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Proximate Composition and Preservation by Combined Methods of Chupandia (*Cyrtocarpa procera*) Pulp

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Abstract: The *Cyrtocarpa procera* pulp was analysed for proximate composition and the effects of combining techniques such as addition of Potassium Sorbate (KS), modification of water activity (a_w) and Heat treatment (H) on the microbiological shelf life of *C. procera* pulp were evaluated during 100 days of storage at 25°C. The experiment was laid out in a randomized 2^3 factorial with three replications. The pulps were periodically analyzed for UFC/g of yeast and moulds, pH, a_w and total soluble solids (°Brix)). The values (%) of proximate analyses were: moisture 83.62, protein 1.61, fat 0.30, ash 0.68, crude fiber 0.38 and carbohydrate 13.41. Metabolizable energy 62.78 kcal/100 g. The addition of 400 ppm KS ($p<0.0001$) and H (60-65°C for 3 min, $p<0.05$) can extend the stability of the pulp up to 100 days at ambient temperature (25°C) without deterioration in quality. The reliability of a model developed to predict the a_w 25°C as a function of pH and °Brix, was assessed through validation with the *C. procera* pulp results, the predictive performance of the model can be considered acceptable. Results indicate that combining heat treatment with addition of KS can be used to extend the shelf life of *C. procera* pulp kept at ambient temperature and that the low carbohydrate and gross energy values may qualify *C. procera* pulp as a good component in food formulation for the obese and diabetics.

Key words: *Cyrtocarpa procera*, combined methods, water activity, pH, total soluble solid, fruits

INTRODUCTION

Fresh fruits and vegetables have both economic and nutritional value. The National Cancer Institute encourages people to eat vegetables and fruits as part of a healthy diet to reduce risk of chronic diseases such as cancer, heart diseases and Alzheimer´s (NCI, 2001). The amount of fruits and vegetables required by an individual per day is at least 400 g (FAO/WHO, 2004). Growing and marketing of fresh fruit and vegetables are complicated by post-harvest losses in quantity and quality between harvest and consumption. Many edible indigenous fruits are nutritious, tasty and contribute to the food security of rural households. But there are very few indigenous fruit products available in the shops. Indigenous fruits are characterized by mass fruiting seasons that last only for a couple of months. This seasonality can cause supply/demand imbalances and a collapse in price at certain times. A critical element in the commercial fruit trade is the inability to store fruit for extended time periods. Kordylas (1990) estimated post-harvest fruit loss to be 20-50% in developing countries. These losses are attributed to a lack of knowledge in fruit handling and marketing. Minimal processing of raw fruits is very important, the essence of processing is to add value to and increase the palatability of fruit. Processing of fresh fruits is necessary, as the fruits´perishability rate is very high, due to lack of cold storage facilities in the rural areas. However, minimally processed foods are good media for growth of microorganisms and represent a potential health risk. The types and counts of microorganisms in minimally processed fruits are affected by the indigenous microflora, the microorganisms contaminating before and after processing, the effects of processing and packing and the intrinsic and extrinsic factors related to the fruit. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF, 1999) have limited to 5 log [CFU/g] the microbial load in minimally processed fruits to consider the product commercially acceptable. The microbial safety and stability as well as the nutritional and sensory quality of most foods are based on an application of combined preservative factors (hurdles). The most important hurdles used in food preservation are temperature, redox potential, water activity, preservatives, modified atmosphere, acidity and competitive microorganisms (Capozzi *et al*., 2009).

Cyrtocarpa procera Kunth is an indigenous Mexican tree that belongs to Anacardiaceae plant family, rarely more than 6 m tall, with twisted limbs, very pale gray bark and pinnate foliage which is native to arroyos in thorn-scrub forest from Jalisco to Puebla and Oaxaca. *C. procera* is used in traditional Mexican medicine (known locally as chupandia or copaljocote), the bark is employed to treat ailments such as diarrhea, dysentery and cough (Argueta *et al*., 1994; Soto and Sousa, 1995). *Cyrtocarpa procera* tree is found growing up to an elevation of

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1500 m. It is an essential nutritious fruit plant as this fruit is tasty and pleasant. The fruit matures in September, is generally round shaped, only about 2 cm in length, purple or yellow to orange at maturity, obliquely obtuse-oblong, styles often persistent, surface often pubescent, exocarp thin, mesocarp fleshy, endocarp bony, with 1-5 opercula; seeds apparently 1, testa with saddle-shaped patch corresponding to the hilum, cotyledons resiform (Mitchell and Daly, 1991). *C. procera* fruits are highly perishable and cannot be stored for more than 96 h at ambient temperature. However, in cold storage (3-4°C) it can be stored for 7 days. It is thus dire need that this fruit should be explored on commercial basis by scientifically assessing its true nutritional values and extending its shelf life through commercially acceptable products. Fruit preservation through processing by combined methods is a suitable combination of various hurdles, which lead to room temperature stable and also low cost fruit (Daza *et al*., 1997). Fruits processed by combined methods can be consumed as if they were fresh or used as components in food formulation such as ice cream, frozen desserts, yogurts, jellies or jams. As far as we know, no works can be found in literature about the proximate composition and the microbiological shelf life of *C. procera* pulp preserved by combined methods.

The aim of this work was to determine the proximate composition of the pulp of *C. procera*, evaluate the effect of combining temperature, water activity and sorbate in the microbiological stability of *C. procera* pulp, to monitor the storage after processing through physicochemical analysis, and assess the validation of a model developed (Gabriel, 2008) to predict the a_w 25°C as a function of pH and °Brix using the actual measure $^{a}a_w$ 25°C in *C. procera* pulp.

MATERIALS AND METHODS

Cyrtocarpa procera fruit was obtained during the month of September in a local market from the community of Acatizapan that belongs to the state of Oaxaca, Mexico. The fruits were transferred to the laboratory in plastic buckets (5 kg/bucket) and selected according to their quality attributes (without damage and in optimal state of ripeness). The sanitation of the process was ensured by personal hygiene and the use of aprons and gloves. All the utensils used were previously sanitized in 200 mg/L on free chlorine. Fruits were washed by immersion in water and given hot water treatment at 95°C for 1 min (Meyer and Paltrinieri, 1990). After blanching, fruits were properly air dried and the pulp was separated from the seeds and the peel with a fruit-peeling machine (Jersa, DRAIOO20). The pulp was used immediately for analysis and processing.

Proximate analysis of *C. procera* pulp: Crude protein, fat and ash, were determined according to AOAC (1997). The moisture content was obtained with a thermobalance (Sartorius, MA45 model), at 100°C for 45 min. The crude fiber content was carried out following procedures described by Kirk (1996). Total carbohydrates content was estimated by difference. Metabolizable energy values (kcal/100 g) were calculated by multiplying the grams of protein, fat and carbohydrates by the factor of 4, 9 and 4 kcal/g, respectively.

Processing of *C. procera* pulp and experimental setup: The experiment was laid out in a randomized 2^3 factorial with three replications. There were three factors-A) the water activity (a_w) of the pulp was lowered from the native value (0.93) to 0.91 by adding sucrose (100 g pulp/60 g sucrose). It has two levels 0.93 or 0.91, B) addition of preservative with two levels (without preservative or with 400 ppm of potassium sorbate) and C) heat treatment with two levels (without heating or heating between 60°C and 65°C for 3 min). As a result of the different processing, 8 treatments were compared (Table 1). Each replication of the treatment is consisted of 50 g pulp. After processing, *C. procera* pulp (50 g) was packaged under air atmosphere in a sterile polyethylene container with twist-off cap. The samples were stored at 25±0.60°C in a fermentation chamber (Industrias Luckie, S.A., Mexico D.F.). Data on shelf life (days), pH, a_w, Total Soluble Solids (TSS) and yeast and mould load were recorded. Data when possible were recorded at first and at 20, 40, 55, 70, 85 and 100 days of storage period.

Table 1: Experimental design for testing combined effects of a_w, antimicrobial and heat treatment in the preservation of *C. procera* pulp

	Particulars		
Treatments	Water activity (a_w)	Antimicrobial (KS)	Heat treatment (H)
T_1	0.93	0	0
T_2	0.91	0	0
T_3	0.93	400 ppm	0
T_4	0.91	400 ppm	0
T_5	0.93	0	60-65°C for 3 min
T_6	0.91	0	60-65°C for 3 min
T_7	0.93	400 ppm	60-65°C for 3 min
T_8	0.91	400 ppm	60-65°C for 3 min

Water activity: The pa_wkit (Decagon Devices, Inc., Pullman, WA, USA) was used to measure the water activity of the samples a 25°C (a_w 25°C). Prior to using the device, the instrument was calibrated using two standards: 6.0 molal NaCl (0.760a_w) and 13.31 molal LiCl (0.250a_w). The a_w 25°C values were obtained with ±0.01 accuracy. Measurements were done in triplicate.

Shelf life (days): The shelf life is a period of time which starts from harvesting and extends up to the start of rotting of fruits (Mondal, 2000).

pH measurement: The pH of samples was measured using a pH-meter (Corning pH-meter 240) at 25°C.

TSS: The total soluble solids were determined using an Abbe refractometer (Vista-C10).

Microbial counts: Yeast and mould counts were using Chloramphenicol Glucose Agar (CGA), according to ISO 7954. Three replicate samples were randomly withdrawn for each treatment. Serial dilutions were prepared by homogenizing 10 g of pulp with 90 ml of 1% sterile peptone water and diluting up to 10^{-4} concentration. Peptone and agar media were purchased from Applichem (Boca Raton FL, USA). Each dilution was plated in duplicate and incubated at 25°C for five days.

Prediction of water activity from pH and °Brix values: The predictive quadratic polynomial equation (1) for a_w 25°C was used to calculate the predicted a_w 25°C ($^{p}a_w$ 25°C) values (Gabriel, 2008) as a function of pH and °Brix values. The a_w 25°C values measured by the a_w meter ($^{a}a_w$ 25°C) were compared with the $^{p}a_w$ 25°C, to assess the predictive performance of the model.

$$a_w\ 25^{\circ}C = [0.95 + 0.03(pH) + 1.02 \times 10^{-3}\ (^{\circ}Brix) + 5.21 \times 10^{-4}\ (pH \times {}^{\circ}Brix) - 3.95 \times 10^{-3}\ (pH^2) - 1.07 \times 10^{-4}\ (^{\circ}Brix^2)]^{1/2} \quad (1)$$

Validation of the model: The performance indices, accuracy factor (A_f) and bias factor (B_f) were calculated using equations (2) and (3) respectively.

$$A_f = \text{antilog}_{10}\left\{\Sigma\left|\log_{10}\left({}^{p}a_w / {}^{a}a_w\right)/n\right|\right\} \quad (2)$$

$$B_f = \text{antilog}_{10}\left\{\Sigma\left[\log_{10}\left({}^{p}a_w / {}^{a}a_w\right)/n\right]\right\} \quad (3)$$

Where n corresponds to the number of replications employed in the model validation process.

Statistical analysis: The experiment was conducted in Factorial Completely Randomized Design (FCRD) with 8 treatments (2^3). Each treatment was replicated thrice. The results were analyzed for statistical significance using the technique of analysis of variance (ANOVA). The statistical procedures were conducted with Design-Expert 6.0.1.0. (Stat-Ease, Inc., Minneapolis, MN, USA).

RESULTS

Proximate analysis of *C. procera* pulp: The proximate composition of *C. procera* pulp is shown in Table 2. The pulp contains a low amount of carbohydrate (13.41±0.32%), very low protein (1.61±0.13%) and extremely low fat (0.30±0.0%). The values (%) of moisture, ash and crude fiber were 83.62±0.03, 0.68±0.01 and 0.38±0.15, respectively. The metabolizable energy was 62.78±1.80 kcal/100 g.

Table 2: Proximate composition of the fruit pulp of *C. procera*

Constituent	Fruit pulp (%)
Moisture	83.62±0.03
Protein	1.61±0.13
Fat	0.30±0.00
Ash	0.68±0.01
Crude fiber	0.38±0.15
Carbohydrate (by difference)	13.41±0.32
Metabolizable energy, kcal/100 g	62.78±1.80

Values are means±SD of triplicate determinations

Yeast and mould counts: The Table 3 shown the yeast and mould plate counts throughout storage of *C. procera* pulp, preserved by combined methods. Initial counts of yeast and mould in processed *C. procera* pulp were lower than 5 log [CFU/g] for all treatments. The reduction of a_w from 0.93-0.91 entailed little benefit on the microbial stability of the pulp. Treatments with addition of sucrose (T_2, T_4, T_6 and T_8) preserved the processed pulp slightly better from microbial deterioration, but this results are no statistically significant (Table 4, p = 0.1540). The heat treatment of the pulp had an immediate lethal effect on the initial microbial counts. Hence, pulps with heating between 60°C and 65°C for 3 min (T_5, T_6, T_7 and T_8) underwent a reduction in their initial microbial loads ($p<0.05$) of at least 1 log [CFU/g] compared to the rest of the samples. The addition of 400 ppm of KS had the most significant (Table 4, $p<0.0001$) and determinant influence on the product stability. The microbiological stability of pulps preserved with addition of KS (T_3, T_4, T_7 and T_8) had the maximum (100 days) shelf life, independently of the other factors involved. What is more, total yeast and mould populations were dramatically reduced during the first 20 days of storage and were kept always below 4 log [CFU/g] throughout the 100 days of storage of pulps with addition of antimicrobial, except for the pulps with a water activity of 0.93 and not heat treatment (T_3). Under the latter conditions microbial count were kept below 5 log [CFU/g] for at least 20 days and then underwent a progressive decrease, reaching 0 log [CFU/g] at 40 days of storage. Within the other involved factors the heat treatment was the most important one (Table 4, p = 0.0462) affecting the stability of the pulps. Pulps without potassium sorbate, a_w = 0.93 and no heat treatment, spoiled rapidly during the first two weeks of storage (T_1, control treatment). The two or three way interaction between aw, KS and heat treatment not showed significant (Table 4, $p>0.35$) effects on the CFU/g content of *C. procera* pulp during the storage period. These results points out that using low concentrations of KS in combination with other hurdles is a feasible way to preserve processed *C. procera* pulp for long storage periods.

Water activity: During storage, a_w varied from 0.79-0.93, comparisons of means of treatments showed (Table 5)

Table 3: Mould and yeast plate counts (Log_{10} FU/g) throughout storage of *C. procera* pulp

	Storage period (days)							Shelf life
Treatments	0	20	40	55	70	85	100	(days)
T_1	4.79±0.045	7.29±0.133	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	14
T_2	3.80±0.00009	6.96±0.916	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	25
T_3	4.63±1.170	4.16±0.072	0*	0	0	0	0	100
T_4	2.61±0.700	0	0	0	0	0	0	100
T_5	1.55±0.625	6.12±0.014	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	19
T_6	1.16±0.087	4.33±1.080	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	Sample spoiled	35
T_7	0	0	0	0	0	0	0	100
T_8	0	0	0	0	0	0	0	100

Values are means±SD of triplicate determinations, *0 = no detected (below the detection limit of the plate-counting method 0.5 log CFU/g)

Table 4: Influence of preservation factors on the total yeast and mould counts of *C. procera* pulp

Factor	Effect	Contribution (%)	Significance (p)
a_w	0.81	1.20	0.1540
SK	-6.12	67.90	<0.0001
H	-1.15	2.39	0.0462
(a_w) (SK)	-0.53	0.50	0.3517
(a_w) (H)	0.057	0.00599	0.9188
(SK) (H)	0.46	0.39	0.4130
(a_w) (SK) (H)	-0.34	0.21	0.5443
Pure error	--------	27.40	--------

that maximum a_w were observed in T_1 (control), T_3, T_5 and T_7, all of them at zero days of storage. These treatments correspond to the high level of a_w (native value) of *C. procera* pulp (0.93). The minimum values of a_w were observed in T_4 (0.79) and T_8 (0.86) at 100 days of storage. These treatments correspond to the low level (0.91) of a_w where sucrose was added. The values of a_w for the treatments T_3 and T_7 were the most stable.

Total soluble solids: Table 5 also shows the effect of treatments on changes in total soluble solids (°Brix) of *C. procera* pulp during storage. The TSS varied from 4.61-56.00 °Brix. It was evident from the data that TSS increased significantly throughout the storage period in the T_3, T_4, T_7 and T_8 treatments. Maximum TSS of 56.00 °Brix was reported in T_4 at 100 days of storage. TSS decreased in the treatments (T_1, T_2, T_5 and T_6) that resulted spoiled. Minimum TSS of 4.61 °Brix was reported in T_1 (control) at 20 days of storage.

pH: The pH of *C. procera* pulp varied during storage from 2.44-3.48. The pH in the fresh pulp was 3.44, from (Table 5) it is found that pH of the T_3, T_4 and T_8 treatments decreased during storage ($p<0.05$). The pH of T_7 did not change significantly ($p>0.05$) during storage. Minimum pH of 2.44 was reported in T_3 after 100 days of storage. The pH level of T_1 (control) and T_5 increased from 3.44 and 3.43-3.52 and 3.50 respectively after 20 days of storage and then the samples were spoiled. The pH values in T_2 and T_6 decreased from 3.42 and 3.32-3.35 and 3.30 respectively after 20 days of storage and then the samples were spoiled.

Estimation of water activity as a function of pH and °Brix: Table 6 presents the predicted and observed water activity values and the bias and accuracy factors appropriate to that data for the model proposed by Gabriel (2008), for the estimation of water activity as a function of pH and °Brix. In all the treatments resulted that ${}^{p}a_w$ 25°C>${}^{a}a_w$25°C. The A_f takes values > 1.00, A_f values calculated from model validation using *C. procera* pulp ranged from 1.04-1.14 (range: 0.10). The calculated A_f and B_f values resulted equal. The Fig. 1 shows the graphical comparisons of the predicted and actual calculated a_w 25°C by plotting ${}^{p}a_w$ 25°C (y) against ${}^{a}a_w$ 25°C (x). The Line of Equivalence (LOE) indicate the region of the plot where ${}^{p}a_w$ 25°C = ${}^{a}a_w$ 25°C. The LOE is the line with an equation y = x and diagonally bisects the plot into two equal regions. All the points fell above of the LOE. In the figure can be seen that only six model predictions had % error values greater than 10% (A_f >1.10).

DISCUSSION

The macro components are generally analyzed for their proximate amounts (Owusu-Apenten, 2005). The pulp of *C. procera* was very high in moisture content (83.62±0.03%) and this way underscore its high perishability and susceptibility to microbial infections; and this is indicative of low solid matter in the pulp. The moisture content was within the range of moisture content for fruits and vegetables [60-83 g/100 g, (FAO, 1968)]. The protein content was low (1.61±0.13%), fruits in general are usually not considered as excellent sources of proteins (Ishola *et al*., 1990). The Recommended Dietary Allowance (RDA) for protein is equal to 0.8 g per kg body weight per day (National Research Council, 1989), an adult man of 70 kg requires 56 g of protein daily, assuming complete protein absorption about 3478 g of *C. procera* pulp will satisfy the daily requirement of an adult. The lipid content of *C. procera* pulp was very low (0.30±0.00%); hence the pulp may not be possible source of oil-soluble vitamins (A, D, E and K). Holloway (1983) showed that the composition of fruits and vegetables dietary fiber were predominantly arabinose, galactose and uronic acid, which are water soluble. The low fiber content of *C. procera* pulp (0.38±0.15%) was indicative of be a bad source of dietary fiber. *C. procera* pulp was low in ash

Table 5: Effect of treatments on changes in water activity, pH and total soluble solids (°Brix) of *C. procera* pulp during storage

		Storage period (days)						
Treatments		0	20	40	55	70	85	100
Water	T_1	0.93±0.005	0.92±0.005	spoiled	spoiled	spoiled	spoiled	spoiled
activity	T_2	0.91±0.000	0.88±0.000	spoiled	spoiled	spoiled	spoiled	spoiled
	T_3	0.93±0.000	0.92±0.000	0.92±0.006	0.92±0.005	0.89±0.000	0.91±0.000	0.91±0.000
	T_4	0.91±0.000	0.87±0.003	0.89±0.006	0.88±0.000	0.84±0.000	0.82±0.000	0.79±0.006
	T_5	0.93±0.000	0.92±0.000	spoiled	spoiled	spoiled	spoiled	spoiled
	T_6	0.91±0.005	0.90±0.000	spoiled	spoiled	spoiled	spoiled	spoiled
	T_7	0.93±0.005	0.92±0.000	0.92±0.003	0.91±0.003	0.92±0.006	0.92±0.000	0.92±0.000
	T_8	0.91±0.005	0.90±0.008	0.90±0.009	0.88±0.000	0.87±0.006	0.87±0.010	0.86±0.000
	T_1	3.44±0.09	3.52±0.10	spoiled	spoiled	spoiled	spoiled	spoiled
	T_2	3.42±0.00	3.35±0.00	spoiled	spoiled	spoiled	spoiled	spoiled
	T_3	3.42±0.00	2.79±0.03	2.60±0.03	2.58±0.02	2.53±0.00	2.59±0.01	2.44±0.00
	T_4	3.44±0.00	3.26±0.02	3.28±0.00	3.25±0.05	3.22±0.00	3.23±0.00	3.06±0.01
pH	T_5	3.43±0.00	3.50±0.02	spoiled	spoiled	spoiled	spoiled	spoiled
	T_6	3.32±0.17	3.30±0.08	spoiled	spoiled	spoiled	spoiled	spoiled
	T_7	3.38±0.15	3.30±0.12	3.43±0.06	3.48±0.03	3.45±0.00	3.43±0.00	3.42±0.00
	T_8	3.43±0.11	3.44±0.13	3.28±0.05	3.26±0.07	3.28±0.01	3.23±0.07	3.12±0.04
	T_1	10.14±0.30	4.61±0.05	spoiled	spoiled	spoiled	spoiled	spoiled
	T_2	44.99±0.00	41.83±0.00	spoiled	spoiled	spoiled	spoiled	spoiled
	T_3	10.00±0.00	10.39±0.25	10.83±0.16	10.75±0.09	10.66±0.28	10.80±0.00	13.89±0.53
°Brix	T_4	44.83±0.00	46.50±0.00	49.94±1.80	50.99±0.00	52.61±0.09	55.27±1.17	56.00±6.92
	T_5	10.17±0.00	7.14±0.10	spoiled	spoiled	spoiled	spoiled	spoiled
	T_6	45.83±0.00	45.00±0.16	spoiled	spoiled	spoiled	spoiled	spoiled
	T_7	10.44±0.19	10.61±0.25	10.94±0.25	11.22±0.19	11.05±0.09	11.14±0.23	11.16±0.16
	T_8	47.05±0.45	47.00±0.72	48.83±0.76	48.61±1.45	48.48±0.83	49.49±1.59	50.33±2.01

Values are means±SD of triplicate determinations

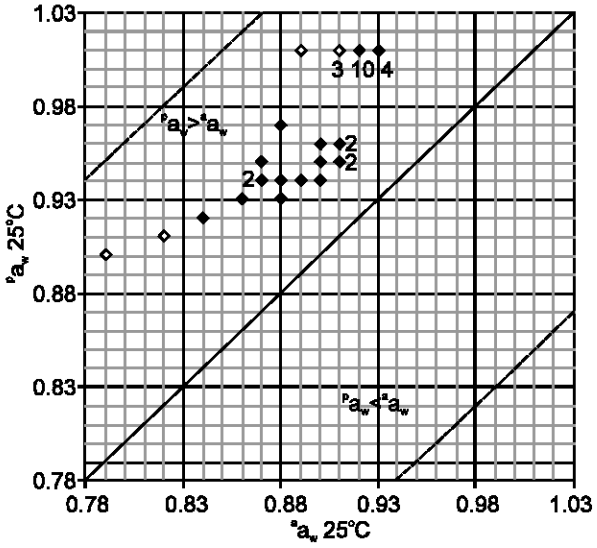

Fig. 1: Graphical comparison between the model-generated $^{p}a_w$ 25°C and actual measured $^{a}a_w$ 25°C in *C. procera* pulp. The numbers placed near the points indicate the number of points that coincided in the same coordinates. The line bisecting the plot is the LOE, while area bound by the dotted lines indicate the ±20% prediction error region where $A_f > 1.20$, ◊ model predictions that had % error values > 10% ($A_f > 1.10$), ◆ model predictions that had % error values ≤ 10% ($A_f \leq 1.10$)

content (0.68±0.01%) and this was indicative of low mineral value, especially the macrominerals. The carbohydrate content of *C. procera* pulp was very low (13.41±0.32%) and this may be responsible for insipid taste. Carbohydrates were the major energy source in the pulp, providing 85% of the energy (53.64 kcal/100 g). The low carbohydrate and gross energy (62.78±1.80 kcal/100 g) values may qualify *C. procera* pulp as a good component in food formulation for the obese and diabetics.

C. procera pulp is a High Moisture Fruit Product (HMFP) and suffers a rapid deterioration after harvest that conducts to a loss of organoleptical quality. Fungal spores and latent infections are either on the surface in the first few cell layers under the peel of the fruit (Barkai-Golan and Phillips, 1991). In order to reduce the initial microbial load by inactivating heat sensitive microorganisms, the *C. procera* fruits were exposed to 95°C for 1 min, blanching has been found to reduce the microbial load from 60-99% (Alzamora *et al*., 1995). In addition, this heat treatment has a sensitizing effect on the survivors, which would be less resistant to the stresses imposed by a_w reduction and by the presence of potassium sorbate.

The pH of the *C. procera* pulp was 3.44 (acid foods have a pH < 4.5) and is not readily spoiled by bacteria but are susceptible to spoilage by yeast and moulds (Wiley, 1997). The initial microflora of the pulp was composed of yeast and mould populations. The initial microbial counts were scarcely diminished by reduction of a_w and

Table 6: Validation of the performance of the predictive model by comparison to *C. procera* pulp

Treatment[A]	Storage period (d)	Food properties[B]		a_w 25°C			Performance indices	
		pH	°Brix	$^{p}a_w$	$^{a}a_w$	Δa_w[C]	A_f	B_f
T_1	0	3.44	10.14	1.01	0.93	0.08	1.09	1.09
T_1	20	3.52	4.61	1.01	0.92	0.09	1.10	1.10
T_2	0	3.42	44.99	0.96	0.91	0.05	1.05	1.05
T_2	20	3.35	41.83	0.97	0.88	0.09	1.10	1.10
T_3	0	3.42	10.00	1.01	0.93	0.08	1.09	1.09
T_3	20	2.79	10.39	1.01	0.92	0.09	1.10	1.10
T_3	40	2.60	10.83	1.01	0.92	0.09	1.10	1.10
T_3	55	2.58	10.75	1.01	0.92	0.09	1.10	1.10
T_3	70	2.53	10.66	1.01	0.89	0.12	1.13	1.13
T_3	85	2.59	10.80	1.01	0.91	0.10	1.11	1.11
T_3	100	2.44	13.89	1.01	0.91	0.10	1.11	1.11
T_4	0	3.44	44.83	0.96	0.91	0.05	1.05	1.05
T_4	20	3.26	46.50	0.95	0.87	0.08	1.09	1.09
T_4	40	3.28	49.94	0.94	0.89	0.05	1.06	1.06
T_4	55	3.25	50.99	0.93	0.88	0.05	1.06	1.06
T_4	70	3.22	52.61	0.92	0.84	0.08	1.10	1.10
T_4	85	3.23	55.27	0.91	0.82	0.09	1.11	1.11
T_4	100	3.06	56.00	0.90	0.79	0.11	1.14	1.14
T_5	0	3.43	10.17	1.01	0.93	0.08	1.09	1.09
T_5	20	3.50	7.14	1.01	0.92	0.09	1.10	1.10
T_6	0	3.32	45.83	0.95	0.91	0.04	1.04	1.04
T_6	20	3.30	45.00	0.96	0.90	0.06	1.07	1.07
T_7	0	3.38	10.44	1.01	0.93	0.08	1.09	1.09
T_7	20	3.30	10.61	1.01	0.92	0.09	1.10	1.10
T_7	40	3.43	10.94	1.01	0.92	0.09	1.10	1.10
T_7	55	3.48	11.22	1.01	0.91	0.10	1.11	1.11
T_7	70	3.45	11.05	1.01	0.92	0.09	1.10	1.10
T_7	85	3.43	11.14	1.01	0.92	0.09	1.10	1.10
T_7	100	3.42	11.16	1.01	0.92	0.09	1.10	1.10
T_8	0	3.43	47.05	0.95	0.91	0.04	1.04	1.04
T_8	20	3.44	47.00	0.95	0.90	0.05	1.06	1.06
T_8	40	3.28	48.83	0.94	0.90	0.04	1.04	1.04
T_8	55	3.26	48.61	0.94	0.88	0.06	1.07	1.07
T_8	70	3.28	48.48	0.94	0.87	0.07	1.08	1.08
T_8	85	3.23	49.49	0.94	0.87	0.07	1.08	1.08
T_8	100	3.12	50.33	0.93	0.86	0.07	1.08	1.08

[A]Only the treatments where was possible to measure the food properties (pH and °Brix)

[B]Values are reported as mean of triplicate determinations

[C]Calculated by subtracting the actual ($^{a}a_w$) from predicted ($^{p}a_w$)

rose rapidly. In our work, the shelf life of untreated pulp (T_1) was found to be hardly 17 days during storage at 25°C. The reduction of a_w from 0.93-0.91 had not significant effect on the stability of the pulp. Moreover, the amount of sucrose needed to get a_w reduction was too high and the pulp resulted too sweet. In addition, Jay (1986) reported that some yeast and moulds, the dominant microorganisms throughout storage, can grow in solutions of 60% sucrose, which limits the use of this hurdle. Many yeasts and moulds are able to proliferate at $a_w < 0.86$, with some osmophilic yeasts and xerophilic moulds capable of slow growth just above 0.6 (Fung, 2009). Several works have demonstrated that heat treatments may reduce the activities of enzymes that are normally enhanced during storage (Sanchez-Ballesta *et al*., 2000; Sapitnitskaya *et al*., 2006; Hirsch *et al*., 2008). In this work we analyzed the possibility of using a combined treatment with heat to delay postharvest deterioration of minimally processed *C. procera* pulp. In the treatments where heat was applied alone or in combination with a_w reduction (T_5 and T_6), there were a significant ($p<0.05$) reduction in the CFU/g in comparison to the control treatment, but was not sufficient to get a good microbiological stability of the pulps. The microorganisms survived the heat treatment, eventually reached the same viable numbers as in the untreated control and spoilage the pulps during storage. It had been demonstrated that reducing the water activity by adding sucrose, increases the resistance of yeast cells to the effects of heat (Beuchat, 1981). The addition of sorbate to the pulps had an immediate lethal effect on the initial microbial counts. Sorbate has been shown to inhibit the growth of yeast, moulds and many bacteria (Sofos and Busta, 1981). Potassium sorbate is

permitted in all countries of the world, since it is considered among the antimicrobial preservatives of low toxicity (Davidson and Juneja, 1990), it can be metabolized similarly to naturally occurring fatty acids. As a result it has received a Generally Recognized as Safe (GRAS) status (FDA, 1978). The pulp of *C. procera* has a low pH (3.44) and the pKa of potassium sorbate is 4.75, this mean that have a higher proportion of antifungal salt in the undissociated form, which is responsible of the antimycotic effect (Gould, 2000). It was obvious from the data that there was an extension of shelf-life (100 days) of *C. procera* pulps when were exposed to 400 ppm of sorbate. Compared to pulps with KS and without heat treatment, pulps with the addition of KS and heat treatment, showed only slight differences in survival of yeast and moulds. Greater lethality, relative to that in pulps without heat treatment was observed. Alzamora *et al*. (1995) and Tapia de Daza *et al*. (1996) observed the same phenomenon in studies with high-moisture fruit products, because the counts of a variety of bacteria, yeast and moulds which survived the mild heat treatment, decreased fast in the products during unrefrigerated storage, since the hurdles applied (pH, a_w, sorbate, sulfite) did not allow growth. It is well documented (Campos *et al*., 1997) that sorbate degrades appreciably as a function of time, temperature, pH and humectants used to depress a_w, during storage of preserved fruits, losing effectiveness as a hurdle. In our work the antimicrobial effect of KS was extended for at least 100 days, this could means that the concentration of KS used may be reduced. Determining the right concentration of a preservative to be used in a food is not easy. Using higher amounts than are needed mean added extra cost to the producer, a negative effect on flavor and possibly a negative effect on health. Using too little preservative has obvious consequences. It was clear that the extent of *C. procera* pulp shelf-life ($p<0.0001$) was mainly due to the addition of KS (T_3, T_4, T_7 and T_8). In T_7 and T_8 the combination with heat treatment contributed ($p<0.05$) to the conservation of the pulps and the addition of sucrose (T_4 and T_8) did not contribute ($p = 0.1540$) to prolong postharvest life of minimally processed *C. procera* pulp. These results also show that, in spite of the determinant influence of heat treatment, pulps can be preserved for 100 days adding only KS (T_3), but the pH of this treatment decreased during storage, from 3.42-2.44 and this affected the flavor significantly. Altogether; the results indicate that the best option is T_7 (native value of a_w, heat treatment and addition of 400 ppm of KS). Moreover, the pH at 100 days of storage for T_7 was 3.42 and did not change significantly during storage, this means that heat treatment and addition of KS were combined to bring additive effect in shelf life improvement and did not entail a negative influence on the sensorial perception of *C. procera* pulp. The pulp of T_7 had excellent visual appearance, flavor and aroma quality after 100 days of storage. In this way it would not be necessary to expend money in the sucrose for depress a_w. The addition of KS and the heat treatment are applicable and affordable for small producers.

The a_w is an important means of predicting and controlling the shelf life of food products. The barrier of a_w change along product storage when sucrose is utilized as humectant. Invertase (ß-D-fructofuranosidase; EC 3.2.1.26) is an irreversible hydrolase and cleaves sucrose into glucose and fructose (Montes de Oca *et al*., 1991). The magnitude of a_w were inversely proportional to the number of storage days, explicitly the longer the pulps were stored, the lower were a_w values. The hydrolysis decreased a_w of the preserved pulps because of the greater capacity of glucose and fructose to reduce a_w. Glucose and fructose have the same a_w lowering capacity (Chirife and Buera, 1996). Moreover, during storage the moisture content of pulps decreased and for this reason a_w may decrease too.

The pulps continue to accumulate TSS during storage (T_3, T_4, T_7 and T_8), the increase could be due to dehydration or hydrolysis of sucrose, because the content of carbohydrate corresponds to 80-95% of the TSS value (Fischer and Martinez, 1999). TSS content may increase also due to the alteration in cell wall structure and breakdown of complex carbohydrates into simple sugars during storage (Rathore *et al*., 2007). When pulp spoilage occurred (T_1, T_2, T_5 and T_6), the TSS decreased substantially, this could be attributable to the respiration process of the pulp (Seyoum, 2002) and microorganisms, the later need the carbohydrate for growth and reproduction (Kays, 2004).

The pH in samples T_1 (shelf-life = 14 d) and T_5 (shelf-life = 19 d) increased, because the only source of carbohydrates was the pulp (13.41±0.32%), there was a poor production of acid, this make the pulps more susceptible to spoilage by yeast and moulds. The pH in samples T_2 (shelf-life = 25 d) and T_6 (shelf-life = 35 d) decreased probably due to decomposition of fermentable substrate especially the carbohydrates in the pulp and the sucrose added to depress a_w, acid production was rapid and low pH slowed down the rate of growth of yeast and moulds (Jay, 1986). The pH of samples T_3, T_4 and T_8 decreased gradually during storage, but the decrease was more in T_3, probably the depressed a_w of samples T_4 and T_8 helped to keep the pH in higher values. The pH of sample T_7 was near the pH of the fresh *C. procera* pulp throughout the 100 days of storage. According to the 2005 version of the Food Code (FDA, 2005), that considers the interaction of a_w and pH in determining if a food is designated as a non-PHF (Potential Hazard Food), the pulp of *C. procera* (T_7) is considered as non-PHF ($a_w > 0.92$-0.95, $pH < 4.6$) throughout the time of storage.

The reliability of the developed model to predict the a_w 25°C as a function of pH and °Brix (Gabriel, 2008), was assessed through validation with the *C. procera* pulp results. A_f values calculated from model validation using *C. procera* pulp ranged from 1.04-1.14 (range: 0.10). Ideally, predictive models should display $B_f = A_f = 1$ (accurate and not biased). From the results of *C. procera* pulp $B_f = A_f > 1$, meaning that the proposed model present little bias, that is, a deviation of values over the LOE. This means that the model overestimates a_w 25°C. Carrasco *et al.* (2006) explained that, a model that forecasts a response from two predictive variables may be expected to have *Af* values that range from 1.20-1.30 or an equivalent % error range of 20-30%. Based on the results obtained from *C. procera* pulp ($A_f < 1.20$), the predictive performance of the established model can be considered acceptable. Six model predictions had % error values greater than 10%. Nevertheless these points were still within a 20% error ($A_f < 1.20$) and hence can still be considered to have acceptable accuracy. As explained by Gabriel (2008), the differences between $^{p}a_w$ 25°C and $^{a}a_w$ 25°C may be due to the influences of food components (carbohydrates, salts, proteins and other soluble components) on the a_w no present in the simulated food solutions used in establishing the model. Results of the validation with *C. procera* pulp showed that the developed model has acceptable predictive performance.

We have demonstrated that shelf life of *C. procera* pulp can be extended up to 100 days at ambient temperature (25°C) without deterioration in quality, by combining practical and inexpensive preservation methods, heating the pulp between 60°C and 65°C for 3 min ($p<0.05$) and addition of 400 ppm KS ($p<0.0001$). The combination of hurdles used was enough to control the growth of yeast and moulds populations that predominate in the indigenous microflora of *C. procera* and due to the low carbohydrate and gross energy values may qualify *C. procera* pulp as a good component in food formulation for the obese and diabetics

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Green Tea Level on Growth Performance and Meat Quality in Finishing Pigs

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Abstract: This study was designed to determinate the effects of green tea on growth performance and meat quality of finishing pigs. Ninety crossbreed "Landrace x Large White" pigs were assigned to 5 treatments a completely randomized design. The five dietary treatments were control (no green tea), antibiotic (30 ppm chlortetracycline) and 0.5, 1.0 and 2.0% of green tea added. The weight gain of pigs fed diets containing 2.0% green tea supplementation was significantly lower than that of the antibiotic supplemented ($p<0.05$). However, the feed intake and feed conversion ratio did not differ among treatments by dietary green tea addition ($p>0.05$). Crude protein in the carcass of pig showed significantly highest value in 1.0% green tea than control and other green levels ($p<0.05$) but similar value with antibiotic. The carcass grade was significantly increased in 0.5 and 1.0% green tea treatments ($p<0.05$) while Thiobarbituric Acid (TBA) value of pork was significantly decreased by 2.0% green tea supplementation ($p<0.05$).

Key words: Pigs, green tea, feed intake, thiobarbituric acid value

INTRODUCTION

It is common practice to add antibiotics to pig diets to improve pig health and productivity. However, it is generally accepted that the use antibiotics may potentially affect human health due to their ubiquitous presence (Levy, 1987; Schwarz *et al*., 2001). But its may result in proliferation of antibiotics-intensive bacteria and thus a decrease in the therapeutic effectiveness of antibiotics used to treat a variety of bacterial infections in humans. This threat to human health has urged European countries to ban antibiotics and alternatives to antibiotics are currently being encouraged (World Health Organization; cited by Humphrey *et al*., 2002). Green tea *(Camellia sinensis*) has been considered as a natural product that is non-toxic. The catechins, the main components of green tea, have many biological and biochemical effects such as anti-carcinogenesis (Mukhatar and Ahmad, 1999), anti-mutation (Okuda *et al*., 1984) and anti-oxidation (Weisburger *et al*., 2001). It has been reported that catechin, a major components of tea polyphenol, has various polyphenol, physiologic modulative activities, such as antibacterial effects, radical scavenging action and inhibitory effect on allergic reactions. Several mechanisms of EGCG's anti-allergic effects have been suggested, such as the inhibition of histamine release from basophilic cells, but the precise mechanisms still remain unclear.

Besides the human consumption, the low graded green tea and green tea wastes were used as feed ingredients for fish (Kono *et al*., 2000), broiler (Kaneko *et al*., 2001; Cao *et al*., 2005), calves (Ishihara *et al*., 2001) and pigs (Suzuki *et al*., 2002) and the positive effects of green tea on animal performance have been discovered already. Yang and Koo (1997) reported that green tea feeding reduced the serum and liver cholesterol contents in rats. Biswas and Wakita (2000) reported that green tea supplementation to layer diet was reduced the cholesterol content of the egg yolk. However, there is limited information available about to using a green tea as feed supplement for pigs, particularly in finishing pigs. Therefore, the objective of this study was to investigate the effects of green tea on growth performance and meat quality in finishing pigs and determine the optimal addition level of green tea to finishing pig diet.

MATERIALS AND METHODS

Animals and design: Ninety crossbreed (Landrace x Yorkshire) finishing pigs with both sexes averaged (70.81±0.14) kg of initial body weight were housed in semi-open concrete floor pens. The pigs were assigned to 5 treatments in a completely randomized design. Each treatment had 3 replicates with 6 pigs per replication. The five dietary treatments were control (no green tea), antibiotic (30 ppm chlortetracycline) and 0.5, 1.0 and 2.0% green tea powder added diets. All diets were formulated to meet or exceed nutrient requirements of finishing pigs, NRC (1994). The formula and chemical composition of basal diet used in this experiment are given in Table 1. The diets and drinking water were supplied *ad libitum*. At the end of the experiment, pigs reached at 110 kg of body weight were

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Table 1: Formula and chemical composition of basal diet (%) (as fed basis)

Ingredients	(%)
Yellow corn	45.15
Wheat (13%)	25.00
Wheat bran	4.00
Soybean meal (40%)	16.00
Limestone	0.78
Calcium phosphate-25/18	1.10
Salt	0.25
Vit-min. premix[1]	0.55
Animal fat	2.50
Molasses	4.50
L-Lysine	0.17
Total	100
Chemical composition[2]	
ME (kcal/kg)	3,160
C. Protein (%)	15.00
C. Fat (%)	4.86
Lysine (%)	0.80
Ca (%)	0.78
Avail. P (%)	0.55

[1]Vit-min. premix : vit A, 6,000IU; vitmin D_3, 800IU; vitmin E, 20IU; vit K_3, 2mg; thiamin, 2mg; riboflavin, 4mg; vitmin B_6, 2mg; vitmin B_{12}, 1mg; pantothenicacid, 11mg ; niacin, 10mg; biotin, 0.02mg; Cu, 21mg; Fe, 100mg; Zn, 60mg; Mn, 90mg; I, 1.0mg; Co, 0.3mg; Se, 0.3mg. [2]Calculated value

transported to the slaughter house located in Naju city, Gwangju, Korea.

Catechin components of green tea: Before mixing green tea to pig diet, the catechin contents of green tea was determined according to the method devised by Ikeda *et al.* (2003). Approximately 100 mg green tea powder was dissolved into 100 ml double distilled water and heated in a water bath at 80°C for 30 min. After cooling, it was filtered through Whatman No.1 paper. Then filtrate was transferred to a separating funnel and the chloroform was added. After washing 3 times with chloroform, the solution was separated into 2 layers. After collected water layer, the catechin component was fractionated with 25 ml of ethyl acetate. Then ethyl acetate fraction was evaporated at 30°C in a rotary evaporator under the nitrogen flow and concentrate was dissolved in methanol and passed through a membrane filter (0.45 μm polyvinylidene diflouride) and Sep-Pack C_{18} cartridge. Finally, the catechin components of green tea powder were isolated by HPLC (Model 501, Waters, Milford, USA). The catechin components of green tea were as follows: total catechin 16.16%, of which Catechin 0.94%, Epicatechin 1.41%, Epigallocatechin 1.67%, Epicatechin gallate 2.46% and Epigallocatechin gallate 9.68% in dry matter basis.

