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Journals

Bagley, L. G., and V. L. Christensen. 1991. Hatchability and physiology of turkey embryos incubated at sea level with in- creased eggshell permeability. *Poult. Sci.* 70:1412–1418.

Buch, L. H., A. C. Sorensen, J. Lassen, P. Berg, J. A. Eriksson, J. H. Jakobsen, and M. K. Sorensen. 2011. Hygiene-related and feed-related hoof diseases show different patterns of genetic correlations to clinical mastitis and female fertility. *J. Dairy Sci*.94:1540–1551.http://dx.doi.org/10.3168/jds.2010-3137.

Chapinal, N., A. M. de Passille, D. M. Weary, M. A. Hayes, B. J., P. J. Bowman, A. C. Chamberlain, K. Savin, C. P. van Tassell, T. S. Sonstegard, and M. E. Goddard. 2009. A validated genome- wide association study to breed cattle adapted to an environment altered by climate change. PLoS ONE 4:e6676.

De Vries, M. J., and R. F. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. *J. Dairy Sci*. 83:62–69.

Jenkins, T. C., E. Block, and P. H. Morris. 2011. Potassium reduces the accumulation of trans-10, cis-12 conjugated linoleic acid and trans-18:1 in continuous cultures of mixed ruminal microorganisms regardless of dietary fat

level. *J. Dairy Sci*. 94(E-Suppl. 1):509. (Abstr.)

VanRaden, P. M. 2008. Efficient methods to compute genomic predictions. *J. Dairy Sci*. 91:4414–4423.

Books

AOAC International. 2012. Official Methods of Analysis. 19th ed. AOAC International Gaithersburg, MD.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Lengemann, F. W., R. A. Wentworth, and C. L. Comar. 1974. Physiological and biochemical aspects of the accumulation of contaminant radionuclides in milk. Pages 159–170 in Lactation: A Comprehensive Treatise. Nutrition and Biochemistry of Milk/ Maintenance. Vol. 3. B. L. Larson and V. R. Smith, ed. Academic Press, London, UK.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Conferences

Barbano, D. M. 1996. Mozzarella cheese yield: Factors to consider. Page 29 in Proc. Wisconsin Cheese Makers Mtg. Ctr. Dairy Res., Univ. Wisconsin, Madison.

National Mastitis Council. 1995. Summary of peer-reviewed publications on efficacy of premilking and postmilking teat disinfections published since 1980. Pages 82–92 in Natl. Mastitis Counc. Reg. Mtg. Proc., Harrisburg, PA. Natl. Mastitis Counc., Inc., Madison, WI.

Talmant, A., X. Fernandez, P. Sellier, and G. Monin.1989. Glycolytic potential in longissimus dorsi muscle of Large White pigs as measured after in vivo sampling. In: Proc. 35th Int. Congr. Meat Sci. Technol., Copenhagen, Denmark. p. 1129.

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Other

Biernoth, G., and W. Merk, inventors. 1985. Fractionation of milk fat using a liquified gas or a gas in the supercritical state. Unilever NV-PLC, assignee. US Pat. No. 4,504,503.

Choct, M., and R. J. Hughes. 1996. Long-chain hydrocarbons as a marker for digestibility studies in poultry. Proc. Aust. Poult. Sci. Symp. 8:186. (Abstr.)

FASS. 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. Federaton of Animal Science Societies, Champaign, IL.

Interbull. 2008. Genetic evaluation. Direct longevity. Accessed Dec. 20, 2012. http://www-interbull.slu.se/longevity/l aug08.html.

Kelly, M. G. 1977. Genetic parameters of growth in purebred and crossbred dairy cattle. MS Thesis. North Carolina State Univ., Raleigh.

Peak, S. D., and J. Brake. 2000. The influence of feeding program on broiler breeder male mortality. Poult. Sci. 79(Suppl. 1):2. (Abstr.)

US Department of Agriculture, Plant and Animal Health Inspection Service. 2004. Blood and tissue collection at slaughtering and rendering establishments, final rule. 9CFR part 71. Fed. Regist. 69:10137–10151.

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Assessment of Rural Dairy Products in North Kordofan State, Sudan

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ABSTRACT

Laboratory cheese making trials were conducted in a rural area of western Sudan (North Kordofan) to study the effects of milk type (goat vs. cow) and cheese type (white soft vs. braided) on cheese characteristics. Randomized complete block design for the cheese samples data and a 2×2 factorial randomized complete block design for the laboratory trials data were used. Goat milk recorded the highest ($P < 0.05$) ash contents, whereas cow milk contained the highest total solids (TS), fat, lactose and protein. Goat milk required longer coagulation time compared with cow milk. White cheese required relatively ($P > 0.05$) more time to coagulate compared with braided cheese. White cheese made from goat milk (WG) required the longest ($P < 0.05$) coagulation time, followed by braided cheese from goat milk (BG), braided cheese from cow milk (BC) and then white cheese from cow milk (WC). Cheese yield was significantly affected by both milk and cheese types ($P < 0.01$) and their interaction ($P < 0.05$). The yield of WC was the highest, followed by WG, BC and the least was for BG. Goat cheese had the highest pH, whereas cow cheese had the highest TS, fat and protein contents. Braided cheese had the highest pH, TS, and protein contents while fat contents were highest in white soft cheese. Efficiency of protein and fat recovery were highest in cow cheese ($P < 0.05$). The highest efficiency of recovery was found in braided cheese. No staphylococcus and Coliform bacteria were detected in milk samples used in cheese making trials. BG recorded the highest scores in color ($P < 0.001$), texture ($P > 0.05$) and flavor ($P< 0.01$). However, taste score was higher ($P < 0.05$) in BC compared to the other three cheese types. Cheese produced under laboratory conditions in this study was of high quality. However, there is a high need to raise the awareness of rural dairy producers on hygiene and public health measures necessary for obtaining safe dairy products. Milk intended for dairy processing should be heated (boiling, pasteurization) in order to control bacterial growth and to ensure good quality dairy products. Moreover, there is also a high need for setting quality standards and measures for the different dairy products. Cheese making from sheep and camel milk should also be tested. Other milk coagulants (natural and/or synthetic) should also be evaluated to reduce cost.

Key words: dairy products, cow, goat

INTRODUCTION

Cheese making in Sudan is the major preservation method for surplus milk in rural area especially during rainy season when plenty of milk is available (El Owni and Hamid, 2008). White cheese is the major type of cheese in Sudan. Other cheese varieties are also produced but in limited scale, these include braided (Mudaffara) and recently Gouda and Mozzarella have been introduced (Ibrahim, 2008). White cheese is usually made from raw milk and without the use of starter culture (Abdalla *et al*., 1993). The gross composition of cheese milk, especially the concentrations of protein, casein and fat, has a major influence on several aspects of cheese manufacture, including rennet coagulability, gel strength, curd syneresis, cheese composition, yield and quality (Guinee *et al*., 2007).

In Kordofan region, traditional cheese making is a seasonal activity. During the rainy season when plenty of milk is available, few people are actively engaged in making cheese. This, perhaps, is the most commonly practiced method of chemical preservation of milk in Sudan (Ibrahim, 1970). Rural development projects in the area have many mobile laboratories or units for cheese making, scattered along the migration routes and camping areas of transhumant tribes in North Kordofan State (Ahmed, 1985). However, no thorough investigation of traditional processing methods and quality of dairy products in the area has been undertaken. This work was carried out with an ultimate objective of assessing rural dairy products situation and quality in North Kordofan, and comparing cheeses made from goat and cow milk.

MATERIALS AND METHODS

Area of study

This study was carried out in western Sudan (North-Kordofan state) under semi-arid conditions; latitude 11° 15’ N, longitude 27° 32’ E. Average temperature varied between 30 – 35°C with peaks above 40°C. Summer rainy season extends from July to October during which many animal herders are engaged in cheese making from surplus milk.

Laboratory cheese products making

At the research station, two types of white cheese (soft and braided) were processed from cows and goats using rennet and table salt (NaCl). The trials were replicated 6 times for 6 consecutive days. Milk from both sources were first heated to 72°C, cooled and divided into two equal portions, one is used to make braided chesses and the other for soft white cheese.

The white cheese was made by heating the milk to 40°C. Table salt (8%) and rennet (One tablet/50 kg milk) were added, stirred for 5 minutes and left to coagulate. The curd was cut into cubes, placed in a mesh and left to drain the whey for 20 – 30 minutes. The whey was kept for further use, whereas the cubes were transferred into clean wooden moulds lined with cheese cloth and pressed overnight. The cubes were finally stored in plastic containers containing the previously preserved whey heated to 72°C for 1 min and cooled. The braided cheese was prepared as the soft cheese but the cubes produced were colded until the required acidity (0.46 –

0.60) for kneading was reached. Ripening was assessed by testing the ability of the curds to be kneaded into ropes. After draining off the whey, the ripened cubes were placed in a wooden plate and cut into slices to which *Nigella sativa* seeds were added. The slices were then hand-kneaded and pulled into braided ropes washed and stored with the whey in plastic containers.

Chemical analyses

Chemical analyses for milk, cheese and whey were carried out: fat was determined by the Gerber method, crude protein (CP) according to Kejldahl method, total solids (TS) and water by the draft oven method (Marshall, 1993). Lactose was determined as outlined by Taylor (1970), while pH and ash were determined according to AOAC (1990).

Bacteriological analyses

Media preparation and chemical tests of bacteria were carried out as shown by Cowan and Steel (1981). Viable bacterial counts were done according to Schalm *et al.* (1971).

Panel test

Ten untrained panelists were chosen to judge the quality of cheese (color, texture, flavor and taste) using hedonic scale of 1 to 4 (Watts *et al*., 1989).

Statistical analyses

Descriptive statistics were used for the questionnaire. For chemical composition data, random complete block design was utilized. The data of the laboratory cheese making trials and panel test used a 2 X 2 factorial randomized block design. Duncan multiple range test was used to test mean separation (Steel and Torrie, 1980).

Results

Chemical Composition and Bacteriological Profile of Dairy Products

Dairy products trials

Fresh milk samples from cows and goats, analyzed for their nutrient contents and chemical composition showed that, cow milk had higher ($P < 0.05$) TS, fat and protein contents, whereas goat milk had higher ($P < 0.05$) pH and ash values (Table 1).

Table 1. Chemical composition of milk

Constituent	Cow milk	Goat milk	SE±
pH value	6.6	6.7	0.04*
Total solids (%)	13.2	12.2	0.28*
Ash (%)	0.7	0.9	0.03*
Fat (%)	3.9	3.5	0.10*
Protein (%)	3.7	3.4	0.09*
Lactose (%)	4. 8	4.5	0.22NS

NS = not significant (P > 0.05), * Significant (P < 0.05), SE = Standard Error

Effects of milk and cheese types on coagulation time and yield, showed that, goats' milk had longer (P <0.05) coagulation time but lesser yield (P<0.001) than cows' milk, whereas braided cheese showed higher yield (P <0.001) than the white cheese. Interactions of type of milk X type of cheese (interaction between type of milk and type of cheese) showed significant (P<0.05) longer time for coagulation of white cheese from goats but at the same time higher yields for the white cheese from goat milk. The lowest yield was obtained for braided cheese of goat milk (Table 2).

Table 2. Effects of milk type and cheese type on coagulation time (hr) and yield of cheese

Factor	Coagulation Time (hr)	Cheese Yield (kg/100 kg milk)
Type of milk		
Cow	6.5	13.7
Goat	10.6	11.9
±SE (Type of milk)	1.06 *	0.12 ***
Type of cheese		
White	9.1	15. 9
Braided	7.9	9. 8
± SE (Type of cheese)	1.06NS	0.12 ***
Type of milk×Type of cheese		
Cow–White (CW)	5.1b	17a
Goat–White (GW)	13.2a	14.8b
Cow–braided (CB)	7.8b	10.8c
Goat–braided (GB)	8.1b	9.2d
± SE (interaction)	1.5*	0.17*

abcdValues within the same column bearing different superscripts vary significantly at (P<0.05).
NS = not significant (P>0.05). *Significant at P<0.05, *** highly significant at P<0.001.

Except for lactose, significant differences due to milk type could be detected, TS, and ash (P <0.01) as well as fat and protein (P <0.05) were higher in cheese produced from cow than those from goats, whereas the pH was higher (P <0.05) in cheese produced from goats. Braided cheese showed higher (P <0.001) pH, TS and protein values than white cheese. Interaction due to type of milk X type of cheese was only significant (P <0.05) for ash where braided cheese from goats was higher than that of both white and braided cheeses from cows (Table 3).

Table 3. Effects of milk type and cheese type on cheese chemical composition.

Factor	pH	TS[1] (%)	Ash (%)	Fat (%)	Protein (%)	Lactose (%)
Type of milk						
Cow	5.3	58.3	7.9	24.6	20.8	4.9
Goat	5.4	55.2	9.1	21.8	19.6	4.8
±SE	0.07*	0.62*	0.17**	0.42**	0.39*	0.38NS
Type of cheese						
White	5.1	54.0	8.5	23.5	16.6	5.4
Braided	5. 7	59.5	8.5	22.8	23.8	4.4
±SE	0.07***	0.62***	0.17NS	0.42NS	0.39***	0.38NS
Type of milk × type of cheese						
Cow-white (CW)	4.9	56.3	8.37bc	24.9	17.3	5.8
Goat-white (GW)	5.1	51.7	8.8ab	22.2	15.9	4.9
Cow-Braided (CB)	5.7	60.3	7.64c	24.2	24.3	4.0
Goat-braided (GB)	5.7	58.7	9.4a	21.3	23.3	4.7
±SE	0.10NS	0. 9NS	0.25**	0.59NS	0.55 NS	0.5NS

abcValues within the same column bearing different superscripts vary significantly at (P<0.05). NS = not significant (P>0.05). *Significant at P<0.05, ** highly significant at P<0.001 *** very highly significant at P<0.001.
[1] total solids

Efficiencies of milk fat and protein recovered were higher (P <0.05) in milk from cows than those recovered from goats. Whereas braided cheese showed higher (P <0.01) protein recovery but less (P <0.01) fat recovery (Table 4).

Table 4. Effects of type of milk used and type of cheese produced on nutrient recovery in different cheeses

Factor	Efficiency of fat recovery (%)	Efficiency of protein recovery (%)	Efficiency of lactose recovery (%)
Type of milk			
Cow	85.9	78.7	14.3
Goat	78.7	74.7	14.8
± SE (type of milk)	2.22*	1.75*	1.23NS
Type of cheese			
White	91.7	72.0	17.4
Braided	72.8	81.4	11.7
± SE (type of cheese)	2.22**	1.75**	1.23**
Type of milk×type of cheese			
±SE (interaction)	3.15NS	2.48NS	1.73NS

NS = not significant (P > 0.05) *Significant at P<0.05, ** highly significant at P<0.001

A first whey characteristic was shown to be significantly affected by both cheese type and interaction of milk type X cheese type. Fat (P <0.01), TS and ash (P <0.001) were higher in braided than white cheese. Interaction was only significant (P <0.05) for the protein content where braided cheese first whey from goat milk had higher protein content than that of the white cheese (Table 5).

Table 5. Effect of milk type and cheese type on first whey (the whey before ripening) chemical composition

Factor	pH	TS[1]%	Ash%	Fat%	Protein%	Lactose%
Type of milk						
Cow	5.8	10.3	4.0	0.08	1.06	5.1
Goat	5.7	11.0	4.6	0.09	1.07	5.3
±SE	0.14^{NS}	0.40^{NS}	0.41^{NS}	0.27^{NS}	0.066^{NS}	0.28^{NS}
Type of milk cheese						
White	5.9	14.5	8.0	0.14	0.99	5.3
Braided	5.6	6.9	0.55	0.02	1.13	5.1
±SE	0.14^{NS}	0.40***	0.41***	0.03**	0.07^{NS}	0.28^{NS}
Type of milk x type of cheese						
Cow-white (CW)	6.0	14.0	7.5	0.13	1.1^{ab}	5.3
Goat-white (GW)	5.8	14.9	8.5	0.15	0.88^{b}	5.4
Cow-braided (CB)	5.7	6.6	0.50	0.02	1.03^{ab}	5.1
Goat-braided (GB)	5.5	7.1	0.59	0.02	1.3^{a}	5.2
±SE	0.19^{NS}	0.57^{NS}	0.58^{NS}	0.03^{NS}	0.09*	0.40^{NS}

[ab]Values within the same column bearing different superscripts vary significantly at (P<0.05). NS = not significant (P>0.05). *Significant at P<0.05, ** highly significant at P<0.001 *** very highly significant at P<0.001.
[1] total solids

Second whey showed higher fat and protein contents (P <0.001) as well as lactose values (P <0.05) in the white cheese whey compared to braided cheese (Table 6). The microbial profiles for the dairy products were not affected by either type of milk, type of cheese or their interaction (Table 7).

Table 6. Effect of milk type and cheese type on chemical composition of second whey (the whey after cheese ripening)

Factor	pH	TS[1]%	Ash%	Fat%	Protein%	Lactose%
Type of milk						
Cow	4.8	13.1	8.4	0.15	1.0	3.5
Goat	4.9	13. 6	9.2	0.24	1.1	3.0
±SE	0.12^{NS}	0.52^{NS}	0.44^{NS}	0.03^{NS}	0.08^{NS}	0.29^{NS}
Type of milk cheese						
White	4.4	14.1	8.1	0.37	1.04	3.9
Braided	5.3	12.6	9.4	0.03	0.45	2.7
±SE	0.12**	0.52^{NS}	0.44^{NS}	0.03***	0.08***	0.29*
Type of milk x type of cheese						
±SE	0.17^{NS}	0.74^{NS}	0.63^{NS}	0.04^{NS}	0.11^{NS}	0.42^{NS}

NS = not significant (P>0.05). *Significant at P<0.05, ** highly significant at P<0.001 *** very highly significant at P<0.001.
[1] total solids

Table 7. The microbiological profile of milk types used and cheese processed in the laboratory

	Staphylococcus	*Bacillus*	*Coliform*	Total Bacteria Count
Milk Type				
Cow	1.2×10^4	0	0	12×10^4
Goat	2.2×10^4	1.3×10^4	0	3.5×10^4
Cheese type x Milk type				
Cow-White (CB)	0	4.9×10^2	0	4.9×10^2
Goat-White (GW)	0	6.1×10^2	0	6.1×10^2
Cow-Braided (CB)	0	3.1×10^2	0	3.1×10^2
Goat-Braided (GB)	0	3.3×10^2	0	3.3×10^2

Organoleptic scoring of white and braided cheese

Cheese of cow milk had higher scores for taste and texture ($P < 0.05$) as well as flavor ($P < 0.001$) than goat milk. However, type of milk did not show significant effect on organoleptic scoring. Interactions showed that white cheese of cow milk had higher ($P<0.05$) flavor score than goat braided cheese (Table 8).

Table 8. Organoleptic scoring of white and braided cheese made from milk of cows and goats

Factor	Color	Texture	Flavor	Taste
Type of Milk				
Cow	2.81	3.53	3.42	3.06
Goat	2.94	3.03	2.50	2.33
±SE	0.214NS	0.153*	0.134***	0.192*
Type of Cheese				
White	3.11	3.36	2.97	2.86
Braided	2.64	3.19	2.94	2.53
±SE	0.214^{NS}	0.153^{NS}	0.134^{NS}	0.192^{NS}
Type of Milk x Type of Cheese				
Cow-White (CW)	2.78	3.44	3.22^{a}	3.00
Goat-White (GW)	3.44	3.28	2.72^{ab}	2.72
Cow-Braided (CB)	2.83	3.61	3.61^{a}	3.11
Goat-Braided (GB)	2.44	2.78	2.29^{b}	1.94
±SE	0.303^{NS}	0.217^{NS}	0.189^{NS}	0.271^{NS}

[ab]Values within the same column bearing different superscripts vary significantly at ($P<0.05$). NS = not significant ($P>0.05$). *Significant at $P<0.05$, ** highly significant at $P<0.001$ *** very highly significant at $P<0.001$.

DISCUSSION

Dairy products assessment

Nutrient contents of fresh and processed milk products showed higher fat, protein and total solid (TS) for cow's milk and milk products than goats milk. This could be attributed to species differences where goats' milk is characterized by small fat globules and low protein content and hence lower TS. Similarly, it has been pointed out that as fat content increases, moisture content decreases (El Erian *et al*., 1976). Also goat's milk showed longer time to coagulate, the poor cheese making ability with goats milk could be due to the specific properties of casein micelles such as composition, hydration and size compared to cows milk (Abdel-Razig, 1966). On the other hand, the high yield of cheese from cow's milk could be attributed to the high TS content (Moneib *et al.*, 1981: Ahmed and Khalifa, 1989).

Bacterial profile

Bacillus was found in all samples of cheese, this was attributed to that the wide range of pH for the growth of *Bacillus* that is 4.9 to 9.3 and salt content of 7.5 to 10% (Buchanon and Gibbons, 1974). The average total bacteria count for cow and goat milk used for cheese manufacturing were 1.2×10^4 and 3.5×10^2 CFU/ml. These were low counts compared with those reported by Zeng and Escobar (1996) who obtained a maximum bacterial count of 6.4×10^5 CFU/ml. Also O'Oconnor (1993) stated that plate count of bacteria should not exceed 50000 bacteria per milliliter. The average of *Staphylococcus* counts found in cow and goat milk were 1.2×10^4 and $2.2x10^4$ per ml, respectively. This count was in the range of the recommended number of *Staphylococcus* < 10^3 to 10^6 CFU/ml, depending on origin of milk (Zeng and Escobar, 1996).

Organoleptic Scores of White and Braided Cheese Made from Cow and Goat Milk

Cow milk cheese recorded the highest score points for texture, flavor, and taste. Generally, white cheese recorded relatively the highest score points in color, texture, flavor and taste compared to braided cheese. White cheese made from cow milk had the highest score points in color, flavor and taste. These results were in accord with the findings of Abdalla and Abdel-Razig (1997) who reported that white cheese made from cow milk significantly scored the best texture and flavor, while the color, saltiness and sourness were not significantly affected by type of milk. It is worth noting here that the cheese obtained in this study was of high standard quality, good color, attractive and glossy with smooth but firm body and texture, with better consistency, richness, much clean and had a good flavor, and without gas holes.

REFERENCE

Ahmed, A. M. 1985. Bacteriological and Chemical Characteristics of Sudanese White Cheese Produced and Stored under Different Conditions, Ph.D. Theses. University of Khartoum, Sudan.

Abdel-Razig, K. A.1996. The Production of White Soft Cheese from Different Milk Sources. M.Sc. Thesis. University of Khartoum. Sudan.

Abdalla, O. M., G. L. Christen, and P. M. Davidson. 1993. Chemical composition of and Listeria monocytogenes survival in White pickled cheese. *J. Food. Prot*., 56:841-846.

Abdalla, O. M. and A. K. Abdel Razig. 1997. Effect of type of milk on the quality of white soft cheese. U.K. *J. Agric. Sci*., 5:147-157.

Ahmed, T. K., and N. A. Khalifa, 1989. The Manufacture of white soft cheese from recombined milk. *Sudan J. Anim. Prod*. 2:63-69.

AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists (AOAC). Washington D.C. USA.

Buchanon, R .E., and N. E. Gibbons. 1974. Bergeys Manual of Determinative Bacteriology (8th Edn.). William and Wilking Co. Baltimore.

Cowan, S.T. and Steel, R.L. 1981. Cowan and Steel's Manual for the Identification of Medical Bacteria. 2nd edition.

El-Erian, A.F.M.; A.Tawab, and, I. E. G. Elrab. 1976. The Effect of Brine Salting. Method on the Quality and Chemical Composition of Domiati Cheese frounsalted Milk. *Agric. Res. Rev*. 54(7):173-179.

El Owni, A. O. O., and I. A. O. Hamid. 2008. Effect of storage period on weight loss, chemical composition, microiological and sensory characteristics of Sudanese white cheese (Gibna Bayda). *Pak. J. Nutr*. 7:75-80.

Guinee, T. P., E. O Mulholland, J Kelly, and D. J .O. Callaghan. 2007. Effect of protein-to-fat ratio of milk on the composition, manufacturing efficiency, and yield of Cheddar cheese. *Journal of Dairy Science*. 90:110–123.

Ibrahim, A. E. 1970. Chemical pasteurization of milk in the Sudan. *J. Food Sci. Technol*. 2:31-32.

Ibrahim, M. N. N. 2008. Effect of manufacturing methods and storage period on characteristics of Mozzarella cheese. M.Sc. Thesis. University of Khartoum.Sudan.

Marshall, R. T. 1993. Standard Methods for the Examination of Dairy Products. American Public Health Association. Washington D.C.

Moneib, A. F., A. A. El-Heiba, A. F. Al Khamy, S. El-Shibiny, and M. H. A. Elsalam. 1981. Pickling soft cheese- making from whole and skim milk powder. *Egyptian Journal of Dairy Science*. 9:37-44.

OConnor, C. B. 1993. Traditional Cheese Making Manual. ILCA (International Livestock Centre for Africa), Addis Ababa, Ethiopia.

Steel, R. G. D., and J. K. Torrie. 1980. Principle and Procedures of Statistics A biometrical Approach (2nd Edn.). McGraw- Hill Books CO. NY. USA.

Schalm, W.O., E. J. Carrol, and N. C. Jain. 1971. Bovine Mastitis, a book. School of Veterinary Medicine, University of California Press, Davis, USA.

Taylor, G. C. 1970. A rapid enzymatic method for the determination of lactose in cheese. *Australian J. Dairy Technology*. 25: 7.

Watts, B. M.; G. L.Ylimaki; L. E. Jeffery, and L. G. Elias. 1989. Basic Sensory Methods for Food Evaluation. International Development Research Centre (IDRC). Ottawa. Ontario. Canada. IDRC-277e. p:160.

Zeng, S. S., and E. N. Escobar. 1996 . Effect of breed and milking method on somatic cell count, standard plate count and composition of goat milk. *Small Ruminant Research*. 19:169-175.

