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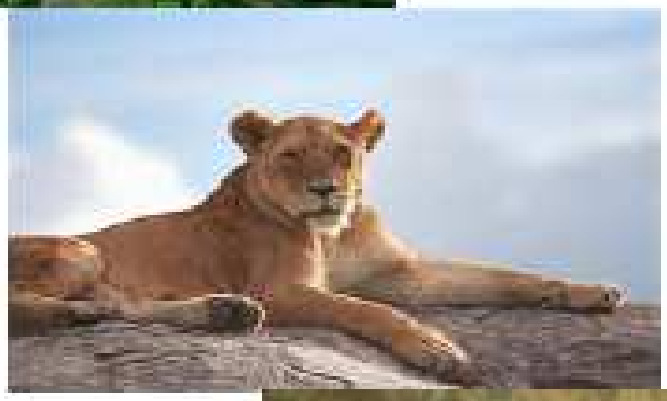

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EFFECTS OF CORN DISTILLERS DRIED GRAINS ON THE PERFORMANCE AND EGG QUALITY OF LAYING HEN

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ABSTRACT

A study was conducted to determine the effects of corn distillers' dried grain (CDDG) on the performance and egg quality of laying hens. The hens were fed dietary inclusions of CDDG at 0, 10, 20, 30 and 40% for a period of eight weeks. Average feed intake, weight gain, feed conversion ratio and nitrogen economy varied significantly (P<0.05) among dietary treatments. Birds fed 30% dietary CDDG had the highest feed intake (127.00 g/bird/day). Feed conversion ratio (FCR) was inversely related to increasing levels of dietary treatments with CDDG. Among hens fed dietary CDDG, those fed 10% dietary the best feed conversion ratio (6.3) and highest weight gain (20.10 g/bird/day). Nitrogen retention was the highest (88.6%) in birds fed control diet and lowest (61.2%) in birds fed 30% dietary CDDG. Hen-day production (HDP) and Haugh Unit (HU) were significantly affected (P<0.05) by dietary treatments. Birds fed 20% dietary CDDG had the highest (61%) HDP, while birds fed 0% CDDG (control diet) had the lowest HDP (52.2%). Birds fed 20% dietary CDDG had the highest HU value (88.7) while birds fed 0% dietary CDDG (control) had the lowest (81.2) value. Birds fed 20% CDDG performed best in terms of HDP, egg quality, cost-benefit ratio. Laying hens can be fed CDDG at 10-20% inclusion level. Inclusion levels of CDDG above this level were observed to have counter-productive effects on the production performance of bird; this observation is as a result of high fibre level of CDDG.

Keywords: Corn distillers' grain, Laying hens, Feed intake, Weight gain, Feed conversion ratio, Nitrogen economy, Egg quality

INTRODUCTION

Poultry production in Nigeria has witnessed a decline primarily due to the astronomical rise in the cost of poultry feeds. Major causes of high cost of feeds are due to high cost of energy and protein feedstuffs. The consequence of the high cost of poultry feeds is the astronomic increase in the price of poultry products (Okoye *et al.,* 2006). Dietary energy constitute up to 50% of a balanced poultry diet. Cereal grains which constitute the major source of energy in poultry diets are also consumed by man (Bolu and Balogun, 1998a). This competition may result in increasing poultry product price and reduced reduce affordability due to increased relative cost of animal production (Leaflets, 2008).

Corn Distiller's dried grains (CDDG) is a by-product of corn milling and fermentation for ethanol production. It contains all the nutrients found in the corn kernel, except starch, which has been fermented to ethanol and carbon dioxide. Thus, CDDG have been useful for animal feed production in the last decade (Cheon, 2008). In addition, CDDG has been reported to be a rich source of energy and protein, and therefore a promising replacement for corn and soybean meal in feed production

(Shurson, 2003; Leaflet, 2008; Cheon *et al.,* 2008). CDDG was used as a feed ingredient in poultry diets, partially due to its ample supply of unidentified growth factors, which can be vitamins and other synthesized products during fermentation (Cromwell *et al.,* 1993; Batal, 2007; Bregendahl *et al.,* 2008). Thus, feeding CDDG resulted in improved overall performance of broilers and laying hens (Lumpkins *et al.,* 2004; 2005). However, the use of CDDG is limited by high fibre content, variability in the compositions due to source and specific amino acids (Belyea *et al.,* 1998; 2004).

Excretion of nitrogen especially as volatilized ammonia lost to the atmosphere is currently a global concern. In the United States, poultry (including laying hens) is one of the largest contributors of atmospheric NH_3 emissions among domestic animal species, accounting for 27% of the total NH_3 emissions in 2002. Ammonia adversely affect the health and production of poultry through deciliation of the trachea, corneal ulcers, impairment of macrophage function, reduced lung function, lower egg production and lower body weight gains, but it may also cause eutrophication of surface water resources and nuisance odours (Spiehs *et al.,* 2002; Noll *et al.,* 2007).

Several research have reported decreased nitrogen excretion and ammonia emissions in laying hens fed high fibre and reduced crude protein, without causing depressed egg production and nitrogen balance though, may lower nutrient digestibility (Parsons *et al.,* 2006; Leaflet, 2008). This study investigated the effects of feeding CDDG on the performance, egg quality and nitrogen balance in laying hens. Cost-benefit ratio of feeding levels of CDDG in poultry was also determined

MATERIALS AND METHODS

Housing and Management: Three hundred and sixty (360), 18-week-old bovan black growers were housed in battery cages. Feed and water were given *ad libitum*. Prior to the experiment, the birds were fed a pre-lay diet until hen-day production was 5%. Thereafter, they were fed the control diet containing 0% CDDG for two weeks until hen-day production became 50%.This was done to acclimatize the birds to the experimental condition, new diet and also to maintain a stable egg production. Thereafter, hens were fed the experimental diets (varying levels of CDDG) at about 21 weeks of age.

Dietary Treatments: Five diets were formulated to meet the NRC (1994) nutrient requirements for laying hens. The diets were formulated to include 0, 10, 20, 30 and 40% corn CDDG (Table 1). Corn CDDG was analysed for proximate composition using the methods outlined by AOAC (1990). Gross Energy (GE) was determined using the bomb calorimeter. Metabolizable Energy (ME) was obtained by deducting the GE faeces from the GE in feed using the following formula: S = 100 (T - B) + B / s, where S = energy value of test ingredient, T = energy value of basal + test ingredient, B = energy value of basal diet and s = level of supplementation of test ingredient in the diet (Bolu and Balogun, 1998b).

Nitrogen was determined using the micro-Kjeldahl method and the crude protein content was calculated as nitrogen × 6.25. Ether extract and ash contents were determined using a Soxhlet extraction method and by wet-ashing in a muffle furnace, respectively (AOAC, 1990).

Samples of formulated diets were also subjected to proximate analysis to determine the contents of metabolizable energy, crude protein, crude fibre, ether extracts and ash. Diets fed were isocaloric (2,600 kcal/kgME) and isonitrogenous (17.5% CP) (AOAC, 1990).

Parameters Measured: Data collected include feed intake, feed conversion ratio, weight gained, egg quality and percentage nitrogen retention. Feed intake was measured every week by collecting left over feed, and deducting from the initial ration supplied. Feed intake was recorded on a weekly basis throughout the period of experiment. Birds were weighed at the beginning of the experiment and on a weekly basis to obtain the weekly weight gained. Feed conversion ratio (feed gain ratio) was calculated as: FCR = Feed consumed / Weight gain. Eggs collected from each dietary

treatment were recorded daily to determine the hen-housed production which was used to obtain the weekly hen-day production.

Hen-day production was then calculated as: HHP = Number of egg produced/Number of hen-days x 100, where Hen-day = Number of hens x Number of days in lay.

Four eggs were collected three times in a week from each treatment, weighed and used for egg quality analyses. Eggs were broken on to a flat surface to measure the heights of the albumen and yolk with the use of a spherometer. The yolk width/diameter was measured using the vernier calliper. Weights of albumen and yolk were also taken using the electronic weighing balance. Haugh unit was calculated from the records of egg weight and albumen height as: HU =100 X Log (H-1.7 X $W^{0.37}$+ 7.56), where HU = Haugh unit, H = albumen height (mm) and W = weight of the egg (g) (Haugh, 1937). Yolk index was calculated thus: YI = height of yolk (HY) / width of yolk (WY). The relative specific density (RSD) of egg was determined by measuring the volume displaced by egg when immersed in water in a graduated cylinder. Relative specific density was then calculated as: RSD = weight of egg (g) / volume of egg (cm^3).

During the last week of the experiment, a total collection of faeces was made for three days. The faecal samples were collected, dried and analysed for nitrogen contents using kjeldahl method (AOAC, 1990). The percentage nitrogen retention was obtained from nitrogen balance and nitrogen in feed. Thus, % nitrogen retention = NI – NE / NI x 100, where NI = nitrogen intake and NE = nitrogen excreted.

A cost-benefit analysis was conducted to determine the cost-benefit ratio (CBR) of the various inclusion levels of CDDG. Cost-benefit ratio was obtained as the cost of feed consumed to produce a dozen eggs. It was calculated as: CBR = Cost (in Naira) of feed consumed to produce 12 eggs / Cost (in Naira) of 12 eggs.

Statistical Analysis: The study employed completely randomised block design. Data were analysed using analysis of variance (ANOVA). Significant differences among treatment means were separated using the least significant difference (LSD). A probability level of $P < 0.05$ was employed in all the analysis.

RESULTS

Average daily feed intake varied significantly ($P<0.05$) among dietary treatments. Birds fed diet containing 40% dietary CDDG had the lowest feed intake (105.00 g/bird/day) while birds fed 30% dietary CDDG had the highest feed intake (127.00 g/bird/day), which was same as birds fed the control diet. Feed conversion ratio (FCR) was inversely related to increasing level of dietary treatments with CDDG. Layers fed control diet (0.00% dietary CDDG) had feed conversion ratio of 5.90 while the lowest feed conversion ratio of 7.50 was recorded for birds fed 30% dietary CDDG. Birds fed diet containing 10% dietary CDDG had the highest feed conversion ratio (6.3) among the dietary treatments with CDDG inclusion. Nitrogen retention was the highest (88.6%) in birds fed control diet and lowest (61.20%) in birds fed 30% dietary CDDG. There was no mortality recorded throughout the period of experimentation (Table 2).

Average number of eggs and Hen-Day Production (HDP) (Table 3) were significantly affected ($P<0.05$) by dietary treatments. Birds fed 20% dietary DDG had the highest (61%) HDP and highest (56) number of eggs per week while birds fed 0% DDG (control diet) had the lowest HDP (52.2%) and birds fed 40% DDG had the lowest (41) average number of eggs per week.

There were significant changes ($p<0.05$) among dietary treatments for egg weight, yolk height, albumen height, albumen weight and yolk weight while yolk width and yolk index were not affected ($p<0.05$) by dietary treatments. Birds fed 30% dietary DDG had the highest albumen height (7.8mm) while birds fed 10% dietary DDG (diet B) had the lowest albumen height (6.9 mm).Birds fed 30% dietary DDG had the highest albumen weight(36.3g) while birds fed 40% dietary DDG (diet E) had the lowest albumen weight (33.4g).

Haugh unit (Table 4) was significantly affected ($p<0.05$) by dietary treatments. Birds fed 20% dietary DDG had the highest value

Table 1: Composition of corn distillers' dried grain based diets fed to laying hen

Ingredients	0%	10%	20%	30%	40%
Maize	55.2	49.6	44.4	38.8	33.2
Wheat	12.0	10.8	9.6	8.4	7.2
Soybean meal	24.0	20.8	18.4	16.0	14.0
Oyster shell	8.0	8.0	8.0	8.0	8.0
Bone Meal	1.2	1.2	1.2	1.2	1.2
Layers' Premix	0.2	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2	0.2
Lysine	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3	0.3
CDDG	0.0	10.0	20.0	30.0	40.0
Total	100.0	100.0	100.0	100.0	100.0
CP (%)	17.0	17.4	17.8	18.1	18.6
ME(Kcal/kg)	2604.0	2623.4	2643.5	2662.7	2682.4

**Layers' Premix supplied per Kg of diets; Vitamin A: 8 x 10^6, Vitamin D_3: 1500 IU, Vitamin E: 101 IU, Vitamin K_3: 1.5 mg, Vitamin B_1: 1.6 mg, Vitamin B_2: 4 mg, Vitamin B6: 1.5 mg, Vitamin B_{12}: 0.0 mg, Niacin: 20 mg, Pantothenic acid: 5 mg, Folic acid: 0.05 mg, Biotin: 0.75 mg, Choline chloride: 1.75 x 10^4 mg, Cobalt: 0.2 mg, Copper: 0.2 mg, Iodine: 1 mg, Iron: 20 mg, Manganese: 40 mg, Selenium: 0.2 mg, Zinc: 80 mg, Antioxidant: 1.25 mg N.B: ME- Metabolizable Energy (calculated), CP-Crude Protein (calculated).*

Table 2: Effects of graded levels of corn distillers' dried grain diets on the performance of laying hen

CDDG inclusion levels	Feed intake (g/bird/day	Weight gain (g/bird/day)	FCR	Nitrogen Retention (%)	Mortality* (%)
0%	127.0 ± 2.04^a	21.5 ± 0.11^a	5.9 ± 0.13^d	88.6 ± 0.48^a	0
10%	126.6 ± 3.22^a	20.1 ± 0.68^b	6.3 ± 0.11^c	83.2 ± 1.22^b	0
20%	108.3 ± 3.25^b	17.1 ± 0.56^c	6.3 ± 0.13^c	84.7 ± 1.42^b	0
30%	127.0 ± 2.15^a	16.9 ± 0.32^c	7.5 ± 0.23^a	61.2 ± 2.26^d	0
40%	105.0 ± 3.21^b	15.6 ± 0.14^d	6.8 ± 0.14^b	72.5 ± 2.68^c	0

*Means in the same column having different superscripts are significantly different at $p<0.05$, * the percentage mortality was not significant.*

Table 3: Effects of graded levels of corn distillers' dried grain diets on laying performance of layers and the cost-benefit ratio of laying hen

CDDG Inclusion level	Average No of Egg	Hen-Day Production	Feed Cost Naira/Kg	Cost Benefit Analysis
0%	48.0 ± 3.26^{b}	52.2 ± 1.84^{a}	82.4 ± 2.56^{a}	0.9 ± 0.38^{a}
10%	49.0 ± 2.18^{b}	53.0 ± 2.62^{ab}	76.1 ± 2.42^{b}	0.8 ± 0.34^{a}
20%	56.0 ± 5.04^{a}	61.0 ± 1.84^{c}	70.8 ± 5.88^{c}	0.6 ± 0.32^{a}
30%	51.0 ± 5.36^{ab}	55.6 ± 2.62^{b}	65.8 ± 4.58^{cd}	0.7 ± 0.36^{a}
40%	41.0 ± 2.28^{c}	52.6 ±1.78^{a}	62.0 ± 3.83^{d}	0.8 ± 0.36^{a}

Means in the same column having different superscripts are significantly different at P<0.05. N.B: Feed cost per kilogram was applicable at the time the experiment was performed.