Measurements and chemical analysis: The body weight of pigs was measured every two weeks from the initial day to the final day of the experiment for calculate the body weight gain. The feed intake of pigs was recorded by offering weighed quantity of feed and weighing their residues in biweekly basis. The feed conversion ratio was calculated on the basis of unit feed consumed to unit the body weight gain of pigs. Carcass quality traits in terms of slaughter weight, back fat thickness, carcass grade were determined according to the methods of Korean Meat Evaluation System procedure. The carcass composition was analyzed by common methods of AOAC (1990). Tiobarbituric Acid (TBA) value of pork was assayed through the methods of Vernon *et al.* (1970). Sensory evaluation of pork meat was organoleptically evaluated by a panel of trained judges on the three point hedonic scale as juiciness, tenderness and flavor.

Statistical analysis: The data from this study was preformed by SAS Package Program (1990) to estimate variance components for completely randomized design. Duncans' multiple comparison tests (1955) were used to compare the significant differences between treatment means. Differences were statistically assessed at p<005%.

RESULTS AND DISCUSSION

Growth performance: Effects of green tea on growth performance in finishing pigs are shown in Table 2. Throughout the first week of experiment, there were no significant differences in weight gain, feed intake and feed conversion ratio of pigs fed diet containing 0.5-2.0% green tea and antibiotics supplementation (p>0.05). At 4-6 week of the experiment, the feed intake of pigs was significantly decreased by dietary supplementation of 2.0% green tea (p<0.05). However, the feed conversion ratio of pigs was significantly higher in 1.0% green tea treatment than that of the control of (p>0.05). In over all periods, the weight gain of pigs was significantly lowered in 0.5 and 2.0% green tea added treatments (p<0.05) except 1.0% green tea which didn't differ from the antibiotic treatment (p<0.05). This significant reduction of weight gain for 0.5 and 2.0% green tea treatment due to the gradually decrease of weight gain throughout the experiment period and the lower final body weight obtained from pigs fed diets containing 0.5 and 2.0% green tea supplementation. Sayama *et al.* (2000) reported that 2.0 and 4.0% Japanese green tea supplementation to the diet was reduced a body weight in rats. Kaneko *et al.* (2001) reported that 0.5% green tea extracts mixed to the drinking water had reducing effects on weight gain of the broilers. However, El-Deek and Al-Harthi (2004) reported that 0.5% of green tea addition to the broiler diet had no adverse effects on weight gain, feed intake of the broilers.

Results of our experiment demonstrated that the feed intake and FCR of pigs was significantly increased in 2.0% green tea treatments compared to that of the antibiotic treatment (p<0.05). Similar results were observed by Biswas and Wakita (2001) who reported that supplemental green tea powder for broiler chicks tended to decrease feed intake and body weight gain with a higher dose, also improved the feed conversion ratio of broilers. Some researchers stated that the

Table 2: Effects of green tea on growth performance in finishing pigs

Treatments			Green tea		
Items	Control	Antibiotics	0.5%	1.0%	2.0%
0~2 weeks					
Weight gain (kg)	12.28	13.67	14.17	13.33	12.94
Feed intake (kg)	41.22	43.94	43.22	41.17	39.72
FCR (feed/gain)	3.35	3.26	3.06	3.09	3.09
2~4 weeks					
Weight gain (kg)	13.28	15.39	14.22	14.22	13.44
Feed intake (kg)	46.11	51.44	47.83	48.17	46.67
FCR (feed/gain)	3.56	3.37	3.37	3.40	3.55
4~6 weeks					
Weight gain (kg)	13.83	13.22	12.89	12.22	12.61
Feed intake (kg)	52.11^{ab}	56.67^{a}	52.50^{ab}	51.94^{ab}	48.67^{b}
FCR (feed/gain)	3.76^{b}	4.32^{a}	4.07^{ab}	4.25^{a}	3.86^{ab}
0~6 weeks					
Weight gain (kg)	39.39^{b}	42.28^{a}	41.28^{ab}	39.78^{ab}	39.00^{b}
Feed intake (kg)	139.44	152.06	143.56	141.28	135.06
FCR (feed/gain)	3.54	3.60	3.48	3.55	3.46

^{a,b,c}Means with different superscripts within same row are significantly different ($p<0.05$)

Table 3: Effects of green tea on meat quality profiles in finishing pigs

Treatments			Green tea		
Items	Control	Antibiotics	0.5%	1.0%	2.0%
Slaughter wt (kg)	83.00	84.78	85.17	83.83	82.06
Back fat (mm)	25.33^{a}	23.67^{ab}	21.94^{bc}	22.11^{bc}	21.22^{c}
Carcass grade	3.00^{b}	3.44^{ab}	3.50^{a}	3.67^{a}	3.22^{ab}
Shear value (kg)	3.37	3.33	3.86	5.02	2.94
Heating loss (%)	32.01	32.88	33.07	34.64	31.64
Meat color					
Lightness (L)	53.44	52.99	48.97	52.12	51.97
Redness (a)	8.66	8.80	7.39	7.69	7.58
Yellowness (b)	4.77	5.03	2.73	3.72	4.08
Sensory evaluation					
Juiciness	4.00	3.93	3.53	3.83	4.13
Tenderness	4.43	4.10	4.17	4.13	4.93
Flavor	4.40	4.17	4.40	4.00	4.47

^{a,b,c}Means with different superscripts within same row are significantly different ($p<0.05$)

catechin contents of green tea, mainly Epicgallocatechin Gallete (EGCg) could inhibited a digestive lipase activity and affect on the lipid metabolism of animals (Yang and Koo, 1997; Sayama *et al*., 2000; Weisburger *et al*., 2001) so, thus reduction of body weight and weight gain in finishing pigs by green tea powder supplementation to the pig diet might be ascribed to green tea catechins. Green tea catechins may favor the slow digestion of carbohydrates which prevents sharp spikes of insulin in the blood and favors fat-burning over fat-storage and prove a reduction body weight and weight gain of animals with ingestion of green tea and their components (INPR, 2000).

Meat quality parameters: Effects of green tea on pig meat quality parameters are presented in Table 3. There were no significant differences in slaughter weight of pigs diets containing 0.5-2.0% green tea and antibiotic supplementations ($p>0.05$). The back fat of thickness was significantly decreased in 1.0 and 2.0% green tea treatments compared to that of the control ($p<0.05$). This reduction on back fat thickness for green tea feeding, clearly caused by low weight gain obtained from pigs fed 1.0 and 2.0% green tea diets. The carcass grade was significantly higher in 0.5 and 1.0% green tea treatments than that of the control ($p<0.05$). Although, there were no significant differences in shear value and heating loss of meat from pigs fed diets containing 0.5-2.0% green tea and antibiotic supplementations ($p>0.05$). There were no significant differences in meat color changes (lightness, redness and yellowness) and sensory evaluation traits in terms of juiciness, tenderness and flavor in different level of green tea and antibiotic treatments ($p<0.05$). Lee (2005) reported that 0.02% green tea addition to diet had no effects on back fat and carcass grade and meat color of the beef cattle (Lee, 2005). However, 2.0% green tea inclusion to layer diet increased the yellowness of the egg yolk.

Carcass composition: The moisture content of Longissimus Muscle (LM) from pig fed a diet containing 1.0% green tea supplementation was significantly

Table 4: Effects of green tea on carcass composition in finishing pigs (%)

Treatments			Green tea		
Items	Control	Antibiotics	0.5%	1.0%	2.0%
Moisture	73.07^{ab}	71.66^{b}	72.84^{ab}	73.33^{a}	72.76^{ab}
Crude protein	22.01^{b}	23.15^{ab}	22.13^{b}	24.02^{a}	22.13^{b}
Crude fat	2.06	2.02	1.65	1.82	1.25
Crude ash	2.09^{b}	2.58^{a}	2.04^{b}	1.86^{c}	1.83^{c}

^{a,b,c}Means with different superscripts within same row are significantly different ($p<0.05$)

Table 5: Effects of green tea on TBA value of meat in finishing pigs (μmol MDA/100g)

Treatments			Green tea		
Weeks	Control	Antibiotics	0.5%	1.0%	2.0%
Fresh	1.08	1.17	1.11	1.11	0.98
1 week	1.91^{ab}	2.12^{a}	2.00^{ab}	1.82^{b}	1.75^{b}
2 week	2.89^{ab}	2.92^{a}	2.86^{ab}	2.83^{ab}	2.74^{b}
3 week	4.09^{a}	4.12^{a}	4.03^{a}	3.82^{b}	3.51^{b}

^{a,b}Means with different superscripts within same row are significantly different ($p<0.05$).
TBA-Thiobarbituric Acid Value, MDA- Malondialdehyde

higher ($p<0.05$) than that of the antibiotics treatment (Table 4). However, the crude protein of LM was significantly increased in 1.0% green tea treatment compared to that of the control ($p>0.05$). The crude fat content tended to decrease with increasing level of green tea addition but without significant differences ($p>0.05$). Ikeda *et al.* (1992) reported that the catechin components of green tea have an inhibitory effect in lipid metabolism in rats. The oolong tea supplementation rat to diet had an inhibition on pancreatic lipase activity and reducing the fat adipose in rats. The crude ash content of pork was significantly lower in 1.0 and 2.0% green tea treatments than that of the antibiotic treatment ($p>0.05$).

Lipid oxidation of pig meat: Effects of green tea on Thiobarbituric Acid (TBA) value of pork meat are shown in Table 5. Green tea studies showed that green tea extracts had a dose-dependent inhibitory activity against end stage lipid peroxide decomposition product formation and early lipid oxidation (Pearson *et al.*, 1998; Yamane *et al.*, 1999). Results of present study demonstrated that TBA value of meat was significantly decreased by dietary 1.0 and 2.0% green tea supplementation ($p<0.05$) at 4°C for one week of storage. After the 2 week of storage at same temperature, the TBA value of meat was significantly lower in 2.0% green tea treatment ($p>0.05$) although the values were significantly lower in 1.0 and 2.0% green tea treatments than that of the antibiotic treatment ($p<0.05$) after preservation of 3rd week. Yoshino *et al.* (1994) stated that the TBA value of blood plasma was reduced by 10% dietary green tea supplementation in rats.

Conclusion: The supplementation of 0.5 and 2.0% green tea to the pig diet slightly reduced the weight gain in finishing pigs. The results of current study indicated that up to 2.0% inclusion level of green tea to pig diet showed adverse effects on growth performance and meat quality characteristics in finishing pig. The incorporation of 1.0-2.0% green tea to pig diet had reducing effects on oxidation degree (TBA value) of pig meat. Based on the results of our study, it can be suggested that 1.0% of dietary addition of green tea is effective in improving growth performance and meat quality in finishing pigs.

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Blood Lipid Profile, Oxidation and Pressure of Men and Women Consumed Olive Oil

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Abstract: Hypercholesterolemia and hypertension is one of the most important risk factors for Coronary Heart Disease (CHD). Recent studies have pointed out the possibility that olive oil may reduce these factors. The present study was designed to assess the effect of three olive oils contained different levels of phenolic comounds on blood lipid profile, oxidation status and pressure of normo cholesterol and pressure men and women. 12 men and 13 women participated in the study. Subjects consumed there habitual diets with low phenol-, medium phenol-, or high phenol- containing olive oil for 4 weeks each with a 4-week washout period between them. Consumption of these oils was nonsignificantly reduced triglycerides, total cholesterol, free cholesterol and cholesterol ester concentrations and no marked effect in phospholipids concentration. However, resulted in significantly reduction in LDL-c and rising in HDL-c concentrations in plasma of men and women. These reduction and rising effects were increased with increasing the phenols content and no worth differences between men and women. Plasma contents of α-tocopherol, β-carotene and retinol were nonsignificantly and squalene and phenol were significantly increased after consumption of olive oils in both sexes compared with the base lines. Plasma malondialdehyde level and blood pressure (systolic and diastolic) were significantly reduced with increasing phenol content in consumed oil. In conclusion, dietary olive oil with high phenol content proved to be helpful in reducing the CHD risk factors and normalize blood pressure systolic pressure of men and women.

Key words: Olive oils, minor components, phenols, TC, LDL-c, HDL-c, MDA, BP

INTRODUCTION

Olive oil production in Jordan is increasing from year to year with increasing growing areas. Jordan's annual production of olive oil is estimated at 32,000 tons. Roughly, Jordan's five million people consume about 22.5 million kg of olive oil every year (4.5 kg/person). As in diets of the Mediterranean populations, the olive oil is an integral part of Jordanian diets. Although figures may vary by time and place, olive oil contributes almost 20% of the total energy intake in Mediterranean menus (Kipnis *et al*., 1993). Thus, the effects of olive oil on health and disease have frequently been assessed through epidemiological studies focusing on the Mediterranean diet (Trichopoulou and Dilis, 2007). It has been proposed that the Mediterranean diet may be closer to the ancestral foods that were part of human development and our metabolism may have evolved to work optimally on such a diet rather than with the current diets richer in saturated fat and highly refined and processed foods. Therefore, it is possible that alleles that are associated with increase disease risk may be silenced in the presence of that more ancestral and traditional diet and lifestyle. This knowledge may provide the basis for successful public health as well individual approaches for disease prevention (Ordovas *et al*., 2007). Traditionally, olive oil has been the only alimentary fat containing primarily monounsaturated fat in its composition. Nowadays, other edible oils contain fatty acid compositions similar to that of the olive oil, namely high-oleic sunflower oil and rapeseed oil. However, olive oil has the exclusivity of being a real juice and its composition includes not only fat but also other multiple minor components with biological properties that are not present in any other edible oil. Chemically, olive oil is composed of triacylglycerols which account for about 98% of its total weight. In addition, it contains about 2% of other, nearly 250 minor components including aliphatic and triterpenic alcohols, sterols, hydrocarbons, volatile compounds and antioxidants (Servili and Montedoro, 2002; Servili *et al*., 2004; Tripoli *et al*., 2005; Covas *et al*., 2006). In particular, among the natural antioxidants of olive oil are carotenoids and phenolic compounds, which have both lipophilic and hydrophilic properties. Tocopherols are known as lipophilics, while phenolic alcohols and acids, hydroxy-isochromans, flavonoids, secoiridoids and lignans constitute the hydrophilic compounds. Natural antioxidants are reported to play a key role in preventing oxidation and have been already correlated to the storage stability of olive oils (Bendini *et al*., 2007; Baccouri *et al*., 2008; Nevado *et al*., 2009). The content of the minor components of an olive oil is strongly affected by many agronomical and technological factors, such as olive cultivar (Tura *et al*., 2007), the place of

cultivation (Vinha *et al*., 2005; Al-Maaitah *et al*., 2009), the climate, degree of maturation (Kalua *et al*., 2005; Dabbou *et al*., 2009), crop season (Gomez-Alonso *et al*., 2002; Ocakoglu *et al*., 2009), irrigation (Tovar *et al*., 2001) and the processing system (Ranalli *et al*., 2001; Ocakoglu *et al*., 2009) employed to produce the types of olive oil currently present on the market: extra-virgin, virgin, refined, ordinary, or pomace (Gimeno *et al*., 2002). Virgin Olive Oil (VOO) is that obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil. It has not undergone any treatment other than washing, decantation, centrifugation or filtration. Oils obtained using solvents, adjuvant, having a chemical or biochemical action, re-esterification process, or any mixture with oils of other kinds are excluded from this category (EEC, 2001). Extra-VOO is VOO with a free acidity, expressed as g of oleic acid/100 g of olive oil, less than 0.8 g. Virgin olive oil with an acidity greater than or equal to 3.3 is submitted to a refining process in which some components, mainly phenolic compounds and to a lesser degree squalene, are lost (Owen *et al*., 2000). By mixing virgin and Refined Olive Oil (ROO) an ordinary olive oil is produced and marketed. After VOO production, the rest of the olive drupe and seed is processed and submitted to a refining process, resulting in Pomace Olive Oil (POO), to which a certain quantity of VOO is added before marketing (International Olive Council, http://ucce.ucdavis.edu/files/filelibrary /2161/34496.pdf). Virgin olive oil is unique among edible vegetable oils because it is consumed without any refining process that would eliminate its natural flavors inherent part of which is the bitter taste. Taste is a very important factor among sensory liking characteristics that affects consumer preference as well as olive oil and food pairing (Boskou *et al*., 2005; Cerretani *et al*., 2008). The oxidative stability, sensory quality and health properties of virgin olive oil stem from a prominent and well-balanced chemical composition (Bendini *et al*., 2007; Esti *et al*., 2009; Inarejos-Garcia *et al*., 2009).

Elevated concentrations of in vivo circulating oxidized low density lipoprotein (oxLDL show a positive relationship with the severity of acute coronary events (Holvoet *et al*., 2001). Circulating oxLDL plasma concentrations were predictors for Coronary Heart Disease (CHD) both in CHD patients and in the general population (Meisinger *et al*., 2005; Wu *et al*., 2006; Ruano *et al*., 2007). The type of fat ingested is a key factor concerning LDL oxidation because it can modulate the susceptibility of LDL to undergo oxidative modification. Poly Unsaturated Fatty Acids (PUFA) rich in double bonds, are more prone to form conjugated dienes than Mono Unsaturated Fatty Acids (MUFA). A linoleic acid account for 90% of the PUFA presents in LDL and is the major substrate for its oxidation (Esterbauer *et al*., 1992; Bos *et al*., 2007). In most studies, oleic acid-rich LDL have been shown to be less susceptible to oxidation than linoleic acid rich LDL (Mata *et al*., 1997; Baroni *et al*., 1999; Fito *et al*., 2007). The oxidative modification of LDL plays a key role in atherosclerosis and CHD development (Hennig and Toborek, 2001). It is currently thought that oxLDL is more damaging to the arterial wall than native LDL (Fito *et al*., 2007). One of the earliest steps in the generation of oxLDL is the lipid peroxidation of PUFA. Tissue membranes that are rich in PUFA are more susceptible to oxidation by free radicals than membranes rich in MUFA (Reaven *et al*., 1994; Shimokawa, 1999) However, lipid peroxidation and its chain reaction in LDL, can be interrupted if LDL lipids are protected from free radicals by antioxidants. Olive oil is rich in MUFA and antioxidant compounds. The concentration of these antioxidants is influenced by the olive oil extraction procedures. Olive oil obtained exclusively by physical procedures, is much more than a MUFA fat because it contains relatively high amounts of antioxidants, mainly phenolic compounds have been shown to protect LDL from lipid peroxidation in vitro experiments (Owen *et al*., 2000; Visioli *et al*., 2000). It has displayed anti-thrombotic effects in cell culture and in vitro studies (Perez-Jimenez *et al*., 1999; Guzik *et al*., 2002; Duvall, 2005; Fito *et al*., 2005; Pacheco *et al*., 2006; Ruano *et al*., 2007; Kasdallah-Grissa *et al*., 2008). Compared with a saturated fat diet, olive oil- rich diet has been found to be associated with lower levels of blood pressure (Ruiz-Gutierrez *et al*., 1996; Ferrara *et al*., 2000). Hypertension is related to endothelial dysfunction which contributes to make the atherosclerotic plaque more unstable, thus increasing the risk of secondary events in CHD patients (Escobar, 2002; Perona *et al*., 2006).

Thus, the aim of the present study was to evaluate the effect of olive oils on lipid profile, oxidative stress and blood pressure in human.

MATERIALS AND METHODS

Subjects: Twenty five Jordanian (12 men and 13 women) from Alkarak city (Mutah and Almerj) participated in the study. Started from October/2008 to March/2009. The average age of men was 45 years (ranged from 37-50 years) with body mass index of 25.2 kg/m^2 (range, 23.4-27.2 kg/m^2) and of women was 39 years (ranged from 33-44 years), with body mass index of 26.3 kg/m^2 (range, 24.6-28.31 kg/m^2). All men and their wives were healthy. None of them showed evidence of chronic diseases (hepatic, renal, thyroid, cardiac), or family history of early onset cardiovascular disease. Venous blood was obtained from the fasting after an overnight period, at the beginning (base line) and end of each period of the study. Blood was collected in heparinized tubes and stored in containers with ice and kept in the dark. Special care was taken to avoid exposure to air, light and ambient temperature. Plasma was separated from whole blood by low-speed centrifugation at 1500 rpm for 20 min at 4°C and frozen at -20°C until analyzed.

Diet and design: Before participating in the study, all subjects were receive instruction on the basic concepts of food composition and characteristics, appropriate portions and recommended cooking techniques. Participants were given strict instructions about what to eat at home and were asked to replace their usual fat intake (butter, ghee, margarine, visible fat on meat and all oils) with the study olive oils. The participants were successively consumed three local, *balady*, virgin olive oils: High Phenolic Component Olive Oil (LPCO), Medium Phenolic Component Olive Oil (MPCO) and Low Phenolic Component Olive Oil (HPCO), purchased from local market in Alkarak city, for four weeks each. A 4-week washout period (basal lines) was included between the three experimental periods, consisting on the habitual dietary fat, comprised of hydrogenated, refined oil and a blend of seed oils. Body weight and height were recorded at the beginning and end of olive oil treatment until completion of the study and BMI were calculated. Before the beginning of the experimental study, the habitual dietary intake of the volunteers was recorded during four consecutive weeks (base line), using three- day- diet recall weekly and food frequency questionnaires. The same procedure was used during washout and experimental periods. This work showed that, there were no worthy differences in habitual diets of participants from week to week. During the experimental period, the only difference between the diets of participants was in the composition of the edible fats, added in the form of olive oils (LPCO, MPCO and HPCO) for cooking, salad dressing and occasionally for spreading on bread slices. The fatty acid composition of the three oils used in the study, as well as the minor component is shown in Table 1.

Intakes of protein, total carbohydrates, total fiber, fat, type of fat and micronutrients were assessed using food composition tables (Pellett and Shadarvian, 1970; Poul and Southgate, 1978). Energy content of daily food was calculated by multiplying the daily eaten carbohydrates, proteins and fats (grams) by 4, 4 and 9 Kcal, respectively Free acidity (% of oleic acid), Peroxide Value (PV) expressed (mEq O_2 kg^{-1} oil), K_{232} and K_{270} extinction coefficients calculated from absorption at 232 and 270 nm and β-sitosterol content were measured, following the analytical methods described in European Regulation, EEC (1991). Fatty acids methyl esters were formed according to the method of Lepage and Roy (1986). The fatty acid composition profiles of the oils were determined by gas chromatography according to the method described in EEC (1991). The concentrations of β-carotene and α-tocopherol in oils and blood lipid extracts were measured by High-performance Liquid Chromatography (HPLC) according to the method of Thurnham *et al.* (1988). Squalene content of the oils and plasma were determined by Gas-Liquid Chromatography (GLC) according to the methods of Miettinen (1988). Total phenols in oils and blood lipid extracts were measured spectnophotometrically according to the method of Singleton and Ross (1965) with phosphomolybdic phosphotungstic acid reagents. Plasma lipid peroxidation was determined by measuring by using the Thiobarbitunic Acid Reactive Substances (TBARS) assay, which measures Malondialdehyde (MDA) equivalents (Buege and Aust, 1978). This technique involves the spectrophotometric measurement of substances that react with thiobarbituric acid. Plasma Total Lipid (TL) concentration was measured according to the method of Sperry and Brand (1995). Total Cholesterol (TC) and Triglycerides (TG) concentrations in plasma were determined using the enzymatic methods from Arab Company for medical diagnostic, Jordan and Bicon, Germany, respectively. Concentrations of plasma Free Cholesterol (FC) and Phospholipids (PL) were measured according to methods mentioned by Tietz (1987). Plasma Cholesterol Ester (CE) concentration was calculated from the difference between TC and CE. Concentration of HDL-c in the plasma was measured by the precipitation technique using magnesium chloride and phosphotungstic acid from Bicon, Germany. Concentration of plasma LDL-c was calculated as the difference between TC and HDL-c using the formula of Friedewald *et al.* (1972), LDL-c = (TC) - (HDL-c) - (TG/5). Blood pressure measurements of participants were performed in the morning, after an overnight fast, at the right brachial artery in seated participants using a mercury-gauge sphygmomanometer. The measurements were recorded by the same nurse at the residential home at the beginning, middle and end of washout and both experimental periods. At each visit two blood pressure measurements were recorded and the average used to determine eligibility.

The data were expressed as Mean±Standard Deviation (SD). Statistical differences were determined by Duncan's multiple range tests at $p \leq 0.05$ by SAS Version (1988).

RESULTS AND DISCUSSION

Table 1 shows the physicochemical quality parameters and the composition of used olive oils. All the analyzed oils were met the legal limit values for VOO required by Regulation European Union (EEC, 2003) (acidity = 0.8%; peroxide value = 20 mequiv. O2 kg^{-1}; K_{270} = 0.22 and K_{232} = 2.5), indicating a low initial oxidation status, as was desired. These results show that the cultivar and factors affecting these analytical parameters had no significant influences (Kiritsakis *et al.*, 1998; Gomez-Alonso *et al.*, 2007). Given the object of this study, olive oil samples were selected on the basis of differences in natural antioxidant contents. The amounts of total phenols were significantly differed among analyzed oils. It ranged from 132 mg kg^{-1} in LPCO to 753 mg kg^{-1} in HPCO. As

Table 1: Quality indices, minor components (mg kg^{-1}) and fatty acid composition (%) of Low, Medium and High Phenols Containing Olive Oils (LPCO, MPC and HPCO) used as lipid sources in the study

Parameters	LPCO	MPCO	HPCO
Quality indices			
F A (% oleic acid)	0.41	0.38	0.37
P V (mEq O_2 kg^{-1})	11.6	10.8	10.2
K_{232}	2.03	1.92.	1.83
K_{270}	0.20	0.18	0.17
Minor components			
α-Tocopherol	264^{b}	221^{c}	319^{a}
β-Carotene	3.72^{b}	4.28^{a}	3.31^{c}
β-Sitosterol	1721	1847	1698
Squalene	3560	3730	3632
Total phenols	132^{c}	368^{b}	753^{a}
Fatty acids			
C14:0	0.3	0.2	0.3
C16:0	10.9^{ab}	10.6^{b}	11.9^{a}
C16:1	0.8	0.7	1.0
C18:0	5.4	5.0	5.2
C18:1(n9), O	71.8	72. 5	70.2
C18:2(n6), L	10.2	10.8	10.7
C18:3(n3)	0.50	0.40	0.30
C20:0	0.3	0.2	0.2
SFA	16.7^{ab}	15.6^{b}	17.8^{a}
MUFA	72.6	73.2	71.2
PUFA	10.7	11.2	11.0
MUFA/SFA	4.30^{ab}	4.65^{a}	3.94^{b}
MUFA/PUFA	6.79	6.54	6.47
O/L	7.04	6.71	6.65

PV: Peroxide Value. FA: Free Acidity. K_{232}, K_{270}: Specific extinction at 232 and 270 nm, respectively. SFA, MUFA and PUFA: Saturated, monounsaturated and polyunsaturated fatty acids, respectively. Significant differences in the same row are shown by different letters (a-c)

reported by different authors, the amount of total phenols is ranging between 50 and 1000 mg kg^{-1}, depending on various factors such as cultivar, climate, location, degree of maturation, type of crushing machine and oil extraction procedures. (Aguilera *et al*., 2005; Aparicio and Luna, 2002; Allalout *et al*., 2009). Phenolic compounds have a strong antioxidant and a free radical scavenging ability (Visioli *et al*., 1998). Moreover, their presence in olive oil contributes to the sensory characteristics, like its bitter, astringent and pungent taste (Gutierrez-Rosales *et al*., 2003). As previous investigations showed, the main determinants of virgin olive oil antioxidant activity are phenolic compounds that share o-diphenolic structures such as hydroxytyrosol and its derivates (Lavelli, 2002). The tocopherol fraction in virgin olive oils consisted mainly of α-tocopherols; these substances exert both vitamin potency and antioxidant action. There were significant differences in α-tocopherol amount between studied oils. In fact, α-tocopherol amounts are ranging from 221 mg kg^{-1} in MPCO to 319 mg kg^{-1} in HPCO. These results are in agreement with observations of Deiana *et al*. (2002) that α-tocopherol content is highly variety-dependent. Several authors reported a high correlation between phenol and α-tocopherol compounds and oxidative stability in virgin olive oil (Gomez-Alonso *et al*., 2002; Gomez-Alonso *et al*., 2007; Allalout *et al*., 2009). Table 1 also shows the pigment contents of studied oils. β-carotene concentration was 3.31 mg kg^{-1} in HPCO, 3.72 in LPCO mg kg^{-1} in and 4.28 mg kg^{-1} in MPCO. These results show that significant differences between the three oils were observed in pigment contents probably because the olives were at different stages of ripeness (Gomez-Alonso *et al*., 2007). These results are in agreement with the findings of Salvador *et al*. (2001), Psomiadou and Tsimidou (2001) and Allalout *et al*. (2009) which reported that the presence of the pigment in the oil depends on several factors, such as the olive cultivar, soil and climatic conditions, fruit ripeness and the processing procedures.

As shown in Table 1, the fatty acid composition, with a high percentage of oleic acid and low linoleic and linolenic acid contents, was in compliance with established limits (EEC, 2003) and with ranges depending to the varieties (Aranda *et al*., 2004). Oleic acid, the major Monounsaturated Fatty Acid (MUFA), showed limit variability among the three oil samples (about 72%). Relatively low percentages of Linoleic acid (about 11%), the major Polyunsaturated Fatty Acid (PUFA), were observed in the analyzed samples. Myristic, palmitoleic, stearic, linolenic and arachidic, showed no significant differences from one olive oil to the other. Levels of these fatty acids in the three tested oils were close to the IOC (2009). Variations in fatty acid composition observed in olive oil samples are probably related to both genetic factors and environmental conditions during the development and maturity of the fruit. These results are in agreement with the findings of other authors (Salvador *et al*., 2001; Morello *et al*., 2004; Allalout *et al*., 2009). They mentioned that several agronomic parameters could modify the fatty acid composition of olive oil. The percentages of Saturated Fatty Acids (SFA), MUFA, PUFA and the oleic acid/linoleic acid ratio (O/L) in the studied olive oils were also evaluated. It was observed that HPCO was rich in total SFA (17.8%) essentially due to its higher content in palmitic acid which represents the major acid of the SFA fraction. Concerning the total MUFA, PUFA and MUFA/PUFA ratio, there were no significant differences from one olive oil to the other. However, the MUFA/SFA ratio was significantly higher in MPCO (4.65) than in HPCO. The O/L ratio was 7.04 in LPCO, 6.71 in MPCO and 6.65 in HPCO. This ratio can be useful to characterize olive cultivars and to have a marked relationship with stability. Allalout *et al*. (2009) reported a positive correlation (r^2 = 0.7, $p \leq 0.001$) between this ratio and the oxidative stability of olive oil. Other authors showed that the O/L ratio was described as the main responsible factor for virgin olive oil oxidative stability (Aguilera *et al*., 2005).

Table 2: Calculated daily total energy, E (kcal) and macronutrients (% of E) intakes of men and women consumed Low, Medium and High Phenols Containing-olive Oils (LPCO, MPCO and HPCO) for 4-week*

Nutrients	Washout period 1	LPCO	Washout period 2	MPCO	Washout period 3	HPCO
Total energy						
Men	2460±35	2520±48	2510±33	2470±41	2490±38	2430±27
Women	2220±32	2270±40	2260±39	2240±30	2230±42	2280±35
Protein						
Men	10.2±0.4	10.5±0.5	10.1±0.8	10.7±0.3	10.3±0.7	10.5±0.3
Women	10.0±0.6	10.2±0.4	9.8±0.5	10.1±0.6	9.7±0.4	10.3±0.6
Carbohydrates						
Men	58.4±4.7	58.9±5.2	57.8±3.7	59.0±4.3	59.2±4.5	58.5±6.1
Women	57.7±5.3	56.6±4.3	57.4±4.5	57.4±5.1	57.3±4.8	57.5±4.7
Total fat						
Men	31.4±1.2	30.6±1.5	32.1±1.8	30.3±1.7	30.5±2.1	31.0±2.3
Women	32.3±1.5	33.2±1.7	32.8±2.4	32.5±1.6	33.0±1.8	32.2±2.0
SF						
Men	9.1±0.5	4.1±0.4**	9.5±0.7	5.1±0.3**	8.3±0.5	4.4±0.3**
Women	10.1±0.6	6.5±0.3**	10.6±0.4	5.5±0.2**	9.9±0.7	4.5±0.4**
MF						
Men	7.6±0.2	20.5±0.8**	7.1±0.4	19.8±0.7**	8.2±0.5	20.7±1.2**
Women	6.8±0.4	20.9±0.9**	7.4±0.2	20.3±1.4**	7.7±0.3	21.2±0.8**
PF						
Men	14.7±0.6	6.0±0.3**	15.5±0.8	5.4±0.4**	14.0±0.9	5.9±0.2**
Women	15.4±0.8	5.8±0.5**	14.8±1.3	6.7±0.3**	15.4±1.2	6.5±0.4**

*Data shown as means± SD. **: Differed significantly from the base line. SF, MF and PF: Saturated, monounsaturated and polyunsaturated fat. Washout Periods: 4-week of habitual diet pre olive oil administration

Table 2 shows the calculated daily energy intake and contribution of macronutrient in total energy of participants during basal and olive oil periods. As shown in this table, there were no significant differences in the calculated of daily energy intake and contribution of protein, carbohydrates and total fat in total energy between periods of olive oils and washouts. However, consumption of the three olive oils resulted in significant increase in monounsaturated fat and decrease in saturated and polyunsaturated fats contribution in total energy compared with washout periods. In general, the men received more energy than women (2480, 2250 kcal/day) and no markedly differences between them and women in contribution of macronutrients in total energy intake. A dietary survey showed that olive oils were consumed in a daily dose of about 70 g. It used at least twice a day, consistently for breakfast and lunch especially with chickpea and thyme and in this study used in cooking. All participants were complied with dietary and lifestyle recommendations and conserve their body weight during the study periods.

Table 3 shows the micronutrient intakes of participants during basal and olive oil periods. This table showed that there were no significant differences in intakes of fiber, cholesterol, β-carotene, vitamin A, α-tocopherol vitamin C and sodium between periods of olive oils and base lines. Consumption of studied olive oils resulted in significant higher intakes of squalene, β-sitosterol and total phenols than there base lines. However, no worth differences between men and women in intakes of micronutrients.

Table 4 shows the effects of intake of three olive oils on plasma lipid profile of men and women. Seven lipid classes were identified and quantified in the plasma of participants, for instance TG, PL, TC, FE, CE, LDL-c and HDL-c. There were no significant differences in TG, PL, TC, FC, or CE concentrations in plasma of participants consumed LMCO, MMCO or HMCO. However, statistically significant differences were observed in plasma LDL-c and HDL-c, HMCO consumption resulted in the highest reduction in LDL-c concentration (-12.2%) and rising in HDL-c concentration (+23.7%), whereas the LMCO consumption resulted in the lowest reduction and rising (-6.4 and +9). However, effects of MMCO consumption in these parameters were at the mid point. There was no worth noting differences in effects of these oils between men and women for that the given data were there mean. Finally, a statistically significant effect was observed upon TC/ HDL-c and LDL-c/HDL-c ratios, these being lowest in the HMCO treatment (3.62 and 2.10 for men; 3.25 and 1.77 for women). Recent works have shown increasing total and LDL-c concentrations after consuming olive oil, rich in oleic acid, compared to dietary oils rich in n-6 fatty acids (Howell *et al*., 1998; Pedersen *et al*., 2000) and others have reported greater cholesterol reductions of this oil (Sirtori *et al*., 1992; Madigan *et al*., 2000). These discrepancies may be due to differences in experimental conditions, including the employment of different varieties of olive oil (Perona *et al*., 2003; Perona *et al*., 2004). Present study showed that the olive oil intake had lowering effect on LDL-c and rising effect on HDL-c concentrations in the plasma of participants and the same observations were reported by Ruiz- Gutierrez *et al*. (1996) and Perona *et al*. (2004).

Table 5 shows the effects of intake of three olive oils on minor components in plasma of men and women.

Table 3: Estimated daily total fiber (g) and micronutrients (mg) intakes of men and women consumed Low, Medium and High Phenols Containing-Olive Oils (LPCO, MPCO and HPCO) for 4-week*

Nutrients	Washout period 1	LPCO	Washout period 2	MPCO	Washout period 3	HPCO
Total fiber						
Men	25.3±3.8	24.1±2.5	23.8±3.1	23.6±4.2	24.2±3.3	25.1±2.7
Women	23.7±4.2	25.0±2.8	24.6±4.0	25.2±3.3	23.4±2.4	24.5±3.5
Cholesterol						
Men	282±15	271±12	291±13	289±18	276±21	267±20
Women	296±18	279±22	305±16	295±15	290±17	278±13
β-Carotene						
Men	3.2±0.1	3.4±0.3	3.3±0.2	3.5±0.2	3.5±0.1	3.7±0.3
Women	3.5±0.4	3.5±0.3	3.4±0.3	3.5±0.2	3.6±0.4	3.6±0.3
Vitamin A (RE)						
Men	410±15	430±13	385±17	428±15	393±11	416±10
Women	392±16	411±14	367±12	403±11	378±16	421±15
α-Tocopherol						
Men	14.2±0.8	14.4±0.7	13.8±1.2	14.1±1.0	14.6±0.9	14.8±1.1
Women	14.4±1.1	14.5±1.0	14.2±1.4	14.6±1.3	14.8±1.2	14.6±0.7
Vitamin C						
Men	55.8±4.3	57.3±3.7	61.4±3.2	58.2±4.5	54.7±3.9	55.1±3.2
Women	56.3±3.5	59.5±4.1	60.2±3.7	59.6±3.3	57.8±2.8	60.2±4.4
Squalene						
Men	160±11	270±14**	175±9	267±15**	158±11	282±12**
Women	143±9	258±15**	131±8	251±13**	154±9	265±11**
β-Sitosterol						
Men	109±5	123±6**	112±7	115±5	105±8	133±10**
Women	102±7	116±5**	107±8	111±7	104±6	123±11**
Phenols						
Men	7±1.2	16±2.5**	6±1.3	34±3.7**	8±2.0	56±4.5**
Women	6±0.9	14±2.6**	8±1.5	27±2.9**	8±2.1	53±5.1**
Sodium						
Men	803±23	764±31	781±34	831±22	745±19	783±27
Women	758±25	803±40	823±18	775±36	767±32	834±30

*Data shown as means± SD. **: Differed significantly from the base line. Washout periods: 4-week of habitual diet pre olive oil administration

Present study aimed at analyzing the effect of the non glyceride fraction of olive oil as a whole. Nevertheless, the three different experimental olive oils employed had different concentrations of β-carotene, α-tocopherol, β-sitosterol, squalene and total phenols. The amount of squalene, the main hydrocarbon in VOO, has been considered hypercholesterolemic compared with other oils (Pedersen *et al*., 2000; Perona *et al*., 2004), in plasma of the subjects was increased after consumption of olive oil especially after HPCO. However, there were no markedly differences in squalene content in the studied oils, it ranged from 3560 mg kg^{-1} in LPCO to 3730 mg kg^{-1} in MPCOO (Table 1) and in estimated squalene intake, it ranged from 259 mg/day for MPCO to 273 mg/day for HPCO (as average for men and women) (Table 3). The effect of oil type on plasma squalene level was took the order: HPCO> LPCO> MPCO (Table 5). Thus, it appears that this constituent had a minor role, if any at all, on the changes in the plasma lipid profile observed (Table 4). The same observation was mentioned by (Mangas-Cruz *et al*., 2001). Nakamura *et al*. (1997), they reported that the oral administration of squalene at different dose has no effect on serum lipid in rats. Table 5 also shows that the plasma levels of α-tocopherol retinol and β-carotene were nonsignificantly increased after consumption of olive oils compared with the beginning line. Back to the Table 1, this table shows that the amount of α-tocopherol in the three experimental oils was took the order: HMCO> LMCO> MMCO and of β-carotene was took the order: MMCO> LMCO> HMCO whereas the effect of these oils on blood lipids was followed the order: HMCO> MMCO> LMCO (Table 4). Moreover the concentrations of both vitamins in the plasma were not significantly affected by either of these oils which suggest that these constituents were not responsible for the effect on plasma lipid concentrations. It is known that the main effect of β-sitosterol is preventing the absorption of dietary cholesterol from the gut. It has a hypocholesterolemic effect on plasma TC and LDL-c (Heinemann *et al*., 1991; Mangas-Cruz *et al*., 2001). As the three olive oils employed were devoid of cholesterol, no such effect was possible in the current study. Thus, among the minor component in olive oil, the phenols could be responsible for the observed effect upon plasma lipid. Data showed that the amount of total phenol compounds in three studied olive oils (Table 1) and in subjects plasma (Table 5) was significantly followed the order: HMCO> MMCO> LMCO and the effects of these oils in LDL-c reduction and HDL-c rising was followed the same order

Table 4: Plasma lipid profile (mg dl^{-1}) of men and women consumed Low, Medium and High Phenols Containing- Olive Oils (LPCO, MPCO and HPCO) for 4 week*

Parameters	Base line	After LPCO (L)	Base line	After MPCO (M)	Base line	After HPCO (H)	Changes (%) after		
							L	M	H
TG									
Men	120±7	119±8	118±6	116±5	124±8	121±5	-0.8	-1.7	-2.4
Women	127±6	124±7	121±5	117±8	123±7	123±6	-2.4	-3.2	-
PL									
Men	109±5	108±7	111±6	109±4	110±5	110±5	-0.9	-1.8	-
Women	110±7	107±6	109±4	110±5	108±5	110±4	-2.7	+0.9	+1.8
TC									
Men	174±7	170±6	172±7	167±9	172±6	166±9	-2.3	-2.9	-3.5
Women	169±8	165±9	170±8	166±7	169±9	167±8	-2.4	-2.4	-1.2
FC									
Men	51±3	49±3	50±3	47±5	51±3	47±4	-3.9	-6.0	-7.8
Women	50±4	47±3	52±3	49±4	52±4	50±3	-6.0	-5.8	-3.8
CE									
Men	122±6	121±6	122±5	120±7	121±6	119±8	-0.8	-1.6	-2.5
Women	119±7	118±5	118±7	117±7	117±8	117±6	-0.8	-0.8	-
LDL-c (L)									
Men	114 ±5	107±4**	111±4	101±5**	110±5	96±6**	-6.1^{a}	-9.0^{b}	-12.7^{c}
Women	104±5	97±4**	105±5	96±6**	103±4	91±8**	-6.7^{a}	-8.6^{ab}	-11.7^{c}
HDL-c(H)									
Men	36.0±3	39.2±3**	37.4±4	42.8±3**	37.2±5	45.8±4**	$+8.9^{c}$	$+14.4^{b}$	$+23.1^{a}$
Women	39.6±4	43.2±4**	40.8±3	46.6±3**	41.4±4	51.4±5**	$+9.1^{c}$	$+14.2^{b}$	$+24.2^{a}$
TC/H									
Men	4.83±0.2	4.33±0.3**	4.60±0.4	3.90±0.2**	4.62±0.3	3.62±0.2**	-10.4^{a}	-15.2^{b}	-21.6^{c}
Women	4.27±0.3	3.82±0.2**	4.17±0.2	3.56±0.3**	4.08±0.3	3.25±0.4**	-10.5^{a}	-14.6^{b}	-20.3^{c}
L/H									
Men	3.17±0.1	2.73±0.2**	2.97±0.3	2.36±0.1**	2.96±0.3	2.10±0.2**	-13.8^{a}	-20.5^{b}	-29.1^{c}
Women	2.63±0.1	2.25±0.1**	2.57±0.2	2.06±0.2**	2.49±0.1	1.77±0.3**	-11.0^{a}	-19.8^{b}	-28.9^{c}

*Data shown as means± SD. **: Differed significantly from the base line. Significant differences in effects of the three oils are shown by different letters (a-c). TG, PL, TC, FC, CE, LDL-c and HDL-c: Triglycerides, Phospholipids, Total Cholesterol, Free Cholesterol, Cholesterol Ester, Low Density Lipoprotein- Cholesterol and High Density Lipoprotein-cholesterol, respectively

(Table 4). The same observation was mentioned by (Mangas-Cruz *et al*., 2001).

