]	4.41±0.22[a]	4.08±0.13[a]	8.02±0.63	5.67±1.23
TC (mmol/l)	4.75±1.50[b]	4.98±1.87[b]	3.78±0.81[b]	3.80±0.71[b]	5.75±2.47	4.12±0.98
Tg (mmol/l)	1.40±0.57[c]	1.73±1.28[c]	1.29±0.19[c]	1.33±0.67[c]	2.35±1.21	1.52±2.01
LDL-C (mmol/l)	3.08±0.78[d]	3.28±1.37[d]	3.15±0.70[d]	3.21±0.15[d]	3.76±1.40	3.54±0.97
HDL-C (mmol/l)	1.03±0.85	1.05±0.66	1.01±0.51	0.83±0.50	1.00±0.68	0.97±0.63

TC: Total Cholesterol; Tg: Triglyceride; LDL-C: Low Density Lipoprotein-Cholesterol; HDL-C: High Density Lipoprotein-Cholesterol.
[1]Values are expressed as mean ± Standard deviation.
[2]Values with the same superscript along the row are significantly ($p<0.05$) different from the diabetic control

Lipoprotein-Cholesterol (LDL-C) were significantly ($p<0.05$) lower in the animals (diabetic and non-diabetic alike) treated with the extract when compared with those untreated. This effect however appeared to be abolished at higher concentration of the extract as evidenced by lower decreases in the lipid fractions at extract concentration of 600 mg/kg body weight against that at 300 mg/kg body weight.

DISCUSSION

Data from the present study show that aqueous extract of spices mixture containing curry, garlic and ginger exert hypoglycaemic and hypolipidaemic effects, but while hypoglycaemic effect was dose dependent, the hypolipidaemic effect tended to be abolished at higher concentration. Studies that investigated effects of spice mixture are scarce. For example, spice mixture of cumin, coriander and red pepper has been reported to affect bacteria metabolism in the serum (Sugawara and Suzuki, 1997). Also, *Amrita Bindu*-a salt-spice-herbal mixture has been found to possess antioxidant potential against free radical-induced oxidative damage (Ntarajan *et al*., 2006). Previously, we have reported both hypoglycaemic and hypolipidaemic effects of spices mixture made of equal proportions of curry, garlic and ginger on normal rats at 2% w/w, a concentration beyond which there were significant increases in both mean plasma glucose and total cholesterol (Ugwuja *et al*., 2008), which is consistent with the findings of the present study. Cinnamon, garlic and ginger has been reported to exert both hypoglycaemic and hypolipidaemic effect individually (Khan *et al*., 2003; Thompson *et al*., 2006; Gorinstein *et al*., 2006; Al-Amin *et al*., 2006). However, one study (Islam and Choi, 2008) has specifically reported that aqueous extract of ginger and garlic lacks effect on plasma lipids but are insulinotropic rather than hypoglycaemic in type 2 diabetes model, with ginger having better antidiabetic effect than garlic. Also, garlic and ginger has been found to provide some help for persons with hyperlipidaemia (Ali *et al*., 2000; Chetty *et al*., 2003). Although the mechanism by which spices such as curry, garlic and ginger lower plasma glucose or/and lipids has not been fully elucidated, laboratory data has proved that garlic for example, contain many biologically and pharmacologically important fat soluble organosulfur compounds such as Diallyl Sulphide (DAS), Diallyldisulfide (DADS), Diallyl Trisulfide (DATS) as well as water soluble S-allylcysteine which are beneficial to individuals suffering from cardiovascular, neoplastic and several other diseases (Agarwal, 1996; Alpers, 2009). Nevertheless, two mechanisms have been suggested by which garlic for instance lowers plasma cholesterol. These include inhibition of cholesterol and fatty acid synthesis (Ojiako and Nwanjo, 2009; Ojiako and Nwanjo, 2006). For instance, garlic and garlic-derived organosulfur compounds, including S-allylcysteine have been found to inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA), a rate limiting enzymes in the biosynthesis of cholesterol (Liu and Yeh, 2002). Also HMG-CoA activity has been reported to be significantly reduced in rat microsomes after garlic consumption (Merat and Fallahzadeh, 1996). Additionally, Inhibition of fatty acid synthesis by reduction in the incorporation of C-acetate into fatty acid has been demonstrated (Ojiako and Nwanjo, 2006). Again, dietary garlic has been reported to inhibit the synthesis of lipids in the liver and increases the levels of serum insulin, thereby increasing the glycogen in the liver and lowering serum glucose (Chang and Johnson, 1980). The reason for the loss of hypolipidaemic actions by the aqueous extract at higher concentration is obscure. However, a whole family of sulphur compounds (terpenoids, phenolic, allicin) known as sulphinyl disulphides in curry, garlic and ginger are known to undergo exchange reaction with SH-groups of enzymes and other proteins in the body spontaneously at physiological pH and temperature and may have inhibitory action on their activities (Rabinkov *et al*., 2000). It is therefore suggested that at higher concentration of the aqueous extract of the spices mixture, these chemical interactions might be strong enough to produce metabolic alterations which is manifested by abolition of the hypolipidaemia. Arguably, toxic effect of this extract at higher concentration can not be said to be responsible for the abolition of hypolipidaemic effect observed in the present study, as the glucose-lowering effect was

enhanced at the same concentration at which hypolipidaemic action seemed to be abolished. Regrettably, neither the liver enzymes nor histological examination was carried out on our subjects to rule out this possibility. However, it has been previously reported that garlic has a hypolipidaemic effect at lower dose, but at higher dose produces hypoglycaemic effect (Thompson *et al*., 2006). In corroboration with earlier study (Ugwuja *et al*., 2008), this study also showed that aqueous extract of spices mixture had no significant effect on body weight of both the diabetic and non-diabetic rats. Study on aqueous extracts of herbs has shown similar results (Proph *et al*., 2006). We therefore conclude that concurrent intake of curry, garlic and ginger at culinary dose in the diet has beneficial effect on plasma glucose and lipids in health and disease and reaffirms the safely of spices combinations as practiced currently.

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Comparison of Plasma Copper, Iron and Zinc Levels in Hypertensive and Non-hypertensive Pregnant Women in Abakaliki, South Eastern Nigeria

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Abstract: With the increasing speculations of the involvement of nutrition, particularly trace elements in the pathogenesis of preeclampsia, a comparative study of plasma copper, iron and zinc levels was carried out between preeclamptic and non-eclamptic Nigerian women living in Abakaliki, Southeastern Nigeria. Data for 40 preeclamptic and 40 non-eclamptic women matched for age, gestational age, Body Mass Index (BMI), parity and socioeconomic status from a cohort of 349 pregnant women recruited at gestational age ≤25 weeks for the assessment of impacts of trace elements' status on pregnancy outcomes were analyzed. In addition to trace elements which were determined by Atomic Absorption Spectrophotometer (Buck Scientific, Model AVG 210), Total White Blood Cell Count (TWBC) and Haemoglobin Concentration (HbC) were determined using standard laboratory techniques. The preeclamptic and the non-eclamptic women had comparable TWBC and HbC with the former having significantly ($p < 0.05$) higher blood pressure. However, although, the preeclamptic women had lower plasma copper, iron and zinc levels than the non-eclamptic women, only copper was found to be statistically significant (6.02±7.23 vs. 10.23±9.84 µmol/l, $p<0.05$). It is thus concluded that preeclampsia is associated with significant decrease in plasma copper. Further research is desired to elucidate the role of trace elements, especially copper in the pathogenesis of pregnancy induced hypertension.

Key words: Pregnancy-induced hypertension, trace elements, proteinuria, oedema, ceruloplasmin, Abakaliki

INTRODUCTION

Pregnancy-induced hypertension (otherwise known as pre-eclampsia), characterized by persistently elevated blood pressure of greater than 140/90 mmHg, proteinuria and oedema (ACOG, 2002) during pregnancy has been described as a transient but potentially dangerous complication of pregnancy that affects approximately 5-10% of pregnancies worldwide (Skjaerven *et al*., 2002; Sarsam *et al*., 2008; Cunningham *et al*., 2007). Pregnancy induced hypertension has been associated with adverse course and outcomes of pregnancy (Dekker and Sibai, 1998; Ziaei *et al*., 2006). For instance, in developing countries, pre-eclampsia accounts for 20-80% of the strikingly increased maternal mortality (Roberts, 1998) and 15% of preterm deliveries (Belizan *et al*., 1983). Although many pathophysiologic factors such as inflammation, cytokine production, dyslipidaemia (Hube, 1998), elevated homocysteine (Laivuori *et al*., 1999), oxidative stress (Roberts and Hubel, 2004), reduced calcium intake and excretion and an imbalance between thromboxane and prostacyclin (Paknahad *et al*., 2008; Williams *et al*., 1999) have been implicated in the aetiology of pre-eclampsia, the complete aetiologies have not been fully elucidated (Golmohammad *et al*., 2008). Although the high rate of pre-eclampsia in developing countries has forced some authors to propose the involvement of nutrition, especially the trace elements in the aetiology of the disorder (Caughey *et al*., 2005; Golmohammad *et al*., 2008), studies on the relationship between maternal plasma trace elements concentrations and pre-eclampsia have produced inconsistent results (Harma *et al*., 2005; Atamer *et al*., 2005; Ilhan *et al*., 2002). For example, while

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Golmohammad *et al*. (2008) reported non-significant difference in the mean serum levels of calcium, magnesium, copper and zinc between non-eclamptic and preeclamptic pregnant women, Paknahad and colleagues (2008) reported increased urinary excretion of copper in preeclamptic women in comparison to their non-eclamptic counterparts. The present study is aimed at comparing the plasma levels of copper, iron and zinc in hypertensive and non-hypertensive pregnant women in Abakaliki, a semi-urban setting with high prevalence of trace element deficiencies (Ugwuja *et al*., 2010a,b).

MATERIALS AND METHODS

This study which was part of a larger study that investigated the impact of plasma copper, iron and zinc status on pregnancy outcomes was carried out among pregnant women attending antenatal clinic of the Department of Obstetrics and Gynaecology of the Federal Medical Centre, Abakaliki, one of the referral tertiary health institutions in the South eastern part of Nigeria. The protocol for this study was approved by the Ethics and Research Committee of the Hospital. The approval was based on the provision of Helsinki Declaration (2000) and on the agreement that patient anonymity must be maintained, good laboratory practice/quality control ensured and that every finding would be treated with utmost confidentiality and for the purpose of this research only. Informed consent of the participants was sought and obtained after which recruitment was carried out. Subjects' selection and detailed methodology has been previously described (Ugwuja *et al*., 2010a,b). Data for forty hypertensive and forty non-hypertensive pregnant women matched for age, gestational age, parity, anthropometrics and socioeconomic status were analyzed. While plasma copper, iron and zinc were determined by Atomic Absorption Spectrophotometer (Buck Scientific, model AVG 210), haemoglobin concentration was determined by Cyanmethaemoglobin method while total white blood cell count was estimated as in a standard haematology textbook (Dacie and Lewis, 1994).

Data analysis: Data were analyzed for mean and standard deviation while comparison between subjects and controls were analyzed using Student's t-test with statistical significance set at $p<0.05$.

RESULTS

From Table 1, the hypertensive and the non-hypertensive pregnant women had comparable ($p>0.05$) Body Mass Index (BMI), gestational age and number of antenatal attendance. Although the hypertensive women appeared to be older (29.45±3.70 vs. 27.55±4.23) than their non-hypertensive counterparts, this was not statistically significant ($p>0.05$). The haematological parameters (haemoglobin concentration and total white blood cell counts) were also comparable ($p>0.05$) between the groups. However, the blood pressure was significantly ($p<0.05$) higher in the hypertensive than in the non-hypertensive pregnant women.

Table 2 shows that hypertensive pregnant women had significantly ($p<0.05$) lower plasma copper when compared with the non-hypertensive women (6.02±7.23 vs. 10.17±9.84). Lower plasma levels were also observed for iron and zinc in the hypertensive women in comparison to the non-hypertensive group, although these were not statistically significant ($p>0.05$).

Table 1: Comparison of maternal characteristics between hypertensive and non-hypertensive pregnant women[1]

Parameters	Non-hypertensive (n = 40)	Hypertensive (n = 40)	p-values
Age (yrs)	27.55±4.23	29.45±3.70	0.076
BMI (kg/m^2)	28.79±3.99	29.19±4.76	0.145
Gestational age (Wks)	21.53±3.73	21.40±3.22	0.873
Parity (n)	1.45±1.40	1.95±1.55	0.134
Number of antenatal attendance	6.88±2.90	6.35±2.42	0.383
HBC (g/dl)	10.24±1.28	10.44±0.97	0.444
TWBC (x 10^9/l)	5.12±1.37	5.48±1.38	0.255
Blood pressure (mmHg)			
Systolic	119.00±23.3	147.00±19.1	0.049*
Diastolic	78.00±12.7	96.00±9.3	0.031*

BMI: Body Mass Index; HBC: Haemoglobin Concentration; TWBC: Total White Blood Cell Count.
[1]Values are mean ± standard deviation, *$p<0.05$

Table 2: Comparison of plasma copper, iron and zinc between hypertensive and non-hypertensive pregnant women[1]

Parameters	Non-hypertensive (n = 40)	Hypertensive (n = 40)	p-values
Copper (µmol/l)	10.17±9.84	6.02±7.23	0.041*
Iron (µmol/l)	11.63±11.03	9.92±7.80	0.430
Zinc (µmol/l)	10.87±10.28	9.97±9.74	0.686

[1]Values are mean ± standard deviation, *$p<0.05$

DISCUSSION

Although study has shown that women with greater Body Mass Index (BMI) in pregnancy are more likely to become hypertensive than those with lower BMI (Pipkin, 2001), the comparable Body Mass Index (BMI), gestational age and number of antenatal attendance observed in the hypertensive and the non-hypertensive pregnant women in the present study ruled out the influence of these parameters on the aetiology or severity of hypertension in the two groups. This study had documented lower plasma copper, iron and zinc in hypertensive pregnant women in comparison to their non-hypertensive counterparts, although only plasma copper was found to be statistically significant ($p<0.05$) between the two groups. The non significant lower plasma zinc in preeclamptic women in comparison to non-eclamptic women recorded in the present study contrast earlier findings (Kumru *et al*., 2003) where serum zinc level was 43% lower in the preeclamptic women. It is also in contrast with 31% higher median leucocyte zinc concentrations in preeclamptic women when compared with controls (Mohamed *et al*., 2000). However, it corroborated the findings of Golmohammad *et al*. (2008) where the difference in serum level of zinc between preeclamptic and non-eclamptic women was found to be non-significant. Zinc insufficiency has been recognized by a number of experts as an important public health issue, especially in developing countries (Prasad, 1998). Zinc has been found to be involved in a number of metabolic processes that are essential for growth and development (WHO, 1996) and zinc deficiency has been associated with a number of chronic diseases such as diabetes (Ugwuja *et al*., 2010a,b), increased prevalence of Coronary Artery Disease (CAD), as well as other risk factors including hypertension and dyslipidaemias (Singh *et al*., 1998). Like zinc, non-significantly lower plasma iron concentration in preeclamptic women when compared to their non-pre-eclamptic controls in the present study suggest a role for iron in the pathophysiology of pregnancy induced hypertension. Although iron deficiency can be a major contributory factor to severe anemia with adverse pregnancy outcomes, such as low birth weight, premature birth and maternal mortality, evidence that iron-deficiency anemia is a causal factor in poor pregnancy outcomes is still lacking (Conlan *et al*., 1990; Eaton-Evans *et al*., 1996). However, elevated hemoglobin, especially in later pregnancy, has been associated with poor pregnancy outcomes. Although we did not encounter any study on plasma iron in pregnancy complicated with hypertension, it is interestingly to note that elevated hemoglobin rather than anaemia in pregnancy was linked to underlying conditions like pregnancy induced hypertension or preeclampsia, which are well known to contribute to poor pregnancy outcomes (Eaton-Evans *et al*., 1996). In the present study, although plasma copper concentration in preeclamptic women was found to be significantly lower than that of the non-eclamptic women, study has shown that while severe copper deficiency results in heart abnormalities and damage (cardiomyopathy) in some animal species, the pathology differs from atherosclerotic cardiovascular disease that is prevalent in humans (Institute of Health, 2001). Although studies in humans have produced inconsistent results and their interpretation is hindered by the lack of a reliable marker of copper nutritional status, outside the body, free copper is known to be a pro-oxidant and is frequently used to produce oxidation of Low Density Lipoprotein (LDL) in the test tube. Moreover, the copper-containing protein ceruloplasmin has been found to stimulate LDL oxidation in the *in vito* (Fox *et al*., 2000), leading some scientists to propose that increased copper levels could increase the risk of atherosclerosis by promoting the oxidation of LDL. However, there is little evidence that copper or ceruloplasmin promotes LDL oxidation in the human body. Additionally, the cuproenzymes, superoxide dismutase and ceruloplasmin, are known to have antioxidant properties, leading some experts to propose that copper deficiency rather than excess copper increases the risk of cardiovascular diseases (Jones *et al*., 1997). However, both epidemiological (Malek *et al*., 2006; Leone *et al*., 2006) and experimental studies (Turley *et al*., 2000; Rock *et al*., 2000) have failed to unequivocally confirm the role of copper in the aetiopathology of pregnancy-induced hypertension. It is therefore concluded that pregnancy-induced hypertension is associated with significant reduction in plasma copper level without significant effect on plasma iron and zinc status. Further research is desired to explain the roles of trace elements, particularly copper in the pathogenesis of preeclampsia.

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Contribution to the Survey of the Food Regime of the Forest Elephant (*Loxodonta africana cyclotis*) in the Peripheral Zone of the National Park Ogooue Leketi

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Abstract: In this survey, the authors analyze the food regime of the forest Elephant (*Loxodonta africana cyclotis*) in a part of the forest sector of the National Park of the Ogooué-Léketi in Republic of Congo. The survey achieved itself on two trails parallel distant of 7.5 km one of the other and covering a total distance of 13.19 km. During two months, of March to April 2010, the authors browsed the 13.19 km while harvesting the data on the plant species consumed by the forest Elephant. Every trail was browsed while using the method of oriented recognition (guided recces) until the discovery of the track of meal. Three methods were used for the collection of the data: opportunist observation of the traces; consistent of the cool tracks borrowed by the Elephant and the analysis of the cool droppings. According to the results of this survey, hundred thirty four (134) species of plants of which sixty ten eight (78) known and fifty six (56) unknown were identified in the rests of food of the Elephant. Otherwise, one notes that the forest Elephant eats the bushes mainly, the trees, the lianas, the monocotyledons and accidentally of ferns. The bush the more clear soup in the zone of survey is *Hugonia sp* 1, a bush lianescent of the family of Linaceae present in the two trails. The parts of the plants preferred by the forest Elephant are the leaves followed the roots, the stems, the fruits and finally the peels. Finally, sixty ten eight (78) droppings were analyzed in which one identified seeds of nine (9) species. Of these species, *Anonidium manii* was present in 50% of the droppings.

Key words: Track of meal, traces, droppings, trail

INTRODUCTION

To find their foods in the different habitats, the Elephants use the zones to strong potentiality of food while borrowing a complex network of the tracks. The tracks of the meals opened by the. Elephants can spread on hundreds of kilometers (Maisels *et al*., 2002; Blake, 2002). These tracks join several points of interest for the Elephants as the big fruit trees, the points of water and the bay named swampy clearings in central Africa (Blake, 2002).

The food regime of the savanna Elephant is relatively very known (Barnes, 1982; Tchamba, 1996). These studies showed the dominance of the herbs in the food of the Elephants during the season of rain and that the leaves, woods and peels become important when rains become rare. They consume the fruits that when they are available (Chapman *et al*., 1992).

The food regime of the forests Elephants is also studied in spite of the fact that this habitat of forest is closed. The studies led in West Africa (Alexandre, 1978; Merz, 1981; Short, 1981) and in Central Africa (Blake, 2002) show that the forests Elephants consume a variety of active food of the leaves, peel, wood, root and fruits. Tsoumou (2008) had found the same food behavior while studying the Elephants of the reserve of fauna of the Léfini, zone of the Tray Batékés of Congo.

To the level of the National Park Ogooué Lékéti, the exploratory polls achieved by the teams of Wildlife Conservation Society (WCS) of which Moukassa and Madzou (1996), Inkamba-Nkulu and Diahouakou (2005), constitute the first given on the food regime of the forest Elephant.

The present survey done in the peripheral forest of the National Park Ogooué Lékéti and neighboring of the fields of the inhabitants of the Simonbondo village, had for global objective, to bring our contribution on the knowledge of the food regime of the forest Elephant.

The specific objectives sum up:

- To produce the list of the plant species consumed by the forest Elephants of the zone of the National Park Ogooué Lékéti,
- To identify the shapes as well as the parts of the plants preferred by the Elephants.

MATERIALS AND METHODS

Localization and characterization of the survey zone

Geographical situation of the survey zone: The survey has been achieved on both sides of the Ogooué river to the level of the Simonbondo village, (Fig. 1), especially in the zone where there is conflict man Elephant and the zone said of conservation. It has been determined for

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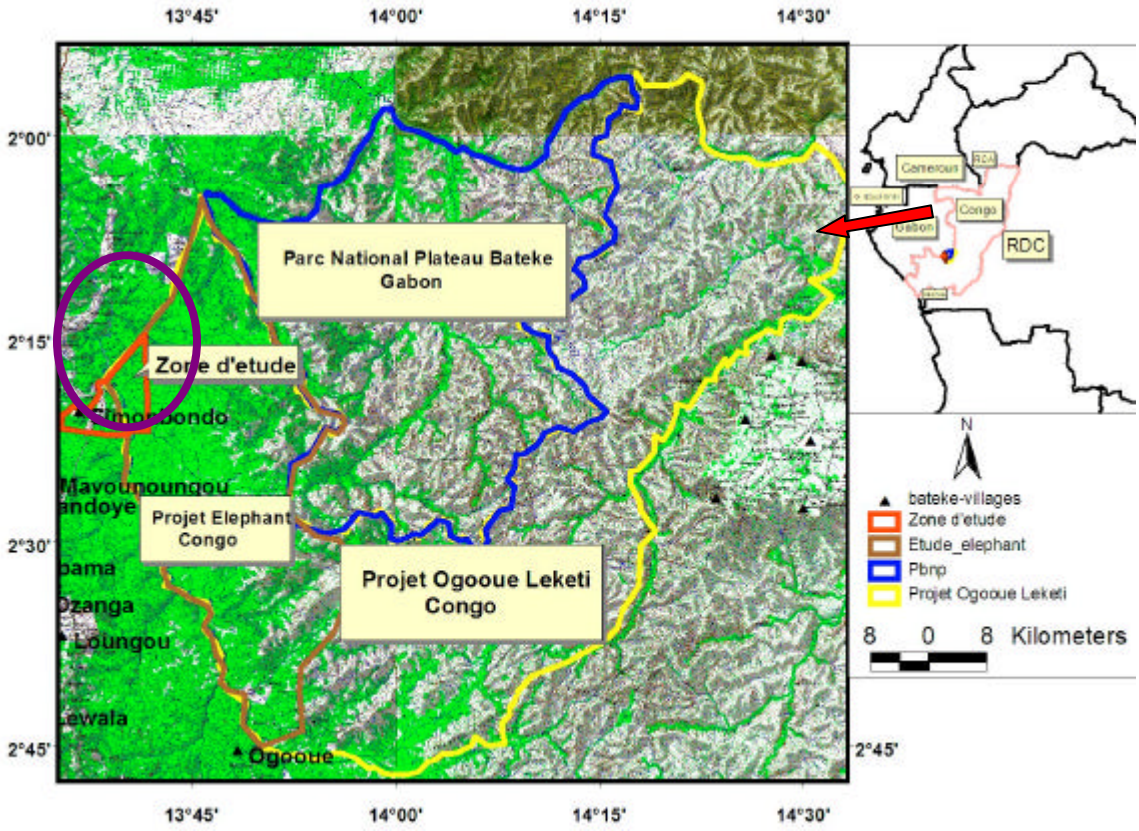

Fig. 1: Presentation of the survey zone

this survey a surface of 81.52 km^2 for the tracking of the Elephants. The main types of habitats that overflow the zone are: open mixed forests, forests mixed closed, flooded forests, savannas, marshes and rivers. These natural factors are susceptible to influence the distribution and the movements of the animals.

The zone of survey is limited at the North is by the National Park Ogooué Lékéti and Republic of Gabon; to the South by Republic of Congo and the banal zone to multiple exploitations. A carriage able road joins the zone of survey to the villages as Mavounoungou, Bandoy, Bambama and other.

Description of the abiotique middle

Climate: The zone of survey is one of the components of the Léconi-Batéké-Léfini landscape that is submitted to a tropical climate of transition of the sub-equatorial type (Elenga and Ikoli, 1996). It is a characterized climate by: of the precipitations of the order of 1200-1700 mm (INRAP, 1976); a dry season that lasts between 1 and 3 months; a yearly thermal amplitude adjoins 5°C; a yearly middle temperature of 25°C.

Geomorphology: The relief of this zone consists of many trays whose altitude oscillates between 600 and 700 m, cut at the bottom of which, sink the rivers (200-300 m). The zone of the National Park is located geomorphologique of the Tray Tékéses on the whole (Elenga and Ikoli, 1996). It individualizes herself/itself in two very different wholes composed of the trays and hills.

Geology and pedology: Soils are clayey sablo freeing the dust or the mud depending on whether one is in dry season or in rainy season. These climatic features and edaphique confer him an essentially forest vegetation (INRAP, 1976). Indeed, the geological cover essentially decomposes itself in two tertiary formations of which will and sandy silts then will and the argilite. these two geological formations drift several types of soils: on the high trays of soils yellow ferralitiques impoverished on the materials sandy argilo; on the low and pouring of the trays, of soils yellow ferralitiques impoverished on sandy materials.

Hydrography: The essential of the hydrography of the survey zone is the presence of the Ogooué river. This river takes the source to Congo and finished his/her/its race in Gabon. All along his/her/its passage, the Ogooué river receives the water of the different affluents of which

Rented it, Moba, Létili and other. The Ogooué rivers and Lékétis constitute the set of the hydrographic network of the water tower that throws himself the some toward the Congo stream and the Mpassa in Gabon.

Description of the biotique middle

Vegetation

Savannas: In the zone of the Park, the savannas cover close to 60% of the surface of the reserve and 40% by the forest. The survey of the vegetation savanicole in the Tray Batékéses has been led by several authors. Three groupings savanicoles exists (Makany, 1976; Moutsamboté, 1998):

- Savanna to *Hyparrhenia diplandra* that associates to *Bridelia ferrugina* on the trays. These savannas are composed on the one hand of the *Hyparrhenias diplandra* and *Nauclea latifolia* and on the other hand by *Hyparrhenia diplandra* and *Annona arenaria*,
- Savanna to *Loudetia demeusei* is characterized by a generally high herbaceous carpet and *Hymenocardia acida* on the flanks of hills,
- Savanna to *Loudetia simplex* and *Monocymbium ceresiiforme*, mainly in the shallows or on the banks inondables.

Forests: The studies of vegetation in the zone of the National Park Ogooué Lékéti is inexistent. A team of Botanical Garden had achieved an inventory floristique in the forest part in 2009 where have been identified close to 300 plant species. The forests of the reserve were classified in three big types:

- The forests on soils hydrornorphes are composed of the forests ripicoles colonizers to *Alchomea cordifolia* or to *Ancistrophyllum secundiflorum*, of the forests ripicoles to *Treculia tracaganta*, *Irvingia gabonensis*, of the swampy forests and inondables to *Lasiodiscus sp*,
- The forests of firm earth regroup the forests of trays and the forests of slopes or dry valleys. These forests are constituted by a diversity of the plant gases among which we can mention: *Aucoumea klaineana*, *Anonidium mannii*, *Piptademiastrum africana*, *Uapaca guinensis*,
- Of the spaces important of the forests monodominantes to *Pterocarpus soyauxii* and *Uapaca guinensis* to the under almost clear wood.

In these forests of firm earth there are places well tightened by lianas that are for most composed of *Laccosperma* rattans, *Ancistrophilums* and *Eremospathas* generally (*Arecaceae*).

In the other places there is the presence of the big woods of which the under wood is either clear is dominated by *Palisota* sp (*Commelinaceae*), *Megaphrinium sp.* (*Marantaceae)*.

Fauna: The inventories mammaliens achieved in the National Park Ogooué Lékéti had counted to the minimum 28 different species going from the leading species to the small mammals (Moukassa and Madzou, 1996; Inkamba and Diahouakou, 2005; Inkamba *et al*., 2009). These species of mammals of which some of fundamental importance for the conservation, include, the Chimpanzee (*Pan troglodytes troglodytes*), the Buffalo of forest (*Syncerus caffer nanus*), the Sitatunga (*Tragelaphus spekei*), the Leopard (*Panthera pardus*), the Jackal (*Canis adustis*), Hippo (*Hippopotamus amphibius*), Céphalophes, small Monkeys and the forest Elephant (*Loxodonta africana cyclotis*).

Although there are not some inventories on the plants and the birds in the National Park Ogooué Leketi, of the studies led on the other side of the border to Gabon by. Christy (2001) had counted 260 species of birds while including an endemic species of *Cisticola;* and beyond 300 species of the plants (Walters, 2005) with at least 2 endemic species in the Tray Batékéses.

Human populations: The zone kept for this survey is localized close to the Simonbondo village (District of Bambama in the Department of the Lékoumou). In this District, outside of the Pygmies (Babongo), the autochthonous Bantus are of two ethnic groups of which the Tékéses and the Mbambases. The other villages that adjoin the Park are localized along the road that joins Sibiti in Congo in Franceville in Gabon. The villages that have a direct influence on the Park are: Ogooué, Loungou, Mavounoungou and Simonbondo.

Collection of the data

Plan of poll of the survey zone: The zone of survey, of a surface of 81.52 km^2, is localized between 13°35,5'-13°41,8'E and 2°14,8'-2°22'S. She/it occupies a part of the Project Elephant of the Tray Batékéses within the future area protected from the National Park Ogooué Lékéti.

The DISTANCE program (Thomas *et al*., 2006) was used to establish the plan of poll in this zone of survey. Two parallel trails of variable length (trail 1 measuring 3.87 km) in the zone close to the Simondondo village where is installed the fields of the peasants and the trail 2, having 9.32 km of length and situated in the zone of the National Park Ogooué Lékéti. The two trails had separated one of the other of 7.6 km (Fig. 2). These two trails had to be browsed while following a direction determined of 152°. This orientation has been chosen in such a way that most fields of the populations of the Simondondo village are represented in order to measure the impact of the Elephants in the different types of vegetation is wild vegetation is of plantation.

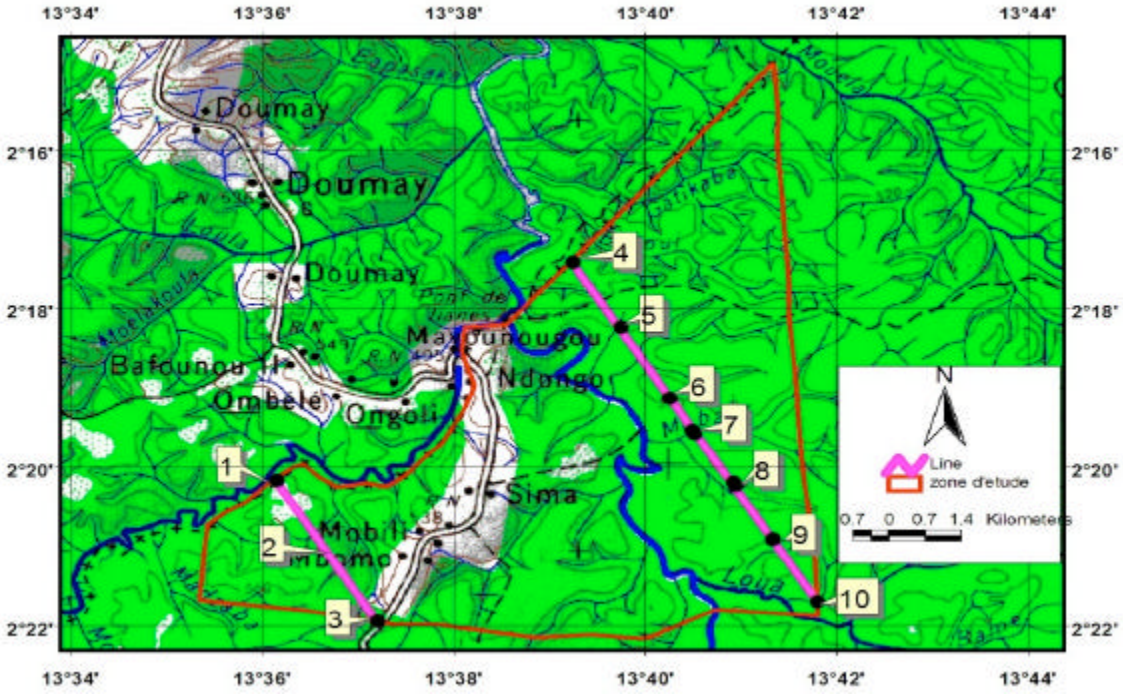

Fig. 2: Plan of poll of the survey zone

Material of land and technique of collection of data: Two missions of lands were organized for the collection of the data in the tracks of meal of the Elephants. The first mission had taken place in the month of March in the trail 1 situated close to the Simondondo village and the second mission was achieved in April in the trail 2 situated in the zone of the National Park Ogooué Lékéti. The observations were made every day between 7h30' and 12h00' then between 13h00' and 17 through the oriented recognition method or recces guided (Blake, 2002).