Table 4: Effects of graded levels of corn distillers' dried grain diets on egg quality traits of laying hen

Treatment	Egg Weight (g)	Yolk Height (mm)	Albumen Height (mm)	Yolk Width (mm)	Albumen Weight (g)	Yolk Weight (g)	Haugh Unit	Yolk Index	Relative Specific Density
0%	52.5 ± 1.24^{b}	15.2 ± 0.20^{a}	7.0 ± 0.41^{cd}	3.7±0.12^{a}	35.2±0.16^{b}	12.1±0.02^{a}	81.7±0.12^{c}	4.0±0.21^{a}	1.2±0.11^{a}
10%	51.9 ± 1.82^{c}	15.4 ± 0.24^{a}	6.9 ± 0.21^{d}	3.6±0.18^{a}	35.2±0.14^{b}	11.5±0.03^{b}	85.5±2.98^{b}	4.1±0.31^{a}	1.2±0.11^{a}
20%	54.0 ± 1.12^{a}	14.9 ± 0.08^{b}	7.6 ± 0.22^{b}	3.6±0.12^{a}	36.1±1.04^{ab}	11.1±0.31^{c}	88.7±0.02^{a}	4.1±0.22^{a}	1.2±0.12^{a}
30%	52.1 ± 1.81^{bc}	14.7 ± 0.11^{c}	7.8 ± 0.12^{a}	3.6±0.21^{a}	36.3±0.21^{a}	10.6±0.40^{c}	86.3±0.71^{b}	4.0±0.13^{a}	1.2±0.12^{a}
40%	50.1 ± 2.02^{c}	14.8 ± 0.18^{bc}	7.4 ± 0.40^{bc}	3.7±0.14^{a}	33.4±0.16^{c}	11.9±0.41^{b}	88.1±0.24^{b}	4.1±0.12^{a}	1.3±0.12^{a}

Means in the same column having different superscripts as significantly different at P<0.05.

(88.7) while birds fed 0% dietary DDG (control) had the lowest (81.2) value. Relative Specific Density (Table 4) was similar (1.2) among dietary treatments during the egg analysis period.

DISCUSSION

The lower feed intake observed with birds fed 40% CDDG could be due to the increasing fibre level and dustiness of the feed as the level of CDDG increased in the diets. It could be probable that at the 30% level of the inclusion of CDDG the tolerable limit of layers for fibre had exceeded the threshold hence the decrease in feed intake. Birds have been reported to eat to satisfy energy requirement (NRC, 1994). Fibre is a nutrient diluent especially for monogastric animal and therefore reduces nutrient density. Birds fed high fibre diets are expected to increase voluntary feed intake (Batal and Dale, 2006; Bolu *et al.,* 2012). Okoye *et al.* (2006) reported decreased feed intake when birds were fed sorghum malt at 30% inclusion level due to dustiness of the feed. Feed intake observed for in this study corroborated the findings of Waldroup *et al.* (1981; 2007) and Leaflets (2008), who suggested 25% and 20% of CDDG inclusion, respectively, as accepted levels in poultry diets. Lowest feed conversion ratio (7.5) observed in birds fed 30% CDDG could be related to their low body weight gained in spite of their high feed intake (Quant *et al.,* 2011). This means birds could not efficiently convert feed consumed into body weight, hence the decrease in feed conversion ratio, which can also be attributed to the increasing fibre level with increasing levels of CDDG. Bolu *et al.* (2012) reported low feed utilisation as a result of high fibre level in broilers fed graded levels of CDDG. Nitrogen retention decreased with increasing levels of CDDG (Fastinger *et al.,* 2006; Roberts *et al.,* 2007). This is contrary to the findings of Leaflet (2008) who reported an increase in nitrogen retention with increasing CDDG levels despite the increased nitrogen excretion, while egg production increased.

Birds fed 20% dietary CDDG performed best in terms of HDP and average number of eggs per week. Leaflet (2008) suggested that CDDG could be fed to laying hens in commercial settings, and can be fed at up to 15 to 20% of the diet with no adverse effects on egg production. However, HDP and average number of eggs per week decreased as dietary CDDG rose above 20%. Ghazalah *et al.* (2011) studied the effects of DDGS as replacement for soya bean and reported a decrease in average egg production as dietary inclusion of DDGS increased.

Since there was significant difference observed with egg weight, yolk height, yolk index yolk weight, albumen weights and heights, it means the variables were affected by the dietary treatment (Roberson *et al.,* 2005). In terms of albumen height and weight, birds fed 30% CDDG diet had the best performance. The higher the Haugh unit value, the better the albumen quality. Birds fed 20% CDDG had highest values for haugh unit and a better albumen quality. Lumpkins *et al.* (2005) and Mahmoud and Sheila (2011) did not observe variations in egg interior qualities as a result of dietary DDG fed to laying hens. However, Jung and Batal (2009) observed that the incorporation of 20% DDGS to laying hens diets significantly increased Haugh units.

Specific gravity, according to Mahmoud and Sheila (2011) is a good indicator of egg shell quality when value is around 1.080 or above. The results in this experiment indicated an average relative specific density of 1.2 during egg analysis period. Lumpkins *et al.* (2005) who reported that laying hens fed 15% corn CDDGS had no negative effect on egg shell specific gravity. In the same vein, Mahmoud and Sheila (2011) reported that similar specific gravity was observed among dietary treatments when birds were fed a graded level of corn CDDGS at 5 – 25%.

Birds fed 20% CDDG had the highest cost-benefit ratio (0.6) which means profit was being maximised at 20% CDDG. This could be related to the high hen-day production recorded in Diet C (20%). In summary, birds fed on 20% dietary CDDG performed best. This agreed with Lumpkins *et al.* (2004) who suggested a maximal inclusion level of 10 to 12% CDDGs may be used in commercial layers' diets.

Conclusion: Birds fed 20% DDG performed best in terms of HDP, Average number of eggs, egg quality, cost-benefit ratio and general performance, it can be concluded that laying hens can be fed DDG at 10 – 20% inclusion level. Inclusion levels of DDG above this level was observed to be counter-productive as it affected production performance of bird; this observation is as a result of the high fibre level of the feed. Inclusion of DDG in poultry diet also minimized the cost of feed in poultry production generally since the cost of feed decreased with increasing DDG inclusion level.

REFERENCES

AOAC (1990). *Official Method of Analysis. Association of Official Analytical Chemist.* 15th Edition, Washington DC, USA.

BATAL, M. (2007). *Use of dried distillers' grains and other alternatives to replace corn.* Thirty-Fourth Annual Carolina Poultry Nutrition Conference, North California.

BATAL, A. B. and DALE, N.M. (2006). True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. *Journal of Appllied Poultry Research,* 15: 89 – 93.

BELYEA, R. L., ECKHOFF, S., WALLIG, M. and TUMBLESON, M. (1998). Variability in the nutritional quality of distillers soluble. *Bio-Research Technology,* 66: 207 – 212.

BELYEA, R. L., RAUSCH, K. D. and TUMBLESON, M. E. (2004). Composition of corn and distillers grains with solubles from dry grind ethanol processing. *Bio-Research Technology,* 94: 293 – 298.

BOLU, S. A., ALLI, O. I. and ESUOLA, P. (2012). Response of broilers to graded levels of distiller's dried grain. *Sustainable Agriculture Research,* 1(1): 147 – 150.

BOLU, S. A. and BALOGUN, O. O. (1998a). Comparative energy value of sorghum distiller's waste, maize cob and shea butter cake for pigs. *Nigerian Journal of Animal Production,* 25: 157 – 162.

BOLU, S. A. and BALOGUN, O. O. (1998b). Performance of Laying hens fed graded levels of locally produced and natural vitamin premix. *Nigerian Journal of Animal Production,* 26: 54 – 59.

BREGENDAHL, K., HAYES, D. J. and LAWRENCE, B. A. (2008). *Using Distillers Grains in the US and International, Livestock and Poultry Industries*. Iowa State University, lowa.

CHEON,Y. J., JANQ, A., LEE, H. L., LEE, S. K., LEE, J. H., LEE, B. D., SHIN, M. H. and SON, C. K. (2008). Effects of corn distiller's dried grains with solubles on production and egg quality in laying hens. *Asian-Australasian Journal of Animal Sciences,* 21: 414 – 419.

CROMWELL, G. L., HERKELMAN, K. L. and STAHLY, T. S. (1993). Physical, chemical and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. *Journal of Animal Science,* 71: 679 – 686.

FASTINGER, N. D., LATSHAW, J. O. and MAHAN, D. C. (2006). Amino acid availability and true metabolizable energy content of corn distillers dried grains with solubles in adult cecectomized roosters. *Poultry Science,* 8: 1212 – 1216.

GHAZALAH, A. A., ABD-ELSAMEE, M. O. and MOUSTAFA, E. (2011). Use of distillers dried grains with solubles (DDGs) as replacement for soya bean meal in laying hen diets. *International Journal of Poultry Science,* 10 (7): 505 – 513.

HAUGH, R. R. (1937).The Haugh units for measuring egg quality. *US Egg Poultry Magazine, 43:* 552 – 555.

JUNG, B. and BATAL, A. (2009). The nutrient digestibility of high-protein corn distillers dried grains and the effect of feeding various levels on the performance of laying hens. *Journal of Applied Poultry Research,* 18: 741 – 751.

LEAFLET, A. S. (2008). Maximum dietary content of corn dried distiller's grains with solubles in diets for laying hens. Effects on nitrogen balance, manure

excretion, egg production and egg quality. Iowa State University, Iowa.

LUMPKINS, B. S., BATAL, A. B. and DALE, N. M. (2004). Evaluation of distillers dried grains with solubles as a feed ingredient for broiler. *Poultry Science,* 83: 1891 – 1896.

LUMPKINS, B. S., BATAL, A. B. and DALE, N. M. (2005). Use of distillers dried grains plus solubles in laying hen diets. *Journal of Applied Poultry Research,* 14: 25 – 31.

MAHMOUD, K. M. and SHEILA, E. P. (2011). *Dried Distillers Grain with Solubles in Laying Hen and Pullet Rations.* University of Nabraska, Nabraska.

NRC (1994). *Nutrient Requirements of Poultry*, 9th Edition, National Reseach Council (NRC), National Academics Press, Washington DC, USA.

NOLL, S. L., BRANNUN, J. and PARSON, S. C. (2007). Nutritional value of corn distiller dried grains with soluble (DDGs): Influence of soluble addiction. *Poultry Science,* 86: 68 – 70.

OKOYE, F. C., UGWUNE, M. C. and UBAEDUONU, L. C. (2006).Effect of the replacement of maize with graded levels of sorghum malt (sorghum bicolour) on the performance of broiler chicks. *Agricultural Journal,* 1: 77 – 80.

PARSONS, C. M., MARTNEZ, V. S. and RADHAKRISHNAN, S. (2006). Modified DDGs For poultry. *In: Proceeding of Multi-State Poultry Feeding and Nutrition Conferences,* Indianapolis.

QUANT, A. D., PESCATORE, A. J., PIERCE, J. L., ROSSI, P., CANTOR, A. H., FORD, M. J. and KING, W. D. (2011). Production performance and egg quality of hens fed diets containing up to thirty percent distillers dried grains with soluble (DDGS) and an enzyme supplement. *Poultry Science,* 90(1): 157 – 158.

ROBERSON, K. D., KALBFLEISCH, J. L., PAN, W. and CHARBENEAU, R. A. (2005). Effects of corn distiller's dried grains with soluble at various levels on performance of laying hens and yolk colour. *International Journal of Poultry Science,* 4: 44 – 51.

ROBERTS, S. A., XIN, H., KERR, B. J., RUSELL J. R. and BREGENDABL, K. (2007). Effects of dietary fibre and reduced crude protein on nitrogen balance and egg production in laying hens. *Poultry Science,* 86: 1716 – 1725.

SHURSON, J. (2003). The value and use of distillers dried grains with solubles DDGS) in livestock and poultry ration. http://www.ddgs.umn.edu. Accessed on January 23, 2011.

SPIEHS, M. J., WHITNEY, M. H. and SHURSON, G. C. (2002). Nutrient database for distiller's dried grains with soluble produced from new ethanol plants. *Animal Science,* 80: 2639 – 2645.

WALDROUP, P. W., OWEN, J. A., RAMSEY, B. E. and WELCEL, D. L. (1981). The use of high levels of distillers dried grains plus solubles in broiler diets. *Poultry Science,* 60: 1479 – 1484.

WALDROUP, P. W., WANG, Z., COTO, C., CERATE, S. and YAN, F. (2007). Development of a standardized nutrient matrix for corn distillers dried grains with solubles. *International Journal Poultry Science,* 6: 478 – 483.

MORINGA PLANT AND IT USE AS FEED IN AQUACULTURE DEVELOPMENT: A REVIEW

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ABSTRACT

Aquaculture, an important source for animal protein utilizes a lot of fishmeal regarded as the best protein source in fish feeds. For making feeds, aquaculture sector alone consumed the equivalent of about 23.8 million metric tons (mmt) of fish or 87% of non-food fish by 2006. By 2011, the non-food uses of world fisheries were 23.2 mmt out of a total fish production of 154 mmt. The use of fishmeal is therefore substantial; 15.0 mmt being used in 2010. In Nigeria for example, small forage pelagic fish used in fishmeal production contributes 51% of total fish supply. Because most of the conventional plant/animal feed sources are equally in great demand for human consumption, there is an urgent need to examine other products from little researched plants such as moringa (Moringa oleifera Lam) as alternative source of protein in aquaculture feeds. There is a dearth of information on the use of moringa leaf or seed meals as fish feed ingredients. An extensive search and analyses of published data on moringa and any of its use in aquaculture were therefore carried out. It was reported to be hardy, high yielding and thrive in diverse ecological zones. Its leaves, the kernel and the fat-free kernel meals contain 26.4 %, 36.7 % and 61.4 % of crude protein, respectively. The kernel contains over 40 % of good quality oil comparable to olive oil. The leaves and pods are rich in vitamins and minerals. Moringa leaves are free from anti-nutritional factors except for saponins and phenols. Studies on the use of moringa in fish feed production are few and far between as is discovered in this review. Combination of seed and leaf meals in desired proportion might result in obtaining a plant-based protein source that could favourably replace fishmeal in fish feeds. Hence, this review on available information on M. oleifera used in fish feeds exposes the need for further research.

Keywords: Fish feed ingredients, Fishmeal, Alternative protein sources, *Moringa oleifera,* Anti-nutritional factors

INTRODUCTION

Naylor *et al.* (2000) reported that as the world's human population continues to increase beyond six billion, there is a corresponding reliance on farmed fish production as an important source of animal protein. FAO (2000) projected world fishery production in 2010 to range between 107 and 144 million metric tons (mmt). By 2008, actual global aquatic animals' production totaled 52.9 mmt. Around three quarters of the world's capture fisheries are fully or overexploited (Huntington and Hasan, 2009). Aquaculture, has been the fastest growing food sector for over 25 years, supplying 49% (8.6 kg/capita) of total global food fish supply (17.6

kg/capita) in 2010 (Tacon, 2011). Considering this increasing global population and recognizing that no additional supplies from marine capture fisheries, it has been estimated that, to maintain the current level of per-capita consumption by 2030, the world will require at least another 23 mmt of aquatic animal food to be provided by aquaculture (FAO, 2012). The volume of farm-produced aquatic animals represented 46.7% of the global food fish supply in that year (FAO, 2012). It is expected that most of the increase in fish production will come from aquaculture, the fastest growing food production sector. It was further predicted that aquaculture sector would contribute more than 50% of total world fish production by the year 2030 (FAO, 2000). Hardy (2000) disclosed that the proportion of global fishmeal production used in fish feeds increased from 10 to 35% since 1985. He predicted that fishmeal needs for aquaculture in 2010 would be 2.8 mmt which he regarded as 44% of the 10-year average global fishmeal production of 6.5 mmt. Hardy (2000), therefore, predicted that about 3 million metric tonnes of fishmeal equivalent alternative protein sources would be required in aquaculture sector by the year 2010 to replace fishmeal, which is considered as an ideal inclusion in fish feed production. The actual aquafeed production by 2009 was 68.3 mmt worth $106 billion (US) (Allan, 2010). Aquaculture which utilizes a lot of fishmeal is set to remain one of the fastest-growing animal food-producing sectors and, in the next decade, total production from both capture and aquaculture will exceed that of beef, pork or poultry (FAO 2012). Fishmeal has been regarded as the best protein source in fish feeds. The proportion of global fishmeal production used in fish feeds has increased from 10 – 35% in the last 15 years. Aquaculture sector alone consumed the equivalent of about 23.8 mmt of fish (live weight equivalent) or 87% of non-food fish in the form of feed inputs in 2006 (Tacon and Metian, 2009). The non-food uses of world fisheries are 23.2 mmt out of a total fish production of 154 mmt by 2011 (FAO, 2012). The use of fishmeal is therefore substantial. In Nigeria for example, small forage pelagic fish used in fishmeal production contributed 51% of total fish supply (Tacon and Metian, 2009).