Table 5 shows the effects of intake of three olive oils on oxidation status of participants' plasma. Lipid oxidation, a process mediated by free radicals, is considered to be important in the development of atherosclerosis. Lipid may be protected against attacks of free radicals by antioxidants in plasma. There are a growing number of studies indicating that antioxidants may be responsible for some of the protective effects of olive oil (Giugliano, 2000; Moline *et al*., 2000). One way of estimating free radical activity is to determine the concentration of MDA in plasma which is byproduct of lipid peroxidation. In this study, olive oil intake resulted in lower plasma MDA concentrations compared with the beginning lines. However, there were significant differences in effects of the three studied olive oils in this oxidation product, there reducing effect followed the order: HMCO> MMCO>LMCO, which parallel there effects in blood lipids. The same observation was obtained from studies in animals (Del Boccio *et al*., 1990; Yu *et al*., 1993) and humans (Yalcin *et al*., 1989; Hargrove *et al*., 2001) have shown that there is a close relationship between lipid peroxidation and hypercholesterolemia. Halliwell and Chirico (1993), demonstrated higher stability of saturated and monosaturated oils in lipid peroxidation as compared to polyunsaturated oils. Lipoproteins rich in MUFA after long term consumption of olive oil have been shown to be less susceptible to oxidation (Hargrove *et al*., 2001). Ohrvall *et al*. (1994) states that MDA concentrations in plasma are inversely correlated to the proportion of PUFA in blood lipoprotein lipids. His findings suggested that other factors, such as, the availability of antioxidants, polyphenols and others, may be of greater importance for intravascular lipid peroxidation. Olive oil has the biophenols (Moline *et al*., 2000; Mangas-Cruz *et al*., 2001), β-carotene and tocopherols (Rosengren *et al*., 1999; Chen *et al*., 2000) which were shown to improve in vivo an antioxidant defenses and protect LDL from oxidant phenomena, a condition necessary for the formation of athermanous plaque.

Table 5 shows the effects of intake of three olive oils upon blood pressure. It is probable that arterial hypertension resulted to be quantitatively the most important risk factor for CHD due to its repercussion on cardiovascular mortality (Aranda, 1996). However, there were increasing evidence showing that olive oil reduces systolic and diastolic blood pressure in normotensive and hypertensive individuals (Ruiz-Gutierrez *et al*., 1996;

Table 5: Plasma malondialdehyde, MDA (μmol L^{-1}l) and antioxidant compounds (μg ml^{-1}) and blood pressure, BP (mmHg) of men and women consumed Low, Medium and High Phenols Containing-Olive Oils (LPCO, MPCO and HPCO) for 4-week*

Parameters	Base line	After LPCO (L)	Base line	After MPCO (M)	Base line	After HPCO (H)	Changes (%) after		
							L	M	H
MDA									
Men	0.92±0.02	0.86±0.03**	0.89±0.02	0.78±0.01**	0.91±0.03	0.76±0.03**	-6.5^{a}	-12.4^{b}	-16.5^{c}
Women	0.93±0.03	0.87±0.01**	0.92±0.01	0.81±0.02**	0.90±0.04	0.74±0.03**	-6.5^{a}	-12.0^{b}	-17.7^{c}
α-Tocopherol									
Men	4.9±0.3	5.0±0.4	5.1±0.3	5.2±0.2	4.8±0.2	4.8±0.3	2.0	2.0	-
Women	4.6±0.3	4.7±0.2	5.0±0.2	5.0±0.4	5.1±0.3	5.0±0.4	2.2	-	-2.0
Retinol									
Men	2.4±0.3	2.5±0.2	2.5±0.1	2.6±0.2	2.7±0.2	2.7±0.1	4.2	4.0	-
Women	2.3±0.1	2.3±0.3	2.6±0.2	2.7±0.2	2.5±0.3	2.5±0.1	-	3.8	-
β-Carotene									
Men	0.27±0.03	0.28±0.01	0.28±0.01	0.29±0.02	0.30±0.02	0.32±0.01	3.7	3.6	6.7
Women	0.28±0.02	0.30±0.01	0.29±0.02	0.30±0.01	0.29±0.01	0.30±0.02	7.1	3.4	3.4
Squalene									
Men	0.32±0.02	0.36±0.04**	0.33±0.04	0.35±0.02	0.34±0.03	0.40±0.02**	12.5^{ab}	6.1^{b}	17.6^{a}
Women	0.34±0.04	0.36±0.02	0.35±0.03	0.37±0.04	0.34±0.02	0.39±0.03**	5.9^{b}	5.7^{b}	14.7^{a}
PC (mmolL^{-1})									
Men	0.28±0.03	0.31±0.03**	0.30±0.04	0.37±0.03**	0.31±0.03	0.41±0.04**	10.7^{c}	23.3^{b}	32.3^{a}
Women	0.27±0.02	0.31±0.04**	0.29±0.02	0.36±0.03**	0.30±0.01	0.41±0.04**	14.8^{c}	24.1^{b}	36.7^{a}
Systolic BP									
Men	136±4	132±7	135±5	126±4**	135±6	124±8**	-2.9^{a}	-6.7^{ab}	-8.1^{b}
Women	135±6	130±5	134±6	126±8**	135±5	125±6**	-3.7	-5.9	-7.4
Diastolic BP									
Men	84±4	80±3	83±6	76±4**	84±5	75±4**	-4.8^{a}	-8.4^{ab}	-10.7^{b}
Women	83±5	80±4	84±5	78±4**	84±4	74±6**	-3.6^{a}	-7.1^{ab}	-11.9^{b}

*Data shown as means±SD. **: Differed significantly from the base line. Significant differences in effects of the three oils are shown by different letters (a-c). PC: Phenolic Compounds

Ferrara *et al*., 2000). The present study was designed to assess the effects of three olive oils with different levels of minor compounds on plasma lipid composition and oxidation and blood pressure of healthy volunteers. Systolic and diastolic blood pressures were reduced in participants after consuming these oils and this reduction was increased with increasing phenols level in the oil. However, some studies failed to find effects of fish oil or n-3 fatty acid supplementation on blood pressure in treated subjects when compared to VOO as a placebo, suggesting that VOO is able to reduce blood pressure to a similar extent. Previously, Ruiz- Gutierrez *et al*. (1996) and Perona *et al*. (2004) had observed a hypotensive effect of dietary VOO compared to oleic acid-rich oil, such as High Oleic Acid Sunflower Oil (HOSO). Although it needs to be elucidated which of the minor constituents of VOO are responsible of these effect, on the basis of the accumulated data, the PC may play a key role in this regard, since these compounds are absent in HOSO. The mechanism of reduction of blood pressure by dietary olive oil has been related to endothelium-dependent vasorelaxation via enhanced nitric oxide (Ruiz-Gutierrez *et al*., 1996; Ferrara *et al*., 2000).

Conclusion: Diet rich in olive oil, containing high minor compounds, especially phenols, produce beneficial modification in plasma lipid profile and oxidation in healthy subjects. Dietary olive oil is helpful in reducing the systolic and diastolic pressures However, among the minor components, the phenolic compounds have the significative beneficial effect in these parameters which imply that the daily ingestion of food rich in these compounds is necessary to produce an accumulative desirable effect.

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Determinants of Malnutrition among the Children under 2 Years of Age

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Abstract: Two hundred and twenty five mothers of Dhaka medical college hospital are interviewed to investigate of determinants of malnutrition. All of our respondents were under two years of age. One hundred fourteen of them were girls and rests 111 were boys. All of the children were from different socio-economic status coming different places of Bangladesh. 28% of the respondents family monthly income were below 3001-5000, 3.6% were below 20,000-25,000 and 16.4% were 10,000-15,000, 21.3% respondents mothers were illiterate, 24.4% mothers were primary level, 32% mothers were secondary level, 11.1% mothers were higher secondary level and 11.1% were graduate. The nutritional status children were not found very from normal to 3rd degree malnourishment. Nearly 45.8% were normal, 1.8% was over nourished and 2.2% were 3rd degree malnourished. In the above context, it can be concluded that a large number of the population of Bangladeshi children are suffering from malnutrition (more than half) and are likely to grow smaller and smaller. This is implication of the fact that we are heading towards a nation that will see its children to be of small status and low weight population. So, we need to give highest priority to child health and nutrition if we hope for a brighter future of our country.

Key words: Malnutrition, gomez classification, prelacteal feed

INTRODUCTION

Malnutrition is widespread in most developing countries and it is particularly prevalent among the children, as they are the most vulnerable group of a society. The problem is more serious in Bangladesh, where poverty and illiteracy are predominating. Using the new WHO 2005 GRS, 40% of children aged < 5 years were underweight, 46% were stunted, 15% were wasted and 1.4% were overweight/obese, according to criteria of the World Health Organization (WHO, 1995). The children also have high rate of diarrhoeal infection, nutritional blindness, morbidity and mortality. These high rates of malnutrition may be attributed to poor environmental sanitation, overcrowding, lack of preventive and curative health services and other socioeconomic, educational and cultural factors. Feeding practices have long been recognized as one potentially important determinant of infant malnutrition (Dettwyler, 1987; Whitehead, 1985).

It is well known that breast-feeding improves child survival by providing protection against malnutrition and infectious diseases. Under conditions in which a mother is motivated, healthy and relaxed, breast milk alone is adequate fully to support infant growth for at least 4 months and often 6 months. Additional foods are needed when the supply of breast milk is no longer sufficient to meet the energy and nutrient needs for the growing infant.

In many areas where prolonged breast-feeding is common, nutritional problems generally arise after the first year of life. Delayed supplementation and inadequate weaning foods are major factors responsible for this. In some populations, a child is weaned so early that he is deprived of breast milk and given substitutes, which may be inadequate in nutrition (Greiner, 1997). The timing of supplementary feeding is thus critical for the infant's health and nutrition.

The general view is that additional food should be given by 4-6 months of age since breast milk alone is insufficient to sustain normal growth beyond this period. Some workers, however, believe that food should be introduced even earlier since it has been observed in some instances that growth faltering occurs by 3 months of age (WHO, 1988; 1981). It is important, therefore to examine how long breast milk alone is adequate for normal growth of infants reared under different ecological conditions. It is recommended in Bangladesh that infants should be exclusively breast-fed up to five months of age and then appropriate weaning foods must be added along with breast-feeding (Whitehead, 1985; Islam and Ali, 1983). Recent evidences show that the young children of Bangladesh are typically breast-fed for as long as two years or even more (Greiner, 1997; Whitehead, 1985; Becker *et al*., 1986). Although this incidence of breast-feeding in Bangladesh is satisfactory, the high rate of child malnutrition signifies the operation of other important factors, such as, inadequate supplementation and delayed weaning.

Successful breast-feeding and weaning practices depend to a large extent on the knowledge and attitude of mothers regarding infant feeding. Pattern of infant feeding, as mentioned above, can have a major effect on the nutritional status, health and growth of a child. Therefore, a first step towards understanding malnutrition and infant health in a particular community

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is to have a through knowledge of the beliefs and practices associated with the infant feeding in that community. In Bangladesh, several studies regarding infant and child feeding practices have been carried out, mostly in rural areas. In urban areas, such type of study has been found scant. Also some other related malnutrition came we can approach like family food frequency, Immunization, safe water, Anthropometry and also other clinical form.

Thus, the present study was undertaken to investigate the pattern of infant malnutrition as well as the principal factors associated with such feeding practices in a population of Dhaka.

MATERIALS AND METHODS

The study compared 225 mother infant pairs from different socio-economic and educational groups. The characteristics of the study population and details of the methodology are given below.

The study place: Data were collected from "Dhaka Medical College Hospital (DMCH)". It was located in the centre of Dhaka city. The place was chosen because adequate facilities are available there and a large number of mother-infant pairs attend the centre for receiving health services.

Subject: The subjects of study belong to middle and lower socio economic classes. They came from different parts of the Dhaka city. Two hundred and twenty five infants and their mothers constituted the study population.

During the study period, approximately 600 mothers together with their infants visited the centre. Out of these, 261 mothers could be approached for interview permitting anthropometric measurements of the infants subsequently. However, some mothers were discarded from the study since they could not give complete information as required. Finally, only 225 mothers' infant pairs were successfully interviewed in the present study.

Data collection: Direct interviewing the selected mothers collected data on a designed questionnaire. Initially a questionnaire was constructed and pre-tested in a pilot survey. The standard questionnaire was developed in which all the requisite information was recorded. Mothers who had at least one child below 2 years of age were selected for interviewing. The data collection continued from April 2004 to February 2005.

Data analysis: All the data were placed in a master sheet to obtain a particular variable readily and to compare it with others. The data were processed with the help statistical software and sometimes by scientific standard statistical methods were followed as required.

Result analysis plan: We want to display our study finding by tables as a descriptive way of presentation to this attempt proper statistical analysis will be adopted as far as this data permit.

Appropriate statistic tests will be applied to establish the linkage between malnutrition and factors under consideration.

RESULTS

Monthly Family Income and effect of family income: The monthly family incomes of the respondents are summarized in Table 1. It shows that 19.1% of the respondent fell in the monthly income group of taka three thousand or less, 28% in three to five thousand, 24.4% in five to ten thousand, 16.4% in ten to fifteen thousand, 8.4% in fifteen to twenty thousand and only 3.6% in twenty to twenty five thousand. The lowest and highest incomes were found TK. 1,500 and 30,000 (per month) respectively (not shows in the table).

Table 1: Distribution of the respondents by monthly family income

Monthly income (T.K)	Respondent (n = 225) Number	%
3,000 or less	43	19.1
3,001-5,000	63	28.0
5,000-10,000	55	24.4
10,001-15,000	37	16.4
15,001-20,000	19	8.4
20,001-25,000	8	3.6

The effect monthly family income on the duration of exclusive and total breast-feeding is given in Table 2, which shows that there was a decreasing trend of the duration of total breast-feeding as the family income increased ($p<0.05$). The variation was found insignificant in case of exclusive breast-feeding ($p>0.05$).

Age and sex of subject infants: The age and sex distribution of subject infant is presented in Table 3. It appears form the table that the subject infants fell within the age group of less than three months. It also appears that the proportion of female child was slightly larger than the male. Out of 225 million infants 111 (49.3%) was male against 114 (50.7%) female. It correspond the proportion of 974 male per thousand of female children.

Exclusive breast-feeding: Table 4 describes the duration of exclusive breast-feeding for male and female infants. The result shows that the duration of exclusive breast-feeding was longer for male than for female children. The mean during for males (3.63 months) was slightly higher than that for female (3.26 months). However, this difference was not significant, as found by the values of standard deviation.

Education Level of the respondents and their husbands and effect of breast feeding: Table 5 describes the result of education levels of infant's

Table 2: Duration of exclusive and total breast-feeding as affected by family income (figures are number of cases with percentage in parenthesis)

Monthly family income (Tk.)	Duration of EXBF (month) <3	3-5	>5
<5,000	19 (8.4)	29 (12.8)	25 (11.1)
5,000-10,000	25 (11.1)	34 (15.1)	22 (9.8)
>10,000	24 (10.7)	28 (12.4)	19 (8.4)
	χ^2 = 2.02	p>0.05	
Monthly family income (Tk.)	Duration of TBF (Month) <12	12-24	>24
<5,000	13 (5.7)	33 (14.7)	31 (13.7)
5,000-10,000	17 (7.6)	42 (18.7)	37 (16.4)
>10,000	15 (6.7)	26 (11.6)	11 (4.9)
	χ^2 = 5.91	p<0.05	

Table 3: Distribution of infants by age and sex

	Girl		Boy	
Age (months)	Number	%	Number	%
<3	16	6.7	14	6.2
3 + to 6	13	5.8	12	5.3
6 + to 9	30	13.3	30	13.3
9 + to 12	25	11.1	23	10.2
12 + to 24	31	13.8	32	14.2
Total (n = 225)	114	50.7	111	49.3

Table 4: Duration of exclusive breast-feeding

	Girl		Boy	
Duration (month)	Number	%	Number	%
<1	12	5.3	10	4.4
1+ to 2	14	6.2	8	3.6
2 + to 3	18	8.0	19	8.4
3 + to 4	27	12.0	25	11.1
4 + to 5	31	13.8	27	12.0
5 + to 6	12	5.3	16	7.1
6 + to 7	-	-	4	1.8
7 + to 8	-	-	2	0.9
Total (n = 225)	114	50.7	111	49.3
Mean Duration ±SD	3.26±0.4 months		3.63±0.4 months	

Table 5: Distribution of infant's parents by education

	Mother (n = 225)		Father	
Education level	Number	%	Number	%
Illiterate	48	21.3	8	3.6
Primary	55	24.4	40	17.8
Secondary	72	32.0	53	23.6
Higher secondary	25	11.1	25	11.1
Graduate	25	11.1	99	44

parents. The result shows that 21.3% and 3.6% mother and fathers respectively were illiterate. The highest percentage was observed at secondary level of education for mother and the highest percentage was observed at graduation level of education for fathers. The graduate level of education was observed only 11.1% among mothers. The rates of literacy and higher education had been found lower among mothers.

Table 6 shows the effect of mother's education on the duration of exclusive and total breast-feeding. There was a decreasing trend of both the duration of exclusive and total breast-feeding as the level of mother's education interested. The variations were significant at 5% level of both exclusive and total breast-feeding as obtained by the chi square-test.

Age of the fathers and mothers and effect of breast feeding: The age distribution of infant's mother along with their husband's is presented in Table 7. It shows that 24% of the mothers had less than 20 years of age. Majority of mothers (38.2%) was of the age group of 20-24 years, followed by the age group of 25-29 years 32% and 30-34 years (5.8%). No mother was found having more than 34 years of age. On the other hand, no father was reported table less than 20 years of age. The highest percentage of fathers was found with in the age group of 30-34 years (39.6%) followed by 35-39 years (24%). The lowest percentage of father (6.2%) was observed within 40-45 years.

Table 8 explains the effect of maternal age on the exclusive and total duration of breast-feeding. It appears that though variations in duration of both exclusive and total breast-feeding were observed among different maternal age of groups, these were statistically in significant, as reveled by the chi square - test.

Prelacteal feeds: Table 9 describes the various Prelacteal feeds given to the neonates before breast-feeding. It appears from the table that in about 70% of cases Prelacteal feed was not given. Sugar water was given in 9.3% of cases. Diluted cow-milk, powdered milk, honey, only water and other's expressed breast milk were reported as Prelacteal feeds.

Colostrums feeding: Practice and attitude of the respondent mothers regarding colostrums feeding are summarized in Table 10. The result reveals that 87.6% of the respondent mothers gave colostrums to their infants and 12.4% discarded it. About half of the respondent mothers who gave colostrums delivered that it was good for infants. Only 5.8% of the mothers gave it for immunity development in infants. Some of the mothers (19.1%) reported that they gave it according to the doctor. Rejection of colostrums was due to social custom (6.7%) or bad for infant's health (5.7%).

Total duration of breast-feeding: Table 11 describes the total duration of breast-feeding. It appears from the table that the mean duration was slightly higher for male (1.92 years) than for female children (1.89 years). The difference was, however, insignificant as observed by the values of standard deviation. About half of the mothers said that they would breast-feed for 2 years. Forty two percent mothers would like to breast-feed for 2 years-feed for as long as till the breast milk exhausted. Only 8% of the mothers would like to give breast for 1 years of less.

Table 6: Duration of exclusive and total breast-feeding as affected by mother's education (figures are number of cases with percentage in parenthesis)

Total years of schooling	Duration of EXBF (Month)			Duration of TBF (Month)		
	<3	3-5	>5	<12	12-24	>24
<6	33 (14.7)	36 (16.0)	26 (11.0)	4 (1.8)	43 (19.1)	42 (18.7)
6-10	28 (12.4)	37 (16.4)	23 (10.2)	9 (4.0)	30 (13.3)	40 (17.8)
>10	17 (7.6)	13 (5.8)	12 (5.3)	5 (2.2)	21 (9.3)	31 (13.8)
	χ^2 = -2.75	p>0.05		χ^2 = 3.74	p<0.05	

Table 7: Distribution of infant's parents by age

Age (year)	Mother (n = 225)		Father (n = 225)	
	Number	%	Number	%
15-19	54	24.0	-	-
20-24	86	38.2	16	7.1
25-29	72	32.0	52	23.1
30-34	13	5.8	89	39.6
35-39	-	-	54	24.0
40-45	-	-	14	6.2

Table 8: Duration of exclusive and total breast-feeding as affected by mother's age (figures are number of cases with percentage in parenthesis)

Mothers age (year)	Duration of EXBF (month)		
	<3	3-5	>5
<21	19 (8.5)	33 (14.7)	14 (6.2)
21-25	36 (13.3)	41 (18.2)	13 (5.8)
>25	23 (10.2)	35 (15.6)	11 (4.9)
	χ^2 = 3.07	p>0.05	
Mothers age (year)	Duration of TBF (Month)		
	<12	12-24	>24
<21	6 (2.7	38 (6.9)	36 (16.0)
21-25	7 (3.1)	39 (17.3)	37 (16.5)
>25	5 (2.2)	32 (14.2)	25 (11.1)
	χ^2 = 0.39	p>0.05	

Table 9: Prelacteal feeds offered to infants (n = 225)

Name of feed	Number of cases	%
Powdered	14	6.2
Cow milk	5	2.2
Sugar water	21	9.3
Honey	12	5.3
Water	9	4.0
Other's breast milk	7	3.1
Total	68	30.2
Not given	157	69.7

Table 10: Mother's practice regarding colostrums feeding

Reason for giving/ discarding colostrums	Given		Discarded	
	Number	%	Number	%
Good for health	117	52.0	-	-
Immunity	13	5.8	-	-
Doctors suggestion	43	19.1	-	-
Not know	24	10.7	-	-
Harmful	-	-	8	3.5
Causes stomach upset	-	-	5	2.2
Custom	-	-	15	6.7
Total (n = 225)	197	87.6	28	12.4

Table 11: Distribution of total duration of breast-feeding

Duration (Year)	Girl		Boy	
	Number	%	Number	%
<6	6	2.7	5	2.2
1	3	1.3	4	1.8
1-1.5	44	19.6	52	23.1
1.5-2	7	31	8	3.6
Till breast milk exhausts	45	20.00	51	22.6
Total (n = 225)	105	46.7	120	53.3
Mean duration±SD	1.89±0.23 years		1.92±0.24 years	

Infants interest towards food: It is important to consider infants' interest towards weaning food since it is intimately related to infants' nutrition and health. Table 12 summarizes the interest of infants towards breast milk and weaning food.

It is evident form Table 12 that almost all the infants were interested to breast milk. Sporadic evidence of infant's disinterest towards breast milk was observed. On the other hand, more than 50% of the infants were disinterested to their weaning food.

Cooking of weaning food: Since weaning is to accustom the infant with normal family diet, it is note worthy the type of cooking of infant's food. Distribution of the respondent mothers according to type of cooking of infant's weaning food is presented in Table 13.

It appears from the table that in (36.9%) of cases the weaning food was cooked separately and in (22.7%) of cases jointly. Mixed type of cooking (sometimes separately and jointly) was reported in (20.9%) of cases. Here "Jointly cooking" implies that infant's weaning food was cooked not separately but along with the adults. About one fifth of the respondent's said that they did not cook weaning food especially for infants, in such cases the children were given from normal adult's meals.

Nutritional status of the subject infants: The nutritional status of the subject infants was assessed through anthropometric measurements. The results are presented in Table 14-17.

It appears from Table 14 that, according to the Harvard standard, about half of the infant populations were under-weight and about 2% of infants were found over height. Rest of the infants would fall within the range of normal weight-for-age. The nutritional status of these

Table 12: Distribution of infants according to their Interest towards breast milk and weaning food (n = 225)

	Type of food			
	Breast milk		Weaning food	
Infant's interest	Number	%	Number	%
Positive	222	98.7	72	32.0
Negative	3	1.3	123	54.7
Not known	-	-	30	13.3

Table 13: Type of cooking of infant's food

	Mother (n = 225)	
Type of cooking	Number	%
Separately	83	36.9
Jointly	51	22.7
Separately and Jointly	47	20.9
Not specially for infant	44	19.5

Table 14: Distribution of infants by height for-age (Harvard Standard)

	Infants (n = 225)	
Standard	Number	%
100%	47	20.9
90%	56	24.9
80%	47	20.9
70%	43	19.1
60%	23	10.2
<60%	5	2.2
120%	4	1.8

Table 15: Nutritional status of infants according to Gomez classification

		Infants (n = 225)	
Gomez Classification	Nutritional status	Number	%
<60.0	3rd grade malnutrition	5	2.2
60.0-74.9	2nd grade malnutrition	66	29.3
75.0-89.9	1st grade malnutrition	47	20.9
90.0-110.0	Normal	103	45.8
>110.0	Over Nourished	4	1.8

infants can easily be assessed by Gomez classification, which is presented in Table 15.

It appears from Table 13 that only 45.8% of the infants were in normal maturational state and 1.8% was over nourished. Majority (5.2%) of the infants suffered from malnutrition of which 20.9% suffered first degree 29.3% second degree and 2.2% from third-degree malnutrition. The nutritional status of infants was also assessed through measuring their Mid-Upper Arm Circumference (MUAC) and head/chest ratio. The results are shown in Table 16 and 17.

According to the measurement of infants MUAC, 40.4% of the infants found in normal nutritional status and rest of the infants was malnourished (Table 16). According to head/chest ratio only 20.9% infants were in the normal group (Table 17).

Table 16: Distribution of infants by their mid-upper arm circumference

		Infants (n = 225)	
MUAC (cm)	Nutritional status	Number	%
<12.5	Severely malnourished	47	20.9
12.5-13.5	Moderately malnourished	87	38.7
>13.5	Above normal nutritional status	91	40.4

Table 17: Distribution of infants by their head/chest ratio

		Infants (n = 225)	
Head/ chest ratio	Nutritional Status	Number	%
<1	Over nourished	74	32.9
>1	Malnourished	104	46.2
1	Normal nutritional status	47	20.9

From the above results, it may be summarized that majority of infants of the study population suffered form malnutrition.

DISCUSSION

In the present study, 225 mothers of various socioeconomic and educational levels were interviewed on a pre-set questionnaire to investigate the infant feeding practices prevailing among them. Some factors, which might influence the feeding practices, such as infant's sex, mother's age and education and family income, were also studied. The important findings are discussed below.

Prelacteal feeding: Breast-feeding is rarely initiated after birth, but is usually receded by Prelacteal feeds. The period immediately following delivery is a time when many women introduce breast milk substitutes to their infants' diet. The introduction of breast-milk substitute before the milk comes in is known as prelacteal feeding. In the present investigation, majority of the mothers started breast-feeding on or after the third day of delivery. It is usual a practice in our country as observed in some studies (Talukder *et al*., 1988). The cause of delay in putting the baby to the breast was 'lack of flow of milk' from the breast, which is a well-known observation in many other studies (Chowdery *et al*., 1995; Das *et al*., 1992; Faruque *et al*., 1992; Jacbson *et al*., 1991).

Colostrums rejection was found only in 12.4 of the cases. In most of the cases mothers accepted the matter as social custom. Although most of the mothers gave colostrums to their babies, only 5.8% of them knew its immunological significance.

Breast-feeding: Breast-feeding is the traditional and ideal form of infant feeding for meeting a child's nutritional needs during the first few months of life. It has been recognized that human milk prevents obesity of infants and has a protective effect against various infectious diseases. It represents the only of available source of the protein containing all the amino acids.

Breast milk, unlike most substitute foods, is easily digestible. Breast-feeding also has some contraceptive effects.

According to most of the investigators, breast-feeding is universal in Bangladesh. Ahamed (1986) reported that the mean duration of breast-feeding of Bangladeshi children was 27.3 months (2.27 years). In our study, the mean duration was found to be 1.89 years for female and 1.92 years for male children. The shorter mean duration of breast-feeding as observed in the present study may be because the study might be due to the fact that the population was urban. It has been found from several studies that urban women breast-feed for shorter duration than rural women (WHO, 1981; 1991).

Mother's education and breast-feeding: The educational level of mother is often found to influence breast-feeding. Educated mothers usually breast-feed their children for a shorter duration than uneducated mothers. The inverse relationship between breast-feeding duration and education was found in most studies carried out in Bangladesh (Ahamed, 1986; Ahamed and Barkat-e-Khuda, 1984). For example, Ahamed and Barkat-e-Khuda observed that the better educated a woman is the less likely she is to start breast-feeding, and if she starts, she breast-feeds for a shorter duration on the average. Our findings (Table 6) substantiate the issue.

Mother's age and breast-feeding: Ahamed (Ahamed, 1986) observed that the overall mean duration of breast-feeding increased with age of the mothers. Some other studies also found that mother's age was positively associated with longer duration of breast-feeding (Cosminsky *et al*., 1993; Forman, 1984; Huffman *et al*., 1980; Jackson *et al*., 1992). In our study, however, we could not find any significant relation between mother's age and breast-feeding duration (Table 8).

Infant's sex and breast-feeding: Several studies in Bangladesh shows (Ahamed, 1986; Ahamed and Barkat-e-Khuda, 1984; Briend *et al*., 1998) that female children are breast-fed for periods shorter than male children, and male children are reared more carefully than female children in the hope of future or old age security. Guldan *et al*. (1993) reported that if the child was a boy, the mother was more likely to be in leisure during breast-feeding, while if the child was a girl, the mother was more likely to be engaged in economic activity. It suggests different degrees of seriousness with which the mothers took breast-feeding children of different sexes. In our study, however, no significant variation in breast-feeding duration was observed between male and female children.

Family income and breast-feeding: Family income is a principal predicator of socioeconomic status of the family. In the present study it was observed that the duration of exclusive and total breast-feeding decreased with increasing family income. Similar results were also reported in many other studies (Janowitz *et al*., 1981; Kalra *et al*., 1982; Piwoz and Viteri, 1985; Thimmayamma *et al*., 1980; Black *et al*., 1982).

Supplementation and weaning: Weaning period is a crucial time in an infant's life when he shifts gradually from exclusive breast-feeding to the adult diet. This transitional period generally begins at about four months and may extend up to two years or even more. The problem of when to begin weaning and what foods are appropriate has been extensively examined (Underwood and Hof Vander, 1982; Wharton, 1989; WHO, 1988).

In appropriate weaning may initiate undesirable consequences. Too early initiation of weaning carries the risk of increased morbidity due to diarrhoea and food allergies, as external challenges are introduced into the immature digestive tract (Wharton, 1989; Whitehead, 1985). It may also result in infant malnutrition due to the normal decrease in maternal milk production, as the baby is withdrawn from the breast (Wharton, 1989; Whitehead, 1985). Weaning too late can lead to faltering growth, malnutrition and decreased immune protection when exclusive breast-feeding becomes inadequate (Whitehead, 1985; Whitehead and Paul, 1984). Inappropriate choice of weaning foods can lead to protein-energy, malnutrition and micronutrient deficiencies.

During the first four months of life, breast milk alone provides optimal nutrition for rapidly growing infants (Popkin *et al*., 1986). As physical and developmental capacities mature, solid foods are introduced. The composition and consistency of the diet are advanced so those by one year the infant can eat a variety of foods from a mixed diet (Jason *et al*., 1984).

An important point should be considered here that our information on supplementation is limited. We only know the age at first supplementation. We do not have information on the adequacy of the supplement or how frequently it was given to infants. In our country infants seldom receive the necessary amounts of supplements to maintain normal growth. The supplements that are given to infants contain only minimal amounts of calories and the infants rely on breast milk as their major source of nourishment even into the second year of life (Islam and Ali, 1983; Malek *et al*., 1986).

Few infants who are fed only breast milk beyond age 6 months remain healthy and grow well. In a study among poor women of India (WHO, 1981), 80% or more of the mothers were found to exclusively breast-feed their

infants without evidence of significant growth faltering for at least 6-7 months. It is noteworthy; however, that slowed growth is prevalent in 1-3 year-old Indian infants. The question here is that, among those poor women in India and among those in similar situations in which mothers rely on prolonged unsupplemented breast-feeding, is whether complementary feeding introduced earlier would have averted the subsequent malnutrition (Mitra *et al*., 1994).

Faltering of growth after 6 months is not only a consequence of too early and insufficient provision of complementary food under unhygienic conditions, but perhaps of equal importance is the failure to stimulate the early acceptance of a varied eating pattern during the weaning process. Our finding supports the view as majority of infants was found to be disinterested to their weaning food (Table 12).

Assessment of nutritional status: We assessed of under 2 years children nutritional status using anthropometric measurements, among them height for age by Harvard standard, Gomez classification, Mid-Upper Arm Circumference (MUAC) and head/chest ratio were respectively. Nutritional anthropometry is concerned with the measurement of the variations of the physical dimensions and the gross composition of the human body at different age levels and degree of nutrition (Jelliffe and Derrick, 1966). Before the recommendations of Indian Harvard as "reference" standard for Academy of Pediatrics for adopting assessment of growth and calculating various grades of Protein Energy Malnutrition, most of the studies in India on growth have been conducted on the low or mixed socio-economic groups, but after this recommendation, a large number of workers have reported growth pattern in higher socio-economic strata of the community indicating growth potential of Indian children, who do not have nutritional and economic constrains (Bhandari and Mandowara, 1982). Although according to the Harvard standard, our data are shown that about half of the infant populations were under-weight (Table 14).

The Gomez classification has been world wide used. Generally it was introduced as a prognostic tool which one is used for hospitalized cases of protein calorie malnutrition. According to Gomez classification, 45.8% infants were normal maturational state and majority of the infants suffered from malnutrition (Table 15). According to the measurement of infants by mid upper arm circumference, most of the infants were malnourished and according to the head/chest ratio also shown that most of infants were suffered malnutrition (Table 16 and 17).

Conclusions: In finally, the present study was conducted to investigate the infant feeding practices that prevailed in a selected hospital of the Dhaka City. Two hundred and twenty five mothers were interviewed on a pre-set questionnaire. The respondent mothers were of various socioeconomic and educational levels.

Even the most educated of the mothers could not be said to follow proper weaning practices. In addition, increased family income was not translated into improved nutritional status of children. The duration of exclusive breast-feeding was alarmingly low. In 74% of cases, the introduction of supplement food was either too early or unduly delayed.

Nevertheless, the prevalence of breast-feeding is still high in urban population but the practices of too early introduction of supplement and weaning food play an important role in initiating and sustaining the vicious cycle of malnutrition and infection. The situation demands improved infant feeding practices. To achieve the goal, following recommendations may be considered:

- The formal education system should be strengthened by an increased emphasis on nutrition and health-related components
- Although the findings of this research emphasize some relationships between formal education and feeding practice, informal education is also recommended

It is important that the medical and health personnel educate the mothers about these practices at every possible contact, so as to ensure proper infant feeding.

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Bacteriological and Polycyclic Aromatic Hydrocarbon Accumulation in Mangrove Oyster (*Crassostrea tulipa*) from Douglas Creek, Nigeria

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Abstract: Bacteriological density and polycyclic aromatic hydrocarbon concentrations were determined in the brackish surface water and mangrove oyster (*Crassostrea tulipa*) from Douglas creek, Nigeria. Total Heterotrophic Bacteria (THBC), Total Vibrio (TVC) and Total Coliform (TCC) counts ranged from 8.8 x 10^4 - 10.8 x 10^4 cfu/ml, 2.1 x 10^4 - 3.8 x 10^4 cfu/ml and 5.2 x 10^4 - 7.2 x 10^4 cfu/ml in water whereas THBC, TVC, TCC in the mangrove oyster ranged from 12.5 x 10^6 - 17.9 x 10^6 cfu/g, 3.9 x 10^5 - 5.8 x 10^5 cfu/g and 8.9 x 10^5 - 9.7 x 10^5 cfu/g. The microbial groups in the water and oyster exhibited varying correlations with r-values (p = 0.05) ranging from -0.3767 to 0.7209. The bacterial isolates were *Staphylococcus saprophyticus, Aerobacter aerogenes, Citrobacter sp, Bacillus cereus, Streptococcus sp, Vibrio parahaemolyticus, Vibrio cholerae, Pseudomonas putida, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis* and *Serratia marcescens, Acinetobacter iwoffii, Chromatium sp, Micrococcus sedentarius, Listeria monocytogenes* and *Klebsiella pneumoniae*. The individual PAH accumulated by the mangrove oyster ranged from 0.02-3.56 mg/kg against background surface water PAH concentration which ranged from 0.02 - 1.00 mg/l. Total PAH concentration in brackish surface water and mangrove oyster tissue was 7.51 mg/l and 11.96 mg/kg respectively. The Bioconcentration Factor (BFC) of the individual PAH in the mangrove oyster ranged from 0.51 [benzo (b) fluoranthene] to 17.8 [dibenzo (a,h) anthracene]. The microbial isolates are of health significance and PAH concentration in the mangrove oyster could biomagnify along the food chain with adverse toxicological effects on ardent consumers of the biota. The mangrove oyster (*Crassostrea tulipa*) could be used as a biomarker of bacterial and PAH contamination of the mangrove ecosystem.

Key words: Bioaccumulation, ecotoxicology, Douglas creek, mangrove oyster, polycyclic aromatic hydrocarbon

INTRODUCTION

The Niger Delta region is richly endowed with both renewable and non-renewable resources with oil and gas accounting for over 85% of Nigeria's Gross Domestic Product (GDP). However, the region remains the poorest due largely to the ecologically unfriendly exploitation of oil and gas and State policies that expropriate the indigenous people (Aaron, 2005). One of the most severe pollution problems in the Niger Delta is from oil and related activities which have been on the increase in recent times largely due to spillage and sabotage, although there are scanty quantitative details on the actual level of contamination. There is fecal pollution of water bodies and wetlands due to inadequate or nonexistent toilet facilities in the Niger Delta riverine communities (World Bank, 1995). Also, municipal solid waste and waste water in open drains and surface runoff which could adversely affect the environment are discharged directly into the water bodies, wetlands, creeks and estuaries. Several studies have reported oil-related pollution of the Niger Delta especially the Qua Iboe River Estuary (QIRE) (Ekwere *et al*., 1992; Asuquo *et al*., 1995; Udotong, 2000; Aaron, 2005; Essien and Antai, 2005; Itah and Essien, 2005; Udotong *et al*., 2008).

PAHs are present as natural constituents in fossil fuels, are formed during the incomplete combustion of organic material and are therefore present in relatively high concentration in products of fossil fuel refining (Bos *et al*., 1984; Deschenes *et al*., 1996; Lee *et al*., 1981; Nestler, 1974; Nishioka *et al*., 1986; Wang *et al*., 1990; Wang *et al*., 1999). Petroleum refining and transport activities are major contributors to localized loading of PAHs into the environment. PAH molecule stability and hydrophobicity are two primary factors which contribute to the persistence of high molecular weight PAHs in the environment (Kanaly and Harayama, 2000). In localized areas such as rivers, estuaries and harbours, the rate of accumulation greatly exceeded the rate of environmental degradation (Jhonson and Ghosh, 1998). Due to their lipophilic nature, PAHs have a high potential for biomagnification through trophic transfers (Lu *et al*.,

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1977; Clements *et al*., 1994; Twiss *et al*., 1999). PAH are also known to exert acutely toxic effects and/or posses mutagenic, teratogenic or carcinogenic properties (Philips, 1983; Cerniglia and Heitkamp, 1989; IARC, 1990).

Oysters are filter-feeding organisms capable of accumulating microorganisms in high concentrations (Silval *et al*., 2004). According to Nunes and Parsons (1998), feeding oysters filter the surrounding water at a rate of 2-5 litres/hour eventually assimilating all the biotic and abiotic contaminants present in their environment. Indigenes of the Niger Delta are exposed to these microbial and chemical pollutants through the consumption of the diverse aquatic biota in the ecosystem. The ingestion of bivalve mollusks has been frequently associated with food-related infectious diseases (Cook *et al*., 2001). Additionally, exposure to contaminated sediments may occur during recreational activities such as wading, fishing, clamming and water sports. Inhalation of volatile contaminants released by sediments is also a potential exposure route. Although these exposure routes are generally not expected to contribute greatly to human health risks compared to consumption of seafood (WSDE, 1990).

Oysters are widely used for environmental monitoring purposes although some physiological factors such as spawning and growth can directly affect their ability to indicate pollution or environmental contamination (Rebelo *et al*., 2005). The inter tidal mangrove oyster *Crassostrea tulipa* cemented to the prop roots of mangrove macrophytes are freely harvested from the environment for domestic and commercial purposes being widely consumed by the coastal and estuarine communities in the Niger Delta as a delicacy and dietary protein supplement. The biota constituting lower level food chain could have elevated tissue burden of the pollutant due to their filter-feeding habit and ultimately be the route for transmission of these pollutants to humans. Several of these PAHs are known for their carcinogenic, mutagenic and teratogenic properties and also implicated in causing reproductive problems (Luch, 2005).

There is an increasing concern about PAHs due to their toxic and carcinogenic properties and the paucity of definite environmental information on these substances in the Qua Iboe River Estuary. This study was undertaken to assess the bacteriological load, PAH accumulation and possible toxicological implications due to the consumption and over-dependence by the indigenes of estuarine communities on the aquatic biota from the chronically polluted ecosystem for their dietary protein supplement.

MATERIALS AND METHODS

Study area: The Niger Delta region of Nigeria is characterized by a humid tropical climate with the Qua Iboe River Estuary (QIRE) located within latitude 4° 30'N and 4° 45'N and longitude 7° 30'E and 8° 45'E as a dominant hydrographic feature. The ecotone has mean minimum and maximum temperatures of 22°C and 30°C with an annual rainfall of 80% (Ukpong, 1995), although the ambient air temperature of Douglas creek during the sampling period was 39±2°C due to its proximity to the flare site. The estuary has a shallow depth ranging from 1-7 m at flood and ebb tide (Ekpe *et al*., 1995). The estuary is comprised of tidal creeks, lagoons, wetlands, and tributaries fringed with mangrove vegetation made up of species of *Avicennia, Rhizophora* and *Nypa* and harbor a rich collection of edible biotopes.

Sample collection/processing: The oyster samples were collected with the aid of machete at six different often exploited natural oyster beds in the creek during the wet season months of July and August, 2008. Samples for microbiological analysis were collected into a sterile isothermal container and transported to the laboratory. Mangrove oyster and surface water samples for chemical analysis were collected separately into Amber glass containers with Teflon-lined screw-cap. Water samples for chemical analysis were spiked with 5 ml of 1:1 HCl acid, stored in the dark at 4°C with a maximum holding time of 2 h before extraction (APHA, 1998). The oyster shells were extensively washed with water and rinsed with normal saline to remove all surface contaminants. The edible parts were removed with a sterile knife and transferred immediately to a sterile blender for homogenization and serial dilution.

Enumeration of Heterotrophic, Vibrio and Coliform bacteria: The counts of Total Heterotrophic Bacteria (THB) in the water and mangrove oyster was enumerated by pour plate technique (Harrigan and McCance, 1990) using diluents prepared with 25% Ringer's solution and cultured on nutrient agar (Difco) whereas Total Coliform (TC) counts were determined on MacConkey agar (Oxoid) and Total Vibrio (TV) counts on Thiosulphate Citrate Bile-salt (TCBS) medium. The nutrient agar was supplemented with cycloheximide (100 µg/ml and benomyl (50 µg/ml) to prevent fungal growth (Kinkle *et al*., 1995). Inoculated TC and TV plates were incubated aerobically whereas THB plates were incubated aerobically and anaerobically at room temperature (28±2°C) for 24 h and thereafter enumerated (Harrigan and McCance, 1990; Amadi and Braide, 2003). Representative bacterial colonies were purified by repeated subculturing and maintained as stock on nutrient agar slants. The identification of the isolates was done by comparing the cultural, morphological and biochemical characteristics of the cultures with the characteristics of known taxa using the Bergey's manual of determinative bacteriology (Holt *et al*., 1994) and Cowan and Steel's manual for the identification of medical bacteria (Barrow and Feltham, 1992).

