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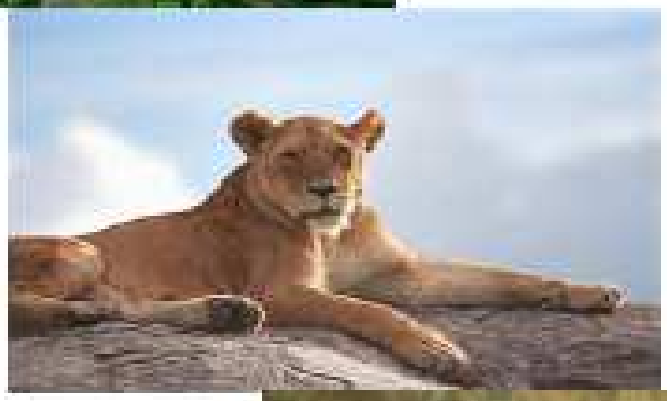

Volume 12 Number 1
April 2015

An International Peer Reviewed Multidisciplinary Open Access Journal Publishing Original Research Involving the Use of Animals and Animal Products

ISSN: 5197-3115 **Website: www.zoo-unn.org**

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INTRAHIPPOCAMPAL ADMINISTRATION OF VITAMIN C AND PROGESTERONE ATTENUATES SPATIAL LEARNING AND MEMORY IMPAIRMENTS IN MULTIPLE SCLEROSIS RATS

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ABSTRACT

It seems antioxidant and sex hormones are able to protect the multiple sclerosis (MS) rats against spatial memory reduction. Since sex hormones and oxidative stress are affective in the process of multiple sclerosis (MS), as well as cognitive functions, the study evaluates the effects of intrahippocampal injection of vitamin C and progesterone, alone or in combination on spatial memory in multiple sclerosis. Sixty-three (63) male Wistar rats were divided into nine groups (n = 7): control, (saline), sesame oil, lesion (ethidium bromide (EB)), vitamin C (1, 5 mg/kg), progesterone (0.1, 1 µg/µl) and combination therapy. In combination therapy, animals were treated with vitamin C (5 mg/kg) + progesterone (0.01 mg/kg). Animals in experimental groups received different treatments for 7 days. Characteristics of learning and spatial memory were assessed using Morris Water Maze (MWM). The results showed that intrahippocampal injection of ethidium bromide destroys MWM significantly (p<0.05). Vitamin C (5 mg/kg), progesterone (0.1 mg/kg) and vitamin C (5 mg/kg) + progesterone (0.1 mg/kg) significantly decreased latency time and travelled distance (P<0.05) in MS or lesion rats. In comparison with control group, the lesion group decreased and progesterone 0.1 mg/kg + vitamin C 5 mg/kg increased the time and distance in the target quadrant after the platform was removed. In comparison with lesion group, vitamin C (1 and 5 mg/kg), progesterone (0.1 and 1 mg/kg) and vitamin C + progesterone effective doses increased the time and distance in the target quadrant after the platform was removed. The results showed that multiple sclerosis rats had a decreased travelled distance and time spent in target quadrant to find the hidden platform in a MWM task. Vitamin C and progesterone alone improved spatial memory in comparison to lesion group. Effective doses of vitamin C + effective dose of progesterone had more improving effect on memory.

Keywords: Neuroscience, Neurosteroid, Antioxidant, Demylination, Progesterone, Learning and memory impairments, Multiple sclerosis rats

INTRODUCTION

Multiple sclerosis (MS) is a multifocal inflammatory disease of the brain and the spinal cord. The main cause of MS is unknown, although genetic and environmental factors have been shown to contribute to its etiology (Sospedra and Martin, 2005). As in many autoimmune diseases there is a higher prevalence of women than men in MS, with a female – male ratio of 2.6:1. This ratio is rising with a disproportional increase of females, especially in the relapse-onset form of the disease (Alonso and Hernan, 2007; Debouverie *et al.,* 2008). Sex hormones are affective in the process of MS disease, for example there is