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Microbial Quality of Beef in the Yendi Municipality of Ghana

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ABSTRACT

The microbial quality of beef, table and apron in five meat retail shops in the Yendi Municipality of Ghana was investigated in order to ascertain their safety. The shops were selected from Central market A (external), Central market A (internal), Central market B, Central mosque and Taxi rank. A total of 45 samples were collected, 9 from each meat shop (retailer). The samples were stored under 4oC for transportation to the laboratory. Microbiological analysis was carried out immediately upon arrival in the laboratory under aseptic conditions. Beef, table and apron samples from Central market B had the highest mean total bacterial count of 5.8×107 cfu/cm2, followed by Taxi rank (9.5×106 cfu/cm2), Central mosque (1.5×106 cfu/cm2), Central market A (external) (1.0×106 cfu/cm2) and Central market A (internal) (8.1×105 cfu/cm2). Mean bacterial count of beef, table and apron were 5.0×106 cfu/cm2, 3.7×107 cfu/cm2 and 3.1×105 cfu/cm2, respectively. Table surface bacterial count from Central market B was significantly higher ($p<0.05$) than bacterial counts from the other samples. Bacterial species identified on the beef, apron and table samples were Staphylococcus spp., Escherichia coli, Streptococcus spp., Pseudomonas spp., Proteus spp., and Bacillus spp. Among the five meat shops/retailers sampled, Central market B was the most contaminated shop. Table surfaces were also the most contaminated source compared to beef and apron. Staphylococcus spp. and Escherichia coli were the most common identified bacteria. There is the need for improvement is the standard of selling meat in the Yendi Municipality.

Keywords: Apron, beef, microbial quality, table, retail shops

INTRODUCTION

Animal production is an integral part of Ghana's agricultural economy and a major source of livelihood for many rural households in the Yendi Municipality. Ruminants such as cattle, goats and sheep and non-ruminants such as poultry and pigs are reared in Yendi (Adzitey, 2013). Animal protein is essential in human diets because the amino acid composition of animal protein

matches closely to that of humans (Warriss, 2010). Even though meat is very important to humans it can also be detrimental when contaminated with pathogenic microorganisms. The food that we eat is also rarely if ever sterile, they carry microbial associations whose composition depends upon which organism gain access and how they grow, survive and interact in food over time (Adams and Moss, 2008). The microorganism present will originate from the natural microflora of the raw material and those organisms introduced in the cause of harvesting\slaughter, processing, storage and distribution (Adams and Moss, 2008).

Some foodborne pathogenic microorganisms that contaminate meat are *Staphylococcus spp*., *Aspergillus spp*., *Salmonella spp*., *Campylobacter spp.*, *Listeria monocytogenes*, *Enterococcus spp*., *Streptococcus spp*., and *Escherichia coli* (Jay *et al*., 2005; Adams and Moss, 2008; Adzitey *et al*., 2010; Adzitey *et al*., 2011; Adzitey *et al*., 2012a; Adzitey *et al*., 2012b; Adzitey *et al*., 2013). Foodborne pathogens cause human illnesses and some deaths in developed and developing countries including Ghana. A good meat ready for consumption should not contain foodborne pathogens or their toxins that will be injurious to human health. It is therefore important to conduct research to determine the microbial quality of beef, to create awareness of the microbial safety of meat. The objective of this study was to determine the microbial quality of beef in the Yendi Municipality of Ghana.

MATERIALS AND METHODS

Location, duration and data collection

Samples were collected from five retail shops in the Yendi Municipality. The Municipal is located in the eastern corridor of the Northern Region of the Republic of Ghana between latitude 9^{o}-35^{o}N, 0^{o}-30^{o}W, and 0^{o}-15^{o}E (Population and Housing Census, 2010). This study took place between January, 2013 and July, 2013. A total of 45 samples were collected from five different meat retail shops in the Yendi Municipality using random sampling. Nine (9) samples were taken from each shop. The retail shops were Yendi market shop A internal, Yendi market Shop A external, Yendi market Shop B, Central mosque shop and Taxi rank shop. Table 1 shows the breakdown of the meat shops, type and number of samples examined. Swabs were taken from the table, apron and the meat. The swabs were transported to the University for Development Studies (U.D.S) Microbiology Laboratory under 4^{o}C and microbiological analysis carried out immediately upon arrival.

Table 1: Meat shop, type and number of samples examined at the Yendi Municipality

Meat shop	Type of sample and number examined		
	Beef	Table	Apron
Central market A (internal)	3	3	3
Central market A (external)	3	3	3
Central market B	3	3	3
Central mosque	3	3	3
Taxi rank	3	3	3
Total	15	15	15

Microbiological analysis and identification of bacteria genera

Swabs were placed in 10 ml sterile peptone water and thoroughly shaked to obtain the neat (10^1). One (1) ml of the neat was transferred into 9 ml sterile peptone water until 10^6 was obtained. Thus serial dilutions were made from 10^1 to 10^6 and were spread plated onto blood and nutrient agar plates. Plates were incubated at 37 ^oC for 24 hours under aerobic condition and the colony forming units were counted to obtain the microbial load. Colony forming unit was calculated using the formula:

$$N = \frac{\Sigma C}{[(1 \times n1) + (0.1 \times n2)] \times (d)}$$

N = Number of colonies per cm^2
ΣC = Sum of all colonies on all plates counted
n_1 = Number of plates in first dilution counted
n_2 = Number of plates in second dilution counted
d = Dilution from which the first counts were obtained

Some colonies with different shape, colour and appearance were picked at random from plate count agar and identified using Gram staining. The morphology and colour of the colonies under the microscope was compared to that of Anonymous (2013) to aid in the identification of the various genera. Other tests like catalase test, oxidase test and growth on McConkey (lactose and sorbitol) agars and blood agar were used to confirm some of the isolates.

Statistical analysis

Data obtained was analyzed using Statistical Package for the Social Sciences (SPSS) version 17.0 at 95% confidence level.

RESULTS AND DISCUSSION

The result obtained from sampling five meat retail shops is presented in Table 2. From Table 1, the total bacteria count for beef, table and apron ranged from 1.3×10^4 cfu/cm^2 to 1.7×10^8cfu/cm^2. The total mean microbial load was 5.8×10^7cfu/cm^2, 9.5×10^6 cfu/cm^2, 1.5×10^6cfu/cm^2, 1.0×10^6cfu/cm^2, and $8.1x10^5$cfu/cm^2 for Central market B, Taxi rank, Central mosque , Central market A (external), and Central market A (internal), respectively. Thus Central market B retail shop had the highest total mean count of 5.8×10^7cfu/cm^2 followed by the Taxi rank retail shop with a total mean count of 9.5×10^6cfu/cm^2 and the least was the Central market A (internal) with a total mean count of 8.1×10^5cfu/cm^2. In general, there were no significant differences ($p>0.05$) among all the type of samples examined except table surfaces from Central market B which was significantly higher ($p<0.05$) than the rest of the samples. The high bacteria count in the Central market B was above 10^6 and meat samples with microbial load above 10^6 is said to be unsatisfactory (Wilson *et al.*, 1981). The high level of contamination in this shop can be attributed to the fact that retailers in this area sell under shade, (exposed to the external environment) and practice unhygienic practices.

Table 2: Total aerobic bacteria count

Meat shop	No. of samples examined	Sample examined (cfu/cm^2)		
		Beef	Table	Apron
Central market A (internal)	9	1.9×10^{5a}	2.2×10^{6a}	2.5×10^{4a}
Central market A (external)	9	1.6×10^{5a}	2.9×10^{6a}	2.5×10^{4a}
Central market B	9	4.1×10^{4a}	1.7×10^{8b}	1.3×10^{4a}
Central mosque	9	2.4×10^{6a}	8.5×10^{5a}	1.3×10^{6a}
Taxi rank	9	2.2×10^{7a}	6.0×10^{6a}	2.1×10^{5a}
Total mean	45	5.0×10^{6}	3.7×10^{7}	3.1×10^{5}

Means (cfu/cm^2) in the same row and column with different superscripts are significantly different ($p\leq0.05$).

Observation made shows that the meat here is placed on tables which are not well cleaned after a day's work and in the open with houseflies hovering around the beef. The butchers themselves pay little concern to their personal hygiene and serve the meats with dirty hands and clothing. Prescott *et al*. (2002) reported that, the muscle tissues of healthy animals are free of microorganisms. However the muscle tissues are easily contaminated with both pathogenic and non-pathogenic microorganisms at the time of slaughter under poor processing conditions. In addition the high nutritive value of meat makes it an ideal medium for bacterial growth.

On comparing the microbial contamination at various retail shops it was observed that those retailers who sell in a closed environment (Central market a internal and Central market A external) had less microbial count than the rests. For instance, Central market A internal had the least average microbial count, and this meat shop was located within a block building housing numerous butchers with their meat displayed on tables. The building is well aerated with covered windows. This seems to reduce the number of flies within the building. The meats sold here are obtained from the abattoir. The main sources of contamination may be the unsterilized tables, apron and the handling of the meat with unsterilized instruments such as knives.

Other researchers have also investigated bacterial counts or types on beef and it related samples. Arthur *et al*. (2004) investigated the prevalence of *E. coli* and the number of aerobic bacteria and enterobacteriaceae at various steps in commercial beef processing plants and reported that 76% of animal hides coming into the plants were contaminated with *E. coli* O15, but no carcasses leaving the cooler were contaminated with *E. coli* O157. They also reported aerobic plate and enterobacteriaceae counts average of 7.8 and 6.2 log cfu/100cm^2, respectively, on hides, and 1.4 and 0.4 log cfu/100cm^2, respectively, on chilled carcasses. Swabs from 48 beef carcasses were all positive for aerobic bacteria with 99.8% of the samples, having total counts of ≤100000 cfu/cm^2 (Bohaychuk *et al*., 2011). Coliform bacteria were isolated from 22.4% beef carcasses (Bohaychuk *et al*., 2011). Goja *et al*. (2013) analyzed 40 samples of fresh meat (beef) randomly selected from Khartoum, Omdurman and Bahri in Khartoum State, Sudan and found that total viable count ranged from 4.78×10^4 to 3.39 x 10^5cfu/g and *Staphylococcus* count ranged from 3.23×10^3 to 8.7×10^3cfu/g. Out of 340 (250 raw meat samples and 90 surface swabs from meat processing equipment samples), 84% were found to be contaminated with bacterial species, including *Klebsiella*, *Enterobacter*, *Staphylococcus aureus* and *Bacillus subtilis* (Ali *et al*., 2010).

Microbial load on beef, table and apron

The genera of bacteria identified from beef, table and apron is shown in Table 3. From Table 3 beef sold in the Municipality was contaminated with various genera of bacteria with *Staphylococcus spp*. and *Escherichia coli* being the most commonly identified bacteria probably due to the poor slaughtering, handling, and environmental conditions. The mean viable count

found on the beef showed that apart from the beef sold in the Central market B retail shop with a mean total bacteria count of 5.8×10^7 cfu/cm^2, all the beef from the retail shops were not spoiled since counts were 10^6cfu/cm^2 or less. Nevertheless the isolation of pathogenic organisms like *Escherichia coli* which is important food-borne pathogen is of public health concern. Consumers are therefore at risk of consuming beef from the various meat shops around Yendi Municipality and adequate cooking will be needed to kill these pathogens.

Table 3: The genera of bacteria identified from beef, table and apron

Meat shop	Beef	Table	Apron
Central market A (internal)	*Staphylococcus spp., Streptococcus spp., Escherichia coli., Bacillus spp.*	*Staphylococcus spp., Proteus spp., Pseudomonas spp., Escherichia coli.*	*Staphylococcus spp., Streptococcus spp., Escherichia coli., Mucor spp.*
Central market A (external)	*Staphylococcus spp., Streptococcus spp., Mucor spp.*	*Staphylococcus spp., Pseudomonas spp., Proteus spp., Escherichia coli,*	*Staphylococcus spp., Mucor spp., Pseudomonas spp., Escherichia coli.*
Central market B	*Staphylococcus spp., Pseudomonas spp., Bacillus spp., Proteus spp.*	*Staphylococcus spp., Pseudomonas spp., Escherichia coli,*	*Staphylococcus spp., Streptococcus spp., Escherichia coli.*
Central mosque	*Staphylococcus spp., Pseudomonas spp., Proteus spp., Escherichia coli.*	*Staphylococcus spp., Proteus spp., Bacillus spp.*	*Staphylococcus spp., Pseudomonas spp., Bacillus spp., Escherichia coli.*
Taxi rank	*Staphylococcus spp., Bacillus spp., Pseudomonas spp., Proteus spp., Escherichia coli.*	*Staphylococcus spp., Proteus spp., Pseudomonas spp., Bacillus spp., Escherichia coli.*	*Staphylococcus spp., Streptococcus spp., Bacillus spp.*

The meat cutting table was highly contaminated with a total mean bacteria count of 3.7×10^7 cfu/cm^2 and Central market B had the highest count of 1.7×10^8 cfu/cm^2. Most retailers in the municipality cover the table with a piece of cardboard instead of washing after the days' work and the continuous addition of pieces of meat provide a source of nutrient for the growth of bacteria. Also, the temperature ranges between 21^{o}C to 36^{o}C which is conducive for bacteria growth. Reynolds *et al*. (2005) stated that about 80% of infectious diseases are spread through hand contact with hands and other objects. Some of the genera of bacteria that were identified on the table were *Staphylococcus spp.*, *Escherichia coli*, *Bacillus spp.* and *Proteus spp*. These Bacteria are easily transferred to the meat due to close contact and continuous turning of meat during cutting (FAO, 1991).

Apron contains total mean count of 3.1×10^5 cfu/cm^2 and Central mosque had the highest value of 1.3×10^6cfu/cm^2. It was observed that most of the retailers only put on their apron when they want to sell bones whilst others use the aprons to clean the cutting edge when about to sell meat. Also the apron is left on the table containing the meat. This gives a clear idea why most of the genera of bacteria on the table are also found on the apron. Rombouts and Nouts (1994) reported that, the clothing or hands of the personnel and the physical facilities are all sources implication of foodborne illnesses.

It was found that *Staphylococcus spp*. runs through all the retail shops. This can be due to contamination from the skin of the animal/humans or other unhygienic place in the abattoir during the process of slaughtering. This is in agreement with report by Postgate (2000) that *Staphylococcus spp*. can be part of the normal flora on the skin of humans and animals which can be transmitted from person to product through unhygienic practices. A similar work done by Adzitey *et al*. (2011) in the Tamale Metropolis revealed that, animals are slaughtered in abattoirs and sometimes in backyards without observing strict hygienic practices. It is also a common practice to see people carrying carcasses just after dressing on their bare shoulders (Adzitey *et al*., 2011). Sulley (2006) reported that, the vehicles and trucks for transporting carcasses are inadequate, and compelling others to use motor-bikes and bicycles as a means of transport. The same researcher reported that, the few transports are not properly cleaned and thus contained high microbial loads. Ansah *et al*. (2009) found various levels and numbers of total bacteria count, *Streptococcus spp., Staphylococcus spp., Bacillus spp., Escherichia coli, Micrococcus spp., Diplococci spp.* and *Corynebacteria spp*. on eggs sold in the Tamale Metropolis.

The general of bacteria identified in this study include many species which are non-pathogenic, and form part of the commensal human microbiome of the mouth, skin, intestine, and upper respiratory tract. However, some species of these genera can be pathogenic or cause food spoilage. For instance, *Escherichia coli* can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and pneumonia (Guentzel., 1996; Jay, 2000; Adams and Moss, 2008; Adzitey, 2011). *Bacillus spp.* includes species that cause anthrax, food spoilage and food poisoning similar to that caused by *Staphylococcus* (Guentzel., 1996; Jay, 2000; Adams and Moss, 2008). *Staphylococcus aureus* and *Pseudomonas aeruginosa* are currently the most common pathogens in nosocomial pneumonia, followed by *Enterobacter* and *Klebsiella* (Guentzel., 1996; Adams and Moss, 2008). *Pseudomonas spp*. also causes food spoilage (Jay, 2000). *Streptococcus spp*. can cause septic sore throat, scarlet fever, septicemia infections, meningitis, endocarditis, erysipelas and necrotizing fasciitis (FDA, 2013). *Proteus spp*. includes pathogens responsible for wound and many human urinary tract infections (Guentzel, 1996). Some *Mucor spp.* can cause mucormycosis which is characterized by thrombosis and tissue necrosis (Badior *et al*., 2013).

CONCLUSION

Beef sold at the Central market B was the most contaminated and contains various genera of bacteria that can be injurious to human health (*Escherichia coli, Staphylococcus spp., Bacillus spp*.). Nevertheless beef sold in the other retail shops were found to be near unacceptable limits and contain some genera of bacteria that are of public health concern. Table sources were the most contaminated, followed by beef and apron. General observation also revealed that beef were under unhygienic conditions. It is recommended that retailers/butchers should be educated on the need to practice personal hygiene.

REFERENCE

Adams, R., and M. O. Moss. 2008. Food microbiology. RSC Publishing. Cambridge. UK.

Adzitey, F. 2013. Animal and Meat Production in Ghana-An Overview. *The Journal of World's Poultry Research*. 3:1-4.

Adzitey, F., G. Rusul, N. Huda, T. Cogan, and J. Corry. 2013. Prevalence, antibiotic resistance and genetic diversity of *Listeria monocytogenes* isolated from ducks, their rearing and processing environments in Penang. *Malaysia. Food Control*. 32:607-614.

Adzitey, F., G. Rusul, and N. Huda. 2012a. Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang. *Malaysia. Food Research International*. 45:947-952.

Adzitey, F., G. Rusul, N. Huda, T. Cogan, and J. Corry. 2012b. Prevalence, antibiotic resistance and RAPD typing of Campylobacter species isolated from ducks, duck rearing and processing environments in Penang, Malaysia. International Journal of Food Microbiology. 154:197-205.

Adzitey, F. 2011. *Escherichia coli*, it prevalence and antibiotic resistant in Malaysia- a mini review. *Microbiology Journal*. 1:47-53.

Adzitey, F., G. A. Teye, W. N. Kutah, and S. Adday. 2011. Microbial quality of beef sold on selected markets in the Tamale Metropolis in the Northern Region of Ghana. *Livestock Research for Rural Development*. 23(1): 2011.

Adzitey, F., G.A.Teye, A.G. Ayim, and S. Adday. 2010. Microbial quality of chevon and mutton sold in Tamale Metropolis of Northern Ghana. *Journal of Applied Sciences and Environmental Management*. 14: 53-55.

Ali, N.H., A. Farooqui, A. Khan, A.Y Khan, and, S.U. Kazmi .2010. Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. *The Journal of Infection in Developing Countries*. 4:382-388.

Anonymous. 2013. Bacteria under microscope. Available at: http://www.bacteriainphotos .com/bacteria%20under%20microscope.html accessed on 18/02/2013

Ansah, T., G. S. K. Dzoagbe, G.A. Teye, S. Adday, and J. K. Danquah .2009. Microbial quality of table eggs sold on selected markets in the Tamale municipality in the Northern Region of Ghana. *Livestock Research for Rural Development*. 21(8): 2009.

Arthur, T.M., J.M. Bosilevac, X. Nou, S.D. Shackelford, T.L. Wheeler, M.P. Kent, D. Jaroni, B. Pauling, D.M. Allen, and M.Koohmaraie. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, enterobacteriaceae, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *Journal of Food Protection*. 67:658-665.

Badior, M., F. Trigo, C. Eloy, and J.E. Guimarães. 2013. Mucor infection: difficult diagnosis. *Clinical Drug Investigation*. 33:S19-21.

Bohaychuk, V.M., G.E. Gensler, and P.R. Barrios. 2011. Microbiological baseline study of beef and pork carcasses from provincially inspected abattoirs in Alberta, Canada. *Canada Veterinary Journal*. 52:1095-1100.

FAO. 1991. Techniques and hygiene practices in slaughtering and meat. Available at: handlinghttp://www.fao.org/docrep/004/t0279 e/t0279e04.htm:accessed on 18/02/2013.

FDA .2013. Foodborne pathogenic microorganisms and natural toxins handbook.

Streptococcus spp. Available at: http://www.fda.gov/Food/FoodborneIllnessCo ntaminants /CausesOfIllnessBadBugBook/ucm070584.ht m accessed on 18/02/2013.

Goja, A.M., T.A.A. Ahmed, S.A.M. Saeed, and H.A. Dirar. 2013. Isolation and identification of *Staphylococcus spp*. in fresh beef. *Pakistan Journal of Nutrition*. 12:114-120.

Guentzel, M.N. 1996. *Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter*, and *Proteus.* Available at: http://www.ncbi.nlm.nih.gov/books/NBK8035 / accessed on 18/02/2013.

Jay, J.M., M.J. Loessner, and D.A. Golden. 2005) Modern food microbiology. Available at: http://www.springer.com/food+science/book/9 78-0-387-23180-8 accessed on 20/011/2012.

Population and Housing Census (PHC). 2010. PHC, Yendi Municipality, Northern Region, Ghana. Available at: http://www:ghanadistricts.com accessed 18/06/2012.

Postgate, J.R. 2010. Microbes and man. Cambridge University, UK. Pp. 373.

Prescott, L.M., J.P. Harley, and D.A. Klein. 2002**.** Food and industrial microbiology. Mc Graw-

Hill Companies, Inc., New York, USA. Pp.125-964.

Reynolds, K.A., P.M. Watts, S.A. Boone, and C.P. Gerba .2005. Occurrence of bacteria and biochemical markers on public surfaces. *International Journal of Environmental Health Research*. 15: 225-234.

Rombout, F.M., and R. Nout. 1994. Food Microbiology and hygiene. *Encyclopedia of Human Biology*. Academic Press. 111: 661-665.

Sulley, M.S. 2006. The hygienic standard of meat handling in the Tamale metropolis. B.Sc. Dissertation. University for Development Studies, Tamale, Ghana. Pp. 23-29.

Warriss, P.D. 2000. Meat science-An introductory text. CAB-International, Wallingford, England. pp: 1-297.

Wilson, N.R.P., E.J. Dyertt, B.R. Hughes, and C.R.V Jones. 1981**.** Meat and meat products, factors affecting quality control, Applied Science Publishers Ltd., England. pp: 81-108.

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Effects of Inclusion of Different Levels of Watermelon Bug Meal In Broiler Diets on Feed Intake, Body Weight Changes and Feed Conversion Ratio

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ABSTRACT

T This study was conducted in El-Obeid, North Kordofan State, Sudan with the objective of evaluating the effects of inclusion of different levels of watermelon bug meal (WMBM) as a substitute for sorghum grains in diets on broiler chick's performance. One day old unsexed 200 broiler chicks with an average weight of 40g/bird were used in an experiment designed as completely randomized design (CRD) with five treatments and four replicates. At the beginning of the experiment the chicks were fed a pre-starter diet for one week and then offered five diets prepared using (WMBM) substitution of sorghum at 0, 15, 30, 45 and 60 percent. The diets were offered twice a day. Chicks were weighed weekly during the experimental period. The collected data were analyzed using analysis of variance. The results indicated that feed intake of broiler chicks increased significantly ($P < 0.05$) with inclusion of WMBM. The chicks consumed 67, 89, 94, 97 and 97 g/day/bird when the WMBM constituted 0, 15, 30, 45 and 60 percent in the diets, respectively. The final body weight followed the same trend where the groups weighed 1332.5, 2130, 2100.6, 1922.5 and 1772.3 g for the birds that consumed diets of 0, 15, 30, 45 and 60 percent (WMBM) respectively. Weight gains and feed conversion ratio were also significantly ($P < 0.05$) improved. It was concluded that WMBM could replace sorghum grains as source of energy in broiler diets and it was recommended that more studies be carried out to investigate effects of inclusion of the WMBM on weight of cuts and meat quality.

Keywords: poultry diets, insect meals, body weight change, feed conversion ratio

INTRODUCTION

Poultry keeping in Sudan is an ancient occupation that started as a traditional practice and it is still dominantly traditional as seen in almost all parts of the country. The concurrent growth of the animals' feed industry was due to the increasing demand of poultry products (Osman, 1988). Poultry production development faced many difficulties that constrained establishment of sustainable indigenous production systems. Highly productive commercial hybrids and feed ingredients were always imported. In traditional and small scale production systems are mainly constrained by feed availability and cost, it represented an especial challenge. For the purpose of obtaining maximum benefits from the imported high yielding commercial hybrids, super concentrates and premix of vitamins and minerals were brought from different countries at very high cost. Importation was sometimes constrained by some noncommercial restrictions such as embargoes and economic sanctions on the country. The poultry industry was seriously affected by the dioxins' contamination of feed at the beginning of the century that necessitated looking for local sources of poultry feed. Sorghum grains are used as source of energy in broiler diets despite of the fact that the crop is a staple food for humans in different parts of the country including North Kordofan state. This created a high competition between man and poultry over this common food resource (Technoserve, 1987).

The bug (*Aspongopus viduatus*) is distributed over the Middle East and Africa in general and has a wide distribution in the Sudan occurring in all regions of the country. Watermelon bug is a notorious pest to cucurbitaceous plants in general and particular to water melon. The crop is considered as one of the main cash crops where its seeds are collected and used internally or exported to Egypt with relatively high prices.

A. viduatus was reported being controlled by several methods but the hand picking method is followed mainly as a control practice in North Kordofan State by the Plant Protection Department (PPD) assisted by World Food Programme (WFP). They used to pay farmers to collect manually and burn the watermelon bugs during the dry season.

Chemical analysis of the meal of watermelon bugs reported by Shamat (2007) has indicated that the insect meal was rich in oil and minerals and though it was found to be low in crude protein, so it can consider as a good source of metabolizable energy which can substitute sorghum grain in broiler diets. Also Mariod *et al.,* (2004) reported that watermelon bug meal contained 46.3% linoleic acid and 41.3% Oleic acid.

The objective of the study was to assist in developing appropriate small scale poultry production systems that could make use of the available resources of feed concomitant with control of some insect pests on cash crops of the region and minimizing poultry production cost by reducing the amount of sorghum grains used in broiler diets formulation since the grains represent 65% of the broiler diet/ ton. Specifically this study is proposed to study, also to study the effects of replacement of sorghum grains by different levels of watermelon bug meal on broilers feed intake, body weight changes, feed conversion ratio and feeding cost.

MATERIALS AND METHODS

The Study Area

The experimental birds were housed in the poultry Farm of the Animal Resources Administration Headquarters in Elobied city. The experiment was carried out during the period extending from June to July 2007.

The experimental birds Housing and treatments

The study used a total of 200 one day old unsexed commercial broiler chicks (ROSS 308) that were produce. The experiment was carried out in an open housing system on a deep litter floor. The house was closed up in three sides with a plastic shelter and divided into 20 pens of $1m^2$ each at the floor space. The pens were separated from each other by wire mesh and pampoo and they were supplied with a source of light for lighting 23 h/ day. Each pen was provided with fountain drinker and a feeder trough.

The chickens were delivered at the experimental site in the second day afternoon (on the 2^{nd} day to the hatchery release). The birds were weighed upon arrival by a scaled balance and every 10 birds were randomly penned as replicate with a mean weight of 40g and a total of 20 replicates for the experiment. The penned chicks were reared for 7 days as an adaptation period. At that period, the birds were fed broiler pre starter feed and were introduced afterwards to the experimental diets for 5 weeks.