Chemical analysis: Mangrove oyster sample was dried with anhydrous Na_2SO_4, extracted with a mixture of Dichloromethane (DCM) and acetone, thereafter solvent-exchanged with hexane. Clean-up and fractionation was done using silica gel permeation chromatography. The water sample was extracted with methylene chloride, dried with anhydrous Na_2SO_4 and solvent-exchanged into hexane. Clean-up and fractionation was done using silica gel permeation chromatography. Final extracts of water and mangrove oyster samples after reconcentration using a rotary evaporator was packed in a 2 ml GC vials and analyzed with a Gas Chromatography (GC), Perkin-Elmer/Clarius 500. GC columns conditions: Column made up of 5% PMS (100/120 mesh) coated with 3% OV-17 packed in a 1.8 x 2 mm ID glass column with helium carrier gas at 40 ml/min flow rate. Column temperature held at 100°C for 4 min, then programmed at 8°C/min to a final hold at 280°C. H_2 and Ar gas were used to light up the FID. Quantification of the PAHs was accomplished using a seven-point, external standard curve (APHA, 1998).

Statistical analysis: Correlation analysis of data were performed using Analyze-It General 1.73 statistical software® on log-transformed estimates of densities of heterotrophic, Vibrio and coliform bacteria in surface water (log cfu/ml) and mangrove oyster (log cfu/g) with levels of significance maintained at 95% for each test. Interpretation was done based on Hinkle *et al.* (1994) rule of thumb for interpreting the size of a correlation coefficient.

Bioconcentration factor determination: The quantification of the accumulation of polycyclic aromatic hydrocarbon in the tissue of the mangrove oyster in mg/kg body mass was made as a dimensionless factor, called Bioconcentration Factor (BCF) (Walker, 1987), expressed as:

$$BCF = \frac{\text{Concentration of the pollutant inside the tissue of the organism}}{\text{Concentration of the pollutant outside the tissue}}$$

"Outside" refers to the sources of the pollutant to which the organism is directly or indirectly exposed. In this study, outside is the brackish surface water in which the mangrove oyster grows and derives its nutritive material.

RESULTS AND DISCUSSION

The enumeration of viable heterotrophic and enteric microorganisms in the surface water and mangrove oyster tissues using different culture media are shown in Table 1. Counts of Total Heterotrophic Bacteria (THBC), Total Vibrio (TVC) and Total Coliform (TCC) ranged from 8.8×10^4 - 10.8×10^4 cfu/ml, 2.1×10^4 - 3.8×10^4 cfu/ml and 5.2×10^4 - 7.2×10^4 cfu/ml in water whereas THBC, TVC, TCC in the mangrove oyster ranged from 12.5×10^6 - 17.9×10^6 cfu/g, 3.9×10^5 - 5.8×10^5 cfu/g and 8.9×10^5 - 9.7×10^5 cfu/g respectively. The trend of counts taken separately for the different microbial groups was in the order THBC > TCC > TVC in both the surface water samples and mangrove oyster respectively. The different microbial groups in the brackish surface water and mangrove oyster exhibited varying correlation ships with r-values ($p = 0.05$) that ranged from -0.3767 to 0.7209 as shown in Table 2. There was a high positive correlation ($r = 0.7209$) in the relationship between counts of Vibrio in the water (TVCw) and oyster (TVCct) samples whereas moderately positive corelationships were exhibited between TVCw and THBCw, TVCw and TCCct, TBCct and TCCct and TVCct and TCCct indicating that an increase of the bacterial count in water directly influenced the load in the mangrove oyster.

The culturable microorganisms were isolated from both water and mangrove oyster tissues irrespective of the proximity of the sample location to human settlements and activities in the estuary, suggesting a widespread distribution of the isolates in the surface water. The heterotrophic, enteric and Vibrio species accumulated by the oyster were one order of magnitude higher in all the sample location than the background count in the surrounding brackish surface water. The bacterial isolates were *Staphylococcus saprophyticus*, *Aerobacter aerogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Streptococcus sp*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio alginolyticus*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Serratia marcescens*, *Acinetobacter iwoffii*, *Chromatium sp*, *Micrococcus sedentarius*, *Listeria monocytogenes*, *Klebsiella pneumoniae* and *Proteus vulgaris*.

In this study, emphasis was on the general occurrence of human pathogens instead of total coliform. This is because many studies (Carlucci and Pramer, 1959; Dutka, 1973; Dawe and Penrose, 1978; Rhodes *et al.*, 1983) have indicated the inadequacy of the coliform as an indicator of fecal contamination of marine ecosystem and the safety of shellfish harvested from sewage impacted areas. They demonstrated that *E. coli* is rapidly eliminated from seawater whereas other pathogenic bacteria in sewage effluents may survive for extended periods.

The occurrence of *Escherichia coli* in the samples indicated recent fecal pollution of human origin (Duffour *et al.*, 1985) and together with other bacterial isolates have been implicated in infectious diseases involving every organ system (Gracey *et al.*, 1982; Burke *et al.*, 1983; Smith and Williams, 1984; Von Graeventz and Altwegg, 1991; Koneman *et al.*, 1992). The most common of these in the QIRE communities are gastrointestinal and bronchopulmonary disorders due to incidental swallowing of the estuarine water during swimming and consumption of inadequately cooked shellfishes.

Table 1: Microbial counts in the water and mangrove oyster samples

Sampling location	Water			*Crassostrea tulipa*		
	THBC (x10^4)	TVC (x10^4)	TCC (x10^4)	THBC (x10^6)	TVC (x10^5)	TCC (x10^5)
L1	9.5 (4.98)	2.7 (4.43)	6.8 (4.83)	13.2 (7.12)	5.1 (5.71)	8.9 (5.95)
L2	8.8 (4.94)	2.1 (4.32)	5.6 (4.75)	12.5 (7.10)	3.9 (5.59)	9.2 (5.96)
L3	9.2 (4.96)	3.4 (4.53)	6.6 (4.82)	13.0 (7.66)	5.2 (5.72)	9.7 (5.99)
L4	10.2 (5.01)	2.6 (4.41)	7.2 (4.86)	17.9 (7.25)	4.2 (5.62)	8.9 (5.95)
L5	10.8 (5.03)	3.8 (4.58)	6.8 (4.83)	17.5 (7.24)	4.7 (5.67)	9.4 (5.97)
L6	9.7 (4.99)	3.5 (4.54)	5.2 (4.72)	14.6 (7.16)	5.8 (5.76)	9.6 (5.98)

Values in parenthesis are Log10

Table 2: Correlation between the microbial groups in water and C*rassosstrea tulipa*

	THBw	TVCw	TCCw	THBct	TVCct	TCCct
THBw	1					
TVCw	0.5755^a	1				
TCCw	0.4569^c	0.0607^d	1			
THBct	-0.1104^d	0.4406^c	0.3315^c	1		
TVCct	0.1283^d	0.7209^a	-0.2343^d	0.2838^d	1	
TCCct	-0.1497^d	0.6689^b	-0.3767^c	0.6784^b	0.5839^b	1

w = water sample, ct = C*rassostrea tulipa*

a = high positive correlation, b = moderate positive correlation, c = low positive (negative) correlation, d = little if any correlation

Water and food-borne infections in the QIRE communities of the Niger Delta has continued largely due to the low literacy level, poor hygienic conditions, historical neglect, apathy, pollution, socio-ecological green washing of the exploration and allied companies operating in the region and the abysmal poverty level of the inhabitants who cannot afford clean, safe drinking water and medicare.

The gastrointestinal illnesses prevalent in the area range from mild to severe stomach distress with symptoms including abdominal cramps, frequent diarrhea, occasional vomiting and dehydration. These illnesses although preventable, are often undiagnosed, life threatening, sometimes fatal and unreported. Food borne infection and intoxication in the study area as a result of consumption of sea foods is particularly worrisome because of the unhygienic environment, handling of the harvested aquatic biota and the mild heat applied during the smoking process which could be stimulatory to the microorganisms. The tissue microbial load in the oyster was above the ICMSF (1986) standard of 10^5 cfu/g for fresh and frozen bivalve molluscs. This is indicative of the health hazard susceptible consumers would be exposed to, if the mangrove oyster is not depurated, hygienically prepared or adequately cooked before consumption. Exposure to food borne infection and intoxication is usually more pronounced in avid consumers who are in the habit of eating uncooked oyster harvested from the estuarine ecosystem (personal communication).

The levels of individual PAH quantified in this study ranged from 0.20-1.00 mg/l and 0.20-3.56 mg/kg in the brackish surface water and mangrove oyster with a total PAH concentration of 7.51 mg/l and 11.96 mg/kg respectively as represented in Table 3. These levels may be regarded as baseline concentration and will be useful in comparing the risk factors in the consumption of other aquatic foods. It will be premature to make categorical statements on the residue levels of PAHs in the estuary considering the inflows and inputs from Qua Iboe River, atmospheric deposition, runoffs, sewage, industrial and municipal waste and petroleum E and P activities contributing significantly to the load and the small sample size.

Several PAHs are the most potent carcinogens known to exist, producing tumors in some organism through single exposures to microgram quantities. PAHs act at both the site of application and at organs distant to the site of absorption; their effects have been demonstrated in nearly every tissue and species tested, regardless of the route of administration (Lee and Grant, 1981). This could influence and negatively impact the biodiversity and densities of the biota in the estuary.

Susceptible human population in the Niger Delta would be exposed to PAHs primarily through the consumption of shellfish and other aquatic resources that are essentially an integral component of every diet of the people in this region. In the Douglas creek, much of the PAHs are released into the atmosphere via soots from gas flaring and eventually reaches the water by direct deposition or by deposition on vegetation in addition to inputs from sewage and used motor oil from diffuse sources. The carcinogenic activity of soots, tars and oils to man is beyond dispute. Benzo(a)pyrene, a PAH, was identified as one of the most carcinogenic compound in coal tar (Dipple, 1985). Sewage effluents usually contain measurable levels of PAHs, although extreme variability between and among sites is common (Eisler, 1987). In addition to the skin cancers, higher incidences of respiratory tract and upper gastrointestinal tract tumors

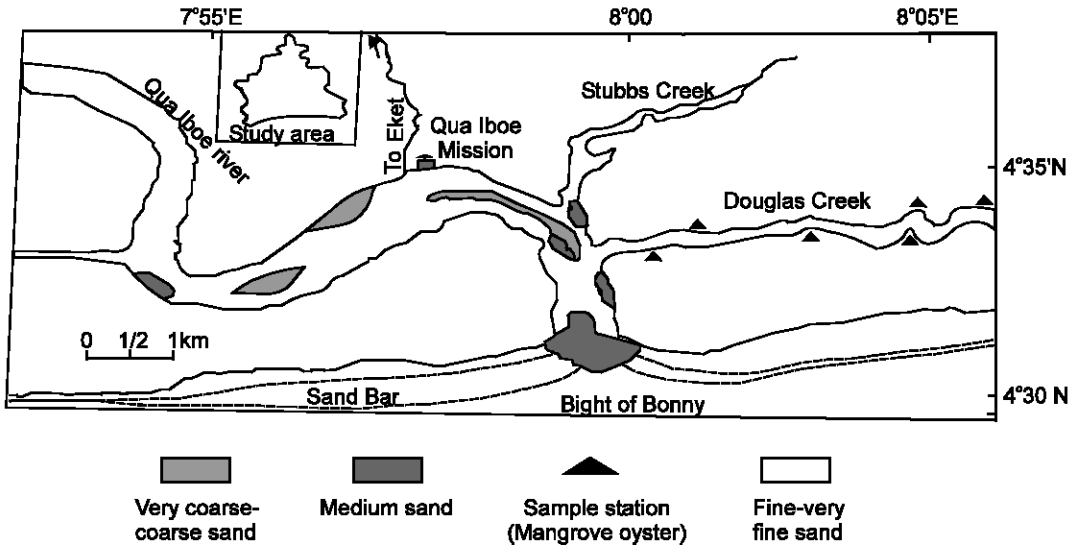

Fig. 1: Map of Qua Iboe River Estuary showing Douglas creek and sampling sites (Inset: Map of Akwa Ibom State)

Table 3: Concentration of polycyclic aromatic hydrocarbon in samples and their Bioconcentration factor

	Concentration		
Parameter	Water (mg/l)	Mangrove Oyster (mg/kg)	BCF
Naphthalene	1.00	1.00	1.00
2-Methylnaphthalene	0.20	0.20	1.00
Acenaphthylene	1.00	1.00	1.00
Acenaphthene	0.70	0.70	1.00
Fluorene	0.70	0.70	1.00
Phenanthrene	0.20	0.67	3.35
Anthracene	0.40	0.40	1.00
Fluoranthene	0.20	0.20	1.00
Pyrene	0.20	0.20	1.00
Benzo(a)anthracene	0.43	0.44	1.02
Chrysene	0.22	0.58	2.64
Benzo(b)fluoranthene	0.49	0.25	0.51
Benzo(k)fluoranthene	0.74	0.82	1.11
Benzo(a)pyrene	0.43	0.36	0.83
Dibenzo(a,h)anthracene	0.20	3.56	17.8
Benzo(g,h,I)perylene	0.20	0.68	3.40
Indeno(1,2,3-d)pyrene	0.20	0.20	1.00
Total PAH	7.51	11.96	1.59

were associated with occupational exposures to these carcinogens (Dipple, 1985). This research finding has strong public health implications although there are no records or data of body burden reflecting exposures of the populace to PAHs or other persistent-lipophilic and bioaccumulative or toxic compounds in the Qua Iboe River Estuary, Nigeria. Apart from microbial causes, gastrointestinal distress prevalent in the area may not be unconnected with direct uptake of PAH through consumption of the aquatic biota.

It has been demonstrated that certain Lower Molecular Weight (LMW), non carcinogenic PAHs, at environmentally realistic levels were acutely toxic to aquatic organisms, or produced deleterious sublethal responses (Neff, 1985). Among the LMW PAH, elevated concentration was recorded for phenanthrene with 0.67 mg/kg above the background level of 0.20 mg/l in water. It is suggestive that the uniform concentration of other low molecular weight PAHs such as 1.00 (naphthalene), 1.00 (acenaphthylene), 0.70 (acenaphthene) and 0.70 (fluorene) in the surface water (mg/l) and those accumulated in the aquatic biota (mg/kg) respectively could have adverse effects on the mangrove biota. The most obvious could be the low population densities of the mangrove oyster compared with other oyster beds in the estuary where the populations of *Rhizophora* and *Avicennia* species in the ecosystem are not asphyxiated. The *Nypa fructican* that proliferate in the study area have a thick and impervious cuticle that seems to excludes the oil from the plant tissues except through direct uptake and does not provide adequate breeding site for the biota. The Douglas creek surface water is darkened with an oily sheen as a result of soot deposition and episodic discharges from oil installations which could have contributed to the accelerated mortality of the mangrove plant by asphyxiation, blocking breathing pores on the mangrove prop roots and pneumatophores of *Avicennia* and by extension the aquatic resources.

Several studies have indicated that bivalve mollusks and some other invertebrates are unable to efficiently metabolize PAHs and excrete them (Neff *et al*., 1976; Jackim and Lake, 1978; Lawrence and Weber, 1984a; Varanasi *et al*., 1985), presumably due to inefficient or missing mixed function oxidase systems (Sirota and Uthe, 1981). Several PAHS are the most potent carcinogens known to exist, producing tumors in some organism through single exposures to microgram quantities. PAHs act at both the site of application and at organs distant to the site of absorption; their effects have

been demonstrated in nearly every tissue and species tested, regardless of the route of administration (Lee and Grant, 1981). There could also be the formation of reactive intermediates such as diol epoxides and phenol epoxides of benzo (a) pyrene both of which are implicated in mammalian mutagenesis and carcinogenesis (Varanasi and Gmur, 1981). The concentration of individual carcinogenic PAH in the mangrove oyster was in the following trend: 0.20 mg/kg [indeno (1,2,3-d) pyrene], 0.25 mg/kg [benzo (b) fluoranthene], 0.36 mg/kg [benzo (a) pyrene], 0.44 mg/kg [benzo (a) anthracene], 0.58 mg/kg (chrysene) and 3.56 mg/kg [dibenzo (a,h) anthracene]. The Bioconcentration Factor (BFC) for most of the PAH were above one order of magnitude in the samples with benzo (b) fluoranthene (0.51) and benzo (a) pyrene (0.83) as the exceptions, having values less the one.

PAHs from drinking water contribute only a small proportion of the average human intake (Harrison *et al*., 1975). The drinking water quality criterion for carcinogenic PAH compounds is based on the assumption that each compound is as potent as benzo (a) pyrene and that the carcinogenic effect of the compound is proportional to the sum of their concentrations (EPA, 1980). Based on an oral feeding study of benzo (a) pyrene in mice, the concentration of this compound estimated to result in additional risk of one additional case for every 100,000 individuals exposed (i.e., 10^5) is 0.028 µg/l. Therefore, with this assumption, the sum of the concentrations of all carcinogenic PAH compounds should be less than 0.028 µg/l in order to keep the lifetime cancer risk below 10^{-4}. The corresponding recommended criteria which may result in an incremental cancer risk of 10^{-6} and 10^{-7} over the lifetime are 0.0028 µg/l and 0.00028 µg/l, respectively. If the above estimates are made for consumption of aquatic organisms only, the levels are 0.311 (10^{-5}), 0.031 (10^{-6}) and 0.003 (10^{-7}) µg/kg, respectively (EPA, 1980).

Though inhabitants of the study area do not drink the brackish estuarine water with a 0.43 mg/l concentration of benzo(a)pyrene, diffused exposure to PAHs may be during recreational activities such as wading, fishing, water sports and inhalation but significant route of the PAHs exposure would be through consumption of diverse aquatic foods, including most especially the mangrove oyster. The elevated concentration of PAHs in the mangrove oyster tissue (11.96 mg/kg) above the background levels in the surface water (7.51 mg/l) with BCF of 1.57 is both suggestive of bioaccumulation and the chronic cancer risk humans in the study area are exposed to, as a result of oyster consumption since the concentration of individual PAHs in the water and mangrove oyster in this study was above the minimum recommended 0.00028 (10^{-7}) µg/l and 0.003 (10^{-7}) µg/kg respectively.

Conclusion: There is an increasing anthropogenic generation, use and deliberate release of waste containing persistent organic pollutants such as PAHs, and sewage into the Douglas creek of the Qua Iboe River estuary where prohibitions and/or restrictions existing on documents of regulatory agencies are rarely or poorly enforced. The concentration of PAHs in the surface water and mangrove oyster from the Douglas creek should be considered alarming in view of the carcinogenic and mutagenic properties of many individual PAHs. The widespread occurrence of pathogenic microorganisms and PAHs in all the samples is indicative of the local influence of the inhabitants and industrial activities in the area. The PAHs level of accumulation was found to be higher in the mangrove oyster than in the surface water and could be regarded as baseline environmental concentration and bioaccumulation in the mangrove oyster tissues respectively. The PAHs level is considered to be bioavailable since it is present in detectable quantities in the surface water and mangrove oyster tissues. The mangrove oyster (*Crassostrea tulipa*) could serve as a bioindex for microbiological and ecotoxicological risk assessment of the various probabilities of adverse effect of pollutants in the ecosystem and the body burden of microbial and organic pollutants accumulation therefore may provide a good indication of the health of the QIRE mangrove ecosystem.

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Antidiabetic and Antihyperlipidemic Properties of *Phyllanthus emblica* Linn. (Euphorbiaceae) on Streptozotocin Induced Diabetic Rats

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Abstract: Diabetes is known to involve oxidative stress and changes in lipid metabolism. The present study was designed to investigate the therapeutic effects of an ethanolic extract of *Phyllanthus emblica* fruits on antidiabetic, antioxidant and lipid profile in plasma and tissues (liver and kidney) of experimental diabetes. Thirty rats were allocated randomly into 5 groups, each of 6 rats. Group I was acted as control group, group II rats were rendered diabetic by intraperitoneal injection of streptozotocin (40 mg/kg bw), group III rats received *Phyllanthus emblica* fruit ethanolic extract (PFEet) (200 mg/kg bw) by using an intragastric tube for 45 days, group IV rats received glibenclamide (600 µg/kg bw), group V rats given PFEet (200 mg/kg bw) alone. Ethanol extracts of *Phyllanthus emblica* fruits was administered orally at doses of 200 mg/kg body weight for 45 days resulted in a significant reduction in blood glucose and a significant increase in plasma insulin in diabetic rats. Diabetic rats had elevated levels of Total Cholesterol (TC), Very Low Density Lipoprotein-Cholesterol (VLDL-C), LDL-cholesterol, Free Fatty Acids (FFA), Phospholipids (PL), Triglycerides (TG) and decreased HDL-cholesterol. Diabetic rats fed PFEet showed a significant reduction in TC, VLDL-C, LDL-C, FFA, PL, TG and an elevation in HDL-C. In conclusion, the observations from this study show that *Phyllanthus emblica* has antidiabetic and its beneficial effects on lipid profile, thus it can be recommended for use as a natural supplementary herbal remedy in patients suffering from diabetes mellitus.

Key words: *Phyllanthus emblica*, streptozotocin-diabetes, plasma insulin, blood glucose, lipid profile

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action (Jayakar and Suresh, 2003). During hyperglycemia excessive production of free radicals is believed to be involved in many diabetic complications including diabetic neuropathy in diabetes mellitus (Sima and Sugimoto, 1999). Besides hyperglycemia, several other factors including dyslipidaemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major causes of morbidity and death (Bennet and Joslin's, 1998). Diabetes mellitus is also associated with hyperlipidaemia with profound alteration in the concentration and composition of lipid (Odetola *et al*., 2006). Changes in the concentration of lipid with diabetes contribute to the development of vascular disease. Excessive levels of blood cholesterol accelerate atherogenesis and lowering high blood cholesterol reduces the incidence of CHD (Grundy, 1986). One of the risk factors for coronary heart disease is elevated Total Cholesterol (TC), Low Density Lipoprotein-Cholesterol (LDL-C) and Lower High Density Lipoprotein-Cholesterol (HDL-C) (Moreno and Mitjavalia, 2003; Schaefer, 2002; Hu *et al*., 2001; Jain *et al*., 2007).

An ideal oral treatment for diabetes mellitus would be a drug that not only controls the glycaemic level, but also prevents the development of atherosclerosis and other complications associated with diabetes mellitus. A different type of oral hypoglycemic agents such as biguanides and sulphonylurea are available along with insulin for the treatment of diabetes mellitus (Holman and Turner, 1991), but has side effects associated with their uses (Kameshwara Rao *et al*., 1997; Valiathan, 1998). There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in and relatively low costs.

In spite of the presence of known antidiabetic medicines in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease possibly because they are considered to be less toxic and free from side effects compared to synthetic one (Momin, 1997). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes (Pushparaj *et al*., 2000), but only a few have received scientific scrutiny. The World Health Organization has recommended the evaluation of the effectiveness of plants in conditions where safe orthodox drugs are scarce (World Health Organisation, 1980).

Phyllanthus emblica Linn. (Euphorbiaceae) commonly known as amla is an ancient herb used in folk medicine for the treatment of variety of human ailments,

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particularly diabetes. A decoction of the fruit with stems of *Tinospora cordifolia* is a well known remedy for various urinary diseases (Jayaweera, 1980). Amla is highly nutritious and could be an important dietary source of vitamin C, minerals and amino acids. The fruit pulp is being used in several indigenous medical preparations against a variety conditions such as headache (Jeena and Kuttan, 1995), liver injury (De *et al*., 1993), atherosclerosis (Thakur *et al*., 1998) and diabetes (Sabu and Kuttan, 2002). Mishra *et al*. (1981) reported that amla has hypocholesterolemic activity. Extract of *Phyllanthus emblica* and quercetin (a flavonoid isolated from *emblica*) for hepatoprotective action was assessed against paracetamol induced liver damage in albino rats and mice (Gulati *et al*., 1995). To the best of our knowledge, no detailed investigations had been carried out to shed light on the antihyperglycemic and antihyperlipidaemic effect of *P. emblica* in diabetic rats injected with STZ. The core aim of the present study was to assess the therapeutic effects of an ethanolic fruit extract of *P. emblica* on blood glucose, non-enzymic antioxidants and lipid profile in plasma, liver and kidney of STZ-induced diabetic rats. The effect of *P. emblica* ethanol extracts was compared to glibenclamide, which is often used as a standard drug.

MATERIALS AND METHODS

Animals: Male Albino Wistar rats weighing (170-220 g) were obtained from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, Annamalai nagar. The animals were kept in clean and dry cages with a bedding of paddy husk, exposed to 12 h dark and night cycle, fed with rat pellets feed (Hindustan lever ltd, Bangalore, India) and water *ad libitum*. The study was conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Consens Statement, 1985). Animal studies in the work have been performed as per the Institutional Animal Ethical Committee of Rajah Muthiah Medical College (Reg no: 465/160/1999/CPCSEA), Annamalai University, Annamalai nagar.

Chemicals: Streptozotocin (STZ) was purchased from Sigma chemical company, St. Louis, Mo, USA. All other chemicals and solvents used were of analytical grade obtained from E-merck and Himedia, Mumbai, India.

Plant material: *Phyllanthus emblica* fruits were collected in and around areas of Chidambaram, Tamil Nadu. The Plant was taxonomically identified and authenticated by Dr. V. Venkatesalu, Reader, Department of Botany, Annamalai University. A voucher specimen was deposited at the Herbarium of Botany.

Preparation of methanolic fruit extract of *Phyllanthus emblica*: Amla fruits were cut into small pieces and ground into uniform powder using a blender. The methanolic extract of amla was prepared by soaking 100 g of dried powdered samples in 250 ml of methanol for 12 h. The extracts are filtered by using Whatman No. 1 filter paper. The filtrate was used for phytochemical screening.

Preliminary phytochemical analysis: Qualitative analysis carried out for *P. emblica* fruit crude extract showed the presence of phytochemical constituents such as flavonoids, tannins, terpenoids, alkaloids, carbohydrates and proteins with absence of steroids.

Chromatographic purification

Thin layer chromatography: The major compounds present in the *P. emblica* fruit extract were identified by using TLC/HPTLC.

Analytical method

Application of the sample in TLC plate: 2 µl of sample was applied on precoated silica gel GF254 aluminium plates (MERCK, 8cm * 7cm size). Develop the plate in Methanol: Chloroform (3:7) mobile phase. Run the mobile phase up to 9 cm of the plate. Remove the plate and dry in air. A spot in TLC was performed using a CAMAG is scanned in 2 Densitometer and linomatt IV applicator.

High Performance Thin Layer Chromatography (HPTLC): The methanol extract was determined in HPTLC/ (CAMAG TLC SCANNER II). HPTLC was performed using a CAMAG HPTLC spectrophotometer provided with a scanner II densitometry a Linomat applicator.

Preparation of *Phyllanthus emblica* alcoholic fruit extract for biochemical studies: The fruits of *Phyllanthus emblica* were dried and powdered. 100 g of powder was extracted with 250 ml ethanol for 72 h. After 72 h the suspension was filtered through a fine muslin cloth and concentrated at 40±5°C. A 13% solid material obtained was stored at 20°C prior to experimentation. Whenever needed, the residual extract was suspended in distilled water and used for treatment.

Experimental design: Diabetes was induced in a group of rats after an overnight fast by single intraperitoneal injection of STZ, which was freshly dissolved in 0.1M citrate buffer (P^H 4.5). The dose was 40 mg/kg body weight. STZ treated animals were allowed to drink 5% glucose solution over night to overcome drug induced hypoglycemia. After 48 h of STZ administration, the blood glucose range above 200-300 mg/dL were considered as diabetic rats and used for the experiment. In the experiment, a total of 30 rats were used. Animals were randomized and divided into five groups of six animals each.

Group A: Control rats received 3% gum acacia.

Group B: Rats were made diabetic by a single intraperitoneal injection of streptozotocin (40 mg/kg body weight) with citrate buffer (pH 4.5).

Group C: Diabetic rats treated with *Phyllanthus emblica* Fruit Ethanolic extract (PFEet) (200 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days.

Group D: Diabetic rats received glibenclamide (600 µg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days.

Group E: Control rats treated with PFEet (200 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days.

At the end of the experimental period, all the rats were kept overnight fast and anesthetized using ketamine chloride (24 mg/kg body weight) by intramuscular injection and sacrificed by cervical decapitation between 8.00-10.00 am. Blood samples collected in potassium oxalate/sodium fluoride-containing tubes were used for the estimation of glucose. Plasma was separated for the estimation of insulin and other biochemical parameters. Liver and kidney tissues were dissected out and transferred to ice cold containers containing 0.9%NaCl and used for various biochemical estimations.

Biochemical investigations: Blood glucose was determined by the method of O-toluidine using the modified reagent of (Sasaki *et al*., 1972). Reduced glutathione in the plasma and tissues was estimated by the method of Ellman (1959). Ascorbic acid in the plasma and tissues was estimated by the method of Roe and Kuether (1943). α-Tocopherol in the plasma and tissues was estimated by the method of (Baker *et al*., 1980). Lipids were extracted from plasma and tissues by the method of (Folch *et al*., 1957). Total cholesterol were analyzed by the method of (Allain *et al*., 1974). Triacylglycerol in the plasma and tissues were estimated using the diagnostic kit based on the enzymic method described by (McGowan *et al*., 1983). Free fatty acids and phospholipids were estimated by the method of (Falholt *et al*., 1973) and Zilversmit and Davis (1950). HDL-cholesterol was estimated using the diagnostic kit based on the enzymic method described by (Izzo *et al*., 1981). Very Low Density Lipoprotein Cholesterol (VLDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) fractions were calculated as VLDL-C = TG/5 and LDL-C = total cholesterol - (HDL-C + VLDL-C), respectively.

Data evaluation: Statistical analysis was performed using SPSS software package, version 11.5. The values were analysed by One Way Analysis Of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). All these results were expressed as mean±SD for six rats in each group: p-values <0.05 were considered as significant.

RESULTS

The qualitative analysis of methanolic crude extracts of *P. emblica* and the results were summarized in Table 1. It shows that flavonoids, tannins, carbohydrates, alkaloids, Proteins, terpenoids were present in extract. Steriods were absent in *P. emblica* extract.

The methanol extract of *P. emblica* fruit shows two major peaks. This indicates that there are two major compounds which may be present in the *P. emblica* fruit extract and it is shown in Table 2. Figure 1 shows HPTLC chromatogram of *P. emblica*.

Table 1: Qualitative analysis on phytochemical constituents in the methanol extract of *P. emblica* fruit

Flavonoids	+
Tannins	+
Carbohydrates	+
Alkaloids	+
Proteins	+
Steroids	-
Terpenoids	+

+ = Presence; - = Absence

Table 2: Analysis of methanol extract of *P. emblica* fruit by HPTLC

Peak	Retention Factor (R_f)	Area (%)
1	0.09	43.66
2	0.20	42.38

The methanol extract of *P.emblica* fruit shows 2 major peaks. This indicates that there are two major compounds may be present in the *P.emblica* fruit extract

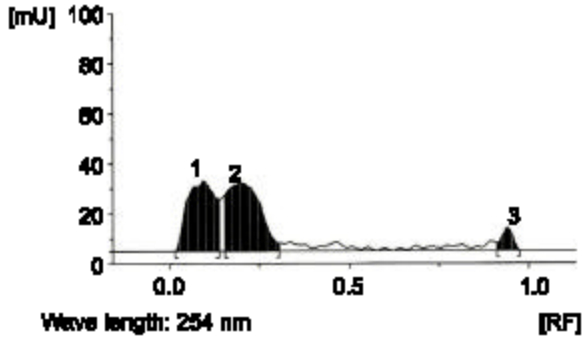

Fig. 1: HPTLC chromatogram of *P. emblica*

The body weight changes in control and experimental groups were illustrated in Table 3. The body weight of diabetic rats were significantly decreased ($p<0.05$) when compared with control group. Supplementation of PFEet and glibenclamide showed a significant improvement in the body weight of diabetic rats. There were no significant changes observed between control (group I) and control treated group (group V).

Table 3: Effect of PFEet on the changes of body weight of control and experimental rats

	Body weight (g)	
Groups	Initial (0 day)	Final (45 days)
Control (3% gum acacia)	175.12±6.50	202.76±8.08[a]
Diabetic control	184.07±4.54	147.06±6.46[b]
Diabetic + PFEet (200 mg/kg bw)	186.20±3.31	171.23±7.42[c]
Diabetic + glibenclamide (600 µg/kg bw)	189.06±5.43	175.43±9.08[c]
Control + PFEet (200 mg/kg bw)	180.21±6.54	203.54±5.70[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 4: Effect of PFEet on the levels of blood glucose, plasma insulin in control and experimental rats

Groups	Blood glucose (mg/dL)	Plasma insulin (µU/mL)
Control (3% gum acacia)	80.52±7.00[a]	15.89±1.50[a]
Diabetic control	266.85±16.43[b]	6.87±0.54[b]
Diabetic + PFEet (200 mg/kg bw)	120.70±8.02[c]	13.92±0.61[c]
Diabetic + glibenclamide (600 µg/kg bw)	91.48±7.59[d]	14.90±0.79[a]
Control + PFEet (200 mg/kg bw)	82.08±4.81[a]	15.60±0.79[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 5: Effect of PFEet on non-enzymatic antioxidants in plasma of control and experimental rats

	Plasma		
Groups	GSH (mg/dL)	Vitamin C (mg/dL)	Vitamin E (mg/dL)
Control (3% gum acacia)	24.94±1.90[a]	1.86±0.13[a]	1.74±0.12[a]
Diabetic control	14.82±2.62[b]	0.95±0.05[b]	0.85±0.06[b]
Diabetic + PFEet (200 mg/kg bw)	22.32±1.46[c,a]	1.12±0.04[c]	1.75±0.05[c]
Diabetic + glibenclamide (600 µg/kg bw)	20.71±1.90[d]	1.80±0.14[a]	1.60±0.03[a]
Control + PFEet (200 mg/kg bw)	22.07±1.28[a]	1.84±0.07[a]	1.65±0.14[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 6: Effect of PFEet on non-enzymatic antioxidants in liver of control and experimental rats

	Liver		
Groups	GSH (mg/100 g wet tissue)	Vitamin C (mg/100 g wet tissue)	Vitamin E (mg/100 g wet tissue)
Control (3% gum acacia)	132.50±8.43[a]	0.67±0.05[a]	5.23±0.21[a]
Diabetic control	46.26±8.41[b]	0.30±0.02[b]	2.12±0.39[b]
Diabetic + PFEet (200 mg/kg bw)	116.86±8.00[c]	0.53±0.07[c]	4.20±0.31[c]
Diabetic + glibenclamide (600 µg/kg bw)	128.63±13.81[c,a]	0.63±0.05[a]	5.08±0.27[a]
Control + PFEet (200 mg/kg bw)	133.72±11.16[a]	0.69±0.04[a]	5.27±0.40[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 7: Effect of PFEet on non-enzymatic antioxidants in kidney of control and experimental rats

	Kidney		
Groups	GSH (mg/100 g wet tissue)	Vitamin C (mg/100 g wet tissue)	Vitamin E (mg/100 g wet tissue)
Control (3% gum acacia)	118.42±8.10[a]	0.58±0.05[a]	3.28±0.10[a]
Diabetic control	47.32±5.14[b]	0.28±0.03[b]	1.12±0.15
Diabetic + PFEet (200 mg/kg bw)	95.53±10.68[c]	0.43±0.06[c]	2.10±0.34
Diabetic + glibenclamide (600 µg/kg bw)	110.21±10.68[a,d]	0.48±0.05[c]	3.01±0.20[a]
Control + PFEet (200 mg/kg bw)	125.86±13.85[a]	0.63±0.04[a]	3.10±0.21[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

To evaluate the effects of PFEet on blood glucose and plasma insulin levels of control and diabetic rats were summarized in Table 4. There was a significant (p<0.05) increase in the blood glucose and decrease in plasma insulin levels in diabetic rats as compared to control. After administration of PFEet and glibenclamide to diabetic rats were significantly reduced blood glucose and elevated plasma insulin levels. No variations were observed between control (group I) and control treated group (group V).

Table 5, 6 and 7 shows the levels of non-enzymatic antioxidants such as, vitamin C, vitamin E and GSH in the plasma and tissues of control and STZ-diabetic rats. The levels of non-enzymatic antioxidants were found to

Table 8: Effect of PFEet on the levels of total cholesterol in plasma and tissues of control and experimental rats

Groups	Plasma (mg/dL)				Total cholesterol	
	Total Cholesterol	HDL-C	LDL-C	VLDL-C	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)
Control (3% gum acacia)	76.32±5.24[a]	45.12±2.92[a]	17.42±1.12[a]	10.40±0.16[a]	3.76±0.13[a]	4.98±0.21[a]
Diabetic control	159.82±8.72[b]	24.53±1.98[b]	60.21±6.32[b]	21.62±1.41[b]	6.62±0.52[b]	7.85±0.56[b]
Diabetic + PFEet (200 mg/kg bw)	94.32±5.65[c]	36.15±2.11[c]	39.53±5.91[c]	18.12±1.14[c]	5.28±0.35[c]	5.63±0.68[c]
Diabetic + glibenclamide (600 µg/kg bw)	83.21±6.43[d,a]	43.10±3.20[a]	27.46±1.15[d]	12.31±0.09[d]	4.05±0.12[d,a]	5.02±0.43[a]
Control + PFEet (200 mg/kg bw)	72.98±5.55[a]	46.35±3.21[a]	12.63±1.04[a]	10.11±0.72[a]	3.42±0.31[a]	4.43±0.29[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 9: Effect of PFEet on the levels of phospholipids in plasma and tissues of control and experimental rats

Groups	Phospholipids		
	Plasma (mg/dL)	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)
Control (3% gum acacia)	78.10±6.57[a]	21.46±1.77[a]	15.10±1.57[a]
Diabetic control	149.64±12.02[b]	56.74±3.89[b]	34.18±3.02[b]
Diabetic + PFEet (200 mg/kg bw)	109.54±0.78[c]	39.58±3.89[c]	20.25±1.39[c]
Diabetic + glibenclamide (600 µg/kg bw)	80.43±6.68[a]	28.56±2.05[d]	16.32±1.68[a]
Control + PFEet (200 mg/kg bw)	76.53±5.42[a]	24.52±1.97[a]	14.56±1.24[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 10: Effect of PFEet on the levels of triglyceride in plasma and tissues of control and experimental rats

Groups	Triglyceride		
	Plasma (mg/dL)	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)
Control (3% gum acacia)	55.45±0.48[a]	4.21±0.38[a]	4.65±0.38[a]
Diabetic control	157.8±0.42[b]	7.2±0.22[b]	7.56±0.32
Diabetic + PFEet (200 mg/kg bw)	115.10±0.43[c]	5.65±0.43[c]	5.98±0.32[c]
Diabetic + glibenclamide (600 µg/kg bw)	60.45±0.43[d]	4.30±0.61[a]	4.03±0.28[d]
Control + PFEet (200 mg/kg bw)	57.34±4.54[a]	4.09±0.35[a]	4.86±0.76

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

Table 11: Effect of PFEet on the levels of free fatty acid in plasma and tissues of control and experimental rats

Groups	Free fatty acid		
	Plasma (mg/dL)	Liver (mg/100 g tissue)	Kidney (mg/100 g tissue)
Control(3% gum acacia)	54.76±0.38[a]	7.54±0.48[a]	3.94±0.23[a]
Diabetic control	120.45±10.30[b]	17.15±1.33[b]	10.56±0.27[b]
Diabetic + PFEet (200 mg/kg bw)	69.54±3.89[c]	9.67±0.79[c]	7.87±0.61[c]
Diabetic + glibenclamide (600 µg/kg bw)	60.57±5.90[a]	7.96±0.69[a]	4.54±0.14[d]
Control + PFEet (200 mg/kg bw)	56.87±0.34[a]	7.25±0.41[a]	3.90±0.26[a]

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT)

be significantly (p<0.05) decreased in the plasma and tissues of diabetic rats. Oral administration of PFEet and glibenclamide for 45 days resulted in the near normalization of the levels of vitamin C, vitamin E and GSH in the plasma, liver and kidney of diabetic rats. No significant changes were observed between control (group I) and control treated group (group V).

The results of the effects of PFEet on plasma and tissue total cholesterol of control and diabetic rats were depicted in Table 8. In diabetic rats, while the levels of TC, Low-Density Lipoprotein Cholesterol (LDL-C) and very Low-Density Lipoprotein-Cholesterol (VLDL-C) levels were found to be increased significantly (p<0.05), the level of High-Density Lipoprotein Cholesterol (HDL-C) was significantly (p<0.05) decreased.

Administration of PFEet and glibenclamide to diabetic rats decreased the TC, LDL-C and VLDL-C levels and significantly increased the levels of HDL-C. No significant changes were observed in the non-diabetic and non-diabetic rats treated with PFEet.

Influence of PFEet on PL, TG and FFA in plasma and tissues of control and experimental rats were presented in Table 9, 10 and 11. The levels of phospholipid, free fatty acid and triglyceride increased significantly (p<0.05)

in plasma and tissues of diabetic rats as compared to those of control rats. On the progression of treatment with PFEet and glibenclamide significantly decreased these parameters in diabetic rats. The control (group I) and control treated group (group V) did not show any significant changes.

DISCUSSION

Streptozotocin induced hyperglycemia in rodents is considered to be a good preliminary screening model. Streptozotocin is well known for its selective pancreatic ß-cell cytotoxicity and has been extensively used to induce Type-1 diabetes in experimental rat model. It interferes with cellular metabolic oxidative mechanisms (Papaccio *et al*., 2000). STZ-induced diabetes is characterized by severe loss in body weight of untreated rats. The characteristic loss of body weight in diabetic rats could be due to degradation and catabolism of fats and proteins (Hakim *et al*., 1997). Increased catabolic reactions leading to muscle wasting may be the cause for weight loss in diabetic rats (Rajkumar *et al*., 1991). In our study, the oral administration of PFEet and glibenclamide normalized the body weight in diabetic rats. This suggests that the protective effect of the extract in controlling muscle wasting is glycolysis.

Phyllanthus emblica is claimed to be useful in diabetes in folklore medicines, the results indicates that the plant extract was found to reduce the blood glucose level in STZ-induced diabetic rats. The observed significant increase in blood glucose was accompanied by a significant decrease in plasma insulin levels in diabetic rats when compared with control rats. Our observations are in complete agreement with the reports by several workers that STZ-induced diabetes mellitus and insulin deficiency lead to increased blood glucose (Chaude *et al*., 2001). This could be due to the result of impairment of peripheral tissues of liver to metabolize glucose. In the present study, diabetic rats treated with PFEet and glibenclamide showed a significant decrease in blood glucose with significant increase in plasma insulin level as compared to diabetic control rats. The possible mechanism by which PFEet bring about its antihyperglycemic action may be by increasing the pancreatic secretion of insulin from remnant ß-cells, which was clearly evidenced by the increased level of insulin in diabetic treated rats. A number of other plants have been reported to exert hypoglycemic activity through insulin release-stimulatory effects (Twaij and Al-Badr, 1988). The effect produced by the extract was compared with standard drug, glibenclamide.

Phytochemical analysis of the fruit revealed the presence of tannins, flavonoids, alkaloids, terpenoids, carbohydrates and proteins. These compounds are powerful antioxidants to scavenge the free radicals induced by hyperglycemia. Herbal extracts containing flavonoids and tannins were reported to demonstrate anti-diabetic activity (Suba *et al*., 2004). Inihibited α-glucosidase enzyme shows reduced blood glucose level due to the polyphenolic compounds such as tannins and saponins (Chakravarthy *et al*., 1982). On the basis of the above evidence, it is possible that the flavonoids and tannins present in this fruit may be responsible for the observed antidiabetic activity.

