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Performance Characteristics of Finisher Broiler Chicks Fed Varying Levels of Exogenous Enzyme Supplemented Bambara Seed [*Vigna subterranean* (L) Verdc] Offal as Replacement for Maize

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Abstract: A 28-day feeding trial was conducted using 240 Anak 2000 strain of finisher broilers fed 0, 15, 30 and 45% levels of exogenous enzyme supplemented Bambara Seed Offal (esBSO) in a completely randomized design where esBSO replaced whole maize weight for weight. Each of four dietary treatments was further replicated four times. Routine vaccination and medication typical of broilers were strictly adhered to. The initial weight, final weight, weight gain, feed intake, feed conversion ratio, feed cost/kg and feed cost/weight gain were measured. Results show that birds on control (0%) and 15% esBSO with weight gains 1.73kg each, did not differ significantly ($p>0.05$) and were superior to those of levels 30 and 45% (1.55 and 1.43 kg respectively). Feed conversion ratio, feed cost/kg and feed cost/weight gain declined as the level of esBSO increased from 0-45%, with 0% having the best FCR.

Key words: Whole maize, finisher broiler, enzyme supplemented bambara seed offal (esBSO)

INTRODUCTION

Livestock is the major source of animal protein for man based on their limiting amino acids profile. Unfortunately, livestock-especially the monogastric type-compete with man for feed ingredients. Consequently, in the present biting global inflation, the livestock production industry appears most hit in terms of scarcity and high cost of feed (Madubuike, 1994).

Thus the monogastric livestock industry, has witnessed a rapid decline in production due to high cost of inputs especially the feed ingredient (Ogundipe, 1987; Ndiforn, 1988; Obioha, 1992). This situation has forced many poultry farmers either to reduce the scope of production or fold up. The need to search for alternative and cost effective ingredients for the monogastric livestock has become imperative if man and his livestock must be saved from starvation and possible extinction.

One of such unorthodox feed ingredient with high nutrient potential is the Bambara seed (*Vigna subterranean* (L) Verdc) offal. The Bambara nut (seed) is an under-utilized tropical legume that is indigenous to Africa. It grows in areas where the cultivation of other legumes such as groundnut is too risky due to poor soil conditions or the threat of drought. It yields as much as 3-5 tons/hectare under conditions (Bamford, 1984).

Oyenuga (1982) and Uwaegbule (1978) described Bambara nut as a legume crop which contains higher crude protein than many other legume grains and therefore recommended its incorporation in livestock feeds. Carbohydrates account for approximately 45% of the total dry seed weight and has a lipid content of 6-8% (Poulter, 1981).

Bambara seed offal is a sievate after extracting the flour for human use and it has no industrial use. The supplementation of enzyme and other probiotics are known to improve the digestibility and utilization of feed ingredients by livestock thereby increasing productivity, (Silva *et al*., 2000). The objective of this study is therefore to evaluate the potentials of enzyme supplemented bambara seed offal as a partial replacer for maize in finisher broiler diets, aimed at reducing cost of production while in turn making table birds affordable for consumers.

MATERIALS AND METHODS

Sitting of the experiment: This research work was conducted at the poultry production unit of the Imo State University Teaching and Research farm, Owerri, Nigeria, situated on longitude $7^{o}0^{11}$ 06^{11} E and $7^{o}03^{1}.00^{11}$ E and latitude $5^{o}28^{1}24^{o}$N cross chick and $5^{o}30^{1}.00^{11}$N.

Preparation experimental diets: Bambara seeds were procured from the local market and subjected to hammer milling and thereafter sieving to separate the flour from the offal. The offal was toasted for 10-15 min at 50-60°C after which it was allowed to cool ambient temperature. Toasting was carried out to destabilized and reduce toxic materials (anti-nutritional factors) present in the seed most of which are heat labile. (Onwudike and Egbuakam, 1994; Apata and Ologhogbo, 1997). However, the proximate composition and nutrient quality of the offal were expected to be affected by the processing method used when preparing the flour for use as human food (Amaefula and Osuagwu, 2005).

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Table 1: Proximate composition of bambara seed (*Vigna subterranean* (L) Verdc) offal

Nutrients	Composition (%)
Crude Protein (CP)	21.28%
Nitrogen Free Extract (NFE)	40.23%
Gross Energy or ME	29856 kcal/kg
Ether Extract (EE)	4.60%
Ash	14.77%
Crude Fibre (CF)	9.43%

The offal was subjected to proximate analysis at the research laboratory of the school of agriculture and agricultural technology, of the federal university of technology, Owerri, according to AOAC, 1984. Other named ingredients were procured from reputable local dealers (crushed where necessary) and mixed according to the formulae shown in Table 2. The enzyme Nutrizyn was used to supplement the BSO.

Procurement and preparation of experiment birds: A total of 260 day old *Anak* 2000 broiler chicks were procured from a local distributor, and brooded for 4 weeks on deep litter poultry building. At the end of the 4 weeks period, 240 birds were selected on the basis of apparent physical soundness and assigned to the dietary treatment having 0, 15, 30 and 45% esBSO, each of which was replicated 4 times in a completely randomized design. Sound management practices of sanitation, appropriate medication and vaccination were adopted.

Experiment design, data collection and analysis: The experiment design was Completely Randomized Design (CRD). Each of four treatments had 60 birds and each replicate had 15 birds. Parameters, measured were weight gain, feed intake, feed conversion ratio, feed cost/kg of weight gain.

The birds were weighed individually using a top loading (10 kg Salter weighing scale in the morning hours before the day's feeding (7.00 am to 8.00am) on weekly interval. Initial body weights of the birds were taken at the start of the experiment and this was used to calculate the weight gain as final weight minus the initial weight. Daily feed intake was also measured by subtracting the weight of leftover feed from the weight supplied.

Feed conversion ratio was calculated as follows:

$$\text{Feed conversion ratio} = \frac{\text{Feed intake}}{\text{Weight gain}}$$

Feed cost per kilogram was calculated by adding prevailing prices of the different ingredients per kilogram at the time of the experiment multiplied by their inclusion levels and divided by one hundred. The cost per kilogram of weight gain was calculated as FCR x cost/kg of feed. All the data were subjected to one way analysis of variance (Steel and Tories 1980), while differences in the treatment means were separated using the Duncan's Multiple Test as outlined by Onuh and Igwemma (1998).

RESULTS

Table 3 shows the effect of replacing whole maize with enzyme supplemented Bambara Seed Offal (esBSO) on the performance of finisher chicken.

Results of the experiment show that there were no significant differences ($p>0.05$) for final body weight gain (2.39 and 2.3 kg), total weight gain (1.73 and 1.73 kg) and average daily weight gain (61.79 and 61.79 g) for birds on control 0% esBSO and 15% esBSO) respectively, and these were superior to those of birds on 30 and 45% esBSO diets. Average daily feed intake differed significantly ($p<0.05$) between treatments and increased progressively from 150.67 g in 0% esBSO to 161.00 g in 45% esBSO diets. Birds on 0% esBSO diet produced the best feed conversion ratio (2.45) while 15, 30 and 45% esBSO diets followed with 2.55, 2.78 and 3.15 respectively. Both feed cost/kg of feed and feed cost/kg weight gain differed significantly ($p<0.05$) between treatments with both decreasing as the percentage inclusion rate of esBSO increased.

DISCUSSION

The initial live-weights of the birds did not differ significantly ($p>0.05$) this precaution was taken to avoid bias arising from wide differences in weight. Significant difference ($p<0.05$) were however observed for final live weights of 2.39, 2.19 and 2.08 kg for 0, 15, 30 and 45% levels of esBSO respectively, decreasing with increasing levels of esBSO. However 0% and 15% esBSO levels were similar ($p>0.05$). the declining trend in performance could be attributed to the decline in metabolizable energy level from 2829.83 kcal in 0% esBSO to 2572.54 kcal/kg in 45% esBSO below the (NRC, 1994) recommendation of 2900 kcal/kg, from 2829.8 kcal/kg in 0% esBSO to 2572.5 kcal/kg in 45% esBSO. This birds consumed more feed as the level of esBSO increased (150.67, 158.00, 59.67 and 161.00 g) for 0, 15, 30 and 45% levels which was exactly different of esBSO respectively which differed significantly ($p<0.05$). the birds must have consumed more feed to make up for the shortfall in energy (Uko *et al*., 2001).

Feed conversion ratio differed significantly ($p<0.05$) between treatments increasing as the inclusion level of esBSO increased from 0% in T1 to 45% in T4 with the control (0% esBSO) birds converting better (2.45) than the others, 2.55, 2.78 and 3.15 for 15, 30 and 45% esBSO respectively.

Feed cost/kg of feed declined, as the inclusion level of esBSO increased and differing significantly between treatments. This is expected due to the wide margin

Table 2: Composition of experiment diets (%)

	Diets			
Ingredients	T_1 (0% esBSO)	T_2 (15% esBSO)	T_3 (30% esBSO)	T_4 (45% esBSO)
Maize	45	30	15	0
BSO	0	15	30	45
Exogenous enzyme	-	0.015	0.015	0.015
Brewers spent grain	10	10	10	10
Soyabean meal	20	20	20	20
Palm kernel cake	8.3	8.29	8.29	8.29
Fish meal	5.0	5.0	5.0	5.0
Bone meal	6.0	6.0	6.0	6.0
Groundnut cake	5.0	5.0	5.0	5.0
Common salt	0.3	0.3	0.3	0.3
Vat/min premix (B/F)	0.25	0.25	0.25	0.25
L-lysine	0.09	0.09	0.09	0.09
DL-Methanione	0.06	0.06	0.06	0.06
Total	100	100	100	100
Calculated nutrient composition				
CP (%)	20.99	22.68	24.37	26.06
ME/kcal/kg	2829.83	2710.73	2591.63	2572.54
Crude fibre	5.23	6.37	7.48	8.89
Ether extract	4.18	4.27	2.35	4.45

Table 3: Performance characteristics of finisher broilers fed varying levels of exogenous enzyme supplement Bambara Seed Offal (esBSO) as replacement for maize (*Zea mays*)

	Diets				
Parameters	T_1 (0% esBSO)	T_2 (15% esBSO)	T_3 (30% esBSO)	T_4 (45% esBSO)	SEM
Initial body wt (kg)	0.66^a	0.69^a	0.64^a	0.65^a	0.000
Final body wt (kg)	2.30^a	2.38^a	2.19^b	2.08^c	0.051
Total wt gain (kg)	1.73^a	1.73^a	1.55^b	1.43^c	0.066
Av. Daily wt gain (g)	61.79^a	61.79^a	55.36^b	51.07^c	2.130
Avg. daily feed intake (g)	150.67^c	158.99^{bc}	159.67^b	161.00^a	1.750
Feed Conversion Ratio (FCR)	2.45^c	2.55^{bc}	2.78^b	3.15^a	0.180
Feed cost/kg of feed (N)	87.21^a	76.71^b	66.21^c	55.71^d	0.090
Feed cost/kg of meat (N)	213.66^a	195.61^b	184.06^c	175.49^d	1.780

Means within the same row with different superscripts are significantly different ($p<0.05$)

between the market prices of esBSO (N25.00/kg) and maize (N62/kg) as at the time of this experiment. Feed cost/kg weight gain followed the same trend with both the feed conversion ratio and feed cost/kg differing significantly ($p<0.05$) between treatments. This is understandable since feed cost/kg gain is equal to feed cost/kg x feed conversion ratio.

Conclusion: Results of the experiment indicate that esBSO can replace whole maize up to 45% with 15% as optimal. Replacing whole maize with esBSO reduced the cost of broiler production thereby making animal protein affordable for consumers.

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Effect of Sulfur Supplementation on *in vitro* Fermentability and Degradability of Ammoniated Rice Straw

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Abstract: *In vitro* studies were conducted to determine effect of supplementation various amount of sulfur (Ammonium sulphate) on fermentability and degradability of ammoniated rice straw. The *in vitro* experiment was carried out following the first stage of Tilley and Terry method. The treatments consisting of four levels of sulfur, were A = ammoniated Rice Straw (RS) (control), B = A + 0.15% Sulfur (S) supplement, C = A + 0.3% Sulfur supplement and D = A + 0.45% sulfur supplement on dry matter. Completely randomized design was used as the experimental design and differences among treatment means were examined using Duncan multiple range test. Variables measured were Ammonia (NH_3) and Volatile Fatty Acid (VFA) concentrations, as fermentability indicators, as well as degradability indicators including degradability of Dry Matter (DM), Organic Matter (OM), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and cellulose. The results indicated that fermentability and degradability of diets consisting ammoniated RS with Sulfur supplementation were significantly higher than the control diet ($p<0.05$). It is concluded that sulfur supplementation is important to improve fermentability and degradability of rations containing ammoniated RS. The best level of sulfur supplementation is obtained at 0.3% on dry matter.

Key words: Ammoniated rice straw, sulphur, fermentability and degradability

INTRODUCTION

The possibility of increasing fiber digestibility of high fiber containing material such as Rice Straw (RS) by ruminants to obtain high protein products is of particular interest in developing countries. In these countries such as Indonesia high fibrous feedstuffs constitute the main or only dietary component for animals since feeds of higher energy and protein values (cereals, legumes, etc.) are reserved for human needs. The utilization of rice straw is hampered by deficiency of nutrients, both for the animal and the rumen microbes. However, ruminants are still capable of using feedstuffs with low nutrient values and its rumen microbes are able to produce protein from simple nitrogen (N) sources such as urea, biuret, etc and Sulfur supplement. Therefore, nutritive value of some straws and other byproduct feeds can be improved simply by adding urea and Sulfur.

In recent years, sulfur content of forage diets has been recognized as a significant factor that affect the size of the rumen microbial population (Akin *et al*., 1983). Deficiency of dietary Sulfur could limit microbial growth in the rumen and hence limited their contribution to plant tissue digestion (Karsli and Russell, 2001). Sulfur was required to support *de novo* synthesis of Sulfur amino acids and thus microbial synthesis (Slyter *et al*., 1996). Bacteria can use carbohydrates as carbon skeletons for protein synthesis in combination with ammonia (Bach *et al*., 2005) and also utilize sulfur (organic or inorganic sulfur) to synthesize sulfur-containing amino acids (Kandylis, 1984) to produce microbial protein. Sulfur was also important for fiber fraction degradation in the rumen as it stimulated specifically growth of cellulolytic bacteria (Bal and Ozturk, 2006).

Sulfur supplementation is important for rumen fermentation and microbial protein synthesis. As Although there is little information explaining how the rumen bacteria and fermentation are influenced by Sulfur supplementation, it has been shown that the supplementation of poor quality fiber diets with Sulfur improved ruminal fiber degradation as well as apparent organic matter digestibility. Therefore, a study was conducted to examine effects of S addition on *in vitro* fermentability and degradability of ammoniated RS.

MATERIAL AND METHODS

The experimental was designed to study the effects of level sulfur on the *in vitro* degradability when rice straw was the substrat. The rice straw was previously treated with 4% urea on dry matter. Ammonium sulphate was used an Sulfur source and added in diet with level 0.15, 0.3 and 0.4% on dry matter respectively in rations B, C and D.

In vitro fermentability and degradability of nutrient were determined following the first stage of Tilley and Terry procedure (1969). Ruminal fluid was obtained from cannula steer. Fermentation tube consisted of 10 ml of

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ruminal fluid and 40 ml of McDougall buffer solution. Samples were incubated in duplicate in 100 ml polyethylene tubes in 39°C in a shaker water bath for 48h. Two fermentation tubes that did not contain diets were also incubated and used as blanks. Fermentation was terminated at 48 h by injecting the tubes with 1 ml of $HgCl_2$. Tubes were then centrifuged at 2000 x g for 15 min and the supernatant was removed. Tubes with residue were dried at 60°C for 48 h and weighed and the data were used for degradability determination. These residues were also analyzed for its DM, OM and N by standard procedures (AOAC, 1990), the NDF, ADF and cellulose of residues were determined by Goering and Van Soest (1970) procedures. Supernatants were used to determined NH_3 concentration (microdifusion Conway method) and total VFA concentration (steams distillation, Strorry and Miller (1965).

A completely randomized design was used as experimental design consisting of four treatments. Variables measured were fermentability indicators (ammonia and total VFA concentration) and degradability of Dry Matter (DM), Organic Matter (OM) and fibrous fractions (NDF, ADF and cellulose). Data were analyzed by ANOVA using the GLM procedure. Differences between the control treatment and S supplementation treatment were analyzed by Duncan Multiple Range Test (DMRT) (Steel and Torrie, 1981).

RESULTS

Fermentability: Table 1 shows results of Sulfur supplementation effects on of rumen fermentability. Effects of treatments were significant ($p<0.05$) on the following variables: ammonia and total VFA concentrations.

Ammonia concentrations decreased from 56.35 mg/100 ml in control diet to 54.63 mg/100 ml, 51.60 mg/100 ml and 49.10 mg/100 ml, respectively in rations B, C and D which consisted of Sulfur supplements of 0.15, 0.30 and 0.45. Differences in ammonia concentration among treatment diets were significant ($p<0.05$). Reverse results from ammonia concentrations were obtained in total VFA concentrations. The addition of Sulfur supplement increased total VFA concentrations from 68.75 mM in diet A to 82.50 mM in diet B, 92.50 mM in diet C and 100.75 mM in diet D. Total VFA concentrations differed significantly between all rations ($p<0.05$), except that between rations C and D.

***In vitro* degradability:** Data on *in vitro* degradability of ammoniated RS are presented in the Table 2. The addition of Sulfur at different level affected all degradability variables ($p<0.05$). Control diet (A) had the lowest DM, OM, NDF, ADF and cellulose degradabilities ($p<0.05$). An increase in S supplementation increased the degradabilities of DM, OM, NDF, ADF and cellulose, and the increase in degradabilities of DM, OM and fibrous fractions followed linier patterns with the levels of Sulfur supplementation.

DISCUSSION

Fermentability: These *in vitro* results indicated that sulfur could limit maximum ruminant utilization of rice straw ammoniated. The supplement sulfur used in this study apparently reduced ammonia concentration. This is consistent with the result of Hegarty *et al.* (1991), who found that high S supplementation tended to reduce NH3 concentration (78 vs. 88 mg N/l) in the rumen fluid. This is indicated that sulphur can promote rumen bacterial growth, because almost rumen bacteria used ammonia as source nitrogen for their growth. Increasing the population of rumen microbe which subsequently increased total VFA concentration. These results had agreed with the works reported by Qi *et al.* (1992).

Comparisons among diets supplemented with Sulfur, the best results in fermentability study had been obtained at 0.3% S supplement. This level was higher than that was obtained by Harrison and McAllan (1980) who suggested that the best 0.2% level. However, the N/S diet ratio in the present study were still in the range of 3:1 up to 18.5:1 that had been recommended by Bal

Table 1: Effect of sulphur supplementation on total NH3 and VFA concentration in the rumen (mean value)

	Treatments				
Variables	A	B	C	D	SE
N-NH_3 (mg/100 ml)	56.35^a	54.63^b	51.60^c	49.10^c	0.678
Total VFA (mM)	68.75^d	82.50^c	92.50^a	100.75^a	1.874

Values within the same rows differ significantly at ($p<0.05$). A = Ammoniated RS, B = A + o.15% S , C = A + 0.3% S and D = A + 0.45% S on dry matter

Table 2: Effect of sulphur supplementation on *in vitro* degradability of ammoniated rice straw (coefficient)

	Treatments				
Variables	A	B	C	D	SE
Dry matter degradability (%)	0.48^c	0.53^b	0.54^a	0.55^a	0.524
Organic matter degradability (%)	0.50^b	0.51^b	0.54^a	0.56^a	0.575
NDF degradability (%)	0.42^c	0.44^b	0.46^a	0.47^a	0.812
ADF degradability (%)	0.40^b	0.41^b	0.44^a	0.45^a	0.552
Cellulose degradability (%)	0.42^c	0.45^b	0.47^a	0.47^a	0.880

Values within the same rows differ significantly at ($p<0.05$). A = Ammoniated RS, B = A + o.15% S , C = A + 0.3% S and D = A + 0.45% S on dry matter

and Ozturk (2006). The different response obtained was due to differences in diet used in this experiment consisting of low quality roughage.

***In vitro* degradability:** Supplementation sulphur was effective in stimulating dry matter, organic matter and fibrous fraction degradability. The increase in degradabilities of DM, OM and fibrous fraction of ammoniater rice straw vs control demonstrate that sulphur addition develops the rumen bacterial growth, resulting in a greater rate of degradability. This study has also shown that ammoniated RS were deficient in Sulfur, and it supplementation is important to improve fibre degradation of fibrous feedstuffs. The present results were in agreements with the results of Little (1986), Komizarczuk and Durand (1991) and Akin and Benner (1988). The last researchers had indicated that improvement in fibre degradation by sulfur supplementation occurred through its specific stimulation on rowth of rumen cellulolytic bacteria and anaerobic rumen fungi. Bacteria can use carbohydrates as carbon skeletons for protein synthesis in combination with ammonia (Bach *et al*., 2005) and also utilize sulfur (organic or inorganic sulfur) to synthesize sulfur-containing amino acids (Kandylis, 1984) to produce microbial protein. The present study has suggested that the best result was obtained by supplementing Sulfur at 0.3% on dry matter.

Conclusion: Supplemental sulphur have effect of fermentability and degradability of ammoniated RS. The effects occurred through reduction in ammonia concentration, increase total VFA concentration and degradabilities of DM, OM and fibrous fraction. The best level of S supplementation is obtained at 0.3% S on dry matter. Further study is required to determine effects of supplementation on *in vivo* experiment.

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Abbreviations: RS, Rice Straw; ADF, Acid Detergent Fibre; DM, Dry Matter; NDF, Neutral Detergent Fibre

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Nutritional Status of Elderly in Asaba, Delta State, Nigeria

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Abstract: This study described three anthropometric measures (height, weight and Body Mass Index [BMI]) of elderly in Asaba, Nigeria. Data was derived from 176 subjects who attended the Medical Lectures of the Ebreme foundation for the elderly in Asaba. The SPSS (Statistic Package for Social Science) version 17.0 was used for data analysis. This study had 62.5% males and 37.5% females. Approximately 18.2% was aged 50-59 years, 43.8% (60-69 years), 29% (70-79 years) and only 9.1% aged 80 years or more. The general population had a mean weight of 70.98±13.98 kg, height 1.61±0.11 m and BMI 27.36±5.60 kg/m^2. The male subjects had significant lower body weight (70.55±12.07 kg) and BMI (25.90±4.2160 kg/m^2) than the females (71.70±16.76 kg and 29.79±6.7060 kg/m^2 respectively) with taller height (1.65±0.08 m) than the women (1.55±0.12 m). The weight and height decreased with age; 50-59 year group had 79.75±15.21 kg, 60-69 years (70.56±12.58 kg), 70-79 years (69.25±13.49 kg) while from 80 years and above had 61.00±10.62 kg. The height decreased from 1.66±0.07 meters (50-59 years group) to 1.54±0.17 meters (from 80 years and above). This study revealed that the nutritional indices of the elderly in Asaba, Nigeria decline with higher magnitude before the age of 60 years and from 80 years. This call for special nutritional and health attention to the aged before and after their 60th and 80th birthday respectively.

Key words: Elderly, anthropometric measures, body weight, height, BMI

INTRODUCTION

Nutritional Status refers to the nutritional state of the body as expressed according to scientifically tested parameters which included weight, height and Body Mass Index (BMI). Height and weight are two of the most easily obtained anthropometric measures and have been used extensively in screening and monitoring programs because overweight and obesity have been considered as risk factors for various diseases (Knowler *et al*., 1991; Lee *et al*., 1995; Solomon and Manson, 1997).

Anthropometry has been used at different ages for assessing health and nutritional well-being. But the distributions of body composition are usually generated for children, adolescents and adults between the ages of 19 and 64 years, most of them from the perspective of nutrition (Kuskowska-Wolk and Bergstrom, 1993; Seidell *et al*., 1995; Al-Isa, 1997). Kuczmarski and his associates (1994) confirmed that the mean values of BMI increased with each 10-year increment until 50 through 59 years of age for both men and women and then decreased with age. However, researchers had called for more attention to the changes in body composition related to aging (Baumgartner *et al*., 1995; Launer and Harris, 1996). For cross-national comparison, Launer and Harris (1996) compared anthropometric data (height, weight and BMI) from 19 geographically and ethnically varied samples of community-dwelling elderly people. Across the studies there were large differences in the prevalence of overweight and underweight, but in all studies the mean values of height and BMI decreased with age.

Therefore, it is important to generate more information on body composition of elderly persons in Nigeria, to contribute more empirical evidence to aging studies.

This present study however, focuses on the body composition of an elderly population sample in Asaba, Nigeria.

MATERIALS AND METHODS

A total of 176 willingly participants completed this study in Asaba. Asaba is the capital of Delta state, South-South Nigeria. The survey was conducted from April to May, 2009 in the Federal Medical Centre, Asaba. Subjects were recruited from the attendees to the April quarterly medical lectures of the Ebreme foundation of the elderly, a non Government Organization. The subjects were grouped in batches giving appointment over a period of 4 weeks for screening and data collection at the Federal Medical Center, Asaba. The Ethical Committee on human study at the Federal Medical Center, Asaba granted approval for this study protocol.

Three major anthropometric measures; body weight, height and BMI were used in the present study. The height and weight of the subjects were measured simultaneously by using standard hospital weighing balance and height measure (SMIC Health Scale, Made

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in China). Body weight was measured to the nearest 0.1 kg, with the participant wearing light clothes and without shoes, jackets, caps and heavy ornaments. Standing height without shoes was measured to the nearest 0.1 cm. BMI was calculated as the weight (in kg) divided by height (in m^2). The criteria of the World Health Organization (Seidell and Flegal, 1997) were adopted to measure the prevalence of overweight and obesity. The subjects falling in the BMI categories between 25 and 29.9 were considered overweight; those with a BMI 30 and above were obese. A descriptive analysis was performed to generate relationship of height, weight and BMI of these elderly persons by age and gender.

Analysis of data: All data were coded and entered into the SPSS (Statistic Package for Social Science) version 17.0. The categorical variables were presented as frequencies and percentages. The differences in mean values of height, weight and BMI among different age groups and between sexes were examined by ANOVA analysis. Statistical significance was set at 95% confidence interval.

RESULTS

The sex and age distributions are shown in Table 1. A total of 176 elderly subjects included in this study had 110 (62.5%) men and 66 (37.5%) women. Approximately 18.2% of the subjects were aged 50-59 years, 43.8% aged 60-69 years, 29% aged 70-79 years and only 9.1% aged 80 years or more.

The mean values of weight, height and BMI are presented in Table 2. The general population had a mean weight of 70.98±13.98 kg, height 1.61±0.11 m and BMI 27.36±5.60 kg/m^2. According to gender, the elderly men had lower body weight (70.55±12.07 kg) and BMI (25.90±4.2160 kg/m^2) than the elderly women (71.70±16.76 kg and 29.79±6.7060 kg/m^2 respectively) but they were taller in height (1.65±0.08 m) than the elderly women (1.55±0.12 m). The difference in height and BMI were statistically different between male and female elderly ($p<0.05$; F-value 43.082 and 22.35 respectively) whereas the difference in weight was not significant (F-value 0.274, p-value 0.601).

Table 1: Sex and age distribution of subjects

	Frequency	Percent
Sex		
Male	110	62.5
Female	66	37.5
Total	176	100
Age group		
50-59	32	18.2
60-69	77	43.8
70-79	51	29
80 and above	16	9.1
Total	176	100

Table 3 described the distributions of weight, height and BMI according to age. The mean value of weight decreased with age; 50-59 year old group had 79.75±15.21 kg, 60-69 years had 70.56±12.58 kg, 70-79 years had 69.25±13.49 kg while 80 years and above had 61.00±10.62 kg. Similar decreasing trends were also found in the height and BMI of the elderly subjects. The height decreased from 1.66±0.07 meters (50-59 years old age group) to 1.54±0.17 meters (elderly age 80 years and above). BMI also decreased from 29.18±5.86 kg/m^2 among the group of 50-59 years old to 26.70±9.15 kg/m^2 among elderly of 80 years and above. The magnitude of decrease in the weight and height were relatively more before the age of 60 years and 80 years. The decrease between the group of 60-69 years and 70-79 years were relatively small. The decreasing rates among the age groups were statistically significant in weight and height ($p<0.05$) while in BMI, the difference was not significant (F-value 1.563; p-value 0.200).

DISCUSSION

Information on body composition of the elderly is needed for proper evaluation of their nutritional and functional status. The potential change in body composition may lead to associated changes in some other risk factors of diseases, especially for elderly persons. However, little is known about the value of anthropometric data for predicting the health status of older people.

As expected, our study found the elderly men taller whereas the elderly women were heavier with higher BMI. The higher value of mean weight can be translated into the higher mean BMI. The finding of higher BMI

Table 2: Mean values of body weight, height and Body Mass Index (BMI) of subjects according to sex

	Male	Female	Total	F-value	p-value
Weight (kg)	70.55±12.07	71.70±16.76	70.98±13.98	0.274	0.601
Height (m)	1.65±0.08	1.55±0.12	1.61±0.11	43.082	0.000
BMI (kg/m^2)	25.90±4.21	29.79±6.70	27.36±5.60	22.35	0.000

Table 3: Mean values of body weight, height and Body Mass Index (BMI) of subjects according to age

	50-59 years	60-69 years	70-79 years	80 years and above	Total	F-value	p-value
Weight (kg)	79.75±15.21	70.56±12.58	69.25±13.49	61.00±10.62	70.98±13.98	8.069	0.000
Height (m)	1.66±0.07	1.61±0.11	1.61±0.09	1.54±0.17	1.61±0.11	4.113	0.008
BMI (kg/m^2)	29.18±5.86	27.25±5.06	26.57±4.61	26.70±9.15	27.36±5.60	1.563	0.200

among the women in this study population is comparable with that of Herng-Chia *et al.* (2000), which also found elderly males taller than the females. A Nigerian study on 65-78 year old subjects from rural and urban areas of the south-western region of Nigeria also reported that male subjects were significantly taller than female subjects (Oguntona and Kuku, 2000). Therefore, the higher body weight among the elderly females could be contributed to fat deposits rather than skeletal weight since the male were taller. A decreasing pattern of height, weight and BMI values with age was demonstrated among the elderly in this our study. The magnitude of decrease in the BMI, weight and height were relatively more before the age of 60 years and after 80 years. This revealed that from the age of 60-80 years the elderly demonstrate minimal change in their nutritional status. Previous studies had reported an increasing trend in BMI only up to older adults (Huang *et al.*, 1992; Curb and Marcus, 1991). Also, Kuczmarski and his associates (1994) confirmed that the mean values of BMI increased with each 10-year increment until 50 through 59 years of age for both men and women and then decreased with age.

Though this study showed significant declined in height and weight with the subjects' age, however, the non significant declined in their BMI with age could be attributed the low magnitude in their decline in weight. This is because Launer and Harris (1996) reported that for BMI to decline with age, weight must also decline and to a greater extent than height.

The mean BMI (27.36±5.60 kg/m^2) of the general population indicated that these elderly were neither underweight nor obese according to WHO classification of BMI (WHO, 2000). This finding is in agreement with an earlier study on food habit of same population where healthy eating habit was portrayed (Odenigbo *et al.*, 2009). These findings suggest that their adequate food intake influenced the overall nutritional status.

Conclusion: The anthropometric indices (body weight, height and BMI) of the elderly in Asaba, Nigeria declined with age. The decline was minimal from age of 60-80 years but had higher magnitude before the age of 60 years and above 80 years. This call for special nutritional and health attention to the aged before and after their 60th and 80th birthday respectively.

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Chemical Composition of *Artocarpus communis* (Breadfruit) Seed flour as Affected by Processing (Boiling and Roasting)

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Abstract: *Artocarpus communis* (Breadfruit) seed flour was subjected to boiling and roasting to determine their effects on the chemical composition. Proximate composition showed a significant difference ($p>0.05$) in the raw and processed flour samples. The moisture content was lowest (14.77%) in the roasted *A. communis* seed flour and highest (24.08%) in the boiled seed flour. Percentage ash, fat and protein were highest (3.66, 3.74 and 4.67%) in the raw while ash and fibre contents were least (2.75 and 1.81%), respectively in the boiled flour. Carbohydrate was highest (87.29%) in the boiled and least (85.60%) in the raw. Boiling and roasting indicated that the vitamin C content and the mineral contents were significantly higher in the raw *A. communis* seed flour. The effects of boiling and roasting with regards to loss and retention of the nutrients differed significantly ($p>0.05$), with only the roasting retaining more of the nutrients than boiled seed flour. Sodium and potassium contents of boiled (0.27 and 0.75 mg/L), respectively and roasted (0.34 and 0.78 mg/L, respectively) *A. communis* seed flours compared well ($p<0.05$) with the raw (0.37 and 0.83 mg/L, respectively).

Key words: Chemical composition, *Artocarpus communis* seed flour, boiling, roasting

INTRODUCTION

Indigenous food crops and edible seeds and plant products which are widely grown but neglected and rarely consumed by people in urban areas are much more highly nutritious than most exotic foods (Okafor and Okolo, 1974; Okigbo, 1975). *Artocarpus communis* (Ukwa bekee) is one of the seeds which is neglected, underutilized, underdeveloped and even going into extinct. *A. communis*, a breadfruit belongs to the Mulberry family Moraceae. The name *A. communis* is derived from the Greek Word Artos, bread and karpus which refer to the bread like quality of breadfruit when baked. The fruit is Achene but not a drupe. Other names of *A. communis*, which can be interchanged are *A. atilis* and *A. incisa*. The seeds are edible and are of high nutritional values (Kaey *et al*., 1989). As a leguminous crop, A. communis is considered as a good source of nutrients such as protein, fats and oils and a reasonable amount of carbohydrates for both man and livestock feeds for animals (Onana, 1995). When, *A. communis* seeds are cooked, they are fair source of thiamine and vitamin C (Amusa *et al*., 2002).

In Nigeria, *A. communis* is regarded as the poor man's substitute for yam (*Dioscorea esculenta* and *Dioscorea cayenensis)* due to the fact that it is used in several traditional food preparations of yam and also costs <1/3 the price of yam at the market (Mayaki *et al*., 2003). The seeds could be cooked for main dish, roasted for snacks or even converted to flour, which can be used for snacks making or as soup thickner (Anazonwu-Bello, 1986). Informations are limited on the processing (boiling and roasting) effects on the nutrients of *A. Communis* seed flour: Consequent upon this, this research aims at evaluating the processing (boiling and roasting) effects on the chemical composition of *A communis* seed flour.

MATERIALS AND METHODS

A. communis seeds were purchased from Assa market in Assa town in Ohaji/Egbema Local Government Area, Imo State, Nigeria.

Production of *Artocarpus communis* seed flour: The seeds of *A. communis* were cleaned and sorted to remove dirts, etc. One thousand, five hundred grams (1,500 g) of the whole seeds were divided into three portions. Two portions of 500 g each were given boiling and roasting treatments for 35 and 40 min, respectively. Boiling was in a distilled water in the ratio of 1:5 w/v at the temperature of 100°C. The seeds of the boiled portion were drained, cooled, dehulled and then dried in a hot air oven at 80°C for 4 h and milled with a hammer mill to produce flour.

Roasting was in a hot air oven at 130°C. The seeds were cooled, dehulled and further milled with a hammer mill to produce flour.

The raw portion (500 g) was dehulled and dried in a hot air oven at 80°C for 4 h before milling with a hammer mill. The flours (boiled, roasted and raw) were sieved using a metal sieve of mesh size 1.0 mm and packaged with a polyethylene bags and kept for analysis.

Chemical analysis: Moisture content was determined by the standard method of AOAC (1995). Carbohydrate (by difference), protein, ash and fat were determined according to the method described by James (1995). Crude fibre was determined by the method described by Pearson (1976). Calcium and magnesium were determined by the Verant EDTA Complexiometric titration method according to Pearson (1976) and James (1995). Sodium and potassium were determined by flame photometry method described by James (1995). Phosphorus was determined by the Vanedomolydrate Colorimetric method according to Udoh and Ogunwale (1986) and James (1995). Iron and vitamin C were determined by the standard method of AOAC (1995).

RESULTS AND DISCUSSION

Proximate composition of *Artocarpus communis* flour from boiled and roasted seeds: The result (Table 1) showed the proximate composition of the raw and processed *A. communis* seed flours. The boiled sample was the highest (24.08%) in moisture content and the least (14.77%) in the roasted. Also, there was a significant difference ($p>0.05$) in moisture content between boiled *A. communis* flour sample and the other two samples. This increase in the boiled sample was due to the absorption of water into legumes by simple diffusion (Rosario and Flores, 1981). The reduced (14.77%) moisture content of roasted sample was as a result of the treatment which caused loss of moisture in legume seeds (Kabirullah *et al*., 1977).

In terms of ash content, the raw *A. communis* seed flour had the highest value of 3.66% and it is significantly different ($p>0.05$) from the boiled and roasted samples. The result of the boiled sample of *A. communis* seed flour decreased (2.75%) in ash content. The loss in ash content of the boiled sample was caused by leaching of nutrients into the water and time involved in boiling (Fox and Cameron, 1984). Roasting increased the crude fibre content from 2.34-2.41%. Statistically, the value (2.41%) of roasted *A. communis* was significantly better ($p>0.05$) than raw and boiled samples. The decrease in crude fibre content of the boiled sample was attributed to loss of solid particles by boiling (Albercht *et al*., 1966). Percentage fat content was highest (3.74%) in the raw sample and least (3.63%) in boiled sample but there was no significant difference ($p<0.05$) among the raw, boiled and roasted samples. Boiling and roasting decreased the protein content of *A. communis* seed flour from 4.67% (raw) to 4.51 and 5.53%, respectively. In other words, the raw *A. communis* seed flour sample was significantly different ($p>0.05$) from the boiled and roasted sample in protein content. The reduced level of protein in boiled and roasted *A. communis* seed flour sample was due to leaching loss and solubility of nitrogen as explained by Edijale (1980) in his research on cowpea.

The processing methods employed (boiling and roasting) affected the carbohydrate content of *A. communis* seed flour. Boiling had an appreciable increase (87.29%) in carbohydrate content of the *A. communis* seed flour than roasting (86.57%). It can be observed that boiled sample was significantly different ($p>0.05$) from the raw and roasted samples. The decrease in carbohydrate content of the roasted sample may be due to heat treatment.

Table 1: Mean values of the proximate composition of *Artocarpus communis* flour samples from boiled and roasted seeds

Parameters	Raw	Boiled	Roasted
Moisture content (%)	21.53±0.10$_b$	24.08±0.37$_a$	14.77±0.00$_c$
Ash content (%)	3.66±0.02$_a$	2.75±0.00$_c$	2.81±0.01$_b$
Fibre content (%)	2.34±0.00$_b$	1.81 0.01$_c$	2.41±0.01$_a$
Fat content (%)	3.74±0.03$_a$	3.63±0.02$_a$	3.67±0.00$_a$
Protein content (%)	4.67±0.01$_a$	4.51±0.01$_b$	4.53±0.00$_b$
Carbohydrate content (%)	85.60±0.02$_c$	87.29±0.01$_a$	86.57±0.00$_b$

Means with the same subscripts in the same row are not significantly different ($p<0.05$)

Table 2: Mean values of the mineral and vitamin c contents of *Artocarpus communis* flour samples from boiled and roasted seeds

Parameters	Raw	Boiled	Roasted
Calcium (mg/L)	0.18±0.00$_a$	0.12±0.00$_c$	0.14±0.00$_b$
Magnesium (mg/L)	0.24±0.00$_a$	0.17±0.01$_c$	0.19±0.00$_b$
Sodium (mg/L)	0.37±0.00$_a$	0.27±0.01$_a$	0.34±0.00$_a$
Phosphorus (mg/L)	0.47±0.00$_a$	0.39±0.00$_b$	0.41±0.01$_b$
Iron (mg/L)	0.85±0.01$_a$	0.38±0.00$_c$	0.44±0.01$_b$
Potassium (mg/L)	0.83±0.00$_a$	0.75±0.01$_a$	0.78±0.00$_a$
Vitamin C (mg)	8.55±0.01$_a$	7.25±0.01$_c$	7.43±0.01$_b$

Means with the same subscript in the same row are not significantly different ($p<0.05$)

Mineral and vitamin C contents of *Artocarpus communis* flour samples from boiled and roasted seeds: The result of mineral and vitamin C contents of raw, boiled and roasted *A. communis* seed flours are shown in Table 2. Boiling and roasting as processing methods decreased the calcium, magnesium, iron and vitamin C contents. In effect, the Calcium (Ca) magnesium (Mg) and iron (Fe) contents and the vitamin C contents of the raw *A. communis* seed flour were significantly better ($p>0.05$) than the minerals and vitamin C contents of the boiled and roasted samples, respectively. The calcium contents decreased from 0.18 mg/L (raw) to 0.12 mg/L (boiled) and 0.14 mg/L (roasted) while, that of magnesium also decreased from 0.24 mg/L (raw) to 0.17 mg/L (boiled) and 0.19 mg/L (roasted).

In the case of the vitamin C content, it decreased from 8.55 mg (raw) to 7.25 mg (boiled) and 7.43 mg (roasted). Although, boiling and roasting decreased the sodium and potassium contents of the raw *A. communis* seed flour, there was no significant difference ($p<0.05$) between the raw and processed samples. Sodium content in raw *A. communis* seed flour decreased from 0.37 mg/L (raw) to 0.27 mg/L (boiled) and 0.34 mg/L (roasted) while, potassium decreased from 0.83-0.78mg/L (roasted). Processing also affected the

phosphorous content of raw *A. communis* by decreasing it from 0.47-0.39 mg/L (boiled) and 0.41 mg/L (roasted). Raw *A. communis* seed flour significantly differed ($p>0.05$) in phosphorus content from the boiled and roasted samples. However, boiled and roasted samples compared well.

Comparatively, it will be observed that boiling decreased the mineral and vitamin C content more than the roasting method. This occurred probably because soluble minerals and vitamin C leached into the processing water with long cooking time and higher temperature. This result agreed with the research of Fox and Cameron (1984) and Edem *et al.* (1994), that soluble minerals get lost by dissolving into cooking water.

Conclusion: Boiling and roasting as processing methods reduced the chemical composition of breadfruit (*Artocarpus communis)* seed flour when compared with the raw sample. It was also observed from the result that processing of *Artocarpus communis* by roasting retained more nutrients than boiling.

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Examination of Physical Properties of Goat Meat

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Abstract: The study was conducted to examine physical properties of goat meat to evaluate the relationship between goat meat in different age groups, group A (≤7 m), group B (8-10 m) and group C (≥11 m). In the first step physicochemical characteristics of goat meat in respect of pH, Water Holding Capacity (WHC), Cooking Loss (CL) and Drip Loss (DL) were determined. A total of 21 goat meat samples were collected equally from three age groups each containing 7 samples. The mean pH value of goat meat of group A, B, and C (6.28, 6.30 and 6.34% respectively) mean WHC (61.77, 63.36 and 63.36% respectively) were not significantly different ($p>0.05$) from each other. WHC of goat meat group B (63.36±028%) and group C (63.36±0.21%) were very similar and significantly ($p<0.05$) higher than meat group A goat (61.77±0.32%). Cooking loss and drip loss in goat meat of group A (38.72±0.60 and 4.93±0.16%, respectively) were higher compared to advanced slaughter age (8-10 m of age: 35.77±0.86 and 4.02±0.10% and ≥11 m of age: 33.40±1.13 and 4.06±0.14%, respectively). The result concludes the meat of goat slaughtered in advanced age may have an extensive advantage to reduce qualitative and quantitative losses of end products and by products with relation to export.