In the guided recces, the team walks on a right line while following the direction indicated in the compass. When a cool track of meal was marked along the main trail, the team should begin the tracking while counting and noting all traces and the rests of food of the Elephant. All signs of the Elephants (droppings and traces of foods) were noted in a strip of two meters (a meter of the observer's side) and all others signs (traces of the humans, direct observations of the animals and all nests of the big Monkeys) are recorded to all distances from the main trail.

The types of vegetation have been described qualitatively as one advanced along the trails and every time that there was a change. To move on the land us used the Global Positioning System (GPS) Garmin XL. All way dawned of the beginning and the end of the trails was recorded in the GPS before beginning the work of land. At the time of the coming down on the land, we used the GoTo function from the GPS to reach the beginning and the end of every trail. We took the care to note a point each 10 min in the trail to permit the establishment of the card of the itineraries followed and to help with the géo référencement. The data were noted in a notebook of land according to the time GMT. The watch and the GPS operated synchronous manner. Thus, all collected information were géo. referenced. These data of land were recorded then in a file meadow prepared of Excel in a portable computer before being submitted to the analyses.

Collection of the traces of the elephants

Data on the tracks of meal: He/it is nearly impossible in the habitat of closed forest to approach an Elephant in order to see what he/it even eats to a distance of less than 10 meters. In this survey we used the signs indirect of the rests of old food of at least 48 h let by the Elephants thus. Three methods were used for the collection of the data: Opportunist observation of the traces, followed of the cool tracks borrowed by the Elephants and the analysis of the cool droppings.

Opportunist observations of the traces: In order to produce an exhaustive list of food of the Elephants, we counted all signs of different ages that testify the damages caused by the Elephants. For what is foliages, he/it was kept to consider that the cool or recent signs of the Elephants associated by the presence of the prints, the dug holes and the damage of vegetation. The nutrition of the peels of the trees was distinctive even though the signs dated of several months.

The identification of the plant species consumed by the Elephants was is made directly on the land by the specialist of the Elephants in the project WCS Tray Batékés. In the event the species dealt with us unknown, a small herbarium of land was conceived while noting the least detail to allow his/her/its identification the laboratory. The works of botanies next one, Letouzey (1969), White and Abernethy (1996) and the list of the plants made by Nsongola *et al*. (2006), allowed us to identify some species of the plants collected. These species of the plants were classified then according to their taxonomie.

Tracking of the cool tracks: The cool tracks of the Elephants were followed through the zone of survey to a distance of 1 km in theory of which 500 meter of every side of the trail.

The method of tracking was to follow the cool track of meal once it was discovered from the main trail. The tracking was made with the aid of a hunter experimented in the hunt of the Elephants. The number of the individuals in the group was estimated from the sizes and the number of their prints in age/sexe (children, juvenile, sub-adult, adult females and adult males) of the Elephants in the group.

The distance browsed in the track of meal was measured while using the topo thread. The change of vegetation and all details on the types of the plants and food behaviors were recorded.

Analysis of the droppings: The cool droppings met in the trails or in the tracks of meal were analyzed while following the method described by Blake (2002). The analysis had started with the measurement (cm) with a flat rule. We used two tips of sticks to see the content of the dropping, the nature and the abundance of the components (leaf, fiber, woods peel, fruit) were discovered thus. The relative abundance of content of the droppings was estimated while using the scale of abundance (1 = rare, 2 = little, 3 = common, 4 = abundant). All seeds were identified on the land by the specialist of the Elephants. The unknown seeds met in the dungs were dried and were placed in the small sachets in plastics for their identification by specialists.

Treatment of the data: To the term of this work, he/it was question to determine the food régime of the Elephants in the two trails followed. The data descended of the tracks of meal were cleaned and were regrouped by families and type of plant. He/it has been established in every trail the first ten plant species the more consumed by the Elephants. Of a general manner, the analyses were made from a software Excel. To be easily assimilated, the percentages have been calculated from the relation:

$$\% = (n/N)*100$$

Where:
% = Percentage,
n = Sum of the signs in a track,
N = Total Sum of all signs.

RESULTS

Types of plant formations in the tracks of meal of the Elephants: In this zone of survey, there was a diversity of types of plant formations in the tracks of meal of the Elephants. This diversity makes appear of the resemblances in their specific compositions in all tracks. The main recorded plant formations were composed of the closed mixed forest (fmf), open mixed forest (fmo), swampy forest (fmr), the savanna (sav) and the flooded forest (fi).

We identified fourteen (14) cool tracks of meal covering a total distance of 7227.7km. Of this distance, the closed mixed forest was represented by 50%, consistent by the open mixed forest 34%. The three other types of plant formations were less represented of which the swampy forest 9%, the savanna 6% and the forest flooded 1% (Fig. 3).

Plant species consumed by the Elephants in the zone of survey: In the fourteen (14) tracks of meal, we counted in food consumed by the Elephants, hundred thirty four (134) species different from plants (Fig. 4) exits of 40 known families and 39 species whose families are unknown. These species were collected from 414 parts different from the plants (leaves, peels, woods, stems, branches, roots and fruits). Of these 134 species different of plants, 78 species were identified either directly on the land is from the botanical works to our disposition. Fifty six (56) other species remained unknown. These unknown samples were placed in a small herbarium of land for their identification ulterior to the level of the survey Center on the Plant Resources (CERVE) of Brazzaville.

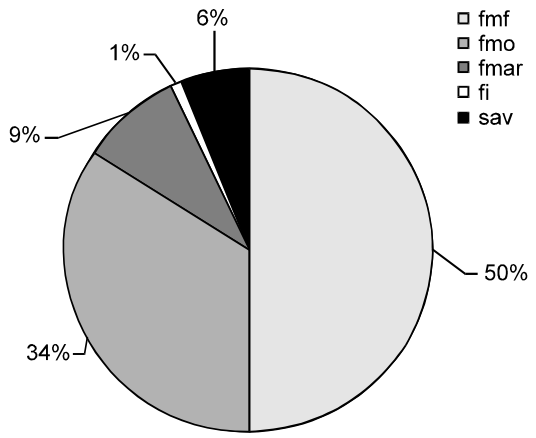

Fig. 3: Percentage of type of plant formations in the tracks of the meals of the Elephants

Table 1: The 10 species the more consumed by the Elephants in the zone of survey

Trail 1	N	% of consumption	Trail 2	N	% of consumption
Hugonia sp1	10	8.13	*Hugonia sp1*	23	7.90
Palisota hirsuta	6	4.88	*Cephaelis peduncularis*	19	6.53
Caloncoba welwitschii	5	4.07	*Chaetocarpus africanus*	16	5.50
Macaranga sp1	4	3.25	*Palisota hirsuta*	15	5.15
Pseudosabicea mildbraedii	4	3.25	*Anonidium mannii*	12	4.12
Bridelia ferruginea	3	2.44	*Pentaclethra macrophylla*	10	3.44
Chaetocarpus africanus	3	2.44	*Haumania liebrechtsiana*	10	3.44
Craterosiphon scandens	3	2.44	*Costus afer*	10	3.44
Eremospatha wendlandiana	3	2.44	*Mammea africana*	7	2.41
Haumania liebrechtsiana	3	2.44	*Manniophyton fulvum*	6	2.06
Total de ces 10 espèces		35.77	Total de ces 10 espèces		43.99
Nbre des espèces		67.00	Nbre des espèces		99.00
Nbre de fois consommé		123.00	Nbre de fois consommé		291.00

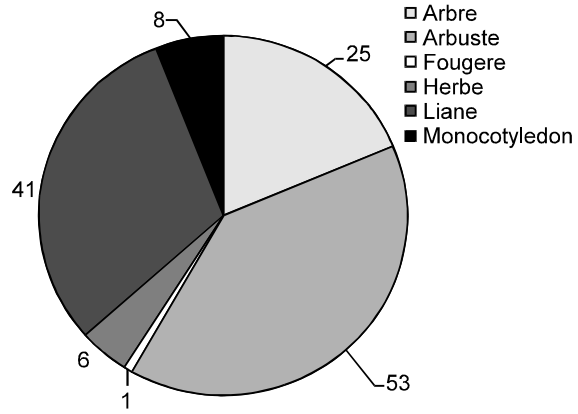

Fig. 4: Number of known plant species (N = 134) consumed by the Elephants in the zone of survey

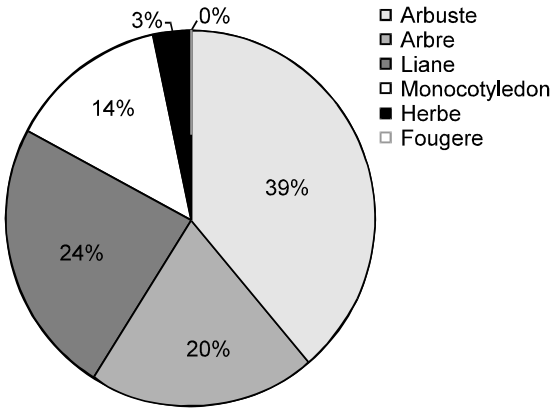

Fig. 5: Percentage of the shapes of the plants consumed by the Elephants

Of the hundred thirty four (134) species of plants, one noted that ten (10) were consumed more by the Elephants (Table 1). Of these ten (10) species, one notes that 4 species were common in the two trails of which *Hugonia sp 1*, *Palisota hirsuta*, *Chaetocarpus africanus* and *Haumania liebrechtsiana*. The other species are present in one or the other trail. The species *Hugonia sp1* is the more consumed with the frequencies of 8.13% in the trail 1 and 7.9% to the trail 2. on the other hand the species the less consumed in the lists of the first 10 species is the *Manniophyton fulvum* with a frequency of 2.06% in the trail 2.

Frequency of shape of plant consumed in the tracks of meal: To make itself/themselves an idea of the preference of shape of plant consumed by the Elephants in this zone, we classified these plants in six shapes: tree, bush, lianas, ferns, monocotylédone and herbs, (Fig. 5). The exam of the Fig. 5 shows that on the whole, the nutrition of the Elephants was composed more of the bushes (39%) consistent by the lianas (24%) and the trees (20%). The three other shapes of plants were less represented of which Monocotylédone (14%), grass (3%) and the fern is consumed accidentally (0.2%).

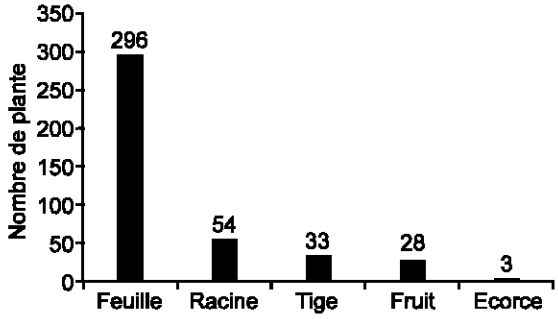

Fig. 6: Frequency of the parts preferred of the plants consumed by the Elephants

Frequency of the consumption of the parts of the plant: The results on the parts of the plant preferred by the Elephants are represented in the Fig. 6. Had to the total 414 parts of plant that the Elephants had eaten in the two trails followed. The exam of the Fig. 6 shows that the nutrition to basis of the leaves was raised very in relation to the peels with a percentage of 71.5% and 0.72% respectively. The other left from the plant of which the roots (13.04%), the stems (7.97%) and the fruits (6.76%) present intermediate values between the leaves and the peels.

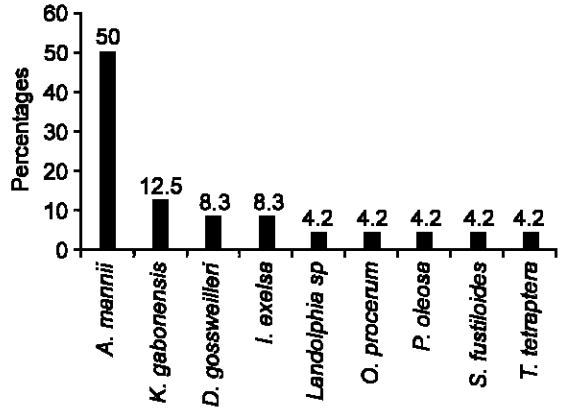

Fig. 7: Plant species found in the droppings of the Elephants

Analysis of the droppings: During this survey, we analyzed 78 cool droppings of Elephants. Of these droppings, we identified seeds of nine plant species. The species the more represented is the *Anonidium mannii* (50%) and the other species present some values lower to 13% (Fig. 7).

DISCUSSION

Of the general surface kept for this survey, the tracks of meal of the Elephants followed were only limited along two distant parallel trails of 7.5 km one of the other while following a declension of 152° degree. This declension appeared us important for the fact that the tracks of meal would owe arrivals in the fields of the populations of the Simondonlo village. This possibility had to allow us to make us an idea of that that the Elephants ate in the fields of the villagers. Unfortunately, the two trails, drawn by the DISTANCES Program moved away of the fields to the point where no track of meal had reached the fields of the villagers. Although the Elephants didn't arrive in the fields, one observed their tracks nevertheless to the level of the trail 1, an easy access zone. Indeed, the zone where is located the trail 1 is a zone of forest to closed canopée with opened one coins wood. This vegetation contains for most cases of the big trees with the abundance of fruit trees eaten by the Elephants like *Irvingia excelsa*, *Anonidium manii* and *Baillonella toxisperma*. During the period of our survey, a lot of fruits walls fallen of these trees dragged to soil and that the feet of these were the places of meeting of several animals including the forest Elephant.

To the level of the open mixed forest had food also for Elephant as well as the lianas of several species consumed. The marshes also contain a particular vegetation with sometimes of the *Maranthacloas sp*.

In the crossing of every track, at least exist a fruit tree appreciated by the Elephants. What comes back to say that these are the Elephants who create these tracks while following the fruit trees. In addition, they maintain them to be used during the seasons of the fruits. This situation lets believe that the tracks of the Elephants in the habitat of forest are not a fact of the luck. Thus, as affirm it (Blake and Inkamba-Nkulu, 2004; Tsoumou, 2008), the tracks of the Elephants accumulate toward the zones where there is an interest, for example the bay of forests or the Elephants eat the mineral salts.

Finally, the Elephants don't only find their food in the habitat of forest but one also meets them in the savanna where they find the herbs and the other plant species very appreciated. The track of meal 1 that crosses a river then arrives in the savanna, is a good example of use and the selection of food by the Elephants in several habitats.

As our results indicate it, 134 plant species were consumed by the Elephants among which ten were consumed more that of others. While making the comparison of the plants the more consumed in every trail, one notes that it is *Hugonia sp 1* that is the species the more consumed. This species is appreciated a lot by the Elephants can be because his/her/its wood stretch. The Elephants eat all parts of the plant then (leaves, stems, roots). other reasons can justify this preference as the abundance of the species in the zone of survey. This hypothesis seems to confirm the results of our survey.

In the 14 tracks of meal, one notes that the Elephants consume six shapes of plants but with a very elevated preference for the bushes the lianas and the trees Three hypotheses can justify this preference of the Elephants. In the first place, one thinks about the type of plant formation crossed by the Elephants. Indeed, depending on whether the Elephants cross a closed mixed forest composed of big tree or a dominated open mixed forest by the bushes and the lianas, it is obvious that these animals will eat what they find on their path. In second place, there is the accessibility to the plant. Indeed, no matter the type of plant formation that the Elephants cross, they have access more easily to the bushes that to the trees. Finally, it is necessary to signal abundance. It is obvious that the habitats crossed by the Elephants don't have the same composition of the point of view of the quantity of shape of plants. Even though the studies on the composition floristique have not been undertaken in this zone, our results let believe that the habitats crossed by the Elephant were the open mixed forests, composed for most bushes and lianas.

Our results on the parts of the plant the more consumed, raise that the Elephants consume more of leaves that the roots, the stems, the fruits and the peels. These results confirm those of (White, 1994) that raise that the consumption of leaves by the Elephants is always more important than the other left of the plant and that the abundance of the leaves in a determined zone, can influence abundance and the distribution of the Elephants. The exam of the droppings confirms our results besides on this very elevated consumption of the leaves even though the consumption of the fruits notably the seeds of the species *Anonidium mannii* and *Irvingia excelsa* that confirm works of (Chapman *et al*., 1992) presents very elevated values.

Conclusion and perspectives: The survey of the food regime of the Elephants while using the recces guided in the trails and the cool tracks of meal were achieved in the forest sector from the National Park of the Ogooué Lékéti to Congo. A total of 14 cool tracks of meal was browsed during two months.

The results of this survey raise that 134 species of plants were consumed by the Elephants. The plant species the more consumed in the zone of survey is *Hugonia sp1* of the family of *Linaceae*.

In the zone of our survey, the Elephants eat the bushes mainly, the lianas, the trees, the monocotyledones and accidentally of the ferns. The parts preferred of these plants are the leaves, consistent the roots, the stems, the fruits and incidentally the peels.

Such works whose results are henceforth accessible can continue around other parks so that to term the authorities concerned can put in place at a time on the one hand coherent programs to fight against the Elephants who ravage the fields of the populations and on the other hand to protect the Elephants who are killed by the populations while putting some mines around their fields. It is for example about cultivating to the periphery of the fields of the species of trees whose Elephants are sausage rolls and that will have the advantage to slow down their impetus to penetrate in the fields of the villagers.

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Effect of Sudanese Marketing Condition on Quality Attributes of Meat Products

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Abstract: This study was carried out to evaluate the raw meat found in Khartoum local markets. Raw meat samples from modern and traditional markets were evaluated as sources for processing fast foods. Beef top side cuts 6 kg were purchased from a modern local meat plant and also from a traditional meat market at tow times of the day, in the morning at 8 am and in the evening at 5 pm. Two meat products (sausage and burger) were processed from these meat sources. The products were evaluated at zero time and at the end of 5 weeks freezer storage (-18°C), for ultimate pH, Water Holding Capacity (WHC), rancidity and cooking loss (%), sausage treatments were significantly different ($p<0.05$) in cooking loss (%). The chemical composition was determined also for sausage and burger treatments. Crude protein content (%) were significantly different ($p<0.05$) in sausage and burger products. The moisture content (%), fat and ash (%) were also determined. The colour measurements of redness (a), yellowness (b) and lightness (L) of the sausage and burger treatments were not significantly different ($p<0.05$). Sensory attributes of sausage and burger as assessed by panelist included colour, flavour, tenderness, juiciness and overall acceptability and were not significantly different ($p<0.05$). Storage loss (%), total viable bacterial count and coliform count (Log_{10} cfu/g) of the various treatments were not significantly different ($p>0.05$).

Key words: Raw meat, fast foods, beef

INTRODUCTION

Among African countries, Sudan is characterized by diverse wealth including cattle, goats and camel. Meat animals in Sudan depend mainly on the natural grazing system which affects meat production (Abugroun, 2000). Sudan has a huge livestock population, estimated by more than one hundred and twenty million heads and classified as follows: 37.1 million heads of cattle, 3.18 million camel, 42.8 million sheep and 37.8 million goats. Therefore, modern aspects of animal production efficiency based on recent scientific developments must be considered, especially slaughter and processing techniques with good control of sanitation and hygiene. These will result in greater yields and higher profits and would also provide incentives for increased production (FAO, 2000).

Meat and meat products are highly perishable and spoil easily and soon become unfit to eat and possibly dangerous to health through microbial growth, chemical changes and breakdown by endogenous enzymes (Judge *et al*., 1990).

In many parts of the world, refrigeration is inadequate for the storage, distribution or processing of meat into manufactured forms. Slaughter schedules in many developing countries are planned to permit consumption of meat within a few hours after slaughter. The procedures vary in developed countries but they result in microbial counts that are characteristically low (Abugroun *et al*., 1993).

Nychas *et al*. (2008) reported that the microbiological quality of meat depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage and distribution. In fact, some of the microorganisms originate from the animal's intestinal tract as well as from the environment with which the animal had contact at some time before or during slaughter (Koutsoumanis and Sofos, 2004).

MATERIALS AND METHODS

Sampling procedure: Beef topside cuts 6 kg were purchased from three different sources. And used for processing in the experiment. Then were divided into three groups, according to source. One group consists of chilled meat purchased from a meat processing plant. The second group consists of meat purchased from a traditional market at 8:00 am in the morning and the third group consists of meat purchased in the evening at 5:00 pm from a traditional market. Sausage and burger samples were processed from the various meat sources and prepared for analysis immediately after processing and after storage. The samples were stored for five-week period by freezing at -18°C.

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Chemical evaluation: Approximately 150 g of products from each treatment were blended for 15s in laboratory blender and were used in all chemical analysis. Each products samples was homogenized and analyzed in triplicate, to determine moisture (drying for 6 h at 105°C), fat (as extractable component in Soxhlet apparatus), protein (Kjeldahl nitrogen) using standard methods (AOAC, 1980). The ultimate pH of products samples determined by sing pH meter. The pH meter was calibrated with buffers 4 and 7.

Meat quality attributes: Fresh sausage and burger products were prepared for colour sensing and covered by polythene sheets. The colour was determined using a Hunter-Lab Tristimulus colorimeter (Model D25 M.Z, Hunter Associated Lab. Inc., Virginia, USA). Hunter (L) lightness, (a) redness and (b) yellowness were recorded before and after storage. Duplicate samples, each of approximately 0.5 gm of two products, were placed on a humidified filter paper (Whatman No. 4 in a desiccator over saturated KCl solution) and pressed between two plexiglass for 1 min at 25 Kg/cm^2. Meat and moisture areas were measured using a compensating planometer. The result was expressed as ratio (Grau and Hamm, 1953). Water Holding Capacity (WHC) = [Loose water area-meat film area] ÷ meat film area, before and after storage. Cooking loss determined as Babiker (1981) by using thermostatically controlled water bath 90°C for 90 min, samples were weighed before and after cooking.

Oxidative rancidity: The oxidative rancidity of processed meat was determined using 2-thiobarbituric acid (TBA) method as described by Hoyland and Taylor (1989).
The storage loss % was determined by taking the initial weight of the products (sausage or burger) after processing immediately and then after the storage period (five weeks). The frozen samples were left overnight in a refrigerator at 4°C for thawing and then weighed.

Microbial analysis: One gram of products (sausage or burger) was homogenized in nine ml of sterile distilled water for 1-5 min. ten fold dilutions of homogenate were prepared in normal saline.

Enumeration of total aerobic mesophilic bacteria: Plating was performed into plate count agar (PCA, OXOID CM 325) from the prepared dilutions by spread plate method. Colonies formed after 48 h incubation at 30°C under aerobic conditions were counted (Swanson *et al*., 1992).

Enumeration of coliforms: Total coliforms were determined by the tubes Most Probable Number (MPN) method. Laury sulphate tryptose broth (LST Broth, OXOID CM 451) and brilliant green lactose bile (2%) broth (BGLB Broth, OXOID CM 31) were used for presumptive and confirmed tests for coliforms, respectively. Results were evaluated according to the MPN tables (Harrigan and McCane, 1976).

Detection of *Staphylococcus aureus*: Spread plate method was performed to plate form pre-determined dilutions onto Baird-Parker agar (BPA, OXOID CM 275) prepared by adding sterile egg yolk tellurite emulsion (OXOID, SR 54). After incubation at 37°C for 48 h, coagulase test was applied to typical black-grey, bright, smooth colonies with clear zones determined accordingly. (The Oxoid manual, 1998).

Presence-absence test of Salmonella *spp*: After anon-selective pre-enrichment at 37°C for 16 h in lactose broth, samples were transferred to Rappaport-Vassiliadis enrichment broth (RV, OXOID CM 669) for selective enrichment and plates were incubated at 42°C for 24 h -Aloopful of sample was streaked onto bismuth sulphite agar (BSA, OXOID CM 201) for selective growth, and was incubated at 37°C for 48 h. Brown-grey-black colonies surrounded by a brown-black zone and yielding metallic sheen were regard as typical suspect salmonella colonies and a appropriate confirmatory tests were performed (Andrews and Hammack, 2003).

Detection of yeast and molds: From the samples of each product, plating was performed by spread plate method onto Rose Bengal Chloramphenicol Agar (RBCA, OXOID CM549) with chloramphenicol selective supplement. Colonies formed at 30°C after 4-5 day incubation was determined (The Oxoid manual, 1998).

Sensory attributes: The sensory evaluation was conducted in the sensory evaluation facilities of the Meat laboratory, Samples were separately cooked from each group of treatment as two methods of cooking and frying by oil and oven cooking at 180°C for 15 min. 11 semi-trained panelists were used to evaluate the sausage and burger samples. The evaluation included, colour, tenderness, flavour and juiciness using an 8-point scale score (hedonic scale) card as described by Cross and Overby (1988).

Statistical analysis: The data obtained were analyzed statistically and the means were tested for significance using Duncan Multiple range test as described by SPSS. v.16 (2008).

RESULTS AND DISCUSSION

The differences in pH level might be due to the changes that occurred after slaughter owing largely to the differences in the amount of glycogen available for transformation into lactic acid (Guingnot *et al*., 1992). Aberle *et al*. (2001) mentioned that a normal pH declines in porcine muscle by a gradual decrease from approximately pH 7.4 in living muscle to a pH of about 5.6-5.7 within 6-8 h postmortem and then to an ultimate pH (reached at approximately 24 h postmortem) of about

5.3-5.7. In other animals muscle pH drops rapidly to around 5.4-5.5 during the first hour after exsanguinations. The results of ultimate pH for processed meat sausage and burger were not significantly different ($p>0.05$). Sausage and burger groups were not significantly different in water holding capacity ($p>0.05$). Water holding capacity is affected by several factors such as pH, species, age and muscle type and function. Price and Schweigert (1987) reported that the water holding capacity of meat could be increased by addition of table salt. Addition of these salts to meat during curing or manufacture of emulsion thus increases the water holding capacity. Water-holding capacity is especially critical in meat ingredients of meat products that are subjected to combinations of heating, grinding and other processes. Weight losses during fabrication processes are largely the results of water evaporation (Aberle *et al*., 2001). W.H.C shows in (Table 1) were not significant differences, nevertheless the products were processed from chilled raw meat had a lower value compared with other groups shown in (Fig. 1).

Rancidity values of Sausage and burger groups in this study reported no significantly different ($p>0.05$). The storage life of post rigor ground products is shortened because of incorporation of oxygen during grinding. Addition of salt to processed meat products also accelerates oxidation (Aberle *et al*., 2001). Isabel and Ana (2005) mentioned that the highest content of capsaicin in hot paprika decreases the rancidity of dry sausages, because of its contents of flavonoids, capsaicinoids, tocopherols and cartenoids (Daood *et al*., 1996). As capsaicin has an important anti-oxidant effect (Lee *et al*., 1995; Kogure *et al*., 2002), the greater the content of pepper fruits the greater the anti-oxidant effect (Perucka and Materska, 2001). Cooking loss values of sausage treatments were significantly different ($p<0.05$). Sausage processed from modern chilled raw meat had lower values for cooking loss and were not significantly different among the burger treatments. Pearson and Dutson (1987) reported that 84% of the total volume of beef psoas muscle was water. Of that amount, 66% was in the myofibrillar element and 18% in the sarcoplasmic space. It is, therefore, apparent that water losses during cooking come largely from myofibrillar fraction. Moreover, Cross and Overby (1988) reported the most drastic changes during heating of meat, such as shrinkage and hardening of tissue and the release of juice, are caused by changes in the meat proteins. Moisture losses are of great monetary importance. Although moisture losses make meat less attractive, they do not significantly influence its eating quality after cooking, except in case of very large losses, which could affect juiciness and tenderness (Hui *et al*., 2004). The results (Table 1) indicate that colour parameters were not significantly different ($p>0.05$) among the treatments. Meltem Serdaroglu (2006) recorded that patty lightness as measured by using the Hunter (L) value increases with increasing amounts of fat in the formulations. The increased fat and reduced lean meat probably caused the difference in colour values. Reitmer and Prusa (1991) indicated that as fat content decreased in raw ground meat the colour intensity decreased because fat contributed a yellow-white colour to fresh meat. Fernandez-Lopez (1988) reported that the; salt content was responsible for increases on this colour coordinate in a dry cured sausage model system, the increase in (a) redness, observed during fermentation may have also been due to moisture loss, which would increase the salt content (on a wet basis). The same authors mentioned that the salt content lowers the value of (b) yellowness, (due to the effect of salt on oxygen solubility in the meat batter).

The study had no significant difference ($p>0.05$) in moisture content (Table 2). Water content is inversely proportional to fat content that is meat with high fat content has a low water content and vice versa (Gaman and Sherrington, 1998). During freezing, storage and thawing, meat loses water by evaporation, sublimation, and exudation, respectively; moisture is also lost during cooking (Hui *et al*., 2004).

Sausage and burger protein content were significantly different among the treatments ($p<0.05$). Pace *et al*. (1989) indicated that in most instances proximate nutrients, such as crude protein and some vitamins and minerals increased due to loss of moisture. The results of the study showed no significant differences ($p>0.05$) in fat content among the treatments and also for ash content. Meltem Serdaroglu (2006) mentioned that several researchers have found that moisture contents of meat batters increased with the addition of soy proteins.

The results show in Table 3 that the treatments did not differ significantly in colour, flavour, tenderness, juiciness and overall acceptability ($p>0.05$). The sausage and burger samples were cooked by two procedures frying and oven. Huffman and Egbert (1990) found no differences in beef flavour intensity over a range of 5-20% fat content of patties. Decreasing fat content in patties from 20-5% reduced texture scores. As protein has a greater influence on texture than fat. Reducing fat meat products can have a greater hardness (Jimenez-Colmenero *et al*., 1995). This relationship has been observed by other authors in various meat product results (Akoh, 1998; Garcia *et al*., 2002; Serdaroglu and Sapanci-Ozsumer, 2003). Adding 4% oat flour to patty formulation increased the juiciness scores. Panelists found these patties more, juicy than other treatments. This is not surprising as adding of additives to meat products results in more moisture retention in the product during cooking. An increase in moisture levels has been reported to increase juiciness in frankfurters (Hung and Carpenter, 1997) and goat patties (Gujral *et al*., 2002). The tenderness, juiciness and flavour were affected by fat level (Meltem Serdaroglu, 2006).

Table 1: Means and standard errors for quality attributes of the various treatments

		Treatment*						
		M		TM		TE		
Storage (weeks)		0	5	0	5	0	5	S.E.
Independent variables								
Sausage	pH	5.38[a]	5.29[a]	5.24[a]	5.28[a]	5.23[a]	5.25[a]	±0.06
	WHC	0.73[b]	0.34[b]	1.84[b]	1.40[b]	1.36[b]	0.70[b]	±0.49
	Rancidity	0.04[c]	0.10[c]	0.02[c]	0.12[c]	0.13[c]	0.16[c]	±0.02
	Cooking loss (%)	7.95[b]	5.20[a]	16.81[f]	11.91[d]	15.66[f]	10.51[c]	±0.40
Colour values	Redness (a)	14.47[a]	10.33[a]	12.50[a]	9.73[a]	13.23[a]	8.43[a]	±0.76
	Yellowness (b)	9.30[b]	6.37[b]	10.37[b]	6.97[b]	9.40[b]	7.13[b]	±0.75
	Lightness (L)	38.67[c]	34.63[c]	39.40[c]	35.73[c]	38.67[c]	35.07[c]	±1.66
Burger	pH	5.38[a]	5.33[a]	5.22[a]	5.28[a]	5.26[a]	5.25[a]	±0.06
	WHC	0.87[b]	0.60[b]	1.67[b]	1.09[b]	1.45[b]	0.92[b]	±0.14
	Rancidity	0.04[c]	0.11[c]	0.08[c]	0.11[c]	0.13[c]	0.17[c]	±0.02
	Cooking loss (%)	16.18[d]	10.65[d]	16.87[d]	11.13[d]	14.49[d]	10.27[d]	±1.40
Colour values	Redness (a)	13.70[a]	9.16[a]	12.6[a]	9.66[a]	11.86[a]	8.86[a]	±1.20
	Yellowness (b)	9.40[b]	6.63[b]	9.40[b]	5.67[b]	9.70[b]	6.63[b]	±0.31
	Lightness (L)	36.27[c]	33.23[c]	41.33[c]	35.27[c]	40.93[c]	37.68[c]	±2.41

[abcd]Means in the same row bearing different superscripts are significantly different ($p<0.05$)

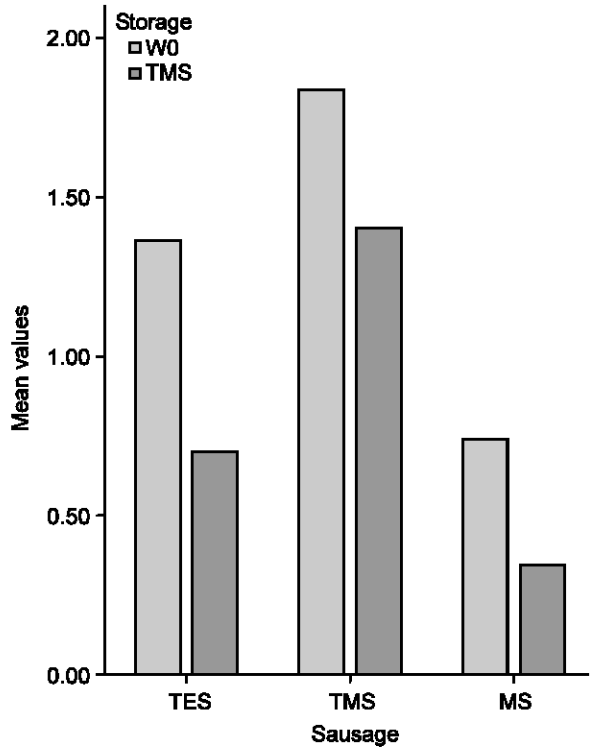

Fig 1: Water holding capacity of sausage treatments

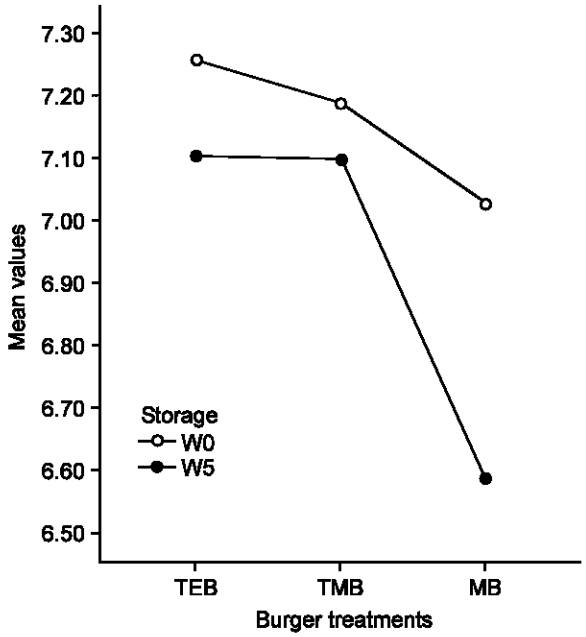

Fig. 2: Total bacterial count (log_{10} cfu/g)

Table 4 and Fig 2 shows total viable bacterial count and coliform count colony forming unit/gm (Log_{10} cfu/g) were not significantly different ($p>0.05$).