Free-radical induced oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficiency of natural antioxidant defenses. The non-enzymatic antioxidant defense systems are the second line of defense against free radical damage. Vitamin C, a potent water soluble non-enzymic antioxidant effectively intercept oxidants in the aqueous phase before they attack and cause detectable oxidative damage (Beter, 1994). Vitamin C plays an important role in the detoxification of reactive intermediates produced by $cytP_{450}$ which detoxifies xenobiotics. In our investigation, the decrease in vitamin C level may be due to increased utilization of vitamin C as an antioxidant defense against reactive oxygen species or to a decrease in GSH level, since GSH is required for recycling of vitamin C (Inofers and Sies, 1988). The observed decrease in the levels of vitamin C in the diabetic condition is consistent with previous reports (Prince and Menon, 1999). Vitamin E is an important radical scavenging antioxidant that interrupts the chain reaction of LPO by reacting with lipid peroxyl radical (Garry and Buethner, 1993). In our experiment, there is a decreased level of vitamin E in plasma and tissues of diabetic rats were noticed. Reduced vitamin E levels may be due to the decreased vitamin C level, because there is well established synergism between vitamin C and vitamin E.

Glutathione is one of the most abundant tripeptide, non-enzymatic antioxidant present in liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Moreover, it is substrate for glutathione peroxidase (Prakash *et al*., 2001). The levels of GSH in plasma and tissues decreases during diabetes represent the increased utilization due to oxidative stress. Treatment with PFEet and glibenclamide showed normalization of enzymic and non-enzymic antioxidant levels, which suggests the efficacy of extract to scavenge the reactive oxygen species overproduction during diabetes. The antioxidant potential of *P. emblica* which may be due to the presence of phenolic compounds and vitamin C. It is reported that phenolic compounds in plants possess strong antioxidant activity and may help to protect cells against the oxidative damage caused by free radicals (Kirakosyan *et al*., 2003).

Lipids play an important role in the pathogenesis of diabetes mellitus. The marked hyperlipidaemia that characterizes the diabetic state may therefore be regarded as a consequence of the uninhibited actions of

lipolytic hormones on the fat depot (Casiglia and Palatini, 1998). In our study, we have noticed that the elevated levels of serum lipids such as Total Cholesterol (TC) and Triglyceride (TG) in plasma and tissues of diabetic rats when compared with that of control animals. This could be due to insulin deficiency, which results in failure to activate the lipoprotein lipase thereby causing hypertriglyceridaemia (Shirwakar *et al*., 2004). Under normal circumstances, insulin activates the enzyme lipoprotein lipase and hydrolyses Triglycerides (TG) (Suresh Kumar and Menon, 1992; Taskinen, 1987). The increased levels of TG and TC in diabetic rats are in agreement with other reports (Tunali and Yanardag, 2006). Upon administration of PFEet and glibenclamide reduced triglyceride in plasma and tissues of diabetic rats, this may be due to low activity of cholesterol biosynthesis enzymes or low level of lipolysis that are under the control of insulin (Sharma *et al*., 2003).

Fattyacids, an important component of cellular membranes are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body (Rajasekaran *et al*., 2006). Apart from the regulation of carbohydrate metabolism, Insulin also plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lipolysis. Since it inhibits the activity of the Hormone Sensitive Lipase (HSL) in adipose tissues and suppresses the release of Free Fatty Acids (FFA) (Cohn and Roth, 1996). In our study it is clear that the plasma and tissues of the diabetic animal shows increased level of free fatty acids. Increased FFA in diabetic rats is mainly due to an increase in the mobilization of free fatty acid from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase (Goodman and Gilman, 1985). Our results are in accord with other reports (Krishnakumar *et al*., 2000). A significant reduction of FFA was observed in plasma and tissues of diabetic rats fed with PFEet and glibenclamide.

Phospholipids (PL) are vital part of biomembrane rich in Polyunsaturated Fatty Acid (PUFA), which are susceptible for free radicals such as O_2° and OH° radicals (Ahmed *et al*., 2001). Our present results indicate that the increased levels of phospholipids in diabetic rats may be due to the elevated levels of free fatty acid and total cholesterol. The phospholipids level tends to back towards normal after treatment with PFEet and glibenclamide.

Cholesterol is a powerful risk factor for many coronary heart diseases. The degree of hypercholesterolaemia is directly proportional to the severity of diabetes (Zavaroni *et al*., 1989). ß-Hydroxy-ß-Methylglutaryl Coenzyme A (HMGCoA) reductase catalyzes the rate-limiting step in cholesterol biosynthesis and its activity correlates closely with the rate of tissue cholesterol synthesis. According to our study, the total cholesterol concentration was remarkably increased in plasma and tissues of diabetic rats which is also associated with an increase in LDL-C, VLDL-C and decrease in HDL-C. In a diabetic state, plasma and tissue cholesterol levels were elevated due to lack of insulin, as the activity of HMG CoA as lowered in insulin deficiency. The increased concentration of cholesterol could be due to a decrease in HDL-C, Since HDL is known to be involved in the transport of cholesterol from tissues to the liver for its catabolism. The increased level of plasma and tissue cholesterol observed in our study is in agreement with the previous findings (Kweiterovich, 2000). Administration of PFEet and glibenclamide attenuated a significant reduction in total cholesterol, LDL-C, VLDL-C and significant increase in HDL-C in diabetic animals.

Conclusion: Our present investigation shows that *P. emblica* fruit extract possesses antidiabetic, antioxidant and antihyperlipidaemic effects in streptozotocin-induced diabetic rats. Therefore, *P.emblica* fruits show therapeutic promise as a protective agent against the development and progression of atherosclerosis and possible related cardiovascular complications in diabetes mellitus. It's worth emphasing that *P.emblica* has considerable potential for improving public health if used on a regular basis. Further pharmacological and biochemical investigations are underway to find out the active constituent responsible for the antidiabetic activity and to elucidate its mechanism of action.

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Hepatoprotective Activity of Desert Truffle (*Terfezia claveryi*) in Comparison with the Effect of *Nigella sativa* in the Rat

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Abstract: Hepatoprotective activity of *Terfezia claveryi* aqueous, methanolic and petroleum ether extracts was evaluated in the rat using a potent hepatotoxin carbon tetrachloride (CCl_4) in comparison with the hepatoprotective activity of a reference plant *Nigella sativa*. The extracts were administrated *via gavage* three days prior to CCl_4 intoxication followed by two additional doses one hour and four hours after CCl_4 injection. Twenty four hours after intoxication, blood samples were collected and serum bilirubin concentration, Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) activities were measured. Body weight was measured then livers were excises and livers were weighed. The aqueous, methanolic and petroleum ether extracts of *T. claveryi* and *N. sativa* lowered all liver function tests significantly. However, the aqueous extract of *T. claveryi* almost normalized the effect of CCl_4 and was as effective as the petroleum ether extract of the reference plant *N. sativa*. Moreover, the aqueous extract of *T. claveryi* normalized CCl_4 induced hepatomegaly, which was comparable to the effect of petroleum ether extract of *N. sativa*. These results demonstrate that aqueous extract of *T. claveryi* possesses a very powerful hepatoprotective activity against CCl_4 and it is as effective as petroleum ether extract of the reference plant *N. sativa*.

Key words: Truffles, *Terfezia claveryi*, *Nigella sativa*, hepatoprotective, bilirubin, ALP, AST, ALT

INTRODUCTION

Truffles grow naturally in many parts of the world including particular localities of the Arabian Desert (Al-Delaimy, 1977). Truffles are considered one of the oldest foods used by the Arabs. They are well known for their nutritional importance especially when compared with meat and fish (Bokhary and Parvez, 1993). The Bedouins use truffles as a substitute for meat in their diet. Its preparation and cooking methods are similar to those of meat (Al-Delaimy and Abu-Ghraib, 1970). Truffles are healthy foods that are low in calories and fat and rich in fiber, proteins, vitamins and minerals. Their protein content is higher than that of most vegetables and their amino acid composition is comparable to that of animal proteins (Gazzani *et al*., 1998a; Gazzani *et al*., 1998b; Murcia *et al*., 2002).

Truffles are traditionally used in folk medicine for the treatment of eye ailments in Iraq, Saudi Arabia and the Eastern Badia of Jordan (Janakat *et al*., 2004). Furthermore, truffles have been used as convalescent for several centuries due to their high content of antioxidants such as vitamin A, C, ß-carotene and many phenolic compounds, which are very specialized scavengers of peroxy radicals and are able to reduce and chelate ferric ions, which induce lipid peroxidation (Gazzani *et al*., 1998a; Gazzani *et al*., 1998b; Murcia *et al*., 2002). The effect of truffles in general and of *T. claveryi* in particular on liver functions was not documented earlier. Since the overall incidence of liver diseases in the general population is about 1% (Rochling, 2001) and since truffles are very rich source of antioxidant then most probably truffles will act as a hepatoprotective agent. Therefore the present study was undertaken to evaluate the hepatoprotective activity of aqueous, methanolic and petroleum ether extracts of *T. claveryi* in comparison with a reference plant *N. sativa* extracts against experimental liver damage inflicted by CCl_4.

MATERIALS AND METHODS

Sample preparation: *Terfezia claveryi* which is dark brown red in color, small in size and round in shape was purchased from local markets of Baghdad. The sample was washed carefully, peeled and preserved at -20°C until use. *Nigella sativa* seeds were purchased from the local market of Irbid. The sample was sorted from impurities, washed and air-dried then was kept at room temperature until use.

Chemicals: Bilirubin, ALP, ALT and AST kits were purchased from Cromatest, Spain. CCl_4 was purchased from Pharmacos LTD, England.

Test animals: Male Wister albino rats weighing 170-200 g were obtained from the Animal House Unit at Jordan University of Science and Technology. The animals were

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housed in suspended screen wire cages in an air-conditioned room at 20±3°C and maintained on tap water and standard diet *ad libitum*. All animal experiments conformed to local animal care regulations.

Preparation of extracts: Frozen Iraqi truffles were homogenized using 1:3 (w/v) of each solvent (distilled water, methanol or petroleum ether), using a household blender on full speed for one minute. Whereas, *N. sativa* seeds were first milled using a household electric mill then the sample was mixed with each solvent using a household blender on full speed. The homogenates were refrigerated overnight, filtered through cheesecloth and then were centrifuged at 4000 rpm for 15 min. The supernatants were then dried using rotary evaporator. The dried matter of the aqueous and methanolic extracts were re-suspended using distilled water while the dried matter of the petroleum ether extracts were re-suspended using paraffin oil and kept at -20°C until use (Nielsen *et al*., 1997; Janakat *et al*., 2004).

Experimental design: Hepatotoxicity was induced in rats using a (1:1) mixture of CCl_4:olive oil, administrated intraperitoneally at a single dose of 2 ml CCl_4/kg body weight (Janakat and Al-Merie, 2002a,b). Rats were divided into groups of five. The control group consisted of normal untreated rats (negative control). The other four groups were intoxicated with CCl_4 as described above. Intoxicated groups were treated either with *T. claveryi* or with *N. sativa* extracts (aqueous, methanolic, or petroleum ether). One intoxicated group did not receive any extracts (positive control). The test groups were treated twice daily with the extracts using intragastric tube for three days. On the fourth day, the rats were intoxicated with CCl_4:Olive oil mixture intraperitoneally, followed by two additional doses of truffle extracts after 1 and 4 h of CCl_4 injection. The negative and positive control groups received distilled water instead of the extracts. Blood samples were collected 24 h after CCl_4 administration (Janakat and Al-Merie, 2002a,b).

Assessment of liver function: Rats were anaesthetized with ether and then decapitated for blood collection. Serum was separated by centrifugation at 3000 rpm for 10 min. The level of total serum bilirubin and the activity of ALP, ALT and AST were assayed according to the methods of Jendrassik and Groff (1938), Bergmeyer and Brent (1974), Reitman and Frankel (1957) and Berger and Rudolf (1963), respectively (Jendrassik and Groff, 1938; Bergmeyer and Brent, 1974; Reitman and Frankel, 1957; Berger and Rudolf, 1963).

Statistical analysis: Data were analyzed using analysis of variance of the complete randomized design (ANOVA) using the General Linear Model (GLM) of the Statistical Analysis System (SAS, 2004). Least significant difference was calculated by Students t-test. Different superscripts differ significantly $p<0.05$.

RESULTS AND DISCUSSION

Effect of *T. claveryi* extracts on liver function tests: Table 1 depicts the effect of *T. claveryi* extracts on liver function tests. As expected the positive control group which was intoxicated with the potent hepatotoxin CCl_4 had significantly higher bilirubin concentration (0.50 mg/dl) in comparison to the negative control group (0.14 mg/dl). This comes in accordance with the all researchers findings since the classical article of Recknagel, 1967 to the present day (Recknagel, 1967; Muchizuki *et al*., 2009). The elevation of these parameters is attributed to significant free radical mediated hepatotoxicity leading to cell necrosis, fibrosis and cirrhosis. The mechanism by which CCl_4 causes damage involves the biotransformation of CCl_4 by cytochrome P450 system into a trichloromethyl free radical (CCl_3), which in turn is transformed into a more reactive trichloromethyl peroxyl radical (CCl_3O_2) leading to lipid peroxidation and hepatocellular injury [18]. Moreover, ingestion of *T. claveryi* extracts caused a strong significant reduction in all liver function tests performed. Serum bilirubin level decreased from 0.5 to 0.16, 0.31 and 0.4 mg/dl in aqueous, methanolic and petroleum ether extracts respectively. Whereas, the activity of ALP decreased from 144-70, 105 and 126 U/L respectively, ALT decreased from 791-111, 356 and 511 U/L respectively and AST decreased from 795-188, 420, and 612 U/L respectively. This can be attributed to the high antioxidants contents in *T. claveryi*, such as vitamin C and ß-carotene (Gazzani *et al*., 1998a; Gazzani *et al*., 1998b; Murcia *et al*., 2002) which stop the mounting of peroxyl radical formation and preventing plasma membrane bleb formation, which conserve the integrity of the plasma membrane from rupturing and cytosolic enzymes such as ALP, ALT and ASP from being released into the blood stream (Mehendale *et al*., 1994).

Effect of *N. sativa* extracts on liver function tests: Table 2 depicts the effect of *N. sativa* aqueous, methanolic and petroleum ether extracts on liver function tests. Elevated bilirubin level induced by CCl_4 decreased significantly when aqueous, methanolic and petroleum ether extracts of *N. sativa* were used (from 0.49-0.34, 0.42 and 0.21 mg/dl respectively). The activity of ALP decreased from 142-105, 133 and 81 U/L respectively, ALT decreased from 781-385, 553 and 196 U/L respectively and the activity of AST decreased from 790-404, 601 and 210 U/L respectively. As evident form the above mentioned results all extracts were hepatoprotective, yet the hydrophobic extract was the most potent, this can be attributed to the volatile oil which is abundant in *N. sativa* seeds that has been

Table 1: Effect of *T. claveryi* extracts on liver function tests

Group	-ve control	+ve control	Aqueous extract	Methanolic extract	Petroleum ether extract
BRN (mg/dl)	0.14±0.003[e]	0.50±0.009[a]	0.16±0.005[d]	0.31±0.011[c]	0.40±0.007[b]
ALP (U/L)	46±1.034[e]	144±1.035[a]	70±1.409[d]	105±1.611[c]	126±1.034[b]
ALT (U/L)	108±1.234[d]	791±2.566[a]	111±1.235[d]	356±2.45[c]	511±1.232[b]
AST (U/L)	170±0.889[e]	795±2.18[a]	188±3.905[d]	420±1.235[c]	612±1.235[b]

ALP; Alkaline Phosphatase, ALT; Alanine Aminotransferase, AST; Aspartate Aminotransferase, BRN; Bilirubin,-ve control; Normal rats, +ve control; CCl_4 intoxicated rats. Values are expressed as mean±SEM (n = 5). P-values were calculated by Students t-test.
Means with superscripts (b,c,d,e) differ significantly from the positive control group, p<0.05

Table 2: Effect of *N. sativa* extracts on liver function tests

Group	-ve control	+ve control	Aqueous extract	Methanolic extract	Petroleum ether extract
BRN (mg/dl)	0.12±0.005[e]	0.49±0.009[a]	0.34±0.041[c]	0.42±0.008[b]	0.21±0.009[d]
ALP (U/L)	46±1.409[e]	142±1.784[a]	105±2.523[c]	133±1.596[b]	81±1.684[d]
ALT (U/L)	111±1.502[e]	781±1.491[a]	385±1.491[c]	553±2.016[b]	196±1.491[d]
AST (U/L)	171±1.235[e]	790±1.491[a]	404±1.127[c]	601±1.008[b]	210±1.127[d]

ALP; Alkaline Phosphatase, ALT; Alanine Aminotransferase, AST; Aspartate Aminotransferase, BRN; Bilirubin,-ve control; Normal rats, +ve control; CCl_4 intoxicated rats. Values are expressed as mean±SEM (n = 5). P-values were calculated by Students t-test.
Means with superscripts (b,c,d,e) differ significantly from the positive control group, p<0.05

shown to contain many antioxidants such as thymoquinone, monoterpenes (El-Tahir *et al*., 1993). *N. sativa* seeds extracts were also found to cause immunomodulatoion (El-Kadi and Kandil, 1987), act as anti-inflammatory agent (Houghton *et al*., 1995) anti-tumor agent (El-Daly, 1998) and prevents liver fibrosis, cirrhosis and decreases liver enzymes elevation induced by the potent hepatotoxin CCl_4 in the rat (Kanter *et al*., 2005; Turkdogan *et al*., 2001; Turkdogan *et al*., 2003), the hepatoprotective effect of *N. sativa* was attributed to the presence of highly potent antioxidants such as thymoquinone, carvacrol, t-anethol and 4-terpineol, phytosterols, phenols and tocopherols, which prevent the transformation of CCl_4 to trichloromethyl free radical and trichloromethyl peroxyl radical (Houghton *et al*., 1995; Ramadan *et al*., 2003; Daba and Abdel-Rahman, 1998; Burits and Bucar, 2000; Dakhakhny *et al*., 2000).

Effect of *T. claveryi* extracts on liver weight/body weight ratio: Figure 1 depicts the effect of *T. claveryi* extracts on Liver Weight/Body Weight Ratio (LW/BW). CCl_4 intoxicated rats developed pronounced hepatomegaly in comparison with the normal control, LW/BW almost doubled in the positive control. This hepatomegaly can be attributed to the action of Constitutive Androstane Receptor (CAR), which is a central regulator of xenobiotic metabolism. CAR activation induces hepatic expression of detoxification enzymes and transporters which increases liver size (Huang *et al*., 2005). The ingestion of *T. claveryi* aqueous extract normalized the effect of CCl_4 on LW/BW ratio, whereas methanolic extract decreased LW/BW ratio significantly while petroleum ether extract was ineffective. This indicates that the quality and quantity of antioxidants in the aqueous extract was superior to that

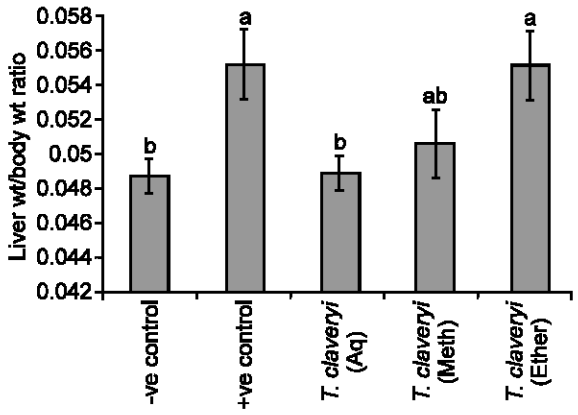

Fig. 1: Effect of *T. claveryi* extracts on Liver weight/body weight ratio. -ve control; Normal rats, +ve control; CCl_4 intoxicated rats. Aq; Aqueous, Meth; Methanolic, Ether; Petroleum ether. Values are expressed as mean±SEM (n = 5). P-values were calculated by Students t-test. Means with different superscripts (a,b) differ significantly (p<0.05)

in the methanolic and petroleum ether extracts, this inhibited the biotransformation and mounting of CCl_4 to $CCl_3^{\cdot}$ and $CCl_3O_2^{\cdot}$ thus decreasing the need for detoxification enzymes and transporters (Recknagel *et al*., 1989; Huang *et al*., 2005).

Effect of *N. sativa* extracts on liver weight/body weight ratio: Figure 2 depicts the effect of *N. sativa* extracts on liver LW/BW ratio. Once again CCl_4 intoxicated rats developed pronounced hepatomegaly in comparison with the normal control which is attributed to the action of CAR which increases the expression of detoxification enzymes and transporters that leads to increased liver size (Huang *et al*., 2005). Aqueous and methanolic

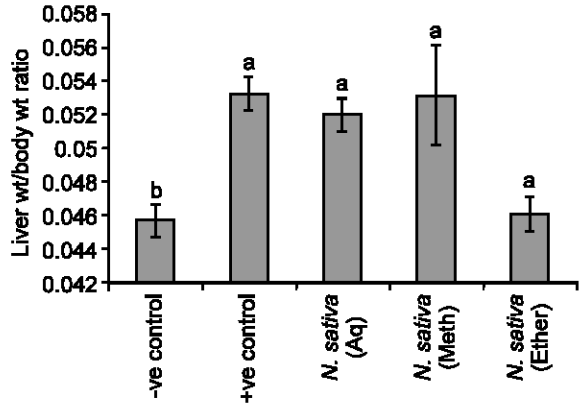

Fig. 2: Effect of *N. sativa* extracts on Liver weight/body weight ratio. -ve control; Normal rats, +ve control; CCl_4 intoxicated rats. Aq; Aqueous, Meth; Methanolic, Ether; Petroleum ether. Values are expressed as mean±SEM (n = 5). P-values were calculated by Students t-test. Means with different superscripts (a,b) differ significantly ($p<0.05$)

extracts of *N. sativa* did not affect the significant increase induced by CCl_4. Whereas, the ingestion of *N. sativa* petroleum ether extract normalized the effect of CCl_4 on liver weight/body weight ratio which indicates the abundance of fat soluble antioxidants such as tocopherols, phytosterols, and phenols in *N. sativa* crude oil plays a major role in the prevention of hepatomegaly (Ramadan *et al*., 2003).

Conclusion: The aqueous extract of *T. claveryi* is as potent as the effect of the reference plant *N. sativa* seeds petroleum ether extract and can be used to prevent liver damage induced by oxidative stress.

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Performance and Linear Measurements of Growing Pigs Fed on Basis of Their Body Weight

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Abstract: The performance of growing pigs fed on basis of percentage of their body weight was evaluated using twenty four growing pigs of Landrace x Large White crosses weighing averagely 35±0.5 kg at the start of the study. The pigs were assigned to four treatments and each treatment was replicated thrice with two pigs per replicate in a Completely Randomized Design. An 18% CP and 11.82 MJME/kg diet was formulated and fed at levels of 10, 8, 6 and 4% of body weights in treatments 1, 2, 3 and 4 respectively. The feeding trial lasted for 56 days. Results showed that treatment effect on the response parameters were significant ($P<0.05$). As percentage of body weight feeding reduced, final body weight of the pigs increased significantly ($P<0.05$). Pigs on treatments 2, 3 and 4 had a final body weight of 68.64, 67.62, 67.68 kg respectively which differed from the 60.03 kg observed for pigs on treatment 1. Pigs fed 4% of their body weight (Treatment 4) showed superiority in the response parameters. Pigs on Treatment 4 had a feed cost/kg gain value of 79.56 naira which differed significantly from 263.21 naira, 161.78 naira and 120.67 naira recorded for pigs on T_1, T_2 and T_3 respectively. Linear body measurement of the pigs followed the same trend as observed in the other response parameters. Pigs on T_4 recorded the highest significant ($P<0.05$) changes in chest girth, height at withers and body length. It is concluded that feeding growing pigs at level of 4% of their body weight leads to optimum performance and better economic returns.

Key words: Performance, linear measurements, pigs

INTRODUCTION

The increases in the prices of feedstuffs have made many pig farmers in Nigeria to embark on indiscriminate feeding of their animals with poor quality feeds. Often garbages form the bulk of ration for these animals. The antecedent impact on the animal is a drastic reduction in growth and reproductive performances. Pigs reared in this manner often take a longer time to reach market weight. What most pig farmers do not realize is that they can feed their pigs with balanced ration using a feeding module based on the body weight of the pigs and still attain recommended market weight faster. Barber *et al.* (1972) had earlier described a feeding module for pigs based on their body weight. The authors observed that although pigs fed on semi-*ad libitum* level grew faster than pigs on scale feeding, the feed to gain ratio of the two groups were not significant. Onyimonyi and Okeke (2007) reported that weaner pigs can be fed on basis of 4% of their body weight without compromising performance. Some authors had equally utilized *ad libitum* feeding module for pigs (Ayuk *et al.*, 2008; Olowofeso, 2002). Linear body measurement such as chest girth, heart at withers and body length are used to relate body dimensions to an animals overall body size or weight. These measurements are frequently used in studies of an animal growth. Sulabo *et al.* (2006) established that there is a positive correlation between linear measurements and body weight in pigs. Brown *et al.* (1973) observed that linear body measurements can be used in assessing growth rate, weight, feed utilization and carcass characteristic in farm animals. According to Tegbe and Olurunju (1988) and Oke *et al.* (2006), changes in linear measurememts are an indication of tissue growth evidenced in the muscle and fat tissues. These parameters tend to increase as the animal grows over time.

The present work was designed to investigate the performance and some linear body measurements of grower pigs subjected to a feeding module based on their body weight.

MATERIALS AND METHODS

This study was conducted at the Pig unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka. Twenty four growing pigs of Landrace x Large White crosses weighing 35±0.5 kg were used for the study. The pigs were assigned to four treatments and each treatment was replicated thrice with two pigs per replicate in a Completely Randomized Design (CRD). Each replicate were housed in an open sided pen measuring 3.2 x 2.75 m. Each pen had inbuilt concrete water and feed troughs. An 18% CP and 11.82MJME/kg diet was formulated and fed to the treatments as follows:

Treatment 1	:	10% of body weight
Treatment 2	:	8% of body weight
Treatment 3	:	6% of body weight
Treatment 4	:	4% of body weight

Table 1: Percentage composition of the grower ration

Ingredients	Quantity (%)
Maize	7
Cassava chips	25
Palm kernel cake	21
Brewers spent grain	30
Groundnut cake	12
Bone meal	2.30
Vitamin mineral premix*	0.25
Lysine	0.10
Methionine	0.10
Common salt	0.25
Total	100
Calculated	
Crude protein	18.06
Energy (MJME/kg)	11.36

*Supplied vitamin A (15,00,000, iμ), vitamin D_3 (3,000,000), vitamin E (30,00 iμ), vitamin K (2, 500 iμ), Thiamin (2,000 mg), Riboflavin (600 mg), Pyridoxine (4000 mg), folic Acid (1000 mg), Biotin (80 mg), chlorine chlonde (500 mg), Manganese (96 g), Zinc (60 g), Iron (24 g), copper (6 g), Iodine (1.4 g), Selenium (24 g), cobalt (12 g), Antioxidant (12 g) per tonne of feed

The feeding trial lasted for 56 days. The initial body weights of the pigs were taken on day 1 of the trial and subsequently on weekly basis. Feed intake record was taken on daily basis. Feed conversion ratio was calculated as ratio of feed/gain. Feed cost/kg gain was computed as the feed cost/kg of feed multiplied by the feed conversion ratio. Weekly measurements of chest girth (cm), height at withers (cm) and body length were taken as earlier described by Onyimonyi (2002).

Data collected were processed and analyzed using the analysis of variance method as outlined by Steel and Torrie (1980). Significantly different means were separated by methods of Duncan's New Multiple Range Test, Duncan (1955).

RESULTS AND DISCUSSION

Results of the performance of the pigs used in this study are presented in Table 2. Results showed that as the percentage of body weight feeding reduced, final body weight of the pigs increased significantly (P<0.05). Pigs on T_1 had a final body weight of 60.03 kg which differed significantly from a value of 68.64, 67.62 and 67.68 kg recorded for pigs on T_2, T_3 and T_4 respectively. Final body weights of the pigs on T_2, T_3, and T_4 were statistically the same (P>0.05). However, pigs on T_2 had the highest numerical final body weight of 68.64 kg. Body weight gain and average daily gain values followed the same trend as final body weight. The effect of treatments on average daily feed intake and feed conversion ratio were significantly different (P<0.05) for all the treatments. Pigs on T_4 had the least average daily feed intake ADFI of 1.40 kg which was significantly different from the values of 3.52, 2.87 and 2.11 observed for pigs on T_1, T_2 and T_3 respectively. The same pigs on T_4 had the best FCR of 2.40 which also differed significantly (P<0.05) from 7.94, 4.88 and 3.64 observed for pigs on T_1, T_2 and T_3 respectively. Feeding pigs at 4% of their body weight (T_4) also lead to a significant reduction in feed cost/kg gain. Pigs at this level of feeding have a feed cost/kg gain value of 79.56 naira which differed significantly (P<0.05) from 263.21 naira, 161.78 naira and 120.67 naira observed for pigs on T_1, T_2 and T_3 respectively. Feed cost/kg gain is a product of feed conversion ratio and feed cost/kg. A feeding regime that enhances better fed utilization will consequently result in better growth and economic performance. This agrees with the earlier view of Fetuga *et al.* (1975) that efficiency of feed conversion in pigs is inversely related to increased feed intake because higher intake allows for increased body fat deposition and body fat deposition requires more energy and protein for the same unit increase in body weight. The superiority of pigs on T_4 furthers corroborates the report of Caspar (1994) that improvement of feed efficiency leads to a lower total cost of production keeping the feed cost constant and signifies a lower nutrient expenditure per unit of production.

Chest girth, height at withers and body length changes were significantly (P<0.05) affected by treatments (Table 3). Pigs on treatment 4 recorded significantly (P<0.05) higher changes in these linear measurements than pigs on the other treatments. Linear body measurements are useful predictors of body weight. This finding is in harmony with earlier view of Sulabo *et al.* (2006) and Brown *et al.* (1973). It also agrees with the view of Tegbe and Olurunju (1988) and Oke *et al.* (2006) that changes in linear body measurements are indications of tissue growth and tends to increase as the animal grows. The practical implication of the present study is that a farmer that feeds his growing pigs on 4% of their body weight will have the pigs reach desired market weight faster. Such a pig farmer will make more

Table 2: Performance of growing pigs fed on basis of percentages of body weight

Parameters	1	2	3	4	SEM
Initial body weight (kg/pig)	35.20	35.70	35.16	35.07	0.46
Final body weight (kg/pig)	60.03^b	68.64^a	67.62^a	67.68^a	0.49
Body weight gain (kg/pig)	24.83^b	32.94^a	32.46^a	32.61^a	0.09
Average daily gain (kg/pig/day)	0.44^b	0.59^a	0.58^a	0.59^a	0.15
Average daily feed intake (kg/pig/day)	3.52^a	2.87^b	2.11^c	1.40^d	1.08
Feed conversion ratio (feed/gain)	7.94^a	4.88^b	3.64^c	2.40^d	0.84
Feed cost/kg gain (naira)	263.21^a	161.78^b	120.67^c	79.56^d	4.39

^{abcd}Row means with different superscripts are significantly different (P<0.05). SEM = Standard Error of Mean

Table 3: Linear body measurements of pigs fed on basis of percentage of body weight

	Treatments				
Parameters	1	2	3	4	SEM
Chest girth (cm)					
Initial	54.05	54.12	54.65	54.39	-
Final	62.30	63.70	64.79	07.70	0.35
Δ in chest girth	8.25^{b}	9.58^{b}	10.4^{ab}	13.31^{a}	1.71
Height withers (cm)					
Initial	48.62	48.51	48.11	48.23	-
Final	59.00	60.67	60.73	61.13	0.96
Δ in body length	10.38^{b}	12.16^{a}	12.82^{a}	12.91^{a}	1.25
Body length					
Initial	56.34	56.66	56.01	56.75	-
Final	76.47	82.67	86.73	87.93	0.56
Δ in body length	20.13^{c}	26.01^{b}	30.72^{a}	31.18^{a}	1.36

Δ change

profit than pig farmers feeding at 10, 8 or 6% of body weight.

Conclusion: It is concluded that growing pigs in Nigeria should be fed 4% of their body weight for optimum performance, superior linear body measurement increases and better economic yields.

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In vitro Gas Production and Dry Matter Digestibility of Tannin-Containing Forges of Semi-Arid Region of North-Eastern Nigeria

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Abstract: A study was conducted to determine the chemical composition, *in vitro* dry matter digestibility and *in vtro* fermentation of four dorminant browse species in the region of experimentation (*Leuceana lecocephala, Moringa Oleafera, Acacia tortilis* and *Ziziphus mucronata*). Crude Protein (CP) (from 13.96% DM for *A. tortilis* to 19.42% DM for *L. leucocephala*) except *M. oleifera* which had the highest (21.42% DM). The range of lignocelluloses (acid detergent fibre, ADF) was from 21.16 g/100 g DM for *A. tortilis* to 31.39 g/100 g DM for *M. oleifera*. The netral detergent fibre ranged from 33.31 g/100 g DM for *M. oleifera* to 58.81 g/100 g DM. CF, EE and ash had a range of 20.00% DM for *Z. mucronata* to 32.53% DM for *M. oleifera*, 3.03% DM for *A. tortilis* to 5.33% DM for *Z. mucronata* and 10.76% DM for *A. tortilis* to 17.76% DM for *L. leucocephala*. The Total Condensed Tannin (TCT) for the browses ranged from 0.25 mg/g DM for *M. oleifera* to 2.96 mg/g DM for *L. leucocephala*. *Z. mucronata* had the highest value for Calcium (Ca), Magnesium (Mg), Sodium (Na) and Potassium (K) except for phosphorus. *In vitro* gas and methane production was highest in *Z. mucronata*. The IVDMD ranged from 70.66-72.00%. CP and TCT showed a positive relationship with IVDMD.

Key words: *In vitro*, Browse, semi-arid, tannin, digestibility, forages

INTRODUCTION

The utilization of browse is limited by the high lignin content and the presence of anti-nutritional factors, which may be toxic to ruminants. Many browse species have chemicals that appear to be produced for the purpose of deterring invasion or consumption of their leaves by microbes, insects and herbivorous animals. The most important cited is tannin, which is shown to decrease the digestibility in browse fodders. Tannins are a group of polyphenol substances with the ability to bind protein in aqueous solution. They are classified into two groups: Hydrolyzable or condensed tannins and are considered to have both adverse and beneficial effects depending on their concentration and nature and also animal species, physiological state of the animal and the composition of the diet (Makkar, 2003). Silanikove *et al*. (1996) concluded that goats have the ability to consume large amounts of tannin rich plants without exhibiting toxic syndromes (due to a detoxifying enzyme in the saliva), which is not the case for other ruminant species. The negative effect of tannin is seen in lowered feed intake, directly due to the astringent properties of tannin rich feed and indirectly by reducing the digestibility of the feed. However, the level of digestibility reduction varied depending on the level and the activities of tannin (Ebong, 1995). A level of tannin below 5% seems to be tolerable for ruminant animals. While tannins are the best known of the anti-nutritional factors of browse, there is a long list of secondary compounds: cyanide, nitrate, fluoroacetate, cyanogenic glucosides, saponins, oxalates, mimosine and various sterols (Leng, 1997). However, the toxic compounds seem to become of significance nutritionally only when the plant constitutes a high proportion of the diet. Hence, the effects of high protein forage could override the effect of the toxic compounds when used as supplement in the diets.

The *in vitro* gas production technique as modified by Menke and Steingass (1988) is widely used to evaluate the nutritive value of feeds resources consumed by ruminants especially tree and shrub legume forages, particularly to estimate energy value of straws (Makkar *et al*. (1999), agro industrial by-products (Krishna and Gunther, 1987), compound feeds (Aiple *et al*., 1996) and various types of tropical feeds (Krishnamoorthy *et al*., 1995). The use of *in vitro* gas method to estimate the digestion of feed is based on measured relationships between the *in vitro* digestibility of feeds and *in vitro* gas production, in combination with the feeds chemical composition (Menke and Steingass, 1988). The main objective of the present study was to investigate changes in chemical composition and *in vitro* digestibility of leaves of semi-arid browses. The present study therefore examines the nutrient composition, *in vitro* gas production and *in vitro* dry matter digestibility of some browsable forages in semi-arid region of Nigeria.

MATERIALS AND METHODS

Forage samples: Four indigenous browse samples (leaves) were used in this study. The species were: *Leuceana lecocephala, Moringa Oleafera, Acacia tortilis* and *Ziziphus mucronata*. All forages were harvested from Gwoza local government area of Borno State, Nigeria. The area is located at 11.05° North and 30.05° East and at an elevation of about 364 above sea level in

the North Eastern part of Nigeria. The ambient temperature ranges between 30°C and 42°C being the hottest period (March to June) while its cold between November to February with temperatures ranging between 19-25°C. The browse forages were harvested from at least 10 trees per each specie selected at random in four locations with the study area at the end of the season. The harvested sample were then pooled for each individual tree species and then oven dried at 105°C for 24 h to constant weight and ground to pass through a 1.0 mm, sieve. The samples were then sub-sample to obtain three samples for each tree species and used for the laboratory analysis.

Chemical analysis: Browse species were analyzed for Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crue Fibre (CF) and ash according to AOAC (2005). The leaves samples were analyzed for Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF), according to Van Soest *et al.* (1991). Total condensed tannin was (Polshettiwar *et al.*, 2007).

***In vitro* gas production:** Rumen fluid was obtained from 3 West African Dwarf (WAD) female sheep through suction tube before morning feed, normally fed with concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal. Incubation was as reported (Fievez *et al.*, 2005) using 120 ml calibrated syringes in three batch incubation at 39°C. Into 200 mg sample (n = 4) in the syringe was introduced 30 ml inoculums containing cheese cloth strained rumen liquor and buffer ($NaHCO_3$ + 3 Na_2 HPO_4 + KCl + NaCl + $MgSO_4.7H_2O$ + $CaCl_2.2H_2O$) (1:4, v/v) under continuous flushing with CO_2. The gas production was measured at 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45 and 48 h. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample.

Methane production: In order to estimate methane production by the substrate and immediately after evacuation from the incubator, 4 ml of NaOH (10 M) was introduced using 5 ml capacity syringe as reported by Fievez *et al.* (2005). The content was inserted into the silicon tube, which was fastened to the 120 ml capacity syringe. The clip was then opened while the NaOH was gradually released. The content was agitated while the plunger began to shift position to occupy the vacuum created by the absorption of CO_2. The volume of methane was read on the calibration.

***In vitro* Dry Matter Digestibility (IVDMD):** After 24 h digestion, the samples were transferred into test tubes and centrifuge for 1h in order to obtain the residues which was then filtered using Whatman No 4 filter paper by gravity and the residues placed in for drying at 65°C for 24 h. The dry residues were weighed and digestibility calculated using the equation as follows:

$$\text{IVDMD (\%)} = \frac{\text{Initial DM input-DM residue-Blank}}{\text{Initial DM input}} \times 100$$

Statistical analysis: Metabolizable Energy (ME) was calculated as ME = 2.20 + 0.136GV + 0.057 CP + 0.0029 CF (Menke and Steingass, 1988). Organic Matter Digestibility (OMD%) was assess as OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA (Menke and Steingass, 1988). Short Chain Fatty Acids (SCFA) as 0.0239 GV-0.0601 (Getachew *et al.*, 1999) was also obtained, where GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash respectively. Data obtained were subjected to analysis of variance. Where significant differences occurred, the means were separated using Duncan multiple range F-test of the SAS (Statistical Analysis System Institute Inc., 1988) options.

RESULTS AND DISCUSSION

The result of the detailed proximate composition of the leaves of the browse forages is presented in Table 1. The Crude Protein (CP) contents of the browses studied had a similar range as those from West Africa (Rittner and Reed, 1992). All the browses used in the current study had a CP content of above 13% DM. The results of the current study, those of Rittner and Reed (1992), Makkar and Becker (1998) and Njidda *et al.* (2009) indicate that most tropical browse species are high in CP and can be used to supplement poor quality roughages to increase productivity of ruminant livestock in tropical regions. Calicium (Ca), Phosphosrus (P) and Magnesium (Mg) are within the range reported by Njidda *et al.* (2009) for semi-arid browses while Sodium (Na) and Potassium (K) falls within the range reported by Bamikole (2003). Generally, the minerals were within the range values and are adequate to meet the requirement for growth, reproduction and milk in sheep and goat as reported by Babayemi (2006).

The highest cumulative gas production was observed in *Ziziphus muconata* and lowest in *Acacia tortilis*. The variation in gas production between the browse species forages can be attributed to compositional differences of the browse forages, especially CP and fibre and may be other anti-nutritional components. These factors influence the amount of substrate OM that is fermented and the Short Chain Fatty Acids (SCFAs) produced upon fermentation. Other reason may be due to low NFE content for browses which has a positive correlation with gas production. On the other hand, cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters. This may tend to reduce the microbial

Table 1: Proximate composition of some selected browse species forages of semi-arid (% DM); NDF, ADF (g/100 g DM); TCT (mg/g)

Browse forages	DM	CP	CF	EE	Ash	NFE	NDF	ADF	TCT
Acacia tortilis	72.33^{c}	13.96^{c}	30.50^{b}	3.03^{c}	10.76^{c}	14.06^{c}	48.62^{b}	21.16^{c}	0.32^{b}
Leucaena leucocephala	89.63^{b}	19.42^{b}	31.50^{a}	3.46^{c}	17.96^{a}	17.29^{b}	58.81^{a}	25.52^{b}	2.96^{a}
Moringa oleifera	88.22^{b}	21.42^{a}	32.53^{a}	4.16^{b}	17.60^{a}	12.51^{d}	33.31^{c}	31.39^{a}	0.25^{b}
Ziziphus mucronata	92.26^{a}	19.23^{b}	20.00^{c}	5.33^{a}	15.43^{b}	29.27^{a}	58.67^{a}	22.89^{c}	0.72^{b}
Means	85.61	18.51	28.63	3.99	15.44	18.28	49.85	25.24	1.06
SEM	2.19	0.73	0.89	0.26	0.95	1.66	3.42	0.97	0.55

^{a,b,c}Means in the same column with different superscript differ significantly ($p<0.05$). Dry matter; CP = Crude Protein; EE = Ether Extract NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; TCT = Total Condensed Tannin

activity through increasing the adverse environmental conditions as incubation time progress. This is consistent with De Boever *et al.* (2005), who reported that gas production was negatively related with NDF content and positively with starch. The tannin content of the browse are low 0.25-2.96 mg/g DM, the tannin values in browse could be higher than the values obtained in this study, since a considerable amount of tannins are bound to either fibre and/or proteins and remain unextracted (Jackson *et al.*, 1996) The beneficial effect of tannins when forages containing low levels of tannins (Barry *et al.*, 1986) are fed could be due to the protection of protein from microbial degradation by tannins, thus increasing the amount of undegraded protein entering the small intestine. In addition, a higher flow of microbial protein to the intestine as a result of higher efficiency of microbial protein synthesis (Getachew *et al.*, 2000) has been observed. However, higher concentration of tannins in the diet is associated with reduction in organic matter digestibility (Silanikove *et al.*, 1997; Waghorn and Shelton, 1997). There are many factors that may determine the amount of gas to be produced during fermentation, depending on the nature and level of fibre, the presence of secondary metabolites (Babayemi *et al.*, 2004) and potency of the rumen liquor for incubation. It is possible to attain potential gas production of feedstuff if the donor animal from which rumen liquor for incubation was collected got the nutrient requirement met. Generally, gas production is a function of and a mirror of degradable carbohydrate and therefore, the amount depends on the nature of the carbohydrates (Demeyer and Van Nevel, 1975; Blummel and Becker, 1997). The *in vitro* gas production pattern of the forages shown in Fig. 1 indicated that more degradation of dry matter were still possible beyond 48 h. The situation here depicted that of typical dry season in Nigeria, when most of the forages are fibrous and therefore take longer time to degrade in the rumen. The highest gas production was obtained from *Ziziphus mucronata* for reason that was not clear since the secondary metabolites are all within the normal ranges as shown in Table 1, although high crude protein in feed enhances microbial multiplication in the rumen, which in turn determines the extent of fermentation. Methane (ml/200 mg DM) production (Fig. 2) ranged from 5-17 among the forages, the least and the highest being from

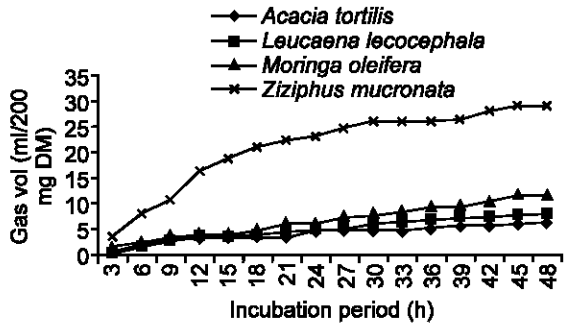

Fig. 1: *In vitro* gas production of semi-arid browse forages

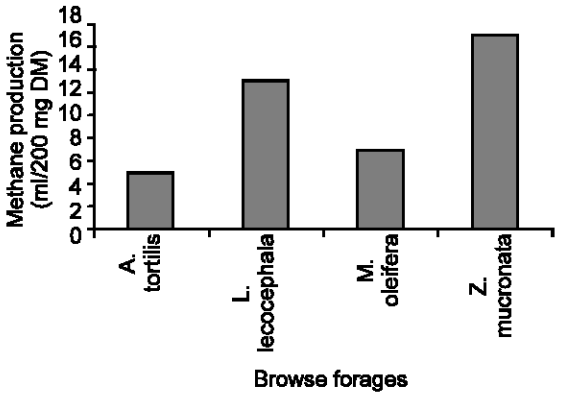

Fig. 2: Methane production of semi-arid browse forages

Z. mucronata and *A. tortilis* , respectively. In most cases, feedstuffs that show high capacity for gas production are also observed to be synonymous for high methane production. Methane production indicates an energy loss to ruminant and many tropical feedstuffs have been implicated to increase methanogenesis (Babayemi and Bamikole, 2006) as an integrated part of carbohydrate metabolism (Demeyer and Van Nevel, 1975).