evidence that estrogen has inhibitory effects on MS disease (Ito *et al.,* 2001). Also during pregnancy in woman suffering from MS, because of the high level of estrogen and progesterone, the severity of the disease is reduced, but after delivery the clinical symptoms were aggravated (Confavreuex *et al.,* 1998). The finding suggests that MS is influenced by sex hormones. Clinically, progesterone produced a moderate delay of disease onset and reduced the clinical scores. Thus, progesterone attenuated disease severity, and reduced the inflammatory response and the occurrence of demyelination in the spinal cord during the acute phase of experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis characterized by demyelination and immune cell infiltration in the spinal cord (Garay *et al.,* 2007). In the spinal cord, progesterone increases motor neuron survival after axotomy or injury, protects cultured neurons against glutamate toxicity and normalizes defective functional parameters of injured neurons (Labombarda *et al.,* 2002). In conjunction with neuronal effects, progesterone strongly influences myelin synthesis in the peripheral and central nervous system (Melcangi *et al.,* 2000). In the oligodendrocytes, the central myelin producing glia, progesterone increases myelination in culture and in cerebellum, as shown by the increased expression of myelin basic protein (MBP) (Ibanez *et al.,* 2003; Schumacher *et al.,* 2004). On the other hand, in MS disease, the axon of nerve fibres is damaged. Axonal damage often produces oxidative stress, nitric oxide, dysfunction of sodium/potassium pump, neurotoxicity induced by glutamate and destruction of myelin's protective agents (Dutta and Trapp, 2007). It has been determined that progesterone has antioxidant and anti-glutamatergic effect. Progesterone is able to induce the re-expression of sodium/potassium pump in axon. Progesterone down regulates the myelin basic protein (MBP) which is an index of axonal damage (Stein, 2008). Other antioxidant such as ascorbic acid (vitamin C) is an essential micronutrient required for normal metabolic functioning of the body (Gey, 1998). Ascorbic acid is a potent antioxidant, which is highly concentrated in the central nervous system (Sanchez-Moreno *et al.,* 2003). In most studies, researchers believed that ascorbic acid prevents memory deficit by its antioxidant effect (Castagne *et al.,* 2004). Since it is widely accepted that cognitive dysfunction occurs in 40 – 70% of MS patients (Sartori and Edan, 2006) and the most common cognitive deficits are memory dysfunction and spatial perception impairment (Glanz *et al.,* 2012), so the aim of present study was to determine the alone treatment of ascorbic acid and progesterone and co treatment of them on spatial memory task in MS induced rats. On the other hand the major question is whether vitamin C, progesterone alone or in combination has the ability to alter spatial memory in male MS rats or not?

MATERIAL AND METHODS

Animals: Sixty three (63) adult male Wistar rats weighing 200 - 250 g were housed in standard hygienic plastic cages under a 12 hour light/dark cycle (lights on at 07:00 a.m.) in a room with controlled temperature (23 ± 2 °C). Food and water were available *ad libitum*. The experiments were carried out during the light phase of the cycle. All animal procedures were performed according to the National Institutes of Health's Guide for the care and use of laboratory animals.

Experimental Demyelination with Ethidium Bromide (EB) and Treatments: The animals were randomly divided into nine groups (7 animals per group): group 1 - control, (no treatment); group 2 - sham, (sesame oil, solvent of progesterone); group 3 - saline (solvent of vitamin C); group 4 - (ethidium bromide, or lesion group); group 5 - (ethidium bromide + 1 mg/kg vitamin C) group 6 - (ethidium bromide + 5 mg/kg vitamin C), group 7 - (ethidium bromide + 1 mg/kg progesterone); group 8 - (ethidium bromide + 0.1 mg/kg progesterone), group 9 - (ethidium bromide + 1 mg/kg progesterone +5 mg/kg vitamin C). For the surgical demyelination procedure, the animals were anaesthetized with intraperitoneal injection of ketamine (100