A total plate count provides an indication of total populations of microorganisms. It can be used to assess microbial loads on or in meat products, in air and water and on equipment and facilities (Aberle *et al.*, 2001). Even though only mentioned in a limited way previously, the microbiology of hot processed beef is an extremely important consideration. Oblinger (1982) extensively reviewed that area and summarized the work of several researchers. It appears that hot processing and electrical stimulation have no significant effects on the contaminating microflora.

Musa (2004) obtained on fresh beef meat with an average load of 1.2×10^6 cfu/g to aerobic plate count and coliform count average load 5.0×10^5 cfu/g and also reported the total plate count for sausage during and after processing by average load and coliform count was 1.1×10^7, 8.2×10^7, 7.1×10^6 and 4.4×10^6 respectively.

Kotula (1981) also indicated that the microbial quality of hot-processed beef need not be of concern if proper sanitation and chilling practices are used. However,

Table 2: Means and standard errors for moisture %, protein%, fat%, and ash% of the various treatments

		Treatment*						
		M		TM		TE		
Storage (weeks)		0	5	0	5	0	5	S.E.
Independent variables								
Sausage	Moisture (%)	63.75[a]	59.98[a]	64.29[a]	61.40[a]	61.45[a]	58.66[a]	±1.32
	Protein (%)	22.67[a]	18.48[b]	24.43[d]	22.10[b]	26.17[b]	22.45[a]	±0.21
	Fat (%)	3.37[b]	2.20[b]	4.28[b]	3.08[b]	7.84[b]	5.20[b]	±0.33
	Ash (%)	3.12[c]	1.38[c]	2.23[c]	1.42[c]	2.08[c]	1.21[c]	±0.44
Burger	Moisture (%)	65.45[a]	61.99[a]	65.98[a]	62.80[a]	62.50[a]	59.20[a]	±0.67
	Protein (%)	22.38[a]	18.36[b]	22.53[a]	19.12[c]	25.31[d]	22.59[a]	±0.12
	Fat (%)	3.02[b]	2.20[b]	3.27[b]	2.26[b]	7.47[b]	7.40[b]	±0.47
	Ash (%)	2.64[c]	1.44[c]	1.55[c]	1.12[c]	1.01[c]	0.51[c]	±0.31

[abcd]Means in the same row bearing different superscripts are significantly different ($p<0.05$)

Table 3: Means and standard errors for sensory attributes of the various sausage and burger treatments

		Treatment*						
		M		TM		TE		
Cooking procedure		Frying	Oven	Frying	Oven	Frying	Oven	S.E.
Independent variables								
Sausage	Colour	5.10[a]	4.24[a]	5.56[a]	5.06[a]	5.21[a]	4.97[a]	±0.49
	Flavour	4.88[b]	4.88[b]	4.64[b]	5.15[b]	4.97[b]	5.18[b]	±0.57
	Tenderness	4.94[c]	4.61[c]	5.33[c]	5.24[c]	5.51[c]	7.18[c]	±0.56
	Juiciness	4.94[d]	4.70[d]	5.49[d]	5.18[d]	5.44[d]	5.21[d]	±0.27
	Overall acceptability	4.92[e]	4.61[e]	5.26[e]	5.16[e]	5.27[e]	5.64[e]	±0.47
Burger	Colour	5.24[a]	4.88[a]	5.33[a]	4.69[a]	5.42[a]	4.21[a]	±0.43
	Flavour	4.21[b]	4.88[b]	5.12[b]	4.54[b]	5.18[b]	3.92[b]	±0.54
	Tenderness	4.66[c]	5.33[c]	5.03[c]	4.21[c]	5.21[c]	4.18[c]	±50
	Juiciness	5.52[d]	5.12[d]	4.57[d]	4.79[d]	5.03[d]	4.91[d]	±0.58
	Overall acceptability	4.91[e]	5.10[e]	5.01[e]	4.56[e]	5.21[e]	4.31[e]	±0.51

[abcd]Means in the same row bearing similar superscripts are not significantly different ($p>0.05$)

workers are cautioned to chill hot-processed meat promptly to avoid excessive time at temperatures that would allow for microbial proliferation. Studies indicate that the bacterial flora of hot microbial proliferation. Studies indicate that the bacterial flora of hot-processed and/or electrically-stimulated meat is not significantly different from that of conventionally-processed meat (Lee *et al*., 1982; Oblinger, 1982). The levels of contamination of hot- and cold-boned beef were similar after cutting. Count of total viable bacteria on the hot-boned beef increased slightly during the 24 h storage period at 10°C, but at the start of storage at 1°C they were similar on hot- and cold-boned joints. After three weeks storage at 1°C, total viable counts were 10-1,000 times higher on hot-boned beef, but counts were similar on hot- and cold-boned beef after 8 weeks storage.

The surface of a beef carcass may carry between 10^2 and 10^4 bacteria/cm^2 and after butchering, joints and pieces of meat for packing are likely to carry considerably higher numbers (Taylor, 1985). During the freezing process, the main effect is on water activity. The aqueous portion of meat remains in its liquid phase until reaching its freezing point at some temperatures below 0°C (Golden and Arroyo-Gallyoun, 1997).

Sausage and burger products of the study were investigated for detection of *Staphylococcus aureus*, *Salmonella* spp., yeasts and molds immediately after processing and after freezing storage at -18°C for 5 weeks. Table 5 indicate that all species of microbes were lightly present at the beginning of storage (zero week) compared with the end of storage (5 week). Aberle *et al*. (2001) reported that the bacterium grows over a temperature range of approximately 7-45°C and a pH of 4.0-9.8, growth rate and toxin production are most rapid above 20°C and in foods having little acidity.

Staphylococcus aureus organisms are quite easily destroyed by heat (66°C for 12 min), but destruction of the enterotoxin requires severe heat treatment (121°C for 30 min) and they reported about Salmonellosis is a food infection resulting from ingestion of any one of numerous species of living *salmonella* organisms.

Salmonellosis continues to prevail as a food borne disease in the world. Raw or improperly cooked meat products are frequently implicated (Bryan, 1980; Carpenter *et al*., 1966; Ewen, 1978). The incidence of *Salmonella* varies widely between and within countries (Guinee and Valkenburg, 1975; Hurt *et al*., 1985). Conflicting reports on the prevalence of this microorganism likely depends on the specimen examined, the food type and the method of analysis. Data on the incidence of *Salmonella* in meat products, such as beef casings and the very popular pasturma

Table 4: Means and standard errors for total bacterial count and coliform count (Log_{10} cfu/g) of the various treatments

		Treatment*						
		---------- M ----------		-------- TM --------		-------- TE ---------		
Storage (weeks)		0	5	0	5	0	5	S.E.
Independent variables								
Sausage	Total bacterial count (Log_{10} cfu/g)	7.17^{a}	6.48^{a}	7.15^{a}	7.21^{a}	7.27^{a}	7.18^{a}	±0.22
	Coliform count (Log_{10} cfu/g)	6.29^{b}	1.77^{b}	6.55^{b}	3.76^{b}	6.58^{b}	2.30^{b}	±1.45
Burger	Total bacterial count (Log_{10} cfu/g)	7.03^{a}	6.59^{a}	7.19^{a}	7.09^{a}	7.26^{a}	7.10^{a}	±0.21
	Coliform count (Log_{10} cfu/g)	6.29^{b}	1.77^{b}	6.55^{b}	3.77^{b}	6.58^{b}	2.30^{b}	±1.12

^{abcd}Means in the same row bearing similar superscripts are not significantly different (p>0.05)

Table 5: The detection of microorganisms in sausage and burger treatments.

			Treatment*					
			--------- M ---------		--------- TM ---------		---------- TE ----------	
Storage (weeks)			0	5	0	5	0	5
Independent variables								
Sausage	R_1	Staphylococcus	-ve	-ve	+ve	+ve	+ve	+ve
		Salmonella spp.	-ve	-ve	+ve	-ve	+ve	-ve
		Yeast	-ve	+ve	-ve	+ve	+ve	+ve
		Mold	-ve	+ve	+ve	+ve	+ve	+ve
	R_2	Staphylococcus	-ve	+ve	+ve	+ve	+ve	+ve
		Salmonella spp.	+ve	-ve	+ve	-ve	-ve	-ve
		Yeast	+ve	-ve	+ve	-ve	+ve	-ve
		Mold	-ve	-ve	+ve	-ve	+ve	-ve
	R_3	Staphylococcus	+ve	+ve	+ve	+ve	+ve	+ve
		Salmonella spp.	+ve	-ve	-ve	+ve	-ve	+ve
		Yeast	-ve	+ve	-ve	+ve	-ve	+ve
		Mold	+ve	+ve	+ve	+ve	+ve	+ve
Burger	R_1	Staphylococcus	-ve	-ve	+ve	+ve	+ve	+ve
		Salmonella spp.	-ve	-ve	-ve	-ve	-ve	-ve
		Yeast	-ve	+ve	-ve	+ve	+ve	+ve
		Mold	-ve	+ve	+ve	+ve	+ve	+ve
	R_2	Staphylococcus	-ve	+ve	+ve	+ve	-ve	+ve
		Salmonella spp.	+ve	-ve	+ve	-ve	+ve	-ve
		Yeast	+ve	-ve	+ve	-ve	+ve	-ve
		Mold	-ve	-ve	+ve	-ve	+ve	-ve
	R_3	Staphylococcus	+ve	+ve	+ve	+ve	+ve	+ve
		Salmonella spp.	-ve	-ve	-ve	-ve	-ve	-ve
		Yeast	-ve	+ve	+ve	+ve	-ve	+ve
		Mold	+ve	+ve	+ve	+ve	+ve	+ve

beef sausages in Iraq are generally lacking one. Pasturma sausages are produced entirely by local butchers using different formulations and are delivered to the shops for retail sale.

There are thus, numerous opportunities for cross-contamination during processing (Abbar and Mohammad, 1989). Also reported chopped meat, spices, or the environment could also have contributed to products contamination.

Abbar and Mohammad (1989) While *Salmonella* may be present in animal tissues, a major source of infection results from cross-contamination of carcasses and meat during slaughter operations. Most cases of Salmonellosis results from cooked or prepared foods contacting raw meat or its juices. Thermal processing conditions normally used to cook meat are sufficient to destroy most species of *Salmonella*, but their resistance to heat increase as water activity decreases (Aberle *et al*., 2001).

The problem encountered in preservation of meats frequently are the same for bacteria, molds and yeasts, the exception being that yeasts and molds can grow at lower pH and need less moisture. They can frequently use nitrates as a source of nitrogen and sometimes live on dried, salted and fermented products; some are able to grow at freezing temperatures. They are destroyed by heat. Molds require oxygen and so often live on the surface of liquids. As molds and yeasts occur principally on the surface of meats, much of the contamination can usually be removed with only a little trim loss, for example, surface molds on hams rarely, if ever, make them unfit to eat. Heavy mold may be trimmed off and the only real damage remaining is in deep cracks (Levie Albert, 1979).

Conclusion: Commercial ground meats and processed meat generally consist of trimming from various cuts and thus represent peaces that have been handled

excessively. The meat grinding provides a greater surface area, which itself accounts in part for the increased flora. Meat handling and display reduce the nutrient value of meat and meat products in Sudan market. All steps of meat processing increase contamination which could result from additives, water, spices and equipment.

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Impact of Birth Weight on the Nutritional Status and Academic Performance of School Age Children

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Abstract: This study compared the nutritional status and academic performance of Low Birth Weight (LBW) and Normal Birth Weight (NBW) school-aged population in Nigeria. A total of 119 subjects were involved in this study from a purposely selected one rural and one urban school in Abia state. Pre tested and validated questionnaires were used in data collection. Academic performance was obtained from their school records, while birth weights and ages were obtained from health/immunization cards. SPSS version 15.0 was used for data analysis. The subjects comprised of 57.1% males and 42.9% females, of 9-12 years old. Low prevalence (14.3%) of LBW was found. All three indicators used for nutritional status assessment (weight for age; height for age; BMI) showed more than 50% of study population as having normal nutritional status (96.64, 74.79 and 63.03% respectively). Stunting was 10.08%, overweight 2.52% and 6.72%, while obesity was 0.84%. This study revealed a significant ($p<0.05$) influence of birth weight on nutritional status with the use of BMI and weight for age indicators. The only child found underweight (<-2SD weight for age) had a LBW. The NBW group had higher percent of normal nutritional status than LBW group (Weight for age: 98.04% Vs 88.24%, BMI 65.69% Vs 47.06%). Stunted was found among 11.77% of the LBW and 9.80% of NBW groups ($p>0.05$). The subjects' birth weight had no significant ($p>0.05$) influence on their academic performance. The findings of this study emphasize more attention to children born with LBW for improvement in their growth and academic performance.

Key words: Children, LBW, nutritional status, academic performance

INTRODUCTION

At birth, fetal weight is an important determinant of the chances of the newborn to survive and experience healthy growth and development. This is because low birth weight has been shown to be directly related to both immediate, long-term and very long-term development and well-being (Wilcox and Skaeven, 1992).

Low Birth Weight (LBW) according to WHO(2004) refers to children who at birth had a weight of 2,500 g or less, which may be caused by preterm birth, Intra Uterine Growth Retardation (IUGR) or both. These children are in a higher risk for mental retardation, sensual, cognitive and developmental defects (Manoochehr *et al*., 2009). At school age, these complications can be observed prominently in VLBW (Very Low Birth Weight = BW of 1,500 g or less) children, in the form of poor physical growth, cognitive function and school performance, which appear to persist into adulthood (Stoll, 2007). There are some studies that evaluated effect of birth weight on growth of children and most of them have reported that birth weight is a significant marker on delayed growth and short stature (Gutbrod *et al*., 2000; Ranke *et al*., 2007; Brandt *et al*., 2005; Takeuchi *et al*., 2001). The growth of children has been reported as a sensitive index of health (Tanner, 1994). Children's health is tomorrow's wealth" is one of WHO's slogans of recent years. However, children's health is to a great extent determined by factors that operate in utero, well before they are born. According to World Health Organization (WHO) and United Nations Children's Fund (UNICEF) in 2004, the prevalence of LBW in developed, developing and least developed (undeveloped) countries are 7, 16.5 and 18.6%, respectively.

However, in Nigeria, the prevalence of LBW is recorded as 12% by United Nations Standing Committee on Nutrition (SCN, 2004). Other researchers revealed LBW prevalence in different parts of country as 12% (Ngwu and Opara, 2009), 11.4% (Ngwu and Ezekiel, 2005) and 12.6% (Olowonyo *et al*., 2006). A comprehensive review (Hack, 1998) of low birth weight follow up studies of children under 18 years of age showed that the children had lower scores or higher rates of mental retardation or learning deficits than their counterparts who were born with normal birth weight. But these differences were sometimes small and not significant (Hawdon *et al*., 1990).

As many developing countries, Nigeria inclusive, have signed on to the World Education Forum declaration promising basic education for all citizens by the year

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2015, the educational impact of poor health and poor nutritional status among school children has been relatively neglected. Nevertheless, governments are making massive efforts to improve basic education, a core component for building development capacity.

However, these efforts raise urgent questions which this study aimed to address on impact of low birth weight on nutritional status and academic performance of school-aged population in Nigeria.

MATERIALS AND METHODS

The study population was purposely selected from a rural and urban school in Umuariaga and Oboro villages respectively in Ikwuano Local Government, Abia State. The subjects, 119 school aged children were simply randomized and included in the study. Sixty of the subjects (50.4%) were pupil in Michael Okpara University of Agriculture, Staff School, Umuariaga community. The remaining 49.6% were from Oboro Primary School, Oboro community.

This study was carried out from May 25-26th, 2009 with a validated questionnaire to elicit information from the subjects. Data on their anthropometric measurements and socioeconomic characteristics were collected with the use of the validated and pretested questionnaire. Data on the subjects' academic performance were obtained from their school records. Their birth weights as well as their ages were obtained from their health/immunization cards. Anthropometric measurements included height, weight, triceps skin fold thickness and mid-upper arm circumference. These indices of each subject was measured twice and the average taken. A 150 cm vertical wooden rod was used for height measurement with a tape rule fixed to the vertical wood. Height measurement was taken with the subject standing erect on bare feet (removing their shoes and stockings). The weight of the subject was taken to the nearest 0.1 kg using a portable bathroom scale (HANSON MODEL). The subjects were weighed standing erect in minimal clothing without shoes. The scales were checked daily with the same known weight to ensure it's in perfect order.

Analysis of data: The nutritional status of children are assessed using anthropometric indices; weight for age, height for age and weight for height indicating underweight, stunting and wasting respectively. However, in this study, the weight for height index was replaced with Body Mass Index (BMI) since it still reflected weight with respect to height and considering the age of the children studied. Body Mass Index (BMI) is a number calculated from a child's weight and height. BMI is a reliable indicator of body fatness for most children and teens. BMI does not measure body fat directly, but research has shown that BMI correlates to direct measures of body fat, such as underwater weighing and dual energy x-ray absorptiometry (DXA). BMI can be considered an alternative for direct measures of body fat. For children and teens, BMI is age- and sex-specific and is often referred to as BMI-for-age. The nutritional status of children was obtained from the National Center for Health Statistics/U.S. Center for Disease Control (NCHS/CDC 2000) BMI for age percentile chart for boys and girls. BMI greater than 95th percentile or 85th percentile was considered obese or overweight respectively while BMI less than 10th percentile was considered underweight (NCHS/CDC 2000).

All data were coded and entered into the SPSS (Statistical Package for Social Sciences) version 17.0. The categorical variables were presented as frequencies and percentages. The differences in mean values of height, weight and birth weight were compared between the LBW and NBW groups by ANOVA analysis. The difference among their class performance was also evaluated with ANOVA. Chi- square analysis was used to comparing their levels of nutritional status (underweight, overweight, stunted, normal) based on their birth weight. Statistical significance was set at 95% confidence interval.

RESULTS

Table 1 revealed that most of the subjects studied were males (57.1%) and majority (62.2%) of the population was within the ages of 9-12 years. Low prevalence of LBW (14.3%) was found in this study and 85.7% were of normal birth weight. The class performance indicated that only 10.1% of the children ranked top 5 in the class academic position while more (35.3% and 25.2%) ranked last 5 and last 10 position respectively in the class.

The nutritional status of these subjects is shown in Table 2. Generally, the three indicators used revealed that more than 50% of study population has normal nutritional status (96.64, 74.79 and 63.03%). However, low prevalence of stunting (10.08%), overweight (2.52%, 6.72%) and very low prevalence of obesity (0.84%) were found in this study. However, weight for age indicator showed a lower prevalence of underweight (0.84%) compared to BMI indicator (29.41%).

Table 3 revealed a significant ($p<0.05$) association of birth weight with nutritional status among the subjects studied only with BMI and weight for age indicators whereas the height for age indicator revealed non significant difference ($p>0.05$). The only child who was underweight among the weight for age indicator had a low weight at birth, while none of the NBW group was underweight. But with BMI as an indicator, same percent (29.41%) were underweight among the LBW and their

counterpart NBW subjects. The percent of normal nutritional status among subjects born with normal weight was higher than those with low birth weight (Weight for age: 98.04% Vs 88.24%, BMI 65.69% Vs 47.06%). However, for Height for age indicator, the reverse was the case but not statistically significant. LBW subjects had higher percent (82.35%) of normal nutritional status than the NBW subjects (73.53%). Stunted was found among 11.77% of the LBW and 9.80% of NBW groups ($p>0.05$). Only one subject (5.88%) among the LBW group was tall while 16.67% were found tall among the NBW group. Prevalence of overweight was higher among the LBW group compared to the NBW group with both indicators (BMI 23.53% Vs 3.92%; Weight for age 5.88 Vs 1.96% respectively).

Table 4 indicated no significant differences ($p>0.05$) in the class performance between the LBW and NBW subjects. The LBW subjects had 11.77% among the first 5 academic position and 29.41% among the last 5 position in class. Among the NBW group, 9.8% took the first 5 academic position and 36.28% the last 5 position in class. Higher percent took the first 10 position among the LBW group (35.29%) compared to the NBW group (28.43%).

Table 1: Characteristics of the subjects

Characteristics	Attributes	Frequency	Percentage
Sex	Male	68	57.1
	Female	51	42.9
Age range (years)	6-8	45	37.8
	9-12	74	62.2
Birth weight	LBW (<2.5 kg)	17	14.3
	NBW (>2.5 kg)	102	85.7
Class performance	First 5	12	10.1
	First 10	35	29.4
	Last 10	30	25.2
	Last 5	42	35.3

LBW = Low Birth Weight, NBW = Normal Birth Weight

Table 2: Nutritional status of the subjects

Indicator	Attributes	Frequency	Percentage
Weight for age	Underweight	1	0.84
	Normal	115	96.64
	Overweight	3	2.52
	Total	119	100.00
Height for age	Stunted	12	10.08
	Normal	89	74.79
	Tall	18	15.30
	Total	119	100.00
BMI	Underweight	35	29.41
	Normal	75	63.03
	Overweight	8	6.72
	Obese	1	0.84
	Total	119	100.00

Table 3: Nutritional status of subjects according to their birth weight

		LBW		NBW		Total	
Indicator	Attributes	Frequency	%	Frequency	%	Frequency	%
Weight for age	Underweight	1	5.88	0.0	0.00	1	0.84
	Normal	15	88.24	100.0	98.04	115	96.64
	Overweight	1	5.88	2.0	1.96	3	2.52
	Total	17	100.00	102.0	100.00	119	100.00
	$Chi^2 = 6.96$, df = 2, p = 0.031						
Height for age	Stunted	2	11.77	10.0	9.80	12	10.08
	Normal	14	82.35	75.0	73.53	89	74.79
	Tall	1	5.88	17.0	16.67	18	15.13
	Total	17	100.00	102.0	100.00	119	100.00
	$Chi^2 = 5.139$, df = 2, p = 0.162						
BMI	Underweight	5	29.41	30.0	29.41	35	29.41
	Normal	8	47.06	67.0	65.69	75	63.03
	Overweight	4	23.53	4.0	3.92	8	6.72
	Obese	0	0.00	1.0	0.98	1	0.84
	Total	17	100.00	102.0	100.00	119	100.00
	$Chi^2 = 9.302$, df = 3, p = 0.026						

LBW = Low Birth Weight; NBW = Normal Birth Weight

Table 4: Relationship between academic performance and birth weight of the subjects

	Academic performance					
	LBW		NBW		Total	
Academic position	Frequency	%	Frequency	%	Frequency	%
First 5	2	11.77	10	9.80	12	10.08
First 10	6	35.29	29	28.43	35	29.41
Last 10	4	23.53	26	25.49	30	25.21
Last 5	5	29.41	37	36.28	42	35.30
Total	17	100.00	102	100.00	119	100.00
$Chi^2 = 0.506$, p = 0.918						

DISCUSSION

The observed prevalence of LBW (14.3%) among these school aged population (6-12 years) in this study is similar to the report of 16.5% prevalence in developing countries (WHO, 2004). In Nigeria, Dawodu and Laditan (1985) reported a prevalence of 8.2% low birth weight. The advances in newborn medical care have greatly reduced the number of deaths associated with low birth weight but Pryor *et al.* (1995) reported that a small percentage of survivors develop mental retardation, learning problems, cerebral palsy and vision and hearing loss.

However, this study found no statistical difference ($p>0.05$) in academic performance between the children born with LBW and their counterpart with NBW. This finding is in agreement with an earlier study by Tong *et al.* (2006). These researchers found a statistically significant association between birth weight and cognitive performance at age 2 years but the magnitude of this association gradually decreased and became statistically non-significant at later childhood.

Another researcher, Stoll (2007) reported that at school age, the effects of LBW in the forms of poor cognitive function and school performance can be observed prominently only in VLBW (Very Low Birth Weight = BW of 1,500 g or less) children. However, our study did not obtain information on VLBW.

The class performance of the general study population indicated that only 10.1% of the children ranked top 5 in the class academic position while more (35.3% and 25.2%) ranked last 5 and last 10 position respectively in the class. The finding of low academic performance of these study population could be attributed to other factors rather than nutritional status and birth weight. This is because majority of the subjects had normal nutritional status, significantly influenced by their birth weight. And their birth weight had no statistical significance on their academic performance in class. The lack of association between LBW and school performance could be because of the small LBW sample size in our study. This study found significant relationship between birth weight and nutritional status ($p<0.05$). The significance was observed in both BMI and weight for age indicators. These results are similar to the findings of Emond *et al.* (2006), Elgen *et al.* (2005), Manoochehr *et al.* (2009), Peng *et al.* (2005), Cooke and Foulder-Hughes (2003). However, the height for age indicator applied in this study revealed non significant difference between birth weight and nutritional status ($p>0.05$). This finding was in contrast to the study of Manoochehr *et al.* (2009) were significant difference was observed in the ratio of height to standard height for age between LBW and NBW.

The higher prevalence of overweight among the LBW groups may be attributed to the catch up growth in the subjects. Children who experience catch up growth are often seen to be heavier than those who did not. The low prevalence of stunting may be an indicator of past insults in the nutritional status of the children. The found no significant influence of birth weight on stunting suggests that birth weight has no effect on children's height.

Conclusion: This study found significant influence of birth weight on childhood growth. We recommend more attention to be paid to nutritional status of children born with LBW for improving growth and academic performance of children in our society.

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Study of the Bioavailability and Clinical Studies of Calcium-Magnesium Tablets

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Abstract: The bioavailability of the trace elements selenium, manganese, zinc, iron etc. has been investigated using human and experimental animals as the test observations. Twenty volunteers were used for this study. All volunteers have a deficient in calcium and magnesium. All patients were administered orally a tablet of combined calcium carbonate 292.5 mg and magnesium carbonate 232.3 mg produced by Commercial Company, Jordan. The mean concentration of blood calcium and magnesium after one week of tablet supplement was 100.8±8.3 and 15.7±1.0, respectively. The results showed that calcium-magnesium tablet is the most important elements to recover patient's deficiency. It is thus concluded that combined calcium-magnesium carbonate are the best form for those elements for people deficiency.

Key words: Calcium, magnesium, supplement, bioavailability

INTRODUCTION

A therapeutic or prophylactic approach to osteoporosis, or to suppression of decrease in bone mass, is calcium supplementation (Proposed Diagnostic Criteria for Osteoporosis, 1993). The underlying mechanism is generally thought to consist in the suppression of parathyroid hormone secretion (Recker, 1981). Recently, stress is laid particularly on the importance of a well-balanced supply of calcium and magnesium rather than simple calcium intake (Esashi, 1992).

In their epidemiological study on the relationship of cardiac disorders to calcium/magnesium intake ratio in 1940, Karppanen *et al.* (1978) pointed out that the number of patients with cardiac disorder was prone to increase with rising calcium/magnesium ratio. Additionally, cardiac disorders were found to be of the highest in incidence in Finland where the intake ratio exceeded 4:1.

It has also been demonstrated by Seelig (1982) in a balance test with a daily magnesium intake of 350 mg and a progressively increasing daily calcium intake of 200 to 1,400 mg that urinary magnesium excretion increased with increasing calcium intake, leading eventually to a negative balance with excessive magnesium excretion over its actual intake. The nutritional requirement for calcium is 600 mg a day and the recommended daily magnesium intake is 300 mg in Japan. It may thus be said that a calcium-magnesium intake ratio of 2:1 is advisable for Japanese (Itokawa, 1990).

Ryukyuan coral is a dietary material approved as a food additive that contains calcium and magnesium in an approximate ratio of 2:1, with their contents of 20 and 10%, respectively. Under the view that it is justified to add this foodstuff to the so-called nutritionally well-balanced foods which satisfy the mineral balance, some researchers were incorporated coral powder into inexpensive, light, tasty crackers. This foodstuff was incorporated into crackers to permit a well-balanced mineral intake of about half the daily requirements of calcium and magnesium (i.e., 300 mg calcium and 150 mg magnesium) by the daily ingestion of 4 crackers (per box) as a snack.

This study was undertaken to evaluate in humans whether mean intestinal absorption of coral-derived calcium incorporated into crackers might be comparable or even superior to mean intestinal absorption of calcium carbonate-derived calcium in crackers.

Magnesium is an essential trace element for humans and animals. The recommended daily allowance is mg of mg per day in diet of a human adult.

There are many different forms of trace elements in supplements for human consumption that are available in the market place. Inorganic salts such as sulphates and carbonates are the most commonly used forms as well as they are the cheapest formula. Also the organic salts such as citrates and gluconates are common. Another form which is thought to be more utilized by the body are the amino acid chelates. These are usually formed by hydrolysis of protein and reaction of the resulting amino acid with an inorganic salt to supposedly form a chelate of the metal with the ligand amino acids.

A third form, yeast, is produced by growing yeast in a nutrient medium containing the inorganic salt. In theory, the yeast absorbs the element by forming a natural chelate between the metal ion and the proteins and/or amino acids of the yeast.

With the advent of atomic absorption spectroscopy twenty years ago, there has been large body of literature on the concentration of trace elements in foods and physiological fluids. While laboratory analysis can

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determine how much of a given material is present in a food, only a well designed bioassay can provide information about the bioavailability of that material. In nutrition, a bioassay is especially important in dealing with essential trace elements because many factors influence their utilization. Among these are digestibility of the carrier food, chemical and physical form of the element, the body's own need for that element and interaction with other nutrients and the role of chelating. Bioavailability was defined by Fritz (1976) as the ration between the qualities of a nutrient in a sample as determined by animal assay to the quantity determined by chemical analysis. There are several methods to determine bioavailability of trace elements. These include:

1) Balance studies with either man or laboratory animals
2) Radiotracer techniques, or
3) Serum metal response following test doses

Although radiotracer studies with mice or animals are the easiest to perform, the results may be criticized because the spiked radiotracer may not behave in the same way as the non-spiked element being tested. This is the case when the form of the element being tested is different to that of the spiked tracer. Animal studies offer the best method of determining bioavailability of a trace element. The Association of Official Analytical Chemists (1975) has adopted a method for measuring bioavailability of iron based on repletion of hemoglobin in anemic rats. In this official method a reference standard is used and other forms of the element compared to it.

MATERIALS AND METHODS

Blood protocol: Twenty (20) volunteers (15 males and 5 females) their ages 30 years, body weight 67 kg and height 169 cm, participated in this study (Table 1). None of the subjects had a history of bone disease, peptic ulcer, enterectomy, regional enteritis, malabsorption, nephrolithiasis, liver cirrhosis, or renal disorder. Subjects had not taken Ca or Mg or Vitamins, or any other drugs that could affect calcium or magnesium metabolism during the week preceding the start of the study. After a subsequent 3 days wash out period. All volunteers have calcium and magnesium element deficiency in their blood. All volunteers were taken for one week a single dose of Calcium carbonate 292.5 mg + Magnesium carbonate 232.3 mg tablet as 67 mg Mg and 117 mg Ca produced by Commercial Company was administered orally to all volunteers which have deficient in both calcium and magnesium level, the supplementation of Cal-Mag. tablet was taken for one week to reduce the deficiency of this element in their blood. Each subject appeared on the experimental day after an overnight fast. The tablets were taken orally with 200 ml of water in the form of Cal-Mag carbonate.

Table 1: Characteristics of the volunteers

Subject	Sex	Age\years	Height\cm	Weight\kg
1	M	23	170	68
2	M	24	165	65
3	M	26	173	63
4	M	29	166	70
5	M	20	174	80
6	M	35	172	78
7	M	32	171	74
8	M	27	167	75
9	M	33	169	73
10	M	29	173	84
11	M	30	168	72
12	M	40	174	69
13	M	24	160	58
14	M	23	164	57
15	F	24	166	60
16	F	26	168	55
17	F	29	170	59
18	F	30	161	60
19	F	32	165	58
20	F	34	165	60
Mean		28.5	168.5	66.90
±SD		4.94	4.11	8.59

Blood samples were collected from all volunteers before treatment and after treatment. Blood (3 ml) was taken by means of intravenous sample. Blood samples were collected in polyethylene test tubes one week before treatment, as well as the blood were collected after 7 days intervals of oral administration after treatment. The samples were centrifuged and the plasma layer was kept frozen until analysis. The experiment was repeated 1 week later with the same volunteers and given the tablets from the same batch of tablets. The blood was analyzed by atomic absorption spectroscopy (AAS 1260, Schimatzu, Japan). Statistics were done using a t-test.