Key words: Goat meat, public health hazards, food borne diseases

INTRODUCTION

Pakistan is the second largest goat producing country in South Asia region having 56.7 million goats contributing about 578, 000 tons of mutton (Anonymous, 2008-9). Goat production in Pakistan has expanded considerably over recent decades as a result population densities have also increased.

Meat is an essential food fit for human consumption obtained after postmortem originating from the live animals after slaughters that are used as food by human. These animals include domesticated cow, buffalo, sheep, goat, camels and some wild animals i.e. dear and rabbit. In addition poultry have become a major meat producing species, while various game animals and birds provide a substantial amount of meat particularly in localized areas. Fish and other sea foods have also important part of human diet since earliest time. However, cow, buffalo, sheep and goat are the main sources of red meat in Asia. No doubt, goat meat one of the staple red meat in human diet, indeed goat meat is acceptable throughout the world but cultural and social tradition and economic condition often influence consumer preferences.

It is still difficult to ensure safety of meat supply to the consumers. The public due to unawareness and due to non enforcement of laws, many time buy meat which can not ensure protection to consumers from the effect of potentially danger of inferior quality meat. High sanitation standards, in the slaughter houses, processing plants and handling of meat at various stages of marketing are of great importance to obtain high quality meat; because the meat is an ideal media for the development and multiplication of microorganisms, particularly bacteria. Many changes occur in the handling, processing and packing of meat in relation to microbiological quality because bacteria reduce shelf life of meat and cause public health hazards. Bacteria which are responsible for food borne diseases contaminate the meat directly or indirectly from animal excreta at the slaughter process, and may also transfer from the surfaces, utensils and other equipments. Slaughtering processing and distribution of meat may produce suitable environment for the contamination of carcass by potentially pathogenic bacteria. Therefore hygienic goal of modern harvesting system is to reduce cross contamination; to the practicable level (Brown *et al*., 2000).

Limited studies on carcass physical properties of goat meat have appeared in literature (Babiker *et al*., 1990; Mahgoub and Lodge, 1996; Babji *et al*., 2000) and no studies have been reported so far on the same aspects of goat meat particularly in Sindh. Therefore keeping in view the importance of the subject, this study is conducted to evaluate the physico attributes of goat meat available in local market of Tando Jam.

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MATERIALS AND METHODS

pH value: Meat sample (10 g) homogenized in distilled water (90 ml) was used to measure the pH value using pH meter (Ockerman, 1985).

Water-holding Capacity (WHC): The method reported by Wardlaw *et al.* (1973) was used to determine the WHC of meat samples. Meat sample (8 g) was mixed with 0.6 M NaCl solution (12 ml) in the test tubes and placed into a water bath (5°C) for 15 min. These were then centrifuged (4°C) at 10,000 rpm for 15 min in a refrigerated centrifuge machine. The supernatant was decanted and measured. WHC was reported as ml of 0.6 M NaCl per 100 g of meat.

Cooking loss: Meat sample (20 g) was placed in polyethylene bag and heated in a water bath at internal temperature of 72°C. Cook-out was drained and the cooked mass was cooled and weighed to determine the weight loss (Kondaiah *et al.*, 1985).

$$\text{Cookingloss}(\%)=\frac{\text{Actualweight}-\text{Cookedmassweight}}{\text{Actualweight}}\text{x}100$$

Drip loss: Drip loss was measured as described by Sen *et al.* (2004). Raw meat samples (50 g) were placed at 4°C for 24 h in a refrigerator under polyethylene sealed covers. After 24 h the sample was wiped and dried with filter paper and weighed. Weight of sample is the drip loss of sample.

$$\text{Driploss}(\%)=\frac{\text{Actualweight}-\text{Weightafterrefrigeration}}{\text{Actualweight}}\text{x}100$$

RESULTS

pH value: The pH value of goat meat was examined and results are presented in Fig. 1. It was observed that pH value slightly varied in different age groups of goat meat. Goat meat of group A (≤7 m) showed pH value in a range between 6.13 and 6.51, while meat of group B (8-10 m) revealed the variation in between 6.12 and 6.42. Where as pH value of meat of group C (≥11 m) appeared in between 6.08 and 6.68. Further, the result revealed that the pH value of goat meat of group C (6.34) is slightly higher followed by group B (6.30) and group A (6.28). Moreover, these differences in the means of pH of goat meat of different age groups are not statistically different (p>0.05).

Water holding capacity: The effect of age on water holding capacity of goat meat was analyzed and the results are presented in Fig. 2. It was found that the meat of young goat has low water holding capacity than old age groups. The water holding capacity varied between 60.50-63.0% (average 61.77±0.32%) in group A goat meat; while in between 62.50-64.50% (average 63.36±0.28%) in group B goat meat and in between 62.50-64.0% (average 63.36±0.21%) in group C goat meat. Furthermore, the results of statistical analysis illustrates that the differences in water holding capacity between three groups of goat meat were highly significant (p<0.001).

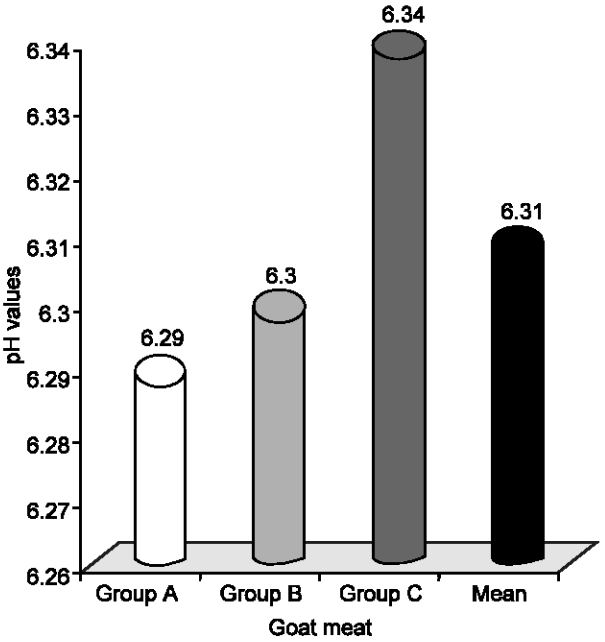

Fig. 1: pH value of goat meat of different age groups

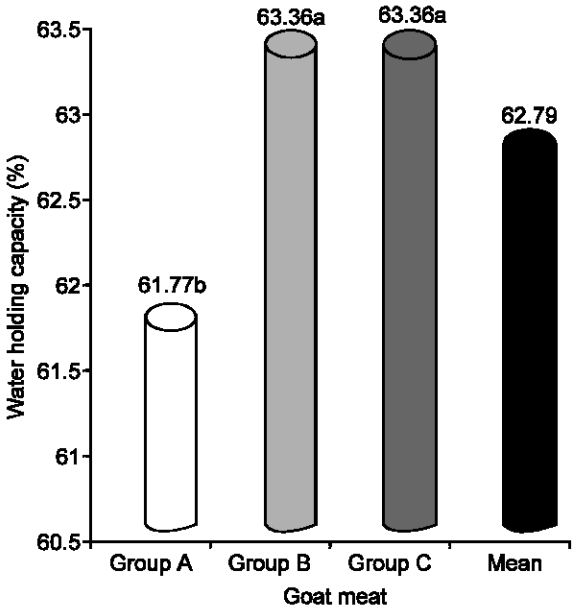

Fig. 2: Water holding capacity of goat meat of different age groups

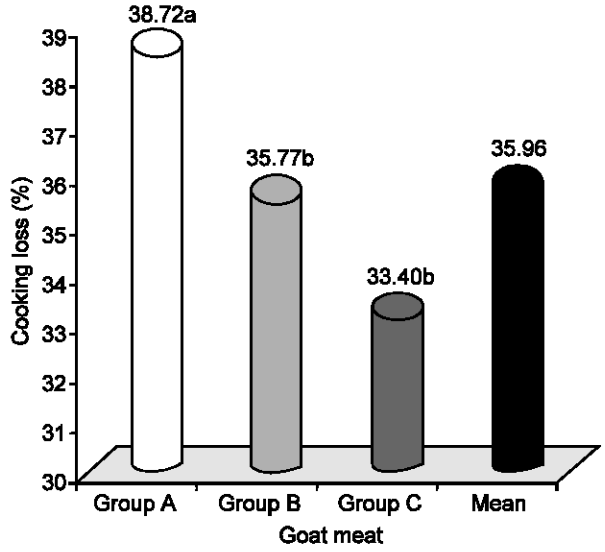

Fig. 3: Cooking loss (%age) of goat meat of different age groups

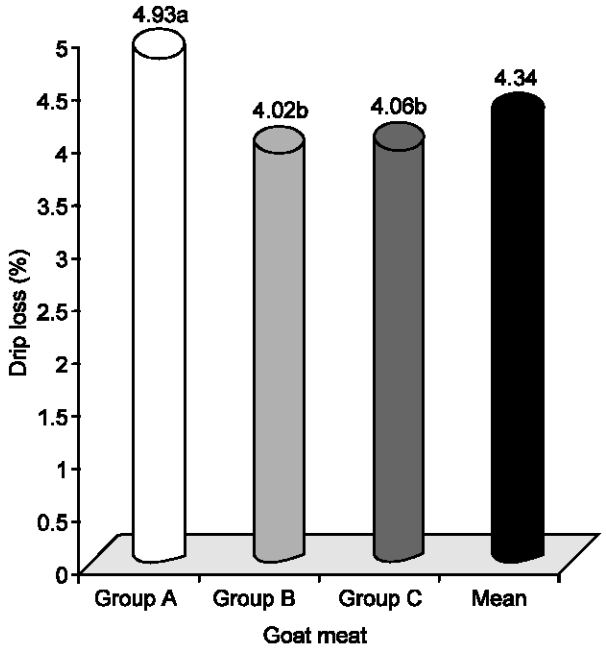

Fig. 4: Drip loss (%age) of goat meat of different age groups

However, when means ware compared (LSD 0.05), the result showed no significant difference ($p>0.05$) in water holding capacity in group B and group C; While meat of group A was significantly different ($p<0.05$) in water holding capacity from other groups.

Cooking loss: The effect of age on cooking loss of goat meat of different age groups was evaluated and results are shown in Fig. 3. It was observed that the cooking loss in meat of young age goat (≤7 m) group A was higher (38.72±0.60%; range 36.45-41.15%) compared to group B (8-10 m) and group C (≥11 m) i.e. 35.77±0.86% (range 32.60-38.25%) and 33.40±1.13% (range 29.65-37.85%) respectively. Statistical analysis (ANOVA) showed significant differences ($p<0.002$) in cooking loss in meat of different age group. Both groups (B and C), of which cooking loss in meat was significantly different ($p<0.05$) from the meat of group A, (≥7 m) goat.

Drip loss: The goat meat of three different age groups was analyzed for drip loss and results are summarized in Fig. 4. The drip loss in goat meat of group A averaged 4.93±0.16% (range 4.48-5.69%), which is remarkably higher than the drip loss in meat of group B 4.02±0.1% (range 3.58-4.42%) and in group C, goats 4.06±0.14% (range 3.58-4.60%). Statistical analysis (AOV) indicates highly significant differences ($p<0.001$) in drip loss in meat of different age groups.

However, the means of drip loss in meat of group B and group C goat were statistically (LSD 0.05) non significant ($p>0.05$); while drip loss in meat of both groups (B and C) goats was significantly ($p<0.05$) lower compared to drip loss in meat of group A goats.

DISCUSSION

pH value of 6.28±0.05 was observed in ≤7 m age group, while it was 6.30±0.04 in 8-10 m age group compared to ≥11 m age group (6.34±0.07). The results in the present study were not significantly different in respect of pH value between three age groups of goat meat. These results are supported by Laskar and Nath (1998). However, Karakaya *et al.* (2006) found significant ($p<0.05$) differences between meat species in respect of pH value in pre and post rigor stages. Pearson and Young (1989) investigated that rigor stage of meat species had significant effect on pH value, this is because of glycolysis, under which the glycogen is largely degenerated and mainly responsible for the formation of lactic acid. Laskar and Nath (1998) reported no significant differences in pH value of either between sexes or between muscle cuts at 24 and 72 h after slaughter.

Water holding capacity of (61.77±0.32%) in group A goat meat was lower compared to goat meat in group B (63.36±0.28%) and in group C goat meat (63.36±0.21%). In a study conducted by Laskar and Nath (1998) found that ultimate pH of meat showed a significant positive correlation with water holding capacity. The relationship between pH and water holding capacity has been well established Warriss *et al.* (1999). In another study Karakaya *et al.* (2006) reported that mutton and goat meat had higher EbC and water holding capacity value in both pre and post rigor stages. The rigor stage of meat species affect significantly, ($p<0.05$) of pH, EbC water holding capacity.

The maximum cooking loss (38.72±0.60%) was observed in ≤7 m age group of goat meat, while gradually decreased with increase in age of goat i.e. (35.77±0.86% and 33.40±1.13% in 8-10 m and ≤11 m age group of goat meat respectively. Karakaya *et al.* (2006) reported the significant differences ($p<0.05$) in cooking loss between the species of pre rigor stage. These results are consistent with those of Aaslyng *et al.* (2003), who showed the significant effect of pH and water holding capacity on cooking loss. In an other study the increase in cooking loss had been attributed with decline in pH value Lawrie (1991).

Drip loss (4.93±0.16%) in goat meat of ≤7 m age group was higher compared to 8-10 m age group (4.02±0.10%) and ≤11 m age group (4.06±0.14%). Significant increase in drip loss towards the time and different muscles had been observed Nagaraj *et al.* (2005). There were different opinions regarding the reason behind the increase in drip loss, namely protein degeneration (Penny, 1977), sarcomere shortening (Honekel *et al.*, 1986) and myosin degeneration (Offer, 1991) resulting in shrinkage of myosin, drawing the thick and thin filaments more closely together.

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Microbiological Safety of Raw Milk in Khartoum State, Sudan: 1-Khartoum and Omdurman Cities

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Abstract: Twenty four random samples of raw cow milk were collected from Khartoum and Omdurman Cities. Samples were analyzed for microbiological population, included Total Plate Count (TPC), Total Coliforms (TC), *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, Lactic Acid Bacteria (LAB) and spore forming bacteria. Microbiological enumeration revealed for the counts of total mesophilic aerobic bacteria, 5.86 log cfu/ml; lactic acid bacteria, 4.47 log cfu/ml; coliforms 2.76 log cfu/ml; *E. coli* 1.63 log cfu/ml; *Staphylococcus aureus*, 1.92 log cfu/ml and 2.38 log cfu/ml Spore forming bacteria. The microbial profiles found had non-conformance to the Standard. Based on the exceedingly high microbial counts found in this study, it could be concluded that this milk type poses a serious health risk in the study areas.

Key words: Milk, human diet, pathogenic bacteria,

INTRODUCTION

Milk is a major component in human diet all over the world, but it also serves as a good medium for growth of many microorganisms, especially pathogenic bacteria. Thus, the quality of milk is considered essential to the health and welfare of a community. Also, all cases of dairy illness continued to be of bacterial origin, pathogens that have involved in communicable diseases associated with the consumption of milk include *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter*, *Yersinia* pathogenic *Escherichia coli* and *Clostridium botulinum* (Adesiyun *et al*., 1995; Hahn, 1996).

The detection of coliform bacteria and pathogens in milk indicates a possible contamination of bacteria either from the udder, milk utensils or water supply used (Olson and Mocquot, 1980; Bonfoh *et al*., 2003).

Fresh milk drawn from a healthy cow normally contains a low microbial load (less than 1000 ml^{-1}, but the loads may increase up to 100 fold or more once it is stored for some time at normal temperatures (Richter *et al*., 1992). However, keeping milk in clean containers at refrigerated temperatures immediately after milking process may delay the increase of initial microbial load and prevent the multiplication of microorganisms in milk between milking at the farm and transportation to the processing plant (Adesiyun, 1994; Bonfoh *et al*., 2003). Contamination of mastitis milk with fresh milk may be one of the reasons for the high microbial load of bulk milk (Jeffery and Wilson, 1987).

In Sudan milk is considered one of the oldest kind of food and so many people depend on its products. Cow's milk is predominant, but some people depend on goat milk where the goats are kept by the families and camel milk by nomadic people.

The aim of this research project was to evaluate the quality of milk available to the consumers, comparing two different regions. The samples of milk were evaluated to determine the bacteriological quality.

MATERIALS AND METHODS

Microbiological analysis

Collection of samples: Twenty four samples of cow milk were collected randomly from different regions of Khartoum and Omdurman. Milking was done manually twice a day at 7.00am and 5.00pm.

The samples collected aseptically in sterile containers and transported to the microbiology laboratory, Food Research Centre, Khartoum North, where the analysis was done.

Sterilization, serial dilution and preparation of the media were done according to Harrigan and MacCance (1976).

Total Bacterial Count (TBC): Total viable count was carried out using the pour plate method described by Harrigan and MacCance (1976). Appropriate dilution (10^{-1} up to 10^{-6}) of the samples was plated on Standard Plate Count Agar (Oxoid). The plates were incubated at 37°C for 48 h.

Enumeration of total Spore forming bacteria: The colony count method to determine the total spore forming bacteria was followed as described by Harrigan and MacCance (1976). A test tube of suitable dilution is heated in water bath at 80°C for 10 min to destroy vegetative cells. The tube was cooled and 1 ml from this dilution was aseptically transferred into sterile Petri dish. To each plate melted Starch Milk Agar (SMA) was added. The plate's inoculums were mixed with the medium and

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allowed to solidify. The plates were incubated at 37°C for 2 days.

Enumeration of total coliforms: Standard multiple tube fermentation technique described by multiple tube system was used for samples examination, which is described into three tests:

Presumptive test: MacConkey Broth (Oxoid) was used. 1 ml was added to MacConkey broth culture medium with a Durham's tube. All tubes were incubated at 37°C for 24 h. For positive tubes, numbers of bacteria were looked out from statistically calculated, MPN prepared tables used for Most Probable Number (Andrews, 1992).

Confirmatory test:

- The medium used for this test was Brilliant Green Bile (BGB) Broth a selective medium for *E. coli* (Oxoid). The tubes were inoculated with positive tubes (MacConkey Broth), incubated at 44°C for 48 h. After incubation, tubes were examined for gas production and colour changing from green to yellow.
- A loopfull of suspension from positive BGB broth tubes were streaked on Eosin Methylene Blue (EMB) agar (Oxoid). The plates were incubated at 37°C for 24 h (Harrigan and MacCance, 1976).

Enumeration of *Staphylococcus aureus*: Enumeration of *Staphylococcus aureus* was performed on Mannitol Salt Agar (Oxoid). The plates were incubated at 37°C for 48 h. yellow colonies were counted and checked for gram and catalase reactions. Also, the isolated colonies were checked for their coagulation on rabbit plasma. Catalase positive Gram negative colonies were spread cultured on Trypticase Soya Agar slants for further characterization (Harrigan and MacCance, 1976).

***Salmonella*:** Detection of *Salmonella* was carried out according to Harrigan and MacCance (1976). Thus, 25 ml of the sample were added to 250 ml of sterile nutrient broth (Oxoid) and incubated for 24 h at 37°C. 2 tubes of Selenite cystein broth (Oxoid) were inoculated with 1 ml from the nutrient broth and incubated for 24 h at 37°C. Positive tubes were streaked on Bismuth Sulfite agar (Oxoid) and incubated at 37°C for 24 h. The pure colonies were then subjected to the confirmatory tests.

Lactic Acid Bacteria (LAB): Numbers of LAB were determined on selective media MRS agar. Appropriate dilutions were plated on MRS agar and incubated anaerobically using the anaerobic jars and the BBL, Gas Pak, anaerobic system envelopes (Becton, Dickinson, Cockeysville, USA) at 37°C for 48 h.

Enumeration of Lactic acid bacteria was determined using MRS medium incubated at 30°C for 48 h. After incubation, colonies were enumerated, recorded as colony forming units (cfu) per milliliter of the products (Harrigan and MacCance, 1976).

Statistical analysis: All microbial counts were converted to the base -10 logarithm of the number of colony forming units per ml of raw cow milk samples (log cfu/ml) and from the means and their standard deviations were calculated. Data were analyzed using Analysis of Variance (ANOVA) through the General Linear Models (GLM) procedure of the statistical analysis system software (SPSS version-11.5, 2003). Least significant differences were used to separate means at $p<0.05$.

RESULTS AND DISCUSSION

The microflora of raw cow milk is presented in Table 1. Difference among milks from different regions of cow raw milk were studied by analysis of variance (Table 1) of the two regions. The highest ($p<0.05$) average loads of TBC, LAB and coliforms were observed with respect to the average counts of *E. coli*.

Raw milk contained an average TBC of 5.86 log cfu/ml. It is a high count of TBC and should be due to inadequate sanitary condition during milking, collection and transport. Raw milk in Italy (Supino *et al*., 2004) had total bacterial counts of 5.23 log cfu/ml, which is of the same order of our data. LAB constituted a major part of the microflora with an average 4.47 log cfu/ml. Boycheva *et al*. (2002) observed that LAB and psychrotrophs predominated in Bulgarian buffalo milk.

The average load of *S. aureus* was 1.92 log cfu/ml. Fook *et al*. (2004) reported considerably higher levels of *S. aureus* in other cow milk, with 35% of samples having 4.2 log cfu/ml. Since S. aureus is potentially hazardous at $>10^4$ cfu/ml (Han *et al*., 2005), all cow milk samples were within an acceptable level.

The contamination of the milk by *S. aureus* is often original but can also occur after handling draft in non-hygienic conditions. *Staphylococcus aureus* is a poor competitor and is readily outgrown by lactic acid-producing microorganisms, so growth is limited in raw milk (Holsinger *et al*., 1997; Asperger, 1994).

On the other hand *Salmonella* was not detected in any samples that collected from Khartoum or Omdurman. The presence of *Salmonella* as pathogenic microorganism is a health problem and this in accord with the finding of Gazzar *et al*. (1992) who reported that *Salmonella* spp., become a major concern for the dairy industry due to out breaks of illness.

The average levels of coliform bacteria and *E. coli* were 2.76 and 1.63 log cfu/ml respectively. These counts were higher than those reported by Desmasures *et al*. (1997), who reported that 84% of samples of French cow milk had coliform counts <100 cfu/ml and 80% had *E. coli* counts $\leq$10 cfu/ml. *E. coli* may be considered an

Table 1: Microbiological loads of cow milk (log cfu/ml) in Khartoum district

Region	TBC	SFB	LAB	Coliforms	*E. coli*	*S. aureus*
Khartoum (morning) [n = 6]	5.17±0.46^{a}	3.08±0.11^{a}	4.53±0.64^{a}	2.41±0.21^{a}	1.63±0.13^{a}	2.15±0.53^{a}
Khartoum (night) [n = 6]	6.40±0.31^{b}	2.44±0.21^{b}	5.01±0.31^{b}	3.36±0.08^{a}	1.65±0.41^{b}	2.34±0.47^{a}
Omdurman (morning) [n = 6]	5.45±0.34^{b}	1.61±0.26^{b}	3.66±0.20^{ab}	2.21±0.29^{a}	1.46±0.17^{b}	1.40±0.72^{b}
Omdurman (night) [n = 6]	6.37±0.14^{b}	2.35±0.41^{a}	4.61±0.61^{ab}	3.09±0.11^{a}	1.89±0.09^{b}	1.81±0.17^{c}
Average	5.86±0.31	2.38±0.27	4.47±0.44	2.76±0.18	1.63±0.20	1.92±0.47

*Means±SD; ^{abc}Means bearing different superscripts in the same column differ significantly ($p<0.05$); TBC: Total Bacterial Count; SFB: Spore Forming Bacteria; LAB: Lactic Acid Bacteria

indicator microorganism of faecal contamination and other enteric pathogens. The presences of large numbers of coliform bacteria are suggestive of unsanitary conditions or practices during production, processing, distribution or storage (Thomas *et al*., 1971).

Pathogenic bacteria may also be present in raw milk as a direct consequence of clinical or subclinical mastitis (Giesecke *et al*., 1994). Among the organisms commonly producing mastitis, *Streptococcus aureus* and *E. coli* are pathogenic for man (Bramley and McKinnon, 1990).

Total bacterial counts or total aerobic colony counts are used to estimate viable bacterial populations in milk and reflect the hygienic practices used in the production and handling of the milk (Houghtby *et al*., 1994).

Generally, fresh raw milks collected from retailers were heavily contaminated. Possible reasons for the counts could be due to infected udders of the cows, unhygienic milking procedures or equipment and/or inferior microbiological quality of water used for cleaning utensils and animals, as well as the milk storage conditions. The milking process, especially the equipment associated with it, introduces the greatest proportion of microorganisms in raw milk (Olson and Mocquot, 1980; Cousin, 1982).

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Cinnamon May Reduce Glucose, Lipid and Cholesterol Level in Type 2 Diabetic Individuals

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Abstract: The effect of cinnamon on blood glucose and lipid profile was studied in type-2 diabetic individuals for 30 days. Fourteen diabetic individuals of both sexes and of age 40 and above were divided in two groups, each having 7 individuals. Group 1 was assigned for 1.5 g cinnamon dose/day while group 2 was assigned for 1.5g placebo dose/day. The doses were given for 30 days. Fasting blood samples were collected at the start of experiment (day 0) and at the end of experiment (day 30). Serum glucose, serum triglycerides, serum total cholesterol, high density lipoprotein HDL and low density lipoprotein LDL cholesterol, of both cinnamon and placebo groups were determined. The results showed that cinnamon dose significantly reduced glucose, triglycerides and cholesterol. HDL and LDL cholesterol were not affected. Placebo did not affect any of the above parameters.

Key words: Cinnamon, blood glucose, lipid profile, type-2 diabetes

INTRODUCTION

Diabetes mellitus is a disorder of glucose metabolism, characterized by high blood sugar level that results from the body's inability to make or use insulin. Diabetes can lead to life threatening complications including blindness, memory problems, kidney diseases, heart disease, nerve damage and amputation (Robinson *et al*., 1986). The most common type is type 2 diabetes, which usually develops because of the body's inability to use insulin properly. It commonly occurs in people of age 40 and above and who are obese (Khan and Ahmad, 1994). Dietary therapy should be the primary approach for the treatment of diabetes. This approach is natural, economical and feasible in developing countries. The broad aims of dietary prescription for people with diabetes remain, first, to abolish the primary symptoms, secondly to minimize the long term macro vascular and micro vascular complications which together results in morbidity and shortened life span with all types of diabetes (Camerini-Davalos and Cole, 1987).

The diabetic diet should contain 60% carbohydrate, 20-25% fat and 15-20% protein. High carbohydrate diet increases the sensitivity of peripheral tissue to both endogenous and exogenous insulin. Such diet improves glucose intolerance and lowers levels of serum insulin (Khan and Ahmad, 1994).

Recently some of the spices like cinnamon, cloves, bay leaves and turmeric have been reported to have insulin potentiating factor which potentiates the function of insulin in glucose metabolism (Khan *et al*., 1990). This study was initiated to confirm the previous findings that cinnamon intake reduces glucose, triglycerides and cholesterol in type 2 diabetic individuals.

MATERIALS AND METHODS

The study was conducted in the Institute of Biotechnology and Genetic Engineering (IBGE), Agricultural University Peshawar, North West Frontier Province, Pakistan. This study was approved by the Ethics Committee and Board of Studies of the University. The criterion for selection was that the type 2 diabetic patients should be of both sexes and of age 40 and above. The blood sugar of these patients must be 125 mg/dl or above and these patients should not be on insulin therapy. Diabetic patients in Abasine Institute of Medical Sciences (AIMS) were screened and 14 type 2 diabetic patients were selected and registered for the study. The registered patients were randomly divided into two groups, each of seven individuals. One group was assigned for 1.5 g cinnamon/day while the other was assigned for 1.5 g placebo dose/day.

The required amount of cinnamon and maize flour (placebo) was purchased from the local market and was ground finely and put in to capsules. Each capsule was containing 0.5 g cinnamon or maize flour. The study was conducted for 30 days. A dose of 1.5 g cinnamon/day and 1.5 g placebo/day in the form of capsules were given for 30 days to the individuals of group1 and group2 respectively. Dose of cinnamon and placebo were spread over the day as breakfast, lunch and dinner. For example 1.5 g dose of cinnamon per day was spread

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over the day as 0.5 g (one capsule) of cinnamon after breakfast, 0.5 g (one capsule) of cinnamon after lunch and 0.5 g (one capsule) after dinner. The placebo dose was spread over the day in the same pattern.

Approximately 5ml fasting blood samples were taken from each individual on day 0 before the experimental trial was started (control) and on day 30, when the experimental trial was completed. Blood samples were transferred to sterilized centrifuge tubes and allowed for clotting at room temperature. The blood samples were centrifuged for 5 min in a centrifuge at 4,000 rpm for serum separation. Separated serums were transferred to eppindorf tubes and were freezed for later analysis.

Glucose was determined by the enzymatic colorimetric method of Tinder and Ann (1969). Triglyceride was determined by enzymatic calorimetric procedure of Werner *et al*. (1981). Cholesterol was determined by the enzymatic colorimetric method of Allian *et al*. (1974). LDL (Low Density Lipoproteins) was precipitated by adding phosphotungstic acid and magnesium ion to the sample. Centrifugation left only the HDL (High Density Lipoproteins) in the supernatant; their cholesterol content was determined by the method of Assmann (1979). Auto analyzer (Express plus, Ciba corning, USA) and Elitech kits were used.

Statistical analysis: Two- way Analysis of Variance and Randomized Complete Block Design (RCBD) was used for statistical analysis (MSTAT-C with MGRAPH, Russell D. Freed, MSTAT, Crop and Soil Sciences Department, Michigan State University, Version 2.00).

RESULTS AND DISCUSSION

Effect of cinnamon and placebo on serum glucose: The effect of cinnamon and placebo on serum glucose in type 2 diabetic individuals is given in Table 1. The mean glucose values on day 0 in Table 1 indicate the fasting serum glucose of diabetic individuals before the start of intake of cinnamon and placebo capsules and were considered as control values for the study.

On the starting day of the experiment (day 0), the mean fasting serum glucose concentration of the diabetic individual of group 1, assigned for 1.5 g cinnamon dose/day was 216.3±52.7 mg/dl. When Diabetic individuals of this group used the above dose of cinnamon for 30 days, their mean fasting serum glucose level was reduced to 163.3±44.9 mg/dl. This reduction in glucose was significant at $p<0.05$. To verify that the drop in the fasting blood glucose was not due to psychological effect of the cinnamon capsules, placebo capsules were given to group 2 in the same pattern as the cinnamon. After 30 days blood samples were collected and analyzed which showed that placebo dose did not affect the glucose level.

Effect of cinnamon and placebo on triglycerides: The effect of cinnamon and placebo on triglycerides in type 2 diabetic individuals is given in Table 2. The triglycerides values on day 0 in Table 2 indicate the fasting serum triglycerides of diabetic individuals before the start of intake of cinnamon and placebo capsules and were considered as control values for the study.

On the starting day of the experiment (day 0), the mean fasting serum triglycerides of the diabetic individual of group 1, assigned for 1.5 g cinnamon dose/day was 186.1±124.2 mg/dl. When the diabetic individuals of this group used 1.5 g cinnamon dose/day for 30 days, their mean fasting serum triglycerides level significantly reduced to 142.6±36.7 mg/dl at $p<0.05$. To verify that the drop in the fasting serum triglycerides was not due to psychological effect of the cinnamon capsules, placebo capsules were given to group 2 in the same pattern as

Table 1: Effect of cinnamon and placebo on serum glucose in type 2 diabetic individuals

		Fasting serum glucose[1,2] mg/dl	
		Before intake of cinnamon/placebo	After intake of cinnamon/placebo
Group of diabetes	Dose of cinnamon/ placebo (1.5 g/day)	Day 0	Day 30
1	Cinnamon	216.3^{A}±52.7	163.3^{B}±44.9
2	Placebo	204.1^{A}±44.7	232.00^{A}±65.0

1. Values given in column 3 and 4 of the respective rows are the means and standard deviations of 7 individuals.
2. Means followed by different letters in the rows are significantly different at $p<0.05$ as determined by analysis of variance

Table 2: Effect of cinnamon and placebo on serum triglycerides in type 2 diabetic individuals

		Fasting serum TGL[1,2] mg/dl	
		Before intake of cinnamon/placebo	After intake of cinnamon/placebo
Group of diabetes	Dose of cinnamon/ placebo (1.5 g/day)	Day 0	Day 30
1	Cinnamon	186.1^{A}± 24.2	142.6^{B}±36.7
2	Placebo	221.9^{A}±124.2	223.00^{A}±103.1

1. Values given in column 3 and 4 of the respective rows are the means and standard deviations of 7 individuals.
2. Means followed by different letters in the rows are significantly different at $p<0.05$ as determined by analysis of variance

the cinnamon. After 30 days blood samples were collected and analyzed which showed that placebo dose have no effect on the serum triglycerides in diabetic individual.

Effect of cinnamon and placebo on serum cholesterol: Effect of cinnamon and placebo on serum cholesterol in Type 2 diabetic individuals is shown in Table 3. The cholesterol values on day 0 in Table 3 indicate the fasting serum cholesterol concentration of diabetic individuals before the start of intake of cinnamon and placebo capsules and were considered as control values for the study.

On the starting day of the experiment (day 0), the mean fasting serum cholesterol concentration of the diabetic individual of group 1, assigned for 1.5 g cinnamon dose/day was 182.4±40.7 mg/dl. When the diabetic individuals of this group used 1.5 g cinnamon dose/day for 30 days, their mean fasting cholesterol concentration dropped significantly ($p<0.05$) to 151.4±29.2 mg/dl. To know that this drop in the fasting blood cholesterol was not due to psychological effect of the cinnamon capsules, placebo capsules were given to group 2 in the same pattern as the cinnamon. The placebo dose did not affect the cholesterol concentration in the diabetic individuals.

Effect of cinnamon and placebo on serum HDL-cholesterol: The effect of cinnamon and placebo on serum HDL-cholesterol in type 2 diabetic individuals is given in Table 4. The HDL values on day 0 in Table 4 indicate the fasting serum HDL of diabetic individuals before the start of intake of cinnamon and placebo capsules and were considered as control values for the study.

On the starting day of the experiment (day 0), the mean fasting serum HDL cholesterol concentration of the diabetic individual of group 1, assigned for 1.5 g cinnamon dose/day was 35.3±3.5 mg/dl. When diabetic individuals of this group used 1.5 g cinnamon dose/day for 30 days, their mean fasting serum HDL cholesterol concentration dropped non significantly to 34.7±3.2 mg/dl. Placebo did not affect HDL-cholesterol in type 2 diabetic individuals.

Effect of cinnamon and placebo on serum LDL-cholesterol: The effect of cinnamon and placebo on serum LDL-cholesterol in type 2 diabetic individuals is given in Table 5. The serum LDL cholesterol values on day 0 in Table 5 indicate the fasting serum LDL values of diabetic individuals before the start of intake of cinnamon and placebo capsules and were considered as control values for the study.

Table 3: Effect of cinnamon and placebo on serum cholesterol in type 2 diabetic individuals

		Fasting serum cholesterol[1,2] mg/dl	
		Before intake of cinnamon/placebo	After intake of cinnamon/placebo
Group of diabetes	Dose of cinnamon/ placebo (1.5 g/day)	Day 0	Day 30
1	Cinnamon	182.4^{A}±40.7	151.4^{B}±29.2
2	Placebo	184.4^{A}±29.6	180.1^{A}±13.2

1. Values given in column 3 and 4 of the respective rows are the means and standard deviations of 7 individuals.
2. Means followed by different letters in the rows are significantly different at $p<0.05$ as determined by analysis of variance

Table 4: Effect of cinnamon and placebo on serum HDL-cholesterol in type 2 diabetic individuals

		Fasting serum HDL[1,2] mg/dl	
		Before intake of cinnamon/placebo	After intake of cinnamon/placebo
Group of diabetes	Dose of cinnamon/ placebo (1.5 g/day)	Day 0	Day 30
1	Cinnamon	35.3^{A}±3.5	34.7^{A}±3.2
2	Placebo	36.1^{A}±3.0	37.6^{A}±2.4

1. Values given in column 3 and 4 of the respective rows are the means and standard deviations of 7 individuals.
2. Means followed by different letters in the rows are significantly different at $p<0.05$ as determined by analysis of variance

Table 5: Effect of cinnamon and placebo on serum LDL-cholesterol in type 2 diabetic individuals

		Fasting serum LDL[1,2] mg/dl	
		Before intake of cinnamon/placebo	After intake of cinnamon/placebo
Group of diabetes	Dose of cinnamon/ placebo (1.5 g/day)	Day 0	Day 30
1	Cinnamon	109.7^{A}±38.1	88.6^{A}±25.1
2	Placebo	100.1^{A}±18.7	99.4^{A}±19.1

1. Values given in column 3 and 4 of the respective rows are the means and standard deviations of 7 individuals.
2. Means followed by different letters in the rows are significantly different at $p<0.05$ as determined by analysis of variance

On the starting day of the experiment (day 0), the mean fasting serum LDL cholesterol concentration of the diabetic individual of group 1, assigned for 1.5 g cinnamon dose/day was 109.7±38.1 mg/dl. When the diabetic individuals of this group used 1.5 g cinnamon dose/day for 30 days, their mean fasting serum LDL cholesterol concentration dropped non-significantly to 88.6±25.1 mg/dl. Placebo did not affect LDL-cholesterol in type 2 diabetic individuals.

Khan and Anderson (2003) studied the effect of cinnamon doses on blood glucose and lipid profile in type 2 diabetic individuals. They reported that the intake of 1, 3, or 6g of cinnamon per day reduced fasting serum glucose (18-29%), triglycerides (23-30%), LDL-cholesterol (7-27%) and total cholesterol (12-26%). In their study they gave the 3 doses of cinnamon for 30 days. They did not report any adverse effect of cinnamon on humans in their 30 days trial. Also people have been using cinnamon in food preparations for centuries, indicating that consumption of cinnamon in reasonable amounts is safe. Our study confirms their findings.

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Studies on Amylolytic Enzyme Synthesized by *Aspergillus flavus* Associated with Mouldy Bread

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Abstract: Studies were carried out on amylolytic enzymes produced by *Aspergillus flavus* isolated from mouldy bread with the aim of establishing some factors that affect its activity. *Aspergillus flavus* grew in synthetic medium containing starch as the sole carbon source and synthesize enzymes which exhibited amylolytic activities. Production of the enzyme increased with increase in days of incubation with optimum activity occurring on the tenth day of incubation. Activity of the enzyme increased at 4mg/ml starch concentration. Activity also increased with increase in temperature reaching a maximum at 40°C. The pH of the reaction mixture influenced the activity of the enzyme, optimum activity being at pH 7.0.

Key words: *Aspergillus flavus*, amylolytic activity, amylase, bread

INTRODUCTION

Moulding is the most common and hence the most important cause of spoilage of bread and most bakery products (Bharat and Hoondal, 1998). However, the temperature attained in the baking procedure is usually high enough to kill all mould spores in and on the loaf, hence it is most probable that moulds reach the outer surface or penetrate the loaves after baking, usually from the air during cooling and thereafter from handling or wrappers (Miller, 2001). Prominent moulds involved in the spoilage of bread are usually referred to as bread-moulds and they include *Rhizopus stolonifer, Penicillium expansum, Penicillium stolonifer, Aspergillus niger* and *Aspergillus flavus* (Duke *et al*., 1996).

Aspergillus flavus is a phytopathogenic fungus able to grow on different crops, but most commonly on corn, cotton and peanuts contaminating them with mycotoxins (William and Dennis, 1998). *A. flavus* can also be pathogenic in animal species, including humans and some domestic animals (Collier *et al*., 1998). The mycotoxins produced by *A. flavus* cause damage during metabolism and this induces vital changes in the chemical constituent of the associated substrate (Anjana and Sinha, 1983). Aflatoxins that are primarily produced by the mould *Aspergillus flavus* and *Aspergillus parasiticus* are among the most toxic and carcinogenic compounds occurring naturally (Lillehoj and Ciegler, 2003).

Amylases are enzymes that hydrolyse starch molecules into polymers composed of glucose units (Reddy *et al*., 2003). Amylases are important enzymes employed in the starch processing industries for the hydrolysis of starch into simple sugar constituents (Mitchell and Lousane, 1990; Akpon *et al*., 1996 El-Saadany *et al*., 2006).

Amylases from plants, animals and microorganisms have been studied since enzymes were discovered (Horikoshi, 1996). Amylases have received a great deal of attention because of their significance especially in biotechnology (Reddy *et al*., 2003). Amylase constitutes a class of industrial enzymes having approximately 25% of the enzyme market world-wide (Sindhu *et al*., 1997). Many *Bacillus* species and thermostable *Actinomycetes* like *Actinomycetes thermomonospora* and *Actinomycetes thermoactinomyces* are versatile producers of amylase (Buzzini and Martini, 2002). The genus *Bacillus* produces a large range of extracellular enzymes of which amylases and proteases are of industrial importance (Bajpai and Bajpai, 1999; Demiorijan *et al*., 2001). Currently two types of amylases, glucoamylase and alpha-glucosidase are important for starch hydrolysis. Glucoamylase attacks α-1, 4-bonds, releasing D-glucose molecules (Guzman-Maldonato and Paredes-Lopes, 1995). This enzyme also attacks α-1, 6 bonds at branching points in the amylopectin molecule but much more slowly than α-1, 4 linkages (James and Lee, 1997). Alpha-glucosidase catalyzes the splitting of alpha-D-glucosyl residues from the non-reducing end of substrates to release alpha-glucose (Pandey *et al*., 2002). In this study we investigated some factors affecting amylase produced by *Aspergillus flavus* isolated from mouldy bread.

MATERIALS AND METHODS

Organism and culture condition: The strain (MIC/OAU/038) of *Aspergillus flavus* used in this work was isolated from mouldy bread. It was obtained from the culture collection of Prof. P.O. Olutiola of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The stock culture was routinely

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grown and maintained on 1% malt-yeast extract agar slants in test-tubes.

Preparation of assay medium: Sterile 50 ml of basal medium was aseptically added to 50 ml of the starch medium and final pH was adjusted to 6.7. Thereafter 1 ml of 10^6 spore suspension was added to each of the 100 ml growth medium.

The content of each flask was monitored daily for amylase activity. Five milliliters were aseptically obtained from each flask and filtered with small disc of Whatman filter-paper.

Enzyme assay: The activity of the enzyme (amylase) was determined by a modification of the dextrinogenic assay of Pfueller and Elliot (1999). It involved the measurement of changes in the blue values of starch-iodine complex due to the decrease in the amount of the starch in the reaction mixture. The reaction mixture consisted of 2ml of 0.4% starch (w/v) and 0.5 ml of enzyme preparation. The control tubes contained only 2 ml of 0.4% starch (w/v). Experimental and control tubes were incubated at 40°C for 30 min.