Prior to commencement of the treatments, the chicks were vaccinated against infectious diseases such as bronchitis, Newcastle, Bursa and with two doses for the mentioned diseases during the rearing period. The birds were also treated for five days by coccidostat and anithlementic dose that was administered as prophylactic doses.

The chicks were subjected to five different level of feeding and were fed the experimental diets till 42 day of age. Throughout the experimental period, feed intake was calculated daily by weighing the remained feed and subtracting it from the feed provided the previous day. Water was provided continuously. The live body weight was recorded weekly. Feed intake and weight gain was used to estimate the feed conversion ratio (FCR) according to the equation: - FCR= (g) feed / (g) gain. Feed cost was calculated by adding price value of each quantity in one metric ton of the diet.

The Experimental Diets

The diets used in this study were isocoloric, isonitrogenic and formulated using Watermelon bug *(Aspongogus viduatus)* meal. The bug meal was used as substitute for sorghum grains as source of energy at five different levels and grouped as B, C, D and E with 15, 30 ,45 and 60 % watermelon bug meal substitute with sorghum and last group A as control with 60% sorghum and zero watermelon bug meal (Table 2) . The insect meal was hand collected, dried and packed in jute sacs and stored prior being ground. The chemical composition of the water melon bug meal (W.M.B.M) and other ingredients incorporated in the diets shown in table (1).

Table 1.The chemical composition of the ingredient used in the experimental diets (%)

Ingredient	CP	CF	E.E	ASH	Ca	P	Na	ME(MJ/Kg)
WMBM	10.9	-	15.1	3.75	0.35	1.6	0.81	1.25
Sorghum	13.23	24.8	-	21.5	0.5	3.1	1.2	14.38
Wheat bran	16.83	129.8	-	54.4	1.7	7.3	0.8	7.91
Groundnut cake	43.5.8	97.2	-	92.5	6.2	3.7	1.5	11.46
Sesame cake	41.57	81.8	-	138.6	20.1	9.3	1.5	11.62
Oyster shell	-	-	-	990.5	375	0.6	-	-
Salt	-	-	-	102.5	64.2	10.6	840	-
Super-concentrate LNB	40	3	-	-	8–11	4.6	1.5	37.6

The percent of ingredients used in formulating diet containing different levels or watermelon bug meal in the experimental diets shown in table (2).). Feed Cost Energy, protein and minerals

contents of diets containing different levels of watermelon bug meals were shown in table (3). Different level of diet that replace with sorghum cost less than sorghum diet.

Table 2.The percent ingredients used in formulating ration containing different levels or watermelon bug meal in the experimental diets (%)

Ingredient%	Level of WMBM %				
	A	B	C	D	E
Sorghum	60	45	30	15	-
Water melon bug	-	15	30	45	60
Wheat bran	10	3.5	3.5	3.5	3.5
Concentrate(LNB)	5	5	5	5	5
Ground nut cake	12.5	19	19	19	19
Sesame cake	10	10	10	10	10
Oyster shell	2	2	2	2	2
Salt	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Table 3. Feed Cost Energy, protein and minerals contents of diets containing different levels of watermelon bug meals

Ingredient%	Level of WMBM %				
	A	B	C	D	E
ME(MJ/KG)	13.13	13.4	13.4	13.4	13.4
CP	22.3	23.6	23.2	22.7	22.3
Ca	1.00	1.00	1.4	1.4	1.5
P	0.3	0.6	0.8	1	1.2
Cost/ton/SDG	547.5	492.4	434.9	377.4	319.9

Chemical and Statistical Analyses

Proximate analysis of watermelon bug, *Aspongopus viduatus* was carried out according to AOAC (2002). After estimation of the total ash of the sample, sodium Na, calcium Ca phosphorous, P, and Potassium were determined according to the methods described also by AOAC, (2002) using flame photometer method. Metabolisable energy estimation was done according to the equation of Lodhi *et al*, (1976).

Metabolisable energy MJ/Kg

ME MJ/kg DM = 1.549+ (CP%×0.0102) + (EE%×0.0275) + (NFE%×0.0148) + (CF%×0.0034).

Where:

ME= Metabolisable energy DM= dry matter CP=Crude Protein EE=Ether Extract NFE= Nitrogen- Free Extract CF= Crude Fiber

The data obtained on feed intake, weight change and feed conversion ratio was considered to complete randomized design and was analyzed via analysis of variance. Where treatments were different least significant difference, LSD, was used to separate means differences (Gomez and Gomez, 1984).

RESULTS

Effects of feeding broilers with diets of different Levels of Watermelon Bug meal on Feed Intake

The weekly mean feed intake (g) for the experimental birds is presented in Table (4). The first week was an adaptation period where the groups were left on pre starter diet. Afterwards the experimental birds were kept on diets containing different levels of watermelon bug meal, WMBM, or sorghum grains. Significant differences (P<0.01) in feed intake were observed during the first week and thereafter and it was significantly (P<0.05) higher for chicks fed diets with different levels of WMBM compared with those fed a diet containing sorghum grains only till the end of the experimental period.

Table 4.The effect of feeding different levels of WMBM on weekly feed intake

Level of WMBM%	Week					
	1st	2nd	3rd	4th	5th	6th
A	97.5^{a}	205^{b}	338.75^{d}	573.75^{d}	643.75^{b}	940^{c}
B	96.9^{ab}	210^{b}	411^{c}	778.13^{c}	1034.13^{a}	1277.25^{b}
C	100^{a}	215^{b}	423.76^{bc}	820^{b}	1051.25^{a}	1344^{b}
D	93.8 ^{b}	220.3^{a}	451^{a}	870^{a}	1107.88^{a}	1414.12^{a}
E	93.8^{ab}	210^{b}	438.75^{ab}	847.5	1103.63^{a}	1452.93^{a}
Total mean	96.4	212.15	412.8	774.31	988.13	1285.73
± SE	0.92	2.09	9.53	25.11	41.10	43.33

Values in the same column with different letters are significantly different at P<0.05
WMBM= Watermelon Bug Meal SE= Standard Error of the mean.

The birds on diets that contained 15 or 30 percent W.M.B.M consumed significantly (P<0.05) greater amount of feed compared to those fed the control diet, while their feed intake was significantly (P<0.05) lower compared to those fed 45 or 60 percent WMBM diets.

Effects of Feeding diets with different Levels of Watermelon Bug Meal on Broilers Body Weight change

The weekly live body weight change of the experimental birds during the experimental period is presented in Table (5). Birds fed diets containing different levels of WMBM had significantly (P<0.05) higher weight gains compared to those on the control diet. Within the WMBM diets, the feed that contained 15, 30 and 45 percent caused significantly (P<0.05) higher weight gains compared to those on a diet containing 60 % WMBM.

Within the different levels of WMBM, consumption of diets containing 15, 30 and 45 percent led to significantly (P<0.05) higher weight gains compared to consumption of a diet containing 60 % WMBM and finally the performance of chicks at 5th and 6th weeks followed the same trend of the 4th week.

Table 5. Mean weekly live body weight of the experimental chicks fed on diets containing different level of water melon bug meal during (0-6 week's period)

Level of WMBM%	Week				
	1st	2nd	3rd	4th	5th
A	328d	258.7c	170c	98.8b	110
B	560.7ab	476a	296a	108.8a	108.75
C	568.5a	429.7a	291.3a	109.3b	105
D	536.8b	478a	303.8a	104.9b	105
E	473.8c	416.25b	252.5b	93.75b	103.75
Total mean	493.5	411.75	262.7	103.10	106.5
± SE	24.1	19.48	12.22	3.21	1.03

Values in the same column with different letters are significantly different at P<0.05
WMBM= Watermelon Bug Meal SE= Standard Error of the mean

Feed conversion ratios of the experimental birds fed diets containing different level of WMBM

Feed conversion ratio of broiler chickens on WMB diets is presented in Table (6). Significant differences (P<0.05) could be observed in feed conversion ratio of chick groups from the first week. The experimental group on sorghum grains had significantly (P<0.05) lower feed conversion ratio values compared with the other four groups that were on diet with varying levels of WMBM. Lower feed conversion ratios were recorded for the group that was offered diets containing 45 and 60 % WMBM than the groups which consumed diet with 15 and 30 % WMBM diets in the second week. No significant differences were detected among birds that consumed diet with sorghum grains and which were on diets containing, 30 or 45 % WMBM.

Table 6. Feed conversion ratios of the experimental birds fed diets containing different level of WMBM during 0-6week's period (kg feed/ kg live weight gain)

Level of WMBM%	Week				
	1st	2nd	3rd	4th	5th
A	0.888 a	1.41a	2.16c	2.34c	1.96a
B	0.872 a	1.96b	1.42a	1.63a	1.75a
C	0.952 b	2.06b	1.45a	1.59a	1.77a
D	0.901 a	2.54c	1.50ab	1.82ab	2.08ab
E	0.916ab	2.24b	1.74b	2.03b	2.42b
Total mean	0.906	2.02	1.066	1.88	1.99
± SE	0.01	0.93	0.072	0.073	0.073

Values in the same column with different letters are significantly different at P<0.05
WMBM= Watermelon Bug Meal SE= Standard Error of the mean

DISCUSSION

Effects of feeding broilers with diets of different Levels of Watermelon Bug meal on Feed Intake

In this experiment, the inclusion of watermelon bug meal W.M.B.M at different levels to the broiler diets has increased the total feed intake when compared with consumption of the conventional diet that was formulated using sorghum grains. Such an improved appetite and greater feed intake might be due to the difference of form of energy source where the energy source of the sorghum diet was starch while energy source of the different levels of WMBM diets

was oil that was rich in essential fatty acids especially oleic and Lenoleic acids (Smith, 1995). Reporting of Eljack,(2004) said that feeding millet, rich in starch, did not have significant positive effect on mean total feed intake when replaced by wheat due to similarity of the source of energy.

The level of watermelon bug meal in the diet has also shown to be of significantly positive effects on total feed intake of the experimental broiler chicks after one week of the experimental diets being introduced i.e. during the 3rd weeks till the end of experiment periods positively correlated. The greater the percentage of WMBM in the diet, the higher was the amount of feed consumed till 45% W.M.B.M. That could be attributed to the increased amount of essential fatty acids consumed. The results obtained here are in line with (Smith, 1995) who reported that diets with fat as energy source were consumed in larger quantities than starch diets.

Eljack (2004) explained improved birds appetite on fat diets according to the body temperature of birds is not constant, and this has been suggested that heat produced after feed is consumed raises the temperature of the blood and hypothalamus, so that the appetite is suppressed in starch diet whereas heat is produced so immediately when birds are fed fat diets. This thermostatic theory would also explain why birds eat less at high ambient temperature and feed with a low heat increment such as fat can cause obesity because it is largely consumed. An alternative is the glucostatic theory. This proposes that there are glucose receptors in the hypothalamus which are sensitive to the rate at which glucose is being utilized by them. Low utilization rates leading to the above theories can possibly explain short term regulation of feed intake. Long term regulation is probably concerned with the prevention of excess fat deposition. According to this theory the hypothalamus is sensitive to concentration of circulating metabolites mobilized from endogenous fat stores. Since the amount of fat mobilized is proportional to the size of fat deposit a lipostatic mechanism keeping fat content controlling body weight. These results are in disagreement with the results reported by Gabriel and Idris, (1997) who reported that locust meal sprayed with insecticide tended to depress feed intake.

Effects of Feeding diets with different Levels of Watermelon Bug Meal on Broilers Body Weight change

These results are consistent with those findings reported by Sulistujanto, *et al, (*1999) who showed that feeding broiler chicks with fat rich diets could increase their final weight. However, Gabriel and Idris, (1997) reported that chicks fed locust meal did not increase in weight than those fed conventional diet of sorghum grains when locust meal was used as a protein source and not energy source as it was in the present study. Live body weight gain was greater in chicks upon consumption of the experimental diets. However the results of this study were in agreement with the results of a demonstration by Sulistujanto, *et al.,* (1999) who reported that among the energy yielding foodstuff, fat sources seem to be better utilized, with no age dependency on growth, weight gain or feed conversion ratio and he concluded that energy utilization of carbohydrates and protein sources during 10 days post-hatch tended to increase with age.

Feed conversion ratios of the experimental birds fed diets containing different level of WMBM

Feed conversion ratio of the broiler chicks was affected by inclusion of W.M.B.M in the experimental diets and the level of the bug meal in the diet. Feed conversion ratios of the groups fed diets that were formulated of W.M.B.M at all levels were significantly ($P<0.01$) better than the group consumed the conventional diet of sorghum grains. The results are in with Sulistujanto *et al.,* (1999) who reported that caused greater weight and lower (better) feed conversion diets

when chicks were fed diets of fat. The results of this were in line with Odunsi *et al.,* (2007) that carried out a study on broilers were

Supplementation with vegetable oil (VO) in his study only gave marginal improvement on performance indices when compared with corresponding wood charcoal (WC) based diets without (VO). The conversion ratio was best (3.37) when the broilers were fed diet without vegetable oil or charcoal whereas the FCR were high when the birds were fed diet with vegetable oil.

REFERENCE

AOAC. 2002. Association of Official Analytical Chemists. The official Methods of analysis, 16th edition. Washington DC.

Eljack B. H. El. 2004. The Effect of Feeding Millet on Growth, Laying and Hatching Performance of Fayoumi Breed. Thesis Ph.D. University of Khartoum.

Gabriel, S. and A. A. Idris. 1997. Utilization of Locust Meal in Poultry Diets .*JONARES*. 1(1):19-23

Gomez, K. A. and Gomez, A. A. 1984. Statistical procedures for agricultural research John Willey and sons. New York U.S.A.

Lodhi, G. N., Singh, D., and I. chponani .1976. Variation in nutrients content of feeding stuffs rich in protein and reassessment of the chemical methods for metabolizable energy estimation for poultry. *J. Agric. Sci*. 69:634-639.

Mariod, A. A., B. Matthäus, and K. Eichner. 2004. Fatty acid, tocopherol and sterol composition as well as oxidative stability of three unusual Sudanese oils. *J. Food Lipids .11*:179-189.

Odunsi, A. A. ; A. A. Rotimi ; E. A. Amao. 2007. Effect of different vegetable protein sources on growth and laying performance of Japanese quails (*Coturnix coturnix japonica*) in a derived savannah zone of Nigeria. *World Appl. Sci. J.* 3 (5): 567-571.

Osman, A. H. 1988. The role of indigenous and exotic genetic resources for poultry development in the Sudan. Proceedings of Symposium. Ministry of Animal Wealth. Khartoum, Sudan.

Shamat, A. .2007.physical and chemical properties of oil extracted from watermelon bugs Veterinary Research Laboratories, Suba. Sudan (unpublished).

Smith, A. J. 1995. The tropical Agriculturalist "Poultry". C.T A.Macmillan. London, UK.

Sulistujanto, B A. Y, and K. Sato. 1999. Energy utilization of Carbohydrates , fat and Protein sources in newly hatched broiler chicks. *British Poultry Science*. 653 – 659.

Technoserve .1987.Credit Component Base line Survey. Technoserve Inc. Agricultural Bank of Sudan, US Agency for International Development, Elobeid, Sudan.

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Performance of Crossbred Dairy Cows Under Small and Medium Scale Farmers' Management in and Around Shashamane City, Southern Ethiopia

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ABSTRACT

The study was conducted in and around Shashamane city to assess feed intake and productive performance of crossbred dairy cows during early lactation under farmers' management. A total of 120 dairy farmers were selected for the study. Structured questionnaire, secondary data sources and field observations were employed to generate data. A total of 48 animals in early lactation and parity from 2 to 6 were used for monitoring study for the period of 90 days. Significant differences were observed in daily intakes of DM, crude protein and ME ($P<0.001$) between production sub-systems and herd size groups. Daily milk yield was also significantly different ($P<0.05$) between production sub-systems and herd size groups ($P<0.01$). Therefore, from the current study it was concluded that productivity of animals on both production sub-systems and farm scale was below their expected genetic potential. Hence, large variation between production sub-systems and farm scale groups showed the opportunities for further improvement with strategic supplementation of energy and protein rich feeds.

Keywords: urban peri-urban dairy, farm scales, nutrient intake, productivity, Shashamane.

INTRODUCTION

The current population of Ethiopia is about 90 million, which is growing at an annual rate of 3.2% (World Fact Book, 2013). Pressure on the agricultural sector is constantly increasing**.** It is expected that an increase in population growth demands a better economic performance than in the past to prevent poverty, create employment and ensure food security (CSA, 2011; MoARD,

2007). The demand for animal products is expected to increase considerably (Mohammed *et al*., 2004). Ethiopia is believed to have the largest livestock population in Africa, of which the contribution of cattle is significant. Cattle population of the country is estimated to be about 52.13 million (CSA, 2012). This estimate excludes cattle population in three zones of Afar and six zones of Somali regions. Dairy production is an important component of livestock production in Ethiopia. It is practiced in almost all parts of the country across all agro-ecological set up. Particularly the mixed crop–livestock system in the highlands offers the best opportunity for dairy development and can support crossbred and pure dairy cattle breeds. However, despite the large livestock resource base and an ecological setting suitable for dairy production, the country is not yet self sufficient in milk production. In Shashamane milkshed, market oriented small and medium scale farms in the urban and peri-urban centers are the two categories of milk production systems (Sintayehu *et al*, 2008). These farms rely on crossbred and exotic cattle breeds under intensive and semi-intensive management with production goal of cash income. Previous study conducted in the area focused mainly on characterization of dairy production, marketing and processing in Shashamane Dilla area (Sintayehu *et al.*, 2008). Whereas the productive performance of crossbred dairy cows, whose contribution has a great role to urban and peri-urban milk production has not been studied. Therefore, it is important to evaluate the current milk production status of small and medium scale dairy farms operating under urban and peri-urban levels in devising appropriate development interventions. This study was therefore, aimed to look into the performance of crossbred dairy cattle with respect to farmers' management in urban and peri-urban areas of Shashamane milkshed.

MATERIALS AND METHODS

The study was conducted on private urban and peri-urban dairy farms in and around Shashamane city. The area is characterized with different altitude ranges of 1900 and 2200 meters above sea level and average minimum and maximum temperature of 12 and 27°C respectively.

Sampling methods

A preliminary visit was conducted in the study area to get general picture of the study sites and to identify the target farms. Two dairy production sub-systems and two herd size groups were identified in the area. Each production sub-system was further stratified into two based on herd size: small holders (farms with <4 cows) and medium level (farms with 4-10 cows); Ike (2002). For this study a total of 120 dairy farms, 60 from each production sub-system were purposively selected. Sixteen farms having crossbred dairy cows, parity ranges between 2 and 6 and were at their last months of pregnancy were selected for the monitoring study. Accordingly, 8 farms from each small and medium scale farms (8 from each urban and peri-urban farm) were selected for the study. A total of 48 dairy cows, two from each small scale and four from each medium farms, were selected for monitoring study. Monitoring study was conducted from October to December 2012.

Data collection procedures

A structured questionnaire was prepared and pre-tested for its applicability before its administration. Monitoring of the utilization of feed resources and milk yield was carried out once a week for the period of 90 days. The amount and type of feed offered to individual cows

was weighed and recorded for each monitoring date using portable spring balance. It was observed that most farms provide concentrates and roughages after wetting it with water or *atela*. Under such conditions, the quantity of feed to be mixed was weighed prior to wetting and divided to the number of dairy cows. Accordingly the refusal of any feed type offered was weighed and recorded. The amount of metabolizable energy (ME) and nitrogen intake over a given period of time was estimated by multiplying the nutrient content of the feed (per kg dry matter) by the daily dry matter intake of the respective feed. Feed utilization for the non collection period was calculated from the average values of the preceding and current measurements. Data were collected by literate individuals. Data collection from the selected dairy cows was started one week after calving. Heart girth of milking cows used for monitoring study was measured in the morning before feed was offered at two weeks interval using a plastic measuring tape for the period of 90 days. Body weight of the cow was estimated from heart girth measurement using the regression equation developed by ILRI as cited by Yoseph (1999).

$Y = -423.405235 + 4.833697x$ ($R2 = 0.86$; $CV = 10\%$).

Where, Y= Estimated body weight, Kg (weight range for prediction was 200-500 kg)

x= Heart girth, cm.

Statistical Analysis

General Linear Model (GLM) procedure of SAS (2004) was used for analyzing data collected during monitoring. Mean comparison was done using the Least Significant Difference (LSD) for parameters with significant difference. Differences were considered statistically significant at 5% level of significant. The model used to analyze the effects of farm scale and parity classes on milk yield, reproductive traits and nutrient intake was:

$Yij = \mu + Ai + Bj + eij$

Where,

Yij = response variables (nutrients intake, productive and reproductive performance of dairy cows)

μ = overall mean

Ai = fixed effect of ith production sub-system (i= 1, 2)

Bj = fixed effect of jth herd size (j= 1, 2)

eij = residual effect.

RESULTS AND DISCUSSION

Feeds and nutrient intake

Basal and supplement dry matter intake was significantly ($P<0.001$) varied between production sub-systems and herd size groups (Table 1). Basal feeds contributed 30.4% and 40.4% of the DM intake in urban and peri-urban farms, respectively. Higher roughage DM intake in peri-urban farms may be due to less availability of concentrate feeds in the area compared to urban areas. The result of the current study was not in agreement with 50% and 36% contribution of basal feed to the total dry matter intake in intra-urban and secondary town dairy farms, respectively, in Addis Ababa milk shed (Yoseph, 1999). The result of the current study was comparable with 9.6kg and 11.4kg daily DM intake by crossbred cows in small and medium scale farms, respectively, in Sebeta Awas area (Dereje, 2012). Basal, supplement and total CP intakes significantly varied ($P<0.001$) between production sub-systems and farm scales. The difference might be related to feeding practices used in different farms. Higher proportion of small scale farms (35%) use *Atela* as protein supplement compared to medium scale (21%) farms. The

energy intake of dairy cows in urban farms was significantly (P<0.01) higher than peri-urban farms. Basal feeds contributed about 24% and 34% of the total energy intake in urban and peri-urban farms, respectively. The difference was related to higher intake of concentrated DM in urban farms than peri-urban farms. Energy intake was also significantly different (P<0.001) between farm scales. The result of the current study was lower than 99.6 MJ ME/cow/day for crossbred cows in small scale farms, but higher than 94.1 MJ ME/cow/day for medium scale farms in and around Dire Dawa city (Fayo, 2006). Higher nutrient intake reported in medium sized farms compared to the small sized dairy farms might be due to generation of larger income from milk sale in medium sized farms which encouraged farm owners to purchase more feed compared to small sized farms.

Milk yield and composition

There was significant difference in daily milk yield between production sub-systems (P<0.05) and herd size groups (P<0.01) (Table 2 and 3). The difference could be attributed to higher nutrient intake originated from concentrate feeds in urban farms compared to the peri-urban dairy farms (Table 2). Mean daily milk yield in the current report was higher than 9.0±3.9 liters/day in urban and 9.0±4.0 liters/day in peri-urban farms reported for crossbred dairy cows in Northern Ethiopia (Gebrekidan *et al*., 2012).

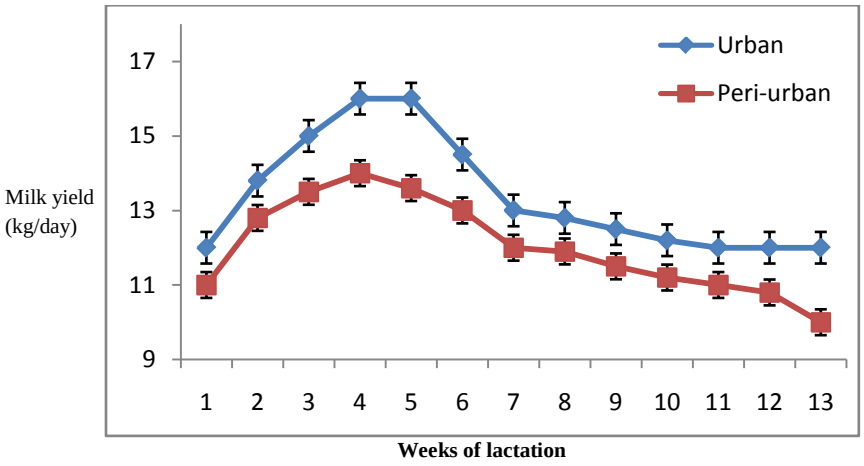

Figure 1. Mean daily milk yield of cows in two different production sub-systems during 13 weeks of lactation.

Cows in the urban farms were showed fast increase of milk yield up to the peak at week 4 and 5, but continuously declined there after (Figure 1). The trend of increase in cows from peri-urban farms was relatively slow and short. The peak yield was attained at week 4 and was declined thereafter. Dairy cows in medium scale farms achieve peak milk yield at week 4 and decline thereafter, while those in small scale farms achieve peak yield at week 5. There was significant difference in fat percentage (P<0.05) between the production sub-systems and herd size groups. The result of the current study was comparable with 4.25% fat, 2.89% protein, 13.2% total solid and 8.53% solid not fat reported for crossbred cows in urban and peri-urban production system (Dereje, 2012). The difference might be attributed to the variation in the level of nutrition in different farms. Fat and protein percent in the current study fall within the acceptable range of 2.5 to 6.0% and between 2.9 to 5.0% for fat and protein, respectively (O'Connor, 1994).

