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Effect of Storage Moulds on the Nutritional Quality of Kolanuts in Nigeria

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Abstract: Proximate analysis of healthy and infested kolanuts (*Cola nitida* and *C. acuminata*) was carried out at the Cocoa Research Institute of Nigeria, Ibadan, Nigeria, to determine the effect of mould attack on the nutritive value of stored kolanuts. The storage moulds encountered on both species of kolanuts in Nigeria were *Lasiodiplodia theobromae, Fusarium pallidoroseum, F. moniliforme, F. cavispermum, Aspergillus niger, A. fumigatus, A. flavus,* and *Paecilomyces variotii*. Proximate analysis of healthy and infested *C. nitida* revealed 0.79% and 1.29% soluble sugar in healthy and infected kolanuts respectively. The healthy *Cola acuminata* has 54% moisture content compared to 23.24% in the infested nuts. The percentage of crude protein in healthy nuts was 4.55% but it was 2.1% in the infested nuts. Macro elements of the kolanuts indicated significant differences in calcium, potassium, magnesium and phosphorus between healthy and infested nuts. There was an increase in caffeine content of diseased *C. nitida* and a significant decrease (p=0.05) in percentage protein and moisture content in such infested kolanuts. The activities of the isolated mould are implicated in the deterioration of these biochemical substances in kolanuts.

Key words: Storage mould, kolanuts, deterioration, nutritive value

INTRODUCTION

Cola popularly called kolanut is indigenous to the tropical rain forest of West Africa, West Indies, Brazil and Java (Rusell, 1955). *Cola nitida* and *C. acuminata* are the most common *Cola* species and *Cola* of commercial value in Nigeria. The effects of chewing the odourless nut with an astringent taste have enhanced its continued use for about 1000 years now in Nigeria (Agatha *et. al*., 1978). The habit if not comparable to the tobacco smoking of Western civilization or opium usage of far Eastern societies certainly shows similarity to the Betel nut chewing of Asiatic communities (Agatha *et al*., 1978). Outside West Africa, cultivation and trade in kolanut have been established in countries like the Caribbean Islands, Mauritius, Sri-Lanka and Malaysia (Oladokun, 1982).

A few hundreds of tonnes of dried kolanuts are exported annually from West Africa to North America and Europe. These are used for the preparation of beverages (e.g. kola chocolate) and pharmaceutical products (e.g. laxatives). The presence of alkaloids and other chemicals in kolanuts such as caffeine, kolanin and theobromine also make them suitable for use in drug preparations (Anon, 1985; Olunloyo, 1979). The geographical and chronological spread in trade and use of kola has inevitably created a high demand for kolanut far in excess of its production.

Storage of kolanuts has always been a laborious and delicate task. For now, kola traders are still using traditional methods of storage. Much of the produce is chewed in its fresh state even though the bulk of the nut is harvested once in a year.

Preserving the freshness of the nuts for many months without storage moulds is therefore a problem, which farmers and traders seek to solve.

A loss of up to 50% per tonne due to storage moulds may be recorded in poorly stored nuts (Olunloyo, personal communication). Kolanut traders often control spoilage by removing infested nuts at intervals during the storage period and throw them away. Thus, the specific objective of this present study was to evaluate the nutritional quality of stored kolanuts through proximate analysis with view of finding alternative uses for infested kolanuts.

MATERIALS AND METHODS

Healthy and infested kolanuts (*Cola nitida* and *C. acuminata*) collected randomly during processing and storage from various locations in Nigeria were used in this study. The culture media used for the fungal isolations and counts were potato dextrose agar (PDA) (Difco, Detroit, USA). The media were sterilized by autoclaving for 15 minutes at 1.05kg/cm^2 (121°C).

Analysis: Healthy and infested kolanut samples were assayed for the percentage moisture content using the oven-dried weight method. Fresh samples of kolanut were dried to constant weight in an air oven at 105°C for 15 hours and the percentage moisture content was calculated as described by Agrawal (1980); AOAC (1990).

The ash content of healthy and infested kolanuts was determined using the method described by Osborne and Voogt, (1978). Fresh samples (10g) of kolanut

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Table 1: Fungal species isolated from *C. nitida* and *C. acuminata*

Genus	*C. nitida*	*C .acuminata*
L. theobromae	+	-
F. pallidoroseum	+	+
F. moniliforme	+	+
F. cavispermum	+	-
A. flavus	+	+
A. niger	+	+
A. fumigatus	-	+
P. chrysogenun	+	+
P. variotii	+	+
Chlamydomyces sp	+	-
Curvularia sp	+	-
M. spinosus	+	+

Table 2: Proximate analysis of healthy and infested *C. nitida* samples

	Biochemical Composition (%)	
	Healthy nut	Infested nut
Moisture content	62.3±1.973	35.6±2.254
Crude Protein	1.36±0.03	0.37±0.02
Fat	0.77±0.10	0.74±0.05
Caffeine	2.1±0.161	2.52±0.002
Sugar	0.79±0.015	1.29±0.075
Ash	1.57±0.153	4.96±0.404

Values were expressed on a dry weight basis as the means ± s.d of 3 determinations.

Table 3: Proximate analysis of healthy and infested C. *acuminata* samples

	Biochemical Composition (%)	
	Healthy nut	Infested nut
Moisture content	54±2.30	23.24±1.23
Crude Protein	4.55±0.05	2.10±1.02
Fat	2.39±0.10	1.17±0.05
Caffeine	0.023±0.001	0.009±0.002
Fibre	11.47±2.015	6.13±1.75
Ash	3.43±0.4.53	1.92±0.340

Values were expressed on a dry weight basis as the means ± s.d of 3 determinations.

were put in pre-weighed crucibles and later in a furnace. The organic matter was burnt off and the inorganic material remaining was cooled and weighed. Heating was carried out in stages, first to drive off the water, then to char the nut samples thoroughly and finally to ash at 600°C in a furnace. The percentage ash content was calculated as described by A.O.A.C. (1990).

The Kjeldahl procedure was used for the determination of the total nitrogen in both healthy and infested nut samples. The amount of crude protein contained in the samples was obtained described by Osborne and Voogt, (1978); Tel and Hargathy, (1984).

The method of extraction of fat of *C. nitida* and *C. acuminata* was adopted from fat extraction methods of Egan *et al*. (1981). The kolanuts were ground using a ceramic pestle and mortar before blending in an electric Philips kitchen blender. An amount of 20g of the ground kolanut was packed into the extraction thimble before covering with a small ball of cotton wool. The thimble was inserted in a quick fit plain body Soxhlet extractor. Petroleum ether in the quantity of 200ml (60-80°C) was poured in a 250ml round-bottom flask of known weight, which was connected to the extractor, and refluxed on an electric thermal heater for 5 hours. The quantity of the fat extracted from the nuts was determined as described by Adekunle and Badejo (2002).

The extractable caffeine in both healthy and infected kolanuts were determined according to A0AC (1990). Samples comprising both healthy and infested kolanuts were analyzed for the calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), and phosphorus (P). The total amount of these minerals contained in a sample was determined by burning away the organic matter. Burning was carried out at a low temperature in order to avoid any loss of volatile mineral content. The individual mineral content of the kolanut sample was then determined as described by Tel and Hagarty (1984) using atomic absorption spectrometer.

RESULTS

Twelve storage rot fungi were isolated from each of infested *Cola nitida* and *Cola acuminata*. The fungi isolated are *L. theobromae, F. pallidoroseum, F. moniliforme, F. cavespermum, Curvularia sp., A. fumigatus, P. chrysogenum, F. cavispermum, M. spinosus, A. flavus, P. variotii, Chlamydomyces sp.* (Table 1).

The results of proximate analysis for both healthy and infested *C. nitida* and *C. acuminata* are shown in Table 2 and Table 3 respectively. The healthy kolanuts has 62.3% moisture content compared to 35.5% in the infested nuts (Table 2). The percentage caffeine was 2.1% in healthy nuts but it was 2.52% in infested nuts. Similarly, the level of sugar in the infected nuts was 1.29% whereas it was 0.79% in healthy nuts.

The percentage of crude protein in healthy nuts was 1.36% but it was 0.37% in the infested nuts. There was a significant difference (P = 0.05) in the values obtained for moisture content, crude protein and ash in both healthy and infected kolanuts.

No significant difference was recorded for values obtained for caffeine and fat contents. Perhaps the storage moulds could not easily degrade the caffeine and fat content of the infested nuts. From the results shown in Table 2, invasion of healthy kolanut by storage moulds generally affect the nutritive quality of stored kolanuts.

The mineral analysis of the healthy and infested *C. nitida* and *C. acuminata* nuts is shown in Table 4. There were variations in the mineral contents of the two species of *Cola*. The calcium content in *C. nitida* was estimated to be 0.69% and 0.417% for the healthy and infested nuts respectively. A similar trend of 0.731% and 0.562% respectively was recorded in *C. acuminata* for both healthy and infected nuts.

Table 4: Mineral Composition of both healthy and infected kolanuts

	C. *nitida*		C. *acuminata*	
Minerals	Healthy	Infested	Healthy	Infested
Copper (Cu)	0.04±0.01	0.02±0.004	0.13±0.18	0.03±0.01
Iron (Fe)	0.06±0.05	0.08±0.004	0.13±0.09	0.06±0.01
Potassium (K)	2.25±1.23	0.49±0.03	1.85±0.43	0.91±0.01
Magnesium (Mg)	0.29±0.01	0.24±0.026	0.41±0.21	0.44±0.01
Manganese (Mn)	0.02±0.01	0.05±0.004	0.05±0.02	0.05±0.01
Sodium (Na)	0.36+0.098	0.14±0.03	0.33±0.12	0.26±0.001
Phosphorus (P)	0.85±0.16	0.57±0.03	1.22±0.28	0.81±0.01
Zinc (Zn)	0.04±0.06	0.07±0.004	0.01±0.001	0.01±0.06

Values were the means ± S.D of the 3 determinations.

The mineral content in the two species of *Cola* is generally high and well distributed. However the mineral composition varied according to species (Table 4). The values obtained for calcium, potassium, phosphorus, magnesium and sodium in healthy *C. nitida* were higher than those obtained in infested nuts. However, potassium is highly concentrated in the healthy nuts of both *C. nitida and C. acuminata*.

The iron content was found to be lower in healthy kolanuts of *C. nitida* when compared with the content in the infested kolanuts. Similar trend was also observed for manganese and zinc (Table 4). However, the value obtained for copper was higher in healthy both *C. nitida* and *C. acuminata* than the value obtained in infected nuts.

A general view of the results revealed that the values obtained for copper, manganese and zinc for both *C. nitida and C. acuminata* were generally low. It is evident from the results presented in Table 4 that healthy kolanuts is rich in major elements such as calcium, potassium, phosphorus, sodium and magnesium. The activities of the storage moulds were implicated in the depletion of these major elements in the stored kolanuts.

DISCUSSION

Neegaard (1977) reported that seed-borne fungi are responsible for deterioration of food reserves in the seeds. As discovered in this study, the results of the proximate analysis of both healthy and infected kolanuts established the deterioration of food reserves in the infected kolanuts when compared to healthy nuts.

Similarly, effects of the storage moulds were also established in the mineral composition of healthy and infected *Cola nitida* and *Cola acuminata*. Reduction in carbohydrate level has been reported in several diseases of other crops (Sempico, 1959). Olofinboba (1968) found that *Botryodiplodia theobromae* cause great depletion in carbohydrate present in *Antiaris africana*; Ogundana *et al.* (1975) found that carbohydrate, protein and lipids were reduced in sweet potatoes infected by *Lasiodiplodia theobromae*. Stavely and Chaplin (1972) observed that tobacco leaves heavily infected by *Cercospora nicotianea* averaged 46% less reducing sugars and 6.5% more total nitrogen than non-infected leaves.

The present study demonstrated the changes due to infection by *L. theobromae and F. pallidoroseum* on the food content of kolanut in Nigeria. There was a decrease of 42.9% in moisture content on the infected nuts compared with healthy nuts. Similarly, there was a decrease of 54.4% in crude protein and fat contents in the infected nut when compared with the healthy nuts.

The results of the present studies corroborate the reports of other workers. Sharma and Bhowmik (1987) reported that fungal infection caused both quantitative and qualitative damages to groundnut (*Arachis hypogea*). Furthermore, the diseased kernels had lower oil percentage but higher ash content. Singh *et al.*, (1972) reported reduction in oil content of sesame (*Sesamum indicum*) seeds infected with *Macrophomina phaseolina*. Also, Lalithakumari *et al.* (1971) reported that groundnut seed-infecting fungi like *Aspergillus flavus*, *Cladosporium herbarum* and *Lasidiplodia* sp. caused alteration in the oil colour.

The present studies clearly showed increase in the content of reducing sugar in the diseased kolanut when compared to healthy ones. This may be due to the susceptibility of cellulose to enzymatic degradation of invading fungi. The large cellulose molecules are hydrolyzed by microbial enzymes into glucose and utilized by the degrading microorganisms (Siu and Reese, 1953; Whitaker, 1957).

Similarly caffeine and ash contents were found to be higher in infected nuts when compared to the healthy nuts. One explanation for this increase in caffeine content could be attributed to the net loss of the other components besides the moisture content. That the protein content of the healthy nut was higher than that of the diseased nuts, suggest the utilization of the nitrogen in the protein component by the pathogenic fungi. Conversely the total sugar in the infected nuts was higher that the quantity recorded in the healthy nuts. One explanation is that the plant, do store their carbohydrate in form of starch. Again, the fungi are well known to have

the ability to hydrolyze starch into reducing sugar. Consequently, higher value was recorded for sugar in the diseased nut. Previous works in support of this observation include those of Sharma and Bhowmik (1987). The values of protein contained in plant tissue may vary in terms of nitrogen content – from 13 percent to 18 percent Tel and Hagarthy (1984). Thus the 1.36% of protein content found in kolanut is very low when compared with 5.4% for oil seed protein, 5.9% for cereal protein and 6.6% for plant leaf protein as reported by Tel and Hagarthy (1984).

Studies have shown that the major elements such as calcium, potassium, phosphorus, sodium, magnesium and iron were well distributed in both *C. nitida* and *C. acuminata* nuts. However, the values obtained for calcium, potassium, phosphorus, magnesium and sodium in healthy C. *nitida* was higher than the values obtained in infested nuts. Conversely, the amount of copper, iron, manganese, and zinc were lower in healthy nuts than in the infested nuts. Thus, this study concluded on the note that storage moulds depreciate the quality of *C. nitida* and *C. acuminata*.

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Response of Broiler Chickens to Palm Kernel Cake and Maize Offal Mixed in Different Ratios

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Abstract: A total of one hundred and twenty (120) unsexed broiler chickens of 5 days of age averaging 59.50±1.00 grams were randomly allocated into 4 dietary treatments designated I, II, III and IV to evaluate the effect of different ratios of palm kernel cake and maize offal mixed in the ratios of 1:1, 3:1 and 4:1 for treatments II, III and IV respectively and these were compared with the control diet at the starter and finishing phases. Each treatment was replicated 2 times. The results at the starter phase show that there were no significant differences ($p>0.05$) in feed intake and efficiency of feed utilization among birds fed all diets. There were also no significant differences ($p>0.05$) in feed cost per unit weight gain among birds fed the control diet and those fed the diets which contained 1:1 and 4:1 ratio of palm kernel cake and maize pap offal. However, the results at the finishing phase show that the diet which contained 3:1 ratio of palm kernel cake and maize pap offal significantly increased ($p<0.05$) feed intake while the diets which contained the ratio of palm kernel cake and maize pap offal of 1:1 and 3:1 significantly ($p<0.05$) supported better weight gain than other diets. Efficiency of feed utilization and feed cost per unit weight gain were however significantly ($p>0.05$) influenced by the dietary treatments. On the basis of the results obtained, it may be recommended that a ratio of 1:1 PKC and maize pap offal maybe fed to both starter and finishing broiler chickens without adverse effect on performance.

Key words: Broilers, maize offal, palm kernel cake, performance

INTRODUCTION

Conventional feed ingredients such as maize and soyabeans serve as staple food for Nigerians and also as good source of energy and protein for livestock and constitute about 90% of the total feed ingredients used in making poultry feeds. This has resulted in feed cost for non-ruminant animals such as pigs and poultry to account for between 70 and 80% of the total recurrent cost of production (Osuwari *et al*., 1995). Agro-industrial by-products such as palm kernel cake and maize offal could be used to spare these conventional feed ingredients in poultry diets because of their low pricing and availability.

Palm Kernel Cake (PKC), is a by-product of hydraulic press oil extraction of the endosperm of oil palm fruit (*Elaeis guinensis*, Jacq) and sells for about 8:00-12:00 naira per kg (Afolabi *et al*., 2008). The production is not seasonal as the oil palm tree produces fruits all year round. However, the peak of production falls between the months of March and May when seasonal protein meal sources are scarce and expensive (Aduku *et al*., 1988). Results of analyses of palm kernel cake (Abonyi and Uchendu, 2005) showed that this by-product contain 90.89, 22.84, 4.02, 12.85, 58.06 and 2.23% of dry matter, crude protein, crude fibre, ether extract, Nitrogen-Free Extract (NFE) and ash respectively depending on the efficiency of oil extraction from the kernel (Onwudike, 1986). Sundu *et al*. (2005) have reported that the cake is moderately rich in metabolizable energy of between 1479 and 2260 Kcal/kg. However, the crude protein content of palm kernel cake is lower than that of all protein concentrate feedstuffs. Successful utilization of PKC in diets of broilers, layers and local chicks have been documented severally in literature (Onwudike, 1986; Onifade and Babatunde, 1998; Abonyi and Uchendu, 2005; Ugwuene *et al*., 2005; Sundu *et al*., 2005; Akpodiete, 2007).

According to Aduku (1993), maize offal contain 13.38, 3.43, 2.58, 78.54 and 2.35% of crude protein, crude fibre, ether extract, Nitrogen-Free Extract (NFE) and ash respectively. Vantsawa *et al*. (2007) reported that maize offal (*dusa*) is a by-product obtained by dehulling maize grain containing the testa, aleurone layer, reasonable quantities of broken endosperm and most of the germ of the maize grain using the locally fabricated machines. These workers also reported that large quantities of maize offal are produced in Nigeria since this cereal grain feature prominently in human diets in many parts of Nigeria. This by-product has been successfully fed to finishing broiler chickens at 10% level of inclusion

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(Onuh, 2006), 40% level of inclusion in broiler chick diets in the presence of 200 mg/kg of Roxazyme enzyme (Onu *et al*., 2006), 100% of *dusa* replaced maize in the diets of egg-type chicks (Vantsawa *et al*., 2007) and equal proportions of maize and maize offal (*dusa*) could be fed to pullets (Vantsawa *et al*., 2008) without adverse effect on performance.

There is a paucity of information in literature with respect to mixture of different ratios of PKC and maize offal in the diets of broiler chickens. This is the thrust of the present study whereby palm kernel cake and maize offal were mixed in the ratios of 1:1, 3:1 and 4:1 respectively and then included in broiler starter and finishing broiler diets and these diets were compared with the control diet.

MATERIALS AND METHODS

The palm kernel cake used in the present study was obtained from a commercial agro-service shop that sells livestock feeds, feed ingredients and veterinary drugs and equipment in Gboko town, Benue State while the maize offal was obtained from local processors who mill maize grain for human consumption in Gboko, Benue State.

Experiment 1: A total of one hundred and twenty (120) unsexed broiler chickens of 5 days of age averaging 59.50±1.00 grams were purchased from a commercial vendor and randomly allocated into 4 dietary treatments of 30 birds in each treatment. Each treatment was replicated 2 times with each replicate having 15 birds. Four (4) diets designated I, II, III and IV were formulated for broiler starter chickens such that the palm kernel cake and maize offal were mixed in the ratios of 1:1, 3:1 and 4:1 and then included in rations for treatments II, III and IV respectively and these were compared with the control diet (Table 1). The birds were reared according to standard procedures (Dafwang and Ogundipe, 1982). The chicks were maintained on these diets and water being provided *ad libitum* until they were four weeks old. Records of feed consumed and live weight changes were kept for 28 days.

Experiment 2: At the end of experiment 1, the birds averaging 539.66±13.80 grams were polled together and randomly allocated into 4 dietary treatments designated I, II, III and IV such that palm kernel cake and maize offal were mixed in the ratios of 1:1, 3:1 and 4:1 and then included in rations for treatments II, III and IV respectively and these were compared with the control diet (Table 2). The birds were given feed and water *ad libitum* until they were eight weeks old, observing standard broiler production practice and keeping records of feed consumed and live weight changes for 28 days.

Table 1: Percent composition of broiler starter test diets

	Dietary treatments			
Ingredients	I	II	III	IV
Maize	40.00	41.50	41.50	41.50
FFRSB[a]	50.00	35.00	35.00	35.00
Palm kernel cake	0.00	10.00	15.00	16.00
Maize offal	0.00	10.00	5.00	4.00
Rice offal	6.00	0.00	0.00	0.00
Bone ash	3.00	3.00	3.00	3.00
Common salt (NaCl)	0.25	0.25	0.25	0.25
Mineral-vitamin premix	0.25	0.25	0.25	0.25
DL- Methionine	0.25	0.25	0.25	0.25
L-Lysine HCl	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein (%)	23.02	22.68	22.73	22.74
ME (Kcal/kg)	3070	2996	2972	2968
Crude fibre (%)	4.99	6.23	7.18	7.37
Methionine (%)	0.66	0.66	0.66	0.67
Lysine (%)	1.77	1.70	1.70	1.70
Meth. + Cystine (%)	0.92	0.93	0.93	0.93
Feed cost/kg (₦/kg)	67.94	63.30	63.50	63.54

ME = Metabolizable Energy (Kilocalories per kg of diet);
a = Full-fat roasted soyabean.
The PKC and Maize Pap offal in treatments II, III and IV were mixed in the ratios of 1:1, 3:1 and 4:1 respectively

Table 2: Percent composition of finishing broiler test diets

	Dietary treatments			
Ingredients	I	II	III	IV
Maize	44.50	41.50	41.50	41.50
FFRSB[a]	40.00	35.00	35.00	35.00
Palm kernel cake	0.00	10.00	15.00	16.00
Maize offal	0.00	10.00	5.00	4.00
Rice offal	12.00	0.00	0.00	0.00
Bone ash	2.50	2.50	2.50	2.50
Common salt (NaCl)	0.25	0.25	0.25	0.25
Mineral-vitamin premix	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25	0.25
L-Lysine HCl	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein (%)	20.04	19.21	19.42	19.46
ME (Kcal/kg)	2953	3045	3011	3004
Crude fibre (%)	5.97	5.78	6.76	6.96
Methionine (%)	0.61	0.60	0.61	0.61
Lysine (%)	1.52	1.41	1.42	1.42
Meth. + Cystine (%)	0.85	0.84	0.85	0.85
Feed cost/kg (₦/kg)	63.70	60.32	61.52	61.77

ME = Metabolizable Energy (Kilocalories per kg of diet);
a = Full-fat roasted soyabean.
The PKC and Maize Pap offal in treatments II, III and IV were mixed in the ratios of 1:1, 3:1 and 4:1 respectively

Data analysis: In each experiment, data on each parameter was subjected to the Analysis of Variance (ANOVA) for Completely Randomized Design (CRD) and where significant differences were indicated, the means were separated using Hsu's MCB (Multiple Comparison with the Best) method (Minitab, 1991).

Table 3: Response of broiler starter chickens fed diets in which Palm Kernel Cake (PKC) and maize pap offal are mixed in different ratios

	Dietary treatments				
Parameters	I	II	III	IV	SEM
Average final weight (g)	671.05	566.67	490.00	541.67	-
Initial average weight (g)	60.50	58.50	60.00	60.50	2.84^{NS}
Average daily weight gain	21.81^{a}	18.15^{ab}	15.36^{b}	17.18^{ab}	5.34
Average daily feed intake	52.84	51.73	49.68	50.92	11.77^{NS}
Feed conversion ratio	2.42	2.85	3.23	2.96	1.06^{NS}
Feed cost per unit weight gain (₦/gain)	164.41^{a}	180.41^{a}	205.11^{b}	188.08^{a}	7.74

^{a,b}Means followed by the same superscript in horizontal rows are not significantly different from one another (p>0.05).
SEM = Standard Error of Mean; NS = Not significantly different from one another (p>0.05)

Table 4: Response of finishing broiler chickens fed diets in which Palm Kernel Cake (PKC) and maize pap offal are mixed in different ratios

	Dietary treatments				
Parameters	I	II	III	IV	SEM
Average final weight (g)	1119.57	1490.48	1428.26	1293.75	-
Initial average weight (g)	541.07	553.45	525.86	543.10	-
Average daily weight gain	20.67^{b}	33.47^{a}	32.23^{a}	26.81^{b}	7.07
Average daily feed intake	86.94^{d}	101.46^{b}	106.85^{a}	92.43^{c}	3.41
Feed conversion ratio	4.21	3.03	3.32	3.45	1.99^{NS}
Feed cost per unit weight gain (₦/gain)	268.18	182.77	204.25	213.12	123.11^{NS}

^{a,b}Means followed by the same superscript in horizontal rows are not significantly different from one another (p>0.05).
SEM = Standard Error of Mean; NS = Not significantly different from one another (p>0.05)

RESULTS AND DISCUSSION

The summary of results of the response of broiler starter chickens fed diets in which Palm Kernel Cake (PKC) and maize pap offal are mixed in the ratios of 1:1, 3:1 and 4:1 for dietary treatments II, III and IV respectively and compared with the control diet are presented in Table 3 while the response at the finishing phase are presented in Table 4.

The results at the starter phase show that there were no significant differences (p>0.05) in feed intake and efficiency of feed utilization among birds fed all diets. The results also show that there were no significant differences (p>0.05) in feed cost per unit weight gain among birds fed the control diet and those fed the diets which contained 1:1 and 4:1 ratio of palm kernel cake and maize pap offal.

The results at the finishing phase also show that the diets which contained the ratio of palm kernel cake and maize pap offal of 1:1 and 3:1 significantly (p<0.05) supported better weight gain than other diets. Finally, efficiency of feed utilization and feed cost per unit weight gain were not significantly (p>0.05) influenced by the dietary treatments.

In the present study, increasing levels of palm kernel cake reduced feed intake and reduced efficiency of feed utilization. The results of the present study are conflicting with those of previous studies. Onwudike (1986) reported that increasing levels of palm kernel cake increases the fibre content, reduces palatability and low availability of amino acids and energy in the diet. However, Odunsi *et al*. (2002) compared Palm Kernel Cake (PKC), Brewers Dried Grains (BDG), Maize Bran (MB) and Wheat Bran (WB) at 30% with maize/groundnut cake based diet and reported that feed intake of the agro-industrial by-products was significantly enhanced while weight gain was not affected.

The better performance of broiler starter chickens fed the control diet and the diets which contained 1:1 and 4:1 palm kernel cake and maize pap offal respectively agrees with the findings of Ezieshi and Olomu (2004) who reported that broiler chickens fed maize offal based diet performed better than those fed palm kernel cake based diet. It is known that the chicken is known to be especially sensitive to dietary energy concentration (Scott *et al*., 1982) and fibre content of the feed (Yaakugh *et al*., 1988). These workers had reported that high fibre diets reduces digestibility of the diet. This may be the case with results of the present study where different ratios of palm kernel cake and maize pap offal increase the crude fibre content of the diets. The better performance of finishing broiler chickens fed diet II (ratio of 1:1 PKC and maize pap offal) could have been due to its superior metabolizable energy content since dietary energy concentration is a function of weight gain for finishing broiler chickens.

On the basis of the results obtained, it may be recommended that diet II (ratio of 1:1 PKC and maize pap offal) maybe fed to broiler starter or finishing broiler chickens without any adverse effect on performance characteristics.

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Comparative Evaluation of Maize and Soyabeans as Energy Sources for Broiler Chickens

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Abstract: A total of one hundred and thirty-five (135) unsexed broiler chickens of 5 days of age averaging 72.22±1.11 grams were randomly allocated into 3 dietary treatments designated I, II and III such that full-fat soyabean and maize respectively each predominate (weight for weight) in diets on the one hand while each of the two ingredients were in equal proportions on the other hand. The results at the starter phase show that feed intake and weight gain were not adversely affected ($p>0.05$) by birds fed all diets. However, the diet which contained the highest level of full-fat soyabean supported insignificantly higher weight gain and efficiency of feed utilization. The results also show that efficiency of feed utilization of birds fed all diets were not significantly affected ($p>0.05$) while feed cost per unit weight gain of the birds fed the diet where soyabean predominated were significantly better ($p<0.05$) than birds fed other diets. The results at the finishing phase however show that there were no significant differences ($p>0.05$) in feed intake, weight gain, efficiency of feed utilization and feed cost per unit weight gain among birds fed diets containing either higher levels of maize or soyabeans. It was observed in the present study that bird fed the diet that contained higher levels of maize had insignificantly ($p>0.05$) better performance characteristics than those fed the diet that contained higher level of soyabeans. On the basis of the results obtained, it may be recommended that either higher levels of maize or full-fat soyabean may be used as energy sources for broiler starter and finishing broiler chickens without any adverse effect on their performance when prices of each feed ingredients do not differ much.

Key words: Maize, full-fat soyabean, broilers

INTRODUCTION

Maize and soyabean have been the most conventionally used sources of energy and protein respectively in the diets of monogastric animals (pigs and poultry). Maize is the widely used cereal grain in human and animal feeding in many parts of the world. It is also a source of industrial gums and recently fuel. Similarly, full-fat soyabean is an excellent source of vegetable oil, containing about 18% (Aduku, 1993). Obioha (1992) reported that fats and oils yield about 2.25 times more energy than equivalent amount of carbohydrate and/or protein. Pond *et al.* (1995) reported that where energy becomes limiting, excess protein in feedstuffs could be deaminated and the carbon skeleton used to support energy. These workers also reported that full-fat soyabean contains an excellent amino acid profile comparable to animal protein.

The metabolizable energy content of full-fat soyabean and maize for many monogastric animals are similar (Carew *et al.*, 2007). Thus, when prices do not differ much, they may become alternatives as energy sources. This is particularly so in most maize and soyabean producing communities like Benue State. According to Aduku (1993), the metabolizable energy content for maize and full-fat soyabean is 3432 and 3300 Kcal/kg respectively. Carew *et al.* (2007) therefore compared maize and full-fat soyabean as energy sources for rabbits and reported that these feed ingredients can be complementarily used as energy sources. This is the reason for the present study where maize and full-fat soyabean respectively each predominate in diets on the one hand and when each of the two ingredients are used in equal proportions on the other hand in diets and evaluating same on the performance of broiler starter and finishing broiler chickens.

MATERIALS AND METHODS

The maize and soyabean used in the present study was obtained from Gboko town, Benue State. The soyabean was manually roasted to rid the feedstuff of the presence of anti-nutritional factors. The particle size of each of maize and soyabean was then reduced by grinding with a hammer mill.

Experiment 1: A total of one hundred and thirty-five (135) unsexed broiler chickens of 5 days of age averaging

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72.22±1.11 grams were purchased from a commercial vendor and randomly allocated into 3 dietary treatments designated I, II and III of 45 birds in each treatment. Each treatment was replicated 3 times with each replicate having 15 birds such that full-fat soyabean and maize respectively each predominate (weight for weight) in diets on the one hand (Diets I and III respectively) while each of the two ingredients were in equal proportions (Diet II) (Table 1). The birds were reared according to standard procedures (Dafwang and Ogundipe, 1982). The chicks were maintained on these diets and water being provided *ad libitum* until they were four weeks old. Records of feed consumed and live weight changes were kept for 28 days.

Experiment 2: At the end of experiment 1, the birds averaging 586.67±1.67 grams were polled together and randomly allocated into 3 dietary treatments designated I, II and III for finishing broiler chickens such that full-fat soyabean and maize respectively each predominate (weight for weight) in diets on the one hand (Diets I and III respectively) while each of the two ingredients were in equal proportions (Diet II) (Table 2). The birds were given feed and water *ad libitum* until they were eight weeks old, observing standard broiler production practice and keeping records of feed consumed and live weight changes for 28 days.

Data analysis: Data on each parameter was subjected to the Analysis of Variance (ANOVA) for Completely Randomized Design (CRD) and where significant differences were indicated, the means were separated using Hsu's MCB (Multiple Comparison with the Best) method (Minitab, 1991).

RESULTS AND DISCUSSION

The summary of results of the response of broiler starter chickens fed diets in which full-fat soyabean and maize respectively each predominate (weight for weight) in diets on the one hand and when each of the two ingredients were in equal proportions on the other hand are presented in Table 3 while the response at the finishing phase are presented in Table 4.

As the results of the response of broiler starter chickens have shown, feed intake and weight gain were not adversely affected ($p>0.05$) by birds fed all diets. In the present study, diet I which contained the highest level of full-fat soyabean supported insignificantly higher weight gain and efficiency of feed utilization. However, feed intake was highest when soyabean predominated in the diet and lowest when equal proportions of maize and soyabean were fed.

The results also show that efficiency of feed utilization (expressed as the ratio of feed intake to weight gain) and feed cost per unit weight gain of the birds fed the diet where soyabean predominated were better than birds fed other diets. However, efficiency of feed utilization of birds fed this diet was not significantly better ($p<0.05$) than those fed the diet where maize predominated. It was observed in the present study that the efficiency of feed utilization and feed cost per unit weight gain of birds fed diets containing when equal proportions of maize and soyabean were significantly inferior ($p<0.05$) to birds fed other diets.

Similarly, as the results of the response of finishing broiler chickens have shown, there were no significant differences ($p>0.05$) in feed intake, weight gain,

Table 1: Percent composition of broiler starter test diets

	Dietary treatments		
Ingredients	I	II	III
Maize	36.00	44.00	52.00
FFRSB[a]	52.00	44.00	36.00
Rice offal	8.00	8.00	8.00
Bone ash	3.00	3.00	3.00
Common salt (NaCl)	0.25	0.25	0.25
Mineral-Vitamin Premix	0.25	0.25	0.25
DL- Methionine	0.25	0.25	0.25
L-Lysine HCl	0.25	0.25	0.25
Total	100.00	100.00	100.00
Crude protein (%)	23.56	21.24	18.92
ME (Kcal/kg)	3020	3028	3036
Crude fibre (%)	5.50	5.22	4.94
Ether Extract (%)	11.36	10.24	9.12
Calcium (%)	1.28	1.27	1.25
Phosphorus (%)	0.98	0.94	0.90
Methionine (%)	0.67	0.63	0.59
Lysine (%)	1.82	1.62	1.41
Meth. + Cystine (%)	0.93	0.88	0.82
Feed cost/kg (₦/kg)	67.02	66.22	65.42

ME = Metabolizable Energy (Kilocalories per kg of diet); a = Full-fat roasted soyabean

Table 2: Percent composition of broiler finisher test diets

	Dietary treatments		
Ingredients	I	II	III
Maize	46.00	43.00	40.00
FFRSB[a]	40.00	43.00	46.00
Rice offal	10.00	10.00	10.00
Bone ash	3.00	3.00	3.00
Common salt (NaCl)	0.25	0.25	0.25
Mineral-Vitamin Premix 0.25	0.25	0.25	
DL-Methionine	0.25	0.25	0.25
L-Lysine HCl	0.25	0.25	0.25
Total	100.00	100.00	100.00
Crude protein (%)	20.04	20.91	18.88
ME (Kcal/kg)	2984	2984	2988
Crude fibre (%)	5.52	5.67	5.38
Calcium (%)	1.26	1.26	1.25
Ether extract (%)	9.74	10.16	9.18
Phosphorus (%)	0.95	0.97	0.95
Methionine (%)	0.61	0.62	0.59
Lysine (%) 1.52	1.56	1.68	
Meth. + Cystine (%)	0.85	0.87	0.82
Feed cost/kg (₦/kg)	64.70	65.00	64.30

ME = Metabolizable Energy (Kilocalories per kg of diet); a = Full-fat roasted soyabean

Table 3: Response of broiler starter chickens fed maize and full-fat soyabeans as energy sources

	Dietary treatments			
Parameters	I	II	III	SEM
Average final weight (g)	656.98[a]	514.77[c]	585.23[b]	19.45
Initial average weight (g)	71.11	72.00	73.33	2.63[NS]
Average daily weight gain (g)	20.93	15.82	18.28	5.26[NS]
Average daily feed intake (g)	50.15	43.14	47.58	9.81[NS]
Feed conversion ratio	2.36[a]	3.76[b]	2.81[a]	0.84
Feed cost per unit weight gain (₦/gain)	158.34[a]	249.15[b]	183.50[a]	55.43

[a,b]Means followed by the same superscript in horizontal rows are not significantly different from one another (p>0.05).
SEM = Standard Error of Mean; NS = Not significantly different from one another (p>0.05)

Table 4: Response of finishing broiler chickens to maize and full-fat soyabeans as energy sources

	Dietary treatments			
Parameters	I	II	III	SEM
Average final weight (g)	1566.33[a]	1138.00[c]	1402.67[b]	-
Initial average weight (g)	588.33	585.00	586.67	-
Average daily weight gain (g)	34.94[a]	19.77[b]	29.13[a]	5.85
Average daily feed intake (g)	108.87[a]	88.63[b]	106.58[a]	9.88
Feed conversion ratio	3.12[a]	4.48[b]	3.66[a]	0.63
Feed cost per unit weight gain (₦/gain)	201.86[a]	291.20[b]	235.34[a]	40.61

[a,b]Means followed by the same superscript in horizontal rows are not significantly different from one another (p>0.05).
SEM = Standard Error of Mean; NS = Not significantly different from one another (p>0.05)

efficiency of feed utilization and feed cost per unit weight gain among birds fed diets containing either higher levels of maize or soyabeans. It was observed in the present study that birds fed the diet that contained higher levels of maize had insignificantly (p>0.05) better performance characteristics than those fed the diet that contained higher level of soyabeans. This performance characteristics were however significantly (p<0.05) depressed when maize and soyabeans were fed in equal proportions.

The metabolizable energy content of the feed is the major determinant of feed intake (Grobner *et al*., 1985). Since the chicken is known to be especially sensitive to dietary energy concentration (Scott *et al*., 1982), the non-significant effect of feed intake and hence weight gain could have been due to the iso-caloric nature of all the diets on the one hand and the fact that maize and full-fat soyabean contain similar levels of metabolizable energy on the other hand. The superior weight gain and efficiency of feed utilization of birds fed diets with the highest level of full-fat soyabean at the starter phase agrees with the previous study of Taiwo *et al*. (2005) who reported that full-fat soyabean has one of the best amino profile of all plant protein sources and therefore has the ability to support optimum performance of broiler chickens. In young, rapidly growing animals, protein is one of the most critical and indispensable nutrients needed for fast growth and body development (Abeke *et al*., 2008). This could have been the case with the results of the present study with respect to the diet containing the highest level of full-fat soyabean supporting the highest weight gain.

The poor performance of birds fed the diet that contained equal proportions of maize and full-fat soyabean could have been due to the high fibre content. The high crude fibre content according to Babatunde *et al*. (1975) dilutes the energy content of the feed and therefore depresses the coefficient of digestibility of energy and protein with subsequent reduction in gain of monogastric animals. Similarly, high crude fibre in diets of monogastric animals also impedes mineral absorption (Nwokolo *et al*., 1985).

On the basis of the results obtained, it may be recommended that either higher levels of maize or full-fat soyabean may be complimentarily used as energy sources for broiler chickens without any adverse effect on their performance. This may be particular so when prices of each feed ingredients do not differ much.

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Proximate Analysis of *Talinum triangulare* (Water Leaf) Leaves and its Softening Principle

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Abstract: The proximate analysis of *Talinum triangulare* was carried out in both wet and dry conditions respectively. The results revealed the presence of carbohydrates (10.87±3.99 mg/g and 12.38±2.76 mg/g), steroids (106.61±2.53 mg/100mg and 11.37±1.19 mg/100g), protein (3.52±0.32% and 18.75±2.72%), oil content (3.52% and 1.44%), b-Carotene (114.5±1.49 mg/g and 40.02±0.50 mg/g) and crude fibre (12.00% and 8.50%) in dry and wet samples respectively. The wet sample was assayed for pectinases at various temperatures. This revealed the presence of pectinases with specific activities of 38.64 units/kg protein, 55.44 units/kg protein, 61.14 units/kg protein and 62.09 units/kg protein at 35, 55, 75 and 95°C respectively. These results indicate that the leaves contain an appreciable amount of nutrients and should be included in our meal for a balanced diet. The high amount of pectinases detected lends credence to the traditional use of water leaf as a softener of other vegetables species and a possible industrial application.

Key words: *Talinum triangulare*, carbohydrates, protein, crude fibre

INTRODUCTION

Leafy vegetables are known to add taste, flavour, as well as substantial amounts of protein, fibre, minerals and vitamins to the diet (Onyenuga and Fetuga, 1975; Adeyemi, 1987). While the amounts of the nutrients constituents in the more commonly used leaf vegetable species in Nigeria have been studied to some extent (Onyenuga, 1968; Kola, 2004), the lesser known regional and local species remain virtually neglected. Lack of information on the specific nutrients in a large number of the native vegetables species with which Nigeria is richly endowed is partly responsible for their under-exploitation especially in areas beyond the traditional localities where they are found and consumed. Among the leafy vegetables in which their proximate analysis and softening principle have not been extensively studied are leaves of water leaf.

Talinum triangulare (water leaf) is an herbaceous, perennial, coalescent and glabrous plant widely grown in tropical regions as a leaf vegetable (Adewunmi *et al*., 1987). It is consumed as a vegetable and constituent of a sauce in Nigeria. In Nigeria, it is widely distributed and consumed as a leafy vegetable in the Southern ecological zones. Its leaves are used as softener of other vegetable species in vegetable soup. However, despite that the leaves are used as a natural softener of other vegetable species during vegetable soup preparation, no information has been published on this role. In order to ascertain the nutritive value of the vegetable species and thereby stimulate interest in its utilization beyond the traditional localities, this study was designed to determine the levels of the major nutrients in the leaves and its softening principle.

MATERIALS AND METHODS

The leaves of *Talinum triangulare* (water leaf) were collected from Ishiagu in Ebonyi State, Nigeria. The leaves were identified and authenticated by a taxonomist Dr. Ibiam, F.O. of the Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria. The leaves were destalked, washed and sun dried by constantly exposing the leaves to sunlight for 2-3 days and turning of the vegetable leaves to avert fungal growth. The leaves were later milled to obtain the Vegetable Leaf Meals (VLMs) using an electric blender, some of the leaves were also ground fresh using electric blender and both were stored in refrigerator in a well labeled air-light container for analysis. Proximate analysis and the softening principle were carried out on dried and wet samples of *T. triangulare* leaves.

Proximate analysis: Proximate analysis was carried out according to the procedure of Association of Official Analytical Chemist (A.O.A.C., 1990) to determine the carbohydrate, protein, oil, crude fibre, b-carotene and steroids components of the sample.

Pectinases assay: The pectinases assay was determined using Somogyi-Nelson methods (Somogyi, 1945; Miller, 1969). Pectinases may be measured by quantification of the reducing groups liberated following enzyme attack in the wet sample.

RESULTS

Table 1 shows the proximate chemical composition of *T. triangulare* leave in both dry and wet samples. It

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Table 1: Proximate chemical composition of *T. triangulare* leaves in both dry and wet samples

Name of the nutrient	Dry Sample	Wet sample
Carbohydrate (mg/g)	10.87±3.99	12.38±2.76
Steroids (mg/g)	106.61±2.53	11.37±1.19
Proteins (%)	3.52±0.32	18.75±2.72
Oil content (%)	3.52	1.42
β-Carotene (mg/g)	114.15±1.49	40.02±0.50
Crude fibre	12%	8.5%

contains considerate amount of b-carotene, (114.15± 1.49 and 40.02±0.50) which indicates high antioxidant effect of the vegetable and low levels of carbohydrate, (10.87±3.99 and 12.38±2.76), protein (3.52±0.32 and 18.75±2.72), oil content (3.52% and 1.42%) and crude fibre (12% and 8.5%) which indicates that water leaves is not a good source of these chemicals compositions. Table 2 present Specific Pectinases Activities at 95°C shows 62.09 units/kg protein as the highest and decreases down to temperature 35°C. This shows that this enzyme is thermo stable and hence its activities increases with increase in temperature. Fig. 1 presents the specific pectinase activities and temperature change.

Table 2: Specific Pectinase Activities at various temperatures

Name of Enzyme	Specific Activities
Pectinase (Units/kg proteins) at 35°C	38.36
Pectinase (Units/kg proteins) at 55°C	55.44
Pectinase (Units/kg proteins) at 75°C	61.14
Pectinase (Units/kg proteins) at 95°C	62.09

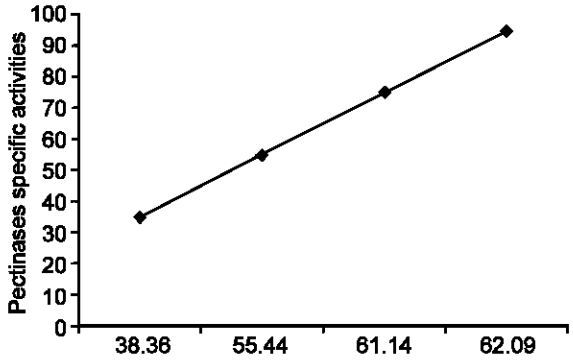

Fig. 1: Specific Pectinase activities against temperature change

DISCUSSION

Proximate compositions of *Talinum trinagulare* were carried out in both dry and wet samples. Its softening principle was equally investigated in the wet sample. Carotenoids, protein, carbohydrates, steroids, crude fibre and among others were revealed to be present in *T. Triangulare* (Table 1), this shows high level of its possible dietary value (Oleyede, 2005).