After incubation, 2 ml of 1 N HCl was added to the experimental tubes to terminate the reaction. To each of the control tube was added 2 ml of 1 N HCl followed by 0.5 ml of the enzyme. The contents of experimental and control tubes were further diluted by adding 3 ml of 0.1 N HCl to each tube. Iodine mixture (0.1 ml) was added to each tube. The content of each tube was mixed thoroughly by using a test-tube mixer (Gallenkemp). Optical density readings were made at 600 nm using colorimeter (Jensway, Essex, UK).

One unit of enzyme activity was arbitrarily defined as the amount of enzyme in 1 ml of reaction mixture which produced 0.01% reduction in the intensity of the blue colour of the starch-iodine complex under the assay conditions.

Characterization of amylase: Effects of growth period i.e incubation period (4-10 days), various concentrations of starch (0.5-4 mg/ml), temperature (10-45°C) and time of heating (0-30 min) at 80°C were tested to characterize the amylase produced.

RESULTS AND DISCUSSION

Aspergillus flavus grew in a synthetic medium containing starch as sole carbon source. During the period of growth, the culture filtrate exhibited amylase activity. Optimum activity was observed on the tenth day of incubation (Fig. 1). *Aspergillus flavus* was able to degrade starch when used as substrate within a concentration range of 0.5-4 mg/ml. Optimum activity was observed at 4 mg/ml starch concentration (Fig. 2). *Aspergillus flavus* exhibited amylase activity within a temperature range of 10-45°C. Activity of the enzyme

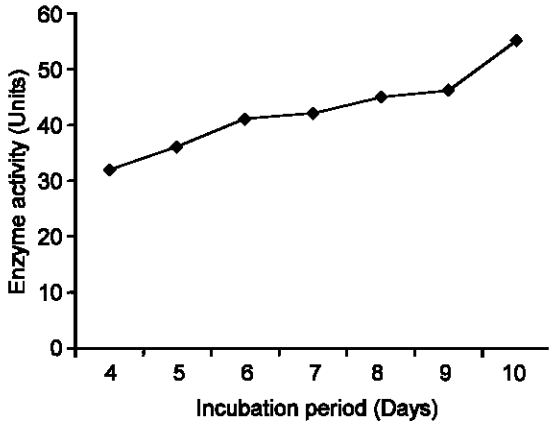

Fig. 1: Effect of growth period on the activity of amylase produced by *Aspergillus flavus*

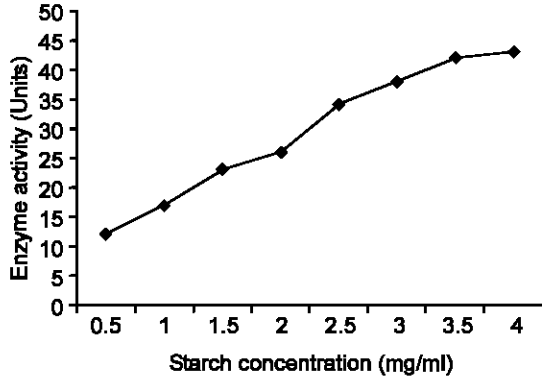

Fig. 2: Effect of starch concentration on the activity of amylase produced by *Aspergillus flavus*

increased as the temperature increased. Maximum activity occurred at 40°C, beyond which no further increase in activity was obtained (Fig. 3). When amylase produced by *Aspergillus flavus* was heated at 80°C, there was a gradual decrease in the activity of the enzyme with increase in period of heating (Fig. 4). When heated for 5 min, approximately 33% loss in enzyme activity was obtained. Heating for 30 min caused a decrease of about 63% in enzyme activity (Fig. 4).

The results of this study showed that *A. flavus* grew in a medium containing starch as the sole carbon source and produced the enzyme amylase required for the hydrolysis of starch. Malankar (2005) reported the production of a raw starch-degrading alpha amylase by a strain of *Aspergillus flavus*.

The results of this work showed that amylase activity increased as the substrate (starch) concentration in the medium increased until a substrate concentration of 4 mg/ml was reached, when no further increase in enzyme activity occurred. Pederson and Nielson (2001) reported similar observations for amylase activity. Dixon and Webb (1997) reported that at low substrate

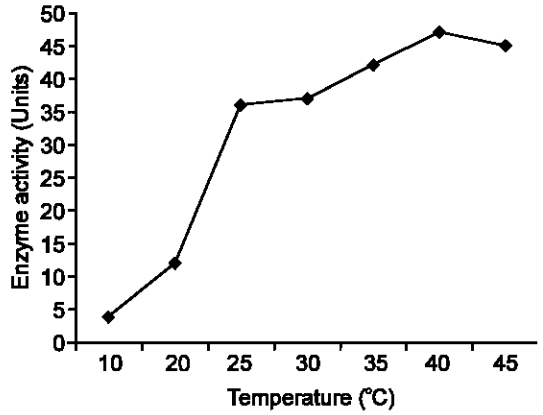

Fig. 3: Effect of temperature on the activity of amylase produced by *Aspergillus flavus*

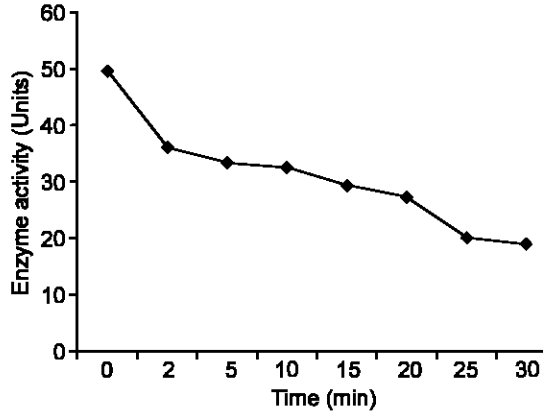

Fig. 4: Effect of time of heating (80°C) on the activity of amylase produced by *Aspergillus flavus*

concentrations the active sites of enzymes are not saturated and hence the activities of such enzymes increased with increase in substrate concentration. Yang and Wang (1999) reported an increase in amylase activity of germinated barley as starch concentration increase. Kuiper *et al.* (1998) reported that maximum activity of alpha amylase enzyme was obtained at 1.67% of substrate (starch) concentration. In addition, Abdel-Rahman (2006) concluded that maximum alpha amylase activity was between 2-3% concentrations.

In this study, the activity of the enzyme was greatly influenced by the pH of the reaction mixture, with optimum activity *occurring* at pH 7.0. Haruyuki-Efugi (1996) reported that amylase activity was affected by the substrate type, temperature, substrate concentration and pH. Kamekura (1997) obtained an optimum pH of 5-6 for amylase from *Micrococcus* sp. However some amylases act best at acidic pH 5.0 and 5.5 (Do and Kin, 1995; Gupta *et al.*, 2008). In the present work, there was a slight decrease in activity of the enzyme beyond pH 7.0. Eke and Oguntimehin (1992) reported that a drastic decline in the activity of amylase at pH above 7.0 indicated a loss of activity at alkaline region.

The effect of pH on the activity of an enzyme has been attributed to a change in the ionic character of the amino and carboxylic acid components of the enzyme, which will in turn affect both the catalytic site and conformational status of the enzyme protein (Rice and Stephen, 2002; Prakash *et al.*, 2009). Extremes of low and high pH values have been protein (Russel and Jacobsen, 1997).

The results of this study showed that temperature greatly affected the activity of amylase synthesized by *A. flavus*. Optimum activity of the enzyme occurred 40°C. This agrees with the work of Khoo *et al.* (1994) who reported an optimum temperature 40-45°C for amylase from *Aspergillus niger*. However, Chakraborty *et al.* (2000) obtained an optimum activity at 50°C for a thermostable alpha amylase.

In this study, enzyme activity was lost with increase in the time of heating at 80°C and within 5 min of heating (80°C), approximately 33% of the activity of enzyme was lost. This agrees with the result of Mulimani and Rudrappa (2002) who reported the inhibition of amylase from pea when subjected to heat and Guerra *et al.* (2005) who showed that amylase activity was inhibited at temperatures above 70°C.

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Determination of Chemical Components of *Memecylon umbellaltum* Burm. - A Medicinal Plant, Karnataka, India

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Abstract: *Memecylon umbellatum* Burm. has been being used not only for treatment of diseases but also for other uses since time immemorial. The different parts of the plant have been used for the preparation of the herbal products which are used orally or for external applications. The leaves of the *M. umbellatum* which have been collected from Koppa and Bannerghatta are subjected for analysis of proximates, micro, macro and toxic elemental composition. The young and mature leaves are separately analyzed. The study reveals that moisture, carbohydrate and crude protein content are the dominant factors in both the young and mature leaf samples. Whereas, crude fat was followed by ash and crude fiber in both young and mature leaf samples of Koppa and in case of Bannerghatta samples crude fat was followed by crude fiber and ash. However, the young leaves are more nutrient than that of mature in both the cases. Among the macronutrients K was dominant which is followed by Ca, Mg, Na and P in both young and mature leaves of Koppa whereas in case of Bannerghatta samples K was followed by Ca, Mg, P and Na, in young leaves but in mature leaves Ca was followed by K, Mg, Na and P in their concentrations. The Fe was highest among the micronutrients of both young and mature leaves of Koppa and Bannerghatta samples. Fe was followed by Mn, Cu and Zn in case of Koppa samples whereas Fe was followed by Mn, Zn and Cu in young leaves and Fe was followed by Mn, Cu and Zn in mature leaves sample of Bannerghatta. There was a significant variation of Cu among the different leaf samples of Koppa and Bannerghatta samples. The Pb content of the young leaves of Koppa is higher than that of Bannerghatta samples and mature leaves of Bannerghatta samples recorded high values of Pb than that of Koppa samples. Between the 2 toxic elements Pb was higher than that of Cd and the mature leaves of both Koppa and Bannerghatta samples recorded lowest concentration of Cd. The results were subjected for statistical analysis.

Key words: Proximate analysis, elemental composition (macro, micro and toxic), medicinal plants of Karnataka, India, *Memecylon umbellatum* Burm.

INTRODUCTION

Memecylon umbellatum Burm. belongs to the family Melastomaceae is a small handsome tree, the local name is Adachare and the English name is Iron-wood tree. The infusion of leaves is used in the treatment of gonorrhoea and leucorrhoea (Nadakarni, 1976; Kirtikar and Basu, 1991). The paste of leaves is used in the treatment of herpes (Maruthi *et al*., 2000). The decoction of the roots is used for the treatment of menorrhagia and the preparation from the bark for the treatment of bruises (Nadakarni, 1976). The seeds are used to cure cough and sedative (Balakrishna Gowda, 2004). The leaf powder is used for the treatment of diabetes (Ayyanar *et al*., 2008). It has hypoglycemic effect in normal and alloxan diabetic mice (Amalraj and Ignacimuthu, 1998). Ram Rastogi and Mehrotra (1991) isolated and characterized the umbellactone -β-amyrin, sitosterol, its glycoside, olenalic and ursolic acids. The first and foremost important use of this tree is the protection of hilly slopes, from which other trees have been removed. The ripe berries of Anjan are edible, are eaten in time of famine and they are quite safe. The leaves have for long been employed in India in the dyeing industry to dye wool, silk and even grass mats can be nicely dyed. The leaves used in conjunction with myrobalans give a deep red colour and when used with tin mordant yield a yellow colour if no mordant is used, they produce light brown colour (http://rajbhavankolkata.gov.in/pdf/occasional%20paper5.pdf). In the present study, an attempt has been made to determine the proximates (ash, moisture, crude fat, crude fiber, crude protein and carbohydrate), nutritive value, macro, micro and toxic elemental composition of young and mature leaves of *M. umbellatum* of different regions of Karnataka.

MATERIALS AND METHODS

Plant collection: The leaves were collected from Bannerghatta and Koppa, Karnataka, India (Map). The mature and young leaves were collected separately. The plant was identified and classified by using taxonomic literature (Flora of British India (Hooker, 1894). Flora of Presidency of Madras (Gamble, 1928), Flora of

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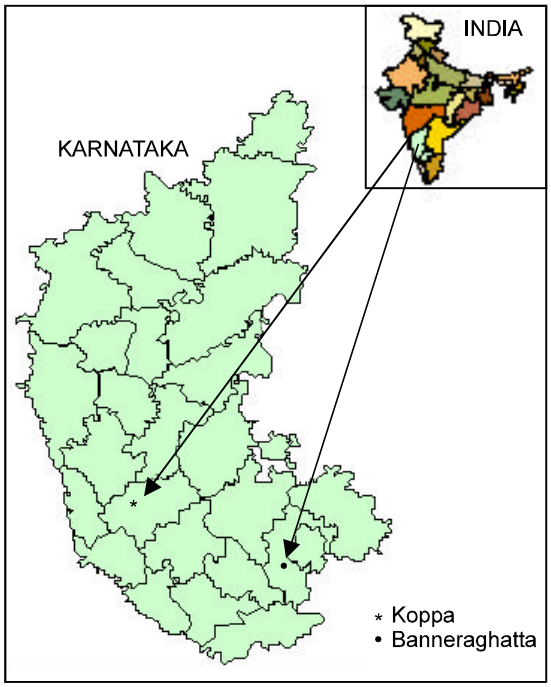

Map: Showing the study area

Davanagere District (Manjunath *et al*., 2002), Flora of Bangalore District (Ramaswamy and Razi, 1973), Flora of Chikmagalore District (Yoganarasimhan *et al*., 1982), Compendium of Indian Medicinal plants (Ram Rastogi and Mehrotra, 1991), Flora of Presidency of Bombay (Theodore Cooke, 1903), The Forest Trees of Travancore (Bourdillon, 1908), Flora of South Indian Hill Stations (Fyson, 1932), Flora of Coorg (Kodagu) (Keshava Murthy and Yoganarasimhan, 1990), Flora of Hassan District (Cecil Saldanha and Dan Nicolson 1976). The voucher specimens are deposited as herbarium in the Department of Applied Botany, Kuvempu University.

Sample preparation: The collected leaves were first washed thoroughly 2-3 times with running tap water and once with sterile water and in alcohol to remove the dust particles, then they are shade dried, powdered and stored in airtight bottles for further investigation.

Proximate analysis: The proximate analysis (moisture, ash, crude fat, crude fiber, crude proteins and carbohydrates) and nutritive values of both the samples were determined and calculated by following the methods of Indrayan *et al*. (2005). All the proximate values were reported in percentage and the nutritive values were expressed in cal/100 g.

Elemental analysis: The macro (P, K, Na, Ca and Mg), micro (Fe, Cu, Zn and Mn) and heavy metals (Pb and Cd) were analyzed by using atomic absorption spectrophotometer and flame photometer by following the procedures of Gali *et al*. (1999) in Central Coffee Research Institute, Balehonnur, Karantaka, India. The results were expressed in percent and ppm for macro, micro and heavy metals respectively.

Statistical analysis: Each experiment was repeated for three times. The results were represented with their means, standard deviation and standard error. The correlation matrix has been calculated.

RESULTS AND DISCUSSION

Proximate analysis: The result of proximate analysis shows variant concentration/proportions of biochemicals and other contents (Table 1). The percentage of moisture content was high in both mature and young leaf samples of Bannerghatta and Koppa. In case of percent of ash content, it was highest in both young and mature samples of Koppa while Bannerghatta sample had comparatively lesser composition. The percent of crude fat was more in young leaf sample of Bannerghatta and mature sample of Koppa. The crude fiber percent was more or less similar in both the samples of Bannerghatta and Koppa. The crude protein percent was highest in Bannerghatta samples than that of Koppa samples. The percentage value of carbohydrate was more in Koppa samples than Bannerghatta samples (Fig. 1). The results revealed that the young leaf samples of both Bannerghatta and Koppa had highest nutritive values when compared to mature leaf samples (Fig. 2).

Among the proximates, mature leaves of Bannerghatta recorded highest percentage of moisture, crude fiber and crude proteins, whereas, mature leaves of Koppa recorded highest percentage of ash and young leaves of Bannerghatta recorded highest percentage of crude fat. The nutritive values ranged between 174.33±0.629 and 218.83±1.010 cal/100g of mature leaves of Bannerghatta and young leaves of Koppa respectively (Fig. 2). When the results are subjected for correlation matrix and it is found that the carbohydrate is negatively significantly correlated with moisture and nutritive value is positively significantly correlated with carbohydrate (Table 2).

Among the components of nutritive value, the moderate values of protein suggests that its contribution to the formation of hormones and other metabolite which are required for the growth and development of plants and Mau *et al*. (1999) emphasized the contribution of low level of proteins to the formation of hormones which controls a variety of body functions such as growth, repair and maintenance of body proteins. According to Michael and David (2002), the ash content is useful in grading the plant material based on their mineral composition. However the leaf samples of *M. umbellatum* record low concentration of ash. Further the crude fat and carbohydrate content indicate the

Table 1: Average values of proximates [(moisture, ash, crude fat, crude fiber, crude protein and carbohydrate (in %) and nutritive values (in cal/100gm)] of *Memecylon umbellatum*

Place	Samples	Moisture	Ash	Crude fat	Crude fiber	Crude protein	Carbohydrate	Nutritive value
Koppa	Mature leaves	54.333±0.577	4.333±0.289	6.333±0.629	4.000±0.500	11.820±0.437	23.18±1.707	197.00±2.250
	Young leaves	47.667±0.289	3.667±0.289	4.833±0.144	2.667±0.289	13.087±0.343	30.75±0.497	218.83±1.010
Bannerghatta	Mature leaves	60.333±0.289	2.333±0.289	5.000±0.250	4.333±0.289	20.767±0.363	11.57±0.830	174.33±0.629
	Young leaves	56.667±0.289	1.333±0.289	7.000±0.500	2.667±0.289	13.627±0.430	21.37±0.529	203.00±3.905

±shows mean and standard error

Table 2: Correlation matrix of proximate parameters

	Moisture	Ash	Fat	Fiber	Protein	Carbohydrate	Nutritive value
Moisture	1						
Ash	-0.570	1					
Fat	0.268	-0.335	1				
Fiber	0.623	0.258	-0.216	1			
Protein	0.689	-0.439	-0.480	0.555	1		
Carbohydrate	-0.966*	0.483	-0.011	-0.720	-0.838	1	
Nutritive value	-0.915	0.272	0.073	-0.859	-0.806	0.974*	1

*Correlation is significant at the 0.05 level (2-tailed)

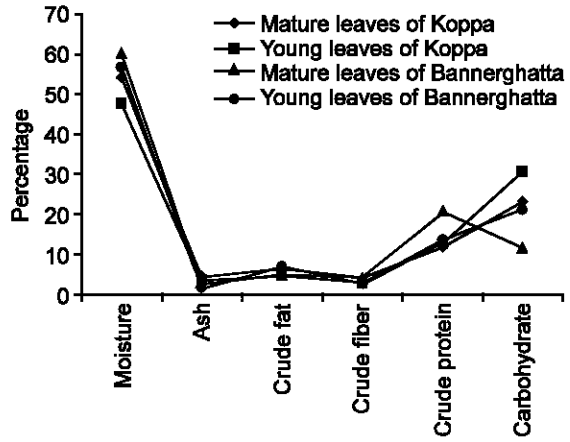

Fig. 1: Average values of components of nutritive value

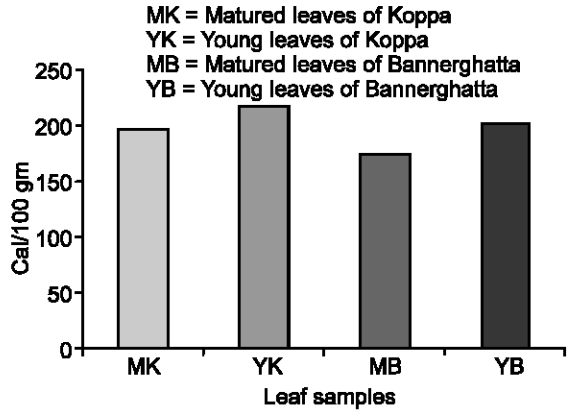

Fig. 2: Average values of nutritive value of Koppa and Bannerghatta

storage and transport of metabolic fuel and energy sources (Alli Smith, 2009). Indrayan *et al.* (2005) included leaves of *Artocarpus heterophyllus*, a medicinal plant based on their nutritive value and high sodium content under fodder. The nutritive value of leaves of *M. umbellatum* is higher than that of *Artocarpus heterophyllus*.

Elemental composition analysis: The average values of macro, micro, toxic elements are given in the Table 3. The K was dominant which is followed by Ca, Mg, Na and P in the leaf samples of Koppa. However in case of young leaves of Bannerghatta K was followed by Ca, Mg, P and Na and in case of mature leaves Ca was followed by K, Mg, Na and P (Fig. 3). The Fe was the dominant microelement which was followed by Mn, Cu and Zn in case of Koppa samples, where as in case of young leaf of Bannerghatta Fe was followed by Mn, Zn and Cu and in case of mature leaves Fe was followed by Mn, Cu and Zn (Fig. 4). The Pb was the highest toxic element in all the leaf samples of Koppa and Bannerghatta (Fig. 5). The correlation matrix of elemental composition reveals that major macroelements do not show any correlations, however among the microelements Fe shows positively correlation with K and Mg, Zn shows positively correlation with K, Mg and Fe and Mn showed positively correlation with K, Fe and Zn respectively (Table 4).

Among the macronutrients, Ca, P, Mg and N are required for repair of worn out cells, strong bones and teeth in humans, building of RBCs and for body mechanisms (WHO, 1996). Their absence in diet might result in weak, stunted growth and poor bone development (Edeoga *et al.*, 2006). Further, Indrayan *et al.* (2005) said that Na and K take part in ionic balance of the human body and maintain tissue excitability. Because of the solubility of salts, Na plays an important role in the transport of metabolites. The K is of importance as a diuretic. Indrayan *et al.* (2005) also mentioned the role of Ca and Mg and said that Ca constitutes a large proportion of the bone, human blood and extracellular fluid, it is necessary for the normal functioning of cardiac muscles, blood coagulation and milk clotting and the regulation of

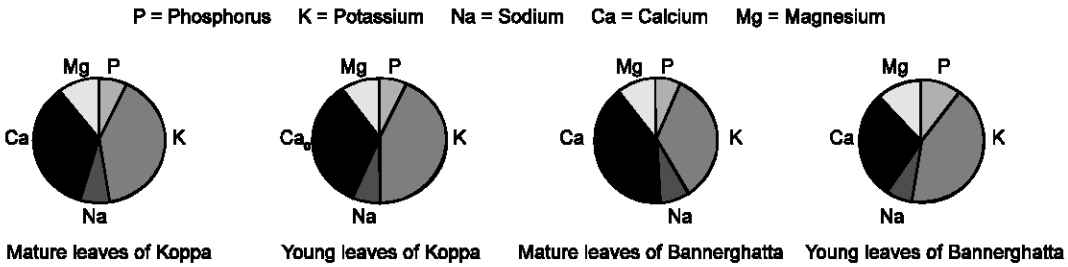

Fig. 3: Macronutrients (in %)

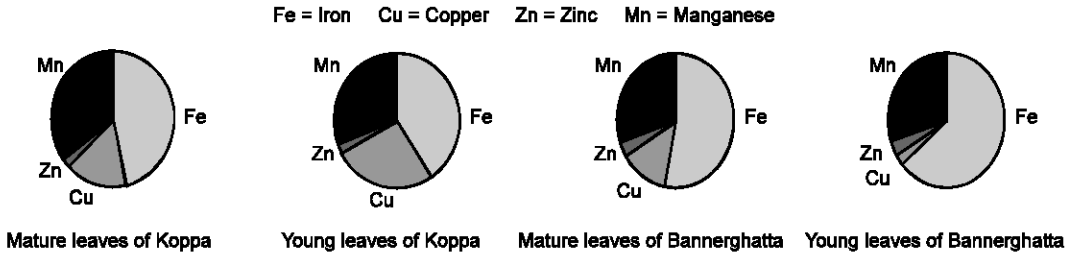

Fig. 4: Micronutrients (in ppm)

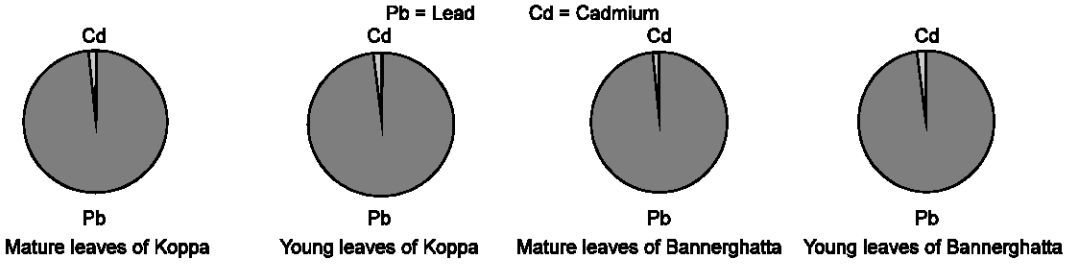

Fig. 5: Heavy metals (in ppm)

Table 3: Average values of elemental composition (macro (in %), micro (in ppm) and heavy metals (in ppm) of *Memecylon umbellatum*

		Macro elements					Micro elements				Trace elements	
Place	Samples	P	K	Na	Ca	Mg	Fe	Cu	Zn	Mn	Pb	Cd
Koppa	Mature	0.167±	1.044±	0.172±	0.901±	0.259±	570.63±	194.85±	29.500±	426.943±	52.923±	0.849±
	leaves	0.013	0.017	0.000	0.062	0.001	1.381	1.874	0.121	2.787	5.808	0.374
	Young	0.169±	1.044±	0.172±	0.835±	0.229±	464.80±	303.89±	25.443±	352.107±	66.420±	1.025±
	leaves	0.014	0.031	0.000	0.036	0.036	1.124	3.638	0.377	2.110	4.151	0.475
Bannerghatta	Mature	0.126±	0.720±	0.143±	0.840±	0.208±	278.78±	67.530±	17.840±	158.040±	68.903±	0.881±
	leaves	0.016	0.012	0.025	0.018	0.016	0.989	5.680	0.234	2.868	16.557	0.486
	Young	0.189±	0.755±	0.115±	0.514±	0.212±	300.29±	10.287±	16.767±	141.280±	55.373±	1.137±
	leaves	0.000	0.010	0.025	0.033	0.007	2.127	0.366	0.338	8.005	9.605	0.577

±shows mean and standard error

Table 4: Correlation matrix of elemental composition

	P	K	Na	Ca	Mg	Fe	Cu	Zn	Mn	Pb	Cd
P	1										
K	0.307	1									
Na	-0.199	0.872	1								
Ca	-0.600	0.567	0.891	1							
Mg	0.246	0.849	0.739	0.561	1						
Fe	0.269	0.950*	0.835	0.598	0.971*	1					
Cu	0.0490	0.907	0.914	0.666	0.591	0.760	1				
Zn	0.142	0.951*	0.902	0.701	0.950*	0.990**	0.808	1			
Mn	0.170	0.968*	0.906	0.685	0.936	0.989*	0.837	0.998**	1		
Pb	-0.697	-0.235	0.128	0.310	-0.568	-0.430	0.193	-0.311	-0.291	1	
Cd	0.707	-0.247	-0.610	-0.875	-0.493	-0.413	-0.245	-0.501	-0.457	-0.115	1

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

cell permeability and Ca plays an important part in nerve-impulse transmission and in the mechanism of neuromuscular system, whereas, Mg is required for plasma and extracellular fluid and it helps in the maintenance of osmotic equilibrium. It is also a metabolic part of the enzyme and it is evident that when the lack of Mg is associated with abnormal irritability of muscle and convulsions and excess of Mg causes depression of central nervous system.

The role of micronutrients varies, Fe is found in all the samples and it is also an important element in the human body and plays a key role in oxygen and electron transport. Cu involves in Fe metabolism and its deficiency results in fragile bone cortices and spontaneous rupture of major vessels (Obiajunwa *et al*., 2002). Mills (1981) said that Cu is a component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin, an iron-oxidizing enzyme in blood. Zn is a component of many metalloenzymes, including some enzymes which play a central role in nucleic acid metabolism, Zn acts as a membrane stabilizer and stimulator of the immune response (Hambidge, 1978). Wang *et al*. (1996) and Calabrese (1981) said that high levels of essential elements such as Fe, Zn, Ca and Mn influence the retention of toxic elements in animals or human beings. Broyer *et al*. (1972) reported 2-6 mg/l of Pb in some plants, but Javid Hussain *et al*. (2009) reported very less amount of Pb in some plant species of Pakistan.

Conclusion: The proximate components, nutritive values, macro, micro and toxic elements of the leaves of different ages of the two regions of Karnataka, India have been determined. The results revealed that young leaves are more nutritive than mature leaves. The macro, micro and toxic elemental content also varied not only with respect to the regions of the plants where they grow, but also with their ages of the leaves. However, the leaf samples recorded low concentration of elemental components which is confirmed by recording low values of ash content. Though, all the leaf samples contained toxic elements, their values were in lowest concentrations. The study play an important role as the proximates and elemental composition influences the quality and the efficacy of the herbal products.

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Evaluation of Production and Economic Feasibility of Using the Recommended Nutrient Requirement for Lactating Cows

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Abstract: An experiment was carried out to evaluate production and economic feasibility of using the recommended nutrient requirements for lactating cows in small scale dairy farms in Khartoum state. The study consisted of two parts; a survey of 90 dairy farms in Khartoum State and an experiment carried out on 16 lactating dairy cows in the Judiciary farm. These cows were divided into two groups: A and B (8 cows in each group). Group A was distributed in eight pens and given balanced ration, whereas group B was fed as practiced in the farm. The experiment lasted for seven months extending from January to July 2007. The survey results revealed that the balanced ration costs were only 23, 38 and 55% of unbalanced ration at 10, 15 and 20 lbs level of milk production respectively. The results of the experiment revealed that the use of balanced ration increased the cow milk production by 27% compared with the unbalanced ration. The four months milk yield of cows fed balanced and unbalanced ration were significantly different ($P<0.05$). The mean cost of four months milk production for cows fed balanced ration was significantly ($P<0.05$) lower than these fed unbalanced ration, being 435 and 578 SDG, respectively. The mean cost of production per pound of milk for cows fed balanced ration was significantly ($P<0.05$) lower than the cows fed unbalanced ration; it was 0.107 and 0.206 SDG, respectively. The balanced ration has a greater trend coefficient of 32.36 compared with 18.56 for the unbalanced ration. In conclusion, the balanced ration increased the level of cow milk production and decreased the cost milk production compared to unbalanced ration.

Key words: Balanced ration, lactating cow, economic feasibility

INTRODUCTION

The dairy cow has special requirements which her diet has to provide. Dairy cows require feed nutrients for maintenance, growth, lactation and reproduction (Orskov, 1998). The dairy cow feeding program affects productivity and profitability more than any other single factor. The effects of good breeding and management program can not be fully realized without good feeding program. Good management of cows with good genetic potential will result in the most efficient response to good nutrition (Krober *et al*., 1999; Niels *et al*., 2003).

Some of the livestock breeders are proved to be accustomed to feed their herds with unbalanced rations which are negatively affected the well being of animals (Shing Field *et al*., 1999) and increased the price of animal products and so adversely affect the revenues and profit gained by the animal owners (Ferquson and Chalupa, 1989; Fadel Elseed *et al*., 2008). Hence the present work was conducted to study the effects of feeding balanced to dairy on milk production and it's economic feasibility.

MATERIALS AND METHODS

Data collection: Data collection was made through the following methods:

- Field investigations (site visit) via structured questionnaire
- Experiment

Field investigation: The study covered 90 milk farms which all located within Khartoum state. The process of data collection continued for three months. Data were collected from Khartoum (namely Jabra , Salama, Jereif west and soba hilla) and from Khartoum North locality (namely shambat, Hillat koko and halfaya) and from Omdurman locality (Fettemat village and Jebel Toriyya).

Experiment: The experiment was carried out in Judiciary farm, in which the herd is housed in barns constructed by Iron bars and Zinc roofs, with soil floor, the water basins were made of Cement and Iron containers, and water is available *adlibitum*. There were barns for calves, elders, dry cows, lactating cows, isolating barns for isolation of sick animals and newly purchased

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animals. Both natural and artificial in seminarian were practiced; the cows almost mated two months after calving, while heifers were mated when they reach puberty. Also there was vaccination against brucellosis and other infectious diseases and medication against external and internal parasites, with routine cleaning and spraying of all premises of the farm by antiseptics. The experiment lasted for four months extending from April to July 2007.

The experiment consisted of one feeding trial in 16 mature cross bred cows with similar average weight, age and production level, which divided to two equal (eight cows for each ration) groups A and B. Group A was given the balanced ration of concentrate according to their nutrient requirements (NRC, 2001), twice daily after milking in morning and evening while group B was given a fixed amount of concentrate irrespective to their nutrient requirement (8 kg per day) twice daily. Ration A composed of 50.9% sorghum grains, 12.6% groundnut cake, 31.8% wheat bran, 1.8% sodium chloride and 2.9% oyster shell, while ration B composed of 24.9% sorghum grain, 28.6% groundnut cake, 23.8% wheat bran, 1.8% sodium chloride and 2.9% oyster shell. Chemical composition of both rations (Table 1) was analyzed according to AOAC (1990). Experimental ration was formulated, based on the locally available concentrate feeding stuffs to contain varying levels of energy and crude protein. In addition roughages (Abu 70, berseem and Bagasse) were also given to these animals. The effect of the feeding of the two rations on Milk yield of cows and economics feasibility of feeding was observed.

Table 1: Chemical composition of ration A and B

Chemical component	Ration (A) %	Ration (B) %
DM	94.91	94.91
Fat	5.09	5.09
CP	18	24.89
CF	20.65	20.65
Ash	12.95	12.95
NFE	36.43	36.43
ME	11.35	10.7

Statistical analysis: The collected data was subjected to statistical T test and standard statistical economic analysis. All analysis was done by the computer program (SPSS, 1998).

RESULTS AND DISCUSSION

Field survey: Table 2 and Fig. 1 shows the percentage of the cost of balanced rations to the cost of unbalanced ones at different levels of milk production. The balanced ration costs are only 23, 38 and 55% of unbalanced ration at 10, 15 and 20 lbs of milk level of productivity respectively. On average, the costs of balanced rations are only 38% at the different levels of milk production.

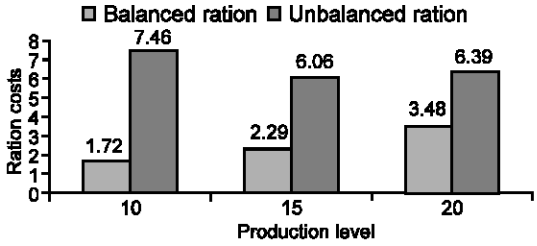

Fig. 1: Ration costs at different level of milk production (Cow/day in SDG)

Table 2: Costs of the balanced and unbalance rations (Cow/day in SDG) in Khartoum State

Productivity level Lbs of milk	Costs of balanced ration (1)	Costs of unbalanced ration (2)	(1) as % of (2)
10	1.716	7.46	23
15	2.288	6.06	38
20	3.484	6.39	55

Source: Field Survey 2007

Table 3: Comparison between four month milk yield of the two experimental groups

Groups	Mean milk yield/Lb	SD
A	3987.41[a]	±348.15
B	3145.12[b]	±536.82

These results agreed favorably with Muller and Fales (1998) and Fadel Elseed *et al*. (2008) who reported that some of the livestock breeders accustomed to feed their herds with unbalanced ration which are known to increase the cost of production due to inefficient use of diet content.

On farm trial: The data in Table 3 illustrated that balanced ration significantly (P>0.05) increased the level of cow milk production by 27% compared to that of unbalanced ration. The recorded values for cows fed balanced and unbalanced ration for four month milk yield were 3987.41±348.15lb and 3145.12±536.82lb, respectively. Similar results were recorded by Ferquson and Chalupa (1989), Krober *et al*. (1999) and Niels *et al*. (2003), who reported that feeding excess protein to requirement reduce energy availability and affect cow productivity and this may be attributed to that excess N will be emeses in the environment.

The data in Table 4 dedicated that the mean cost of four months milk production for cows fed balanced ration was significantly (P>0.05) lower than that of cows fed unbalanced ration. The recorded values for the cost of milk production for cows fed balanced ration were 435.00±83.41 and 578.43±37.49 SDG, these results agreed favorably with Muller and Fales (1998) who reported that total mix ration system is more profitable over most changes in feed cost or milk revenues. The data in Table 5 illustrated that the mean cost of production per pound of milk for cows fed balanced

Table 4: Comparison between the cost/of four months milk production of the two experimental groups

Groups	Mean cost/SP	SD
A	435.00[a]	±83.41
B	578.435[b]	±37.49

Table 5: Comparison between the cost/Lb for the two experimental groups

Groups	Mean cost/SP	SD
A	0.107[a]	±0.0
B	0.206[b]	±0.08

*Mean in the same column carrying similar superscripts are not significantly different at (P<0.05) level

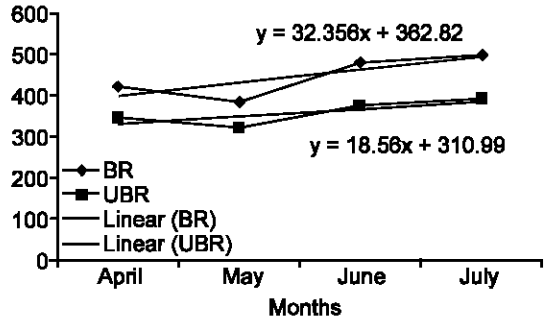

Fig. 2: Milk production trend for balanced and unbalanced rations

ration was significantly (P>0.05) lower than that of cows fed unbalanced ration it almost half of it, the recorded values for the cost per pound of milk for cows fed balanced and unbalanced rations were 0.107±0.00 and 0.206±0.08 SD.

Figure 2 show that both rations showed a ppositive trend of milk production. How ever the balanced ration has a greater trend coefficient of (32.36) compared to (18.56) for the unbalanced ration this result illustrated that balanced ration significantly increase the milk production with advancement of the time and this will ultimately lead to lower price for consumer and higher benefit for producer.

Conclusion and recommendations: The balanced ration increased the level of cow milk production and decreased the cost milk production when compared with unbalanced ration. So it is highly recommended to use balanced ration in feeding of dairy cows.

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Effect of Environmental Changes on Phytic Acid Content of Wheat (*Triticum aestivum*)

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Abstract: Wheat (*Triticum aestivum*) is one of the most important food grain crops in all South Asian countries especially in India and Pakistan. These countries have diversifying soil and climatic conditions inserting measurable effect on nutritional as well as anti-nutritional parameters of wheat. Wheat varieties included in this study are collected from different agro-ecological zones of Pakistan. Myoinositol hexa-phosphate (phytic acid) one of major anti-nutritional factors wheat. Phytic acid of collected samples was determined to facilitate the crop breeders and agronomists, so that they would also consider this factor while conducting research works. It was observed that wheat varieties showed different levels of phytic acid at different locations. At one location (Islamabad), a variety (Pari-73) showed the highest value of Phytic acid (1.343%) and at other location (Faisalabad), same variety the showed lowest phytic acid (0.74%). This maximum variability (44%) also indicated that there was significant effect of change in location on phytic acid contents of wheat varieties. It is mainly due to presence of available phosphorus reserves in soil as phytate has direct relation to soil phosphorus.

Key words: *Triticum aestivum*, wheat varieties, Myoinositol hexa-phosphate phytic acid, environmental changes

INTRODUCTION

Wheat may be the first crop to be domesticated and acquired by most number of people as a foodstuff of life. It occupies a unique position in human diet since ancient times and is consumed in various forms in all part of the world (Anjum *et al*., 1998). Wheat is Asian most important cereal and staple food and demand has been growing much faster than for the rice. Wheat grains contain carbohydrates (60-80%) mainly as starch, protein (8-15%), which contain adequate amount of all essential amino acids except lysine, tryptophane and methionine, fat (1.5-2%) and vitamin B complex and vitamin E (Simmonds, 1976).

Even though its importance in the human nutrition is un doubtable, it also possesses anti-physiological factors such as phytic acid (Reddy *et al*., 1982) and polyphenols (Bressani and Elias, 1980) that tends to impair its nutritional quality. Phytic acid is known to interfere with the bioavailability of minerals in the diet and interacts with proteins (Bassirz and Nahapetian, 1976) and inhibits several proteolytic enzymes and amylases (Singh and Krikorian 1982; Sharma *et al*., 1978).

Phytate concentration in typical wholegrain cereal and oilseeds ranges from 1% (dry basses) for corn rice and wheat to 5% for defatted sesame meal (Erdman and Forbes, 1977).

Presence of phytic acid is considered detrimental to the nutritional quality of grain. It has been observed that variation in the environmental and organic factors has a greater influence on the concentration of various nutrients (Bassirz and Nahapetian, 1976; Sattar *et al*., 1985). Phytic acid and total phosphorous has great relationship with each other (Lolas *et al*., 1976) Similarly effect of varietal and environmental changes has great influence on the phytic acid content durum wheat's and their milled products (Tabekhia and Donnelly, 1982).

Phytic acid is chemically myo-inositol hexaphos-phate) having molecular formula of phytic acid is $C_6 H_{18} O_{24} P_6$ (MW. 660.03). Inositol is chemically hexahydroxycylohexane i.e. stereoisomeric alcohols. When OH^- group in inositol is replace with six phosphate groups than inositol hexaphosphate is form, which is commonly named as myoinositol hexaphosphate for its presence in muscles tissues. When two hydrogen atoms from phosphate group in myoinositol hexaphosphate are replaced with Ca, Fe, Zn, Mg etc result in formation insoluble salts of phytates as shown in (Fig. 1) and the minerals are not available (Erdman and Forbes, 1977).

Environmental and varietal difference exerted great effect on phytate content, which is significantly correlated with total phosphorus concentration. Harvesting year tends to influence the total phosphorus in the soil and ultimately to phytate content of the wheat (Kim *et al*., 2002).