Table 1. Mean (±SE) daily feed intake by dairy cows in urban and peri-urban dairy production systems in Shashamane milk shed (n=48)

Parameters	Dry matter intake (kg DM/day)			Crude protein intake (g/day)			Energy intake (MJ/day)		
	Total	Roughage	Concentrate	Total	Roughage	Concentrate	Total	Roughage	Concentrate
Overall	10.8±0.3	3.8±0.1	7.0±0.2	1500.6±54.9	243±6.8	1257.5±51.8	98.7±2.7	28.2±0.86	70.5±2.3
Production system	NS	***	***	***	***	***	**	***	***
Urban (n=24)	11.2±0.4	3.4±0.1^{b}	7.8±0.3^{a}	1679±57.9^{a}	223.8±7.7^{b}	1454.9±50^{a}	105±3.6^{a}	25.2±0.9^{b}	80±2.8^{a}
Peri-urban (n=24)	10.4±0.4	4.2±0.16^{a}	6.2±0.2^{b}	1322±78.8^{b}	263±9.9^{a}	1060±71.3^{b}	92.2±3.6^{b}	31.3±0.8^{a}	60.9±2.4^{b}
Herd size	***	***	***	***	***	***	***	**	***
Small (n= 16)	8.9±0.3^{b}	3.0±0.09^{b}	5.8±0.3^{b}	1132±88.5^{b}	201±5.8^{b}	931±88.5^{b}	81.5±3.4^{b}	23.2±0.7^{b}	58.3±3.2^{a}
Medium (n=32)	11.7±0.3^{a}	4.1±0.1^{a}	7.6±0.2^{a}	1685±40.9^{a}	264±7.5^{a}	1421±40.5^{a}	107±2.6^{a}	30.5±0.9^{a}	77±2.4^{b}

n= number of dairy cows monitored; ^{ab} means in the same column with different subscript letters were significantly different;
NS = non significant; **= P<0.01; ***=P<0.001; DM= dry matter, MJ= mega joule

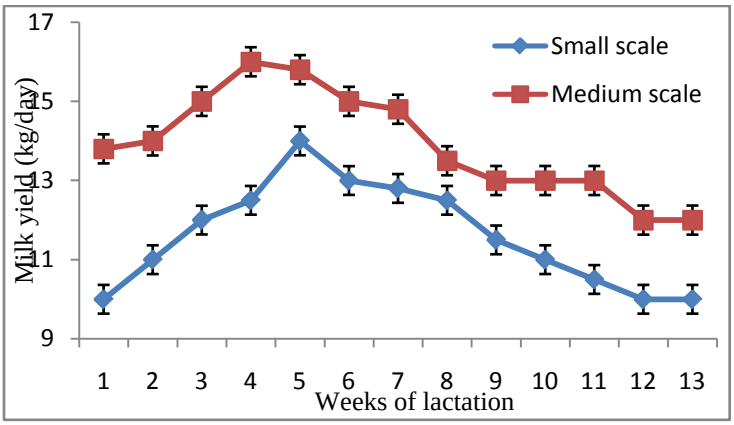

Figure 2. Mean daily milk yield of dairy cows in two different farm scales during 13 weeks of lactation.

Table 2. Mean daily milk yield and composition under urban and peri-urban farms.

Variables	Production subsystems		
	Urban	Peri-urban	Significance
Number of cows	24	24	
Average milk yield (kg/cow/day)	13.4±0.34	12.2±0.33	*
Milk composition (%)			
Fat	3.92±0.08	4.13±0.09	*
Protein	2.91±0.0	2.87±0.01	NS
Total solid	12±0.08	12.2±0.15	NS
Solid not fat	8.1±0.1	8.3±0.1	NS

*= P<0.05; NS= not significant

Table 3. Mean daily milk yield and composition under small and medium farms.

Variables	Production sub-systems		
	Small	Medium	P
Number of cows	16	32	
Average milk yield (kg/cow/day)	11.5±0.35	13.8±0.25	***
Milk composition (%)			
Fat	4.3±0.08	3.77±0.07	*
Protein	2.91±0.0	2.89±0.0	NS
Total solid	12.35±0.15	11.94±0.1	*
Solid not fat	8.06±0.12	8.16±0.09	NS

*= P<0.05, ***= P<0.00; NS= not significant

CONCLUSION

The productivity of animals on both production sub-systems and farm scales was below their expected genetic potential, where in peri-urban and small scale farm was critically low as compared to some parts of the tropics. The amount of crude protein (gram/day/head) consumed was above the requirement for the observed actual milk output except in small scale farms. However, the amount of ME (MJ/day/head) consumed was below the requirement for the observed actual milk output in both production sub-systems and farm scales.

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REFERENCE

AOAC. 1990. AOAC (Association of Official Analytical Chemists), 1995. 16th edition. Official Methods of Analysis. pp: 5-13. Washington DC.

CSA (Central Statistical Agency). 2011. Agricultural sample survey 2010/11. Volume II. Report on livestock and livestock characteristics.

CSA (Central Statistical Agency). 2012. Agricultural Sample Survey 2011/12. Report on Livestock and Livestock Characteristics, Volume II, Statistical Bulletin. 532. Addis Ababa, Ethiopia.

Dereje Shibru. 2012. Evaluation of the Performance of Crossbred Dairy Cows and Heifer Calves in Urban and Peri-urban Dairy Systems in Sebeta Awas Woreda, Oromia, Ethiopia. An MSc. Thesis, Haramaya University, Dire Dawa, Ethiopia. 114p.

Fayo Dubiso. 2006. Assessment of Milk Production, Marketing, Feeds and Feeding System of Dairy Cow in and around Dire-Dawa Town. MSc. Thesis, Alemaya University. Dire Dawa. Ethiopia. 85p.

Garwe, E.C. 2001. Reproductive Performance of Crossbred Cattle Developed for Milk Production in the Semi Arid Tropics and the Effect of Feed Supplementation. A Ph.D thesis submitted to University of Zimbabwe, Zimbabwe. 163p.

Gebrekidan T.W., Z. M. Zeleke, and S. K. Gangwar. 2012. Reproductive and Productive Performance of Dairy Cattle in Central Zone of Tigray, Northern Ethiopia. *International Journal of advanced biological research*. 2(1):58-63.

Ike, A. 2002. Urban dairying in Awassa, Ethiopia. MSc thesis, University of Hohenheim, Stuttgart-Hohenheim, Germany. 113p.

Ministry of Agriculture and Rural Development (MOARD). 2007. Livestock Development

Master Plan Study. Phase I Report – Data Collection and Analysis. MOARD, Addis Ababa. Ethiopia.

Mohamed A. M., A. S. Ehui, and Y. Asefa. 2004. Dairy Development in Ethiopia. EPTD discussion paper No. 123. International Food Policy Research Institute. Washington. DC. U.S.A. 41p.

Mosi, A. K. and M. H. Butterworth. 1985. The voluntary intake and digestibility of diets containing different proportions of tef (Eragrostis tef) straw and Trifolium tembense hay when fed to sheep. *Tropical animal production* .10:19-22.

National Research Council. 1989. Nutrient Requirement of Dairy Cattle. 6th ed., National academy Press. Washington, D.C. 157p.

O'Mahoney. 1988. Options for small holders milk processing. *World Animal Review*. 62:16 - 30.

SAS. 2004. Statistical Analysis System software, Version 9.0. SAS Institute. Inc. Cary. NC. USA.

Sintayehu Y., B. Fekadu, T. Azage and G. Berhanu. 2008. Dairy production, processing and marketing systems of Shashemene, Dilla area, South Ethiopia. IPMS Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 9. ILRI (International Livestock Research Institute), Nairobi, Kenya. 61p.

Sisay Amare. 2006. Livestock Production Systems and Available Feed Resources in Different Agro-ecologies of North Gonder Zone. Ethiopia. M.Sc. Thesis. Alemaya University. Dire Dawa. Ethiopia. 95p.

Solomon Bogale. 2004. Assessment of Livestock Production Systems Feed Resource base in Sinana Dinsho district of Bale highlands. Southeast Oromya, An MSc. Thesis. Alemaya University. Dire Dawa. Ethiopia.

Solomon B., S. Mengistu, and A. Yami. 2008. Potential Use of Crop Residues as Livestock Feed Resources under Smallholder Farmers Conditions in Bale Highlands of Ethiopia. *Journal of Tropical and Subtropical Agroecosystems*. 8:107-114.

VanSoest, P. J., 1982. Nutritional Ecology of the Ruminants: Ruminant metabolism,Nutritional strategies, the cellulolytic Fermentation and the Chemistry of Forages and Plant Fibers. Ithaca. New York. 373p.

Yoseph Mekasha, 1999. Impact of feed resources on productive and reproductive performance of dairy cows in the urban and peri-urban dairy production system in the Addis Ababa milk shed and evaluation of non- conventional feed resources using sheep. MSc. Thesis. Alemaya University. Dire Dawa. Ethiopia. 197p.

Zewdie W., M. Yoseph, and W. Bram. 2011. Assessment of productive and reproductive performance of dairy cattle nexus with feed availability in selected peri-urban areas of Ethiopia. *Journal of Cell and Animal Biology* 5(15):308-315. 15 December, 2011 Available online at http://www.academicjournals.org/JCAB ISSN 1996-0867.

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Bioaccumulation Pattern of Heavy Metals in Commercially Important Fishes in and Around Indian Sundarbans

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ABSTRACT

Concentrations of Zn, Cu, Pb and Cd were determined in edible finfish species (*Polynemus paradiseus*, *Tenualosa ilisha*, *Liza parsia*, *Liza tade* and *Stolephorus commersonii*) collected from four stations in and around Indian Sundarbans. Levels of the selected heavy metals were determined in the muscle of edible finfish species in the Gangetic delta region using a Perkin-Elmer Sciex ELAN 5000 ICP mass spectrometer and expressed as mg kg^{-1} dry weight. To determine whether heavy metal concentrations varied significantly between sites and species, Duncan multiple range test was performed. In finfish species the concentrations of Zn, Cu, Pb and Cd ranged from 15.89±0.58 – 124.12 ±1.63 ppm, 13.68±0.83 – 75.91±0.49 ppm, 3.34±0.90–19.89±0.54 ppm and BDL–4.01±0.03 ppm respectively. For Zn and Cu, accumulated metal concentration in Stn. 4 is significantly lower than accumulated metal concentration in Stn. 1 and Stn. 2. For Pb, significant difference between stations was not found. Among the selected species, lowest metal accumulation values was found for *S. commersonii* ($p<0.05$). The selected heavy metals in finfish muscle (except Zn in *Liza parsia* and *Liza tade* in Stn. 1) were also within the permissible limits for human consumption as indicated by the Food and Agricultural Organization.

Keywords: Finfish; Heavy metals; Indian Sundarbans

INTRODUCTION

The Indian Sundarbans in the lower Gangetic delta, at the apex of Bay of Bengal is recognized as one of the most diversified and productive ecosystems of the Tropics. The deltaic lobe is unique for its wilderness, mangrove gene pool and tiger habitat. However due to intense industrial activities in the upstream zone, and several anthropogenic factors, the western part of the deltaic complex is exposed to pollution from domestic sewage and industrial effluents leading

to serious impacts on biota (Mitra and Choudhury, 1992). The presence of Haldia port-cum-industrial complex in the downstream region of the River Ganga (also known as the Hooghly River) has accelerated the pollution problem with a much greater dimension (Mitra, 1998). The organic and inorganic wastes released from these industries and urban units contain substantial concentrations of heavy metals. The central part of the delta (encompassing the surroundings of Matla River) is relatively less stressful in terms of industrial discharge. Due to siltation of the Bidyadhari channel the area does not receive any water supply from the Hooghly River in the western sector and is therefore tide-fed in nature receiving the tidal flux from the Bay of Bengal (average salinity= ~32 psu). 85 percent of the people in this area consume fishes that are caught from the Gangetic delta region.

The information which is available in the literature suggests that the concentrations of toxic metals in the ecosystem are reaching unprecedented levels. Due to the steady load of contaminated dust in overcrowded cities, the ambient concentrations of toxic metals are now among the highest ever being reported. Metals are elements that accumulate and do not break down further into less harmful constituent (Kennicutt *et al.*, 1992). With increasing urbanization and industrialization, there is a rapid increase of environmental pollution, which is attracting the attention of people around the world. The major sources of contamination in surface water can be traced to industrial discharges, domestic waste disposal and application of agrochemicals on farmlands (Kennicutt *et al.*, 1992). The pollutants like heavy metals after entering into aquatic environment accumulate in tissues and organs of aquatic organisms. The amount of absorption and assembling depends on ecological, physical, chemical and biological condition and the kind of element and physiology of organisms (Jaffa, 1998). These metals ultimate consumed by human, after accumulation by the body of aquatic organisms enter into food chain (Agbozu, 2007). Water - borne chemicals are absorbed in the gills, skin and digestive tract in fish and then transported by the blood to either a storage point such as the bone, or to the liver for transportation. If transported by the liver, it may be stored there, excreted in the bile, or passed back into the blood for possible excretion by the kidney or gills or stored in extra hepatic tissues such as fat (McNicol and Scherer, 1991).

Most of people in West Bengal are fish lover, so, the present paper aims to highlight the concentration of selective heavy metals (Zn, Cu, Pb and Cd) in the muscle tissue of five common finfish species namely *Polynemus paradiseus*, *Tenualosa ilisha*, *Liza parsia*, *Liza tade* and *Stolephorus commersonii* from four stations distributed in two sectors (western and central Indian Sundarbans) of the lower Gangetic region.

Description of the study site

Two sampling sites were selected each in the western and central sectors of Indian Sundarbans, a Gangetic delta at the apex of the Bay of Bengal. The deltaic complex has an area of 9630 sq. Km and houses 102 islands. The western sector of the deltaic lobe receives the snowmelt water of mighty Himalayan glaciers after being regulated through several barrages on the way. The central sector on the other hand, is fully deprived from such supply due to heavy siltation and clogging of the Bidyadhari channel since the late 15^{th} century (Chaudhuri & Choudhury, 1994). The western sector also receives wastes and effluents of complex nature from multifarious industries concentrated mainly in the upstream zone. On this background four sampling stations (two each in western and central sectors) were selected (Table 1 and Fig. 1) to analyze the concentrations of heavy metals in the common edible finfish and shellfish species inhabiting the zone.

Table 1: List of sampling stations with coordinates and salient features

Station	Coordinates	Salient Features
Nayachar Island (Stn.1)	88° 15′ 24" E 21° 45′ 24" N	It is located in the Hooghly estuary in the western sector of the lower Gangetic delta and faces the Haldia port-cum-industrial complex that houses a variety of industrial units.
Sagar South(Stn.2)	88° 01′ 47" E 21° 39′ 04" N	Situated at the confluence of the River Hooghly and the Bay of Bengal in the western sector of Indian Sundarbans, the station is an important navigational channel for the major ports of the area.
Gosaba(Stn. 3)	88° 39′ 46" E 22° 15′ 45" N	Located in the Matla Riverine stretch in the central sector of Indian Sundarbans.
Annpurin Satjelia Island (Stn. 4)	88° 50′ 43" E 22° 11′ 52" N	Located in the central sector of Indian Sundarbans. Noted for its wilderness and mangrove diversity; selected as our control zone.

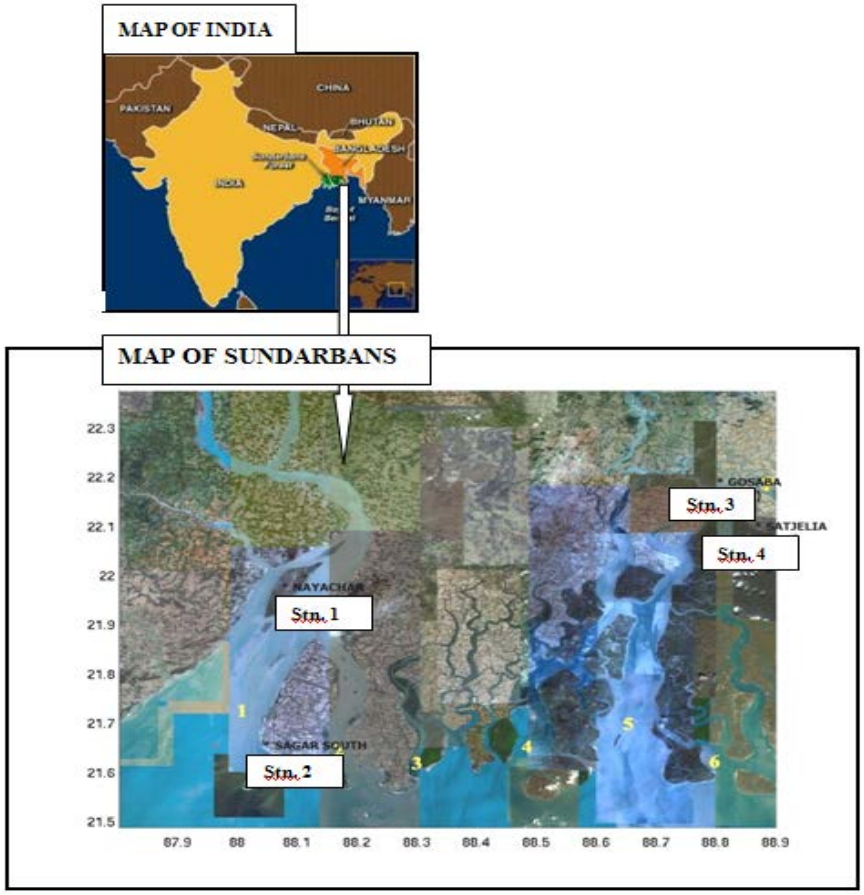

Fig.1. Location of sampling stations

MATERIALS AND METHODS

Sampling of Specimen

Five commonly edible finfish species Paradise fish (*Polynemus paradiseus*), Hilsa (*Tenualosa ilisha*), Parsey (*Liza parsia*), Tade Mullet (*Liza tade*) and Anchovy (*Stoleophorus commersonii)* were collected during low tide condition from the selected stations (Table 1) during a rapid EIA study from 15th October to 30th October, 2013. The collected samples were stored in a stored container, preserved in crushed ice with 1:1 fish to ice ratio and brought to the laboratory for further analysis.

Analysis

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) is now - a - day accepted as a fast, reliable means of multi-elemental analysis for a wide variety of sample types (Date & Gray, 1988). A Perkin-Elmer Sciex ELAN 5000 ICP mass spectrometer was used for the present analysis. A standard torch for this instrument was used with an outer argon gas flow rate of 15 L/min and an intermediate gas flow of 0.9 L/min. The applied power was 1.0 kW. The ion settings were standard settings recommended, when a conventional nebulizer/spray is used with a liquid sample uptake rate of 1.0 mL/min. A Moulinex Super Crousty microwave oven of 2450 MHz frequency magnetron and 1100 Watt maximum power Polytetrafluoroethylene (PTFE) reactor of 115 ml volume, 1 cm wall thickness with hermetic screw caps, were used for the digestion of the collected biological samples. All reagents used were of high purity available and of analytical reagent grade. High purity water was obtained with a Barnstead Nanopure II water-purification system. All glasswares were soaked in 10% (v/v) nitric acid for 24 h and washed with deionised water prior to use.

The analyses were carried out on composite samples of 10 specimens of each species having uniform size. This is a measure to reduce possible variations in metal concentrations due to size and age. 20 mg composite sample from each species of finfish and shellfish were weighed and successively treated with 4 ml aqua regia, 1.5 mL HF and 3 ml H_2O_2 in a hermetically sealed PIFE reactor, inside a microwave oven, at power levels between 330-550 Watt, for 12 min to obtain a clear solution. The use of microwave-assisted digestion appears to be very relevant for sample dissolution, especially because it is very fast (Nadkarni, 1984; Matusiewicz & sturgeon, 1989; De la Guardia, 1990). After digestion, 4 ml H_2BO_3 was added and kept in a hot water bath for 10 min, diluted with distilled water to make up the volume to 50 ml. Taking distilled water in place of biological samples and following all the treatment steps described above the blank process was prepared. The final volume was made up to 50 ml. Finally, the samples and process blank solutions were analyzed by ICP-MS. All analyses were done in triplicate and the results were expressed with standard deviation.

The accuracy and precision of our results were checked by analyzing standard reference material (SRM, Dorm-2). The results indicated good agreement between the certified and the analytical values (Table 2 and 3).

Statistical analysis

A logarithmic transformation was done on the data to improve normality. Duncan multiple range tests was performed to assess whether heavy metal concentrations varied significantly between sites and species; possibilities less than 5% ($p < 0.05$) were considered statistically

significant. All statistical calculations were performed with SPSS 21.0 for Windows. Superscripts were used to show the statistically significant difference between stations and species.

RESULTS AND DISCUSSION

The species-wise variation was not uniform for all the metals. Zn accumulated as per the order *Liza parsia* > *Liza tade* > *Tenualosa ilisha* > *Polynemus paradiseus* > *Stolephorus commersonii* (Table 2a, 2b and Fig. 2). Cu accumulated as per the order *Liza parsia* > *Tenualosa ilisha* > *Polynemus paradiseus* > *Liza tade* > *Stolephorus commersonii* (Table 2a, 2b and Fig. 3). Pb accumulated as per the order *Liza parsia* > *Liza tade* > *Tenualosa ilisha* > *Polynemus paradiseus* > *Stolephorus commersonii* (Table 3a, 3b and Fig. 4). Cd was BDL in all the stations except station 1, where the order was *Liza parsia* > *Liza tade* > *Tenualosa ilisha* (Table 3a, 3b and Fig. 5). For Zn and Cu, an accumulated metal concentration in Stn. 4 is significantly lower than accumulated metal concentrations in Stn 1 and Stn 2. For Pb, significantly stational difference between stations was not found. Between all studies fish species, lowest metal accumulation values was found for *S. commersonii* ($p<0.05$).

Heavy metals are non-biodegradable and once discharged into water bodies; they can be absorbed on sediments particles or accumulated in aquatic organisms (Kotze *et al.*, 1999). Especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards (Jezierska and Witeska, 2001). Accumulation of metal in different species may be the function of their respective. Fish species mostly absorbed heavy metals from its feeding diets, sediments and surrounding waters resulting to their accumulation in reasonable amounts (McCarthy and Shugart, 1990). Microhabitat utilization, feeding habits, age, sex and fish species also determine the accumulation pattern of heavy metals (Kotze *et al.*, 1999). Bioaccumulation is species-dependent and therefore feeding habits and life style can be strongly related to the sediment exposure (Chen and Chen, 1999). On the other hand, bioavailability of metals can be influenced by inorganic and organic factors that control metal speciation and thereby bioaccumulation (Henry *et al.*, 2004). The metal accumulation in different fish organs depends on their physiological role, behavior and feeding habits, as well as regulatory ability, as reported by Clearwater *et al.* (2002). Other factors, such as sex and size may also influence metal bioaccumulation (Al-Yousuf *et al.*, 1999; Canli and Atli, 2003).

Heavy metals are stable and persistent environmental contaminants of aquatic environments. They occur in the environment both as a result of natural processes and as pollutants from human activities (Garcia-Montelongo *et al.*, 1994; Jordao *et al.*, 2002). Some metals like Zn and Cu, which are required for metabolic activity in organisms, lie in the narrow "window" between their essentiality and toxicity. Other heavy metals like Cd and Pb, may exhibit extreme toxicity even at low levels under certain conditions, thus necessitating regular monitoring of sensitive aquatic environments (Cohen *et al.*, 2001; Fergusson, 1990; Peerzada *et al.*, 1990).

From an environmental point of view, coastal zones can be considered as the geographic space of interaction between terrestrial and marine ecosystems that is of great importance for the survival of a large variety of plants, animals and marine species (Castro *et al.*, 1999). The coastal zone receives a large amount of metal pollution from agricultural and industrial activity (Usero *et al.*, 2005). Adverse anthropogenic effects on the coastal environment include eutrophication, heavy metals, organic and microbial pollution and oil spills (Boudouresque & Verlaque, 2002).

Table 2a. Zn and Cu concentrations (in ppm dry wt.) in finfish muscles (station wise)*

Species	Zn				Cu			
	Stn. 1	Stn. 2	Stn. 3	Stn. 4	Stn. 1	Stn. 2	Stn. 3	Stn. 4
Polynemus paradiseus	$76.89^{d} \pm 1.20$	$67.89^{c} \pm 1.56$	$41.26^{d} \pm 1.49$	$15.89^{d} \pm 0.58$	$62.73^{c} \pm 0.45$	$44.12^{d} \pm 1.15$	$21.81^{d} \pm 0.19$	$15.34^{d} \pm 0.91$
Tenualosa ilisha	$93.46^{c} \pm 1.64$	$78.12^{b} \pm 1.34$	$46.35^{c} \pm 1.34$	$18.22^{b} \pm 0.35$	$66.12^{b} \pm 0.48$	$46.56^{c} \pm 1.48$	$25.57^{c} \pm 0.38$	$17.98^{c} \pm 0.33$
Liza parsia	$124.12^{a} \pm 1.63$	$94.63^{a} \pm 1.21$	$61.21^{a} \pm 1.67$	$27.67^{a} \pm 1.89$	$75.91^{a} \pm 0.49$	$54.39^{a} \pm 1.24$	$28.12^{a} \pm 0.67$	$21.01^{a} \pm 0.73$
Liza tade	$103.45^{b} \pm 1.49$	$91.25^{a} \pm 1.45$	$53.98^{b} \pm 1.23$	$25.45^{a} \pm 1.12$	$43.89^{d} \pm 0.36$	$50.38^{b} \pm 1.67$	$27.91^{b} \pm 0.55$	$18.65^{b} \pm 0.88$
Stolephorus commersonii	$45.03^{e} \pm 1.02$	$25.16^{d} \pm 1.63$	$19.26^{e} \pm 1.15$	$16.32^{c} \pm 0.65$	$16.12^{e} \pm 0.35$	$14.90^{e} \pm 0.73$	$13.68^{e} \pm 0.83$	$13.91^{e} \pm 0.91$
WHO (1989) level for Zn and Cu in food		100 ppm					30 ppm	
FAO (1992) level for Zn and Cu in fish		30-100 ppm					10-100 ppm	

*Means in a whole column with different superscripts (a–e) are significantly different ($p < 0.05$, Duncan multiple range test).

Table 2b. Zn and Cu concentrations (in ppm dry wt.) in finfish muscles (species wise)*

Species	Zn				Cu			
	Stn. 1	Stn. 2	Stn. 3	Stn. 4	Stn. 1	Stn. 2	Stn. 3	Stn. 4
Polynemus paradiseus	$76.89^{a} \pm 1.20$	$67.89^{b} \pm 1.56$	$41.26^{c} \pm 1.49$	$15.89^{d} \pm 0.58$	$62.73^{a} \pm 0.45$	$44.12^{b} \pm 1.15$	$21.81^{c} \pm 0.19$	$15.34^{d} \pm 0.91$
Tenualosa ilisha	$93.46^{a} \pm 1.64$	$78.12^{b} \pm 1.34$	$46.35^{c} \pm 1.34$	$18.22^{d} \pm 0.35$	$66.12^{a} \pm 0.48$	$46.56^{b} \pm 1.48$	$25.57^{c} \pm 0.38$	$17.98^{d} \pm 0.33$
Liza parsia	$124.12^{a} \pm 1.63$	$94.63^{b} \pm 1.21$	$61.21^{c} \pm 1.67$	$27.67^{d} \pm 1.89$	$75.91^{a} \pm 0.49$	$54.39^{b} \pm 1.24$	$28.12^{c} \pm 0.67$	$21.01^{d} \pm 0.73$
Liza tade	$103.45^{a} \pm 1.49$	$91.25^{b} \pm 1.45$	$53.98^{c} \pm 1.23$	$25.45^{d} \pm 1.12$	$43.89^{b} \pm 0.36$	$50.38^{a} \pm 1.67$	$27.91^{c} \pm 0.55$	$18.65^{d} \pm 0.88$
Stolephorus commersonii	$45.03^{a} \pm 1.02$	$25.16^{b} \pm 1.63$	$19.26^{c} \pm 1.15$	$16.32^{d} \pm 0.65$	$16.12^{a} \pm 0.35$	$14.90^{b} \pm 0.73$	$13.68^{d} \pm 0.83$	$13.91^{c} \pm 0.91$
WHO (1989) level for Zn and Cu in food		100 ppm				30 ppm		
FAO (1992) level for Zn and Cu in fish		30-100 ppm				10-100 ppm		

*Means in a whole column with different superscripts (a–e) are significantly different ($p < 0.05$, Duncan multiple range test).