Generally the dry sample showed higher level of these nutrients than the wet sample except for protein and carbohydrates (Table 1). The reason may be that the nutrients are not volatile compounds and hence have high dried weight. These results are in correlation with the findings of Akindahunsi (2005). High level of β-Carotenoids (114.15±1.49 mg/100 g 40.02±0.50 mg/ 100 g) in Table 1, showed that the vegetable is good for the management of cardiovascular diseases and oxidative stress, since carotenoids are biologic antioxidants. Antioxidants are compounds that protect cells against the damaging effects of reaction oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxy nitrile. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Burton and Ingold, 1984). Oxidative stresses have been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury and neuro degenerative diseases (Palozza, 1998). Carotenoids may help provide protection against these diseases by contributing along with antioxidant vitamins and enzymes, to the total antioxidant defense system to the human body. Epidemiological studies have shown, that flavonoids and carotenoids intake, are inversely related to mortality from coronary heart diseases and to the incidence of heart attacks (Donald and Cristobal, 2006).

The oxidation of Low-density Lipoproteins (LDL) has been recognized to play an important role in atherosclerosis, immune system cells called macrophages recognize and engulf oxidized LDL, a process that leads to the formation of atherosclerotic plagues in the arterial wall, LDL oxidation can be induced by macrophages and can also be catalyzed by metal ions like copper. Several studies have shown that certain flavonoids can protect LDL from being oxidized (Donald and Cristobal, 2006).

The result of proximate analysis in Table 1 shows that *T. triangulare* has appreciable level of carbohydrates (12.38±2.26 mg/g and 10.87±3.99 mg/100 g), steroids (11.35±1.90 mg/100 g and 106.61±0.73 mg/100 g), proteins (1.39±0.60% and 18.75±2.72%) and crude fibre (12% and 8.5%). The result of this study indicated that *T. triangulare* is a good source of these nutrients. Low oil content and crude fibre obtained in this study confirms that *T. triangulare* is not a good source of oil and crude fibre.

Conclusion: The results of this study revealed that leaves of *T. triangulare* contain an appreciable amount of proteins, carbohydrates, steroids, carotenoids, among others and low level of oil content, etc. Since it contains substantial amount of nutrients, it can therefore be concluded that *T. triangulare* leaves can contribute significantly to the nutrient requirements and health management of man and should be recommended in our diet. Equally, its high specific activity of pectinases that enables it to be used traditionally as a softener of other vegetables species and a possible industrial application for its validity.

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Phytochemical Composition of *Talinum triangulare* (Water Leaf) Leaves

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Abstract: The qualitative and quantitative analyses of *Talinum triangulare* (water leaf) leaf which is commonly used as vegetable in Nigeria were carried out on both dry and wet samples. The result of dry and wet samples revealed the presence of bioactive compounds namely flavonoids (69.80±4.42 mg/100 g and 58.33 ± 9.00 mg/100 g), alkaloids (55.56±5.00 mg/100 g and 13.89±5.00 mg/100 g), saponins (1.48±0.20 mg/100 g and 1.37±0.60 mg/100 g) and tannins (1.44±0.73 mg/100 g and 1.09±0.26 mg/100 g) respectively. The results indicate that the leaves contain an appreciable amount of bioactive compounds. Medically the presence of these phytochemicals explains the use of this vegetable in ethnomedicine for the management of various ailments.

Key words: Quantitative, qualitative, bioactive, phytochemicals

INTRODUCTION

Vegetables serve as indispensable constituents of the human diet supplying the body with minerals, vitamins and certain hormone precursors, in addition to protein and energy (Oyenuga and Fetuga, 1975). Several vegetable species abound in Nigeria and most West African countries where they are used partly as condiments or spices in human diets or as supplementary feeds to livestock such as rabbits, poultry, swine and cattle (Aletor and Adeogun, 1995). These vegetables are harvested at all stages of growth and fed either as processed, semi-processed or fresh to man while they are usually offered fresh to livestock. Leafy vegetables are known to add taste and flavour, as well as substantial amount of proteins, fibre, minerals and vitamins to the diet (Oyenuga and Fetuga, 1975; Adewunmi, 1987).

While the amounts of the nutrient constituents in the more commonly used leaf vegetable species in Nigeria have been studied to some extent (Kola, 2004), the lesser known regional and local species remain virtually neglected. Lack of information on the specific nutrients and phytochemicals in a large number of the native vegetables species with which Nigeria is richly endowed is partly responsible for their under exploitation especially in areas beyond the traditional localities where they are found and consumed. Among the leafy vegetables in which their phytochemicals and nutrients have not been extensively studies are leaves of water leaf. *Talinum triangulare* (water leaf) is an herbaceous perennial, caules cent and glabrous plant widely grown in tropical regions as a leaf vegetable (Ezekwe *et al*., 2001).

It is consumed as a vegetable and constituent of a sauce in Nigeria. Nutritionally, water leaf has been shown to possess the essential nutrients like B-carotene, minerals (such as calcium, potassium and magnesium), pectin, protein and vitamins (Ezekwe *et al*., 2001). Water leaf has been also implicated medically in the management of cardiovascular diseases like stroke, obesity, etc. (Adewunmi and Sofowora, 1980) and traditionally it is used as softener of other vegetable species.

With recent wave of economic depression and its attendant effect on the purchasing power of the population of less developed nations, it has become obvious that the local food stuffs will play increasing role in the food, nutrition and health security of the rural people and the increasing urban poor. As popular as this vegetable is in Nigeria, There is still paucity of information on the phytochemical constituents of *Talinum triangulare*. Hence the present study was carried out to evaluate the phytochemical constituents of *Talinum triangulare* (water leaf) leaves.

MATERIALS AND METHODS

Collection and preparation of samples: The leaves of *Talinum triangulare* (water leaf) were collected from Ishiagu, Ebonyi State, Nigeria and were identified by taxonomist Dr, Ibiam, F.O, of the Department of Applied Biology of Ebonyi State University, Abakaliki, Nigeria. The leaves were destalked, washed and sun dried by constantly exposing the leaves to sunlight for 2-3 days and turning of the vegetable leaves to avert fungal growth. The leaves were later milled to obtain the Vegetable Leaf Meals (VLMs) using an electric blender, some of the leaves were also ground fresh using electric blender and both were stored in refrigerator in a well labeled air-light containers for analysis.

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Qualitative phytochemical screening of *Talinum triangulare*: Phytochemical screening procedures carried out were adopted from Oloyed (2005). This analysis determines the biologically active compounds that contribute to the flavour, colour and other characteristics of vegetable leaves.

Test for alkaloids: About 2 g of the ground sample were pounded separately on a mortar. 0.2 g was boiled with 5 ml of 2% hydrochloric acid on a steam bath for 5 min. The mixture was allowed to cool and filtered and the filtrate was shared in equal proportion into 3 test tubes and labeled A, B, C. One (1) ml portion of the filtrate was treated with 2 drops of the following reagents respectively. With Dragendroff's reagent a red precipitate was shown. With Mayer's reagent a creamy white coloured precipitate indicated the presence of alkaloid (Harborne, 1973; Trease and Evans, 1989).

Test for flavonoids: 0.5 g of the macerated sample of *Talinum triangulare* was introduced into 10 mls of ethyl acetate and heated in boiling water for 1 min. The mixture was then filtered and the filtrate used for the following test. 4 ml of the filtrate was shaken with 1 ml of 1% aluminum chloride solution and kept. Formation of a yellow colour in the presence of 1 ml dilute Ammonia solution indicated the presence of flavonoids (Harborne, 1973; Igwe, 2004).

Test for saponins: 0.1 g of the sample was boiled with 5 ml of distilled water for 5 min. Mixture was filtered while still hot and the filtrate was then used for the following tests (Trease and Evans, 1989). To 1 ml of the filtrates, 2 drops of olive oil was added, the mixture was shaken and observed for the formation of emulsion. 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was vigorously shaken and then observed on a stand for stable froth (Trease and Evans, 1989).

Test for the presence of tannins: Into 2 g of the ground sample was added 5 ml of 45% ethanol and boiled for 5 min. The mixture was cooled and filtered. 1 ml of the filtrate was added 3 drops of lead sub acetate solution. A gelatinous precipitates were observed which indicates the presence of Tannins. Another 1 ml of the filtrate was added 0.5 ml of bromine water. A pale brown precipitates were observed indicating the presence of Tannins (Trease and Evans, 1989).

Test for glycosides: 2 g of the sample was mixed with 30 ml of distilled water and it was heated for 5 min on a water bath, filtered and used as follows: five mls of the filtrate was added to 0.2 ml of fehling solution A and fehling solution B until it turns alkaline and heated in a water bath for 2 min. A lightish blue colouration was observed (instead of brick red precipitate) which indicates the absence of glycosides (Oloyed, 2005).

Quantitative phytochemical analysis of *Talinum triangulare*

Determination of alkaloids: 0.5 g of the sample was dissolved in 96% ethanol -20% H_2SO_4 (1:1). 1 ml of the filtrate was added to 5 ml of 60% tetraoxosulphate (VI), and allowed to stand for 5 min. Then, 5 ml of 0.5% formaldehyde was added and allowed to stand for 3 h. The reading was taken at absorbance of 565 nm (Harborne, 1976).

Determination of flavonoids: Flavonoid in the test sample was determined by the acid hydrolysis of spectrophotometric method. 0.5 g of processed plant sample was mixed with 5 ml of dilute HCl and boiled for 30 min. The boiled extract was allowed to cool and filtered. 1 ml of the filtrate was added to 5 mls of ethyl acetate and 5 mls of 1% NH_3. This was then scanned from 420n-520nm for the absorbance. (Harborne, 1976).

Determination of saponins: 0.5 g of the sample was added to 20 ml of 1NHCl and was boiled for 4 h. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5 ml of acetone ethanol was added to the residue. 0.4 mls of each was taken into 3 different test tubes. 6 ml of Ferrous sulphate reagent was added into them followed by 2 ml of conH_2SO_4. It was thoroughly mixed after 10 min and the absorbance was taken at 490 nm (Oloyed, 2005).

Determination of tannins: 5 g of the ground sample was shaken constantly for 1 min with 3 ml of methanol in a test tube and then poured into a Buchner funnel with the suction already turned on. The tube was quickly rinsed with an additional 3 ml of methanol and the content poured at once into the funnel. The filtrate was mixed with 50 ml of water and analyzed within an hour. For aqueous extractions, 5 ml of water was used for the extraction and for the rinse and the filtrate was added to 50 ml of water. 3 ml of 0.1 ml $FeCl_3$ in 0.1 NH_4Cl was added to 5 ml of the extract and followed immediately by timed addition of 3 ml of 0.008 ml K_2, Fe $(CN)_6$ The absorbance was taken at 720 nm spectrophotometrically (Onwuka, 2005).

RESULTS

The results of qualitative analysis of *Talinum triangulare* (water leaf) leaves in dry and wet samples are shown in Table 1. The results obtained showed the presence of alkaloid, saponins, flavonoids, tannins and absence of glycosides.

Results of quantitative analysis of *Talinum triangulare* are presented in Table 2. The results of phytochemicals analysis (quantitative) of *Talinum triangulare* (water leaf) leaves in both dry and wet samples show higher levels in the dry sample than wet sample.

Table 1: Qualitative phytochemical data of dry and wet samples of *Talinum triangulare*

	Dry sample	Wet sample
Alkaloid	+ve	+ve
Flavonoids	+ve	+ve
Saponins	+ve	+ve
Tannins	+ve	+ve
Glycosides	-ve	-ve

Table 2: Quantitative phytochemical data of dry and wet samples of *T. triangulare*

Name of the phytochemical	Dry sample	Wet sample
Flavonoids (mg/100g)	69.8±4.42	58.33±9.00
Aikaloids (mg/100g)	55.56±5.00	13.89±5.00
Saponins (mg/100g)	1.48±0.20	1.37±0.60
Tannins (mg/100)	1.44±0.73	1.09±0.26

DISCUSSION

Phytochemical analysis is very useful in the evaluation of some active biological components of some vegetables and plants (medicinal). The qualitative and quantitative analyses of *Talinum triangulare* were carried out in both dry and wet samples. Alkaloids, flavonoids, saponins, tannins, were revealed to be present in *T. triangulare* (Table 1 and 2 respectively). This shows high level of its possible medicinal and dietary values (Oloyed, 2005). Although, some of these analyzed constituents of the vegetable species may be completely harmful to both man and farm animals and some are species specific as observed in the case of tannins (Odebiyi and Sofowora, 1979). Some of these active components have been demonstrated to possess anti nutritional effects, following their ability to reduce palatability and digestibility of feedstuff (Odebiyi and Sofowora, 1979).

In Table 2, the levels of these phytochemicals (bioactive compounds) were shown. Generally, the dry sample showed higher levels of these bioactive compounds than the wet sample. The reason may be that the bioactive compounds are not volatile compounds and hence have a high dried weight. These results are in correlation with the findings of Akindahunsi (2005). High levels of flavonoids (69.80±4.42 mg/100 g and 58.33 ±9.00 mg/100 g) in Table 2 showed that the vegetable is good for the management of cardiovascular diseases and oxidative stress, since flavonoids are biologic antioxidants. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrile. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Burlon and Ingold, 1984). Oxidative stresses have been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury and neuro degenerative diseases (Parkinson's and Alzheiner's) (Palozza, 1998). Flavonoid may help provide protection against these diseases by contributing along with antioxidant vitamins and enzymes, to the total antioxidant defense system to the human body. Epidemiological studies have shown that flavonoids and carotenoids intake are inversely related to mortality from coronary heart diseases and to the incidence of heart attacks (Donald and Cristobal, 2006).

The oxidation of Low-density Lipoproteins (LDL) has been recognized to play an important role in atherosclerosis, immune system cells macrophages recognize and engulf oxidized LDL, a process that leads to the formation of atherosclerotic plagues in the arterial wall, LDL oxidation can be induced by macrophages and can also be catalyzed by metal ions like copper. Several studies have shown that certain flavonoids can protect LDL from being oxidized (Donald and Cristobal, 2006).

The presence of saponins (1.48±0.20 mg/100 g and 1.37±0.60 mg/100 g) and alkaloids (55.56±5.00 mg/100 g and 13.89±5.00 mg/100 g) (Table 2) in *T. triangulare* contribute to its medicinal value. Saponins inhibit Na^+ efflux by the lockage of the entrance of the Na^+ out of the cell. This leads to higher Na^+ concentration in the cells, activating a Na^+-Ca^{2+} anti porter in cardiac muscle. The increase in Ca^{2+} in flux through this anti porter, which strengthens the contractions of heart muscle (Schneider and Woliling, 2004).

The valuable pharmaceutical properties in *T. triangulare* may be attributed to the presence of bioactive compound like alkaloid (55.56±5.00 mg/100 g and 13.89±5.00 mg/100 g). Alkaloid has been used as CNS stimulant, topical anaesthetic in ophthalmology, powerful pain relievers, anti puretic action, among other uses (Heikens *et al*., 1995). The result of anti nutrient composition (Table 2), revealed low value of tannins (1.44±0.05 mg/100 g and 1.09±0.26 mg/100 g). This is not high enough to constitute human poison. The lethal value is above 5% (Adebayo *et al*., 2000).

Results of this study revealed that leaves of *T. triangulare* contain an appreciable amount of flavonoids, alkaloids, saponins, among others and low level of toxicants like tannins, since it contains substantial amount of bioactive compounds. It can therefore be concluded that *T. triangulare* leaves can contribute significantly to the health management of man and should be recommended in our daily nutritional need.

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Acceptability Studies on Bread Fortified with Tilapia Fish Flour

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Abstract: This study was conducted to assess the acceptability of bread samples produced by fortification of wheat flour with Tilapia Fish Protein Flour (TFPF) in varying proportions (5, 10, 15 and 20% by weight of wheat flour). Bread produced from 100% wheat flour served as the control. The fortified bread and the control samples were subjected to proximate and organoleptic analysis. The result of proximate analysis revealed that the incorporation of TFPF into wheat flour resulted in improvement in the nutritional value of bread sample. The protein content increases as more and more TFPF was added. The protein content for the control sample was 9.08% while the values for fortified bread samples ranged between 18.01 and 10.59% with sample coded 411 (80% wheat flour: 20% TFPF) having the highest value. The result of organoleptic analysis revealed that there was no significant difference among the samples in term of taste, aroma, crust and crumb colour and overall acceptability. Acceptable fortified bread could therefore be produced from wheat and TFPF.

Key words: Wheat flour, bread, fortification, tilapia fish protein flour

INTRODUCTION

Bread is a baked food produced from flour that is moistened, kneaded, proofed with the addition of yeast. Other raw materials for bread making apart from wheat flour include sugar, baking fat, yeast, vegetable oil, salt and water. Hard wheat flour is used for bread making because of gas produced by yeast during proofing and baking (Famosinpe, 2001).

Bread is highly nutritious eaten in one form or another by nearly every person on earth. An excellent source of vitamins, protein and carbohydrates bread has been an essential element of human diets for centuries in all regions (Ryan, 2005). Bread is a solid foam, a typical bread has the crust with the characteristic golden brown colour and white crumbs. Bread has a short life due to its chemical composition and moisture content compared to other baked products.

Nutritionally, bread contains high percentage of carbohydrate and fat both of which are needed for energy production and source of calories. Other nutrients like vitamins, mineral and protein are relatively in small proportion. The problems of malnutrition in Nigeria although different in magnitude and severity among different areas are due to protein, vitamins, iron and other mineral deficiency (Adebooye, 1996). Many Nigeria consume bread without any nutrient supplement like butter, geisha etc suggesting the problem of malnutrition. Therefore there is the need to enrich or fortify bread in order to improve its nutritional value.

Food fortification is the addition of one or more essential nutrients to food weathers or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency with one or more nutrient in the population or specific population group (Brekkan, 1996). A nutrient or substance is considered an appropriate fortifier when and only the nutrient is stable in the food under normal conditions of storage, distribution, and use. Fortification of food has become a means of ensuring nutritional adequacy of the diet. Example of appropriate food fortifiers include soy flour, fish protein concentrate which are rich in protein.

Fish protein concentrate is produced from edible parts of fish, which is properly dehydrated and milled into flour. It is otherwise referred to as fish flour. It has attractive colour, pleasant flour, and of reduced moisture content which make it to have relatively longer shelf life. Nutritional studies have shown that fish protein concentrate can be added to weaning food growing infants and nursing mothers.

This paper is therefore on the acceptability of bread fortified with Tilapia Fish Flour in order to ensure the nutritional adequacy of bread. The present study is therefore aimed at proximate and organoleptic analyses of fortified bread and the control samples.

MATERIALS AND METHODS

The materials used for this project work include wheat flour, granulated sugar, yeast, shortening agent all of which were purchase at Osogbo Osun State. The Tilapia Fish used for the production of fish protein concentrate was purchased at Eko-Ende Dam also in Osun State. Equipment used include Oven, weighing balance, mixer, kjedahi apparatus, soxhlet apparatus, muffle furnace. All the chemicals used were of food grade.

Production of tilapia fish protein concentrate: Tilapia fish that was purchase at Eko-Ende Dam was sorted and graded; the fish was washed thoroughly with

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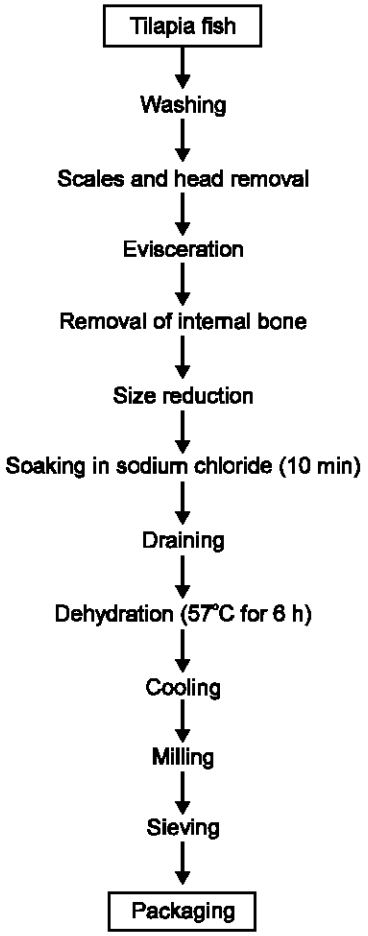

Fig. 1: Flow chart tilapia fish protein concentrate production (Ihekeronye, 1985)

Sample codes and designation

Sample codes	Designation
426	100% WF : 0% TFPF
591	95% WF : 5% TFPF
477	90% WF : 10% TFPF
499	85% WF : 15% TFPF
411	80% WF : 20% TFPF

WF: Wheat Flour; TFPF: Tilapia Fish Protein Flour

potable water so as to remove all extraneous matters. The scales and heads were removed and thereafter the fish was eviscerated and bones were removed. The fish was washed again in order to remove blood and the intestinal waste from the fish.

The eviscerated fish was them sliced into different sizes and was then soaked in sodium chloride (Salt) at room temperature 27±2°C for 10 min to reduce the moisture content through Osmotic dehydration and to improve taste. Thereafter it was drained and drying was done for 6 h. After cooling inside desiccators, the dried was milled to powdery form sieved to have fine powder and was stored in an air-tight container for further use.

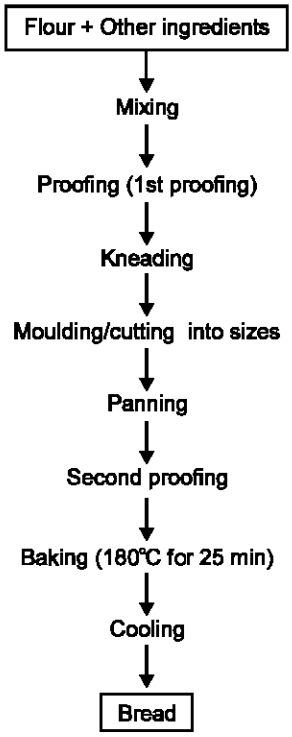

Fig. 2: Flow chart for production

Production of bread fortified with fish protein concentrate: All ingredients were weighed i.e. flour, salt, sugar, fish flour, butter, yeast, shortening agent using a precision mettler balance. The ingredients were added to the mixer and were thoroughly mixed. The mixed dough was then placed in large metal containers or trough and held in an insulated room at about 27°C and in an atmosphere of high humidity to allow fermentation. During fermentation the mass of dough is kneaded several times to allow the escape of some air which is produced continuously during the fermentation.

After fermentation, the dough was divided into pieces of sizes which will eventually make up the finished loaf. This was done by a machine which measures the dough by volume and cuts of pieces of the desired size. The fermenting dough was moulded and dropped into baking pans which had been formerly cleaned and rubbed with oil. It was then baked in an oven. During the first stages in the oven, the dough continues to ferment and increase in volume. The bread was baked at 180°C for about 25 min. The baked loaves were cooled to permit efficient slicing and to prevent moisture formation under the bread wrapper. The bread was thereafter packaged and stored for further research work.

Proximate analysis: The proximate analysis of the samples and the control were carried out using the method described by Association of Officer Analytical

Chemist (AOAC, 1990). Carbohydrate determination was done by difference between all the proximate constituent and 100.

Organoleptic analysis: Organoleptic analysis was carried out using multiple comparison test. Nine panelists were used to evaluate the sample for taste, crust and crumb colours, texture, flour and overall acceptability. The data collected were thereafter analyses statistically to determine if there is any significant difference among the samples in terms of all the parameters.

RESULTS AND DISCUSSION

Proximate analysis results: The results of proximate analysis on the five bread samples are as presented in Table 1.

Results are means of duplicate values: Table 1 shows the result obtained for proximate analysis of fortified bread and that of reference sample. From the table the moisture content for fortified bread samples ranged between 29.10 and 26.28% with sample coded 591 having the highest value. The moisture content for the control sample was 25.00%. The relatively high moisture content observed for fortified bread samples might be due to the incorporation of Tilapia Fish Protein Flour (TFPF). Fish is high in water content; equally protein has a high water holding capacity. Bread, as a product belong to the class of Intermediate Moisture Food (IMF), hence, there can be a little bit of moisture in the product. The products however fall within the acceptable limit of IMF product (Ihekoronye, 1985). The fortified bread samples recorded higher values for protein. This is due to the addition of the TFPF. The values for protein ranged between 18.10 and 16.45% with sample coded 411 having the highest value. The protein content for control sample was 9.08%. The fish protein concentrate used to fortify the bread contains high quality protein more than the control sample which leads to the increase in the percentage protein recorded in fortified bread samples. Proteins serves as antibodies, they serve as primary sources of amino acids, the building block of cellular protein. The fat content was found to be on the increase as more and more TFPF was added. Sample coded 426 which is the control had the least value of fat of 2.15% Bread sample with 5% TFPF had 2.19% followed by sample with 10% TFPF recorded 2.30% while sample with 20% TFPF had the highest value of 2.88%. the ash content of the control sample and the fortified samples were very close. Sample coded 411 with 20% TFPF recorded the highest value 2.88%. Ash content of food product gives an insight to the content of food products. The crude fibre content of the fortified bread samples ranged between 0.85 and 0.73%. Control sample recorded 0.88%. The values are very close. As more and more TFPF was added the crude fibre is on the decrease. Crude fibre through its water absorption capacity aid peristalsis movement of food through the digestive tract. The result of carbohydrate content revealed that the control sample had the highest value ranging from 58.00-48.9%. As more and more TFPF was incorporated. The carbohydrate content is on the decrease. This might be due to the effect of starch dilution through the incorporation of the TFPF.

However, fortified bread samples had appreciable quality of carbohydrate. Their values were very close to that of the control. Samples coded 591 and 477 recorded values that were very close to that of the reference sample 58.00 and 57.86% respectively. These samples equally had higher proximate constituents than the control. Hence, these samples can really compete with the control. Nutritionally, these samples stand out. All the fortified bread samples recorded dry matter (g/100 g) values less than the control through very close. The control sample had a value of 74.75 (g/100 g) while the fortified samples values ranged between 73.75 and 70.90 (g/100 g).

Fortification in this regard therefore leads to production of more nutritious, nourishing and more acceptable bread sample.

Organoleptic analysis result: The result of organoleptic analysis carried out on the bread samples is as presented in Table 2.

From the table, the F-values obtained for the bread samples were less than Q-factor (3.01) which indicates that there is no significant differences among the sample in term of all the parameter evaluated at 5% significant level. This therefore signifies that acceptable bread sample can be produced from fortification of

Table 1: Proximate analysis result for the bread samples

Sample code	Protein (%)	Moisture content	Ash (%)	Crude fibre (%)	CHO (0%)	Fat (%)	DM (g/100 g)
426R	9.08	25.00	2.46	0.88	60.20	2.15	74.75
591	10.59	29.10	2.38	0.85	58.00	2.19	70.90
477	12.14	26.28	2.38	0.83	57.86	2.30	73.75
499	16.45	26.78	2.39	0.78	48.91	2.59	71.30
411	18.01	26.75	2.58	0.73	49.05	2.88	73.13

DM = Dry Matter (g/100 g)

Table 2: Organoleptic analysis result table

Parameter	F-Sample	Q-Factor
Texture	1.62	3.01
Taste	1.13	3.01
Crust and crumb colour	1.00	3.01
Aroma	1.00	3.01
Overall acceptability	1.30	3.01

wheat flour with Tilapia Fish Protein Flour in bread production using any of the blend ratios.

However, sample coded 411 i.e 80% wheat and 20% TFPF received the least preference.

Conclusion: From this work, it has been established that production of acceptable bread sample from wheat flour fortified with Tilapia Fish Protein Flour is technically feasible. The proximate analysis results showed increment in the proximate constituents of bread as more and more TFPF was added which make the product to meet the dietary requirements.

Fish protein concentrate is therefore an excellent fortifier in bread production as well as other bakery products. In order to improve the nutritional values of bakery products especially bread which is a 'staple' in Nigeria diet Tilapia Fish Protein Flour could be used. This will lead to production of more nutritious, nourishing and acceptable bread.

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Functional Properties of Wheat and Sweet Potato Flour Blends

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Abstract: In this study the functional properties of wheat and sweet potato tuber (*Ipomea batatas*) flour blends were investigated. The sweet potato tubers were brought from local farm in Offa Kwara State. The tubers were thoroughly sorted, washed, peeled, sliced, blanched, soaked, drained, sundries and milled into flour. Wheat flour used was purchased at Orisumbare market in Osogbo Osun State. The wheat and sweet potato flour were blended using the following ratios (WF:SPF:100:0, 90:10, 85:15, 80:20, 75:25, 0:100). These samples were thereafter subjected to functional properties analysis. The results revealed that as more and more sweet potato flour was added to wheat flour, there was significant effect on the functional properties.

Key words: Wheat, sweet potato, functional properties

INTRODUCTION

Flour is fine powder made from cereals or other starch based produce. It is most commonly made from wheat. Flour is the key ingredient in bread production which constitutes a staple in the diet of many countries. Therefore, the availability of adequate supply of flour has often been a major economic and political issue. Flour can also be made from legumes and nuts, root and tubers such as yam, cassava, sweet potato etc. flour produced from non wheat sources is otherwise known as composite flour.

Composite flour includes yam, cassava, cocoyam, sweet potato, instant yam flour to mention but few. Attempts to improve the chemical and sensory qualities of composite flour especially instant yam flour has been reported by various authors (Ngoddy and Onuoha, 1983; Ofi, 1983; Sanni *et al*., 2006). These flour has been incorporated with wheat flour in production of bakery goods such as cookies, brand and cake. Through this, diversified, reduced cost of production and acceptable products can be produced since wheat cannot survive in Nigeria's soil. These flours have their properties that enhance their wide utilization which include water and oil absorption capacity, foaming capacity, foam stability, bulk density, gelation capacity, emulsion capacity etc (Adeyeye *et al*., 1994; Abbey and Ibeh, 1988). Incorporation of composite flour into wheat flour for bakery goods production is expected to produce effect in the functional properties of the blended samples. Several studies have indicated the possibility of incorporating hulless barley, soya bean, sorghum, cowpea flour into wheat flour at various level and the rheological and baking properties have been reported (Oftman and Garba, 1997; Kinsella, 1979; Sathe and Salkhe, 1981).

In this paper, functional properties of wheat and sweet potato flour blends were investigated. The two flour were blended using the following blend ratios (WF:SPF 100:0, 90:10, 85:15, 80:20, 75:25, 0:100).

MATERIALS AND METHODS

The sweet potato tubers used for this work were bought from a farm in Offa Kwara State, Nigeria. Commercial wheat flour was purchased at Orisumbare market in Osogbo Osun State, Nigeria. Equipment used include milling machine, mechanical sieve, pH meter, oven, desiccators, centrifuge, balance, Rapid Visco Analyzer (RVA) and stirrer. Other materials used include knife, water, pipettes crucibles, bowls and napkin.

Production of sweet potato flour: The sweet potato tubers were thoroughly sorted to remove bad ones from the lot. The sorted tubers were washed to remove adhering soil, dirts and extraneous materials. The tubers were thereafter peeled and sliced to facilitate fast rate of drying and ease milling operations. The sliced tubers were then blanched in order to inactivate enzymes that may cause browning reaction. These were then cooled and drained followed by drying. Following drying, the tubers were milled, sieved into fine flour and packaged for further use.

Functional properties determination

Swelling power: 1 g of the sample was weighed into a conical flask. It was hydrated with 15 ml distilled water, shook for 5 min with mechanical shaker at low speed. Heating was done for 40 min at 80-85°C with constant stirring in a water bath. The content was transferred into a clean, dried and pre-weighed centrifuge tube.

7.5 mil of distil water was added and centrifuged at 2200 rpm for 20 min. The supernatant was decanted into a pre-weighed can and dried at 100°C to a constant weight. The sediment was weighed in the centrifuge. Swelling power and solubility were calculated viz:

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Solubility = % starch dissolved in the sediment

Viscosity measurement: Viscosity was determined using the Bradender amylograph. The procedure involved dispensing 200 g suspension of 10% (w/v) preparation of each sample into the equipment and monitoring the viscosity of the slurry as the temperature increases.

pH determination: The pH of the samples was measured with a pH meter. 10 g of each sample collected especially were homogenized in 50 ml of distilled water. The resulting suspensions were decanted and their pH determined using pH meter already standardized with buffer solutions of pH 4.0 and 7.0.

Total solids and moisture determination: 2 g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into an oven set at 100°C to dry to a constant weight. At the end of the drying, the crucible plus sample was removed from the oven and transferred to desiccators, cooled for 10 min and weighed.

$$\text{Moisture (\%)} = \frac{W_1}{W_1} - \frac{W_3}{W_0} \times \frac{100}{1}$$

Where,
W_1 = Weight of crucible plus wet sample
W_0 = Weight of empty crucible
W_3 = Weight of crucible plus dried sample

Emulsification capacity: 2 g sample was blended with 25 ml distilled water for 30 sec in a blender at 1600 rpm. After complete dispersion, refine corn oil was added from a burette and blended until there was a separation into two layers of water and fat. Emulsifying capacity was expressed as ml of oil emulsified by 1 g of flour.

Water absorption capacity: 15 ml of distilled water was added to 1 g of the flour in a weighed 25 ml centrifuge tube. The tube was agitated on a vertex mixer for 2 min. It was centrifuged at 4000 rpm for 20 min. The clear supernatant was decanted and discarded. The adhering drops of water was removed and the reweighed. Water absorption capacity is expressed as the weight of water bound by 100 g dried flour.

Fat absorption capacity: 10 ml refined corn oil was added to 1 g of the flour in a weighed 25 or 80 ml centrifuge tube. The tube was agitated on a vertex mixer for 2 min. It was centrifuged at 4000 rpm for 20 min. The volume of free oil was recorded and decanted. Fat absorption capacity is expressed as mil of oil bound by 100 g dried flour.

Foaming capacity and foaming stability: 2 g flour sample and 50 ml distilled water was mixed in a blended at room temperature. The suspension was stirred for 5 min at 1000 rpm. The total volume after 30 sec was recorded. It was allowed to stand at room temperature for 30 min and the volume of foam recorded. The percentage increase in volume after 30 sec is expressed as foaming capacity.

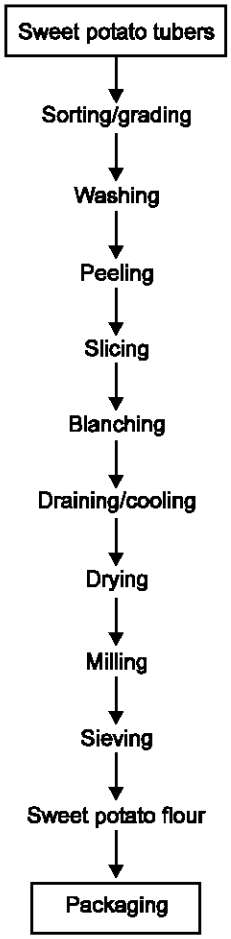

Fig. 1: Flow chart of sweet potato flour production

Sample codes designations

Sample codes	Designation
A	100% WF
B	90% WF : 10% SPF
C	85% WF : 15% SPEF
D	80% WF : 20% SPF
E	75% WF : 25% SPF
F	100% SPF

WF = Wheat Flour; SPF = Sweet Potato Flour

Bulk density: 50 g flour sample was put into a 100 ml measuring cylinder. The cylinder was tapped several times on a laboratory bench to a contant volume. The volume of sample is recorded.

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Weight of Sample}}{\text{Volume of sample after tapping}}$$

Table 1: Functional properties of Wheat Flour (WF) and Sweet Potato Flour (SPF) blends

Sample	MCM (%)	pH	EC (%WF)	GC (%)	WA (g^{s-1})	FA (g^{s-1})	FC (%)	BD (g/cm^3)	WP	V (B.U)	SS
A	3.07	6.01	14.68	10.75	2.45	2.15	4.12	7.47	12.75	73	8.63
B	2.75	5.70	9.68	9.70	1.83	1.75	3.68	6.75	7.40	68	6.85
C	2.60	5.70	10.57	8.75	1.55	1.57	3.30	5.80	6.85	61	6.75
D	3.24	5.63	11.40	6.45	1.24	1.43	2.35	5.73	6.50	55	6.40
E	3.60	5.40	12.70	5.40	1.18	0.70	1.17	5.25	6.10	49	6.35
F	3.68	5.50	25.40	3.60	1.27	0.65	1.28	6.83	5.73	35	6.01

Key: WF = Wheat Flour; SPF = Sweet Potato Flour; A = 100% WF; B = 90% WF : 10% SPF; C = 85% WF : 15% SPF; D = 80% WF : 20% SPF; E = 75% WF : 25% SPF; F = 100% SPF; B.U Brabender Unit.

MCM (%) = Moisture Content M (%) EC = Emulsion Capacity (%WF) GC = Gelation Capacity (%) WA = Water Absorption (g^{s-1})
FA = Fat Absorption (g^{s-1}) FC = Foaming Capacity (%) BD = Bulk Density (g/cm^3) WP = Welling Power
V = Viscosity (B.U) SS = Swelling Solubility

Gelation capacity: 2-20% suspension was prepared with 5 ml distilled water in test tube. The tubes containing the suspension was heated for 1 h in a boiling water bath. It was cooled rapidly under running cold water. The test-tube was cooled for 2 h at 4°C. The test was inverted to see if content will fall or slip off. The least gelation concentration is that concentration when the sample from the inverted test tube does not fall or slip.

RESULTS AND DISCUSSION

Functional properties of wheat and sweet potato flour blends: The functional properties determine the application and use of food material for various food products. The results of functional properties of wheat and sweet potato flour blends are as presented in Table 1. From the table the moisture content of Sweet Potato Flour (SPF) was higher than that of Wheat Flour (WF). This might be due to the drying method used for SPF. The moisture content for WF was 3.07 while 3.68 was recorded for SPF. However, the two products are below the minimum limit of moisture content for flour (Ihekeronye and Ngoddy, 1985). The moisture content of the flour was on the increase as more and more SPF was added to WF. The moisture content of food products goes a long way in suggesting the shelf life of the product. Sample E (75% WF : 25% spf) recorded the highest value for moisture content among the blended samples. All the values of the blended sample fall within the acceptable limit of dry products (15%).

The results obtained for pH of 100% WF was 6.01 while that for 100% SPF was 5.50 signifying that sample E is slightly acidic compared to sample A. The pH values for other blends ranged between 5.40 and 5.70. as more and more SPF was added to WF the pH value was tending toward slight acidity. Acidic products are more shelf stable that non acidic counterpart (Ihekeronye and Ngoddy, 1985). Emulsion capacity results revealed that SPF had 25.40% which was higher than that of WF (14.68%). Emulsion properties play a significant role in many food system where the protein have the ability to bind fat such as in meet products batter, dough and salad dressing (Sathe and Salkhe, 1981). The emulsion capacity of the blends increased as more SPF was added with sample E recording the highest emulsion capacity of 12.70% among the blended samples.

The gelation capacity of sample A (100% WF) was 10.75% while it was 3.60% for sample F (100% SPF). The low gelation value recorded for SPF suggests that it may not be a good gel forming agent. This indicates that more flour will be needed to form a gel with SPF because of its low gelation capacity (Adebowale *et al*., 2005). The gelation capacity of the blended samples was on the decrease as the percentage of SPF incorporation increase. The values for the blended samples ranged between 9.70 and 5.40 Variations in the gelling properties of different flours may be due to variations in the ratio of different constituents such as carbohydrates, lipids and proteins that make up the flours (Abbey and Ibeh, 1998).

The water absorption capacity of sample A is 2.45 gs^{-1} while that of sample E is 1.27 gs^{-1} indicating that sample A has higher water absorption capacity. Sample A therefore has higher affinity for water which is informed by its lower moisture content 3.07%. The water absorption capacity of the blended samples is on decrease as more and more SPF was added to WF. The fat absorption capacity for sample A is 2.15 gs^{-1} while that of sample E is 0.65 gs^{-1}. The fat absorption capacity equally decreased as more and more was incorporated indicating diluting effect of SPF on WF fat absorption capacity. The mechanism of fat absorption is attributed mainly to the physical entrapment of oil and the binding of fat to the apolar chain of protein (Wang and Kinsella, 1976). Wheat flour recorded the highest foaming capacity of 4.12% while sweet potato flour had 1.28%. The foaming capacity of all the blended samples followed the same trend as other properties discussed earlier. Sample with the higher percentage of sweet potato flour (i.e. sample E) recorded the least foaming capacity of 1.17%. Bulk density value for WF (100%) was 7.47 g/cm^3 while SPF recorded 6.83 g/cm^3. As more and more SPF was incorporated into WF, the bulk density was on the decrease. The values for the samples ranged between 5.25-6.75 g/cm^3 with sample E recording the least value. Bulk density is generally

affected by the particle size and density of the flour and it is very important in determining the packaging requirement, material handling and application in wet processing in the food industry (Karuna *et al*., 1996). Swelling power is an indication of the water absorption index of the granules during heating (Loos *et al*., 1981). The swelling power for WF was 12.75% which was greater than that of SPF of 5.73%. The same trend was observed for swelling solubility and their values were on the decrease as more and more SPF was incorporated into WF. Sample A (100% WF) was more viscous than SPF. The viscosity value for WF was 73B.U while SPF recorded 35B.U. This might be due to the higher gluten content in wheat flour.

Conclusion: The study showed that blending sweet potato flour with wheat flour had significant effect on the functional properties of the flour blends. Blending SPF with WF up to 20% level produced samples which can be used for production of bakery goods with improved functional properties and reduced retro-graduation, staling rate and production time.

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Seasonal Variation in Nitrate Levels in Hand Dug Wells in Makurdi Metropolis

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Abstract: Groundwater quality response to changes in season is documented in several studies. In this study, seasonal variation in nitrate levels in hand dug wells in Makurdi metropolis is examined. A total of 15 water samples were collected from hand dug wells and analyzed for nitrate level for both wet and dry seasons. The analysis was done according to standard method of water examination. The results of analyses show that 80% of the wells have nitrate levels above WHO guide limit for drinking water for the wet season as against 67% for the dry season. This implies that consumers of water from these hand dug wells especially children stands a very high of metheamogolineamia. Generally nitrate level in these hand dug wells were noted to be higher in wet season than in the dry season. Source of nitrate in these wells is attributed to use of chemical fertilizers on farms, improper disposal of animal and human wastes and influence of season. All land use activities capable of polluting water should be properly controlled. Water from these wells may be used for other domestic purposes other than drinking. Boiling of water from these should be encouraged to reduce the risk of contracting illness.

Key words: Nitrate, season, variation, hand dug wells, water quality

INTRODUCTION

A wide range of water problems faces nations and individuals around the world. These problems include international and regional disputes over water, water scarcity and contamination, unsustainable use of groundwater, ecological degradation and the threats to climate change. The failure to provide safe drinking water and adequate sanitation services to all is perhaps the greatest development failure of the 20th century. If no action is taken to address unmet basic needs for water, as many as 135 million will die from these diseases by 2020 (Gleick, 2002). Water-related diseases are a human tragedy, killing millions of people each year, preventing millions of people more from leading healthy lives and undermining development efforts (Nash, 1993). About 2.3 billion in the world suffer from diseases that are linked to water (Kristof, 1977; United Nations, 1997).

In Nigeria, urban dwellers faces serious threats to the quality of life and safety. With urbanization resulting into high degree of population densities and concentration of socio-economic activities, it has become increasingly difficult to meet all the water requirements both in quantity and quality. The public water supply is generally inadequate and in most cases inaccessible, the supply is intermittent and unreliable, thus resulting into high dependency on unsafe supplementary sources such as streams, hand dug wells and ponds (Kakulu and Osibanjo, 1992; Olajire and Imeppeoria, 2001; Nnodu and Ilo, 2002; Owuama and Uzoije, 2005; Ocheri, 2006). Concern over the quality of water harnessed especially from the hand dug wells have received wide attention among researchers (Ovrawah and Hymore, 2001; Ehinola and Coker, 2002; Nnodu and Ilo, 2002; Ogunbadewa, 2002; Omofonmwam and Eseigbe, 2009). Consistent in their findings is that water from hand dug wells are polluted through physical processes, geochemistry of the environment and anthropogenic activities. Consequently, consumers of such waters are exposed to series of health risks.

Health problems from nitrates in water sources are becoming a serious problem every here. In over 150 countries, nitrate from fertilizers seeped into wells, fouling the drinking water (Maywald *et al*., 1988) Nitrate is one of the most commonly identified groundwater contaminants (BGS, 2003). It is primarily regulated in drinking water because excessive levels can cause methaemoglobineamia (blue-baby syndrome). This is a condition whereby nitrate is reduced to nitrite in the stomach of infants thus inhibiting the transport of oxygen round the body. The consequence is shortness of breath (Cyanosis), blueness of skin and atimes death (BGS, 2003). Several studies on nitrate pollution of groundwater have been carried in different parts of Nigeria urban areas (Gbodi and Atawodi, 1986; Ezeonu, 1988; Adelana, 2006; Ogbu and Echebiri, 2003). None of these studies focused on the effect of seasons on the level of nitrate in groundwater. In this study therefore, attempt is made to assess the level of nitrate in hand dug wells among seasons in Makurdi urban area. Hand

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dug wells are well systems whereby groundwater is abstracted at shallow depths. They are generally more vulnerable to pollution than boreholes. Due to the general inadequacy and inaccessibility of public water supply in Makurdi use of water from the hand dug wells is common in the residential area.

The study area: The study area is Makurdi town, the capital city of Benue state in north central Nigeria. Makurdi lies between Lat. 7^{o} 44^{N} and Long. $8^{o}54^{N}$. It is located within the flood plain of lower River Benue valley. The physiographic characteristics span between 73-167 m above sea level. Due to the general low relief sizeable portions of Makurdi is water logged and flooded during heavy rainstorms. This is reflected in the general rise in the level of groundwater in wells during wet season. The drainage system is dominated by River Benue which traverse the town into Makurdi North and South banks.

Temperatures are generally high throughout the year due to constancy of insolation with the maximum of 32^{o}C and mean minimum of 26^{o}C. The hottest months are March and April. The rainfall here is convective, and occurs mostly between the months of April and October and is derived from the moist and unstable southwest trade wind from St. Helena Subtropical Anticyclones (STA). Mean annual rainfall total is 1190 mm and ranges from 775-1792 mm. Rainfall distribution is controlled by the annual movement and prevalence of Inter-Tropical Discontinuity (ITD).The mean monthly relative humidity varies from 43% in January to 81% in July-August period (Tyubee, 2009).

The geology is of cretaceous sediments of fluvio-deltaic origin with well-bedded sandstones of hydrogeological significance in terms of groundwater yield and exploitation (Kogbe *et al*., 1978). Makurdi town which started as a small river port in 1920 has grown to a population of 297,393 people (NPC, 2006).