RESULTS AND DISCUSSION

The results of the Cal-Mag. supplementation study are shown in Table 2 and 3. The results showed that the differences of calcium and magnesium in serum after treatment was highly significant than the level before the treatment by about 47% and 33% respectively. The volunteers were recovered some of their deficiency from 68.7-100.7 ppm for calcium throughout one week of supplementation treatment, while the level of magnesium increased from 11.7-15.6 ppm (Tables 2 and 3).

We probably need more Calcium and Magnesium than any other vitamin or mineral because they are the primary building blocks of bones, teeth and muscles. Many foods contain these valuable minerals, especially calcium, so many people supplement their diets with Calcium...yet Calcium remains high on the list of minerals in which people are deficient. It is not how much Calcium and Magnesium you obtain from the foods you eat and supplements you take, but rather how much your body absorbs and utilizes. Very simply, Calcium is difficult to absorb for most people. Life Plus

Table 2: Concentrations of Magnesium (ug\ml) in serum volunteers before and after treatment of calcium magnesium tablets

No. of volunteers	Before treatment ug\ml	After treatment ug\ml
1	10.12	14.50
2	11.34	14.67
3	10.45	14.00
4	11.04	15.45
5	12.75	15.85
6	10.56	14.60
7	11.67	16.35
8	10.79	15.25
9	13.00	16.00
10	12.50	15.80
11	13.00	16.75
12	13.25	17.25
13	12.65	15.80
14	11.85	15.00
15	10.60	14.50
16	10.30	14.60
17	12.48	16.15
18	11.74	15.88
19	11.56	16.24
20	13.25	18.15
Mean	11.745	15.6395
±SD	1.064	1.0393

Table 3: Concentrations of Calcium (ug\ml) in serum volunteers before and after treatment of calcium magnesium tablets

No. of volunteers	Before treatment ug\ml	After treatment ug\ml
1	75.80	105.85
2	65.98	95.35
3	70.45	98.25
4	69.15	97.65
5	65.65	93.68
6	77.14	110.75
7	82.70	120.12
8	85.13	117.45
9	60.60	92.34
10	67.45	98.15
11	64.50	94.60
12	71.56	100.00
13	73.25	106.35
14	55.70	90.55
15	59.80	92.80
16	58.00	93.75
17	63.00	99.10
18	74.15	108.35
19	64.76	102.50
20	68.35	97.80
Mean	68.656	100.7695
±SD	7.81951	8.30605

understands that the name of the game is absorption and utilization; therefore, Cal-Mag PLUS is more than just a straight Calcium supplement. It includes an abundant amount of Magnesium and other specific nutrients your body needs in order to absorb and utilize both Calcium and Magnesium to its fullest potential.

Your body needs adequate amounts of Magnesium in order to absorb calcium and it also needs adequate amounts of Calcium to utilize magnesium. Vitamin D-3 is also included because it is necessary for both the absorption and utilization of Calcium and Calcium is needed to utilize Vitamin D-3. Any calcium supplement that does not contain calcium, Magnesium and Vitamin D is simply not of much value in terms of absorption and utilization in the body (Itokawa, 1990; The Declaration of Helsinki, 1988).

Many Calcium supplements contain only inexpensive, inorganic forms of Calcium, such as oxides or carbonates. In general, most of these products are not worthwhile because most of the Calcium is not absorbed. This is why Life plus Cal-Mag PLUS contains numerous sources of Calcium and Magnesium including citrates, chelates, aspartates, gluconates, ascorbates, glycerophosphates and lactates (Deborah and Canyon, 2007; Hanzlik *et al*., 2005; Pak *et al*., 1987). Individuals who are allergic to dairy products (a major source of Calcium in the American diet) can easily become deficient in Calcium and may need Cal-Mag PLUS to make up for the loss of this source of Calcium. During pregnancy, mothers need a great deal of calcium and magnesium to ensure the proper development of the fetal bones, muscles and teeth and to ensure that the mother's own mineral stores are not depleted. For the same reason, lactating mothers should continue to supplement their diets with Cal-Mag PLUS. Growing children need extra calcium in their diets to ensure continuing development of muscles, bones and teeth. If they maintain proper levels of calcium, they will have less tendency toward cavities and broken bones. Elderly people find it increasingly difficult to absorb calcium and in general don't seem to get enough magnesium. They often develop osteoporosis, a condition where calcium erodes from the bones causing them to become weak and brittle. Early and continuing calcium supplementation can be helpful in prevention of osteoporosis (Janet *et al*., 2005; Birge *et al*., 1969).

Directions: Five tablets twice a day-supply 1,000 mg of Calcium and 500 mg of Magnesium, plus other synergistic nutrients for optimal utilization.

This unique product contains PolyCalPlex™ and PolyMagPlex™ proprietary blends of high quality Calcium and Magnesium sources. Cal-Mag PLUS also contains Betaine HCl and L-Glutamic Acid to enhance Calcium absorption, plus Alfalfa, Kelp and Dulse as sources of trace elements synergistic with Calcium and Magnesium. Cal-Mag PLUS is formulated in the exclusive PhytoZyme™ base of plant enzymes for bioavailability and over 30 synergistic fruit, vegetable and herbal concentrates for "extra" phytonutrient cofactors (Van Dokkum *et al*., 1996; Suzuki *et al*., 1997). It contains no artificial preservatives, sugar, starch, caffeine, salt, wheat, gluten, yeast, corn, milk, egg, shellfish, soya derivatives, artificial sweetening, flavoring or coloring agents.

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Nutritional Status of Newly Enrolled Primary School Children in Jos-Plateau, Nigeria

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Abstract: Nutritional assessment of newly enrolled school children is needful to identify children with poor nutritional status. Poor nutrition as evidenced by poor growth and small stature could affect development, intellectual performance and intellectual achievement. Poor nutrition in school aged children is also likely to negatively affect their participation in normal school activities. Seven hundred and sixty four apparently healthy newly enrolled pupils were randomly selected using a multi stage proportionate sampling from both public and private schools. Their weights and heights were measured using standard methods. Undernutrition was determined using Z scores less than - 2 standard deviations of the NCHS/WHO international reference standard. Pupils from private schools were significantly taller (118.2±6.52) than their public school counterparts (115.7±8.44), p = 001. The prevalence of underweight, stunting and wasting was 10.3, 11.1 and 2.4% respectively. Stunting occurred in a higher proportion of boys than girls. Poor nutritional status was significantly commoner in public school pupils compared with private school pupils. These findings suggest that malnutrition (underweight, wasting and stunting) is not uncommon among newly enrolled school children and it underscores the need for institution and sustenance of a food program among school children.

Key words: Nutritional status, school feeding, anthropometric measure, Jos

INTRODUCTION

The nutritional status of children is an important determinant of child health (Okolo *et al*., 2003). Its assessment in groups of children is necessary in monitoring the health of a community, planning and implementing programmes to reduce malnutrition associated morbidity and mortality (Rabasa *et al*., 1998; Osibogun, 1998). Until recently, the focus of nutritional status assessment has been in children considered more at risk of malnutrition. This group of children are under-five years of age and are generally referred to as pre-school children (World Food Programme, 2002). In the developing world (especially in Sub-Saharan Africa and South Asia) there is widespread undernutrition in this group of children with attendant high under-five mortality rates (WHO, 2005).

In Nigeria, the recommended age for school entry is six years (Federal Ministry of Information, 1981), which is just a year after the risk period of undernutrition. So, many children beginning school are survivors in an environment of very high under-five mortality rates resulting from both micro and macro-nutrition deficiencies and multiple infections that plagued them in early childhood (Oduntan, 1973). Late effects of poor nutrition such as poor growth and small stature have been associated with impaired development and poor intellectual performance (Abidoye *et al*., 1991; Pollitt *et al*., 1993). Also, a relationship between growth status with school performance and intellectual achievement has been documented (Martorell *et al*., 1992). The implication of this is poor nutrition in school aged children would most likely affect their learning and participation in normal school activities.

Primary school children form a sizeable proportion of our population (Federal Ministry of Health, 1991). So it is important to assess nutritional status of not only pre-school children but also school aged children especially at enrolment into primary school. At the point of school entry, simple anthropometric measurement (to determine their nutritional status) would help identify those with poor nutritional status. To the best of our knowledge, there is a paucity of information on nutritional status of school children at the point of school entry. A nutritional status assessment of these school children when they start school will help in detecting those with various forms of malnutrition or the late effects of malnutrition. Ideally every child should undergo routine medical examination while at school-first at school entry, midway through school and at completion of school (Akani and Nkanginieme, 1999). This can be carried out by teachers and school nurses through the school health programme. Those with malnutrition can be easily identified, evaluated and referred for appropriate treatment. Thus nutritional status assessment of school children, at the start of school, can serve as a screening tool to identify children who may need nutritional intervention and so prevent further

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deterioration in their nutritional status and reducing the risk of poor performance in school.

This study was therefore designed to assess the nutritional status of new primary school children at the point of school entry in Jos, North Central Nigeria, in order to ascertain the burden of the problem in this locality.

MATERIALS AND METHODS

This was a descriptive cross-sectional study carried out in Jos North Local Government Area (LGA) of Plateau State, Nigeria between October 2002 and May 2003. There are twenty three public and forty six private primary schools in the town. Children from the lower social class usually attend the free government public schools and such schools are heavily populated. In contrast, children from the middle and upper social classes attend the fee paying private schools run either by religious bodies or private individuals and have a lower population density per class and per school compared to public schools. Ethical clearance was obtained from the ethical committee of the Jos University Teaching Hospital and permission was obtained from the Local Government Education Commission. Written informed and signed consent was obtained from the parents/guardians of children.

The subjects were newly enrolled primary school entrants who were apparently healthy asymptomatic children. During the academic year 2002/2003 8,380 children were newly enrolled in public schools in Jos North LGA and 3,872 children were in private schools giving a total of 12,242 newly enrolled pupils. A sample size of 764 was calculated using a standard formula (Oyejide, 1989). Through a multistage stratified randomization procedure and proportionate sampling, 519 pupils were selected from public schools and 245 pupils were selected from private schools.

The pupils had a complete physical examination in the morning and their anthropometric measurements taken. Height was measured using an ACCUSTAT™ ROSS stadiometer as described by Paynter and Parkin, 1991. The measurements were recorded to the nearest 0.1 cm. The weight of the pupils was measured after the height using a Weylux scale, which has an accuracy of 0.5 kg (Paynter and Parkin, 1991). The weight was measured to the nearest 0.1 kg. The measurement was done with pupils standing bare footed and with light underclothes only. The scale was periodically calibrated to ensure accuracy using standard weights.

Socio-economic status of the subjects was calculated using the socio-economic indices of the parents (fathers' occupation and mothers' level of education) as described by Olusanya *et al.* (1985). Father's occupation was scored 1 for professional, top civil servant, politician or businessman; 2 for middle level bureaucrat, technician, skilled artisan, well to do trader and scored 3 if an unskilled worker or income is below national minimum wage. Mother's educational status was scored as 0 if up to university level, 1 if up to secondary or tertiary level below university e.g college of education, school of nursing and 2 if no schooling. The total score of both parents gives the socioeconomic index of the child. Score of 1-2 is Upper social class, 3 is middle class and 4-5 is lower social class.

Percentile curves were constructed for the weight-for-age and height-for-age for subjects and compared with standard age and sex specific National Centre for Health Statistics (NCHS) growth charts, the international reference recommended by the World Health Organisation (WHO) then. The percentile curve for the NCHS were generated using the Cole's LMS method (Kuczmarski *et al.*, 2000).

Data analysis: Data obtained were analyzed using the EPI Info 2000 1.1.2a statistical software. Mean values and standard deviations expressed as means ± Standard Deviation (SD) of age, weight and height were calculated.

The student t test was used to compare means of variables while the chi-square was used to test for significance of association. P values <0.05 was considered significant.

RESULTS

A total of seven hundred and sixty four pupils (764) were studied, majority (68.1%) of whom attended public primary schools while 244 (31.9%) attended private primary schools. Three hundred and fifty six (356) were males while four hundred and eight (408) were females, giving a male to female ratio of 0.9:1. Majority (93%) of pupils in the public schools were from low socio-economic class while the private schools (95%) had pupils from the middle and high socio-economic class. Their ages ranged from 5 to 12 years with a mean age of 6.7±1.3 years. Although pupils from private schools were younger than those from public schools, the mean weight and height were greater in the former when compared with the latter and this was significantly so for height p = 0.0001 (Table 1). The mean age, weight and height measurements were not significantly different when compared by gender (p>0.05).

Table 1: Comparison of mean age, weight and height of newly enrolled private and public school pupils

Parameters	Private school	Public school	p-value
Age (years)			
Range	5-11	5-12	
Mean±SD	6.3±0.98	6.8±1.43	0.01
Weight (kg)			
Range	12-43	12-35	
Mean±SD	20.4±3.24	20.1±3.71	0.2
Height (cm)			
Range	99-145	95-142	
Mean±SD	118.2±6.52	115.7±8.44	0.001

Table 2: Prevalence of malnutrition of newly enrolled pupils

Nutritional indices	Z-scores	Type of school		Total
		Private	Public	
Underweight (W/A)	< - 2SD	14 (1.8)	65 (8.5)	79 (10.3)
Stunting (H/A)	< - 2SD	9 (1.2)	76 (9.9)	85 (11.1)
Wasting (W/H)	< - 2SD	8 (1.0)	10 (1.3)	18 (2.4)
Both stunting and wasting	< - 2SD	3 (0.4)	31 (4.1)	44 (5.8)

*Figures in parenthesis are percentages

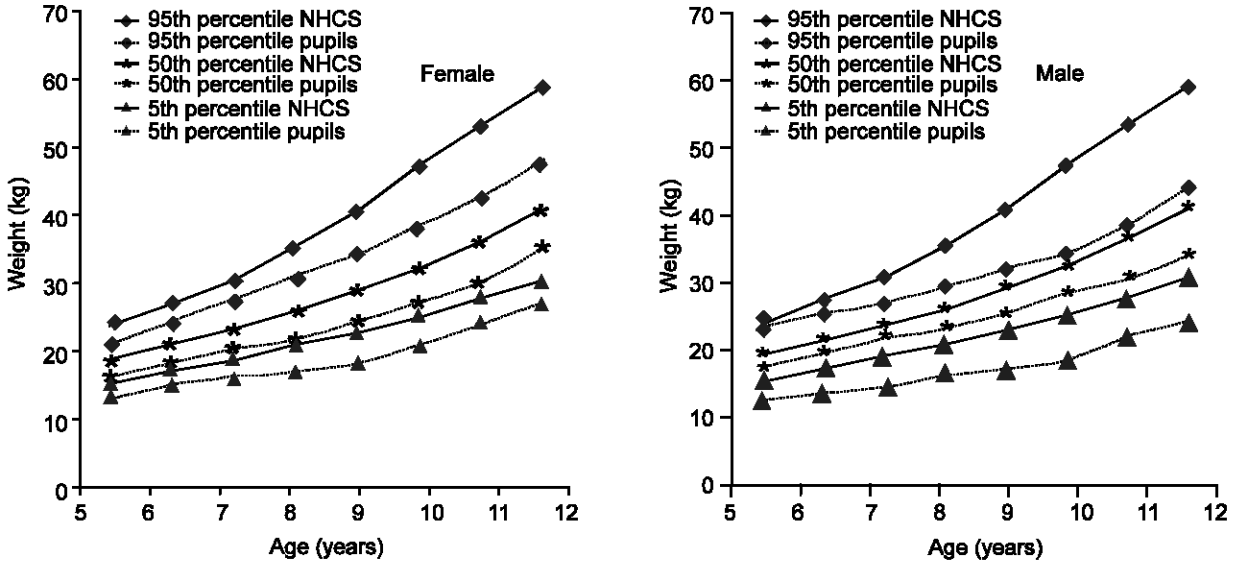

Fig. 1: Weight for age percentile curves of pupils compared to age and gender specific NCHS percentiles

The measured height and weight were used to assess the nutritional status of the pupils by using Z scores less than - 2 standard deviations below the mean weight for age (underweight), weight for height (wasting) and height for age (stunting) on the NCHS/WHO international reference standard. Prevalence of underweight, stunting and wasting was 10.3, 11.1 and 2.4% respectively (Table 2). On the whole nutritional indices were worse off in pupils from public schools, significantly so for underweight and stunting. A higher proportion (58%) of the stunted pupils was boys while there was no gender difference for underweight malnutrition. Stunting and wasting occurring together was recorded in 5.8% of the study population.

Weight and height percentile curves plotted for the study population and compared to age and sex specific NCHS reference standard, showed the study subjects had much lower values of all measurements (Fig. 1 and 2).

DISCUSSION

Our study assessed the nutritional status of apparently healthy newly enrolled primary school children in an urban Nigerian city. The mean age of new primary school entrants was 6.6 years with a wide range of 5 to 12 years. The mean age of the subjects is comparable to the recommended national policy and suggested age of school readiness (Federal Ministry of Information 1981 and Ikefuna and Iloeje, 2002). However the wide age range including older children of up to 12 years negates this recommendation. Although we did not search for reasons for late enrolment of the older children it may not be unconnected with previous undernutrition for example stunting, wasting and small size of child delaying start of school. Parents may deem shorter children to be younger and not physically large enough to attend school (Jukes *et al*., 2002). There is also the practice of late enrolment of house helps by more affluent families in our setting.

This study found that male subjects were heavier and taller than their female counterparts increasingly until 9 to 10 years when the trend changes with females being heavier and taller than males. This change in the trend could be explained by the pre-pubertal growth spurt that occurs earlier in females than males (Nkanginieme, 1999). The prevalence of underweight, stunting and wasting in these apparently healthy school children were low compared to the national rates of 27, 46 and 12% in under-five children (UNICEF, 2003) Recent reports from neighboring North Western state of Kaduna showed that less than 32% of children were severely stunted which

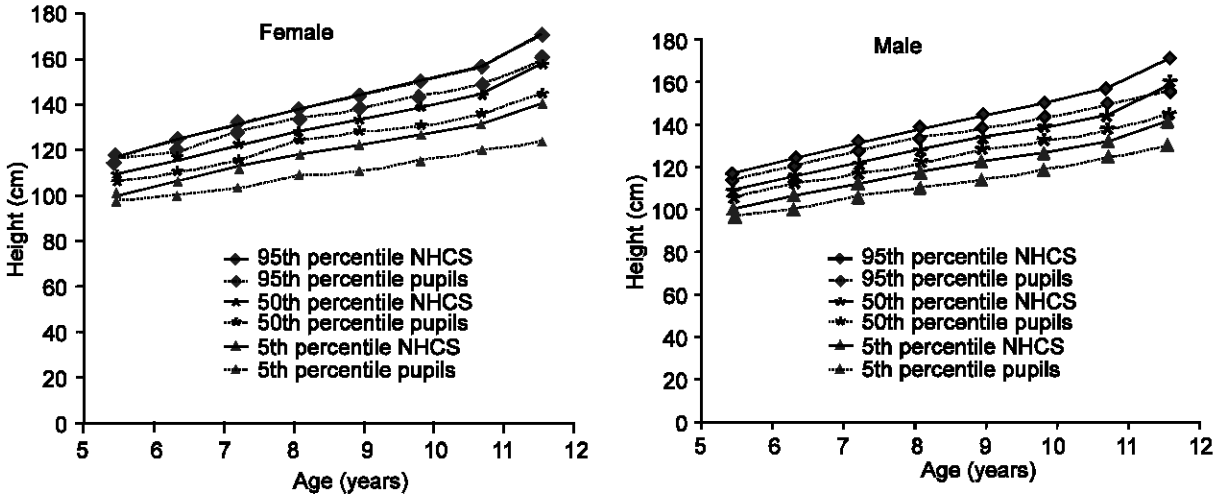

Fig. 2: Height for age percentile curves of pupils compared to age and gender specific NCHS percentiles

is lower than the national average (Matthew *et al*., 2009), although this was in younger children. Our study also had a lower prevalence probably because they were apparently healthy older children; however the pattern of stunting being the commonest form of poor nutrition and wasting the least is similar to the national pattern.

Stunting occurring more in male pupils is consistent with several studies of childhood malnutrition in sub-Saharan Africa. In a meta-analysis of 16 demographic and health surveys in this region it was shown that male children under five years of age are more likely to become stunted than female children (Wamani *et al*., 2007). There were two demographic and health surveys in Nigeria in 2003 and 2008 but these surveys are unable to capture the important issue of malnutrition among school going children as only under five years children are surveyed. Our study investigated children aged 5 to 12 years with majority in the 5-6 year old range. The fact that stunting which indicates past or chronic malnutrition was still prevalent in the study group would suggest that our school children are likely survivors of past malnutrition and so possibly suffering from the late effects of chronic malnutrition (Oduntan, 1973). The occurrence of both stunting and wasting in some of the school children indicates an on-going acute on chronic malnutrition process.

The most significant finding in this study was the occurrence of underweight and stunting in a higher proportion of public school pupils compared to private school pupils. Pupils from public schools had poorer socioeconomic background and individuals from this socioeconomic class are least nourished (Amuta and Houmsou, 2009). This also explains the rather low percentiles observed among the study population when their weight and height percentile curves were compared to corresponding age and sex specific percentiles of the NCHS reference population. The median values for 5^{th}, 50^{th}, 90^{th} and 3^{rd}, 50^{th}, 90^{th} percentiles weight for age and height for age respectively amongst the pupils were much inferior compared to the same age and gender of the corresponding NCHS reference values. The implication of this is that our study population was much lighter and shorter than the standard reference group. While our study population was generally a poor sub-Saharan population the latter was from a western developed country. Under nutrition is widespread among school children (particularly in South Asia and Africa) and their nutritional status often deteriorates during their school years (World Food Programme, 2002). This makes identification of this problem early, at the start of school, important. Remedial steps such as institutionalising school feeding at least once during school day in primary schools would be needful.

At the time of the study there was no government supported school feeding programme in the country but in 2005 a pilot school feeding programme was initiated by the then Nigerian president in a neighbouring state. This is yet to be widely implemented nationally.

Conclusion: This study shows that malnutrition is present even among apparently healthy school children and so is still a problem in school aged children in Nigeria. Since the nutritional status of school children has an impact their cognitive development and school performance it is suggested that school feeding programmes be established in primary schools and this should be fully supported by the government and private partners. This should be targeted especially at primary schools where the burden of the problem lies. In addition the school health programme should be revived and empowered to conduct regular medical examination including nutritional status assessment and improve

nutrition of school children by providing micronutrients and mass de-worming programs. This will help improve nutritional deficiencies and thus school performance in these primary school children.

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Isolation and Identification of *Aspergillus oryzae* and the Production of Soy Sauce with New Aroma

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Abstract: Soy sauce is a dark brown salty liquid with a peculiar and a meaty taste. It is the chief savory-seasoning agent used in Oriental cookery, but it is becoming increasingly popular in many other regions of the world. The purpose of this study was to isolate *Aspergillus oryzae* strain from contaminated rice, soybean and wheat for using in soy sauce production with new aroma of thyme and dill. Samples of rice, soybeans and wheat assumed to be contaminated with *Aspergillus oryzae* were used in the isolation. Pure cultures obtained by culturing and subculturing on Potato Dextrose Agar (PDA) were maintained on PDA slant. All isolates were inoculated on *Aspergillus flavus* and *Parasiticus agar* (AFPA) medium to differentiate them from *Aspergillus flavus* and *Aspergillus parasiticus* based on reverse color. These isolates and the reference strain were inoculated on Czapack Yeast Extract Agar (CYA) and the macroscopic characteristics amongst these strains were compared. Slide cultures for these strains were prepared and their microscopic characteristics were compared. The preparation of the soy sauce was carried out by two stages. The first stage was Koji, which was prepared by mixing the isolates and the reference strain separately with steamed soybeans and the crushed millet was incubated for three days. The second stage involved the preparation of brine which consists of a koji and salt solution. The obtained data were analyzed using SPSS program. The results of analysis of soy sauce encouraged the use of the isolates, especially the rice isolate in soy sauce production and the addition of dill or thyme gave a specific aroma to the final product.

Key words: *Aspergillus oryzae*, soy sauce, Aroma, thyme, dill

INTRODUCTION

Aspergillus oryzae is a member of the *A. flavus* group. The *A. flavus* group also includes *A. sojae*, *A. nomius and A. parasiticus*. They are defined by the production of spore chains in radiating heads which range in color from yellow-green to olive brown. *A. flavus* and *A. parasiticus* are known to produce the potent carcinogen aflatoxin. *A. oryzae* and *A. soji* have been used for producing food grade amylase and fermentation of oriental foods for centuries (Sooriyamoorthy *et al*., 2004; Geiser *et al*., 1998).

Koji fermentation, provides enzymes such as α-amylase to liberate sugars from substrate, thereby facilitating yeast fermentation. Normally a strain of *A. oryzae* is used at 25-30°C (Sooriyamoorthy *et al*., 2004; Waites *et al*., 2001). Moromi fermentation begins by combining the fermented soy bean wheat mixture with salt brine (Sugiyama, 1984).

Soy sauce is a dark brown salty liquid with a peculiar aroma and a meaty taste. It is the chief savory-seasoning agent in Oriental cookery, but it is becoming increasingly popular in many other regions of the world (Yue, 1990).

Soy sauce has been extensively produced and studied worldwide. However, to our knowledge no soy sauce production unit or previously published research has been conducted in Gaza strip. Soy sauce is usually exported to Gaza strip. The present study shows that high quality, low priced, aromatic soy sauce can be produced in Gaza strip.

All chemical ingredients in the soy sauce were investigated. This study was undertaken to isolate and characterize the *A. oryzae* strain. This strain has been used in the production of a new aromatic soy sauce of either thyme or dill.

MATERIALS AND METHODS

Soy beans (*Glycine max*) and rice (*Oryza sativa L*) were purchased from Egypt, the wheat (*Triticum aestivum*), millet (*Panicum miliaceum*) and spices were obtained from Palestinian Ministry of Agriculture. The chemicals used in this study were purchased from Merck Chemical Company (Deisenhofen, Deutschland) and Sigma Chemical Company (N.Y., USA). The other media were purchased as the follow: *Aspergillus* differentiation agar (base) (Sigma; India), Potato dextrose agar (Himedia; India), Chloramphenicol supplement (Liofilchem; Italy).

Microorganism: The reference *Aspergillus oryzae* was obtained from Thailand (Faculty of Science, Mahidol University).

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Isolation: Samples, such as rice, wheat and soybeans assumed to be contaminated with *A. oryzae* were collected. The International Seed Testing Association Techniques (ISTA) especially (Agar plate method) was used to detect the *A. oryzae* (Khan, 1992). Isolates spores, (assumed to be *A. oryzae*) were inoculated on PDA and incubated at 30°C until sporulation (Sooriyamoorthy *et al*., 2004). Five pure cultures of isolates were obtained by sub-culturing and maintained on PDA slants for further identification.

Differentiation on AFPA selective medium: Isolates and the reference strains were inoculated in triplicates on AFPA selective medium and incubated at 30°C for 48-72 h and observed for reverse color. All the plates were incubated at 30°C and observed every two days for one week and any changes of the reverse color were recorded (Sooriyamoorthy *et al*., 2004).

Morphological characteristics: The isolates and the reference strain were plated on CYA (Czapek conc. 1 ml, K_2HPO_4 1 g, yeast extract 5 g, sucrose 30 g, agar 15 g and distilled water 1L) at 25°C for 7 days (Sooriyamoorthy *et al*., 2004). After the incubation period, all the plates were observed for macroscopic culture, such as colony diameter, colony color, conidial color, mycelial color, colony reverse, colony texture and nature of spores (Sooriyamoorthy *et al*., 2004). The microscopic characteristics were observed by preparing slide cultures, as described by Leck (1999).

Starter and Koji: The starter was prepared from spores of *A. oryzae*, 5 g of crushed millet and 2 ml of distilled water. 0.4 g of each starter was suspended in distilled water and the spores were counted using haemocytometer chamber. Different numbers of *A. oryzae* spores were determined (1.2×10^7 spores/ml for the reference *A. oryzae*, 1.33×10^7 spores/ml for the soybean isolate and 2×10^7 spores/ml for the rice isolate). In koji preparation, 0.4 g of each starter was taken and mixed with 330 g of soybeans, washed and soaked for 15 h, crushed and steamed for 30 min and drained for 3.5 h. Then, 300 g of millet was roasted for 15 min and crushed. The mixture was incubated for 72 h at 30°C and stirred twice daily (Ueki *et al*., 1994).

The brine: During the brine preparation (for the isolates and the reference), 276 g of koji, 61.4 g of NaCl and 375 ml of H_2O were mixed in a glass container. The resulting brine was incubated at 30°C for 3 months and stirred daily. Thereafter, the brine was filtered through sterile cotton cloth and the filtrate was pasteurized for 30 min, followed by the addition of thyme (*Thymus vulgaris*) or dill (*Anethum graveolens*) and held at room temperature until cool and then stored in sterilized bottles. The filtrates were analyzed for pH, ash, moisture, total solids, nitrogen, protein, salt, ethanol and calcium. The pH was measured by a pH meter (HANNA PH 211). Ash, moisture, total solids and salt percentages were determined as described by James (1996). Nitrogen and protein levels were determined using Kjeldahl method (Olvera-novoa *et al*., 1994). Ethanol percentage was determined using distillation method (Ault, 1998). Calcium levels were measured using atomic absorption spectrophotometer (A Analyst 100 Perkin-Elmer).

Data analysis: Data were analyzed using SPSS version-13. ANOVA test was used to differentiate between different numerical obtained data and any difference less than 0.05 was considered significant.

RESULTS AND DISCUSSION

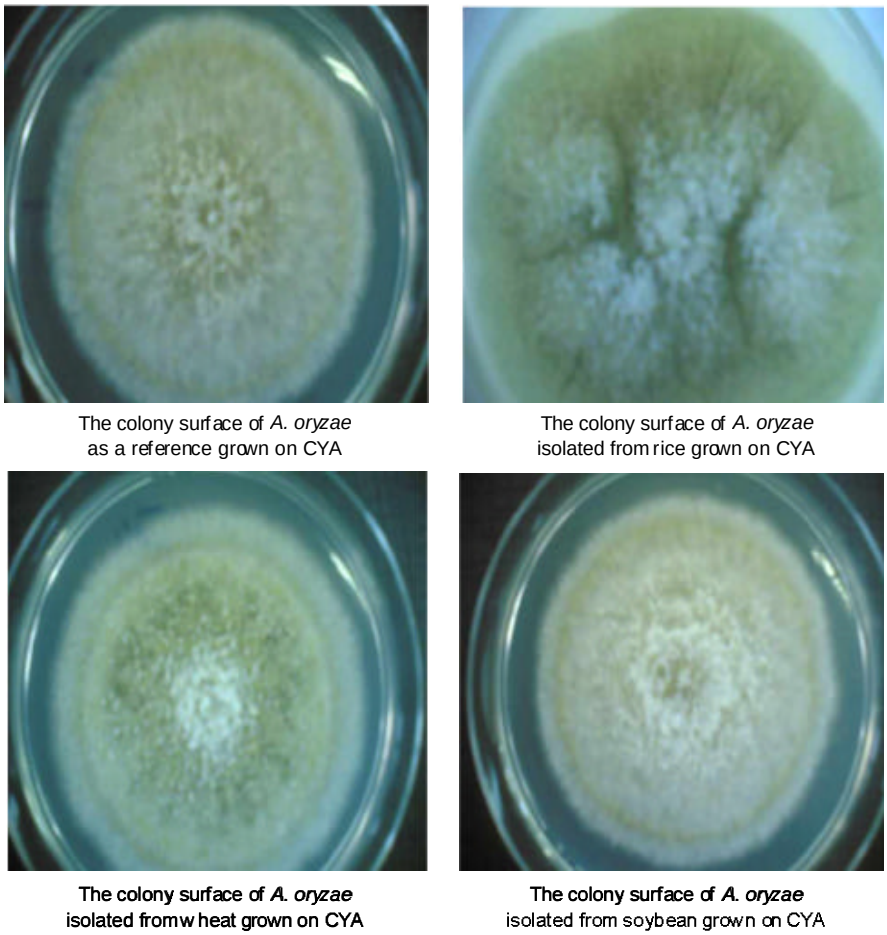
Isolation: Contaminated rice, wheat and soybeans were treated by ISTA, using agar plate method. All pure cultures on PDA varied, gradually becoming white centre, green yellow periphery and colony color. The isolates produced creamy reverse color on AFPA medium within 48 h of incubation at 30°C. The reverse color of the isolates and the reference did not change after the additional incubation period for one week. The isolates and the reference strain were compared.

Morphological characteristics: The macroscopic characteristics of the isolates and the reference were reported as shown in Table 1 and Fig. 1.

All the macroscopic characteristics of the isolates and the reference were identical, indicating that the isolates are most likely strains of *A. oryzae*.

The microscopic characteristics of the isolates were similar to that of the reference (Fig. 2), whereby the conidia, phialides, vesicles and mycelia were identical. In addition, cream reverse color was produced within 48 h when the isolates and the reference samples were inoculated on AFPA. This was in agreement with Sooriyamoorthy *et al*. (2004) and Jernejc and Cimerman (2001). From these comparable characteristics it could be concluded that the isolates are strains of *A. oryzae*.

Koji preparation: Koji was incubated for 72 h at 30°C. After 24 h of incubation, the heat of koji was raised gradually to 35°C and it started to drain and draining was continued for 60 h. *A. oryzae* started to grow during the initial 36 h and continued to grow rapidly. Firstly, the color of the fungus throughout the incubation became white and then yellowish and lastly the yellow greenish color was dominant. These changes were combined with the release of a very clear volatile aroma. Aeration was necessary for the fungi to grow and the fungi would die under anaerobic conditions as agreed with Shankar and Mulimani (2007).