***In vitro* dry matter digestibility:** The result of the IVDMD is shown in Fig. 3. IVDMD was higher for all browses with *M. oleifera* having the highest (72%). In tree leaves, tannins are present in the NDF and ADF fractions and are tightly bound to the cell wall and cell protein and seem to be involved in decreasing digestibility (Reed *et al.*, 1990). The higher IVDMD observed may be due to the low level of tannin in the browse plants which suggest that it could be a valuable protein supplement

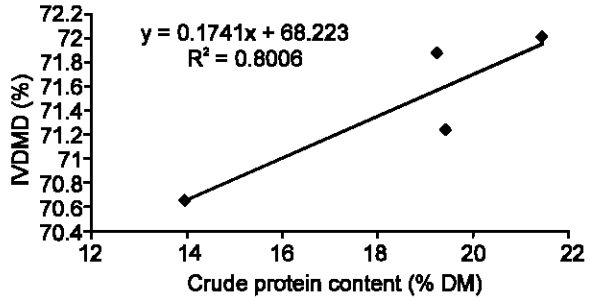

Fig. 3: Relationship between IVDMD and CP of semi arid browses

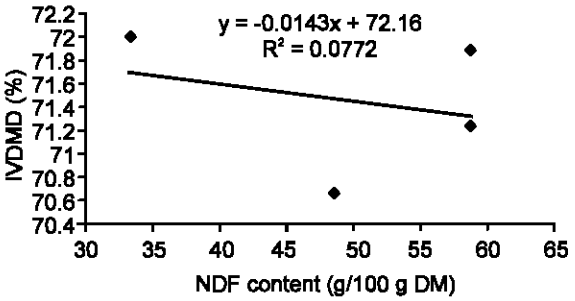

Fig. 4: Relationship between semi arid IVDMD and NDF of semi-arid browses

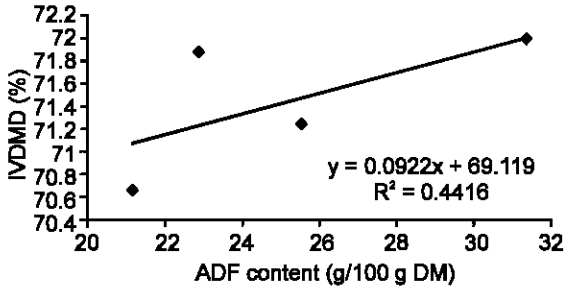

Fig. 5: Relationship between IVDMD and ADF of semi-arid browses

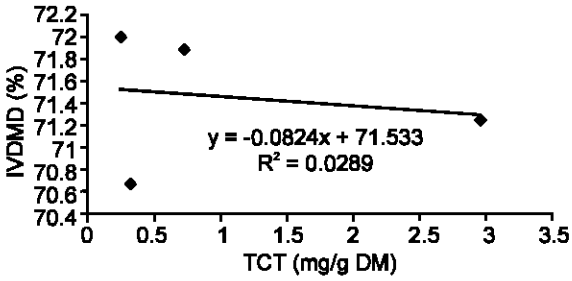

Fig. 6: Relationship between IVDMD and TCT of semi-arid browses

in ruminant diets (Aganga and Mosase, 2001). This was further manifested in methane production (Fig. 2) where methane production showed inhibitory features. This suggest that feeds containing high level of CP and low levels of tannin could generate more methane in the rumen. The *in vitro* DMD of leaves of all the browses under study were generally higher.

IVDMD and its relationship to chemical composition: Crude protein was positively correlated to IVDMD (R^2 = 0.0289, n = 4). CP in the present study is in the level permissible for optimal feed intake and rumen function considering the ranges of IVDMD (70.66-72.00%). A positive correlation between IVDMD and CP indicate that as the crude protein increase, there was an improvement in IVDMD. There were significant ($p<0.05$) negative correlations between *in vitro* DMD and cell wall contents (NDF and ADF) (Fig. 4 and 5). This result is consistent with the findings of Seresinhe and Iben (2003) and Ammar *et al.* (2004), indicate that the cell wall indices in the present group of samples were relatively poor predictors of IVDMD. Madibela and Modiakgotla (2004) reported that ADF has a negative effect on energy content of forages and this was consistent with a highly negative correlation observed between ADF and IVDMD. Irrespective of the maturity stage, leaves were always more digestible than stems, in agreement with Lambert *et al.* (1989). It is well accepted that forage degradation in the rumen is mainly affected by the cell wall content and its lignification, as lignin is indigestible fraction and acts as a barrier limiting the access of microbial enzymes to the structural polysaccharides of the cell wall. Ammar (2002) reported that NDF, ADF and lignin were significant and negatively correlated with *in vitro* digestibility. It is well established that a low content of poorly digestible cell wall components (ADF and ADL) and a high CP content are indicators of a good forage quality (Van Soest, 1994). Therefore, at the light of our results, leaves have a higher nutritive value than stems. There was a positive correlation between *in vitro* DMD and TCT (r = 0.98, n = 4) (Fig. 6). The result is consistent with findings of Frutos *et al.* (2002) and Seresinhe and Iben (2003).

Gas production parameters: Metabolizable Energy (ME), Organic Matter Digestibility (OMD) and Short Chain Fatty Acids (SCFA) of the browse forages are presented in Table 3. The value for the ME, OMD and SCFA ranged from 3.31 in *L. leucocephala* to 6.23 (MJ/Kg DM) in *Z. mucornata*, 30.64 in *L.leucocephala* to 55.44 (%) in *Z. mucornata*, -0.03 in *L.leucocephala* to 0.55 (mmol) *Z.mucornata*, respectively. There were significant differences ($p<0.05$) among the forages in ME, OMD and SCFA. The values obtained in the present study were similar to those reported for tropical browses (Getachew *et al.*, 2002) but lower to those reported for forages by Babayemi (2007). Feedstuffs that are inherent with certain anti-nutritive factor had been reported to be low in metabolizable energy and organic matter digestibility (Aregheore and Abdulrazak, 2005).

Table 2: Composition of macro minerals (% DM)

Browse forages	Ca	P	Mg	Na	K
Acacia tortilis	0.78^{bc}	26.57^{b}	0.25^{c}	0.20	2.00
Leucaena leucocephala	0.90^{b}	10.25^{c}	0.17^{d}	0.30	2.20
Moringa oleifera	1.06^{a}	30.52^{a}	0.40^{b}	0.30	2.10
Ziziphus mucronata	1.24^{a}	11.25^{c}	0.72^{a}	0.50	2.22
Means	0.99	19.64	0.39	0.32	2.13
SEM	0.04	3.52	0.05	0.06NS	0.54NS

^{a,b,c}Means in the same column with different superscript differ significantly ($p<0.05$).
Ca = Calcium; P = Phosphorus; Mg = Magnesium; Na = Sodium and K = Potassium

Table 3: Net Gas volume, metabolizable energy, organic matter digestibility, short chain fatty acid of semi-arid browse forages

	Gas production parameters				
Browse forages	NGV	ME	OMD	SCFA	IVDMD
Acacia tortilis	2.83^{c}	3.47^{c}	30.68^{b}	0.01^{c}	70.66
Leucaena leucocephala	1.16^{c}	3.31^{c}	30.64^{b}	-0.03^{d}	71.24
Moringa oleifera	8.16^{b}	4.33^{b}	40.94^{a}	0.13^{b}	72.00
Ziziphus mucronata	25.50^{a}	6.23^{a}	55.44^{a}	0.55^{a}	71.88
Means	9.41	4.33	39.42	0.17	71.44
SEM	2.43	0.28	3.35	0.06	0.19NS

Net Gas Volume (NGV = ml/200 mg DM), Metabolizable Energy (ME = MJ/Kg DM), Organic matter digestibility (OMD = %), Short Chain Fatty Acids (mmol), IVDMD = *In vitro* dry matter digestibility (%), CH_4 = ml/200 mg DM)

The predicted ME profile were varied in four forages particularly high in *Z.mucronata*. *A. tortilis* and *L. leucocephala* had significantly lower values of ME. The data showed that there was no significant difference among *A. tortilis and L. leucocephala*. There was a positive correlation between metabolizable energy calculated from *in vitro* gas production together with CP and fat content with metabolizable energy value of conventional feeds measured *in vivo* (Menke and Steingass, 1988). The Organic Matter Digestibility (OMD) was highest for *Z. mucronata* and lowest for *A. tortilis* and *L. leucocephala* though there was no significant difference ($p<0.05$) between *M. Oleifera and Z. mucronata*. The OMD was no significant difference among *M. Oleifera and Z. mucronata* and *A. tortilis* and *L. leucocephala*. Menke *et al.* (1979), Steingass and Menke (1986), Menke and Steingass (1988) and Chenost *et al.* (1997) concluded that the prediction of ME is more accurate when based on gas and chemical constituents measurements as compared to calculations based on chemical constituents only. Also, there are significant correlation between *in vitro* gas measurement and *in vivo* digestibility. There was a positive correlation between ME calculated from *in vitro* gas production together with CP and fat content with ME value of conventional feeds measured *in vivo* (Menke and Steingass, 1988). Ranges of gas production characteristics reported in this study may partly due to difference in CP, NDF and ADF contents. The correlation between *in vitro* gas productions measured after 24 h incubation of tropical browses and that calculated from SCFA was similar to that reported for conventional feeds (Blummel *et al.*, 1999). About 94% of the variation in the *in vitro* gas production on incubation of browse leaves was explained by SCFA produced, which mainly comes from carbohydrate fermentation. These results suggest that from browses with a wide range of CP contents, the SCFA production from sources other than carbohydrates is negligible. Cone and Van Gelder (1999) used different proportions of casein and carbohydrate sources (glucose and starch) and reported a poor correlation between gas measured and calculated from SCFA. These poor correlations could be due to the highly fermentable carbohydrate sources that drastically changed the molar proportions of SCFA, indicating the pattern of fermentation of pure substrate does not reflect the normal fermentation pattern that occurs in the rumen.

Conclusion: Chemical composition and *in vitro* digestibility can be considered useful indicators for the preliminary evaluation of the likely nutritive value of previously uninvestigated shrubs. Semi-arid browses are forages with high protein concentration and effective *in vitro* DM digestibility. As such, they have potential as a forage for farmers during the long period of dry season when feed is scarce.

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A Comparative Study on Diet Supplementation with a Mixture of Herbal Plants and Dandelion as a Source of Prebiotics on the Performance of Broilers

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Abstract: In this study a mixed herbal plants (Anise, Cinnamon, Peppermint) and Dandelion were added to the basal diet during the experimental period of 6 weeks. The study aimed to find out the effect of these treatments on productive and physiological traits of 200 broilers unsexed Hubbard chicks at the age of one day. These chicks were randomly divided into five groups equally (40 chicks each). Each group was subdivided into two equal subgroup, and fed with the following treatments. The first group (T1) was fed the basal diet and kept as a control, the second group (T2) was fed the same diet with adding 0.25% of mix herbal plants, where as the third group (T3) was fed the basal diet with adding 0.25% of Dandilion, while the fourth group (T4) was fed the basal diet with adding 0.50% from the mixed herbal plants and the fifth group (T5) was fed the same basal diet with adding 0.50% of Dandilion. The feeding period for all groups lasted for 42 days. The results appeared that there is an improvement in performance traits for all treated groups compared with the control group. However, the chicks fed with 0.25% Mix herbal plants performed better than those fed with 0.50% concerning weekly body weight gain, live body weight and mortality rate. However chicks fed with Dandelion (0.50%) were better in body weight gain, feed conversion ratio, live body weight and mortality rate than those fed with 0.25%. No significant effect was noticed on the addition of Mix herbal plants or Dandilion to the diet on blood traits and stress coefficient as compared with the control group. There was no significant effect on dressing percentage or edible giblet organs.

Key words: Dandelion, herbal plants, prebiotics, performance

INTRODUCTION

Herbs, spices and various plant extracts have received increasing attention as possible Growth Promoters (GP) additives references. There is an evidence to suggest that some of these components have different active substances. Essential oils in aromatic plants are used extensively in medicine and in food and cosmetic industries references. In addition to their antimicrobial activity and antioxidants increasing production of digestive enzymes and improving where also demonstrated absorption of digestive products through enhancing liver functions (Langhout, 2000; Williams and Losa, 2001; Hernandez *et al*., 2004). To be effective on a practical scale, it is likely that these compounds will be needed to be provided in more concentrated form than as found in their natural sources (Bill, 2002). As an aromatic plants, Anise has been used as a stimulating effect of digestion (Cabuk *et al*., 2003) antibacterial (Singh *et al*., 2002; Tabanca *et al*., 2003), antifungal (Soliman and Badea, 2002) and antipyretic (Afifi *et al*., 1994). Furthermore, it has been shown to have anticonvulsant effects and it has also been used for the treatment of constipation (Curtis *et al*., 1996; Pourgholam *et al*., 1999; Chicouri and Chicouri, 2000). They also possessed muscle relaxant effect (Albuquerque *et al*., 1995), As for Cinnamon, the cinnmaldehyde in the cinnamon bark's essential oil acts as antibacterial, fungistatic and promotes motility. It also increases gastric secretions slightly and is an insecticide. (Medical Economical Company, 2000). The plant peppermint (menthe piperita) is commonly used for treatment of losing appetite, common cold, bronchitis, sinusitis, fever, nausea and indigestion as an herbal agent (Akdogan *et al*., 2004). Some times Peppermint is also used as antibacterial (Moreira *et al*., 2005), acarisidal (Kim *et al*., 2004), anti-inflammatory (Carmin *et al*., 2000), antioxidant (Runnie *et al*., 2004), insecticide (Rajaa *et al*., 2001), antispasmodic (Carmin *et al*., 2000), vasodilatory (Runnie *et al*., 2004).

Of particular interest is the use of a new termed Nondigestible Oligosaccharides (NDO). Due to their unique chemical structure some of these carbohydrates resist digestion by the human and animal alimentary tract. Consequently, it reaches the caeco-colon essentially as intact molecules, not providing the body with digestible monosaccharides.

Because these carbohydrates enter the colon as intact molecules it elicits systemic physiological functions and act as fermentable substrates for colonic microflora-influencing the species composition and metabolic characteristics of the intestinal microflora and therefore provide an important health attributes.

Inulin is a natural NDO extracted in large quantities from chicory with potent use in producing physiological functional foods and promoting human health. Evidence of the growing interest in

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inulin is led to increase researches in both short-chained Fructooligosaccharides (FOS) and the longer chained inulin . A number of review have been published dealing with various aspects of the health benefits of FOS and inulin These include: Gibson and Roberfroid, 1995; Gibson *et al*., 1996; Roberfroid, 1999) The aim of the present study is to determine the effect of different medical plants (herbs) used in the diets as a possible feed additives in enhancing and promoting growth of broilers chicks.

MATERIALS AND METHODS

Two hundred a day-old chicks (Hubbard) were divided into 5 groups of 40 chicks. They were randomly assigned to 5 treatments diets. The experiment were carried out in 42 days. Each Treatment group was further more sub-divided into 2 replicates of 20 chicks per replicate. The chicks were fed on the starter and grower diets. The ingredients and chemical compositions of the diets are presented in Table 1, were analyzed using AOAC (1996) procedure. Feed and water were provided *ad libitum* during the experiment. In control group, chicks were fed the basal diet. Two different levels of the mix herbal plants (0.25% and 0.50%) and the Dandelion (0.25% and 0.50%) were added to the basal diets, A continuous light of 24 hours for the 6 weeks was applied. The chicks were weighted individually on days 1, 7, 14, 21, 28, 35 and 42 per pen. The average live body weights, body weight gains, feed intake and conversion ratio were measured on a weekly basis.

Mortality for each treatment were recorded. Birds were slaughtered by cutting the throat and jugular vein using a sharp knife near the first vertebra. From each replicate (10 bird group); were picked for eviscerating to calculate the dressing percent without the edible giblets (Heart, Liver and Gizzard). After recording their weight, two birds from each replicate were slaughtered and blood samples were collected from the bronchial vein to determine the RBC, WBC count, PCV and Hb. The blood samples were collected in test tubes with an anticoagulant (Sodium Eythllene Ditetra amino).

So Data were analyzed by using the General Linear Model Procedure of SAS Institute (2001). The means were compared by the Duncan's Multiple Range Test at 5% probability (Steel and Torrie, 1980).

Table 1: Composition of the basal diets used in the periods of the experiment

Ingredients (%)	Finisher 1-21 day	Starter 22-42 day
Yellow corn	58.0	64.0
Soybean meal (45% protein)	38.0	32.0
*Premix	3.0	3.0
Oil (8900 kcal/kg)	0.5	0.5
Salt (NaCl)	0.3	0.3
Methionine	0.1	0.1
Lysine	0.1	0.1
Total	100	100
Calculated chemical analysis		
ME (Kcal/kg)	2850	2900
Crude protein (%)	22.4	20.2
Calcium (%)	0.13	0.23
Avial.Pho. (%)	0.17	0.16
Methionine + Cystien	0.80	0.75
Lysine	1.22	1.15

Premix: (1%) provided the following (per kg of complete diets), 1400IU Vit. A, 3000 IU Vit. D3, 50 mg Vit. E, 4 gm Vit. K, 3 mg Vit. B6, 6 mg Vit. B12, 60 mg niacin, 20 mg pantothenic acid, 0.2 mg folic acid, 150 mg choline, 4.8 mg Ca, 3.18 mg P,100 mg Mn, 50 mg Fe, 80 mg Zn, 10 mg Cu, 0.25 mg Co, 1.5 mg Iodine. Requirements were adjusted according to NRC, 1994

RESULTS AND DISCUSSION

At the end of the experiment, all treatments recorded an increase in their weight as compared with the control group (Table 2). A highly significant ($p>0.05$) increases were shown in treatment T2 (0.25%) mixed herbal plants and T5 (0.5%) Dandelion they were (2585.94) gm and (2622.06) gm respectively. Also the accumulative weight gain showed that all treatments recorded a highly significant ($p<0.05$) body weight as compared with the control. The highest live body weight gain was recorded at T5 (0.5%) dandelion (2582) gm and T2 (0.25%) mixed herbal plants (2545.9) g. Results showed no significant differences among treatments, in feed intake and feed from the other hand conversion ratio.

Mortality percent in which the control group was higher than the rest of the other groups lower was shown in group T2 (0.25%) mixed herbal plants (Anise, Cinnamon, Pepperment) followed T5 (0.50%)

Table 2: Effect of different level of Mix herbal plants (Anise, Cinnamon and Peppermint) and Dandelion on live body weight, body weight gain, feed intake, feed conversion ratio and mortality rate on broiler ±SE

Items	T1 Control	T2 (0.25%) (Mix herbal plants)	T3 (0.25%) (Dandelion)	T4 (0.50%) (Mix herbal plants)	T5 (0.50%) (Dandelion)
Live body weight (gm)	2428.38±52.0^{b}	2585.94±57.9^{a}	2559.72±42.4^{ab}	2486.84±46.5^{ab}	2622.06±55.9^{a}
Body weight gain (gm)	2388.38±52.0^{b}	2545.94±57.91^{a}	2519.72±42.4^{ab}	2446.84±46.5^{ab}	2582.06±55.9^{a}
Feed intake (gm)	679.77±20.7^{a}	679.59±1.9^{a}	671.95±6.2^{a}	621.21±45.3^{a}	640.07±16.3^{a}
Feed conversion ratio	1.71±0.07^{a}	1.58±0.06^{a}	1.58±0.06^{a}	1.56±0.05^{a}	1.48±0.07^{a}
Mortality rate (%)	12.5±5.3^{a}	5.0±3.5^{a}	7.5±4.2^{a}	7.5±4.2^{a}	5.0±3.5^{a}

$^{a-b}$Means with different superscripts in each column differ significantly ($p<0.05$)

Dandelion. These results are in agreement with several studies which showed the addition of prebiotics to the diets of broiler, improve their performance through improving gut microflora (Pelicano *et al*., 2004; Spring *et al*., 2000; Xu *et al*., 2003). On the other hand Rehman *et al*. (2007) found that the supplementation of inulin resulted in an increase in the villi height of jejunal mucosa of broilers and it is speculated that may increase the absorptive area then enhanced digestion and absorption.

Further more the improvement provided by the mixed herbal plants may be due to its active ingredient such as anethol in Anise, menthol in Peppermint, cinnamaldihyd in Cinnamon. References so the Anise is considered a digestive stimulant (Cabuk *et al*., 2003) while cinnamon act as antimicrobial (Tabak *et al*., 1999) whereas Peppermint acts as digestibility accelerator. These compound enhance gut microbial ecosystem and stimulating secretion of digestive system (Lovkova *et al*., 2001; Williams and Losa, 2001; Cross *et al*., 2007).

In Table 3 the treatments T3, T4, T5 showed a significant increase in dressing percent compared with the control group except T2 (0.25%) mix herbal plants, that showed no significant increase in dressing percent compared with control group. Our result agreed well with (Ocak *et al*., 2008) who reported that the carcass weight and dressing percentage were not significantly affected by peppermint. Also (Durrani *et al*., 2007) reported that the use of 40mL L^{-1} of wild mint infusion in drinking water had a significant ($p<0.05$) effect on mean dressing percentage, as compared with prebiotic. Akinleye *et al*. (2008) reported that the effects of symbiotics on carcass and organs weights were not significantly ($p>0.05$) differ among treatments. Mean weight of heart and gizzard showed no significant difference among treatments but there was a significant difference in liver weight among them. Liver weight of the control group was higher than that of others. This result was in contrast with Debersac *et al*. (2001) who reported that the use of essential oil extract increases the relative liver weight in rats. Our findings could be supported by the findings of Hernandez *et al*. (2004). Elangovan *et al*. (1996) who reported that dietary treatments with herbal plants (alkali-treated) did not cause any pathological change in liver, heart, gizzard and intestine which showed no differences in the mean weight of these organs and abdominal fat were reported.

In Table 4 the haematological parameter was not significantly ($p<0.05$) different between treatments and the values were in correspond with the normal range for healthy birds stated by Mitruka and Rawnsley (1977), No significant difference was noticed in all types of WBC except in Hetrophil, a remarked increase was shown in control group which was about (27.0) while the lowest appeared in T4 Mix herbal plants (0.50%) which was about (12.3), a remarked significant increase difference was also noticed in Esinophil in T3 Dandelion which was (1.0). No significant effect in H/L ratio among treatments but in treatment T5(0.50%) Dandelion and T3 (0.25%) Dandelion there was a little decrease in H/L ratio compared with control group. Our result agreed with AL-Kassie (2008) who reported that the use of *Aspergillus niger*, as a source of probiotic and *Taraxacum officinali* as a source of prebiotic caused a significant decrease ($p<0.05$) in H/L compared with control group, this may be due to the effect of prebiotic

Table 3: Effect of different level of Mix herbal plants (Anise, Cinnamon and Peppermint) and Dandelion on dressing% liver weight% heart weight% and gizzard weight% of broiler ±SE

Items	T1 Control	T2 (0.25%) (Mix herbal plants)	T3 (0.25%) (Dandelion)	T4 (0.50%) (Mix herbal plants)	T5 (0.50%) (Dandelion)
Dressing %	87.18±1.07^{b}	70.82±0.41^{b}	79.21±1.32^{a}	77.05±1.55^{a}	76.50±0.96^{a}
Liver %	2.91±0.5^{a}	2.52±0.31^{ab}	2.27±0.07^{b}	2.31±0.16^{b}	2.20±0.01^{b}
Gizzared %	2.01±0.3^{a}	2.22±0.18^{a}	2.14±0.14^{a}	2.07±0.12^{a}	2.15±0.1^{a}
Heart %	0.64±0.05^{a}	0.59±0.06^{a}	0.63±0.12^{a}	0.66±0.67^{a}	0.67±0.01^{a}

$^{a-b}$Means with different superscripts in each column differ significantly ($p<0.05$)

Table 4: Effect of adding Mix herbal plants (Anise, Cinnamon, Peppermint) and Dandelion in different level on blood picture on broiler chicks ±SE

Items	T1 Control	T2 (0.25%) (Mix herbal plants)	T3 (0.25%) (Dandelion)	T4 (0.50%) (Mix herbal plants)	T5 (0.50%) (Dandelion)
PCV%	31.33±0.88^{a}	28.00±3.72^{a}	29.25±1.70^{a}	28.50±4.87^{a}	30.50±1.76^{a}
Hb g/%	11.03±0.39^{a}	9.83±1.30^{a}	9.58±0.92^{a}	9.90±1.49^{a}	10.50±0.59^{a}
RBCs Cell/mm^{3}	5.6000±0.10^{a}	4.8500±0.64^{a}	5.5500±0.19^{a}	5.0500±0.86^{a}	5.5000±0.35^{a}
WBCs Cell/mm^{3}	266.00±40.45^{a}	297.50±30.06^{a}	251.00±20.94^{a}	235.50±7.85^{a}	306.50±35.62^{a}
Lymphosit	72.5±0.29^{a}	81.3±7.67^{a}	73.0±1.15^{a}	85.0±3.79^{a}	72.67±2.85^{a}
Hetrophil	27.0±0.58^{a}	16.3±8.3^{ab}	24.0±1.5^{ab}	12.3±3.93^{b}	25.3±3.76^{ab}
Eosinophil	0.0±0.0^{a}	0.0±0.0^{a}	1.0±0.58^{a}	0.0±0.0^{a}	0.0±0.0^{a}
Basophil	0.0±0.0^{a}	1.3±0.3^{a}	0.3±0.3^{a}	1.0±0.58^{a}	0.67±0.67^{a}
Monocyte	4.3±3.84^{a}	1.3±0.67^{a}	2.0±0.58^{a}	1.67±0.3^{a}	1.3±0.88^{a}
H/L	0.37±0.01^{a}	0.23±0.14^{a}	0.32±0.01^{a}	0.15±0.05^{a}	0.35±0.06^{a}

$^{a-b}$Means with different superscripts in each column differ significantly ($p<0.05$)

which could be inhibit the nutrition stress or any stress that cause the increase in H/L ratio, because the stress could increase the stimulation of adrenal gland to produce some hormones such as estrone which has a direct effect to analyze a lymphatic cell which causes increase in H/L ratio (Gross and Siegel, 1983). Little increase was also noted in T2(0.25%) mix herbal plants and T4(0.50%) mix herbal plants. This may be due to the effects of the most important activities of essential plant oils which cause improve the endogenous enzymes secretion and stimulation of appetite, digestibility and nutrients absorption. Improvement of the microflora balance and the decrease of *E. coli* and *Clostridium* population and stimulating of the *Lactobacillus* spp. Proliferation. Were also concerned in the advantage of these oils. Intestinal villi layer production, antibacterial, antiviral and anti diarrhea activity and stimulation of the immune system were also enhanced (Horobowicz, 2000; Jamaroz *et al*., 2004).

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Effect of Feeding Cumin (*Cuminum cyminum*) on the Performance and Some Blood Traits of Broiler Chicks

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Abstract: This study was conducted to identify the effect of using cumin (*Cuminum cyminum*) on the performance and some blood traits of broilers. Two hundred one day-old unsexed broiler chicks (Arbor-Acres) were divided into four groups of 50 birds each and assigned to four feeding treatments. Group 1 is considered as a control group where there was no addition of *Cuminum cyminum*. Group 2, 3 and 4 involved the addition of 0.5, 1 and 1.5% of *Cuminum cyminum* respectively. The results showed that group 2 and group 3 performed significantly higher ($p<0.05$) in the average live weight, weekly weight gain, feed consumption, mortality rate and feed conversion ratio. Results showed that chicks in treatment 3 developed a significant decrease in the level of cholesterol in blood serum compared to other treatments.

Key words: *Cuminum cyminum*, performance, blood traits

INTRODUCTION

The huge development in poultry industry that interests researchers and rears to find different methods and means to maintain the development and continuity of this industry, to the increase in productive capacity of poultry projects and then increasing the concentration on nutritional and hygienic aspects of birds. This in turn increase birds resistance against different outbreaks which of mortality causes a high economic losses (Siegel, 1995).

One of the methods is to introduce medical plants to poultry through their diets as a nutritional and medical sources for different purposes. Cumin plant is considered as one of these sources because of its nutritional and medical properties. Scientific information from American Ministry of Agriculture has shown that cumin contains most dietary nutrients such as carbohydrates, fat of both saturated and unsaturated fatty acids, proteins. Moreover minerals, vitamins and water. Jazani *et al*. (2008) have indicated the potential use of cumin essential oil for the control of some diseases caused by *Pseudomonas aeruginosa* infections. An antimicrobial activity of cumin ethanol extract that inhibits growth of *lactobacillus, LP plantarum* was detected by Jonas *et al*. (2007). Sema *et al*. (2007); Friedman *et al*. (2004); Dorman and Deans (2000) were able to identify the antimicrobial activity against *E. coli* infections. Ahmed *et al*. (2004) concluded that the addition of animal manure to soil can enhance the essential oils production and improve chemical structure of cumin oil. Many experiments indicated moderate effects of cumin essential oils on inhibiting microbial activity, mainly, G-positive especially from seeds. *Cuminum cyminum* .It has seen that analytical contents are moisture 11.9; protein 18.7; ether extraction 15.0; carbohydrates 36.6; mineral matter 5.8; calcium 1.08; phosphorus 0.49%; iron 31.0 mg/100 g; carotene calculated as vitamin A 870 I.U./100 gm and vitamin C 3.0 mg/100 g.

The seeds on distillation yield a volatile oil (2.0-4.0%) having an unpleasant characteristic odor, spicy and somewhat bitter taste. The oil is colorless or yellow when fresh, turning to dark where it is stored.

Nutritionally, inclusion the broiler diets with cumin seed meal induces an increase in the relative weight of the crop. An improvements in the absorption process as a result of increasing diet fibers was also noticed (Mansoori *et al*., 2006).

Other researchers proved an increase in body weight , feed conversion ratio; with decreasing in Hematological values (Hb, PCV, RBC) when using 2% of cumin in broiler diets (Ibrahim *et al*., 2007).

As mentioned above it has become very clear that there is a quite bite of benefits of cumin as a medical and nutritional resource to be used for poultry. This study therefore had came out from the understanding that this medical plant can improve the performance of broiler chicks under our environmental conditions. However the literature is not rich dealing with the subject and we think that further studies are required to quantify and characterize the parameters that involved this field. This study is a trial along this direction.

MATERIALS AND METHODS

A total of two hundred one day old unsexed broiler chicks (Arbor Acres) were divided into 4 groups of 50 birds each and assigned to 4 treatment diets. The experiment was carried out in 42 days. Each treatment group was further more sub- divided into 2 replicates and fed on a starter and finisher diets. Cumin seeds were purchased from local market, grounded separately to a fine powder and then mixed with the basal diet Table 1. Feed and water were provided *ad libitum* during the experiment. In

Table 1: Composition of the basal diets in different periods of the experiment

	Starter	Finisher
Ingredients (%)	1-28 day	29-42 day
Yellow corn	58.0	64.0
Soybean meal (45% protein)	38.0	32.0
*Premix	3.0	3.0
Oil	0.5	0.5
Salt	0.3	0.3
Methionine	0.1	0.1
Lysine	0.1	0.1
Total	100	100
Calculated chemical analysis of the diet		
ME (Kcal/kg)	2850	2900
Crude protein (%)	22.4	20.2
Calcium (%)	0.13	0.23
Avail. Pho. (%)	0.17	0.16
Methionine + Cystine	0.80	0.75
Lysine	1.22	1.15

Premix: (1%) provided the following (per kg of complete diets), 1400IU Vit. A, 3000 IU Vit. D3, 50 mg Vit. E, 4 mg Vit. K, 3 mg Vit. B6, 6 mg Vit. B12, 60 mg niacin, 20 mg pantothenic acid, 0.2 mg folic acid, 150 mg choline, 4.8 mg Ca, 3.18 mg P, 100 mg Mn, 50 mg Fe, 80 mg Zn, 10 mg Cu, 0.25 mg Co, 1.5 mg Iodine

control group the birds were fed the basal diet with no added cumin (T_1). The other three groups(T_2, T_3 and T_4) were given cumin in diets at levels of 0.5, 1 and 1.5% respectively.

Standard management practice (lighting, ventilation and spaces) of commercial broiler production was applied. Chicks were vaccinated against New-castel and infectious Bronchitis diseases.

Average body weights, body weight gains and feed conversion ratios (kg feed/kg gain) for each group were recorded weekly. A number of 4 birds from each group were slaughtered at the end of the experiment. Blood samples was collected from each bird for hematology and serum analysis. Hemoglobin (Hb) concentration, Packed Cell Volume (PCV), Red Blood Cell (RBC) counts, were measured by standard methods (Schalm *et al*., 1975). Cholesterol and uric acid were measured according to the methods of Ellefson and Garaway (1967).

Data were analyzed by using the General, Linear Model procedure of SAS (2002). Duncans multiple ranges test was used to detect the differences at level of ($p<0.05$) among different means (Steel and Torrie, 1980).

Cumin powder is added to this basal at levels of 0.5, 1 and 1.5% in the treatments T_2, T_3 and T_4 respectively.

All nutrients are provided to chicks according to their requirements as shown in NRC (1994).

RESULTS AND DISCUSSION

Body weight changes: Data of broiler chicks body weight, body weight gain and feed conversion ratio are presented in Table 2. Chicks fed diet supplemented with 1% *Cuminum cyminum* had significantly ($p<0.05$) higher weight and body weight gains compared to other treatment groups, followed by group 2 that received a diet with 0.5%, *Cuminum cyminum*. This improvement may be attributed to the biological functions of cumin that are essential for growth (Cowieson *et al*., 2003; Ghazalah *et al*., 2005). Also that may be due to its role as a stimulant, carminative, digestion, anti-microbial properties and the prevention of gastric toxicity (Jones *et al*., 1997; El-Husseiny *et al*., 2002).

The case of depression in the growth which appeared for group 4 when high level of *Cuminum cyminum* (1.5%) was used could be explained as due to the damage to the intestine, liver and kidneys, due to mechanisms by which the plant constituents may cause a damage to body tissues (Ibrahim *et al*., 2007).

Scientific evidence demonstrated that many of these herbs and spices do have medicinal properties that alleviate symptoms and may prevent diseases (Srivastava, 1989; Jalali-Heravi *et al*., 2007). Also the high intake of herbal plant may cause poising due to its strong bitter test.

Cumin is one of the popular spices that regularly used as a flavoring agent and an alternative antimicrobial agent that is safe for human applications (Janahmadi *et al*., 2006).

Finally this improvement in body weight leads to an improvement in feed conversion in treatment 2 and 3 in Table 2, in spite of the low consumption compared with other groups, the fact that this herb plant may provide some compounds that enhance digestion and absorption of some nutrients in the diet.

Table 3 showed the hematological and serobiochemical changes in chicks fed *Cuminum cyminum*, These parameters indicate that the low values are related to the high level of cumin in which a significant decrease is resulted when compared with the control group or low levels of cumin supplementation. In treatment 4 a severe decrease ($p<0.05$) in the above parameters may be due to the high level of cumin used that leads to the damage of the intestines, liver and kidneys. These results suggest that these plants may contain materials involved in the derangement of the haemopoietic process of the body (Ibrahim *et al*., 2007).

The decrease in the level of cholesterol in treatment 3 compared with other groups is expected to be due to the active compound that found in cumin which acts as inhibitors to the active enzyme hepatic 3- hydroxyl-3 methyglutaryl coenzyme A (HMG-CoA) that synthesized the cholesterol (Crowell, 1999). Furthermore this reduction in blood cholesterol could be contributed in some cases to the reduction in some hormones secreted by the cortex of the adrenal glands, which in turn causes the reduction in the secretion of fatty acids from the adipose tissues or the reduction of fat oxidation, which leads to the reduction of the level of fatty acids including blood cholesterol (Ganong, 2005).

Table 2: The effect of added cumin (*Cuminum cyminum*) to the diet on broiler performance for 6 weeks

	Diets			
Parameters	1 Control	2 (0.5%) cumin	3 (1%) cumin	4 (1.5%) cumin
Average body weight (g)	2321.7±41.3^{c}	2456.3±38.9^{ab}	2560.7±42.5^{a}	2410.9±35.4^{b}
Average body weight gain(g)	2280.0±40.9^{c}	2414.5±39.6^{ab}	2518.8±40.6^{a}	2369.3±36.3^{b}
total feed consumption (g/bird/day)	4491.5±0.91^{b}	4321.8±0.86^{b}	4382.7±1.02^{b}	4833.4±1.42^{a}
Feed conversion ratio g.feed/g.gain	1.94±0.03^{a}	1.79±0.02^{b}	1.74±0.02^{b}	2.04±0.01^{a}
Mortality rate (%)	7.8^{b}	4.1^{c}	3.4^{c}	10.5^{a}
Dressing percent (%) with out edible parts	68.9±2.5^{b}	75.6±1.9^{a}	77.4±1.3^{a}	70.3±1.8^{b}

Values are mean±SE. Means within row with no common on letter are significantly different (p<0.05)

Table 3: Hematological and Serobiochemical changes in chicks fed *Cuminum cyminum* for 6 weeks

	Diets			
Parameters	1 Control	2 (0.5%) cumin	3 (1%) cumin	4 (1.5%) cumin
Haematological				
Hb (g/dL)	10.2±0.31^{a}	9.8±0.38^{a}	9.6±0.27^{a}	7.62±0.08^{b}
RBC (10^{6}/mm^{3})	3.6±0.32^{a}	3.5±0.03^{a}	2.9±0.2^{b}	2.3±0.07^{c}
PCV (%)	35.7±0.47^{a}	32.6±0.31^{b}	31.8±0.41^{bc}	29.9±0.35^{c}
WBC	23.8±0.15^{a}	22.2±0. 2^{a}	20.3±0.3^{b}	19.6±0.04^{b}
H/L ratio	0.29±0.01^{a}	0.28±0.02^{ab}	0.27±0.03^{b}	0.27±0.04^{b}
Serobiochemical				
Cholesterol (mg /dL)	149.0±3.5^{a}	147.3±4.2^{a}	145.6±3.8^{b}	148.3±4.1^{a}
Uric acid (mg /dL)	4.2±0.3^{a}	4.3±0.2^{a}	3.9±0.3^{a}	5.3±0.7^{b}

Values are mean±SE. Means within row with no common on letter are significantly different (p<0.05)

This could be explained by the fact that these herbal plants can act as an antioxidant agents for chick diets.

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Consumer Knowledge, Attitude and Practice Towards the Use of Monosodium Glutamate and Food Grade Bullion Cubes as Dietary Constituents

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Abstract: The knowledge attitude and practice in use of Monosodium Glutamate (MSG) and food Grade Bullion Cubes (FGBCs) by 240 respondents randomly selected from the rural and urban areas of Enugu and Nsukka in Enugu state were investigated. Information was obtained by means of questionnaires feeding regime and focus group discussions. The feeding regime involved a sample of 15 subjects randomly selected from university students who previously complained of some problem after consumption of MSG. The results showed that 98.3% of the respondents were currently using food flavour enhancers (MSGs and FGBCs) in cooking stews, soups, pottages, pepper soups, moi moi, sauce and others. Consumption of FGBCs in terms of number of households was 95.8% and MSG 49.6%. Consumption was on a daily basis. The low and high category of users of FGBCs consumed 14 g and 24 g/person/week respectively while for MSG it was 0.9 g and 6.6 g/person/week, respectively. In addition, a total of 82.5% and 42.5% have knowledge of FGBCs and MSG respectively. The major medium of information was Radio (50%). Results also showed that 95% used FGBCs because it is affordable, while 54.6% used MSG because it is generally good. A total of 71.3% were aware of the health problems associated with MSG. Out of this number, 45% have experienced it. The 14-day feeding trials showed no health problems on the subjects investigated. Nutrition education, information on safety of MSG, fortification of MSG and consumption of fortified foods are recommended.

Key words: Monosodium glutamate, food flavour enhancers, consumer knowledge

INTRODUCTION

Addressing the global crisis of hidden hunger and its consequences including nutritional anemia and the double burden of malnutrition is paramount in the agenda of many countries. Evidence suggests that vitamins and minerals deficiencies create public health problem that affects over 2 billion people (Borwankar *et al*., 2007; Kraemer *et al*., 2007). Nigeria, like most third world countries is faced with a serious problem of micronutrient malnutrition (WHO/UNICEF, 1994). This problem has been controlled successfully in developed countries (Mehansho *et al*., 2003) and some other countries like Haiti and Kenya (Van Hees *et al*., 2008); the Philippines and Indonesia by fortification (single, double and multiple) of food vehicles like MSG and bullion cubes (Nnanyelugo, 1998).

In Nigeria, the Federal Government/United Nations Children's Fund (FGN/UNICEF) consultative group has selected FGBCs and MSG as the best food vehicles for fortification with micronutrients at the industrial level. The selection was largely on taste and low cost -a critical advantage in reaching the population at risk. However, there has been controversy on the safety of MSG. Efforts to show that MSG posed no significant health hazard need to be strengthened in order to ease the constraint in food fortification strategy for micronutrient deficiency control. It becomes pertinent at this point to investigate the consumer knowledge, attitude and practice towards the use of MSG and FGBCs as dietary constituents.

Objectives: The main objective is to determine the consumer knowledge, attitude and practice towards the use of FGBCs and MSG as dietary constituents.

Specific objectives:

- To determine the consumer knowledge about MSG and FGBCs
- To assess the availability and cost of these FGBCs and MSG
- To determine the mode of utilization and storage of FGBCs and MSG
- To find out the frequency and amount of consumption of FGBCs and MSG
- To identify the common meals used by consumers in consuming these FGBCs and MSG
- To find out some of the problems encountered by consumers in using these flavour enhancers

MATERIALS AND METHODS

The study was conducted in two towns of Enugu state-Enugu and Nsukka, which were purposefully selected because they represent the different socio-economic groups needed for the research. The sub-areas include the urban and rural areas. Information on the knowledge, attitude and practice in use of FGBCs and

MSG of 240 respondents randomly selected from the sub-areas were obtained by means of questionnaires and focus group discussions. More information was also obtained by means of feeding regimes and weighed food intake that involved a sample of 15 subjects randomly selected from university students who previously complained of some problems following the consumption of MSG. Values from food consumption tables were used to calculate daily food intakes and mean daily nutrient intake. Adequacy of diets was determined using recommended intake of nutrients. The responses to the questionnaires and focus group discussions were subjected to statistical analysis and presented.