The varieties selected for present study to check the effect of environment and variety are constantly used in

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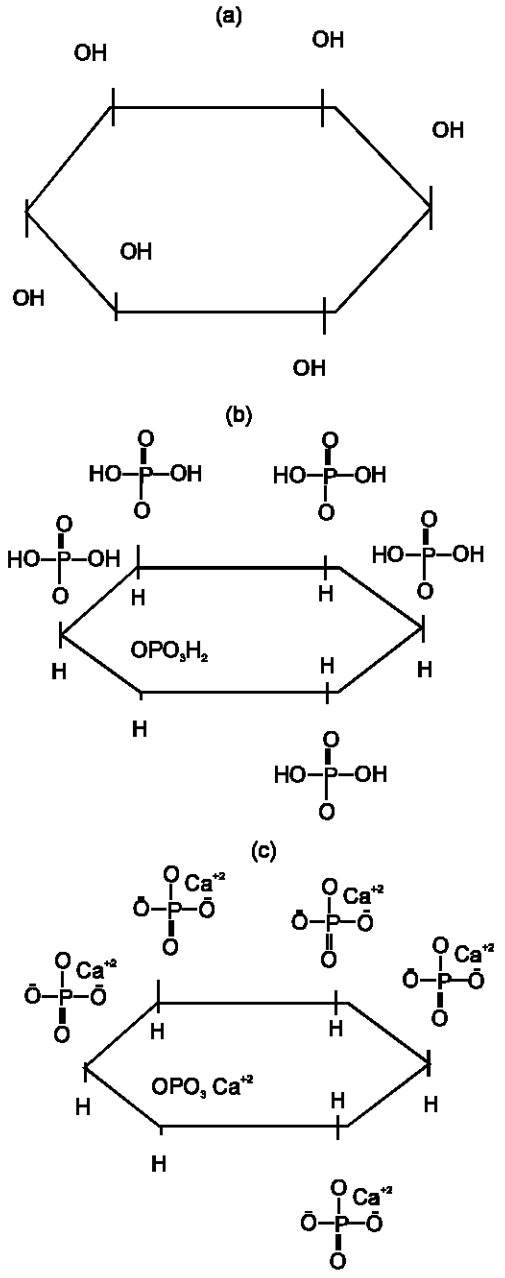

Fig. 1: Representative structure of (a) Myo-inositol (b) Myoinositol hexaphosphate (Phytic acid) (c) Calcium phytate

our crop breeding and improvement programs due to certain special features. These special features are short duration or early maturity (Sonalika, Blue silver), good bread/chapatti quality (Indus-79, Pak-81, Sindh-81, Lyp-73, Sandal, Lu-26), drought tolerance (Zamindar-80, Zarghoon, ZA-77), salt tolerance (Lu-26), high protein content (SA-75), etc.

In view of the nutritional significance of phytate, the present investigation was imitated to determine the effect of variety and environment on the phytic acid content of some wheat varieties grown in Pakistan.

MATERIALS AND METHODS

Wheat varieties used for crop quality improvement and breeding programs running at different parts of the country were collected from National Agricultural Research center, Islamabad, Ayub Agriculture Research institute, Faisalabad, Agriculture Research institute, Pirsolac, Nowshera (NWFP), Arid zone Research Institute, Quetta (Balochistan) and Tandojam University Sindh. Collected samples were stored at 10°C in polyethylene bags. Samples were grounded by using Cyclotec Mill (Cyclotec 1093, Tecator Sweden) according to the AACC (2000) method No 64-70A.

Phytic acid determination: Phytic acid was analyzed according to Haug and Lantzech (1983) method. According to this method an appropriate amount (0.8-1.0 gram) of sample was extracted with 0.2N HCl by taking 25 ml of 0.2N HCl in conical flask and shake for 1 h on shaker at 30°C and 80 revolutions per minute. 0.5 ml extract was taken into test tube fitted with a ground glass stopper. 1 ml of acidic ammonium iron-III sulphate solution of known iron content was added and the tubes were covered with a stopper and fixed with a clip. Tubes were heated in a boiling water bath for 30 min after cooling in ice water for 15 min; tubes were allowed to reach at room temperature. Contents of the tube were mixed and centrifuged for 30 min at 3000 revolutions per minute. 1 ml of the supernatant was transferred to another test tube and 1.5 ml of 2,2-bipyridine solutions was added, light pinkish color appeared. The absorbance was measured at 519 nm against distilled water. Decrease in iron, in the supernatant was measure for phytic acid contents. Standards of known phytic acid concentration were prepared and absorbance was measured at 519 nm. Standard curve was made taking phytic acid concentration in micrograms on X-axis and Optical Density (O.D) on Y-axis (Fig. 2). Phytic acid was calculated by using following formula:

$$\frac{\text{Micro gram from graph x dilution factor x 0.354 x 100}}{\text{Wt of sample x ml sample taken x 1000000}}$$

Statistical analysis: Data obtained was analyzed statistically by using complete randomized design with two factors factorial (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Phytic acid contents of 20 wheat varieties were compared for analyzing effect of environmental and varietal changes. Wheat in Pakistan is grown in planes of the Indus basin (Punjab and Sindh); coastal belt (Sindh and Balochistan); upland mountains (North-West

Table 1: Phytic acid contents of wheat varieties used in breeding program at NARC (Islamabad) and AARI (Faisalabad)

		Islamabad (NARC)		Faisalabad (AARI)		
S. No	Varieties	Phytic acid (%)	Standard Deviation	Phytic acid (%)	Standard Deviation	Variation (%)
1	Blue Silver	1.113^{c}	±0.002	0.89^{ghi}	±0.001	21
2	Pari-73	1.343^{a}	±0.036	0.74^{k}	±0.015	44
3	BWP-79	1.043^{ef}	±0.010	1.10^{cdf}	±0.001	5.1
4	Sandal	1.043^{ef}	±0.001	0.85^{ij}	±0.002	17
5	Aari-83	0.933^{g}	±0.017	0.83^{j}	±0.002	10
6	Lu-26	0.89^{ghi}	±0.002	1.053^{def}	±0.035	15
7	Indus-79	0.92^{g}	±0.035	1.0 03^{f}	±0.020	08
8	Punjab-81	0.86^{hij}	±0.012	1.210^{b}	±0.003	28
9	LYP-73	0.91^{gh}	±0.025	1.00 ^{f}	±0.004	09
10	Pak-81	1.103^{cd}	±0.002	1.100^{cdf}	±0.035	0.2
Variability (%) among varieties:			36 (%)		39 (%)	

*Values are mean±SD of three replications. *Means followed by same alphabets are non significant from one another for p = 5

Table 2: Phytic acid contents of wheat varieties used in breeding programs at NARC and Quetta

		Islamabad (NARC)		Quetta (Balochistan)		
S. No	Varieties	Phytic acid (%)	Standard Deviation	Phytic acid (%)	Standard Deviation	Variation (%)
1	Sonalika	0.80^{e}	±0.002	1.12^{c}	±0.001	28
2	Zarghoon	0.93^{d}	±0.015	1.10^{c}	±0.002	18
3	Pavon	0.90^{d}	±0.012	1.42^{a}	±0.025	36
4	Zamindar-80	0.86^{de}	±0.030	1.21^{b}	±0.015	28
Variability (%) among varieties:			14 (%)		22.50 (%)	

*Values are mean±SD of three replications. *Means followed by same alphabets are non significant from one another for p = 5

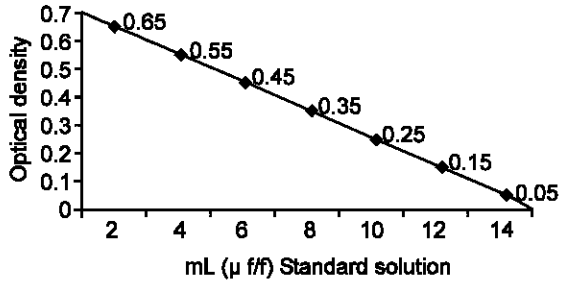

Fig. 2: Standard curve for phytic acid determination

Frontier Province (NWFP) and Balochistan) and northern areas. Wheat quality is greatly effect by these agro-ecological zones.

Phytic acid contents of wheat varieties used in breeding program at NARC (Islamabad) and AARI (Faisalabad): Comparison of overall range and mean values of phytic acid contents of wheat varieties grown at NARC and AARI was presented in Table 1. Phytic acid is between the range of 1.343-0.86% for Pari-73 and Punjab-81 respectively at NARC, Islamabad. Where as it is 1.21- 0.74% for Punjab-81 and Pari-73 respectively. Results also revealed that at one location (Islamabad), a variety (Pari-73) showing the highest value of phytic acid (1.343%) and at other location (Faisalabad), same variety showing the lowest phytic acid (0.74%). This maximum variability (44%) also indicated that there was significant effect of change in location on phytic acid contents of wheat varieties. It is mainly due to presence of available phosphorus reserves in soil as phytate has direct relation to soil phosphorus (Asada *et al*., 1969). Results also support the finding of kim *et al*. (2002) who described that Phytate contents was significantly correlated with total phosphorous concentration (r = 0.97, p<0.001). Harvest year also tends to influence the total phosphorous and phytate contents (p = 0.079 and p = 0.082), respectively.

Phytic acid contents of wheat varieties used for breeding programs at NARC (Islamabad) and Quetta (Balochistan): The results of the four varieties grown at two locations NARC (Islamabad) and Quetta (Balochistan) are shown in Table 2. The range of phytic acid was between 0.8-0.93% for Islamabad and 1.1-1.42% for Zargoon and Pavan at Quetta. Maximum variability was observed in Pavon (36%) having 0.90% phytic acid at NARC (Islamabad) and same variety showed highest levels of phytic acid (1.42%) at Quetta indicating location difference had significant effect on phytic acid contents. That was mainly because of change in soil conditions. Results are in line with (Bassirz and Nahapetian, 1976; Sattar *et al*., 1985). They have mention that variation in the environmental and organic factors has a greater influence on the concentration of various nutrients. Phytic acid and total phosphorous has great relationship with each other (Lolas *et al*., 1976).

Table 3: Phytic acid contents of wheat varieties used in breeding programs at NARC, Quetta and Pirsabak (NWFP)

		Islamabad (NARC)		Quetta (Balochistan)		Pirsabak (NWFP)		
S. No	Varieties	Phytic acid (%)	Standard Deviation	Phytic acid (%)	Standard Deviation	Phytic acid (%)	Standard Deviation	Variability
1	Sarhad-82	0.95^{d}	±0.002	0.99^{c}	±0.012	0.78^{k}	± 0.010	21 (%)
2	LYP-73	1.00^{cd}	±0.001	1.1b^{c}	±0.024	1.11^{bc}	± 0.014	10 (%)
3	Pak-81	1.00^{cd}	±0.025	1.21^{b}	±0.014	1.27^{b}	± 0.015	21 (%)
Variability (%) among varieties:				2.93 (%)		8.94 (%)		23.72 (%)

*Values are mean±SD of three replications. *Means followed by same alphabets are non significant from one another for p = 5

Table 4: Phytic acid contents of wheat varieties used in breeding programs at NARC and Tandojam

		Islamabad (NARC)		Tandojam (Sindh)		
S. No	Varieties	Phytic acid (%)	SD	Phytic acid (%)	SD	Variability (%)
1	Blue Silver	1.113^{c}	±0.001	1.22	±0.003	7%
2	Pavon	0.90^{d}	±0.001	0.87	±0.020	3%
3	Tj-83	0.99^{c}	±0.025	0.82	±0.001	17%
4	Za-77	0.86^{de}	±0.015	0.91	±0.010	5.5%
Variability (%) among varieties:			22 (%)		25 (%)	

*Values are mean±SD of three replications. *Means followed by same alphabets are non significant from one another for p = 5

Phytic acid contents of wheat varieties used in breeding programs at NARC, Quetta and Pirsabak: Phytic acid contents of three wheat varieties grown at three locations are listed in Table 3. Phytic acid ranged from 0.95-1% at NARC (Islamabad), 0.99-1.21% at Quetta (Balochistan) and 0.78-1.27% at Pirsabak (NWFP). Maximum variability was observed in varieties grown at Pirsabak (23.72%) followed by Quetta (Balochistan) (8.94%) and Islamabad (2.93%). Statistical analysis showed that neither variety nor location had a significant effect on phytic acid contents of the whole wheat but location mean showed that phytic acid level were slightly higher at Quetta than at Pirsabak and both had higher levels than Islamabad.

Phytic acid contents of wheat varieties used in breeding programs at Islamabad (NARC) and Tandojam: The results of the four varieties grown at two locations NARC Islamabad and Tandojam (Sindh) are shown in Table 4. The range of phytic acid was between 0.86-1.113% for Islamabad and 0.91-1.22% for Tandojam (Sindh) Maximum variability (25%) for varieties grown at Tandojam (Sindh) was observed while for Islamabad the variability was (22%). Blue Silver had highest phytic acid content (1.22%) from Tandojam (Sindh) and lowered values observed for TJ-83 (0.82%). Results showed that varietal difference had non-significant effect on phytic acid content but location had a significant effect on phytic acid levels. That was mainly because of change in soil conditions.

Conclusion: Environmental and varietal difference exerted great effect on phytate content, which is significantly correlated with total phosphorus concentration. Harvesting year tends to influence the total phosphorus in the soil and ultimately to phytate content of the wheat The varieties selected constantly used in our crop breeding and improvement programs due to certain special features. These special features are short duration or early maturity (Sonalika, Blue silver), good bread/chapatti quality (Indus-79, Pak-81, Sindh-81, Lyp-73, Sandal, Lu-26), drought tolerance (Zamindar-80, Zarghoon, ZA-77), salt tolerance (Lu-26), high protein content (SA-75), etc.

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Innovative Approach of Active Packaging in Cardboard Carton and its Effect on Overall Quality Attributes Such as Weight Loss, Total Soluble Solids, pH, Acidity and Ascorbic Acid Contents of Chaunsa White Variety of Mango at Ambient Temperature During Storage

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Abstract: Significant effect ($p<0.05$) of Innovative approach of Active packaging in Cardboard Carton (APCC) on overall quality attributes such as weight loss, Total Soluble Solids (TSS), pH, Titrate-able Acidity (TA) and Ascorbic Acid (AA) contents of Chaunsa white variety of Mango was investigated at ambient temperature (28-33°C and 56.7-69.7% relative humidity) during storage. It was observed that the uncoated fruit packed in carton had comparatively higher percent weight loss (10.96%) than control (9.39%); however, after application of APCC system same packaging had significantly reduced the percent weight loss up to (6.89%). It was also noted that mango fruit undergone through APCC system showed a slower increase of TSS (16.44-20.76%), pH (3.98-4.83), slower decrease in TA (0.51-0.92%), or slower increased of AA (23.06-40.83 mg/100 g) during ripening with an average mean of 8.10%, 17.73%, 4.28, 0.75%, 25.47 mg/100 g respectively at later stage of storage as compared to control sample (T_1) had higher weight loss (9.39%), TSS (20.83%), highest pH value (4.91), lowest acidity (0.44%), highest AA (42.06 mg/100 g), respectively at much earlier during storage. It is clear from these studies that Innovative approach of APCC with other protective chemicals such as coating emulsions having fungicide, ethylene absorbent and anti-ripening agent had extended storage life up to 25 days and played a very effective and vital role to control compositional changes by delaying the ripening process and with a minimum quality loss, as compared to control sample had greater compositional changes with maximum quality loss during storage at ambient temperature. Due to unattractive skin, brown pulp color and poor taste the control fruit was unacceptable within two weeks of their storage.

Key words: Fruit, Chaunsa mango, active packaging, ascorbic acid, organoleptic, physico-chemical composition, quality characteristics

INTRODUCTION

Active Packaging (AP) is a modern development consisting of a group of techniques in which the package is self-motivated and is actively involved with food products or act together with internal atmosphere to extend the shelf life while maintaining quality and safety. Active packaging is some time referred to as interactive or smart packaging which is planned to sense internal or external environmental changes and to take action by changing its own properties or attributes. Potential techniques used in active packaging are the use of oxygen scavenging/carbon dioxide, ethylene and moisture absorbing systems by placing sachets, incorporation of antimicrobial agents into polymer surface coatings or in plastics films, sheets or on materials and into the pads for fresh produce (John, 2008). Atmosphere in packaging is actively established by replacing the packaging atmosphere with desirable atmosphere through the use of absorbing substances in the package to scavenge O_2, CO_2, H_2O and C_2H_4 and for this purpose $CaCl_2$, sorbitole and Xylitol in package is used to absorb H_2O, molecular sieves and hydrated lime is used to absorb CO_2 while $KMnO_4$ is used to scavenge the ethylene to adjust and maintain the proper atmosphere (Kumar *et al*., 2009). The ripening process of mango fruit involves a series of biochemical reactions or metabolic activities that cause chemical changes, increased respiration, ethylene production, change in structural polysaccharides causing softening, degradation of chlorophyll develops pigments by carotenoids biosynthesis, changes in carbohydrates or starch conversion into sugars, organic acids, lipids, phenolics, volatile compounds, etc thus leading to ripening of fruit with softening of texture to acceptable quality (Herianus *et al*., 2003). Mango is packaged from very simple baskets of bamboo, pigeon pea (*Cajanus*) or mulberry with paddy straw as cushioning material because of their easy availability and low cost. This type of packaging was found to be unsatisfactory because of uneven ripening of fruit, excessive shrinkage, bruising

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and stacking was also a problem with the use of baskets. However, ventilated Low-Density Polyethylene (LDPE) linings have also being found to be beneficial, as this material maintains humidity, which results in less shrinkage during storage (Tharanathan *et al*., 2006). The recent studies showed that coated fruit having other protective chemicals when packaged in polyethylene had played a very effective role to control compositional changes by delaying the ripening process and with a minimum quality loss during 30 days of storage at ambient temperature, as compared to the control sample that had greater compositional changes with maximum quality loss during storage at ambient temperature (Rathore *et al*., 2009). Polyethylene wrapping of $CaCl_2$ treated apple proved very useful for reducing weight loss and shriveling and retained consumer acceptability even after 60 days of storage (Hayat *et al*., 2005). In Israel MAP technique of polyethylene perforated and non-perforated sealed packaged coupled with low temperature at 14°C for 3 weeks and then at 20°C for 4 days when applied to Tommy Atkins during storage showed no decay until opening in non-perforated pack, and then rotted rapidly. Data obtained on shelf life, weight loss, spoilage and retention of Vitamin C indicated that cool chamber was an ideal storage technique (Pal, 1998). The removal of ethylene with ethysord adsorbent, from CFB carton packaged alphonso mango had extended life up to 16 days as compared to its 8 days normal life and controlled black spots completely by washing with 0.01% $KMnO_4$ (Raje *et al*., 1997). In Israel the A. *alternata*, *Phomopsis* sp. or *Lasiodiplodia* sp. attacking at the stem-end of the fruit were controlled recently on commercial level by a combined hot water (55°C) spray with 225 ug/ml prochloraz and fruit brushing, followed by 900 ug/ml prochloraz sprays before waxing for 15-20 sec. The normal life of Baneshan mango is 4 days at ambient temperature, was extended from 6-9 days by low temperature and further extended 8, 12 and 23 days at room temperature, cool chamber and cold storage when treated with bavistin fungicide, parafin wax and wrapping with HM-film that provide modified atmosphere coupled with low temperature (Narayana *et al*., 1996). The previous studies revealed that different techniques have been applied for improvement or maintenance of the quality of fruit during storage. However the innovative approach of Active Packaging in Cardboard Carton (APCC) and its effect on overall quality attributes such as weight loss, total soluble solids, pH, acidity and ascorbic acid of Chaunsa white variety of mango at ambient temperature during storage is not available in the literature. Therefore the present study was designed for the determination of the effect of innovative approach of APCC on the quality parameters such as weight loss, TSS, pH, Titrate-able Acidity and Ascorbic Acid of chaunca white during storage.

MATERIALS AND METHODS

Collection of sample: For present research studies Chaunsa white very important commercial varieties of mango was selected and for this purpose unripened, matured, hard green and uniform size of fresh arrival fruit were purchased from wholesale fruit market in Islamabad.

Hot water treatment: Chaunsa white variety of mango were immediately transferred from wholesale market to post harvest laboratory of Department of Food Technology in University of Arid Agriculture Rawalpindi and after careful sorting, fruits in cotton bags were subjected to hot water treatment at 53°C for three minutes and immediately cooled by dipping in cold water at 20°C and were dried in air. Coatings were prepared according to the concentrations as described by Rathore *et al*. (2009).

Grading, coating, packaging and storage: Chaunsa white late commercial variety of mango fruit was graded according to their size and total 180 selected fruit were divided into 6 groups having 30 mangoes in each group respectively. These groups were under gone into following 6 treatments viz; Control (T_1), Carton (T_2), Wax-CMC having NaOCl coated fruit packed in Carton (T_3), Wax-CMC Coated fruit with $KMnO_4$ package in Carton (T_4), Wax-CMC Coating having 2,4,5-T in Carton (T_5), H_3BO_3 and 2,4,5-T having oil treated fruit packed in Carton (T_6) and then were stored at ambient temperature (28-33°C and 56.7-69.7% relative humidity) for a storage period of 30 days.

Physico-chemical and sensory evaluations: Physico-Chemical parameters such as weight loss, total soluble solids, pH by using HANAS pH meter no. 210, titratable acidity according to standard procedures as mentioned in AOAC (1990). Ascorbic acid was determined by standard method as described by Awan and Rehman (1999). The data obtained were statistically analyzed for Analysis of Variance (ANOVA) by using 2-Factorial Complete Randomized Design (CRD) and Duncan's Multiple Range Test (DMRT) was applied to compare the mean values obtained according to the method described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

It was investigated that Chaunsa white variety of mango under gone through innovative approach of active packaging in cardboard carton had affected the overall quality attributes such as weight loss, total soluble solids, ph, acidity and ascorbic acid contents of chaunsa white variety of mango at ambient temperature during storage.

Weight loss: Table 1 illustrates that statistically, treatments and their interactions had significant difference on percent weight loss during 30 days of their storage. The uncoated fruit packaged in Carton T_2 (10.96%) had comparatively more weight loss than control T_1 (9.39 %) might be due to decreased in the relative humidity or more temperature in side the pack and comparatively dried atmosphere created by lower percent relative humidity increased loss, however this loss was much reduced to 8.10% when coated fruit was packaged in same packaging after 30 days of storage as compared to first day of their storage having 0.00% weight loss. The present investigations showed that Chaunsa white mango coated with Wax-CMC with $KMnO_4$ package in carton (T_4) had minimum weight loss (6.89%), followed by 2,4,5-T and H_3BO_3 having oil treated fruit packaged in carton T_6 (8.01%), Wax-CMC having Sodium Hypochlorite packaged in Carton T_3 (8.37%), Wax-CMC having 2,4,5-T coated fruit packaged in carton T_5 (9.15%) as compared to controlled T_1 (9.39%) had more weight loss whereas, Carton T_2 (10.96%) had the maximum weight loss at ambient temperature during 18 days of storage (Table 1). The effectiveness of carton packaging with the combination of coating, ethylene absorbent used, fungicide or antiripening agent to minimize the loss was varied, may be due to the difference in created Modified Atmosphere in Packaging (MAP), CO_2 levels and decreased O_2 levels which reduce respiration rates, maintained mango quality, slowed down the transpiration as result prevented water loss and decay was effectively controlled by fungicide. These results are generally in agreement with those reported by Ladaniya and Sonkar (1997). It is evident from our studies that combination of wax coating with $KMnO_4$ had reduced the weight loss, might be due to creation of modified atmosphere by removing ethylene with ethylene absorbent and decreased rate of respiration by degrading ethylene produced by the fruits into carbon dioxide and water. These results are agreed with the findings of Yuniarti and Suhardi (1992) they used calcium chloride, wax emulsion, wrapping mangoes in perforated polyethylene bags containing $KMnO_4$ and reported that lower weight loss was observed in case of treated mangoes as compare to control. The increasing trend of weight loss in treated fruit is also reported by other scientists (Carrillo *et al*., 2000; Chitarra *et al*., 2001; Hayat *et al*., 2005). Therefore, it is understandable that Chaunsa white after coating having ethylene absorbent or antiripening agent or antiripening agent with oil and disinfectant packaged in carton played a vital role in delaying of ripening with minimum percent weight loss during storage.

Total soluble solids (TSS): It is obvious from Table 1 that treatments and their interactions had highly significant effect on percent total soluble solids contents of mango except T_2 and T_3 (20.76%, 19.18%) with insignificant effect, however a significant difference in total soluble solids of these treatments to others were found during 18 days of their storage. The TSS of coated fruit packaged in carton ranged from 16.44-20.76% with an average means of 17.73% as compared to first day of their storage having minimum percent of total soluble solids (10.0%). The increasing trend of the percent total soluble solids contents of fruit during storage that could be attributed mainly due to breakdown of starch into simple sugars during ripening along with a proportional increase in TSS and further hydrolysis decreased the TSS during storage. The maximum percent total soluble solids contents of late Chaunsa white mango were observed in T_2 (20.76%), followed by T_3 (19.18%), T_4 (18.54%), T_6 (16.78 %) and T_5 (16.44%) however, all of the treatments showed a slower increasing rates or the more retaining trend in percent total soluble solids contents for longer period as compared to control T_1 (20.83%). The effectiveness to minimize or the retained the percent total soluble solids contents was varied may be due the difference in the modified atmosphere created by coating-carton packaging, having ethylene absorbent, fungicide or antiripening agent used had slowed down the metabolic activities and delay ripening with reduced TSS contents during storage (Table 1). These results are in line with earlier findings (Kittur *et al*., 2001; Raje *et al*., 1997; Rosa *et al*., 2001; Ladaniya and Sonkar, 1997). Therefore, coating-carton combination could be more effective in delaying ripening of mango by slower increase of TSS contents during storage.

pH value: The Table 1 reveals that the pH value of coated fruit packaged in carton was 3.98-4.83 with an average means of 4.28 as compared to control having higher pH (4.91) after 18 days of storage at ambient temperature. The treatments and their interactions had highly significant effect on pH value of mango during storage except T_5 and T_6 (4.03 3.98) with insignificant effect, however a significant difference of pH value of these treatments to others were found during 18 days of their storage. The fluctuations of pH might be due to the variations in titratable acidity or temperature of storage and decline of acidity is attributed due to increased activity of citric acid glyoxylase during ripening or reduction in acid content may be due to their convertion into sugars and further utilization in metabolic process during storage. These results are correspond with Srinivasa *et al*. (2002) who described that pH values of Alphonso mango had an increasing trend from 4.06-6.73 on 12th day in control fruit at ambient temperature 27±1^{o}C at 65% RH. Doreyappy-Gowda and Huddar (2001) also observed that Green mature Alphaso and other 7 varieties of mango fruit stored at 18-34^{o}C under gone a series of physico-chemical changes during

Table 1: Effect of coating having fungicide, ethylene absorbent and antiripening agent packaged in cardboard carton on the physico-chemical composition of chaunsa white variety during storage

	Treatments						
Parameters	T_1	T_2	T_3	T_4	T_5	T_6	Overall effect of Carton-coat
Weight loss (%)	9.39b	10.96a	8.37d	6.89f	9.15c	8.01e	8.10
Total soluble solids (%)	20.83a	20.76b	19.18b	18.54c	16.44f	16.78e	17.73
pH	4.91a	4.83b	4.47d	4.66c	4.03fg	3.98g	4.28
Titratable acidity (%)	0.44l	0.51k	0.67i	0.61j	0.92bc	0.80gh	0.75
Ascorbic acid (mg/100 mg)	44.06a	40.83b	23.06i	26.11g	26.3f	26.41e	25.47

Mean values with different letters in same row are significantly different to each other at ($p<0.05$).

T_1 = Control T_2 = Carton T_3 = Coat + NaOCl + Carton

T_4 = Coat + $KMnO_4$ + Carton T_5 = Coat+2,4,5-T + Carton T_6 = Coat+2,4,5-T having oil + H_3BO_3-$CaCl_2$ + Carton

ripening and the major changes were considerably increased in pH from 2.85-4.38 during ripening. These results are comparable with those of Hayat *et al.* (2005) who reported that there was a gradual increased of pH from 4.22-4.78 in Banky apple during storage at ambient temperature. These results are not agreed with those of Manzano *et al.* (1997) who reported that pH value showed decreasing trend from 4.82-3.82 during 20 days of their storage, however there was an agreement with their 2nd part of statement that temperature of storage also affected pH value and lower pH value 4.21 at 12°C as compared to higher pH value 4.67 at 25°C was observed during 20 days of storage. These results are in line with the findings of Kudachikar *et al.* (2001) who described that the pH value of Neelum mango was decreased (3.0) and acidity increased (1.9%) upto 90 days after the fruit set. Later, pH slightly increased (3.1) and acidity slightly decreased (1.5%) at 110 days after fruit set which is optimum stage of maturity after the fruit set. The maximum pH value of coated late Chaunsa white mango were observed in T_2 (4.83), followed by T_4 (4.66), T_3 (4.47), T_5 (4.03) and T_6 (3.98) as compared to first day with very low pH value (3.85, however, these treatments having comparatively lower pH as compared to control with highest pH value T_1 (4.91), after 18 days of storage at ambient temperature. The control had significantly higher pH value than other treatments after 18 days of their storage might be due to free atmospheric conditions of temperature, O_2 and relative humidity that caused more oxidation and degradations of acids as compared to coated fruit. The treated fruit with CMC-Bee-wax polysaccharide based coating having NaOCl (T_3), $KMnO_4$ (T_4) or 2,4,5-T (T_5) packed in carton respectively had lower pH value might be due to the slower metabolic process of conversion of sugar or degradation of acids compared to other treatments that might be due to a difference in the modified atmosphere created by different types of coatings or might be due to formation of carboxylic acid by dark fixation of CO_2 or due to high internal CO_2 levels. The ripening process was more effectively controlled in those treatments having NaOCl fungicide as compared to H_3BO_3. Similar pattern was observed by Carrillo *et al.* (2000) who reported that Haden mangoes coated with different concentrations of Semperfresh had lower pH (4.75) as compared to noncoated fruit (5.66) at the end of storage at 13°C during 32 days of storage and coating was more effective in maintaining a lower pH during storage. These results are confirmed by (Manzano *et al.*, 1997) who reported that pH value of Hadden mango treated with waxes coating were depending on types of coating and had no significant effect on pH content in between Prolong and control (4.60, 4.54) or Fomesa and Primafresh (4.23, 4.25), however a significant difference in between three groups Fomesa and Prolong (4.23, 4.54) or Fomesa and control (4.23, 4.60) or Primafresh and control (4.25, 4.60) was noted during storage. These results are in line with those Baldwin *et al.* (1999) who observed that pH value depends on type of coating and showed significantly lower pH (4.6, 4.7) in Natural-Seal (NA) a polysaccharide-based edible coated fruit or Tropical Fruit Coating (TPC) respectively compared to control (5.5) at 10 or 15°C with 90-95% RH for 19 days, followed simulated marketing conditions at 20°C with 56% RH for 4 days. These results are comparable with Hayat *et al.* (2005) who reported that apple had higher pH (4.60) in control than paraffin wax coating (4.47) or polyethylene (4.42) during storage. This might be due to less oxidation of the fruits and calcium decrease in the degradation of acids thus maintaining the integrity of cells and polyethylene to delay the metabolic changes in fruits. Similarly polyethylene bags were sealed so air was not available for various chemical reactions resulting in less increase in PH. These investigations are similar to those (Srinivasa *et al.*, 2002) Who described that pH values of Alphonso mango was higher in control (4.06-6.73) as compared to chitosan (5.04) coating or LDPE film (5.79) treated with 500 ppm Carbendazim fungicide in carton boxes on 12th day at ambient temperature 27±1°C at 65% RH during ripening. The results of the present studies shows that Chaunsa white packaged in carton had higher pH value, however coating with anti-ripening agent (T_5) and anti-ripening agent with oil and disinfectant (T_6) respectively when packaged in carton had maintained lower pH value very near to first day pH value (3.85) shows that

these treatments might be controlled the ripening process due to that pH value was maintain at lower level.

Titratable acidity: It is obvious from Table 1 that all of the of treatments had a significant difference in overall percent of titratable acidity ranged from 0.51-0.92%, with an average means of 0.75% after 25 days of storage as compared to first day with very high percent acidity (1.28%). The maximum percent acidity of late Chaunsa white mango were observed in T_5 (0.92%), followed by T_6 (0.80%), T_3 (0.67%), T_4 (0.61%) and T_2 (0.51%) however, these treatments maintained comparatively higher percent acidity compared to control T_1 (0.44%) had very low percent acidity after 25 days of storage at ambient temperature or first day with very high percent acidity (1.28%) that might be due to higher CO_2 and lower levels of O_2 in the internal atmosphere, an aerobic respiration produced carbonic acid and as result increased in acidity. These results are in lined with those (Baldwin *et al*., 1999) who observed that Tomy Atkins mango treated with Natural Seal (NA) a polysaccharide-based edible coating had higher TA (0.28) than TFC (0.21) or uncoated (0.16) fruit at 10 or 15°C with 90-95% RH for 19 days, followed simulated marketing conditions at 20°C with 56% RH for 4 days. These results are confirmed by (Manzano *et al*., 1997) who reported that Hadden mango treated with waxes coating stored at different temperatures had no significant effect on titratable acidity percent in between Prolong and control (0.33%) or Fomesa and Primafresh (0.40%), however a significant difference in between three groups Fomesa and Prolong (0.40, 0.33%) or Fomesa and control (0.40, 0.27%) or Primafresh and control (0.40, 0.27%) was noted during storage. However our research studies disagree with this statement that titratable acidity percent showed increasing trend from 0.18-0.57% during 20 days of their storage. These results are in correspond with (Srinivasa *et al*., 2002) who found that Titratable acidity values also showed a decreasing trend, the initial value of 2.17% being reduced to 0.08% in control fruit on 12th day in desapped, washed with tap water then dipped Alphonso mango in 500 ppm Carbendazim fungicide for 15 min and after drying fruit were kept in carton boxes whose top was covered with Chitosan (100 gauge) or with low-density polyethylene(100 gauge) or kept as such as control at ambient temperature 27±1°C at 65% RH. Similar changes were noted by (Kudachikar *et al*., 2001) in Neelum mango which had optimum stage of maturity 110 days after the fruit set and pH value decreased (3.0) and acidity increased (1.9%) upto 90 days after the fruit set . Later, pH slightly increased (3.1) and acidity slightly decreased (1.5%) at 110 days after fruit set. It is obvious from the results of the present studies that cardboard corten with combination of coating, antiripening agent with or without fungicide, or ethylene absorbent is very effective to maintain the maximum percent acidity and delay ripening process.

Ascorbic acid: It is evident from Table 1 that treatments and their interactions had highly significant effect on ascorbic acid contents of mango during storage and the ascorbic acid content in coated fruit packaged in carton was ranged from 23.06-40.83 mg/100 g with an average means of 25.47 mg/100 g during 25 days of storage at ambient temperature compared to very low ascorbic acid contents at first day (13.49 mg/100 g). The treated fruit showed a slower increasing trend of ascorbic acid contents during ripening might be due to the slower changes in the atmospheric conditions of modified packaging and the un-ripened fruit was going to optimum ripening stage that caused an increase in AA contents and after that the contents was reduced with passage of time might be due to degradation of AA by oxidation. These results are confirmed by Kudachikar *et al*. (2001) who reported that the ascorbic acid content of Neelum mango fruit increased from 42 mg/100 g at 30th days after the fruit set to a maximum of 74 mg /100 g on fresh weight bases at 70th days, there after it decreased to 70.5 mg/100 g at 110th days that was the optimum stage of maturity of Neelum mango after the fruit set. It is apparent from Table 1 that statistically there was a significant difference of ascorbic acid contents among treatments was found during 25 days of their storage at ambient temperature. The maximum ascorbic acid contents of late Chaunsa white mango in coated fruit packaged in polyethylene were observed in T_2 (40.83 mg/100 g), followed by T_6 (26.41 mg/100 g), T_4 (26.11 mg/100 g), T_5 (26.30 mg/100 g) and T_3 (23.06 mg/100 g) as compared to control with maximum ascorbic acid contents T_1 (44.06 mg/100 g) or having very low ascorbic acid contents at first day (13.49 mg/100 g) during storage that might be due to free atmospheric conditions, oxidation of AA was higher in control that caused rapid reduction in AA content compared to others coated fruit retained more AA might be due to slower decrease of AA in the higher concentration of CO_2 inside the fruit package after 18 days of storage at ambient temperature. These results are in line with the findings of Rana *et al*. (1992) who reported that decrease in ascorbic acid content was observed when sweet oranges were treated with oil emulsion stored in wooden box with a polyethylene bag. These results are comparable with (Carrillo *et al*., 2000) Who examined that ascorbic acid had decreasing trend in Haden mangoes coated with different concentrations of Semperfresh at 13°C during 32 days of storage but decrease was slower in coated fruit as compared to noncoated fruit. These results further support the findings of Raje *et al*. (1997) in India who prescribed that the ascorbic acid content of alphonso mangoes in CFB

carton depends on type of absorbent used and was higher in Halogen releaser 66.62-93.98 mg/100 g, followed by control 88.49 mg/100 g and in $KMnO_4$ dipped 75.42 mg/100 g after 8th day at 32-36°C and RH of 70-75% during storage. There was a gradual decline in the ascorbic acid content during storage, however, the maximum retention of ascorbic acid was noted in $KmnO_4$ treated fruit (9.53 mg/100 g), followed by ethysord (8.49 mg /100 g) after 16th day of storage period as compared to the combination of ethysord and SO_2 releaser (1.90 mg/100 g), Oxidizer (1.85 mg/100 g) and halogen releaser (1.49 mg/100 g) having less retention of ascorbic acid during storage as compared to control that was spoiled after 8th day of their storage. It is obvious from the results that Chaunsa white packaged only in cardboard carton packaged was higher in ascorbic acid contents might be due to earlier ripening as compared to coated fruit packaged with ethylene absorbent, antiripening agent or antiripening agent with or without disinfectant were very effective in delay ripening as a result lower contents of ascorbic acid during storage.

Conclusion: Keeping in view the results of the present studies, it is concluded that innovative approach of Active Packaging in Cardboard Carton (APCC) is successful approach in which Chaunsa white either packaged in cardboard carton alone or with combination of coating, antiripening agent with or without fungicide, or ethylene absorbent or antiripening agent with oil and disinfectants had played a vital role in delaying of ripening process and increased the shelf life, with minimum percent weight loss, slower increase of TSS contents, maintained lower pH or maximum percent acidity and ascorbic acid at later stage during storage. Further development in this packaging system is possible by including the wrapping of coated fruit in polyethylene and then packing in carton combination may be more effective in delaying ripening and maintenance of freshness or quality of the fruit. Therefore further research studies for new innovations in this packaging system are required in near future.

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Prevalence of Stunting, Underweight and Obesity in School Aged Children in Uyo, Nigeria

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Abstract: There is a paucity of data on the prevalence and trends of the risk factors associated with diet related chronic diseases in school-aged children in Nigeria. Using international reference standards, we determined the prevalence of underweight, stunting and obesity in school aged children in both privately owned and public schools in a fast growing state capital in the South-south region of Nigeria in a cross sectional prevalence survey. The height and weight measurements of 985 children aged 31-150 months (2½-14 years) were taken; food preference and socio-demographic characteristics of children were determined using a semi-structured questionnaire. BMI was calculated from the data. The results showed that the prevalence of underweight, stunting and obesity were 27.3, 17.1 and 11.1% respectively in the private schools and 39.4, 25.3 and 0.2% in the public schools. The food preference showed the predominance of refined highly processed foods. This study provides evidence of the co-existence of both undernutrition and obesity in the study population. While underweight and stunting remained high in the public schools, obesity existed alongside underweight and stunting in the privately owned schools, presumably patronized by the elite and medium or high socio-economic groups.

Key words: Underweight, stunting, obesity, school-aged children

INTRODUCTION

Anthropometric indices reflect the health and welfare of individuals and communities and can predict academic performance, health outcomes as well as reflect the socio-economic profile of the population (Prista *et al*., 2003; Chatuverdi *et al*., 1993; Cogil, 2003). Globally, studies on anthropometric profile of populations, suggest an increasing incidence of obesity and the co-existence of under nutrition and obesity in transitional countries (Thiam *et al*., 2006; Gales-Camus, 2006; Eggal and Lopriore, 2006; Shrimpton, 2006). In most developing countries of the world like Nigeria, the basic issues in nutrition are those of lack, inadequacy, undernutrition, micronutrient deficiencies and attendant high levels of communicable disease (NDHS, 2004; FMOH, 2005; Chizuru Nishida, 2006; WHO, 2005; Thiam *et al*., 2006; Cannon, 1996).

However among affluent individuals/groups, as well as the elite in developing countries, an increasing body of evidence, suggests that appropriate nutritional interventions are now needed to reduce morbidity and mortality from diet related chronic diseases. The emerging health crises due to the coalescing of the health effects of undernutrition and overnutrition in the same countries, communities and even households, has been termed the double burden of malnutrition (Thiam *et al*., 2006; Gales-Camus, 2006; Eggal and Lopriore, 2006; Shrimpton, 2006). This phenomenon has been attributed to the polarized model of epidemiological nutrition transition from stage 1 to stage 2, creating an obesogenic environment (Kennedy *et al*., 2007; Uauy and Solomons, 2006; Egger and Swinburn, 1997). To stem this current and projected epidemic of morbidities and premature mortalities due to diet-related chronic disease, there has been a call for the protection of foetal and childhood growth, which is central to the prevention of both under and over nutrition (Barker, 2004; Barker *et al*., 2005; Ene-Obong, 2001; McGuire, 1996; Tomkins, 1998; Tara Gopaldas, 1998; Bruno De Benoist and Yun Ling, 1998). There is a call for the surveillance of trends of the major risk factors for the double burden of mal-nutrition such as stunting, underweight, obesity, dietary patterns etc (Rolland-Cachera *et al*., 1997; Siervogel *et al*., 1991; Serdula *et al*., 1993; Shumei *et al*., 2002; Thiam *et al*., 2006).

Recently, consensus has emerged on the use of the modified BMI in the definition of childhood obesity (Cogil, 2003). This study was therefore undertaken to use the international reference standards to document the prevalence of stunting, underweight and obesity in school-aged children in a fast growing state capital in the south-south region of Nigeria. It is well established that health and nutrition of school-aged children is of critical importance in determining the prevalence of diet related chronic diseases in adulthood (Canning *et al*., 2004).

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MATERIALS AND METHODS

The study was a cross sectional prevalence survey, carried out in Uyo, capital city of Akwa Ibom State, in South-south region of Nigeria in March 2009. Akwa Ibom State has 31 local government areas, a population of 3,902,081 with more males than females. Sixty-five per cent of the population are youths aged 15-34 years and 85% of the population live in the rural areas. Uyo, the capital city is a fast growing metropolis with many public and privately owned primary schools. Due to systemic decay, the public schools are patronized by the urban poor, while the rich and the elite, patronize the privately owned schools.

Using random sampling 2 private and 2 public schools were chosen. Sample size was determined by the method of Snedecor and Chochran (1972):

$$n = pqz/d^2$$

Where z = 1.96 corresponding to 95% confidence level d at 5% acceptable margin of error p = .383 proportion of stunted Nigerian children (FMOH, 2005):

$$q = 1-p$$

This came to 385 per group and 500 children each were chosen from public and private schools to allow for drop out rate. Exclusion criteria included all selected pupils who were ill, had a limb deformity, whose ages could not be ascertained or who had attained puberty (breast development in girls and voice breaking in boys).

Using stratified random sampling (based on age and sex distribution in each class) 495 children (260 boys and 235 girls) were chosen from the private schools. While 490 (250 boys and 240 girls) were chosen from the public schools. Information on age was obtained from the class registers. (The schools insist on sighting birth certificates before admission of pupils). A semi structured questionnaire completed by parents/subjects was used to obtain information on; maternal level of education, parents socio-economic status (determined by the method of Oyedeji, 1985) birth order and food preference of the selected children.

Measures: The main variables studied were subjects weight, height, age, sex, socioeconomic status, birth order and food preference. Body adiposity was estimated using BMI (weight/height^2). measurements were made while observing standard precautions (Cogil, 2003).

Definitions of stunting, underweight and obesity (Cogil, 2003)

Stunting: was defined as <-2SD of the WHO 2003 reference standard height Z-score for age and sex.

Underweight: was defined as <-2SD of WHO 2003 reference BMI Z-score for age and sex.

Obesity: was defined as >+2SD of BMI Z-score for age and sex.