MATERIALS AND METHODS

In this study, we relied on the analyses of water samples collected from hand dug wells across the residential area of Makurdi town. Two sets of water samples were collected from 15 hand dug wells in the months of September for wet season and January for dry season. The essence is to enable us ascertain the effect of seasons on the level of nitrate in these wells. To ensure quality assurance, adequate measures such as the use of sterilized containers in water sample collection, proper preservation and storage at temperature of 4^{o}C before laboratory analyses.

The analyses of the water samples collected was done according to standard methods of water examination using calorimetric techniques (APHA-AWWA-WPCF, 1985). The technique is based on the principle that the amount of energy absorbed is proportional to the concentration of nitrate ion present in the sample. The ion has its own characteristic absorption wavelength. Ultraviolet spectrophotometer UNICAM Sp 6-550 model was used to determine the ionic concentrations in water sample. Nitrate concentrations as it affects the quality of drinking water is based on the WHO 2006 prescribed limit.

RESULTS AND DISCUSSION

The results of analyses of nitrate level in hand dug wells for rainy and dry season are presented below for discussion.

From Table 1, 12 out of 15 wells (80%) have nitrate concentration levels above the WHO prescribed limit of 45 mg/l for drinking water for the rainy season. For the dry season, 10 out 15 wells (67%) have elevated nitrate concentration levels above the WHO limit. This shows that nitrate concentrations in hand dug wells in Makurdi during the rainy season are higher than in the dry season. For instance w7, w11, w14 and w15 have very high nitrate concentrations of 148, 120, 128 and 132 mg/l in rainy season as against 89, 98, 110 and 115 mg/l for the dry season. Nitrate concentrations in these well may be explained within the context of the season characteristics, hydrogeochemistry of the environment, well characteristics and associated land use activities. For instance, during rainy season groundwater is recharged through precipitation via percolation leading to general rise in the level of the water. This makes them highly susceptible to pollution and run off activities as elements in soils and rocks are easily released into the water. Beside, depths of the wells are important because contaminants generated through various land uses activities can easily get into them. Average well depth is 3 m and distance to latrines and soak ways is 3.5 m makes them highly vulnerable to pollution activities. The source of nitrate in these wells could be attributed to agricultural fertilization as urban farming is common in the wetland areas coupled with animal grazing. Use of chemical fertilizers on farms has been identified as one of the main causes of nitrate in ground waters (Calabresa, 1971; Laftouch *et al*., 2003; Kumar and Shah, 2004). Beside, indiscrimate disposal of human and animal wastes from grazing animal on land result in leaching of residual nitrate thereby causing high nitrate concentration in groundwater. According to Matthes (1976) Foster *et al*. (1982) use of chemical fertilizers, improper disposal of human and animal wastes are sources of nitrogen containing compounds that are converts to nitrate in soil.

Table 1: Nitrate level in hand dug well in Makurdi

Well code	Well depth (m)	Depth to water level (m)	Nitrate (mg/l) rainy season	Nitrate (mg/l) dry season
W1	2.30	1	50	39
W2	2.70	1.45	48	40
W3	2.70	1.45	46	47
W4	7.30	1.00	58	60
W5	5.20	1.65	62	53
W6	4.33	4.33	85	66
W7	5.80	3.43	148	89
W8	3.77	2.37	40	33
W9	1.87	1.87	49	35
W10	2.93	1.30	43	45
W11	3.70	3.50	120	98
W12	9.15	7.55	87	80
W13	2.90	2.00	86	40
W14	1.00	1.00	128	110
W15	1.00	1.00	132	115

Conclusion: The study has apparently demonstrated that hand dug wells in Makurdi urban area are highly polluted with nitrate. About 80% and 68% of the wells have nitrate concentrations above WHO prescribed limit in drinking water for both the wet and dry season. This implies that consumers of water from these wells especially children stands a very high risk of methaemoglobineamia (blue-baby syndrome). Nitrate level was noted to be higher in these wells in the wet season than in the dry season. The source of nitrate in these is attributed to the use of chemical fertilizers in urban farming, indiscriminate animal grazing, improper disposal of animal and human wastes and seasonal influence. To reduce the rate of contamination, there should be control of all land use activities capable of polluting well. Water from these wells may used for other purpose other than drinking. These water should be boiled for domestic uses.

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Evaluation of the Growth Performance of Snails Fed Different Forages under Intensive Management

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Abstract: A six-week old experiment was conducted during the early rainy season of the year to investigate the growth performance of snails fed different forages under intensive rearing. The different forages used in the study constituted the treatment groups and they included: Fluted pumpkin (*Telferia occidentalis*) leaves (T_A), African Spinach (*Amaranthus sinensis*) leaves (T_B), Pawpaw (*Carica papaya*) leaves (T_C) and formulated mash (T_D). Thirty two (32) snails of 3 weeks old were divided into the four treatment groups of eight (8) snails each in a completely randomized design. Each treatment group was replicated twice. Result of data analysis showed that there was significant difference ($p<0.05$) in all the parameters studied which included feed intake, weight gain, feed conversion ratio and shell length increase. Feed intake was highest in T_A followed by T_C and then T_D. The highest weight gain recorded in T_D differed significantly ($p<0.05$) with those of T_A and T_B. There was no statistical difference between T_A and T_C. Feed conversion ratio was highest in T_B followed by T_C and least in T_D. Shell length increase was significantly lower ($p<0.05$) in the T_B than the other treatment groups that showed no statistical difference with each other. With T_A and T_C giving the highest weight gain after T_D and high overall feed intake, it may be advisable that heliculturists should use fluted pumpkin or Paw paw leaves in intensive snail rearing. Use of formulated ration may only be in time of forage shortages as may be occasioned by seasonal changes.

Key words: Growth performance, African giant snails, forages, intensive rearing

INTRODUCTION

The scarcity of meat for human protein requirement has necessitated the need for intensive rearing of some unconventional livestock such as the snail. Onoja (2005) stated that snail meat tastes good and is of high nutritive value. It serves special delicacies at homes and restaurants (Oyenike, 2008). This implies therefore, that snail can constitute a significant proportion of human diet. Imovbore and Ademosun (1988) reported that the dry matter of snail meat consists of high quality protein with high contents of important amino acids like lysine, leucine, arginine and tryptophan. In addition, snail meat has high level of calcium and phosphorus but low level of cholesterol thus making it unharmful. The proximate composition of snail meat compared to other conventional meat products is as shown in Table 1.

Ajayi *et al.* (1978) stated that apart from the nutritive value of snail meat, snail also supply valuable source of nutrition to livestock and other animals for instance, the non-edible parts (viscera and shell) can be recuperated for feeding monogastric animals.

Snails being nocturnal animals carry out most of their biological activities especially feeding and mating during the night, but during the day, they remain quiescent and hide away from predators (Akinnusi, 1997). He further stated that snail feed on a wide range of materials. While Esobe (1986) reported that *Achatina achatina* feed on both fruits and leaves of plants, Okoroh (1988) reported a disparity in preference for leaves and fruits between the young and adult snails. He said that the young snail like the leaves whereas adults relish the fruits more than leaves.

Growth is a major factor in assessment of performance and Plummer (1975) reported a direct proportional relationship between increase in body weight and increase in shell length. Okwuani (2002) stated that the growth of snail is inversely proportional to the age, with the younger ones growing more than the old ones. Ajayi *et al.* (1978) pointed out that even though snail feeds indiscriminately, not all food effect appreciable change in growth. They observed that some food may even lead to a retrogressive growth, cause some pathological conditions and loss of body weight.

In spite of the growing importance of snail, best production system as well as best nutrient materials for optimum production within a limited time has not been well documented. The research therefore is aimed at investigating the best nutrient or food item for growth under intensive production system.

MATERIALS AND METHODS

Location and duration of the study: The study was conducted at Orji farm situated at Umueze-Awkunanaw, Nkanu West Local Government Area, Enugu State,

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Table 1: Nutrient contents of snail meat compared to other meats

		Amino acids g/16g N			Minerals mg/100 g dry sample			
		Le	Ly	Tr	Ca	P	K	Fe
Snail meat	8.31	9.36	9.21	1.29	185.70	61.24	63.30	1.40
Beef	6.98	8.39	9.30	1.00	53.00	24.05	81.44	1.25
Broiler meat	8.90	7.75	9.22	0.80	55.60	28.29	74.14	0.90
Chevon	7.01	8.28	10.06	0.90	65.30	28.08	97.21	0.19
Mutton	6.40	8.85	10.28	1.32	55.20	22.45	82.83	0.85
Pork	7.22	7.90	9.96	0.70	64.00	34.42	83.45	0.90
Fish (Clarias)	5.78	9.45	11.44	1.40	69.12	26.13	61.48	0.55
Fish (Tilapia)	7.27	9.33	11.49	1.24	103.38	30.81	75.69	0.52

			Percentage of total		Proximate composition dry weight basis	
	CL	FS	SFA	UFA	CP	EE
Snail meat	20.28	0.42	28.71	39.67	88.37	1.64
Beef	76.84	1.06	46.19	31.92	92.75	4.59
Broiler meat	95.84	0.84	32.52	56.12	92.21	4.34
Chevon	69.00	0.76	39.72	35.11	86.63	3.01
Mutton	87.46	0.62	48.51	39.49	86.34	4.20
Pork	61.54	1.02	42.59	455.00	82.42	13.6
Fish (Clarias)	61.54	0.92	28.00	49.56	91.99	3.18
Fish (Tilapia)	65.20	0.39	32.38	42.58	90.81	3.35

Source: Imovbore and Ademosun (1988). Le = Leucine; Ly = Lysine; Tr = Tryptophan; CL = Cholesterol level; FS = Mg/100 fresh sample; SFA = Saturated fatty acid; UFA = Unsaturated fatty acid; CP = Crude protein; EE = Ether extract

Nigeria. The area lies between latitude 07° 4^1 North and 08° 2^1 South with longitude 06° 8^1 East and 07° 6^1 West. The area posseses such factors that could favour growth of snails. These include availability of giant trees for shade and cover, good radiation and availability of water and feed resources. The study lasted for six weeks (September to October, 2008).

Experimental snails: Thirty-two (32) snail hatchlings of three (3) weeks old were procured from Songhai farms, Amukpe-Sapele, Delta State, Nigeria. On arrival to the experimental site, all snails were allowed an acclimatization period of two (2) weeks and were generally fed and provided with water daily. Efforts were made to simulate the natural environment by covering the snail with leaves.

Experimental diets: The experimental diets consisted of fluted pumpkin (*Telferia occidentials*), African spinach (*Amaranthus sinensis*), Paw paw (*Carica papaya*) leaves and formulated mash feed. The choice for the use of these feed items was based on two main factors namely:

- Animal preference on the feed materials during the acclimatization period and
- Ready availability of the feed material

The composition of the mash used is shown in Table 2.

Experimental layout: The experimental snails were housed in a pen constructed with palm fronds. The pen was built under a pear tree (which provided constant shade) and near a well-water (for easy access to water for the animals).

Table 2: Materials used in compounding mash feed

Feeding ingredients	Nutrients supplied	Quantity (gram)
Wheat offal	Energy	320
Maize gluten	Protein	110
Fish meal (local)	Protein	55
Bone meal	Mineral	15
Salt	Mineral	2.5
Premix	Vitamin	2.5

At one side of the pen were some openings where the baskets could be brought out and taken back. The space provided avenue for attending to the snails with ease.

Procedure and management: Four (4) local baskets of 34 cm in diameter each were placed in the pen. Each basket was divided into two with wire gauze to provide two replicate groups per treatment. The experiment therefore had 4 treatments with 8 replicates. The baskets were tightly covered with metal-like mosquito nets. After recording the initial body weight and shell length of the individual snails, they were randomly assigned to the 4 baskets that represented the treatment groups. Each of the treatment which consisted of 8 animals was replicated twice.

Known weight of feed for each treatment was given to the snails daily in plastic saucer plate. The feed were fluted pumpkin (*Telferia occidentials*) leaves T_A, African spinach (*Amarathus sinensis*) (T_B), Pawpaw (*Carica papaya*) leaves (T_C) and formulated mash (T_D). Water was sprinkled on the baskets twice daily to ensure a cool and humid surrounding. Body weight and shell length were taken on weekly basis.

Experimental design and data analysis: A Completely Randomized Design (CRD) was used in the experiment since only treatment effect was evaluated. There were four treatments and eight snails were randomly assigned to each of the treatments. Each treatment was further replicated two times with four per replicate.

Data collected on feed intake, weight gain, shell length and feed conversion ratio were subjected to one-way Analysis of Variance (ANOVA) using the statistical method of Steel and Torrie (1980). The Least Significant Difference (LSD) was used to separate the difference of various means.

RESULTS

Table 3 shows that there was significant difference ($p<0.05$) in food intake of snails between some treatment groups. Feed intake was highest in T_A followed by T_C and lowest in T_D. However, there was no significant difference ($p>0.05$) in feed intake between T_A and T_B. Table 4 shows that there was significant difference ($p<0.05$) in weight gain between some treatment groups. Weight gain was highest in T_D followed by T_A but lowest in T_B. No significant difference ($p>0.05$) was observed between T_A and T_C groups.

There was a significant difference ($p>0.05$) in feed conversion ratio between some treatment groups as shown in Table 5. Feed conversion ratio was highest in T_B followed by T_C but least in T_D. No statistical difference was observed between T_A and T_B.

Table 6 shows that shall length was statistically lower ($p<0.05$) in T_B than any other treatments. No significant difference ($p>0.05$) existed between T_A, T_C and T_D.

Table 3: Mean weekly feed intake (grams)

Age (Weeks)	T_A	T_B	T_C	T_D
3	4.875	3.75	3.0	2.5
4	4.625	3.875	2.625	2.0
5	6.625	4.625	3.875	2.5
6	6.625	5.375	4.625	2.75
7	9.75	6.875	4.5	2.0
8	7.75	7.375	5.5	2.25
ΣX	40.25	31.875	24.125	14.0
X	6.71^a	5.3125^a	4.02^b	2.33^c
SE	±0.7081	±0.5696	±0.3649	±0.4669

Means with rows having different superscripts are statistically different ($p<0.05$)

Table 4: Mean weekly weight gain (grams)

Age (Weeks)	T_A	T_B	T_C	T_D
3	2.375	1.875	1.625	2.0
4	1.375	0.5	1.0	1.875
5	1.25	0.875	1.375	1.5
6	1.125	1.25	1.875	1.75
7	1.375	1.375	1.5	2.5
8	1.375	1.625	8.75	2.125
ΣX	8.875	7.5	8.75	11.75
X	1.48^b	1.25^c	1.46^b	1.96^a
SE	±0.1677	±0.1755	±0.1089	±0.1273

Means with rows having different superscripts are statistically different ($p<0.05$)

Table 5: Mean Weekly feed conversion ratio

Age (Weeks)	T_A	T_B	T_C	T_D
3	2.06	2.02	1.75	1.27
4	3.45	9.50	2.63	1.06
5	5.09	6.85	2.85	1.67
6	6.15	4.42	2.25	1.90
7	7.07	5.12	3.32	1.01
8	5.55	4.50	3.92	0.95
ΣX	29.37	32.41	16.99	7.86
X	4.89^a	5.40^a	2.83^b	1.31^c
SE	±0.6851	±0.9455	±0.2754	±0.1454

Means within rows having different superscripts are statistically different ($p<0.05$)

Table 6: Mean Weekly shell length increase (cm)

Age (Weeks)	T_A	T_B	T_C	T_D
3	0.40	0.30	0.39	0.31
4	0.37	0.23	0.34	0.25
5	0.36	0.11	0.26	0.26
6	0.21	0.19	0.21	0.13
7	0.25	0.22	0.25	0.21
8	0.18	0.20	0.41	0.29
ΣX	1.77	1.25	1.86	1.29
X	0.295^a	0.208^b	0.31^a	0.242^a
SE	±0.0346	±0.0230	±0.0305	±0.0240

Means within rows having different superscripts are statistically different ($p<0.05$)

DISCUSSION

The higher feed intake in T_A and T_B groups compared to T_C and T_D could be as a result of a difference in succulence, palatability and digestibility of the feed materials used. It is possible that the *Telferia occidentalis* (Fluted pumpkin) and the *Amaranthus simensis* (African spinach) in T_A and T_B respectively are more succulent, palatable and digestible than the others used in T_C and T_D. The Pawpaw leaves used in T_C was more fibrous to touch and probably bitter to taste, hence the less intake. The formulated mash of T_D was very dry and powdery. Perhaps, the very low water content of the mash made it unattractive to the snails.

The highest weight gain in T_D could be attributed to the nutrient composition of the mash. Being a formulated feed, it is expected to have a better balance of nutrients. Therefore, a little intake of food would give a corresponding gain in weight. That was not true for the forage used in T_A, T_B and T_C that have their nutrients predetermined by nature. It is possible therefore that the nutrients may not have been in the right proportion to effect significant weight increase as in T_D. Weight gain in T_A, T_B and T_C can only be effected by the available nutrients in the forages which can now be converted to flesh. This agrees with the report of Ajayi *et al.* (1978) who stated that not all food materials can exert appreciable change in snail growth.

The highest feed conversion ratio evidenced in T_A and T_B than in the other groups is reflective of the highest feed

intake recorded for them it can therefore be believed that the greater the feed intake, the greater the feed conversion ratio.

The shell length increase was statistically lower ($p<0.05$) in the T_B group than any of the other. It is possible that African spinach is relatively low in calcium and Phosphorus-both of which are very essential for shell growth and development. This finding agrees with the earlier report by Plummer (1975) that there is a proportional relationship between body weight and shell length increases.

Conclusion and Recommendations: In conclusion, best growth performance of snail could be achieved in intensive rearing with the use of formulated mash.

However, use of formulated mash at all-year-round should not be encouraged for economic reason except during the dry season when forages are generally unavailable.

I recommend the use of Paw paw leaves especially during the rainy season since it gave the best shell increase and second best weight gain. In addition, it is very easily available and affordable especially in the rainy season. Also, it suffers no competition between animals and man.

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Air-Oven Drying of Pre-Treated Fruit Slices: A Promising Solution to Post-Harvest Losses

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Abstract: Reports have shown that over 25% of fruit are lost during their season and virtually out of the market during off season due to their high moisture content couple with poor preservation methods practiced in Nigeria. Smooth cayenne pineapple specie obtained from Ajanla Farms, Ibadan was used for the study. A two factor-factorial experimental design of 3-levels of 3 pre-treatment methods (sucrose, blanching and sulphiting) and 3-levels of drying conditions (80°C/8 h, 70°C/10 h and 50°C/16 h) resulting into 81 treatments was used. The treatments were reduced to 18 by stratified sampling method from the results of the moisture and ascorbic acid content obtained from the pre-treated slices before drying. The slices were dried in a cabinet dryer; the moisture and ascorbic acid content of the dried slices were monitored during drying and after. All the samples exhibited combinations of falling and increase in drying with increasing in drying time. The dried slices were stored for 18 weeks, at which they were analyzed at 3 week intervals. The results obtained revealed that there were significant effects ($p<0.05$) of the pre-treatments on the ascorbic acid and moisture content of the dried slices. The 60%S/60°CB/2500ppm SO_2 at 70°C/10 h had the least value of moisture content of 8.75% and 25.16 mg/100 g of ascorbic acid. Furthermore, the results indicated that drying at 70°C/10 h is suitable for drying of pineapple slices than other drying conditions. During storage, there was continuous reduction in moisture content during the early weeks of storage. However, after 9 weeks of storage the moisture content of the samples increased showing significant differences between the mean values of the samples at different weeks of storage. Though, the samples pretreated with SO_2 showed a little variation over the storage period. Drying condition of 70°C/10 h and pre-treatment of 2500 ppm SO_2/60% sucrose was found to have minimal reduction of quality and thus the most appropriate treatment for air-oven drying of pineapple slices. The study therefore, recommends that production of pre-treated dried fruit slices could be used to reduce the enormous post-harvest losses of fruits.

Key words: Pineapple, sulphiting, sucrose, blanching, food security

INTRODUCTION

In the tropics considerable research and extensive efforts made towards fruit production have started to yield results. However, much of these products are available only during their season and about 25% are lost after harvesting due to inadequate and poorly developed storage facilities (NIHORT, 1990). Thus, increasing fruit production is no more the real goal for food nutrition and security, but how these fruits may be preserved. Several preservation methods for extending shelf-life of fruits have been proposed and successfully utilized. Large capital investments and high skilled personnel that are justified only in a buoyant economy hampered the applications of these storage methods. Simple methods of preservation are a pressing need in developing nations. One of such method is air-oven drying, which is reported to be appropriate for fruits and vegetables preservation (Karim *et al*., 2008, Pappas *et al*., 1999).

Air dehydration of fruits had been reported to be limiting in some factors especially on the quality of the dried fruits (McMinn and Magee, 1997). These can be categorized as nutritional, physical and chemical in nature, which are been imposed as moisture is been removed. To alleviate these problems in air-dehydration, different pre-treatment methods have been developed and successfully used for many temperate fruits and few tropical fruits (Karim *et al*., 2008, Arthey and Dennis, 1990, Raoult-Wack *et al*., 1991).

Among the reported pre-treatment methods are sulphiting (Atkinson and Strachan, 1962; Sapers, 1993), blanching (Alvarez *et al*., 1995) and sucrose osmosis (Spaizzi and Mascheroni, 1997). Pineapple (*Ananas comosus*) is a common non-citrus fruit and a very rich source of vitamin C and organic acids (Bartolomew *et al*., 1995). About 25% losses annually were recorded from total production due to its poor storability life (NIHORT, 1990; FAO, 1990). This study was therefore designed to assess the suitability of combination of these pre-treatment methods for air-oven dehydration of pineapples slices.

MATERIALS AND METHODS

Freshly harvested pineapple fruits (*Smooth cayenne*) obtained from Ajanla Farms; Ibadan, Nigeria was used for the study.

Experimental design: A 2 factor-factorial experimental design of 3-levels of 3 pre-treatment (sucrose, blanching and sulphiting and 3-levels of drying condition (80°C/8 h, 70°C/10 h and 50°C/16 h) resulting into 81 treatments was used for the study. These were reduced to 18 samples by stratified sampling method from the result obtained on the pre-treated samples.

Pre-treatment: The fruits were hand peeled and sliced into spherical shape of 5 mm thickness and 20 cm radius. A batch each of 2 kg of slices were pre-treated accordingly with sucrose osmosis method by immersing fresh slices into aqueous solution of 40 or 60% w/w of sucrose (Food grade of 98% purity) for 10 min. The samples were drained on wire mesh and re-weighed. The blanching pre-treatment were done by immersing the fruit in warm water at 60°C held in a water bath to maintain the temperature and steam generated from boiling water for 5 and 3 min, respectively at atmospheric pressure. The sulphur dioxide pre-treatment was done by dipping the slices in 1500 and 2500 ppm sulphur dioxide solution made from potassium-meta bisulphate solution for 6 min.

The pre-treated pineapple slices were dried in cabinet dryer (Gallen Kamp hot box) under standard conditions on perforated stainless steel trays, of tray loading 1 kg with cross-through air flow. The dried samples were analyzed for moisture and ascorbic acid content after drying using AOAC (1992) methods. The results were used to select 18 samples that were packaged into high density polythene bag of 0.028 mm thickness and stored in the laboratory at ambient temperature (30±2°C) for 18 weeks. The samples were also analyzed for moisture and ascorbic acid content at 3 week intervals.

Statistical analysis: Mean separation was obtained by Duncan Multiple Range Test and analysis of Variance ANOVA was conducted on the mean values to determine the significance of any differences between the samples (Duncan, 1955).

RESULTS AND DISCUSSION

The initial moisture and ascorbic acid content of fresh slices were 81.31% and 31.46 mg/100 g respectively. The result of the moisture and ascorbic acid content obtained from the 81 freshly dehydrated samples were subjected to stratified random sampling to obtain 18 samples that were used for further study.

The effect of pre-treatment methods on the moisture and ascorbic content of 18 pre-treated dehydrated slices is shown in Table 1. The results showed that there were significant differences ($p<0.05$) between the mean values of the moisture and ascorbic acid content. The moisture content range between 15.39 and 8.46% for control sample dehydrated under 50°C/16h and sample pre-treated with 40% sucrose and 60°C blanching and sulphiting at 2500 ppm SO_2 and dehydrated at 70°C/0 h. The result indicated that the pre-treatment methods influenced moisture removal. The samples that were sulphated recorded the low moisture content when compared with the samples that were blanched and osmotically pre-treated. This effect might be due to the effect of SO_2 pre-treatment on physical and chemical changes on the water binding components of these slices and on the cellular membrane permeability as reported by Karim (2005) and Karim and Adebowale (2009). The high moisture content of the osmotically pre-treated samples may be due to the severe ultra structural damage of the cell walls. From the report of Alvarez *et al.* (1995) such material exhibit a reduced optical density, which is due to the fact the binding force between the cell wall and the higher concentration of hydrozium ions present in the high acid fruit may accelerate the breakdown of the binding materials. While the blanching pre-treatment was found to enhance the moisture removal due to elimination of the cell membranes resistance to water diffusion (the membrane integrity is destroyed by heat treatment) and decrease on the resistance of the cell walls to water flux (Del Valle *et al.*, 1998; Alvarez *et al.*, 1995).

The ascorbic acid content ranged between 5.54 and 29.95 mg/100 g for the control sample dehydrated under 50°C/6 h and sample pre-treated with 40% sucrose, 60°C and sulphiting at 2500 ppm SO_2 and dehydrated at 70°C/10 h. This indicated that the pre-treatment methods protected the ascorbic acid of the fruit, most especially the sulphiting method. Furthermore, the reduction in ascorbic acid indicates the instability of ascorbic acid of

Table 1: Moisture and Ascorbic acid content of pre-treated and air-dehydrated pineapple slices

Pre-treatment/Drying conditions	Moisture (%)	Ascorbic acid (mg/100 g)
Control/ D_1	15.39a	5.54h
60% S/Steam B/D_1	9.34gh	8.48f
60% S/Steam B/D_2	8.99h	9.05f
Steam B/2500 ppm SO_2/D_3	9.34gh	25.35b
40% S/2500 ppm SO_2/D_2	9.36gh	21.16c
60% S/1500 ppm SO_2/D_2	10.96d	19.36d
60% S/2500 ppm SO_2/D_3	10.53e	27.35a
2500 ppm SO_2/D_2	10.42e	27.58b
40% S/60°C B/2500 ppm SO_2/D_2	8.46j	25.12b
40% S/60°C B/2500 ppm SO_2/D_3	9.17h	29.95a
60% S/60°C B/2500 ppm SO_2/D_2	8.75i	25.16b
40% S/Steam B/1500 ppm SO_2/D_1	9.66g	13.48d
60% S/Steam B/2500 ppm SO_2/D_2	9.24h	28.53a
60% S/Steam B/2500 ppm SO_2/D_3	8.97h	27.78a
2500 ppm SO_2/D_3	10.41e	25.58b
40% S/D_2	10.41f	5.97h
Steam B/D_1	11.93c	10.48e
Control/D_2	14.67b	6.56g

S-Sucrose; B-Blanching; D_1-50°C/16 h Drying, D_2-70°C/10 h Drying, D_3 -80°C/8 h Drying. Means with the same alphabet in the column are not significantly different at 5% level

Table 2: Effect of pre-treatment on moisture content of dehydrated pineapple slices

	Moisture content%/Week						
Treatments	*0	*3	*6	*9	*12	*15	*18
Control/D_1	15.39c	14.89d	13.97e	13.63e	14.99d	17.58b	19.05a
60% S/Steam B/D_1	9.34a	9.32b	9.17b	9.13b	9.07b	9.14b	9.82a
60% S/Steam B/D_2	8.99hc	8.93e	8.91c	8.83c	8.84c	11.18b	13.93a
Steam B/2500 ppmSO_2/D_3	9.34a	9.32a	9.17a	9.13a	9.07a	9.04a	9.02a
40% S/2500 ppm SO_2/D_2	9.36a	9.31a	9.23a	9.21a	9.18a	8.79b	8.79b
60% S/1500 ppm SO_2/D_2	10.96a	10.88a	10.56a	10.42a	10.12a	9.38b	9.18b
60% S/2500 ppm SO_2/D_3	10.53a	10.59a	10.60a	10.52a	10.28a	10.23a	10.07a
2500 ppm SO_2/D_2	10.42a	10.37a	10.29a	9.73ab	9.31b	9.74ab	10.44a
40% S/60°C B/2500 ppm SO_2/D_2	8.46ba	8.72a	8.72a	8.55a	8.40a	8.39a	8.45a
40% S/60°C B/2500 ppm SO_2/D_3	9.17a	8.46b	8.50b	8.79ab	8.12b	9.39a	9.34a
60% S/60°C B/2500 ppm SO_2/D_2	8.75a	8.70a	8.80a	8.90a	8.98a	9.12a	9.32a
40% S/Steam B/1500 ppm SO_2/D_1	9.66a	9.22ab	9.05ab	8.86b	8.67b	8.39b	8.45b
60% S/Steam B/2500 ppm SO_2/D_2	9.24a	8.97a	8.92a	8.80a	8.79a	8.36a	8.52a
60% S/Steam B/2500 ppm SO_2/D_3	8.97b	9.23a	9.18a	9.14a	9.16a	9.09a	9.05a
2500 ppm SO_2/D_3	9.21a	9.17b	9.09b	9.19ab	9.23a	9.30a	9.36a
40% S/D_2	10.14b	10.11b	9.90b	8.68a	11.43c	13.47d	15.05e
Steam B/D_1	11.93c	11.76d	11.58d	11.29d	12.06c	14.10b	16.87a
Control/D_2	14.67d	14.58d	14.24d	14.13d	15.52c	16.35b	17.42a

S-Sucrose; B-Blanching; D-Drying. Each value represents mean scores of three replicates. *-Significant difference at <0.05%
Mean values having the same letter within row are not significantly different at <0.05%

food during drying. The sucrose osmosis pre-treatment did not show much protective effect on the ascorbic acid loss, while the blanching pre-treatment showed a better retention of ascorbic acid content, which was due to the earlier observed protective effect of blanching pre-treatment on ascorbic acid content of food (Levi *et al*., 1980). The SO_2 pre-treatment exhibited the greatest protection on ascorbic acid content which is in line with earlier reports of Levi *et al*. (1980) and Karim (2005).

Also noticed on the result is the influence of the dehydration condition of time and temperature. Drying of fruit between 8-10 h using 60-70°C temperature is been reported to be adequate for good product quality (Karathanos and Belessiotisi 1997; Jayaraman and Das-Gupta, 1992; Maskan, 2001). This might have influenced the rate of moisture and ascorbic acid removal and supported the differences observed in the values of the samples pre-treated under the same condition.

The result of moisture content of pre-treated and dehydrated pineapple slices during 18 weeks storage is presented in Table 2. The results revealed that there was continuous reduction in moisture content during the early weeks of storage. However, after 9 weeks of storage, the moisture content increased. The results therefore showed that there were significant differences ($p<0.05$) between the mean values of the samples at different week of storage. The sample pre-treated with 2500 ppm SO_2 had the highest retention of moisture content with no significant difference ($p<0.05$) between the mean values across the storage weeks. While the samples pre-treated with 2500 ppm SO_2 at drying condition of 70°C/10 h and 80°C/8 h had 9.36 and 8.45% respectively at the end of drying. Maximum moisture and ascorbic acid content retention recorded in samples pre-treated with SO_2 may be due to the protective effect of SO_2 on the moisture content during storage (Bhardwaj and Kaushal, 1990). The effect was also complemented with sucrose-osmosis pre-treatment. The variation in moisture content might also be influenced with the air-impermeable polyethylene bag used for packaging.

The trend of ascorbic acid losses (Table 3) during storage clearly supported the fact that, it is not a stable component during storage. The result is likened to the earlier findings by Atkinson and Strachan (1962) on the uses of SO_2 for food preservation. Sapers (1993), Sapers *et al*. (1994) also showed that SO_2 for protection of vitamins and organic acids of fruits and vegetables cannot be nullified.

The result indicated that as the storage period increased the level of ascorbic acid reduced. This was also noticed with the significant differences ($p<0.05$) between the values obtained across the storage period. The variation in moisture and ascorbic acid content might also be influenced with the air-impermeable polyethylene bag used for packaging.

It has been reported that it does not provide good barrier against moisture loss or gain (Jenkins and Harrington, 1991). However, the samples pre-treated with SO_2 indicated a better retention of ascorbic than others. The sample pre-treated with 2500 ppm SO_2 had the highest value of 19.20 mg/100 g ascorbic acid at the end of 18 weeks storage.

Table 3: Effect of pre-treatment on ascorbic acid content of dehydrated pineapple slices

	Ascorbic acid content (mg/100 g)/Week						
Treatments	*0	*3	*6	*9	*12	*15	*18
Control/ D_1	5.54[a]	3.45[b]	2.17[c]	1.35	0.21	0.19	0.10
60% S/Steam B/D_1	8.48[a]	7.12[b]	5.62[c]	5.57	3.68	2.51	2.24
60% S/Steam B/D_2	9.05[a]	6.56[b]	5.54[c]	4.61	3.21	2.03	1.68
Steam B/2500 ppmSO_2/D_3	25.35[a]	23.56[b]	20.54[c]	16.61	18.21	12.03	11.68
40% S/2500 ppm SO_2/D_2	21.16[a]	19.12[b]	15.62[c]	15.57	13.68	12.11	11.41
60% S/1500 ppm SO_2/D_2	19.36[a]	17.22[b]	15.12[c]	14.23	12.93	10.48	9.12
60% S/2500 ppm SO_2/D_3	27.35[a]	26.03[b]	25.38[c]	22.61	21.32	19.28	18.52
2500 ppm SO_2/D_2	25.58[a]	23.91[b]	20.41[c]	19.39	18.71	17.91	15.67
40% S/60°C B/2500 ppm SO_2/D_2	25.12[a]	24.97[a]	24.75[a]	23.41	20.24	19.36	12.39
40% S/60°C B/2500 ppm SO_2/D_3	29.95[a]	24.61[b]	24.32[b]	23.12	22.43	20.21	17.21
60% S/60°C B/2500 ppm SO_2/D_2	25.16[a]	24.28[b]	23.84[b]	21.38	17.81	16.42	15.21
40% S/Steam B/1500 ppm SO_2/D_1	13.48[a]	12.88[a]	12.01[ab]	10.43	8.63	7.88	7.61
60% S/Steam B/2500 ppm SO_2/D_2	28.53[a]	27.79[a]	27.41[a]	26.36	23.22	22.25	16.03
60% S/Steam B/2500 ppm SO_2/D_3	27.78[a]	27.11[a]	26.01[b]	24.91	23.18	21.36	18.43
2500 ppm SO_2/D_3	25.58[a]	23.95[b]	22.31[c]	21.42	20.61	19.53	19.20
40% S/D_2	5.97[a]	3.32[b]	1.72[c]	1.08	0.84	0.72	0.62
Control/D_2	6.56[a]	5.85[b]	3.13[c]	1.33	0.77	0.74	0.62
Steam B/D_1	10.48[a]	6.65[b]	3.67[c]	1.64	1.02	0.87	0.81

S-Sucrose; B-Blanching; D-Drying. Each value represents mean scores of three replicates. *-Significant difference at p>0.05

Conclusion: The study could be concluded with the following findings that blanching pre-treatment alone was not found suitable for the drying of pineapple slices, while sulphiting pre-treatment protected the moisture and ascorbic acid loss during storage. Also pre-treatment with 60%S/2500 ppmSO_2 and 2500 ppm SO_2 and drying condition of 70°C/10 h will be suitable for production of dehydrated pineapple slices. Thus, this study suggests that production of pre-treated dried pineapple slices could be encouraged as a means of reducing the high post-harvest losses of pineapple fruit. This could be an alternative way of boosting food and nutrition security in Africa.

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Evaluation of Protein Quality of Unfermented and Fermented Blends of Cereal Based Complementary Food Using Rats

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Abstract: Weanling male rats of 45-55 g were used to compare the protein quality of the unfermented and fermented blends of cereal based complementary food. Paddy rice, parboiled rice, soybean and crayfish were obtained from Jos main market, Nigeria. The paddy rice malted for 72 h and all the foodstuffs were processed into flours. Parboiled rice and soybean mix was formulated in a standard ratio of 70:30 g (PR:DSB_O). A modified standard formulation of parboiled rice, soybean, malted rice and crayfish mix in the ratio of 65:25:5:5 g (PR:DSB:MR_{72}:CFo) was made. From the formulation fermentation of different blends at varying periods 24, 48, 72, 96 and 120 h was carried out. Protein content of the unfermented and fermented blends was determined by the standard assay technique. Seven different diets were fed. Five rats were assigned to each diet for 28-days growth studies and a 7-day N balance period. The rats fed the unfermented modified standard (PR:DSB:MR_{72}:CFo) blend had the highest food intake, weight gain, N intake, retained N, BV, and NPU values (69 g, 27 g, 2.96 g, 2.23 g, 83.5 and 75.47, respectively) than the rest of the groups. The result appeared to suggest that fermentation affected the protein quality of the fermented blends negatively making the protein quality of the unfermented modified standard (PR:DSB:MR_{72}:CFo) blend the best.

Key words: Fermentation, malting, rats complementary

INTRODUCTION

Fermentation has been used for several thousand years as an effective and low cost means to preserve the quality and safety of foods (Parveen and Hafiz, 2003). In opinion of many consumers, fermentation of foods makes the nutrients more readily available than the unfermented. There is quite a lot of evidence suggesting that fermentation enhanced micronutrients and amino acids (Odumodu, 2007; Odumodu, 2008, respectively) than the unfermented foods. However, there are almost no published papers on the animal feeding trials comparing nutrients content of unfermented and fermented foods.

The major objective of this study was to determine the protein quality of the unfermented and fermented blends of the cereal based complementary food.

MATERIALS AND METHODS

A 28 day study was conducted. It consisted of a 21 day growth period, and a 7 day Nitrogen (N) balance period.

Animal and housing: Thirty five male albino weanling rats (supplied by the Animal House, University of Jos), (45-55 g) were divided into seven groups of five rats each on the basis of body weight. The rats were weighed prior to access to the test diets and at weekly intervals to determine weight gain. The animals were housed in individual metabolism cages and fed the diets and tap water ad libitum for 28 days. The cages were of the stainless steel and screen-bottom type equipped to separate urine and feces (Waham ann Laboratory Animal Cages, Timonium, MD 21093, USA).

Diets: Paddy and parboiled rice (*Oryza-sativa)*, soybean (*Glycine max L*) and crayfish (*Astacus fluviatilis)* were purchased from Jos Main Market. The grains and crayfish were manually cleaned to remove foreign materials.

Production of Amylase Rich Flour (ARF): Amylase rich flour was produced by washing 1 kg raw rice grain in 5% (w/v) sodium chloride to prevent growth of mould. It was soaked in tap water in a ratio of 1:3 (w/v), grains to water for 12 h. The grains were spread on a wet jute bag in a basket, covered with a moist muslin cloth and allowed to germinate for 72 h at room temperature (30±3°C). The grains were watered at regular intervals of 12 h. The germinated grains were dried at 80°C for 24 h, devegetated by rubbing between palms, winnowed and dehulled mechanically. The malt was milled in a laboratory hammer mill to fine flour (300 µm mesh screen) and packaged in a low density name labeled polyethylene bag. It was placed in a plastic bucket with a lid and stored in a deep freezer (-18°C) for subsequent use.

Production of soybean, parboiled rice and crayfish: One kilogram of raw soybean was placed in 20 litres of boiling water containing 50.0 g sodium bicarbonate. The soybean was boiled for 10 min and the water drained off.

It was dried in the oven at 80°C for 24 h and dehulled mechanically using laboratory hammer mill.

Five kilograms parboiled rice was washed in tap water and allowed to drain water. It was dried in the oven at 80°C for 24 h. One kilogram of crayfish was measured, dried in an oven at 95°C for 50 min.

Each of the foodstuffs was separately milled in a laboratory hammer mill into a fine flour (300 micrometer mesh screen) and packaged separately in a low density name labeled polyethylene bag. The bags were placed in plastic buckets with covers and stored in a deep freezer at -18°C for analysis.

Formulation of rice-soybean mix 70:30 g ratio (FAO/WHO/UNU, 1985): Rice-soybean mix (70:30 g w/w) was formulated and thoroughly mixed using a laboratory hammer mill to ensure evenness. It was packed in a low density name labeled polyethylene bag and stored in a deep freezer (-18°C) for analysis.

Dough preparation using parboiled rice, dehulled soybean, malted rice and crayfish mix (65:25:5:5 g w/w ratio) fermented at varying periods: A blend of parboiled rice (65 g), soybean (25 g), malted rice (5 g) and crayfish (5 g) flour was prepared and divided into six equal parts. Five parts were fermented for 24, 48, 72, 96 and 120 h, respectively, after mixing with tap water to form a dough by bringing the moisture content to 50%. The other part served as a control. At the end of each fermentation time the blend was taken out and dried at 80°C in the oven for 24 h. The dried blend was remilled in a laboratory mill to fine flour and packaged in low density name labeled polyethylene bag. The bags were placed in plastic buckets with covers and stored in the deep freezer (-18°C) for analysis.

Laboratory analysis: Carmine red was fed on the morning of day 21 and day 28. Coloured feces appeared beginning on days 22 and 29. The coloured feces excreted on day 22 were included in the pool fecal sample and those excreted on day 29 were excluded. Urine was collected from 7.00am of day 22 through the morning of day 29 (7days) and food consumption was recorded for the same 7 day period. Hydrochloric acid (0.1 N) (0.5 ml) was used as a preservative to the pooled urine samples for each group. The urine samples were made to a volume of 200 ml with distilled water and refrigerated until analyzed for nitrogen. Individual fecal collection was dried at 85°C for 3 h, weighed and ground into a fine powder.

Statistical analysis: All data collected were statistically analyzed using analysis of variance and Duncan's new multiple range test as described by Steel and Torrie (1960). Significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION

The crude protein contents of the blends used in this study as sources of N were as follows; PR:DSBo 20.81 g; PR:DSB:MR_{72}:CFo 21.78 g; PR:DSB:MR_{72}:CF_{24} 22.42 g; PR:DSB:MR_{72}:CF_{48} 23.23 g; PR:DSB:MR_{72}:CF_{72} 22.95 g; PR:DSB:MR_{72}:CF_{96} 22.96 g and PR:DSB:MR_{72}:CF_{120} 23.48 g. Values obtained for food intake, weight grain, N intake, fecal N, urinary N, retained N, Biological Value (BV) and Net Protein Utilization (NPU) of the rats are presented in Table 1. The PR:DSBo and the PR:DSB:MR_{72}:CFo were the unfermented standard and modified standard blends, respectively. The food intake, weight gain and N intake of the group of rats fed the PR:DSB:MR_{72}:CFo blend were highest (69.00, 27.00 and 2.96g, respectively) and that of rats fed the PR:DSB:MR_{72}:CF_{120} blend were the lowest (24.00, 6.00 and 0.90 g, respectively). There were significant differences ($p<0.05$) in food intake, weight gain and N intake values between the groups of rats fed the standard and modified standard blends. The differences could be attributed to effects of the malted rice and crayfish supplements which improved the flavour, aroma and palatability of the PR:DSB:MR_{72}:CFo blend as well as improved the essential amino acid pattern of the blend. The improved pattern of the essential amino acids was utilized by the rats for the synthesis of tissue protein. There were significant differences ($p<0.05$) in food intake, weight gain and N intake values between the group of rats fed the PR:DSB:MR_{72}:CFo blend and all the groups fed the fermented blends except for N intake values for the PR:DSB:MR_{72}:CF_{24} and the PR:DSB:MR_{72}:CF_{96} blends that were insignificantly different ($p>0.05$). The differences could be as a result of (a) effect of fermentation which reduced the flavour, aroma and palatability of the blends therefore decreasing the food intake of the rats. Odumodu (2008) reported that prolonged fermentation reduced the sweetness of the blends to sourness. (b) leaching of amino acids into the fermentation media as well as utilization of some of the amino acids by the micro-organisms for metabolic activities which resulted in low weight gain of the rats.

The group of rats fed the modified standard (PR:DSB:MR_{72}:CFo) blend had similar fecal nitrogen, higher urinary and retained nitrogen (0.26, 0.47 and 2.23 g, respectively) than the group fed the standard (PR:DSBo) blend (0.22, 0.34 and 0.57 g respectively). This could be as result of higher protein content (21.78 g) of the blend and food intake of the rats (69 g) which resulted in higher nitrogen retention (2.23 g). On the other hand, the groups fed the PR:DSB:MR_{72}:CF_{24} and the PR:DSB:MR_{72}:CF_{120} blends had similar fecal and urinary nitrogen values except that the latter had negative nitrogen value (-0.11 g). The negative nitrogen value could be due to lower food intake (24.0 g).