There is no doubt that many studies are on-going to identify the needed protein alternative sources be it animal or plant products. However, Tacon and Forster (2001) have advocated the development of non-human grade feed resources whose growth can cope with the projected and desired fast growth of aquaculture sector to minimize any conflict with human food security interests. It is worthy of note that causes of recent outbreaks of diseases in livestock have been linked to feeding animal products to animals that do not normally consume them and this prediction has cast doubt concerning the suitability of feeding animal-derived proteins to non-carnivorous species such as tilapia.

In the search for alternative protein sources, we advocate that greater efforts should be directed towards the use of plant ingredients to replace fishmeal particularly for non carnivorous species. There have been many fish feed trials in the past using a number of plant-derived protein sources to test their suitability for some fish species (Heller, 1996; Hossain and Becker, 2001; Siddhuraju and Becker, 2001, Richter *et al.,* 2003; Ogbe and Affiku, 2011; Sirimongkolvorakul *et al.,* 2012). Many of these trials have concentrated on plant species such as groundnut, palm kernel cakes, soybean, sunflower seed, rapeseed, cotton seed meals, corn and wheat gluten (Francis *et al.,* 2002). Realizing that these plant feed sources are equally in great demand for human consumption, there is an urgent need to examine other products from little researched and unknown plants as aquaculture feed ingredient alternatives. Some of these plants include moringa plant (*Moringa oleifera*), *Jatropha curcas, Sesbania* spp. and *Mucuna puriens*. Unfortunately, ingredients from these plants contain high levels of anti-nutritional factors such as glucosinolates, phytates, protease inhibitors, non-starch polysaccharides (NSPs), saponins, tannins, lectins and gossypols which have been found to have a bitter taste that could result in poor acceptability of feed to the fish under trial. Francis *et al.* (2001) reviewed the effects of anti-nutrients on finfish

and revealed that the use of hydrothermal treatment and soaking with water is efficient in removing high levels of anti-nutrients. Hardy (2000) also suggested the inclusion of phytase to high phytate diets to increase the availability of dietary phosphorus to various fish species. Siddhuraju *et al*. (2002) advocated the use of γ ray irradiation in neutralizing the negative effects of certain anti-nutrients such as saponins. Such treatments are likely to come in handy in for *Moringa* used in feed feeds. This paper reviews available information on *Moringa* plant regarding its potentials to contribute to fish feed ingredients.

MATERIALS AND METHODS

An extensive literature search and analyses of published data on *Moringa* and any of its use in aquaculture were carried out to effect this review.

RESULTS AND DISCUSSION

***Moringa oleifera* as Possible Fish Ingredient:** *Moringa oleifera* Lam or "drum stick" (derived from the shape of the pods) is regarded as a "miracle or wonder plant". This plant has many domestic names depending on the geographical location. In Nigeria, the Igbos call it "*okwe oyibo*", the Yorubas call it "*ewe-igbale*" while the Hausas call it "*sogele*" (Isaac, 2012). According to Isaac (2012) *Moringa* plant contains weight for weight four times the calcium in milk, four times the vitamin A in carrots, two times the protein in milk, three times the potassium in banana and seven times the vitamin C in oranges. *Moringa* plant is non–toxic even at high concentration. It is easily digestible, easy to conserve and easy to use as supplement or on most foods. *Moringa* plant or its processed products has no caffeine like other beverages, thus escaping adverse effects on health.

Moringa plant is native to the sub-Himalayan regions of Northwest India (Foidl *et al*., 2001; Isaac, 2012). The plant now thrives in many countries of Africa, Arabia, South East Asia, the Pacific and Caribbean Islands as well as South America, producing flowers and fruits at all seasons (Isaac, 2012). It can grow in variety of soil conditions, from well drained sandy or loamy soils to heavier clay soils. Currently the young seeds and pods are used as vegetables, the extracted oil from the kernels for industrial purposes, the water extract as a water purifier, the seed cake as fertilizer and feed and various parts (e.g. roots, bark, sap, leaves, oil and flowers) of the plant are used in traditional medicine in several countries (Foidl *et al*., 2001). Moreover, *Moringa* micronutrient liquid is a natural anti-helmintic product and Fahey (2005) reported that because *Moringa* plant is full of leaves at the end of the dry season in the tropics, when other foods are typically scarce, this plant is specially promising as a traditional food source in Africa. Adesina *et al.* (2008) also reported that seeds of *Moringa* are effective as natural coagulant in water. Similarly, Adesina and Omitoyin (2011) reported that moringa fresh root-bark extract is effective as organic piscicide in aquaculture pond management. *Moringa* extracts have also been proven to show potentials of sanitizers or preservatives by inhibiting the growth of the test organisms, which range from food-borne pathogens to spoilage causing organisms in foods (Bukar *et al.,* 2010).

Moringa plant is fast growing and high yielding. Makkar and Becker (1999) reported a high biomass production of up to 120 tons dry matter (DM)/ha/yr in eight cuttings after planting one million seeds/ha. The plant starts to bear pods 6 – 8 months after planting but regular fruiting commences after the second year. The plant fruits for 30 – 40 years.

Nutrient Composition: Makkar and Becker (1997) worked extensively on the chemical composition of *M. oleifera* parts (Table 1). They reported that moringa leaves, the kernel and the fat free kernel meals contain 26.4%, 36.7% and 61.4% of crude protein, respectively. This has placed *Moringa* plant parts as potential protein source. *Moringa* kernel contains over 40% by weight of oil – the fatty acid composition is said to be similar to that of olive oil. The seed oil contains 3% palmitic acid, 7.4% stearic acid, 8.6% behenic acid and 65.7% of oleic acid among other fatty acids.

Table 1: Moringa oleifera nutritional value of leaves and pods

Nutrients	Pod	Leaves	Leave powder
Moisture (%)	86.9	75.0	7.5
Calories	26	92	205
Protein (g)	2.5	6.7	27.1
Fat (g)	0.1	1.7	2.3
Carbohydrate (g)	3.7	13.4	38.2
Fiber (g)	4.8	0.9	19.2
Minerals (g)	2.0	2.3	-
Ca (mg)	30	440	2,003
Mg (mg)	24	24	368
P (mg)	110	70	204
K (mg)	259	259	1,324
Cu (mg)	3.1	1.1	0.57
Fe (mg)	5.3	7	28.2
S (mg)	137	137	870
Oxalic acid (mg)	10	101	1.6%
Vitamin A - B carotene (mg)	0.11	6.8	16.3
Vitamin B -choline (mg)	423	423	-
Vitamin B1 -thiamin (mg)	0.05	0.21	2.64
Vitamin B2 -riboflavin (mg)	0.07	0.05	20.5
Vitamin B3 -nicotinic acid (mg)	0.2	0.8	8.2
Vitamin C -ascorbic acid (mg	120	220	17.3
Vitamin E -tocopherol acetate (mg)	-	-	113
Arginine (g/16g N)	3.6	6.0	1.33%
Histidine (g/16g N)	1.1	2.1	0.61%
Lysine (g/16g N)	1.5	4.3	1.32%
Tryptophan (g/16g N)	0.8	1.9	0.43%
Phenylanaline (g/16g N)	4.3	6.4	1.39%
Methionine (g/16g N)	1.4	2.0	0.35%
Threonine (g/16g N)	3.9	4.9	1.19%
Leucine (g/16g N)	6.5	9.3	1.95%
Isoleucine (g/16g N)	4.4	6.3	0.83%
Valine (g/16g N)	5.4	7.1	1.06%

Analysis of Moringa pods, fresh (raw) leaves and dried leaf powder have shown them to contain the above per 100 grams of edible portion. **Source:** Fahey (2005)

According to Makkar and Becker (1997), in addition to high macronutrient content, *Moringa* leaves and pods are rich in vitamins and minerals such as calcium, phosphorus, magnesium, ascorbic acid and tocopherol. The high true protein content of leaves (26.4% in DM), the presence of adequate levels of essential amino acids (higher than levels present in the FAO (2000) reference protein), and low levels of anti-nutrients are sure indicators of their high nutritive quality.

The high pepsin soluble nitrogen (82 – 91%) and the low acid detergent insoluble protein (1 – 2%) values for the seed meal suggest that most of the protein in the meal is available to most animals (Makkar and Becker, 1997). However, the seed meal is deficient in lysine, leucine, phenylalanine + tyrosine and threonine when compared to the standard FAO (2000) protein. Interestingly, the high content of these deficient amino acids in the leaf meal adequately compensates for them in the seed meal. Combination of seed and leaf meals in desired proportions might result in obtaining a plant-based protein that would favourably replace fishmeal in fish feeds.

Moringa Anti-nutrient Contents: Reports by Makkar and Becker (1997) strongly indicate that moringa leaves are free from anti-nutrients except for saponins (8.1%) and phenols (4.4 %). However, the concentration of phenols is much below the toxic threshold levels reported for animals. Saponins are inactive as far as haemolytic properties are concerned.

Makkar and Becker (1999) further reported that glucosinolates, lectins and alkaloids that form the major anti nutrient substances in

moringa seed meal could be easily removed by water extraction, although this method is capable of removing some soluble nutrients.
Bau *et al.* (1994) reported that the solid state fermentation of the seed meal by the use of *Rhizopus oligosporus* could be considered in *Moringa* studies since this mould has been found to degrade glycosinolates in defatted rapeseed meal.

Moringa in Fish Feed Trials: In agriculture, the use of *Moringa* in feed production is known especially for poultry feeds (Du *et al*., 2007; Oduro *et al*., 2008; Olugbemi *et al*., 2010; Zanu *et al*., 2012). There are virtually little known clear reports of utilizing *Moringa* seed or leaf meals as fish feed ingredients for replacement of fishmeal. However, Richter *et al*. (2003) in their preliminary laboratory feeding trials using *Tilapia niloticus* indicated that there was growth reducing effect at high levels (more than 50%) of inclusion of raw leaf meal of moringa. They suggested that moringa leaf meal could be included up to 10% of dietary protein in Nile tilapia. In their fishmeal replacement study, Afuang *et al*. (2003) fed *Oreochromis niloticus* (initial weights of 15.5 – 17.0 g) on varying amounts and extracts of moringa leaf meals to replace fishmeal and found that the relative liver weight was significantly influenced ($p < 0.05$). They reported that the hepatosomatic index (HSI) ranging from 1.5 to 2.7 correlated with body lipid incorporation and was obviously influenced by dietary nutrient intake and availability. In another study with methanolic extract of moringa not as replacement for fishmeal but as replacement for wheat meal, Dongmeza *et al*. (2006) conducted a research with diets 1 (control without any moringa product), 2, 3 (containing 10.6 and 17.7% of moringa leaf meal methanol extract), 4, 5 (containing 9.3 and 15.4%, respectively of a tannin-reduced fraction), 6, 7 (containing 2.6 and 4.3%, respectively of a saponin-enriched fraction), 8 and 9 (containing 7 and 11.6% of a tannin- and saponin-reduced fraction, respectively).

They reported that at the end of the experiment, a significant reduction ($P < 0.05$) of the growth performance of all the fish fed diets containing 80% methanolic extract of moringa or the extract fractions was generally observed when they were compared to the fish fed with the control diet. The whole body moisture, ash and crude protein of the fish fed diets containing moringa crude extract or extract fractions were not significantly different ($p > 0.05$) to those of the control group. Body lipid was significantly reduced for the fish fed the diets when compared to control. Muscle and plasma cholesterol levels were generally reduced for the fish fed diets containing moringa extract and extract fractions (except for the diet containing 15.4% of a tannin-reduced fraction of the methanolic extract of moringa (group 5) which showed higher muscle cholesterol than that of the control). The fish in the 10.6% moringa leaf meal methanol extract (groups 2) and 15.4%, respectively of a tannin-reduced fraction (group 5) had significantly lower hepatosomatic indices when compared to control. On the other hand, the intestinal somatic indices (ISI) of the groups 2, 3, 4, 5, 6 and 7 were generally higher than the control group and the groups 8 and 9 had lower ISI than the control group. They concluded that the relatively high total phenolics and saponins in diets 2 to 9 may have contributed to the poorer growth performance in these groups.

From more recent feed studies, it was discovered that pre-feeding of *Puntius altus* with leaf semi-powder extract of *M. oleifera* would be protective in reducing lead burdens in fish exposed to environments contaminated with waterborne lead (Sirimongkolvorakul *et al.,* 2012). Yuangsoi and Masumoto (2012) also worked on partial replacement of soybean meal not fishmeal with moringa leaf for fancy carp (*Cyprinus carpio*) (Table 2). The study indicated that the tested moringa leaf diet contained ingredients that could be used for fancy carp diets with possibly not over 20 g/kg soybean protein replacement without negative effect on growth and digestibility.