Statistical analysis: We examined the mean weight for age and sex, mean height for age and sex for the public and private schools. We calculated the BMI values. We then compared the mean BMI for age and sex and mean height for age and sex against the WHO reference standards BMI z-scores and height z-score, in order to apply the cut off definitions for underweight, stunting and obesity. Comparison of values between public/private, male/female was done using z test for two proportions. Pearson's correlation was used to determine association of variables with stunting, underweight and obesity. All calculations were performed on Statistical Package for Social Sciences (SPSS) version 15.0.

RESULTS

Sociodemographic characteristics of the surveyed children: There were 985 children. Of these 490 were in public schools while 495 were in private schools, made up as follows; 250 (25.4%) males and 240 (24.4%) females in the public schools, while there were 260 (26.4%) males and 235 (23.9%) females in the private schools. Majority (54.9%) of surveyed children were of medium socio-economic status. Of these 41.4% were in private schools, while 13.5% were children in public schools. Thirty-eight per cent of mothers had university education (or its equivalent), 5.1% of such mothers had their children in public schools, while 32.8% had their children in private schools. About 15% of mothers had primary education, 14.4% of them had children in public schools, while 0.9% had children in private schools. The children in public schools had higher mean birth orders (5.4 males; 5.8 females) than those in private schools (3.4 males; 3.2 females) Table 1.

Anthropometric profile of school-aged children: The youngest (31-40 months) girls had higher mean weight than boys and this pattern persisted until age 61-70 months. By 91-110 months girls recorded higher mean weights than boys. Thereafter boys almost always recorded higher mean weights than girls of the same age. For both sexes a fall in mean weight occurred, at age 111-120 months for girls and at age 141-150 months for boys.

In the private schools, the boys recorded higher mean weights than girls of the same age up to 61-70 months. At age 71-120 months, girls recorded higher mean weights than boys. A fall in weight occurred at age 111-120 in girls and at age 131 months and above in boys. The differences in weight by age and by type of school being attended was statistically significant ($p<0.05$).

The boys in public schools, recorded higher mean height values than girls of the same age in majority of the classes. There was a relative fall in mean height for

Table 1: Sociodemographic characteristics of surveyed school-aged children in public and private schools in Uyo, Nigeria (March 2009)

	Public		Private		Total
	Male n = 250 (25.4%)	Female n = 240 (24.4%)	Male n = 260 (26.4%)	Female n = 235 (23.9%)	985 (100.00%)
Parents (SES)					
Low	178 (17.1)	179 (18.3)	2 (0.2)	1 (0.1)	360 (36.5)
Medium	72 (7.3)	61 (6.2)	208 (21.1)	200 (20.2)	541 (54.9)
High	0	0	50 (5.1)	34 (3.5)	84 (8.5)
Maternal level of education					
Primary	70 (7.1)	72 (7.3)	5 (0.5)	4 (0.4)	151 (15.3)
Secondary	150 (15.1)	147 (14.2)	80 (8.1)	71 (7.3)	460 (46.7)
University	30 (3.0)	21 (1.2)	163 (16.5)	160 (16.2)	374 (38)
Mean birth order	5.4	5.8	3.4	3.2	
Age (months)	**n = 250**	**n = 240**	**n = 260**	**n = 235**	**985**
31-40	8	6	6	6	26 (2.63)
41-50	16	14	14	13	57 (5.79)
51-60	30	24	46	32	122 (15.4)
61-70	37	20	40	34	131 (13.3)
71-80	24	26	22	26	98 (9.33)
81-90	30	24	29	31	114 (11.6)
91-100	27	28	30	26	111 (11.3)
101-110	24	24	20	28	96 (9.74)
111-120	26	30	30	19	105 (10.7)
121-130	13	20	10	11	54 (5.5)
131-140	8	14	8	4	34 (3.5)
140-150	5	10	5	5	25 (2.5)
150-160	2	0	-	-	2 (0.2)
	250 (25.4)	240 (24.4)	260 (26.4)	235 (23.9)	985 (100.0)

Table 2: Distribution of surveyed school-aged children in public and private schools by age, mean (height and weight), Uyo, Nigeria (March 2009)

	Public school				Private school			
	Male		Female		Male		Female	
Age (Months)	Mean height (cm)	Mean weight (kg)	Mean height (cm)	Mean weight (kg)	Mean height (cm)	Mean weight (kg)	Mean height (cm)	Mean weight (kg)
31-40	100.5±4.6	13.3±2.9	100.5±4.6	13.9±4.1	98.6±5.0	13.8±1.6	98.0±3.6	13.6±0.8
41-50	97.7±2.3	13.1±4.3	97.0±3.8	14.5±3.8	103.1±3.5	16.1±1.9	101.1±4.7	15.3±0.9
51-60	108±3.2	14.5±1.2	105.7±2.8	15.7±3.5	109.9±4.5	18.8±2.6	104.4±19.7	17.0±2.0
61-70	113.9±3.0	17.5±0.6	111.8±0.9	16.6±1.9	114.8±6.7	22.5±1.6	117.1±5.3	21.4±3.9
71-80	115.5±4.9	18.8±11.8	114.2±3.1	17.6±3.0	120.9±5.2	22.5±3.5	122.6±5.1	24.1±3.4
81-90	122.6±4.7	21.5±3.1	121.0±6.2	21.0±3.7	125.1±4.9	23.7±3.5	126.3±6.1	23.9±3.2
91-100	125.4±4.1	22.9±2.2	125.7±6.7	23.3±4.2	132.8±6.3	29.2±5.0	130.7±10.4	28.1±6.8
101-110	127.2±3.9	23.7±4.4	135.1±7.5	28.8± 4.0	134.6±8.0	29.1±7.0	137.8±8.0	45.6±5.7
111-120	136.0±4.8	30.5±3.9	132.0±5.7	26.2±2.7	138.2±6.6	32.4±7.2	140.3±4.0	35.5±7.1
121-130	134.7±9.7	29.8±8.9	135.7±3.5	27.6±5.3	141.4±9.1	37.1±10.8	141.7±6.6	34.7±6.8
131-140	140.9±6.0	31.1±1.8	137.8±4.1	30.0±4.4	145.7±4.1	36.7±6.0	138.7±10.7	30.5±8.7
141-150	137.8±9.2	29.8±4.1	140.5±4.1	30.6±3.3	143.8±7.8	35.1±4.9	144.5±5.76	35.6±6.1
151-160	143.8±7.1	30.2±5.8	-	-	-	-	-	-

age at 141-150 months for boys and at age 110-120 months only for girls.

Among children in private schools, boys recorded higher mean height values than girls of the same age; except between ages of 61-90 months (23.1%). A relatively low value in mean height for age occurred for girls at 141-150 months and for boys at 150-160 months. The differences in height between boys and girls was statistically significant ($p<0.05$) as was the differences in height between public and private schools ($p<0.05$).

Prevalence of underweight: The prevalence of underweight was 39.4% in the public schools, being higher 23.1% in boys than girls (16.3%). The 51-76 months old were the worst affected, the least affected age groups was 91-100 months (21.8%) (Table 3).

The prevalence of underweight in the private schools was 27.3%, being higher in girls (15.6%) than boys (11.7%). The worst affected, were children aged 101-120 months while the least affected in the private schools were children aged 131-140 months (Table 3).

Sex and type of school being attended had the highest association with being underweight, while food preference had the least association (Table 4).

Prevalence of stunting: The prevalence of stunting was 25.3% in the public schools, with boys more affected (14.1%) than girls (11.2%). Children aged 91-100

Table 3: Prevalence of stunting, underweight and obesity in surveyed school-aged children in public schools in Uyo, Nigeria (March, 2009)

	Age in months							
Both sexes	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Total
Total No.	14	30	54	57	50	54	55	490 (100.0)
No. of Stunted	4 (28.6)	81 (26)	17 (31.5)	13 (22.8)	15 (30)	9 (16.7)	18 (32.7)	124 (25.3%)
No. of underweight	5 (35.7)	10 (33.3)	30 (55.6)	28 (49.1)	18 (36)	20 (37)	12 (21.8)	193 (39.4%)
No. of obese	1	0	0	0	0	0	0	1 (0.2%)
Girls								
Total No.	6	14	24	20	26	24	28	240 (49%)
No. of Stunted	2	5	7	6	7	4	8	55 (11.2%)
No. of underweight	3	8	10	10	8	8	5	80 (16.3%)
No. of obese	1	0	0	0	0	0	0	1 (0.2%)
Boys								
Total No.	8	16	30	37	24	30	27	250 (51.0%)
No. of Stunted	2	3	10	7	8	5	10	69 (14.1%)
No. of underweight	2	2	20	18	10	12	7	113 (23.1%)
No. of obese	0	0	0	0	0	0	0	0 (0.0%)

	Age in months						
Both sexes	101-110	111-120	121-130	131-140	141-150	151-160	Total
Total No.	48	56	33	22	15	2	490 (100.0)
No. of Stunted	12 (25)	13 (24.5)	8 (24.2)	4 (18.2)	3 (20)	0	124 (25.3%)
No. of underweight	22 (45.8)	23 (43.4)	12 (36.4)	6 (27.3)	7 (46.7)	0	193 (39.4%)
No. of obese	0	0	0	0	0	0	1 (0.2%)
Girls							
Total No.	24	30	20	14	10	0	240 (49%)
No. of Stunted	6	5	3	1	1	0	55 (11.2%)
No. of underweight	10	10	4	2	2	0	80 (16.3%)
No. of obese	0	0	0	0	0	0	1 (0.2%)
Boys							
Total No.	24	26	13	8	5	20	250 (51.0%)
No. of Stunted	6	8	5	3	2	0	69 (14.1%)
No. of underweight	12	13	8	4	5	0	113 (23.1%)
No. of obese	0	0	0	0	0	0	0 (0.0%)

months were the most affected, while those aged 81-90 months were the least affected (Table 3). In private schools, the prevalence of stunting was (17.1%) with boys more affected (9.7%) than girls (7.9%). Children aged 121-130 months were the most stunted in private schools, while those aged 51-60 months were the least stunted (Table 4).

Prevalence of obesity: Obesity was near absent in public schools (0.2%) (Table 3). The prevalence in private schools was 11.1% with girls being more likely to be obese (6.9%) than boys (4.2%). The youngest age group 31-40 months were the most likely to be obese (25%), followed by those aged 101-110. There was no obesity in children aged 130 months and above (Table 3).

Food preference: Rice was the food most preferred by children in public schools (60.4%). This was followed by Garri and soup (22.2%), Beans (10.4%) and Noodles (2.2%). Among children in private schools, noodles was mentioned as the most preferred food (49.3%), this was followed by rice (23.8%), yam (8.5%) and bread and beverage (6.4%) (Table 6).

DISCUSSION

This study has shown that the children of higher socio-economic status and maternal education, had a better chance at education, since majority of such children were enrolled in private schools, where the facilities are better and the teachers are more committed, though not necessarily better paid (Chatuverdi *et al*., 1993; Tara Gopaldas, 1998; Studert and Soekirman, 1998; Prista *et al*., 2003). It was observed that even some children (5.1%) whose mothers had university education or its equivalent, were enrolled in public schools. This may have to do with prevailing unemployment situation in the country (FMOH, 2005). Previous studies suggest an association between socio-economic status/maternal education and anthropometric indices of children (Guthric and Picciano, 1995; English, 1998; Bruno De Benoist and Yun Ling, 1998; Ene-Obong, 2001; Armstrong *et al*., 2003; Schmidhuber and Shetty, 2008). This study revealed that the youngest girls in public schools recorded a higher mean weight than both their male counterparts and children of the same age and sex in private schools; who are of higher socio-economic status. This observation may be linked to the possibility that comparable children in private schools, may have

Table 4: Prevalence of stunting, underweight and obesity in surveyed school-aged children in private schools in Uyo, Nigeria (March, 2009)

	Age in months						
Both sexes	31-40	41-50	51-60	61-70	71-80	81-90	Total
Total No.	12	27	78	74	48	60	495 (100.0)
No. of Stunted	2 (16.7)	5 (18.5)	8 (10.3)	13 (17.7)	8 (16.7)	14 (23.3)	85 (17.1%)
No. of underweight	3 (25)	8 (29.6)	14 (17.9)	18 (4.3)	15 (31.3)	18 (30)	135 (27.3%)
No. of obese	3	5	8	2	4	8	55 (11.1%)
Girls							
Total No.	6	13	32	34	26	31	235 (47.5%)
No. of Stunted	2	3	5	8	4	6	39 (7.9%)
No. of underweight	1	5	7	10	9	10	77 (15.6%)
No. of obese	2	3	5	2	2	5	34 (6.9%)
Boys							
Total No.	6	14	46	40	22	29	260 (52.5%)
No. of Stunted	0	2	3	7	4	8	48 (9.7%)
No. of underweight	2	3	7	8	6	8	58 (11.7%)
No. of obese	1	2	3	0	2	3	21 (4.2%)
	Age in months						
Both sexes	91-100	101-110	111-120	121-130	131-140	141-150	151-160	Total
Total No.	56	48	49	21	12	10	-	495 (100.0)
No. of Stunted	11 (19.6)	9 (18.8)	6 (12.2)	5 (23.8)	2 (16.7)	2 (2.0)	-	85 (17.1%)
No. of underweight	13 (23.2)	18 (37.5)	19 (38.8)	5 (23.8)	2 (16.7)	2 (2.0)	-	135 (27.3%)
No. of obese	8	11	4	2	0	0	-	55 (11.1%)
Girls								
Total No.	26	28	19	11	4	5	-	235 (47.5%)
No. of Stunted	3	3	2	1	1	1	-	39 (7.9%)
No. of underweight	8	10	10	3	2	2	-	77 (15.6%)
No. of obese	4	7	3	1	0	0	-	34 (6.9%)
Boys								
Total No.	30	20	30	10	8	5	-	260 (52.5%)
No. of Stunted	8	6	4	4	1	1	-	48 (9.7%)
No. of underweight	5	8	9	2	0	0	-	58 (11.7%)
No. of obese	4	4	1	1	0	0	-	21 (4.2%)

Table 5: Pearson correlation (r) of factors associated with underweight, stunting and obesity

	Underweight	Stunting	Obesity
Age	0.26	0.48	0.04^{S}
Sex	0.56	0.68	0.62^{S}
Food preference	0.26	0.01	0.07^{NS}
Maternal education	0.2	0.31	0.08^{S}
Parents socio-economic status	0.25	0.28	0.15^{S}
Type of school	0.57	0.53	0.26^{S}

Significant level = $p<0.05$

been attending school longer than those in public schools, this will translate to higher energy expenditure. The observation in this study that at certain ages, girls recorded higher weights than boys of the same age/school may be explained by differentials in physical activity pattern and maturation rate of both sexes. Similar differences in weight by sex has been observed in other studies (English, 1998; Wang *et al*., 2002; Cordeiro *et al*., 2006; Salih and Abdel-Aziz, 2007).

The observation in this study of an age related fall in mean heights and weights for both sexes could not be explained. It could be postulated that the younger children may be exhibiting a higher height potential than older children which may then be linked to improving levels of socio-economic development. Further research is needed to explain this finding, in our setting, though there are existing theories of post infancy nadir of BMI (Rolland-Cachera *et al*., 1997; Siervogel *et al*., 1991; Serdula *et al*., 1993).

Underweight, stunting and obesity: The observed prevalence of underweight and stunting (39.4% and 25.3% respectively) is near the figures reported elsewhere in Nigeria (FMOH, 2005). Sex and age related variation found in this study had been reported by other authors (English, 1998; Wang *et al*., 2002; FMOH, 2005; Cordeiro *et al*., 2006). However, the existence of underweight and stunting (27.3% and 17.1% respectively) in private schools was an unexpected finding, since these schools are patronized by the elite, the middle class and the rich. The finding may be a reflection of the tendency of the middle class families to 'over-school' their children, while cutting down on food intake. It can further be attributed to poor social services and poor health services in developing economies, alluded to by other authors (Thiam *et al*., 2006; Wang *et al*., 2002; Dewey, 2006; Gales-Camus, 2006). Stunting

Table 6: Food preference of surveyed school-aged children in Uyo, Nigeria (March 2009)

Public schools		Private schools	
Rice	296 (60.4%)	Indomie noodles	244 (49.3)
Garri and soup	10 (22.2%)	Rice	118 (23.8)
Beans	51 (10.4%)	Plantain	22 (4.4)
Indomie	11 (2.2%)	Yam	42 (8.5)
Pap	7 (1.4%)	Beans	19 (3.8)
Others like plantain, yam, rice and beans, ekpang	4 (0.8%) each	Beans and rice	1 (0.2)
		Garri and soup	1 (1.2)
		Ekpang nkukwo	3 (0.6)
		Bread and beverage	32 (6.4)
		Spaghetti	3 (0.6)
		Potato/potato chips	10 (2.0)
Total	100%		100%

and underweight are recognized as risk factors for diet related chronic diseases in adulthood (Uauy and Solomons, 2006, Barker *et al*., 2005; Barker, 2004).

Co-existence of obesity, underweight and stunting: This study provides evidence of the co-existence of both obesity and underweight/stunting even among the poor, though, a low (0.2%) in children enrolled in public schools. The prevalence of obesity was higher among children of the educated, middle and high socio-economic classes, who were enrolled in the private schools (11.1%). WHO (2005) reported that 10-20% of men and 15-45 of women in West Africa were either overweight or obese. In this study, the prevalence of obesity was higher in girls (6.9%) than boys (4.2%) and was highest (12.5%) in the youngest children (31-40 months). These findings are consistent with those of other authors (Eggal and Lopriore, 2006; Shumei *et al*., 2002; Armstrong *et al*., 2003; Chizuru Nishida, 2006). This study found high levels of underweight (39.4%, public schools and 27.3% in private schools) and stunting (23.3% in public and 17.1% in private schools). The co-existence of under and over nutrition in the same population, is postulated to bring about a potentiation of their adverse health effects, translating to rising morbidities and premature mortalities from diet-related chronic disease (Reilly *et al*., 1999; WHO/FAO, 2003; Armstrong *et al*., 2003; Canning *et al*., 2004; Tremblay *et al*., 2002; Schmidhuber and Shetty, 2008). Obesity in childhood is believed to persist into adulthood (Vaska and Volkmer, 2004; Serdula *et al*., 1993; Thornburn, 2005), leading to an increasing incidence of certain types of cancers, osteoarthritis, hypertension, Type 2 Diabetis, cardio vascular Disease, etc. stunting in childhood predisposes to obesity in adulthood due to metabolic changes.

The presence of obesity in this population of school aged children may be attributed to their food preference. Even among the low socio economic status children enrolled in public schools, but more especially among children in private schools where highly processed foods such as noodles, rice, bread and beverage were the most preferred and eaten foods. Sometimes constituting the three main meals in the 24 hr dietary recall. The traditional foods such as Beans, Garri and soup, Ekpang Nkwukwo (a native dish made from cocoyam tubers, leaves and sea foods) were the least mentioned as preferred or eaten. Similar findings were reported by Anyika and Uwaeghute (2005) on the snacks and nutrient intake of secondary school girls. Nutritional transition to the "western diet" and the emergence of an obesogenic environment in urban poor and in developing countries has been associated with the double burden of malnutrition (Kennedy *et al*., 2007; Rolland-Cachera *et al*., 1997; Siervogel *et al*., 1991).

Food preference and dietary patterns are associated with many factors such as, parental influences, home environment, food insecurity, media influence, globalization of the food industry (Campbell *et al*., 2007; Hood *et al*., 2000; Birch and Fisher, 1998; Sharma, 1996; Shrimpton, 2006; Feinberg *et al*., 2008; Ene-Obong, 2001).

We conclude that the findings of these study suggest an escalation of the present and projected epidemic of diet-related chronic diseases in Nigeria, where 24% of the disease burden is attributed to chronic disease (WHO, 2005). It is therefore recommended that already established remedies (Reilly *et al*., 1999; Gales-Camus, 2006; Thiam *et al*., 2006; Dewey, 2006; Lobstein *et al*., 2004; Rudiger Von Kries *et al*., 1999) be put in place to contain this upsurge.

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Effect of Graded Levels of Lemongrass (*Cymbopogon citratus)* on Oxidative Stability of Raw or Cooked Pork Patties

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Abstract: The antioxidative potential of ground lemongrass (*Cymbopogon citratus*) was evaluated at graded levels on raw and cooked pork patties, under refrigeration for 9 and 6 days for raw and cooked respectively, in a 5 x 2 x 4 factorial experiment. In 200 g pork patties 0, 0.5, 1 or 1.5% lemongrass was added and a reference control was prepared with 200 mg α-tocopherol acetate in 200 g pork patties. Results showed that raw pork patties with lemongrass had lower TBARS values than the control or α-tocopherol treated pork patties. Raw pork patties treated with any level of lemongrass had lower TBARS values than their cooked counterparts. Addition of 1.5% lemongrass was effective in reducing lipid oxidation in raw patties under refrigeration. Lemongrass can conveniently replace synthetic antioxidant such as BHA, BHT and TBHQ that consumers have worry for due to their health safety.

Key words: Lemongrass, α-tocopherol acetate, lipid oxidation, raw or cooked pork patties

INTRODUCTION

Lipid oxidation is one of the major problems encountered in meat processing industry leading to quality deterioration, manifesting in adverse changes in colour, flavour and nutritive values couple with accumulation of deleterious compounds causing various human diseases, such as cancer, atherosclerosis and heart problems (Nawar, 1996; Cordon, 2001; Wasowicz *et al*., 2004; Choi and Lee, 2009). Lipid oxidation is an important determinant of shelf life of meat and meat products. Meat deterioration starts following animal slaughter. Pork is an excellent source of protein, but it is very high in lipids thus making it susceptible to oxidative deterioration. The control of lipid oxidation in fresh and processed meat products is the focus of meat scientists. The addition of synthetic antioxidants such as 3-tert-butyl-4-methoxyphenol (that is, butylated hydroxyanisole, BHA); 2, 6-di-tert-butyl-4-methlphenol (that is, butylated hydroxytoluene, BHT) and Tert-Butylhydroquinone (TBHQ) has been used extensively in the meat industry to ensure product preservation and shelf life improvement (Khalil and Mansour, 1998; Byrd, 2001). However, due to the toxicological effects of synthetic antioxidants, consumers have shown preference for the use of natural antioxidants (Namiki, 1990; Madhavi and Salunkhe, 1996; Byrd, 2001; Johnston *et al*., 2005). α-Tocopherol is a lipid soluble natural biological antioxidant which scavenges oxygen and protects against oxidation of double bonds in Poly Unsaturated Fatty Acids (PUFA) (Nunez de Gonzalez *et al*., 2008). In recent times several studies have been carried out on the antioxidant potentials of naturally occurring plant extracts such as ginseng, aloe vera, sage, rosemary, tea catechins, fenugreek, soya protein, mustard and plum (McCarthy *et al*., 2001; Nunez de Gonzalez *et al*., 2008).

Lemongrass (*Cymbopogon citratus)* is a perennial, aromatic tall tropical grass that is commonly used as herbs for flu, headache, malaria, coughs elephantiasis, pneumonia digestive problems, diarrhea, stomach upsets and vascular disorders (Ozer *et al*., 1995). It is also used as a stimulant, diuretic, antispasmodic and a mild irritant (Simon *et al*., 1984). The plant grows in a dense clump up to 2 meters in diameter and has leaves up to 1 meter long, with densely tufted fibrous roots. The fresh stalks and leaves have a clean lemon-like odour. The oil of this plant is sherry coloured with pungent taste, it has citral as the principal constituent. Other constituents of the oil are terpineol, β-myrcene, citronellol, limonene, geranoil, dipentene, methyl heptenon and nerol (Simon *et al*., 1984). Ojo *et al*. (2006) report that lemongrass and green tea inhibited paracetamol-induced lipids peroxidation in rats. Little research has been carried out on this particular cheap and common plant as an antioxidant in meat or meat products, thus this study was designed to evaluate the antioxidative potential of lemongrass in both raw and cooked pork patties and to also determine the optimum level of its use.

MATERIALS AND METHODS

The lemongrass leaves were harvested from homes in Tanke area, a neighbourhood of the University, Ilorin. The leaves were rinsed with water, chopped and then

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oven dried at 40°C for 2 days. The dried leaves were finely ground in a dry cup grinder.

Four kilogram pork meat (ham) was bought from a reputable meat shop. The skin, bones and fat trimmings were manually done; the meat was then cut into small pieces before mincing in a food processor (National MK-5080M Matsushita Electric Industrial, Japan). Into 200 g minced pork meat 0, 0.5, 1 or 1.5% of lemongrass powder was added and mixed thoroughly. A positive reference control was prepared by mixing 200 mg α-tocopherol acetate with 200 g minced pork meat. Each treatment was prepared for both raw and cooked and was subdivided into 20 g wrapped in cellophane and formed into patties using a locally fabricated patty mould designed by the Faculty of Engineering, University of Ilorin. The samples for cooking were done in a microwave oven for $1^{1}/_{2}$ min. All the samples were stored in a refrigerator for 9 or 6 days for raw or cooked respectively. Evaluation was done at 3 or 2 days interval for the raw and cooked samples respectively.

Analysis of lipid oxidation: Thiobarbituric Acid-Reactive Substances (TBARS) assay was performed in triplicates using the method of Pikul *et al.* (1984).

Statistical analysis: The study was a 5 x 2 x 4 factorial experiment with antioxidant (control, 0.5, 1, 1.5% lemongrass or 200 mg α-tocopherol) meat state (raw or cooked) storage days (9 or 6 evaluated at 3 or 2 days intervals for raw or cooked respectively). Each treatment was replicated 3 times. Data were subjected to Analysis of Variance (ANOVA) using the Genstat 5 program (Pane *et al.*, 1987). Fishers Least Significant Difference (LSD) test ($p<0.05$) was used to separate the treatment means.

RESULTS AND DISCUSSION

The addition of 0.5, 1 or 1.5% lemongrass to raw pork patties produced a significantly ($p<0.05$) lower TBARS values than the control or 200 mg α-tocopherol treated pork patties (Fig. 1). The reduced TBARS values recorded for raw pork patties with increasing concentration of lemongrass could be due to a synergistic effect with the endogenous antioxidant enzyme (especially catalase) in the tissue (Rhee *et al.*, 1996; Pradhan *et al.*, 2000). The 200 mg α-tocopherol treated raw samples had the highest TBARS values though this was not significantly ($p>0.05$) different from the control. This indicates that the antioxidative effect of α-tocopherol is weaker than that of lemongrass at any level of inclusion in raw pork patties. However, the opposite was the case in cooked pork patties as added α-tocopherol recorded the least ($p<0.05$) TBARS values which was only matched statistically by the 1% lemon grass. This contradicts the report of Higgins *et al.* (1998) that direct addition of α-tocopherol did not significantly ($p>0.05$) affect lipid oxidation in cooked turkey meat, as exogenous α-tocopherol added postmortem can only be

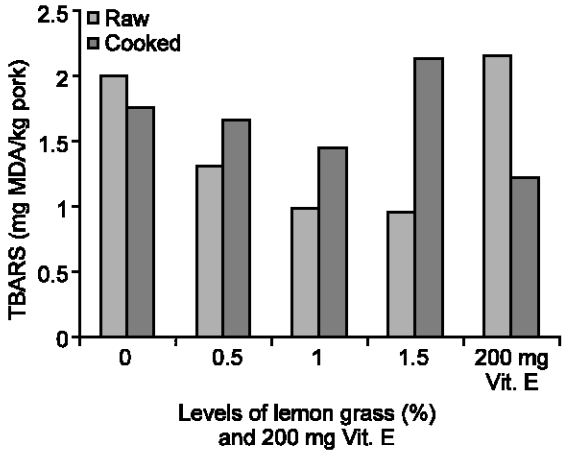

Fig. 1: Effect of graded levels of lemon grass (%) or 200 mg α-tocopherol acetate on oxidative stability of pork meat

incorporated into the neutral fraction thus having a low inhibition on oxidation. The cooked pork patties with 1.5% lemongrass inclusion had surprisingly the highest TBARS of 2.14 mgMDA/kg meat which was higher ($p<0.05$) than any other treatment including the control. The cooked pork patties with any level of added lemongrass had higher ($p<0.05$) TBARS values than their corresponding raw samples. Different reasons have been given for the higher TBARS values observed for cooked pork patties over the raw patties these include (1) disruption of muscle membrane systems (thus, leading to loss of structural integrity) which occurs during cooking, thus exposing membrane lipids to lipid oxidant catalysts, (2) increase in ionic iron concentration from heat-induced release of protein-bound iron after cooking,(3) inactivation of antioxidant enzymes in meat due to cooking,(4) the formation of the hypervalent ferrylmyoglobin (or activated metmyoglobin during cooking (Harel and Kanner, 1985; Asghar *et al.*, 1988; Rhee, 1988; Mei *et al.*, 1994). But the reverse was the case with the 200 mg α-tocopherol treated and the control meat samples. Liu *et al.* (1994) observed that the antioxidant effect of α-tocopherol in cooked meat was less than that of the raw meat. This contradicts our observation in this study as addition of exogenous α-tocopherol significantly ($p<0.05$) reduced lipid oxidation in cooked pork patties than the raw. Nunez de Gonzalez *et al.* (2008) report an increase in TBARS of precooked sausage pork patties treated with dried plum refrigerated or frozen over the raw. Ahn *et al.* (2002) report that rosemary and α-tocopherol retarded oxidation during and immediately after cooking, lemongrass could not be said to play such a role here.

The effect of storage days was also found to have a significant ($p<0.05$) on the oxidative stability of both raw and cooked pork patties (Fig. 2). In the raw pork patties,

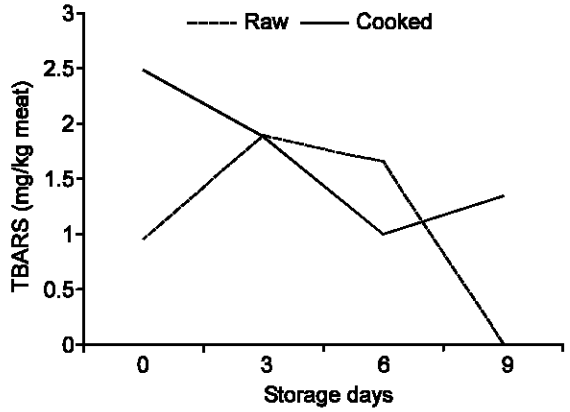

Fig. 2: Effect of storage days on oxidative stability of raw and cooked pork patties

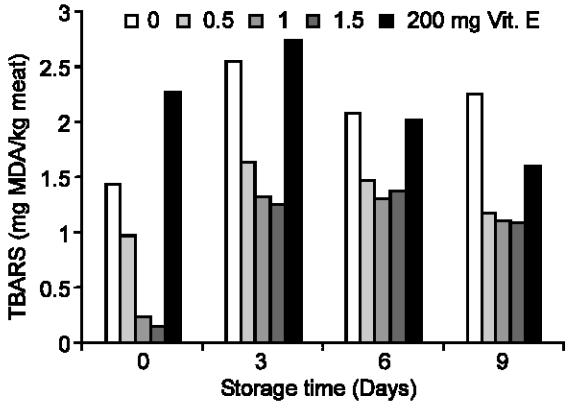

Fig. 3: Relationship of graded levels of lemongrass or 200 mg α-tocopherol acetate and storage days on oxidative stability of raw pork patties

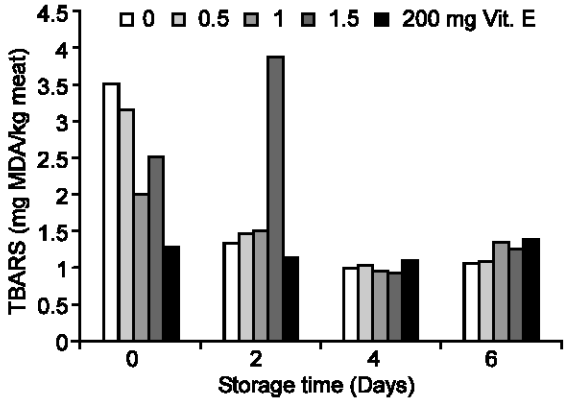

Fig. 4: Relationship between graded levels of lemon grass or 200 mg α-tocopherol acetate and storage days on oxidative stability of cooked pork patties

lipid oxidation increased sharply from the beginning till day 3, it then began to reduce steadily from day 6 to day 9. But in the cooked pork patties a decrease in oxidation was noticed from day 2 to day 4 by day 6 it has started to rise.

The effect of graded levels of lemongrass or 200 mg α-tocopherol and storage days on oxidation of raw pork patties is shown on Fig. 3. Both addition of lemongrass or α-tocopherol and storage time were found to influence ($p<0.05$) oxidation of raw pork patties. Interaction of these two factors was also noticed ($p<0.05$). The antioxidant effect of lemongrass was very pronounced on raw pork patties on day 0, with the meat patties treated with 1.5% lemongrass recording the least TBARS values which was not statistically different from those with 1% lemongrass. The raw pork patties treated with α-tocopherol had TBARS values that were significantly ($p<0.05$) more than other treated meat patties including the control on day 0 but were not significantly ($p>0.05$) different from the control on day 3. However, by day 6 of storage the antioxidative potential of α-tocopherol has started to manifest in raw patties this continues till day 9 where its TBARS values were not significantly ($p>0.05$) different from the raw pork patties treated with lemongrass. Oxidation was observed to increase sharply on day 3 irrespective of the treatment, but by day 6 a decline in oxidation was seen with the α-tocopherol, control and 0.5% lemongrass included patties. On day 9 of storage while all the treated meat patties were experiencing reduction in rancidity the control samples were in increasing in oxidation. Raw pork patties treated with any level of lemongrass was significantly lower ($p<.0.05$) than the control or 200 mg α-tocopherol treated pork patties throughout the storage period except on day 0 when the 0.5% lemongrass treated meat was not statistically different from the control.

Figure 4 shows the effect of graded levels of lemongrass or 200 mg α-tocopherol and storage days on oxidative stability cooked pork patties. There was an interaction between antioxidant agents and storage days. On like the raw pork patties the antioxidant potential of α-tocopherol was more effective in the cooked pork patties. The varied effect of α-tocopherol observed in this study could probably be attributed to the reasons given by Huang *et al.* (2005) that α-tocopherol could act as an antioxidant or as a prooxidant depending on the test system, the concentration, the oxidation time and the method used to determine lipid oxidation. The high TBARS values observed on day 2 for cooked pork treated with 1.5% lemongrass (3.87 mg MDA/kg meat) which was significantly higher ($p<0.05$) than any other treatment could not be explained. McCarthy *et al.* (2001) report on the antioxidant properties of tea catechins used at 0, 0.01, 0.05, 0.10, 0.25, 0.50, or 1% level in fresh or previously frozen pork patties that the optimum working concentration of tea catechins ranged from 0.25-1% (2500-10,000 mg/kg) and that optimum concentration of tea catechins was 0.25% (2500 mg/kg).

Mitsumoto *et al.* (2005) report a lower concentration of 400 mg tea catechins per kg of meat to work well in retarding the development of rancidity. In this study it was observed that raw meats were more prone to lipid oxidation than the cooked meats stored under the same storage conditions, except on day 0.

Conclusion: Inclusion of lemon at any level (0.5, 1 or 1.5%) was found to inhibit lipid oxidation better than exogenous 200mg α-tocopherol acetate in raw pork patties under refrigeration. Lemongrass inclusion at 1.5% in raw pork patties was found to more effective than 200mg α-tocopherol acetate. Treating cooked pork with any antioxidant agent did not improve lipid stability during the storage period examined.

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Isolation, Characterization and Production of Phytase from Endophytic Fungus its Application for Feed

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Abstract: Thirty four isolates of endophytic fungus produce phytases were isolated from leaf, stem and root fragments of soybean. Two isolates were the best of phytases enzyme producer and identified as *Rhizoctonia* sp. and *Fusarium verticillioides*. The phytase production was induced by phytate in medium used. The crude preparations were used in subsequent characterization studies, pH and temperature optimum and compared to other phytases tested and is thus a promising candidate for animal feed applications. The results showed that optimal production of phytase from *Rhizoctonia* sp. were pH 4.0 and temperature 50°C and pH 5.0, temperature 50°C for *Fusarium verticillioides*.

Key words: Endophytic fungus, phytase, soybean, *Rhizoctonia* sp., *Fusarium verticillioides*

INTRODUCTION

Phytate (*myo*-inositol-hexaphosphate) is the major form of phosphorus stored in cereals, pollens, legumes and oil seeds. Phytate is known as an anti-nutrient factor, since it chelates various metal ions such as Mg^{+2}, Ca^{+2}, Zn^{+2}, Fe^{+2}, Fe^{+3} and forms complex with proteins (Pallauf and Rimbach, 1996; Martin *et al*., 2005; Cao *et al*., 2007; Liu *et al*., 2007). Morever, phytate is not metabolized by monogastric animals, which have low levels phytate-degrading enzymes in their digestive tracts, Thereby, inorganic phosphate has to be added to feeds to ensure a sufficient phosphate supply for these animals. Consequently, the phytate in animal feeds is discharged in feces of these animals into waterways, which contributed to eutrophication for surface waters, particularly in areas of livestock production (Takizawa, 1998).

One way to enhance phosphate utilization from phytate is the use of phytase. To obtain a good source of phytase, a variety of microorganisms, animals tissue and plant have been screened for enzyme. Several plant phytases in wheat, barley, bean, corn, soybean, rice and cotton have been studied extensively (Greiner and Konietzny, 2006). Microbial sources are *Bacillus* sp. (Poward and Jagannathan, 1982), *Eschericia coli* (Greiner *et al*., 1993a,b), *Enterobacter* (Yoon *et al*., 1996) *Raoutella* sp. (Greiner *et al*., 1997; Shah and Parekh, 1990), *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus* (Howson and Davis, 1983) and ruminal bacteria (Yanke *et al*., 1998). Several types of fungal phytase are available on the market from several companies such as Gist Brocades Co, Novo Nordisk Co and Kyowa Hakko Kogyo Co (Takizawa, 1998). At the end of 20th century, annual sales of phytase as feed additive were estimated at US$ 500 million and are continuing to rise (Vats and Banerjee, 2004). In Indonesia, the phytase has been new research. The possibility of using these phytases in industry has not investigated. However, more work needs to be done to obtain superior enzyme for industrial applications. It includes screening for strain that produce high phytase with better physicochemical properties, including high thermostability and suitable pH, along with gen cloning. The objectives of this study were to isolate, characterization production of phytase enzyme from endophytic fungus and its applications for feed.

MATERIALS AND METHODS

Calcium phytate was made in the laboratory by adding phytic acid into a saturated calcium hydroxide solution. Sodium phytate and sodium dodecyl sulfate were sourced from Sigma. All other reagents were domestic products of analytical grade.

Isolation of endophytic fungus: Isolation of phytase producers was performed by the agar plate of method Quan *et al*. (2001). Leaf, stem and root fragments sample of soybean [*Glycine max* (L.) Merril] were obtained from a farmer garden in Padang, Indonesia. All leaf stem and root samples were washed twice in distilled water then surface sterilized by immersion for 1 minute in 70% (v/v) ethanol, 4 minutes in sodium hypochlorite [3% (v/v) available chlorine] and 30 sec in 70% (v/v) ethanol and then washed three times in sterilized distilled water for 1 min each time. After surface sterilization, the samples were cut into 5-7 mm pieces and aseptically transferred to plates containing 0.1% Ca-phytate; 1.5% glucose; 0.2% NH_4NO_3; 0.05% KCl; 0.05% $MgSO_4 \cdot 7H_2O$; 0.03% $MnSO_4 \cdot 4H_2O$; 0.03% $FeSO_4 \cdot 7H_2O$ and 1,5% agar. The final pH was adjusted

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to 5.5. Cultivation carried out at 28°C for 2-5 days. Fungal colonies, capable of hydrolyzing Ca-phytate which can be recognized by their surrounding clear halo, were selected and repeatedly streaked onto solid Potato Dextrose Agar (PDA) plates. Colonies which developed on the plates were inspected for their morphology. Pure colonies were obtained by replating single colonies. Identification of fungal phytase was determined with using of methods Samson and Van Reenen-Hoekstra (1988); Barnett and Hunter (1972).

Screening of endophytic for phytase produser: Each of isolated strains was grown in 50 ml of liquid medium (0.1% Ca-phytate; 1.5% glucose; 0.2% NH_4NO_3; 0.05% KCl; 0.05% $MgSO_4 \cdot 7H_2O$; 0.03% $MnSO_4 \cdot 4H_2O$; 0.03% $FeSO_4 \cdot 7H_2O$, pH 5.5) in 500-ml Sakaguchi flask and incubated at 28°C for 48 h on reciprocal shaker (200 rpm). Cells collected from 1 ml of culture by centrifugation at 5000 x g for 10 min in cood room (4°C). Then, the collected cells were resuspended in acetate buffer (0.2 M, pH 5.5) and used for the phytase activity assay.

Measurement of enzymatic activity: The phytase activity assay was determined by measuring the amount of liberated inorganic phosphate according to a method of Quan *et al*. (2001). Reaction mixture consisted of 0.8 ml acetate buffer (0.2 M, pH 5.5) containing I mMNa-phytate and 0.2 ml of cell suspension. After incubation for 30 min at 37°C, the reaction was stoped by adding 1 ml of trichloroacetic acid. A 1 ml aliquot was analyzed for inorganic phosphate liberated by method Kim and Lei (2005). One unit of enzyme activity was defined as the amount of enzyme liberating I nmol of inorganic phosphate per minute.

Enzymatic characterization studies: The effect of the pH on the activity of phytase was examined from pH 2.0-8.0 in 100 mM buffer. The buffers used were as follows: pH 1.0-3.5: Gly-HCl; pH 3.5-6.0: NaAc-NaOH; pH 6.0-7.0: Tris-HAc; pH 7.0-8.0: Tris-HCl. Temperature versus enzyme activity was measured over a range of 28-80°C.

RESULTS

Identification of isolates: A total 34 endophytic fungal strains were screened for their ability to produce extracellular phytase. Only two strains, forming clear peripheral zones on turbid agar plate, were isolated from root samples and their activities were determined using liquid culture. According to the results of morphological observation were classified as fungi. They are *Rhizoctonia* sp. and *Fusarium verticillioides* (Fig. 1). The changes of phytase activity in fermentation were shown in Fig. 2. The phytase activity of *Rhizoctonia* sp. was 0.77-2.72 U/ml and *F. verticillioides* was 0.79-6.11 U/ml.