RESULTS

Demographic and housing characteristics: Of the 240 respondents interviewed, most (52.10%) were mothers. The study populations was made up of large families whose sizes ranged from 1-13 with mean (SD) of 6.4 (2.2). With regards to the respondents housing characteristics, most (61.3%) reside in modern houses, 39% in mud houses while 8.8% live in raffia huts. Also, 45% live in owner-occupier houses, 35.8% in normal rent and 14.2% in normal/subsidized while only 5% in free houses. The major source of water was tanker/truck/vendor (37.9%), followed by pipe-borne (22.9%), stream (18.3%) rain (15.4%) and borehole/well (5.4%). Electricity supply was mostly by PHCN only 59.2%, followed by rural electricity (17.9%), PHCN/Rural Electricity/Generating set (1.3%) and generating set (0.4). The remaining 21.3% had no electricity.

Food consumption patterns of households: The responses indicated that the study of households consume a total of 615 meals per day, with a mean (SD) of 2.6 (0.5). On the whole, 32.1% have adequate meals, 67.1% have meals distributed collectively and 98.3% consumed food flavour enhancers (FGBCs and MSG). The frequency of consumption of food flavour enhancers indicates that consumption was on a daily basis. Most respondents (32.6%) consume FGBCs 2 days per week; MSG most (38.7%) consume it 5 days per week (Fig. 1 and 2).

The quantitative consumption profile shows an average of 21 g FGBCs per person per week; MSG, 3.9 g per person per week. FGBCs and MSG was consumed most often with soup, stew or pottages. In addition to these regular dishes, many of the respondents prepared delicacies such as pepper soup, *moi moi* and tapioca sauce with them. Of the respondents that consume food seasonings 86.7% store FGBCs while 29.2% store MSG. Only 14% store food seasonings in refrigerators; the remaining 86% store at room temperature. The availability and affordability of FGBCs and MSG shows that they are obtainable in the whole study area (Fig. 3).

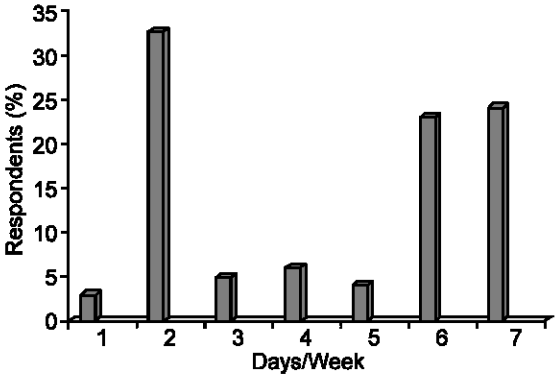

Fig. 1: Weekly frequency (%) of consumption of FGBCs

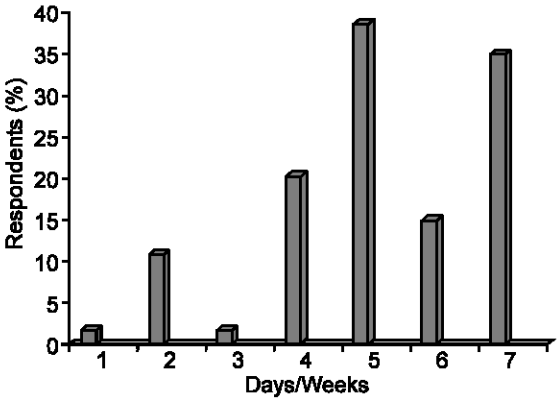

Fig. 2: Weekly frequency (%) of consumption of MSG

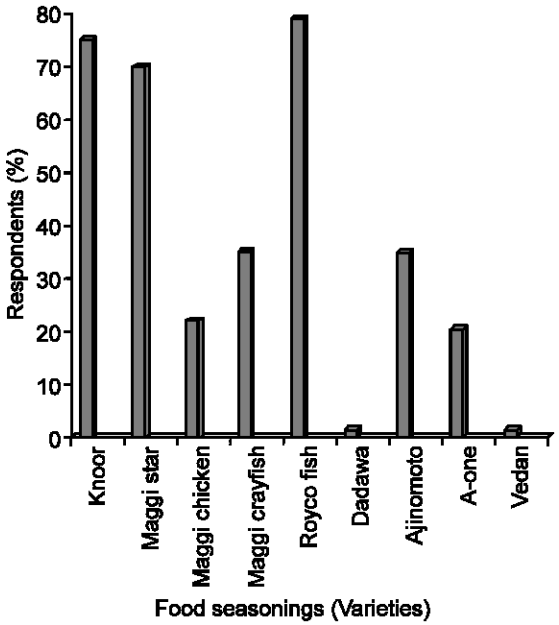

Fig. 3: Availability and affordability of food seasonings

Most (93.3) will increase purchase of food seasoning with increased income (Fig. 4).

Table 1: Knowledge of FGBCs and MSG

Food flavour enhancer	Variety	Heard of		Seen		Used	
		Number	%	Number	%	Number	%
FGBCs	Knoor	239.0	99.6	238.0	99.2	198.0	82.5
	Jumbo	190.0	79.2	130.0	54.2	6.0	2.5
	Maggi star	238.0	99.2	237.0	98.8	118.0	78.5
	Maggi chicken	232.0	96.7	223.0	92.9	86.0	35.0
	Royco fish	238.0	99.2	236.0	96.3	197.0	82.1
	Doyin	76.0	31.2	69.0	28.8	3.0	1.3
	Dadawa	126.0	52.5	115.0	47.9	9.0	3.8
MSG	Ajinomoto	230.0	95.8	223.0	92.9	102.0	42.5
	A-one	222.0	92.5	218.0	90.8	51.0	21.3
	Vedan	104.0	43.3	40.0	16.7	3.0	1.3

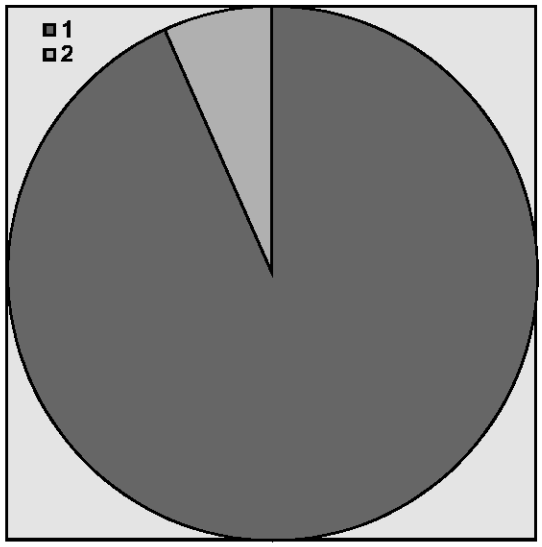

Fig. 4: Effect of extra money on purchase of food seasoning. 1-will increase purchase (93.3%); 2-will not increase purchase(6.7%)

Knowledge, attitude and practice in used of FGBCs and MSG: Knowledge was determined by heard of, seen and used. Many have knowledge of the various food seasonings (Table 1).

The major source of information was Radio (50%), Television (47.7%), friends (47.1%) and relations (35.8%). The least was market display (0.8%) (Fig. 5).

The main reason for the use of FGBCs was because it was affordable (95%), while for MSG, other reasons like desirable and causes no disease arises (54.6%). The remaining 45.4% do not use it because it is not desirable, cause disease and not affordable (Table 2).

The respondents' attitude towards FGBCs is positive as 80.8% will encourage its use and 82.5% will consume it if fortified with micronutrients. But for MSG, most (68.3%) will not encourage its use though an appreciable number (57.5%) will consume it if fortified. Majority (67.5%) adds the food flavour enhancer during cooking, 32.5% before cooking and none after cooking.

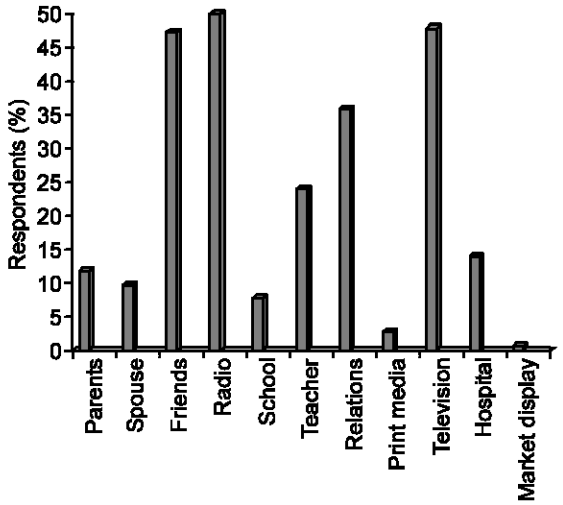

Fig. 5: Medium of first information about FGBCs and MSG

Table 2: Reasons for use and non-use of FGBCs and MSG

Flavour enhancer	Reasons for use and non-use	Respondents	
		Number	%
FGBCs	Affordable	112	93.3
	Not affordable	8	6.7
MSG	Affordable, desirable and causes no disease	131	54.6
	Causes diseases	69	28.8
	Not desirable	31	12.9
	Not affordable	1	0.4
	Not good at all	8	3.3

FGBCs, MSG and health of consumers: Nearly three quarters of the respondents were aware of the belief that FGBCs (MSG) causes health problem. Some had experienced it (45%), heard of it from friends (47.9%) or family members (32.9%). The most frequent health problems encountered was diarrhoea (54%) (Table 3).

The feeding trial on a sample of 15 university students in which the quantity of MSG in meals was increased progressively from 0-1.3 g/meal showed that the consumption of MSG in meals produced no health problem, rather, it improved their appetite and makes

Table 3: Health problems repeatedly associated with FGBCs and MSG

Type of Health Problem	Respondents	
	Number	%
Abdominal upset	38	28.3
Headache	1	0.8
Vomiting	29	22.0
Diarrhoea	54	40.9
Worm	10	7.6

good food taste better. The final BMI of most (93.3%) respondents improved at the end of the feeding trial.

The correlation analysis between knowledge and use of FGBCs and MSG shows that knowledge of food seasonings significantly determines it use ($R = 0.9170$; $p>0.05$); as knowledge increases, the usage also increases. Furthermore, the consumption was affected by availability ($p>0.05$). T-test between respondents that have health problems and those that do not showed that health was not affected by consumption of FGBCs and MSG ($p<0.05$).

DISCUSSION

The results showed that most of the respondents interviewed were in the middle and low-income groups when their housing characteristics were considered. This could explain the inadequate meals collectively consumed by most of the families (67.1%). A greater proportion (98.3%) of the study population consumed FGBCS and MSG. This popularity of food seasonings is similar to the situation in countries like Philippines and Taiwan, where MSG is consumed by essentially all households (Giacometti, 1979; Solon *et al*., 1985; Egbuta, 1993; Ajinomoto, 1994; Schiffman, 1996). The fact that many consume FGBCs and MSG indicates a positive mark for their use in food fortification with micronutrients as this will go along way to reducing or eliminating the micronutrients deficiency problem in the area.

The high frequency of consumption of FGBCs and MSG may be attributed to preference or the fact that they are gaining wider acceptability and may be loosing the image of causing health problems. This is supported by the positive relationship between knowledge and use of FGBCs and MSG. The average quantitative consumption of FGBCs (21 g) in the study area is high compared with that of the entire country which is 13g per person per week (Report of vitamin A Bench Mark Survey, 1997). For MSG 3.9 g per person is lower than that of the national survey (7 g/person/week).

These differences may be due to the size of people used in the studies or the activity of the sales promoters of MSG or the presence of the information on the health effect slogan as it were of MSG by the study population. Furthermore the fact that the study population consumes more of FGBCs than MSG shows the awareness of this belief. This indicated a strong need for nutrition advocacy and massive dissemination of information about the safety of MSG to the public. The high frequency of consumption of these food seasonings to some researchers like Olney (1969) and Olney *et al*. (1973) may pose the risk of toxicity. But since the quantity consumed by the study population is less than the RDA 1-9 g of MSG for 60 kg man per day (FAO/WHO, 1974) and is lower than that consumed in Taiwan, 3 g per person per day (Giacometti, 1979) the problem of toxicity does not arise. Moreso, MSG is consumed as an additive in food and the effect of meal and water on plasma glutamate have been demonstrated by several workers like Anantharaman (1979) and Tung and Tung *et al*. (1980). The study population used FGBCs and MSG only in cooking soup and stew. These culinary utilization data is important information for food processors.

The major storage condition (room temperature) used by the study population gives an insight as to whether the potency of fortificants will be maintained under these conditions. The respondents reported that these seasonings are available throughout the year and all over the area. This is line with the report o vitamin A Bench Mark Survey (FGN/UNICEF, 1997). The respondents showed the desire to purchase more food seasonings with extra money this is not surprising since it is known that as income increases, the purchasing power of the consumers increases.

Many of the respondents have either heard, seen or used the FGBCs and MSG. This is in line with the report of vitamin A Bench Mark Survey (FGN/UNICEF, 1997). The presence of reasons like “not healthy, not desirable, causes diseases” in using MSG goes to show the extent of ignorance about MSG by the study population. These views expressed by some of the respondents were contrary to the scientific report on the safety of MSG. (FAO/WHO, 1974; Filer *et al*., 1979; Tung and Tung, 1980; JECFA, 1988; SCF, 1991; AMASCA, 1992; FDA, 1992; Ajinomoto, 1994; FASEB, 1995; Schiffman, 1996; Reeds *et al*., 1997).

Many had either experienced health problem or heard of it from friends or from family members. This could mean that for them the health problem may reduce the level of consumption somewhat but not be a substantial constraint.

The results of the semi-controlled feeding trial support the report of research on safety of MSG in that all the trial subjects did not experience any discomfort or health problems when MSG meals were fed. They reported that MSG meals improved their appetite. This explains the increase in weight of majority of the subject during the feeding trial.

Conclusion: Nutrition education, information on the safety of MSG, concerted action to raise the consciousness of involved professionals, drawing their

attention to the magnitude of the problem as well as its consequences for individuals and for public health, timely approval of fortification of MSG with micronutrients, political actions such as specific laws to establish clear mechanisms of control and supervision of existing and new programmes, industry compliance with public health policies on the subject and consumption of fortified foods are recommended.

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Assessment of Trace Metal Composition in Fish Samples from Nworie River

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Abstract: This investigation surveyed heavy metal content of fish samples from Nworie river. The elements studied were Pb, Fe, Cd, Mn, Hg, Cu and Zn. The fish samples were collected from different locations in the river. The fish samples were analyzed for heavy metals using Atomic Absorption Spectrophotometer (AAS). The elemental toxicants Fe, Cd, Mn were identified in fresh fish specie *Tilapia guineensis* of mean values 3.275, 0.048 and 0.103 ppm, respectively, whereas Pb was below detection level. The analysis also shows Cu and Zn level of mean values 1.247 ppm and 3.241 ppm in *Tilapia guineensis* respectively. Also Hg was below detection level in *Tilapia guineensis*. The analysis of frozen fish samples purchased from Ekeonunwa market located 3 km from the river shows Pb, Fe and Cd levels of mean values 0.50, 4.73 and 0.05 ppm respectively. Also frozen fish analysis shows concentration of Mn, Hg, Cu and Zn of mean values 11.82, 0.0083, 8.00 and 1.02 ppm, respectively. There are three institutions that discharge untreated waste products into Nworie river. In view of this, there is need to determine the level of contamination of the river, since the inhabitants depend on the river for fishing and other domestic uses. This study is aimed at determining the level of heavy metal contaminants in fish samples from Nworie river. The effects of these elemental toxicants and the associated health hazards were examined.

Key words: Associated health hazards, heavy metals, fish samples, Nworie river

INTRODUCTION

Contamination of rivers, water bodies and aquatic animals by heavy metals have been a global problem especially in developing countries such as Nigeria. The rate of industrialization has affected the level of pollution of rivers and aquatic animals. This may be attributed to discharge of untreated waste products into the river and water bodies. Nworie river flows in Owerri the Imo State of Nigeria. It flows through the Federal Medical Centre, (FMC), Owerri; Alvan Ikoku Federal College of Education (AIFCE), Owerri and Holy Ghost College, Owerri. All these institutions discharge their untreated waste into Nworie river. The river acts as a source of drinking water, fishing and other domestic uses for the inhabitants. In view of the activities of these institutions, which discharge their untreated waste products into the river, it is necessary to investigate the level of contamination of fish in the river, since the inhabitants carry out fishing activity in the river for protein requirement.

Fishes are major sources of protein. They constitute major components of most aquatic habitats and they act as bio-indicator of heavy metal levels in aquatic environment. They have been recognized as good bio-accumulators of organic and inorganic pollutants (King and Jonathan, 2003). Heavy metals gain access into the aquatic environment from natural and anthropogenic sources and distributed in the water bodies, suspended solids and sediments during the course of their transportation (Olajire and Imeokparia, 2000). Reports have shown that heavy metal pollution of ecosystems is more in sediments and aquatic animals than in elevated concentrations in water (Luinnik and Zubenko, 2000). Elemental toxicants could enter fish either directly through the digestive tract due to consumption of contaminated water and food or non-dietary routes across permeable membranes such as gills (Burger *et al*., 2002).

Obodo (2004) working on Anambra river in Nigeria reported heavy metal contamination of fish samples such as Pb, Cu, Zn, Mn and Fe. These heavy metals may be ingested directly by eating the fish contaminated with elemental toxicants. Different fish samples from Kaduna river in Nigeria have been analyzed for toxic elemental contaminants such as Hg, Cd, Ph, V, Zn and Fe were identified in appreciable amount in all the fish samples studied (Nwaedozie, 1998). These contaminants cause unhealthy effects to the fish and this may be transferred to man by eating the fish that is contaminated. Odoemelam (2005) reported accumulation of heavy metals such as Ni, Cu, Mn, Pb, Zn, Fe, Hg, Cr, V and Cd in fish samples from Oguta lake in Nigeria. Some of the detrimental effects attributed to heavy metal-ingestion include mercury poisoning in fish sample in the Minamata Bay (Irukayama, 1964).

MATERIALS AND METHODS

Collection of fish samples: The fresh fish samples were collected from the river as shown in Fig. 1. Only fresh fish Tilapia species (*Tilapia guineensis*) were found in the river. The frozen fish Scale mullet (*Liza*

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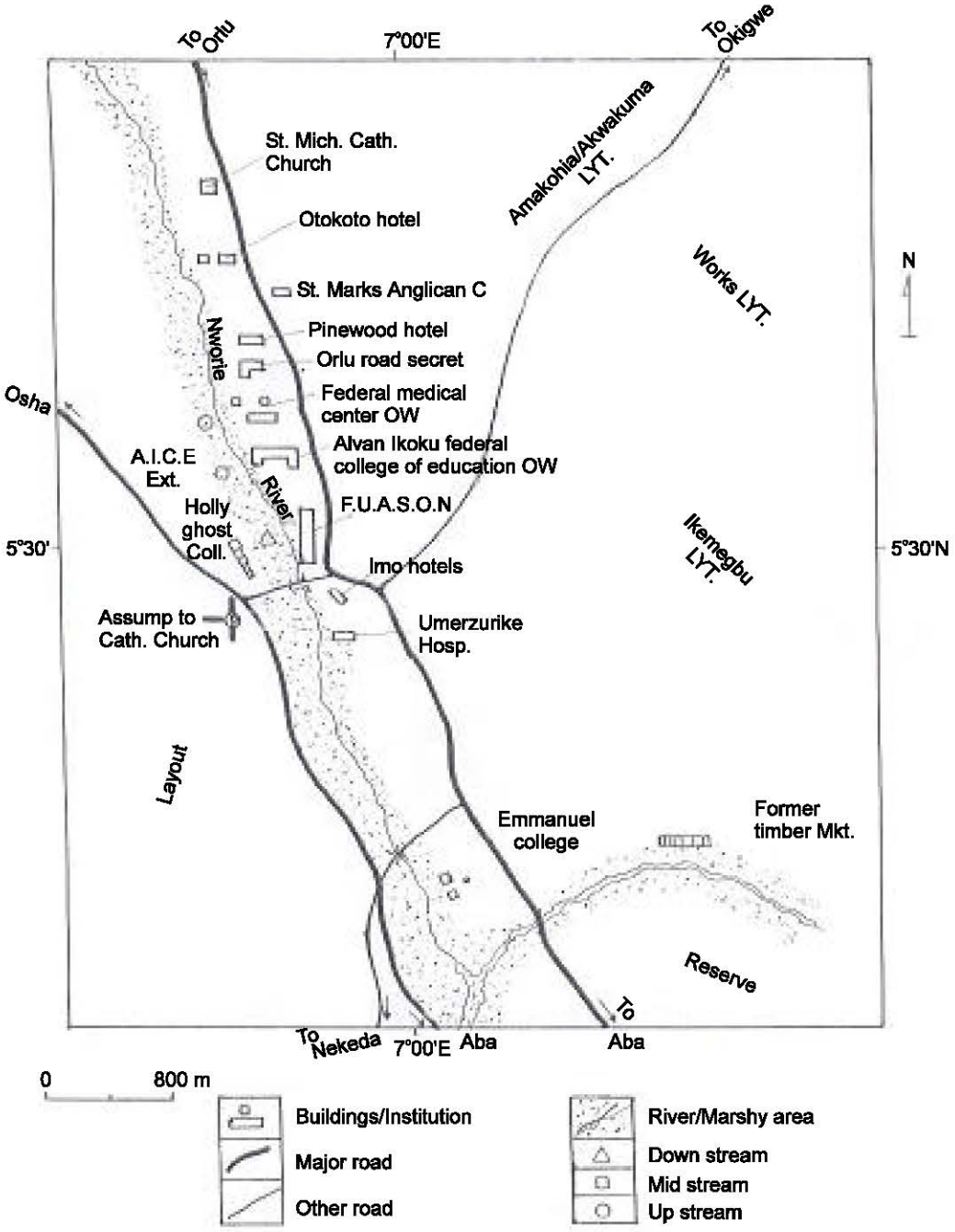

Fig. 1: Location map of the study area

grandisaquamis), Sole (*Synaptura insitanica*) and Barracuda (*Sphyraena sphyraena*) were purchased from Ekeonunwa market 3 km from the river. The fish samples were collected from the bank of the river during the wet season of the year precisely between July and August. Three samples of fish were collected with locally made wire net of 2.5 mm diameter. The samples were stored below 4°C in a refrigerator until use.

Analysis of fish samples: The fish samples were dried in an electric oven at 70-80°C for 3 days. The fish samples were ground and 2 g homogenized samples were weighed in an analytical balance and ashed in a furnace at 550°C. The samples were digested in a flask with 10 ml each of concentrated HNO_3 and HCl for 2-3 h in a fume cupboard until brown fumes ceases to evolve (Cappon, 1987).

The digested samples were filtered through a whatman GFK glass filter and solution made up to 100 ml mark with deionized water and kept ready for Atomic Absorption Spectrophotometer (AAS) analysis. The digested samples were analyzed using Unicam 919 AAS according to the technique as described by (Frank *et al*., 1992). All chemicals used were of analytical reagent grade.

RESULTS AND DISCUSSION

Table 1 shows the levels of heavy metal contamination in fish samples obtained from Nworie river and frozen fish samples sold at Ekeonunwa market about 3 km from the river. Results of the analysis shows that Pb concentration in *Tilapia guineensis* found in the river were below detection level, whereas Pb level in frozen *Liza grandisaquamis* and *Synaptura insitanica* were of mean values 0.3125 ppm and 0.8215 ppm, respectively. The frozen Barracuda fish has the highest accumulation of Pb concentration of mean value 0.50 ppm. Odoemelam (2005) working on Oguta lake reported Pb concentration of mean value 10.90 ppm in *Alestes nurse*. Nwaedozie (1998) working on Kaduna river reported Pb concentration of mean value 0.94 ppm in *Labeo cabic*. The Australian National Health and Medical Research Council (ANHMRC) standard (Bebbinton *et al*., 1997) for Pb in seafood is 2.00 ppm. This study shows that Oguta lake is contaminated with Pb, whereas Nworie river is not contaminated with Pb when compared to ANHMRC standard. The high accumulation of Pb in fish samples from Oguta lake and Kaduna river may be attributed to wastes being discharged into the lake and waste from Kaduna refinery being discharge into kaduna river. However, fish samples purchased from Ekeonunwa market were contaminated with Pb. These frozen fishes being a common source of protein for the inhabitants poses health hazards. Pb is a well-known toxicant and it has deleterious effect even at low concentration on human being. It reduces neuro-psychological function leading to intelligence quotient deficiency and also it leads to reduction in nerve conduction (Waldboh, 1978).

The result of the analysis shows that Fe level in frozen fish were higher than the fresh fish. Barracuda has the highest concentration of Fe of mean value 4.73 ppm. Whereas Sole fish and Scale mullet have Fe level of mean values 3.50 ppm and 4.25 ppm, respectively. Fresh *Tilapia guineensis* has the least concentration of Fe of mean valued 3.275 ppm. Alinnor (2005a) working on Aba river reported Fe concentration of mean value 9.419 ppm in *Hetretis niloticus*. Oboh and Edema (2007) have reported Fe concentration of mean value 2.447 ppm in *Citharinus citharus* from River Niger. This study shows that fish samples from Aba river is contaminated with Fe when compared to values obtained from Nworie river and River Niger. High concentration of Fe found in fish samples from Aba river may be attributed to industries that manufacture soaps, glasses, beverages and breweries that discharge their untreated wastes into Aba river. Iron is one of the essential components of haemoglobin, which is responsible for the transportation of oxygen in the body. Studies have shown that fish generally concentrate metallic iron in their body organism directly or indirectly through ingested food (Vinikour and Goldstein, 1987; Kakulu *et al*., 1980).

Table 1 shows the level of Cd in *Tilapia guineensis* and *Synaptura insitanica*. The concentrations of the two species of fish were similar of mean values 0.048 ppm and 0.05 ppm for *Tilapia guineensis* and *Synatpura insitanica* respectively. Whereas *Liza grandisaquamis* and *Sphyraena sphyraena* have Cd level of mean values 0.042 ppm and 0.019 ppm, respectively. Abdulrahman and Tsafe (2004) working on Sokoto Rima river in Nigeria reported Cd concentration of mean value 0.013 ppm in *Synodontis clarias*. Also (Ibok *et al*., 1989) working on streams in Ikot Ekpene area of Nigeria reported Cd level of mean value 0.45 ppm in A. *fasciatus*. The ANHMRC acceptable level for Cd in seafood is 2.0 ppm. The result of this study indicate that Cd in fish samples from Nworie river, Sokoto Rima river and Ikot Ekpene streams were below ANHMRC standard, but long period of accumulation of Cd in fish poses health hazards. Cadmiam shows no indication of being an essential element in biological processes, instead it is toxic. It causes slight anaemia due to competition between Fe and Cd in the body resulting to iron deficiency (Lauwerys, 1979).

This study identified low concentration of Mn in fresh Tilapia fish of mean value 0.103 ppm, whereas the frozen fish samples have Mn concentration of mean values 9.99, 11.82 and 9.00 ppm for Scale mullet, Soles fish and Barracuda fishes, respectively. Alinnor (2005b) working on Aba river reported Mn concentration of mean value 0.861 ppm in Oriochronis niloticus, whereas (Obodo, 2001) identified Mn concentration of mean value 0.081 ppm in fresh fish obtained from River Niger. This study revealed that Aba river is contaminated with Mn when compared to values obtained from Nworie river and River Niger because of industries that discharge their untreated wastes into Aba river. Manganese in trace amount is an essential element. Eating fish contaminated with Mn can result in manganese poisoning. Mn poisoning results in chronic manganism, which is the disease of the central nervous system and this can be transferred to man on consumption of fish contaminated with Mn. One of the first toxic effects of Mn is its interference with iron metabolism, specifically haemoglobin formation.

Mercury concentration in *Liza grandisaquamis* and *Sphyraena sphyraena* were of mean values 0.0083 ppm and 0.0083 ppm respectively. Hg was below detection level in fresh Tilapia fish and frozen Soles fish. Nwaedozie (1998) working on kaduna river reported Hg

Table 1: Concentration (ppm) of some heavy metals in fish Samples from Nworie River

Name of fish	English Name	Nature	Fish Sample	Pb	Fe	Cd	Mn	Hg	Cu	Zn
Tilapia guineensis	Tilapia		A	Nd	3.285	0.048	0.101	Nd	1.174	3.401
		Fresh	B	Nd	3.175	0.034	0.105	Nd	1.262	2.910
			C	Nd	3.365	0.061	0.103	Nd	1.302	3.412
Liza grandis aquamis	Scale Mullet		D	0.2904	4.01	0.031	9.86	0.0071	5.20	0.46
		Frozen	E	0.2957	4.34	0.058	10.37	0.0092	4.86	0.41
			F	0.3515	4.39	0.037	9.75	0.0086	5.55	0.58
Synaptura Insitanica	Soles		M	0.2814	3.10	0.041	11.63	Nd	4.50	0.73
		Frozen	N	0.3041	3.82	0.062	10.92	Nd	3.95	0.80
			P	0.3520	3.58	0.047	12.90	Nd	5.95	0.75
Sphyraena Sphyaena	Barracuda		R	0.45	4.76	0.017	8.50	0.0078	8.46	1.05
		Frozen	S	0.58	4.68	0.021	9.50	0.0096	7.31	1.01
			T	0.52	4.74	0.019	9.00	0.0074	8.23	0.99

Nd = Non-detectable (below detection level)

level of mean value 0.32 ppm in *Lates niloticus*. Odoemelam (2005) has shown that Hg level in *Synodontis nigritis* from Oguta lake was of mean value 0.08 ppm. The ANHMRC standard for Hg in seafood is 0.50 ppm. The result indicate that Hg level in Nworie river is below ANHMRC permissible limit. The high concentration of Hg in fish samples from Kaduna river may be due to textile industries, refineries, breweries and automobiles that discharge their untreated waste products into the river. This study also shows that two species of fish purchased from Ekeonunwa market were contaminated with mercury. In view of this, the inhabitants that buy fish from the market may be accumulating mercury in their body through the consumption of fish purchased from the Market. Mercury is not required in the body even in trace amounts. Mercury serves as a poison in the body. The presence of mercury in the body affects the nervous system which includes anxiety, depression, lack of concentration (Holtzclaw and William, 1988). Mercury affects the reproductive system causing spontaneous abortion (Waldboh, 1978). Mercury alter enzymatic and metabolic processes in organisms and interferes with functions of brain, kidney and liver. Therefore, eating fish contaminated with mercury poses health hazards.

The result of this study showed that all the fish samples both fresh and frozen were contaminated with copper. The fresh fish Tilapia has copper concentration of mean value 1.247 ppm. Whereas frozen Soles fish, Scale mullet and Barracuda have copper concentrations of mean values 4.80, 5.20 and 8.0 ppm, respectively. Okoye *et al*. (2002) working on Warri river in Nigeria reported Cu level of mean value 2.02 ppm in aquatic organism. Alinnor (2005a) also reported Cu concentration of mean value 0.229 ppm in *Orichronis niloticus* from Aba river. The level of Cu in aquatic organism from Warri river is high as compared to Nworie river probably due to waste discharged from Warri refinery into Warri river. The ANHMRC permissible limit for Cu in seafood is 30.0 ppm. In view of this, fish samples from Nworie river were not contaminated with Cu. Copper is an essential element and it enhance the enzymatic activity of the body. However, if poses health hazard when ingested in large amount.

Zinc level of fresh Tilapia fish has a mean value 3.24 ppm as shown in Table 1. whereas *Liza grandisaquamis*, *Synaptura insitanica* and *Sphyraena sphyraena* have Zn levels of mean values 0.48, 0.76 and 1.02 ppm, respectively. This study indicate that fresh Tilapia fish obtained from Nworie river is contaminated with Zn when compared to values of frozen fish sample from Ekeonunwa market. Odoemelam (2005) reported high accumulation of Zn in *Synodontis nigritis* of mean value 156.0 ppm from Oguta lake. Williams and Kasali (2008) reported Zn level of mean value 316.02 mg/kg in *Chrysichthys nigrodigitatus* obtained from Lagos Lagoon in Nigeria. The ANHMRC standard for Zn in seafood is 1000 ppm. This result indicate that fish samples from Nworie river is below ANHMRC standard. Nwaedozie (1998) reported that zinc concentration has effect on the hepatic distribution of other trace metals in fish. This is due to heavy metals such as Zn, Cu and Mn, which are essential elements that exhibit similar atomic structure and could therefore compete for the same site.

Conclusion: This study shows that untreated waste products are being discharged into Nworie river by various institutions located near the river without consideration of the aquatic life. Also frozen fish samples purchased from Ekeonunwa market were contaminated with heavy metals. These elemental toxicants will be transferred to man on consumption of fish obtained from the river and the market. These heavy metals transferred to man through the consumption of fish poses health hazards because of their cumulative effect in the body. As a result of contamination of Nworie river by heavy metals only one specie of fish is found in the river. This report is significant because it gives an idea to the mechanism of depletion and possible extinction of fish species in Nworie river that contains heavy metals. In view of these findings strict method of waste disposal control should be adopted to ensure the safety of the environment and safeguard our aquatic life.

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Growth Response and Feed Conversion Efficiency of *Tor putitora* (Ham.) Fry at Varying Dietary Protein Levels

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Abstract: The aim of this work was to study the effect of different dietary protein levels Viz. 30, 35, 40, 45 and 50% on growth and Feed Conversion Efficiency (FCE) of *Tor putitora* fry. Five iso-caloric diets containing different levels of protein such as 30, 35, 40, 45 and 50% were prepared in pellet form and fed to *Tor putitora* fry for a period of 120 days to determine the optimum protein requirement in laboratory conditions. Specific growth rates were 0.719, 0.696, 0.538, 0.486 and 0.364 in 45, 50, 40, 35 and 30% protein diet respectively. Food conversion efficiency was minimum at 30% (7.62), it further increased with increasing protein levels up to 45% (14.46) but beyond this it again decreases i.e. at 50% (13.85). On the basis of weight gain the following trend emerges 45%>50%>40%>35%>30%. The results confirm the best protein level for optimum growth of *Tor putitora* seems to be 45% and it is not significantly different from that achieved by 50% ($p<0.001$) protein diet.

Key words: Dietary protein levels, feed conversion efficiency , *Tor putitora*

INTRODUCTION

Golden mahseer or *Tor putitora* recognized as a king of mountain streams is a highly prized, delicious food fish of India, Pakistan, Bangladesh, Nepal, Afghanistan and Myanmar. It has been a source of intense thrill and fascination to the anglers, environmentalist and fisherman in view of its amazing size, leaping capacity and playful habits. However, from the last two decades there has been a lot of hue and cry regarding the dwindling population of mahseer species all over the country. Depletion of mahseer has been reported by many workers (Joshi, 1988; Nautiyal, 1994; Islam and Tanaka, 2004). The aquaculture potential of this fish has been identified only recently. Very little information is currently available on the nutrient requirement of *Tor putitora*. So nutritional studies are important from view point of culture related to conservation and propagation. Dietary protein plays a major role in determining the rate of fish growth. Accurate information on the protein requirement of fish is crucial for any aquaculture initiative owing to cost of protein ingredients that are usually required at high levels by most fishes (NRC, 1983). Information on the effects of dietary protein requirements of *Tor putitora* is scarce, reported by only a few workers (Joshi *et al*., 1989; Sunder *et al*., 1998; Islam, 2002; Islam and Tanaka, 2004). Therefore protein requirement studies are usually one of the first fish nutrition experiments to be conducted for intensive culture. The objective of the present study was therefore to assess the optimum protein level leading to optimum growth of mahseer, *Tor putitora* at fry stage.

MATERIALS AND METHODS

Five iso-caloric diets containing different levels of protein such as 30, 35, 40, 45 and 50% were prepared in pellet form using fish meal as a major source of protein as it is generally recognized that purified proteins, such as casein, are deficient with respect to certain amino acids and it is being expensive protein source for the average fish nutritionist in developing countries and this has led present investigator to formulate practical diets using cheaper locally available feed ingredients. Proportion (%) of different ingredients used in the formulated diets are shown in Table 1.

Table 1: Proportion (%) of different ingredients used in formulated diets for fry

	Diets				
Ingredients	30%	35%	40%	45%	50%
Fish meal	32.82	42.31	51.78	61.26	70.75
Rice bran	27.62	21.29	14.98	8.66	2.33
Mustard oil cake	10.94	14.11	17.26	20.42	23.59
Wheat flour	27.62	21.29	14.98	8.66	2.33
Vitamin and Mineral Premix*	1	1	1	1	1

a*Nutrimin Super forte (Rejuvenating combination of multivitamin and Multi minerals, AROSOL Chemicals PVT. Limited)

Vitamin A	700,000I.U	Vitamin D_3	140,000I.U
Vitamin E	250 mg	Folic acid	100 mg
Niacinamide	1000 mg	Iron	1500 mg
Iodine	325 mg	Cobalt	150 mg
Magnesium	6000 mg	Manganese	1500 mg
Zinc	3000 mg	Selenium	10 mg
Potassium	100 mg	Sulphur	7.2 gm
Calcium	270 gm	Phosphorous	130 gm
Copper	1200 mg	Fluorine	300 mg

Proximate composition of the feed ingredients and experimental diets were determined in the laboratory using standard methods. The crude protein content of

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Table 2: Proximate composition (%) of formulated diets for fry

Diets	Moisture	Dry matter	Crude protein	Crude fat	Ash	Crude fibre	Nitrogen free extract	Calorific content KJ/g
30%	9.79	90.21	29.06	5.39	14.32	9.28	32.16	13.51
35%	9.46	90.54	34.06	5.76	14.98	7.58	28.16	14.03
40%	9.73	90.27	38.92	5.92	13.06	7.02	25.35	14.63
45%	9.27	90.73	44.03	6.26	14.20	6.02	20.22	14.97
50%	9.40	90.60	48.99	6.97	15.24	5.98	13.42	15.14

feed ingredients was determined by microkjeltec method and the value obtained was multiplied by the factor 6.25 to obtain crude protein value. The crude lipid content was determined by extraction using petroleum ether in a soxhlet extraction apparatus for 16 h. The moisture content was determined by heating samples in oven at 105°C for 24 h. The ash content was determined by first igniting the sample and then heating it in a muffle furnace at 550°C (±10°C) for 6 h (AOAC, 1995). Crude fiber was determined by acid and alkali digestion (Pearson, 1976). Nitrogen free extract which was considered as carbohydrate was calculated by difference method (Hasting, 1976). The calorific value of the feed was calculated in terms of KJ/g using the energy value of 9Kcal/g for fat, 4 Kcal/g for carbohydrate (Hasting, 1976) and 5 Kcal/g for protein (Smith, 1975; Viola, 1977). Proximate composition of the experimental diets given in Table 2.

Tor putitora fry weighing (0.442 ± 0.009 g) were used for the experiment. The fry were collected from Govt. Anji fish farm, Reasi (J and K) in oxygen filled water bags. Before dividing the fish for conducting experiment, they were acclimatized in the laboratory for about 2 weeks. During that period the fish were fed (rice bran and mustard oil cake 1:1) *ad libitum*. The experiment was conducted in lab conditions in 100 l plastic tubs under flow through system along with aerators. *Tor putitora* fry were divided in five groups with 20 fish each. The fry were fed once daily in the morning at the rate of 5% of body weight during the period of 120 days and the fed quantity was readjusted after every fifteen day sampling, based on the growth of fishes.

Sampling and growth measurements: The fishes from each tub were captured once in fifteen days and were weighed individually and their growth was assessed by calculating following growth parameters.

Percentage weight (% WG): It was calculated by using the formula:

$$\%WG = [(W_f - W_i) / W_i] \times 100$$

Where W_f is the final weight of the fish and W_i is the initial weight of fish.

Specific growth rate: The formula used for calculating SGR was:

$$SGR = \frac{(\ln \text{final weight} - \ln \text{initial weight})}{\text{No. of days of experiment}} \times 100$$

Feed conversion ratio: The FCR was calculated by using the formula:

$$FCR = \text{Feed fed} / \text{Gain in weight of fish}$$

Feed conversion efficiency FCE (%): It was calculated by using the formula:

$$FCE (\%) = [(\text{Gain in wet weight of fish} / \text{Feed Fed})] \times 100$$

Protein Efficiency Ratio (PER): It was calculated using formula:

$$PER = \text{Increment in body weight (g)} / \text{Protein intake (g)}$$

Statistical analysis: A one way analysis (ANOVA) was conducted in each and every experiment, using the computer software 'Analyse it'.

RESULTS AND DISCUSSION

The present study on relative growth performance of mahseer, *Tor putitora* at fry stage, in response to diets with varying levels of protein viz. 30, 35, 40, 45 and 50% for a period of 120 days shows that fish fry fed on 45% protein diet attained best growth, while 30% protein diet exhibited least growth. On the basis of net weight gain the following trend emerged 45%>50%>40%>35%> 30%.

The average net weight gain of fry fed on different protein diets was 0.610, 0.581, 0.406, 0.354 and 0.236g at 45, 50, 40, 35 and 30% respectively (Table 3). However, there was insignificant difference ($p>0.001$) in net weight gain between 45% and 50% protein diets.

Similar to present observation protein requirement of 45.6% has been recorded in case of grass carp fry by Dabrowski (1977). Siddiqui *et al.* (1988), while working on Nile tilapia, *Oreochromis niloticus* fry, obtained best growth with 40% dietary protein followed by the diet containing 50, 30 and 20% protein. AlHafedh (1999) obtained significantly higher growth for *Oreochromis niloticus* fry fed on a practical diet containing 40% protein. Jana *et al.* (2006) reported significantly higher growth in terms of live weight gain and specific growth rate in milkfish *Chanos chanos* fry fed at 40% protein

Table 3: Showing percentage survival, net weight gain, Percentage Weight Gain (%WG), specific growth rate, food conversion ratio, food conversion efficiency, protein efficiency ratio of mahseer, *Tor putitora* fry at different protein levels in the diet

Protein levels	Experimental sets	Percentage Survival	Net weight gain (gm)	Percentage weight gain (%WG)	Specific growth rate (%)	Food conversion ratio	Food conversion efficiency	Protein efficiency ratio
30%	1	95	0.226	56.079	0.370	13.098	7.634	0.262
	2	95	0.287	64.639	0.415	11.187	8.938	0.307
	3	90	0.196	44.646	0.307	15.895	6.291	0.216
	Average	93.33	0.236	55.121	0.364	13.393	7.621	0.262
	SD	2.826	0.046	10.030	0.054	2.367	1.323	0.045
35%	1	90	0.365	82.766	0.502	9.877	10.124	0.297
	2	90	0.320	70.796	0.446	11.23	8.903	0.261
	3	90	0.379	84.409	0.509	9.385	10.654	0.312
	Average	90	0.354	79.324	0.486	10.164	9.894	0.290
	SD	0	0.030	7.430	0.030	0.955	0.897	0.026
40%	1	85	0.399	90.476	0.536	9.385	10.654	0.273
	2	90	0.412	91.15	0.539	8.996	11.115	0.285
	3	90	0.409	91.091	0.539	9.036	11.066	0.284
	Average	88.33	0.406	90.905	0.538	9.139	10.945	0.281
	SD	2.886	0.006	0.373	0.001	0.213	0.252	0.006
45%	1	95	0.612	140.366	0.73	6.857	14.581	0.331
	2	100	0.599	134.004	0.708	7.053	14.178	0.322
	3	100	0.620	137.168	0.719	6.833	14.633	0.332
	Average	98.33	0.610	137.179	0.719	6.914	14.464	0.328
	SD	2.886	0.010	3.181	0.011	0.12	0.249	0.005
50	1	90	0.556	127.522	0.685	7.547	13.249	0.27
	2	95	0.601	134.451	0.71	7.007	14.271	0.291
	3	100	0.587	129.867	0.693	7.124	14.036	0.286
	Average	95	0.581	130.614	0.696	7.226	13.852	0.282
	SD	5	0.023	3.524	0.012	0.284	0.534	0.01

level. Diyaware *et al*. (2009) showed that all the growth indices Mean Final Weight (MFW), Mean Weight Gain (MWG), Specific Growth Rate (SGR), Protein Index (PI), Mean Daily Weight Gain (MDWG), Apparent Protein Efficiency Ratio (APER), Food Conversion Ratio (FCR), Nitrogen Metabolism (NM), Relative Growth Rate (RGR) and percentage survival (%SR) were higher in hybrid catfish (*Heterobranchus bidosalis* x *Clarias anguillaris*) fry fed 50% crude protein.