Table 1: Food intake, weight gain, nitrogen balance of rats fed the blends

Blend treatment	Food intake (g)	Weight gain (g)	Nitrogen intake (g)	Fecal nitrogen (g)
PR:DSBo 70:30	34.0^{d}±3.5	14.8^{d}±1.0	1.13^{c}±0.18	0.22^{a}±0.03
PR:DSB:MR_{72}:CFo 65:25:5:5	69.0^{a}±5.8	27.0^{a}±1.30	2.96^{a}±0.3	0.26^{a}±0.06
PR:DSB:MR_{72}:CF_{24} 65:25:5:5	56.2^{b}±4.1	22.1^{b}±2.4	2.66^{a}±0.2	0.29^{a}±0.04
PR:DSB:MR_{72}:CF_{48} 65:25:5:5	50.2^{c}±43.6	18.0^{c}±0.9	1.87^{b}±0.2	0.30^{a}±0.03
PR:DSB:MR_{72}:CF_{72} 65:25:5:5	29.0^{e}±2.3	10.4^{e}±0.8	1.05^{c}±0.13	0.16^{b}±0.05
PR:DSB:MR_{72}:CF_{96} 65:25:5:5	55.8^{b}±5.6	14.2^{d}±0.73	2.05^{a}±0.1	2.28^{a}±0.04
PR:DSB:MR_{72}:CF_{120} 65:25:5:5	24.0^{f}±2.7	6.0^{f}±1.5	0.90^{d}±0.2	0.35^{a}±0.06
LSD	1.2	1.56	0.23	0.17
Blend treatment	Urinary nitrogen (g)	Retained nitrogen (g)	Apparent biological value	Net protein utilization
PR:DSBo 70:30	0.34^{c}±0.08	0.57^{d}±0.18	69.61^{e}±7.0	50^{d}.44±7.3
PR:DSB:MR_{72}:CFo 65:25:5:5	0.47^{b}±0.19	2.23^{a}±0.031	83.5^{a}±10.0	75.47^{a}±6.0
PR:DSB:MR_{72}:CF_{24} 65:25:5:5	0.62^{a}±0.07	1.75^{b}±0.19	67.38^{b}±8.3	65.79^{b}±5.0
PR:DSB:MR_{72}:CF_{48} 65:25:5:5	0.36^{c}±0.01	1.21^{c}±0.18	80.35^{b}±2.7	64.11^{c}±7.1
PR:DSB:MR_{72}:CF_{72} 65:25:5:5	0.29^{d}±0.05	0.60^{d}±0.11	72.33^{d}±10.7	57.14^{c}±4.2
PR:DSB:MR_{72}:CF_{96} 65:25:5:5	0.41^{b}±0.15	1.36^{b}±0.16	76.61^{c}±15.1	66.34^{b}±3.2
PR:DSB:MR_{72}:CF_{120} 65:25:5:5	0.66^{a}±0.05	-0.11^{e}±0.14	25.46^{g}±5.0	-12.22^{c}±0.1
LSD	0.14	0.47	2.15	2.21

Values with the same superscript in the column are not significantly difference (p>0.05). Values are means±standard deviations of triplicate determinations. LSD = Least Significant Difference.

PR:DSBo 70:30 = Parboiled rice 70% and 30% dehulled soybean (unfermented)

PR:DSB:MR_{72}:CFo = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (unfermented)

PR:DSB:MR_{72}:CF_{24} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 24 h)

PR:DSB:MR_{72}:CF_{48} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 48 h)

PR:DSB:MR_{72}:CF_{72} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 72 h)

PR:DSB:MR_{72}:CF_{96} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 96 h)

PR:DSB:MR_{72}:CF_{120} = Parboiled rice 65%, dehulled soybean 25%, malted rice (72 h) 5% and crayfish 5% (all fermented for 120 h)

The BV value for the group fed the modified standard (PR:DSB:MR_{72}:CFo) blend was higher (83.5) than the value for the standard (PR:DSBo) blend (69.61). This revealed the protein of the blend to be superior than the standard blend. In the same vein the group fed the PR:DSB:MR_{72}:CF_{24} had higher BV value (80.35) than the rest of the groups fed the fermented blends. This also suggested the PR:DSB:MR_{72}:CF_{24} blend to be more superior than the rest of the fermented blends.

The group of rats fed the modified standard (PR:DSB:MR_{72}:CFo) blend had the highest NPU value (75.47). The groups fed the PR:DSB:MR_{72}:CF_{24}, the PR:DSB:MR_{72}:CF_{48} and the PR:DSB:MR_{72}:CF_{96} blends had similar NPU values (65.79, vs 64.11 vs 66.34) while the group fed the PR:DSB:MR_{72}:CF_{120} blend had a negative value (-12.22).

The higher NPU value for the group fed the PR:DSB:MR_{72}:CFo could be due to lower fecal and urinary N excretion and higher N retention. The similar NPU values for the groups fed the PR:DSB:MR_{72}:CF_{24} and the PR:DSB:MR_{72}:CF_{96} blends could be attributed to their comparable nitrogen retention values (1.75 vs 1.36). The negative NPU value for the group fed the PR:DSB:MR_{72}:CF_{120} blends could be as a result of low food intake and nitrogen retention.

Conclusion: The group of rats fed the PR:DSB:MR_{72}:CFo blend had higher food intake, weight gain and N intake than the rest of the groups. The higher retained N, BV and NPU values for the same group revealed the blend to have the best protein quality than the rest of the blends. However the PR:DSB:MR_{72}:CF_{24} blend fermented for 24 h was the second best.

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Pasting Characteristics of Wheat and Sweet Potato Flour Blends

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Abstract: This study was conducted to investigate the pasting characteristics of wheat and sweet potato tuber (*Ipomea batatas*) flour blends. The sweet potato tubers were bought from a local farm in Offa, Kwara State. The tubers were thoroughly sorted, washed, peeled, sliced, balanced, drained, sundried and milled into flour. Commercial wheat flour was used and purchased from Igbona market in Osogbo, Osun State of Nigeria. The wheat and sweet potato flour were blended using the following ratios (WF : SPF : 100 : 0, 90 : 10, 85 : 15, 80 : 20, 75 : 25, 0 : 100). These samples were thereafter subjected to pasting characteristics analysis. The results revealed that as more and more sweet potato flour was added to wheat flour there was improvement in the pasting properties.

Key words: Wheat, sweet potato, flour pasting characteristics

INTRODUCTION

Flour is fine powder made from cereals. It can equally be produced from other starch based produce such as cassava, maize, yam, nuts etc. Flour is the major ingredient in bakery goods production and most especially bread which constitutes a staple in the diet of many countries. The availability of adequate supply of flour has often been a major economic and political issue. Flour produced from non wheat sources are otherwise known as composite flour.

Wheat as the chief raw material in the production of wheat flour cannot thrive or survive in Nigeria soil cum weather conditions prevalent in our region therefore wheat flour has to be imported. This lead to relatively high prize of the commodity with the overall effect on the price of bakery goods. Attempt to incorporate composite flour into wheat flour resulted in net saving of income and reduced cost of bakery goods. Cassava flour has been successfully blended with wheat flour up to 15% with acceptable result.

Attempts to improve the chemical and sensory qualities of composite flour before their utilization especially instant yam flour has been reported by various authors (Ngoddy and Onuoha, 1983; Ofi, 1983; Sanni *et al*., 2006). These flour has been incorporated with wheat flour in production of bakery goods such as cookies, bread and cake. Through this, diversified, reduced cost of production and acceptable products can be produced. Since cassava flour has been successfully incorporated with wheat flour this work therefore investigates the utilization of underutilized root especially sweet potato.

Composite flours have their properties that enhance their wide utilization which include water and oil absorption capacity, foaming capacity foam stability, bulk density, gelation capacity, emulsion capacity etc. (Adeyeye *et al*., 1994; Abbey and Ibeh, 1988). Incorporation of sweet potato flour into wheat flour for bakery goods production is expected to produce effect in both the functional properties and pasting characteristics of the blended samples. Several studies have indicated the possibility of incorporating hulless barley, soya beans, sorghum, cowpea flour into wheat flour at various levels and the rheological and baking properties have been reported (Oftmann and Garba, 1997; Kinsella, 1979; Sathe and Salekhe, 1981).

This paper investigates the pasting properties of wheat and sweet potato flour blends. The two flour were blended using the following blend ratios (WF : SPF : 100 : 0, 90 : 10, 85 : 15, 80 : 20, 75 : 25, 0 : 100).

MATERIALS AND METHODS

The sweet potato tubers used for this work were bought from a local farm in Offa Kwara state Nigeria. Commercial wheat flour was purchased at Igbona market in Osogbo, Osun State Nigeria. Equipment used include milling machine, mechanical sieve, oven, desicators, balance, stirrer and Rapid Visco Analyzer (RVA). Other materials used include knife, water, bowls and napkin.

Production of sweet potato flour: The sweet potato tubers were thoroughly sorted to remove bad ones from the lot. The sorted tubers were washed to remove adhering soil, dirts and extraneous matters. This was followed with peeling. After peeling, the tuber were sliced to facilitate rate of drying and ease milling operation. The sliced tubers were blanched at 60°C for 15 min in order to inactive enzymes that may cause browning reaction. These were then cooled, drained and followed by drying. After blanching, the chips were spread out uniformly on a stainless steel perforated tray and dried in cabinet dryer at 65°C for eight hours. Following drying, the sliced tubers were milled, sieved into fine flour and packaged for further use.

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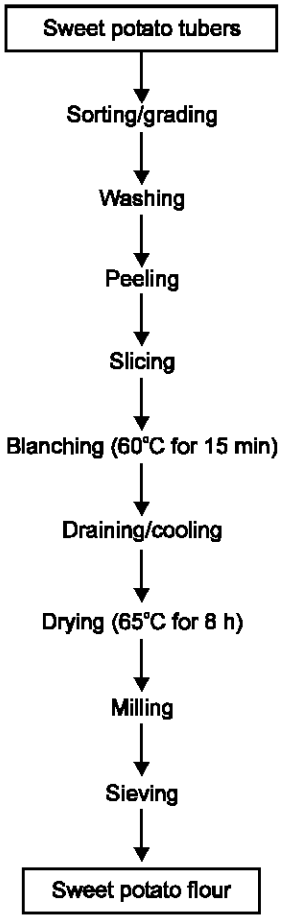

Fig. 1: Flour chart of sweet potato flour production

Sample codes and designations

Sample codes	Designation
A	100% WF
B	90% WF : 10% SPF
C	85% WF : 15% SPF
D	80% WF : 20% SPF
E	75% WF : 25% SPF
F	100% SPF

WF = Wheat Flour; SPF = Sweet Potato Flour

Pasting characteristics analysis: Pasting properties were determined using a Rapid Visco Analyzer (RVA) (Model RVA 3D +; Network Scientific, 5 Australia) described by Adebowale *et al.* (2005).

RESULTS AND DISCUSSION

Result of pasting properties of wheat and sweet potato flour blends: The results of pasting properties of wheat and sweet potato flour blends are as presented in Table 1. When starch-based foods are heated in an aqueous environment, they undergo a series of changes known as gelatinization and pasting. These are two of the most important properties that influence quality and aesthetic considerations in the food industry, since they affect texture and digestibility as well as the end use of starchy foods (Adebowale *et al.*, 2005). Peak viscosity, which is the ability of starch to swell freely before their physical breakdown (Sanni *et al.*, 2004) ranged from 131.42-271.08 RVU. Hundred percent sweet potato flour recorded the highest value of 271.08 RVU. High peak viscosity is an indication of high starch content (Osungbaro, 1990). It is also related to the water binding capacity of starch (Adebowale *et al.*, 2005). The high peak viscosity displayed by 100% SPF implies that the flour may be suitable for products requiring high gel strength and elasticity (Adebowale *et al.*, 2005). As more and more Sweet Potato Flour (SPF) was added to Wheat Flour (WF), the peak viscosity is on the increase. The trough, which is the minimum viscosity value in the constant temperature phase of the RVA profile and measures the ability of paste to withstand breakdown during cooling ranged between 86.42 and 161.08 RVU. 100% SPF had the highest value of 161.08 RVU. 100% WF recorded the lowest value of 86.42 RVU. As more and more SPF was incorporated, the trough value was on the increase. Among the blends, sample with 75% WF and 25% SPF had the highest trough value of 105.42 RVU. The breakdown viscosity value is an index of the stability of starch (Fernande and Berry, 1989). The value for the breakdown viscosity ranged between 45.00 and 110.00 RVU. 100% SPF recorded the highest value suggesting higher stability of starch. The final viscosity, which is the change in the viscosity after holding cooked starch at 50°C ranged from 166.67-246.33. Final viscosity is the most commonly used parameter to define the quality of a particular starch-based sample,

Table 1: Result of pasting properties of wheat and sweet potato flour blends

Sample	PV (RVU)	T1 (RVU)	B (RVU)	FV (RVU)	S (RVU)	PT (min)	PT (°C)
100% WF	131.42	86.42	45.00	166.67	80.25	6.00	82.35
90% WF : 10% SPF	135.58	87.50	48.08	173.75	86.25	5.80	84.45
85% WF : 15% SPF	144.83	90.00	54.88	185.00	95.00	5.53	81.54
80% WF : 20% SPF	142.17	94.50	47.67	179.33	84.83	5.47	84.25
75% WF : 25% SPF	150.58	105.42	45.17	187.33	81.92	5.73	83.35
100% SPF	271.08	161.08	110.00	246.33	85.25	4.33	80.90

Key, WF : Wheat Flour; SPF : Sweet Potato Flour.
PV = Peak Viscosity (RVU) T1 = Trough 1 (RVU) B = Breakdown (RVU) FV = Final Viscosity (RVU)
S = Setback (RVU) PT (min) = Peak Time (min) PT (°C) = Pasting Temperature (°C)

as it indicates the ability of the material to form a viscous paste or gel after cooking and cooling as well as the resistance of the paste to shear force during stirring (Adeyemi and Idowu, 1990). As more and more SPF was added to WF, the final viscosity was on the increase suggesting higher resistance of past to shear force during stirring. The setback value of 100% SPF was 85.25 RVU and it is higher than that of 100% WF (80.25 RVU). The higher the setback value, the lower the retrogradation during cooling and the lower the staling rate of the products made from the flour (Adeyemi and Idowu, 1990). The introduction of SPF into WF for bakery goods production thus resulted in increase in setback value as more and more SPF was added to WF. This will therefore reduce the retrogradation and stalling rate of bakery goods produced from their blends. The peak time, which is a measure of the cooking time, ranged between 4.33-6.00 min. 100% WF recorded the highest value of 6.00 min suggesting more processing time. SPF recorded the lowest value for peak time (4.33 min). As more and more SPF was added to the WF, the processing time for the blended samples was on the decrease. Blending of WF with SPF will therefore lead to a significant reduction in cooking or processing time.

The pasting temperature for 100% WF was 82.35°C while it was 80.90°C for 100% SPF. Pasting temperature gives an indication of the gelatinization time during processing. It is the temperature at which the first detectable increase in viscosity is measured and it is an index characterized by the initial change due to the swelling of starch (Eniola and Delarosa, 1981). Pasting temperature has been reported to relate to water binding capacity. A higher pasting temperature implies higher water binding capacity, higher gelatinization and lower swelling property of starch due to a high degree of association between starch granules (Eniola and Delarosa, 1981; Numfor *et al*., 1996).

Conclusion: The study showed that blending Wheat Flour (WF) with Sweet Potato Flour (SPF) improved the pasting properties of the blending samples. There was reduction in the retrogradation and staling rate, reduction in processing time, higher starch stability, reduced water holding capacity and longer shelf life of bakery products produced from the blends. Therefore SPF which can easily be processed from available raw material (Iponea batatss) will go a long way in saving cost of production and improved product quality.

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Performance of Finisher Broiler Chicks Fed Varying Replacement Levels of *Chromolaena odorata* Leaf for Soyabean Meal

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Abstract: Sixty 5-week old Marshall broiler chicks were used in a 28-day feeding trial in a Completely Randomized Design, in a deep litter house to evaluate the effect of *Chromolaena odorata* Leaf Meal (COLM) on the growth performance of finisher broiler chicks. The chicks were grouped into four treatments having 0, 2.5, 5 and 7.5% COLM which were replicated thrice. Feed and water supply were *ad libitum*. Also medication, vaccination scrupulous sanitation, regular disinfection of the pens and other standard management practices were adopted. Initial weights of the birds, 633.00, 636.67, 630.00 and 585.67 g for treatments 0, 2.5, 5 and 7.5% COLM respectively did not vary significantly ($p>0.05$). However, significant differences ($p<0.05$) occurred between the final weights 2120,00, 2096.67, 2003.33 and 1506.67 g for treatments 0, 2.5, 5.0 and 7.5% COLM respectively. Daily weight gain, daily feed intake and feed conversion ratio, showed similar trends for birds on 0, 2.5 and 5.0% COLM which were similar in values ($p>0.05$) but differed significantly ($p<0.05$) from the values for birds on 7.5% COLM. Results confirmed that COLM could substitute soya bean as feed ingredient for broiler chicks up to 7.5% but 5.0% is optimal.

Key words: *Chromolaena odorata*, leaf meal, soya bean, finisher broiler, performance

INTRODUCTION

Animal protein demand and supply gap among Nigerians has continued to widen, resulting in sub-optimal animal protein intake and consequently pre-disposing the people to deplorable animal protein malnutrition. This nutritional adversity is traceable to geometric increase in the Nigerian human population without a commensurate increase in livestock production, over dependence on imported livestock products, ignorance, inadequate technical skills, diseases and parasites, environmental stress and high cost of ingredients used to formulate monogastric livestock feeds. Also, the increasing awareness of people on the need for animal protein intake further increases their demand.

Similarly, the competition between man and livestock for feed grains (Tegbe *et al*., 1984 and Madubuike, 1992) and inadequate production of farm crops to meet human and livestock needs (Babatunde *et al*., 1990) have been implicated as remote causes of poor animal protein intake among Nigerians. Consequently, research reports show that feed is a critical input in monogastric livestock production as it constitutes 70-80% of cost of monogastric production (Madubuike and Ekenyem, 2001). It becomes imperative to identify means of reducing the cost of feed items with a view to reducing the cost of livestock products and by extension make them available to and affordable by consumers.

Efforts have been made to reduce the dependence on conventional sources of protein e.g. soyabean and groundnut cake which are major protein sources and maize which is the major energy source as their prices have continued to gallop, resulting from competition of monogastric livestock with man for these materials. Leaves of many legumes, shrubs and weeds used in feeding monogastric livestock reduced their cost of production. For instance, *Leucaena leucocephala* (Udedibie and Igwe, 1989), *Microdesmis puberula* (Esonu *et al*., 2003), *Chromolaena odorata* (Ekenyem *et al*., 2009), *Ipomoea asarifolia* (Ekenyem, 2004), *Alchornia mudiflora* (Opara, 1996) fed the leaves of the various plants on monogastric animals and reduced their cost of production. However, the effective utilization and overall digestibility of the leaves are low as a result of the presence of anti-nutritional factors and high fibre contents (Cheek and Myer, 1975), causing depressed feed intake, poor growth and production of watery droppings (Onwudike and Oke, 1998).

Chromolaena odorata commonly called siam weed is a perennial shrub which belongs to the family *Austeracea*. The plant can grow up to 7 m tall and has white to mauve flowers. The leaves have opposite arrangement, triangular in shape with three conspicuous main veins which are coarsely toothed. It is a prolific seeder (Pink, 2004). In Nigeria, the siam weed is a stubborn weed which smoulders crops in the farm.

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It is plenty in supply and cost little or nothing to procure and prepare as feed material. This feeding trial was carried out to replace the costly soyabean with the readily available low-cost *chromolaena odorata* leaf as a means of reducing the cost of finisher broiler production, making the products affordable by consumers. Thus with 16.67% crude protein and 10.07% crude fibre, COLM appears to have the capacity to partially replace soya bean as a protein source in broiler production.

MATERIALS AND METHODS

Location of the experiment: The experiment was carried out at the Imo State University, Teaching and Research Farm, Owerri, Nigeria, located on Longitudes 07^{o} 01^{1} 06^{11}E and 7^{o} 03^{1} 00^{11}E and latitudes 5^{o} 28^{1} 24^{11} N and 5^{o} 30^{1} 00^{11} N in the humid tropical region of West Africa.

Collection and preparation of *Chromolaena odorata* leaf meal: The *chromolaena odorata* leaf meal was harvested from stems of maturing *Chromolaena odorata* plants (before flowering). The leaves were hand-plucked from stems and put directly into jute bags and later spread out on concrete floor for sun-drying until they become crispy but still retained their green colour. The dried leaves were then milled. A sample of the milled leaves was sent to the Animal Nutrition Laboratory of the Federal University of Technology, Owerri for proximate analysis using the standard methods of AOAC (1995), to determine ash content, crude fibre, crude protein, ether extract, nitrogen free extract and moisture content (Table 1).

Table 1: Results of proximate analysis of COLM

Nutrient	Composition (%)
Crude fibre	10.07
Crude protein	16.67
Ash	8.58
Ether extract	1.87
Nitrogen free extract	54.47
Moisture content	8.65

Formulation of experimental diet: The result of the proximate analysis of COLM was the basis for formulation of the experimental diets. COLM was incorporated at levels 0, 2.5, 5.0 and 7.5% for treatments 1, 2, 3, 4 respectively to replace soyabean weight for weight. Each treatment diet was fortified with vitamin premix, methionine and lysine (Table 2).

Experimental birds: Sixty 4 weeks old Marshall broiler chicks were selected from a brooding stock and allotted to four treatment groups in a deep litter house, which was further divided into three replicates in a completely randomized design.

Management of experimental birds: The birds were fed with commercial finisher broiler diets (top feed) for one week to stabilize them before introducing the experimental diets.

The birds were fed *ad libitum* and supplied with potable water usually between 700-800 h for morning and 1700-1800 h for evening. Record of feed intake was always taken by subtracting the feed left over from the feed supplied the previous day before daily feeding. Vaccination programme against new castle, disease, gumboro, fowl pox and marex diseases were applied appropriately while medication, scrupulous sanitation and disinfection and regular replacement of dirty litter were observed in the properly ventilated deep litter house. The zinc roofing and concrete floor provide adequate shade and ease of drainage and cleaning respectively.

Experimental design: The experimental design used for the experiment was completely randomized design in which sixty 4 week old Anak broiler chicks were allotted to four dietary treatments each having three replicates in a deep litter house.

Data collection and analysis: After one week stabilization, data collection started at the 5^{th} week of their age. Initial weight was taken per replicate at the start of the experiment using salter weighing scale. Live weights were also measured weekly to the end of the experiment to determine their final weight. The weight gain was calculated by subtracting the initial weight from the final weights of the birds. The daily feed intake was calculated by subtracting feed left over from the quantity supplied daily. Feed conversion ratio was calculated by dividing the daily feed intake by the daily weight gain. Feed cost per kg feed was calculated as the total cost of all ingredients included to make 1 kg of each diet.

All data from the experiment were subjected to one way analysis of variance (Steel and Torrie, 1980), while the differences between the treatment means were compared using Duncan's multiple range test as outlined by (Onuh and Igwenma, 1998).

RESULTS

The results of performance characteristics of the experimental birds are shown in Table 3. Initial weights were statistically similar ($p<0.05$). The final weights of birds on 0, 2.5 and 5% COLM were significantly higher ($p<0.05$) than those on 7.5%COLM. Also the results of the daily feed intake shows that birds on 0% and 2.5 COLM consumed equal amount of feed within the period of the experiment, while birds on 5% COLM took less feed ($p<0.05$) than those 0 and 2.5% COLM. And durther less was taken by birds fed 7.5% COLM. Feed conversion ratio was similar ($p>0.05$) in treatments 0, 2.5 and 5% COLM but superior in that of 7.5% COLM.

Table 2: Ingredient composition of experimental diet treatment

Ingredient	T_1 (0% COLM)	T_2 (2.5% COLM)	T_3 (5.0% COLM)	T_4 (7.5% COLM)
Maize	45.00	45.00	45.00	42.00
Soyabean	7.50	5.00	2.50	0.00
Leaf meal (COLM)	0.00	2.50	5.00	7.50
PKC	12.00	12.00	12.00	12.00
Fish meal COLM	5.00	5.00	5.00	5.00
CONC	14.80	14.80	14.80	14.80
BSG	8.00	8.00	8.00	8.00
Bone Meal	7.00	7.00	7.00	7.00
Common Salt	0.30	0.30	0.30	0.30
Premix	0.25	0.02	0.25	0.02
L-lysine	0.09	0.09	0.09	0.09
Methionine	0.06	0.06	0.06	0.06
Total	100.00	100.00	100.00	100.00
Crude protein (%)	20.72	1998.00	19.09	18.49
ME (Kcal/kg)	2805.43	2783.33	2779.23	2757.13
Crude fibre (%)	5.49	5.79	6.12	6.49
Ether extract (%)	5.06	5.03	4.99	4.95

Table 3: Performance of finisher broiler chicks fed varying levels of *Chromolaena odorata* leaf meal

Parameter	0% COLM	2.5% COLM	5.0% COLM	7.5% COLM	SEM
Initial weight (g)	633.00	636.67	630.00	585.67	57.11
Final weight (g)	2120.00	2096.67	2003.33	1506.67	74.89
Daily feed intake (g)	161.67	161.67	151.00	134.67	11.45
Weight gain					
Daily weight	53.11	52.14	49.05	32.86	0.02
Feed conversion ratio	3.04	3.10	3.08	4.10	
Fed cost/kg (N)	58.84	56.89	54.33	52.08	2.28

Means with different superscript in the same horizontal row are significantly different ($p<0.05$)

Feed cost per kg feed was significantly lowest ($p<0.05$) on birds fed 7.5% COLM.

DISCUSSION

The similar ($p>0.05$) initial weights of all the experimental birds was to reduce bias which could arise from using birds of different weights and to further guarantee the reliability of the results. The final live stocks of the birds on 0, 2.5 and 5% COLM were similar ($p>0.05$) but significantly ($p<0.05$) varied with those birds on 7.5% COLM. This suggests high level of acceptability of the leaf meal. It further assures the high nutritional profile of the leaf meal as well as its ability to effectively replace soya bean, producing comparable results. However, the significant ($p<0.05$) differences with the birds fed in 7.5% of inculcate of the fact that the fibre level has reached a point where the birds can still make the efficiency of the feed utilization of the advantage of 7.5% COLM. Similar cases of depressed growth with increasing levels of feed with higher fibre levels leaf meal are documented (D'Mello *et al*., 1987; Ekenyem, 2004) the feed intake of the birds were highest among birds on 0, 2.5 and 5% COLM which were similar ($p>0.05$) but differed ($p<0.05$) with birds also on 7.5% which fed the least. This difference could be attributed to decrease in palatability of the feed as the levels of the leaf meal increased. This depressed body weight could be attributed to lower feed intake arising from higher fibre content of the leaf meal which caused the insufficient consumption of digestible nutrients particularly protein and energy required to sustain rapid growth.

Also the daily weight gain was statistically similar among birds on 0, 2.5 and 55% COLM while birds on 7.5% COLM has significantly least ($p<0.05$) gain. Weight is usually an indication of the efficiency of feed utilization and the nutritional safety level. This result confirms earlier reports that *Chromolaena odorata* leaves contain safe levels of toxic factors that could impede feed utilization (Biller *et al*., 1994; Irobi, 1997). However the lower in feed intake with increasing levels of COLM 5 and 7.5% groups could also be attributed to anti-nutritional factors inherent in the plant (Nwokolo, 1987) and also agrees with Onwudike and Oke (1998) who worked on alfalfa leaf meal.

The feed conversion ratio was similar ($p>0.05$) for birds on 0, 2.5 and 5% COLM but significantly differed from birds on 7.5% COLM. It must also have contributed to the levels of weight gain observed away the experimental birds. However, the feed conversion ratio for birds on 7.5% COLM may be attributed to high fibre level of the diet.

The cost of feed per kg gain showed a consistent significant difference ($p<0.05$) between treatments. It appears that 5% is the optimum substitution level of COLM for soya bean in broiler diets.

Conclusion: *Chromolaena odorata* leaf meal could be used as a cheap feed ingredient in broiler diets. It could substitute soya bean up to 7.5% in broiler diets. But 5%

level appears optimum considering the results on the final weight, weight grain and cost per kg gain

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Haemoglobin and Serum Ferritin Levels in Newborn Babies Born to Anaemic Iranian Women: a Cross-Sectional Study in an Iranian Hospital

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Abstract: The purpose of this study was to assess the haemoglobin and serum ferritin levels in newborn babies of anaemic mothers and to determine relationship between maternal iron status with neonatal biochemical and anthropometric characteristics. A total of 70 pregnant women aged between 17 and 40 years were divided into two groups based on their pre-delivery Haemoglobin (Hb) and Serum Ferritin (SF) concentrations; anaemic mothers Hb<11 g/dl and SF $\geq$ 10 ng/ml; normal control group Hb $\geq$ 11 g/dl and SF $\geq$ 10 ng/ml. Maternal biochemical assessments were obtained before delivery and neonatal anthropometric and biochemical measurements were obtained immediately after delivery. Mean maternal haemoglobin and serum ferritin levels were 11.2±1.16 (g/dl) and 45.8±20.8 (ng/ml), respectively. Incidence of anaemia among Iranian pregnant women in this study was 51.4%. Mean neonatal weight, length and head circumference born to anaemic mothers was 2.8±0.23 kg, 47.9±1.0 cm and 31.5±0.9 cm, respectively, while, among neonates born to normal mothers were 3.3±0.1 kg, 49.6±0.8 cm and 33.7±0.6 cm, respectively. No significant correlation were found between neonatal and maternal serum ferritin concentration. Significant differences were found between neonatal haemoglobin levels from normal and anaemic mothers ($p<0.001$). Maternal Hb level showed significant ($p<0.001$) positive correlation with neonatal birth weight ($r = 0.729$), length ($r = 0.665$) and head circumference ($r = 0.762$). However, similar positive correlations were not found between maternal serum ferritin concentration and pregnancy outcome. Neonatal haemoglobin levels were positively correlated with that of their mothers' ($r = 0.423$, $p<0.001$). In conclusion, this study showed that maternal haemoglobin concentration had strong influence on neonatal parameters.

Key words: Anaemia, haemoglobin, ferritin, pregnant women, newborn babies, Iran

INTRODUCTION

Anaemia is a serious problem for women of child-bearing age and it can have devastating effects on their babies. Anaemia is usually caused by deficiencies of iron and, rarely, of folic acid (folate). Some people have anaemia due to more than one of these factors (Collins, 2008). Blood loss is the most common cause of anaemia, especially iron deficiency anaemia. Some factors such as blood loss due to heavy menstrual periods, bleeding of the digestive tract and surgery would result in the reduction of iron stores and consequently, gradual development of anemia (Turgeon, 2004). Women, in general, have smaller stores of iron than men and experience blood loss through menstruation. Therefore, anaemia is more common in women than in men. During pregnancy, iron stores need to support the needs of mother and her growing fetus, which is the required iron for the development of red blood cells, blood vessels and muscles (Turgeon, 2004).

The World Health Organization estimated that about 40% of the world's population (more than 2 billion individuals) suffers from anaemia (World Health Organization, 2000). In Asia, the prevalence of anaemia was estimated to be 44% in non-pregnant women and 60% in pregnant women (Rush, 2000).

Iranian Ministry of Health and Medical Education (1995) reported that the prevalence of anaemia among Iranian women aged 15-49 is 33.34% but there is limited information regarding the prevalence of anaemia in pregnant women. Epidemiological studies which were limited determine the prevalence rate of anaemia on pregnant women, however some showed a rate 51% pregnant women; Childbearing age women generally suffer from anaemia (Abel *et al*., 2000).

This study was carried out to assess the prevalence of anaemia among Iranian pregnant women and determine the haemoglobin and serum ferritin levels in newborn babies from anaemic mothers. This study also determined the relationship between maternal iron

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status with neonatal biochemical and anthropometric characteristics.

MATERIALS AND METHODS

Selection and description of participants: The cross-sectional study was carried out in Aban Hospital (a referral hospital that serves all pregnant women referred from clinics, health care centers and medical practices) in Lahijan, in the north part of Iran in 2008. Of a total of 78 pregnant women, who were referred to the Obstetrics and Gynecology Division, only seventy pregnant women aged 17-40 years consented to participate in the study. All participants were admitted to the labor ward after 37 weeks gestation. Mothers who had hepatitis or renal disorders, thalassemia, parasitic infections and obstetrical complications like preeclampsia, gestational diabetes, preterm delivery and abortion were excluded from the study. The research protocol was approved by the Ethical Committee of Guilan University of Medical Sciences; Iran and written informed consent were obtained from all subjects before inclusion in to the study.

Subjects were divided into two groups, anaemic and non-anaemic, according to their pre-delivery Haemoglobin (Hb) and Serum Ferritin (SF) concentrations. Based on the World Health Organization (2001) reference, mothers with Hb<11 g/dl, SF $\geq$ 10 ng/ml were categorized in the anaemic group and those with Hb $\geq$ 11 g/dl, SF $\geq$ 10 ng/ml were in the non-anaemic group.

Demographic data including maternal age, maternal residency, education, income, parity, cigarette smoking and alcohol consumption, first prenatal visit and obstetrical history were gathered by interviewing the mothers.

Maternal anthropometric and biochemical data included; height, pre-pregnancy weight, pre-pregnancy BMI, pre-delivery weight, blood pressure, haemoglobin, hematocrit and serum ferritin was measured before delivery. While anthropometric and biochemical assessments of the neonates included length, head circumference, weight, haemoglobin and serum ferritin was obtained after delivery from cord blood.

The mother's body weight was measured to the nearest 0.1 kg using a digital scale (model 782 Seca, Germany). The measurement of height was aided by Seca body meter (model 240 Seca, Germany). According to WHO classification (1998), Body Mass Index (BMI) was calculated from the measured pre-delivery body weight divided by the height squared.

The neonate's weight was measured by using a Salter spring balance (CMS Weighing Equipment Ltd, UK) to the nearest 20 grams. The crown-heel length and head circumference were measured by Portable Pedobaby Babymeter (ETS JMB, Belgium) to the nearest 0.1 cm and fiber glass tape to the nearest 0.1 cm (CMS Instruments, UK), respectively.

Blood sampling: Peripheral vein-puncture blood samples were collected from the pregnant subjects for the determination of haemoglobin and serum ferritin levels before delivery by an experienced laboratory technician. Accordingly, cord blood was collected immediately after placenta delivery for measuring neonate's haemoglobin and serum ferritin levels. Analysis of maternal and cord serum were performed on the same day of collection. Haemoglobin was measured by the standard cyanmethemoglobin technique (PATH, 1996) while, serum ferritin was analyzed by Immune Radio Metric Assay (IRMA) technique (Flowers *et al*., 1986). Serum ferritin was standardized according to the international standards included with radioimmunoassay kit (Radim Co, Italy) and a gamma counter (Hewlett Packard, Wilmington, Del, USA).

Data analysis: Statistical analyses were performed using SPSS for windows, version 16. The normality was tested by Kolmogrov-Smirnov test. Differences in qualitative variables mean between the two studies groups (anaemic and non-anaemic groups) were tested by using non-parametric test such as Mann-Whitney U test. Quantitative variables were tested by T-test and chi-square test. All data were expressed as mean±S.D. The correlations among maternal iron status, cord blood composition and pregnancy outcome were analyzed by Pearson's correlation analysis. Multiple regressions were carried out to determine any significant relationship between different variables in order to specify the most important risk factors influencing maternal anaemia. A probability value of $p<0.05$ was considered to indicate statistical significance.

RESULTS

The socio-demography of the subjects is presented in Table 1. The mean age of women was 25.6±4.9 years with a range of 17-40 years. Most of women (34.2%) had diploma and 51.4% were employed and considered economically active. Some 48.6% of the subjects were housewives. Most of them were from urban area (54.2%) and were from the middle income groups (50.5%). Mean parity status was 1.4 and 35.7% women were multipara. The mean haemoglobin and serum ferritin levels were 11.2±1.2 g/dl and 45.8±20.8 ng/ml, respectively; while mean value of hematocrit was 33.5±4.6% which was lower than normal range (Table 2).

Table 3 presents the characteristics of mothers by iron status. Based on the WHO (2001) cut off points, 36 of mothers (51%) were classified as anaemic while 34 were non-anaemic (49%). There were significant differences in relation to parity with haemoglobin and hematocrit levels (Table 3). Parity in anaemic mothers was more than non anaemic ones ($p<0.039$). The multiple regression showed that multi-parity was an influencing factors on maternal anaemia (B = -1.446). Haemoglobin and hematocrit concentrations in anaemic

Table 1: Socio-demography of mothers (n = 70)

Characteristics	Value
Age (y)	25.6±4.9 (17-40)
House hold size	3.1±1.8 (2-7)
Parity	1.4±0.6 (1-3)
Education (%)	
Primary	24.3
High school	24.3
Diploma	34.2
Academic education	17.2
Occupation (%)	
Employed	51.4
House wife	48.6
Income (%)	
Low income <306$	31.4
Middle- income 357-510$	50.5
High income >510$	18.1
Residential status (%)	
Rural	45.7
Urban	54.2

Values are presented as mean±SD (range) 1USD = 9800 Rs

Table 2: Pre-delivery Maternal Iron Status (n = 70)

Characteristic	Mean± SD	Range	Reference range
Haemoglobin(g/dL)	11.2±1.1	9.3-14.2	11-16[1]
Serumferritin(ng/ml)	40±20	5.5-88.3	9-136[2]
Hematocrit (%)	33.5±4.6	15.7-42	37-48[3]

Sources: [1,3]Huch (1992). [2]Jackson *et al.* (2001)

Table 3: Age, parity and iron status of mothers (mean±SD)

Characteristics	Anaemic n = 36	Non-anaemic n = 34
Age (years)	25.6±5.3	25.2±4.2
Parity	1.5±0.6	1.2±0.5
Haemoglobin (g/dl)	10.2±0.4	12.1±0.8
Hematocrit (%)	30.8± 4.6	36.2±2.4

women were significantly lower than non-anaemic subjects. These values were 10.2 g/dl, 30.8% in anaemic and 12.1 g/dl, 36.2% in non-anaemic group.

The prevalence of anaemia among newborn babies from 36 anaemic pregnant women was 22.2% which three of neonates were female while five of them were male. There were also significant differences in neonatal weight, length and head circumference among anaemic and non-anaemic groups ($p<0.001$). As shown in Table 4, the neonatal anthropometric parameters from normal mothers were significantly higher than anaemic group. Neonates from normal mothers were 514 g heavier in weight than those from anaemic mothers and their length and head circumference also were 16.9 cm and 21.9 cm more than those in anaemic group.

Neonatal iron status showed that neonatal haemoglobin was significantly lower in anaemic than non-anaemic mothers ($p<0.001$). On the other hand, serum ferritin concentration in neonates from non anaemic mothers was not significantly different ($p = 0.59$).

As shown in Table 5 there was a significant relationship between maternal haemoglobin and hematocrit levels with neonatal anthropometric parameters ($p<0.001$). Accordingly, as maternal haemoglobin and hematocrit

Table 4: Neonatal anthropometrics and iron status (mean±SD)

Neonatal anthropometric and biochemical assessment	Anaemic mother	Non-anaemic mother
Weight (kg)	2.8 ±0.2*	3.3 ±0.1
Length (cm)	47.9±1.0*	49.6±0.8
Head circumference (cm)	31.5±0.9*	33.7±0.6
Haemoglobin (g/dl)	15.0±1.1*	16.4±1.2
Serumferritin (ng/ml)	207.7 ±108.2	184.8±61.8

SD = standard deviation; *Significant at $p<0.001$

levels rise, a considerable increase is observed in the weight, length and head circumference of neonates born to normal mothers than those to the anaemic ones. However no significant relationship was found between maternal serum ferritin level and neonates' anthropometric parameters in these groups ($p>0.001$).

Correlation of neonatal iron status with maternal iron status illustrated that there was significant correlation between neonatal and maternal haemoglobin ($r = 0.423$, $p<0.000$). In fact, such a positive relationship indicates that neonatal haemoglobin level depends on maternal Hb concentration. This variable would increase with a rise in mothers' Hb levels and vice versa. Positive correlation also found among neonatal haemoglobin and maternal Hct but this relationship was not significant ($p>0.05$). However no significant correlation ($p>0.05$) was identified between neonatal Hb and maternal SF (Table 4). Not significant relationship ($p>0.05$) was found between maternal iron status (haemoglobin, hematocrit and serum ferritin) and neonatal SF (Table 5).

DISCUSSION

In this study more than half of the pregnant women were anaemic. This prevalence was high and was similar with a report from India (Ackurt *et al.*, 1995). In two separate studies in South Africa and Israel, prevalence of anaemia in pregnant women was 3.0% and 21.6%, respectively (Patwardhan, 1996).

Parity, maternal haemoglobin and hematocrit were statistically significant different between the two groups. Multi-parity and short birth interval (less than 2 years) between pregnancies created a large demand for iron, which was needed to develop the fetus and placenta. Additional iron was lost with blood at delivery and it can appear maternal anaemia. These findings were consistent with another study (Veghari *et al.*, 2007). In contrast, in another study, women with parity ≥ 2 had higher mean haemoglobin concentration than nulliparous ones (Chandyo *et al.*, 2006).

Our findings regarding significant relationship between neonatal anthropometric characteristics and maternal iron status were consistent with (Gomber *et al.*, 2002) which studied Indian mothers. They observed that birth weight, crown heel length and head circumference of the neonate increased significantly with rising maternal haemoglobin levels. Previous researcher (Singla *et al.*,

Table 5: Correlation of neonatal anthropometric characteristics and neonatal iron status with maternal iron status

	Maternal Hb (g/dl)	Maternal Hct (%)	Maternal SF (ng/ml)
Neonatal weight	0.729*	0.521*	0.022
Neonatal length	0.665*	0.423*	0.038
Neonatal head-circumference	0.762*	0.568*	0.097
Neonatal Hb	0.423*	0.228	0.061
Neonatal SF	-0.057	0.008	0.013

*Correlation is significant at the 0.001 level (2-tailed)

1997) have reported that the birth weight, head circumference, chest circumference, mid-arm circumference and crown heel length were significantly lower in infants born to mothers with moderate and severe anaemia, in comparison to infants born to non-anaemic mothers. In contrast, another study showed that the babies of iron-deficient anaemic mothers had greater head circumference and were heavier than those from non-anaemic non-iron-deficient mothers (Emamghorashi and Heidari, 2004). It may be interpreted that the effect of maternal anaemia on intra-uterine growth is attributed to chronic deprivation of oxygen to the developing fetus (Pollack and Divon, 1992). In addition, maternal anaemia may be a marker for nutritional, social and environmental deprivations which may independently influence fetal growth.

Another interesting finding of the present study indicated a significant relationship between neonatal haemoglobin ($p<0.001$) but not serum ferritin ($p = 0.59$) from anaemic and non anaemic mothers ($p<0.001$). Our results were partially similar to other studies (Singla *et al*., 1996). They found that the levels of haemoglobin, serum iron; transferrin saturation and ferritin were significantly low in the cord blood of anaemic women than non anaemic ones, indicating that, iron supply to the fetus was reduced in maternal anaemia. In contrast Kilbride *et al*. (1999) showed that iron content in cord blood were similar in anaemic and non-anaemic pregnant women, with no significant difference in haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration or plasma ferritin.

Our study could show significant relationship between maternal haemoglobin and hematocrit with neonatal body composition ($p<0.001$). However, no significant relationship was found between maternal serum ferritin and neonates' body composition. This relationship was demonstrated by Msolla and Kinabo (1997) and Bai *et al*. (2002). Nahum and Stanislaw (2004) found that birth weight correlates negatively with maternal haemoglobin concentration. This was consistent with the well-known effect of high-altitude exposure during pregnancy, which increases both hematocrit and blood viscosity and lowers birth weight. These findings indicate that birth weight of newborn is dependent of multiple factors such as maternal iron status during pre-pregnancy, body size, general nutritional status. Although for women who enter pregnancy with low iron stores, insufficient intakes of iron during pregnancy can produce undesirable pregnancy outcome.

Our results showed similar significant relationship between neonatal length and maternal iron status as was reported by Lee *et al*. (2006). Among Korean and Indian women similar outcome were also shown (Gomber *et al*., 2002; Singla *et al*., 1997).

Our result regarding positive correlation between neonatal Hb and maternal haemoglobin and hematocrit are in keeping with previous study. Although correlation between neonatal Hb and maternal Hct and SF was not significant. A study by Singla *et al*. (1978) showed that maternal haemoglobin had a linear correlation with haemoglobin and iron levels in the cord blood and placental tissue. Some studies showed negative association of hematocrit, haemoglobin and plasma iron measures between mothers and infants (Sichieri *et al*., 2006). Our results were different from Turkey *et al*. (1995) which did not find correlation between maternal haemoglobin and ferritin at 16 and 34 weeks' gestation and newborn haemoglobin parameters. It may be interpreted that, iron stores in the fetus are not adversely affected by mild-to-moderate anaemia in the mother; thus supporting the theory that (for women with mild-to-moderate anaemia) the placenta and fetus have a special affinity for iron in the mother's circulation and iron is transported through the placenta irrespective of the concentration gradient (Turkey *et al*., 1995).

Our results regarding significant correlation between neonatal serum ferritin and maternal iron status was similar with Emery and Barry (2006), however did not support Mexican study (Vásquez-Molina *et al*., 2001).

Conclusion: Prevalence of anaemia is high in the north of Iran and parity is one of factors that could have an influence on anaemia. Maternal haemoglobin and hematocrit concentrations during pregnancy have strong influence on neonatal anthropometric parameters and neonatal haemoglobin, while, such relationship was not seen between maternal and neonatal serum ferritin. New strategies are need for ensuring that mothers take iron supplementation regularly and nutrition education may be beneficial to improve the dietary intake of pregnant mothers.

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Effect of the Different Feed Formulas on Physiological Changes and Milk Production Performance of Holstein-friesian Crossbred Dairy Cows

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Abstract: The effect of 3 different feed formulas on some physiological changes, haematological changes, cortisol levels and milk production performance of Holstein-Friesian crossbred dairy cows were studied during the early period of the first lactation, by Randomized Complete Block Design with 4 replications and 3 treatments. Twelve cows were randomly assigned to each of the 3 feed formula groups as follows; (1) Commercial Feed as control group, (2) Cooperative-mixed Feed and (3) Cooperative-mixed Feed with the supplement of 2 kilograms of whole cottonseed/head/day. They were raised and managed in Sakon Nakhon Livestock Breeding and Research Station. The significant effects of feed formulas on physiological responses (rectal temperature, respiration rate, pulse rate and heat tolerance), haematological changes, cortisol levels and Average Daily Milk Yield (ADMY) were observed ($p<0.05$). The cows in group 3 showed higher Heat Tolerance Coefficient (HTC) than the cows in groups 1 and 2. The ADMY of cows in group 3 (7.89±0.62 kg/d) was higher than the cows in group 1 and 2 (7.72±0.32 and 7.70±0.64 kg/d), respectively. But milk composition was found only significant difference ($p<0.05$) in milk fat percentage.