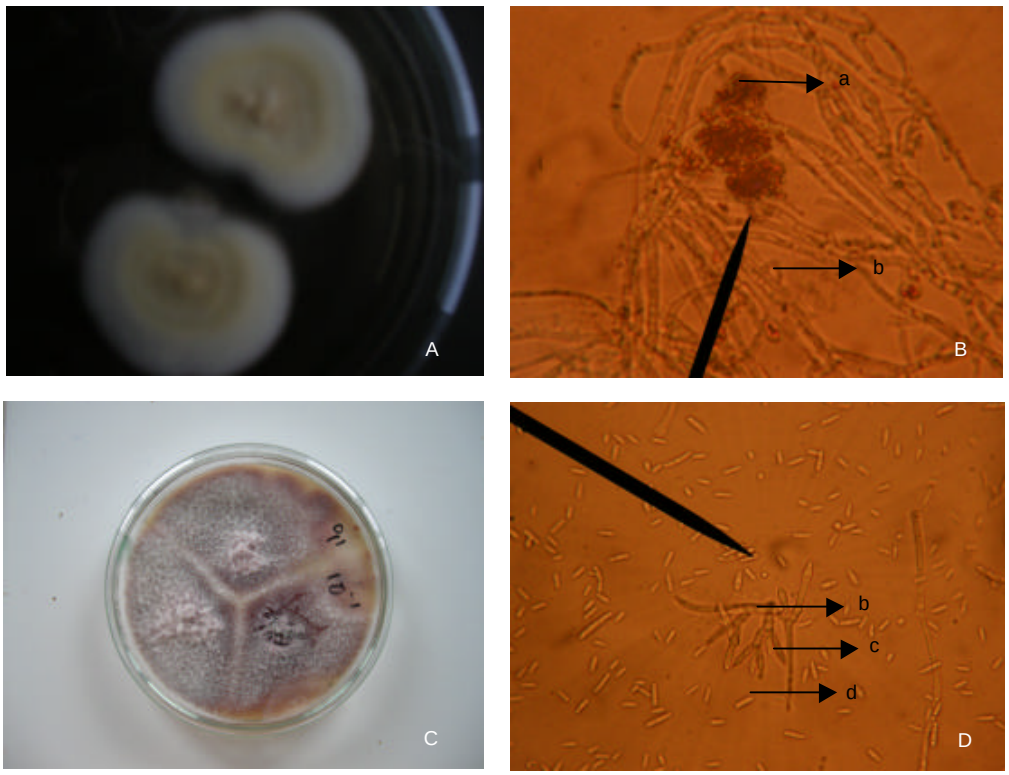

Fig. 1: Morphology of endophytic fungal phytases. A-B = *Rhizoctonia* sp.; C-D = F. *verticillioides*; (100x); a = sclerotia; b = hifa; c = phialid; d = macroconidia

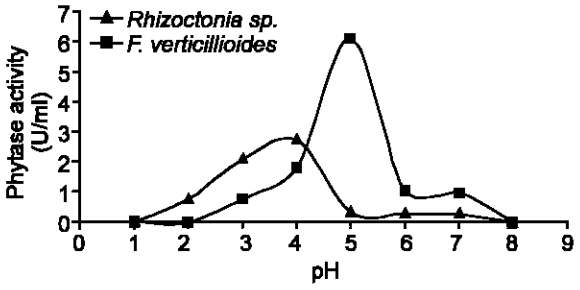

Fig. 2: pH versus activity profiles of phytase from *Rhizoctonia* sp. and F. *verticillioides*

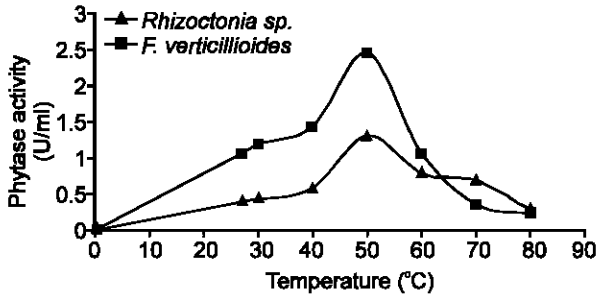

Fig. 3: Temperature versus activity profiles of phytase from *Rhizoctonia* sp. and F. *verticillioides*

Enzymatic characteristics: Phytase production in culture of *Rhizoctonia* sp. and *F. verticillioides* by submerged fermentation reached its stationary growth phase after cultivation for 24 h. During exponential growth phase, its produced small amounts of phytase. Phytase production occurred in late stage of the exponential growth phase and the activity phytase increased gradually with increasing incubation time. Both of enzyme activity increased abruptly and reached the maximal value of 0.46 U/ml and 0.77 U/ml respectively.

pH: The phytase activity of *Rhizoctonia* sp. reached the highest point at pH 4.0 for 15 min and phytase activity of *F. verticillioides* at pH 5.0 (Fig. 2).

Temperature: Both fungal phytase displayed maximum activity were at temperature of 50°C (Fig. 3). At pH 8.0 and at temperature 80°C almost all enzymatic activity of both fungal were lost.

DISCUSSION

Endophytic fungal phytase was isolated from leaf, stem and root fragments sample of soybean and identified as *Rhizoctonia* sp. and *F. verticillioides* was identified for further study. This is the first report of *Rhizoctonia* sp. and *F. verticillioides* are extracellular phytase and exhibiting high phytase activity. The phytase synthesis in *Rhizoctonia* sp. and *F. verticillioides* by phytate in the culture medium. It may be concluded that only phytate induced these enzymes. Many other phytase producing strains as *Eschericia coli*, *Pseodomonas* sp and *Raoutella* sp. also were induced by phytate (Shah and Parekh, 1990; Konietzny and Greiner, 2004). Enzyme induction is due to physiological change in a whole microbial population and it involves an accelerated rate of renewed formation of enzyme in response to a relatively specific nutritional stimulus (Rhodes and Fletcher, 1966). The phytase was induced early phase of cultivation. It seems that the phosphates are released from phytate.

Phytase often has a low-pH optimum range (pH 4.5-6.0) with a rapid drop in activity at pH value above 6.0. Yeast phytases also have an optimal range 4.0-5.0 (Cao *et al*., 2007; Quan *et al*., 2001; Nakamura *et al*., 2000). The phytase in both *Rhizoctonia* sp. and *F. verticillioides* have pH 4.0 and 5.0 respectively and most stable at pH range 2.0-7.0. were very compatible with the internal environment of monogastric animals' stomach such as in pigs and poultry. Compared to many other phytase producing strains which exhibit low enzyme activity at pH values associated with the upper digestive tract, the *Rhizoctonia* sp. and *F. verticillioides* phytase activity is significantly higher, reaching levels of commercial acceptability. The optimum temperature of the these phytase did not reveal differences between phytase from *Aspergillus niger* N-3 and Natuphos phytase, the latter exhibiting maximum activity at 50°C (Martin *et al*., 2005). At present, a major drawback to the widespread use of phytases in animal feed is the constraint of thermal stability required for these enzymes to withstand inactivation during the feed-pelleting or expansion processes (Cao *et al*., 2007). Both of phytases exhibited maximum activity as high as Natuphos and pGP209 phytase, were at 50°C (Martin *et al*., 2005). This phytase reached levels of commercial acceptability. This phytase is worthy of further research as retains activity over a wide range of pH values characteristic of digestive tract and could conceivably be more suited to increasingly higher feed processing temperatures currently employed in the animal feed industry.

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Barymetries Formulos and Control of Growth of Breed Cattle at Dihessé Breeding Farm in Congo Brazzaville

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Abstract: The follow-up of the growth of the Ndama bovines through the barymetric formulas has feta approached with Congo Brazzaville, in the Technical Center of Support of Dihessé which aims at the multiplication and diffusion of the animals in the country medium and the repopulation of the official breeding. This study makes a development of the barymetric equations of females and males of Ndama of birth in 20 months of age. By age group, the thoracic perimeter (Pt) and the live weight (PV) have collected for 253 males and 405 females. The males are born with an average weight from 19.5±0.5 kg and females with 18.14±0.5 kg (td> 1.96). In the 16th month the live weight is not different but significant (114.5±10 kg and 90.1±5 kg). The thoracic perimeters were 62.2±6 cm and 60.7±11 cm with the birth and 110.5±3 cm and 103.8±6 cm in the 16th month (td>1.96).The coefficients of correlation enters the perimeter thoracic and the live weight is higher than 0.80 in the three phase of growth 'observed. This evaluation corresponds more to one series of segments of straight regression line. The equations of regression are relatively higher in the males than in the females (Y = 0.797 X-5.741 et Y = 1.899 X-111.918) to 10 months of age.

Key words: Cattle, growth, barymétry, Congo

INTRODUCTION

The majority of the bovine breeding in Congo Brazzaville have like main objective the production of meat. The meat, part consumable of the carcass is the result of the growth and the development of animal (Soltner, 2001). Facing to the growth of the needs and the constant one unbalanced between offer and asks it, with that the blaze of the prices of the foodstuffs has added, appeared for a few years the d' need; to improve the performances of growth of the pets so to increase the livestock and the number by kilograms of meat per unit of area. The control of the weight of the young animals prove necessary birth until with the adult weight; what allows the follow-up of the Ponder growth of the young animals until with adulthood and identification of the possible ponder disturbances (Coulomb, 1976; Delage *et al*., 1955). This operation particularly constitutes the base of any improvement of the livestock of the beef breeds. This knowledge of weight allows the proportioning of the drugs and rationing according to the weight and also appreciation of the precocity of the young animals (Coulomb, 1976; Huxley, 1932; Johansson and Hildeman, 1954). Thus we initiated the method estimate of ponder growth of weight with the help of the barometric formulas are equations of prediction in the center d' technical support of dihessé, a center creates for the multiplication and the diffusion of the animals in the breeding of Congo thanks to the Prodder project, initiated by Congolese state.

MATERIALS AND METHODS

Area study and pastures: The center is located in the oriental party of the plain of Niari, under the low Congolese climate characterized by one season of rains (October to May) and a dry season (June at September). The season of rains is intersected a small season dries between January and February (Samba, 2002). A formation schistocalcaire gives to the center an argillaceous heavy ground more or less satirized (Gattolin, 1986), on which it has 20.000 hectares of shrubby savanna, with Hyparrenia prevalence will diplandra, forming a natural pasture of variable quality C following the seasons (Diamouanga, 2001; Samba, 2002). Several courses of' water with permanent mode sprinkle this plain and constitute for the cattle of the sources; watering and of the corridors of pasture and service. The center exploits 1800 hectares for a livestock of 677 bovines of race Ndama trypanotolerante, that is to say 0.37 bovine per hectare and year. The rotations of pasture coupled with the practice of fires allow to obtain, even in season dries of the fodder of quality (Landais, 1983). The value of this pasture varies from 1600-2000

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UF/ha and 4 with 12 kg of MAD/ha, according to the seasons and especially from the vegetative state (Boudet, 1984).

Control of the herd: The animals graze all the day and the evening they join the park of night or sorting where they undergo various treatments and prophylactic handling. The cattle population of the ranch occurs in endogamy. It set out again in small herds according to the physiological state of the animals. Go up are directed have rate/rhythm of the returns of the colors. The villages are spread out throughout the year. The first calf cows are observed towards 36 months age. A food complement composed of mixed is of sound of corn is distributed in the park of night, especially in dry season when the vegetation decreases. Stone to be licked is distributed permanently like source of rock salt and vitamins, and also to preserve the docility of the animals and to facilitate their control. This stone to be licked is consisted of rock salt, limestone, the vitamins and cement.

Data-gathering: A population of 219 animals, including 80 males and 139 females from 0-19 month, constituted the biological material of this study. The weighting took place with 0, 6, 12 and 16 month age. Only the Thoracic Perimeter (Pt) is retained in this study like measurement of work because of the facility of measure to the village breeding and its more convenient linear adjustment and more precise with the Live Weight (statement) according to certain authors (Delage *et al*., 1955; Poivey *et al*., 1980). This thoracic perimeter is measured with help of; a ribbon circumference with the passage of the straps (Symoens and Hounsou-Ve, 1991). The statistical analysis of the classified data by sex, by age is made with the Excel software (c) and Stat View (c). What license: - the establishment of the curves of ponder growth and the evaluation of the thoracic perimeter of the animals; - the study of connection thoracic weight sharp-perimeter by simple correlation then by regression linear (PV = aPT+b) and regression logarithmic curve (attention they are not the same coefficients, between the simple linear regression and the passage to the logarithm LnPV = aLnPT + b).

RESULTS AND DISCUSSION

Evolution of live weight and thoracic perimeter: The live weights and the average thoracic perimeters as well as the standards errors be the standard deviations calculated starting from the couples of data (PV-PT) of the animals are presented to the Tableau 1. Like all the data of ground, those unequally set out again in the age classes, with coefficients of variation going from 4-30%. The males are born with an average weight from 19.59±0.50 kg and the females with 18.14±0.52 kg, the difference not being significant (td<1.96).

At 6 months, the males reach 66.58 kg then 82.22 kg in 12 months and 114.50 kg in 16 month; while the females have 61.80 kg in 6 months, 79.31 kg in 12 months and 90.10 kg with 16 month of age. The variations of live weight between males and females in 6 months and 16 month are significant with the threshold of 5% (td<1.96) birth in the 16th month. Weights of birth of 17.7 kg for the males and 16.7 kg for the females of race Ndama; rammed were recorded at the research centre zoo technical of Minankro in Ivory Coast (Coulomb, 1976). These calves reach respectively, with the age of a year 129.7 kg for the males and 102.7 kg for the females; then, at two years: 227.7 kg for the males and 109.9 kg of live weight for the females. Weaker weights of birth at the Ndama bovines; rammed villagers herds of the north of the Ivory Coast respectively: 15 and 14 kg in the males and the females (Landais, 1983) were recorded. The growth of the bovines of our study is thus in the average of that of the of the same animals race. Their weights of birth are slightly higher, which could be a characteristic of Ndama Central Africa, since weights of birth from 20-25 kg were observed village of the democratic republic of Congo (former Zaire) (Schmitz, 1985). In Mali, the animals which are born to 13 kg reach 63 kg of live weight in 7 months. The conditions d' breeding of these animals are certainly element of difference in this ponder growth. With that the technique of barometry used adds which often can miss precision. That can also depend on the growing period during which measurements were taken (Boudet, 1984; Landais, 1983). The established curves of growth (Fig. 1), present in the two sexes an evolution in teeth of saw that is to say, a synchronous succession of ascending segments, particularly in the section ages of 4-7 months. But this growth of blows by blows is also characteristic of the animals in difficult medium because it mainly reflects the strong interactions between genetic factors and factors of environment such as the climate, the vegetation and the technique of control and food with weaning. It is possible to note two significant inflections: towards the age of 7 then in 10 months, subdividing the postnatal growth of the bovines in three phases: from 0-7 months, the curve is overall ascending with profits of weight of 249.5 g/j at the males and 224.33 g/j in the females. This catch of fast weight must be caused by mother's milk and can be with the good vegetation which allows the starting of weight; especially that weaning intervenes that around 9 months. From 8-10 months the curve presents a plate: a deceleration of growth certainly caused by the reduction in the maternal dairy production whereas the calves are not yet able to make up the deficit by an additional consumption of fodder. From 11-16 month at the males and month with 19 mois in the females, it occurs a renewal of growth which seems to indicate that these animals become ready to find their food in this medium after the stress of with weaning.

Table 1: Live weight and thoracic perimeter of ndama cattle

	Male			Female		
Age (month)	n	PV (kg) M±m σ	PT (cm) M±m σ	n	PV (kg) M±m σ	PT (cm) M±m σ
0	44	19.59±0.50 (3.36)	62.25±0.52 (3.51)	50	18.14±0.52 (3.72)	60.70±0.59 (4.22)
1	36	29.51±0.99 (5.97)	71.66±0.81 (4.89)	38	25.80±0.84 (5.20)	68.05±0.72 (4.46)
2	26	36.32±1.65 (8.43)	76.50±1.22 (6.27)	21	29.85±1.07 (4.94)	72.80±0.97 (4.47)
3	15	42.20±3.26 (12.66)	81.20±2.06 (7.98)	13	40.07±2.46 (8.90)	79.76±1.67 (6.04)
4	6	53.66±5.42 (13.29)	87.66±2.91 (7.15)	6	43.16±4.18 (10.26)	81.66±3.37 (8.27)
5	5	54.33±4.65 (10.42)	88.32±2.80 (6.28)	4	44.00±6.13 (12.26)	81.75±3.57 (7.15)
6	2	66.58±4.41 (6.25)	95±00 (00)	5	61.80±8.87 (19.85)	91.20±3.93 (8.81)
7	5	72±5.01 (11.22)	96.60±2.47 (5.53)	4	65.25±7.03 (14.07)	94.75±3.97 (7.94)
8	7	72.14±2.33 (6.17)	99±1.16 (3.07)	10	70.70±6.60 (20.90)	96.30±3.36 (10.64)
9	11	72.18±5.01 (16.63)	96.54±2.41 (8.02)	25	70.60±4.87 (24.37)	97.12±2.28 (11.42)
10	19	73.05±3.55 (15.50)	97.15±1.26 (5.50)	35	75.31±4.42 (26.20)	97.88±1.94 (11.51)
11	21	84.75±5.15 (23.64)	101.52±2.11 (9.69)	39	77.41±3.68 (22.99)	97.88±2.90 (18.16)
12	18	82.22±4.98 (21.13)	100.61±1.83 (7.97)	35	79.31±3.68 (21.78)	100.37±1.53 (9.07)
13	14	87.42±4.10 (15.37)	103.57±1.82 (6.84)	37	75.70±3.21 (19.56)	98.70±1.47 (8.96)
14	13	88.30±5.42 (19.57)	104.46±2.25 (8.12)	35	81.22±3.39 (20.08)	100.54±1.47 (8.72)
15	9	90.66±6.38 (19.16)	106.33±2.39 (7.19)	24	87.16±3.62 (17.78)	102.83±1.62 (7.95)
16	2	114.50±10.96 (15.50)	110.50±3.18 (4.50)	10	90.10±5.64 (17.86)	103.80±2.20 (6.98)
17				6	98.50±7.60 (18.62)	107.11±2.91
18				5	94.40±5.98 (13.39)	107.20±1.98 (4.44)
19				3	103.66±10.92 (18.92)	112.33±3.40 (5.90)

N = Nombre de bovines par classe; M = moyenne arithmétique; ± m = erreur standard; σ = écart type

The conditions prevailing breeding of M'Passa do not allow a total expression of the ponder capacities of the Ndama bovines; rammed. The males presented 62.25±0.52 cm of turn of chest to the birth, 95±00 cm in 6 months, 100.61±1.83 cm in 12 months and 110.50±3.18 cm in 16 month, against 60.70±0.59 cm with the birth, 91.20±3.93 cm in 6 months, 100.37±1.53 cm in 12 months and 103.80±2.20 cm in 16 month, for the females. The Ndama bovines are generally animals of intermediate size with turns of chest oscillating around 120-130 cm in 2 months ages (Coulomb, 1976; Planchenault *et al*., 1984). Figure 2 shows an evolution of the thoracic perimeter comparable with that of sharp pea: even general pace, relative superiority of the males on the females with deviation always no significant. In spite of the number very disparate of the data by age class and of the work conditions in the center, which are far from the rigor of control in station, these studied measurements keep real indicative characteristics of the animals and conditions of their breeding? These Ndama bovines; rammed are of small size with slow growth (Coulomb, 1976; Planchenault *et al*., 1984; Planchenault *et al*., 1986). Linear regressions and logarithmic curves of the live weights on the thoracic perimeters by phase of growth Linear regressions and logarithmic curves were applied to this classified data by phase of growth to obtain the barometric formulas of Table 2 and 3. The 658 couples of collected data translate a connection LW-TP very strong, as well in the males as in the females. Those vary with l' age but do not seem to be influenced by the sex of the animals. The coefficients of correlation regularly increase birth in

Table 2: Linear regression of live weight (Y) to thoracic perimeter (X) by growth phase

Age	Sex	N	PV (cm)	PT (cm)	Coefficient de corrélation (r)	Regression equations Y = a X + b
Birth	M	44	19.59±0.50 (3.36)	62.25±0.52 (3.51)	0.83	Y = 0.872 X-34.735
	F	50	18.14±0.52 (3.72)	60.70±0.59 (4.22)	0.86	Y = 0.821 X-31.748
1-7 Months	M	90	38.42±1.55 (14.73)	77.62±0.98 (9.37)	0.93	Y = 1.524 X-79.885
	F	91	34.43±1.50 (14.38)	74.76±1.01 (9.10)	0.90	Y = 1.412 X-71.143
8-10 Months	M	37	71.94±2.19 (13.34)	97.94±1.20 (7.34)	0.84	Y = 0.797 X-5.741
	F	70	73.07±3.01 (25.25)	97.37±1.36 (11.45)	0.74	Y = 1.899 X-111.918
11-19 Months	M	78	86.50±2.37 (21.00)	103.02±0.97 (8.63)	0.85	Y = 2.242 X-144. 529
	F	194	81.52±1.53 (21.38)	101.06±0.67 (9.40)	0.88	Y = 2.133 X-134.107

Table 3: Logarithm regression of live weight LnY to thoracic perimeter Ln X by growth phase

Age	Sex	N	Ln PV	Ln PT	Coefficient of correlation	Equation de regression LnY = aLnX + b
Birth	M	44	2.961±0.025 (0.17)	4.129±0.007 (0.05)	0.86	LnY=2.840LnX-8.769
	F	50	2.881±0.028 (0.20)	4.103±0.009 (0.07)	0.89	LnY= 2.742LnX-8.373
1-7 Months	M	90	3.587±0.035 (0.34)	4.344±0.011 (0.11)	0.94	LnY= 2.861LnX- 8.842
	F	91	3.470±0.036 (0.35)	4.307±0.012 (0.12)	0.91	LnY= 2.768LnX-6.570
8-10 Months	M	37	4.258±0.031 (0.19)	4.581±0.011 (0.07)	0.84	LnY = 2.363LnX- 6.570
	F	70	4.229±0.043 (0.36)	4.571±0.013 (0.11)	0.70	LnY= 2.569LnX-7.515
11-19 Months	M	78	4.430±0.027 (0.24)	4.631±0.009 (0.08)	0.86	LnY= 2.756LnX- 8.334
	F	194	2.686±0.153 (2.15)	2.834±0.160 (2.24)	0.99	LnY = 3.043LnX-9.744

19 month while passing by a maximum on the phase from 8-10 months, then an unquestionable increase in the last phase. This evolution corresponds to a series of straight regression lines with a model curvilinear; connection LW-TP appears stronger in the ascending phases of the curve of growth. The higher coefficient of correlation during lactation than during translated weaning can be the fact that for certain animals weaning does not occur very well like the habituation with bleaches on grass. The calculated coefficients of correlation are always higher than 80.0. Apart from the phase from 8-10 months, the coefficients of regression of the two types; equations are relatively higher in the males. That wants to say that for females even thoracic variation of perimeter, the males tend to gain plod weight that the females. The difference remaining despite everything no significant. The barometric formulas suggested remain valid for the bovines of Dihessé and in the village herds where the same conditions prevail; breeding. They are simple tools in their handling and useful, fault of adequate equipment, to follow the two-phase or three-phase growth of the bovines. They make it possible to pose the preliminary acts of selection without risk to be mistaken. Growth of these bovines having to rectify itself with the improvement of the breeding conditions, a constant reactualization of these equations will allow to increase their effectiveness. The period of growth of this rustic race of the tropical mediums is prolonged until 4 years as also certain authors (Coulomb, 1976; Gattolin, 1986) report it. This study thus relates to the phase of rapid growth, characterized by a deceleration during the time of weaning. Natural pastures and stone to lick sufficient step to meet the needs for growth. It is possible to remove inflection of 7 months growth with an adequate food and to quickly reach 150 kg of live weights required to the heifers for their setting with the reproduction and that can decrease significantly; age of first projection fixed at 30 months. The organization of the reproduction and the improvement of food should push the bovines with a better externalization of their genetic potentialities. The production; simple tools for effective control of

growth in the villagers herds is possible by mathematical exploitation of relation LW-TP. since weights of birth from 20-25 kg were observed village of the democratic republic of Congo (former Zaire) (Schmitz, 1985). In Mali, the animals which are born to 13 kg reach 63 kg of live weight in 7 months. The breeding conditions of these animals are certainly element of difference in this ponder growth. With that the technical of barometry used adds which often can miss precision. That can also depend on the growing period during which measurements were taken (Boudet, 1984; Landais, 1983). The established curves of growth (Fig. 1), present in the two sexes an evolution in teeth of saw that is to say, a synchronous succession of ascending segments, particularly in the section ages of 4-7 months. But this growth of blows by blows is also characteristic of the animals in difficult medium because it mainly reflects the strong interactions between genetic factors and factors of environment such as the climate, the vegetation and the technical of control and food with weaning. It is possible to note two significant inflections: towards the age of 7 then in 10 months, subdividing the postnatal growth of the bovines in three phases: from 0-7 months, the curve is overall ascending with profits of weight of 249.5 gram a day (g a day) at the males and 224.33 g a day in the females. This catch of fast weight must be caused by to the mother's milk and can be with the good vegetation which allows the starting of weight; especially that weaning intervenes that around 9 months. From 8-10 months the curve presents a plate: a deceleration of growth certainly caused by the reduction in the maternal dairy production whereas the calves are not yet able to make up the deficit by an additional consumption of fodder. From 11-16 month at the males and until with 19 months in the females, it occurs a renewal of growth which seems to indicate that these animals become ready to find their food in this medium after the stress of with weaning. The conditions of breeding prevailing at dihesse do not allow a total expression of the ponder capacities o the Ndama bovines; rammed. The males presented 62.25±0.52 cm of turn of chest to the birth, 95±00 cm in 6 months, 100.61±1.83 cm in 12 months and 110.50±3.18 cm in 16 month, against 60.70±0.59 cm with the birth, 91.20±3.93 cm in 6 months, 100.37±1.53 cm in 12 months and 103.80±2.20 cm in 16 month, for the females. The Ndama bovines are generally animals of intermediate size with turns of chest oscillating around 120-130 cm in the age of 2 months (Coulomb, 1976; Planchenault *et al*., 1984). Figure 2 shows an evolution of the thoracic perimeter comparable with that of sharp pea: even general pace, relative superiority of the males on the females with deviation always no significant. In spite of the number very disparate of the data by age class and of the work conditions in the center, which are far from the rigor of control in station, these studied

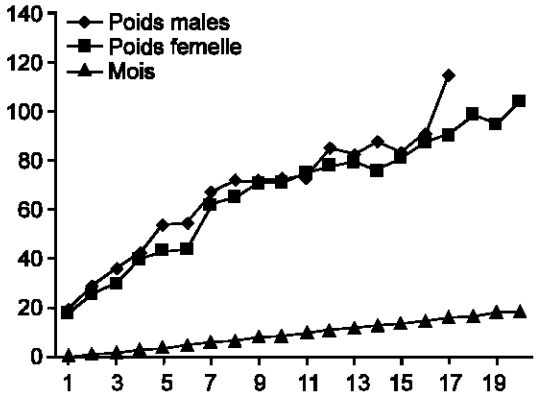

Fig. 1: Growth curves of ndama cattle

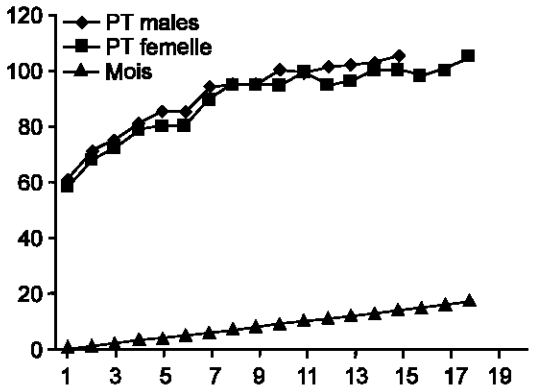

Fig. 2: Thoracic perimeter of ndama cattle

measurements keep real indicative characteristics of the animals and conditions of their breeding. These Ndama bovines; rammed are of small size with slow growth (Coulomb, 1976; Planchenault *et al*., 1984; 1986).

Linear regressions and logarithmic equations of the live weights on the thoracic perimeters by phase of growth: Linear regressions and logarithmic curves were applied to this classified data by phase of growth to obtain the barometric formulas of Table 2 and 3. The 658 couples of collected data translate a connection LW-TP very strong, as well in the males as in the females. Those vary with age but do not seem to be influenced by the sex of the animals. The coefficients of correlation regularly increase birth in 19 month while passing by a maximum on the phase from 8-10 months, then an unquestionable increase in the last phase. This evolution corresponds to a series of straight regression lines with a model curvilinear; connection LW-TP appears stronger in the ascending phases of the curve of growth. The higher coefficient of correlation during lactation than during translated weaning can be the fact that for certain animals weaning does not occur very well like habituation with bleaches on grass. The calculated

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Investigating the Quality Changes of Raw and Hot Smoked *Oreochromis niloticus* and *Clarias lazera*

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Abstract: This study deals with the effect of hot smoke curing by using two types of hard wood (*Acacia seyal* and *Citrus lemon*) on the proximate composition, microbial load and sensory evaluation of two species of fresh water fishes (*Oreochromis niloticus* and *Clarias lazera*). Highly significant differences ($p<0.05$) were observed on the proximate composition between the two species and the two types of wood, the percentage of total protein, lipid and ash contents increased, due to the decrease in the moisture content to the range 64,15±0.130 and 54.42±0.173%, respectively. The total viable counts of bacteria in fresh fish used as raw material (*Oreochromis niloticus* and *Clarias lazera*) were 281.5 x 10^3 and 183.7 x 10^3, respectively. After smoking the total viable counts of bacteria of *O. niloticus* reached 2 x 10^3, while of *Clarias lazera* was reduced to 6 x 10^3. The bacteria isolated before and after smoking was *Staphylococcus aureus*. With respect to the quality of the products related to colour, taste, texture and flavour, significant differences ($p<0.05$) in the parameters measured were observed, generally the products well accepted.

Key words: Hot smoke, hard wood, protein

INTRODUCTION

Fish is a very important food stuff, especially in developing countries, due to its high protein content and nutritional value of unsaturated fatty matter. However fish is greatly perishable, quality losses might occur very rapidly after catch, especially in hot climates and tropical areas where cold preservation techniques are often missing. Traditional fish processing, such as salting/brining, drying, smoking, allow better preservation and storage and increase fish availability to the consumers.

The waters of Sudan (100.000 km^2 fresh water and 750 km length of coastal marine waters on the Red Sea) have been fished for centuries by many generations. It has been estimated that 26000-29000 tons of fish have been taken from them annually (Yousif, 1988). This represents about 29% of the estimated annual potential i.e. 104,000 tons (Henderson, 1975). More recent estimates of production were in the range of 60,000 ton/year (Federal Fisheries Administration Department, Annual Reports). In the Sudan, nearly 70% of the total fish landings are consumed fresh; the rest is cured either by salting, fermentation or sun-drying. Some of the local fish supply is smoked in southern Sudan where smoked and very dry fermented fish products are very popular among the local community (FAO, 1992).

Smoking is the process of applying wood smoke to impart a smoky or smoked flavour and to partially dry a fish, or part of fish such as fillets, to produce a smoked fish product and also to extend the shelf life of the product under some conditions. In many parts of the world, preservation is still the main purpose of smoking. Any preservative effect of the smoke itself is probably largely due to the presence of a range of phenolic compounds, nitrites and formaldehyde.

The objective of this work is to carry out a comparative study on the use of two types of wood (*Acacia seyal* and *Citrus lemon*) to smoke two types of fish (*Oreochromis niloticus* and *Clarias lazera*) using one type of oven (steel), to conclude and recommend on better smoking of the fish.

MATERIALS AND METHODS

Sampling: Fresh fish of this study were purchased from Jebl-Aulia reservoir, 45 Km south of Khartoum. Fifteen samples of *Oreochromis niloticus* and ten samples of *Clarias lazera* were collected in polyethylene bags (Cold stored) and transported (early morning) to the Fisheries Research Center (Shagara), where fresh samples from raw material, for chemical and microbiological analyses were immediately prepared.

Processing: The pre-smoking process included washing of fresh fish, which was eviscerated; washed again and transferred to baskets to dry up while a thin cloth cover was placed in order to keep away insects. Then fishes were weighed to the nearest gram using a dial balance (KRUPS type 875).

Oreochromis niloticus and *Clarias lazera* were smoked using steel kiln whose fuel was composed of *Acacia*

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seyal and *Citrus lemon* weighing 14.6kg and 13.0kg respectively. Best quality products were obtained according to the following process regimes.

Brining in 10% brine for 15 min, drying phase for 30 min at 30°C; cooking phase 30 min at 60°C and the intensive smoking phase 30 min at 80°C, smoked samples were removed from the kiln and exposed to air to cool and dry.

Chemical analysis: Moisture, protein, fat, NaCl and ash contents were determined according to AOAC (1980).

Microbial examination: Microbial examination of the smoked fish was carried out by the following standard methods.

Total Viable Counts (TVC): Using Pour plate technique as described by Harrigan and MacCance (1976), dilution from 10^{-1} to 10^{-6}, in Nutrient agar and incubated at 37°C for 24 h. Colonies were counted by making the colony on the opposite side of the plate on its position in the colonies counter apparatus.

Isolation and identification of colonies: The samples of fish were first inoculated in nutrient Broth medium and incubated at 37°C for 24 h. In MacConkey's agar and blood agar were cultured from Broth medium by streaking method and incubation at 37°C for 24 h of the samples were done to isolate a single colony. The identification of purified isolates was carried out according to Cowan and Steel (1974).

Statistical analysis: The mean and standard deviation (mean ± SD) for the obtained results were calculated using SPSS soft ware (Version 10).

Organoleptic assessment: End products were submitted to 20 people test panel from Fisheries Research Center staff, fishermen and some students of Department of Fisheries, College of Natural Resources, University of Juba and judged in comparison of smoked fish. Comparison was carried out in terms of organoleptic characteristics, such as colour, flavour, taste and texture. The panel was requested to rate each organoleptic feature of the end products according to a 10 point scale (9 = excellent; 8-9 very good; 6.5-7.9 good; 5-6.4 fair; <5 bad), using the score method as reported by (Afolbi *et al*., 1984).

RESULTS AND DISCUSSION

Proximate composition of fresh fish: The proximate composition of *Oreochromis niloticus* and *Clarias lazera* are given in Table 1. In the two species studied, although *Oreochromis niloticus* showed higher average of moisture, ash content, protein and lower average of fat than *Clarias lazera* results revealed no significant differences ($p>0.05$). Similar results were obtained by various researchers, namely Thurston (1962) who worked on two subspecies of lake trout, Awouda (1984) on two species including *O. niloticus* and Mahmmoud *et al*. (1989) on *Eutropius niloticus*.

Table 1: Proximate composition (g/100 g) of two freshwater fish species

Fish species Parameters	*Oreochromis niloticus*	*Clarias lazera*
Moisture	75.1±2.740	70.0±0.740
Ash	2.1±0.358	1.8±0.10
Protein	19.8±0.10	15.0±0.368
Fat	1.5±0.10	2.1±0.10

Note: Values represent pooled means and standard deviations of triplicate determinations of wet weight

The high moisture contents recorded for the two species are comparable to that reported in other fresh water fish species such as *Alestes nurse, A macrolepidotus*, *Hydrocynus brevis* and *Hepsetus odoe* (Abdullahi, 2000a), *Labeo coubie, L senegalensis* and *Barbus occidentalis* (Abdullahi, 2000b).

The crude protein content was 19.8% and 15% on wet bases for *Oreochromis niloticus* and *Clarias lazera* respectively. This is probably due to the high moisture content. These results agree with those obtained by other investigators for common Nile fishes, (Mahmmoud, 1977; Iskander, 1982; Ssali, 1988).

Crude lipids contents were slightly higher in fresh *Clarias lazera* 2.1% than in *O. niloticus* 1.5% on wet basis. It can be seen that both species belong to the category of low fat classified by Ackman (1989) having fat content below 5%.

Ash content was slightly higher in fresh *O. niloticus* 1.8% than in *Clarias lazera*, 1.0%. These results are in accordance with those obtained by Mahmmoud (1977) and Ssali (1988).

Proximate composition of hot smoked cured products: Table 2 shows the percentage of proximate composition (w/w) of two species treated by two types of wood for smoking. In the corresponding smoked products, the percentage of total protein, lipid and ash contents increased due to water loss during smoking. Similar findings were reported by Aminullah Bhuiyan *et al.* (1986) in Atlantic mackerel and Unlusayin *et al*. (2001) in European eel, pike perch and rainbow trout. Industrial specifications for "smoked finished products" generally is recommended with water content in the fish flesh of less than 65% (Cardinal *et al*., 2001). Goulas and Kontominos (2005) reported that the moisture content of smoked chum Mackerel samples were 58.1 and 59%. Kolodziejska *et al*. (2002) also reported that moisture content of smoked Mackerel was 56.7%. This is in agreement with our result of 60.78% for *C. lazera* and 62.35% for *O. niloticus* moisture content.

Table 2: Proximate composition (g/100 g) of two smoked freshwater fish species treated by two types of wood

Fish species	*Oreochromis niloticus*		*Clarias lazera*	
Parameters	*Acacia seyal*	*Citrus lemon*	*Acacia seyal*	*Citrus lemon*
Moisture	62.3±0.183[b]	61.4±1.105[c]	54.42±0.173[d]	64.15±0.130[a]
Ash	8.58±0.222[c]	7.25±0.129[d]	9.74±0.149[b]	12.63±0.174[a]
Protein	22.15±0.129[c]	21.15±0.129[d]	23.15±0.129[a]	22.55±0.129[b]
Fat	5.23±1.296[d]	7.33±0.129[b]	6.23±0.136[c]	9.03±1.296[a]
NaCl	0.435±0.175[b]	0.658±1.296[a]	0.263±0.231[d]	0.375±0.231[c]

*Values represent pooled means and standard deviations of triplicate determinations of wet weight.
**Values with different superscript letters horizontally in rows are significantly different ($p<0.05$)

Table 3: Total viable counts (cfu/gm) and bacteria isolated from fresh and smoked fish sample

	Oreochromis niloticus		*Clarias lazera*		
Fish species	Fresh	Smoked	Fresh	Smoked	Bacteria species
Wood type	281.5 x 10^3	-	183.7 x 10^3	-	*Staphylococcus aureus*
Acacia seyal	-	-	-	6 x 10^3	*Staphylococcus aureus*
Citrus lemon	-	2 x 10^3	-	-	*Staphylococcus aureus*

Table 4: Means sensory scores of organoleptic tests of smoked fish using two types of wood

Fish species	*Oreochromis niloticus*		*Clarias lazera*	
Parameters	*Acacia seyal*	*Citrus lemon*	*Acacia seyal*	*Citrus lemon*
Colour	7.450±0.129[b]	7.150±0.129[b]	8.150±0.129[a]	8.135±0.175[a]
Texture	9.250±0.129[a]	8.950±0.129[a]	8.02±1.231[b]	8.150±0.129[b]
Flavour	7.435±0.358[b]	6.650±0.129[d]	7.850±0.407[a]	7.033±0.129[c]
Taste	7.250±0.129[d]	8.178±0.171[a]	7.950±0.129[c]	8.150±0.129[b]

*Values with different superscript letters horizontally in rows are significantly different ($p<0.05$)

Also from the result presented Table 2 shows that the increase in the crude protein was about 7.85% for *Clarias lazera* and 2.45% for *O. niloticus*. The variation of crude protein is highly significant in difference ($p<0.05$) between the two species. The impact of species is a highly effective factor, as both species were subjected to the same condition during processing, this means that the *Clarias lazera* muscle are susceptible to lose more water than the *O. niloticus* (Doe, 1998).

Salt content increased in the two species and significant differences ($p<0.05$) were recorded with respect to wood type as well as fish species.

The increase in ash content was correlated mostly with the increase in salt content during the smoking process. Ash content of *O. niloticus* and *C. lazera* was 2.1% and 1.0% (on wet basis), respectively, before processing, while after smoking, it increased to average 7.91% and 11.18% respectively. Karra (1978) found that ash content increased in dogfish from 1.19-4.75% after smoking.

Changes in the microbial load as affected by processing: The results presented in (Table 3) show the Total Viable Counts (TVC) as well as bacterial species. The results revealed that the flesh of the *O. niloticus* had a total microbial load reaching 2 x 10^3 and 6 x 10^3 per gram for *C. lazera*. Shewan (1952) reported that the effect of salt was due to the restriction of microbiological activity as a result of the decrease in water content of the tissues and to the direct action of NaCl on the putrefactive micro-organisms. Karra (1978) reported that smoking caused a decrease in total microbial count by an average of 94.7% of the original number in dogfish fillets.

Table 3 shows that no coliforms were detected in the two species before and after smoking, no pathogenic micro-organisms like *E. coli* and Salmonella were found. However, other microflora isolated was *Staphylococcus aureus*.

Sensory evaluation of smoked products: Sensory evaluation results of smoked products are presented in Table 4. The mean scores showed that all the characteristics of the products were moderately liked. Overall acceptability mean scores indicate that the products were generally well accepted. However, the *C. lazera* treated by *Citrus lemon* appeared to be more acceptable of all the products.

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Effects of Age and Method of Drying on the Proximate Composition of Housefly Larvae (*Musca domestica* Linnaeus) Meal (HFLM)

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Abstract: The effects of age at which House Fly Larvae (HFL) is harvested and method of drying the larvae, on its proximate values were studied. Larvae were harvested on three different days representing three different ages and the harvested larvae were dried using both oven and sun light. Results revealed that protein content of HFL processed into meal after drying significantly ($p<0.05$) reduced as the age of larvae increased from 55.4% on 2 day old, through 50.2% on three-day old, to 47.1% on four day old. On the other hand, fat content increased with increase in age of HFL, from 20.8% at age of 2 days, through 22.2% at age of 3 days to 25.3% at the age of 4 days. Fibre content minimally increased with age. Oven-dried maggots had mean higher protein content (50.9%) and less fat (22.8%) than sun dried maggots (47 and 26.4% respectively). Therefore, for maximum protein yield, HFL should be harvested at 2 days old. However, where maggot fat is needed in a diet, increased biomass and or ease of harvesting, processing at later age (4 days old) became desirable. Oven-drying is recommended for superior protein and in rainy season, while sun drying produced higher fat and was cheaper.

Key words: Age, housefly larvae meal, oven-dried, proximate composition, sun-dried

INTRODUCTION

Housefly Larvae Meal (HFLM) like other maggot meals has been found to contain good quality protein for poultry and fish production (Sheppard, 2002; Awoniyi *et al*., 2003; Fasakin *et al*., 2003). However, there are differences in the proximate values reported by various researchers (Teguia *et al*., 2002). These differences were attributed to factors such as age, method of processing and source of maggot (Teotia and Miller, 1994; Fasakin *et al*., 2003, Teguia, 2005). Fasakin *et al*. (2003) attributed the variation in crude protein contents of maggot meal (43.3-46.7%) to drying methods. Atteh and Ologbenla (1993) attributed variation in the chemical composition of the housefly larvae meal to differences in time of harvesting.

Maggots are primarily harvested for its food value which is dependent upon their chemical composition. Since this chemical composition is effected by both age of larvae and method of harvesting, there is great need to identify the actual mature age and method of drying that will give maximum food value (proximate composition). This study is therefore aimed at identifying the optimal age at which maggots will yield maximum food value and the best drying method for processing maggots.

MATERIALS AND METHODS

About 75 kg of Whole Undiluted Blood (WUB) was mixed with 15 kg of Wheat Bran (WB) and spread equally on three open floor spaces (Treatments) of 1.44 cm^2 each, to a thickness of 3.75-5 cm and exposed for biodegradation. This was replicated 3 times. From each of the three replicates, 200 g maggots were separately harvested as follows: treatment one (T1) was harvested on day two of maggot formation, treatment two (T2) on day three while treatment three (T3) was harvested on day four. Each 200 g sample was divided into 2 equal parts of 100 g each, one part was oven dried while the other was sun dried.