By processing moringa leaf meal (MLM), cassava leaf meal (CLM) and cassava root meal (CRM) in an attempt to remove the most significant anti-nutritional factors and substituting each ingredient for fishmeal in

Table 2: Ingredients and chemical composition of practical moringa leave meal based diets used in aquaculture

Ingredient (g Kg-1)	Protein replacement in soybean meal by moringa leaves (g kg-1)		
	0	**200**	**500**
Fish meal	320	320	320
Soybean meal	230	184	115
Moringa leaves	0	88	220
Wheat Flour	120	120	120
Cellulose	160	117.6	54.1
Fish oil	35	35	35
Soybean oil	35	35	35
Guar gum	10	10	10
Dicalcuim phosphate	20	20	20
Premix	70	70	70
L-Methionine	0	4	9
Total	1000	1000	1000
Nutrient composition by analysis (g kg-1 dry weight on basis)			
Protein	35.63 ± 1.95	34.67 ± 0.03	35.12 ± 1.07
Fat	9.39 ± 0.01	9.42 ± 0.04	9.37 ± 0.14
Fiber	2.11 ± 0.78	2.10 ± 0.17	2.18 ± 0.25
Dry matter	67. 60 ± 0.23	68.04 ± 0.28	67.68 ± 0.25
Ash	11.79 ± 0.09	11.52 ± 0.05	11.93 ± 0.45
Amino acid composition (g kg-1dry weight on basis)			
Histidine	2.50 ± 0.01	2.58 ± 0.02	2.74 ± 0.01
Arginine	20.38 ± 0.01	18.50 ± 0.01	15.86 ± 0.10
Asparagine	3.28 ± 0.03	3.83 ± 0.02	5.88 ± 0.03
Glutamic acid	3.19 ± 0.01	3.44 ± 0.04	4.45 ± 0.04
Alanine	4.36 ± 0.09	4.08 ± 0.01	4.03 ± 0.01
Proline	2.52± 0.02	3.30 ± 0.04	3.87 ± 0.05
Methionine	1.27 ± 0.03	1.08 ± 0.01	0.79 ±0.01
Valine	3.33 ± 0.03	3.30 ± 0.03	3.52 ± 0.04
Trytophane	n.d.	n.d.	n.d.
Leucine	9.33 ± 0.06	9.30 ± 0.04	9.53 ± 0.04
Lysine	6.11 ± 0.02	9.08 ± 0.01	10.53 ± 0.02
Cysteine	10.06 ± 0.10	8.62 ± 0.03	8.86 ± 0.07

Source: *Yuangsoi and Masumoto (2012)*

isonitrogenous (30g $100g^{-1}$), isolipidic (10g $100g^{-1}$) and isoenergetic (18 kJ g^{-1}) diets containing graded levels of the processed ingredients, Madalla (2008) fed to *Oreochromis niloticus* to their apparent appetite but not exceeding 10% of their body weight for a period of 8 weeks. He reported that:

a. inclusion of either of the leaf meals, even at the lowest level of 15g $100g^{-1}$ of total dietary protein, led to a significant reduction in feed intake, growth and feed utilization.

b. Liver and small intestine did not show any histopathological changes which could be linked to dietary treatment. Conversely, cassava root meal could replace up to 75% of wheat meal in the diet without significantly affecting performance.

c. The performance of leaf meals was marginally improved by a combination of blending and feeding stimulants, whereby a blend containing 1 part MLM and 2 parts CLM could provide up to 20g $100g^{-1}$ of dietary protein without significantly reducing performance.

d. Biological and economic performance of practical diets containing 30 – 50 g $100g^{-1}$ of dietary protein from moringa and cassava blends (LMB) with feeding stimulants was significantly lower than a fish meal (FM) based diet but comparable to a soybean meal-based diet (SBM).

e. The suitability of MLM and CLM as novel protein sources in *O. niloticus* diets will depend on i) improving reduction/removal of inherent anti-nutritional factors in MLM.

Reporting on the use of temperature treatment on moringa used in fish diets, Tagwireyi *et al.* (2009) reported that heat treatment methods they employed might have increased the digestibility of proteins and other dietary components such as starch related compounds leading to high FCR of 1.1 and PER of 1.9 - 2.0 in fish fed with moringa-treated diets. Also, they reported that steam treatment employed in their study might have resulted in little protein being denaturated thus making more quality protein to be made available in steamed leaves than in boiled leaves. They additionally reported that though boiling broke cell components like cell walls and cell membranes of plants cells, some of the nutrients not specifically mentioned within the cells of boiled moringa leaves were lost to boiling water during the heat treatment process. The soluble cell components such as soluble proteins and glucose molecules might have dissolved in water during boiling. This could have caused the reduction of essential amino acids (EAA) in diets. Boiling was thought to have caused the inactivation of anti-nutrients such as saponins, phytates, phenols and tannins that bind some quality proteins and inhibit digestion in fish. Some of their conclusions though speculative is interesting. Hussien *et al.* (2012) reported that *M. oleifera* leaf powder in the diet and Levamisol HCl as freshwater bath in addition to being a feed ingredient was useful in the treatment of the nematode *Anguillicola crassus which* infests eels (*Anguilla anguilla*).

Conclusion: We are face to face with a non timber forest product (NTFP), *Moringa oleifera* Lam regarded as a "wonder or miracle plant". In agriculture, particularly in aquaculture, there is virtually little known data for the integration of this plant in fish nutrition research. Hence, this plant presents an important area for aquaculture nutrition research.

REFERENCES

ADESINA, B. T., OMITOYIN, B. O., AGBEJA, B. O., ADEBISI, L. A. and ADEYEMO, A. A. (2008). Effectiveness of *Moringa oleifera* Lam. seeds as natural coagulant in public water treatment. *Obeche Journal,* 26(1): 68 – 77.

ADESINA, B. T. and OMITOYIN, B. O. (2011). Potential of *Moringa oleifera* Lam. fresh root-bark extract as an organic piscicide in aquaculture pond management. *Egyptian Journal of Biology,* 13: 8 – 13.

AFUANG, W., SIDDHURANJU, P. and BECKER, K. (2003). Comparative nutritional evaluation of raw, methanol extract residues and methanol extract of Moringa (*Moringa oleifera* Lam.) leaves on growth performance and feed utilization in Nile Tilapia (*Oreochromis niloticus* L.). *Aquaculture Research*, 34: 1147 – 1159.

ALLAN, G. (2010). Sustainable Aquaculture Feeds. Global Outlook for Aquaculture Production, Kuala Lumpur, 2010. http://www.gaalliance.org/update/GOAL10/Allan.pdf Assessed December, 2012.

BAU, H. M., VILLAUME, C., LIN, C. F., EVVRARD, J., QUEMENER, B., NICOLAS, J. P. and MEJEAN, L. (1994). Effect of a solid-state fermentation using *Rhizopus oligosporus* sp. T-3 on elimination of anti nutritional substances and modification of biochemical constituents of defatted rapeseed meal. *Journal of Science of Food and Agriculture,* 65: 315 – 322.

BUKAR, A., UBA, A. and OYEYI, T. I. (2010). Antimicrobial profile of Moringa oleifera Lam. extracts against some food-borne microorganisms. *Bayero Journal of Pure and Applied Sciences*, 3(1): 43 – 48.

DONGMEZA, E., SIDDHURAJU, P., FRANCIS, G. and BECKER, K. (2006). Effects of dehydrated methanol extracts of moringa (*Moringa oleifera* Lam.) leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (*Oreochromis*

niloticus (L.)). *Aquaculture*, 261(1): 407 – 422.

DU, P. L., LIN P. H., YANG, R. Y., FAN, Y. K. and HSU, J. C. (2007). Effects of dietary supplementation of *Moringa oleifera* on growth performance, blood characteristics and immune response in broilers. *Journal of the Chinese Society of Animal Science,* 36(3): 135 – 146.

FAHEY, J. W. (2005). *Moringa oleifera.* http://wapedia.mobi/Moringa oleifera#3. Accessed on August 22, 2010.

FAO (2000). Yearbook of Fishery Statistics 1998. Aquaculture production. Food and Agricultural Organization (FAO) Statistics Series No. 154 and Fisheries Series No. 56, FAO, Rome.

FAO (2012). The State of World Fisheries and Aquaculture 2012. Food and Agriculture Organization of the United Nations, Rome.

FOIDL, N., MAKKAR, H. P. S. and BECKER, K. (2001). The potential of *Moringa oleifera* for agricultural and industrial uses. Pages 45 – 76. *In*: FUGLIE, L. J. (Ed.). *The Miracle Tree: the Multiple Uses of Moringa.* CTA, Wageningen, The Netherlands.

FRANCIS, G., MAKKAR, H. P. S. and BECKER, K. (2001). Anti-nutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture,* 199: 197 – 227.

FRANCIS, G., MAKKAR, H. P. S. and BECKER, K. (2002). Products from little researched plants as aquaculture feed ingredients. Agrippa – FAO online Journal (www.fao.org/agrippa. Accessed on November 2, 2012.

HARDY, R. W. (2000). New developments in aquatic feed ingredients, and potential of enzyme supplements. Pages 216 – 226. *In*: CRUZ-SUÁREZ, L. E., RICQUE-MARIE, D., TAPIA-SALAZAR, M., OLVERA-NOVOA, M. A. Y. and CIVERA-CERECEDO, R. (Eds.). *Avances en Nutrición Acuícola V. Memorias del V. Simposium Internacional de Nutrición Acuícola*, 19 – 22 Noviembre, 2000. Mérida, Yucatán, Mexico.

HELLER, J. (1996). Physic nut *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops I. Institute of Plant Genetics and Crop Plant Research, Gatersleben. International Plant Genetic Resources Research Institute, Rome.

HOSSAIN, M. A. and BECKER, K. (2001). Nutritive value and anti-nutritive factors in different varieties of *Sesbania* seeds and their morphological fractions. *Food Chemistry,* 196: 105 – 123.

HUNTINGTON, T. C. and HASAN, M. R. (2009). Fish as feed inputs for aquaculture – practices, sustainability and implications: a global synthesis. Page 61. *In:* HASAN, M. R. and HALWART, M. (Eds). *Fish as Feed Inputs for Aquaculture: Practices, Sustainability and Implications. FAO Fisheries and Aquaculture Technical Paper,* Number 518, FAO, Rome.

HUSSIEN, A. M. O., ABD EL-MOHSEN, H. and MOHAMED, A. (2012). Studies on anguillicoliasis in cultured *Anguilla anguilla* fish farms in Delta region, Egypt with special reference to hematological, biochemical changes and treatment. *Researcher* 4(11): 77 – 83.

ISAAC, N. (2012). Reaping the gains of R&D in *Moringa oleifera*. http://leadership.ng/nga/articles/31240/2012/07/31/reaping_gains_rd_moringa_oleifera.html. Assessed November 28, 2012.

MADALLA, N. (2008). Novel Feed Ingredients for Nile Tilapia (*Oreochromis niloticus* L.) A thesis submitted for the degree of Doctor of Philosophy, Institute of Aquaculture, University of Stirling, Scotland, United Kingdom.

MAKKAR, H. P. S. and BECKER, K. (1997). Nutrients and antiquality factors in different morphological parts of the *Moringa oleifera* tree. *Journal of Agricultural Science,* 128: 311 - 322.

MAKKAR, H. P. S. and BECKER, K. (1999). Plant toxins and detoxification methods to improve feed quality of tropical seeds –

Review. *Asian–Australian Journal of Animal Science*, 12 (3): 467 - 480.

NAYLOR, R. L., GOLDBERG, R. J., PRIMAVERA, J. H., KAUSTSKY, N., BEVERIDGE, M. C., CLAY, J., FOLKE, C., LUBCHENCO, J., MOONEY, H. and TROELL, M. (2000). Effect of aquaculture on world fish supplies. *Nature*, 405: 1017 – 1024.

ODURO, I., ELLIS, W. O. and OWUSU, D. (2008). Nutritional potential of two leaf vegetables: *Moringa oleifera* and *Ipomoea batata* leaves. *Scientific Research and Essay*, 3(2): 57 – 60.

OGBE, A. O. and AFFIKU, J. P. (2011). Proximate study, mineral and anti-nutrient composition of Moringa oleifera leaves harvested from Lafia, Nigeria: potential benefits in poultry nutrition and health. *Journal of Microbiology, Biotechnology and Food Sciences,* 1(3): 296 – 308.

OLUGBEMI, T. S., MUTAYOBA, S. K. and LEKULE, F. P. (2010). Effect of moringa (*Moringa oleifera*) inclusion in cassava based diets fed to broiler chickens. *International Journal of Poultry Science*, 9(4): 363 – 367.

RICHTER, N., SIDDHURAJU, P. and BECKER, K. (2003). Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L). *Journal of Ethnopharmacology,* 79: 325 – 329.

SIDDHURAJU, P. and BECKER, K. (2001). Preliminary nutritional evaluation of mucuna seed meal (*Mucuna pruriens* var. *utilis)* in common carp (*Cyprinus carpio* L): an assessment by growth performance and food utilization. *Aquaculture,* 196: 105 – 123.

SIDDHURAJU, P., MAKKAR, H. P. S. and BECKER, K. (2002). The effect of ionizing radiation on anti nutritional factors and the nutritional value of plant materials with reference to human and animal food. *Food Chemistry,* 48: 6048 – 6060.

SIRIMONGKOLVORAKUL, S., TANSATIT, T., PREYAVICHYAPUGDEE, N., KOSAI, P. JIRAUNGKOORSKUL, K. and JIRAUNGKOORSKUL, W. (2012). Efficiency of *Moringa oleifera* dietary supplements reducing lead toxicity in *Puntius altus. Journal of Medicinal Plants Research*, 6(2): 187 – 194.

TACON, A. G. J. and FORSTER, I. P. (2001). Global trends and challenges to aquaculture and aquafeed development in the new millennium. http;//www.seaweb.org/AAAS/trends html. Accessed on July 3, 2001.

TACON, A. G. J. and METIAN, M. (2009). Fishing for feed of fishing for food – Lenfes Ocean Program. *Ambio*, 38(6): 294 – 302.

TACON, A. G. J. (2011). The role of rendered products in aquaculture. http://cdn.harmonyapp.com/assets/4f26fba7dabe9d49a7063c1c/9_albert_tacon_rendered_products_in_aquaculture2.pdf. Assessed January 1, 2013.

TAGWIREYI, T., MUPANGWA, J. F., JEPSEN, J. and MWERA P. (2009). Effect of feeding *Moringa oleifera* leaf meal on the growth performance of *Oreochromis niloticus* fry. www.appropriatetech.net/files/3rd_ICAT_Proceedings_Part_5.pdf . Assessed on November 30, 2012.

YUANGSOI, B. and MASUMOTO, T. (2012). Replacing moringa leaf (*Moringa oleifera*) partially by protein replacement in soybean meal of fancy carp (*Cyprinus carpio*). *Songklanakarin Journal of Science and Technology*, 34(5): 479 – 485.

ZANU, H. K., ASIEDU, P., TAMPUORI, M., ABADA, M. and ASANTE, I. (2012). Possibilities of using moringa (*Moringa oleifera*) leaf meal as a partial substitute for fishmeal in broiler chickens diets. *Online Journal of Animal Feed Research*, 2(1): 70 – 75.

ENTOMOREMEDIATION - A NOVEL *IN-SITU* BIOREMEDIATION APPROACH

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ABSTRACT

In this paper entomoremediation as a novel concept was critically projected as a bioremediation technique that needs to be harnessed in line with global realities of involving organisms like microorganisms and earthworms in soil decontamination. Entomoremediation is defined as a type of remediation in which insects are used in order to decontaminate a degraded soil. The candidacy of collembolans, ants, beetles and termites in entomoremediation is advocated because of their role as ecosystem engineers. The need for mass rearing of the insects to be used in proposed bioremediation is discussed. Bioremediation as a measure that requires interdisciplinary approach is emphasized. The need to use insects that are neither threatened or endangered in entomoremediation in order to achieve overall healthy balance of the soil environment is stressed.

Keywords: Entomoremediation, Bioremediation, Insects, Decontaminate, Degraded soil, Ecosystem

INTRODUCTION

Bioremediation is defined as the use of biological processes to degrade, break down, transform and / or essentially remove contaminants or impairments from soil and water (Donlon and Bauder, 1980). A lot of interest has been generated in developing *in situ* strategies for remediating environmental contaminants (Gerhardt *et al*., 2008). The advantages of *in situ* bioremediation techniques have been outlined by Donlon and Bauder (1980) while the overview of *ex situ* decontamination techniques for polluted soil has been discussed (Pavel and Gavrilescu, 2008) with their merits and demerits outlined.

Hitherto, bioremediation has traditionally been viewed as a technique that will stimulate the growth of microorganisms or provide conducive environment for their activity (Pavel and Gavrilescu, 2008) alongside the organisms involved in the bioremediation (Chukwura, 2012).

Soil is a fragile ecosystem Soil however can be contaminated by a number of factors including chemicals (e.g. petroleum products like hydrocarbons), pesticides and heavy metals and even anthropogenic factors in which the nature of the soil can be altered. A contaminated soil can constitute hazard not only to human health but can pose a threat to the ecosystem, with a lot of techniques emerging to remediate the soil on-site (Gimsing *et al*., 2004). In this paper the concept of entomoremediation as an *in situ* soil decontamination technique is advocated to add to the existing *in situ* and *ex situ* soil remediation techniques being harnessed all over the world.