Key words: Whole cottonseed, physiological changes, holstein-friesian crossbred cows, milk production performance

INTRODUCTION

The tendency of dairy production efficiency in Sakon Nakhon province nearly remained to static ADMY situation due to the milk productive performance of dairy cows did not increase. The farmers mostly expected the increase of raw milk price only when comparing the production costs and the price of raw milk. The dairy farming condition under the management of small-holders faced serious problem on the exacberating of concentrate price. The solutions were aimed to the modified feed formulas (local feedstuff usage) for reducing concentrate costs which served as alternative and appropriate for feeding dairy cows in tropical area. The effects of tropical condition influenced the productivity of dairy cows such as poor fertility due to the cows had long calving-interval or low conception rate, which were relatively influenced by heredity, environment and raising management.

This research aimed to study the effect of different feed formulas on some physiological changes and milk production performance of Holstein-Friesian crossbred dairy cows under the raising and management condition in Sakon Nakhon Livestock Breeding and Research Station by comparing among 3 feed formulas: commercial feed, cooperative-mixed feed and cooperative-mixed feed with the supplement of whole cottonseed, which would be useful and practical approach for small-holders to increase milk yield of dairy cows and for sustainable dairy production efficiency.

MATERIALS AND METHODS

Twelve Holstein-Friesian (HF) crossbred dairy cows (75 or over 75% of HF blood with uniform body weight) were studied during the early period of the first lactation, by Randomized Complete Block Design (RCBD) with 4 replications and 3 treatments. All data were analyzed by ANOVA and treatment mean comparison was determined by Duncan's New Multiple Range Test (Umpapol, 2005). The experimental feeds were the 3 different feed formulas as follows; (1) Private Company Commercial Feed as control group, (2) Phupan Dairy Cooperative-mixed Feed and (3) Phupan Dairy Cooperative-mixed Feed with the supplement of 2.0 kilograms of whole cottonseed per head per day. All groups of dairy cows acquired the same feeding practice and management, 18% crude proteins concentrate

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(NRC, 2001) and ruzie grass soilage was fed to the cows.

Temperature-Humidity Index (THI), black globe temperature, respiration rate, pulse rate, body temperature, Heat Tolerance Coefficeincy (HTC), haematological changes, cortisol levels and milk yield were collected for study.

RESULTS AND DISCUSSION

General data of the ambient environment: The general ambient environment during the experiment were studied on maximum and minimum temperature, the different temperature between max.-min. temperature and THI. The results of analysis showed that THI mean was 88.97±3.37 (Table 1) which indicated that the experimental area was tropical zone (Vajrabukka, 1996; Umpapol, 2002).

The solar radiation effected on the temperature of dairy cow experimental house, the study revealed that solar radiation mean was 2.35±0.13 which affected on THI mean into 82.64±0.12 throughout the experiment period (Table 2), the intense of sunlight caused the heat increase in the house (Eley *et al*., 1978; Collier *et al*., 1981; Beede *et al*., 1987).

Table 1: Effects of environment on experimental house

Item	Mean
Maximum temperature (°C)	34.97±0.36
Mean temperature (°C)	29.61±0.19
Minimum temperature (°C)	24.12±0.13
Different temperature (°C)	10.85±0.24
Relative humidity (%)	83.92±0.31
Black globe temperature (°C)	48.66±0.74
Ambient temperature (°C)	37.04±0.39
Radiation (°C)	11.45±0.47
THI	88.97±3.37

Table 2: Effect of solar radiation on experimental house

Environment factor	Value
Black globe temperature (°C)	36.25±0.34
Ambient temperature (°C)	33.90±0.31
Radiation (°C)	2.35±0.13
Air temperature (°C)	33.86±0.13
Maximum temperature (°C)	34.97±0.31
Mean temperature (°C)	29.54±0.19
Minimum temperature (°C)	24.12±0.13
Different temperature (°C)	10.85±0.24
THI	82.64±0.12

Physiological changes: The effect of the 3 different feed formulas on physiological changes of Holstein-Friesian crossbred dairy cows. The feed formulas were: (1) commercial feed as control group, (2) cooperative-mixed feed and (3) cooperative-mixed feed with the supplement of 2 kilograms of whole cottonseed/ head/day and they were raised and managed under the condition in Sakon Nakhon Livestock Breeding and Research Station. The results of physiological changes were as follows.

General physiology: The 3 different feed formulas effected on rectal temperature, pulse rate and respiration rate of dairy cows with no significant differences ($p>0.05$), but HTC and sweating rate found significant differences ($p<0.05$) (Table 3).

When THI mean was raised up over 72 it caused heat stress to dairy cows (Wiersma and Stott, 1966). The dairy cows were raised in heat stress condition (Johnson *et al*., 1960) the THI mean would increase that affected on body heat balance and caused the physiological changes for regulating the increased body temperature (Yousef, 1985). When body heat accumulation was increased as the rules of Van't Hoff effect, so the cows would regulate to static body temperature by many means, the most outstanding responses were the increase of respiration rate, pulse rate and rectal temperature (McDowell, 1972; Bucklin *et al*., 1988; Bond and Laster, 1975; Eley *et al*., 1978). The results were shown on Table 3.

The cows fed with the supplement of whole cottonseed showed HTC mean higher than the other two groups ($p<0.01$), but the comparison between Group 1 and Group 2 were not significantly different ($p>0.05$) (Sirvastana and Sindhu, 1977). The whole cottonseed supplement could reduce efficiently the heat from SDA of cow body, therefore they could regulate and balance the body heat even THI value was increased (Vajrabukka, 1996) so it enhanced the cows to reduce heat stress effectively (Umpapol *et al*., 2001).

Haematological changes: The results showed that haematological mean of average haematocrit percentage of the cows in Group 1, 2 and 3 were not significantly different before the experiment ($p>0.05$), but during the experiment, at the end of experiment and throughout the experiment the average haematocrit percentage were significantly different ($p<0.05$). The results were shown on Table 4.

The cow body could operate a mechanism of body heat regulation under the hot climate by sweating and increased water intake. While the water outside was absorbed into the blood vessels gradually, which caused diluted red blood cell or increased plasma volume so the haematocrit percentage was lower (Hafez, 1968; Garg and Nangia, 1981).

The results showed the average value of haemoglobin concentration of the cows in Group 1, 2 and 3 were not significantly different ($p>0.05$) before the experiment, but during the experiment, at the end of experiment and throughout the experiment the average value of haemoglobin concentration were significantly different ($p<0.05$) (Table 5).

The value of haemoglobin concentration had a positive correlation with the number of red blood cell due to haemoglobin is an important component of red blood cell. Likewise haematocrit, when the ambient

Table 3: The effects of feed formulas on general physiology of dairy cows

	Feed formulas		
General physiology	1	2	3
Rectal temperature (°C)	39.46±0.03	39.45±0.03	39.09±0.02
Pulse rate (b/min)	82.43±4.76	82.31±2.73	79.59±4.53
Respiration rate (br/min)	82.18±5.73	82.00±6.73	78.34±3.51
Heat tolerance coefficient (%)	80.95±4.60[a]	80.94±6.03[a]	87.65±4.38[b]
Sweating rate (ml/m^2/h)	1020.40±46.72[a]	1050.27±00.28[a]	881.15±25.74[b]

Mean within row with different superscript differ significantly ($p<0.05$)

Table 4: Average of haematocrit in this experiment

	Haematocrit (%)			
Feed formulas	Before experiment	During experiment	End of experiment	Throughout experiment
1	31.34±0.89	36.52±0.81[a]	41.04±0.92[a]	36.36±0.80[a]
2	31.23±0.96	37.30±0.24[b]	41.70±0.94[b]	36.88±0.61[b]
3	31.35±0.04	39.68±0.40[a]	46.84±0.27[a]	40.05±0.64[a]

Mean within column with different superscript differ significantly ($p<0.05$)

Table 5: Average value of haemogoblin in this experiment

	Haemoglobin (g/100 ml blood)			
Feed formulas	Before experiment	During experiment	End of experiment	Throughout experiment
1	42.00±0.82	50.63±0.47[a]	56.00±0.82[a]	49.81±0.84[a]
2	42.50±0.29	52.13±0.98[a]	55.50±0.94[a]	50.56±0.64[a]
3	42.25±0.26	55.50±0.34[b]	62.25±0.67[b]	53.88±0.64[b]

Mean within column with different superscript differ significantly ($p<0.05$)

temperature was increased the cows obtained heat stress, cow body could operate a mechanism of body heat regulation under the hot climate by sweating and increase water intake, which increased water volume or plasma volume of blood circulatory ways so the value of haemoglobin concentration became lower (Umpapol, 2002).

Cortisol levels: The result of this study showed that cortisol levels of the cows in Groups 1, 2 and 3 were not significant different before the experiment ($p>0.05$), but during the experiment, at the end of experiment and throughout the experiment, they were significant different ($p<0.05$) (Table 6).

The result of the study revealed that the average of cortisol levels in cow serum were increased due to the response of adrenalcortical, when sensory nerve of the cow skin sent neural message to hypothalamus and anterior pituitary gland that resulted on the secretion of cortisol from adrenal cortex (Christison and Johnson, 1972; Hafez, 1968). Heat stress caused on reduction of blood volume that circulated to rectum, but increased blood volume to flow to adrenal cortex and medulla, whereas metabolism including catecholamine and cortisol secretion were increased. The effect of high ambient temperature stimulated sensory nerve of skin and sent neural current via neural connections to hypothalamus and anterior pituitary gland that caused on the increase of cortisol secretion from adrenal cortex (Christison and Johnson, 1972; Wise *et al*., 1988; Umpapol *et al*., 2001; Umpapol, 2002).

Blood glucose levels: The result of this study found that blood glucose levels of the cows in Groups 1, 2 and 3 were not significantly different in each period viz. before the experiment ($p>0.05$), during the experiment ($p>0.05$), at the end of experiment and throughout the experiment, they were not significantly different ($p>0.05$) (Table 7).

Blood urea nitrogen: The result of this study found that average values of blood urea nitrogen of the cows in Groups 1, 2 and 3 were not significantly different in each period viz. before the experiment ($p>0.05$), during the experiment ($p>0.05$), at the end of experiment and throughout the experiment, they were not significantly different ($p>0.05$) (Table 8).

Milk production performance: The result of this study showed that the cows in Group 1, 2 and 3 were highly and significantly different in roughage intakes ($p<0.01$), but were not significantly different in concentrate intakes ($p>0.05$). The exact ADMY found highly significant difference, but 4%FCM and daily milk fat weights were not significant ($p>0.05$). The study on body condition score found no significant difference in both pre-experiment and post-experiment ($p>0.05$).

The result of milk composition showed that the cows in Group 1, 2 and 3 were significantly different in milk fat ($p<0.05$), but were not significantly different in protein, lactose, Solid Not Fat (SNF) and Total Solid (TS) percentages ($p>0.05$). The result was shown on Table 9.

Table 6: Effect of feed formulas on cortisol levels of dairy cows

	Average of cortisol level (ηg/ml)			
Feed formulas	Before experiment	During experiment	End of experiment	Throughout experiment
1	96.00±2.58	89.88±2.85[a]	80.25±1.26[a]	89.00±6.27[a]
2	96.75±1.71	80.38±4.34[b]	72.25±1.26[b]	82.44±9.71[b]
3	96.25±1.71	76.13±3.44[b]	69.25±0.96[b]	79.44±0.73[b]

Mean within column with different superscript differ significantly (p<0.05)

Table 7: Average blood glucose values in this experiment

	Average of blood glucose (mg/100 ml)			
Feed formulas	Before experiment	During experiment	End of experiment	Throughout experiment
1	53.23±0.45	54.48±0.68	56.32±0.87	54.63±1.30
2	53.17±0.74	54.69±0.99	56.64±0.92	54.80±1.53
3	53.56±1.12	55.27±1.10	57.94±0.68	55.51±1.88

Mean within row with different superscript differ significantly (p<0.05)

Table 8: Average values of blood urea nitrogen of the cows in this experiment

	Average of blood urea nitrogen (mg/100 ml)			
Feed formulas	Before experiment	During experiment	End of experiment	Throughout experiment
1	14.04±0.25	15.17±0.41	15.66±0.32	15.66±0.32
2	14.06±0.27	15.09±0.51	16.12±0.14	16.12±0.14
3	14.06±0.09	14.97±0.36	16.11±0.37	16.11±0.37

Mean within column with different superscript differ significantly (p<0.05)

Table 9: Milk production performance of dairy cows in this experiment

	Feed formulas		
Item	1	2	3
Voluntary feed intake			
Roughage (kg)	6.64±0.25	6.48±0.32	6.72±0.08
Concentrate (kg)	5.02±0.02	5.06±0.01	5.04±0.01
Milk performance production			
Milk yield (kg/d)	7.72±0.32	7.70±0.64	7.98±0.62
FMC 4% (kg/d)	7.34±0.34[a]	7.30±0.63[a]	7.80±0.68[b]
Body condition score			
Pre-experiment	2.76±0.04	2.72±0.03	2.74±0.03
Post-experiment	2.72±0.03	2.66±0.03	2.68±0.02
Milk composition			
Fat (%)	3.32±0.20[a]	3.30±0.12[a]	4.60±0.18[b]
Protein (%)	3.26±0.08	3.20±0.06	3.38±0.05
Lactose (%)	4.34±0.19	4.28±0.07	4.66±0.11
Solid not fat (%)	8.85±0.51	8.76±0.58	9.05±0.58
Total solid (%)	12.84±0.57	12.76±0.62	12.96±0.42

Mean within row with different superscript differ significantly (p<0.05)

The dairy cows decreased milk yield when they were under the ambient climate of THI of 72 or over (Johnson *et al*., 1963) due to the mechanism of body heat balance of cows (McDowell, 1972; Yousef, 1985; Johnson and Givens, 1961), for ventilating the body heat by means of evaporative heat loss which needed more energy in this process and affected the energy utilization for body maintenance. Likewise, McDowell *et al*. (1969) concluded that the process of metabolic heat production in body would decrease continuously when the cows were under high ambient temperature for long time because they reduced feed intakes that caused low energy obtaining and the same as reports of Johnson *et al*. (1966); Curran and Okantah (1982); Smith (1984) which concluded that high ambient temperature influenced directly to the functions of hypothalamus and anterior pituitary gland causing the mechanism of cortisol secretion from adrenal cortex increase. But when the cows faced heat stress continuously for long time, the cortisol concentration would decrease which was the mechanism of adaptation of body to prevent the over metabolic heat from food combustion due to the influence of cortisol function, or the adjustment of cortisol metabolism. Therefore, feed intake, metabolism and metabolic heat were all decreased. However, the efficiency of energy that the cows obtained during hot

climate, could utilize less for milk production due to the cost of maintenance energy was higher than 20% when compared between 35°C and 20°C.

It concluded that the heat stress condition effected to beget the stress in dairy cows which could determine from the values of general physiological changes that were mostly increased (Singh and Bhattacharya, 1990; Legates *et al*., 1991; Kabuga, 1992) so that effected haematologic values (Hafez, 1968; Umpapol, 2002), especially haematocrit percentage and haemoglobin concentration value were increased (Abilay *et al*., 1975), which were the indicators that caused HTC values became lower (Sirvastana and Sindhu, 1977) then effected to endocrine system functions particularly the cortisol concentration levels would be increased (Abilay *et al*., 1975) for reducing metabolic heat due to decrease feed intake (Smith, 1984; NRC, 2001). And later on the adjustment of body heat balance occurred and related to cortisol reducing including finally the dairy cow would decrease milk production (Johnson *et al*., 1960; Wayman *et al*., 1962; McDowell, 1972; Thatcher *et al*., 1974; Yousef, 1985) to encourage cow body to normal condition.

The dairy cows fed whole cottonseed would acquire the efficiency increment in heat ventilation of body so to keep the body heat balance efficiently and would be one way to increase energy obtaining for milk production (Hafez, 1968; Eley *et al*., 1978; Bucklin *et al*., 1988). The management of a mechanism of body heat balance for increasing heat ventilation efficacy of the dairy cows would cause in feed intake quantity and feed energy gain more than the mitigated heat stress cows. Similar results from the experiments that were conducted to reduce heat stress of cows by comparison of the untreated groups (without shelter addition or hair cut) ensued lower milk yield than the treated groups (Johnson *et al*., 1960; Thatcher *et al*., 1974) or lower milk yield than the hair cut groups (Boonprong, 1999).

Whole cottonseed supplement would role similarly by-pass fat feedstuff in concentrate, which caused ruminal heat production deceleration (Yousef, 1985) and meanwhile the cows acquired the increased energy (Wrenn *et al*., 1978) particularly maintenance energy for the utilization of body heat ventilation (Church, 1979) so to remain the normal body temperature and increasing net energy gain for more milk production (Andrew *et al*., 1991; Kim *et al*., 1993), likewise Harrison *et al*. (1995) reported that the supplement of by-pass fat feed as Ca-LCFA as 5% level in TMR to Holstein dairy cows, found that dry matter feed intake were similar ($p>0.05$) as 20.50 and 19.70 kilograms per day in control group and treated group, respectively (Kim *et al*., 1993).

The results of this experiment found that the milk composition was not significantly different ($p>0.05$) which was similarly related to Azizan and Phipps (1997) used bST with the supplement of calcium soap in Sahiwal Friesian dairy cows could increase milk production compared to the untreated group, but milk composition was not significantly different ($p>0.05$) due to milk composition would vary on heredity. Therefore, this experiment found that the milk composition was not significantly different ($p>0.05$) due to lower milk producing cows adapted body under the high ambient temperature by regulation of body heat balance, which cortisol level would initially increase ensuing decrease for reducing body heat and metabolism by decrease of feed intake so that milk yield became lower than the treated group, while milk composition was not effected due to the dairy cows adjusted to reduce only milk production but the milk composition was not significantly different.

Conclusion and recommendations: Based on this experiment, it could be concluded that ambient environment effected to experimental house of dairy cows, causing the increase of THI to 72 or over that affected the general physiological changes, haematocrit and hemoglobin changes, cortisol levels and milk production performance. In addition, the dairy cows acquired the whole cottonseed supplement could regulate body heat balance which caused the changes of the general physiology, haematocrit and hemoglobin levels and cortisol levels that showed lower values than commercial feed and cooperative-mixed feed groups. The dairy cows obtained the whole cottonseed supplement could produce the highest average daily milk yield, ensuing the commercial feed and cooperative-mixed feed groups which both of them were not significant different. However, digestibility and utilization of whole cottonseed should be studied for the use in feed formulas of dairy cows efficiently. Furthermore, the influential and related factors should be jointly studied such as housing adjustment, dairy cow management for improving milk production performance especially in summer or hot season.

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Waste Water Treatment Using Sequential Batch Reactor and Development of Microbiological Method for the Analysis of Relative Toxicity

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Abstract: Pharmaceutical industry is a very progressing sector in Pakistan with over 400 Pharmaceutical manufacturing in the country. The pharmaceutical industry in Karachi is discharging thousands of gallons of waste per day carrying with itself huge quantities of antibiotics, other pharmaceutical synthetics and solvents as its major constituents. These substances are not only harmful to the aquatic flora and fauna but may also contribute to development of resistance to antibiotics. We designed a sequential batch reactor (SBR) after a series of experiments. The effluent met the NEQS specifications after 21 days of treatment in the SBR. The changes in pH, BOD, COD, TDS, TSS, Ammonia levels, Oil and grease levels were found to be significant ($p < 0.05$).

Key words: Biodegradation, bioremediation, toxicity, waste, sequential batch reactor

INTRODUCTION

Karachi has country's biggest industrial estate, the Sindh Industrial Trading Estate (SITE), with an estimated 2000 industrial units. Industries are also located in Federal-B area, North Karachi, Landhi and Korangi Industrial area. The industries include textile, chemical, tanneries, pharmaceuticals, etc. Under the Factories act (1934), the industrialists are expected to discharge only treated industrial effluent in the city infrastructure (UNIDO, 2000).

The body load of Lyari and Malir rivers is very high from this waste water and to the tune of 1000 tons/day and 500 tons/day respectively. Sewage flows in Lyari river channel have reduced tremendously during last two years due to intercepting trunk sewer laid by KW&SB in the river bed taking sewage to Mauripur Treatment Plant (TP-3) - commissioned in December 1997. The sewers connected with storm water drains terminating Layri / Malir river have also been rapidly switched upon trunk sewers under Khushal Pakistan Program.

To improve the environmental conditions in the city and to dilute concentration of raw sewage flowing into sea, three (03) sewage treatment plants (STPs) are operating in the city:

TP-1 at SITE, TP-2 at Mehmoodabad and TP-3 at Mauripur (source: Karachi water and sewage board).

Pharmaceutical industry is a very progressing sector in Pakistan. This industry is experiencing positive growth since last one decade. In Karachi alone, there are over 100 registered Pharmaceutical manufacturing units, whereas Pakistan resides over 400 Pharmaceutical manufacturing units which produce over 19,000 dosage forms. The industry in Karachi is dumping approximately thousands of gallons of waste per day.

This growth is being achieved at the cost of continuous addition of potent and hazardous chemicals into our waste waters. In turn, these chemicals prove to be deleterious for the natural flora and fauna. While many of the countries started working on small and large scale treatment plants for specific sectors, as early as in 1980s and 90s (Reed, 1990), we are yet to establish treatment options and expertise in this vast field of environmental science. Keeping in pace with the modern day needs, we have undertaken the task of designing and experimenting with a most suitable treatment option keeping in mind, the constituents of the waste and the fate of the effluent. We cannot over look the fact that the pharmaceutical effluent also carries with it, antibiotics into wastewater stream. The emergence of resistance to antibiotics can also be attributed to the fact that the pharmaceutical and hospital waste is carried into natural water via sewage without being treated.

MATERIALS AND METHODS

Effluent collection: The effluent under consideration was collected from a Pharmaceutical finished product manufacturing facility. The plant was mainly involved in the manufacture of anti-TB products, multi-vitamins, antacids, antibiotics, anti-tussive and attapulgite. The constituents of effluent were; Rifampicin, Isoniazid, Pyrazinamide, Ethambutol, Aluminum oxide, Magnesium oxide, Gel magma, multi vitamins such as B_1, B_2, B_6, B_{12} and sugar syrups (liquid glucose, malt extract).

Table 1: NEOS specifications

Parameters	NEOS standard values*
pH[†]	6-9
Biological Oxygen Demand (BOD)	250 (mg/L)
Chemical Oxygen Demand (COD)	400 (mg/L)
Total Sedimentation Solids (TSS)	400 (mg/L)
Total Dissolved Solids (TDS)	3500 (mg/L)
Ammonia	40 (mg/L)
Oil & Grease	10 (mg/L)

*Pakistan National Environmental Ouality Standards; [†]pH adjusted between 6-9. Note: The values are based on 24 hours period.

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Table 2: Summary of results (commercial scale batch reactor)

	pH[1]	BOD (mg/L)	COD (mg/L)	TSS (mg/L)	TDS (mg/L)	Ammonia (mg/L)	Oil and Grease (mg/L)
NEOS specifications	6-9	250	400	400	3500	40	10
Control	6.62	799.00	907.00	592.33	2976.67	14.77	2.94
7 days post treatment	6.69	462.33	592.33	298.33	2876.67	14.17	2.63
14 days post treatment	6.79	300.33	370.33	202.00	2916.67	12.97	2.33
21 days post treatment	6.90	250.33	302.00	191.00	2800.00	12.40	1.94

Table 3: Statistical analysis of NEOS results for SBR (at 14 days)

One-sample statistics	N	Mean	SD	SEM
pH	5	6.7240	0.11524	0.05154
BOD	5	499.6000	273.43244	122.28271
COD	5	585.2000	295.98936	132.37046
Total sedimentation solids	5	356.6000	212.08206	94.84598
Total dissolved solids	5	2936.0000	29.66479	13.26650
Ammonia levels	5	13.6400	1.35019	0.60382
Oil and Grease levels	5	2.5620	0.44690	0.19986

Sample collection: Waste water analysis was carried out at different production times with full production load. In case of lab scale experiment, observations were based on 24-hour values, while samples were collected and tested at different time intervals.

In case of pilot scale and commercial scale reactors, composite samples were collected on 24-hour basis and submitted for testing against NEQS specifications. Samples from batch reactors were drawn for consecutive 3-months.

The experiment was scaled up to a commercial scale Sequential batch reactor (SBR) after successful design qualification using lab and pilot scale designs. The process comprised of aeration, settling, reacting and decanting (Metcalf and Eddy, 2003). The reduction in toxicity was measured using National Environmental Quality Standards (1999).

Design of sequential batch reactor (SBR): A sequential batch reactor (SBR) was first designed on lab-scale to determine the functional specifications. This lab scale project was then expanded to a pilot scale project for the determination of effects of various factors on the biodegradation and waste treatment process on a comparatively larger scale, after which a full scale SBR was constructed and made operational. The general design of SBR (Fig. 1) consisted of the following:

i Equalization tank
ii. Neutralization tank
iii. Sequential batch reactor
iv. Drying bed

Equalization tank: Homogenization of waste water was achieved in 4000L concrete tank with air supplied through SS304 pipes for mixing.

Neutralization tank: Waste from equalization tank was transferred to neutralization tank (of 4000L capacity), where pH adjustment was performed using 1N HCl and/or 1N NaOH. pH was adjusted in a range of 6 to 8.

Sequential batch reactor: The waste was then transferred from neutralization tank to SBR. Aeration was performed for 8 hours on daily basis using atmospheric air. Dissolved oxygen was maintained at 2.0 mg/L in the flask during operation.

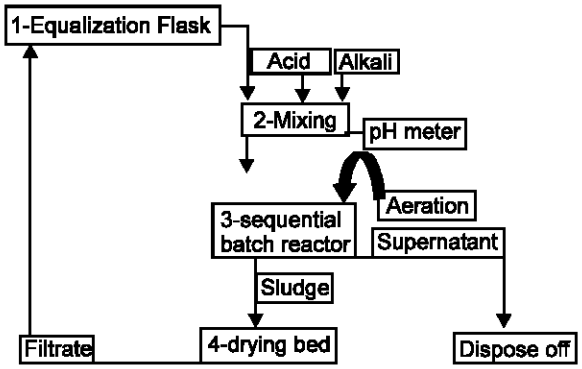

Fig. 1: Design of sequential batch reactor

Sedimentation/Clarification: The liquid was allowed to settle in order to facilitate solid-liquid separation, thus providing a clear supernatant. It took almost 2 days for settlement.

Decant: The supernatant was then carefully decanted using level switch pump, back into the equalization tank.

Drying bed: The sludge was manually lifted and placed on concrete base where it was manually pressed so that the filtrate was collected and circulated back to the equalization tank, using a drain pipe while sludge was dried under sun-light, thereafter it was disposed off.

Commercial scale plant was built totally under covered condition, as that of pilot scale condition.

NEQS testing: Testing of effluent for NEQS specifications were analyzed at different time points. Treated and untreated wastes were analyzed for NEQS specification at baseline, 7 days, 14 days and 21 days. The NEQS specifications are listed in Table 1.

RESULTS

The samples were found to be in compliance with the NEQS (1999) specifications at 21 days for pilot scale and commercial scale study designs. Preliminary information was collected from the lab scale designs.

In pilot scale studies, the effluent samples were found to be in compliance with the NEQS specifications. The changes in BOD, COD and Total sedimentation solids were observed, though they were not statistically significant. However, all results were found within NEQS limits after the treatment period.

The NEQS compliant results were observed after a period of 2 weeks upon using the same filtrate as obtained from the commercial scale experiment (Table

Table 4: One sample t-test for NEOS results of SBR (at 14 days)

	Test value = 0.05					
	t	df	Sig. (2-tailed)	Mean Difference	95% confidence interval of the Difference	
One-sample test	Lower	Upper	Lower	Upper	Lower	Upper
pH	129.501	4	0.000	6.674	6.5309	6.8171
BOD	4.085	4	0.015	499.550	160.0388	839.0612
COD	4.421	4	0.012	585.150	217.6307	952.6693
Total sedimentation solids	3.759	4	0.020	356.550	93.2153	619.8847
Total dissolved solids	221.306	4	0.000	2935.950	2899.1163	2972.7837
Ammonia levels	22.507	4	0.000	13.590	11.9135	15.2665
Oil and Grease levels	12.569	4	0.000	2.512	1.9571	3.0669

2). These results showed statistically significant improvement ($p < 0.05$) at 14 days (Tables 3 and 4). Significant change in pH, BOD, COD, Total sedimentation solids (TSS), Total dissolved solids (TDS), Ammonia and Oil and grease levels were observed (Mahvi *et al.* 2004). The analysis was performed on SPSS version 15.0 using one sample t-test. These changes are consistent with the findings of Mahvi *et al.* (2004); Sandhya (2004).

In all study designs, a discoloration of effluent was also observed, from dark red color (characteristic of Rifampicin) to almost colorless (Elias, 2000).

DISCUSSION

The toxicity levels were reduced and observed to be within the limits defined by NEQS (1999) using Sequential batch reactor (SBR) as a waste water treatment model.

The preliminary findings from lab scale experiments revealed that the selected design is rational. This led to further experiments by scaling up the design to pilot scale and then commercial scale. The effluent samples were found to be in compliance with the specifications laid down by NEQS. The test results improved and the duration of treatment was also reduced as we inoculated the filtrate into the re-circulation stream. The results are consistent with the previous studies carried out by different scientists in their different environmental conditions meeting their local regulatory requirements. Most of the studies suggest that there is a reduction in the levels of BOD, COD, Ammonia, Total sedimentation solids (TSS) and Organic pollutants and in few cases discoloration may also be observed (Buitron *et al.,* 2003; Elias, 2000; Sandhya *et al.,* 2004).

The statistical analysis of results suggests that there is a significant change ($p<0.05$) in BOD, COD, TSS, Ammonia and Oils and Grease levels (Mahvi *et al.* 2004).

Generally, Sequential batch reactors are suitable for areas for confined spaces, stringent treatment requirements, and small wastewater flows. This system is very useful for treating pharmaceutical, brewery, dairy, pulp and paper, and chemical wastes. SBRs are also suitable for sites that need minimum operator attendance and have a wide range of inflow and/or organic load. Industries with high BOD load, such as chemical or food processing plants, find SBRs useful for treating wastewater. These systems are also suitable for facilities requiring nitrification, denitrification, and phosphorous removal. Most significantly, SBRs are applicable for areas where effluent requirements can change frequently and become stricter, as these systems have tremendous flexibility to change treatment options (US-EPA fact sheet, 2000).

Work on biodegradation of pharmaceutical effluent has not been very wide and spread throughout the globe especially in Pakistan where there is no data that describes the model of SBR for the treatment of effluents. This work provides a good starting model of different study designs and their implementation and outcome. It also highlights the use of aerobic treatment of waste and development of new and economical methods for analysis that are comparable with conventional methods and consistent with NEQS specifications.

It is now very much imperative that other industries also establish a central waste management site, and take initiative towards greener environment so that the effluent is treated to a safer level before introduction into natural water sources.

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Incidence and Severity of Crown Gall Disease of Cherry, Apple and Apricot Plants Caused by *Agrobacterium tumefaciens* in Nagar Valley of Gilgit-Baltistan, Pakistan

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Abstract: The crown gall is a world wide tumor forming disease of the plants and are a major problem for plant nursery industries. This disease is caused by pathogenic species of soil borne bacteria *Agrobacterium* and cause great economic loss in fruit plants. From July to November in 2008 an extensive survey was conducted in five villages (Chalt, Skindarabad, Gulmit, Askurdas and Nagarkhas) of the Nagar valley of Gilgit-Baltistan randomly by walking in a zigzag pattern and examined the plants for typical curly top symptoms to determine the incidence and severity of crown gall on cherry, apple and apricot plants. A total of 6100 cherry, 6900 apple and 8000 apricot plants were inspected and the mean incidence of crown gall on cherry plants was found to be 5360 (87.87%), apple plants 6069 (87.96%) and in apricot plants 00 (00.00%). In cherry plants, the severity of the disease observed varied from 33.80-40% in mild, 20-34.49% in moderate and 27.85-40% in severe. While in the apple plants it varied from 36.81-42.5% in mild, 29.14-34.49% in moderate and 27-30.31% in severity. There was no infestation of crown gall in apricot plants. A total of 35 samples (15 soil samples; 1 from each orchard of each village and 20 tumor samples; 2 from each orchard of cherry and apple infected plants) from each inspected village were cultured on modified selective medium (3-Ketolactose agar). The growth of *Agrobacterium tumefaciens* was identified on the basis of colony characteristics and biochemical tests based on Bergey's Manual of determinative Bacteriology. A 100% infestation of *A. tumefaciens* was observed from both the plant and soil samples except for the soil sample cultures from the apricot orchard. All the 30 *A. tumefaciens* strains isolated from the soil specimens and infected cherry and apple plant tumors were tested against six different antibiotics by disc diffusion method. All the strains were resistant against Lincomycin, Amoxycillin, Ampincillin and Cloxacillin while Cephradine, Tetracycline and Dioxycycline, showed intermediate sensitivity.

Key words: Crown gall diseases, crown gall in cherry, apple and apricot trees, incidence of *A. tumifaciens*

INTRODUCTION

Crown gall is a worldwide plant disease of economic significance in nurseries, vineyard and fruit orchards (Abussaoud and Al-Momani, 1992; Keane *et al*., 1970; Wang *et al*., 1991; Panagopoulas and Psallidas, 1973; Schroth and Moller, 1976; Sule, 1978).

In fruit plants, crown gall is caused by a rod shaped flagellated, gram negative soil borne bacterium, *Agrobacterium tumefaciens* (Smith and Townsend, 1907).

The galls provide a nutrient rich environment for the growth of *A. tumefaciens* and it returns to the soil as the galls decompose (Gillman, 2005). In the soil, survival of the organism is well documented (Burr and Katz, 1983). From the soil, with the help of flagella they swim towards photoassimilates that accumulate in the rhizosphere around roots. Some strains may chemotactically move towards chemicals that indicate a wounded plant cell, such as acetosyringone, where they colonize on the plant's wounded tissue. These wounds may be made by use of agriculture tools, nematode, insect feeding and/or frost injury. The attachment of the bacteria to the plant is a two step process; following an initial weak and reversible attachment, the bacteria synthesize cellulose fibrils that anchor them to the wounded plant cell. *A. tumefaciens* manages to survive in the rhizosphere on materials that leak from wounded host plant stems and roots, these are sugars and phenolic compounds and attract the motile crown gall bacteria. The bacterium primarily affects the plant by attachment to the cell and insertion of the plasmid (Ti plasmid) with genes that code for production of plant growth-regulating hormones by horizontal gene transfer. The bacterial plasmid genes induce the production of higher than normal concentrations of plant hormones (auxins and cytokinins) that favor bacterial growth at the expense of the plant. Thus, the crown gall genes induce the plant cells to grow more profusely and to a larger size than they would normally grow, thereby forming galls.

Galls appear within weeks at above 70°F temperature and latent infection typically develops into galls in a later growing season.

Agrobacterium is a causal agent producing crown gall disease in over 600 species of trees (Wang *et al*., 2000). Its virulent strains infect dicotyledonous plants that belong to about 90 different families and few

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monocotyledonous plants causing crown gall disease throughout the world (De Cleene and Deley, 1976).
The infected plants become weakened, stunted and unproductive. Yield loss from the disease occurs primarily at nurseries, where galled plants should be discarded (Al-Momani, 1987; Moore and Cooksey, 1981). Crown gall disease can also cause severe stunting of the mature plants (Agrios, 1978).

MATERIALS AND METHODS

Survey area: This extensive survey of fruit trees (cherry, apple and apricot) was conducted in five villages of Nagar valley by walking in a zigzag pattern from July to November, 2008 and the samples were collected for culture in sterilized polythene bags.

Disease assessment: Plants were randomly inspected for the assessment of crown gall disease using two parameters; disease incidence and its severity level as shown below:

$$\text{Disease incidence} = \frac{\text{Number of infected plant}}{\text{Total number (Healthy and infected)}} \times 100$$

Severity level was assessed as:

Size of Infection	
Mild	1-3 cm
Moderate	3-6 cm
Severe	above 6 cm

Sample cultures: Twenty tumor samples (2 from cherry plants, 2 from apple plants of each orchard inspected) from each village and fifteen soil samples (1 sample from each orchard of cherry, apple and apricot) were cultured on modified selective medium (3-Ketolactose agar) and incubated at 28°C for 48 hours.

Preparation of tumor samples for culture: In the laboratory, the surface of the tumor tissue was disinfected by dipping for 1-3 min (depending on the sensitivity of tissue) in 10% household bleach (1 part bleach: 9 parts water). A 10 to 20 gram piece of tissue was cut with a sterilized surgical blade, homogenized in 10 ml sterile distilled water using mortar and pestle, mixed by vortexing and 100 µl of the sample was cultured on the modified selective medium (3-Ketolactose agar).

Preparation of soil sample for culture: The soil samples from different orchards of each surveyed village were collected from the top 20 cm, sieved to remove particles larger than 2 mm. One gram of the soil particles were suspended in 100 ml of sterile distilled water. After vigorous shaking, serial dilution up to 10^5 was prepared and 0.1 ml of the appropriate dilution was spread on the selective medium plate and incubated at 28°C for 48 hours.

Identification of *Agrobacterium tumefaciens*: Bacterial colonies with smooth, glistening, translucent, convex, circular and colony colour ranging from light blue to olive green were selected. Further identified by Gram staining, motility, oxidase, catalase, urease, citrate utilization tests and alkaline and acid production from Tripple Sugar Iron (TSI) and H_2S production from cystine, based on Bergey's Manual of Determinative Bacteriology (Kersters and Deley, 1984) were performed.

Sensitivity against antibiotics: Two to three isolated colonies were picked and mixed in 2 ml nutrient broth, vortexed to resuspend the cells and spread onto the sensitivity test agar surface of Mueller-Hinton agar plates (MHA, Difco) by disc diffusion method (Bauer *et al*., 1966).
The *Agrobacterium tumefaciens* strains were subjected to sensitivity tests against seven antibiotics at varying concentrations by placing sterile discs with Lincomycin 10 µg, Amoxycillin 10 µg, Tetracycline 30 µg, Cephradine 30 µg, Ampincillin 10 µg, Cloxacillin 5 µg and Doxycycline 30 µg obtained from Oxoid/Difco suppliers and sensitivity was monitored after overnight incubation of the plates at 28°C.

RESULTS

Disease incidence: Table 1 shows that 6100 cherry plants from different villages of Nagar valley were randomly inspected for the incidence of crown gall and 5360 plants were found infected and the mean infestation of the disease in cherry plants was 87.87%. Of 1500 cherry plants inspected from Chalt, 1200 from Skindarabad, 700 from Gulmit and Askurdas each and 2000 from Nagarkhas, the highest infestation of the disease was found in Chalt 1370 (91.34%) followed by

Table 1: Incidence of crown gall on cherry, apple and apricot in different villages of Nagar Valley

	Name of village					
	Chalt	Sikandarabad	Gulmit	Askurdas	Nagarkhas	Total
Cherry plants inspected	1500	1200	700	700	2000	6100
Cherry plants diseased	1370	988	600	580	1822	5360
Disease incidence %	91.34	82.34	85.72	82.86	91.1	87.87
Apple plant inspected	1400	1200	900	1000	2400	6900
Apple plants disease	1270	1000	777	800	2222	6069
Disease incidence %	90.72	83.34	86.34	80.00	92.59	87.96
Apricot plants inspected	1600	1400	800	1200	3000	8000
Apricot disease	00	00	00	00	00	0000
Disease incidence %	00	00	00	00	00	00.00

Nagarkhas 1822 (91.1%), Gulmit 600 (85.72%), Askurdas 580 (82.86%) and Skindarabad 988 (82.34%). Similarly, 6900 apple plants were randomly inspected and 6069 were found infected with a mean infestation of the disease on the apple plants of 87.96%. Of 1400 apple plants from Chalt, 1200 from Skindarabad, 900 from Gulmit, 1000 from Askurdas, and 2400 from Nagarkhas, the highest infestation was observed in Nagarkhas 2222 (92.59%) followed by Chalt 1270 (90.72%), Gulmit 777 (86.34%) and Sikandarabad 1000 (83.34%).

Interestingly, the 8000 apricot plants from Nagar valley inspected for the infestation of crown gall showed no infestation throughout this study.

Table 2 shows that from Chalt, 1370 cherry and 1270 apple plants were inspected; in cherry plants the incidence of the disease was mild with 548 (40%) plants, moderate in 274 (20%) plants and severe in 548 (40%) plants. While, in apple plants it was mild in 515 (40.55%), moderate 370 (29.14%) and severe in 385 (30.32%) plants.

From Skindarabad, 988 cherry and 1000 apple plants were inspected; in cherry plants, the incidence of the severity level of the disease was mild in 380 (38.46%), moderate in 318 (32.19%) and severe in 290 (29.35%) plants. While, in apple plants it was mild in 425 (42.5%), moderate in 300 (30.0%) and severe in 275 (27.5%) plants.

In Gulmit, 600 cherry and 700 apple plants ware inspected and the incidence of the disease in cherry plants was mild in 214 (35.66%), moderate in 204 (34%) severe in 182 (30.34%) plants, whereas, in apple plants, out of 777 inspected plants, 286 (36.81%) with mild, 256 (32.95%) moderate and 235 (30.25%) were observed.

From Askurdas, 580 cherry and 800 apple plants were inspected; in cherry plants, mild incidence of the disease was found in 196 (33.80%), moderate 200 (34.49%) and severe in 184 (31.73%). In apple plants, the mild incidence was in 298 (37.25%), moderate in 274 (34.25%) and severe in 228 (28.5%) plants.

From Nagarkhas, 1822 cherry and 2222 apple plants were inspected; in cherry plants, the incidence was mild in 720 (39.52%) plants, moderate in 594 (32.57%) and severe in 508 (27.85%) plants. While, in apple plants, mild incidence in 926 (41.68%), moderate 696 (31.33%) and severe in 600 (27%) plants was recorded.

Table 3 shows the number of colonies of *A. tumefaciens* from the soil of cherry, apple and apricot orchards. The infestation in the soil of cherry orchards is high in all the villages as compared to apple orchards. There is no observed infestation of *A. tumefaciens* in the soil from the apricot orchards in all the villages.

Table 4 shows the growth of *A. tumefaciens* from tumors of cherry and apple plants from each village of the Nagar valley. All the cultured samples show growth of *A. tumefaciens*.

Table 2: Severity level of crown gall disease in cherry apple and apricot in different villages of Nagar valley

	No. of Plants infected	Disease severity		
		Mild	Moderate	Severe
Chalt				
Cherry	1370	548	274	548
Percentage (%)		40%	20%	40%
Apple	1270	515	370	385
Percentage (%)		40.55%	29.14%	30.32%
Apricot	0	0	0	0
Skindarabad				
Cherry	988	380	318	290
Percentage (%)		38.46%	32.19%	29.35
Apple	1000	425	300	275
Percentage (%)		42.5%	30.0%	27.5%
Apricot	0	0	0	0
Gulmit				
Cherry	600	214	204	182
Percentage (%)	-	35.66%	34.0%	30.34%
Apple	777	286	256	235
Percentage (%)	-	36.81%	32.95%	30.25%
Apricot	0	0	0	0
Askurdas				
Cherry	580	196	200	184
Percentage (%)	-	33.80%	34.49%	31.73%
Apple	800	298	274	228
Percentage (%)	-	37.25%	34.25%	28.5%
Apricot	0	0	0	0
Nagarkhas				
Cherry	1822	720	594	508
Percentage (%)	-	39.52%	32.57%	27.85%
Apple	2222	926	696	600
Percentage (%)	-	41.68%	31.33%	27%
Apricot	0	0	0	0

Table 3: Number of colonies grown from soil samples of orchards of cherry, apple and apricot from different villages of Nagar Valley

Name of villages	Number of colonies		
	Cherry	Apple	Apricot
Chalt	480	300	00
Sikandarabad	430	300	00
Gulmit	410	280	00
Askurdass	400	390	00
Nagarkhas	450	380	00

Table 5 shows the antibiotic sensitivity pattern of *A. tumefaciens* isolated from the plant tumors and soil samples. All the isolated strains are resistant to lincomycin, Amoxacillin, Ampincillin and Cloxacillin. Only 22 (73.34%) strains showed sensitivity to Cephradine, 28 (93.33%) strains to Tetracycline and 26 (86.66%) strains were sensitive to Doxycycline.

DISCUSSION

The main fruits of Gilgit-Baltistan are apple, almond, apricot, cherries, peaches, grapes, pomegranate and pears, which are infected by many pests and by infectious diseases. Yet many of these diseases have not been reported or recognized and they pose a

Table 4: Infestation of *Agrobacterium tumefaciens* from tumor of crown gall

Name of villages of Nagar valley	Name of plants and number of specimens cultured				
	Cherry specimens cultured (10)		Apple specimen cultured (10)		Apricot specimens cultured (10)
	Growth	No growth	Growth	No growth	Not processed
Chalt	02 (100%)	00 (00%)	02 (100%)	00 (00%)	Not processed
Skindarabad	02 (100%)	00 (00%)	02 (100%)	00 (00%)	Not processed
Gulmit	02 (100%)	00 (00%)	02 (100%)	00 (00%)	Not processed
Askurdass	02 (100%)	00 (00%)	02 (100%)	00 (00%)	Not processed
Nagarkhas	02 (100%)	00 (00%)	02 (100%)	00 (00%)	Not processed

Table 5: Antibiotic resistance pattern of *Agrobacterium tumefaciens* from the soil and plant samples

Antibiotics	No. of specimen investigated	Resistant	Sensitive
Lincomycin (10 µg)	30	30 (100%)	00 (00%)
Amoxycillin (10 µg)	30	30 (100%)	00 (00%)
Tetracycline (30 µg)	30	02 (6.67%)	28 (93.33%)
Cephradine (30 µg)	30	08 (26.66%)	22 (73.34%)
Ampincillin (10 µg)	30	30 (100%)	00 (00%)
Cloxacillin (5 µg)	30	30 (100%)	00 (00%)
Doxycycline (30 µg)	30	04 (13.34%)	26 (86.66%)

potential threat to plants in the horticultural industry in this region.