Each sample was subjected to proximate analysis using the method described by the Association of Official Analytical Chemist (AOAC, 1990) to determine effects of age and drying method on their chemical composition namely; dry matter, ash, crude protein, fat and crude fibre. Nitrogen was determined using the Kjeldahl procedure, fat was determined by petroleum ether (bp 40-60^{o}C) extraction in a Soxhlet apparatus. Crude fibre determination involved dissolution of starch and protein constituents of the sample through boiling with acid and then sodium hydroxide. The residue was fibre. Ash determination was by ignition of sample at 550^{o}C to burn off organic materials. Data collected were subjected to analysis of variance as described by Steel and Torrie (1980) and means were separated using the multiple test of Duncan (1955).

RESULTS

Results of proximate analysis of HFLM harvested at 2-4 days old and processed dry weight by both sun and oven

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Table 1: Effects of larvae age and method of drying on the proximate composition of housefly larvae meal (%)

Larvae age	Drying method	Dry matter	Crude protein	Fat	Crude fibre	Ash
2 days	Ovum dried	92.7	55.4	20.8	6.2	6.23
p-value	Sun dried	92.8	51.3	23.4	6.3	6.24
3 days	Ovum dried	92.7	50.2	22.2	6.7	6.23
p-value	Sun dried	92.9	47.7	26.0	6.7	6.23
4 days	Ovum dried	92.7	47.1	25.3	7.0	6.25
p-value	Sun dried	92.9	42.3	29.7	7.1	6.26

	Analysis table (%)			
Method	Day 2	Day 3	Day 4	Mean
CP				
Oven-dried	55.4±0.053^{c}	50.2±0.0247^{b}	47.1±0.043^{c}	50.9
Sun-dried	51.0±0.14^{a}	47.7±0.038^{b}	42.3±0.743^{c}	47.0
Fat				
Oven-dried	20.8±0.141^{c}	22.2±0.138^{b}	25.3±0.35^{a}	22.8
Sun-dried	23.4±0.14^{c}	26.0±0.14^{b}	29.7±0.35^{c}	26.4

$^{a, b, c}$means within the same row with the same superscripts are significant ($p<0.05$). P = Proximate

(Table 1), revealed that protein content of HFLM significantly reduced as the age of larvae increased (55-4, 50.2 and 47.1%), while fat contents significantly increased with increased age (20.8, 22.2 and 25.3%). The result also showed that method of drying significantly influenced the proximate composition of HFLM, especially protein and fat. Oven-dried maggot had higher protein than sun dried (51:3%), but lower ether extract (20.8%) in oven-dried than the 23.4% recorded for sun-dried maggot meal.

DISCUSSION

Results of proximate analysis of samples harvested at different ages showed that fat deposit increased with larval age and this had direct effect on the crude protein content. It implied that fat content is inversely related to protein in HFLM. The result is consistent with the findings of Atteh and Ologbenla (1993) that the nearer the larvae are to pupa stage, the lower the protein content and the higher the fat content. It appears that age slightly influenced fibre content. Two days old larvae appeared tenderer with less fibre (6.2-6.32) than 4 days old larvae (7.0-7.1%)

The 2 drying methods applied in this study (oven and sun) tended to affect the fat content and thus protein. It appeared that some level of defatting took place during oven drying, which reduced fat contents and thus increased protein content.

Therefore, the wide range of percentage protein contents of maggot meal (39.0-63.0%) reported between 1971 and 2003 in previous investigations could be attributed to variations in drying methods, age of larvae, growing environment (organic matter), species of insect and method of processing (defatted or full fat). Calvert *et al.* (1971) reported 63%, Gado *et al.* (1982) 45%, Atteh and Ologbenla (1993) 39-54%, Awoniyi *et al.* (2003) 55.1% and Fasakin *et al.* (2003) reported 43.3-46%. Since, the result of this study has collaborated the earlier results, it is concluded that for a maximum protein value, maggots should be harvested on the 2nd day of maggot formation especially where protein is the major dietly need or in a diet where high fat is undesirable. However, where maggot fat is needed in a diet, harvesting at a later age becomes desirable. Similarly, oven drying produced higher protein and less fat while sun drying produced less protein and high fat. Therefore, other than economic considerations in terms of drying cost, where sun drying has comparative advantage, farmers needs and other available options could determine the choice of drying method.

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Manganese Deficiency in Bovines: Connection Between Manganese Metalloenzyme Dependent in Gestation and Congenital Defects in Newborn Calves

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Abstract: In order to determine the relationship between diet low in mineral manganese and birth of calves with bone and joint abnormalities of all complexion in the skeleton develop congenital ataxia offspring, study was conducted in nine regions of the center western of São Paulo State. Were determined the mineral composition of forage plants used for beef cattle in nine regions between the years 1984-1992 and clinical observations of animals were carried out to date in the of year 2009. The forage *Brachiaria decumbens* used in cutting farm was studied during the four seasons of the year writing the collection of sampling the twelve months of the year. We used a completely randomized design with three replicates per season and each of the nine municipalities studied, resulting in the total sample of 108 samples of forage grasses to evaluate the behavior of the macrominerals: Ca, P and Mg and trace elements Cu, Zn, Fe, Mn and Co from the analysis by atomic absorption spectrophotometry and P by molecular absorption of forage available for grazing cattle during the seasons spring, summer, autumn and winter. Still, we sampled animal tissue (liver) and soil to determine the mineral profile. The variation in averages were: Macro 0.36 g to 0.40 g% Ca, 0.05 g to 0.10 g% P, 0.14 g to 0.20 g% Mg. Microminerals: 30 ppm to 40 ppm Zn, 3 ppm to 8 ppm Cu, 0.06 ppm to 0.09 ppm Co and 40 ppm to 80 ppm Mn. Adjusting the requirements supplementation, through dry matter, all small macro: Ca, P and Mg and trace elements Cu, Co and Zn must also be supplemented second animal growth, reproduction and lactation. Trace minerals concentration in liver varied: 152.38 ppm Cu, 219.67 ppm Zn, 6.36 ppm Mn, 260.18 ppm Fe and 0.132 ppm Co. The results found here suggested that the matrix of epiphiseal growth plate cartilage was affected during embryogenesis by manganese deficiency in the diet of the animals causing reproductive malformation and birth of calves with congenital defects in the skeletal tissues manly and articulate.

Key words: Congenital disorder, teratogenic defects, malformation, joint bony abnormality, bovine

INTRODUCTION

Early workers used the adjective "trace" for those elements present in such small amounts in living tissues that they could not be measured with the methods available (Anonymous, 1969). Thus, the term "trace element" was born. Deficiency of an essential trace element results in a characteristic deficiency syndrome in a manner analogous to a specific vitamin or hormone deficiency. The deficiency syndrome is associated with specific structural, functional, biochemical, or physiological abnormalities. These abnormalities, in turn, are prevented or reversed after administration of the deficient element (Mcdowell, 1985, 1992; Hurley and Keen, 1987).

Manganese (Mn) is known for its strong causal relationship with birth congenital and teratogenic defects in animal species rabbits, guinea pigs, pigs, cattle, mice and rats (Hurley *et al*., 1958; Huan-Chang and Everson, 1967; Hurley, 1968, 1976a,b). Experimentally, researchers have promoted the birth of fetuses with abnormalities seen since the internal organs and external joints and bone structure that was extremely affected by Mn deficiency. The defect linked mainly to manganese deficiency in the diet of the parents and teratogenic changes in the structure of DNA or RNA molecule binding Mn has been diagnosed in many animal species newborn (Erway *et al*., 1970; Erway *et al*., 1971; Shrader *et al*., 1973).

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Has been possible to prove a link between the deficiency of Mn in the conception and gestation with birth of calves, mice, rabbits, mice, guinea pigs and pigs with congenital ataxia. Lawrence *et al.* (1971) studied strain of mice predisposed to pale birth of progeny with neonatal ataxia prevent the birth of newborns ataxia Mn supplementation to the mothers' diets. However, the relationship between teratogenicity and deficiency of trace elements in specific disability manganese is attributed to the effect of Mn metaloenzyme linking DNA and RNA polymerase for protein synthesis and amino sugars that will form structures mucopolysaccharides, key components of tissue chondrocyte of supply for the calcification and bone therefore stretching the bones of animals (Bell and Hurley, 1973; Shrader *et al.*, 1973; Song and Hunt, 1988). The growth plate shows flattened and there may be abnormalities in the formation of joint tissues connective (cartilage and tendons) and calcification of bone tissue can result in stunting and ataxia. If anomaly on DNA is irreversible will then be installed on the teratogenic effect of Mn on the tissues in the following generations (Hurley, 1976a,b).

The only now-proven manganese metalloenzyme is pyruvate carboxylase (Baly *et al.*, 1985a; Baly *et al.*, 1985b). Various metal ions are exchanged at the active site of enzymes and because manganese may function as an enzyme activator it is probable that it, too, interchanges with other divalent elements and consequently a manganese deficiency may be associated with other trace-element effects. These effects include increased or decreased concentrations of an element or actual exchange of the element with manganese in an enzyme. This possibility has not been thoroughly investigated. Manganese seems to be intimately involved in synthesis of protein, DNA and RNA. The DNA-manganese complex was first reported by Wiberg and Neuman (1957). They concluded from its dissociation constant that manganese binds to DNA more strongly than do other metals. Since minute quantities of manganese were detected during the isolation of RNA and DNA, it was suggested that manganese may bear a functional relationship to protein synthesis and the transmission of genetic information.

Manganese and other cations stabilize the secondary structure of DNA by their electrostatic interaction with the negatively charged phosphate group. Extensive studies relating conformation and reactivity to DNA interaction with manganese ion have recently been reported (Luck and Zimmer, 1972). The results of these investigations indicate that manganese has an important role in initiating protein synthesis by stimulating RNA polymerase and DNA polymerase activities in the mammalian system (Wiberg and Neuman, 1957; Song and Hunt, 1988).

In kwashiorkor, there was a definite correlation between decreased hepatic manganese content and decreased hepatic protein content in the protein-calorie malnutrition (Scrutton *et al.*, 1966). Thus, manganese is a specific nutrient that affects expression of the mutant gene without altering subsequent transmission of the mutation to future generations of mice (Erway *et al.*, 1971).

Effects of large proportions of the animal organism to a particular trace element such as manganese which at first seems so insignificant, often goes unnoticed in its importance on fetal development and after birth. Teratogenic effects due to disorders in the metabolism of Mn may be linked to the synthesis of RNA or DNA (Dyer and Rojas, 1965; Hurley *et al.*, 1963; Hurley, 1969; Hurley, 1976a; Hurley, 1976b; Petukhova, 1971; Hurley, 1981a; Hurley, 1981b; Shrader *et al.*, 1973; De Rosa *et al.*, 1980; Leipold *et al.*, 1983; Hurley *et al.*, 1982; Hidiroglou *et al.*, 1990; Cave *et al.*, 2008).

In the syndrome deficiency of Mn, the congenital chondrodystrophy neonatal calves, attributed to a maternal deficiency manganese. The epiphyses growth plate chondrocytes has improperly aligned and short columns and reducing the width of the zone of hypertrophy when compared to a normal calf. Examinations of numerous calves were performed and pathology focused on the musculoskeletal and joint system (Leach, 1968; Leach and Muenster, 1962; Leach *et al.*, 1969).

The manganese with atomic mass equal to 55, transition metal, in animal tissue actively participates in the formation and activation of enzymes in the body system. As co-factor in catalize enzyme or strongly linked to enzymes metalloenzymes. Enzymes avimanganin, 1 Mn atom, pyruvate carboxylase, 4 atoms of Mn and superoxidismutase, 2 atoms of Mn /mole of protein and the first two liver bird. The Mn catalyzes the formation of structures mucopolysaccharides allow the connection between carbohydrates and amino acids to form complex structures responsible for the functionality of any type of tissue where there is the composition including amino sugars noble fabrics feature in the annexes of the joints and terminal ends of the joint members in the sensory cells responsible for auditory perception present in the mastoid of the hearing aid and a host of other functional cells present in the animal organism. Chondroitin sulfate is the most affected mucopolysaccharide in Mn deficiency (Leach, 1969; Howes and Dyer, 1971). The chondrogenesis rather than osteogenesis is affected by the dysfunction of the synthesis of mucopolysaccharides.

According to Underwood (1976, 1977), the mineral in number of twenty-two elements representados por macrominerals: calcium, phosphorus, sulphur, potassium, sodium, chlorine, magnesium and trace minerals: iron, iodine, copper, manganese, zinc, cobalt, molybdenum, selenium, chromium, tin, vanadium, fluorine, silicon, nickel and arsenic are essential animal

life. Dietary imbalances of many of these elements and their interactions with other minerals are described and their functions and requirements by farm animals are outlined. For some species is included arsenic, boron, silicon and vanadium, have been essential to one or more but there is no evidence that these minerals are of practical importance in beef cattle. The function, signs of deficiency, factors affecting requirements, sources and toxicity of each essential mineral and the many interrelationships between minerals determine complex responses in the animal organism (Underwood, 1983). Mineral nutrients are either positively charged (cations) or negatively charged (anions). Cations are derived from metals, including calcium, cobalt, chromium, copper, iron, magnesium, manganese, molybdenum (as molybdate), potassium, selenium (as selenate), sodium and zinc. Non-metallic elements yield anions: iodine as iodide; sulfur as sulfate; phosphorus as phosphate; chlorine as chloride and fluorine as fluoride. Combinations of anions and cations yield salts such as sodium chloride, calcium phosphate and sodium iodide. The cation manganese is mineral nutrients with positively electric charge (Underwood and Mertz, 1987). Such “borderline” deficiencies are both the most costly and the most difficult to manage, often go unnoticed and unrectified, and yet they may result in poor and expensive gains, impaired reproduction, or depressed production (Conrad *et al*., 1985; Underwood, 1981).

The NRC requirements often do not take into account that in disease, certain minerals are needed at higher-than-recommended levels for response. Minerals play a major role in the immune response, the body's defense system against infectious disease, mineral supplementation above requirements is required for optimal immune responses (Mcdowell, 1994). Mg, P, Na, Cl, Zn, Cu, Fe and Se have been shown to improve an animal's ability to cope with infections. Metabolic interactions among the mineral elements influenced by stress, interactions between minerals and productive stage can determine changes in the minimum requirement of minerals (Underwood, 1966).

Manganese functions as a component of the metalloenzymes pyruvate carboxylase, arginase and superoxide dismutase and as an metal activator for a number of enzymes (Hurley and Keen, 1987). Enzymes activated by manganese include a number of hydrolases, kinases, transferases and decarboxylases. Of the many enzymes that can be activated by manganese, only the glycosyltransferases are known to specifically require manganese (Scrutton *et al*., 1966).

The general pattern of the deformities observed in the manganese deficient guinea pig at birth suggested a defect in cartilagenous tissue and stimulated an interest in the relationship of manganese deficiency to disorders of the tissues of mesenchymal origin hexosamine content occurred primarily in the galactosamine fraction (Huan-Chang and Everson, 1967; Shrader and Everson, 1967; Hurley *et al*., 1958; Hurley and Everson, 1959).

There is a shortening of the long bones, enlargement and malformations of the joints and deviations in the shape of the skull. A defective development of the rib cage is also observed with anterior posterior flattening of the chest. The composition of the ground substance was therefore investigated, beginning with the Acidic Mucopolysaccharides (AMPS) present. The principal compounds in the AMPS group which have been identified in connective tissue are: hyaluronic acid; chondroitin 4-sulfate (chondroitin sulfate A); chondroitin 6-sulfate (chondroitin sulfate C); dermatan sulfate (chondroitin sulfate B) and heparin (Hurley and Everson, 1963; Hurley *et al*., 1960; Hurley, 1967).

At one moment of conception or birth defects in which there is deficiency of Mn can be impaired chondrogenesis in the formation of the fetus. As a result, experimental animals, rats, mice and tested experimental calves under natural breeding, born with various deformities and some are clinically visible, and were specifically described by Dyer *et al*. (1964) in calves because they have curves or joints twisted inward members and or forelegs twisted and rear pasterns in permanent flexion. Slight enlargement of knees and twisted rear legs also has been observed. Rojas *et al*. (1965) observed enlarged joints, stiffness, twisted legs and a general physical weakness were observed in calves from cows fed the low manganese rations. Deficiencies marginal or borderline deficiencies that may go unnoticed because they are asymptomatic.

Dyer and Rojas (1965) and Rojas *et al*. (1965) reported a positive relationship between a low manganese intake of gestating cows and the incidence of neonatal deformities in their calves. The manganese content of different bovine tissues (including liver, kidney, gonads, and blood) was determined. Leg deformities with "overknuckling" and dwarfism in calves, infertility and frequent abortions in cows grazing manganese-depleted pastures were reported for Rojas *et al*. (1965).

Manganese is essential to all known living organisms; it activates numerous enzyme systems including those involved with glucose metabolism, energy production and superoxide dismutase; it is a major constituent of several metalloenzymes, hormones and proteins of animals and humans (Shrader and Everson, 1967).

Manganese is part of the developmental process and the structure of the fragile ear bones and joint cartilage. Deficiency diseases of Mn are very striking ranging from severe birth defects (congenital ataxia, deafness, chondrodystrophy), asthma, convulsions, retarded growth, skeletal defects, disruption of fat and carbohydrate metabolism to joint problems in new born Leach, Muenster and Wien (1969), Leach (1969) and Leach and Nesheim (1972).

Overall Mn deficiency, animals may have diseases such defects of chondroitin sulfate metabolism with poor cartilage formation, shortened long bones, chondrodystrophy, congenital ataxia, slipped tendon, deafness for malformation of otolithes, asthma, chondromalacia, convulsions, infertility for failure to ovulate or testicular atrophy, stillbirths or spontaneous abortions, loss of libido in males and females, glucose intolerance and retarded growth rate (Leach and Muenster, 1962).

Manganese is an essential trace mineral nutrient. Manganese is needed for normal brain and muscle function, building bones, blood clotting, cholesterol synthesis, fat synthesis and DNA and RNA synthesis. Manganese activates the enzyme responsible for the formation of urea, the waste product of protein degradation. In carbohydrate metabolism manganese is required for the synthesis of glucose from non-carbohydrate substances (gluconeogenesis). Manganese assists the action of superoxide dismutase, which degrades superoxide, a free radical and a highly damaging form of oxygen. In addition, manganese is required to synthesize components of mucopolysaccharides (glycosaminoglycans), components of connective tissue (Hurley, 1968). A manganese-dependent enzyme of the brain synthesizes the amino acid, glutamine, as a way of removing ammonia, a toxic product of nitrogen metabolism. Conditions possibly associated with manganese deficiency include osteoporosis, rheumatoid arthritis, lupus erythrematosis, allergies, alcoholism and diabetes (Burch *et al*., 1975).

Biochemical function, manganese is both an activator, and a constituent of several enzymes. Those activated by manganese are numerous and include hydrolases, kinases, decarboxylases and transferases, but most of these enzymes can also be activated by other metals, especially magnesium. This does not apply, however, to the activation of glycosyltransferases or possibly to that of xylosyltransferase. Manganese metalloenzymes include arginase, pyruvate carboxylase, glutamine synthetase and manganese superoxide dismutase (Shrader *et al*., 1973).

The enzymatic activity is the main function of the manganese binding proteins-metalloenzymes -, or activating enzymes. Like other essential trace elements, Mn can function both as an enzyme activator and as a constituent of metalloenzymes. Manganese-containing enzymes include arginase, pyruvate carboxylase and Mn-superoxide dismutase. While the number of Mn metalloenzymes is limited, the enzymes that can be activated by Mn are numerous. They include hydrolases, kinases, decarboxylases, and transferases (Groppel and Anke, 1971). Whether an activator or a component of the enzyme proper, Mn is often the priority cation, but another cation, especially Magnesium (Mg), can partially substitute for Mn with little or no loss in enzymatic activity. Thus, biotin-dependent enzymes such as pyruvate carboxylase continue to fix CO, during Mn deficiency because Mg substitutes for Mn in the enzyme.

Bone growth, Mn-deficient bones are considerably shortened and thickened. Manganese is essential for development of the organic matrix of the bone, which is composed, largely of mucopolysaccharide. Impairment in mucopolysaccharide synthesis associated with Mn deficiency has been related to the activation of glycosyltransferases (Leach, 1971). These enzymes are important to polysaccharide and glycoprotein synthesis, and Mn is usually the most effective of the metal ions required.

Effects on reproduction were among the first signs of Mn deficiency to be observed. The deficiency can cause an irreversible congenital defect in young calves, chicks, rats and guinea pigs characterized by ataxia and loss of equilibrium. Shils and McCollum (1943) found several stages of Mn deficiency in female rodents: (1) birth of viable young with ataxia; (2) nonviable young that die shortly after birth and (3) disturbance of estrus, with no reproduction. Impaired or irregular estrus has also been observed in cattle and swine. Hidiroglou (1975), on the basis of Mn tissue-distribution studies of the reproductive tract of normal and anestrus ewes, has suggested that Mn has a role in corpus luteum functioning. In laying hens, Mn deficiency has resulted in a decreased rate of egg production, poor shell quality, reduced hatchability and an embryonic deficiency called chondrodystrophy. Testicular degeneration has been reported in Mn-deficient rats, mice and rabbits (Leach and Lilburn, 1978).

The main manifestations of manganese deficiency include a high neonatal death rate, impaired growth, abnormal skeletal development, congenital ataxia, disturbed or depressed reproductive function and defects in lipid and in carbohydrate metabolism. Many of these gross manifestations of manganese deficiency are now believed to be due to a defect in the synthesis of mucopolysaccharides. Although available information on manganese deficiency in man is limited, these findings suggest that manganese may play a role as one potential factor in the development of intrauterine malformations.

Lipid metabolism, association between Mn and choline has been known for some time. Fatty liver in rats induced by Mn deficiency is alleviated by either Mn or choline. Also, Mn deficiency increases fat deposition and backfat thickness in pigs. Both Mn and choline are needed for prevention of perosis in poultry. Manganese is involved in the biosynthesis of choline. Furthermore, the changes in liver ultrastructure that arise in choline deficiency are very similar to those in Mn deficiency (Bruni and Hegsted, 1970). Deficiencies of Mn and choline both appear to affect membrane integrity.

Manganese also has a role in cholesterol biogenesis (Davis *et al*., 1990). We may postulate a number of ways in which manganese may play a role in lipid and lipoprotein metabolism, which may be ultimately related to the development of atherosclerosis. Furthermore, manganese, by being a cofactor of MnSOD, protects membranes from free radical formation and preserves the integrity of its lipid components. Glycoproteins are integral components of the arterial extracellular matrix and play an important role in maintaining structural integrity and normal function of the arterial wall including regulating permeability and retention of plasma components, controlling vascular cell growth and interacting with lipoproteins. Manganese, as a specific activator of glycosyltransferases, may also affect glycosylation of glycoproteins on cell membranes including receptors. This would alter receptor composition and structural properties and affect lipoprotein binding and their ultimate metabolic fate (Hurley, 1981a,b).

Manganese may also affect lipoprotein composition and metabolism by its role in stabilizing lipoprotein structure due to its high affinity in complexing with the polar heads of lipoprotein phospholipids and amino acid residues. Furthermore, manganese may modify intramolecular interaction of the lipoprotein particle with its receptor by bridging the anionic groups of cell membrane glycosaminoglycans with certain amino acid residues and phospholipids on the surface of the lipoprotein. Finally, manganese may play a crucial role in the glycosylation of plasma apolipoproteins in the liver Golgi apparatus by specifically activating glycosyl transferases and manganese deficiency may result in abnormal lipoprotein formation and impairment of lipoprotein secretion from the liver, thus resulting in fatty liver formation observed in many studies (Stock and Latshaw, 1981; Hurley *et al*., 1982; Hurley, 1976a,b).

Carbohydrate metabolism, glucose utilization is impaired by Mn deficiency. Necropsy has revealed gross abnormalities in the pancreas such as aplasia or marked hypoplasia of all cellular components, so Mn may in some way be involved in insulin formation or activity (Leach, 1967). Rats deficient in Mn had fewer insulin receptors per cell compared to controls (Baly *et al*., 1988). Biosynthesis of glycoproteins may be impaired in Mn-deficient animals. Prothrombin is a glycoprotein whose synthesis has long been known to be controlled by vitamin K. Manganese is also required, and a Mn deficiency reduces the vitamin K-induced clotting response (Doisey, 1974).

Manganese deficiency in experimental animals results in a diabetic-like glucose intolerance. This may result in part from alterations in processes comprising glucose homeostasis including pancreatic insulin synthesis, secretion and degradation, as well as peripheral insulin action on target tissues. Interestingly, diabetes itself may result in marked changes in manganese metabolism. Abnormalities in cell function and ultrastructure, particularly involving the mitochondria, occur in Mn deficiency (Hurley and Keen, 1987). Manganese deficiency caused alterations in cell membrane integrity in the liver, pancreas, kidney, and heart in aged mice (Bell and Hurley, 1973). Chondrodystrophy and dwarfism is abnormality is characterized by a disproportionate shortening of the long bones.

MATERIALS AND METHODS

To study the seasonal behavior of the forage in *Brachiaria decumbens* used for grazing cattle in the center-west of the state were randomly selected nine counties represented by Arealva, Duartina, Bauru, Lucianopólis, Cabralia Paulista, Avaí, Piratininga and Ubirajara. Research project to rural sampling were performed at the Pathology Laboratory Animal Unit of Research and Development of Bauru. The mineral analysis were carried out in the Section of Metabolic and Deficiency Diseases of Instituto Biologico of São Paulo. Samples of the grass *Brachiaria decumbens* were harvested within twelve months of the year to analyze the macrominerals: Ca, P and Mg and microminerals: Cu, Zn, Co, Fe, Mn (AOAC, 1990). The Dry Matter (DM) of the standardization sample was obtained after drying in the oven at 65°C for 24 h with standardization of DM in 90%. After grinding in Wiley mill type knife with stainless steel and free from contamination by minerals, the samples were transferred to Erlenmeyer flask and subjected to acid digestion in a hot plate with the aid of digestor wrapped inside chapel of exhaust gases. The eleven liver samples after crushing and treated with solvents to extract the fat has been kiln dried and mineralized by acid hot digestion. The extract obtained by wet digestion was transferred to volumetric flask and measured the volume with distilled and demineralized water obtained in deionized and distiller apparatus, both with internal circuit entirely of glass. Then the samples were injected into the atomic absorption spectrophotometer Varian® mark and properly calibrated with standards in reading and hollow cathode lamp specifies the mineral to be analyzed. O Phosphorus (P) was read by molecular absorption spectrophotometry. The reading obtained for the mineral in question was applied the formula to calculate the concentration according to the volume used in the dilution of concentrated extract obtained. The farms with animals with birth defects were registered and systematically analyzed forage, total diet and animal tissue of the newborn for the minerals studied. Were selected nine regions with the birth of animals with deformities in joint and musculoskeletal to sampling of soil, forage and total ration in the farms with cows pregnant and lactating. They were accompanied the birth and calves born with deformities were registered and monitored in their development. Animals born with

deformities and problems worsened during growth were sacrificed and preceded the macroscopic evaluations and mineral analysis of hepatic tissue.

Statistical analysis: A completely randomized design were used for sampling 108 the total samples taken in nine municipalities and four distinct seasons, namely: spring, summer, autumn and winter to determine the three main macro: Ca, Mg and P and five trace minerals: Cu, Zn, Co, Fe and Mn in grass *B. decumbens* in the region studied. Were applied the model of analysis of variance for difference of mean seasons and regions for each mineral analyzed and ingredients to rations by SAS software (SAS, 1994). The Tukey test to compare means at 5% level of significance was used.

RESULTS

The figures above illustrate the use of diet deficient Mn progenitors during conception or pregnancy can lead to end the birth of progeny of calves with teratogenic defects.

The levels of trace manganese in grazing *B.decumbens* showed high standard deviations and of coefficients of variation for the mean that ranged widely according to the times of the seasons of autumn, winter, spring and summer. However, the highest mean of 89.15 mg of Mn (winter) did not differ ($p>0.05$) the lowest average equal to 79.41 mg Mn (winter), 81 mg Mn (autumn) and 83.48 mg Mn/kg. In contrast, the lowest average (winter) had the highest coefficient of variation - 30.78% - and the largest deviation from the average - 20.01 - (Table 1).

The levels of trace manganese in animal tissue, dry liver newborn calves, ranged from 4 ppm to 8 ppm, with an average 6 ppm and a standard deviation 1.79 (Table 2).

The final composition of the diet for cows' feed consisted of forage base of napier grass (*Pennisetum purpureum*) and sugar cane (*Saccharum officinarum*) crushed a mean equal to 36.67 ppm Mn (consumption, sd±2.31). The animals consumed an average of 7.50 kilograms DM/day (sd±1.31). After receiving the roughage in the time between 7-10 am, the animals were managed for grazing *B. decumbens* where they remained until the following day. The average was 56 ppm Mn in the pasture and 36.67 ppm Mn in the final diet of the animals (Table 3). After weaning, calves, cows and bulls, were transferred to pasture, where were offered in troughs sodium chloride additive of limestone. No other mineral supplement was offered to the reproduction herd.

A group of newborn calves were monitored during the first months of development and another group, after autopsy, was used for the macroscopic and microscopic observations. We observed numerous skeletal and joint deformities in newborn calves (Fig. 1-8). Many newborn animals had permanent flexion of the forelimbs, angular deformities and limb shortening. With the growth and weight gain in some animals showed lack of support at the tip of the hull of the front and support on the tarsus articulation radius. Macroscopically, in addition to joint deformities, the legs showed marked shortening of the bones of the forelimbs, curvature and thickening.

DISCUSSION

The birth of calves with severe skeletal deformities, musculoskeletal and knuckle joint in this research are similar in physical conditions equal those described by Dyer *et al.* (1964) in studies conducted in the area Texana the United States and by and in subsequent studies by Dyer and Rojas (1965) and Rojas *et al.* (1965) on manganese deficiency in the etiology of deformed calves. In subsequent research the authors mentioned above reported that after careful study of soil, forage and total ration, levels of 15.8 ppm Mn in the total diet, were increased to 25 ppm in the diet of cows, corrected the problem of the birth of calves with abnormalities. The deficiency Mn in this study, the authors above were associated with the birth of calves with birth defects, confirming the clinical suspicion of nutrients that are in inadequate amounts in the diet deficient in Mn.

The final composition of the diet of the animals showed insufficient levels of the nutrient mineral manganese (Table 3). Although the data of leaf analysis in *B. decumbens* reveal average levels adequate Mn, it is unbalanced or deficient for the other nutrients for the daily needs of cattle grazing. Still, it was observed that all nutrients including high Mn showed high coefficients of variation and standard deviation for the different seasons (Table 1). According Underwood (1983, 1977) in mineral nutrition is a common occurrence of interaction between different nutrients in the gastrointestinal tract and often antagonistic interrelations may occur in the animal organism and result in worsening of the deficiencies of certain trace element that can determine framework pathognomonic of a particular nutrient deficiency.

In this study were observed that the mean of 36 mg Mn/kg DM - "bordeline" deficiency - showed up near the levels of Mn indicated in the NRC (1996) of 40 ppm for adult cattle. However, in some specific conditions such as dependence on the true availability of the element in the diet, several authors considered that the requirements can reach up to 70 ppm Mn (Bentley and Phillips, 1951; Rojas *et al.*, 1965; Grace, 1983; NRC, 1996). In these research should also take into account that the properties that had calves born with birth defects coincidentally registered the addition of significant amounts of Ca as limestone of the total ration of dairy cattle. Breeding bulls and cows in gestation and lactation were above 4 g Ca/kg DM in the total ration added directly to the basal diet of the animals.

Table 1: Means of the mineral content in the pasture *Brachiaria decumbens* by season of year in the regions studied

Variables[1]	Ca (g%)	P (g%)	Mg (g%)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Co (ppm)
Autumn								
μ	0.36[A]	0.070[A]	0.15[A]	4.11[A]	41.18[A]	81.00[A]	132.89[A]	0.096[A]
sd	0.03	0.013	0.02	0.39	7.73	11.94	16.63	0.006
cv	20.51	42.90	21.09	21.42	30.96	23.25	26.95	14.37
Winter								
μ	0.37[A]	0.077[A]	0.15[A]	4.11[A]	42.93[A]	89.15[A]	143.15[A]	0.101[A]
sd	0.05	0.024	0.02	0.65	8.75	20.01	37.04	0.004
cv	20.47	44.07	20.54	28.89	32.28	32.67	47.92	10.65
Spring								
μ	0.39[A]	0.090[A]	0.16[A]	4.78[A]	45.15[A]	79.41[A]	144.00[A]	0.090[a]
sd	0.03	0.020	0.02	0.74	6.06	13.92	29.06	0.006
cv	23.91	47.55	18.00	27.89	22.20	30.78	35.45	13.28
Summer								
μ	0.36[A]	0.080[A]	0.16[A]	4.93[A]	48.82[A]	83.48[A]	129.67[A]	0.084[A]
sd	0.03	0.010	0.01	1.05	7.82	12.54	19.18	0.005
cv	18.24	44.96	18.52	29.75	24.08	26.34	28.13	10.84

*Means in the column with the same superscript denote no significant (p<0,05) difference.
[1]μ = means of the analysis of three months of collection of forage in grazing beef cattle in the regions studied; sd: standart deviation; cv: coefficient of variation

Table 2: Concentrations of Mn and other minerals in the animal tissue of newborn calves with defects in farms of various regions

	Dry matter of liver (mg/kg)							
Region	Ca	P	Mg	Cu	Zn	Mn	Fe	Co
Arealva	160	8000	400	42	140	4	102	0.150
Avaí	280	10500	200	65	365	4	148	0.150
Bauru	400	9000	400	187	160	8	272	0.150
Bauru	800	7000	600	8.20	90	6	246	0.090
Duartina	600	8600	200	12	94	6	340	0.100
Cab Paulista	700	11000	600	335	125	8	232	0.095
Iacanga	120	9000	200	75	200	8	22	0.165
Iacanga	250	11000	200	272	114	8	640	0.130
Lucianopólis	800	6000	200	290	600	4	117	0.090
Piratininga	800	6000	200	290	600	4	128	0.115
Ubirajara	120	11000	106	70	78	6	172	0.170
μ	457.27	8827.27	300.55	149.65	233.27	6.00	219.91	0.128
dp	287.27	1925.66	172.52	126.39	197.86	1.79	165.53	0.031
	Reference value in the liver[1]							
	200	12000	600	100-400[2]	100	10-14	150.00	0.150
	Mineral analysis of soil[3]							
Soil	-	-	-	0.30-0.50	0.70-1.30	41-70	112-125	-

[1,2]Underwood (1969) and NRC (1984); [3]pH = 4.2

Table 3: Composition of the ration of the herd bull and cow in milk production on farms with calves with birth teratogenic malformations

Ingredients in ration	Consumption DM (kg/animal/day)	Mn (ppm/kg DM)	Ca (g/animal/day)	Ca (g/kg DM)
Pennisetum purpureum	4.50	11±6.25		
Saccharum officinarum	2.00	6±2.52		
Pasture[1]	1.00	56±9.51		
	Consumption DM (total/kg/day)	Mn (μ)* (ppm in DM)		
μ±sd	7.50±1.31	36.67±2.31		
Limestone (38% Ca)			30.40-38.00	4.05-5.07
Sodium chloride			45	

*μ = means of Mn in the DM (mg x kg^{-1}); 1- estimated value of consumption of 1 kg DM/day

The additional excess calcium in the diet in some circumstances can intervene with the absorption of trace elements in the small intestine. Absorption of manganese from ^{54}MnCl in lactating dairy cows was less than 1% (Van Bruwaene *et al*., 1984) and little is known concerning dietary factors that may influence manganese absorption. Some evidence suggests that high dietary calcium and phosphorus may increase manganese requirements (Hawkins *et al*., 1955; Dyer *et al*., 1964; Lassiter *et al*., 1972; Alfaro *et al*., 1988). Biliary excretion of manganese plays an important role in manganese homeostasis but little excretion of manganese occurs by the route of urine (Hidiroglou, 1979). From the clinical point of view is a well

Fig. 1: Calf of a month with three supporting members after birth deformities in the joints of the limbs and stiffness and twisted legs of the left foreleg

Fig. 2: Calf of Figure 1 after sacrificed and necropsy showed bone deformities of the skeleton and joints of the forelimbs and hindlimbs

known fact over the table pathognomonic of osteopathy and many other references in the literature since the scientific publications of Hurley *et al.* (1963), Dyer and Rojas (1965) and Hurley (1981a,b) with proven experimental studies in guinea pigs, rats and mice.

The results of this study were similar to those found in the vast literature diponível to deficiency of the trace element manganese which were analyzed retrospectively to the results of research and the NRCs published in different years and which are reported

Fig. 3: Calf in Figure 1 after showing deformities of the feet, permanent flexion and limb shortening of bone of the left foreleg

Fig. 4: Calf of the one month with deformities of the forelimbs and hindlimbs by manganese deficiency

below. Thus, the NRC (1984) mentions requirements Mn of 40 ppm in animal reproduction and the levels in the diet should be increased to 40-70 ppm in stressed animals. Agreeing with these values reported the increased need for 70 ppm and recommended 100-150 ppm referring to the Mn deficiency is to be installed when the soil pH is above 6.0 (Groenewald, 1960). The study of soils can provide indications of possible deficiencies. As the pH increases, the availability and use by plants of iron, manganese, zinc, copper and cobalt decreases, while concentrations of phosphorus, calcium, magnesium, molybdenum and selenium rise (Howes *et al.*, 1973; McDowell and Conrad, 1977).

Fig. 5: Observation of the posterior calf of months with joint deformities grossly of the forelimbs radius carpus metacarpus joint and hindlimbs tibio tarsusmetatarsus articulation enlargement in the manganese deficiency

Fig. 6: Observation of the anterior calf of months with joint deformities of the forelimbs radius carpus metacarpus joint and hindlimbs tibio metatarsus articulation thickening in the manganese deficiency showing the difficulty of balancing the locomotor apparatus

Analysis of forage is limited by the difficulty of representative samples of animal diet, the possibility of contamination of soil and lack of quantity consumed by the animal.

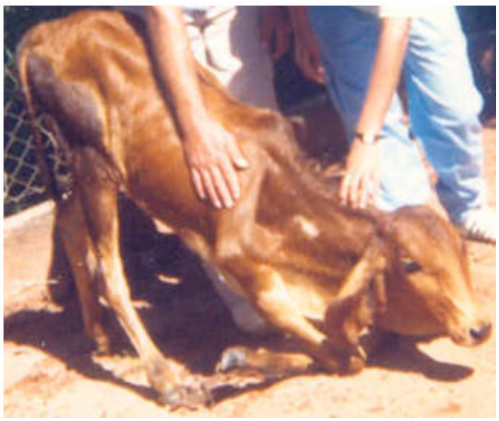

Fig. 7: Persistent flexed forelegs twisted in calf two months age with no support on foreleg and support on the radius carpus metacarpus joint (enlargement of knees) by manganese deficiency

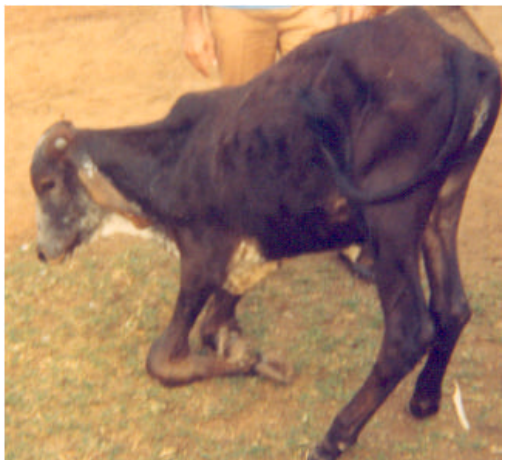

Fig. 8: Angulation deformity of the forelimbs of calf three months of age with lack of support from former members and support on the radius carpus metacarpus joint (enlargement of knees) in the manganese deficiency

The analysis of the concentrations of minerals in tissues may ultimately provide an indication of the environment as a whole and the state (s) element (s) deficient (s) in the diet. However, it is desirable to confirm the diagnosis of mineral deficiency by adequate supplementation of mineral or minerals found deficient in the diet, aiming to observe the response in animal health or performance. The main sources of supplemental are: manganese carbonate: 47.80%, manganese chloride: 27.76%; manganese sulfate: 32.50% and manganese oxide: 77.45% of Mn.

The manganese requirement for growing and finishing cattle is approximately 20 mg Mn/kg diet. Skeletal abnormalities were noted in calves from cows fed diets containing 15.8 mg Mn/kg but were not present when diets were supplemented to contain 25 mg Mn/kg (Rojas *et al*., 1965). The quantity of manganese needed for maximum growth is less than that required for normal skeletal development. Manganese requirements for reproduction are higher than for growth and skeletal development, and the recommended concentration for breeding cattle is 40 mg/kg. Cows fed a diet containing 15.8 mg Mn/kg had lower conception rates than cows fed 25 mg Mn/kg (Rojas *et al*., 1965; Howes *et al*., 1969; Howes and Dyer, 1971). Heifers fed 10 mg Mn/kg exhibited impaired reproduction (delayed cycling and reduced conception rate) compared to those fed 30 mg Mn/kg, but growth was similar for the two groups (Bentley and Phillips, 1951).

The manganese has a direct effect on reproduction, influencing conception rate (Hidiroglou, 1975; Hidiroglou *et al*., 1978). Experimentally, DiCostanzo *et al*. (1986) with the addition of 14 mg Mn/kg on a diet of corn silage containing 32 mg Mn/kg, reduced conception services ratio from 1.6 to 1.1.

Deformations of the skeleton similar to those found in this research (Fig. 1-8) were mentioned in various considerations addressed by several authors in the literature. The calves born to mothers with diet containing manganese bordeline deficiency showed pathognomonic signs of Mn deficiency equal to guinea pig, mice, pig and rat.

In this study, calves born with skeletal abnormalities showed low Mn content in the liver (Table 2 and Fig. 1-8). These results agreed with com Rojas *et al*. (1965) when determined experimentally that mothers receiving 21 ppm manganese born calves content of 11.8 ppm Mn in the liver versus low 6.60 ppm (low) Mn or 7.28 ppm Mn. Cave *et al*. (2008) reported congenital chondrody-strophy in forty-seven holstein calves with a dwarf-like appearance, born in South East Australia. They pointed to manganese deficiency during fetal development as the most likely cause against the evidence prognosis.

Deficiency of an essential trace element results in a characteristic deficiency syndrome in a manner analogous to a specific vitamin or hormone deficiency. The deficiency syndrome is associated with specific structural, functional, biochemical, or physiological abnormalities. These abnormalities, in turn, are prevented or reversed after administration of the deficient element (Leach and Gay, 1987).

The evidence of skeletal abnormalities in calves found in this study and in several studies mentioned in the literature shown to have biochemical and genetic mechanisms in common which are described below. Thus, Hurley and Keen (1987) after numerous experimental researchs were convinced, that manganese functions as a component of the enzymes pyruvate carboxylase, arginase and superoxide dismutase and as an activator for a number of enzymes. Enzymes activated by manganese include a number of hydrolases, kinases, transferases and decarboxylases. Of the many enzymes that can be activated by manganese, only the glycosyltransferases are known to specifically require manganese.