The discharge of these wastes without adequate treatment often contaminate the estuarine and coastal water with conservative pollutants (like heavy metals), many of which accumulate in the tissues of resident organisms like fishes, oysters, crabs, shrimps, seaweeds *etc*. In many parts of the world, especially in coastal areas and on smaller islands, fish is a major part of food, which supplies all essential elements required for life processes in a balanced manner (Iyengar, 1991). Hence, it is important to investigate the levels of heavy metals in these organisms to assess whether the concentration is within the permissible level and will not pose any hazard to the consumers (Krishnamurti & Nair 1999).

Table 3a. Pb and Cd concentrations (in ppm dry wt.) in finfish muscles (station wise) *

Species	Pb				Cd			
	Stn. 1	Stn. 2	Stn. 3	Stn. 4	Stn. 1	Stn. 2	Stn. 3	Stn. 4
Polynemus paradiseus	$10.99^{c}\pm 0.26$	$9.71^{c}\pm 0.35$	$9.04^{d}\pm 0.70$	$7.20^{d}\pm 0.48$	BDL	BDL	BDL	BDL
Tenualosa ilisha	$14.01^{b}\pm 0.15$	$13.12^{b}\pm 0.98$	$11.30^{c}\pm 0.65$	$9.39^{c}\pm 0.53$	$1.35^{c}\pm 0.82$	BDL	BDL	BDL
Liza parsia	$19.89^{a}\pm 0.54$	$15.16^{a}\pm 0.53$	$14.85^{b}\pm 0.67$	$13.92^{a}\pm 0.46$	$4.01^{a}\pm 0.03$	BDL	BDL	BDL
Liza tade	$15.77^{b}\pm 0.45$	$13.51^{b}\pm 0.47$	$15.79^{a}\pm 0.90$	$11.65^{b}\pm 0.42$	$1.95^{b}\pm 0.16$	BDL	BDL	BDL
Stolephorus commersonii	$4.65^{d}\pm 0.04$	$3.69^{d}\pm 0.89$	$5.01^{e}\pm 0.44$	$3.34^{e}\pm 0.90$	BDL	BDL	BDL	BDL
WHO (1989) level for Pb and Cd in food	2 ppm				1 ppm			
FAO (1992) level for Pb and Cd in fish	0.5 – 6.0 ppm				0.05 – 5.5 ppm			

BDL means below detectable level
*Means in a whole column with different superscripts (a–e) are significantly different ($p < 0.05$, Duncan multiple range test).

Table 3b. Pb and Cd concentrations (in ppm dry wt.) in finfish muscles (species wise)*

Species	Pb				Cd			
	Stn. 1	Stn. 2	Stn. 3	Stn. 4	Stn. 1	Stn. 2	Stn. 3	Stn. 4
Polynemus paradiseus	$10.99^{a}\pm 0.26$	$9.71^{b}\pm 0.35$	$9.04^{b}\pm 0.70$	$7.20^{c}\pm 0.48$	BDL	BDL	BDL	BDL
Tenualosa ilisha	$14.01^{a}\pm 0.15$	$13.12^{b}\pm 0.98$	$11.30^{c}\pm 0.65$	$9.39^{d}\pm 0.53$	1.35± 0.82	BDL	BDL	BDL
Liza parsia	$19.89^{a}\pm 0.54$	$15.16^{b}\pm 0.53$	$14.85^{c}\pm 0.67$	$13.92^{d}\pm 0.46$	4.01 ± 0.03	BDL	BDL	BDL
Liza tade	$15.77^{a}\pm 0.45$	$13.51^{b}\pm 0.47$	$15.79^{a}\pm 0.90$	$11.65^{c}\pm 0.42$	1.95 ± 0.16	BDL	BDL	BDL
Stolephorus commersonii	$4.65^{b}\pm 0.04$	$3.69^{cd}\pm 0.89$	$5.01^{a}\pm 0.44$	$3.34^{d}\pm 0.90$	BDL	BDL	BDL	BDL
WHO (1989) level for Pb and Cd in food	2 ppm				1 ppm			
FAO (1992) level for Pb and Cd in fish	0.5 – 6.0 ppm				0.05 – 5.5 ppm			

BDL means below detectable level
*Means in a whole column with different superscripts (a–e) are significantly different ($p < 0.05$, Duncan multiple range test).

Heavy metals have contaminated the aquatic environment in the present century due to intense industrialization and urbanization. The Gangetic delta is no exception to this usual trend. The rapid industrialization and urbanization of the city of Kolkata (formerly known as Calcutta), Howrah and the newly emerging Haldia complex in the maritime state of West Bengal has caused considerable ecological imbalance in the adjacent coastal zone (Mitra & Choudhury, 1992; Mitra,

1998). The Hooghly estuary, situated on the western sector of the Gangetic delta receives drainage from these adjacent cities, which have sewage outlets into the estuarine system. The chain of factories and industries situated on the western bank of the Hooghly estuary is a major cause behind the gradual transformation of this beautiful ecotone into stinking cesspools of the megapolis (Mitra & Choudhury, 1992). The lower part of the estuary has multifarious industries such as paper, textiles, chemicals, pharmaceuticals, plastic, shellac, food, leather, jute, tyres and cycle rims (UNEP, 1982). These units are point sources of heavy metals in the estuarine water. Due to toxic nature of certain heavy metals, these chemical constituents interfere with the ecology of a particular environment and on entering into the food chain they cause potential health hazards, mainly to human beings. It was reported by several workers that the discharge of heavy metals into the sea through rivers and streams results in the accumulation of pollutants in the marine environment especially within fishes (Yusof *et al.*, 1994). Thus fishes can be used for monitoring potential risk to humans because these are directly consumed by a large population (Subramanian & Sukumar, 1988).

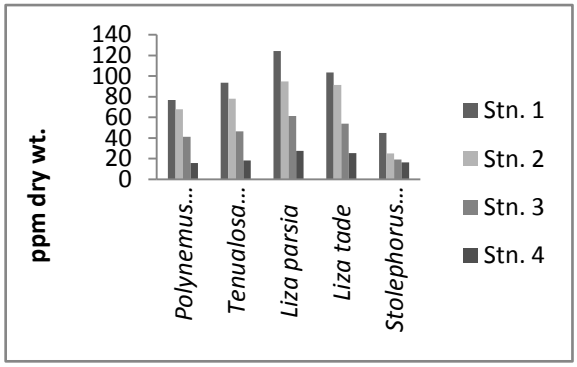

Fig. 2. Zn concentrations in finfish muscle

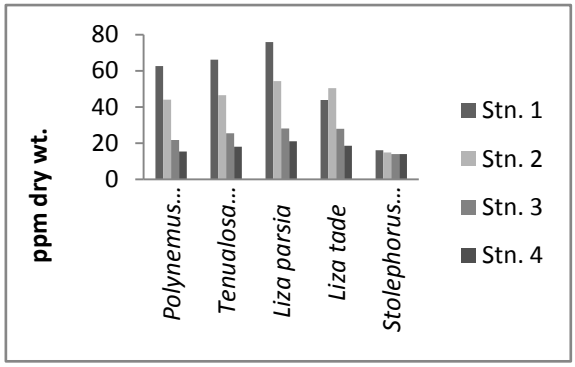

Fig. 3. Cu concentrations in finfish muscle

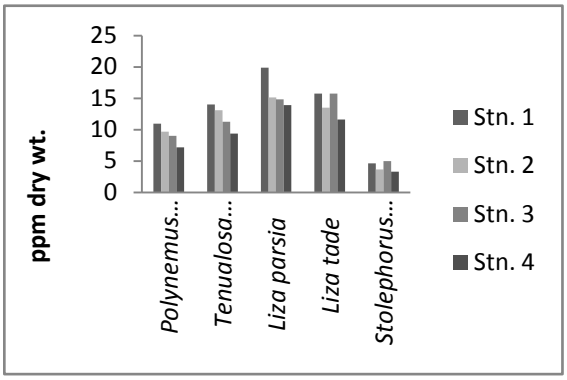

Fig.4. Pb concentrations in finfish muscle

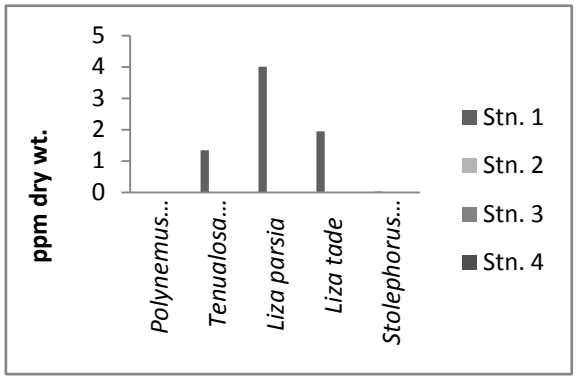

Fig. 5. Cd concentrations in finfish muscle

The selected finfish species in the present study have different food preference and different behavioral pattern e.g., *Liza parsia*, *Liza tade*, *Polynemus paradiseus*, and *Stolephorus commersonii* are resident fish species in the study area, while *Tenualosa ilisha* exhibit migration

from coastal region (~ salinity = 20 psu) to freshwater system in the upstream zone of the River Ganga for breeding. These factors may be attributed to species-wise variation of heavy metals in the study zone. The spatial variation of bioaccumulation followed the order station 1 > station 2 > station 3 > station 4, which may be related to different degree of contamination in different location. All the heavy metals (except Zn in *Liza parsia* in station 1) were found to be lower than the recommended maximum level allowed in food as prescribed by the World Health Organization (WHO, 1989). Furthermore the selected heavy metals in finfish muscle (except Zn in *Liza parsia* in station 1) were also within the permissible limits for human consumption as indicated by the Food and Agricultural Organization (FAO, 1992).

Of the four metals studied in the present work, Zn and Cu are essential elements while Pb and Cd are non-essential elements for most of the living organisms. The concentrations of Zn and Cu in all the finfish and shellfish species were relatively higher, compared to the concentration of other metals in same samples. It can be explained because these metals (Cu and Zn) are essential elements required by animals for metabolic process. Zn and Cu appear to diffuse passively (probably as a soluble complex) the gradients created by adsorption of membrane surfaces and are found in blood proteins metallothioneins. Carbonell and Tarazona (1994) concluded that different tissues of aquatic animals provide and/or synthesize nonexchangeable binding sites resulting in different accumulation levels. The primary sources of Zn in the present geographical locale are the galvanization units, paint manufacturing units and pharmaceutical processes, which are mainly concentrated in the Haldia industrial sector (opposite to station 1). Reports of high concentrations of Zn were also highlighted in the same environment by earlier workers (Mitra and Choudhury, 1992; Mitra and Choudhury, 1993; Mitra, 1998).

The main sources of Cu in the coastal waters are antifouling paints (Goldberg, 1975), particular type of algaecides used in different aquaculture farms, paint manufacturing units, pipe line corrosion and oil sludges (32 to 120 ppm). Ship bottom paint has been found to produce very high concentration of Cu is sea water and sediment in harbours of Great Britain and southern California (Bellinger & Benham, 1978; Young *et al.*, 1979). In the present study area, the major source of Cu is the antifouling paints used for conditioning fishing vessels and trawlers apart from industrial discharges (that is predominant around station 1). This is the reason why Cu was detected in the fish samples of stations 3 and 4, even there are no existence of industries. The complete siltation of the Bidyadhari River also does not permit the industrial effluents released in the Hooghly River to mix with the rivers in the central sector of the deltaic complex (location zone of stations 3 and 4).

Pb is a toxic heavy metal, which finds its way in coastal waters through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries (Mitra, 1998). Antifouling paints used to prevent growth of marine organisms at the bottom of the boats and trawlers also contain Pb as an important component. These paints are designed to constantly leach toxic metals into the water to kill organisms that may attach to bottom of the boats, which ultimately is transported to the sediment and aquatic compartments. Lead also enters the oceans and coastal waters both from terrestrial sources and atmosphere and the atmospheric input of lead aerosols can be substantial. Station 1 is exposed to all these activities being proximal to the highly urbanized city of Kolkata, Howrah and the newly emerging Haldia port - cum - industrial complex, which may be attributed to high Pb concentrations in the finfish species.

The main sources of Cd in the present geographical locale are electroplating, manufacturing of Cd alloys, production of Ni-Cd batteries and wielding (Mitra, 1998). No trace of Cd was recorded

in the fish muscle from stations 3 and 4, which are located almost in industry-free zone surrounded by mangrove vegetation.

A part from industrial discharges, significant difference in metal concentrations in biological samples between stations ($p<0.01$) may also be related to contrasting physico-chemical characteristics between the western and central part of the Gangetic delta. The western part of the Gangetic delta is connected to Himalayan glacier through Bhagirathi River. Researchers pointed out that the glaciers in the Himalayan range are melting at the rate of 23 m/yr (Hasnain, 1999; 2000; 2002). This along with Farakka discharge has resulted in gradual freshening of the system (Mitra *et al.*, 2009), which has role in elevation of dissolved metal level in the aquatic phase by way of lowering the salinity, pH and enhancing the process of dissolution of metallic species (Mitra, 1998). The central sector on contrary is derived from freshwater supply of Ganga-Bhagirathi system, and the Matla River is now tide fed with an increasing trend of salinity. This results in the precipitation of dissolved metals on the sediment bed (an event of compartmentation due to variation in metallic species) making the availability of metal low to the tissue of fish species thriving in the system (Mitra and Choudhury, 1992; Mitra and Choudhury, 1993; Mitra, 1998). Similar result has been found my many researchers (Ambedkar and Muniyan, 2011; Kamaruzzaman *et al.*, 2011; Heidary *et al.*, 2011)

This study reflects in some zones in and around Indian Sundarbans ecosystem, the bioaccumulation of certain heavy metal has touched the alarming level. Hence, strict regulations should be implemented to control industrial waste from the point sources both in the upsteam and downstream areas of the Indian Sundarban deltaic complex.

REFERENCE

Ambedkar,G., and M.Muniyan .1999. Accumulation of metals in the five commercially important freshwater fishes available in vellar river, Tamil Nadu, India. *Scholars Research Library*. 3(3):261-264.

Al-Yousuf, M.H., M.S. El-Shahawi, and S.M. Al-Ghais. 1999. Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. *Sci. Total Environ*. 256:87-94. DOI:10.1016/S0048–9697 (99)00363–0.

Bellinger, E., B. Benhem .1978. The levels of metals in Dockyard sediments with particular reference to the contributions from ship bottom paints. *Environ. Poll. Assess*. 15(1): 71-81.

Boudouresque, C.F., M. Verlaque. 2002. Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. *Mar. Poll. Bull*. 44: 32-38.

Canli, M. and G. Atli .2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environ. Pollut*. 121: 129-136. DOI:10.1016/S0269–7491 (02) 00194–X.

Carbonell, G., and J.V. Tarazona .1994. Toxicokinetics of Cu. Aqua. Toxicol. 29: 213-221.

Castro, H., P.A. Aguilera, J.L. Martinez, and E.L. Carrique. 1999. Differentiation of clams from fishing areas an approximation to coastal quality assessment. *Environ. Monit. Assess*. 54: 229-237.

Chaudhuri, A.B., and A. Choudhury. 1994 Mangroves of the Sundarbans, India, Vol-I., first ed. IUCN.

Chen, M.H., and C.Y. Chen.1999. Bioaccumulation of sediment-bound heavy metals in grey mullet, *Liza macrolepis*. *Mar. Pollut. Bull*. 39:239-244.DOI:10.1016/S0025–326X (99)00027–2.

Clearwater, S.J., A.M. Farag, and J.S. Meyer. 2002. Bioavailability and toxicity of dietborne copper and zinc to fish. Comparative biochemistry and physiology part C: *Toxicol. Pharmacol.*132:269-313.DOI:10.1016/S1532–0456 (02)00078–9.

Cohen, T., S. Hee, and R. Ambrose. 2001. Trace metals in fish and invertebrates of three

California Coastal Wetlands. *Mar. Poll. Bull*. 42: 232-242.

Date, A.R., and A.L. Gray (Eds.) .1988. Applications of Inductively Coupled Plasma Source Mass Spectrometry, Blackie, Glassgow.

De la Guardia, M. (Ed.) .1990. Empleo de losHornos de Microondas en Quimica, University of Valencia, Spain.

Enoyer, E.R., .1992. Semi–quantitative analysis of environmental materials by laser-sampling inductively coupled plasma mass spectrometry. *J. Anal. Atom. Spectro*. 7: 1187.

Fergusson, J.E. 1990. The heavy elements: Chemistry, environmental impact and health effects, Pergamon Press, New York. p: 614.

FAO .1992. FAO/WHO, Food standard programme. 2nd ed. Codex Alimentarius Commission. Vol. 1. pp: 114-190.

Garcia-Montelongo, F., L.Galindo, M.S. Larrechi, X. Rius .1994. Heavy metals in three fish species from the coastal waters of Santa Cruz de Tenerife (Canary Islands). *Scientia Marina*. 58: 179–183.

Goldberg, E.D. 1975. The mussel watch – a first step in global marine monitoring. *Mar. Poll. Bull*. 6: 111.

Grosell, M., K.V. Brix. 2005. Introduction to the special issue on mechanisms in metal toxicology. *Aqua. Toxicol*. 72: 3-4.

Hasnain, S.I.,.1999. Himalayan Glaciers: Hydrology and Hydrochemistry. First ed, Allied Publication Limited, New Delhi.

Hasnain, S.I. 2000. Status of the Glacier Research in the HKH region. ICIMOD, Kathmandu, Nepal.

Hasnain, S.I. 2002. Himalayan Glaciers Meltdown: Impact on South Asian Rivers. IAHS; 7**:** 274.

Heidary, S., J. Imanpour Namin, and F. Monsefrad. 2011. Bioaccumulation of heavy metals Cu, Zn, and Hg in muscles and liver of the stellate sturgeon (*Acipenser stellatus*) in the Caspian Sea and their correlation with growth parameters. *Iranian Journal of Fisheries Sciences*.11(2): 325-337.

Henry, F., R. Amara, L. Courcot, D. Lacouture, and M.L. Bertho. 2004. Heavy metals in four fish species from the French coast of the Eastern English Channel and Southern bight of the North Sea. *Environ. Int*. 30:675-683.DOI: 10.1016/j.envint.2003.12.007.

Islam, M.D., M. Tanaka. 2004. Impact of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. *Mar. Poll. Bull*. 48: 624-649.

Iyengar, G.V. 1991. Milestones in biological trace elements research. *Sci. Total Environ*. 1: 100.

Jaffa, M., M. Ashraf, and Rasoal. 1998. Heavy metals contents insome selected local freshwater fish and relevant water. *Pakistan Journal of Scientific and Industrial Research*. 31:189-193.

Jezierska, B., and M. Witeska .2001. The metal uptake and accumulation in fish living in polluted waters. Department animal Physiology. University of podlasie. Pursa. 12. 108-110 Siedlce. Polant. pp :107.

Jordao, C. P., M.G. Pereira, C. R. Bellato, J.L. Pereira, A.T. Matos. 2002. Assessment of water systems for contaminants from domestic and industrial sewages. *Environ. Monit. Assess*. 79: 75–100.

Kamaruzzaman, B.Y, M.C. Ong, and S.Z. Rina. 2011. Heavy metal accumulation in commercially important fishes of South West Malaysian Coast. *Research Journal of Environmental Science*. 5(6): 595-602.

Kennicutt, M.C, T.L. Wade, and B.J. Presley .1992. Texas A&M University. Assessment of Sediment Contamination in Casco Bay. Casco Bay Estuary Project. p:113.

Kotze, P., H.H. Du Preez, and J.H. Van Vuren. 1999. Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga. *South Africa. Water SA.* 25 (1) 99-110.

Krishnamurti J.A. and R.V. Nair. 1999. Concentration of metals in shrimps and crabs from Thane- Bassein creek system, Maharastra. *Ind. J. Mar. Sci*. 28: 92-95.

Matusiewicz, H.,and R.E. Sturgeon.1989. Present status of microwave sample dissolution and decomposition for elemental analysis. *Prog. Anal. Spectro*. 12: 21.

McCarthy, J.F., and L.R. Shugart. 1990. Biomarkers of environmental Contamination Lewis Publishers, Chelsea, Mich. 3-16.

McNicol, R.E., and E. Scherer.1991. Behavioral responses of lake whitefish (*Coregonus clupeaformis*) to cadmium during preference-avoidance testing. *Environ. Toxicol. Chem*. 10: 225-234.

Mitra, A., A. Choudhury. 1992. Trace metals in macrobenthic molluscs of the Hooghly estuary. *India. Mar. Poll. Bull. UK*. 26 (9): 521-522.

Mitra, A., and A. Choudhury. 1993. Seasonal variations in metal content in the gastropod

Nerita articulata (*Gould*). *Ind. J. of Env.Hlth. NEERI*. 35 (1): 31-35.

Mitra, A. 1998. Status of coastal pollution in West Bengal with special reference to heavy metals. *J. Ind. Ocn. Stud*. 5(2): 135 -138.

Mitra, A., K. Banerjee, K. Sengupta, and A. Gangopadhyay (2009) Pulse of Climate change in Indian Sundarbans: A myth or reality? Natl. *Acad. Sci. Lett*. 32(2):1-2.

Nadkarni, R.A. .1984. Applications of microwave oven sample dissolution in analysis. *Anal. Chem*. 56: 22-33.

Peerzada, N., L. McMorrow, S. Skiliros, M. Guinea, and P. Ryan. 1990. Distribution of heavy metals in Gove Harbour, Northern Territory, Australia. *Sci.Total Environ*. 92: 1–12.

Romeo, M., Y. Siaub, Z. Sidoumou, M. Gnassia-Barelli. 1999. Heavy metal distribution in different fish species from the Mauritania coast. *Sci. Total Environ*. 232: 169-175.

Subramanian R, and A. Sukumar. 1988. Biological reference materials and analysis of toxic elements. *Fresenius Z Anal. Chem*. 332: 623-626.

UNEP. 1982. Pollution and the marine environment in the Indian Ocean. UNEP Regional Seas Programme Activity Centre. Geneva, Switzerland.

Usero, J., J. Morillo, and I. Graccia. 2005. Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. *Chemosphere*. 59: 1175-1181.

World Health Organization. 1989. Heavy metals environmental aspects. Environmental Health Criteria. No. 85. Geneva, Switzerland.

Young, D.R., G.V. Alexander, and D. McDermott-Ehrlich. 1979. Vessel related contamination of southern California harbours by copper and other metals. *Marine Pollution Bulletin*. 10: 50-56.

Yusof AM, N.F. Yanta, and A.K.H. Wood. 2004. The use of bivalves as bio-indicators in the assessment of marine pollution along a coastal area. *J. Radioanal. Nuclear Chem*. 259(1): 119-127.

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Comparative Study on Rabbit Breeds for Post Weaning Growth Traits in the Humid Tropics of Nigeria

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ABSTRACT

Rabbit meat is a cheap source of high quality animal protein which could be reared in any agro-vegetational zone without any cultural and religious constraints. The rate of growth and reproductive ability of the different breeds differ, however. The aim of the present study was to determine the post weaning growth traits of exotic rabbit breeds which are commercially available in Nigeria. The two rabbit breeds used for this study were New Zealand White and California White. The study commenced from when the rabbits were 8 weeks old and lasted till 30th week of age. Analyzed data revealed that both breeds were not significantly ($P>0.05$) different in live body weight and linear measurements. Age of rabbits has significant effect ($P<0.01$) on all the traits evaluated, that is, all the body dimensions measured increased in size as the animals advanced in age. In addition, there was statistically significant ($P<0.01$) positive phenotypic correlations between live weight and linear measurements. This implies that all the body dimensions were good indicators of live weight and anyone of them could be used to predict its value. Age of rabbits also has significant positive phenotypic correlation with all the traits. There was significant breed x age interaction effects on all the traits measured. In this study, it was revealed that either of the two breeds could be used to cross with our local breeds of rabbits in order to improve their productivity.

Keywords: Breeds, growth, productivity, phenotypic correlation, rabbit, trait.

INTRODUCTION

Rabbit production is gradually becoming an important source of income and employment generation in Nigeria. It can also serve as alternative source of cheap animal proteins to mitigate the negative impacts of malnutrition in infants and adults which are prevalent in the developing countries. The only limiting factor reported to be affecting growth and productivity of rabbits in tropical and arid climates according to McNitt *et al*. (2000) is calorie stress associated with high

ambient temperature. Rabbit meat provides a cheap source of meat which is characterized by a high protein and low fat cholesterol content (Aduku and Olukosi, 1990), and it is considered a delicacy and a healthy food product (Dalle Zotte, 2000). Rabbits have the potentials to supply good and high quality animal proteins, and are comparable to domestic chickens which have short gestation and generation interval, highly prolific, lack of taboos to its production and consumption, and can subsist on domestic waste and succulent leaves. In the tropics where there is stiff competition for grains and legumes between man and animals, rabbits can conveniently be reared in small or large scale since they can survive on forages and agricultural by-products not consumed by man. According to Lukefahr and Cheeke (1990), rabbit production occupies a vital role in the utilization of fibrous by-products that are not suitable for poultry or swine, and forages that may be available in insufficient quantities for raising ruminants. The most popular breeds used in commercial rabbit production are medium-sized ones such as New Zealand White and California (Ozimba and Lukefahr, 1991; Shemeis and Abdallah, 1998). Piles *et al*. (2000) and Shahin and Hassan (2002) documented that selection for high growth rate in rabbits had improved slaughter performance, but that it carries a high risk of lowering the quality of meat. Crossbreeding according to Nofal *et al*. (1997) is one of the fast tools offered to the breeders to improve many traits in farm animals. In a study involving New Zealand White and California, Maj *et al*. (2009) observed that crossbred rabbits were heavier than purebred animals. In Egypt, significant differences in body weights at various ages of local breeds had been documented (Khalil, 1997). In addition, McNitt and Lukefarh (1993) found that New Zealand White had significantly higher market weight and lower age than California, Palomino and White Satin breeds.