During present investigation growth of fry was proportional to the dietary protein levels up to 45% beyond which it was not proportional i.e. percentage weight gain of fry increased with an increase in the dietary protein levels up to 45% and thereafter the growth increment was insignificant ($p>0.001$) Fig. 1. Similar growth pattern has been reported for mrigal fry (Singh *et al*., 1987), walking cat fish fry, *Clarias batrachus* (Chuapoehuk, 1987) and for *Heterobranchus* (Jamabo and Alfred-Ockiya, 2008).

During the present investigation the value of SGR was highest for fry fed with 45% protein (0.719%) and lowest for 30% dietary protein in fry (0.364%), Table 3, Fig. 1. SGR increases with increasing dietary protein content up to 45% in fry (Fig. 1) and above optimum protein level SGR decreased (Fig. 1). These results agree with those of Jauncey (1982b) who postulated that the decrease in specific growth rate at protein level above the optimum may be due to a reduction in the dietary energy available for growth to deaminate and excrete excess absorbed amino acid.

In the present investigation, FCR obtained with different diets having different levels of protein ranged from 6.91 in 45% protein diet to 13.39 in 30% protein diet in fry. Low feed conversion ratio at 45% protein level clearly reflects that these diets were utilized more efficiently. Further, in the present investigation the Food Conversion Ratio (FCR) decreased with increasing protein levels although not significantly above 45% in fry (Fig. 1). Similar to the present results, Siddiqui *et al*. (1988) and AlHafedh (1999) have all reported that FCR values decreases with increasing protein level.

In the present investigation, the Food Conversion Efficiency (FCE) was higher (14.46%) in fry fed with diet containing 45% protein level showing the best utilization of the diet and lowest for 30% dietary protein i.e. 7.62% (Fig. 1).

Present observation further reveals that food conversion efficiency increased with increasing protein level in the diet up to 45% in fry and in fingerling up to 35% protein level then decreased afterwards. Support for this can be drawn from Siddiqui *et al*. (1988) who also reported that feed conversion efficiency increased with increasing protein level up to 40% and then decreased for the diet containing 50% protein in Nile tilapia, *Oreochromis niloticus*.

In addition, feed conversion efficiency of fish fed with the varying levels of dietary protein in the present study indicated that the optimum dietary protein requirement was 45% in fry. Below and beyond these ranges, feed

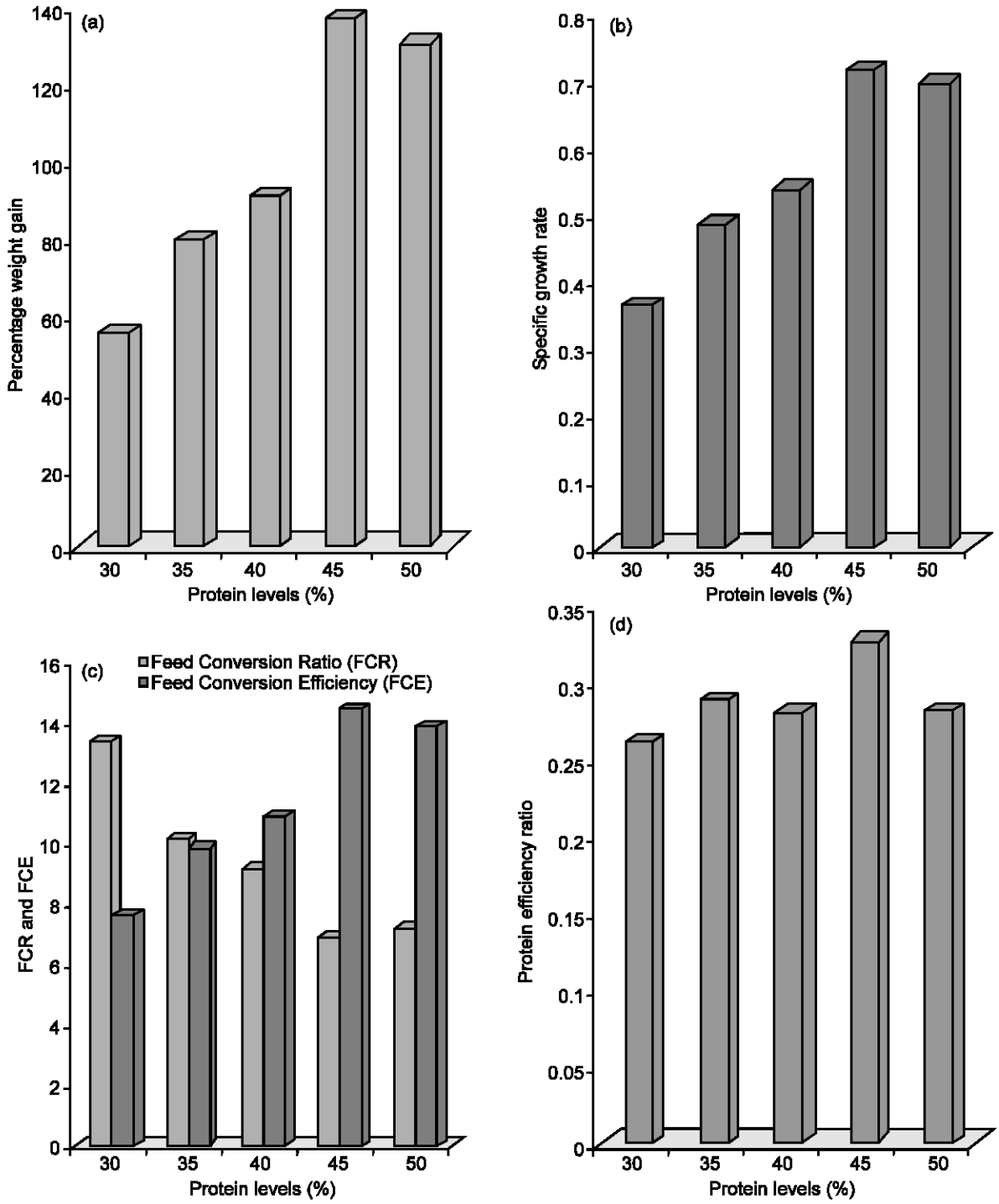

Fig. 1: Bar diagrams showing growth response of *Tor putitora* fry at different protein levels in the diet (a) Showing percentage weight gain (%WG); (b) Showing specific growth rate (SGR); (C) Showing food conversion ratio (FCR) and feed conversion efficiency (FCE); (d) Showing protein efficiency ratio (PER)

efficiency reduced. The present trend of FCE is similar to that reported for bagrid catfish, *Mystus nemurus* (NG *et al*., 2001).

Thus the present results clearly indicate that present fish species i.e. *Tor putitora* fry require higher protein level i.e. 45% or less than 50% when fed artificially, for better

growth. It was interesting to find that weight gain, SGR and FCE were lowered if protein in feed was higher then the required level of 45% by the present fish species.

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Trace Metal Distribution in Nigerian Leafy Vegetables

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Abstract: Trace metal distribution in some selected Nigeria leafy vegetables were determined. All the minerals investigated were found present in all the components of the selected vegetables. On the average, zinc was the most abundant metal with averages of 2.82, 1.97 and 2.08 (mg/g) in roots, stem and leaf of the vegetables, while lead was the leafy with averages of 0.07, 0.06 and 0.07 (mg/g), in roots, stems and leaves respectively. Trace metal distribution number (TMDN) indicates that the roots concentrate most of the metals than the stems and leaves.

Key words: Leafy vegetables, trace metal, human health

INTRODUCTION

Environmental impact of some trace metals cannot be over emphasized. While some of these metals (Pb, Cd, Hg e.t.c) have been reported to be extremely dangerous to human health without any known useful biochemical functions even at low concentration, some (Fe, Zn, Cu, e.t.c) are essential nutrient that are required in enzymatic biochemical activities in the body (Silvia *et al*., 2007; Asaolu *et al*., 1997; Shannon *et al*., 2009).

Apart from the fact that some of these metals could be naturally present in a greater amount (through weathering of the rocks and soils) in the area where the vegetables are grown, some of the metals have been reported to be introduced into our environment through municipal and industrial discharges, urban run off and atmospheric precipitation and deposition (Yuan Gao *et al*., 2007; Ipinmoroti *et al*., 1997).

Knowing the toxicity effect and the essentialities of some of the trace metals in the environment on human health, it is of paramount importance for food hygienist and health authorities to be familiar with available information on some of the trace metal content of our foods like the leafy vegetables. Also information on the distribution of some of these metals in the various components of the vegetables could be a useful guide as to what part of the leafy vegetables contains the highest level of the minerals and in accessing the nutritional value of the various components for appropriate application. Equally, any component could serve as pollution indicator for some metals. To this end, the concentration of iron, zinc, nickel, cadmium and lead have been determined in the various components of five Nigeria leafy vegetables.

MATERIALS AND METHODS

Sampling: Five commonly and widely consumed Nigeria leafy vegetables were selected for analysis. The varieties are: *Corchorus oliterius, Grassocephilum crepodes Ammaranathus caudatus, Talium tragulare and Senecio biofrae*. The vegetables were purchased from local farmers at Ago/ibira located close to University of Ado-Ekiti.

Sample preparation: The roots, stems, and leaves were separated in each case and the components were cuts into pieces, washed, air dried and then dried in the oven at 80°C for six hours. About 10g of the dried materials of each component were powdered in a hammer mill. 1.0g of each powdered component were weighed and digested as reported by Asaolu (1995). The digest were analyzed for the mineral content by atomic absorption spectroscopy (Buck scientific model-210).

RESULTS AND DISCUSSION

Table 1 presents trace metal distribution vegetables (TMD); while Table 2 presents the trace metal distribution number (TMDN) for the components of the vegetables.

All the minerals examined were found present in the various components of the selected vegetable. On the average, zinc is the most abundant metal with averages of 2.82, 1.97 and 2.08 (mg/g) in root, stem and leaves respectively, while lead is the least in the vegetables with averages of 0.01,0.06 and 0.07 (mg/g) in root, stem and leaf respectively. Also *Talium triangulase* seems to concentrate some of the metals better than the other vegetables (Table 1). The difference in the mineral content of the vegetable plant products might be due to the soil compositions and the rate of uptake of minerals by individual plant (Asaolu, 1995; Tanner and Beevers, 2001). In most cases, TMDN indicates that the root concentrates most of the metals than the stem and leaf respectively, while clear distinction could not be made in mineral distribution between the stem and the leaf.

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Table 1: Trace metal Distribution in Vegetable (TMD) (mg/g) dry matter

	Fe			Zn			Ni			Cd			Pb		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
V_1	1.87	1.69	1.57	3.10	1.79	1.85	1.04	1.01	0.94	0.12	0.21	0.14	0.08	0.07	0.05
V_2	2.20	1.81	0.84	1.67	2.14	2.30	1.12	1.20	1.12	0.20	0.19	0.19	0.06	0.07	0.07
V_3	1.19	1.64	1.53	2.90	1.49	1.17	1.03	1.07	1.03	0.11	0.16	0.18	0.07	0.04	0.09
V_4	2.00	1.86	1.10	3.50	2.60	3.12	1.01	0.78	1.05	0.20	0.17	0.10	0.07	0.05	0.07
V_5	1.90	1.70	1.72	2.93	1.81	1.98	1.10	1.11	0.97	0.21	0.16	0.12	0.06	0.06	0.05
M	1.98	1.74	1.75	2.82	1.97	2.08	1.07	1.03	1.02	0.17	0.18	0.15	0.07	0.06	0.07
SD	0.13	0.19	0.23	0.69	0.42	0.71	0.04	0.16	0.07	0.05	0.02	0.04	0.01	0.01	0.02
CV%	6.8	5.3	13.0	24.0	21.0	34.0	4.0	5.0	6.9	27.0	12.0	26.0	12.0	22.0	24.0

V_1 = Corchorus olitorius V_2 = Grassocephilium crepodes V_3 = Ammaranthus caudus V_4 = Talium triangulase
M = Mean SD = Standard deviation CV = Coefficient of variation V_5 = Senecio biodrae

Table 2: Trace Metal Distribution number (TMDN) n vegetable parts

	Fe			Zn			Ni			Cd			Pb		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
V_1	1.00	0.90	0.84	1.00	0.58	0.60	1.00	0.97	0.90	0.57	0.21	0.67	1.00	0.88	0.65
V_2	1.00	0.82	0.84	0.73	0.93	1.00	0.93	1.00	0.93	1.00	0.95	0.95	0.86	1.00	1.00
V_3	1.00	0.86	0.80	1.00	0.51	0.40	1.00	0.99	0.95	0.61	0.89	1.00	0.78	0.44	1.00
V_4	0.95	0.89	1.00	1.00	0.74	0.89	0.96	0.74	1.00	1.00	0.85	0.50	1.00	0.74	1.00
V_5	1.00	0.89	0.91	1.00	0.62	0.68	0.99	1.00	0.87	1.00	1.00	0.83	1.00	1.00	0.83

V_1 = Corchorus olitorius V_2 = Grassocephilium crepodes V_3 = Ammaranthus caudus
V_4 = Talium triangulase V_5 = Senecio biodrae

However, from this observation, the root might be acting as storage or these minerals after up take from the surrounding soil before translocation occurs.

Situation where there is high concentration of the metals in the roots, and the lower part of the stem might be an advantage for the consumers most importantly in the case of toxic metals since in processing vegetables plant for human consumption, the root and the lower part of the stem are usually discarded. However, in this study TMDN indicates that the leaf of *Grassocephilium crepodes Ammaranthus caudatus and Senecio biofrae* have the highest lead storage and the leaf of *Ammaranthus caudatus* has the highest cadmium storage (Table 2). Although, in this case, the lead and cadmium concentration in the leafy part of some of the vegetables seems not to be alarming except in a case of excessive consumption. The high storage of iron and zinc as indicated by TMDN in the leafy part of some of the vegetables might be advantageous for their useful biochemical functions in human system.

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Dietary Patterns Associated with Risk for Metabolic Syndrome in Urban Community of Karachi Defined by Cluster Analysis

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Abstract: Dietary trends have been found to be related with metabolic syndrome in various studies. To identify dietary patterns and study associations between the dietary patterns of subjects with high and low risk of metabolic syndrome in a Karachi based community. A group of 871 men and women were selected randomly from 532 households. Data about consumption of specific foods was available for 867 adults. Participants completed a health and lifestyle questionnaire and 363 subjects provided fasting blood samples for glucose and lipids. Dietary intake was assessed by a questionnaire to identify consumption of 33 specific food items and the dietary patterns categorized into 6 food groups was assessed by cluster analysis. Five dietary patterns were identified through cluster analysis. Cluster 1 had the lowest proportion of persons with metabolic syndrome i.e. 42.7% while cluster 2 had the highest percentage of metabolic syndrome subjects (56.3%) ($p = 0.09$). Consumption of fat and caloric dense foods was significantly higher among highest risk group (cluster 2) compared to lowest risk group (cluster 1) ($p = 0.0001$). The consumption of food groups containing fruit, milk and meat was also more than twice in high risk compared to low risk group ($p = 0.0001$). Even within the same population there are marked differences in dietary patterns and these apparently contribute to the risk of developing metabolic syndrome. Dietary pattern studies will help elucidate links between diet and disease and contribute to developing healthy eating guidelines.

Key words: Dietary patterns, cluster analysis, metabolic syndrome, South Asians

INTRODUCTION

High prevalence of metabolic syndrome and Cardiovascular Disease (CVD) risk factors have been reported worldwide especially in South Asians (Ramachandran *et al*., 2003; Wierzbicki *et al*., 2005; Basit *et al*., 2002; Jafar *et al*., 2005). Metabolic Syndrome (MS) has been shown to be a good marker of future disease risk and it is estimated that subjects with metabolic syndrome are three times more likely to have and twice as likely to die from a heart attack or stroke compared to people without the syndrome (Sarkar *et al*., 2006). Similarly, people with metabolic syndrome have a five-fold increased risk of developing type 2 diabetes. Although dietary intake has been linked to individual components of MS or the outcome diseases such as diabetes and cardiovascular diseases, the dietary patterns which may lead to the development of metabolic syndrome have not been specified. In recent years there has been increasing interest in the identification of dietary patterns as consumed by populations to better understand the association of diet with chronic diseases (Schwerin *et al*., 1982; Randall *et al*., 1990). During the last two decades, there has been significant changes in society's life style habits with increase in unhealthy eating, sedentary activities and smoking (Panagiotakos *et al*., 2003). These habits have fueled the epidemic of obesity, which is an important risk factor for diabetes, cardiovascular diseases, hypertension and dyslipidemia all of which may be preceded by metabolic syndrome (Basit and Shera, 2008).

The 1990-1994 National Health Survey of Pakistan showed that overall 25% of the population was overweight or obese. The factors significantly associated with obesity were increasing age, being female, higher education, urban residence, high economic status and a high intake of meat (Jafar *et al*., 2006). Knowledge of specific food patterns is important for relating diet to nutritional status and for the identification of groups at risk of under-or over consumption of specific food items (Tucker *et al*., 1992).

Several studies have shown that adopting a dietary pattern characterized by high intake of red meat, refined grains, snacks, sweets and fried foods contribute to the

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increased prevalence of type 2 diabetes (Song *et al*., 2004; Schulze *et al*., 2003; Van *et al*., 2002). Whilst adopting a dietary pattern characterized by high consumption of non-refined cereals, fruits and vegetables, a moderate intake of dairy products, poultry and fish and a low intake of red meat contribute towards a reduced prevalence of type 2 diabetes, metabolic syndrome and cardiovascular disease (Kris-Etherton *et al*., 2001; Trichopoulou *et al*., 2003; Chrysohoou *et al*., 2004).

Thus understanding the food patterns around which diets are formed is important for meal planning and nutritional counseling. Cross-sectionally, dietary intake rich in whole-grain foods have been linked to a lower prevalence of metabolic syndrome (Sahyoun *et al*., 2006; McKeown *et al*., 2004; Esmaillzadeh *et al*., 2005). Dairy intake has been inversely associated with metabolic syndrome (Azadbakht *et al*., 2005; Mennen *et al*., 2000; Pereira *et al*., 2002). Greater intakes of fruit and vegetables have been associated with a lower prevalence of metabolic syndrome (Esmaillzadeh *et al*., 2006). No association has been found between metabolic syndrome and intakes of meat and fish (Mennen *et al*., 2000).

In cross-sectional dietary pattern analysis, a greater prevalence of MS was found among consumers of empty calorie dietary patterns, whereas a lower prevalence was found among those consuming a healthy dietary pattern (Esmaillzadeh *et al*., 2007; Sonnenberg *et al*., 2005).

There are no clear recommendations regarding dietary guidelines for the prevention of metabolic syndrome in persons at risk. The present study will help to evaluate the relationship between dietary intake and the risk of developing MS. Cluster analysis offers advantages over the alternative quantitative approaches as it aims to identify distinct, relatively homogeneous groups based upon selected attributes (the dietary variables) (Hu, 2002).

The aim of the present study is to identify dietary patterns within a general population sample of urban Pakistani subjects. We also aim to report the associations between dietary patterns and prevalence of metabolic syndrome which is a precursor for the development of Cardiovascular Disease (CVD) and glucose intolerance.

MATERIALS AND METHODS

The survey was conducted from July 2004 to December 2004 over a period of 6 months. The Lyari Town Geographical Information System (GIS) was used in this survey which ascribed unique identification numbers to 85,520 households in Lyari, where the study on prevalence of metabolic syndrome amongst selected households was undertaken (Hydrie *et al*., 2009). The ethical approval for the Lyari survey was given by the Institutional Review Board (IRB) of Baqai Institute of Diabetology and Endocrinology. The survey activities were divided into two phases, the household interview based on questionnaire and blood sample collection. The questionnaire included demographical details, diet and physical activity questions and anthropometric measurements.

Around 532 households were randomly selected through the GIS software and maps. All adults older than 25 years were invited to participate after providing signed consent. By following this procedure, a total of 871 persons were approached, out of which 867 persons participated in the survey (response rate: 99.5%). These people were interviewed by the field teams and their anthropometric measures taken. Of these, 363 persons gave blood samples, producing a response rate of 42% for blood collection.

Anthropometry: Weight, height, waist, and hip circumference were measured with the subjects in standing position wearing light clothes and no shoes. The weight was taken to the nearest 0.1 kg by a digital bathroom scale and height was taken to the 0.1 cm. Body Mass Index (BMI) was calculated as a ratio of weight (kg) to height in meters squared. Waist circumference was measured at the minimum circumference between the lower border of the ribs and iliac crest on the midaxillary line and hip circumference was measured at the greatest protrusion of the buttocks just below the iliac crest The measurements were taken in centimeters and the Waist-to-Hip Ratio (WHR) was calculated as waist/hip circumference. Blood pressure was measured twice by using a mercury sphygmomanometer, with individuals requested to sit for 10 min before measuring the blood pressure as a special precaution to minimize blood pressure variations and a mean value taken for the final measurements.

Laboratory assays: All subjects were asked to undertake an 8 h fast for blood tests (fasting blood glucose and lipid profile) that were collected at home on weekends (Hydrie *et al*., 2009). All selected parameters of blood lipids (total cholesterol, triglycerides, High Density Lipoprotein Cholesterol [HDL-C] and Low-Density Cholesterol [LDL-C]) and blood glucose estimation were performed using a Vitalab Selectra autoanalyzer. Fasting blood glucose and lipid profiles were done by the glucose oxidase GOD PAP method and cholesterol CHOD PAP method, respectively.

Criteria for metabolic syndrome: Diagnostic criteria for the metabolic syndrome were taken from the American Heart Association (AHA)/National Heart, Lung and Blood Institute (NHLBI) (Table 1) (Grundy *et al*., 2005).

Table 1: AHA/NHLBI diagnostic criteria for metabolic syndrome

Measure (any three of the five criteria below constitute a diagnosis of metabolic syndrome)	Categorical cut points
Elevated waist circumference	U.S. population: ≥102 cm in men, ≥88 cm in women; lower cut points for insulin-resistant individuals or ethnic groups. For South Asians: ≥90 cm in men, ≥80 cm in women
Elevated triglycerides	≥150 mg/dl (1.7 mmol/l) or on drug treatment for elevated triglycerides
Reduced HDL cholesterol	<40 mg/dl (1.03 mmol/l) in men, <50 mg/dl (1.29 mmol/l) in women
Elevated blood pressure	≥130 mmHg systolic blood pressure or ≥85 mmHg diastolic blood pressure or on drug treatment for hypertension
Raised fasting glucose	Fasting plasma glucose ≥100 mg/dl (5.6 mmol/l) or previously diagnosed type 2 diabetes

Dietary data: Dietary consumption was assessed by a 33 food items interviewer-administered semi quantitative food-frequency questionnaire. The food items were categorized into 6 major food groups: Dairy, meat, fat and sweet, cereals, vegetables and fruits groups. Out of the 363 subjects assessed for metabolic syndrome 362 completed the food-frequency questionnaire.

Statistical analysis: We used cluster analysis to identify dietary patterns and to segregate subjects based on the similarity of diet. We chose food variables because we wanted to identify food patterns clusters. K-means cluster analysis was used to define clusters of subjects using the cluster analysis option in SPSS. This procedure attempts to identify relatively homogeneous groups of cases based on selected characteristics.

In K-means cluster analysis, the homogeneity of cases within a cluster is measured by the total within-cluster sum of squares. Cluster memberships are determined by sequentially moving cases from one cluster to another so that the total within-cluster sum of squares is minimized.

The algorithm requires the number of clusters to be specified prior to analysis. It is possible to identify seeds using information derived from previous research.

Five clusters were defined. We investigated metabolic syndrome prevalence for each cluster and compared the dietary patterns of the clusters with the lowest and highest prevalence of metabolic syndrome.

RESULTS

We identified five distinct groups in this population on the basis of cluster analyses. A total of 75 participants (20.7%) were in cluster 1, 71 (19.6%) in cluster 2, 64 (17.8%) in cluster 3, 85 (23.5%) in cluster 4 and 67 (18.5%) in cluster 5. Frequency of consumption of each food group in all the clusters is shown in Table 2.

Analyzing for proportion of subjects with metabolic syndrome in each cluster it was observed that cluster 1 had the lowest proportion of persons with metabolic syndrome while cluster 2 had the highest percentage of metabolic syndrome subjects (42.7% vs. 56.3%) with a p value of 0.09 compared to the other clusters as shown in Fig. 1.

Table 2: Frequency of consumption of food groups in clusters (%)

	Clusters				
	1	2	3	4	5
Milk group	24	69	32	57	29
Meat group	35	79	61	61	56
Fat group	13	70	20	42	44
Cereal group	76	91	90	92	81
Vegetables group	72	94	83	93	82
Fruit group	34	74	45	59	46

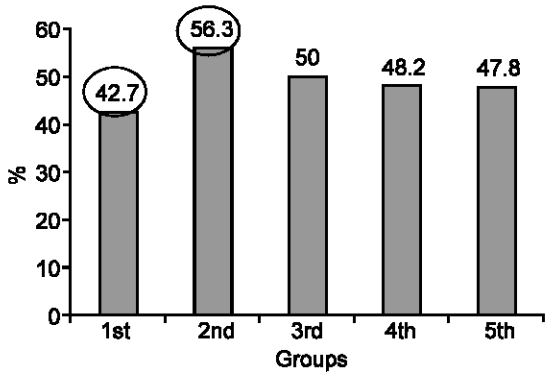

Fig. 1: Metabolic syndrome in five clusters according to modified ATP III definition

Comparing the food items in milk group it was observed that the consumption in cluster 2 (high risk group) was twice compared to cluster 1 (low risk group), the greatest consumption was in cream\custard (7.6 times) and ice cream\sweet lassi (5 times) as shown in Fig. 2.

In meat group the consumption of red meat, organ meat, prawns and eggs in cluster 2 was 3-5 times compared to cluster 1 as shown in Fig. 3.

There was five times increased consumption of the sweet and fat group in cluster 2 compared to cluster 1 as shown in Fig. 4.

In the cereal group there was not much difference in the consumption of legumes and fried rice in both the clusters but around 1.6 times more consumption of naans (refined grain) was seen in cluster 2 compared to cluster 1 (Fig. 5).

In the vegetable group there was also not much difference in the consumption of cooked vegetables and

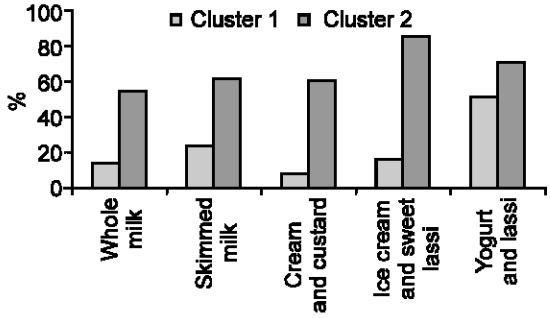

Fig. 2: Comparison of groups with regards to milk group

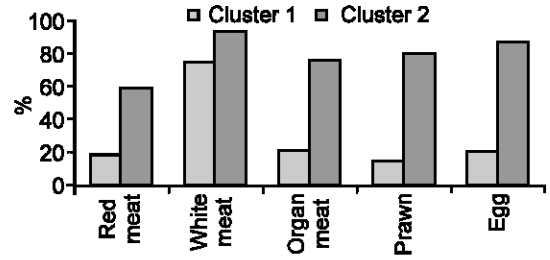

Fig. 3: Comparison of groups with regards to meat group

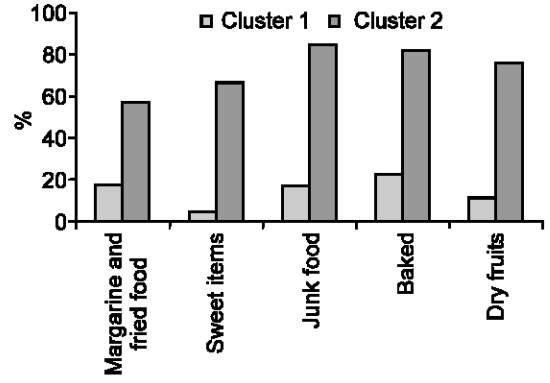

Fig. 4: Comparison of groups with regards to fat and sweet group

cooked potatoes in both clusters but the consumption of raw vegetables was almost double in cluster 2 compared to cluster 1 (Fig. 6).

In the fruit group both clusters showed high consumption of fruits but 8 times more consumption of fruit juices was seen in cluster 2 compared to cluster 1 as shown in Fig. 7.

DISCUSSION

Metabolic Syndrome (MS) has been identified as a precursor of predicting future disease and understanding how MS can be influenced by overall dietary pattern as an entity is valuable.

No individual dietary component is wholly responsible for the association of diet with metabolic syndrome and its components. Rather it is the interaction between different components of diet as well as the consumption of different food items which contribute to the risk for metabolic syndrome. Thus overall dietary trends needs to be observed as individuals consume a mixture of different food items in a single meal, rather than isolated groups.

To our knowledge, this is the first investigation to look into major dietary patterns and its association with the metabolic syndrome by cluster analysis.

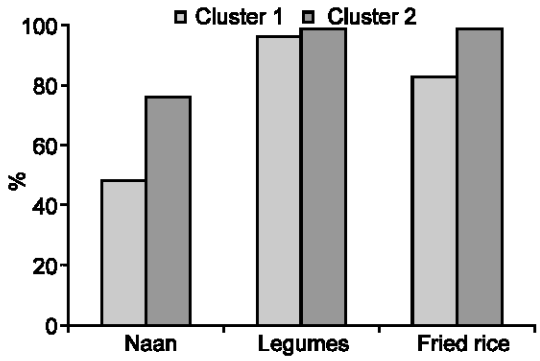

Fig. 5: Comparison of groups with regards to cereal group

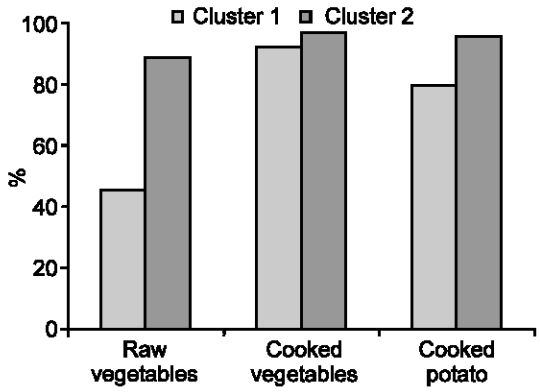

Fig. 6: Comparison of groups with regards to vegetable group

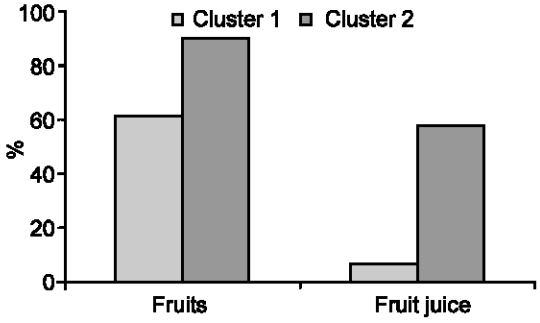

Fig. 7: Comparison of groups with regards to fruit group

In this study low MS risk group (cluster 1) had lowest consumption of all the food groups while the high MS risk group (cluster 2) had highest consumption in most of the food groups. This high food consumption may also contribute to the high prevalence of MS as seen in cluster 2.

Looking at the food groups individually it appears that the food items which were the most energy-dense had the highest consumption in cluster 2 and this probably had the most influence in creating an unhealthy dietary pattern which may lead to increased prevalence of MS.

It has been observed in other studies that the consumption of traditional food (low in saturated fat, low in simple sugars and high in fibre) has declined recently and energy-dense food (high in calories, carbohydrates and saturated fats and low in fibre) and non-traditional energy-dense fast food are being increasingly consumed in South Asia (Misra *et al*., 2009; Misra and Khurana, 2008).

Studies have shown that South Asians have a high consumption of dairy products and sugar compared to other populations (Misra *et al*., 2009; Popkin, 2001). Although dairy consumption has been inversely related to MS in some studies (Azadbakht *et al*., 2005; Mennen *et al*., 2000; Pereira *et al*., 2002) more than twice dairy consumption was seen in the high risk group. Looking further at the individual food items in the milk group it was observed that the highest consumption was in cream\custard and ice cream\sweet lassi; items which have a high fat and sugar content. Coincidently a high intake of fat, milk products and sugars in various regions in India has also shown to be associated with increased cardiovascular mortality (Gupta *et al*., 2006). Thus a combination of dairy products, with high fat and sugars may influence the individual properties of the food and produce a positive association with metabolic syndrome. In our study these factors probably made dairy consumption lose its protective effect in our subjects as documented elsewhere.

Red meat, organ meat and prawns from the meat group were consumed 3-5 times more in cluster 2 compared to cluster 1. All of these food items are known to be high in saturated fat, which has been adversely associated with cholesterol (Schaefer, 2002), blood pressure (Appel *et al*., 2006), obesity and diabetes risk (Parillo and Riccardi, 2004).

Similarly all the food items in fat and sweet group were consumed five times more in cluster 2 compared to cluster 1. Sweet products were consumed at an alarming 13 times more in cluster 2 and they probably influenced the increased prevalence of MS in cluster 2 with their load of empty calories in the diet.

South Asians consume more carbohydrates compared to Europeans and this may lead to hyperinsulinemia, postprandial hyperglycemia, hypertriglyceridemia and low HDL cholesterol levels, all of which is probably due to insulin resistance (Burden *et al*., 1994). Processed cereals, such as refined grains have been shown to be associated with an increased risk of the components of the metabolic syndrome in The Malmö Diet and Cancer Study (Wirfalt *et al*., 2001). Similarly in our study refined grains were consumed nearly twice in the high MS risk group (cluster 2).

Almost double consumption of raw vegetables was seen in cluster 2 compared to cluster 1. Similarly the overall double consumption of the fruit group was seen in cluster 2. An inverse association between prevalent MS and intakes of fruit and vegetables has been reported previously (Esmaillzadeh *et al*., 2006). Also consumption of diets high in fruit and vegetables has been associated with lower blood pressure (Appel *et al*., 2006) and a better lipid profile (Lichtenstein *et al*., 2006). Looking at the individual food items in the fruit group it was observed that the consumption of fruit juices which accounts to empty calories was 8 times more in cluster 2 compared to cluster 1. As mentioned earlier empty calories in diet may lead to increased prevalence of MS; the increased consumption of fruit juices probably undermined the protective effect which vegetables and fruits may have in cluster 2.

In summary the dietary pattern in cluster 2 was loaded with both healthy (milk, legumes, vegetables and fruits) and unhealthy (refined grains, potatoes, meat and meat products, high fat dairy products, snacks, sweet items and fruit juices) foods. Although the healthy foods have been reported to be protective against the metabolic syndrome, the cluster's unhealthy diet constituents have adverse effects on metabolic markers which may lead to increased prevalence of MS.

A limitation to consider in the interpretation of our results is the use of an FFQ containing only 33 items, thus restricting the number of food items needed to characterize usual dietary intake. Furthermore, for some food groups such as dry fruits, low consumption and a narrow range of values among consumers may have prevented us from detecting a relationship if one was present. Moreover, reporting biases may have occurred. Although we acknowledge these limitations, other studies have indicated that there is reasonable validity and reliability of food groups and major dietary patterns obtained from FFQs.

Another limitation of our study is its cross-sectional nature. Thus, the association observed between these dietary patterns and the metabolic syndrome needs to be confirmed in prospective analyses. Furthermore we cannot generalize our findings to Pakistani populations, since only one area within an urban city was used for the sample population.

However, participants in the current study reflected almost all major ethnic groups of Pakistan so that a broad range of dietary habits were represented. Most previous studies relating MS to diet have focused on a

single food group. Thus, a major strength of our study is that all six major food groups have been covered in the FFQ.

Thus we need to further explore the development of a method which accurately measures an individual's overall diet quality and quantity and this is a prerequisite for further research regarding the relationship between diet and metabolic syndrome. Further research is required in larger prospective populations to be able to validate the findings of this study and improve our understanding of the association of diet with MS.

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Blood Progestrone Level of Intensively Managed Savanna Brown Does Fed Maize Bran and Protein Concentrate Diet

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Abstract: The effect of feeding Maize Bran and 10% crude protein concentrate diet on the blood progesterone level of intensively managed savanna brown doe was investigated. Twelve nulliparous savanna brown does with mean body weigh of 8.70±1.12 kg were used for the experiment which lasted for a period of eight weeks. The animals were randomly assigned into two groups (A and B) with a replicate each. Does in treatment A were fed diets containing 10% CP while those in treatment B had Maize Bran. Blood progesterone level were monitored 18 days prior to and 30 days following oestrus. It was observed that levels of progesterone in both groups were low prior to oestrus and mating but increased following oestrus and mating. The rate of increase was slightly higher in the group that had 10% CP diet particularly between day 21 and 27 post oestrus. Even though level of hormone in the serum of does in both groups did not differ significantly ($p>0.05$). It was concluded that protein have an effect on the level of circulating progesterone in the blood of Savanna Brown does. Therefore incorporation of protein into the ration of Savanna Brown does is highly recommended.

Key words: Blood progesterone, intensive, savanna brown does, maize bran and concentrate

INTRODUCTION

The role of protein in the reproductive performance of most domestic animals particularly ruminants cannot be overemphasized. The inherent property of the biochemical breakdown of protein allows it participate in the precursor of most reproductive hormones particularly progesterone (Sonderman *et al*., 1987).

In the reproductive organ, particularly uterus, a progesterone binding receptor site is present. This progesterone receptor can be induced by oestrogen since inhibitors of Ribonucleic Acid (RNA) and protein synthesis administered 15 min before the hormone prevented its effect. An assertion of the stimulation of the uterine progesterone binding activities may account for the synergistic action of oestradiol and progesterone on the uterus (Carroll *et al*., 1990).

The need to ensure sufficient amount of protein in the ration of ruminant become inherent. Most nutritional materials have ingredients which could be precursor or even elicit effect that conventionally known reproduction hormone (Sonderman and Larson, 1989). Therefore, this study was conceived to evaluate the effect of feeding Maize Bran and a 10% CP concentrate diet on the level of blood progesterone in Savanna Brown does.

MATERIALS AND METHODS

Twelve Savanna Brown does with mean body weight of 8.70±1.12 kg were used to determine the effect of feed type on the level of progesterone in the blood. The animals were allowed a pre-treatment period of two weeks to enable them acclimatize following which they were randomly divided into two groups with a replicate each. Does were allowed to run with buck throughout the study period of eight weeks.

Animals in the treatment group were given compound protein concentrate feed containing 10% CP while those in the control group had Maize Bran (Table 1). Mango leaves at the rate of 1.5 kg per group per day was fed as supplement to the animals throughout the study period. Water and salt lick were also supplied ad-libitum.

10 mls of blood was collected from the jugular vein of does every other day (18 days) prior to and 30 days following oestrus in clean plastic test tubes which were immediately transferred into test tube rack placed in an ice chest. Blood samples were later taken to the laboratory and the serum was separated from the plasma by centrifugation at 5,000 revolutions per minute for ten minutes. This was done in order to minimize enzymatic progesterone degradation. The serum collected was stored at a temperature of -20^{o}C until ready for radio-immunoassay.

Data generated from this study were subjected to t-test using a computer package "minitab" for window release 9.2". Graph of progesterone profile was plotted using Microsoft Excel.

RESULTS AND DISCUSSION

The composition of experimental diets and the proximate composition of diets are presented in Table 1 and 2 while the effect of type of diet on the serum progesterone profile of Savanna Brown does is presented in Fig. 1 and Table 3.

Table 1: Composition of concentrate portion of experimental diet

	Composition	
Ingredient	T	C
Maize	67.03	Maize bran
R. celusk	24.00	-
Soya bean	6.38	-
Blood meal	1.59	-
Premix	0.50	-
Salt	0.50	-
Total	100.00	100.00
Calculated CP (%)	11.06	-

Maize bran not compounded, supplemented with salt lick,
T = Treatment; C = Control

Table 2: Proximate composition of experimental diet

Component %	T	C	ML
Dry matter	90.70	84.07	48.42
Crude protein	10.83	7.10	10.00
Ether extract	5.44	5.00	6.28
Crude fibre	6.80	9.93	21.30
Ash	7.48	7.50	13.37
Nitrogen free extract	60.15	70.47	50.04

T = Treatment group (concentrate); C = Control (maize bran);
ML = Mango Leave

Serum progesterone concentrate (ng/ml) were similar for both groups from day 18 to day 6 prior to oestrus. On day 3 after oestrus, this trend was remarkably altered as does on 10% CP diet recorded very sharp increase in the level of serum progesterone. This declined appreciably between the 12th and 15th day post oestrus and rose again to about twice the level of hormone in the Maize Bran fed group from day 18-30 following oestrus. However, the differences in hormone level were not statistically significant ($p>0.05$).

The generally low level of progesterone in serum prior to oestrus and mating is a normal phenomenon in the oestrus cycle (McElroy *et al*., 1995). The author noted that during the pro-oestrus phase, growths of follicles are generally controlled by oestrogen. Following oestrus the effect of both diets became obvious. The level of progesterone in circulation increased and ranged from 0.50 at oestrus to a peak of 5.50 ng/ml in the group that had Maize Bran and 0.20 at oestrus to a peak of 9.10 ng/ml in the group that had the compound concentrate feed. However, this did not differ significantly ($p>0.05$) among both groups.

Although Maize Bran appear to be inferior in CP than the compound concentrate feed, Shiawoya and Mohammed (1999) observed that the crude protein content of Maize Bran obtained from Minna was above the minimum of 8% CP (9.48±0.13) required by the rumen microbial population to function efficiently and also above the average requirement, in Nigeria for maintenance of small ruminants (8.9%) hence its ability, to compare favourably with the compound protein diet.

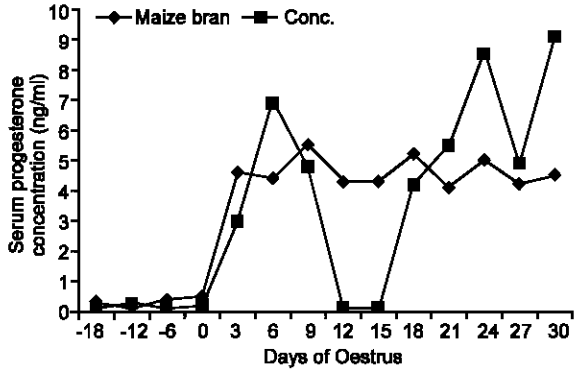

Fig. 1: Progesterone concentration of serum obtained from savanna brown does fed maize bran and 10% CP concentrate diet

Table 3: Progesterone concentration of serum obtained from savanna brown does fed maize bran and 10% CP concentrate diet

Day of oestrus	Serum progesterone A (Maize bran)	Concentration (mg/ml) B (10% Cp concentrate)
-18	0.30	0.10
-12	0.10	0.30
-6	0.40	0.10
0	0.50	0.20
3	4.60	3.00
6	4.40	6.90
9	5.50	4.80
12	4.30	0.10
15	4.30	0.10
18	5.20	4.20
21	4.10	5.50
24	5.00	8.50
27	4.20	4.90
30	4.50	9.10

0-Day of Oestrus

The level of protein in both diets helped to trigger oestrus which was immediately followed by mating in both groups. This was more pronounced in the group that had 10% CP in their diet. The progesterone profiles appear to confirm pregnancy since the levels were maintained beyond the normal duration of oestrus cycle.

Conclusion and recommendations: Results obtained from this study show that the level of protein has an effect on the level of progesterone in the blood of Savanna Brown does. Therefore, incorporation of protein into the ration of Savanna Brown does is recommended.

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