In Gilgit, the occurrence of crown gall disease in the local nurseries of (Jalalabad) in almond seedling was first reported (Anonymous, 1991) and on the bases of this report conducted a (symptom based) survey of existing fruit trees nurseries in some selected localities of Northern Areas (Gonar farm, Jalalabad, Skarkoi, Thasuit, Murtazabad, Singal, Gakutch, Pokara, Yasin and Skardu and found 90% nurseries are infected with as high as 80% infestation (FAO report, 1992).

The present investigation was conducted during July to November 2008 in fruit nurseries of different villages of Nagar Valley in the District Hunza-Nagar to record the incidence, severity level and causative agent of the Crown gall disease in stone fruits especially cherry, apple and apricot. The cherry and apple plants were severely affected. In Algeria, Bouzar and his colleagues in (1991) conducted a survey and found 99% of the plant nurseries were infected with crown gall. The results of this study in Gilgit-Baltistan nurseries are not unexpected. Crown gall is considered universal in its distribution, occurring wherever stone fruits are grown. The cultivation of stone fruits in the area for centuries and the propagation and distribution of the plants from these nurseries throughout the area for more than 40 years would have provided ample opportunity for the introduction and dissemination of pathogen in the area as the disease spreads by transplantation and grafting of the infected section of the plants (Hafiz, 1986). In India Sharma *et al*. (2002) conducted a survey in Sirmour, Solan, Shimla, Kullu and Mandi districts of Himachal Pradesh during 2001-2002 to record the prevalence of crown gall disease in stone fruit nurseries. They also found the cherry and peach rootstock Colt, were worst affected with the disease incidence of 100% on cherry plants of Palsehar in Mandi.

Seventeen nurseries in England were surveyed for crown gall (Agrobacterium tumefaciens). The disease was detected in nine of 101 apple root-stock beds examined: five were on the only nursery surveyed that was on peat soil (pH 5.9) and 49-84% of these beds were galled; 1-4% was recorded in single beds on four other nurseries. It was found on five of seven nurseries with F12/1 cherry rootstock beds: three of four beds examined had 6, 6 and 43% of stools affected and two of three layer beds had 4 and 12 gall clusters/20 yd (18.3 m) run of bed. Slight infection (one gall cluster in 20 yd of bed) was found in one of 17 plum beds on seven nurseries and 16% of stools affected were in one of 14 quinces A beds on 11 nurseries. The presence or amount of crown gall could not be related to type of bed, to age of bed, to soil pH or to type of loam or silt soil (Lelliott, 2007).

In our study the sensitivity of the isolated strains of *A. tumefaciens* is very low. Only the tetracycline has 94% sensitivity and the cephradine and doxycycline is 73-86%. The lincomycin, amoxicillin, ampincillin and cloxacillin are 100% resistant. In a study conducted by Hafiz (1986) proved quite effective the treatment of terramycin and venomycin against tumor formation.

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Optimization of Indoor Production of Fresh Water Rotifer, *Brachionus calyciflorus,* b: Feeding Studies

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Abstract: *Brachionus calyciflorus* is commonly found in fresh water ponds. Its production depends on unrelenting supply of *Chlorella* in sufficient quantity. In the current studies a water sample was collected from fish culture ponds by Wisconsin plankton net (64 µm mesh). The freshly collected stock was concentrated and fractionated by passing through 600, 200, 125, 75 and 38 µm sieves arranged vertically with gradual decrease in pore size. The pure *Brachionus calyciflorus* were fed on *Chlorella* available in the laboratory. Algae was gradually replaced by yeast to reduce dependency on labour intensive live food. Maximum number of rotifers 413 ml^{-1}, was observed when they were fed on 160:32 yeast:algae ratio by weight combination. Ciliates and cyclops posed a major threat during culture and frequent crashes were observed due to this menace. Cyclops were selectively eradicated from the rotifer culture at 0.09 DDVP after 20 h of exposure but not ciliates. There was no selective mortality in ciliates at any stage. Nevertheless both ciliates and rotifers were dead at 0.2 ppm. Simple method of cyst preservation is mentioned.

Key words: *Brachionus calyciflorus, Chlorella* sp., yeast

INTRODUCTION

The larvae of culturable Indian and Chinese major carps hatch in relatively undeveloped state. They depend on the nutrients stored in the yolk sac for the first few days. On completion of yolk sac absorption, they demand immediate external food supply for nourishment. Microorganisms present in rearing environment, serve as the first food to fish larvae during the initial period of their life after they hatch from eggs and soon after mouth opening (Lubzens *et al*., 2001). In wild these organisms are available in the form of variety of zoo and phytoplankton but in captive fish culture environments, they need to be provided either from outside sources or from laboratory cultures. Hence culture of live food, rotifer for example, is an important component of a successful fish hatchery (Lee *et al*., 2002). Non-availability of appropriate food at this stage is a major cause of larval losses.

Rotifers have been widely used as essential food source in raising freshwater and marine fish larvae due to its unique characteristics (Lubzens, 1987; Dhert, 1996). It is easily digestible, has appropriate size, can survive in high stocking densities and swims slowly giving an ample opportunity to its predator for prey (Qie *et al*., 1997; Lubzens *et al*., 2001). It possesses apposite biochemical composition that suits the nutritional requirements of larval fish. In addition to the above it has the potential for enrichment with essential nutrients like fatty acids and vitamins if required and various therapeutants for production of healthy fish (Maragelman *et al*., 1985). Therefore, successful culture of fish and shrimp in various parts of the world can be attributed partly if not totally to successful mass cultivation of rotifers.

Rotifera (Rotatoria) belong to the smallest metazoan of which over 1000 species have been described, 90% of which inhabit freshwater habitats. *Brachionus* is one of the most common genera (Dhert, 1996). This genus is important zooplankton species as a primary live food source for early life of both marine and freshwater animal species. Its body is covered with a distinct cuticle, has bilateral symmetry and possesses sexual dimorphism. *Brachionus rotundiformis* and *Brachionus plicatilis* are euryhaline and common in marine environment while *Brachionus calyciflorus* and *Brachionus rubens* are common in freshwater environment.

With the remarkable developments in larval rearing technology of important food fishes, demand for rotifers has increased considerably. *Brachionus* is intensively used to cultivate marine fish larvae due to its essential role in first feeding of fish. Accordingly research on rotifers has enormously increased which is primarily devoted to the needs of aquaculture industry. Major focus is always on high stocking density culture, identification of appropriate food species and control of bio chemical factors which hinder their mass production. Not much attention has been given on its culture in

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freshwater in general and in our local environment in specific.

Our environment is not exception and neither our fish culture practices are. Similar terms and conditions apply to these fishes as applicable to those present in other parts of the world. We need this minuscule in abundance to make our fish hatcheries a successful venture. Therefore to address these concerns, we planned a study to introduce its culture and tried to replace algae, widely used rotifer feed, with cheaper feeds/ feed combinations without compromising its growth and production. Provision of good quality and sufficient micro algae as the sole food over an indefinite period is usually considered not only labor-intensive but expensive too.

MATERIALS AND METHODS

Experimental site: The studies were based on freshwater rotifer, *Brachionus calyciflorus* and were conducted simultaneously at two different places; Fish Hatchery Faisalabad and Fisheries Research and Training Institute, Lahore.

Preparation of stock culture of rotifers: Mixed population of zooplankton comprising of copepods, rotifers and cladocerans was collected from the wild. The water containing rotifers was sifted through 600, 200, 125, 75 and 38 µm mesh sieves arranged one above the other in a decreasing downward order. The material collected in the bottom most screen, was observed under microscope at 10x for confirmation of desired fauna which was saved for future rotifer culture.

Starter culture: The starter culture consisted of a static system and was limited to 500 ml Erlenmeyer's flasks. The inoculated flasks were placed at 2 cm from fluorescent light tubes (500 lux). The temperature was approximately constant at 28°C. Sufficient aeration was provided through the perforated stones based at the bottom of the flasks. The rotifers were inoculated @ 30 rotifers ml^{-1} and fed on fresh *Chlorella* containing 1.6 x 10^6 cells ml^{-1}. Rotifer culture was maximized to meet the requirement of subsequent trials.

Feeding trials: Density and production of rotifers are dependent on food availability and quality. The subsequent trials were on various food options. The *Chlorella vulgaris* was cultured in laboratory while the baker's yeast, *Saccharomyces cerevisiae* and vitamins were purchased locally. Based on algal density (cells ml^{-1}), quantities of algal supplies were determined by haemocytometer and algal volume was calculated following Nhu (2004):

$$V_1 = V\frac{N - N_2}{N_1 - N_2}$$

Where V_1 = Volume of algae supplied, V = Volume of rotifer culture, N = Target density of algae, N_1 and N_2 = Density of algae before and after inoculation of rotifers.

Supplementation of algae with vitamin C and B vitamins: Nine jars were arranged in order. They were randomly allotted to three treatment groups, three per treatment. Control group received only *Chlorella* while *Chlorella* in treatment 2 and 3 were supplemented with vitamin C and B respectively in a fixed proportions. All the three groups were fed thrice a day at 8.00, 12.00 and 16.00 h for 15 days. At the end of experimental duration each jar was randomly sampled for rotifer estimation (Table 1).

Algae-yeast trials: There were 4 treatment groups and a control. Control group was totally nourished on fresh *Chlorella*. In the subsequent treatment groups *Chlorella* was gradually replaced with baker's yeast. The sequence of treatments designed is given hereafter; (algae: *Chlorella*; 80:40, 64:80, 48:120, 32:160). Algae was fed in the morning while yeast in the afternoon. The yeast was well mixed with tap water and blended to form a uniform suspension before dispensing into respective jar for homogeneous particle dispersal. *Chlorella* was taken on wet weight while baker's yeast on dry weight basis (Table 2). Though various algae: yeast combinations were used but feeding rate was always 0.5 µg $rotifer^{-1}$.

Quantitative estimation of rotifers: All the culture water was poured and was filtered through a 45 µm Millipore membrane filter and rinsed well with several washings to remove the extraneous material. Rotifers were counted on Sedgewick-Rafter counting chamber (APHA, 2005) under Labomed light microscope model CX3. One ml of sample from each jar was transferred to Sedgewick-Rafter counter and cells were counted within 10 squares chosen randomly. The total cells were calculated using the following mathematical expression (Stirling, 1985; Rahman and Afzal Hussain, 2008 and modified by Ashraf, 2009 unpublished):

$$N = \frac{A \times 1000 \times C}{V \times F \times L \times 1000}$$

Where

N = Number of plankton cells ml^{-1} of original water
A = Total number of rotifer counted $field^{-1}$
C = Volume of final concentration of samples in ml
V = Volume of field cubic meter
F = Number of fields counted
L = Volume of original water in liters (optional)

Control of ciliates and cyclops: Ciliate contamination in algae or rotifers is common but their density depends on the hygiene and type of feed applied to the rotifers. Various dilutions of DDVP were prepared and applied to rotifer culture containers to control ciliates and cyclops (Table 3a, b and 4a, b, c).

Table 1: Effect of vitamin C and B on rotifer production in jars. Total water volume was 5 L and inocula size was 30 rotifers ml^{-1}. Number of algal cells provided $rotifer^{-1}$ were ~35000±4500 while Vitamin 'C' and 'B' were supplemented @ 8 g jar^{-1} in treatment 2 and 3 respectively

Rotifer data	Water quality data							
Treatment #	No. of rotifers ml^{-1}	Temp. °C	DO (ppm)	pH	Alkali-nity (ppm)	Hardness (ppm)	NH_3 (ppm)	CO_2 (ppm)
1	289±15.7[a]	26±2	5.6±0.4	8.9±0.3	900±50	450±34	0.02	7.8±0.6
2	310±14.5[a]	26±3	4.8±0.6	8.9±0.2	880±56	370±36	0.025	8.1±0.4
3	340±18.2[b]	26±3	4.6±0.5	8.8±0.3	720±34	440±32	0.025	8.5±0.5

Table 2: Effect of various combinations of algae and yeast on the production of rotifers. Yeast was provided @ 0.5 µg $rotifer^{-1}$

Rotifer data	Water quality data								
Treatment #	Algae to yeast ratio	No. of rotifers ml^{-1}	Temp. °C	DO (ppm)	pH	Alkali-nity (ppm)	Hardness (ppm)	NH_3 (ppm)	CO_2 (ppm)
1	80:40	211±15.3[a]	26±2	5.0±0.4	8.3±0.5	850±60	440±30	0.02	7.9±0.6
2	64:80	350±20.0[a]	26±3	4.5±0.3	8.7±0.4	875±52	370±25	0.02	7.9±0.7
3	48:120	221±19.1[a]	26±2	4.4±1.0	8.9±0.5	780±40	380±36	0.021	8.0±0.4
4	32:160	413±24.1[b]	26±1	4.2±0.4	8.9±0.3	780±45	445±39	0.025	8.1±0.4
5	96:0	180±11.3[a]	26±3	5.2±0.3	8.9±0.4	840±47	390±26	0.015	7.7±0.5

Daily protocol: Daily stirring of culture media, suspended the dregs at the bottom of the tank, which contained uneaten algae, unhealthy rotifers and accumulated fungal growth. All of this material was kept out of the harvest to maintain the quality of future cultures and preventing its accumulation in larval fish containers. Daily feeding was strictly monitored and excess was avoided. The water clarity (cloudiness) was gauged visually before each feeding. All the trials were conducted indoor and system was exposed to 16h:8h light: dark duration.

Water quality parameters: Temperature was recorded daily by mercury thermometer. Total ammonia and dissolved organic nitrogen were analyzed by water analysis kit (Hach, USA) after filtration of water sample through 0.45 µm filter. Un-ionized ammonia fraction was computed from the data of temperature and pH following Emerson *et al.* (1975) because analytical procedures do not differentiate between the two forms of ammonia in solution:

$$NH_3\text{-}N = TAN \times f$$

Where

$$f = 1/10^{pKa\text{-}pH} + 1$$

$$10^{pKa\text{-}pH} = (NH_3)(H^+)/(NH^+_4)$$

and

$$pKa = 0.09018 + 2729.92/ T + 273.15.$$

Oxygen was determined by YSI-D.O. meter (51B), pH by pH meter WTW model 735 and other parameters by Hach kit (Table 1 and 2). Any significant decrease in DO was immediately compensated with additional air stones to minimize DO fluctuations and to avoid accidental crashes.

Statistical analysis: Differences between groups were analyzed by one-way Analysis of Variance (ANOVA) and differences among treatment means were distinguished using Duncan's Multiple Comparison test at $\alpha = 0.05$ level. The results are presented as means ± SEM. The analysis was performed using SPSS statistical package (version 14).

RESULTS

Feeding studies were conducted on rotifers to maximize their production and make the culture cost effective. Algae was also supplemented with vitamin C and B in the first trial. In the second trial *Chlorella* and yeast were applied in various proportions. Rotifers produced were observed, counted and recorded.

Effect of vitamin C and B: Rotifers fed on *Chlorella* supplemented with vitamin B produced highest rotifer density (340 ml^{-1}) significantly($p<0.05$) higher than control and treatment 2. Vitamin C supplemented group was the second highest though not different from that of control group at $p<0.05$ (Table 1).

Effect of algae and yeast: All the feeding combinations performed equally except treatment group 4 (Algae:yeast; 32:160) which produced the highest number of rotifers(413 ml^{-1}) significantly ($p<0.05$)higher than its counterparts (Table 2).

Control of ciliates: Though various doses of DDVP (0.05-0.5 ppm) were applied to control ciliates but none of them was effective. Mortality was not selective and the death point was same both for ciliates and rotifers (Table 3a, b).

Control of cyclops: Similar to ciliate control various doses of DDVP (0.05-0.09 ppm) were devised and

Table 3a: Effect of various doses of DDVP on ciliates

	DDVP dose (ppm)				
Time	0.2	0.25	0.3	0.4	0.5
10 min	Rotifers + ciliates both alive	Rotifers + ciliates both alive	Rotifers + ciliates both alive	Rotifers + ciliates both dead	Rotifers + ciliates both dead
20 min	Both alive	Both alive	Rotifers dead ciliates present	Both dead	Both dead
30 min	Both alive	Rotifers dead, ciliates present	Both dead	Both dead	Both dead
1 h	Rotifers dead, ciliates present	Both dead	Both dead	Both dead	Both dead
2 h	Both dead	Both dead	Both dead	Both dead	Both dead

Table 3b: Effect of various doses of DDVP on ciliates

	DDVP dose (ppm)				
Exposure time	0.05	0.1	0.15	0.2	0.25
10 min	Rotifers + ciliates alive	Rotifers + ciliates alive	Rotifers + ciliates alive	Rotifers + ciliates both alive	Both alive but rotifers inactive
30 min	-do-	-do-	-do-	Rotifers were partially alive ciliates present	Rotifers dead ciliates alive
3 h	-do-	-do-	Rotifers dead, Ciliates alive	Both dead	Both dead
16 h	-do-	-do-	-do-	-do-	-do-

applied. DDVP dose of 0.09 showed the best results. It completely controlled cyclops permitting the normal growth and survival of the rotifers (Table 4a, b, c).

Water quality parameters: The range of physico-chemical parameters during culture of rotifers such as water temperature, pH, NH_3, Dissolved Oxygen (DO) and others did not vary much from treatment to treatment and remained within the suitable ranges during the course of experiment (Table 1 and 2).

DISCUSSION

Feeding studies were bifurcated in 2 trials. In trial 1 *Chlorella* was supplemented with vitamin C and B individually while in trial 2 with baker's yeast in different ratios.

Trial 1: Vitamin B supplementation gave the highest rotifer density significantly ($p<0.05$) higher than its counterparts (Table 1). Maruyama *et al.* (1990) indicated that B12 was essential for the population growth of rotifers. Rotifers cultivated with B12-enriched thraustochytrids (algae) contained 3.1% (w/w) of DHA and enhanced the population growth of rotifers. Lee and Park (2003) even got higher rotifer density when they supplemented condensed algae with vitamin B12. According to Shirasaka *et al.* (2005), propionyl CoA, a primer for odd numbered fatty acids was converted to succinyl CoA and it was consumed in TCA cycle in thraustochytrids, cultivated with vitamin B12. Vitamin B12-enriched thraustochytrids strain mh0186, enhanced the population growth of rotifers fed on the cells as sole feed (Hayashi *et al.*, 2007). Takao *et al.* (2005) investigated that the incorporation of Cobalt (Co) to green water produced higher number of rotifers and 3 times more vitamin B12. Yoshimatsu *et al.* (2006) further confirmed in their studies that addition of cobalt has increased the population of *Brachionus rotundiformis* by indirectly enhancing B12 production when added @ 0.01 mg ml^{-1}. Though our research work was not at cellular/molecular level which could explain all the working mechanisms of B12 nor we studied fatty acid levels in algae or rotifers, nevertheless like previous findings vitamin B12 did improve production of rotifers in current studies.

Rotifer density of vitamin C supplemented group was equal to control. Hapette and Poulet (1990) in their studies observed vitamin C in 26 species of the major zooplankton taxa. This confirms the ubiquity of this essential micronutrient in eukaryotes. Copepods and their fecal pellets were found substantial carriers of vitamin C constituting a potential pathway from phytoplankton to consumers. Treece and Davis (2000) in their findings further confirmed that vitamin A and B as dietary essentials for rotifer production but not vitamin C. Previous studies endorse ours with the affirmation that vitamin C is not a dietary essential for rotifers rather they meet their requirements from algae, the potential vitamin C producer for zooplanktons. Interesting results were however, found when all these groups were exposed to higher temperatures. All the rotifers in *Chlorella* and vitamin B12 group perished immediately at 34°C but not vitamin C group which survived up to 40 ^{0}C temperature. Role of vitamin C in enhancing the resistance capability of higher organisms is well documented (Ashraf *et al.*, 2008) but was not clear in zooplanktons. Hien *et al.*

Table 4a: Effect of various doses of DDVP on control of cyclops

Exposure time (min)	DDVP dose (ppm) 0.05	0.1	0.15
10	Cyclops and rotifer both alive	Both alive	Both alive
20	-do-	-do-	-do-
30	-do	-do-	-do-
60	-do-	-do-	Both were dead

Table 4b: Effect of various doses of DDVP on control of cyclops

Exposure time (min)	DDVP dose (ppm) 0.11	0.12	0.13	0.14	0.15
10	Both alive	Both alive	Both alive	Both alive	Both alive
30	-do-	-do-	-do-	-do-	Cyclops dead + rotifer alive
60	Both dead	Both dead	Both dead	Both dead	Both dead

Table 4c: Effect of various doses of DDVP on control of cyclops

Exposure time	DDVP dose (ppm) 0.05	0.06	0.07	0.08	0.09
10 min	Rotifers+ cyclops both alive	Rotifers + cyclops both alive	Rotifers + cyclops Both alive	Rotifers + cyclops both alive	Rotifers + cyclops Both alive
30 min	Both alive	Both alive	Both alive	Both alive	Both alive
1 h	Both alive	Both alive	Both alive	Both alive	Both alive
2 h	Both alive	Both alive	Both alive	Both alive	Both alive
5 h	Both alive	Both alive	Both alive	Both alive	Both alive
11 h	Both alive	Both alive but cyclops inactive	Both alive but cyclops inactive	Rotifers alive cyclops half dead, half alive	Rotifer alive cyclops mostly dead. Some alive but inactive
20 h	Rotifers alive cyclops dead	Rotifers alive cyclops dead	Rotifers alive cyclops dead	Rotifers alive cyclops dead	Rotifer alive cyclops dead

(1999) determined the effects of various concentrations of vitamin C in diets, on the survival, metamorphosis and resistance capability of larval freshwater prawn (*Macrobrachium rosebergii*). Vitamin C enhanced survival up to 500 mg, with no effect on metamorphosis and improved resistance to bacterial infection up to 1000 mg incorporation kg^{-1} of diet. These studies substantiate our findings and further open new avenues for comprehensive investigative work on these lines.

Trial 2: Rotifers were cultured on *Chlorella vulgaris* alone and also with yeast supplementation. Purpose was to gradually replace or at least minimize the inclusion of expensive and labor intensive production of *Chlorella* ((Lubzens, 1987). Significantly ($p<0.05$) higher rotifer density (413 ml^{-1}) was observed at 32:160 *Chlorella:* yeast combination than its counterparts. There was no gradual trend in the increase of rotifer density with the increase in the ratio of yeast which demands further research. Oie *et al.* (1994) stated that quality and quantity of diet is the most important criteria that affects density and production of rotifers. Sarma *et al.* (2001) reported that *Brachionus calyciflorus* grew better on *Chlorella* alone while *Brachionus patulus* grew equally well when fed only on *Chlorella* or when mixed with *Saccharomyces cerevisiae* in equal proportions. *Chlorella* produced 100-150 individuals ml^{-1} while addition of baker's yeast enhanced it up to 562 individuals ml^{-1} meaning that species cultured also has lot of bearing on production. Previously Watanabe *et al.* (1983) found out that sudden rotifer crashes and larval losses can be prevented by culturing rotifers on both yeast and then feeding on marine *Chlorella*. Recently Mostary *et al.* (2007) obtained contradictory results to previous and our studies when he used dried, fresh *Chlorella* and baker's yeast for culture of rotifers. The mean population densities of *Brachionus angularis* recorded in treatment 1, treatment 2 and treatment 3 were 30.1±12, 37.4±14.6 and 21±6.1 ind. ml^{-1} respectively highest on fresh *Chlorella* while the lowest on yeast.

The rotifer density observed in current study was well close to the above observations which further validate our findings. Even copepods are not exception and show similar behaviour when cultured in captivity. Rhodes (2003) reported that copepod population fed formulated feed grew significantly faster and achieved significantly higher population densities ($p<0.01$) (the highest intrinsic growth rates than those fed on live algal food only). Use of baker's yeast is not something unusual but even ground shrimp meal, flour, rice bran, dried frozen algae and formulated diets have been extensively used

for culture of rotifers (Lubzens *et al*., 1995) because rotifers can ingest food particles of up to 30 µm, including bacteria, baker's yeast, *Saccaromyces cerevisiae*. Baker's yeast has as such no nutritional value for rotifers but bacteria associated with the yeast is the source of nutrition for rotifers (Alessandro *et al*., 1999). Still much higher rotifer densities can be achieved with better hand on bio-chemical factors and maintenance of proper hygienic conditions.

The range of physico-chemical parameters during culture of rotifers such as water temperature, dissolved oxygen, pH, NH_3 and others were within the acceptable limits and more or less similar in all the treatments. Though there were slight variations in some water quality parameters but that might not have any bearing on the integrity of findings (Table 1 and 2).

Ciliate contamination was a major problem during rotifer culture which badly affected stability and production of rotifers not only in our attempts but it was more prominent in the past studies (Hino, 1993) (Watanabe *et al*., 1983). Halotricha and Hypotricha cilciates, such as *Uronema* sp. and *Euplotes* sp., are not desired in intensive cultures since they compete for feed with the rotifers. The appearance of these organisms is generally due to sub-optimal rearing conditions. They decrease the performance of rotifers and increase the chances of competition for food and air. Ciliates produce metabolic wastes which increase the NO_2-N level in the water and cause a decrease in pH. In current studies NH_3 remained in the normal range that might be due to low rotifer density hence its effects were not discernable. Dhert (1996) however, has different view point and states that presence of ciliates in the culture medium is not necessarily harmful except at high concentrations. He however, did not differentiate the high or low levels of ciliates in rotifer culture media. Nhu (2004) nullifies the Dhert (1996) and says that excessive presence of ciliates in mass cultures lead to considerable reduction in yield because of their activity which brings about an aggregation of food and thereby reduce the availability to rotifers. A lot were present in our cultures too which really has negative impact on rotifer proliferation. High concentrations of ciliates were related to low fertility of culture medium, low rotifer density and finally mass mortality in ours as well as in previous studies (Dhert, 1996). To achieve pure culture of the rotifer, *Brachionus calyciflorus*, Arimoro and Ofojekwu (2004) recommended the use of 'Basudine' an organophosphoric acid ester applied at the rate of 1.5 mg l^{-1}. At this concentration copepods and cladocerans and aquatic insects including mosquito larvae failed to flourish thereby permitting the rotifers to multiply. Ludwig (1993) found Trichlorfon, an organophosphate parasiticide that inhibits cholinesterase, very toxic to free swimming copepods and their nauplii but does not kill rotifers at dosages of 0.25 ppm (active ingredient). Arimoro (2006) renewed inoculum containing ciliates and other undesired insects with 10 ppm Oxyteteracycline 30 mg l^{-1}, Sarafloxacin or Lincospectin 30 mg l^{-1}. We used DDVP in our studies for control of ciliates and cyclops. Ciliates survived up to 0.3 ppm. They started dying at 0.2 ppm and totally died at 0.4 ppm but unfortunately at this concentration rotifers could not survive too (Table 3a and b). Cyclops however, proved much easier to control. They were selectively controlled at 0.09 ppm for 30 min exposure only without harming the rotifer stock (Table 4a, b and c).

Nevertheless we were able to attain 413 rotifers ml^{-1} on combination feed which has been achieved in the past in selected cases under different experimental and environmental conditions. We were able to control all the cyclops at 0.09 ppm DDVP which was far less than used by Arimoro (2006) which can increase the operational cost of the hatchery and may induce toxic effects to both rotifers and fish. Although with several accomplishments there were lot of failures too but it is really a major breakthrough in indoor culture of rotifers in this country. Presently rotifers are totally produced in wild and fish fry is left at the mercy of that unknown food sources whose both quality and quantity are uncertain. Further we have devised easy and cheaper methods for purification of rotifer culture. However, still lot needs to be done in this field especially in our local environment.

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Radical Scavenging Capacity of Rwandan CTC Tea Polyphenols Extracted Using Microwave Assisted Extraction

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Abstract: Extracts of Cutting, Tearing and Curling (CTC) black and green teas from Rwanda were extracted using decoction and Microwave Assisted Extraction (MAE). Green Tea Extract from Microwave Assisted Extraction (GTMAE) had higher concentration of total tea polyphenols than others; (GTMAE26%>GTD21%>BTMAE16%>BTD14%). The higher the concentration of the total tea polyphenols, the higher was free radical scavenging activity. Antioxidant activity of the teas extracts is expressed by the DPPH radical scavenging with the decreased absorbance in the order: green tea microwave assisted extraction > green tea decoction > black tea microwave assisted extraction > black tea decoction extraction. Consequently, MAE method was found to much more effective than decoction in total polyphenolics content, scavenging capacity, time and energy consumption for both CTC black and green tea.

Key words: Tea extracts, CTC tea, total polyphenols, decoction, microwave assisted extraction, scavenging capacity

INTRODUCTION

The tea plant *Camellia sinensis* (L.) Kuntze (family Theaceae) is native to the East Asia region and is grown in Rwanda and in many more countries worldwide. Tea is consumed worldwide, although in greatly different amounts; it is generally accepted that, next to water, tea is the most consumed beverage in the world (Hasan and Nihal, 2000).

Tea has been consumed for many thousands of years, but it is only in the last few decades that we are beginning to understand the full potential of this widely enjoyed beverage (Huafu Wang *et al*., 2000).

Tea Flavonoids, or tea extracts have been linked to benefits in reducing the risk of certain cancers and cardiovascular diseases in experimental animals (Huafu Wang *et al*., 2000).

Catechins dominate in green tea and theaflavins and thearubigins predominate in black tea. These kinds of tea flavonoids are thought to have the strongest chemopreventitive effects in animal models at the concentrations usually consumed by humans (Dreostic *et al*., 1997).

Tea polyphenols have a strong affinity for proteins and minerals and thus may affect nutritional status. The various phenolic groups of tea can bind to more than one place on a protein via hydrophobic interactions and hydrogen bonding. Polyphenols have a strong affinity for proteins with high proline content, such as milk caseins, gelatin and salivary proline-rich proteins. Whether tea consumption impairs protein absorption in humans remains to be investigated. Because of the strong binding affinity of tea polyphenols to metal ions, the possible effects of tea on the absorption of these nutrients is of importance (Chung and Janelle, 2000).

Most of the green tea polyphenols are flavonoids known as catechins. Catechins constitute about 25% of the dry weight of the tea leaf, although the total catechin content varies widely depending on clonal variation, growing location, seasonal/light variation and altitude (Balentine *et al*., 1998). In Fig. 1, are some major catechins: (-)-epigallocatechin-3- gallate (EGCG), (-) - epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), (-)-epicatechin (EC). In the manufacturing of black tea, the monomeric flavan-3-ols undergo polyphenol oxidase dependent oxidative polymerization leading to the formation of flavonols known as thearubigins and theaflavins and other oligomers in a process known as “oxidation”. Theaflavins possess benzotropolone rings with dihydroxy or trihydroxy substitution systems which give the characteristics color and taste of the black tea. About 10-20% of the dry weight of black tea is due to the thearubigins, which are even more extensively oxidized and polymerized, have a wide range of molecular weights and are less characterized (Lin *et al*., 2003).

The studies found that Total Phenolic Content (TPC) of green tea was higher than that of black and oolong tea due to the reduction of catechins during fermentation process and that also affected radical scavenging activity of the tea (Yokozawa *et al*., 1998) and confirmed by Atoui *et al*. (2005).

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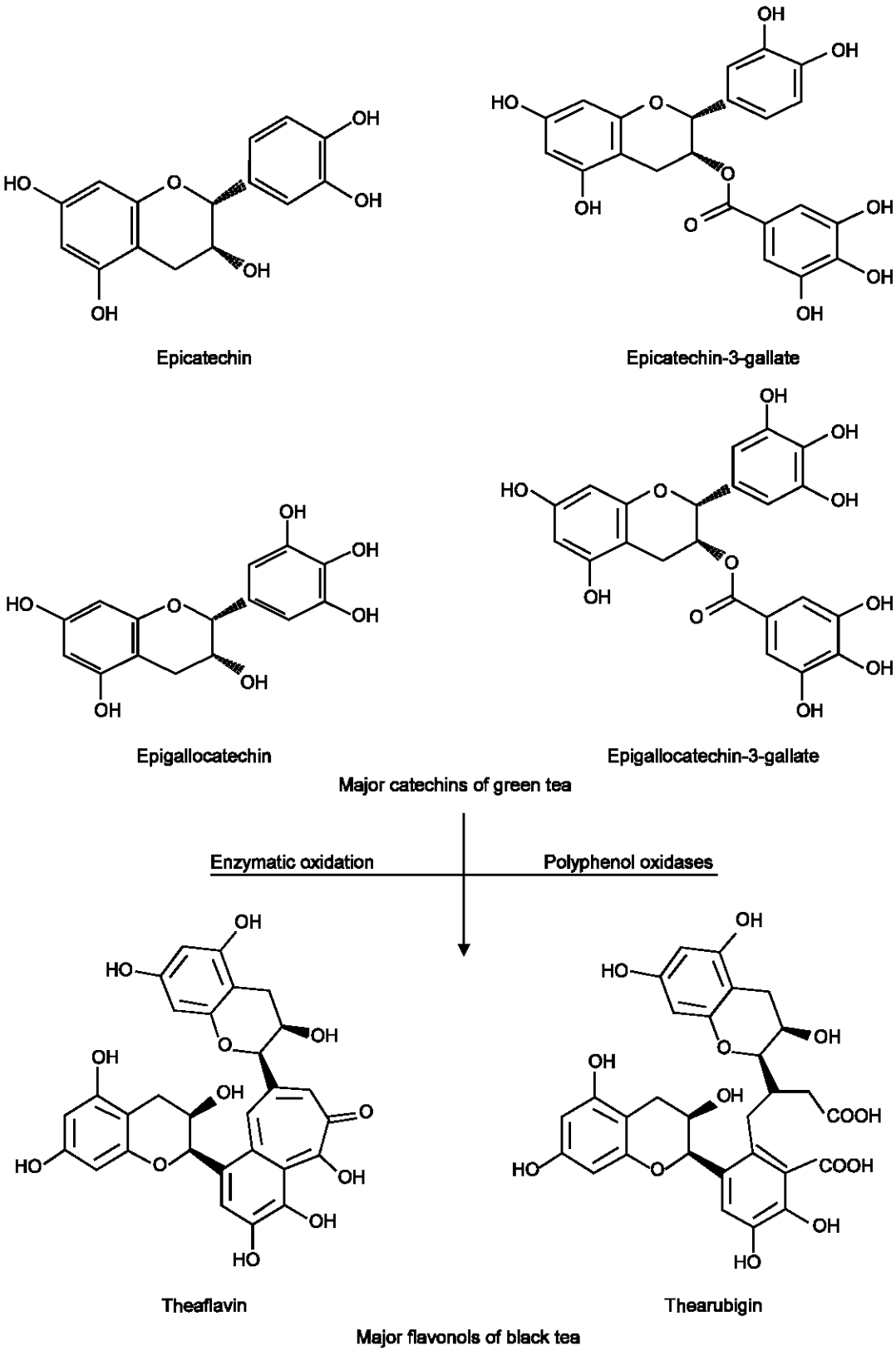

Fig. 1: Oxidation of catechins of green tea into flavonols of black tea

Microwave digestion of matrices for their eventual elemental analysis has been routinely used for several years (Kingston and Jassie, 1988). Recently, Microwave-assisted Extraction (MAE) has been used for the extraction of biologically active compounds, such as extraction of essential oils from the leaves of rosemary and peppermint (Chen and Spiro, 1994), extraction of taxanes from Taxus biomass (Mattina *et al*., 1997), extraction of ergosterol and total fatty acids from fungal hyphae and spores, mushrooms, filtered air, artificially contaminated corn, naturally contaminated grain dust and soil (Young, 1995) and the extraction of azadirachtin-related limonoids from neem seed kernel (Dai *et al*., 1999).

The aim of this study was to quantify the total polyphenolic content and the scavenging capacity of Rwandan CTC black and green teas, using new technology of extraction which uses the microwave and ultrasonic energies.

Results are reported here, on the scavenging capacity and contents of total phenolics in teas commonly processed and consumed in Rwanda.

MATERIALS AND METHODS

CTC (cutting, tearing and curling) black and green teas were purchased in Rwanda and brought to Jiangnan University and were processed and analyzed in food safety and quality control laboratory of the school of food science. Their moisture content was measured using a laboratory scale apparatus (SHANGPING DHS16-A). All chemicals used in the experiments were of analytical grade and available from the chemical department of Jiangnan University.

Tea extraction methods

Decoction or hot water extraction: In brief, 10 g of dried sample were extracted with 300 ml of distilled water at a temperature 77°C in a water bath for 30. Extracts were cooled at room temperature and filtered using whatman No.40 filter paper under vacuum. Samples are concentrated using rotary evaporator .final extracts were kept at 4°C for further use.

Microwave assisted extraction (MAE): A laboratory scale microwave extraction apparatus (CW-2000; Xintuo Technology Company. Shanghai, China) operated at atmospheric pressure with microwave frequency was used for the extraction purpose. The apparatus was equipped with a digital controlled system for temperature, time and power. The microwave power was of 450 W and the radiation was done for 120 sec to keep temperature not to rise above 70°C. 10 g of dried sample were extracted with 300 ml of distilled water as previous experiments. Final extracts were kept at 4°C for further use.

Total tea polyphenols determination: The amounts of total phenolic content in tea extracts were determined according to Yuanyuan *et al*. (2005).with modification. Triplicate mixtures of 2 ml extract filtrate, 8 ml distilled water and 10 ml iron tartrate solution were prepared in volumetric flask and toped to 50 ml with 0.05 mol/l sodium phosphate buffer solution at pH 7.5. The absorption values of the extracts were measured spectrophotometrically at a wavelength of 540 nm. Pure tea polyphenols were used as standard. The calibration equation for pure tea polyphenols was:

$$y = 0.356x + 0.010$$
$$R^2 = 0.998$$

DPPH free radical scavenging activity of tea extracts: The antioxidant activity of tea extracts were measured, as described by Yi *et al*. (2008) with some modifications, in terms of radical scavenging ability or hydrogen donating, by using the stable radical 2, 2-diphenyl-1-picrylhydryzyl (DPPH).

An aliquot of 1.5 ml of sample solution (2 mg/ml) was mixed with 1.5 ml of methanolic solution of DPPH (0.2 mM). The reaction mixture was incubated for 30 min in the darkness at room temperature. The absorbance of the resulting solution was measured with spectrophotometer at 517 nm. Methanol instead of sample solution was used as a control. DPPH scavenging capacity of the tested samples was measured as a decrease in the absorbance and calculated using the following equation:

$$\text{DPPH scavenging capacity (\%)} = (1-A_{sample}/A_{control}) \times 100$$

Where A_{sample} and $A_{control}$ are the absorbance at 517 nm of the sample and control, respectively.

Statistical analysis: Determinations were carried out in three triplicate and data subjected to analysis of variance. Analysis of variance was performed using the ANOVA procedure. Statistical analyses performed according to SAS software. Significant differences between means were determined by Duncan's multiple range tests. p values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Moisture content was brought to 3% and this facilitates the pre processing of tea. Green tea was grinded into powder, this reached to breaking cells to facilitate the extraction.

Effect of cutting, tearing and curling (CTC) on extraction and black tea fermentation on total polyphenol content (TPC): Processing routes can be seen to result in a significant compositional of the green

Table 1: CTC black tea manufacturing and size fractions

Grade	Nominal classification	Particle size range
Broken Pekoe (BP)	Large	1.7-1.18 mm
Pekoe Fannings (PF)	Medium	1.18-500 µm
Pekoe Dust (PD)	Small	500-250 µm

From Conrad *et al.* (2001)

and black tea products. Differences in leaf size have an influence on the extraction efficiency of the tea component due to cell destruction during the CTC process and this result to exposure and extraction of tea components.

During green tea production, the main polyphenols (the catechins) remain relatively intact during the process, this is due to the enzymes, which catalyze their oxidative polymerization, are deactivated by heat treatment soon after plucking. Black tea production involves a leaf disruption step to promote the enzymatic oxidation of the flavonols (catechins) present in fresh green tea leaf to produce polymeric flavonoids (theaflavins and thearubigins), Robertson (1992) . The total polyphenol in green tea (26% GMAE and 21%GTD) are higher than in black tea(16%BMAE and 14% BTD); due to the different molecular properties of the green tea polyphenols (mainly catechins) and black tea polyphenols (mainly polymeric thearubigins), which have an influence on total polyphenols results. Fig. 2 shows the variation of total polyphenol level within the green and black tea samples using different methods of extraction.

DPPH free radical scavenging activity of tea extracts: DPPH radical scavenging show the antioxidant activity of different tea extracts. Figure 3 illustrates the decrease in absorbance of the DPPH radical due to the scavenging ability of soluble solids in the different tea extracts.

All tea extracts showed decrease in absorbance, where by Green Tea Extract Using Microwave Assisted Extraction (GTMAE) exhibited the fastest decrease compared to other extracts.

This shows the hydrogen donating ability as evaluated by DPPH radical scavenging method and the decreasing is in the order: GTMAE>GTD>BTMAE>BTD.

Green tea extracts (GTMAE and GTD) showed the fastest decreasing due to availability of tea polyphenols especially catechins.

Black tea extract (BTMAE and BTD) showed slow decreasing in absorbance due to less content in tea catechins compared to those in green tea extracts. Ki Won Lee and Hyong Joo Lee (2002) conclude that green tea has more health benefits than an equal volume of black tea in terms of antioxidant capacity. This can be explained by the fact that each tea is different in terms of composition and concentration of antioxidant compounds.

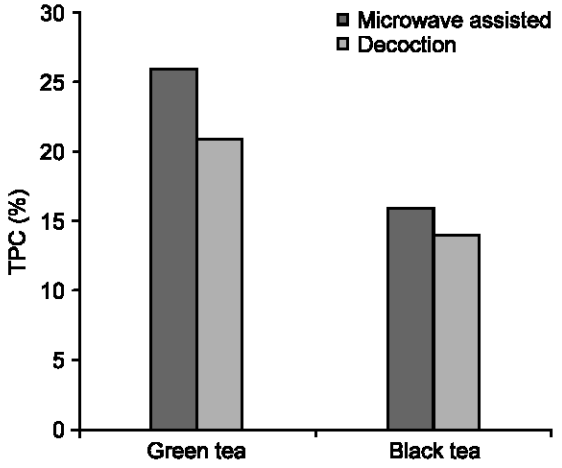

Fig. 2: The Total Polyphenolic Content (TPC) of tea extracts using different extraction methods

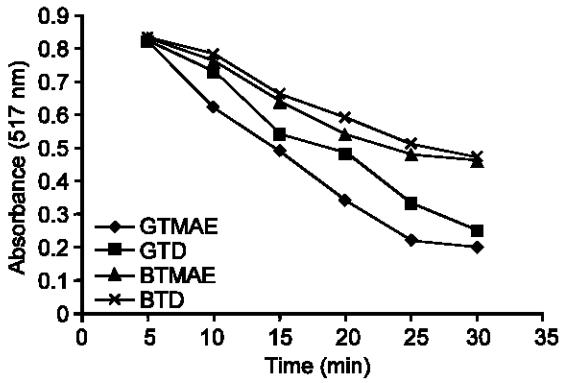

Fig. 3: The decrease in absorbance of the DPPH radical

During the fermentation process in black tea processing, catechins are oxided in thearubigins and theaflavins which leads to less scavenging capability.

Conclusion: Extracts obtained from Rwandan CTC black and green tea showed difference between green tea and black tea in total polyphenol content using different methods of extraction. Green tea extracts showed higher content in total polyphenols content compared to black tea extract for both methods of extraction; microwave assisted extraction and decoction with 26% and 21% for green tea and 16 and 14% for black tea respectively.

High content in polyphenols mainly catechins of the green tea extracts, showed fastest decreasing in absorbance of the DPPH radical, this was due to the scavenging capability of the catechins. therefore, MAE method was found to much more effective than decoction in total polyphenolics content ,scavenging capacity, time and energy consumption for both CTC black and green tea.

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Application of GnRH Administration at Post Artificial Insemination in Synchronized Estrus Heifer and Dairy Cows by $PGF_{2\alpha}$ Induction on Conception Rate in Phupan Dairy Co-operative, Sakol-Nakon Province, Thailand

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Abstract: This experiment was conducted to study the effects of GnRH on conception rate of 32 crossbred Holstein-Friesian dairy cows. All cows were uniform of body condition score and were taken care by the management of small holders in Phupan dairy co-operative, Sakol-Nakon. They were randomly placed in 2 x 2 factorial in randomized complete block design. Factor 1 was for dairy heifers and lactating cows comparison and factor 2 was assigned to compare between distilled water and GnRH injection on day 5 post insemination under the treatments. Blood samples were collected and analyzed for progesterone concentration examination by RIA technique. The environment effects, general physiology and hematology changes were recorded in the entire period of the experiment. The result showed that, the Temperature Humidity Index (THI) was over than 72, which affected the increase of general physiology changes, co-efficient of heat tolerance, sweating rate, hematology and cortisol level concentration that were over standard value. However, there were no significant differences ($p>0.05$). The examination of serum progesterone concentration on day 11, 17 and 24 post artificial insemination including the conception rate were significantly different ($p<0.01$).

Key words: GnRH, $PGF_{2\alpha}$, synchronized estrus, AI, conception rate, phupan dairy co-operative

INTRODUCTION

The project of dairy cow promotion has been one approach of the government that expects to improve the national dairy cow production by establishing a good systemic plan for dairy cow production and management. However, there were still many problems for dairy cow raising in some area such as the Project of dairy cow promotion in Pattaloong province. Within 4 years of the said project operation the farmers had to cancel the dairy cow raising due to losses as much as 37 per cent of business cost (Thamawasorn and Luengwatanawilai, 1992). Another is the poor fertility of dairy cows that were raised in tropical zone (Jantalakana, 1994; Umpapol, 2002), anoestrus; silent heat and infertility affected the decrease of milk yield and calf population (Janpongsaeng, 1996). The imbalance nutrients related to gonadotropin secretion, estrous cycle or ovulation, breeding management, heat detection, breeding techniques and semen quality could affect the absolute fertility (Janpratheep, 1996).

The efficient feed value improvement (Janpongsaeng, 1996) which related with prostaglandins application for estrous cycle control or for indicating the suitable breeding time could be one means to reduce the problems of poor fertility on cows due to corpus luteum regressed in luteal phase and followed by follicular phase that caused the cows become in heat in about 45 day post-parturition. The $PGF_{2\alpha}$ (cloprostenol) administration by intramuscular injection for dairy cows in luteal phase could induce normally estrus of the cows (Iamlamai, 2003; Patarajinda, 2003).