There are genetic and environmental factors affecting the post weaning growth rate of rabbits, and these include breed (Lukefahr *et al.*, 1983b; Ozimba and Lukefahr, 1991), sex (Afifi *et al*., 2000), season or month (Afifi and Emara, 1990). Oke *et al*. (2010) and Isaac *et al*. (2010) also observed significant differences in growth traits among breeds of rabbits. In addition, variations in growth rate or weight gain of rabbit within the same breed or among different breeds could be attributed to environmental factors such as nutrition, disease, hormone and general management. The documented breed differences in growth rate for exotic breeds could be exploited and used in breeding programme to develop a fast growing indigenous strain adaptable to hot environment. Adequate information is required in development and general recommendations for pure breeding or crossbreeding programmes on genetic and environmental factors affecting growth traits of both purebred and crossbred rabbits reared in a variety of climates. It is worthy to note that post weaning growth rate of an animal has effect on its reproductive activity, survival, age at market weight and other economic traits and value. The present investigation was therefore, undertaken to assess breed and age effects on post weaning growth traits of New Zealand White and California White. The aim was to identify and recommend the breed that can be used to upgrade and improve the growth potential of our local unimproved rabbits. The study also estimates the relationship between body weight and linear measurements so as to be able to predict the value of the former from the latter.

MATERIALS AND METHODS

Study location

The study was carried out at the Animal Breeding Unit, Teaching and Research Farm, Ekiti State University, Ado-Ekiti, between July, 2010 and December, 2010. Ado-Ekiti is situated along

latitude $7^0 31^1$ and $7^0 49^1$ North of the Equator and longitude $5^0 71^1$and $5^0 27^1$ East of the Greenwich Meridian. The city falls under Derived Savannah zone. The city enjoys two separate seasonal periods namely, Rainy (May-October) and Dry (November-April) seasons.

Population and Management of Rabbits

A total number of 10 rabbit kits (weaners) comprising of five kits each of New Zealand White and California White were purchased at 6 weeks old from a breeder farm, and kept in separate hutches made of wooden materials. The hutches were properly disinfected before the arrival of the rabbits. They were allowed two weeks to adapt to the new environment. They were dewormed and given necessary medications during the experimental period. Fresh and green leaves of *Tridax procumbens*, *Talinium triangulae* and *Aspergillia africana* were given, while pelletized feeds as supplements were also given at regular intervals. Fresh water was given *ad libitum.*

Traits Studied

Evaluation criteria in which breed-type comparisons were based included body weight at 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 30 weeks. Linear body measurements taken were body length, ear length, trunk length, abdominal circumference, nose to shoulder and tail length, all in centimetres. Body weight was taken using a top loading scale, while other traits were measured using tailor's tape rule. All the animals were starved overnight, carefully restrained before they were measured in order to get accurate weight of the body.

Data Analysis

Data were collected on breed basis on all the studied traits and were subjected to T-test and significant differences between means were determined as per SAS (2001). Also, correlations between body weight and linear measurements were determined by Pearson Correlation analysis as per SAS (2001) computer package.

The appropriate statistical model used was:

$Y_{ijk} = \mu + G_i + A_j + (GA)_{ij} + €_{ijk}$

Y_{ijk}= observation of the k^{th} population, of the j^{th} age and of the i^{th} genotype

μ = common mean

G_i = fixed effect of i^{th} genotype (i=2)

A_j= fixed effect of j^{th} age (j=12)

$(GA)_{ij=}$ genotype x age interaction effect

$€_{ijk=}$ residual error

RESULTS

Breed and age effects

Table 1 shows the effects of breed and age on post weaning growth traits. There were no significant breed ($P>0.05$) differences in live body weight and linear measurements at 8^{th} week of age between New Zealand White and California White. The weanling rabbits were of the same age and size at the commencement of the experiment. Also from 10^{th} – 30^{th} week of age, the two breeds recorded similar mean values in live body weight and linear measurements.

Table 1. Least square means showing breed and age effects on body weight and linear measurements of two rabbit breeds

Age (weeks)	Breeds	Traits							
		Body weight	Body length	Ear length	Trunk length	Tail length	Abdominal circumference	Nose-shoulder	P-value
8	CLN	0.65	28.30	8.35	10.57	5.75	10.83	9.50	N.S
	NZW	0.65	27.67	8.97	11.63	5.65	10.50	9.75	N.S
10	CLN	0.83	28.73	9.08	11.60	6.53	11.53	9.85	N.S
	NZW	0.80	29.12	9.22	12.62	6.45	11.92	10.18	N.S
12	CLN	0.98	30.80	10.02	13.30	7.23	13.5	11.23	N.S
	NZW	0.93	30.47	9.65	13.45	7.07	13.0	11.15	N.S
14	CLN	1.28	33.50	10.32	14.07	7.52	14.28	11.60	N.S
	NZW	1.10	32.62	10.12	14.17	7.70	13.83	11.65	N.S
16	CLN	1.38	35.62	10.60	14.50	8.08	15.12	12.05	N.S
	NZW	1.28	33.88	10.40	14.53	7.97	14.68	12.18	N.S
18	CLN	1.53	37.17	11.05	14.90	8.37	16.02	12.73	N.S
	NZW	1.43	35.53	10.72	15.03	8.23	15.47	12.70	N.S
20	CLN	1.76	38.92	11.38	15.65	8.95	16.73	13.45	N.S
	NZW	1.63	37.30	11.03	15.48	8.73	16.50	13.15	N.S
22	CLN	1.83	40.40	11.60	16.33	9.18	17.48	13.83	N.S
	NZW	1.74	38.77	11.35	15.87	9.17	17.65	13.85	N.S
24	CLN	2.00	41.75	11.82	16.82	9.65	18.08	14.25	N.S
	NZW	1.95	40.20	11.55	16.27	9.60	18.32	14.00	N.S
26	CLN	2.10	42.53	12.10	17.27	10.22	18.63	14.80	N.S
	NZW	2.14	41.22	11.77	16.70	9.85	18.82	14.35	N.S
28	CLN	2.15	43.02	12.40	17.43	10.43	18.80	15.32	N.S
	NZW	2.22	42.33	11.95	17.07	10.25	19.10	15.13	N.S
30	CLN	2.37	44.30	12.73	17.70	10.77	20.13	16.30	N.S
	NZW	2.42	43.52	12.57	17.43	10.72	19.95	15.87	N.S

N.S: means along columns without superscripts are not significantly different (p>0.05). CLN: California White NZW: New Zealand White

Table 2 presents the combined analyses of phenotypic correlations between live body weight and linear measurements on one hand, and among various linear measurements on the other hand. Body weight has statistically significant (p<0.01) positive phenotypic correlations with body length (0.977), ear length (0.924), trunk length (0.918), tail length (0.911), abdominal circumference (0.966) and nose to shoulder (0.933). Furthermore, there were statistically significant (p<0.01) positive phenotypic correlations among the linear body measurements.

Table 2. Phenotypic correlations between live weight and linear measurements of rabbits (pooled data)

Traits	Age	Body weight	Body length	Ear length	Trunk length	Tail length	Abdominal circumference	Nose-shoulder
Age	1.00	0.925**	0.922**	0.882**	0.916**	0.897**	0.938**	0.953**
Body weight		1.00	0.977**	0.924**	0.918**	0.911**	0.966**	0.933**
Body length			1.00	0.947**	0.947**	0.930**	0.957**	0.954**
Ear length				1.00	0.937**	0.916**	0.903**	0.927**
Trunk length					1.00	0.922**	0.925**	0.956**
Tail length						1.00	0.909**	0.918**
Abdominal Circumferene							1.00	0.925**
Nose-shoulder								1.00

**P<0.01

In this study (Table 3), breed-basis analyses showed there were positive significant phenotypic correlations between live weight and linear measurements. In both California White and New Zealand White, age has positive and high significant phenotypic correlations with body weight, body length, ear length, trunk length, tail length, abdominal circumference and nose-tail.

Table 3. Phenotypic correlations between live weight and linear measurements in two rabbit breeds

Traits	Age	Body weight	Body length	Ear length	Trunk length	Tail length	Abdominal circumference	Nose-shoulder
Age	1.00	0.892**	0.929**	0.922**	0.946**	0.929**	0.915**	0.966**
Body weight	0.956**	1.00	0.976**	0.926**	0.902	0.908**	0.962**	0.915**
Body length	0.924**	0.981**	1.00	0.946**	0.944	0.948**	0.969**	0.952**
Ear length	0.844**	0.925	0.948**	1.00	0.933	0.952**	0.933**	0.926**
Trunk length	0.877**	0.947	0.963**	0.950**	1.00	0.946**	0.938**	0.960**
Tail length	0.867**	0.916	0.916**	0.883**	0.907	1.00	0.915**	0.951**
Abdominal circumference	0.962**	0.972	0.950**	0.873**	0.917	0.904**	1.00	0.923**
Nose-shoulder	0.942**	0.954	0.961**	0.931**	0.953	0.887**	0.929**	1.00

**P<0.01 Above diagonal- California White Below diagonal- New Zealand White

DISCUSSION

The non-significant (P>0.05) breed differences recorded in all the traits evaluated between the two breeds suggest that the two breeds have similar genetic background. The obtained results contradicted the findings of Oke *et al*. (2010) and Isaac *et al*. (2010) who found significant breed differences in linear measurements at different ages with the exception of shoulder to tail. The present study corroborates the findings of Ozimba and Lukefahr (1991) and Roberts and Lukefahr (1992) who asserted that there was small to non-significant breed differences for postweaning growth traits in breed comparison studies involving three breeds. The two breeds however, increased in body size and other body dimensions as the animals grew in age. The rate of change was high initially but slowed down from 16th week to 22nd week. This type of growth has been called a convex-shaped growth curve as reported in broilers (Marks, 1979).

The result of phenotypic correlation analyses implies that all linear body measurements are good determinants of body weight. That is, body weight could be predicted with greater accuracy using the values of anyone of body dimensions. In agreement with this study, Ige *et al*. (2005) found that linear body measurements are useful in live weight determination. The observed findings also presuppose that the growth in any body dimension will invariably result to increase in live weight. Similarly, the positive, significant phenotypic correlations recorded among linear body measurements indicate strong relationships between the various traits that are connected with animal growth. According to El-Labban (1999), positive relationships between these traits were as a result of pleiotrophic effects of genes and linkage effects which operate on these traits. Therefore, any attempt to perform phenotypic selection for one trait will consequently result in improvement of the other.

Breed-basis analyses indicate that the two breeds grew in size and linear parts with advancing age under normal conditions. Moreover, phenotypic correlation values between body weight and other linear parts were very high in the two breeds. This suggests possible strong and positive relationship between these traits, and the likelihood of pleiotrophic effect of genes operating on them. Therefore, any attempt to select for one trait in a breeding program will

automatically result to improvement on other traits. Previous studies have indicated positive and significant correlations between live weight and body dimensions in farm animals, that is, body dimensions are good indicators and can be used to predict the body weight of an animal. The current study was in agreement with the findings of Ige *et al*. (2007) in local fowls, Kolawole and Salako (2010) in cane rat and Elamin and Yousif (2011) in Sudanese rabbits. In addition, age of the rabbits across the two breeds was found to have significant positive correlation with body weight and linear measurements. There was significant breed x age interaction effects on all the traits measured. This implies that growth traits in rabbits are breed and age dependent.

CONCLUSIONS

Since there were no significant breed differences in live body weight and linear measurements, anyone of the two breeds could be used to cross with our local breeds to improve their genetic potentials and productivity. The local breeds are hardy and heat tolerant but low in mature body weight in comparison with exotic breeds. The crossbreds will no doubt combine the quality traits of both the local and exotic rabbit breeds. Also, it was indicated in this study that body weight has significant positive phenotypic correlation with linear measurement, that is, all the linear parts are good determinants of body weight in rabbits. The phenotypic correlations between body weight and linear body parts on one hand were high and positive. On the other hand, there were significant positive correlations among the various linear measurements evaluated. This implies that improvement in any part will lead to increase in body weight and other linear body dimensions.

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REFERENCE

Aduku, A.O., and J.O. Olukosi. 1990. Rabbit management in the tropics production, processing, utilization, marketing, economic practical training, research and future prospects. Living Book Series, G. U. Publications, Abuja.

Afifi, E.A., and M.E. Emara. 1990. Breed group and environmental factors influencing post weaning daily gain in weight of purebred and crossbred rabbits. *J. Applied Rabbit Res*. 13: 114-118

Afifi, E.A., A. Abd El-Ghany, and E.G. Ahmed. 2000. Reproductive profile of New Zealand white and California rabbits under semi arid environmental conditions. *Egypt Poul. Sci*. 20(1): 145-155.

Dalle Zotte, A. 2000. Main factors influencing the rabbit carcass and meat quality. Proc. Of the 7th World Rabbit Congress, Valencia, Spain. pp.1-32.

Elamin, K.M., and I.A. Yousif. 2011. Evaluation of litter traits in Sudanese rabbits. *Livestock Res. for Rural Dev*. 25-30.

El-labban, A.F.M. 1999. Comparative studies on phenotypic performance of body measurements and carcass characteristics in males of some local strains of chickens. *Egypt Poul. Sci*. 19: 419-434.

Ige, A.O., A.E. Salako, L.O. Ojedapo, T.A. Adedeji, A. Yakubu, S.R.Amao, A.O.Animasahun, and O.A. Amao. 2007. Prediction of body weight on the basis of body measurements in mature indigenous chickens in derived savannah zone of Nigeria. Proc. 32nd annual conference, Nigeria Society for Animal Production, 18-21 March, 2007, Calabar, Nigeria, Pp185-187.

Ige, A.O., A. Akinlade, L.O. Ojedapo, O. Oladunjoye, S.R. Amao, and A.O. Animasahun. 2006. Effect of sex on interrelationship between body weight and

linear measurements of commercial broilers in a derived savannah environment of Nigeria. Proceedings 11th Annual Conference, Animal Science Association of Nigeria, September 18-21, 2006 held at I.A.R. & T, Ibadan. Pp:231-233.

Isaac, L.J., P.M. Eko, J.S. Ekpo, E. Ekanem, and G.B. Essien. 2010. Effect of breed on performance of rabbit in feed", Proc. 35th conf., Nigerian Soc. for Animal Production, 14-17, March 2010, University of Ibadan. pp.18-19.

Khalil, M.H. 1997. Model for the description of rabbit genetic resources in Mediterranean countries. Application to the Egyptian breeds Giza white and Baladi. CIHEAM. IAMZ. Zaragora 41 pp.

Kolawole, A., and A.E. Salako. 2010. Phenotypic Characterization of the cane rat (Thryonomys swinderianus). Proc. 35th conf., Nigeria Society for Animal Production 14-17 March, 2010, University of Ibadan, Nigeria. Pp92-94

Lukefahr, S.D., W.D. Hohenboken, P.R. Cheeke, and N.M. Patton. 1983b. Characterization of straight bred and crossbred rabbits for milk production and associative traits. J. Anim. Sci. 57: 1100.

Lukefahr, S.D., and P.R. Cheeke. 1990. Rabbit Project planning Strategies for Developing countries. (2): Practical considerations. *Livestock Res. for Rural Dev*. 2(3):2. http://www.lrrd.org/lrrd2/3/cheeke2.htm

Maj, D., J. Bieniek, P. Lapa, and I. Sternstein. 2009. The effect of crossing New Zealand white and Californian rabbits on growth and slaughter traits. *Archiv Tierzucht.* 52(2): 205-211.

Marks, H.L. 1979. Growth rate and feed intake of selected and non-selected broilers. Growth, 43: 80-90.

McNitt, J.I., and S.D. Lukefahr. 1993. Breed and environmental effects on post weaning growth of rabbits. *J. Anim. Sci*. 71: 1996-2005.

McNitt, J.I., N.M.Patton, S.D. Lukefahr, and P.R. Cheeke. 2000. Rabbit Production. Interstate Publishers (7thedition) Danville, IL .p:493.

Nofal, R.Y., S. Toth, and G. Virag. 1997. Evaluation of seven genetic groups of rabbits for carcass traits. *Archiv Tierzucht.* 40: 61-67.

Oke, U.K., U. Herbert, C. Nwichi, O.M. Onyiro, and C.N. Okocha. 2010. Effect of breed of sire on growth performance of crossbred rabbits in a humid tropical environment. Proc. 35th conf., Nigerian Soc. for Animal Production. 14-17, March 2010, University of Ibadan. pp:15-17.

Ozimba, C.E., and S.D. Lukefahr. 1991. Comparison of rabbit breed types for post weaning litter growth, feed efficiency and survival performance traits. *J. Anim. Sci*. 69: 3494-3500.

Piles, M., A. Blasco, and M. Pla. 2000. The effect of selection for growth rate on carcass composition and meat characteristics of rabbits. *Meat Sci*. 54: 347-355.

Roberts, J.D., and S.D. Lukefahr. 1992. Evaluation of California, Champagne D'Argent, New Zealand White and Palomino as potential sire breeds: 1. post weaning litter traits. *J. App. Rabbits Res*. 15:274.

Shahin, K.A., and N.S. Hassan. 2002. Changes in sources of shared variability of body size and shape in Egyptian local and New Zealand white breeds of rabbits during growth. *Archiv Tierzucht.* 45: 269-277.

Shemeis, A.R., and O.Y. Abdallah. 1998. Selection indexes for increased marketing body weight and advantageous body composition in New Zealand white rabbits. *Archiv Tierzucht.* 41: 597-605.

Statistical Analysis System (SAS, 2001). SAS Users Guide. Statistics, 8th edition, SAS Institute Cary, NC, USA.

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The Effect of 'Prekese' (Tetrapleura Tetraptera) Pod Extract on the Sensory and Nutritional Qualities of Pork Sausage

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ABSTRACT

This study was carried out to determine the effect of 'prekese' (Tetrapleura tetraptera) pod extract (PPE) on the sensory characteristics and nutritional qualities of smoked pork sausages. Four kilograms of minced pork was used. The pork was divided into four equal parts (1kg per treatment). Each product contains the following: (T1) control (without PPE), (T2) 10 ml of full pod PPE/1kg of pork, (T3) 10 ml of chopped pod PPE/1kg of pork, and (T4) 10 ml of ground pod PPE/1kg of pork. Sensory analysis was conducted to determine the effect of the 'prekese' pod extract on the sensory characteristics of the product. Crude fat, crude protein and moisture content were determined to find out the effect of the pod extract on the nutritional qualities of the products. There were no significant differences in the sensory characteristics. There were significant differences in the nutritional qualities of the products in terms of crude protein, crude fat, and pH and moisture contents. The inclusion of PPE in the sausage at 10ml/1kg of pork improved the protein content of the products.

Keywords: pork sausage, 'prekese', sensory characteristics, nutritional qualities.

INTRODUCTION

Lawrie and Ledward (2006) defined meat as the flesh of animals used as food. Meat is recognized as a 'functional food' that can beneficially affect physiological processes in the consumer and thereby potentially mitigate or prevent diseases (Jiminez-Colmenero *et al.*, 2001). Meat processing refers to procedures such as addition of ingredients and/ or mechanical action that convert intact meat into specific products such as bacon, minced meat, fresh or raw sausages, liver sausage, scalded sausages and cook sausages (Teye, 2007). Sausages consist of ground lean

meat, animal fat, herbs or spices with sometimes other ingredients and usually packed in casing and preserved in a way by curing or smoking (Kumar, 2007).

Spices are esoteric food adjuncts that are used as flavouring agents and as preservatives in meat products (Srinivisan, 2005). Platel and Srinivasan (2000;2001) reported on the beneficial effects of spices on human health, nonetheless there have been reported mixed feelings from consumers over health issues such as hypertension, cancer and obesity resulting from excessive use of artificial food additives and preservatives in meat products including pork sausages (Lawrie and Ledward, 2006). In order to replace artificial spices, some indigenous plants such as 'Akokobesa' (*Ocimum basilicum*), 'Dawadawa' (*Parkia biglobosa*) and 'Prekese' (*Tetrapleura tetraptera*) whose parts are used as spices in the preparation of local dishes can be used as replacement for seasoning meat products. *Tetrapleura tetraptera* plant has many medicinal uses ranging from it leaves, fruit, bark and pod (Steentoft, 1988). In Northern Nigeria for example, the fruits are used to prepare food for mothers from the first day of delivery to prevent postpartum contraction (Nwamu and Akah, 1986). Okwu (2003) reported on the chemical evaluation, nutritional and flavouring properties of 'Prekese' which contains varying amount of crude protein, crude lipids, crude fat, carbohydrate and energy.

A recent study involving the use of 'prekese' pod powder as a spice in sausage and hamburger, had shown a promising result in the sensory characteristics and nutritional qualities of the products (Lartey, 2012). For example, there were increases in the protein and fat contents of the products (sausages) but the colour of the products became darker as inclusion levels of the 'prekese' pod powder increased in the sausages (Lartey, 2012). The effect of prekese' pod extract (PPE) on sensory characteristics is not known; therefore this study seeks to determine:

- The effect of 'prekese' pod extract on the sensory characteristics of pork sausage.
- The crude protein, crude fat, pH and moisture contents of pork sausage.

MATERIALS AND METHODS

Study area

This study was conducted at the Meat Processing Unit of the University for Development Studies (UDS), Nyankpala Campus. Chemical analysis of the meat products were conducted at the Spanish laboratory of UDS, Nyankpala.

Preparation of pod extract

Tetrapleura tetraptera (prekese) pod extract was obtained by:

i) Weighing three pods of 'prekese', 50g each with a length of about 25cm each.
ii) The first pod was left fully intact.
iii) The second pod was chopped into five pieces with each piece measuring about 5cm long.
iv) The third pod was ground into powdery form and the weight retaken to ensure it is 50g.
v) 0.5 litres of water was used to soak the three different forms of the pod in plastic containers for 24 hours to get the extract. Figure 1 shows the various forms of the processed *Tetrapleura tetraptera* pod.

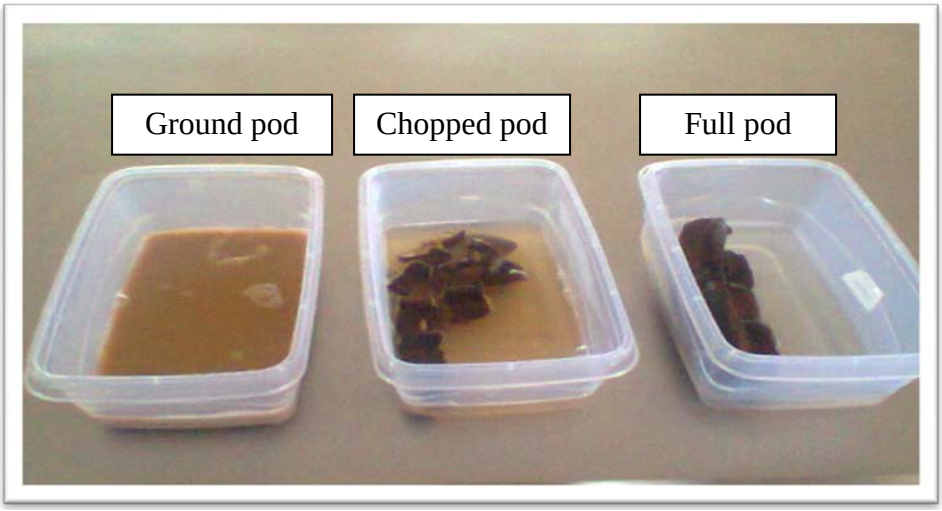

Figure 1: Pod extract in different forms of the pod after 24 hours

Sausage preparation

The meat used were thawed overnight at a temperature of 4^{o}C, cut into smaller pieces and minced using a 5mm sieve table top mincer (Talleras Rammon, Spain). The inclusion levels of test spice, (PPE) in millilitres per 1kg of meat, were as follows:

a. Treatment one (T1-Control): 2g/kg meat of Adobo and no PPE.
b. Treatment two (T2): 2g/kg meat of Adobo with 10 ml of PPE (full pod).
c. Treatment three (T3): 2g/kg meat of Adobo with 10 ml of PPE (chopped pod).
d. Treatment four (T4): 2g/kg meat of Adobo with 10 ml of PPE (ground pod).

The meat (4kg) was weighed and divided into four groups of 1kg each and placed in separate containers. The containers were labeled Treatments 1 to 4. The spices as indicated above were added to their respective treatments and manually mixed thoroughly. They were then stuffed into natural casings, using a hydraulic stuffer (Talleres Rammon, Spain) and manually linked into similar length of about 10cm. The sausages were hung on labeled smoking racks and smoked for an hour, after which the sausages were allowed to cool under room temperature.

Products preparation for sensory evaluation

For the sensory evaluation, the sausages were removed from the refrigerator and allowed to thaw for two hours. They were then grilled in an electric oven and sliced into uniform length (about 2cm). A total of 15 panelists were trained based on the British Standard Guide (BSI, 1993) to effectively carry out the sensory analysis. Each panelist was served with a piece of bread and water to serve as a neutralizer and was given a five-point category scale to evaluate the products for sensory characteristics.

Proximate analyses of the products

The sausages were analyzed for moisture, crude protein (Kjeldhal method) and fat contents (Soxtec apparatus**)** according to the methods of the International Association of Official Analytical Chemist (AOAC, 1999) and the pH of the products were determined (using the pH meter). All reagents were of analytical grade.

Statistical analysis

Data obtained was analyzed using Analysis of Variance (ANOVA) of the Minitab Statistical Package, Version 15.

RESULTS AND DISCUSSION

Sensory characteristics of the smoked pork sausages

The results obtained from the sensory evaluation of the pork sausages are presented in Table 1. There were no significant differences in colour, aroma, 'prekese' flavour, flavouring liking and acceptability of the products.

Table 1- Sensory characteristics of the smoked pork sausages

Parameters	T1	T2	T3	T4	Sed	Sig
Colour	2.60	2.60	2.50	2.40	0.4577	N.S
Aroma	3.60	3.60	3.60	3.40	0.5142	N.S
'Prekese' Flavour	2.50	2.90	3.40	3.00	0.4702	N.S
Flavour liking	1.90	2.00	2.10	1.60	0.3815	N.S
Acceptability	2.00	1.90	1.80	1.70	0.3543	N.S

N.S = not Significant, Sed = Standard error of difference, Sig = Significance.

The colour of meat and meat products is an important quality attribute that influences consumers' acceptance of the product and usually consumers like bright-red raw meats, brown-gray cooked meats and pink cured meats (Cornforth, 1994). It was expected that the dark brown colour of the pod extract would be imparted to the product, but that was not the case when the pod extract was used in this study. The results therefore differed from that of Lartey (2012), who indicated that there were significant differences between the colours of products prepared with pod powder (PPP). This is an indication that it will be better to use the PPE instead of PPP. The insignificant differences ($P > 0.05$) suggest that sausages prepared with 'prekese' pod extract at an inclusion level of 10ml per kilogram meat have similar colour as the control product and could be patronized equally by consumers.