The colony surface of *A. oryzae* as a reference grown on CYA

The colony surface of *A. oryzae* isolated from rice grown on CYA

The colony surface of *A. oryzae* isolated from wheat grown on CYA

The colony surface of *A. oryzae* isolated from soybean grown on CYA

Fig. 1: The colony surface of the reference and the isolates strains

Table 1: Macroscopic characteristics of the isolates compared with the reference strain of *A. oryzae* observed after 7 days of incubation at 25°C on CYA medium

Characteristics	Soybean isolate	Rice isolate	Wheat isolate	Reference
Colony diameter	55 mm	75 mm	60 mm	56 mm
Colony color	White centre green yellow periphery	White centre green yellow periphery	White centre green yellow periphery	White centre green yellow periphery
Colony reverse	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Colony texture	Wet	Wet	Wet	Wet
Conidial color	Yellow green	Yellow green	Yellow green	Yellow green
Nature of pore	Powdery	Powdery	Powdery	Powdery

Brine preparation: In the brine solution, the *Aspergillus* enzymes of koji continued to hydrolyze the soybeans and millet and as a result an excess of different kinds of sugars and amino acids were produced. These sugars and amino acids were consumed by natural microorganisms, such as salt tolerant lactic-acid bacteria (*Tetragenococcus halophila*) and yeasts (*Zygosaccharomyces rouxii* and *Candida versatilis*) during the so-called brine fermentation (Der sluis *et al*., 2001). During brine fermentation, the color was changed gradually to dark brown and a different aroma was released. Glutamic aroma was originated and an additional attractive new aroma was produced by the addition of dill or thyme. The brine was analyzed to observe the capacity of the isolates. The amount of protein in rice isolate soy sauce was higher than that in the reference strain and soy bean isolate soy sauce. Rice isolate soy sauce also contained higher levels of nitrogen, calcium, NaCl and pH (Table 2). This increase may be due to the rapid growth of this strain. Consequently, acid protease of this isolate might increase the proteolytic hydrolysis in brine fermentation,

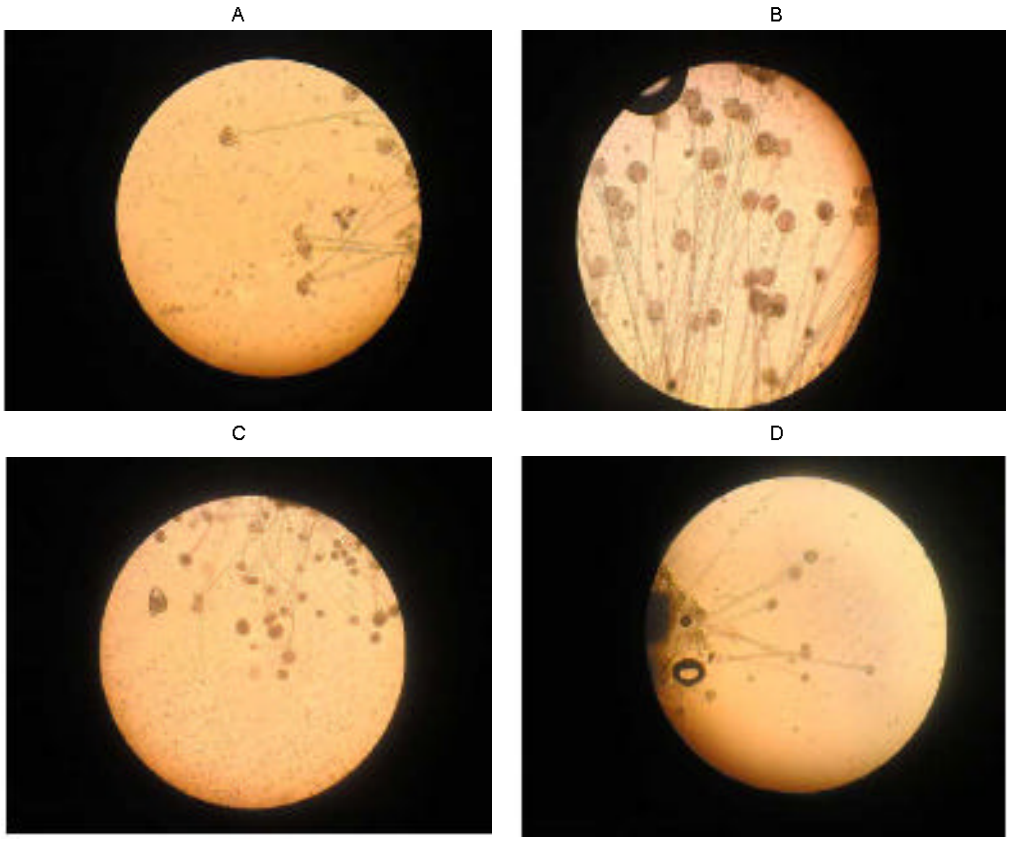


Fig. 2: Fruiting heads of A- reference strain, B- wheat isolates, C- rice isolates, D- Soybean isolates

Table 2: Chemical analyses of three kinds of soy sauce products

Types of chemical analysis	Reference soy sauce	Rice isolate soy sauce	Soy bean isolate soy sauce
pH	$4.16^{b}\pm(0.12)$	$4.65^{a}\pm(0.20)$	$4.25^{b}\pm(0.09)$
Ash %	$10.1^{b}\pm(0.10)$	$12.5^{a}\pm(0.13)$	$12.5^{a}\pm(0.14)$
Protein %	$8.1^{b}\pm(0.07)$	$9.8^{a}\pm(0.08)$	$7.2^{c}\pm(0.10)$
Nitrogen %	$1.3^{b}\pm(0.04)$	$1.5^{a}\pm(0.02)$	$1.1^{b}\pm(0.01)$
Moisture %	$76.6^{a}\pm(1.2)$	$43.3^{b}\pm(1.1)$	$78.3^{a}\pm(1.4)$
Total solids %	$23.4^{b}\pm(1.1)$	$56.7^{a}\pm(1.7)$	$21.7^{b}\pm(1.2)$
Ethanol %	$0.11^{c}\pm(0.01)$	$0.57^{b}\pm(0.08)$	$0.92^{a}\pm(0.11)$
NaCl %	$14.04^{c}\pm(1.12)$	$16.38^{a}\pm(1.52)$	$15.21^{b}\pm(1.23)$
Ca (mg/100 g)	$123.2^{a}\pm(2.63)$	$127.1^{a}\pm(3.35)$	$102.3^{b}\pm(4.12)$

Note: Wheat isolate was not used in soy sauce production and values of different superscripts in the same raw differ significantly ($p<0.05$)

resulting in good soy sauce (Iizuka and Aishimal, 1999). Nitrogen content is important parameter used for grading the quality of soy sauce product. According to the Chinese National Standard, grade A soy sauce should contain total nitrogen and amino nitrogen of more than 1.4 and 0.56%, respectively (Chou and Ling, 1998). The concentration of NaCl of the products was sufficient to stop bacterial growth. These results were also confirmed by the results of Iizuka and Aishimal (1999). They found that sodium chloride helps to destroy staphylococci in soy sauce. However, Soy sauce with salt solution containing 10-17% sodium chloride, pH 4.7 destroyed 90% of the staphylococci cells in 10% NaCl solution at 980-1440 min and of 17% at 460-530 min. For moisture and total solids, there was an opposite relation between them may be due to the strength of hydrolysis in the brine. The less moisture, the higher total solids. So, good soy sauce is produced with the available quantity of protein, nitrogen and calcium. These components were close to those of commercial soy sauce (Chou and Ling, 1998). This study indicates that rice isolate would be the best industrial starter for the production of a new aromatic soy sauce.

Conclusion: The following specific objectives were achieved:

- Isolation of the *A. oryzae* from different contaminated sources.
- Characterization of the *A. oryzae*.
- Production a new aroma of soy sauce with *Anethum graveolens* and *Thyme vulgaris*.
- Identification of the chemical gradients of the new product.

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Analysis of Nutrition Habits of the Teachers and Nurses

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Abstract: Nutritional habits are an important factor in terms of quality of life and the use of existing sources. The purpose of this study was to determine the nutritional habits of teachers and nurses. A face-to-face questionnaire survey was applied to 471 participants, 186 of whom were nurses and 285 of whom were teachers. The findings indicated that the nutritional habits of the teachers were more positive than those of nurses ($p = 0.001$). In addition, it was found that there was a significant difference between age and nutrition habits ($p<0.001$) and that as the person aged, nutrition habit scores increased. No significant difference was detected in terms of gender ($p>0.05$).

Key words: Nutrition habits, nutrition habits score, teachers, nurses

INTRODUCTION

Urbanization, economic development and globalization have led to transitions in nutrition with the rapid changes in lifestyle and diet. Transition in nutrition is related to the consumption of foods with high energy content (foods with low fiber-content foods, containing sugar or sweetened foods), low levels of physical activity and sedentary lifestyles (Pekcan, 2008). Balanced and sufficient nutrition is of great importance for maintaining good health and for pursuing a quality life. The relationship that has been demonstrated between unhealthy nutritional habits and many chronic diseases emphasizes the preventive effect of correct nutrition. Previous research has demonstrated that a wide range of factors can affect individual nutritional habits, including: Characteristics such as age (Oakes, 2003), gender (Oakes and Slotterback, 2001b; Kroshus, 2008; Delores, 2009), living place (Volatier and Verger, 1999; Bovell-Benjamin *et al*., 2009), family structure, ethnic origin, cultural factors, socio-economic status, occupation, beliefs and psychological factors (Bellisle *et al*., 1999; Gibney *et al*., 2004). However, previous studies indicated that individuals have only a limited perception and knowledge about a healthy diet (Oakes and Slotterback, 2001a; Oakes and Slotterback, 2001b; Oakes, 2003; Oakes *et al*., 2005). Women, in particular, tend to associate a healthy diet with the energy values of foods (Carels *et al*., 2007). Recent years have seen a global rise in rates of obesity and obesity-related health issues (Timperio *et al*., 2002; Thorpe *et al*., 2004; Musingarimi, 2008; Stewart *et al*., 2009). However, it would be a mistake to associate unhealthy or poor diet solely with obesity. In contrast, it is constantly emphasized that insufficient energy and food intake is a factor in the development of various diseases and that dietary arrangements have an important effect on in the treatment of diseases (Baysal *et al*., 1999).

Within Turkey, one reason for an inadequate and unbalanced diet is the common unhealthy nutrition habits, related to various factors, that prevent the most effective use of existing sources. Changes in nutrition habits are important in terms of individuals' health and the risk of diseases such as cardiovascular diseases, diabetes mellitus, osteoporosis etc (Wallace *et al*., 2001; WHO, 2003; Ren, 2004; Sanlier, 2005; Uyar, 2007).

In order to attain the desired quality of life in the rapidly globalizing world, awareness should be raised about nutrition and the emphasis on healthy diet should be adopted as a lifestyle choice. Adequate and balanced diet is important for physiological needs throughout life and for satisfying psychological and sociological needs. For this reason, priority should be given to national policies which provide effective nutritional education (Moron, 2006). Nutrition awareness has a positive impact on an individual's nutritional preferences (Tepper *et al*., 1997). Reliable channels of communication, which can reach large groups are required for effective educational programs (Moron, 2006).

Institutions which have a significant influence on the society are important sources for the acquisition of positive societal behaviors in terms of nutrition habits. Thus, health personnel and teachers who pioneer the society as educators can be considered as potential leaders in this role. Teachers inform their students in terms of food, nutrition, growth and development, body

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image, weight control and help them acquire positive attitude and behaviors (O'dea, 2002). Nurses also have an important role in offering preventive health services and public information.

For those reasons, the it should be thought that nutritional habits and awareness of healthy diet amongst both occupational groups is of great importance. The purpose of the present study is therefore to determine the nutrition habits of teachers and nurses and to compare these two professional groups in terms of nutrition habits.

MATERIALS AND METHODS

Research design: The study was carried out in Ankara between March-September 2008. The study group consisted of 471 people, 186 of whom were nurses and 285 of whom were teachers. Initially, the aim was to include an equal number of nurses and teachers (nurses = 300 and teachers = 300, total = 600). However, as the nurses were involved in a busy working program, 200 volunteer nurses were included in the study. A further 15 teachers, 14 nurses (total 29), who initially agreed to participate, left the study during the survey process. Nurses and teachers were compared in terms of nutrition habits, in addition, the effect of gender and age in relation to nutrition habits were evaluated.

Participants: The study group comprised nurses and teachers working in Ankara, Turkey's capital city. The participants were informed about the subject, purpose and the rules of the study. The questionnaire was administered to the volunteered participants through a face-to-face interview technique. The average age of the participants was 31.11±10.11 years. 98.4% of the nurses were female; 55.4% of the teachers were male and 44.6% of the teachers were female. Although nursing department of universities is highly promoted to encourage males to choose this profession, the number of male nurses is quite low. Nursing is still perceived as a female profession in Turkey.

Instrumentation: The data was collected between May-August 2008. In data collection, the questionnaire form was used. The gender and ages of the participants were identified as the independent variables. In order to determine nutritional habits, a scale consisting of 25 statements was developed, with reference to various previous studies (Tepper *et al*., 1997; Sanlier and Unusan, 2007a; Sanlier and Unusan, 2007b; Beydoun and Wang, 2008). Each statement about nutrition habits was scored as follows: "always = 3", "sometimes = 2" and "never = 1". In negative statement (8 statements), the scoring was the reverse. The range of possible scores was between 25-75 (Tavsancil, 2006).

The pilot test: The reliability of the questionnaire was also determined by a pilot study on 50 adults. As a result of the item analysis, several test questions were modified to improve clarity. The reliability coefficient of the test, conducted after the real application, was calculated to be 0.694.

Data analysis: Statistical analysis was conducted using SPSS for Windows (version 11.0, 2001, Chicago, IL). The level of statistical significance was $p<0.05$. The gender, age and occupation distribution of the participants are given in percentages. For each statement about nutrition habits, the percentage distribution of the responses of the teachers and the nurses were calculated and the difference was evaluated using a Chi^2 test. Demographic characteristics and nutrition habit total scores t test were evaluated according to ANOVA and Pearson Correlation Coefficient (Buyukozturk, 2008).

RESULTS

The demographical characteristics of the participants: The demographic characteristics of the participants are given in Table 1.

65.8% of the participants were female, 34.2% were male. 37.2% of the participants were between the ages of 20-23 ($\bar{X}$ = 31.11±10.11 years). 60.5% of the participants were teachers and 39.5% were nurses (Table 1).

Table 1: Demographic characteristics of teachers and nurses (n = 471)

Demographic characteristics	n	(%)
Gender		
Male	161	(34.2)
Female	310	(65.8)
Age (year)		
20-23	52	(11.0)
24-30	175	(37.2)
31 and over	244	(51.8)
Occupation		
Nurse	186	(39.5)
Teacher	285	(60.5)

Nutrition habits of teachers and nurses: The nutrition habits of the teachers and nurses are given in Table 2. 69.1% of the teachers reported that they prefer plant oils rather than animal fat; 22.1% reported that they regularly eat fish on a weekly basis; 71.9% reported that they did not prefer to eat fast-food products instead of regular food; 42.5% reported that they always consume a minimum of 2 glasses of milk, yoghurt or 2 matchbox size pieces of cheese per day; 51.9% reported that they avoid eating food containing additives and 72.3% reported that they read the labels when buying food and beverages. These values were found to be 48.9, 11.3, 37.1, 28.5, 29.0 and 52.7% respectively in nurses and there was a statistically significant difference between

Table 2: Distribution of nutrition habits of teachers and nurses (%)

	Always		Sometimes		Never			
Statement	T	N	T	N	T	N	X^2	p
I have breakfast every day	61.1	53.2	34.0	43.5	4.9	3.2	4.64	0.098
I do not skip meals	29.5	16.7	63.2	76.9	7.4	6.5	10.79	0.005*
I prefer to eat animal products such as meat, milk and egg every day minimum for one time	53.3	38.7	36.8	46.8	9.8	14.5	9.91	0.007*
I prefer black bread made of whole-wheat/whole wheat flour rather than white bread	21.8	18.8	51.9	44.6	26.3	36.6	5.59	0.061
I prefer plant oils in food rather than fats/animal fats	69.1	48.9	22.5	42.5	8.4	8.6	22.37	0.000**
I like to eat every kind of food	60.0	47.3	30.9	43.0	9.1	9.7	7.98	0.019*
I consume minimum one portion of vegetable, fruit and salad in my daily nutrition	54.4	43.0	40.0	47.8	5.6	9.1	6.53	0.038*
I prefer to eat fast-food like hamburger and cheeseburger rather than regular food in meals	2.5	3.8	25.6	59.1	71.9	37.1	56.68	0.000**
I do not generally drink beverages such as cola and soda pop for meeting my daily fluid need	49.5	46.8	21.1	34.4	29.5	18.8	12.85	0.002*
If I feel that I put on weight, I take up physical activity (exercise)	23.2	25.3	52.3	44.6	24.6	30.1	2.84	0.241
If I feel that I put on weight, I eat less	30.9	30.6	50.2	41.9	18.9	27.4	5.25	0.072
I avoid food that contain additives	51.9	29.0	39.6	60.2	8.4	10.8	24.38	0.000**
I regularly eat fish each week	22.1	11.3	58.2	51.6	19.6	37.1	21.18	0.000**
If the bread is fresh but is moldy, I prefer to eat the fresh bread instead.	78.2	81.7	12.3	12.4	9.5	5.9	1.94	0.379
I do not eat while watching television	21.1	9.7	56.5	58.1	22.5	32.3	12.95	0.002*
I drink a minimum of 8 glasses (1.5 liters) of water a day	47.7	46.2	41.8	44.6	10.5	9.1	0.49	0.785
I eat 2 glasses of yoghurt, milk or 2 matchbox size pieces of cheese a day	42.5	28.5	42.5	55.4	15.0	16.1	9.97	0.007*
I avoid consuming alcohol	64.2	76.3	22.5	12.4	13.3	11.3	8.98	0.011*
I do not smoke	49.8	67.2	16.1	10.8	34.0	22.0	13.85	0.001*
I eat food at regular meal intervals	21.8	29.0	66.3	63.4	11.9	7.5	4.70	0.095
I read the labels when I buy food and beverages	72.3	52.7	23.5	43.0	4.2	4.3	20.41	0.000**
I do not use ketchup, mayonnaise, salad sauce etc in food	37.9	27.4	42.1	58.6	20.0	14.0	12.27	0.002*
I eat dry legumes 1-2 times a week	45.3	49.5	50.9	44.1	3.9	6.5	3.05	0.218
I eat granola, muesli etc and milk at breakfast	7.7	4.8	30.9	29.6	61.4	65.6	1.80	0.408
I do not eat the skin when I eat chicken, turkey or fish	48.1	55.4	29.1	29.0	22.8	15.6	4.12	0.128

T: Teacher, N: Nurse, *p<0.05, **p<0.001

the nurses and teachers (p<0.001) (Table 2). 29.5% of the teachers reported that they never skip meals 22.5% reported that they eat while watching television. With regard to the statement "I consume a minimum of one portion of vegetables, fruit and salad in my daily nutrition" it was found that only 54.4% of the participants answered "always" (the values in nurses were 16.7%, 32.3%, 43.0% respectively) and there was a statistically significant difference (p<0.05). for the responses to the statements "I prefer to eat animal products such as meat, milk and eggs at least once per day " and "I do not drink beverages such as cola and soda pop for meeting my daily fluid need" there was a significant difference between the teachers and nurses (p<0.05). The teachers were found to have more positive habits in terms of these statements. In terms of the statements "I avoid consuming alcohol" (p<0.05) and "I do not smoke" (p = 0.001), it was found that the nurses had more positive habits and that there was a statistically significant difference between the two groups (Table 2).

Evaluation of nutrition habit scores according to demographic characteristics: Arithmetic average and standard deviations of nutrition habit scores according to gender and occupation are given in Table 3.

Table 3: Nutrition habit scores according to gender and occupation

Variables	$\bar{x}$ ±SD	t	p-value
Gender			
Male	56.11±5.56	-1.312	0.190
Female	56.84±5.89		
Occupation			
Nurse	55.49±5.81	-3.374	0.001*
Teacher	57.31±5.66		

*p<0.05

Table 4: Correlation of nutrition habit score between age, gender and occupation (r)

Variables	Nutrition habits score
Gender	0.060
Age (year)	0.185**
Occupation	0.154**

**p<0.01

No statistically significant difference was found between nutrition habit scores and gender. Nutrition habit scores of the teachers (57.31±5.66) were found to be higher than those of nurses (55.49±5.81) and the difference was found to be statistically significant (Table 3).

It was found that there was a significant relationship between nutrition habits score and age (r = 0.185, p<0.01) and occupation (r = 0.154, p<0.01). Examination

of the relationship between professional groups and nutrition habits when the age factor was kept under control did not produce a statistically significant result ($r = -0.0109$, $p = 0.814$). This result shows that age is a more important factor.

DISCUSSION

The role of a nurse includes protecting and improving community health and the role of a teacher includes promoting positive attitudes amongst students. The appropriate nutritional habits of both these occupational groups have an important role in positively influencing wider societal nutritional habits and behaviors. In addition, the nurses, who receive health education in parallel with their occupational training, would be expected to have higher scores. Despite that, the average nutrition scores of the teachers were found to be higher ($p<0.05$, Table 3). According to the correlation results, occupation is a significant variable in nutrition habits (Table 4). This may be due to the fact that, in Turkey, nurses generally work in 3 shifts and 24-h shift patterns, thus having lifestyle that is less conducive to taking regular meals and maintaining healthy eating behaviors; while the teachers work for a regular 6-8 h per day under more positive conditions (Bilazer *et al*., 2008).

More than half of the teachers and nurses reported that they have breakfast every morning. Pearson *et al*. (2009) reported that parents' habit of having breakfast had a positive impact on the breakfast habits of children. Considering that the adults comprising the population of this study are parents/candidate parents, their positive habit of having breakfast is important for themselves and for their children. In a study by Berg *et al*. (2009) there was a relationship between obesity and skipping breakfast and lunch and eating at night. We can conclude that, in addition to causing insufficient and unbalanced diet, skipping meals is a negative habit posing a risk for obesity. In the present study, it was found that the majority of the participants have the habit of skipping meals (Table 2).

There is a common recognition that, in terms of health benefits, white meat should be consumed instead of red meat. However, in the present study the, statement "I regularly eat fish each week" received noticeably low scores, which indicates that this awareness of healthy nutritional choices is not reflected in the eating habits of the study group. In their studies, Yen *et al*. (2008) found that greater nutritional awareness amongst consumers led to reduced red meat consumption but did not affect white meat consumption. Previous studies indicated that there was a positive relationship between consuming fish and age (Olsen, 2003; Myrland *et al*., 2000). Another study indicated that the community culture had a significant effect on widespread fish consumption in Vietnam (Tuu *et al*., 2008). Although Turkey is a peninsula and is rich in sea products and fish sources and although fish is cheaper than red meat, the present study found that only half of the participants sometimes eat fish (Table 2). This may be due to the fact that Turkish people have no habit of consuming fish and sea products. Similarly, in their studies, Yen *et al*. (2008) reported that males consumed more fish and meat than females and that meat consumption decreased with age. The fact that the teachers in the present study consumed more animal products such as meat and egg when compared to the nurses ($p<0.05$) may be related to the fact that the majority of the nurses in the present study were female. A previous study found that adults perceived foods with high fat content, cholesterol and sodium as unhealthy and perceived the foods with high fiber, vitamin/mineral and protein content as healthy (Oakes and Slotterback, 2001a). This finding is supported by the results of the present study, in which the teachers have more positive behaviors and healthy diet perceptions than nurses in terms of their preferences for consuming daily meat, egg, milk and dairy products, vegetables and fruits and consuming liquid oil instead of fats (Table 2). The occupational duty of the teachers, which is based on encouraging their students to acquire positive behaviors may have an effect on adopting accurate nutritional habits.

The finding that the participants have very little preference for fast-food is a positive behavior. Tepper *et al*. (1997) found that individuals with greater knowledge of nutrition consume more healthy food and less fast-food. In our study it was found that the nurses preferred fast-food more than the teachers ($p<0.001$) (Table 2). In addition, it was found that the teachers have more positive nutrition habits than the nurses (Table 3 and 5). The results of Tepper *et al*. (1997) support our findings. Since sauces such as ketchup and mayonnaise were recently introduced to Turkish cuisine, these sauces are generally consumed with fast-foods in Turkey. The finding that, parallel to higher fast-food consumption, the nurses in our study consume more sauces such as ketchup and mayonnaise (Table 2) verifies that these sauces are not widely used in traditional food, or by older people. According to the FDA, in relation to the energy consumption of American people (1200-1300 calories), an individual is recommended to eat 5-13 portions of vegetables per day (Anon, 2005). Previous studies indicated that as the vegetable and fruit consumption of an individual decreases, energy and fat intake increases (Derwnowski *et al*., 1997; Becker, 1999; Haraldsdottir, 1999). According to a study carried out in France, fat intake was found to be high, while vegetable and fruit consumption was found to be low (Volatier and Verger, 1999). Fisher *et al*. (2002) found that vegetable and fruit consumption by parents had an effect on children's habit of consuming similar foods. Considering the results of previous studies on

recommended daily vegetable and fruit intake and the effects of vegetable and fruit consumption, the fact that 54.4% of the teachers and 43.0% of the nurses reported that they consumed these foods "always" can be regarded as a challenging result.

In a study of women, it was indicated that a two-hour increase in watching television per day increased the risk of obesity by 23.0% and every two-hour increase in sedentary work per day increased the obesity risk by 5.0% (Hu *et al*., 2003). In another study, it was found that watching television while having lunch significantly increased the amount of food that is consumed in meal intervals in the afternoon (Higgs and Woodward, 2009). In accordance with the findings of previous studies, we conclude that an inactive life style and consumption of snacks-generally foods with high energy content are preferred while watching television- have a significant effect on the prevalence of obesity (Dogan and Yildiz, 2001). When the habit of eating while watching television was questioned in the study, it was found that the teachers gave more positive responds ($p<0.01$), but the majority of the participants reported that they ate while watching television (Table 2).

Smoking and alcohol consumption are totally undesired habits due to their harmful effects on health. The education the nurses received may influence the finding that nurses smoked less and consumed less alcohol than the teachers. In a study of university students it was found that appetite increased after alcohol consumption and during the term of the study, parallel to the amount of consumed alcohol, BMI also increased and there was a statistically significant BMI increase in the group which drank large quantities of alcohol (Lloyd-Richardson *et al*., 2008). The fact that the majority of the participants in both groups report that they avoid drinking alcohol (Table 2) is a positive habit that has a significant effect on maintaining body weight (within the healthy limits). In addition, smoking and alcohol consumption is more common among males. High smoking and alcohol consumption rates of the participant teachers can be associated with the fact that nearly all study nurses were females and most of the male participants were teaches. As a matter of fact, gender-based evaluation of smoking and alcohol consumption showed that males consumed such products more than females ($p<0.05$). It shows that gender rather than profession is determinant in smoking and alcohol consumption.

As a consequence, correcting the in appropriate nutrition behaviors within society and encouraging a societal shift towards appropriate and healthy nutrition habits is only possible by conducting social education on the subject of nutrition. Educating and raising societal awareness about healthy diet is of great importance during the entire life cycle. (Anon, 1997). In today's rapidly globalizing world, educators and health-professionals have important roles in terms of attaining the desired quality of life, by raising awareness of nutritional and dietary issues and promoting better nutrition as a healthy lifestyle choice. For these reasons, both professional groups have to be a role model for the society with their right nutrition habits.

Limitation of the study: Because the population of this study consisted of nurses and teachers in central Ankara, the results should not be generalized to cover all nurses and teachers, age groups or to the entire country. Although the reliability coefficient was found to be high, this research measured self-reported behaviors; self-reportage is subject to individual bias. However, this study pioneers further studies with higher number of participants.

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Ingredients, Proximate Composition, Colour and Textural Properties of Commercial Malaysian Fish Balls

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Abstract: Fish balls are the popular value-added products in Malaysia. This study was carried out to determine quality characteristic associated with fish balls available in Malaysia markets. A total of six brands of Malaysian fish balls were collected, recorded and analyzed for list of ingredients and some physicochemical properties which include proximate composition, color and texture (folding test and Texture Profile Analysis). The results showed that there was a wide variation in the types of ingredients used in producing of fish balls. The study also reveals that there is a new trend of using surimi as substation for fish meat in Malaysian fish balls production. The price of currently fish balls varying from US$ 1.29-2.78/kg, respectively. The proximate composition from different brands of fish balls analyzed were found to be significantly different ($p<0.05$). The moisture, protein, fat, ash and carbohydrate contents of fish balls samples varied between 73.80-88.71%, 7.54-9.89%, 0.13-1.75%, 1.61-3.40% and 1.17-13.58%, respectively. The color value for lightness (L), redness (a) and yellowness (b) of the fish balls also significantly different ($p<0.05$), ranging from 69.61 - 77.96, -2.02 - 0.33 and 15.66 - 19.70, respectively. All of samples of fish balls showed AA grade of folding test which indicated that the texture of fish balls were acceptable for consumer preference. This result showed that Malaysian fish balls produced from different manufacturers were different in ingredient used, chemical composition and color, however these differences was not effect to the folding test of the samples.

Key words: Fish balls, commercial products, physicochemical properties, folding test

INTRODUCTION

Meatballs are among popular meat products in Malaysia. The types of meatballs can be found in Malaysia are including chicken ball, beef ball, fish ball, prawn ball and squid ball. However, the most popular and commonly consumed by Malaysian are fish ball, chicken ball and beef ball. As for fish ball, generally white fish ball is more preferred by Malaysian as it indicates the freshness of the fish ball. Chilled fish ball which appears glossy or little transparent is now becoming a popular among Malaysian.

Fish ball production in Malaysia is usually initiated by small family-based enterprises. However, in recent years many factories have invested in modern machinery to increase the production of fish balls. Fish ball production is the second largest processed fish-based production in Malaysia after fish cracker production. The contribution of fish ball production to the total fish-based processed food products in Malaysia has reached approximately 15-20%. Fish ball production increased from 7875 tonnes in 1996 to 16470 tonnes in 2008 (Department of Fisheries, 2010). Although fish balls are popular food among Malaysian, but there are increasing concerns among the consumer regarding the nutritive value of these fish balls. The consumers also prefer to have real meats than processed meat in the products. Many researchers had been conducted on the nutritional and quality aspects of fish balls. Yu and Yeang (1993) and Yamprayoon *et al.* (1991) conducted the effect of ingredient on quality of fish balls. Previously Huda *et al.* (2000) reported on chemical composition and quality of commercial Malaysian fish balls. The purpose of this study is to up date the information regarding the ingredients, proximate composition, colour and textural properties associated with fish ball available in Malaysia market.

MATERIALS AND METHODS

Sample collection: Samples 6 different brands of fish balls (FB1, FB2, FB3, FB4, FB5 and FB6) were collected and picked from local supermarket and wet market around Penang, Northern part of Malaysia. The label and information's on the package with the ingredient lists were recorded.

Proximate composition: The proximate composition was determined according to AOAC (2000) methods. Moisture content was determined by drying samples

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overnight at 100°C until constant weight was achieved (Memmert UL 40, Germany). Crude protein content was determined using the Kjeldahl method. Crude lipid content was determined by the Soxhlet Extraction method. Ash content was determined by ashing samples overnight at 550°C (Thermolyne Sybranm model: 6000, USA). The carbohydrate content was calculated by difference.

Colour: The colour of fish ball sample was measured using a colourimeter (Minolta spectrophotometer CM 3500d, Japan). The colour reading includes lightness (L), redness (a) and yellowness (b).

Folding test: Folding test was determined according to Lanier (1992). The fish ball samples were cut into a 3 mm thick round-shape slice at the middle of the ball and evaluated by a five-stage method, as follow: 1 (D) = Breaks by finger pressure, 2 © = Cracks immediately when folded in half, 3 (B) = Cracks gradually when folded in half, 4 (A) = No crack after folding in half and 5 (AA) = No crack after folding twice.

Textural properties: Texture measurement on meatballs was conducted by using a computer-assisted Stable Micro Systems TA-XT2i Texture Analyzer. The procedures to operate Texture Analyzer were stated in the Standard Operating Procedure (SOP).

Three types of test were carried out in order to compare texture profile of meatballs obtained from different test. First is Texture Profile Analysis (TPA) which was used to determine hardness, cohesiveness, chewiness, elasticity and gumminess (Bourne, 1978). This test was carried out by using compression platen with 75 mm diameter. TA-XT2i setting for TPA test was load cell 25 kg; pre-test speed 2.0 mm/s; test speed 2.0 mm/s; post-test speed 5.0 mm/s; distance 50% and trigger type Auto-30 g.

Second type of test is blade shear test which used knife blade to determined shear force required to cut through sample. TA-XT2i setting for this test was load cell 50 kg; pre-test speed 2.0 mm/s; test speed 2.0 mm/s; post-test speed 5.0 mm/s; distance 40 mm and trigger type Auto-30 g. Third test is penetration test which used a 2 mm diameter penetration probe to determine force required to penetrate through sample. TA-XT2i setting for this test was load cell 50 kg; pre-test speed 2.0 mm/s; test speed 2.0 mm/s; post-test speed 5.0 mm/s; distance 75% and trigger type Auto-10 g.

Statistical analysis: Data obtained is analyzed by using SPSS (Statistical Package for Social Science) software version 12.0 (SPSS Inc., Illinois, USA). Duncan test is used with significant level at $p<0.05$.