The integration of $PGF_{2\alpha}$ and GnRH application at post artificial insemination would be one of the methods to solve the problems of poor fertility of cows during summer because of low level of progesterone. Both hormones could induce the production of accessory corpus luteum and could increase the tissue of corpus luteum and the progesterone level that caused effectively on endometrium preparation for the implantation of zygote in blastocyst phase; reducing the contraction of uterus and stimulating the uterine milk secretion for nourishing zygotes; but the early regression of corpus luteum could cause the embryonic mortality to lower the level of progesterone (Shelton *et al*., 1990). If the

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function of corpus luteum failed it caused the reduction of progesterone level that affected on the conception and gestation (Wiebold, 1998). If the level of progesterone was low after mating it could negatively interfere on zygote implantation and acceptable response of cows for zygote implantation (Inskeep, 2004).

This research aimed to study the conception rate of dairy heifers and lactating cows of the farmers in Phupan Dairy Co-operative (PDC) which raised under small holder management and the animals were induced for synchronized estrus by application of $PGF_{2\alpha}$ (Dinoprost trometamol) and GnRH injection on day 5 post artificial insemination for investigation the effects for improving and increasing the conception rate of dairy cattle under heat stress condition.

MATERIALS AND METHODS

Holstein-Friesian (HF) crossbred dairy heifers and milking cows (82.5% or over of HF blood with uniform body score), under the similar management condition of small holders who were the members of PDC, by randomization the problematic dairy cattle in conception of the voluntary small holders in the number of 32 heads (16 dairy heifers and 16 lactating cows). The experiment was conducted by 2 x 2 factorial in RCBD, factor 1 aimed to heifer and lactating cow comparison (2-4 lactation cows were used) and factor 2 was assigned to compare between 2 ml of distilled water and GnRH (Receptal ®) injection on day 5 post artificial insemination by intramuscular injection.

The synchronized estrus induction was conducted by $PGF_{2\alpha}$ intramuscular injection of 2.5 ml/head at the rump region because they were in estrus on different days but it was very close period therefore the injection was done 2 times. The first injection was conducted on day 9 post-estrus and the second injection followed 11 days thereafter. The cattle would respond to the second injection and artificial insemination could be done during 72-96 h after application. Generally, the cattle would respond to $PGF_{2\alpha}$ injection around 4-5 days before estrus and until 4-5 days after estrus. The estrus would occur 2-5 days after the injection (Willard *et al*., 2003), by observing the estrus symptom and followed by the artificial insemination services (Iamlamai, 1999).

The data collection was conducted with concerns on ambient environment and environmental effects, Temperature-Humidity Index (THI), general physiological changes, haematology, co-efficient of heat tolerance, sweating rate (Umpapol, 2002), pedigree, milk yield, ovary examination by rectal palpation, blood sampling for progesterone examination and pregnancy test by collecting blood on day 0, 5, 11, 17, 24 and 42 post artificial insemination (day 0 meaned a proestrus day before estrus and day 5 indicated a GnRH injection day) (Shelton and David, 2002) and confirmed the certain pregnancy by the results of rectal palpation on day 60 post artificial insemination.

RESULTS

The general condition of the ambient environment: The results of the study on the general ambient environment in the entire duration of the experiment found that maximum, optimum and minimum temperatures, and the different temperature between maximum and minimum temperatures were 34.80±0.40, 29.40±0.60, 24.20±0.80 and 11.20±0.70°C, respectively. The relative humidity was 84.40±4.40 (%) and THI was 86.40±5.02 (Table 1).

Table 1: Effect of the ambient environment on temperature, relative humidity and THI in experiment

Environmental	Mean±STD
Maximum temperature (°C)	34.80±0.40
Optimum temperature (°C)	29.40±0.60
Minimum temperature (°C)	24.20±0.80
Different temperature (Max. - Min.) (°C)	11.20±0.70
Relative humidity (%)	80.40±4.40
Temperature Humidity Index (THI)	86.40±5.02

Table 2: Effect of $PGF_{2\alpha}$ injection on the synchronized estrus induction in heifers and lactating cows

Item	Heifers	Cows
Number of animals (head)	16 (100.00)	1616 (100.00)
Response on estrus induction (head)	16 (100.00)	16 (100.00)
• Standing heat (head)	16 (100.00)	16 (87.50)
• Silent heat (head)	-	2 (12.50)
Anoestrus (head)	-	-

Effect of $PGF_{2\alpha}$ injection on the synchronized estrus induction: The results of the synchronized estrus induction by $PGF_{2\alpha}$ injection found that all of the dairy heifers and lactating cows showed estrus symptom and two of the lactating cows were in silent heat (12.50%) (Table 2).

Effect of the ambient environment on the general physiological changes: The study on the effect of the ambient environment on the general physiological changes among Group 1 (distilled water injected heifers), Group 2 (GnRH treated heifers), Group 3 (distilled water injected lactating cows) and Group 4 (GnRH treated lactating cows), were; the rectal temperatures were 39.38±0.20, 39.40±0.12, 39.42±0.40 and 39.42±0.20°C, skin temperatures were 36.80±0.80, 36.80±0.84, 36.70±0.74 and 36.48±0.92°C, pulse rates were 72.60±2.60, 72.42±1.48, 72.80±2.80 and 73.64±2.02 times/minute, respiration rates were 71.20±2.76, 70.60±1.48, 70.60±3.02 and 72.40±3.46 breathing/minute, sweating rates were 920.20±30.20, 916.20±31.48, 904.40±33.70 and 922.80±40.20 ml/square metre/hour, respectively with non-significant differences ($p>0.05$) (Table 3).

Table 3: Effects of environment factors on general physiology

	Heifers		Lactating cows	
General physiology	Distilled water	GnRH	Distilled water	GnRH
Rectal temperature (°C)	39.38±0.20	39.40±0.12	39.42±0.40	39.42±0.20
Skin temperature (°C)	36.80±0.80	36.80±0.84	36.70±0.74	36.48±0.92
Pulse rate (times/min)	72.60±2.60	72.42±1.48	72.80±2.80	73.64±2.02
Respiration rate (breaths/min)	71.20±2.76	70.60±1.48	70.60±3.02	72.40±3.46
Sweating rate (ml./m^2/h)	920.20±30.20	916.20±31.48	904.40±33.70	922.80±40.20

Means within row with different superscript differ significantly ($p<0.05$)

Table 4: The values of hematology and cortisol level

	Heifers		Cows	
Hematology	Distilled water	GnRH	Distilled water	GnRH
Hematocrit (%)				
• Before experiment	32.20±0.80	32.10±0.34	34.02±0.20	31.80±0.40
• During experiment	33.40±0.64	34.24±0.42	34.68±0.60	32.80±0.46
Hemoglobin (g./100 ml. blood)				
• Before experiment	9.60±0.50	10.64±0.62	9.48±0.42	11.04±0.40
• During experiment	11.20±0.34	11.96±0.74	11.28±0.50	13.04±0.62
Cortisol (ηg/mg)				
• Before experiment	12.14±0.40	12.68±0.64	12.60±0.60	13.04±0.42
• During experiment	12.82±0.26	13.26±0.34	13.68±0.76	13.40±0.62

Means within row with different superscript differ significantly ($p<0.05$)

Effect of the ambient environment on the haematological changes: The haematological values of the experimental cattle in Group 1 (distilled water injected heifers), Group 2 (GnRH treated heifers), Group 3 (distilled water injected lactating cows) and Group 4 (GnRH treated lactating cows) were studied on haematocrit percentages. In the preliminary period of the experiment (before experiment value) the percentages were 32.20±0.80, 32.10±0.34, 34.02±0.20 and 31.80±0.40% but during the experiment (during experiment value) the percentages were 33.40±0.64, 34.24±0.42, 34.68±0.60 and 32.80±0.46. The hemoglobin concentrates in the preliminary period of the experiment had the levels of 9.60±0.50, 10.64±0.62, 9.48±0.42 and 11.04±0.40 gm/100 ml of blood but during the experiment, it had the levels of 11.20±0.34, 11.96±0.74, 11.28±0.50 and 13.04±0.62 gm/100 ml of blood. The cortisol concentration in the preliminary period had the levels of 12.14±0.40, 12.68±0.64, 12.60±0.60 and 13.04±0.42 ηg/ml but during the experiment it had the levels of 12.82±0.26, 13.26±0.34, 13.68±0.76 and 13.40±0.62 ηg/ml, respectively with no significant differences ($p>0.05$) (Table 4).

The comparison of progesterone levels (ηg/mg) at post artificial insemination: The comparative results of progesterone levels of the experimental cattle in Group 1 (distilled water injected heifers), Group 2 (GnRH treated heifers), Group 3 ((distilled water injected lactating cows) and Group 4 (GnRH treated lactating cows) showed that the progesterone levels on day 0 post artificial insemination were 0.12±0. 04, 0.14±0.02, 0.10±0.20 and 0.18±0.03 ηg/mg respectively and on day 5 post artificial insemination were 1.28±0.12, 1.06±0.20, 1.12±0.14 and 1.24±0.32 ηg/mg respectively with no significant difference.

But the comparison of progesterone levels on day 11 post artificial insemination were 2.90±0.20, 4.62±0.16, 2.80±0.24 and 4.20±0.42 ηg/mg respectively, on day 17 post artificial insemination were 3.70±0.26, 6.70±0.28, 3.40±0.30 and 6.60±0.39 respectively and on day 24 post artificial insemination were 2.20±0.32, 5.80±0.24, 2.60±0.24 and 5.78±0.46 ηg/mg respectively with significant difference ($p>0.05$) (Table 5).

Pregnancy examination: The results of pregnancy examination of the experimental cattle in Group 1 (distilled water injected heifers), Group 2 (GnRH treated heifers), Group 3 ((distilled water injected lactating cows) and Group 4 (GnRH treated lactating cows) by rectal palpation on day 60 post artificial insemination found that the numbers of examined pregnant cattle were 8, 8, 8 and 8 heads respectively, but the real pregnant cattle were 3, 7, 2 and 6 heads respectively and the conception rate were 37.5, 87.5, 25.0 and 75.0% respectively with significant differences ($p<0.05$) (Table 6).

DISCUSSION

The influences of ambient environment: The results of this study indicated that the climate was tropical zone (Vajrabukka, 1996). The influence of temperature and relative humidity were correlated in term of Temperature-Humidity Index (THI) (Johnson, 1985) and when THI mean was raised up to 72 it would cause the heat stress to the dairy cows (McDowell, 1972).

Table 5: The comparison of progesterone level (ηg/mg) at post artificial insemination

Day on test (day) (Post artificial insemination)	Heifers		Cows	
	Distilled water	GnRH	Distilled water	GnRH
0	0.12±0.04	0.14±0.02	0.10±0.20	0.18±0.03
5	1.28±0.12	1.06±0.20	1.12±0.14	1.24±0.32
11	2.90±0.20	4.62±0.16[a]	2.80±0.24	4.20±0.42[a]
17	3.70±0.26	6.70±0.28[a]	3.40±0.30	6.60±0.39[a]
24	2.20±0.32	5.80±0.24[a]	2.60±0.24	5.78±0.46[a]

Means within row with different superscript differ significantly (p<0.05)

Table 6: The results of pregnancy examination and conception rate at day 60 pot artificial insemination

Item	Heifers		Cows	
	Distilled water	GnRH	Distilled water	GnRH
Number of cattle (head)	8	8	8	8
Checked pregnant cattle (head)	8	8	8	8
Actual pregnant cattle (head)	3	7	2	6
Conception rate (%)	37.5[b]	87.5[a]	25.0[b]	75.0[a]

Means within row with different superscript differ significantly (p<0.05)

Particularly, the climate in PDC areas and the adjacent areas that raised the dairy cows in Sakol-Nakorn province had THI value of 82.20±4.50 which were affected on the heat stress to the dairy cows (Wiersma *et al*., 1984). The heat stress affected the general physiological changes, haematological values, hormones, fertility and milk production performance (Yousef, 1985).

The impact of the ambient environment on general physiological changes: The responses of the dairy cows under the condition of high THI, would adjust to the mechanism of heat expulsion and heat regulation for static body temperature control by which the changes of general physiology of the body such as rectal temperature, pulse rate, respiration rate and sweating rate increased so the cows reduced the voluntary feed intake (Stermer *et al*., 1986). Similarly, their related to Smith (1984) reported that feed intake reduction (NRC, 2001) was the response of body to the high temperature environment (Maust *et al*., 1972). For decreasing heat combustion from metabolism and for increasing respiration rate and water consumption, and changing the behavior such as movement reduction which related to Thermo-regulatory system under the control of hypothalamus (Ruckebusch *et al*., 1991). The changes of haematology includes such as the values of haemoglobin and heamatocrit were reduced but cortisol level was increased (Abilay *et al*., 1975) that affected the feed intake for regulating the body temperature balance (Umpapol, 2002).

The impact of the ambient environment on endocrine system: The heat stress affected on endocrine gland system, interfered negatively to the mechanism of gonadotropin secretion due to the responses through the hypothalamo-pituitary-gonad axis that were less effective (Iamlamai, 1999). It caused the reduction of estrogen secretion and directly affected to the condition of fallopian tube and uterus (Pilachai, 2005). Luteinizing Hormone (LH) level was lower than normal level, glucorticoid and serum progesterone were also low (Wolfenson *et al*., 2000).

Effect of $PGF_{2\alpha}$ application on the estrus induction: The application of $PGF_{2\alpha}$ could effect on the decrease of LH secretion from anterior pituitary gland and corpus luteal capillaries. They were slightly contracted during the day 6-18 of estrous cycle that caused the progesterone level to rapidly reduce within 12 h and at the least level within 24 h. The estradiol level would elevate highly during 48-72 h and the cows became in estrus around 72 h (+ 24 h) after the $PGF_{2\alpha}$ injection. However, $PGF_{2\alpha}$ application had the limited usage in each case, considering the cow condition of $PGF_{2\alpha}$ could influence the corpus luteum regression. Furthermore, the progesterone level was reduced but estrogen level was elevated and the cows would turn back to their natural behavior and condition (Iamlamai, 1999; 2003).

The responses of GnRH application at post artificial insemination: This experiment was conducted while THI value was raised up over 72 so it caused the heat stress to the dairy cattle during the experiment. The plasma progesterone was decreased due to the functions of corpus luteum which was also decreased as well as the follicle size was smaller including the decrease of granular cell number and androstinidiol secretion from thaca cell. These were the causes of progesterone secretion reduction (Howell *et al*., 1994; Wolfenson *et al*., 2002). These causes affected the reproductive system viz. shortening the estrus period, low conception rate, reducing the development and growth of follicle, extension of corpus luteum regression, high embryonic mortality and abnormal functions of gonadotropin occurred (Jorden, 2003). Furthermore, it affected the ovarian functions of the cows because when the cows

were under the ambient temperature of 29°C and relative humidity of 60% that which interfered negatively to lengthen the duration of the system of corpus luteum regression, because when estrogen concentration was reduced. It could stimulate corpus luteum regression because the endometrium epithelial decreased the building of enzyme that synthesis of prostaglandins (Pilachai, 2005).

However, when ovulation or scar follicle regression was reduced and progesterone level was also decreased (Wilson *et al*., 1998), it affected to the GnRH and LH secretion. In addition, the decrease of estrogen secretion during estrous period because the follicle became smaller than normal and the ovulation system was delayed, silent heat or undetected heat, prolonged luteal phase. This is due to the low level of prostaglandin secretion from endometrium epithelial because of the increase of high temperature would cause the endometrium epithelial secreted less level of prostaglandins (Fabio and Scaramuzz, 2003).

The synthesis of progesterone by motivating in corpus luteum functions and the developing the new corpus luteum by GnRH application in early luteal phase would effect on LH function in inducing the ovulation of the first wave of follicle. Ovulation could occur by the present and influence of LH and later effected on new corpus luteum forming (Techakampu, 2000), progesterone level on day 11 and 17 post artificial insemination of the GnRH treated cattle were highly and significantly different ($p<0.01$) and they were pregnant (Schmitt *et al*., 1996).

The application of GnRH effected on LH secretion and the mechanism of LH function would begin by LH united with LH receptors on SLC wall and became the second messenger that stimulated the function of adnylate cyclase to synthesize increasingly the cyclic AMP from ATP. Later on, the cyclic AMP would stimulate the function of PKA which was the enzyme for catalyzing in adding the phosphate group with some protein that caused the increase of cholesterol transferring into the mitochondria. So cholesterol changed into progesterone more than the direct synthesis from SLC with similar results as Schmitt *et al*. (1996). It concluded that the responses of hCG or GnRH by intramuscular injection of GnRH (Burserelin) 8 μg or hCH (Chorulon®) 3,000 IU compared with normal saline on day 5 post artificial in dairy heifers and lactating cows found that the hormone treated groups formed accessory corpus luteum 93% of the cattle and could elevate progesterone level in the blood but there was no significant difference in pregnancy result of both dairy heifers and lactating cows in summer (Stevenson and Mee, 1991; Thatcher *et al*., 1993).

Conclusion: The ambient environment that caused THI value over than 72 could effect the heat stress to dairy cattle by resulting in the changes of general physiology, haematology and cortisol level. In addition, the application of $PGF_{2\alpha}$ effected to stimulate the dairy heifers and lactating cows under small holder management in Phupan Dairy Co-operative areas and Sakol-Nakorn province region found that they became in clear about estrus symptom. The dairy heifers and lactating cows under the ambient environment of THI value over than 72, were treated with $PGF_{2\alpha}$ for estrus induction and further treated with GnRH on day 5 post artificial insemination could effect to elevate the plasma progesterone that enhanced well to conception rate.

Recommendations:

- A study on conception rate in dairy cattle which was low in heritability characteristics but the influence of environment was higher therefore the consideration for control of many factors that could affect the study should be recognized such as feed and feeding management, house and equipments, climate and the sufficient number of experimental cattle for accurate results of the experiment.
- The health management of dairy cattle must be complete for the efficiency of hormone effects, particularly the lactating cows should be perfect in nutritional condition when application of hormone were being done for stimulating the body.
- The improvement of environment, feed quality and management would effect to the efficiency of hormone when the hormone was applied.

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Estimated Intakes of Iron, Zinc and Selenium of Jordanians as Obtained from Data of Jordanian Household Expenditures and Income Survey (JHEIS) 2006

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Abstract: This paper evaluates the intakes of the trace elements: iron, zinc and selenium as obtained from food consumption calculations based on the most recent data of JHEIS, 2006. The estimated mineral intake was analyzed using a nutrition analysis software program (Food Processor SQL, 2008). The nutrient intake of these 3 trace elements for different governorates was calculated. The results showed that the means of daily *per capita* intake of iron, zinc and selenium were 21.2 mg, 9.3 mg and 154.4 µg, respectively. Chicken meat was the first animal food among the richest top 10 foods in iron; it provided small amount of iron (0.9 mg, 6% of total consumption). The rest food sources of iron were of plant origin, of which bread topped the list (8.3 mg) followed by molukhyia (*Corchorus olitorius* L.) (1.0 mg). Also results showed that bread provided 2.7 mg zinc (29% of total consumption). Similarly, bread was the first source of selenium (86.7 µg, 56% of consumption). There was a variation in the estimated intake of these nutrients among different governorates. It is obvious that bread is the leading food of the top ten food sources of iron, zinc and selenium; this might be due to the fact that wheat flour is fortified with vitamins and minerals, including iron and zinc. However, it should be noted that bioavailability of minerals such as iron and zinc from plant food sources is low.

Key words: Iron, zinc, selenium, Jordan, JHEIS

INTRODUCTION

The food consumption pattern has changed in Middle Eastern countries, including Jordan in the last half century (Musaiger, 2007; Musaiger, 2010). Studies and surveys conducted, in Jordan, on food consumption in the previous half century have shown that the contribution of different food groups to energy and nutrients has changed (Alwan and Kharabsheh, 2006; FAO, 2003). This trend has been attributed to many factors including change in life style and changes in socioeconomic status leading to nutrition transition for Middle Eastern countries including Jordan (Alwan and Kharabsheh, 2006; Popkin, 2004). The subsidization of basic food commodities by the government has been canceled since 1997 and the poverty line has increased (USAID, 2005). There has been an improvement in health indicators; crude death rate has been decreased to 7/1000 population, the infant mortality rate has been decreased to 24.0/1000 live births and the life expectancy has increased to 71.7 years. As shown in Table 1, the urban population has increased from 46.3% in 1960 to 82.6% in 2006, whereas, the rural and Bedouin population have decreased from 53.7% in 1960 to 17.4% in 2006 (DOS, 2008). The consumption of fast foods as well as the number of meals taken outside home has increased (Musaiger, 2009; FAO/WHO, 2006).

Table 1: Demographic and socioeconomic indicators in Jordan in 2006*

Demographic indicator	Value
Total population (1000)	5,600
Urban (%)	82.6
Rural (%)	17.4
Population growth rate (%)	2.3
Average household size	5.4
Average life expectancy (yr)	71.7
Male	70.8
Female	72.5
Crude birth rate (per 1,000 pop)	29.1
Crude death rate (per 1,000 pop)	7.0
Infant mortality rate (per 1,000 live births)	24.0
Literacy rate (age 15+ yrs)-both sexes	90.7
Male	94.9
Females	86.3
Per capita GDP (JD)	1805.1

*DOS, 2007

Trace element deficiency is a worldwide problem and affects nearly half of humanity (WHO/EMRO, 2009; MOH, 2002; Mason *et al*., 2001). The WHO (WHO/EMRO, 2009) has identified iron deficiency anemia as a significant health problem in all countries of the Middle East and North Africa including Jordan (Alwan and Kharabsheh, 2006; Faqih *et al*., 1996; Tukan, 1996; WHO and UNICEF, 1996). To overcome this problem, the Jordanian government in collaboration with world health

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agencies has initiated a national program of fortification of wheat flour with 9 vitamins and minerals (MOH, 2006) besides introducing a school feeding program that included enriched biscuits for almost all schools (Takruri, 2004). Since the launching of wheat flour fortification in April, 2006 (MOH, 2006) there have been no studies concerning the positive potential effect of this fortification.

Iron is needed in the synthesis of hemoglobin and myoglobin, oxidative process and other vital biological functions. Clinical signs of iron deficiency include fatigue, impaired temperature regulation and decreased resistance to infection. Severe deficiency causes iron deficiency anemia (Mahan and Escott-stump, 2008).

Zinc as an important essential trace element participates in biological functions as a cofactor of many enzymes involved in energy metabolism, protein synthesis, immune function, sex maturation and sensation of taste and smell. Prolonged zinc deficiency during growth periods causes retarded physical growth (dwarfism). Other deficiency symptoms include retarded sex maturation (in males), impaired taste and smell and poor wound healing (Prasad, 2003).

Selenium is associated with vitamin E as an antioxidant that prevents oxidative stress due to generation of reactive oxygen species and thus protects lipid in cell membrane (Brenneisen *et al*., 2005). Selenium deficiency causes disease conditions such as Keshan disease, which is manifested by heart muscle failure and Kashin Beck which is, manifested by blood vessel abnormalities (Arthur *et al*., 1994). Selenium is also thought to protect against cancer through its role in induction of apoptosis and enhancement of immune system (Arthur *et al*., 2003; Wolf and Green, 1999).

Zinc deficiency has been reported in many countries of the Middle East (Mason *et al*., 2001) and it has been reported that there is a marginal zinc deficiency in Jordan (Alwan and Kharabsheh, 2006). In this paper, it has been thought important to evaluate the nutritional status of the trace elements, iron, zinc and selenium from food consumption calculations based on the most recent data of Jordanian Household Expenditures and Income Survey (JHEIS, 2006) (DOS, 2008). In addition, the obtained data on these trace elements were compared with the recent DRI recommendations (IOM, 2001; IOM, 2000). Despite the limitations of depending on such surveys for evaluating the per capita food consumption of nutrients, it is used internationally as an indicator of food consumption and nutrient intake when data from nutritional surveys are lacking.

MATERIALS AND METHODS

Data in this paper were based on the JHEIS 2006, which aimed at collecting detailed data on the household expenditures and income and correlating these data with the demographic, social and economic changes in Jordan. The raw data collection of this survey extended from July, 2006 to January, 2007 (DOS, 2008). The annual per capita food consumption data of a representative sample of all Jordanian households was calculated. The included 12768 households were proportionally distributed among the different governorates of the Kingdom using two-stage cluster stratified sampling method in light of the housing census frame, 2004. A questionnaire was distributed to households included in the study. The questionnaire contained data of the expenditure on different food and non food categories. Each category included a number of food items. The data on food items was analyzed using a nutrition analysis software program (Food Processor SQL, 2008) which included details on the contents of energy and nutrients for each food item. In case a food item was not included in the database of the mentioned program, the nutrient makeup of this food was obtained from other food analysis sources such as Food Composition Tables for Use in the Middle East (Pellett and Shadarevian, 1970) and Food Composition Tables of the Gulf Region (Musaiger, 2006). Such foods and their analysis were introduced to the Food Processor database. Then the daily intakes of iron, zinc and selenium were calculated and the daily per capita intakes of these nutrients were obtained using the provided household expenditure data for 6 months. The nutrient consumption values obtained for the different governorates were compared with the highest DRI of the 3 nutrients to assure that needs were met for all age groups (IOM, 2001; IOM, 2000).

RESULTS AND DISCUSSION

Table 2 shows the consumption of iron in the 12 governorates and the whole country (Kingdom). The consumption (mg/day) ranged between 17.3 for Tafilah and 23.5 for both Irbid and Jarash, while the Kingdom consumption was 21.2. When compared to the highest DRI of iron (18 mg/day), 10 governorates had higher consumption than this DRI, whereas the consumption of the other 2 governorates, Tafilah and Aqaba, were lower but close to this DRI. These figures are higher than those reported by Tukan (1996) based on JHEIS 1992 data, who found an average intake of iron for the Kingdom to be about 18.3 mg/day. This difference could be explained by the fact that flour was fortified with iron since 2002.

Based on these statistics, Jordan governorates, except Mafraq, should not suffer from iron deficiency. However, it was documented that some age groups of the Jordanian children suffer from iron deficiency anemia. To combine the fact of the presence of iron deficiency

Table 2: Fe, Zn and Se intakes in different governorates

Governorate	Fe (mg/day)	Zn (mg/day)	Se (µg/day)
Amman	20.7	9.6	148.1
Balqa	21.8	8.7	154.0
Zarqa	20.9	9.0	147.7
Madaba	22.8	9.9	184.9
Irbid	23.5	10.0	166.5
Mafraq	18.5	7.9	152.7
Jarash	23.5	8.9	151.8
Ajlun	22.1	9.2	165.5
Karak	18.9	8.3	163.5
Tafilah	17.3	8.5	140.5
Ma'an	21.6	8.2	149.0
Aqaba	17.9	8.6	148.9
Kingdom	21.2	9.3	154.4

Table 3: The top 10 food sources of Fe in the Jordanian diet

Food item	Consumption (mg/day)
Bread, all types	8.3
Molukhyia	1.0
Chicken	0.9
Thyme	0.8
Parsley	0.8
Spearmint	0.8
Beans	0.7
Rice, all types	0.6
Tomatoes	0.5
Potatoes	0.4
Total	14.8

anemia and the high iron consumption, it is possible that the deficiency is due to the presence of many antinutritional factors that hinder iron bioavailability and the presence of other factors that interfere with the bioavailability of iron such as vitamin C in the diet, heme and nonheme food sources of iron and tannin contents of foods.

Table 3 shows the top 10 food sources of iron in Jordan. It is noticed that these 10 food sources provide 14.8 mg out of 21.2 mg (=70%) of the total iron consumed. Except for chicken, all of the foods in this list (which provide 94% of the consumed iron) are plant foods, thus all of the iron contributed by the plant sources is in the form of nonheme iron. It is also noteworthy that the plant foods which are rich in iron (molukhyia, thyme, parsley, spearmint and legumes) are rich sources of antinutritional factors that hinder the iron bioavailability (Fairweather-Tait and Hurrell, 1996).

Table 2 also shows the consumption of zinc in the different governorates of Jordan. The consumption range (mg/day) was between 7.9 (in Mafraq) and 10.0 (in Irbid) with an average of 9.3 mg/day for the Kingdom. Like iron, the lowest consumption of zinc was in Mafraq. When compared to the highest DRI of zinc (11 mg/day), the estimated zinc intakes of all of the governorates and the Kingdom were below this DRI. The apparently low levels of zinc intake might be important and deserves investigation since marginal zinc deficiency has been reported in the country (Tukan, 1996). It is noticed from Table 4 that the main sources of zinc among the top 10 ones are of plant origin with bread being the highest contributor to the total zinc consumption (29%) and chicken coming next to it. Furthermore, the contribution of zinc from plant foods accounts for 67% of the total zinc; it is well-known that the bioavailability of zinc from plant food sources is low in comparison with animal food sources (Lönnerdal, 2000). The contribution of bread and other cereals is 40% of the total zinc intake in the current study, while their contribution was reported to be 56% in the JHEIS 1992 (Tukan, 1996). This difference could be ascribed to the decrease in bread intake in the recent survey.

The consumption of selenium in the 12 governorates and the Kingdom (µg/day) ranged from 140.5 (Tafilah) to 184.9 (Madaba), while the Kingdom consumption was 154.4 (see Table 2). The highest DRI of selenium is 55 µg/day. It is very clear that Jordan as a whole and all the governorates consumption of selenium exceed the recommended requirements by at least 2.5 folds, therefore the deficiency is unexpected. At the same time, such levels do not reveal any adverse impact on health as the highest UL for selenium is 400 µg/day (IOM, 2000). Table 5 shows the top ten food sources of selenium. Five of these top 10 foods were of plant origin. Bread occupies the leading one and provides 86.7 µg/day which equals 56% of the total consumption of selenium in Jordan. It is well known that wheat is among the high sources of selenium (Rayman, 2000) and wheat bread is the stable food in Jordan (DOS, 2008). Foods of animal origin provide 30.1 µg of selenium per day which equals 19% of the Kingdom consumption and 22% of the total top ten consumption. It is noteworthy that the selenium content fluctuates in the same food depending on the type of soil, soil pH and source of food whether it is of plant or animal origin. Therefore, the apparently high intake of selenium in the Jordanian diet, as estimated in this study, may not reflect the actual nutritional status of this trace element (Navarro-Alarcon and Cabrera-Vique, 2008).

These apparently high levels of selenium intake should be confirmed by studying the selenium content of local food intakes, as well as of imported foods, including wheat which is the stable food in Jordan. This is of paramount importance since selenium deficiency is known to be common in different parts in the world and since selenium status is an important issue in human health.

It is obvious from the present study that bread is the leading food of the top ten food sources of iron, zinc and selenium; wheat flour fortification is the explanation of this finding, in case of iron and zinc. Wheat flour fortification in Jordan has begun in 2006 by adding

Table 4: The top 10 food sources of Zn in the Jordanian diet

Food item	Consumption (mg/day)
Bread, all types	2.7
Chicken	1.1
Rice, all types	1.0
Lamb meat	0.6
Beef meat	0.5
Milk, all types	0.3
Yogurt	0.3
Eggs	0.2
Seeds, dried	0.2
Goat meat	0.1
Total	7.0

Table 5: The top 10 food sources of Se in the Jordanian diet

Food item	Consumption (µg/day)
Bread, all types	86.7
Chicken	16.8
Rice, all types	11.1
Eggs	6.2
Pasta, macaroni	5.0
Flour, all types	3.9
Lamb meat	3.7
Milk	1.9
Luncheon meat	1.5
Beans	1.0
Total	137.8

9 vitamins and minerals, including iron and zinc, to the flour (*Mowahad* type) (MOH, 2006). Deficiencies of these 9 nutrients are the most common in Jordan and many other parts of the world. It is expected that the incidence of deficiencies of the added nutrients will be reduced in the next few years. It is concluded that assessment of flour fortification program is very essential.

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Impact of Xylitol Replacement on Physicochemical, Sensory and Microbial Quality of Cookies

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Abstract: Effect of replacement of xylitol on physicochemical, sensory and microbiological parameters of cookies were studied. Sucrose was replaced with xylitol at various levels ranging from 25-100%. Physico-chemical, microbiological and sensory evaluation of cookies at different intervals of storage i.e. 0, 15, 30, 45 and 60 days was carried out to find out the best treatment for commercialization. The results pertaining to sensory evaluation of cookies indicated that T_2 (50% sucrose + 50% xylitol) got the highest score for fresh cookies which subsequently decreased but remained the highest during storage after 60 days. There was a significant change in moisture content while non-significant changes were recorded in fat, ash, protein, fiber and NFE contents. In cookies increasing trend in moisture content and decreasing trend in other parameters with storage was observed. Physical analysis revealed that hardness was observed the highest in cookies containing 100% sucrose which decreased significantly with increase in replacement of sucrose with xylitol. Fructurability values increased from cookies containing 100% sucrose to cookies containing 100% xylitol showing that cookies with sucrose were more crunchy. Color and water activity of cookies increased significantly in cookies containing 100% sucrose. It decreased with an increase of xylitol in cookies. Microbiological load was also maximum in cookies having 100% sucrose as compared to cookies containing 100% xylitol.

Key words: Xylitol, replacement, physico-chemical, sensory, microbiological

INTRODUCTION

Low caloric foods available to consumer shelves on the market are products prepared with low energy sweeteners. These products are very popular among weight and health conscious consumers (Abdullah and Cheng, 2001).

Intense sweeteners such as aspartame, cyclamate, saccharine and thaumatin are low energy sweeteners and are used in very small amounts as a sweeteners source in food products. On the other hand, bulk sweeteners provide less energy weight for weight compared to sucrose while having the same bulk volume which includes sorbitol, mannitol, lactitol and xylitol (Bond and Dunning, 2006).

Xylitol has been recently attracting global interest due to its sweetening power equivalent to sucrose. The energy provided by xylitol is only 2.4 kcal/g which is 40% less energy from sucrose. This property makes it a good sugar substitute for producing reduced energy foods (Russo, 1977; Emodi, 1978; Faria *et al*., 2002).

Moreover, slow adsorption and entry into metabolic pathways independently of insulin and without rapid fluctuation of blood glucose levels, support the use of xylitol as a diabetic sweetener (Rolla *et al*., 1987).

Xylitol has also some other health benefits as it is regarded as a non-cariogenic sweetener, because it can not be utilized by acid producing bacteria of human oral cavity (Olinger and Pepper, 2001).

At equivalent concentrations, xylitol has a lower water activity than sucrose, contributes to microbial stability and shelf life of a product.

Xylitol has high affinity for water and is more soluble than sucrose at elevated temperature, which allows the formation of very high solid content. This property is very particularly beneficial in hard coating procedure (Bond and Dunning, 2006).

Another remarkable characteristic of xylitol is its negative heat of dissolution which produces a feeling of vaporization in the oral and nasal cavity (Kamijo *et al*., 1988) and is used in part of confectionary and pharmaceutical products (Pepper and Olinger, 1988) and in the formulation of dietary complements such as amino acids, vitamins and trace elements.

Xylitol exerts a number of beneficial effects as sweetener when used alone or on combination with other sugars in yougurts (Hyvonen and Slotte, 1981), jams (Hyvonen and Torma, 1981), chewing gum (Scheinin *et al*., 1975; Olinger and Pepper, 2001) and hard candy (Voirol and Brugger, 1976).

In jams, jellies and marmalades sugars acts as a preserving agent, xylitol, in addition to non-fermentability of carbohydrates by mold, yeast and bacteria, is an

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effective agent due to high osmotic pressure even at low temperature.

The pleasant taste profile and cooling effect with no unpleasant aftertaste make it a desirable ingredient for chewing gums (Olinger and Pepper, 2001).

Unlike sucrose, xylitol can not invert in to glucose units. This very positive advantage of xylitol can be used in manufacturing of sweets together with fruit acids without any alteration (Winkelhausen *et al*., 2007).

In sugar cakes, xylitol proves to be a good substitute for sucrose. The color and texture of xylitol cakes closely resembles those of sucrose cake (Hyvonen and Espo, 1981).

Being a nearly inert substance, it can be heated to melting point (95°C) without causing Maillard browning (Winkelhausen *et al*., 2007). However, if crust formation, caramalization or non enzymatic browning is required, the addition of reducing sugar is necessary (Olinger and Pepper, 2001).

Considering the beneficial effects of xylitol in other food products, attempt has been made to explore the effect of replacement of sucrose with xylitol on physico-chemical, sensory and microbiological parameters of cookies.

MATERIALS AND METHODS

Procurement of raw material: Raw materials were purchased commercially from local market. Xylitol added in replacement of cookies was prepared in Lab. All the reagents were purchased from Sigma Aldrich.

Chemical analysis of wheat flour: Wheat flour was analyzed for moisture, crude protein, crude fat, crude fiber, nitrogen free extract and total ash content according to the methods described in AACC (2000).

Sample preparation: Cookies evaluated in these experiments were prepared from commercially available flour with sucrose and xylitol in the ratios of 100:0 (T_0), 75:25 (T_1), 50:50 (T_2), 25:75 (T_3) and 100:0 (T_4).

Cookies were prepared according to the method given in AACC (2000) with certain modifications. The recipe followed is flour (500 g), shortening (250 g), sweetener (250 g), eggs (2) and baking powder (5 g).

The ingredients were weighed accurately. Then creaming of shortening and sugar was done, followed by the addition of eggs. The flour and baking powder were added to the creamy mass and mixed to a homogenous mass by mixer (Mod. A-200, Hobart, USA) for 30 min. The batter was then rolled out with rolling pin to a thickness of 3 inches having 1 inch diameter cut with the help of a biscuit cutter. Cookies were placed on a baking tray inch distance and were baked at 425°F in a baking oven for 10 min.

After cooling at ambient temperature, cookies were packed in polyethylene bags and stored for 60 days at ambient temperature. The cookies were analyzed for physical, chemical, sensory and microbiological analysis at 0, 15, 30, 45 and 60 days interval.

Chemical analysis: The cookies were analyzed for moisture, crude protein, crude fat, crude fiber, NFE and ash content according to the methods described in AACC (2000).

Physical analysis

Texture analysis of cookies: Texture of cookies was determined at different storage intervals according to Piga *et al*. (2005) by using a texture analyzer (Mod. TA-XT2 Stable Microsystems, Surrey, UK) with a 5 kg load cell. The Texture Expert program version 1.21 was used for data analysis. Textural determinations were made by using a 3 bend ridge for a bend test. The cookies were bent in order to determine whether any structural was happened as a result of force exerted on cookies. Both the load cell and probes were calibrated before each test. Hardness measurement of samples by bending involved plotting force (g) versus distance (mm). The maximum force (g) was used as an index of hardness for the bend test.

Color measurement of cookies: Color of cookies at different storage intervals was determined according to the method described by Piga *et al*. (2005) with the help of colorimeter (Color Test-II Neuhaus Neotec). The colormeter was calibrated by using standards (54 CTn for dark and 151 CTn for light). The color of the cookies was determined by placing the cookies under the photocell.

Water activity of cookies: Water activity of cookies was determined at different storage intervals by using an electronic hygropalm water activity meter (Model Aw-Win, Rotronic, equipped with a Karl-Fast probe). Hygropalm water activity meter was calibrated and cookies were analyzed according to Piga *et al*. (2005).

Sensory evaluation: The cookies were evaluated by a panel of judges for color, taste, flavor, texture, mouth feel and overall acceptability at 0, 15, 30, 45 and 60 days storage intervals (Meilgaard *et al*., 1991).

Microbiological analysis: Colony forming unit was carried out by serial dilution according to the method of Awan and Rehman (2005).

Statistical analysis: The data was analyzed by using analysis of variance with the help of statistical package 8.1.

RESULTS AND DISCUSSION

Chemical composition of wheat flour: The results regarding chemical composition of wheat flour indicated

that wheat flour contained moisture 11.17%, crude protein 10.15%, crude fat 1.10%, crude fiber 0.29%, ash 0.53% and nitrogen free extract 76.76%. The results are in close agreement with the findings of Pasha *et al.* (2002).

Chemical analysis of cookies: Effect of different treatments on the means of chemical analysis is given in Table 1. There is a significant change in moisture contents from T_0 (3.29) having lowest score to T_4 (4.04) having highest score.

During the storage, cookies showed significant changes in moisture contents and non-significant changes in fat, ash, protein, fiber and NFE contents (Table 2).

The increase in moisture contents and decrease in other parameters during storage was observed in cookies. The increase in moisture contents can be associated with the more hygroscopicity of xylitol than sucrose (Bond and Dunning, 2006). The phenomenon of moisture absorbtion is also supported by Wade (1988). The chemical analysis revealed that moisture content, ash content, crude protein, crude fat, crude fiber and nitrogen free extract were ranging between 3.01-4.08, 0.44-0.45, 6.44-6.45, 23.43-23.44, 0.094-0.1 and 66.19-65.46%, respectively. These results are in close agreement with Pasha *et al.* (2002).

Physical analysis: The mechanical properties of cookies are important when evaluating the quality attributes from the point of view of consumer acceptance. Amongst other things, mechanical characteristics of cookies depend on properties of its matrix.

Results obtained for physical characteristics of cookies are presented in Table 3, 4.

Cookies made with 100% sucrose were significantly harder, drier and crunchier than cookies made with replacement of xylitol. Cookies made with 100% xylitol (T_4) were significantly softer than cookies made with 100% sucrose (T_0).

The hardness of a cookie results in part from the development of a gluten network to form the cookie structure. Gluten must interact with water molecules to promote development of the network, but sugars interfere with this by preferentially attracting water. After the baked cookie cools, sugars may crystallize, which will also contribute to cookie hardness (Taylor *et al.*, 2008). Xylitol, being the more soluble at elevated temperature and hygroscopic than sucrose, has a very high affinity for water and thus interferes the most with gluten development. Xylitol would also crystallize during cooling, less gluten and less crystallization together

Table 1: Effect of different treatments on the means of proximate composition of cookies

Treatments	Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Fiber (%)	NFE (%)
T_0	3.29^{d}	23.459	0.458	6.470	0.130	66.193
T_1	3.57^{c}	23.459	0.458	6.467	0.129	65.917
T_2	3.61^{c}	23.457	0.458	6.466	0.128	65.885
T_3	3.82^{b}	23.455	0.456	6.466	0.126	65.675
T_4	4.04^{a}	23.456	0.453	6.463	0.123	65.465

Table 2: Effect of storage period on the means of proximate composition of cookies

Storage (Days)	Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Fiber (%)	NFE (%)
0	3.07^{e}	23.460	0.460	6.470	0.130	66.41
15	3.57^{d}	23.459	0.459	6.469	0.129	65.917
30	3.76^{c}	23.458	0.458	6.467	0.128	65.729
45	3.88^{b}	23.456	0.454	6.464	0.126	65.62
60	4.06^{a}	23.454	0.452	6.461	0.124	65.449

Table 3: Effect of different treatments on the means of physical characteristics of cookies

Treatments	Hardness (g)	Fracturability (mm)	Color (CTn)	Water activity (aW)
T_0	2302^{a}	68.76^{e}	172.48^{e}	0.24^{a}
T_1	2163^{b}	70.57^{d}	174.76^{d}	0.23^{b}
T_2	2069^{c}	71.28^{c}	178.71^{c}	0.22^{c}
T_3	2003^{d}	72.43^{b}	184.47^{b}	0.22^{c}
T_4	1832^{e}	73.37^{a}	186.45^{a}	0.20^{d}

Table 4: Effect of storage on the means of physical characteristics of cookies

Storage (Days)	Hardness (g)	Fracturability (mm)	Color (CTn)	Water activity (aW)
0	2127^{a}	70.913^{a}	179.77	0.216^{d}
15	2100^{b}	71.043^{cd}	179.87	0.220^{cd}
30	2064^{c}	71.324^{bc}	179.19	0.226^{bc}
45	2050^{cd}	71.519^{ab}	179.05	0.230^{ab}
60	2027^{d}	71.631^{a}	178.99	0.239^{a}

would result in softer cookies. Olinger and Velasco (1996) also investigated that cookies made with polyols are softer than cookies made with sucrose.

The influence of storage on the physical attributes of cookies is given in Table 4. Hardness of cookies decrease progressively with increase of storage period. Maximum score was observed at 0 days (2127 g). This change can be associated with increase of moisture contents due to more hygroscopicity of xylitol.

Fracturability (Table 3) indicates the crispiness of product. The product having lower value is more crispy than product having high value. Fracturabilty increase from T_0 having lowest score (68.76 mm) to T_4 (73.365 mm) having highest score. This could be attributed to more soft texture of cookies with sucrose than cookies with xylitol. Fracturabilty of cookies is significantly effected by storage (Table 4). Minimum score observed was at zero day which increase gradually with storage period. This change can be associated with the increase of moisture content of cookies.

The color values of the samples are shown in Table 3. These values are indicative of the lightness of samples. Lower color values indicate a darker surface color. The mean values for cookies made with 100% sucrose and 100% xylitol were 172.48CTn and 186.45CTn respectively, which were significantly lower than the mean value for cookies made with 100% sucrose. Xylitol is chemically quite inert because of the lack of an active carbonyl group. It cannot participate in browning reactions. This means that there is no caramalization during heating, as is typical of sugars (Olinger and Pepper, 2001). Because xylitol does not form Maillard reactants, so color of cookies made by replacement of sucrose are lighter in color. Zoulias *et al.* (2000) also observed the same results for polyols. Storage period has non significant influence on the color of cookies (Table 4).

The water activity of bulk sweetener can influence product microbial stability and freshness. Water activity of cookies is given in Table 3. T_0 (0.24) got the highest score while T_4 (0.20) has lowest score.

Owing to its molecular weight, xylitol exerts a higher osmotic pressure and therefore, provides a lower water activity than equivalent solution of sucrose, meaning that it effectively exerts a greater preservative effect in solution than sucrose. This makes xylitol a particularly useful sweetener to increase the solids and therefore, the microbial stability of liquids (Bond and Dunning, 2006). Water activity is significantly effected by storage (Table 4).This change could be due to the hygroscopic nature of xylitol.