There was no significant difference ($P > 0.05$) in the aroma of the sausages. Aroma gives an indication of the degree of attraction or repulsion of consumers to food substance. The panelists described the aroma as pleasant (Table 1) and this indicates that 'prekese' pod extract could be a good spice at an inclusion level of 10 ml per kilogram meat in meat products. The 'prekese' flavour of the sausages was not significantly affected. This result therefore indicates that 'prekese' flavour obtained from using the pod extract is not as stronger as the powder. Lartey (2012) reported significant differences between products prepared with PPP in terms of 'prekese' flavour. This could be possibly due to the fact that, soaking the pod for 24 hours may not be enough to release the pungent aroma of 'prekese' fully into the solution as described by Aladesanmi (2007). The insignificant difference in flavour liking and acceptability of the products indicate that sausages prepared with PPE at 10 ml per kilogram meat would be equally accepted just as the standard meat products on the market.

The results of the analysis of the pork sausages to determine the crude protein, crude fat, moisture content and pH level is shown in Table 2. There were significant differences in the crude protein content of the products (Table 2). T2 was significantly higher, than T3, T4 and T1. T3 and T4 protein content were similar. Okwu (2003) reported that, the composition of protein in *Tetrapleura tetraptera* ranges from 7.44-17.50%. This might have contributed to the significant

and marginal increases in the protein contents of the test products. This indicates that the inclusion level of 'prekese' pod extract in pork products may improve the nutritional qualities of the products.

Table 2- Proximate composition of smoked pork sausages

Parameters	T1	T2	T3	T4	Sed	Significance
Protein	12.02^{b}	16.08^{a}	13.58^{ab}	13.45^{ab}	0.030	***
Moisture	45.30^{b}	44.85^{b}	46.10^{b}	50.12^{a}	0.934	*
Fat	53.84^{b}	51.00^{c}	62.24^{a}	47.27^{d}	0.724	**
pH	6.15^{c}	6.11^{d}	6.24^{b}	6.29^{a}	0.008	***

Sed= Standard error of differences. Means on the same row with different superscripts are significant
*= P<0.05, **= P<0.01 and ***=P<0.001

The moisture content of the products increased significantly in T4 ($P < 0.05$). However the moisture content of T3 and T2 were similar to the control product. The moisture content in meat is a good indicator of its relative components of protein and lipids (Aberoumad and Pourshafi, 2010). It can be observed from table 2 that as T4 has the highest moisture content, the level of crude protein and fat in it were lower when compared to T3 and T2, respectively. There were significant differences in the fat content of the products, although there was no trend, with T3 having the highest fat content followed by T1, T2 and T4. The reasons for the differences in fat content are unknown but may be due to the fat content of the type of meat used. There were significant differences in the pH of the products (Table 2). There was no clear trend that PPE contributed to the increase in pH although T3 and T4 suggest a possibility. According to FAO (2007) a typical pH value for pork and its product ranges from 5.50 to 6.20. The importance of measuring the pH of meat and meats product is to evaluate the keeping quality (FAO, 2007). It can be suggested from the result that T3 and T4 may deteriorate faster than T1 and T2 due to their pH level creating a favourable environment for bacterial growth.

CONCLUSION

This study reveals that, the use of 'prekese' pod extract had no adverse effect on the sensory characteristics of the smoked pork sausages at an inclusion level of 10 ml/1kg of meat. However, there were effects on the moisture, fat and protein contents, and the pH of the products. There was also an improvement in the nutritional quality of the test products in terms of protein content. It is recommended that different extraction methods and higher inclusion levels of the 'prekese' pod extract be evaluated.

REFERENCE

Aberoumad, A., and K. Pourshafi. 2010. Chemical and Proximate composition properties of different fish species obtained from Iran. *World Journal of Fish and Marine Sciences.* 2:237-239.

Aladesanmi, J.A. 2007. *Tetrapleura Tetraptera:* Molluscicidal activity and chemical constituents. *African Journal of Traditional, Complementary and Alternative Medicines.* 4: 23-36.

AOAC. 1999. Official method of analysis. 17th edition, Association of Analytical Chemists. Washington DC. USA. pp: 56-132.

British Standard Institution. 1993. Assessors for Sensory Analysis. Guide to Selection. Training and Monitoring of Selected Assessors. BS. 17667. British Standard Institution London. United Kingdom.

Cornforth, D. 1994. Colour- its basis and importance: Quality attributes and their measurement in

meat, poultry and fish products. *Advance in Meat Research Series*. 9:34-78.

FAO. 2007. Meat processing technology for small to medium scale producers. pp: 9-149.

Jiminez-Colmenero, F., J. Carballo, and S. Cofrades. 2001. Healthier meat and meat products: their role as functional foods. *Meat Science*. 59:5-13.

Kumar, R. 2007. History of sausage. Available at: http://www.ifood.tv/blog/sausages. Accessed on 18/02/2013.

Lartey. 2012. The effect of prekese pod powder on the sensory characteristics and nutritional qualities of pork products. BSc. Dissertation, University for Development Studies. pp: 1-29.

Lawrie, R.A., and D.A. Ledward. 2006. Lawrie Meat Science, 7th Edition. Wood head Publication Press and CRC Press. UK.

Nwawu, J.I., and P.A. Akah. 1986. Anti-convulsant activity of the volatile oil from the fruit of *Tetrapleura tetraptera*. *Journal of Ethnopharmacology*. 18:103-107.

Okwu, D.E. 2003. The potentials of *Ocimum gratissimum, Penrgularia extensa* and *Tetrapleura tetraptera* as spice and flavouring agent. *Nigeria Agriculture Journal*. 35:143-148.

Platel, K., and K. Srinivisan, 2000. Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. *Nahrung*. 44:42- 46.

Platel, K., and K. Srinivisan, 2001. A study of the digestive stimulant action of selected spices in experimental rats. *Journal of Food Science Technology*. 38:358-361.

Steentoft, M. 1988. Flowering plants in West Africa. Cambridge University Press. ISBN 0-521-26192-9. pp: 1-364.

Teye, G.A. 2007. Manaul on small scale pork processing. Faculty of Agriculture. University for Development Studies, Tamale, Ghana. pp: 1-4.

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Dietary Supplementation of *Silybum marianum* or *Curcuma spp* on Health Characteristics and Broiler Chicken Performance

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ABSTRACT

This study was carried out to investigate the effect of *silybum marianum* (SM), *Curcuma spp* (CP), or their mixture (PM) on intestinal microflora and broiler chicken performance. A total of 180 unsexed broiler chicken (Ross-308) were randomly assigned to 4 diets with 3 replications of set 15 chickens in each. Diets were included control or the inclusion of SM, CP, or PM (equal amount) at level of 0.5% in diets. Feed intake, body weight gain, and feed conversion ratio were significantly increased by diets contained CP and PM rather other diets at 42 days of age ($P<0.05$). In contrast, the inclusion of SM and PM in diets resulted in significantly decreases in total number of bacteria, gram-negative bacteria, and coliforms bacteria in ileum rather other diets at 42 days of age ($P<0.05$). The inclusion of tested medicinal plants in diets led to significantly decreases in pH value in ileum and significantly increases in intestinal weight and length rather control ($P<0.05$). The results of present study have shown that the inclusion of 0.5% *Curcuma spp* or *Silybum marianum* in diets boost up broiler chicken performance and reduced ileum pathogenic bacteria. The equal mixture of tested medicinal plants showed mutual effects which could help to improve intestinal health and well being of poultry.

Key words: broiler chicken, intestinal microflora, medicinal plant

INTRODUCTION

Antibiotic resistance and unreliable antibiotic therapy in poultry (Joerger, 2002) have lead to ban the use of antibiotic in many countries (Patterson and Burkholder, 2003). Increasing investigations regarding alternatives to antibiotics were widely carried out to achieve gut health and best growth performance. It is well documented that effect of antibiotics alternatives are mediated by intestinal microflora (Joerger, 2002). Today, there is accelerating trend toward the

development of using alternative ingredients particularly those from plants which are perceived as natural and safe ingredients. Medicinal plants are being used as feed additive to improve growth performance, to manipulate gut functions and microbial habitat of domestic animals (Panda *et al*., 2000). The positive effects of medicinal plants were shown on health condition and production performance (Yakhkeshi *et al*., 2012; Elmakki *et al*., 2013). A variety of medicinal plants have been widely used to maintain and improve health of birds (Yakhkeshi *et al*., 2012; Jadalla *et al*., 2014) as prohibitory the growth of inward baneful bacteria through the digestion system (Hernandez *et al*., 2004). They can modulate microflora population and improve growth performance (Chen *et al*., 2003; Garca *et al*., 2007; Yakhkeshi *et al*., 2012).

Curcuma longa is a perennial herb, and a member of *Zingiberacae* family. *Curcuma longa* has been reported to have antimicrobial, anti-inflammatory, anti-viral, antioxidant, and anti-cancer effect (Gandhi *et al*., 2011). The main active component of *Curcuma longa* is curcumin (diferuloylmethane) which commonly used in Iran and Indian cuisine as a spice and food-coloring.

Silybum marianum is a member of *Asteraceae* family. *Silybum marianum* have been used as a natural medication for the liver and biliary duct since ancient times. Pharmacologically effective substance of silymarin includes four main ingredients: silybin, silychristin, silydianin, and isosilybin (Ding *et al*., 2001) which have hepatoprotective, anti-inflammatory, cytoprotective and anti-carcinogenic effects (Manna *et al*., 1999).

Although, there are many inconsistent results regarding substitution of medicinal plants for antibiotic and clarifying roles of these additives in poultry production, but some of these additives have been reported to have a great potential to replacement of antibiotics. Therefore, the present study was carried out to determine whether *Silybum marianum* and *Curcuma spp* or their mixture would influence the growth performance and intestinal microflora of broiler chicken to get health condition.

MATERIAL AND METHODS

Experimental Design and Birds

A total of 180 unsexed 1-day-old broiler chickens (Ross 308) were randomly divided to 4 treatments with 3 replications of 15 birds in each. Treatments were included of control (without medicinal plant) and the inclusion of *Silybum marianum* (SM), *Curcuma spp* (CP), or equal amount of plant mixture (PM) at level of 0.5% in diets. Diets were designed as starter (1 to 21 days of age) and grower (22 to 42 days of age) based on NRC (1994) recommendations to meet their nutrient requirements (Table 1). Feed and water were offered *ad libitum* in all period of experiment. Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality were measured. The lighting schedule was 23 h light / 1 h darkness at 32°C the first day. This was subsequently reduced 3°C each week until third week. Thereafter it was constant.

Intestinal pH and Characteristics

Two birds from each replicate were randomly selected and sacrificed by cervical dislocation at 42 days of age. Weights and length of small intestinal as well as pH value of intestinal were measured. The digesta diluted nine-fold (w/v) with distilled water were stirred for 5 min and the pH of the suspensions were measured using a calibrated pH meter.

Microbial Sampling and Incubation

On day 42 of the experiment, 2 birds from each replicate were slaughtered by cervical dislocation and ileum contents were collected. Contents were gently removed into sterile sampling tubes and immediately transferred on ice to the laboratory. Serial dilutions of 1 g sample (10^{-4} to 10^{-7}) were made. Selective media of Nutrient Agar, MacConkey Agar, Eosin-methylene Blue Agar, and Xylose Lysine Deoxycholate Agar were inoculated to detect the total number of bacteria, coliforms, gram-negative bacteria, and Salmonella, respectively. Total number of bacteria and coliforms were counted after aerobic incubation for 24 h at 37°C. Gram-negative bacteria were counted after incubation for 48 h at 37°C and Salmonella were counted after incubation for 24 h at 37°C.

Table 1. Diet composition at different periods of the experiment[1]

Ingredient (% or as stated)	Starter (1-21 d)	Grower (22-42 d)
Corn grain	53.80	60.74
Soybean meal (45% CP/kg)	38.70	32.22
Soybean oil	3.0	3.0
Calcium carbonates	1.63	2.05
Dicalcium phosphate	1.72	1.15
Premix[2]	0.50	0.50
Common salt	0.44	0.23
DL-Met	0.14	0.06
L-Lys	0.07	0.05
Calculated Analysis		
Metabolizable energy (kcal/kg)	3,000	3,055
Crude protein	21.54	19.09
Calcium	0.93	0.85
Available phosphorus	0.45	0.33
Calcium: Phosphorus	2.07	2.57
Energy: Protein	139.27	160.03

[1]*Silybum marianum*, *Curcuma spp*, or plants mixtures (equal amount) were added to the basal diet at 0.5% to make the respective diets for each experiment, respectively. [2]Supplied the following per kilogram of diet: vitamin A (retinyl acetate), 8,000 IU; vitamin D_3 (cholecalciferol), 3,000 IU; vitamin E ($_{DL}$-alpha-tocopheryl acetate), 25 IU; menadione , 1.5 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.1 mg; folacin (folic acid), 1 mg; niacin (nicotinic acid), 50 mg; pantothenic acid, 15 mg; pyridoxine (pyridoxine_HCl), 4 mg; riboflavin, 10 mg; and thiamin, 3 mg (thiamin mononitrate); 10 mg of copper ($CuSO_4$); 1.0 mg of iodine Ca (IO3) 2; 80 mg of iron ($FeSO_4\_H_2O$); 100 mg of manganese ($MnSO_4\_H_2O$); 0.15 mg of selenium ($NaSeO_3$); 80 mg of zinc ($ZnSO_4\_H_2O$); and 0.5 mg of cobalt ($CoSO_4$).

Statistical Analyses

All data were analyzed for normal distribution using the NORMAL option of the UNIVARIATE procedure of GLM procedure of SAS software (SAS Inst. Inc., Cary, NC). A completely randomized design was employed. Pen was used as the experimental unit and data were analyzed by GLM procedure. Logarithmic (log10) transformation was applied for microbial colony forming unit (CFU). Duncan's multiple range test were used for comparison of means ($P<0.05$).

RESULTS

The effect of dietary treatments on broiler chicken performance is shown at Table 2. The results indicated that the diets contained CP or PM led to significantly increases in FI and BWG as well as significantly decreases in FCR rather other diets ($P<0.05$). Diets contained medicinal plants resulted in significantly decreases in mortality rather control ($P<0.05$). Table 3 showed the effect of diets on ileum microflora population at 42 days of age. Diet contained SM or PM caused

to significantly decreases in total number of bacteria, gram-negative bacteria, and coliforms bacteria rather other diets (P<0.05). Moreover, none of samples had shown Salmonella. The effect of diets on the intestinal characteristics, pH, intestinal weight, and length, are presented at Table 4. The inclusion of tested medicinal plants in diets led to significantly decreases in pH value in ileum rather control (P<0.05). Same diets induced significantly increases in intestinal weight and length rather control (P<0.05).

Table 2. Effect of diets on broiler chicken performance at 42 days of age

Treatment	FI[1] (g/d per bird)	BWG[2] (g/d per bird)	FCR[3]	Mortality (%)
Control	112.9[b]	50.4[b]	2.2[b]	3.3[a]
SM[4]	103.2[c]	45.3[c]	2.3[c]	2.0[b]
CP[5]	116.2[a]	53.3[a]	2.2[a]	1.7[b]
PM[6]	116.0[a]	53[a]	2.2[a]	1.7[b]
SEM	2.82	1.28	0.06	0.35
P value	0.023	0.051	0.081	0.011

Means with common letters in the same column are not significantly different (P<0.05). SEM: Standard error of the means. [1]Feed intake, [2]Body weight gain, [3]Feed conversion ratio. [4]*Silybum marianum*, [5]*Curcuma spp*, and [6]Plants mixture.

Table 3. Ileum microflora in response to diets at 42 days of age (Log10 cfu/g of digesta)

Treatment	Total number of bacteria	Gram-negative	Coliforms	Salmonella
Control	10.00[a]	9.33[a]	9.00[a]	Negative
SM[1]	8.33[b]	7.33[b]	6.00[c]	Negative
CP[2]	9.50[a]	9.33[a]	7.17[b]	Negative
PM[3]	8.83[b]	7.83[b]	6.50[c]	Negative
SEM	0.23	0.21	0.18	NA[4]
P value	0.0001	0.0001	0.0001	NA

Means with common letters in the same column are not significantly different (P<0.05). SEM: Standard error of the means. [1]*Silybum marianum*, [2]*Curcuma spp*, [3]Plants mixture, and [4]Not applicable.

Table 4. Effect of diets on the intestinal characteristics of birds at 42 days of age

Treatment	pH	Weight (g)	Length (cm)
Control	6.69[a]	71.70[b]	198.50[b]
SM[1]	5.58[b]	82.80[a]	225.60[a]
CP[2]	5.87[b]	80.50[a]	219.60[a]
PM[3]	5.82[b]	85.30[a]	232.70[a]
SEM	0.15	2.19	5.41
P value	0.004	0.003	0.004

Means with common letters in the same column are not significantly different (P<0.05). SEM: Standard error of the means. [1]*Silybum marianum*, [2]*Curcuma spp*, and [3]Plants mixture.

DISCUSSION

Various parameters such as plant parts, physical properties, genetic variation, age, used dosage, extraction method, harvest time, and compatibility with the other ingredients can influence performance of broiler chicken fed with medicinal plant (Yang *et al*., 2009). Based on obtained results, it posses that SM and CP had different effect on measured traits. The inclusion of CP in diets (as single or mixed with SM) could promote performance traits (FI, BWG, and FCR), while the inclusion of SM in diets (as single or mixed with CP) could decrease detrimental bacteria colonization in ileum. Dietary supplementation of CP exhibited a significantly positive

effect on FI, BWG, and FCR which is in accordance with several reports (Durrani *et al*., 2006; Kumari *et al*., 2007; Rajput *et al*., 2013), who validated positive effect of *curcuma longa* / curcumin on broiler performance. These positive effects might be due to the well reported antiinflammatory, antioxidant, antibacterial activities (Chattopadhyay *et al*., 2004), prebiotic like effects (Niamsa and Sittiwet, 2009), enhanced secretions of amylase, trypsin, chymotrypsin, and lipase enzymes by curcumin (Platel and Srinivasan, 2000). Moreover, Suchy *et al*. (2008) reported that the *Silybum marianum* treated groups of broiler showed significantly better performance as compared to the untreated group which is in agreement with current study. On the other hand, it is reported that mixture of medicinal plants resulted in better performance in broiler chicken (Mushtaq *et al*., 2013). It is appear that inclusion of PM in diets had an intermediate effect as midway of tested medicinal plants.

Many medicinal plant oil and extracts have been reported to have antimicrobial properties (Lawless, 1995). It is proposed that plant antibacterial properties are related to their lipophilic characters (Farag *et al*., 1989). The major mechanism of medicinal plants is adhesion and thrust of bacterial membrane which inhibits bacterial enzymes activation (Shapiro and Guggenheim, 1995; Stiles *et al*., 1995). These reactions can reduce pathogenic populations in the intestine which was also seen in the present study by reducing gram-negative bacteria, coliforms, and total number of bacteria in ileum by the inclusion of SM or PM in diets. Results are in agreement with other studies (Guo *et al*., 2004; Sarica *et al*., 2005). One another possible mechanism of antimicrobial effect of medicinal plants is reducing of intestinal pH which confirm by obtained results of present study. Any factor that increases the activity of an organ above threshold levels can lead to increases in organs weight and length by hypertrophy and hyperplasia of the related organs (Yakhkeshi *et al*., 2012). It seems that tested medicinal plants induce intestinal activation and increases intestinal weight and length.

CONCLUSION

The results of present study have shown that the inclusion of 0.5% *Curcuma spp* in diets improve broiler chicken performance while the inclusion of 0.5% *Silybum marianum* in diets reduce ileum pathogenic bacteria. The equal mixture of tested medicinal plants showed mutual effect.

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REFERENCE

Chattopadhyay, I., K. Biswas, U. Bandyopadhyay, and R.K. Banerjee. 2004. Turmeric and curcumin: Biological actions and medicinal applications. *Curr. Sci*. 87:44-53.

Chen, H. L., D.F. Li, B.Y. Chang, L.M. Gong, J.G. Dai, and G.F. Yi. 2003. Effects of Chinese herbal polysaccharides on the immunity and growth performance of young broilers. *Poult. Sci*. 82:364-370.

Ding, T.M., S.J. Tian, Z.X. Zhang, D.Z. Gu, Y.F. Chen, Y.H. Shi, and Z.P. Sun. 2001. Determination of active component in silymarin by RP-LC and LC/MS. *J. Pharm. Biomed. Anal*. 26:155-161.

Durrani, F.R., M. Ismial, A. Sultan, S.M. Suhail, N. Chand, and Z. Durrani. 2006. Effect of different levels of feed added turmeric (*Curcuma longa*) on the performance of broiler chicks. *J Agric. Biol. Sci*. 1:9-11.

Elmakki, A.M., A.K. AbdelAtti, M.B. Dousa, A.A.H. Elagib, E.E.H. Malik, and M. K. Elamin. 2013. Effect of treated cowpea seeds on broiler chicken. *Global Journal of Animal Scientific Research*. 1(1):61-68.

Farag, R.S., Z.Y. Daw, F.M. Hewed, and G.S.A. El-Barory. 1989. Antimicrobial activity of some egyptian spice essential oils. *J. Food Protec*. 52:665-667.

Gandhi, P., K. Khan, and N. Chakraverty. 2011. Soluble curcumin: a promising oral supplement for health management. *J. Appl. Pharm*. Sci. 1:1-7.

Garca, V., P. Catala-Gregori, F. Hernandez, M.D. Megıas, and J. Madrid. 2007. Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. *J. Appl. Poult. Res*. 16:555-562.

Guo, F.C., R.P. Kwakkel, J. Soede, B.A. Williams, and M.W. Verstegen. 2004. Effect of a chinese herb medicine formulation, as an alternative for antibiotics, on performance of broilers. *Br. Poult. Sci*. 45(6):793-797.

Hernandez, F., J. Madrid, V. Garcia, J. Orengo, and M.D. Megias. 2004. Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. *Poult. Sci*. 83:169-174.

Jadalla, J.B., D. M. Mekk, I. Bushara, and A.M.H. Habbani. 2014. Effects of inclusion of different levels of watermelon bug meal in broiler diets on feed intake, body weight changes and feed conversion ratio. *Global Journal of Animal Scientific Research*. 2(1):18-25.

Joerger, R.D. 2002. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. *Poult. Sci*. 82:640-647.

Kumari, P., M.K. Gupta, R. Ranjan, K.K. Singh, and R. Yadav. 2007. *Curcuma longa* as feed additive in broiler birds and its patho-physiology effect. *Indian J Exp. Bio*. 45:272-277.

Lawless, J. 1995. The illustrated encyclopedia of essential oils. Element Books Ltd, Shaftesbury, UK.

Manna, S.K., A. mukhopadhyay, N.T. Van, and B.B. Aggarwal. 1999. Silymarin suppresses TNF-induced activation of NF-kappa B, c-Jun N-terminala kinase, and apoptosis. *J. Immunol*. 163(12):6800-6809.

Mushtaq, M, N. Sh, S. Khan, S. Ur-Rehman, and R. Ullah Khan. 2013. *In vivo* effect of *Berberis lyceum* and *Silybum marianum* on production performance and immune status in broiler chicks. *Archiv. Tierzucht*. 56:91.

Niamsa, N., and C. Sittiwet. 2009. Antimicrobial activity of curcuma longa aqueous extract. *J. Pharmacol. Toxicol*. 4:173-177.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington. DC.

Panda, A.K., M.R. Reddy, S.V. Rama Rao, M.V.L. Raju, and N.K. Praharaj. 2000. Growth, carcass characteristics, immunocompetence and response to escherichia coli of broilers fed diets with various levels of probiotic. *Eur. Poult. Sci*. 64:152-156.

Patterson, J.A., and K.M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. *Poult. Sci*. 82:627–631.

Platel, K., and K. Srinivasan. 2000. Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. *Nahrung*. 44:42-46.

Rajput, N., N. Muhammad., R. Yan, X. Zhong, and T. Wang. 2013. Effect of dietary supplementation of curcumin on growth performance, intestinal morphology and nutrients utilization of broiler chicks. *J. Poult. Sci*. 50: 44-52.

Sarica, S., A. Ciftci, E. Demir, K. Kilinic, and Y. Yildirim. 2005. Use of an antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broilet diets. *S. Afri. J. Anim*. Sci. 35: 61-72.

Shapiro, S., and B. Guggenheim. 1995. The action of thymol on oral bacteria. Oral Microbiol. Immunol. 10:241-246.

Stiles, J. C., W. Sparks, and R. A. Ronzio. 1995. The inhibition of candida albicans by oregano. *J. Appl. Nutr*. 47:96-102.

Suchy, Jr. P., Straková, E, V. Kummer, I. Herzig, V. Písaříková, R. Blechová, J. Mašková 2008. Hepatoprotective effects of milk thistle (*Silybum marianum*) seed cakes during the chicken broiler fattening. *Acta. Vet. Brno*. 77:31-38.

Yakhkeshi, S., Rahimi, S., Hemati Matin, H.R., 2012. Effects of yarrow (*Achillea millefolium* L.), antibiotic and probiotic on performance, immune response, serum lipids and microbial population of broilers. *J. Agr. Sci. Tech.*, 14, 799-810.

Yang, Y., P. A. Iji, M. Choct. 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World. Poult. Sci. J*. 65:97-114.

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Pathophysiology of Cerebral Ischemia

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ABSTRACT

Cerebrovascular accident (CVA) is the sudden interruption or decrease of blood supply (oxygen and glucose) to the brain resulting in cerebral infarction, permanent neurological damage, severe functional limitations and death. Stroke is the second most common cause of death worldwide and the leading cause in Brazil. The risk factors for CVA include systemic arterial hypertension and other vascular diseases, diabetes mellitus, sedentarism, dyslipidemia, and smoking. These risk factors are at high prevalence, globaly, increasing the prospects for new incidents of the disease. Currently, the treatment options for CVA are limited, partially because many promising medicines presented intolerable side effects or limited therapeutic effects in the clinical trials. In the acute and subacute phases of the CVA the therapeutic goals are to protect the neurons at risk, increase the endogenous capacity of the central nervous system (CNS) to regenerate itself, and diminish functional sequelae. The knowledge regarding the role of the molecular mechanisms underlying CVA is the key for new therapeutic discoveries aiming at neuroprotection, neuroregeneration and neurogenesis.

Key words: Cerebral Ischemia, Primary Brain Lesion, Secondary Brain Lesion.