MATERIALS AND METHODS

A comprehensive search was made from the Internet, various journal articles and textbooks for reports on the use of insects, insect parts and biochemicals for bioremediation in various parts of the world.

Such articles were assembled, studied and represented in this review.

RESULTS AND DISCUSSION

Entomoremediation – A Novel In Situ Soil Decontamination Technique: Literature is rich regarding the use of *in situ* and *ex situ* bioremediation measures for decontamination of the soil (Gimsing *et al.,* 2004; Gavrilescu, 2006; Pavel and Gavrilescu, 2008; Chukwura, 2012) but no mention has been made on the possibility of use of insects in soil decontamination. No name has also been given to describe the measure. This remediation measure can be called entomoremediation and the insects so utilized entomoremediators. Entomoremediation can be defined as a type of remediation in which insects are used in order decontaminate a degraded soil.

The candidacy of collembolans, beetles and termites is advocated in the proposed entomoremediation. These four groups of insects - collembolans, ants, beetles and termites have been classified as ecosystem engineers (Jones *et al*., 1994; Folgrait, 1998; Badejo *et al.,* 2004; Badejo, 2012; Ewuim *et al.,* 2012). The dung beetle, for example, which have also been implicated as ecosystem engineers and have been protected in several countires (Figure 1) (Boze *et al*., 2011; Ewuim *et al.,* 2012), can contribute to ecosystem health by enhancing nutrient cycling and fertilizing by aerating soil (Halffter and Matthews, 1966; Boze *et al.,* 2011). Entomoremediation for instance can be useful in decontaminating a soil polluted with heavy metals, after soil ecotoxicological risk assessment, already discussed by Van Gestel (2012). Just like plants are known to sequester certain elements in their tissues (Marscher, 1995; Justin *et al.,* 2011), most soil invertebrates have the capacity to sequester at least a portion of their heavy metal burden at least in such a way that it does no longer pose a risk (Van Gester, 2012). Soil invertebrates like collembolans and beetles use the midgut epithelium for storing metals (Van Gester, 2012). During moulting the renewal of the midgut epithelium allows these organisms to excrete excess metal (Hopkin, 1989; Van Gester, 2012).

Figure 1: Dung beetles are extremely important environmental engineers and even protected in various parts of the world (Garrison, 2002)

Mass Rearing of the Insects is Implicit: After the study of biological indices for example as noted by Knoepp *et al*. (2000) and the investigation of the soil ecotoxicity already reported by Van Gester (2012), mass rearing of the insect of choice is necessary either in the laboratory or field cages. The insect(s) of choice may be one or a combination of desired species from the four insects groups already advanced – collembolans, ants, beetles (e.g. dung beetles), and termites before their introduction into the field for decontamination. Mass rearing of these insects of choice in entomoremediation will achieve sustainability of the desired insect species that will be released in the field for decontamination. Apart from the dung beetles which have been mass reared (McKay, 1976; Hayakawa and Kamashita, 1990) and which have witnessed large scale release in the field (McKay, 1976) after rearing, termites including the African giant termites (*Macrotermes jeanneli*) have been mass reared in the laboratory and in the vivarium (Leuthold

et al., 2004). Instance of mass rearing of collembolans e.g. *Anurida granaria* by Lynch (2001) indicate that *A. granaria* is cosmopolitan and ideal, safe and harmless for import/export and invariably not threatened or endangered. Literature is however copious on the various insects so mass reared for various purposes but cannot be exhausted here.

Entomoremediation versus Vermi-remediation: It is being advanced that if earthworms which have been classified as ecosystem engineers by Badejo *et al*. (2004), Badejo (2011) and Ewuim *et al.* (2011) can have the capability of converting a 'wasteland' into 'wonderland' in vermiculture (Sinha *et al.,* 2008), the inclusion of entomoremediation for decontamination of soils polluted even by heavy metals is advocated. Earthworms also can bio-accumulate high concentrations of metals including heavy metals in their tissues without affecting their physiology (Ireland, 1983; Sinha *et al*., 2008).

As vermiremediation may prove very cost-effective and environmentally sustainable in handling polluted soils and sites contaminated with hydrocarbons even in few weeks to months (Sinha *et al.,* 2008), entomoremediation has the potential of decontaminating soils polluted with heavy metals and hydrocarbons provided that any routine ethical issues in the use of these invertebrates in the novel entomoremediation are observed and that the insect species involved are not threatened or endangered species as applicable in vermiremediation studies.

Conclusion: In total, bioremediation measures require interdisciplinary approach. Many organisms ranging from microorganisms to the earthworms that have played their role in the recovery of the degraded soils clearly demonstrate a universal effort to evolve cost-effective methods of utilizing the potentials in these groups of organisms in salvaging the soil environment. Harnessing of the potentials of insects that are not threatened or endangered in entomoremediation will further enhance the restoration of a healthy balance of the soil environment.

REFERENCES

BADEJO, M. A., TIAN, G. and OKOH, I. O. (2004). Litter transformers an ecosystem engineers in sustainable agro ecosystems. Pages 86 – 106. *In.* BADEJO, M. A. and TOGUN, A. O. (Eds.) *Strategies Tactics of Sustainable Agriculture in the Tropics.* Volume 2, College Press Ibadan and Enproct Consultants, Lagos, Nigeria.

BADEJO, M. A. (2012). Ecosystem Engineering as a concept: The significance of functional classification of the true engineers. *Environtropica,* 8: 194 – 199.

BOZE, B. G. V., HERNANDEZ, A. D., HUFFMAN, M. A. and MOORE, J. (2011). Parasites and dung beetles as ecosystem engineers in a forest ecosystem. *Journal of Insect Behaviour,* 25(4): 352 – 361.

CHUKWURA, E. I. (2012). Microbial transformation of biospheric wastes for economic growth. Pages 1 – 93. *In: Inaugural Lecture Series Number 21,* Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

DONLON, D. L. and BAUDER, J. W. (1980). A general essay on bioremediation of contaminated soil. *waterquality.montana.edu/docs/metha.* Retrieved on 21st November, 2012.

EWUIM, S. C., AKUNNE, C. E. and FANIRAN, O. J. (2012). The role of soil insects as allogenic ecosystem engineers in Nigeria. *Environtropica,* 8: 187 – 193.

FOLGARAIT, P. J. (1998). Ant biodiversity and its relationship to ecosystem functioning: a review. *Biodiversity* and *Conservation*. 7: 1221-1244.

GARRISON, L. (2002). Photos obtained online February 14, 2012 at http://0.tqn.com/d/cruises/1/0/o/a/4/Addo_Elephant_Park_02.JPG

GERHARDT, K. E., HUANG, X., GLICK B. R. and GREENBERG, B. M. (2008). Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Science,* 176: 20 – 30.

GIMSING, A. L., HANSEN, J. B., PERMILD, E., SCHWARZ, G. and HANSEN, E. (2004).

In-Situ Bioremediation of Oil Contaminated Soil-Practical Experiences from Denmark. www.eugris.info/news downloads/green. Obtained online February 14, 2012.

HALFFTER, G. and MATHEWS, E. G. (1966). The natural history of dung beetles of the subfamily Scarabaeinae (Coleopera: Scarabaeidae). *Folia Entomologia,* 12(14): 312.

HAYAKAWA, H. and HAMASHITA, N. (1990). Studies in mass rearing methods of the dung battle *Onthophagus gazella* 4. A preliminary testing. *Annual Report of the Society of Plant Protection of North Japan,* 41: 182 – 183.

GAVRILESCU, M. (2006). Overview of *in situ* remediation technologies for sites and groundwater.*Environmental Engineering and Management Journal,* 5: 79 – 114.

IRELAND, M. P. (1983). Heavy metal uptake and tissue distribution. Pages 245 – 265. *In:* SATCHEL J. E. (Ed). *Earthworm Ecology, from Darwin to Vermiculture*. Chapman and Hall, London.

JONES, C. G., LAWTON, J. H. and SHACHAK, M. (1994). Organisms as ecosystem engineers *Oikos*, 69: 373 – 386.

JUSTIN, V., MAJID, N. M., ISLAM, M. M. and ABDU, A. (2011). Assessment of heavy metal uptake and translocation in *Acacia mangium* for phytoremediation of cadmium contaminated soil. *Journal of Food, Agriculture and Environment,* 9(2): 588 – 592.

KNOEPP, J. D., COLEMAN, D. C., CROSSLEY, JR. D. A. and CLARK, J. S. (2000). Biological indices of soil quality: an ecosystem case study of their use. *Forest Ecology and Management,* 138(1-3): 357 – 368.

LEUTHOLD, R. H., TRIET, H. and BERN, B. S. (2004). Husbandry and breeding of African giant termites (*Macrotermes, jeanneli*) at Berne animal park. *Der Zoologische Garten,* 74: 26 – 37.

LYNCH, T. A. (2001). Methodology and applications for the collection, rearing and handling of *Anurida granaria* (Nicolet, 1884) as a universal, safe transportable species ideally suited for teaching and demonstrating basic entomological techniques and exhibit of bioluminescence in collembolan. http: //www.byteland.org/bioluminus/geo.ht ml. Obtained online February 14, 2012.

MARSCHNER, H. (1995). *Mineral Nutrition of Higher Plants.* 2nd Edition, Academic Press, New York.

MCKAY, A. (1976). The industrious dung beetle, surprise and enterprise. *In:* WHITE, F., and KIMPTON, D. (Eds.), *Fifty Years of Science for Australia's CSIRO*, CSIRO Publishing, Australia.

PAVEL, L. V. and GAVRILESCU, M. (2008). Overview of *ex situ* decontamination techniques for soil *cleanup. Environmental Engineering and Management Journal,* 7(6): 815 – 834.

SINHA, R. K., BHARAMBE, G. and RHYAN D. (2008). Converting wasteland into wonderland by earthworms; a low-cost nature's technology for soil remediation: a case study of vermiremediation of PAHs contaminated soil. *The Environment,* 28: 466 – 475.

VAN GESTEL, C. A. M. (2012). Soil ecotoxicology: State of the art and future directions. *Zookeys,* 1766: 275 – 296.

EFFECT OF VITAMIN C TREATMENT ON SERUM PROTEIN, ALBUMIN, BETA-GLOBULIN PROFILES AND BODY WEIGHT OF *Trypanosoma brucei*-INFECTED *Rattus norvegicus*

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ABSTRACT

The effect of vitamin C supplements on serum protein profile and body weight of Trypanosoma brucei-infected rats was investigated. The rats were inoculated with trypanosomes intraperitoneally and samples were collected on fourth, eight, twelfth and sixteenth days of post infection (pi). Sixty (60) parasite free-albino rats were used, which were divided into four groups. Group A (control) was left uninfected with trypanosomes, group B and C were infected with Trypanosomes and treated with 40mls and 60mls of ascorbic acid (Vitamin C), respectively. Trypanosoma brucei infection caused significant ($p<0.01$) decreases in serum total proteins, albumin, beta globulin and body weight levels in untreated rats. Consumption of Vitamin C, however, prevented these disease-induced anomalies in the treated infected rats. Analyses of the sera using Bradford method and cellulose acetate electrophoresis showed that Vitamin C infected the state of serum protein, albumin and gamma globulin in the trypanosome-infected treated rats. It was concluded that consumption of the Vitamin C ameliorated the pathological changes in serum protein and body weight of T. brucei – infected rats.

Keywords: *Trypanosoma brucei, Rattus norvegicus,* Ascorbic acid, Body weight, Serum protein

INTRODUCTION

Trypanosomiasis is still a major factor retarding the growth of livestock in Africa. It is one of the most important livestock diseases in sub-Sahara Africa (Morrison *et al.,* 1981). Economic loss due to this disease runs into hundreds even higher in other parts of Africa (Doko *et al.,* 1991). Trypanosome is known to attack red blood cells and vascular endothelium. It concentrates more in the peripheral circulation (Jackson, 1979). The first wave of parasitaemia is accompanied by depressed packed cell volume, neutropenia and thrombocytopenia (Schoral *et al.,* 1981). Trypanosomiasis is characterized by tissue and organ degenerative changes. One factor implicated in the pathogenesis of the disease is oxidative stress (Vray *et al.,* 1991) imposed by trypanosome and macrophageal activities. Oxidative stress has been alleviated in experimental infections with various species of trypanosomes (Umar *et al.,* 1999a; 2000) by administration of exogenous antioxidants, such as ascorbic acid and/or Vitamin E to infected rats and rabbits (Umar *et al.,* 1999b; 2000; 2008). This vitamin therapy considerably reduced the degree and rate of degeneration of tissues and organs and in some instances

significantly reduced the parasitaemia and anaemia in the trypanosome-infected animals (Umar *et al.,* 1999b). This study was designed to evaluate to what extent this ascorbic acid can influence the state of serum protein profile and body weight in trypanosome-infected rats.

MATERIALS AND METHODS

Sixty adult male albino rats (*Rattus norvegicus*) weighing approximately 145 ± 2.13g, were used for this experiment. The rats were marked for identification and held in stainless wire rats cages in clean experimental animal house. The cages were labeled A to D corresponding to four (4) groups, replicated thrice with each replicate having five (5) rats. Rats in cage A were not infected while rats in cages B, C and D were infected with *Trypanosoma brucei*. One rat was first inoculated with trypanosome of NITR type from Veterinary Medicine Faculty, University of Nigeria, Nsukka. It was isolated from other animals and after 14 days of inoculation, the blood of that rat was used to inoculate others. Each experimental rat was intraperitoneally infected with about 10^6 cells of *Trypanosoma brucei* in 0.5 ml of cold saline diluted blood from the donor rat, using a matching chart to determine the level of parasitaemia (Herbert and Lumbsden, 1976). Infected animal were monitored for 7 day for the establishment of *Trypanosoma brucei.* Rats in cages A and D served as control groups. Diet 1 given to rats in cage A contained 1 kg of chick mash without Vitamin C (control). Diet 2 was given to rats in cage B and contained 1 kg of chick mash mixed with 40 ml of Vitamin C. Diet 3 was used to feed rats in cage C and had 1 kg of chick mash mixed with 60 ml of Vitamin C and Diet 4 was used to feed rats in cage D which contained 1 kg of chick mash without any Vitamin C. Each experimental set up was replicated three times. The rats had unlimited supply of clean water. Five (5) ml of the blood of the rats were collected at four days intervals for sixteen days experimental period to determine the total serum albumin and gamma globulin concentrations. The collected blood was allowed to clot for about 30 minutes at room temperature. Then each sample was centrifuged at 3, 000 rpm for 15 minutes and then serum was removed. The sera were used for total serum protein and serum fractions determination using Bradford method and cellulose acetate electrophoresis (Herbert and Lumbsden, 1976). The absorbance of the solutions was read at 520 nm-wavelengths using spectrophotometer. As an index of the physical status of the animals, the weight of each experimental rat was monitored over the period of study. Initial weights and interval weights of all experimental rats were taken at day 0, 4, 8, 12 and 16. The data were analyzed by one – way ANOVA, significant differences of treatment means were established using F-LSD at $p < 0.05$.

RESULTS AND DISCUSSION

The result obtained indicated that administration of Vitamin C positively influenced the serum profile and body weight of trypanosome infected rats. The lowest level of total serum protein of 49.12± 5.22g/l, albumin of 17.77± 3.52g/l and beta – globulin of 3.46± 0.82g/l was observed in infected untreated rats (cage D rats). Followed by 50.79± 4.01 g/l total serum protein, 20.76± 3.52 g/l albumin, 5.16± 0.54 g/l beta – globulin observed in infected rats treated with 40 ml of Vitamin C per kg of chick mash (cage B rats). Infected rats treated with 60 ml of Vitamin C per kg of chick mash had 52.71± 4.02 g/l albumin and 5.38 ± 0.63 g/l beta – globulin (cage C rats). The highest serum biochemical level was seen in cage A rats which had 60.99 ± 0.48 g/l total serum protein, 32.24 ± 0.43 g/l albumin and 8.26 ± 0.23 g/l beta – globulin (Table 1). The body weight were 120 ± 7.07, 129.75 ± 7.05, 133.50 ± 4.35 and 153.00 ± 5.12 grams corresponding to treatments D, B, C and A, respectively (Table 2).