Sensory evaluation: Results pertaining to sensory evaluation of cookies are presented in Table 5. Analysis of variance explicit that cookies differed significantly regarding various sensory attributes like color, taste, texture, flavor, mouth-feel and overall acceptability, due to treatments. The results regarding each sensory attribute are discussed one by one.

Treatments have significant effect on color of cookies (Table 5). T_2 got maximum score (8.13) while T_4 obtained the lowest score (4.16). T_0 and T_1 got fairly high score which showed that T_2 (50% sucrose + 50% xylitol) was preferred by the judges because it gave the desired color to the cookies which distinguished it from others, yet T_0 and T_1 were also acceptable. Taste of cookies showed highly significant differences among the treatments. Judges ranked T_2 (7.60) at the first position and T_4 (3.53) at the last position, when averaged over all means. The results concerning with the score for texture of cookies disclosed a highly significant difference among treatments. T_2 got the maximum score 7.86 while T_4 was at the bottom obtaining 4.33 score. Judges placed T_2 (7.86) at the first position and T_4 (4.83) at the last position, when averaged over all means for flavor of cookies. T_2 (6.66) was also favored by the judges. The quality score in response to mouth-feel of the cookies depicted that T_2 got maximum score (7.73) while T_4 obtained the lowest score (5.36). T_1 and T_3 got fairly high score which showed that T_2 (50% replacement of xylitol) was preferred by the judges because it gave the desired mouth-feel to the cookies which distinguished it from others.

Overall acceptability was determined on the basis of quality scores obtained from the evaluation of color, taste, flavor, texture and mouth feel of the cookies. T_2 got the maximum score 7.20 while T_4 was at the bottom obtaining 3.73 score.

The results of this study are in close agreement with the findings of Winkelhausen *et al.* (2007), which reveals that xylitol addition effect sensory properties of cookies. The type and quantity of sweeteners to be added has significant effect on appearance, flavor and texture of biscuits (Matz, 1968).

Storage has significant effect on color of cookies (Table 6). The maximum score 6.80 (Average of 5 treatments) was obtained at 0 days by all the cookies which was significantly decreased as the storage increased. The minimum score of 5.10 (average of 5 treatments) was obtained at 60 days storage. The deterioration in color of biscuits might be due to the absorption of moisture from the atmosphere and oxidation of fats. These results are in close agreement with the findings of Iftikhar (2002).

As regarding taste of cookies, maximum score was obtained by fresh cookies (0 days) which was gradually decreased with storage days. The range between 0 days and 60 days was 6.60-4.66.

The results concerning with the score for texture of cookies disclosed maximum score was obtained by fresh cookies (0 days) which was gradually decreased with storage days. The range between 0 day and 60 days was 6.73-4.33. Winkelhausen *et al.* (2007) also

Table 5: Effect of different treatments on the means of sensory characteristics of cookies

Treatments	Color	Taste	Texture	Flavor	Mouth-feel	Overall acceptability
T_0	6.06^{c}	5.53^{bc}	6.00^{c}	6.20^{bc}	5.93^{cd}	5.66^{c}
T_1	7.73^{b}	6.00^{b}	6.73^{b}	6.66^{b}	6.40^{bc}	6.40^{b}
T_2	8.13^{a}	7.60^{a}	7.86^{a}	7.86^{a}	7.73^{a}	7.20^{a}
T_3	4.80^{d}	5.60^{c}	5.06^{d}	5.86^{c}	6.66^{b}	4.46^{d}
T_4	4.16^{e}	3.53^{d}	4.33^{e}	4.83^{d}	5.36^{d}	3.73^{e}

Table 6: Effect of storage period on the means of sensory characteristics of cookies

Storage (Days)	Color	Taste	Texture	Flavor	Mouth-feel	Overall acceptibilty
0	6.80^{a}	6.60^{a}	6.73^{a}	7.20^{a}	7.33^{a}	6.20^{a}
15	6.53^{a}	5.90^{b}	6.50^{a}	6.73^{a}	6.80^{a}	5.96^{ab}
30	5.93^{b}	5.46^{bc}	6.13^{ab}	6.13^{b}	6.36^{bc}	5.66^{ab}
45	5.53^{bc}	5.10^{cd}	5.63^{b}	5.96^{b}	6.00^{cd}	5.10^{c}
60	5.10^{c}	4.66^{d}	5.00^{c}	5.40^{c}	5.60^{d}	4.53^{d}

Table 7: Effect of xylitol on microorganism of cookies

	Treatments (CFU/g)					
Storage (Days)	T_0	T_1	T_2	T_3	T_4	Means
0	0	0	0	0	0	0
15	45	39	32	28	22	33
30	102	98	92	88	70	90
45	160	139	122	120	93	127
60	216	196	160	134	118	164
Means	105	94	81	74	61	

found the significant effect of storage on texture of cookies made with xylitol.

Flavor of cookies disclosed that maximum score was obtained by fresh cookies (0 days) which was gradually decreased with storage days. The range between 0 days and 60 days was 7.20-5.40. The loss in flavor might be attributed to absorption of water that resulted in fat oxidation.

The quality score in response to mouth-feel of the cookies depicted that maximum score 7.33 (Average of 5 treatments) was obtained all the fresh cookies (0 days) which was decreased significantly as the storage increased. The minimum score of 5.60 (average of 5 treatments) was obtained at 60 days.

Overall acceptability was determined on the basis of quality scores obtained from the evaluation of color, taste, flavor, texture and mouth feel of the cookies.

Analysis of variance disclosed a highly significant effect of storage on overall acceptability of cookies. As a whole the maximum score was obtained by fresh cookies (0 days) which gradually decreased with storage days. The range between 0 days and 60 days was 6.20-4.53. In earlier studies, a gradual decrease in overall acceptability of biscuits during storage was reported by Pasha *et al*. (2002) who attributed it to moisture absorption, increase in peroxide value and free fatty acid contents in biscuits.

Microbiological analysis: Microbiological evaluation, as an objective and widely used test in studying the food quality, was performed. The results of microbiological tests are presented in Table 7. The cookies made with replacement of xylitol had lower microbial load than cookies made with sucrose. The number of CFU decrease among treatments from T_0 having highest CFU to T_4 having lowest number of CFU. The analysis of freshly made cookies showed no growth at zero day. However, the number of CFU increased with storage period. The maximum number of CFU were at 60 days in T_0 (100% sucrose), while T_4 (100% xylitol) had least microbial load at 60 days.

The presence of low number of CFU in the cookies made with replacement of xylitol is associated with rather rare ability of microorganisms to metabolize xylitol compared with the microbial utilization of hexoses (Winkelhausen and Kuzmanova, 1998). In view of these observations, the baked products with xylitol are not only microbiologically safe but their shelf-life could be much longer too.

Conclusion: The present study demonstrated that the cookies containing xylitol in replacement of sucrose are sensorially acceptable and microbiologically safe with tendency to have extended shelf-life. This makes xylitol not only promising sugar substitute but alternative sweetener with real practical applicability in this type of products.

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Median Regression Analysis of Body Mass Index of Adults in Pakistan

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Abstract: Body Mass Index (BMI) is considered to be the most popular measure for overweight and obesity. Numerous studies of BMI are limited to compute and interpret different percentiles of BMI and do not account for many other covariates affecting BMI. Conventional regression methods are used for estimating how covariates are related to mean values of the dependent variable but in many situations, we are interested in quantiles rather than in mean values as in the case of BMI analysis. The present study addresses the same using median regression. Some important covariates such as gender, age, marital status, daily working hours, daily exercise routine and number of meat-eaten days per week are included in the study and found to be significant.

Key words: Obesity, quantile, quantile regression

INTRODUCTION

Overweight is a result of an imbalance between energy intake and expenditure while the term, obesity is used to describe body weight that is much greater than what is considered healthy. It is well established that obesity is associated with adverse health effects, e.g., gall bladder disease, hypertension, sleep apnea, gout, breast and endometrial cancer, colorectal cancer and osteoarthritis etc. (Bray *et al*., 1998; Marion and Jacobson, 2000; Ferris, 2007). There are approximately 350 million obese people and over 1 billion overweight people in the world. Over all about 2.5 millions deaths are attributed to overweight/obesity worldwide (Siervo *et al*., 2007). In developed countries, obesity is one of the aggravated public health problems (Zohoori *et al*., 1998; Mokdad *et al*., 2002; Peytremann-Bridevaux, 2007). In the US alone, it was estimated (see Golditz, 1999) that excess weight and physical inactivity accounted for 300,000 premature deaths per year and for 9.4% of all direct health care costs ($70 billion attributable to obesity). According to Bovet *et al*. (2004), the prevalence of overweight has increased greatly in developed countries over the last two to three decades in adults, children and infants. Although few data are available in developing countries, the epidemic of obesity is also occurring in these countries and Pakistan is one of them. Many researchers have focused their interest to study the overweight/obesity prevalence in Pakistan, see for example, Bharmal, 2000, Pappas *et al*., 2001; Nanan, 2002; Afridi and Khan, 2004 etc. and recently, Aslam *et al*. (2010).

Usually, weight status is determined by a person's BMI, defined as the ratio of weight (kg) to squared height in meters (m^2). According to WHO's standards, a person is overweight if BMI $\geq$ 25 and is obese if BMI $\geq$ 30. Unlike the many earlier studies in Pakistan (Kiyani *et al*., 2002; Rehman *et al*., 2003; Shah *et al*., 2004; Khan *et al*., 2008) on BMI, Aslam *et al*. (2010) use the new recommendation of WHO (2000) for Asia Pacific Region. According to this recommendation, in Pakistan, a person will be underweight (if BMI $\leq$ 19), normal (if 20 $\leq$ BMI < 23), overweight (if 23 $\leq$ BMI < 25) and obese (BMI $\geq$ 25) (see, also Nanan, 2002; Leung *et al*., 2008; Jaleel, 2009). Aslam *et al*. (2010) report that more than 46% people are overweight or obese in Multan. The common point in all the studies, discussed above, is that all of them rely on just computing BMI and percentiles. They do not take into account different covariates, responsible for obesity. According to Kan and Tsai (1993), the possession of knowledge on obesity's health risks prevents an individual from being overweight so it is useful to see the impact of different responsible covariates on BMI. This fact motivates the present article and thus, it is a continuation of the work done by Aslam *et al*. (2010). Following Chen (2005) and Ruhm (2006), we make use of median regression, a special case of quantile regression, for studying the effects of different covariates on BMI.

Median regression: According to Chen (2005), the percentiles of BMI for a specified age are of particular interest in light of public health concerns. The empirical percentiles with grouped age provide a discrete approximation for the population percentile so it is more plausible to employ some regression methods to study the effects of different factors on obesity prevalence. He adds further that for the obesity prevalence rates with the relation to different factors, usual regression methods

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do not fulfill the desired objectives. Traditional regression methods are useful for estimating how covariates are related to mean values of the dependent variable but, without strong parametric assumptions, will not accurately indicate changes at other points in the distribution. Such assumptions are unlikely to be justified since BMI increases more over time at the high than the low end of the distribution. Kan and Tsai (1993) also report, in their study, that there is a steeper increase in the BMI towards the right tail of the distribution. As an alternative, quantile regression methods are used for such type of analysis. Quantile regression, proposed by Koenker and Bassett (1978), minimizes the weighted sum of the absolute deviations of the error term, unlike regression models that minimize the sum of the squared residuals. According to Koenker and Hallock (2001), the purpose of the quantile regression is to estimate conditional quantile functions, where quantiles of a response variable's distribution are specified as functions of observed covariates (see also, Flegal and Troiano, 2000).

To briefly recall the ordinary quantile, consider a real valued random variable Y characterized by the following distribution function,

$$F(y) = \text{Prob}\,(Y \leq y)$$

The τ-th quantile of Y is defined as the inverse function

$$Q(\tau) = \inf\{y: f(y) \geq \tau\}$$

Where $0 < \tau < 1$. In particular, the median is Q(1/2).

The τ-th sample quantile $\hat{\xi}(\tau)$, which is an analogue of $Q(\tau)$, may be formulated as the solution of the optimization problem,

$$\min_{\xi=R} \sum_{i=1}^{n} \rho_\tau(y_i - \xi)$$

Where $\rho_\tau(z) = z[\tau - I(z < 0)]$, $0 < \tau < 1$, is usually called the check function.

When covariates X (e.g., age, gender etc.) are considered, the linear conditional quantile function, $Q(\tau | X = x) = x'\beta(\tau)$, can be estimated by solving,

$$\hat{\beta}(\tau) = \text{argmin} \sum_{i=1}^{n} \rho_\tau(y_i - x'_i\beta) \qquad (1)$$

for any $\tau \in (0,1)$. The quantity $\hat{\beta}(\tau)$ is called the regression quantile. The case $\tau = 1/2$, which minimizes the sum of absolute residual, is usually known as median regression. For more details about median regression, see Koenker and Hallock (2001), Buhai (2004), Martins and Pereira (2004) and Chen and Wei (2005).

MATERIALS AND METHODS

A cross-sectional data comprising of 2000 adult (aged 14 years or more) individuals, both males and females were taken from Multan city from January 1, 2007 to December 31, 2008 as a case study of Pakistan (see, Aslam *et al*., 2010 for more details about the data).

For the present study, following Gortmaker *et al*. (1993), Chen (2005) and Ruhm (2006), we take data on different variables, including the factors responsible for obesity determination. These variables, with respective codes and values shown in parentheses, are Gender (GENDER: 1 = male and 2 = female), Age in years (AGE, rounded to next year), Marital Status (MSTAT: 0 = single, 1 = married), Weight in Kg. (WT), Height in inches (HT), Hours Worked in Field per day (FWH: No. of daily working hours in field), Hours Worked at Home per day (HWH: No. of daily working hours at home), Daily Exercise Routine (EXER: 0 = no exercise, 1 = irregular exercise, 2 = regular exercise) and No. of Meat-eaten Days per Week (MWK: No. of days per week when any type of meat is taken in the meal of the respondent).

BMI of the individuals are calculated as weight in kilograms divided by height in meters squared. BMI is taken as dependent variable (y_i) and vector of regressors, X_i including AGE, MSTAT, FWH, HWH, EXER, and MWK for median regression equation, defined in (1). Since men and women have different growth patterns (Chen, 2005), median regression analysis of BMI is split into two sets, one for males and other for females. Chen (2005) uses SAS QUANTREG procedure but we use the software package, STATA 10.0 for the computations.

RESULTS AND DISCUSSION

In our data set of 2000 individuals, 1123 are males (56.2%) and 877 are females (43.8%). The details of summary statistics about the age, marital status, weight, height, BMI, BMI percentiles and obesity status can be had from Aslam *et al*. (2010) while the same about the rest of variables is presented here.

Table 1 reports the first quartile (Q1), median, third quartile (Q3), mean and standard deviation (SD) for age, weight, height and BMI of both the genders. The empirical value of median for BMI will be used next to compare with the estimates of median regression.

Table 2 shows daily working hours of the respondents working in field and at home. This table reflects the physical exertion of the respondents when working. However, the table does not reflect the nature of the work and is not gender discriminating as males are expected to do more in field as compared to do at homes and for

Table 1: Summary statistics

Variable	Q1	Median	Q3	Mean	SD
Males					
Age	20.00	24.00	30.00	25.92	7.61
Weight	62.00	68.00	74.00	68.11	10.64
Height	65.00	67.00	69.00	67.06	3.02
BMI	20.91	23.48	25.81	23.51	3.63
Females					
Age	19.00	21.00	23.00	22.33	5.41
Weight	48.40	54.00	61.00	55.23	9.69
Height	61.00	62.00	64.00	62.38	2.63
BMI	19.33	21.53	24.27	22.05	3.92

Table 2: Daily working hours

Working hours	In field		At home	
	Frequency	%age	Frequency	%age
<4	357	17.85	1593	79.65
5-9	1193	59.65	339	16.95
10-14	438	21.90	67	03.35
15 and above	12	00.60	01	00.05
Total	2000	100.00	2000	100.00

females, this fact is vice versa. It is noted that majority of the respondents (59.65%), work for 5-9 hour daily in field while only 0.60% do work for 16 or more hours daily in field. On the other hand, about 80% of the respondents do work for less than 4 hour at home.

The daily exercise routine of the respondents is given in Table 3. The table shows that majority of the respondents (about 70%) do not take any exercise while just 12% take some regular exercise.

Table 4 shows the no. of days in which the respondents take meat in their meals per week. It can be noted that only 1.2% of the respondents do not take meat in their foods at all while about 20% take meat daily. It is also reported that about 43% of the respondents take meat in 3 or less days of a week. These figures, however, do not give any information about the quantity, type and form of the meat taken.

Table 3: Daily exercise routine

Daily exercise	Frequency	%age
No exercise	1394	69.7
Irregular exercise	367	18.4
Regular exercise	239	12.0
Total	2000	100.0

Table 4: No. of meat-eaten days per week

No. of days	Frequency	%age	Cumulative %age
0	24	01.2	01.2
1	109	05.5	06.7
2	305	15.3	21.9
3	426	21.3	43.2
4	378	18.9	62.1
5	214	10.7	72.8
6	153	07.7	80.5
7	391	19.6	100.0
Total	2000	100.0	

The parameter estimates of median regression for males and females are given in Table 5 and 6, respectively. It is noted that majority of the coefficients are statistically significant at 1% level of significance except EXER (at 5%) for females while MSTAT, FWH and EXER are significant at 5% level of significance for males. By using these estimates, one can easily estimate 50^{th} percentile of BMI for any adult male or female. For illustration purpose, suppose one considers an unmarried male (MSTAT = 0) of age 22 years who works for 6 hour in field daily (FWH = 6) and for 1 hour at home (HWH = 1), does not take any regular exercise (EXER = 0) and eats meat four days in a week (MWK = 4). Using Table 5, the 50^{th} percentile of BMI for such kind of males, is 22.49. Thus, median regression estimates the 50^{th} percentile (i.e., median) to be 22.49 by incorporating all the above stated significant covariates while the empirical median of BMI for all ages is 24 (Table 1). A similar practice can readily be done to compute 50^{th} percentile for females using Table 6.

Table 5 : Parameter estimates with median regression ($\tau = 1/2$) for males

	Coeff.	Std. Error	t	P > t	95% Confidence Interval	
Constant	17.52	0.724	24.19	0.0000	16.10	18.94
MSTAT	0.81	0.409	1.99	0.0470	0.01	1.62
AGE	0.16	0.025	6.52	0.0000	0.11	0.21
FWH	0.01	0.005	2.27	0.0234	0.00	0.02
HWH	-0.22	0.060	-3.59	0.0000	-0.34	-0.10
EXER	-0.06	0.197	2.05	0.0402	-0.45	0.32
MWK	0.40	0.071	5.61	0.0000	0.26	0.54

Table 6 : Parameter estimates with median regression ($\tau = 1/2$) for females

	Coeff.	Std. Error	t	P > t	95% Confidence Interval	
Constant	14.85	0.811	18.32	0.0000	13.26	16.45
MSTAT	2.67	0.465	5.74	0.0000	1.76	3.58
AGE	0.16	0.031	5.02	0.0000	0.09	0.22
FWH	0.19	0.049	3.95	0.0000	0.10	0.29
HWH	0.23	0.054	4.25	0.0000	0.12	0.34
EXER	-0.22	0.110	-2.04	0.0416	-0.44	-0.01
MWK	0.29	0.075	3.82	0.0000	0.14	0.43

Conclusion: Obesity is considered to be epidemic health problem worldwide. Being overweight is recognized as a significant risk factor for numerous diseases. Consequently, the prevalence of obesity needs to be observed, seriously. Unlike usual studies, the present work focuses on evaluating the dependence of BMI on many significant covariates including age, marital status, physical exertion reflected by daily working hours in field and at homes, routine about daily exercise and meat intake per week. The results show that more than 60% people work in field for 5 or more hours and a majority (about 80%) does less than 4 hour at homes. Majority of the respondents does not take any regular exercise. It is also reported that only 1.2% of the respondents do not take any meat in their foods while about 20% take meat daily. The median regression analysis shows that all the above stated covariates play a significant role in the prevalence of obesity. These estimates are obtained for both, males and females, separately and one can find 50^{th} percentile of BMI for males and females using these estimates against given covariates. With the help of quartile regression, we can estimate other percentiles choosing appropriate τ $(0 < \tau < 1)$.

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Development of Salinity Stress Tests for Larval Striped Bass, *Morone saxatilis* and Inland Silver Sides, *Menidia beryllina,* Used in Nutritional Studies

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Abstract: Salinity "stress tests" have previously been developed to detect subtle differences in physiological condition of larval fish between treatment groups in nutritional studies, when no differences exist in survival and growth. Methods that have been described in literature for Asian species include a) netting the fish out of water for a few seconds and measuring recovery time after its re-immersion and b) subjecting larvae to salinity stress for 2 h and measuring differences in mortality. In this study, we attempted to define the conditions for 15-h salinity stress tests with larval striped bass and 2-h salinity stress tests with inland Silver sides where a sharp delineation exists between survival and mortality and finally to use this test in a study of fatty acid requirements of the Silver sides. Striped bass larvae were cultured in an aquarium containing 5‰ water and fed on Reference *Artemia* nauplii for two weeks. Silverside larvae were reared in 30‰ sea water and fed on fatty acid enriched or un-enriched Great Salt Lake *Artemia* nauplii for three weeks. Striped bass were then exposed to 10, 20, 30 and 40 ‰ salinities while inland Silver sides to 40, 60, 65, 70, 75, 80 and 100‰ salinities and their survival was determined at ½ h intervals. Striped bass exhibited maximum survival at 20‰ and below. About 15% died in 30‰ water while all were dead in 40‰ at the end of 2-h period. Mortality count was increased when exposure time was prolonged. Fish larvae displayed significant ($p<0.05$) differences in mortality when duration between feeding and their exposure to stress was increased. Silverside larvae displayed maximum survival at 60‰ salinity and below but those raised on fatty acid enriched nauplii performed significantly ($p<0.05$) better than the controls in various stress tests.

Key words: Striped bass, inland silverside, salinity stress tests

INTRODUCTION

Brine shrimp (*Artemia*) nauplii are used as a convenient source of food for larval rearing of fish and crustaceans. These nauplii are hatched from dormant cysts which are collected from various places all over the world. As the quality of these cysts is very much dependent upon the environmental factors, their nutritional value varies from source to source (Leger *et al*., 1986). This variability in nutritional quality of *Artemia* nauplii can affect the physical condition of the larvae (Dhert *et al*., 1990). Therefore, larval survival, growth and tolerance to unfavourable environmental factors in the culture ponds, is very much dependent on *Artemia* quality (Kuhlmann *et al*., 1981; Leger *et al*., 1986; Watanabe *et al*., 1978, 1980; Kontara *et al*., 1995). This persistent variation in the quality of the larvae and their unpredictable future has led the aquaculturists to assess the quality of the larvae, before introduction into the ponds; one of the pre-requisite for a sustained growth of aquaculture industry. Only quality seeds can guarantee high growth and resistance to stress when exposed to unfriendly environment (Santhanakrishnan and Visvakumar, 1995). Larvae therefore, are examined for colour, activity and muscle development (Tackaert *et al*., 1989).

Salinity stress tests are commonly applied in shrimp hatcheries to estimate the quality of Post Larvae (PL) to be used during grow out (Placios *et al*., 2004). Stress tests in which larvae are netted from an aquarium and exposed to air for a few seconds and then returned to the water (Watanabe *et al*., 1982) are difficult to standardize and analyze statistically. In recent years the problem of finding a simple procedure to evaluate the quality of post larvae produced in commercial hatcheries has led to the development of stress tests, most of them using salinity (Tackaert *et al*., 1989; Rees *et al*., 1994) or pH shocks (Arellano, 1990) or formalin shocks (Bauman and Jamandre, 1990). Recently attempts have been made to standardize stress tests for fish larvae by exposing them to very high salinity for 2 h, determining mortality and assessing physical state of the larvae (Dhert *et al*., 1990). The development of similar methods for other species cultured in the various parts of the world might be useful not only for research purposes such as evaluation of diet effectiveness, but also as a

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simple tool for the farmer to assess the quality of the fry before stocking.

Hence it was decided to develop a stress test in which striped bass (*Morone saxatilis*) and inland silverside (*Menidia beryllina*) larvae were subjected to salinity shock. Survival was observed on fed and unfed state in the former while in the later response of fish was determined when fed on enriched and un-enriched *Artemia* nauplii. Survival percentage of the larvae after 2 h period was assumed to be an indicator of their physiological state and nutritional effectiveness of the diet provided during rearing.

MATERIALS AND METHODS

Holding conditions: Striped bass larvae obtained from the University of Maryland's Crane Aquaculture Facility, were transferred to a 75-L aquarium and acclimated for three days. Four hundred and fifty fish (11 d old) were randomly transferred from that aquarium into an adjacent 75-L aquarium (5 ‰) for experimentation. One hundred fifty larvae were equally divided into three 75-L(2 larvae L^{-1}) aquaria and fed on Reference *Artemia* II nauplii (RAC II) (Bengtson *et al*., 1985) for two weeks. Every day 4 g of dry *Artemia* cysts were placed in a 500 ml separatory funnel. They were incubated in filtered sea water (25±1°C) for 36 h under continuous aeration and constant light. After 36 h *Artemia* nauplii were harvested on a 150 µm sieve, rinsed with clean sea water and fed to larval fish.

Three hundred silverside larvae (10 d old), hatched from a single batch of eggs, spawned in laboratory were equally divided into 2 75-L (2 larvae L^{-1}) aquaria. Silver sides in one aquarium served as control and were fed on 48-h-old Great Salt Lake *Artemia* nauplii. The second group was offered *Artemia* nauplii from the same batch of cysts after enrichment with a commercial HUFA-enrichment product (Selco, *Artemia* Systems, Ghent, Belgium) hereafter referred to as *Artemia* Enrichment Product (AEP). These nauplii contained higher HUFA concentrations (Table 1). Group 1 was fed un-enriched Great Salt Lake *Artemia* while 2nd on HUFA enriched nauplii for three weeks.

Every day 3 g of Great Salt Lake cysts were put in two separatory funnels containing filtered sea water (30 ‰). The cysts were incubated for 29 h under constant fluorescent light and vigorous aeration. After 29 h, aeration in one separatory funnel, was stopped, *Artemia* nauplii were harvested and rinsed thoroughly with distilled water. Pre-weighed 1.2 g AEP was poured into a 2-L beaker containing 1 liter filtered sea water and homogeneously mixed. The nauplii were then transferred to the freshly prepared AEP solution, which was then poured into clean 2-L separatory funnel. The aeration was reinstated. After 19 h nauplii from both funnels (enriched + un-enriched), were harvested, rinsed with de-ionized water and fed to the respective treatment groups.

Both fish species received an *ad libitum* diet of live *Artemia* nauplii once a day, seven days a week. The fish in the aquaria received ambient photoperiod and continuous aeration. Every morning, before feeding, the aquaria were cleaned. Uneaten food, dead *Artemia* nauplii, cysts, any dead fish and accumulated debris, were siphoned out. Every day ¼th of the total aquarium water was removed and replaced by new sea water of the appropriate salinity.

Table 1: Fatty acid composition of reference *Artemia* cysts II (RAC II), great salt lake *Artemia* (GSL), *Artemia* enrichment product; selco (AEP) and AEP-enriched GSL *Artemia* nauplii (values are in mg fatty acid/100 mgs of total fatty acylmethylesters (FAME)

FAME	RAC II nauplii	GSL *Artemia* nauplii	AEP	AEP enriched GSL *Artemia* nauplii	Level of incorporation
13:0 (Tridecanoic)	-	10.0±0.9	-	-	-
14:0 (Tetradecanoic)	1.2±0.1	6.5±0.6	6.7± 0.3	2.0± 0.1	-4.5
15:0 (Pentadecanoic)	0.7±0.0	0.5±0.0	0.4± 0.0	0.8± 0.0	+0.3
15:1	0.5±0.1	-	ND	0.3± 0.0	-
16:0 (Palmitic)	13.7± 1.0	12.8±0.0	15.6± 1.3	11.0± 0.7	-1.8
17:0 (Margaric)	3.2± 0.0	1.0±0.0	ND	6.3± 0.1	+5.3
16:1ω7 (Palmitoleic)	13.6± 1.4	4.7±0.2	7.5± 0.4	6.3± 0.1	+1.6
16:2ω4 (Palmitolenic)	4.5± 0.3	1.0±0.0	0.9± 0.0	1.1± 0.0	+0.1
18:0 (Stearic)	4.4± 0.4	5.8±0.0	2.4± 0.0	4.2± 0.5	-1.6
18:1ω9 (Oleic)	36.9± 0.8	28.5±0.2	30.8± 2.5	31.4± 0.1	+2.9
18:2ω9 (γ-Linoleic)	-	4.9±0.3	-	-	-
18:2ω6 (α-Linoleic)	11.0± 0.0	-	7.0± 0.3	7.2± 0.0	+7.2
18:3ω3 (α-Linolenic)	0.5 ±0.0	-	3.0±0.0	17.4± 0.0	+17.4
18:3 (γ-Linolenic)	-	18.4±0.1	-	-	-
20:0 (Arachidonic)	ND	0.3±0.0	ND	ND	-
20:1ω9 (Gadoleic)	ND	3.0±0.3	3.0± 0.0	3.9± 0.3	+0.9
20:3ω6	-	1.0±0.2	-	-	-
22:1ω11 (Erucic)	4.7± 0.4	-	ND	ND	-
20:4ω6 (Arachidonic)	ND	0.5±0.0	0.6± 0.0	0.8± 0.2	+0.3
20:5ω3 (EPA)	4.8± 0.5	2.1±0.1	10.0± 0.3	9.0± 0.8	+6.9
22:6ω3 (DHA)	ND	-	5.6± 0.4	2.3± 0.1	+2.3

Note: ND = Not Detected EPA = (Eicosapentaenoic) DHA = (Docosahexaenoic)

Fatty acid analysis: Fatty acid composition of RAC II (Reference *Artemia* cysts), AEP (*Artemia* Enrichement Product) and Great Salt Lake *Artemia* with and without AEP, was determined using gas chromatography. Crude lipids were estimated by Bligh and Dyer (1959) lipid extraction method as modified by Kates (1986). Fatty acid profile was determined using the protocol of the National Marine Fisheries Service (NOAA) (1988) for the analysis of marine fish oil. Fatty acids were saponified and esterified with boron triflouride methanol (BF_3 methanol) reagent to form fatty acid methyl esters (FAME; AOAC, 1995). (Folch *et al*., 1957), The FAMEs were analyzed using Carlo Erba Strumentazione Series 4160 gas chromatograph. A 30 m fused silica glass capillary column (I.D. 0.32 mm) coated with SP-2330 0.2 mm thick film from Supelco Inc., Bellfonte, P.A., was used. The free fatty acids were detected by flame ionization detector. Helium gas with a flow rate of 25 ml/min at a column pressure of 0.57 kg/cm^2 was used as a carrier gas. Injector and detector temperature was 220°C. Oven temperature was held at 150°C for 8 min and programmed up to 190°C at 3°C with a linear velocity of 20 cm/min. Detector response was integrated and quantitated with a Hewlet-Packard 3390A integrator. After dilution with 10% chloroform, the samples containing FAME, were injected and separated on a above described fused silica capillary column. Fatty acyl methyl esters were identified by comparison of their retention times to those of standards (Spelco Inc., Bewllfonte, P.A.). The results have been expressed as area percent Fatty Acid Methylesters (FAME) (Table 1).

Development of stress test and data collection: Twenty-five-day-old striped bass and 31 day old inland silverside larvae were exposed to 10, 20, 30 and 40‰ and 40, 60, 65, 70, 75, 80, 85 and 100‰ salinity respectively (Table 2, 3). Striped bass exposures were subdivided into a) 1-h post-feeding, b) 4-h post feeding and c) 8-h post feeding states. In 1-h post-feeding state, the fish were fed on *Artemia* nauplii to satiation. When sufficient nauplii were seen in the gut of the fish, they were harvested and exposed to the higher salinity levels mentioned above. Mortality was monitored every ½ h for 2 h, then monitored again after 15 h of exposure. In the 4-h post-feeding, larvae were fed as above but then were not disturbed for 4 h after feeding. Nauplii were then harvested and exposed to the same salinities as explained earlier. At this time nauplii in the gut were partially digested and still some nauplii were visible through the transparent gut. In the 8-h post feeding

Table 2: Growth and survival of striped bass and inland Silver sides at the end of 2nd and 3rd week of rearing period respectively under various feeding regimes

Species	Fed on	Initial weight (mg)	Final weight (mg)	Initial length (mm)	Final length (mm)	Survival (%)
Striped bass	Great Salt Lake *Artemia* naupli	2.8±0.3	16.63±0.8	2.7±0.31	13.58±0.3	85.0±4.5
Inland Silver sides	Un-enriched *Artemia* nauplii	5.2±0.3^a	32.0±2.1^b	11.0±1.0^c	18.4±1.5^d	97.2±1.5^e
	Enriched *Artemia* nauplii	5.3±0.4^a	33.1±1.9^b	11.0±1.5^c	19.5±2.0^d	98.1±1.2^e

Table 3: Mortality (%) of striped bass larvae exposed to higher salinities

	Salinity ppt			
Time (h)	10	20	30	40
a) 1-h post-feeding				
½	0	0	0	30±7.0
1	0	0	0	85±12.0
1 and ½	0	0	15±3.5	90±9.5
2	0	0	15±4.0	100
15	20±4.0	40±6.5	80±8.5	100
b) 4-h post feeding				
½	0	0	0	0
1	0	0	0	0
1 and ½	0	0	15±4.0	80±5.0
2	0	10±5.0	20±8.0	100
15	0	10±6.5	90±9.8	100
c) 8-h post feeding				
½	0	0	0	0
1	0	0	0	0
1 and ½	0	0	10±3.0	30±5.0
2	0	0	20±4.0	80±6.0
15	0	20±7.0	50±9.0	100

Total number of fish used = 10 fish/replicate x 3 replicates x 4 treatments x 4 stress tests
= 360 fish

group, the larvae were fed as above but were collected after 8 h and exposed to the same salinity levels as in "a" and "b". There were 30 fish per treatment with three replicates in each treatment (salinity level). Silverside exposures were also further subdivided into a) range finder 1, b) range finder 2 and c) definitive test. In range finder 1, the silverside larvae from holding tank were exposed to salinities of 40, 60, 80 and 100 ppt. The survival was monitored during 2 h exposure. The data collected were used to set up the salinity range for range finder 2. In range finder 2, the overall salinity range was narrowed and fish was exposed to only 60, 70 and 80‰ salinity for 2 h. The survival data acquired from this test was used to arrange the salinity levels for the definitive test, in which the fish were exposed to 65, 70, 75 and 80‰ salinity. The various salinities used in each test are given in Table 3. There were three fish in each replicate three replicates for each treatment in range finder 1 and 5 fish in each replicate for range finder 2 and for definitive test. Replicate number was uniform throughout the stress test.

In both experiments sea water was evaporated by continuous heating to raise salt concentration up to desired level. The hot water was brought to room temperature and aerated vigorously. Where required, distilled water was added and required dilutions were prepared. The salinity levels were continuously monitored by a temperature compensated Refractometer. The fish were not netted but were collected individually from the aquaria with a small bowl. They were concentrated in a strainer immersed in rearing water. When a required number was achieved, they were transferred to a higher salinity treatment and held there for 2 h. The exposure time was further prolonged for striped bass (Table 2). The survival was monitored at ½ h intervals during the 2 h exposure. Dead fish were removed and counted (Table 2, 3).

Statistical analysis: Mortality values were analyzed by one-way ANOVA using statistical software (SPSS 11.0 version). Duncan's Multiple Range Test was used to compare means between treatments for their statistical significance. Differences were considered significant at $p<0.05$.

RESULTS

Fatty acid composition of enriched Artemia nauplii: The fatty acid composition of *Artemia* nauplii has been summarized in Table 1. The fatty acids such as 15:0, 17:0, 16:1ω7, 16:2ω4, 18:1ω9, 18:2ω6, 18:3ω3 and 20:1ω9 showed considerable increments but elevated levels of Polyunsaturated Fatty Acids (PUFA) arachidonic acid (20:4ω6), eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3) were more prominent. Fatty acids 14:0, 16:0 and 18:0 exhibited decline in concentrations. Fatty acids 13:0, 18:2ω9, 18:3, 20:0 and 20:3ω6 were though present in *Artemia* nauplii before enrichment but were totally lost after enrichment. Pentadecanoic acid (15:0) could not incorporate itself at all.

Striped bass: Striped bass was reared on RAC nauplii. Survival was 85%. During the 2 weeks of the feeding trial fish increased in mass from 2.8±0.3 mg to 16.63±0.8 mg (means±SEM) (Table 2). No deformity or disease was observed during rearing. After two weeks of *Artemia* feeding, fish were exposed to various salinity levels to determine their salinity tolerance. Each salinity levels higher than 20‰ gave some mortalities within two hours but there was considerable variation in mortality data when it was compared from salinity to salinity. Significant ($p<0.05$) differences were apparent not only at different salinities but also over exposure time (Table 3). Increased exposure time significantly ($p<0.05$) increased mortality, but this increase was less perceptible at lower salinities. Four hour post-feeding and 8-h post feeding fish performed significantly ($p<0.05$) better than 1-h post-feeding fish (Table 3a, 3b and 3c).

Inland Silver sides: Neither any deformity or disease was observed, nor was there any difference in growth and survival between treatments at the end of the feeding trials. Enrichment of *Artemia* did not induce any additional effects upto this stage (Table 2). However, after three weeks when fish were exposed to various elevated levels of salinities to determine their resistance capability, pronounced differences were observed. All the fish survived at 60‰ salinity and below and almost all were dead at 80‰ and above. Mortality counts were similar in range finder 1 [Table 4(i)] regardless of the nutritional status of food organisms (enriched or un-enriched). Range finder 1 pointed out that 60 ‰ or lower salinity did not kill the fish in 2 h. But 80‰ salinity killed all individuals. Both these extremes were therefore not suitable to detect differences in physiological condition of fish larvae. Therefore, in range finder 2 extreme salinity levels (40‰ and 100‰), were dropped and then larvae were exposed to 60, 70 and 80‰ salinities. The treatment groups were significantly ($p<0.05$) different at 70‰ but not at 60 and 80‰ in range finder 2 [Table 4(ii)]. In the definitive test, significant differences between treatments were observed at 65‰ [Table 4(iii)], with fish fed AEP-enriched nauplii showed significantly better survival. No differences were observed at 70, 75, or 80‰ after 2 h. Number of survivors significantly ($p<0.05$) declined and all fish died within the first half of experiment.

Table 4: Mortality (%) of inland Silver sides exposed to higher salinities fed AEP-enriched vs. unenriched *Artemia* nauplii

	Salinity (ppt)			
i) Range finder 1				
Time (h)	40	60	80	100
a) Unenriched				
½	0	0	100	100
1	0	0	100	100
1 and ½	0	0	100	100
2	0	0	100	100
b) AEP-enriched				
½	0	0	100	100
1	0	0	100	100
1 and ½	0	0	100	100
2	0	0	100	100
ii) Range finder 2				
Time (h)	60	70	80	
a) Unenriched				
½	0	0	0	
1	0	10±10	10±10	
1 and ½	0	20±0	30±10	
2	0	20±0	30±10	
b) AEP-enriched				
½	0	0	0	
1	0	0	20±10	
1 and ½	0	0	20±10	
2	0	0	20±10	
iii) Definitive test				
Time (h)	65	70	75	80
a) Unenriched				
½	0	0	0	0
1	0	10±10	20±20	30±10
1 and ½	40±40	80±20	60±20	70±10
2	80±20	89±10	90±10	100
b) AEP-enriched				
½	0	0	0	0
1	0	0	10±6	100
1 and ½	10±10	60±10	60±10	100
2	30±10	90±10	90±10	100

Total number of fish used

i) Range Finder 1 = 3 fish/replicate x 3 replicates x 4 treatments x 2 groups = 72 fish

ii) Range Finder 2 = 5 fish/replicate x 3 replicates x 3 treatments x 2 groups = 90 fish

iii) Definitive test = 5 fish/replicate x 3 replicates x 4 treatments x 2 groups = 120 fish

Total i + ii + iii = 282 fish

DISCUSSION

The present studies defined a salinity range for 25 day old striped bass in which differences between maximum survival and mortality can be ascertained. These studies further confirmed that immediate exposure or transfer to a stressful environment, after feeding, can lead to high mortality. Pecha *et al.* (1983) demonstrated in his studies that fish with full digestive tracts needed more oxygen to combat stress due to production of undigested excreta which took much of the oxygen of the water. Fish hence became more susceptible to stress. Ammonia built up due to protein metabolism of oxygen uptake inhibited the ability of hemoglobin to combine with oxygen, altering the oxygen carrying-capacity of the blood (Comogolio *et al.*, 2008). The metabolism of blue gourami was found to decrease with increasing duration of starvation (Chow *et al.*, 1994). The stress resistance of guppies starved for 1 day was significantly higher than that of fish starved for two days indicating that starvation for this fish should not exceed 1 day (Chuan Lim *et al.*, 2003). In our studies those striped bass whose feed was withheld long enough (15 h) and they have voided their stomachs and intestine, did not face this difficulty and survived longer than their counterparts. The fish were reared on Reference *Artemia*, it is not clear that higher or lower levels of HUFA in the diet could affect tolerance ability of striped bass to a stressful environment.

Sorgeloos *et al.* (1988) reported the essentiality of HUFA for culture of larval fish. Lisac *et al.* (1986), Franicevic *et al.* (1987) and Watanabe *et al.* (1982) confirmed HUFA requirement for European sea bass, sea bream and other bream species for growth, survival, advancement in metamorphosis (Babitha *et al.*, 2006) and during activity test. Forty day old Japanese flounder, *Paralichthys olivaceus*, has shown better growth and survival and increased tolerance when exposed to higher salinity levels when fed on HUFA enriched

Artemia nauplii (Furuita *et al*., 1999). DHA, however, showed superiority over EPA in inducing resistance to stress. Like other fish species, striped bass has shown better performance in growth and survival when fed on *Artemia* nauplii containing HUFAs (Webster and Lovell, 1990). Like Asian sea bass (Dhert *et al*., 1990) striped bass survival under stressful conditions might be improved by feeding diets high in HUFA contents. But presently it will not be discussed further for striped bass because their effect was not tested. In the current studies response of fish was observed when exposed to higher salinities and its relationship was developed between duration from last feeding with elevated salinity level.

Unlike striped bass, *Menidia beryllina*, were fed on HUFA-deficient and HUFA-rich *Artemia* nauplii. Then salinity range for salinity stress tests for this species was determined. Moreover nutritional effectiveness of HUFA supplementation in *Menidia* food was evaluated. Prior to the stress test, there were no differences in growth and survival between *Menidia* larvae fed HUFA-deficient or HUFA-rich *Artemia*. However, fish fed high HUFA diet exhibited better survival in the salinity stress test than did those fed un-enriched *Artemia*. To detect quality of *Penaeus monodon* post larvae fed on n-3 HUFA enriched *Artemia*. Rees *et al*. (1994) subjected the larvae to 0, 5 and 10‰ salinity and found that the n-3 HUFA enriched *Artemia* fed groups resisted the stress significantly ($p<0.05$) better than those of fed un-enriched *Artemia*. Citarasu *et al*. (2002) after 30 days of culture observed marked differences in the stress resistance ability of *Penaeus* post larvae when exposed to osmotic shock, pH and formalin stress. The un-enriched *Artemia* fed group succumbed to death within 80 min while the enriched *Artemia* fed group was able to tolerate it to a maximum of 140 min. The beneficial effect of HUFA supplementation in the diet on survival to salinity stress test is partially related to modification of fatty acid composition of gills and to a larger gill area which in turn enhances osmoregulatory mechanisms, namely Na^+/K^+ ATPase and carbonic anhydrase activities (Palcios *et al*., 2004). Examination of fatty acid levels in live feeds and larval tissue confirmed the physiological incorporation of fatty acids relative to dietary levels. Better resistance was noticed in *Penaeus indicus* fed on HUFA enriched *Artemia* (Immanuel *et al*., 2001). Studying the effect of n-3 HUFA, Horstmark *et al*. (1987) demonstrated that erythrocytes of rats fed HUFA rich cod liver oil achieved a higher resistance to hypo osmotic shock, an effect that probably resulted from a higher incorporation of n-3 HUFA in cell membranes. If similar phenomenon also occurs in crustaceans, the better resistance of fish fed HUFA enriched *Artemia* may be attributed to the increased osmotic resistance of their cells, delaying the onset of irreversible damage in some essential tissues (Rees *et al*., 1994). When sea bream and Atlantic salmon (*Salmo salar*) were subjected to confinement to induce a stress response (McCormick, 2001; Iversen *et al*., 2005)), the fatty acid composition of the gills was affected. The PUFA level decreased in gill tissue while saturates and mono un-saturates decreased. Catechol amines released due to this physiological turnover (Koven *et al*., 2003) can lead to an increased permeability of the branchial epithelium leading to influx of ions in fish in a hyper-osmotic environment. This in turn stimulates the ionic extrusion by the chloride cells, located mainly in the opercula and gills. Larval summer flounder, *Paralichthys dentatus* fed on fatty acid enriched rotifers were better able to survive the salinity tolerance test (Willey *et al*., 2004). Jalali *et al*. (2008) observed better growth, survival and stress resistance in those beluga (*Huso huso*) larvae which were fed on HUFA and vitamin E enriched *Artemia urmiana*. Probably during other stressful conditions such as transportation, transfer from hatchery to culture ponds or when fish are under other stressful conditions (e.g. oxygen deficiency, poor feeding and drastic temperature fluctuations) high HUFA-enriched diets could improve the physiological fitness of the larvae and make them more resistant to unfavourable environments (Dhert *et al*., 1990).

The present studies have demonstrated marked changes in the stress response due to feeding HUFA enriched *Artemia* nauplii for 3 weeks which emphasizes the physiological impact of these fatty acids. It can therefore be concluded that where extensive fish handling or other stressful conditions are expected, incorporation of HUFA in the diet is highly important. Moreover, a practical tool (stress test) has been developed during these studies which can provide very useful criteria for research purposes to evaluate diet effectiveness and could be beneficial for aquaculturists to evaluate the physical condition of the larvae.

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