INTRODUCTION

Currently, the ischemic cerebrovascular accident (ICVA) is the leading cause of death in Brazil (Camargo *et al*., 2005; Minelli *et al.*, 2007), the second most common cause of death globally, and the major cause of adult disability in the United States (Feigin, 2005). Among the risk factors are systemic arterial hypertension and other cardiovascular diseases, diabetes mellitus, sedentarism, dyslipidemia, and smoking, increasing the prospects for new incidents of the disease (Campos-Souza *et al*., 2007). The encephalic lesion extends to adjacent areas where the reperfusion mechanisms are trying to repair the damage, hence diminishing subsequent sequelae (Dinargl *et al*., 1999).

Recently, many treatment strategies are being proposed, and despite the improvement of patients suffering from acute neurological damage, there are no specific treatments for CVA capable of preventing the progress of the cerebral disorder, ultimately resulting in permanent neurological damage, severe functional limitations, coma and death (Hankey, 1999; Muntner *et al.*, 2002). Therefore, the knowledge and understanding of the physiopathological mechanisms in cerebral ischemia are essential to create new strategies for tissue repair and neuroprotection. The goal of this review is to present the cellular events triggered in the CNS after cerebral ischemia.

Pathogenesis of cerebral ischemia

OICVA is caused by the interruption of the blood supply, in a particular arterial branch, by thromboembolic or hemodynamics mechanisms, causing metabolic imbalance (high demand versus low supply of oxygen and glucose) in the brain ultimately determining cell death (Feigin, 2005).

At the cellular level, the reduction of cerebral blood flow (CBF) and subsequent oxygen depletion trigger biochemical events resulting in phosphorylation and anaerobic metabolism. The anaerobic glycolysis is insufficient and determines the depletion of phosphate reservoir, including ATP accumulating lactic acid (Wyatt *et. al*., 1989), calcium (Ca+), and water. Further, the cell membrane depolarize and excitatory neurotransmitters are released, particularly glutamate in the axonal endings. Meanwhile, in the cytoplasm, will occur accumulation of free fatty acids, arising from the disintegration of the phospholipidic membrane undergone oxygen peroxidation, free radicals which in turn promotes the formation of more free radicals inside the mitochondria, with the aid of prostaglandins, xanthine and uric acid, and finally in some neurons Ca+ will induce nitric oxide (NO) production (Shalak and Perlman, 2004).

The effects from disruption of cellular energy balance, Ca+ accumulation, lipid peroxidation, acidosis, glutamate release, intense production of free radicals, and NO neurotoxicity culminate in cell death (Figure 1).

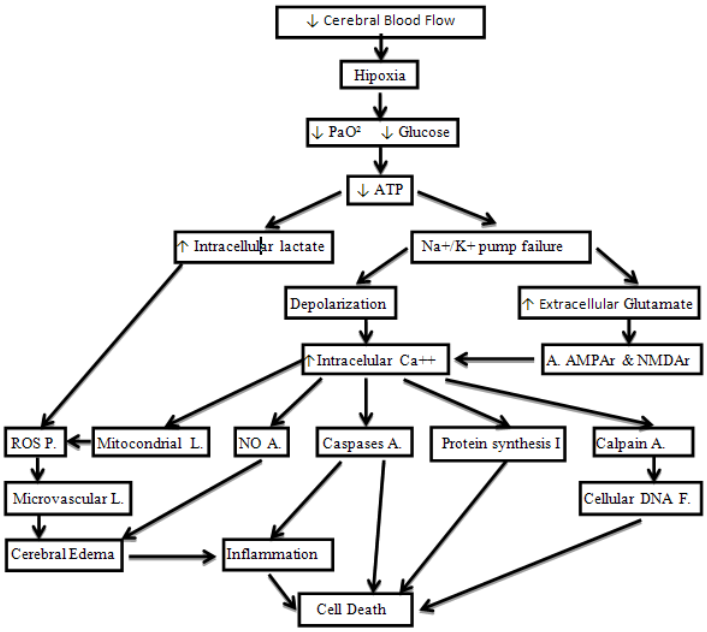

Figura 1. Physiopathological evolution of cerebral ischemia. (P) Production. (L) Lesion. (A) Activation. (I) Interruption. (F) Fragmentation. (ROS) Reactive oxygen species. (NO) Nitric oxide. (DNA) Deoxyribonucleic acid (Modified from Shalak and Perlman, 2004).

Secondary cerebral lesion

Neuropathological analysis reveals that brain ischemia leads to two distinct areas of ischemia: the core zone which is an area of severe ischemia, and the penumbra zone, the term used to describe ischemic, but still viable cerebral tissue. In the central zone, severe reductions of blood supply to the brain causes metabolic collapse, reduction of cell energy and ionic homeostasis, subsequent loss of cellular integrity resulting in cell death in a few minutes. Meanwhile, in the penumbra zone there will be hypoperfusion, i.e., low blood flow compensated by collateral arteries anastomosing with branches of the occluded vascular tree resulting in neurophysiological functional losses, but the cellular metabolism and structure will remain preserved (Sharp *et al*., 2000 and Hossmann, 2009). An ischemic event is dynamic (Figure 2).

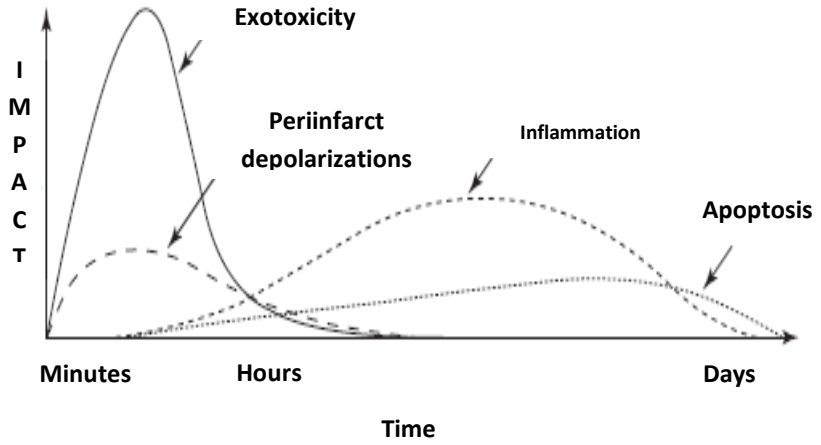

Figure 2. Cascade of harmful events in focal cerebral ischemia. Minutes after deficits in focal perfusion, exotoxic mechanisms lead to lethal damage to neurons and glial cells. In addition, exotoxicity triggers a series of events that contribute to enhancing tissue injury. Such events include periinfarct depolarization and mechanisms that promote inflammation and apoptosis. The x-axis reflects the evolution of the cascade over time, while the Y axis shows the impact of each event in the cascade of neuronal death.

While blood supply is low, but sufficient to attend the demands of the ionic channels electrical activity the cerebral tissue remains alive. Paradoxally, reperfusion initiates an inflammatory cascade with free radicals, released from dead cells; worsening the ischemic injury.

It is estimated that initially, the area of penumbra corresponds to 50% of the tissue that later will progress into infarct (Dinargl *et al*., 1999). The understanding of these mechanisms in the penumbra zone is essential to preserve function and promote the survival of nervous cells after reperfusion, and could potentially be an area of new discoveries of more effective treatments.

Glutamate release

Astrocytes regulate neuronal excitability and synaptic activity by releasing gliotransmitters such as glutamate, which is the most important excitatory neurotransmitter of the CNS. The neurons are only exposed to small amounts of these neurotransmitters because of efficient mechanisms of removal and absorption responsible to free the neurons from its toxic effects (Kostandy, 2012). During cerebral ischemia, blood flow is severely diminished along with adenosine triphosphate (ATP) resulting in energy failure and collapse of the ATP-dependent

cell metabolism, such as ionic channels (Kimelberg and Mongin, 1998). Depolarization of the cell membrane, stimulated by Ca^{++} entry, release the glutamate retained in the intracellular vesicles, which is then eliminated by exocytose. The extracellular glutaminase released by the injured neurons increases the hydrolysis of glutamine producing extracellular glutamate (Mena *et al*., 2000).

The disruption of the electrochemical gradient of the astrocytes causes the glutamate transporters to operate in the reverse direction leading to excess extracellular glutamate. The accumulation of extracellular glutamate stimulates the neuronal receptors *N*-Methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAr) and kainite. These receptors are linked to ionic channels in the membrane and determine the influx of Na^{+} and Ca^{++} together with water resulting in cytotoxic edema (Furukawa, 1997).

Activation of the NMDAr is sufficient to destroy the majority of the neurons, being preponderant in the mediation of at least some of the glutamate neurotoxicity, because allows Ca^{++} influx and its entry determines mitochondrial dysfunction, caspase-3 activation through the action of calpain, and production of NO and ROS leading to neuronal death (Dobrek and Thor, 2011).

Intracellular Calcium accumulation

The intracelular Ca^{++}, in low concentrations, participate in many cytoplasmic reactions. During an ischemic injury Ca^{++} will influx the cells of nervous tissues through NMDA receptors stimuli, a Ca^{++} agonist regulated by glutamate, and the Ca^{++} efflux is paralyzed. Calcium is also released by the mitochondria and sarcoplasmic reticulum. These alterations, resulting in the increase of intracellular Ca^{++} interfere with many enzymatic reactions including the activation of lipases, proteases, endonucleases and phospholipases, and in the formation of ROS derived from xanthine and prostaglandins synthesis. The accumulation of cytoplasmic Ca^{++}, after ischemia, determines irreversible cerebral injury (Grow and Barks, 2002).

Increase of free radicals

The nervous tissue is too vulnerable to the action of free radicals because holds some peculiarities such as being rich in lipids and unsaturated fatty acids which may react with ROS to form peroxyl radicals that determine the lipid oxidation of the membrane of neurons (Porter, 1984). Additionally, the brain has low to moderate catalase, and glutathione activity which eliminate the hydrogen peroxidase (H^2O^2) reducing oxidation (Cooper and Kristal, 1997), and has elevated metabolic activity using up to 20% of the consumed O_2 by the body, although the brain constitutes only 2% of the total body mass. The combination of these factors makes the CNS totally vulnerable to oxidative damage (Dringen, 2000).

Reactive Oxygen Species

Reactive oxygen species (ROS) are molecules of short life span originated as part of the normal cell metabolism and defense system. At low levels they play a role in the regulation of cell growth, differentiation, proliferation and apoptosis through actions in different receptors, genes, ionic channels, enzymes, proteins and nuclear transcription factors (Poli *et al*., 2004; Liu *et al*., 2005). In cerebral ischemia the production of ROS is increased, but in reperfusion its production is accelerated because of cytotoxic events stemming from lipid peroxidation, protein oxidation and fragmentation of deoxyribonucleic acid (DNA) (Crack and Taylor, 2005). The ROS are produced through the reduction of molecular oxygen (O_2) to form

superoxide (O_2^-) mediated by NADPH oxidase, xanthine oxidase or mitochondrial electron transport chain (Droge, 2002). There is a balance between the production of ROS and antioxidant defense system which is essential for normal metabolism. In this balance, the cells produce superoxide dismutases (SODs) that convert the superperoxide to hydrogen peroxide (H_2O_2) and oxygen, and the catalase and glutathione peroxidase convert H_2O_2 to water. In the presence of transition metals, may produce the radical hydroxyl (OH^-). The accumulation of ROS, due to excessive production or depletion of antioxidant enzymes, generates a synergic cascade activating signals that could lead to cell injury. This damage results from DNA alteration, lipid peroxidation, protein oxidation and rupture of structural functions and cellular integrity, such as ability of molecular transportation, energy production, and ionic balance (Olmez and Ozyurt, 2012).

Nitric Oxide

The free radical, nitric oxide, is synthesised from l-arginine by the enzyme NO synthase (NOS) to l-citrulline. The NOS is heavily activated during ischemia, as well as during reperfusion, and its production lasts long periods of time in the neurons, glial and endothelial cells. The nitric oxide has neuroprotective and neurotoxic properties. The activation of NOS during the ischemia of neurons causes the death of neurons by combining with superoxide yielding peroxynitrite, a potent radical that activates lipid peroxidation and increases glutamate release. The activation of NOS in the endothelial cells is neuroprotective because NO production acts relaxing the adjacent smooth muscle cells leading to vasodilation and increase of blood flow in the affected cerebral region. Conversely, the NO production, in the astrocytes, occurs during ischemia and has uncertain effects, but it is believed that in excess can be both neurotoxic and neuroprotective (Bolaños and Almeida, 1999).

During reperfusion, the O_2 concentration may exceed the mitochondrial ability to reduce O_2 to H_2O, and therefore the production of superoxide anion (O_2^-) can be increased. The superoxide reacts with the ON and form peroxynitrite ($ONOO^-$), which is an irreversible inhibitor of mitochondrial function in addition to being a pro-oxidant that damages lipids, proteins and DNA, determining neuronal cell death (Radi *et al*., 1991).

Elevation of free iron

Iron (Fe) is essential to most living things because of its unique property of reverse cyclic oxidation and reduction. Easily donate and receive electrons, alternating between Fe_3^+ and Fe_2^+ ions and therefore is considered to be a source of free radicals, particularly the hydroxyl radical (OH^-). Iron is also an enzymatic co-factor and as such is a key participant in the mediated oxygen toxicity, being involved in the generation O_2 e H_2O_2, through the acceleration of the non-enzymatic oxidation of many molecules, including adrenaline and glutathione (Gutteridge and Halliwell, 2000).

The majority of the iron present in the body is found in the hemoglobin, nevertheless inside the cells, the ferritin is the main reservoir, in the form of iron oxide, and is utilized to synthetize cofactors in the respiration and DNA synthesis. The release of iron from the ferritin requires reduction from ferric to ferrous form in the serum by an chelating agent. The role of ferritin is controversial since it acts both as a neuroprotector, since in ischemia it is capable of chelating the iron released from proteins, but also ferritin donate free Fe which increases the oxidative stress contributing to a larger neurological deterioration. The ability to damage cerebral tissue is linked to increased systemic reservoir of iron, because when elevated the prognosis is worse in the initial phases of cerebral ischemia, increasing the oxidative stress and leading to necrosis of parts of the ischemic penumbra (Carbonell e Rama, 2007).

Inflammatory mediators

In ischemia, after the collapse of the blood-brain barrier (BBB) the membrane permeability changes and intensifies the leucocyte extravasation, and the production of ROS from damaged cells, triggers oxidative stress and inflammatory cascade. Endogenous molecules, known as damage-associated molecular patterns (DAMPs) are released from the injured tissue and activate the immune system infiltrated cells. In the ischemic brain, Heat shock proteins, β-amyloid (Aβ), hyaluronan, heparin sulfate, dNa or RNa immune complexes, oxidized low-density lipoproteins, and several other molecules, have been considered as possible DAMPs. Among them, high mobility group box 1 (hmGB1) is a well characterized damp in ischemic brain injury that increases Blood-Brain- Barrier (BBB) permeability or promote its breakdown. HMGB1, is localized in cell nuclei in the normal brain, translocates to the cytoplasm increasing the vascular permeability and promoting the disaggregation of BBB during ischemia (Zhang *et al*., 2011).

The DAMPs stimulate Toll-like receptors (TLRs), TLR2 and TRL4, involved in the innate immune-mediated response to non-infectious injury, including ischemic brain injury (Shichita *et al*., 2012). Several inflammatory cytokines and mediators are produced. Among them, IL-β signal or activates other molecules such as capase-1, a protein known to participate in the inflammasome complex and is present in the neurons, glial cells, microglia, and macrophages, which can be activated with hypoxia, ATP reduction, or through endogenous molecules produced by injured cells (Shichita *et al*., 2012). IL-1β is considered to be a neurotoxic mediator that promotes neuronal cell death and increases the chemokine in the microglia and astrocytes, and its inhibition is associated with reduction of ischemic lesion (Allan *et al*., 2005). The TNFα is also involved in ischemia; it is expressed in the cerebral tissue during hypoxemia within 1 hour after reperfusion and causes neuronal cell death through neurotoxic effects, but also, may be considered to a neuroprotector mediator because it participates in the mechanisms of suppression of inflammatory signs (Hallenbeck, 2002). The IL-6 is important in many types of inflammation, but in cerebral ischemia acts as a neuroprotective cytokine contributing to angiogenesis, favoring the survival of nervous cells (Jung *et al.*, 2011).

Chemokines are also important potentiating of inflammation post ischemia. The monocyte chemotactic protein-1 and IL-8 act in the leucocytes infiltration and increases the area of ischemic injury (Shichita, *et al.*, 2012). The intercellular adhesion molecule 1 (ICAM-1), produced by endothelial cells, is essential for chemotaxia and for leucocyte infiltration, it is elevated in cerebral ischemia favoring the augmentation of the inflammatory processes. The matrix metalloproteinases (MMPs) are important mediators of the inflammation post ischemia and intensifies the permeability of the BBB. The MMP-9 has neurotoxic action (Shichita, 2012).

The T-cells produced cytokines act in the inflammatory regulation during ischemic lesions. The IFN-γ is neurotoxic and acts in the neurons inducing their death. The release of IL-4 e IL-5 induce the production of neurotrophic factors by the astrocytes, which has a neuroprotetive signaling role through inhibition on the expression of cytokines induced by ONS (Butovsky et al, 2005). The IL-10 is an immune suppressor and exert a neuroprotective effect by suppressing the neurotoxic actions of TNF-α e IFN-γ (Liesz *et al*., 2009).

Cell Death

The neuronal cell death induced by ischemic injury has been, traditionally, characterized as necrosis. The high indices of extracellular glutamate stimulate the influx of Na^{+} e Ca^{++} through the NMDAr, AMPAr and kainite receptors and caring water with it to the inside of the cell resulting in cytotoxic edema (Furukawa, 1997). In necrosis there is edema, rupture of cytoplasmic organelles, loss of the membrane integrity, and lysis of the neuronal cells activating the inflammatory process (Shalak and Perlman, 2004). However, in models of

cerebral ischemic injury, there are morphological and biochemical evidences that the cell death occurs through apoptosis. Apoptotic neurons are more easily detected in the penumbra from the beginning of the ischemic lesion and during the reperfusion period and probably those nerve cells that maintains a minimum level of metabolic activity (Yuan and Yankner, 2000). The apoptosis, programmed cell death, is characterized by capases activation through extrinsic or mitochondrial via. The extrinsic via is induced by the activation of the death receptor on the surface of the cell FAS (FasR), also known also known as apoptosis antigen 1 (APO-1 or APT). The oligomerization of death receptors recruit adaptive molecules involved in caspase-8 activation. The intrinsic or mitochondrial activation, in ischemia, is initiated by high levels of glutamate, intracellular calcium, reactive oxygen species (ROS), and DNA damage. When the mitochondria receives proper apoptotic signaling or suffers an irreversible damage, pro-apoptotic molecules such as cytochrome C are released to the cytoplasm. Accompanied by the ATP, the cytochrome C forms the complex apoptotic protease activating factor 1 (Apaf-1), also known as apoptosome. This cleaves the procaspase-9, which in turn releases caspase-9. The caspases 8 and 9 activate, among others, the caspase-3 which cleaves the amyloid precursor protein (APP), resulting in increased production of the amyloid ß-peptide, which in turn increases caspase-3 activation, initiating the cell death by apoptosis (Carbonell and Rama, 2007).

CONCLUSION

At the cellular level, the reduction CBF and subsequent oxygen depletion trigger biochemical events responsible for the primary and secondary lesions that will overtake the CNS and perpetrate morphofunctional losses. An ischemic event is dynamic and the knowledge of the physiopathological mechanisms underlying the cerebral ischemia is essential to discover new pathways to re-establish the microenvironment and conditions needed for damaged brain neurons to inhibit intrinsic undesirable programmed processes of cell death, and thus offer more definite neuroprotection.

REFERENCE

Allan S.M., P.J. Tyrrell and N.J. Rothwell. 2005. Interleukin-1 and neuronal injury. *Nat. Rev. Immunol.* 5:629–640.

Bolaños, J.P. and A. Almeida. 1999. Roles of nitric oxide in brain hypoxia-ischemia. *Biochim Biophys Acta.* 1411:415–436.

Butovsky, O., A.E. Talpalar, K. Ben-yaakov and M. Schwartz. 2005. Activation of microglia by aggregated beta-amyloid or lipopolysaccharide impairs MHC-II expression and renders them cytotoxic whereas IFN-gamma and IL-4 render them protective. *Mol. Cell. Neurosci.* 29:381–393.

Camargo, E.C., L.A. Bacheschi and A.R. Massaro. 2005. Stroke in Latin America. *Neuroimaging Clin. N. Am.* 15(2):283-296.

Campos-Sousa, R.N., V.Y. Soares, K.J. Almeida, L.I. Carvalho, K.S. Jacobina, A.E. Athayde Netto, E.A. Macedo and L.A. Veloso. 2007. Knowledge of stroke among a Brazilian urban population. *Arq. Neuropsiquiatria.* 65:587-591.

Carbonell, T. and R. Rama. 2007. Iron, Oxidative Stress and Early Neurological Deterioration in Ischemic Stroke. *Current Medicinal Chemistry.* 14:857-874.

Cooper A.J. and B.S. Kristall. 1997. Multiple roles of glutathione in the central nervous system. *Biol. Chem.* 378(8):793-802.

Crack, P.J. and J.M. Taylor. 2005. Reactive oxygen species and the modulation of stroke. *Free Radic. Biol. Med.* 38:1433–1444.

Dirnagl, U., C. Iadecola and M.A. Moskowitz. 1999. Pathobiology of ischaemic stroke: na integrated view. *Trends Neurosci.* 22:391-397.

Dobrek, L. and P. Thor. 2011. Glutamate NMDA receptors in pathophysiology and pharmacotherapy of selected nervous system diseases. *Postepy. Hig. Med. Dosw.* 65:338–346.

Dringen, R., 2000. Metabolism and functions of glutathione in brain. *Prog. Neurobiol.* 62(6):649-671.

Droge W. 2002. Free radicals in the physiological control of cell function. *Physiol. Rev*. 82:47-95.

Feigin, V.L. 2005. Stroke epidemiology in the developing world. *Lancet*. 365(9478):2160-2161.

Furukawa, K., W. Fu, Y. Li, W. Witke, D.J. Kwiatkowski And M.P. Mattson. 1997. The actin-severing protein gelsolin modulates calcium channel and NMDA receptor activities and vulnerability to excitotoxicity in hippocampal neurons. *J. Neurosci*. 17:8178–8186.

Grow, J. and D.E. Barks. 2002. Pathogenesis of hypoxic–ischemic cerebral injury in the term infant: current concepts. *Clin. Perinatol.* 29:585–602.

Gutteridge, J.M. and Halliwell, B. 2000. Free radicals and antioxidants in the year 2000. A historical look to the future. *Ann. N. Y. Acad. Sci*. 899:136-47.

Hallenbeck, J.M. 2002. The many faces of tumor necrosis factor in stroke. *Nat. Med.* 8:1363–1368.

Hankey, G.J. 1999. Stroke prediction and prevention by carotid endarterectomy: keep an eye on the doughnut and not just the hole. *Cerebrovasc. Dis.* 9(6):345-350.

Hossmann, K.A. 2009. Pathophysiological basis of translational stroke research. *Folia Neuropathol.* 47:213-227.

Jung, J.E., G.S. Kim and P.H. Chan. 2011. Neuroprotection by interleukin-6 is mediated by signal transducer and activator of transcription 3 and antioxidative signaling in ischemic stroke. *Stroke***.** 42:3574–3579.

Kimelberg, H.K. and A.A. Mongin. 1998. Swelling-activated release of excitatory amino acids in the brain, relevance for pathophysiology. *Contrib. Nephrol.* 123:240–257.

Kostandy, B.B. 2012. The role of glutamate in neuronal ischemic injury: the role of spark in fire. *Neurol. Sci.* 33:223–237.

Liesz, A., E. Suri-Payer, C. Veltkamp, H. Doerr, C. Sommer, S. Rivest, T. Giese and R. Veltkamp. 2009. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat. Med.* 15:192–199.

Liu, H., R. Colavitti, I.I. Rovira and T. Finkel. 2005. Redox-dependent transcriptional regulation. *Circ. Res.* 97: 967–974.

Mena, F.V., P.J. Baab, C.L. Zielke and H.R. Zielke. 2000. In vivo glutamine hydrolysis in the formation of extracellular glutamate in the injured rat brain. *J. Neurosci. Res.* 60:632–641.

Minelli, C., L.F. Fen and D.P. Minelli. 2007. Stroke incidence, prognosis, 30-day, and 1-year case fatality rates in Matão, Brazil: a population-based prospective study. *Stroke*. 38(11):2906-2911.

Muntner, P., E. Garret, M.J. Klag and, J. Coresh. 2002. Trends in stroke prevalence between 1973 and 1991 in the US population 25 to 74 years of age. *Stroke*. 33(5):1209-1213.

Olmez, I. and H. Ozyurt. 2012. Reactive oxygen species and ischemic cerebrovascular disease. *Neurochemistry International.* 60:208–212.

Poli, G., G. Leonarduzzi, F. Biasi and, E. Chiarpotto. 2004. Oxidative stress and cell signalling. *Curr. Med. Chem.* 11:1163–1182.

Porter, N.A. 1984. Chemistry of lipid peroxidation. *Methods Enzymol.* 105:273-82.

Radi, R., J.S. Beckman, K.M. Bush and B.A. Freeman. 1991. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J. Biol. Chem.* 266:4244-4250.

Rivest, S. 2009. Regulation of innate immune responses in the brain. *Nat. Rev. Immunol.* 9:429–439.

Shalak, L. and J.M. Perlman. 2004. Hypoxic-ischemic brain injury in the term infant-current concepts. *Early Human Development*. 80:125-141.

Sharp, F.S., A. Lu, Y. Tang and D.E.J. Millhorn. 2000. Multiple molecular penumbras after focal cerebral ischemia. *J. Cereb. Blood Flow Metab.* 20(7):1011-32.

Shichita, T., T. Ago, M. Kamouchi, T. Kitazono, A. Yoshimura and H. Ooboshi. 2012. Novel therapeutic strategies targeting innate immune responses and early inflammation after stroke. *J. Neurochem.* 123 Suppl 2:29-38.

Wyatt, J.S. and A.D. Edwards, D. Azzopardi, E.O.R. Reynolds. 1989. Magnetic resonance and near infrared spectroscopy for investigation of perinatal hypoxic–ischemic brain injury. *Arch. Dis. Child.* 64:953-63.

Yuan J, and B.A. Yankner. 2000. Apoptosis in the nervous system. *Nature.* 12;407(6805):802-809.

Zhang, J., H.K. Takahashi and K. Liu. 2011. Anti-high mobility group box-1 monoclonal antibody protects the blood-brain barrier from ischemia-induced disruption in rats. *Stroke.* 42:1420–1428.

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