Several scientific researches have been done on trying to identify and standardize active food supplement that would be active in treatment of trypanosomiasis. Trypanosomiasis is characterized by tissue and organ degenerative changes. One factor implicated in the pathogenesis of the disease is oxidative stress imposed by trypanosome and

Table 1: Total serum protein, albumin and beta-globulin concentrations in *Trypanosoma brucei* - infected *Rattus norvegicus* administered vitamin C for 16 days

Duration of experiment (days)	(Cage A) Uninfected rats + 0 ml Vitamin C (-ve control)	(Cage B) Infected rats + 40 ml Vitamin C	(Cage C) Infected rats + 60 ml Vitamin C	(Cage D) Infected rats + 0 ml Vitamin C (+ve control)
		Serum protein (g/l)		
4	60.60 ± 0.46[a]	59.60 ± 0.78[a]	59.84 ± 0.43[a]	58.80 ±0.45[a]
8	59.82 ± 0.46[a]	55.86 ± 0.24[a]	56.24 ± 0.46[a]	54.20 ± 0.50[a]
12	61.68 ± 0.49[a]	50.88 ± 0.45[a]	53.46 ± 0.84[a]	48.82 ± 0.45[a]
16	61.86 ± 0.48[a]	36.80 ± 0.53[a]	41.31 ± 0.71[a]	34.64 ± 0.46[a]
Group Mean	60.99 ± 0.48[a]	50.79 ± 4.01[b]	52.71 ± 4.02[c]	49.12 ± 5.22[d]
		Albumin (g/l)		
4	32.70 ± 0.48[a]	30.02 ± 0.26[a]	30.22 ± 0.46[a]	28.00 ± 0.28[a]
8	30.94 ± 1.06[a]	20.40 ± 0.01 [a]	23.90 ± 0.47[a]	15.62 ± 0.01[a]
12	32.68 ± 0.50[a]	19.74 ± 0.48[a]	22.06 ± 0.46[a]	15.70 ± 0.48[a]
16	32.64 ± 0.01[a]	12.88 ± 0.23[a]	17.08 ± 0.46[a]	11.74 ± 0.01[a]
Group Mean	32.24 ± 0.43[a]	20.76 ± 3.52[b]	23.32 ± 2.70[c]	17.77 ± 3.52[d]
		Beta-globulin (g/l)		
4	7.88 ± 0.01[a]	6.44 ± 0.01[a]	6.92 ± 0.01[a]	5.84 ± 0.00[a]
8	8.66 ± 0.00[a]	5.20 ± 0.01[a]	5.78 ± 0.01[a]	3.20 ± 0.01[a]
12	8.18 ± 0.01[a]	4.22 ± 0.01[a]	4.88 ± 0.02[a]	2.68 ± 0.02[a]
16	8.33 ± 0.01[a]	4.78 ± 0.02[a]	3.92 ± 0.00[a]	2.11 ± 0.02[a]
Group Mean	8.26 ± 0.23[a]	5.16 ± 0.54[a]	5.38 ± 0.63[a]	3.46 ± 0.82[a]

Table 2: Body weight (g) of *Trypanosoma brucei* - infected *Rattus norvegicus* administered vitamin C for 16 days

Duration of experiment (days)	(Cage A) Uninfected rats + 0 ml Vitamin C (-ve control)	(Cage B) Infected rats + 40 ml Vitamin C	(Cage C) Infected rats + 60 ml Vitamin C	(Cage D) Infected rats + 0 ml Vitamin C (+ve control)
4	142.00 ± 0.94[a]	150.00 ± 0.94[a]	146.00 ± 1.70[c]	138.00 ± 1.25[d]
8	147.00 ± 0.82[a]	128.00 ± 2.62[b]	132.00 ± 1.25[c]	122.00 ± 1.25[d]
12	164.00 ± 1.70[a]	123.00 ± 1.70[b]	130.00 ± 1.63[c]	116.00 ± 2.50[d]
16	159.00 ± 1.25[a]	118.00 ± 3.40[b]	126.00 ± 1.25[c]	104.00 ± 1.25[d]
Group Mean	153.00 ± 5.12[a]	129.80 ± 7.05[b]	133.50 ± 4.35[c]	120.00 ± 7.07[d]

macrophageal activities. Oxidative stress has been alleviated in experimental infections with various species of trypanosomes by administration of exogenous antioxidants, such as ascorbic acid to infected rats and rabbits (Umar *et al.,* 1999b; 2000; 2008). The observed effect of vitamin C supplement from this study on the serum protein of trypanosome infected rats was attributed to its effect on the haemopoietic system (Vray *et al.,* 1991). Its effect on the infected treated rats when compared with the infected untreated rats showed that vitamin C had positive influence on the defense capacity of infected treated rats (Schoral *et al.,* 1981). Therefore, this study has provided evidence that vitamin C has potential for influencing the state of hypoproteinaemia in

the trypanosome-infected rats. Even if vitamin C cannot destroy the trypanosomes, it can ameliorate the stress of trypanosomiasis and boost the host's immune system to fight the invaded pathogens.

Conclusion: From the research, we thus conclude that consumption of dietary vitamin C enhances the immune system of animals. It is advisable to include vitamin C in the feed of livestock because it boosts the immune system of animals. Also in the treatment of trypanosomiasis, it is advisable to add vitamin C to infected animals' feed for quick recovery.

REFERENCES

DOKO, A., GUECLEGDE, B., BACIMANS, R., DRBEY, I., DIAVE, N., PANDEY, V. S. and VERHUISI, A. (1991). Trypanosomiasis in different breeds of cattle from Benin. *Veterinary Parasitology,* 40: 1 – 7.

HERBERT, W. J. and LUMBSDEN, W. H. R. (1976). *Trypanosoma brucei.* A rapid matching method for estimating the host's parasitaemia. *Experimental Parasitology,* 40: 427 – 431.

JACKSON, G. J. (1979). *Trypanosoma congolense*: inheritance of susceptibility to infection in inbred strains of mice. *Experimental Parasitology,* 48: 378 – 383.

MORRISON, W. I., MURRARY, M. and MCLNTYRE, W. I. M. (1981). Bovine trypanosomiasis. Pages 469 – 497. *In:* RISSTIC, M. and MCLNTYRE, W. I .M. (Eds), *Disease of Cattle in the Tropics.* Martins Nijhoff Publishers, The Hague, The Netherlands.

SCHORAL, C. J., TORMEY, W. P., BROOKS, G. H., ROBERTS LAW, A. M., YOUNG, G. A., TALUKDAR, R., and KELLY, J. F. (1981). The effect of vitamin C supplements on body weight, serum proteins, and general health of an elderly population. *American Journal of Clinical Nutrition,* 34(5): 871 – 876.

UMAR, I. A., TOH, Z. A., IGBALAJOBI, F. I., IGBOKWE, I. O. and GIDADO, A. (1999a). The effect of orally administered vitamin C and E on the severity of anaemia in *T. brucei*-infected rats. Tropical Veterinarian, 18: 71 – 78.

UMAR, I. A., WURO-CHEKKE, A. U., GIDADO, A. and IGBOKWE I. O. (1999b). Effects of combined parental vitamin C and E administration on the severity of anaemia, hepatic and renal damage in *T. brucei*-infected rabbits. *Veterinary Parasitology,* 85: 43 – 47.

UMAR, I. A., TOH, Z. A., IGBALAJOBI, F. I., GIDADO, A. and BURATAI, L. B. (2000). The role of vitamin administration in alleviation of organ damage in rats infected with *T. brucei.* Journal of Clinical Biochemistry and Nutrition, 28: 1 – 7.

UMAR, I. A., RUMAH, B. L., BULUS, S. L., KAMLA, A. A., JOBIN, A., ASUELIMAN, B. I., MAZAI, M. H., IBRAHIM, M. A. and ISAH, S. (2008). Effect of intraperitoneally administration of vitamin C and E or A and E combinations on the severity of *Trypanosoma brucei* infection in rats. *African Journal of Biochemistry Research,* 2(3): 88 – 91.

VRAY, B., DEBAETSELIER, P., OUAISIS, A. and CARLIER, Y. (1991). *Trypanosoma cruzibut* not *T. brucei* fails to induce a chemiluminescent signal in a macrophage hybridoma cell line. *Infections and Immunology,* 59: 3303 – 3308.

HISTOLOGICAL STUDIES OF SOME ORGANS OF SQUIRRELS (*Xerus erythropus*) IN TROPICAL ECOLOGICAL ZONE

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ABSTRACT

The functional morphology of some organs of squirrels was investigated through histological observations. A total number of thirty two (32) ground squirrels (Xerus erythropus); 15 males with mean weight 220.0 ± 2.0 g and length 40.0 ± 0.2 cm, and 15 females with mean weight 229.8 ± 2.0 g and length 39.0 ± 2.0 cm were used. The ground squirrels were trapped using rodent traps, anesthetized identified to species level and dissected to expose the viscera. The organs (liver brain, heart, gonads (testes and ovaries) were dissected out, clean off ceolomic fluid, fixed in normal buffered saline prior to the histological studies of the tissues. The organs of squirrel from different zones were examined at the light microscopy. The results compared clearly showed normal tissues without degenerations, the tissue histology showed almost the same pattern and arrangement of the cells in the tissues. The liver develops embryologically outgrowth of the gut. The endocardium, with all its endothelial lining and supporting tissues, accommodates movement of the myocardium. The cerebellar cortex forms a series of deeply convoluted folds or folia. The spermatozoon is absorbed into the lumen of the tubule, where they are drawn into the epididymal walls of the testis while the primary oocyte is surrounded by a single layer of flattened follicular cells.

Keywords: Histology, Liver, Heart, Brain, Testes, Ovary, Squirrel

INTRODUCTION

Squirrels belong to a large family of small or medium-sized rodents called *Sciuridae* (Steppan *et al.,* 2004) which includes; tree squirrels, ground squirrels (Friggens, 2002), chipmunks (Baack, and Paul, 2003), marmots (including woodchucks), flying squirrels, and prairie dogs (Foltz and Hoogland 1981). Ground-dwelling species are generally social animals, often living in well-developed colonies, but the tree-dwelling species are more solitary (Milton, 1984). The ground species (*Xerus erythropus)* are typically diurnal, while flying squirrels tend to be nocturnal - except lactating flying squirrels and their offspring, which have a period of diurnality during the summer (Thorington and Hoffman, 2005). They are cosmopolitan in their distribution, except in the Polar Regions. In the wild, most species of squirrels are threatened or endangered. Human activities are responsible for direct (i.e. hunting

squirrel for food and trapping of same for research or display), or indirect (i.e. habitat destruction) decimation of wild populations. Although about 9,000 squirrels are still being imported into the United States of America annually for research, there is increasing emphasis on obtaining "bred for purpose" animals for research. Squirrels feed primarily on seeds and plant. Difference in diet is thought to be a key factor responsible for the rapid increase of squirrel distribution, and may significantly or partially explain their success. Specifically, the meat of squirrel is usually considered a favoured meat in certain regions of the United States and the United Kingdom, where it is listed as wild game. The meat can also be exchanged for rabbit or chicken in some recipes as the meat is low in fat content, unlike most game meat (Bradley, 1968; Davidson, 1999; Muser, 2007; Musser *et al*., 2010).

Thus, a thorough knowledge of normal histology is essential for the understanding of the altered structure seen in the various conditions of disease (Copenhaver, 1964; Banks, 1993). Diseases can also be a significant problem; with squirrel pox virus (sometimes colloquially referred to as parapox or simply pox), caused by a protozoan parasite *Toxoplasma gondii* being common and mange ringworm being quite rare. Mortality varies between species and populations and is strongly correlated with the mast crop, with higher survival during good mast years. Mortality sources for squirrels include predators (domestic dogs and cats), starvation and road vehicles, with the latter being the most recent common in the Isle of Wight where out of 158 animals found dead between September 2008 and October 2009, 123 (78%) had been hit by cars (Wikipedia, 2010). Liver disease is not very common in squirrel. Multinucleated hepatocytes are occasionally seen as an incidental finding in squirrel as well as in chimpanzees and gorillas. Hepatic hemosiderosis is a common "incidental" finding in marmosets, tamarins, owl monkeys and other species of New World primates and in lemurs and gorillas (Lowenstine, 2003). Experimentally, diets high in iron caused mortality due to infections is common in marmosets. Hepatocellular iron storage may become severe enough to lead to alterations in hepatocellular function (hemochromatosis) compromising pharmacologic studies (Lowenstine, 2003). In lemurs, hepatocellular iron is thought to play a role in the development of spontaneous hepatocellular carcinomas, but recently a virus has also been identified. The circulatory system is a closed hydraulic system powered by a pump-the highly efficient heart. The micro-architecture of the heart-arteries, veins, and capillaries-is reflected in their functions. The close and strikingly obvious correlation between structure and function in the heart and vessels of the blood vascular system greatly simplifies understanding their histologic organization. Caprette and Senturia (1984) reported that hearts from winter-active ground squirrels developed greater pressures than those from winter-hibernating and summer-active animals. Contractility of the seasonal hibernator's heart is influenced by both season and hibernation itself possibly through shifts in myocardial metabolism. However, seasonal adaptations appear not to be required to confer the special resistance of the seasonal hibernator's heart to the deleterious effects of low temperature. Hoque *et al.* (2011) reported that ground squirrel's brain loses many vital neural connections, but it has evolved a way to recuperate. Understanding that process might help scientists treat Alzheimer's diseases. Evidence from other hypoxic-tolerant species suggests that some of the adaptations are similar but perhaps not sufficient to promote ischemic tolerance. For example, turtle brain is highly resistant to anoxia, but inhibition of glycolysis (as it would occur during ischemia) renders this species highly vulnerable to injury. Hoque *et al*. (2011) reported that during selection of breeding buck, special attention should be given on age, body weight, soundness of the sexual organ especially testis and quality of semen.

The structural alterations are minimized when tissues and cells for microscopic examination are fixed in quality fixatives. The objectives this study was to describe the histological arrangement of tissues of some organs of squirrels (liver, brain, heart and gonads (testes and ovaries)) sampled from four

villages (Ede-Oballa, Obukpa, Ibagwa and Eha-alumona) in Nsukka agro-ecological zone.

MATERIALS AND METHODS

The 32 ground squirrels used in this study were wild-caught from four villages in Nsukka in agro-ecological zone. Animals were cared for in accordance with guidelines on animal experimentation of the Committee on Care and Use of Laboratory Animal Resources, under an animal use protocol approved by the University of Nigeria, Nsukka. This concentration on a particular species was a consequence of their commonality and their gregarious nature in these areas. Twenty-two ground squirrel were caught from their dens using specialized catching traps, and eleven with land traps. All specimens were collected between May and July, 2012 from four villages (Ede-Oballa, Obukpa, Ibagwa and Eha-alumona) in Nsukka agro-ecological zone, Enugu, Nigeria. Collected squirrels were identified to species level as *Xerus erythropus* (Milton, 1984). They were then transported to the Histology Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, where specimens were anesthetized using chloroform fumes and the viscera dissected to expose the internal organs. The organs were dissected out, cut and fixed in 10% neutral-buffered formalin for 36 hours (Onuoha, 2010) and transferred to 70 - 98% ethanol for dehydration before being processed for routine paraffin embedding, sectioning, staining re-dehydration and mounting (Weiss *et al*., 2010). Several 4 – 5 µm sections were made from each tissue and stained with the Hematoxylin and Eosin. The tissues were processed using standard histological methods (Onuoha, 2010).