RESULTS AND DISCUSSION

Table 1 shows information's on fish ball samples collected for the analysis. Most of the fish ball samples were packaged and labeled with the ingredients used in the formulation, except two samples (FB5 and FB6) which purchased from traditional wet market. The retail prices for fish balls were ranged from US$ 1.29-2.78/kg, respectively. This retail price is lower compared with the retail price of fish balls reported by Huda *et al*. (2000) which ranged between US$ 1.97-3.47/kg. The decreasing of retail price maybe was related with the modernization of machinery and large-scale of fish balls production which will decrease the cost of production.

The ingredients used were also different for different brand names. The type and species of fish used was not mentioned. Based on the products labels, the main ingredients commonly used in Malaysian fish balls were quite similar which include fish or surimi, starch, sugar, salt, flavour enhancer and permitted food conditioner. The flavour and food conditioner used in fish balls were not clearly stated. Surimi is refers to stabilized myofibrillar proteins obtained from mechanically deboned fish meat that is washed with water and blended with cryoprotectants. Surimi is an intermediate product used in variety of products ranging from traditional product to shellfish substitutes (Park and Lin, 2005). The survey found that there is a new trend of using surimi as substitute to fish meat in Malaysian fish balls nowadays. Development of this trend is mainly because the price of surimi is more economical compared with fish meat which can cut down processing productions time. In addition, using surimi rather than whole fish can ensure standard quality supply in fish ball processing as well (Park, 2005).

The result of proximate composition of fish ball is showed in Table 2. All fish balls samples showed significant differences in protein, fat and ash content. The result were similar to the data reported by Huda *et al*. (2000), the moisture content of Malaysian fish balls ranged from 72.5-89.9%. This indicated that in term of moisture content, the Malaysian fish balls do not change much. Protein content of fish balls obtained in this project ranged from 7.54-9.89%. However, the results were in contrast with the report by Huda *et al*. (2000)

Table 1: Sample code and relevant information for fish ball samples

Code	Ingredients	Price ($US/kg)
FB 1	Surimi, water, modified starch, salt, monosodium glutamate (E621), flavour.	$US 2.04
FB 2	Fish meat (surimi), wheat flour, sugar, salt, MSG and vegetable oil.	$US 2.78
FB 3	Fish meat (surimi), water, starch, salt, sugar, vegetable oil and permitted flavour additive.	$US1.29
FB 4	Fish (surimi), starch, sugar, salt, sodium polyphosphate, flavour and flavour enhancer.	$US1.85
FB 5	N/A	$US 2.78
FB 6	N/A	$US 2.78

N/A-Not available

Table 2: Proximate composition of Malaysian fish balls (% wb)

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
FB 1	81.08^{c}±0.57	7.54^{a}±0.49	0.70^{bc}±0.09	2.27^{b}±0.03	8.59^{c}±0.31
FB 2	88.71^{e}±0.30	7.81^{ab}±0.08	0.13^{a}±0.07	2.12^{b}±0.05	1.17^{a}±0.14
FB 3	73.80^{a}±0.13	9.86^{d}±0.14	0.47^{ab}±0.10	2.28^{b}±0.10	13.58^{e}±0.21
FB 4	79.31^{b}±0.68	8.23^{b}±0.51	1.75^{d}±0.53	1.61^{a}±0.01	9.26^{d}±0.57
FB 5	81.50^{c}±0.26	9.89^{d}±0.15	1.06^{c}±0.08	3.18^{c}±0.10	4.31^{b}±0.08
FB 6	84.60^{d}±0.32	8.92^{c}±0.31	0.85^{bc}±0.44	1.63^{a}±0.18	3.98^{b}±0.17

Means in the same column with different superscripts are significantly different ($p<0.05$)

Table 3: Colour and folding test score for Malaysian fish balls

	Colour			Folding test	
Sample	L	a	b	Score	Grade
FB 1	75.94^{d}±0.34	-2.04^{d}±0.08	5.86^{c}±0.08	5.0^{b}±0.00	AA
FB 2	74.97^{c}±0.36	-3.87^{a}±0.04	-0.80^{a}±0.22	4.6^{a}±0.55	AA
FB 3	71.09^{b}±0.28	-0.37^{f}±0.12	11.21^{e}±0.17	5.0^{b}±0.00	AA
FB 4	79.19^{f}±0.79	-1.74^{e}±0.12	5.75^{c}±0.46	5.0^{b}±0.00	AA
FB 5	68.35^{a}±0.72	-2.58^{b}±0.09	6.98^{d}±0.29	5.0^{b}±0.00	AA
FB 6	78.06^{e}±0.23	-2.35^{c}±0.16	4.11^{b}±0.76	5.0^{b}±0.00	AA

Means in the same column with different superscripts are significantly different ($p<0.05$)

Table 4: Textural properties of Malaysian fish balls

	Texture profile analysis				Blade knife	Penetration
Sample	Hardness (kg)	Cohesiveness	Elasticity (mm)	Chewiness (kg mm)	Shear force (kg)	Force (kg)
FB 1	1.76^{a}±0.03	0.73^{c}±0.01	13.10^{c}±0.39	16.86^{c}±0.33	0.93^{b}±0.07	0.12^{b}±0.03
FB 2	3.01^{c}±0.13	0.80^{e}±0.00	8.25^{a}±0.14	19.77^{d}±0.76	1.29^{cd}±0.22	0.16^{b}±0.01
FB 3	1.84^{a}±0.16	0.70^{b}±0.01	11.40^{b}±0.14	14.66^{ab}±1.36	0.70^{b}±0.24	0.06^{a}±0.01
FB 4	1.71^{a}±0.12	0.66^{a}±0.01	11.53^{b}±1.01	13.09^{a}±1.63	0.24^{a}±0.18	0.07^{a}±0.03
FB 5	2.46^{b}±0.15	0.77^{d}±0.00	10.83^{b}±0.39	20.53^{d}±1.24	1.24^{c}±0.22	0.21^{c}±0.01
FB 6	1.77^{a}±0.11	0.76^{d}±0.03	11.50^{b}±0.61	15.42^{bc}±1.45	1.55^{d}±0.45	0.15^{b}±0.03

Means in the same column with different superscripts are significantly different ($p<0.05$)

where the protein content were higher, range from 8.3-14.6%. This might be due to some manufacturers had cut down production cost by reducing amount of fish meat and replace with other extenders such as starch. This also can be linked with the carbohydrate content of present fish balls (1.17-13.58%) which was higher than the result (0.2-11.7%) reported by Huda *et al*. (2000).

For the fat content the ranged was from 0.13-1.75%. This is similar to study reported by Huda *et al*. (2000) ranged from 0.1-1.9%. This showed that the amount of fat content in fish balls do not change much. Ash content determined in this project was ranged from 1.61-3.18%. This is slightly higher than values reported by Huda *et al*. (2000), which ranged from 1.1-2.7%.

Colour values of fish ball samples are given in Table 3. The L values of fish balls which analyzed in this project ranged from 68.35-79.19, a values were ranged from -3.87 - (-0.37) and b values were ranged from -0.80 - 11.21. These results were slightly different from previous report by Huda *et al*. (2000) where the commercial Malaysian fish balls L values were ranged from 62.1-76.8, a values were ranged from -3.4 - (-0.9) and b values were ranged from 2.7-10.7. The increasing of L value was related with utilization of surimi as raw material for current fish ball production in Malaysia. Washing treatment during surimi preparation resulting white fish meat which increasing the lightness of surimi-based product such as fish balls. Yu (1994) found similar result and concluded the washed treatment not only reduces fat content, but also improved the fish balls quality, including making it whiter in colour, more elastic and firm thus giving an improved mouth feel.

Overall, the texture quality of fish balls was higher. The folding test for fish balls samples were ranged from 4.6-5.0. Previous report by Huda *et al*. (2000) also mention that all of commercial fish ball samples tested showed AA grade for folding test which was point 5.0 in this analysis. According to Yu (1994), using washed minced fish meat in fish ball formulation will result higher grade of texture quality. Due to time constrain so far only simple texture analysis was performed on the fish balls samples. Texture profile analysis is more suitable to determine the detail information of textures qualities of fish ball.

Table 4 shows textural properties of commercial fish balls. Sample FB 2 was the hardest fish balls with hardness 3.01 kg. However, fish ball that required maximum shear force to cut through was FB 6 (1.55 kg) and fish ball that required maximum force to penetrate through was FB 5 (0.21 kg). All fish balls were different

slightly in their hardness, shear force and force values as can be seen in table above. The cohesiveness of all fish balls were quite similar, ranged from 0.66-0.80. Elasticity of fish balls were quite different, the lowest was only 8.25 mm in FB 2 while the highest was 13.10 mm in FB 1. All fish balls also very different in chewiness where the highest was 20.53 kg mm in FB 5 but the lowest was only 13.09 kg mm in FB 4.

According to Serdaroglu *et al.* (2005), factors responsible for textural properties in comminuted meat proteins such as fish balls are degree of extraction myofibrillar proteins, stromal protein content, degree of comminuting and type and level of non-meat ingredients. Apart from amount of connective tissue, types and amount of extenders such as starch will play a decisive role on hardness of fish balls as well. As an example, addition of legumes flour can slightly increase toughness of meatballs (Serdaroglu *et al.*, 2005).

According to Yu and Yeang (1993), higher values for all the five parameters measured in TPA (hardness, cohesiveness, elasticity and chewiness) do not necessary mean better quality. There is a cut-off point above which the texture of meatballs would be unacceptable. Therefore, determination of good textural qualities of meatballs should be done together with sensory test in order to find out the most suitable range which is preferable by consumers.

Conclusion: The nutritional value in fish ball is consider as high because it has approximately quite high content of protein and carbohydrate although it has low content of fat. These compositions are desirable in human growth and in maintaining daily nutrient supplies for metabolism of body. The texture of all the fish balls samples are tender and gained AA grade for folding test.

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Eating Disorders and Body Image Perception among University Students

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Abstract: This study aimed to determine the prevalence of eating disorders and body image among university students undertaking nutrition education. Subjects included 577 students from Gazi University, Faculty of Vocational Education, Department of Food and Nutrition Education (Group 1 n = 299) and other departments (Group 2 n = 278) which did not involve nutrition education. The Eating Attitudes Test (EAT-40), body cathexis index and anthropometric measurements were used to assess eating attitudes. The results showed that 75.9% of nutrition educated students and 83.5% of others had normal BMI (18.5-24.9 kg/m^2). Thirty-four of the 577 students had an EAT-40 score over the cut-off level of 30. Thus, 14% of the nutrition educated were at high risk and 9.7% at moderate risk. Among others, 7.2% were at high risk and 13.3% at moderate risk. It was also seen that 30.1% of the nutrition educated and 29.5% of others were dissatisfied with their bodies. The mean EAT-40 score of all participants was 15.1±10.68; body cathexis score was 147.9±21.48. The difference between the two index means was not significant ($p>0.05$). A weak negative relationship was found between BMI, EAT-40 and the body image perception score. The results indicated that the prevalence of abnormal eating attitudes and behaviors was 5.9% among students. Nutrition education was found to have no effect on the occurrence of abnormal eating attitudes and behaviors.

Key words: Nutrition education, EAT-40, anthropometric measurements, body cathexis

INTRODUCTION

The changing social, economic, cultural and traditional structure of a society may also affect the bodily perceptions of individuals. In Western societies where the concept of beauty is based on body weight and shape, having a "thin" body is considered beautiful and consequently, eating disorders are on the rise. Print and visual media throughout the world encourage people to stay thin and they often publish special "recipes" for this purpose. As a result, many people follow special diets and exercises in order to lose weight. Seen almost exclusively among white middle class women until recently, eating disorders are now more common across all social classes and in all countries (Basterzi *et al*., 2003). Having both physical and psychosocial dimensions, eating disorders reveal themselves in one's thoughts about food and body weight and image, as well as in their eating behaviors (Becker *et al*., 1999). This study examined the relationship among anthropometric measurements, eating disorders and body cathexis in Turkish university students who were and were not undertaking education in nutrition science.

MATERIALS AND METHODS

Participants: Subjects consisted of 577 students enrolled in Gazi University, Faculty of Vocational Education, Department of Food and Nutrition Education (Group 1 n = 299) and other departments (Group 2 n = 278) which do not offer nutrition education. Gazi University is located in the capital city of Turkey, Ankara. The main goal of the Department of Food and Nutrition Education is to offer undergraduate and graduate programs to equip individuals with technical knowledge and skills in the fields of hygiene in mass nutrition systems, quality (HACCP), preparation of food in experimental practices, cooking and preserving principles, mother and child nutrition, nutrition during diseases, Turkish cuisine and upholstery and scientific subjects.

Among the students, 51.8% were in their 3rd and 4th year of university undertaking nutrition education and the remaining 48.2% were undertaking other classes. As for accommodation, 53.6% were living in dormitories whereas the remaining 46.4% were living in off-campus housing. The mean age of study population was 21.4±2.18 years (range 18-30 years). Data was collected by face to face interviews that took place for 6 months between January and June 2008.

The questionnaire: The questionnaire contained demographic items including sex, age, smoking and alcohol intake.

The eating attitudes test (EAT-40): The risk estimations were obtained from the criteria used by Garner and Garfinkel (1979) and later addressed by Savasir and Erol (1989). The questionnaire contained 40 questions related to eating habits, which were graded as follows: always, very often, often, sometimes, rarely, never. Answers marked as never, rarely and sometimes

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carried zero points whilst often = 1, usually = 2 and always = 3 points (Garner *et al*., 1982). In our study, the reliability (Cronbach's alpha) of the EAT-40 was found to be 0.814. In the scale, the scores were classified as follows: a score of 30 and above indicated high risk (abnormal eating behavior), between 21 and 30 indicated moderate risk and below 21 indicated low risk.

Body cathexis: Developed in 1953 by Secord and Jourard, the scale was tested for validity and reliability and adapted to the Turkish society in 1989 by Hovardaoglu (Hovardaoglu, 1990). The scale contains 40 items, each of which is related to an organ, a part of the body (such as arms, legs, or the face) or a function (such as sexual activity level). For each item, scores range between 1 to 5 across the alternatives "Don't like it at all", "Don't like it", "Undecided", "Like it" and "Like it a lot". The total score ranges between 40 and 200, with a higher score indicating a higher level of satisfaction. In our study, the reliability (Cronbach's alpha) of the test was found to be 0.929 and the mean score of the scale was 147.95±21.48. The resulting grouping indicated that a value within ±1/2SD to the mean (137.21-158.69) showed moderate risk, below 137.21 indicated high risk and over 158.69 represented low risk.

Anthropometric measurements: All anthropometric measurements were conducted according to the World Health Organization standards and made in triplicate by nutritionists. Body Mass Index (BMI) was calculated from measurements of height and weight. Participants were classified according to BMI and a value between 18.5-24.9 kg/m^2 was classified as normal weight while 25 or greater was considered overweight (WHO, 1987). Fat mass was determined by bioelectrical impedance analysis with a TBF-300 Body Composition Analyzer (TANITA, Tokyo, Japan) according to the manufacturer's internal algorithm.

Statistics: Chi Square test was used to determine the difference between female participants' age, BMI, cigarette and alcohol intake, physical activity level, EAT-40 and body cathexis index distributions with respect to taking nutrition education. Student's t-test was utilized to test the difference between body fat mass, BMI, EAT-40 and body cathexis index scores of those who did and did not undertake nutrition education. Multiple regression analysis (stepwise) was used to identify how well cathexis index score is predicted by body fat mass and EAT-40 scores. The data was analyzed by using "Statistical Package for Social Sciences" (SPSS for Windows 15.0).

RESULTS

As shown in Table 1, 65.0% of participants were between 18-21 years old and the BMI of 79.5% was found to be within normal limits (18.5-24.9 kg/m^2). The prevalence of current smoking was 11.1% and 4.5% of participants were alcohol users. The level of physical activity was low (%16.6) in all groups. Overall, the EAT-40 scores of 5.9% of participants suggested high risk. The body cathexis scores also indicated high risk for 29.8% of participants in all groups.

Table 1: Baseline characteristics of respondents (n = 577)

	Group 1 (n = 299)		Group 2 (n = 278)		Total (n = 577)			
Age (Years)	n	%	n	%	n	%	χ^2	p-value
18-21	193	64.5	182	65.5	375	65.0	0.258	0.879
22-25	94	31.4	87	31.3	181	31.4		
26-29	12	4.0	9	3.2	21	3.6		
BMI (kg/m^2)								
<18.5	42	14.0	33	11.9	75	13.0	7.101	0.029
18.5-24.9	227	75.9	232	83.5	459	79.5		
>25	30	10.0	13	4.7	43	7.5		
Cigarette smoking								
Smokers	27	9.0	37	13.3	64	11.1	2.675	0.102
None-smokers	272	91.0	241	86.7	513	88.9		
Alcohol intake								
User	12	4.0	14	5.0	26	4.5	0.350	0.554
Non-user	287	96.0	264	95.0	551	95.5		
Physical activity								
Yes	55	18.4	41	14.7	96	16.6	1.381	0.240
No	244	81.6	237	85.3	481	83.4		
EAT-40 score								
Low risk (<21)	256	85.6	221	79.5	477	82.7	3.837	0.147
Moderate risk (21-30)	29	9.7	37	13.3	66	11.4		
High risk (>30)	14	4.7	20	7.2	34	5.9		
Body cathexis score								
High risk (<137)	90	30.1	82	29.5	172	29.8	0.989	0.610
Moderate risk (137-159)	119	39.8	121	43.5	240	41.6		
Low risk (>159)	90	30.1	75	27.0	165	28.6		

Table 2: Mean anthropometric measurement, EAT-40 and body perception scores with respect to undertaking nutrition education

	Group 1 (n = 299)	Group 2 (n = 278)	Total (n = 577)	t-score	p-value
Anthropometric measurements					
Fat (%)	21.4±6.89	21.2±6.36	21.3±6.64	0.444	0.658
BMI (kg/m^2)	21.5±2.97	21.0±2.81	21.2±2.90	2.142	0.033
EAT-40	14.4±10.01	15.9±11.32	15.1±10.68	-1.648	0.100
Body cathexis	148.4±21.36	147.5±21.64	147.9±21.48	0.492	0.623

Table 3: Regression analysis results for the dependent variable, i.e. body cathexis and the independent variables, i.e. BMI and EAT-40 test scores

Model summary[c]

					Change statistics				
Model	R	R^2	Adjusted R^2	Std. error of the estimate	R^2 change	F change	df 1	df 2	Sig. F change
1	0.114[a]	0.013	0.011	21.36216	0.013	7.615	1	575	0.006
2	0.144[a]	0.021	0.017	21.29907	0.008	4.412	1	574	0.036

[a]Predictors: (Constant), BMI
[b]Predictors: (Constant), BMI, tope EAT40
[c]Dependent variable: Body cathexis

Coefficients[a]

							Correlations			Collinearty statistics	
Model		Unstand. coeff. (B)	Std. error	Stand. coeff. (Beta)	t	Sig.	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	165.901	6.564	-	25.273	0.000	-	-	-	-	-
	BMI	-0.845	0.306	-0.114	-2.760	0.006	-0.114	-0.114	-0.114	1.000	1.000
2	(Constant)	169.630	6.781	-	25.014	0.000	-	-	-	-	-
	BMI	-0.896	0.306	-0.121	-2.925	0.004	-0.114	-0.121	-0.121	0.994	1.006
	Tope EAT40	-0.175	0.083	-0.087	-2.100	0.036	-0.077	-0.087	-0.087	0.994	1.006

[a]Dependent variable: Body cathexis, Unstand. = Unstandardized, Stand. = Standardized, Coeff. = Coefficients

Table 2 shows that the BMI of participants who were undertaking nutrition education (Group 1) (21.5±2.97 kg/m^2) was higher than those who were not (21.0±2.81 kg/m^2) ($p<0.05$). The difference between the body fat (%), EAT-40 and body cathexis scores of those who were (Group 1) and were not undertaking nutrition (Group 2) education was insignificant ($p>0.05$).

Multiple regression analysis was conducted by using the body image perception scale as the dependent variable and the EAT-40 score and BMI as independent variables. Variables were selected with the stepwise method. Among independent variables, BMI and EAT-40 scale seemed to be the most important ones predicting the body image perception score. Anthropometric measurements did not meet the variable selection criteria and were consequently excluded. BMI accounted for 1.3% of the total variance in body image perception scale scores and the EAT-40 test scores accounted for 0.8% ($p<0.05$). The regression coefficient showed that a negative relationship existed between the body image perception scale score and the variables of BMI and EAT-40.

DISCUSSION

School-based nutrition education programs and services can offer a systematic and efficient venue for promoting health-enhancing eating behaviors among youth. These behaviors constitute a complex phenomenon occurring as a result of arranging motor, cognitive, social and emotional developments with the help of central and peripheral factors. People do not eat only for biological development and physiological functions. On the contrary, eating is related to the development of all social relations ever after the mother-baby relationship. It is indeed associated with many satisfying and painful experiences (Saygili, 1999). A great majority of people with eating disorders find themselves overweight even though they are not (Borgen, 2001). A high score on the EAT-40 scale shows increased negative eating behaviors. The mean EAT-40 score was found to be 15.1±10.68 in this study and no meaningful difference was found between the EAT-40 mean scores of students who were (Group 1) and were not undertaking nutrition (Group 2) education ($p<0.05$). In previous studies conducted with university students in Turkey, the following mean EAT-40 scores were reported: 14.66±8.98 (Asçi *et al*., 2006), 16.74±10.45 (Oral, 2006), 20.3±14.3 (Sanlier *et al*., 2008), 20.7±10.5 (Uzun *et al*., 2006), 14.4±7.27 and 20.07±13.80 (Ilhan *et al*., 2006). Our finding was similar to these studies. In our sample, 5.9% of students were at a high risk of eating disorders. However, in other studies on young people in Turkey, the rate of disordered eating attitudes varied between 2.2 and 22.8% (Altug *et al*., 2000; Uzun *et al*., 2006; Asci *et al*., 2006; Kugu *et al*., 2002; Ilhan *et al*., 2006; Sanlier *et al*., 2008; Bas *et al*., 2004; Bas *et al*., 2006). Even among students who received nutrition education professionally, the rate of abnormal eating

attitudes and behaviors was 9.4% (Odabasi *et al*., 2007). In the present study, we found eating disorders in 4.7% of nutrition educated students (Group 1). The results of studies conducted in Western societies show the prevalence of disordered eating attitudes to be between 9.5 and 24.6% among students (Janout and Janoutova, 2004; Toro *et al*., 2006, O'dea and Abraham, 2001).

Designed to measure how satisfied people are with the different parts and functions of their bodies, the body image scale implies a higher rate of satisfaction as the score increases (Secord and Jourard, 1953). In the present study, the mean body cathexis score was found to be 147.9±21.48. The mean body cathexis score did not differ between students who were educated in nutrition (Group 1) and who were not ($p>0.05$). In other studies, the mean scores were 84.9±19.9 (Sanlier *et al*., 2008) and 135.81± 27.26 (Pinar, 2002). The rate of poor body image was 29.8% among all students who participated in this study. Özmen *et al*. (2007) reported that 56.7% of the females in his study were not satisfied with their bodies. In the present study, the percentage of participants dissatisfied with their bodies was found to be lower than the studies conducted by Sanlier *et al*. (2008) and Özmen *et al*. (2007). In yet another study conducted by Odabasi *et al*. (2007), self-evaluation score was found to be 148 (83-200) among students undertaking professional nutrition and dietetic education. In a study (Espina *et al*., 2002) conducted on 11 to 18-year-old children, the results showed that 32% of females were concerned over their body shape, which increased even more with age. On the other hand, the rate of dissatisfaction among males was much lower at 8.9%. However, there is a positive relationship between body shape concern and body weight, irrespective of gender.

The changes in body image and the increased tendency for eating disorders have been associated with social structure changes. Globalization is a comprehensive process which also includes nutrition. The rapid spread of globalization and its influence on societies bring changes in nutrition-related areas (Aslan, 2004). The present study found no difference between the EAT-40 test and body image scores of either group (Group 1, Group 2).

In a study on the relationship between BMI and possible eating disorders (Ilhan *et al*., 2006), 85,1% of participants with eating disorders were observed to actually be within normal body weight limits. This has implications for the relationship between eating disorders and body image perception. Altug *et al*. (2000) reported that eating behavior disorders were correlated with body shape and body weight. BMI has a significant correlation with body image (Hrabosky and Grilo, 2007). However, in our study, BMI accounted for only 1.3% of the total variance related to body image perception score and the EAT-40 scores accounted for 0.8% ($p<0.05$). The relationship between BMI and EAT-40 and body image perception scale score was weak and negative in this study. High BMI and EAT-40 scores reveal less satisfaction with the various parts and functions of the body. Similarly, Erol *et al*. (2000) and Maor *et al*. (2006) found no significant correlation between BMI and eating attitude scale. Furthermore, nutrition knowledge was found to have no effect on the occurrence of abnormal eating attitudes and behaviors in another study conducted by Odabasi *et al*. (2007).

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A Comparative Study on the Physicochemical Parameters of Milk Samples Collected from Buffalo, Cow, Goat and Sheep of Gujrat, Pakistan

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Abstract: This research work was carried out to compare the physicochemical parameters of milk samples of four different species like buffalo, cow, goat and sheep. Milk samples were collected from the different areas of Gujrat, Pakistan and analyzed for different physiochemical parameters, including pH, specific gravity, titratable acidity, total solids, ash, fat, protein and lactose. It was recorded that buffalo milk had 6.75±0.15 pH, 1.033±0.002 specific gravity, 0.21±0.03% titratable acidity, 18.45±0.85% total solids, 0.81±0.09% ash, 7.97±0.44% fat and 4.36±0.23% protein and 5.41±0.54% lactose. Cow milk had 6.64±0.02 pH, 1.029±0.001 specific gravity, 0.17±0.02% titratable acidity, 12.94±0.97% total solids, 0.60±0.13% ash, 4.00±0.43% fat, 3.37±0.32% protein and 4.51±0.38% lactose. Goat milk had 6.55±0.06 pH, 1.030±0.001 specific gravity 0.16±0.01% titratable acidity, 12.84±0.56% total solids, 0.75±0.13% ash, 3.97±0.51% fat, 3.15±0.32% protein and 4.39±0.34% lactose. Sheep milk contained 6.63±0.04 pH, 1.034±0.002 specific gravity, 0.23±0.01% titratable acidity, 18.13±0.21% total solids, 0.88±0.07% ash, 6.49±0.23% fat, 5.30±0.29% protein and 4.77±0.31% lactose. All the tested parameters were higher in buffalo and sheep milk than cow and goat milk.

Key words: Physicochemical parameters, buffalo milk, cow milk, goat milk, sheep milk, Gujrat

INTRODUCTION

Milk, which is the secretion of the mammary glands, is the only food of the young mammal during the first period of its life. The substances in milk provide both energy and the building materials necessary for growth. Milk also contains antibodies which protect the young mammal against infection (Bylund, 1995). Milk plays a tremendous role in building a healthy society and can be used as vehicle for rural development, employment and slowing down the migration of the rural population (Sarwar *et al*., 2002).

In the year 2008-2009, Pakistan produced 43,562 million tons of milk; of which 62.04% was contributed by buffaloes, 34.39% by cows, 1.65% by goats, 0.08% by sheep and 1.83% by camels (Anonymous, 2009).

Buffalo is the most valuable animal and is being highly liked by the people of the sub-continent. Buffalo milk is preferred more than the cow's milk (Bilal *et al*., 2006). Buffalo milk is a valuable nutrient with high content of milk proteins, lipids, vitamin and other biologically active substances (Mikailoglu *et al*., 2005).

Cow have contributed greatly to human welfare, supplying draft power, milk, meat, hides, fuel and a variety of other products (Hodgson, 1979). Cow's milk has long been considered a highly nutritious and valuable human food and is consumed by millions daily in variety of products (Heeschen, 1994).

Goats play a special role in the life of smallholder farmers. Their small size makes it possible for farmers to keep a large herd in small area (Boylan *et al*., 1996). Goat has been referred as the "poor man's cow" due to his great contribution to the health and nutrition of the landless and rural poor (Dresch, 1988). Goat milk differs from cow or human milk in having better digestibility, alkalinity and buffering capacity (Park, 1994).

Sheep milk is an excellent raw material for the milk processing industry especially in cheese production (Park *et al*., 2007). Sheep milk has higher specific gravity, viscosity, refractive index, titratable acidity, and lower freezing point than average cow milk (Haenlein and Wendorff, 2006).

The aim of present study was to assess and compare the physicochemical parameters of milk samples collected from buffalo, cow, goat and sheep of Gujrat, Pakistan.

MATERIALS AND METHODS

Collection of samples: Forty fresh milk samples were collected in sterile bottles from four species like buffalo, cow, goat and sheep (ten milk samples of each species) of Gujrat, Pakistan. Milk samples after collection were brought to the Laboratory of Chemistry Department, University of Gujrat, Gujrat, Pakistan. Physicochemical analysis of milk samples was carried out in laboratory.

Physicochemical analysis: The pH was measured using a digital pH-meter (InolabWTW Series 720) calibrated with pH 4 and 7 buffers. Specific gravity was determined by using pycnometer as described by

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AOAC (2000). Titratable acidity was determined by titrimetric method as described by AOAC (2000). Total solids content was determined according to the method of AOAC (2000). Ash content was determined by gravimetric method using a muffle furnace at 550°C as described by AOAC (2000). Fat content was determined by Rose-Gottlieb method as described by AOAC (2000). Protein content was estimated by formal titration method (Davide, 1977). Lactose content was determined by using Fehling's solution method (Triebold, 2000).

Statistical analysis: The statistical analysis was carried out using SPSS program (Statistical Package for Social Sciences version 16). The significant differences between means were calculated by one-way Analysis of Variance (ANOVA) using Tukey range test.

RESULTS AND DISCUSSION

pH: pH of milk samples collected from different species was determined at the time of sampling. The values of pH of milk samples of different species are shown in Table 1. The results showed that pH values were in the range of 6.53-7.00 in buffalo milk, 6.59-6.67 in cow milk, 6.48-6.64 in goat milk and 6.55-6.68 in sheep milk. pH values of buffalo milk were significantly ($p<0.05$) higher than that of cow and sheep milk. pH values of goat milk were lower than that of buffalo milk at a highly significant ($p<0.001$) level. The results showed that pH values of milk sample collected from cow, goat and sheep were non-significantly ($p>0.05$) different from each other.

pH values found in buffalo milk were in accordance with the findings of Braun and Stefanie (2008), Kanwal *et al.* (2004) and Imran *et al.* (2008). pH values found in cow milk were in agreement with the findings of Kanwal *et al.* (2004) and Enb *et al.* (2009). pH values of goat milk were similar to that reported by Sawaya *et al.* (1984). pH values of sheep milk were similar to that reported by Kurkdjian and Gabrielian (1962); Haenlein and Wendorff (2006).

Specific gravity: Specific gravity of milk samples collected from buffalo, cow, goat and sheep is given in Table 2. Specific gravity was found in range of 1.030-1.035 in buffalo milk, 1.027-1.031 in cow milk, 1.028-1.032 in goat milk and 1.032-1.037 in sheep milk.

Specific gravity of buffalo milk was higher than that of cow and goat milk at highly significant ($p<0.001$) level. Specific gravity of sheep milk was also higher than that of cow and goat milk at highly significant ($p<0.001$) level. There was non-significant ($p>0.05$) difference between the specific gravity of buffalo and sheep milk, cow and goat milk.

The specific gravity of buffalo milk was similar to the findings of Franciscis *et al.* (1988). The specific gravity of cow milk was similar that cited by Jenness *et al.* (1974).

Table 1: pH values of milk samples collected from buffalo, cow, goat and sheep

	pH values			
Source of milk	Min.	Max.	Mean	SD(±)
Buffalo	6.53	7.00	6.75	0.15
Cow	6.59	6.67	6.64	0.02
Goat	6.48	6.64	6.55	0.06
Sheep	6.55	6.68	6.63	0.04
Significance				
Buffalo milk v/s Cow milk	*			
Buffalo milk v/s Goat milk	***			
Buffalo milk v/s Sheep milk	*			
Cow milk v/s Goat milk	n.s			
Cow milk v/s Sheep milk	n.s			
Goat milk v/s Sheep milk	n.s			

Significance: *** = $p<0.001$, * = $p<0.05$, n.s = $p>0.05$,
Min. = Minimum, Max. = Maximum, SD = Standard Deviation

Table 2: Specific gravity of milk samples collected from buffalo, cow, goat and sheep

	Specific gravity			
Source of milk	Min.	Max.	Mean	SD(±)
Buffalo	1.030	1.035	1.033	0.002
Cow	1.027	1.031	1.029	0.001
Goat	1.028	1.032	1.030	0.001
Sheep	1.032	1.037	1.034	0.002
Significance				
Buffalo milk v/s Cow milk	***			
Buffalo milk v/s Goat milk	***			
Buffalo milk v/s Sheep milk	n.s			
Cow milk v/s Goat milk	n.s			
Cow milk v/s Sheep milk	***			
Goat milk v/s Sheep milk	***			

Significance: *** = $p<0.001$, n.s = $p>0.05$,
Min. = Minimum, Max. = Maximum, SD = Standard Deviation.

The specific gravity of goat milk was in accordance with that reported by Juarez and Ramos (1986). The specific gravity of sheep milk was quietly similar to that reported by Kurkdjian and Gabrielian (1962); Haenlein and Wendorff (2006). Specific gravity of sheep milk high was due to its high content of solids-non-fat.