Manganese seems to be intimately involved in synthesis of protein, DNA, and RNA. The DNA-manganese complex was first reported by Wiberg and Neuman (1957). These authors concluded from its dissociation constant that manganese binds to DNA more strongly than do other metals. Since minute quantities of manganese were detected during the isolation of RNA and DNA, it was suggested that manganese may bear a functional relationship to protein synthesis and the transmission of genetic information. Studies with zinc induced Sever and Emanuel (1973) to believe in the connection between the metal effect on DNA and RNA and birth defect in humans similar to that described for Mn. Mammalian cells exhibit two types of RNA polymerase activity, one of which requires manganese. Manganese also affects the DNA polymerase system. The RNA-dependent DNA polymerase activities in human placenta and rat liver nuclei are stimulated predominantly by manganese. Manganese and other cations stabilize the secondary structure of DNA by their electrostatic interaction with the negatively charged phosphate group. Extensive studies relating conformation and reactivity to DNA interaction with manganese ion have recently been reported (Luck and Zimmer, 1972). The results of these investigations indicate that manganese has an important role in initiating protein synthesis by stimulating RNA polymerase and DNA polymerase activities in the mammalian system. A study comparing the effect of manganese to that of other ions showed a slight but significant increase in protein biosynthesis, attributable to manganese, in isolated rat liver nuclei. In kwashiorkor, there was a definite correlation between decreased hepatic manganese content and decreased hepatic protein content in the protein-calorie malnutrition (Underwood, 1981).

Manganese is necessary for optimal growth in mice, rats, and other species. Swine, guinea pigs, and calves do not show impaired growth with manganese deficiency (Asling and Hurley, 1963).

Perhaps the most remarkable discovery was made by Erway *et al*. (1966), who demonstrated that a manganese dietary supplement fed to pregnant mutant mice who develop congenital ataxia would result in ataxia-free offspring. This study demonstrated for the first time that supplementation with an essential nutrient, manganese, can prevent development of a genetically predetermined phenotype. The mutation had not been abolished, because offspring of the ataxia-free mice

developed ataxia. Ataxia in mice, whether attributable to the mutant gene "pallid" or to a maternal dietary manganese deficiency, is identical; however, a high manganese concentration (1 mg) in the diet of mutant mice during pregnancy completely prevented the congenital defect and altered the mutant expression without changing the genetic constitution (Hurley, 1968). Thus, manganese is a specific nutrient that affects expression of the mutant gene without altering subsequent transmission of the mutation to future generations of mice according Erway *et al.* (1971). These offspring exhibited shortening of the radius, ulna, tibia and fibula from birth to maturity. Results similar to this research can be found in several experimental studies developed by Leach (1967, 1968, 1969, 1971), Leach and Muenster (1962), Leach *et al.* (1969), Leach and Nesheim (1972), Leach and Liburn (1978), Leach and Gay (1987) and Leach and Harris (1997).

Thereby, these researchers mentioned that perosis, or "slipped tendon," may also occur in manganese-deficient chicks. It is characterized by enlargement of the hocks, short and twisted tibiae and slipping of the gastrocnemius tendon from its condyles. The discovery that perosis is related to changes in the mucopolysaccharide content of the epiphyseal cartilage focused attention on the chemical composition of the organic matrix of cartilage and bone (Leach and Muenster, 1962). The Mn deficiency can leads to abnormalities in glucose utilization with hypoglycemic and gluconeogenesis effect.

The decrease in chondroitin sulfate in manganese deficiency is the result of impaired mucopolysaccharide synthesis, manganese being a necessary cofactor for the enzymes involved in chondroitin sulfate synthesis. The above studies have demonstrated that manganese affects the primary sites of chondroitin sulfate synthesis. Because the chondroitin sulfate-protein complex is necessary to maintain the rigidity of connective tissue, these findings provide a biochemical explanation for the skeletal abnormalities observed in manganese deficiency. The association of manganese deficiency in experimental animals and the bony abnormalities were find resulting from the inhibition of mucopolysaccharides synthesis extremely fascinating in relation to the mucopolysaccharidoses occurring in humans. In humans, these diseases are characterized by bony abnormalities, mental retardation, and accumulation of tissue mucopolysaccharides. Even if manganese deficiency or an abnormality of manganese metabolism is not subsequently shown to be involved in the human mucopolysaccharidoses, the manganese-deficient animal seems to be an ideal model for the elucidation of the biosynthetic pathways involved in mucopolysaccharide synthesis, pathways that are ill-defined and poorly understood at present (Shrader *et al.*, 1973; Hurley and Keen, 1987).

About teratogenicity, studies confirming manganese as an essential nutrient were reported simultaneously by several investigators Orent and McCollum (1931). Animals on manganese-deficient diets develop numerous biological and physical symptoms, such as decreased manganese concentrations in tissues and milk, suboptimal growth, decreased testicular and ovarian function, accumulation of fat and diabetic-like glucosetolerance curves (Everson and Shrader, 1968). Moreover, the risk of intoxication by Mn this is of 25 times or around (NRC, 1980) the maximum tolerable concentration of manganese was set at 1.000 mg/kg, at least on a short-term basis (Jenkins and Hidiroglou, 1991).

Manganese is needed for the glycosyltransferases that function in the synthesis of the glycosaminoglycan side chains of the proteoglycan (Leach *et al.*, 1969). Other nutrient deficiencies such as deficiencies in choline, niacin, zinc, biotin and folic acid also resultin skeletal deformities grossly similar to manganese deficiency. Although these deficiencies result in a narrow growth plate, they apparently do not share a common mode of action with manganese. The alterations in cartilage proteoglycans are specific for manganese deficiency and are not observed with other deficiencies (Leach, 1969; Stock and Latshaw, 1981).

Manganese deficiency, this research, affect the reproductive performance of animals (or delay cycling estrus, silent estrus and reduced rates of conception), bone deformities and contractures (shortening) of tendons in newborn calves, enlargement of joints and reduced birth weight. There is some evidence that excessively high calcium in the diet predisposes to manganese deficiency. The positive interaction of manganese and choline has been identified in preventing of the "fat cow syndrome".

Thus manganese deficiency can cause reproductive failure, it is recommended to provide the supplemental manganese at levels recommended for the animals. The analysis of the chemical composition of the diet will determine the levels of manganese in the diet. The requeriment for cow and calf is above 20 ppm (70 ppm for stressed calves) and 40 ppm, respectively, and maximum tolerable of 1000 ppm for the two categories (Table 4).

The concentration of manganese in forages varies greatly depending on plant species, soil pH and soil drainage (Minson, 1990). This element is not usually be needed in mineral mixtures of the savannas, where the concentrations are high. Analysis of native pastures and cultivated in this region has always shown concentrations above the maximum level of demand for cattle (Underwood, 1981).

Thus, the above authors, just as this research, showed that experimental diets for calves born of dams receiving 15.8 ppm Mn had forelegs, knees, twisted inward,

Table 4: Mineral requirements and maximum tolerable concentrations

		Requeriment			
			Cows		Maximum tolerable concentration
Mineral	Unit	Growing and finishing cattle	Gestating	Early lactation	
Manganese	mg/kg	20	40	40	1000

NRC (1996)

forelegs twisted and rear pasterns in permanent flexion, twisted forelegs, weak, slight enlargement of knees versus calves born normal of mothers given a diet of 25,1 ppm of Mn. In this study, cows fed diet deficient in Mn (Table 1-3) originated calves with deformities of the limbs, twisting of the legs, standing flexion of the forelimb and hindlimb deformities (Fig. 1-8), similar to those described for animals born in the research Dyer *et al*. (1964).

Likewise, in studies of Dyer *et al*. (1964) the liver manganese content of the control calf was 11.84 ppm compared with 6.94 ppm for two deficient calves, show that the cow's requirement is manganese in excess of 16 ppm rather than 6-10 ppm as suggested by NRC (1966). The requirement is probably about 20 ppm, since all calves from cows on the ration containing 15.8 and 16.9 ppm manganese were deformed. Agreeing with the above authors, this study, averaging 6 ppm in liver of newborn calves and media ranging from 4-8 reflected low levels of Mn in the diet of cows in all seasons of the year (Table 2).

Howes and Dyer (1971) utilized Mn^{54} marked and found that the newborn calf preferentially stores manganese in the liver. With 13 ppm Mn in the diet of mothers, calves had 4.48 ppm in the liver and 6.36 ppm to the seventh day versus 7.96 (the birth) and 9.43 (seventh day) ppm in the liver of calves from mothers with diet of 21 ppm Mn.

Supplementing the newborn calf with manganese resulted increase in liver manganese concentrations when compared to calves given no supplemental manganese. Authors also found that calves responded directly to the administration of Mn. Thus, addition of 1.5% Ca plus manganes in milk from calves raised the level in the liver to 942.67 (with 14 ppm Mn in milk) and 410.87 ppm (21 ppm Mn in milk) Mn in the liver of calves to the seventh day.

Hansen *et al*. (2006) observed signs of Mn deficiency in calves born to heifers fed 16.6 mg of Mn/kg of DM: disproportionate dwarfism (three in each seven), unsteadiness/weakness at birth (three in each seven) and superior brachygnathism (five in each seven). The heifers supplemented with 50 ppm Mn, the calves were born normal ($p \leq 0,03$).

The most recent beef cattle NRC (1996) recommends 40 mg of Mn/kg of DM for reproducing beef cattle. Recently, Weiss and Socha (2005) estimated, based on Mn intake and fecal excretion, that dry and lactating dairy cows, respectively, require approximately 2.7 and 1.6 times more Mn for their maintenance requirements than values calculated using the current dairy cattle NRC model. The present study was designed to observe the effects of long term low-Mn diets on gestating heifers and their offspring. As an essential trace element, Mn plays a crucial role in several enzymes in the body, such as glycosyltransferases. Glycosyltransferases are a group of enzymes that are involved in the metabolism of cartilage proteoglycans, affecting the biosynthesis of glycosaminoglycan and oligosaccharide sidewere randomly selected at the conclusion of the previous side chains (Leach and Harris, 1997). The role of Mn in cartilage formation makes it essential to the formation of the epiphyseal growth plate, which directly affects longitudinal bone growth. Thus, the most frequently observed sign of Mn deficiency in young animals is skeletal malformation (Leach and Muenster, 1962). This signs may indicate that low dietary Mn decreased glycosyltransferase activity.

Huan-Chang and Everson (1967) administered experimentally purified diet containing less than 3 ppm Mn. Defects in biosynthesis of the organic matrix of cartilage during fetal development were observed. When the adult female were fed with less than 3 ppm of Mn throughout gestation there was a significant reduction in the concentration of all Acid Mucopolysaccharides (AMPS) tested in rib and epiphyseal cartilage. The mixture of chondroitin sulfates which makes up the major constituent of the total AMPS present was significantly reduced, with chondroitin 4-sulfate and chondroitin 6-sulfate being affected about equally in epiphyseal cartilage.

According to Leach (1971) and Leach and Harris (1997) the main compounds in the AMPS group which have been identified in connective tissue are: hyaluronic acid; chondroitin 4-sulfate (chondroitin sulfate A); chondroitin 6- sulfate (chondroitin sulfate C); dermatan sulfate (chondroitin sulfate B) and heparin. The precursors of these AMPS compounds are mainly hexosamine and hexuronic acid which have been studied in manganese-deficient poultry.

Rib cartilage and epiphyseal cartilage of mice born to mothers with Mn deficient diet: hyaluronic acid, chondroitin sulfate, chondroitin sulfate A e C (ribs cartilage) and heparin to normal guinea pigs and Mn deficient diet.

The data suggest that manganese is involved in a more general step in metabolism essential for all AMPS. The skeletal abnormalities observed in the manganese-

deficient guinea pig at birth are believed to be related to flaws in the metabolism of cartilage matrix.

Compared to the results of this research, several authors working with other species experimentally reported that the consequences of deficiencies of Mn were also reasons of extensive research conducted by Shrader and Everson (1967) using guinea pigs as an experimental model. Analyzed 13 puppies born of 25 guinea pigs in reproduction were subjected to the control diet with normal levels of Mn and 14 puppies from mothers with Mn deficient diet. We observed a high incidence of postural defects, types of otolith abnormalities and of deformities of the semicircular canals and ampullae. Postural defects were observed in none of the control animals. Of the unsupplemented deficient animals, 60% had observable postural abnormalities, whereas 71% of the deficient animals that had received postnatal supplementation with manganese showed head tilting or retraction. These structural abnormalities of the ear are linked to prejudice the synthesis of sulpho-mucopolysaccharides. According to the authors above, were the numerous complications in the labyrinth and labyrinthitis changing postural balance. According to Leach and Gay (1987) the epiphyseal growth plate plays a key role in skeletal development and factors that influence the metabolism of this tissue can lead to abnormal skeletal development. Abnormalities of epiphyseal plate may be in cellular differentiation may exist where Mn deficiency or shortly after cell differentiation begins when the development of hypertrophic growth area where demand for mucopolysaccharide synthesis catalyzed by Mn metalloenzymes is dependent on intense training for the epiphyseal plate normal. At this stage the board is also sensitive to deficiency of other nutrients such as Ca, P and vitamin D. After the zone chondrocytes formed the board this should receive the minerals responsible for ossification mainly Ca and P (Leach *et al*., 1969).

The bone growth will be normal if the support structure ossification, zone chondrocytes, is fully formed. Abnormal cartilage development is associated with chondrodystrophy, tibial dyschondroplasia and rickets. Manynutrient deficiencies result in chondrodystrophy, which is characterized by shortened, thickened bones and a narrowing of the epiphyseal growth plate. Tibial dyschondroplasia is a condition in which the prehypertrophic cells fail to hypertrophy and vascularization is aborted. This abnormality is found in genetically predisposed animals and its occurrence is altered by subtle changes in calcium, phosphorus and electrolyte content of the diet. Calcium and vitamin D deficiencies cause rickets, which is characterized by an increase in the width of the prehypertrophic zone of the epiphyseal growth plate (Leach, 1969).

The differential diagnostic of rickets is that the anomaly by Mn deficiency in the formation of growth epiphyseal plate chondrocytes region presents malformed and there is little or no available tissue calcification, whereas in rickets that can by no region available calcification or malnutrition of Ca and P in some phase of growth. It is observed flattening of the epiphyses weakening of the bones that can demonstrate it fragile and brittle. Manganese is needed for the glycosyltransferases that function in the synthesis of the glycosaminoglycan side chains of the proteoglycan. Burch *et al*. (1975) mentioned that the evidence of trials indicated that manganese has an important role in initiating protein synthesis by stimulating RNA polymerase and DNA polymerase activities in the mammalian system. Experimentally induced manganese deficiency has been produced in various domestic animals. The pathogenesis of symptoms in this deficiency is unknown and all attempts to explain their etiology have been unsuccessful.

Manganese is necessary for optimal growth in mice, rats, swine, guinea pigs and calves and other species. Skeletal abnormalities, but subsequent experiments demonstrated that manganese deficiency retarded endochondral bone growth per se, not osteogenesis. The discovery that perosis is related to changes in the mucopolysaccharide content of the epiphyseal cartilage focused attention on the chemical composition of the organic matrix of cartilage and bone (Leach and Muenster, 1962). The galactosamine-containing polysaccharides were drastically diminished by manganese deficiency in the chick.

Skeletal abnormalities have been studied extensively in manganese deficiency. In rats, mice and rabbits the primary skeletal effects are shortening and bowing of the forelegs. In rats, these effects are seen only in the offspring of manganese-deficient mothers. These offspring exhibited shortening of the radius, ulna, tibia, and fibula from birth to maturity.

In addition, as observed in this study, poor development of the tibial epiphysis resulted in abnormalities of the knee joint and other skeletal abnormalities in calves and other species have been extensively discussed by Rojas *et al*. (1965), Leach (1969), Dyer *et al*. (1964). The above studies have demonstrated that manganese affects the primary sites of chondroitin sulfate synthesis. Because the chondroitin sulfate-protein complex is necessary to maintain the rigidity of connective tissue, these findings provide a biochemical explanation for the skeletal abnormalities observed in manganese deficiency. Tibial dyschondroplasia is a condition in which the prehypertrophic cells fail to hypertrophy and vascularization is aborted. Leach and Muenster (1962) showed that the concentration of amino sugars hexoseamine, glucoxamine plus galactosamine, components of mucopolysaccharides were found in the amount of 1.54 mg/kg in the diet deficient in Mn (0 ppm Mn) versus 3.93 mg/kg tissue (100 ppm Mn) forming mucopolysaccharides. In the research, showed that choline deficiency did not result in any substantial

change in the mucopolysaccharide content of the epiphyseal cartilage that are observed with manganese deficiency. According to Cave *et al.* (2008), the same way in this research, the histopathology of affected skeletal samples showed chondrodysplasia, strengthening the evidence that the cause of the deformities could be a manganese deficiency during foetal development (Hurley, 1976a,b).

Conclusion: The deficiencies of macro and trace elements in cattle grazing in the region subjected to the nine counties studied supplemented shall at all times and in all seasons. The study revealed a worsening of disability in the dry season of the year (autumn, winter and transition to spring) when the deficiencies highlighted by the low quality and quantity of dry matter and consequently of all nutrients and therefore imposing the need for supplementation of dry matter and correction of nutrient for the provision of adequate energy, protein, minerals and vitamins.

Manganese deficiency was found in pastures and the addition of sugar cane and napier grass in the trough does not replace that with the requirements.

Adding mineral supplementation balanced mainly manganese, discontinued the birth of calves with abnormalities of the skeleton.

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Emerging Dietary Patterns from Daily Food Intake Pattrens of Young Children under Five

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Abstract: Modern lifestyle extends the umbrella of social responsibility for provision of appropriate nutritionally balanced foods to children of all age groups in particular the children under 5 years of age of all socio-economic groups of civil society which starts from home leads to the health professionals at all health outlets, Nutritionists, Dieticians, schools and the food industry. Dietary pattern established in early childhood significantly influence the probability of having less tendency towards junk food which certainly result into mal nutrition whether under/over weight or obesity. This review paper will open an avenue of understanding of how children's food preferences are acquired during infancy and early childhood, which are helpful in developing strategies to improve the quality of children's food based dietary pattern and nutritional status. This study shall also be useful for those food companies engaged in manufacturing/ producing ready to serve diets for infants and children. Moreover, it will contribute in developing food-based dietary guidelines for children (under 5 years of age).

Key words: Childhood, dietary patterns, food neophobia, nutritionist, nutritional status, meal patterns, food preferences

INTRODUCTION

Like many other domains of development, young children's eating patterns are largely influenced by learning about what is edible and non-edible and what is acceptable within a family and the culture.

A carefully timed development exists between children's early physiological and their increasing nutrition needs. The literature regarding early childhood nutrition has focused upon children's changing nutrient requirements for growth and health and has neglected the social developmental framework from which children's eating behaviours and intake patterns emerge. The purpose of this paper is to review the research and identify research gaps in the development of children eating behaviour, with a focus on how children develop early experiences, particularly within family eating environments and to shape food consumption patterns belonging to different socio economic groups.

Appropriate nutrition during the childhood is essential for the maintenance of normal growth and good health (Ministry of health, 1997). Before we review and discuss matters related to the children nutritional needs and their emerging dietary patterns, let us understand what dietary pattern stands for?

Dietary patterns versus food intake patterns: In recent years, dietary pattern, vaguely defined as multiple dietary attributes as a single exposure, have emerged as an alternative or adjunct to traditional approaches for the study of the association of diet and health (Kant, 1996, 2004; Hoffman *et al*., 2002). Daily eating pattern emerges from daily eating practices broadly refer to daily food in-take during the proper meals or between the meals or on any particular eating occasion. Eating patterns influence nutrient intake; for example, Dwyer found that as the number of eating occasions increased, so did the overall energy intake (Dwyer, 1995). Dietary patterns are the outcome of meal patterns of the individuals that are determined from short term intake of an individual food or composite food or combination of foods on any particular time.

Many dietary studies attempts to explain the relationship between a single dietary component and health outcomes or antecedents influencing consumption. Although these studies are valuable but they do not represent the interplay of all the individual food choices that describe a complete food pattern (Popkin *et al*., 1999; Messina *et al*., 2001; (Kant, 2004). Over all diet quality depends on the time. An analysis featuring a total diet approach may explain how foods substitute for (e.g. skim milk for whole milk) or complement each other (e.g., milk and cereal). It provides a complete assessment of the combinations of foods consumed and may be useful in identifying emerging dietary patterns in a population, dietary pattern inconsistent with dietary recommendations or dietary patterns related to specific health outcomes (Tucker *et al*., 1992; Wirfalt and Jaffery, 1997; Green-wood *et al*., 2000; Millen *et al*., 2001; Wirfalt *et al*., 2001; Tucker *et al*., 2002; Quatromoni *et al*., 2002; Rasanen *et al*., 2002; Newby *et al*., 2003).

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The dietary patterns of infants influence their health during infancy and later in childhood and adulthood (Davis, 2001).

Stages of early childhood: Young children are one of the most vulnerable groups in any society. They have many special dietary needs, which are quite different from those of healthy adults. Childhood is a time of change between the infant diet and adult diet. Hilton (2004) has classified childhood as follow:

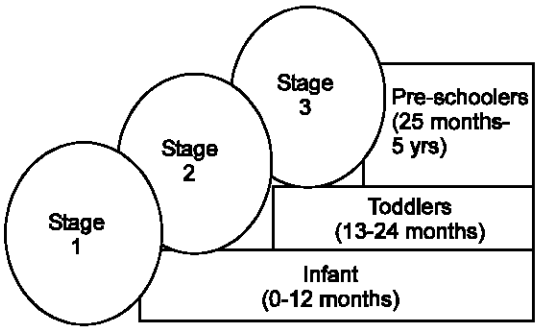

Fig. 1: Stages of early childhood (Adapted from Hilton, 2004)

Infants grow most rapidly, with an average rate of weight gain of about 9% per month, but once the first birthday is reached this rate drops to about 1% per month. Many parents become concerned at about this time, because they are unaware of the different weight gain rates of different ages. The infant lays down more body fat as compared with the high proportion of lean tissues accumulated by the preschool child. This body fat gain by infants requires more energy per gram of body weight. Hence, the 2-5 years old child requires only half as much energy to gain per gram of body weight as compared with infant (Forbes, 1991).

Inborn abilities and preferences: The feeding interaction is perhaps the most important experience for infants as it supplies nutrients for growth, establishes the mother-infant bond, provides a sense of security and pleasure for the infant and presents repeated opportunities for learning and social exchange.

Transition to complementary foods: The developmental period during which the transition to solid foods is initiated is a dynamic period of growth and learning. Dietary pattern change more during this period than any other time of life. This transition necessitates rapid learning about flavours, foods, etiquette and social exchange. An infant's first experience with flavours may, in fact, occur before birth as flavours from a pregnant woman's diet are transmitted to amniotic fluid (Mennella *et al*., 1995). Human milk also conveys flavours from the maternal diet to the breast fed infant; in contrast, the formula fed infant has a very different feeding experience with respect to exposure to flavour from food.

Current findings in the area of gustatory research suggest that an infant's early experience with flavours in human milk may positively influence the transition to, and acceptance of, solid foods (Sullivan and Birch, 1994; Mennella and Beauchamp, 1997). Figure 2 shows that breast-fed infants consumed greater amount of novel food than formula-fed infants at three points during an experimental study on food acceptance of the first transition foods offered and consume greater quantities of cereals prepared with human milk.

Therefore, the learning associated with repeated exposure to flavours from human milk may predispose the infant to accept solid foods more readily.

An unnoticed development in young children's eating behaviour is food neo phobia that refers to the fear of new foods. As infants enter toddler-hood, the types and amounts of foods offered to them also change, they begin to indicate, verbally and behaviourally, likes and dislikes for certain foods (Carruth *et al*., 1998). Their food dislikes may result in avoidance of particular foods or groups of foods, thus limiting dietary variety (Falciglia *et al*., 2000) and potentially, the major sources of essential nutrients. Moreover, consistent avoidance of foods may result in lifelong unhealthy food habits.

Toddlers are predisposed to neophobia, which typically presents between 13-24 months of age. Children previously judged as "good eaters" in infancy often begin to reject new foods and exhibit refusals of formerly accepted, familiar items. This is a particularly confusing and worrisome time for parents who fear that the child will suffer growth or health problems that stem from a less than balanced diet. Escalating parents-child power struggles during mealtime can perpetuate the problem. Parental confusion and anxiety developed by children's noephobic tendencies get worse by the lack of clear nutrition guidelines for this period of rapid development and growth. There is growing recognition for the construction of practice guidelines to be used by health care professionals to support parents in helping their established healthy eating behaviours (Picciano *et al*., 2000). Children's acceptance of new foods is not instantaneous and it requires repeated exposure and experience with new foods to overcome neophobia and enhance acceptance (Birch and Marlin, 1982). Research indicates, that children may require 5-10 exposures to a new food before changes in liking are expressed.

After repeated experience with one version of a novel food, children show clear preferences for the version to which they have been repeatedly exposed over an unfamiliar version (Sullivan and Birch, 1990).

Mothers and care givers at home who struggle with their children's picky eating may not be aware that neophobia is a normal stage of development and the consistency,

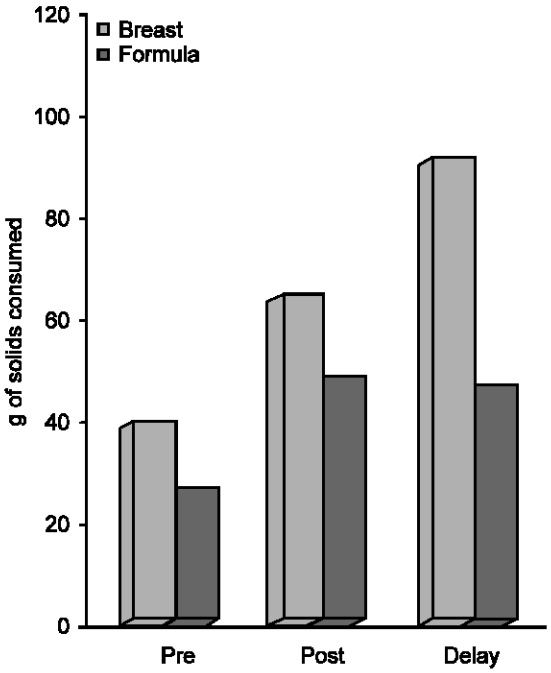

Fig. 2: Comparison of breast-fed and bottle-fed infant's consumption before and after repeated experience with first solid food. Source: Sullivan and Birch (1994)

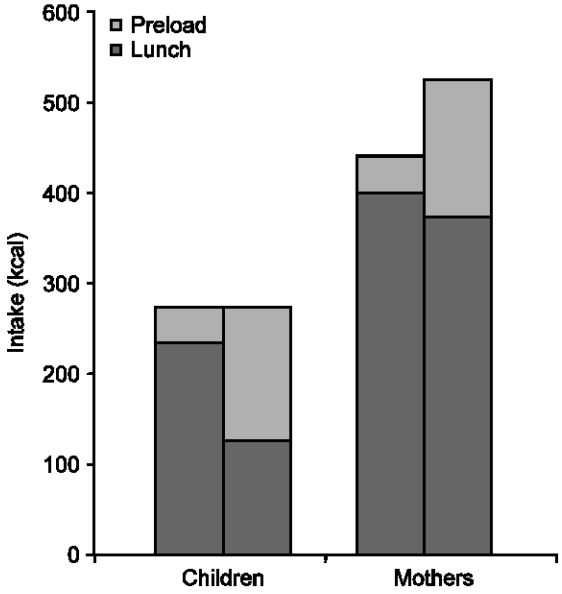

Fig. 3: Children's and mother's energy intake at lunch (after the ingestion of high and low calorie "preloads" or snacks). Children adjust energy intake to reflect the energy content of the preload. In contrast, adult mothers showed little evidence of compensation. Source: Birch and Deysher, 1986

patience and endurance are the keys to helping children increase the variety in their diets. Research findings stress the importance of early experience and opportunities for learning. The most consistent findings reveal that children like what they know and come to accept new foods if provided repeatedly, in a non-coercive manner.

Self-regulation of energy intake in children: Conventional wisdom holds that natural patterns of intake among young children are erratic, where children eat "like birds" one day and "like horses" the next. Research provides evidence to the contrary. Fomon and colleagues demonstrated that infants could self-regulate energy intake-consuming consistent energy from formula over the course of a 24-h period when formula energy density and/or energy source (carbohydrate or fat) were modified (Fomon *et al*., 1969, 1975).

Subsequent research has indicated that preschoolers also have the ability to respond to energy dense cues within a meal and to adjust their food intake in relation to energy density. Using single meal protocols in which a preload and a main course are offered (Birch and Deysher, 1986) have consistently demonstrated that children consume less during a meal after ingesting high energy preloads than after low energy preloads. As shown in Fig. 3 children exhibited a greater responsiveness to the energy content of the foods consumed than did their mothers as adults who consumed roughly the same amount of food at lunch whether given a low or high energy preload.

Children need enough energy to sustain optimal growth. Young children need to consume energy-dense foods in order to meet their energy requirements.

Reinforce children's ability to self-regulate energy intake: Individual differences in the extent to which children self-regulate energy intake clearly exist and are systematically related to differences in children's weight and adiposity; preschool-aged children who show less evidence of self-regulating energy intake tend to be heavier (Fisher and Birch, 1999).

Factors affecting dietary patterns of children (under 5 years): There are many factors that influence on "Dietary Patterns" of children. Dietary patterns of children are shaped by some of the endogenous factors and others are environmental factors (see Fig. 4).

As Katherine describes children's food choices are shaped by individual, societal and cultural factors. Some are endogenous to the individual child; but others are environmental. These include the foods made available to children inside and outside the home and the modelling of food behaviours by parents or the caregivers (Katharine *et al*., 2001).

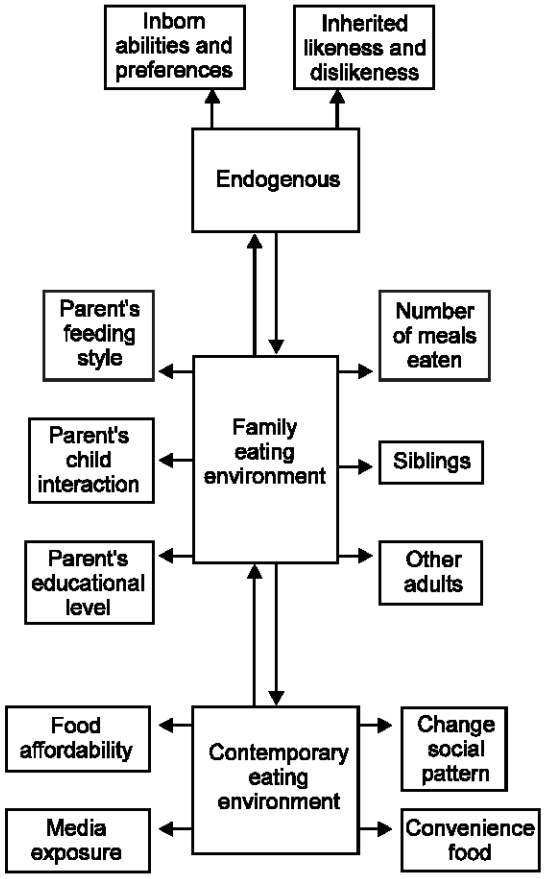

Fig. 4: Factors affecting dietary patterns of young children (under 5 years of age). Adapted from Katherine *et al.* (2001)

Parents provide their children with both genes and the environment where eating occurs. Genetic influences on eating are apparent at birth, in the observation that infant possess an unlearned preference for the taste of Sweet (Desor *et al.*, 1975).

The family eating environment: Parents and other influential care givers like grandparents, Aunts, Uncles living with the family and siblings profoundly influence the eating environment in which children's preferences and intake regulation patterns develop they determine the availability and composition of the child's diet, provide a model eating behaviour and guide the child's eating through feeding practices (Birch and Fisher, 1998).

Many health authorities, therefore, recommend that parents, grand parents, teachers and other influential adults such as day-care staff should guide children in developing healthy eating patterns and acquiring information on nutrition and diet-health relationships. Some studies have explored the affects of parent's nutritional knowledge on their children's knowledge and dietary practices. Contento found that Latin mother's nutritional knowledge and attitudes toward nutrition were positively correlated with nutrient intakes of their 4-5 years old children (Contento *et al.*, 1993). An association was also found between complementary feeding practices and mother's education by Liaqat *et al.* (2007). Parents have a major influence on their children's eating patterns. Oliveria found nutrient intakes in aggregation families with the strongest associations between mothers and their children (Oliveria *et al.*, 1992).

Composition of the family diet: By selecting the foods that come into home, parents have direct control over the foods to which children are repeatedly exposed. This point is particularly important as familiarity and repeated exposure to foods facilitate the acquisition of food preferences. This is true in case of Pakistani meals where mixed/composite foods are frequently used in daily meals (Liaqat *et al.*, 2009).

Stanek found a positive correlation between the qualities of the home environment and the nutrient adequacy of the children's diets. Children who ate with parents, siblings, or both at mealtime also had better diets, defined as taking more servings from the five basic food groups (Stanek *et al.*, 1990).

Family eating behaviour as model for young children: The second role that parents play in the development of children's eating behaviour is serving as models, both in terms of what and how much is consumed.

A recent study provides evidence that modelling effects may be most significant for high energy containing foods (Jansen and Tenney, 2001).

Children's eating patterns is influenced by routine with the family Unit, such as number of meals eaten together (Vauthier *et al.*, 1996). Young children who eat meals with other family members eat more healthy, nutrient-dense foods. Further, children who have companionship at meal times tend to eat more serving of foods from the basic five food groups (Stanek *et al.*, 1990).

Finally, children learn manners and adopt cuisine rules from observing their parents, such as whether foods are eaten with a spoon or with their hands and at what occasions foods are normally consumed. For instance, children tend to prefer particular foods at the times of the day when those foods are most commonly consumed within a given culture (Birch *et al.*, 1984).

Child feeding practices: The social contacts in which foods are presented also influences whether they are accepted; young children who observe adults eating a certain food is more likely to eat it, like wise, using a food as a reward are presenting it with some attention from the adults also enhances a child's acceptance of it (Birch *et al.*, 1995).

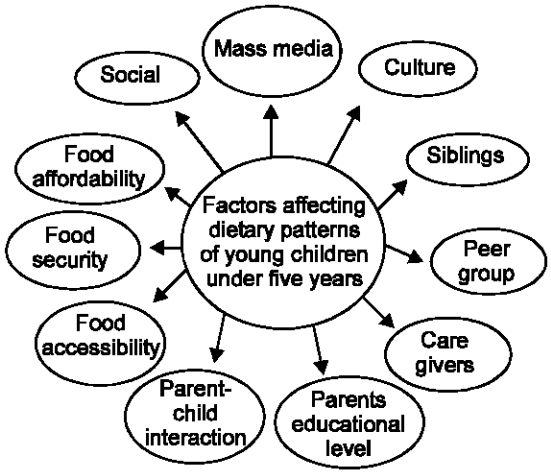

Fig. 5: Factors affecting on dietary pattern of young children (under 5 years) in Pakistan

Horne describes the relationship between using reward contingencies to encourage intake, however, is not straightforward. A series of experiments has shown that reward can promote the consumption of foods and vegetables when paired with modelling and praise (Horne *et al*., 1995).

Parent's feeding styles are also associated with children's food habits. Feeding styles represent parent's approaches to maintain or modify children's behaviours with respect to choosing and/or eating foods.

Permissive feeding: It is characterized by a lack of structure in child feeding, the child is simply allowed to eat choice foods of any quantities however these choices are limited only by what is available. Permissive feeding has been associated with drinking less milk and lower consumption of all nutrients except fat (Cullen *et al*., 2000).

Authoritarian feeding: Authoritarian feeding including behaviours such as restricting the child from eating certain foods and forcing the child to eat other foods. In the long-term, authoritarian feeding has been associated with lower intake of fruit, juices and vegetables (Cullen *et al*., 2000). According to Birch and Fisher parents employing stringent controls during mealtimes may influence their child's preference for high fat, energy dense foods and inhibit their preference for a variety of healthy foods (Birch and Fisher, 1998).

Authoritative: It represents a balance of authoritarian and permissive feeding and sets the stage for children to make healthful eating choices in the future. Adults determine which foods are offered and children determine which foods (and how much) are eaten. Authoritative feeding practices include: asking the child to make decisions about the type of food eaten, giving small portions when introducing a new food, involving the child in decisions about new foods, explaining the health benefits of foods perceived as healthy and praising the child for eating healthy foods. Authoritative feeding is associated with greater fruit and vegetable availability, higher intake of fruits and vegetables and lower intake of foods with less nutritive value (Gable and Lutz, 2000).

Trendy eating environment: While the family is undoubtedly the first and the most central environment in which children's eating develops, families do not parent there in isolation of the larger societal and physical environment in which they live. Environmental constraints on parent's ability to promote healthful patterns of eating include food availability, accessibility, affordability, time demands in family life, loss of the family meal, television, dinning out and child care.

The dietary patterns of young children from families in which television viewing is a normal part of meal routines include fewer fruits, vegetables, more pizzas, snack foods and sodas than the dietary patterns of children from families in which television viewing and eating are separate activities (Katharine *et al*., 2001).

Increasing reliance on convenience foods and meals consumed outside the home is associated with higher intake of dietary fat and calories and with lower intake of fruits and vegetables. This could result in lower intakes of fibre, calcium, iron and other nutrients that are important for children's growth and development. (French *et al*., 2001).

These children consume fewer fruits and vegetables, more fried food and soda, more saturated and trans fat, higher glycemic load and less fibre and micronutrients (Stanek *et al*., 1990) may preclude preparation of healthful dinners at home.

Dietary pattern that result in high intakes of fats and saturated fats and low intakes of fruits and vegetables are linked to increase risks of coronary heart disease, certain cancers, diabetes, hypertension and obesity. (Frazao, 1998). Although these diseases `typically manifest themselves in midlife or later, diet-influenced physiologic variables associated with chronic disease track from childhood into adulthood and evidence suggests a positive association between obesity in adolescence and morbidity in later life (Freedman *et al*., 1987). For these reasons there is considerable interest in deeping our understanding of the influences on children's diet (Must and Strauss, 1999).

Changing social patterns and socio economic factors influence whether meals are cooked and served at home and how frequently these have been prepared. Today's parents are working longer hours and many children have either single-parent families or two parents working outside the home. Thus parents increasingly rely on convenience foods or home meal replacements, including those from restaurants (NPD, 2000).

Table 1: Indicators of child malnutrition

Indicators	(NNS 2001-02)	(NNS 1985-87)
Malnourished (low weight-for-age)	37.8%	47%
Stunting: (low height-for-age)	37%	46%
Wasting: low weight-for-height	13.2%	15%

Pakistani perspective on dietary pattern of children (under 5 years): Nutrition Survey of West Pakistan (1965-66) and National Micro-Nutrient Survey (1976-77) had focused on important nutritional problems, particularly Protein-Energy Malnutrition (PEM) and hidden hunger among children of all ages. Similarly National Health Survey of Pakistan 1985-87 highlighted the nutritional status of the various population groups with particular emphasis on at risk groups National Nutrition Survey 2001-02 however focus on the children under five and their indexed mothers, highly vulnerable segment of the society, who could be prevented in coming decades.

During 2001-02 survey, the food intake was obtained using food frequency method for a given period of time in combination with the 24-h recall method for the previous day. Frequencies indicate that the food consumption pattern and use of selective food groups over a given period of time.

Pakistan being developing country have diversified scenario of Nutritional problems e.g. according to various nutritional surveys conducted from time to time at national level indicate that the prevalence of malnutrition among Pakistani children remains high. In spite of sustained food supply Pakistan is one of the few Asian Countries that has shown little evidence of improvement in nutritional status of young children under five over the last two decades. This has been documented in the past twenty years (Table 1). The information is drawn from two major national nutritional surveys I-e the National Nutrition Survey (NNS) conducted in 1985-87, the National Nutrition Survey Conducted in 2001-2002 and UNICEF report at a glance 2003.

Persistently high malnutrition indicators are responsible for hidden retardation among its population and as well as the country's development. Poor nutritional status of children can lead to poor education performance, poverty and consequently affect the country's overall potential economic growth.

Factors affecting dietary pattern of Pakistani children under five years: Despite the environmental and technological constraints, total food availability in the country has been sufficient to meet the overall national requirement of the whole country's population. On the other hand, poor health and poor nutritional status continues to be a major public health concerns among children and their mothers. This indicates general food availability issues leading to food affordability at the household level as one of the major cause of poor nutritional status in Pakistan. The factors affecting the dietary pattern of young children in the long run are summarized in Fig. 5 About 18.86 million children under five fall in age group of which 42% are underweight, representing an alarming condition of food insecurity.

Conclusion: It can be concluded that dietary habits are the resulting affect of broad spectrum dietary patterns emerging from the daily food consumption patterns and meal patterns over a longer period of time and are developed due to availability, accessibility and affordability and effected geographical, cultural, psychological and religious and media influences.

There is a dire need to design and conduct research studies with an entire dietary pattern approach including daily intake of mixed/composite foods prepare with different recipes and their meal patterns to identify and assess the overall dietary pattern of young children under 5 years of age and to study the various combinations of foods consumed along with the assessment of their nutritive values. A dietary pattern also covers the routine or timings at which child takes a meal, number of meals, frequency of meals and snacks (eating between main meals) intake patterns or profiles of intake of multiple or single nutrient in the form of foods. Thus research studies need to be planned and conducted with the objectives of exploring target areas to establish relationship between dietary patterns and nutritional status of young children living in different socio-economic situations with an aim to contribute in developing food base dietary guidelines for children's under 5 years at national level.

The association need to be established among different dietary patterns with socio economic groups of the regions thereby finding an opportunity to assess and evaluate the prevailing dietary standards and to recommend measures for improving dietary pattern of young children (under five years of age) in all socio economic groups.

These studies are highly effective in getting an understanding of children's food preferences, which are essential in developing strategies to improve the quality of children's dietary intake and will contribute, in developing dietary guidelines for children (under 5 years), as research in nutritional deficiencies and food availability issues in order to modify and promote healthy eating habits of vulnerable groups is the key to convince and generate consciousness not only among policy makers at national and provincial levels but also among the consumers families and communities at large.

To combat with the existing malnutrition mixed/ composite foods for toddlers based on indigenous foods need to be processed and manufactured locally to compete the cost of the imported complementary food products. Such complementary foods locally processed and manufactured from indigenous foods could be highly cost effective. The Government, food factory owners multinational food industries would want to undergo the manufacturing and marketing these composite complementary food products.

Local private food industry might find some studies interesting to become active and more productive to meet the demand of ready to eat or ready to cook complementary composite foods. As the overall food availability in accordance with desired dietary pattern on an affordable price is the ultimate solution to the malnutrition problem among children under five.

Indigenous food items for young children would be convenient for improving dietary status of children of low economic groups if these could be prepare and manufactured under hygienic conditions. This method will control the diarrhoea/ cholera and food borne diseases which are caused by the unhygienic food items and contaminated water.

In addition the proposed studies could be highly useful in designing intervention programs to promote, protect and support optimal breast-feeding and complementary feeding practices and discourage/minimize bottle-feeding.

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