RESULTS

Liver: In squirrels, the liver develops embryologically as a glandular outgrowth of the primitive gut. The liver cells (hepatocytes) are separated by wide vascular channels, sinusoids (S). It has a hepatic portal vein (Figures 1 – 4) from were absorbed food products pass directly from the gut to the liver. The larger branches of the hepatic artery and vein are separated by fibrous tract, the portal tract (T), usually arranged around a terminal hepatic venule. There were no differences in the architecture of the liver tissue of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 1 – 4).

Heart: This constitutes the circulatory system of squirrels. It mediates the continuous movement of their body fluids, its principal functions being the transport of oxygen and nutrients to the tissues. It has two functional components, the blood vascular system and the lymph vascular system. It comprises three layers: an inner lining comprising a single layer of extremely flattened cells called endothelium (E), forming the Tunica intima; an intermediate muscular layer, the Tunica media; an outer supporting tissue layer, the Tunica adventitia. Furthermore, the *Tunica intima* forms the endocardium (innermost); the *Tunica media* forms the myocardium, the *Tunica adventitia* forms the epicardium (Epi), enclosed by a fibrous sac, the pericardium (the parietal pericardium) (Figures 5 – 8). The endocardium, with all its endothelial lining and supporting tissues, accommodates movement of the myocardium without damage to the endothelium and may also contain a small amount of adipose tissue for insulation. The mesothelial cells of the epicardium secrete a small amount of serous fluid, which lubricates the movement of the epicardium on the parietal pericardium. There were no differences in the architecture of the heart tissue of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 5 – 8).

Brain: In squirrels, the cerebellar cortex forms a series of deeply convoluted folds or folia, supported by a branching central medulla (M) of white matter. It consists of the outer molecular layer (ML) which contains relatively few neurons and large numbers of unmyelinated fibres, the inner granular cell layer (GL) which is extremely cellular Purkinje cells (P) found between the two layers (Figures 9 – 12). It has very large cell bodies, a relatively fine axon extending down

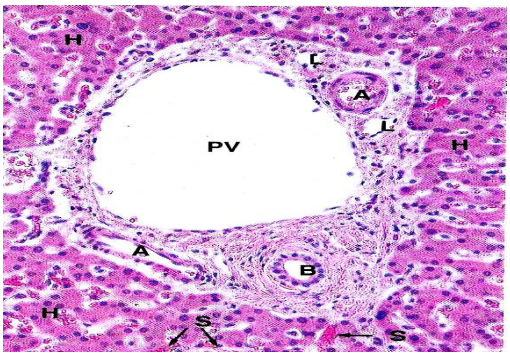

Figure 1: Transverse section of squirrel liver from Ede-Oballa, showing a branch of the hepatic portal vein PV, plates of hepatocytes H, a branch of hepatic artery A, lymphocytes L, hepatic sinusoids S and bile ductulus B. H&E x 100

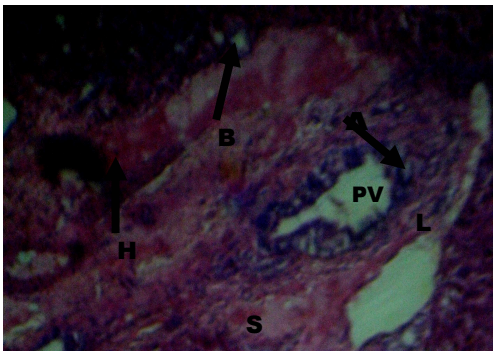

Figure 2: Transverse section of squirrel liver from Eha-Alumona, showing the hepatic portal vein PV, plates of hepatocytes H, lymphocytes L, hepatic sinusoids S and bile ductulus B. H&E x 100

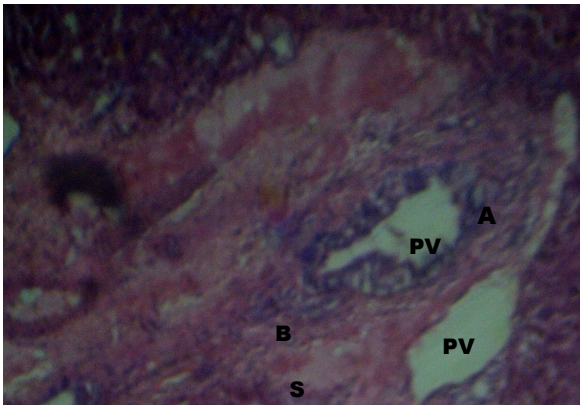

Figure 3: Transverse section of squirrel liver from Obukpa, showing the hepatic portal vein PV, hepatic artery A, bile ductulus B and hepatic sinusoids S. H&E x 100

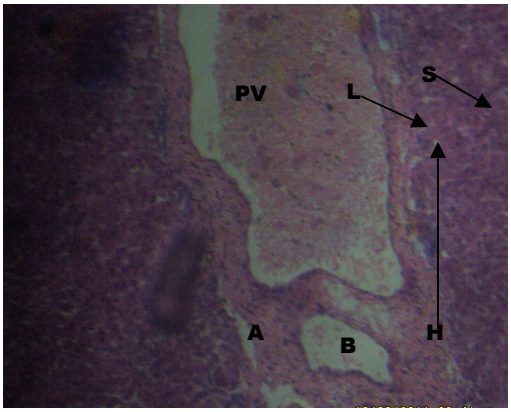

Figure 4: Transverse section of squirrel liver from Ibagwa showing the hepatic portal vein PV, bile ductulus B, hepatic artery A, plates of hepatocytes H, hepatic sinusoids S and lymphocytes L. H&E x 100

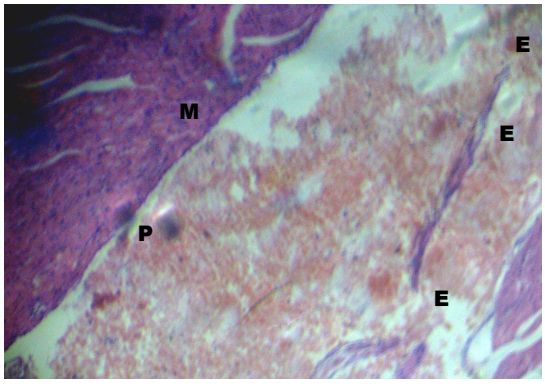

Figure 5: Transverse section of squirrel heart from Ede-Oballa, showing the tunica media M, the flattened cells pericytes P and endothelial cells E. H&E x 100

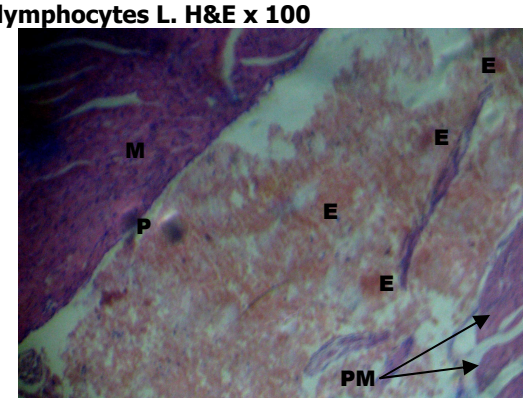

Figure 6: Transverse section of squirrel heart from Obukpa, showing endothelial cells E, the Tunica media M, flattened cells pericytes P, papillary muscles PM of the ventricle. H&E x 100

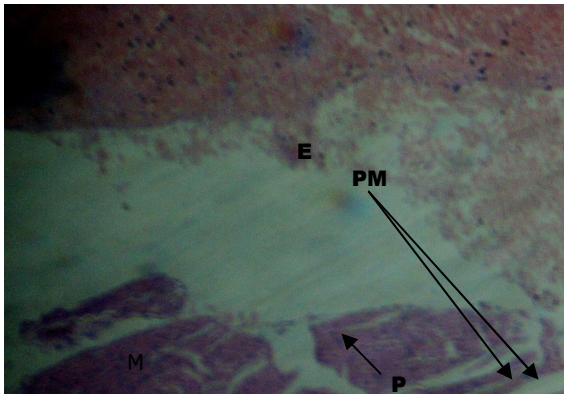

Figure 7: Transverse section of squirrel heart from Ibagwa, showing the papillary muscles PM, the tunica media M, flattened cells pericytes P. H&E x 100

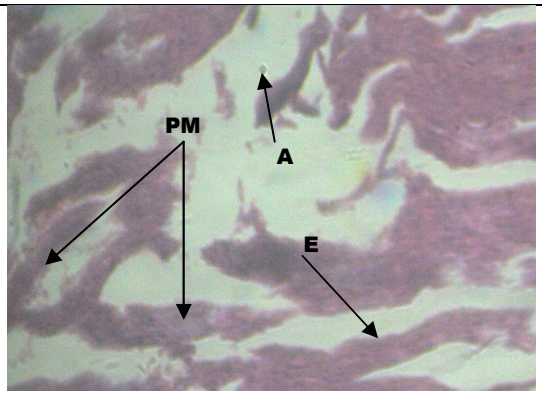

Figure 8: Transverse section of squirrel heart from Ede-Oballa, showing the papillary muscles PM, the endocardium (tunica intima), branch of coronary artery A. H&E x 100

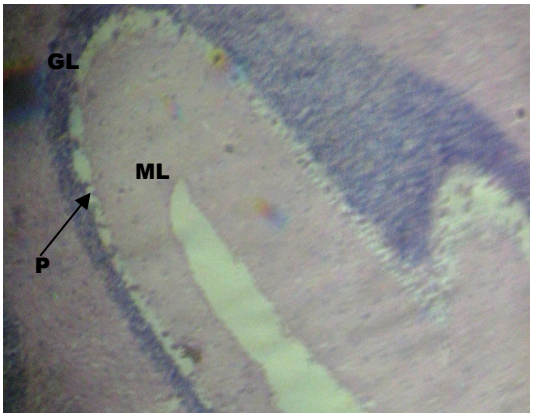

Figure 9: Transverse section of squirrel brain from Eha-Alumona, showing the outer molecular layer ML, an inner granular layer GL, and purkinje cell of the cerebellar cortex. H&E x 100

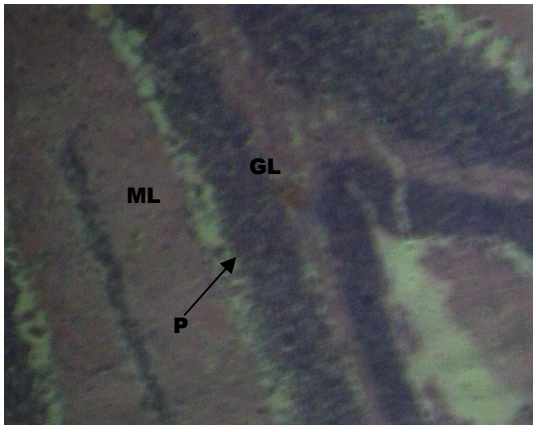

Figure 10: Transverse section of squirrel brain from Ibagwa, showing the outer molecular layer ML, an inner granular layer GL, and purkinje cell of the cerebellar cortex. H&E x 100

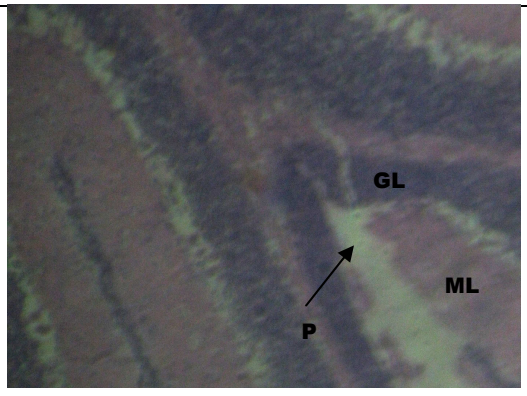

Figure 11: Transverse section of squirrel brain from Obukpa, showing the outer molecular layer ML, an inner granular layer GL, and purkinje cell of the cerebellar cortex. H&E x 100

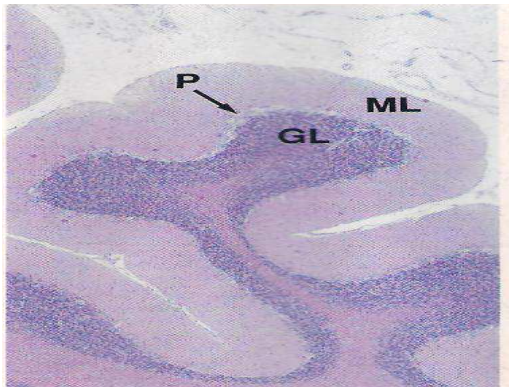

Figure 12: Transverse section of squirrel brain from Ede-Oballa, showing the outer molecular layer ML, an inner granular layer GL, and Purkinje cell of the cerebellar cortex. H&E x 100

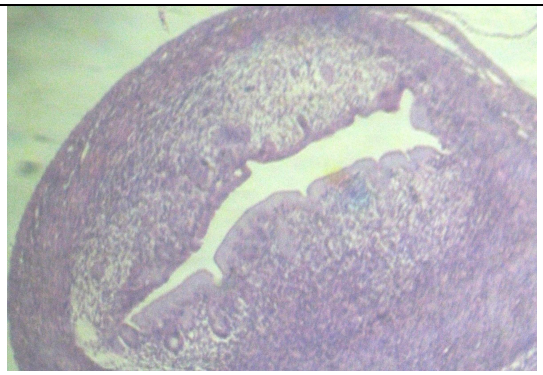

Figure 13: Transverse section of squirrel testis from Eha-alumona H&E x 100

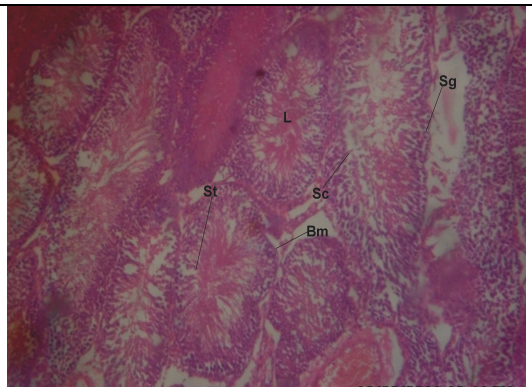

Figure 14: Transverse section of seminiferous tubule of squirrel testis from Eha-alumona, showing basement membrane BM, spermatogonia Sg, spermatid St, lumen L. H&E x 100

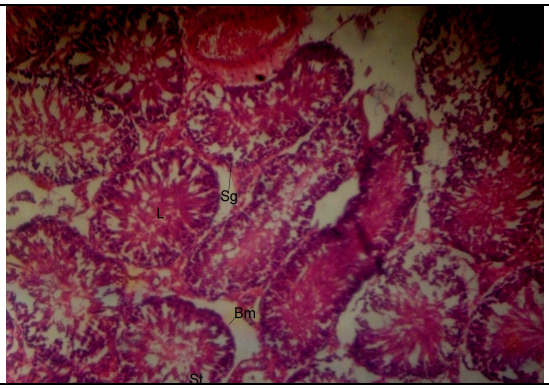

Figure 15: Transverse section of seminiferous tubule of squirrel testis from Obukpa, showing Basement membrane BM, spermatogonia Sg, spermatid St, lumen L. H&E x 100