Titratable acidity: The values of titratble acidity of milk samples collected from buffalo, cow, goat and sheep are given in Table 3. It was observed from results that the values of titratble acidity were in the range of 0.17-0.26% in buffalo milk, 0.14-0.19% in cow milk, 0.14-0.18% in goat milk and 0.21-0.26% in sheep milk.

The values of titratable acidity of buffalo milk were higher than that of cow and goat milk at highly significant ($p<0.001$) level. The values of titratable acidity of sheep milk were also higher than that of cow and goat milk at highly significant ($p<0.001$) level. It was observed that difference in the values of titratable acidity in buffalo and sheep milk was significant ($p<0.05$). Difference between the values of titratable acidity of cow and goat milk was non-significant ($p>0.05$).

Table 3: Titratable acidity of milk samples collected from buffalo, cow, goat and sheep

	Titratable acidity (%)			
Source of milk	Min.	Max.	Mean	SD(±)
Buffalo	0.17	0.26	0.21	0.03
Cow	0.14	0.19	0.17	0.02
Goat	0.14	0.18	0.16	0.01
Sheep	0.21	0.26	0.23	0.01
Significance				
Buffalo milk v/s Cow milk	***			
Buffalo milk v/s Goat milk	***			
Buffalo milk v/s Sheep milk	*			
Cow milk v/s Goat milk	n.s			
Cow milk v/s Sheep milk	***			
Goat milk v/s Sheep milk	***			

Significance: *** = p<0.001, * = p<0.05, n.s = p>0.05,
Min. = Minimum, Max. = Maximum, SD = Standard Deviation.

Table 4: The concentration of total solids in milk samples collected from buffalo, cow, goat and sheep

	Total solids (%)			
Source of milk	Min.	Max.	Mean	SD(±)
Buffalo	16.99	20.18	18.45	0.85
Cow	11.23	14.26	12.94	0.97
Goat	12.00	13.73	12.84	0.56
Sheep	17.94	18.53	18.13	0.21
Significance				
Buffalo milk v/s Cow milk	***			
Buffalo milk v/s Goat milk	***			
Buffalo milk v/s Sheep milk	n.s			
Cow milk v/s Goat milk	n.s			
Cow milk v/s Sheep milk	***			
Goat milk v/s Sheep milk	***			

Significance: *** = p<0.001, n.s = p>0.05,
Min. = Minimum, Max. = Maximum, SD = Standard Deviation.

The values of the titratable acidity in buffalo milk were in accordance with the findings Rehman and Salaria (2005). The values of titratable acidity in cow milk were in line with that reported by Enb *et al.* (2009) and Mahboba and Zubeir (2007). The titratable acidity values of goat milk were similar to the findings of Sawaya *et al.* (1984). The values of titratable acidity of sheep milk were similar to that reported by Kurkdjian and Gabrielian (1962), Haenlein and Wendorff (2006). Acidity of milk is due the presence of lactic acid, citric acid and phosphoric acid (Bylund, 1995).

Total solids: The concentration of total solids in milk samples collected from buffalo, cow, goat and sheep is given in Table 4. These results illustrated that the concentration of total solids was in range of 16.99-20.18% in buffalo milk, 11.23-14.26% in cow milk, 12.00-13.73% in goat milk and 17.94-18.53% in sheep milk.
The concentration of total solids in buffalo milk was higher than that in cow and goat milk at highly significant (p<0.001) level. The concentration of total solids in sheep milk was also higher than that in cow and goat milk at highly significant (p<0.001) level. Statistical analysis showed non-significant (p>0.05) difference between the concentration of total solids in buffalo and sheep milk, cow and goat milk.
The concentration of total solids found in the buffalo milk was similar to that reported by Zaman *et al.* (2007), Braun and Stefanie (2008) and Bei-Zhong *et al.* (2007). The concentration of total solids found in cow milk during this investigation was in line with the findings of Imran *et al.* (2008), Enb *et al.* (2009) and Mahboba and Zubeir (2007). The concentration of total solids found in goat milk was similar to that reported by Kanwal *et al.* (2004) and Imran *et al.* (2008). The concentration of total solids found in sheep milk was similar to the findings of Talevski *et al.* (2009).

Ash: Ash content in milk samples collected from buffalo, cow, goat and sheep is given in Table 5. The results of this study revealed that the ash content was in the range of 0.69-0.98% in buffalo milk, 0.40-0.80% in cow milk, 0.56-0.99% in goat milk and 0.78-0.98% in sheep milk. Amount of ash content in cow milk was lower than that in buffalo and sheep milk at highly significant (p<0.001) level. There was significant difference (p<0.05) between the amount of ash content in cow and goat milk. There was non-significant (p>0.05) difference between the amount of ash content in the milk samples collected from buffalo, goat and sheep.
Amount of ash content found in buffalo milk was in agreement with that reported by Enb *et al.* (2009), Khan *et al.* (2007), Imran *et al.* (2008) and Bei-Zhong *et al.* (2007). Amount of ash content found in cow milk was in accordance with that reported by Enb *et al.* (2009) and Imran *et al.* (2008). Amount of ash content found in goat milk during this study was in line with the findings of Bhosale *et al.* (2009) and Keskin *et al.* (2004). Imran *et al.* (2008) reported higher ash content in goat milk. Ash content found in sheep milk during this research work was similar to that reported by Adewumi and Olorunnisomo (2009) and Bylund (1995).

Fat: Fat content in milk samples collected from buffalo, cow, goat and sheep is given in Table 6. Results illustrated that fat content was in the range of 6.99-8.41% in buffalo milk, 3.44-4.96% in cow milk, 3.16-4.73% in goat milk and 6.09-6.80% in sheep milk.
The amount of fat content in buffalo milk was higher than that in the milk of other species at highly significant (p<0.001) level. The amount of fat content in sheep milk was higher than that in milk of cow and goat but lower than that in buffalo milk at highly significant (p<0.001) level. There was non-significant (p>0.05) difference between the amount of fat content in cow and goat milk. Fat content found in buffalo milk was in accordance with that reported by Khan *et al.* (2007). Fundora *et al.* (2001) reported lower fat content in buffalo milk than present investigation. Amount of fat content in cow milk was in

Table 5: Ash content in milk samples collected from buffalo, cow, goat and sheep

	Ash (%)			
Source of milk	Min.	Max.	Mean	SD(±)
Buffalo	0.69	0.98	0.81	0.09
Cow	0.40	0.80	0.60	0.13
Goat	0.56	0.99	0.75	0.13
Sheep	0.78	0.98	0.88	0.07
Significance				
Buffalo milk v/s Cow milk	***			
Buffalo milk v/s Goat milk	n.s			
Buffalo milk v/s Sheep milk	n.s			
Cow milk v/s Goat milk	*			
Cow milk v/s Sheep milk	***			
Goat milk v/s Sheep milk	n.s			

Significance: *** = p<0.001, * = p<0.05, n.s = p>0.05,
Min. = Minimum, Max. = Maximum, SD = Standard Deviation.

Table 6: Fat content in milk samples collected from buffalo, cow, goat and sheep

	Fat (%)			
Source of milk	Min.	Max.	Mean	SD(±)
Buffalo	6.99	8.41	7.97	0.44
Cow	3.44	4.96	4.00	0.43
Goat	3.16	4.73	3.97	0.51
Sheep	6.09	6.80	6.49	0.23
Significance				
Buffalo milk v/s Cow milk	***			
Buffalo milk v/s Goat milk	***			
Buffalo milk v/s Sheep milk	***			
Cow milk v/s Goat milk	n.s			
Cow milk v/s Sheep milk	***			
Goat milk v/s Sheep milk	***			

Significance: *** = p<0.001, n.s = p>0.05,
Min. = Minimum, Max. = Maximum, SD = Standard Deviation.

Table 7: Protein content in milk samples collected from buffalo, cow, goat and sheep

	Protein (%)			
Source of milk	Min.	Max.	Mean	SD(±)
Buffalo	4.01	4.78	4.36	0.23
Cow	2.98	3.87	3.37	0.32
Goat	2.38	3.48	3.15	0.32
Sheep	4.56	5.50	5.30	0.29
Significance				
Buffalo milk v/s Cow milk	***			
Buffalo milk v/s Goat milk	***			
Buffalo milk v/s Sheep milk	***			
Cow milk v/s Goat milk	n.s			
Cow milk v/s Sheep milk	***			
Goat milk v/s Sheep milk	***			

Significance: *** = p<0.001, n.s = p>0.05,
Min. = Minimum, Max. = Maximum, SD = Standard Deviation.

Table 8: Lactose content in milk samples collected from buffalo, cow, goat and sheep

	Lactose (%)			
Source of milk	Min.	Max.	Mean	SD(±)
Buffalo	4.56	6.21	5.41	0.54
Cow	4.01	5.00	4.51	0.38
Goat	3.70	4.88	4.39	0.34
Sheep	4.37	5.22	4.77	0.31
Significance				
Buffalo milk v/s Cow milk	***			
Buffalo milk v/s Goat milk	***			
Buffalo milk v/s Sheep milk	**			
Cow milk v/s Goat milk	n.s			
Cow milk v/s Sheep milk	n.s			
Goat milk v/s Sheep milk	n.s			

Significance: *** = p<0.001, ** = p<0.01, n.s = p>0.05,
Min. = Minimum, Max. = Maximum, SD = Standard Deviation.

line with the findings of Kanwal *et al*. (2004), Samia *et al*. (2009) and Mahboba and Zubeir (2007). Lingathurai *et al*. (2009) reported higher fat content in cow milk. Amount of fat content found in goat milk during this investigation was similar to that cited by Strzalkowska *et al*. (2009) and Bhosale *et al*. (2009). Amount of fat content found in sheep milk during this research work was lower than that reported by Adewumi and Olorunnisomo (2009), Talevski *et al*. (2009) and Pavic *et al*. (2002).

Protein: Protein content in milk samples collected from buffalo, cow, goat and sheep is given in Table 7. According to these results protein content was in range of 4.01-4.78% in buffalo milk, 2.98-3.87% in cow milk, 2.38-3.48% in goat milk and 4.56-5.50% in sheep milk. The amount of protein content in sheep milk was higher than that in the milk of other species at highly significant (p<0.001) level. The amount of protein content in buffalo milk was higher than that in the milk of cow and goat but lower than that in sheep milk at highly significant (p<0.001) level. There was non-significant (p>0.05) difference between the amount of protein content in cow and goat milk.

It was observed that protein content found in buffalo milk was in accordance with the findings of Imran *et al*. (2008). Higher protein content in buffalo milk was reported by Braun and Stefanie (2008) and Fundora *et al*. (2001). Protein content in cow milk was in line with the findings of Imran *et al*. (2008), Enb *et al*. (2009) Mahboba and Zubeir (2007) and Samia *et al*. (2009). Protein content found in goat milk during this investigation was similar to the findings of Strzalkowska *et al*. (2009) and Aneja *et al*. (2002). Protein content found in sheep milk during this research work was lower than that reported by Pavic *et al*. (2002). The reduction might be due breed difference, health status of the udder and stage of lactation.

Lactose: Lactose content in milk samples collected from buffalo, cow, goat and sheep is given in Table 8. Results illustrated that the lactose content was in range of 4.56-6.21% in buffalo milk, 4.01-5.00% in cow milk, 3.70-4.88% in goat milk and 4.37-5.22% in sheep milk. The amount of lactose content in buffalo milk was higher than that in cow and goat milk at highly significant (p<0.001) level. By comparing lactose content of buffalo and sheep milk moderately significant (p<0.01)

difference was obtained. There was non-significant ($p>0.05$) difference between the amount of lactose content in cow, goat and sheep milk.

Lactose content found in buffalo milk was similar to that cited by Imran *et al.* (2008) and Khan *et al.* (2007). Lactose content found in cow milk during this research work was similar to the findings of Samia *et al.* (2009) and Lingathurai *et al.* (2009). Lactose content in goat milk was in accordance with that reported by Imran *et al.* (2008), Strzalkowska *et al.* (2009), Bhosale *et al.* (2009) and Sawaya *et al.* (1984). Lactose content in sheep milk was similar to that reported by Pavic *et al.* (2002) and Bylund (1995).

Conclusion: All the tested parameters were higher in buffalo and sheep milk than cow and goat milk. Specific gravity, titratable acidity, ash and protein in sheep milk were higher than that in buffalo milk but pH, total solids, fat and lactose in sheep milk were lower than that in buffalo milk. All the tested parameters were similar in cow and goat milk except ash which was higher in goat milk.

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Influence of the Biotope on the Food Regime of *Chrysichthys ornatus* Boulenger 1902 and *Chrysichthys punctatus* Boulenger 1899 of the Basin of the Léfini River (Congo)

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Abstract: The objective of this survey was to know the influence of the biotope on the food regime of two species of catfishes of the basin of the Léfini river having an interest aquacade or economic. To arrive to this end, we captured and analyzed the stomachs of seventy six (76) specimens belonging to two species that are: *Chrysichthys ornatus* Boulenger 1902 (26 specimens) and *Chrysichthys puntatus* Boulenger 1899 (50 specimens). The two species have been captured in two different biotopes to know: the savanna and the forest. To appreciate the food régime according to these two biotopes, we calculated some parameters and food indications notably: the percentage of Occurrence (OC), the volumetric percentage (V) and the food indication (IA) of Lausanne. The done analyses show that the two studied species have a plastic food regime. This one varies according to the biotope. In savanna, the food regime of *Chrysichthys ornatus* has been varied more than in forest with a content stomacal where one identified eight (8) different preys against five (5) where dominate the scales cycloids with a percentage of occurrence of 75%. At *Chrysichthys puntatus*, on the other hand, the regime has been varied very in forest that in savanna with a content stomacal understanding five (5) types of preys against three (3) dominated by plant particles with a percentage of occurrence of 87%.

Key words: Catfishes, biotope, prey, scales cycloids

INTRODUCTION

Fish is a very important natural resource in tropical environment and to this title he has a reminiscent and mobilizing power that of other aquatic groups don't have. It is about a zoological group that presents the advantage to offer the varied biologic models and that has a patrimonial value recognized in some cases. He/it is also a group very threatened by the human activities. From where the necessity to know the biology and the ecology of these fish, particularly those to high value bargain.

The studies targeted on fish require research of accompaniment on their preys as well as on the surroundings in which they live, it following the place that they occupy in the networks trophiques (Daget *et al*., 1986, Leveque, 1994).

The catfishes are fish with naked skin provided with wattles. Their pisciculture, with the exception of some species as *Clarias gariepinus*, *Pangosius spp*, is not again to the point like the Cichlideses.

In pisciculture, the knowledge of the food regime of fish is necessary because the production depends on some. If food misses or if it is not appropriated, fish don't grow so much in captivity that in liberty. The administrators of the fisheries and the pisciculturists cannot ignore the relative problems to the food of fish. Besides, the persons responsible of the planning of the continental fishings use the knowledge of the nature of food, of the zones where one meets them in abundance to orient the fishers (Daget *et al*., 1991; Teugels *et al*., 1991).

It suits to recall that to Congo, very little study have been achieved on the food regime of the catfishes.

The present survey that constitutes a complement to the survey of the biology of these fish, aim to know the influence of the biotope on the food régime of two species of the basin of the Léfini river having an interest aquacole and economic.

MATERIALS AND METHODS

Localization of the survey zone: The analyzed specimens come from the captures achieved in the waters of the basin of the Léfini river. The Léfini river is an affluent of the Congo stream that is located in totality in the Trays téké as definite by Makany (1976) (Fig. 1).

Collection of the data: The present survey has been achieved during four expeditions. During this period, twelve (12) stations have been prospected of which six (6) in zone of savanna and six (6) other in zone of forest.

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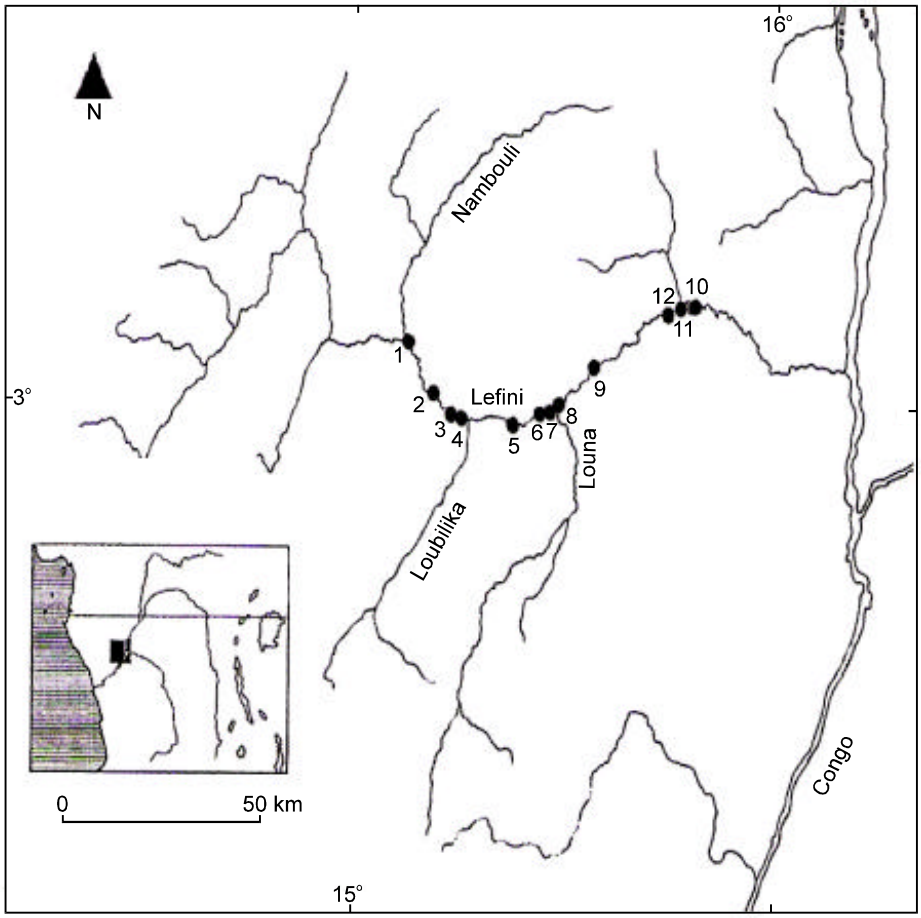

Fig. 1: Localization of the survey zone

These stations present the following features:

- Station n°1: Léfini river, Léfini-Nambouli confluent, left strand, bank to herbs, coordinates: 02°53.764'S; 015°06.846'E; altitude: 336m.
- Station n°2: Léfini river, 2.4 km upstream of the Malina camp, right strand, bank forest, coordinates: 02°59.231'S; 015°11.755'E; altitude: 318 m.
- Station n°3: Léfini river, more or less 3 km upstream of the LéfiniLoubilika confluent, right strand, bank forest, coordinates: 03°01.188'S; 015°14.239'E; altitude: 327 m.
- Station n°4: Léfini river, more or less of 2 km of the Loubilika upstream, strand left bank to herbs, coordinates: 03°01.356'S; 015°15.619'E; altitude: 336 m.
- Station n°5: Léfini river, more or less 3km upstream of the Mount Epopé, left strand, bank to herbs, coordinates: 03°01.971'S; 015°23.707E; altitude: 324 m.
- Station n°6: Léfini river, Mount Epopé, right strand, bank to herbs, coordinates: 03°01971'S; 015°23.707E; altitude: 324 m.
- Station n°7: Léfini river, Pool to snakes, right strand, 2 km upstream of the camp Project Protection Gorilla (PPG) Louna-Léfini confluent, bank forest; coordinated: 03°00.484'S; 015°28.869'E; altitude: 320 m.
- Station n°8: Léfini river, in front of the camp Project Protection confluent Gorilla, left strand, bank forest, coordinates: 02°59.649'S; 015°29.751'E; altitude: 321 m.
- Station n°9: Léfini river, Mondo camp, about 4km upstream of Mbouambé, left strand, bank forest, coordinates: 02°55.789'S; 015°34.570'E; altitude: 312 m.
- Station n°10: Léfini river, Obanga camp, downstream Mbouambé, right strand, bank to grass, coordinates: 02°49.084'S; 015°48.770'E; altitude: 303 m.
- Station n°11: Léfini river, to 600 m upstream of the Oténi camp, left strand, bank to herbs, coordinates: 02°49.480'S; 015°47.507E; altitude: 312 m.
- Station n°12: Léfini river, more or less 500m upstream of the Oténi camp, left strand, bank forest, coordinates: 02°50.251'S; 015°46.787E: altitude: 308 m.

Material of identification: For the identification of the preys contained in the stomachs, we used the material next one: a stereo microscope of mark Carl Zeiss; a foot to slide; a rule stepped up of 60 cm; a kit to dissection understanding a scalpel, of scissors, of the small pans in plastic; a balance of precision (0.01 g) having a range of 410 g; of the gloves made of latex; two glasses of watch' to put the content stomacal at the time of identification, in order to not to damage the stereo microscope, a test-tube of 1/10 ml of precision and a capacity of 10 ml permitting to have the volume of the preys considered; a syringe having a capacity of 1 ml with a precision of 1/100 ml for the obtaining of the volume of the smallest preys and the aluminum paper.

Method of identification of the preys: The used method is the one of the presence-absence of a type of prey in the stomachs, from which one calculates the percentage of occurrence that is the report of the number of stomachs where the prey is present in relation to the number of stomachs studied (Leveque, 1994).

After the opening of the stomach, the different preys have been identified with the help of the stereo microscope and the two volumes of Fauna and aquatic Flora of the Africa Sahélo-Soudanienne (Durand and Leveque, 1980; Leveque, 1994). once the identified preys, we weigh them and we take the volume of these. The weighing and the evaluation of the volume made themselves by categories of preys.

To appreciate the influence of the biotope on the food regime of the two species of the parameters and indications have been calculated. It is about the coefficient of emptiness and the food indication (IA) of Lausanne.

These parameters define themselves respectively of the following manner:

a) Coefficient of emptiness (V%):

$$V\% = \frac{\text{Number of empty digestive tubes}}{\text{Total number of tubes digestives examined}} \times 100$$

b) Food Indication (IA) of Lausanne:

The calculation of the indication food IA of Lausanne permits to use two methods:

The method of occurrence or percentage of occurrence that consist in enumerating the number of stomachs examined containing an i item given. She/it has for formula:

$$F = \frac{n_e}{N_t} \times 100$$

Where, n_e = number of stomachs containing the i prey; N_t = number of studied stomachs.

The ponderal method (p) or volumique (v) that consists to sort out then to determine the weight or the volume of every prey category for the whole sample. His/her/its formula is:

$$P = \frac{P_i}{P_t} \times 100 \quad \text{or} \quad V = \frac{V_i}{V_t} \times 100$$

Where, P_i = weight of the i preys and V_i = volume of the i preys; P_t = total weight of the preys and V_t = total volume of the preys.

The food indication of Lausanne (IA) that associates the food preferences of a species (F = frequency) and the relative importance of every category of preys (V = volume) has for formula:

$$IA = \frac{F \times V}{100}$$

This indication varies from 0 to 100
So, IA <10 it is about accidental preys,
10 <IA <25 the preys are non negligible,
25 <IA <50 it is about essential preys,
IA> 50 the preys are main.

RESULTS

Food regime of *Chrysichthys ornatus* Boulenger 1902 according to the biotope

Percentage of occurrence, volumetric percentage and food indication of the preys identified in savanna: The analysis has been made on twenty six (26) specimens of which ten nine (19) female and seven (7) males. On the set of the stomachs of studied fish for this species, eight (8) were empty and ten eight (18) contained preys of different natures, either a rate of emptiness of 30.76%. While examining the representative (Fig. 2) of the savanna, one notes that the food regime of *Chrysichthys ornatus* was qualitatively very rich and quantitatively. He/it was composed of eight types of preys: scales cycloids (75, 10.35 and 7.76%), the Décapodeses (25, 61.85 and 15.46%), larvas of Odonates (25, 20.61 and 5.15%), the plants (50, 2.06 and 1.03%), the non identified bugs (n.i) (25, 2.06 and 0.51%), the non identified preys (25, 1.03 and 0.25%), the larvas of bugs non identified (n.i) (25%) and finally the scales chéloïdes (25%).

Percentage of occurrence, volumetric percentage and food indication of the preys identified in forest: The Fig. 3, representative some forest shows that the food regime of *Chrysichthys ornatus* was qualitatively poor. He/it was composed of five (5) types of preys only: the fish-preys (66.66, 40.50 and 26.99%), the plants (66.66, 25.31 and 16.87%), the Décapodeses (33.33, 31.64 and 10.54%), the larvas of Odonates (33.33, 2.53 and 0.84%) and finally the feathers (33.33%).

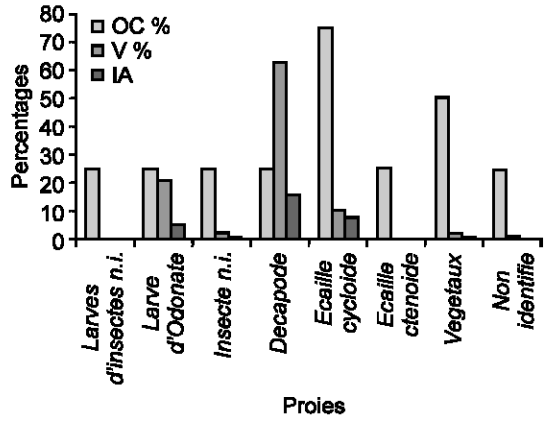

Fig. 2: Food specter of *Chrysichthys ornatus* in savanna

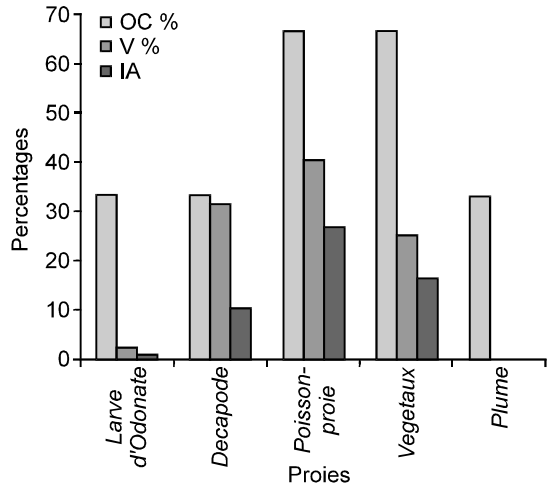

Fig. 3: Food specter of *Chrysichthys ornatus* in forest

Food regime of *Chrysichthys puntatus* Boulenger 1899 according to the biotope

Percentage of occurrence, volumetric percentage and food indication of the preys identified in savanna: The analysis has been made on fifty (50) specimens of which twenty six (26) female and twenty four (24) males. On the set of the stomachs studied for this species, twenty five (25) were empty and twenty five (25) contained preys of different natures, either a rate of emptiness of 50%.

The Fig. 4, representative some savanna shows that the food régime of *Chrysichthys puntatus* was so much qualitatively poor that quantitatively. He/it was composed of three (3) types of preys only: the larvas of Trichoptères (50, 100 and 50%), the plants (100%) and the scales cycloids (50%).

Percentage of occurrence, volumetric percentage and food indication of the preys identified in forest: The Fig. 5, representative some forest raises that five (5) types of preys have been identified composed of: plants

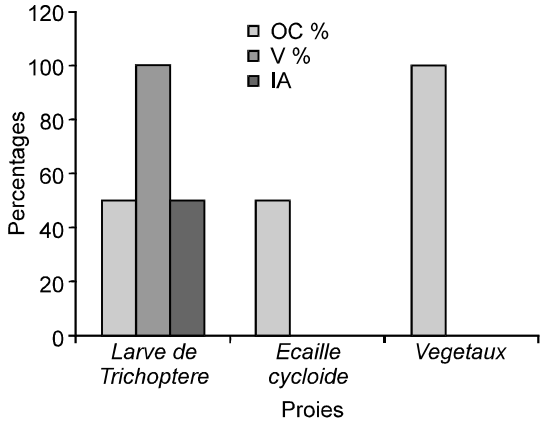

Fig. 4: Food specter of *Chrysichthys punctatus* in savanna

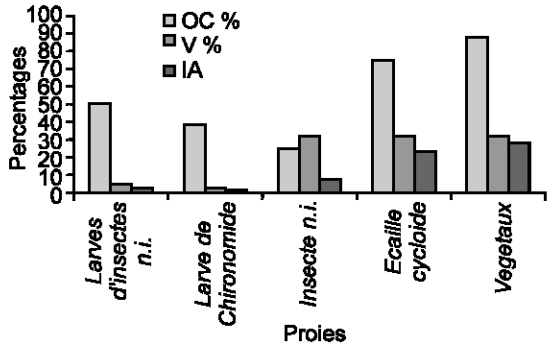

Fig. 5: Food specter of *Chrysichthys punctatus* in forest

(87, 31.57 and 27.62%), the scales cycloids (75, 31.57 and 23.67%), the non identified bugs (n.i) (25, 31.57 and 7.89%), the larvas of bugs non identified (n.i) (50, 5.26 and 2.68%) and finally the larvas of Chironomides (37.5, 2.63 and 0.98%).

DISCUSSION

Many researchers underlined the importance of the analyses stomacales to acquire valid and numerous knowledge on the food habits of fish and on the essential of their food.

Indeed, if the direct observation informs on the food habits, the nature of the preys absorbed and their possible repercussion on the habitat, one cannot get any precise information on the other hand on the plant and animal species constituent the food of a fish species, as well as on their relative importance that by an indirect method to know the analysis of the content of the digestive tube.

The exam of the contents stomacaux shows that the composition of the menu of the studied fish depends closely on the biotope. Indeed, this one is so much susceptible on the qualitative plan that quantitative to influence the development of the preys.

On the qualitative plan, one knows for example that the savanna and the forest are two different biotopes capable to encourage the types of preys differently. To suppose that the same types of preys develop themselves without distinction in the two surroundings, he/it is obvious that the fish who meet them in their habitat, will consume them without discernment. It is certainly the case of the larvas of Odonates, the Décapodeses and plants that develops himself at a time in savanna and in forest and that are identified in the digestive tubes of the two species in savanna and in forest. In the contrary case, if the biotope doesn't encourage the development of a prey given, it is obvious that such a prey won't be consumed except if fish does a migration in search of the favorite prey.

In addition to the qualitative aspect, the quantitative aspect also intervenes to orient the preferences of fish. Here the middle is put again, to strong contribution to justify the preferences of fish.

Indeed, a given middle can encourage the abundant development of individuals. In our case, one notes that every biotope tends to encourage the development of more than organisms of which eat fish in relation to the other. In these conditions, fish is going to consume as many preys as the nature placed at its disposal.

Thus, at *Chrysichthys ornatus*, the food regime has been varied more in savanna that in forest with eight (8) preys against five (5). On the other hand, at *Chrysichthys puntatus* one notes that the food regime has been varied more in forest that in savanna with five (5) preys against three (3) only in savanna.

Conclusion and perspectives: The survey of the food régime of the catfishes constitutes an element important of the knowledge of the biology of the freshwater fish of Congo in general and of the basin of the Léfini river in particular. She/it should allow term us to manage the stocks of this group better and to exploit it to good knowledge.

The done analyses show that the two studied species have a plastic food régime that varies according to the biotope. This situation seems to be bound to the presence and the abundance of food that, according to the cases, develop himself and accumulate in such biotope (savanna) or in such other (forest). The preferences observed for some preys by one or the other species seem bound also to the availability of these preys in the biotopes.

This survey should integrate other parameters as the seasons and the zones in order to have some points of support having to permit us to explain the variations of growth, some aspects of reproduction, the migrations and the behaviors of research and hold of food.

Otherwise, this analysis of contents stomacaux that informed us on the nature of the natural food as well as on the food habits of the studied species, could be completed by the experimentations on the nutrition of these species in order to see on the one hand what is the absorbed value nourishing of the various food, on the other hand what are the real food needs of these species. This acquaintance is us necessary to be able to estimate the capacity of production so much in fish of a stock determined of food in the nature that in raisings to compose highly nourishing rations.

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Isolation and Identification of Lactic Acid Bacteria from Raw Cow Milk, White Cheese and Rob in Sudan

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Abstract: The cultural, physiological and biochemical of fifty two strains of Lactic Acid Bacteria (LAB) isolated from twenty samples of Sudanese fermented milk (Rob), white cheese and raw milk were investigated. Three genera were found, *Lactobacillus* (69.23%), *Lactococcus* (19.23%) and *pediococcus* (11.53%). The dominate species were *Lactobacillus xylosus* (10 strains), *Lactococcus lactic* sub. *cremoris* (10) strain from raw cow milk. *Lactobacillus delbrucckii* (8 strains) from Rob and *Pediococcus cereviasae* (6 strains) from white cheese. *Lactobacillus casei* sp. Comprise 53.3% of cheese sample and 13.3% of raw milk sample (M_2). Other isolates were, *Lacobacillus fermentum* from rob, *Lactobacillus brevis* from white cheese and unidentified *Lactobacillus* spp. from rob. This study suggests that dairy products in Sudan may be a rich source of lactic acid bacteria.

Key words: Lactic acid bacteria, raw cow milk, fermented dairy products, health benefits, Isolation, Identification

INTRODUCTION

Lactic Acid Bacteria (LAB) have played along and important role in Food technology and have a long history of use by man for food production and food preservation. LAB are Gram positive, non spore forming bacteria, grow under anaerobic condition, *cocci* or *rods* which produce lactic acid as the major end product during the fermentation of carbohydrate (Axelsson, 2004). LAB divided into two groups based on the products produced from the fermentation of glucose. Homofermentative lactic acid bacteria and heterofermentative organism. LAB is generally associated with habitat rich in nutrients such as milk, cheese, meat, beverages and vegetables. They could be also isolated from soil, lakes, intestinal tract of animals and humans (Tserovska *et al*., 2002; Chen *et al*., 2005; Schillinger and Luke, 1989). LAB are widely used as starter cultures in the manufacture of fermented products including milk products such as yoghurt, cheese, meat products, bakery products wine and vegetable (Axelsson, 2004). Many recent studies have shown the health effects of various strains of lactic acid bacteria in humans and animals and have tried to describe their action mechanism in the digestive tract. A number and a variety of potential beneficial effects have been published. Some of these effects have already been described such as the improvement of lactose digestion and the treatment of diarrheal disorders (Gillilard, 1990; Drouault and Corthier, 2001). LAB can act as cell factories for production of food additive and aroma compounds and they have a potential use for the establishing the so called functional food (Halzapfel *et al*., 2001). The samples used in this study are traditional fermented milk (Rob) which made from pasteurized milk with addition of small amount of yoghurt, left at room temperature until it tastes sour. The white cheese which is popular in the country is made from raw unpasteurized cow milk. The processing of white cheese like feta cheese, semi hard and is kept in brine (Helen *et al*., 1981). The third sample was raw cow milk. The aim of the study was the isolation of LAB from raw milk, Rob and white cheese manufactured in Sudan so as to be preserved in cultural national bank. To ower knowledge this the first study for Isolation of lactic acid bacteria from raw milk and traditional white cheese in Sudan.

MATERIALS AND METHODS

Samples: A total of ten samples of Rob (R = 4 samples) raw cow milk (M = 2 samples) and white cheese (Ch = 4 samples) were collected from the local market inside ice box. The samples were transferred immediately to the laboratory for microbiological analysis.

Isolation of lactic acid bacteria: Twenty five grams from each sample were inoculated into 1% peptone water (Oxoid) and shacked thoroughly. Serial dilutions (1 ml) of these samples were spread on sterile Petri dishes of MRS agar. Incubation was carried out anaerobically at 30°C for 48 h. Colonies with distinct morphological differences such as color, shape and size were selected and purified by streaking at least three times in MRS agar.

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Identification of bacterial strains: All strains were maintained by weakly sub culturing on MRS agar. All tests were carried out from 48 h MRS agar cultures. The morphological characteristics of isolates were examined after staining by Gram stain (Harrigan and MacCance, 1976). Growth characteristic were monitored daily at 15, 30, 45 and 50°C in tubes of MRS broth over a 7 day period. Salt tolerance was assessed after 3 days of incubation at concentration of 4% and 6.5% Nacl in MRS broth. Catalse test was carried out by transferring a drop of MRS broth culture onto a clean slide, flooded with a drop of H_2O_2 and observed for production of effervescence. CO_2 production, from glucose in Gibson semi solid medium, after 1-7 days of incubation at 37°C. Production of ammonia form arginine was detected using Nessler's reagent. Milk curdle characteristic was tested by inoculating isolates in Litmus medium (Schillinger and Luke, 1989).

Carbohydrate fermentation: All strains were grown overnight at 37°C in MRS broth, but glucose and meat extract were omitted. Solution of 1% of the test carbohydrates were sterilized by membrane filtration (0:13 mum; pore size = 0.45 µm Adventec, Tokyo) and added to sterilized medium at a final concentration of 20 gm $litre^{-1}$. Carbohydrates utilization was assessed at 24 h, 48 h and 7day after incubation of the tested isolates at 37°C.

RESULTS AND DISCUSSION

Eighty strains were isolated from raw milk, white cheese and Rob. The Gram-negative and catalase positive strains were regarded as non-LAB (Sharpe, 1979) and were not tested further. After original characterization and grouping fifty two lactic acid bacterial cultures were isolated. Generally the LAB isolated from the three samples, belonged to the genera *Lactobacillus*, *Lactococcus* (previously) *streptococcus* and *pediococcus*. The genus *lactobacillus* was dominant in all samples tested (69.23%) followed by the genus *Lactococcus* (19.23%) and the genus *pediococcus* (11.53%). Strains M1, M2, R1, R2, R4, ch1, ch3 and ch4 were referred to genus *Lactobacillus*. They are Gram positive, non spore forming rods, catalase negative. The morphological, physiological and biochemical characteristics were tested. The data are shown in Table 1 and 2.

Most strain (83.3%) isolated from sample M1 were identified as *L. Xylosus*, which has been reclassified as *L. lactic* subs *lactic* (Schleifer *et al*., 1985). Cells were coco bacilli in shape, grew at 15, 30 and 45°C. It can tolerated 4% and 6.5% Nacl and Curdle the milk. All carbohydrates tested were fermented except arabinose, salicine and sorbitol (Table 2). Strain M2, ch3 and ch4 are similar in their morphological and physiological characteristics except strain M2 which grew at all temperature tested (15, 30, 45°C) and unable to grow at 6.5 NaCl. These strains referred to *L. casie* and can be differentiated by carbohydrate fermentation (Table 2). They were identified as *L. casie* subsp. *aloctosus* and *L. casie* subsp. *Casie* respectively. Sub species of L*actobacillus casie* can be differentiated according to growth temperature and lactose fermentation (Garvie, 1986). *Lactobacillus casie sp*. comprise 53.3% of cheese samples isolates (Table 3) and 13.3% from raw milk samples (M2). Strain R1 and strain ch1 are hetero

Table 1: Morphological and physiological characteristics of the isolated strains

	Strains									
Characteristics	M1	R1	Ch1	M2	Ch2	Ch3	R2	Ch4	R3	R4
Cell morphology	Coccbacilli	Rods	Rods	Rods	Cocci tetrads	Short rod	Rod	Rod	Cocci	Rod
Gram strain	G+	G+	G+	G+	G+	G+	G+	G+	G+	G+
Spores formation	-	-	-	-	-	-	-	-	-	-
Colony morphology	1-2 mm white	1 mm white	<1 mm white	<1 mm white	1 mm white	1 mm white	1-2 mm white	1-2 mm white	2 mm white	1-2 mm white
Catalase activity	-	-	-	-	-	-	-	-	-	-
Gas production from Gibson semi solid media	-	+	+	-	-	-	-	-	-	-
Growth at different temperatures										
15°C	+	-	-	+	+	+	-	+	-	+
30°C	+	+	+	+	+	+	-	+	+	+
45°C	+	+	-	+	-	-	+	-	-	+
50°C	-	-	-	-	-	-	+	-	-	-
Growth in NaCl										
4%	+	-	-	+	+	+	+	+	-	+
6.5%	+	-	-	-	+	+	-	+	-	+
Gelatin liquefaction	-	-	-	-	+	-	-	-	-	-
Milk curd	+	+	+	+	+	+	+	+	-	+
NH_3 from Arginine	-	+	+	-	+	-	-	-	-	-

Table 2: Biochemical characteristic of the tested strains

	Strains									
Carbon source	M1	R1	Ch1	M2	Ch2	Ch3	R2	Ch4	R3	R4
Arabinose	-	-	-	-	-	-	-	-	-	
Cellobiose	-	-	-	+	+	-	+	+	-	+
Esculin	+	-	-	+	+	-	+	-	-	+
Fructose	+	+	-	+	+	-	-	-	-	-
Glactose	+	-	+	+	+	+	-	+	+	+
Glucose	+	+	+	+	+	-	+	+	+	+
Lactose	+	+	-	+	+	-	-	+	-	-
Maltose	+	-	-	+	+	-	-	-	-	-
Mannitol	+	-	-	+	-	-	-	-	-	-
Mannose	+	+	-	+	-	-	+	-	-	+
Raffinose	+	+	+	-	-	-	-	-	-	+
Ribose	+	+	-	+	+	+	-	+	+	-
Salicine	-	-	-	-	-	-	-	-	-	-
Sorbitel	-	-	-	-	-	-	-	-	-	-
Sucrose	+	-	-	+	-	+	-	-	-	+
Trehalose	+	-	-	+	+	-	-	-	-	-
Xylose	+	-	-	-	-	-	-	-	+	+

Table 3: The group identities and number of isolates

Sample	Group	Identities of isolates	Number of isolates
Milk	M1	*Lactobacillus xylosus*	10
	M2	*Lactobacillus casie subsp. Pseudoplanturum*	2
Total			12
Rob	R1	*Lactobacillus frementum*	4
	R2	*Lactobacillus delbrucckii*	8
	R3	*Lactococcus lactis subsp. Cremoris*	10
	R4	*Lactobacillus sp.*	3
Total			25
Cheese	ch1	*Lactobacillus brevis*	3
	ch2	*Pediococcus cerevisiae*	6
	ch3	*Lactobacillus casie subsp. aloctosus*	3
	ch4	*Lactobacillus casie subsp. casie*	3
Total			15
Total number of lactic acid bacteria isolates			52

fermentative, produce CO_2 from Gibson semisolid medium. They are similar in their morphological and physiological characteristics except growth temperature. Strain ch1 grew at 30°C but strain R1 grow at 30°C and 45°C. The two strains could ferment glucose and raffinose. These strains were characterized as *L. fermentum* and *L. brevis* respectively. Strain R2 which isolated from Rob, could grow at high temperature (45 and 50°C) and tolerated growth in 4% NaCl and curdle the milk. It could ferment cellobiose, Esculin, glactose and ribose.This strain was referred to *L. delbrucckii*. It comprised 32% of the total isolates of rob. Other strain isolated from rob (R4) were not identified to species level.

Cells of strains R3 were cocci in shape. They were isolated from Rob and strains ch2 isolated from white cheese, the morphological of its cells formed tetrads. They were Gram +ve, nonspore forming, non motile, catalase negative and facultative anaerobes. The morphological, physiological characteristics are shown in Table 1 and biochemical characteristic in Table 2. They were homofermentative, not producing CO_2 from Gibson semisolid medium. Strains R3 could grow only at 30°C while strains ch2 grew at 30°C and 15°C. Strain ch2 tolerate growth at 4% and 6.5% NaCl but strain R3 was not.

The two strains R3 and ch2 were different in carbohydrate fermentation (Table 2). A according to Bergey's Manual (Gravie, 1986) strain R3 considered as *Lactococcus Lactis* subsp. *Cremoris* (formerly recognized as *streptococcus cremoris* (Axelsson, 2004). Strain ch2 were referred to as *Pedicococcus cerevisiae*. Results of the microbiological analyses, obtained from this study showed that, LAB isolated from raw milk, Rob and cheese are the normal flora of these products. Axelsson (2004) reported that LAB is generally associated with habitats rich in nutnents however *Lactococcus lactis* is actually used in dairy technology. It is well known that LAB produces a variety of antimicrobial substances with potential importance for food fermentation and preservation. Topisirovic (2006) isolated lactocci and lactobacilli from home made cheese, which were bacteriocin producer. They showed antimicrobial activity against some spoilage organism.

The same species were isolated from Rob samples, which act as normal preservative for Rob and cannot spoiled easily when stored at room temperature. The lactic acid bacteria isolated from Rob sample in this study are almost like the finding of Abdelgadir *et al.* (2001) and Hamza *et al.* (2005) who were studying lactic acid bacteria of Sudan fermented milk (Rob).

Pedicoccus cerevisiae represented 60% of total isolates of white cheese; these organisms are used for conservation of meat and vegetable foods and play an essential role as components trade starter culture by Sausage production (Raccah, 1987).

In this study *Lactococcus lactis subsp. cremoris* isolated from Rob, but plants are thought to be their original ecological niche and have been recovered from many different plants (Klijn *et al.*, 1995; Salama *et al.*, 1995). All *Lactobacillus* species isolated from the samples tested are associated with dairy industry. Hegazi and Elnaga (1980) isolated *L. brevis, L. casei subsp. pseudo plantarum, L. Casei sub sp alactosus* and *L. xylosus* from *Awshari* cheese in Iraq.

The strains which are isolated from raw milk, Rob & white cheese are of industrial uses in dairy industry. *L. lactis subsp. Cremoris*, used as dairy starter culture (Klijn *et al.*, 1995). *L. fermentum* was used with other mixed culture as a raw mixed starter. It is thermophilic cheese culture, used in traditional cheese making in Switzerland (Annika and Marc, 2004). *L. casei* strains are used as probiotic bacteria (Shah, 2002).

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The Potentials of Lime (*Citrus aurantifolia*) for Improving Traditional Corn Fermentation for Probiotic Lactic Acid Bacterial Proliferation

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Abstract: Lactic Acid Bacteria (LAB) as probiotic organisms can be sourced from fermented diary and vegetable products. The problem in Nigeria is their affordability to the greater populace, as the common sources are relatively expensive. The study was aimed at sourcing for a low cost meal rich in probiotic LAB by utilizing raw lime juice for the fermentation of corn. Washed yellow corn variety (6%) was fermented using prepared lime solution (pH 5.1-5.5). The lime-fermented corn was wet-milled, sieved and the resulting substrate was enumerated for LAB and coliform on DeMan Rogosa Sharpe' agar and MacConkey agar plates respectively. The predominant LAB were characterized as *Lactobacillus fermentum, Lactobacillus plantarum* and *Lactobacillus acidophilus*. At their predetermined optima growth periods (18 h, 22 h and 24 h), they were evaluated for antimicrobial potentials against *Salmonella typhi, Shigella dysenteriae* and *Candida albicans*. Their resistance to acid, bile and antibiotics was determined. The LAB ($\geq 3.80 \times 10^{8} \pm 012$ cfu/ml) recovered from lime-treated samples were significantly ($p<0.05$) higher than that from untreated samples ($\leq 5.70 \times 10^{6} \pm 003$ cfu/ml). The pH (≤ 5.3) and coliform counts ($\leq 0.50 \times 10^{1} \pm 013$) were also reduced in the treated sample. Although their antibiotic resistance was very low, the test LAB especially *L. acidophilus* demonstrated efficient probiotic properties by inhibiting the test pathogens, maintaining >78% acid stability at pH 3 and withstanding up to 20% bile concentration at 51.4% survival rate. These results reveal that low cost lime-fermented corn is a rich source for probiotic LAB and thus can be recommended as a food supplement.

Key words: Lime, corn fermentation, probiotics, lactic acid bacteria

INTRODUCTION

Fermentation of various food stuffs by Lactic Acid Bacteria (LAB) is one of the oldest forms of bio-preservation practiced by mankind. During the past 20 years, much of the research on LAB focused on diary Lactococci but now investigations include different LAB involved in a wide variety of fermentation processes (Soomro *et al*., 2002).

The LAB have demonstrated a number of desirable properties that are beneficial to mankind. Their potential benefits range from the prevention of harmful bacterial growth, antibiotic-associated diarrhea, colon cancer to immune support and management of lactose intolerance in the body (Anukam *et al*., 2008; Ouwehand *et al*., 2002; Brady *et al*., 2000). Based on these probiotic potentials, they are sometimes recommended by doctors and more frequently by nutritionists after a course of antibiotic to booster the body immune systems.

The crucial issue in this regard is the availability and affordability of the functional LAB for use. Much attention has been directed towards the use of diary products such as yogurt or kefir and other fermented vegetables such as sauerkraut which are not readily affordable by the greater populace. There is therefore considerable need to extend the range of foods harboring probiotic organisms from diary foods to infant formulae, baby foods, fruit juice and cereal based-products (Lee and Salminen, 1995). These functional foods may not contain only probiotic LAB culture but also the prebiotic substrates that favor their growth. The fermented drink "Fyos (Nutricia)" for instance, is a combination of probiotic culture of *Lactobacillus casei* and prebiotic oligo-fructose (Soomro *et al*., 2002). The traditional fermented corn ("Akamu") which serves as a weaning food in Nigeria has also been associated with LAB (George-Okafor *et al*., 2007; Achi, 2005; Amusa *et al*., 2005).

Akamu, as a LAB food supplement is likely to be effective in the treatment and prevention of acute diarrhea and dental caries in children, as observed by most supplements (infant formulae) (Weizman *et al*., 2005). Although affordable by the populace, the limitation for its wide use has been traced to its unpleasant odor and the health risk posed by the presence of pathogenic microflora (Chukwuemeka *et al*., 2006; Adeyemi and Beckkley, 1986).

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The application of sterility measure during the corn steeping had a positive impact on reducing the problem (George-Okafor *et al*., 2007). The critical issue, however, is the adoption of such practice by the commercial producers (local producers). Hence, there is need to seek for alternative measure for improvement, not only for odor reduction but also for probiotic enrichment, since antimicrobial properties of fermented foods seem to be their most interesting quality. The study therefore, was aimed at utilizing raw lime for the 72 h-corn fermentation with a view of creating suitable environment (e.g. low pH, vitamins) for probiotic LAB proliferation and subsequent inhibition of pathogenic flora.

MATERIALS AND METHODS

Preparation of raw lime solution: A freshly plugged lime fruits were used for the study. The fruits were thoroughly washed with tap water. Thereafter, the skin and the seeds were removed prior to mechanical homogenization. The homogenized substrate was filtered by pressure with sterile muslin cloth to obtain the 'must'. The 'must' (pH 2.0-3.0) was diluted with tap water (pH 8.0) and the resulting lime solution of pH 5.1-5.5 (optimum for most LAB) (Jay, 1992) served as the fermentation medium.

Fermentation process: A well sorted and washed yellow corn variety (6%) was steeped in a steel-made fermenter (2-liter capacity), containing the fermentation medium (1000 ml). The fermentation medium was maintained at 28-38°C during steeping (Wollowski *et al*., 2001).
Fermentation was carried out for 72 h with intermittent mechanical agitation and 48 h constant change of lime solution at 12 h intervals. The fermented corn was wet-milled (Disc Afriton Mill Model 10-2A, India) and sieved under pressure with muslin cloth to obtain the filtrate which served as the substrate for the recovery of LAB. The control sample was the filtrate from the corn steeped with only tap water (pH 8.0) and fermented under similar condition.

Bacterial strains recovery: The test and control samples were serially diluted with sterile water and 1% of each type was inoculated into DeMan Rogosa Sharpe' (MRS) medium and MacConkey broth respectively and incubated at 37°C/24 h. Thereafter, 0.1 ml of each culture was sub cultured in triplicates into the agar form of the same medium under the same experimental conditions. The pH of the fermented liquor was determined using pH meter (Model PHM 92, Copenhagen). The grown microbiomes were estimated using Quebec Colony Counter. The mean LAB counts from the two samples were compared and the significant difference at $p<0.05$ was tested using Analysis of Variance (ANOVA).

The dominant strains of LAB were identified by morphological and biochemical tests as described by Holt (1994) and Collins *et al*. (1991). They were subjected to the following assays for proof of their probiotic characteristics for *in vivo* application.

Acid tolerance assay: A modified method of Brizuela *et al*. (2001) was adopted. Each LAB isolate (10%) was incubated under reduced O_2 content, with agitation (150 rpm) at 37°C in MRS broth adjusted with 1 N HCl for pH 2.0 and 3.0 respectively. At every 6 h interval for 24 h, the viable cells were estimated spectrophotometrically (LKB) at 600 nm. The cell density (%) was expressed in relation to the mass of the control sample (Unadjusted MRS) which was taken as 100%.

Bile tolerance assay: Freshly processed bile from a young rat gall bladder was used. The bile tolerance capacity of the isolates was tested as described for the acid except that the MRS was supplemented with the bile (5-25%) and incubated only for 24 h. The MRS medium not supplemented with the bile was the control.

Antibiotic resistance assay: The antibiotics used were Erythromycin (10 µg/ml) and Chloramphenicol (10 µg/ml) as stipulated by Denou *et al*. (2008). The prepared antibiotic solutions (1%v/v) were respectively incorporated into the MRS broth and similar conditions were applied for inoculation and incubation as earlier described. The medium (MRS) without the antibiotics was the control.

Time-course determination on LAB growth rates: The young cultures (10%) of the identified LAB (*Lactobacillus acidophilus, Lactobacillus plantarum* and *Lactobacillus fermentum*) were respectively grown in freshly prepared MRS broth for 30 h. At every 2 h, a culture of each type was pooled and the viable cell density determined as earlier described. The cell density at 0 h served as the control. The time corresponding to the highest cell density was recorded as the optimum.

Assay for antimicrobial potentials: *Salmonella tyhi, Shigella dysenteriae* and *Candida albicans* obtained from University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu, were the test pathogens utilized for the assay. A loopful of each LAB culture (10%), was streaked vertically in triplicates on Nutrient agar plates and pre-incubated at 37°C for 18 h (for *L. fermentum*), 22 h (for *L. plantarum*) and 24 h (for *L. acidophilus*). Thereafter, equal concentrations (10%) of each the test pathogens were cross-streaked and incubated for 24-48 h for haloes development around the contact points. The diameters of the haloes were measured and the mean results obtained.

Table 1: Bacterial growth profile from 72 h-lime-fermented and non-lime-fermented corn

	Lime-fermented-corn			Non-lime-fermented-corn		
Sample no.	pH	LAB counts ($x10^8$ cfu ml^{-1})	Coliform counts ($x10^1$ cfu ml^{-1})	pH	LAB counts ($x10^6$ cfu ml^{-1})	Coliform counts ($x10^3$ cfu ml^{-1})
1	4.9	3.80±012	0.50±003	6.5	4.50±021	1.14±003
2	5.0	4.20±033	0.40±003	6.2	5.70±030	2.90±001
3	5.0	4.70±001	0.40±013	6.7	3.80±001	7.80±014
4	5.2	5.80±005	0.50±020	6.3	5.10±005	3.40±004
5	5.3	5.30±011	0.30±015	6.6	2.60±017	5.30±011

LAB: Lactic Acid Bacteria

RESULTS AND DISCUSSION

Bacterial recovery: The growth of LAB in MRS medium inoculated with lime-fermented corn filtrate was higher ($\geq$3.80 x 10^8±012 cfu/ml) than that recovered from non-lime- fermented corn filtrate (Table 1). The growth difference and the changes in pH after the fermentation (72 h) was significant ($p<0.05$). The significant difference in the LAB counts is an indication that lime supported the profuse growth of LAB. The support could be linked to both the acidic nature of the lime (which aided in the maintenance of adequate pH level for the LAB growth) and the supply of vitamins (B6 and C) and minerals which are essential for the metabolic activities of LAB (Okafor, 1987). The lime utilization could also be responsible for the very low pH of the lime-fermented liquor (Table 1), since LAB metabolic activities always result in high yields of acids which reduce the pH (Akpapunam and Sefa-Dedeh, 1995). Coliform count which is a measure of the safety of a product was significantly ($p<0.05$) low in the lime-fermented corn. This is an additional advantage on the utilization of lime for corn fermentation.

The LAB yield recovered from our study was higher than that (5.0 x 10^5 cfu/g) recovered from Nixtamalized maize prepared by boiling whole maize in 1% lime solution for 30 min prior to fermentation (Sefa-Dedeh *et al*., 2004). The variation could be attributed to the different fermentation methods utilized.

The dominant LAB isolates were identified to be *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus acidophilus*. These organisms especially *L. plantarum* have been severally isolated from spontaneous fermented cereal-based meals (George-Okafor *et al*., 2007; Edema and Sanni, 2006; Sefa-Dedeh *et al*., 2004).

Growth rate profile of LAB isolates: Specific growth rates of the three dominant isolates are shown in Fig. 1. *L. acidophilus* exhibited the highest growth rate but at a longer time. It achieved its optimum growth at 24 h while *L fermentum* and *L. plantarum* demonstrated their optima growths at 18 h and 22 h respectively. The observed variation in their optima growth rates could be explained from their varying physiological characteristics as LAB grow and thrive with the production and activity of their desirable enzymes (Herrero *et al*., 1996).

The LAB optima growth periods were determined in order to expose the LAB to the test pathogens when some or most of their antimicrobial products must have been produced. For instance, most bacteriocins (natural antibiotics) being secondary metabolites, are produced almost at the end of the log phase or at the onset of the stationary phase (Okafor, 1987).

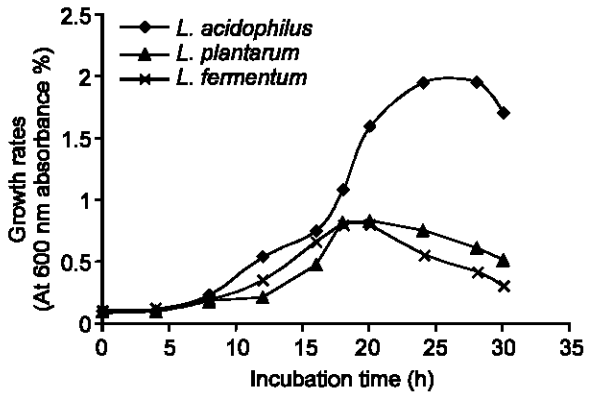

Fig. 1: Time-course on the growth rates of the lactic acid bacterial isolates

Antimicrobial sensitivity: The antimicrobial potentials of the three isolates are stipulated in Table 2. *L. acidophilus* demonstrated the highest antimicrobial activity against *Salmonella typhi, Shigella dysenteriae* and *Candida albicans* (>6.2±002 mm), followed by *L. plantarum* (>3.4±004 mm). *L. fermentum* exhibited only activity against *Candida albicans* (8.1±002 mm).

The observed antimicrobial potentials of *L. acidophilus* and *L. plantarum* are similar to other reports (Wang *et al*., 2004; McCarthy *et al*., 2003; Simakachorn *et al*., 2000). However, limited information exists on the antimicrobial potentials of *L. fermentum* against the test pathogens and the susceptibility of *Shigella dysenteriae* towards *L. acidophilus* and *L. plantarum* which were observed in this study.

The susceptibility of the test pathogens to *L. acidophilus* and *L. plantarum* could be linked to stronger antimicrobial substances produced by them. For instance, *L. acidophilus* has been implicated for the production of extracellular substance which inhibited several enteropathogens (*in vivo* and *in vitro*), including *Salmonella enterica* var *typhimurium* (Coconnier *et al*., 2000). It inhibited the adhesion of *Salmonella typhimurium* by secreting cell-surface proteins

Table 2: Antimicrobial potentials of the LAB isolates

LAB isolates	Zones of inhibition of the test pathogens (mm)		
	Salmonella typhi	*Shigella dysenteriae*	*Candida albicans*
Lactobacillus acidophilus	12.2±001	6.2±002	18.6±030
Lactobacillus plantarum	6.6±002	3.4±004	10.4±010
Lactobacillus fermentum	-	-	8.1±020

(Gueimonde *et al*., 2006). The antimicrobial activity of *L. plantarum* could be due to the presence of plantaricin which has been reported to be lethal to most microorganisms including *Entercoccus faecalis* (Knoll *et al*., 2008). On the other hand, the resistance of *Salmonella typhi* and *Shigella dysenteriae* towards *L. fermentum* could be explained by the presence of strong acidic polysaccharide outer layer of their cell walls which tend to protect the organisms against any bactericidal actions (Cruickshank *et al*., 1980). It could also be as a result of the development of a great variety of toxic substances that include several acids, ammonia and digestive enzymes.

***In vitro* acid and bile resistance:** *L. acidophilus, L. plantarum* and *L. fermentum* showed unrelated resistance patterns over exposure to acids (Fig. 2 and 3). Although the acid treatment reduced the Lactobacilli viable counts, yet *L. acidophilus* was able to achieve 43% and 81.2% stability at pH 2 and 3 for 18 h, while *L. fermentum* which exhibited the least resistance, maintained about 24% and 51% stability within the first 12 h. At 24 h acid exposure (pH 2), only *L. acidophilus* could withstand the harsh acidity which is similar to the strong acid level of an empty stomach (fasted condition). The acid tolerance of *L. acidophilus* could be through mediation by membrane ATPase as described by Lorca and Font de Valdez (2001).

Generally, the viable counts of the 3 *Lactobacillus* sp., were higher at pH 3 than at pH 2 (Fig. 2 and 3); an indication that their survival will be higher in non-fasted stomach condition (*in vivo*) than the fasted one (pH of 1 to 2). This observation is similar to the report that the decreased acidity following the consumption of food favored the increase in the viable cells of Bifidobacterium and Lactobacillus such that their resistance was significantly high (Saxelin *et al*., 1999; Hull *et al*., 1996). It is then important that the probiotic microorganisms are able to reach the Gastrointestinal Tract (GIT) and remain viable there for 4 h or more (Conway *et al*., 1987).

The relatively reduced viable counts observed by the isolates in this study is contrary to the result obtained for *Lactobacillus* strain LB-12 which achieved small cell concentration increments at pH 3 under 24 h exposure (Brizuela *et al*., 2001). However, our result is similar to the acid stability profiles exhibited by *Lactobacillus* strain B/103-1-5 at pH 3 and other *Lactobacillus* and *Bifidobacterium* species at pH 4.0 (Brizuela *et al*., 2001; Charteris *et al*., 1998).

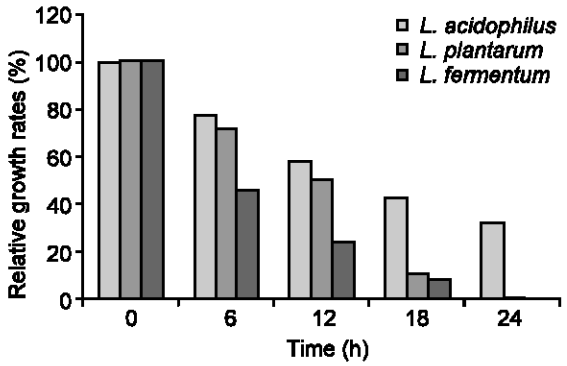

Fig. 2: Acid tolerance (pH 2) pattern of LAB

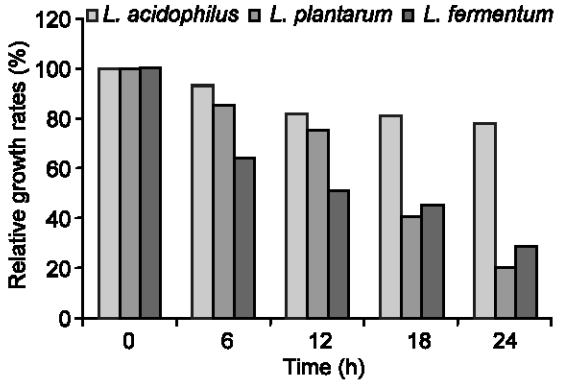

Fig. 3: *In vitro* acid effect (pH 3) on LAB growth

Table 3: Effect of bile concentrations on LAB growth

Bile Concn. (%)	Relative growth rates (%)		
	L. acidophilus	*L. plantarum*	*L. fermentum*
0	100.0	100.00	100.00
5	83.4	60.10	55.40
10	83.3	44.70	14.90
15	65.9	0.94	0.05
20	51.4	-	-
25	41.8	-	-

The resistance pattern of our LAB isolates to 5-25% of bile concentration (Table 3) is similar to that of acid stability profile. As the bile concentration increased, the viable cell concentration decreased. However, *L. acidophilus* was still able to maintain 51.4% stability at 20% bile concentration which was found to be within the normal range in human GIT (Charteris *et al*., 1998).The observed result is encouraging as most LAB were reported to be susceptible to bovine and porcine bile

Table 4: Resistant pattern of LAB to test antibiotics

	Relative growth rates (%)					
	L. acidophilus		*L. plantarum*		*L. fermentum*	
Time (h)	Ery	Chlo	Ery	Chlo	Ery	Chlo
0	100.00	100.00	100.00	100.00	100.00	100.0
6	53.46	48.74	45.35	32.94	21.30	24.1
12	18.85	16.51	14.31	8.21	0.50	-
18	3.27	0.57	1.03	-	-	-
24	-	-	-	-	-	-

Ery = Erythromycin; Chlo = Chloramphenicol

in vitro but resistant to human bile (Dunne *et al*., 2001). The *L. acidophilus* bile resistance appeared to be mediated by bile salt hydrolysis which usually results in the precipitation of cholesterol (Ahn *et al*., 2003; Ashar and Prajapathi, 1998). This reaction could be responsible for the decrease of serum cholesterol in patients treated with probiotics (Ashar and Prajapathi, 2000). The inability of *L. fermentum* and *L. plantarum* to withstand the bile concentration at 20-25% could be due to the absence or limited number of strong genes encoding bile salt hydrolysis which were identified in *L. acidophilus* (McAuliffe *et al*., 2005).

Influence of antibiotics on the LAB isolates: The inhibitory effects of antibiotic treatment on the isolates were more than that observed for acid and bile treatment. Their resistance varies with time (Table 4). At prolonged exposure (24 h), no growth was detected especially with Chloramphenicol which exerted more cidal effect than Erythromycin. The observed cidal effect of the test antibiotics on the investigated LAB, especially at prolonged time complies with the report that the prolonged use of antibiotics has the effect of destroying LAB from digestive system and as such stimulates the development and predominance of harmful microorganisms (Hull *et al*., 1996). We then hypothesized that LAB may not have possessed strong antibiotic resistant encoded genes which Hull *et al*. (1996) associated with different kinds of enterobacteria. Our result on antibiotic resistance differs from that reported by Mattila-Sandholm *et al*. (1999) and Brizuela *et al*. (2001), which stipulated that many *Lactobacillus* strains were able to survive in the presence of antibiotics. However, the *in vivo* antibiotic assay with mice (Denou *et al*., 2008), produced results similar to that of the present study. The implication of this result is the proper avoidance of indiscriminate or prolonged use of antibiotics for effective protection of probiotic LAB in the digestive system.

Conclusion: The results obtained in relation to the assayed parameters gave an insight that lime which is easily affordable richly improves the fermented corn for use as a dietary probiotic supplement. Its in-take will enrich the digestive system with adequate LAB for proper body functioning as it has mixed population of LAB with different antimicrobial characteristics. It will also improve the safety and storage stability of the product. Hence, it will be important to utilize it properly during corn fermentation for increase in LAB yields that have probiotic potentials.

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