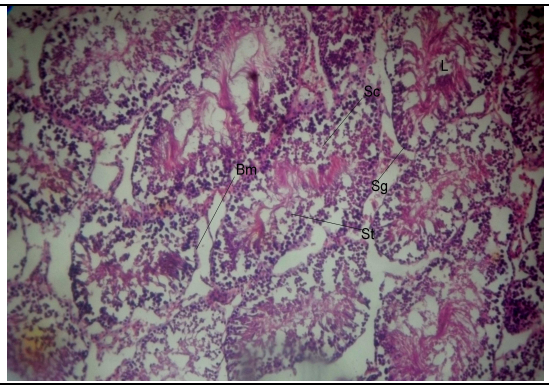

Figure 16: Transverse section of seminiferous tubule of squirrel testis from Ibagwa, showing basement membrane BM, spermatogonia Sg, spermatid St, lumen L. H&E x 100

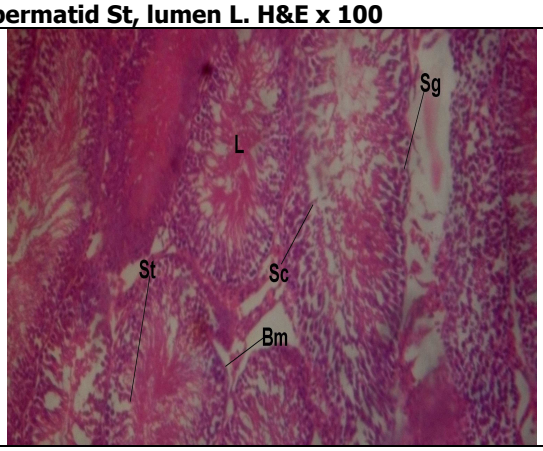

Figure 17: Transverse section of Seminiferous tubule of Squirrel Testis from Ede-Oballa, showing basement membrane BM, spermatogonia Sg, spermatid St, lumen L. H&E x 100

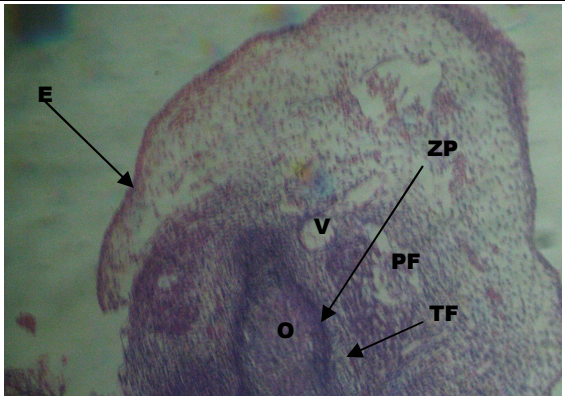

Figure 18: Transverse section of squirrel ovary from Ede-Oballa, showing the primordial follicles PF, zona pellucida ZP, oocyte O, a blood vessel V, theca folliculi TF, columnar epithelial cell E. H&E x 100

through the granular cell layer and an extensively branching dendrites system, which arborises into the outer molecular layer. There were no differences in the architecture of the brain tissue of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 9 – 12).

Testis: A section of the seminiferous tubule consists of a basement membrane (BM) which serves as the tubule's membrane, mitotic spermatogonia (Sg) which undergo active mitosis to give rise to the primary spermatocytes (Sc). The primary spermatocytes undergo the first meiotic division to form the secondary spermatocytes. They in turn undergo a second meiotic division to form the spermatids (St) (Figures 13 – 16) which undergo maturation to form the spermatozoon. The newly formed spermatozoon is absorbed into the lumen of the tubule where they are drawn into the epididymal walls of the testis for temporary storage. There were no differences in the architecture of the seminiferous tubules of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 13 – 16).

Ovary: The ovaries of all mammals have a similar basic structure. Their overall appearance, however, varies considerably in accordance with the species differences in ovarian cycle and the stage in the cycle at which the ovary was examined. A transverse section of the ovary showed a primordial follicles (PF), which is composed of primary oocytes (O) (Figures 17 and 18) surrounded by a single layer of flattened follicular cells. This PF exists in a mature ovary as undeveloped follicle. Also, the zona pellucida (ZP), a thick homogenous layer of glycoprotein and acid proteoglycans, develops between the Oocyte and follicular cells and serves as the eggs membrane. The theca follicle (TF) is an organized layer around the follicle and is separated from the granulose cells by a basement membrane. The epithelial cell (E) is found on the surface of the ovary. The blood vessel (V) also helps in the circulation of their blood. There were no differences in the architecture of the ovaries of squirrels sampled from the four villages in Nsukka agro-ecological zone (Figures 17 and 18).

DISCUSSION

In the present study, the squirrels' livers were normal and develops embryologically as outgrowth of the primitive gut. The hepatic portal tracts were normal and were in line with the report of Robert *et al*. (2004). Cullen and Marion (1996) reported that a few of the portal tracts had inflamed liver, the inflammatory infiltrate penetrated the limiting plate and extended into the adjacent parenchyma. Robert *et al*. (2004) reported centrilobular hepatocytic degeneration or necrosis occurred in the liver in the IP-infected animals. Inflammatory cell infiltration in the lobules was minimal. The hepatic portal veins the organ, from which absorbed food products pass directly from the gut to the liver. Oxygen required to support liver metabolism in squirrels is supplied through the hepatic artery and the hepatic vein, for venous drainage. The liver metabolizes glycogen, (gluconeogenesis), synthesizes albumin and clotting factors, helps in the destruction of spent red cells and reclamation of their constituents (Young and Heath, 2000). Lowenstine (2003) reported that hepatic hemosiderosis was a common finding in marmosets, tamarins, owl monkeys and other species of new world primates and in lemurs and gorillas. Hepatocellular iron deficiency may become severe enough to lead to alterations in hepatocellular function (hemochromatosis). The heart was normal without degenerations and constitutes the circulatory system of squirrels (Young and Heath, 2000). The cerebellar cortex forms a series of deeply convoluted folds or folia, supported by a branching central medulla (M), of white matter in the brain. Kunjan *et al*. (2006) reported that the Arctic ground squirrel (*S. parryii*) had eosinophilic cytoplasm, dark-staining triangular-shaped nucleus, and eosinophilic-staining nucleolus. The low partial pressure of oxygen (PO_2) levels found in AGS during euthermia is accompanied by increased hypoxia-inducing factor–1α protein levels in brain, suggesting that this species experiences mild, chronic hypoxia attributable to low respiratory drive. Kunjan *et al*. (2006) also reported that learning how the ground squirrel's brain recuperates could not only help scientists understand the brain's plasticity, but also suggest new ways to reverse or prevent cellular damage in neurodegenerative diseases. In this study, the testes showed a section of the seminiferous tubule and consist of a basement membrane (BM), which serves as the tubule's

membrane. The newly formed spermatozoon, then, is absorbed into the lumen of the tubule, where they are drawn into the epididymal walls of the testis for temporary storage. This study was similar to the report of Hoque *et al.* (2011), that testicular parenchyma of ground squirrel is composed of seminiferous tubules (from which spermatozoa is formed) and leydig cells or interstitial cells. The sertoli cells are responsible for phagocytosis of residual cytoplasm cast off during maturation of spermatocytes and for synthesis of androgen-binding protein which is essential for proper germ cell differentiation and leydig cells secrete testosterone responsible for male sexuality (Young and Heath, 2000; Hoque *et al.,* 2011). Sever and Sengel (2006) reported that the spermathecal secretions may serve to attract and prolong the viability of sperm, but sperm that become enmeshed in the secretions or epithelium are phagocytized. They also reported that the bundles of sperm are aligned in parallel clusters and showed similar orientation. Even when sperm are not crowded into a tubule, sperm can be found with their nuclei embedded in the secretion matrix bathing the surface of the spermathecal epithelium. Abney and Keel (1989) reported damage to the germinal epithelium and resulting to infertility in humans and experimental animals as well as the degree of damage to the different stages of germ cell development. They also reported morphological alterations in Sertoli and Leydig cells in terms of cellular hypertrophy and hyperplasia. Shackelford and Goetz (2007) reported that in man as sperm cells mature they move between sertoli cells from the basal toward the adluminal compartment of the seminiferous tubule. Occluding junctions that interconnect adjacent sertoli cells shield secondary spermatocytes, spermatids and spermatozoa from autoimmune recognition. Nieschlag *et al*. (2006) reported that amphibians and most fish do not possess seminiferous tubules, instead the sperm are produced in spherical structures called sperm ampullae. Under a tough membranous shell, the tunica albuginea, the testis of amniotes and some teleost fish, contains very fine coiled tubes called seminiferous tubules. The developing sperm travel through the seminiferous tubules to the rete testis located in the mediastinum testis, to the efferent ducts, and then to the epididymis where newly created sperm cells mature. The sperm move into the vas deferens, and are eventually expelled through the urethra and out of the urethral orifice through muscular contractions. Nieschlag *et al*. (1989) *reported* that the testes of the non-boreotherian mammals such as the monotremes, armadillos, sloths, elephants remain within the abdomen. There are also some boreoeutherian mammals with internal testes, such as the rhinoceros. The ovaries of all mammals have a similar basic structure. In this study, a transverse section of the ovary showed a primordial follicles which is composed of a primary oocytes surrounded by a single layer of flattened follicular cells. This study is similar to the report of Young and Heath (2000). Walker *et al.* (2009) reported that in ovaries of aging squirrel monkeys (*Saimiri sciureus*), clusters of granulosa cells occur that resemble granulosa cell tumours in humans. These appear to be a normal change with age in this species. Elene *et al*. (2006) reported that the ovary of the squirrel monkey consist of a large almond-shaped structure with the lateral margin of the ovary covered by a simple cuboidal epithelium known as the germinal epithelium. However, the germinal epithelium actually is a continuation of the layer of cells that lines the peritoneal cavity.

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REFERENCES

ABNEY, T. O. and KEEL, B. A. (1989). *The Cryptorchid Testis.* CRC Press, 08-31.

BAACK, J. K. and PAUL, V. S. (2003). Alarm calls affect foraging behaviour in eastern Chipmunks (*Tamias striatus*, Rodentia: Sciridae). *Ethology,* 106: 1057 – 1066.

BANKS, W. J. (1993). *Applied Veterinary Histology.* Third Edition, Mosby Books, USA.

BRADLEY, W. G. (1968). Food habits of the antelope ground squirrel in southern Nevada. *Journal of Mammalogy,* 49(1): 14 – 21.

CAPRETTE, D. R. and SENTURIA, J. B. (1984). Volumetric performance of isolated ground squirrel and rat hearts at low temperature. *American Journal of Physiology,* 247(4): 722 – 727.

COPENHAVER, W. M. (1964). *Bailey's Textbook of Histology.* The Williams and Wilkins Company, Baltimore.

CULLEN, J. M. and MARION, P. L. (1996). Neoplastic liver disease associated with chronic ground squirrel hepatitis virus infection. *Hepatology,* 23: 1324 – 1329.

DAVIDSON, A. (1999). *Squirrel - Oxford Companion to Food.* Oxford University Press, Oxford.

ELENA, S., SHU-YUAN, X., PATRICK, C. N. and ROBERT, B. T. (2007). Comparative pathology of north American and central African strains of monkey pox virus in a ground squirrel model of the disease**.** *American Journal of Tropical Medicine and Hygiene*, 76(1): 155 – 164.

FOLTZ, D. and HOOGLAND, J. L. (1981). Analysis of the mating system in the black-tailed prairie dog, *Cynomys ludoricianus,* by likelihood of paternity. *Journal of Mammalogy,* 62(4): 706 – 712.

FRIGGENS, M. (2002). Carnivory on desert cottontails by Texas antelope ground squirrels. *The Southwestern Naturalist,* 47(1): 132 – 133.

WIKIPEDIA (2010). *Squirrel.* http://en.m.wikipedia.org/wiki/Squirrel, Accessed: 18th April, 2011.

HOQUE, S. A. M., KABIRAJ, S. K. and (YAHIA KHANDOKER M. A. M. (2011). *Morphometry and Histology of the Testis of Black Bengal Buck: Age Depended Changes on Morphometry and Histology of the Testis of Black Bengal Buck*. LAP Lambert Academic Publishing, GmbH and Company, Saarbrücken, Germany

KUNJAN, R. D., RICARDO, P., AMI, P. RAVAL, M. A. and PEREZ, P. (2006). The arctic ground squirrel brain is resistant to injury from cardiac arrest during euthermia. *Stroke,* 37: 1261 – 1265.

LOWENSTINE, L. J. (2003). A primer of primate pathology: Lesions and nonlesions. *Toxicological Pathology,* 31: 92 – 102.

MILTON, K. (1984). Family Sciuridae. Pages 612 – 621. *In:* MACDONALD, D. (Editor), *The Encyclopedia of Mammals.* Facts on File, New York.

MUSER, G. G. (2007). Squirrel*. Encyclopaedia Britannica Online Academic Edition*. Encyclopaedia Britannica, Chicago. Retrieved: 20th February, 2010.

MUSSER, G. G., DURDEN, L. A., HOLDEN, M. E. and LIGHT, J. E. (2010). Systematic review of endemic Sulawesi squirrels (Rodentia, Sciuridae), with descriptions of new species of associated sucking lice (Insecta, Anoplura), and phylogenetic and zoogeographic assessments of sciurid lice. *Bulletin of the American Museum of Natural History,* 339: 125 – 129.

NIESCHLAG, E., HERMANN, M. B., AHLEN, H. V., SKINNER, M., MCLACHLAN, R. and BREMNER, W. (1989). Stimulation of sertoli cell inhabits secretion by the testicular paracrine factor PModS. *Molecular and Cellular Endocrinology,* 66(2): 239 – 249.

ONUOHA, E. O. (2010). *A Practical Guide for the Preparation of Histological, Histopatho-logical and Cytological Slides.* Fepam Ventures, Nigeria.

ROBERT, B. T., DOUGLAS, M. W., ELENA, S., MARINA, S., VSEVOLOD, L. P. and SHU-YUAN, X. (2004). Experimental infection of ground squirrels (*Spermophilus tridecemlineatus*) with monkey pox virus. *Centre for Disease Control and Prevention,* 10(9): 13 – 16.

SEVER, D. M. and SIEGEL, D. S. (2006). Sperm aggregations in the spermatheca of the red back salamander (*Plethodon cinereus*). *Acta Zoologica,* 87: 331 – 340.

SHACKELFORD, T. K. and GOETZ, A. T. (2007). Adaptation to sperm competition in

humans. *Current Directions in Psychological Science,* 16: 47 – xx.

STEPPAN, S. J., SCORZ, B. L. and HOFFMANN, R. S. (2004). Nuclear DNA phylogeny of the Squirrels (Mammalia: Rodentia) and the evolution of arboreality from C-myc and RA 91. *Molecular Phylogenetic and Evolution,* 30(3): 703 – 719.

THORINGTON, R. W. and HOFFMAN, R. S. (2005). Family Sciuridae. Pages 754 – 818. *In:* WILSON, D. E. and REEDER, D. M. (Editors), *Mammal Species of the World: A Taxonomic and Geographic Reference.* Johns Hopkins University Press, Baltimore.

WALKER, M. L., ANDERSON, D. C., HERNDON, J. G. and WALKER, L. C. (2009). Ovarian aging in squirrel monkeys (*Saimiri sciureus*). *Reproduction,* 138(4): 793 – 799.

YOUNG, B. and HEATH, J. W. (2000). *Wheaters Functional Histology: A Text and Colour Atlas*. Fourth Edition, Harcourt Publishers Limited, London.

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