mg/kg) and xylazine (20 mg/kg) and placed on the rat stereotaxic instrument (Stoelting, USA) in the skull-flat position. Hair of the corresponding skull surface was shaved and then, an incision was made to expose the skull. Two holes were drilled in the skull according to appropriate coordinates to achieve cornu ammonis (CA1) of hippocampal formation (3.8 mm dorsal to the bregma, 2.4 mm deep from the dorsal surface and ± 2.2 mm laterality) (Paxinos and Watson, 1986). Two guide cannulae (21 gauges) were inserted into the holes and fixed using dental cement. After the surgery, dummy inner cannulae were inserted into the guide cannulae and left in place until the injections were made. All animals were allowed to recover for 1 week before starting the microinjections. Demyelination was induced bilaterally by direct single injection of 3µl of 0.01% ethidium bromide (EB) in sterile 0.9% saline (Goudarzvand *et al.,* 2013). Animals in experimental groups 4 – 9 received vitamin C or progesterone and vitamin C + progesterone with above mentioned doses for 7 days post lesion (Hooshmandi *et al.,* 2011). The animals from groups 2 and 3 were injected equal volume of sesame oil or sterile 0.9% saline. Injections for all groups were made at the rate of 1µl/minute using a 10-µl Hamilton syringe, and the needle was kept in the guide cannulae for an additional 60 second in order to facilitate the diffusion of the drug.

Morris Water Maze Test: The Morris water maze was black circular pool (136 cm in diameter and 100 cm in height). The pool was filled to a depth of 60 cm with water (20 ± 1 °C) and divided into four quadrants of equal area (NE, SE, SW and NW). A platform (10 cm in diameter) was centred in one of the four quadrants of the pool and submerged 1 cm below the water surface so that it was not visible at water level. The swimming was monitored by a video camera, which was positioned directly above the centre of the pool. The pool was located in a test room, which contained various prominent visual cues (Moosavi *et al.,* 2006).

One week after surgery, the rats were trained in the water maze. The single training session consisted of eight trials (in two blocks) with four different starting positions that were equally distributed around the perimeter of the maze. The task requires rats to swim to the hidden platform guided by distal spatial cues. After mounting the platform, the rats were allowed to remain there for 20 seconds, and then were placed in a holding cage for 30 seconds until the start of the next trial. Rats were given a maximum of 60 seconds to find the platform and if it failed to find the platform in 60 seconds, it was placed on the platform and allowed to rest for 20 seconds. Latency to platform and distance travelled were collected and analyzed. After completion of the training, the animals were returned to their home cages until retention testing 24 hours later. The probe trial consisted of 60 seconds free swim period without a platform and the time swum in the target quadrant was recorded. In order to assess the possibility of drug interference with animal sensory and motor coordination or the animal motivation, the capability of rats to escape maximum of 60 seconds to find the platform and if it failed to find the platform in 60 seconds, it was placed on the platform and allowed to rest for 20 seconds, the capability of rats to escape to a visible platform was tested in this study. Latency to platform and distance travelled were collected and analyzed later. After completion of the training, the animals were returned to their home cages until retention testing 24 hour later. The trained rats were given four trials for visual-motor coordination on the visible platform (Castagne *et al.,* 2004; Mohaddes *et al.,* 2009).

Data Analysis: SPSS 13.0 software was used for statistical comparisons of data and data were expressed as means ± SEM. For comparisons between Block 1 and Block 2 in each group, a paired-sample t-test was used. The statistical analysis of the data between groups was carried out by one-way ANOVA followed by Turkey test. In all comparisons, $p<0.05$ was the criterion for statistical significance.

RESULTS AND DISCUSSION

In comparison of block 1 and block 2, vitamin C (5 mg/kg), progesterone (0.1 mg/kg) and vitamin C (5 mg/kg) + progesterone (0.1 mg/kg) significantly decreased latency time ($p<0.05$) and travelled distance ($p<0.05$) in MS or lesion rats (Figures 1 and 2). Probe test data were compared between groups. One-way ANOVA of the distance travelled in the target quadrant revealed significant differences ($p<0.05$) between groups. In comparison with control group, the lesion group decreased and progesterone 0.1 mg/kg + vitamin C 5 mg/kg increased the time and distance in the target quadrant after the platform was removed. In comparison with lesion group, vitamin C (1 and 5 mg/kg), progesterone (0.1 and 1 mg/kg) and vitamin C + progesterone effective doses increased the time and distance in the target quadrant after the platform was removed (Figures 3 and 4). No treatments significantly changed swimming speed in the target quadrant.

In the present study vitamin C, progesterone, and vitamin C + progesterone were used for the first time in evaluating memory impairment of ethidium bromide (EB) - induced multiple sclerosis (MS). The results showed that MS rats had decreased travelled distance and time spent in target quadrant to find the hidden platform in a Morris Water Maze task. Vitamin C (1 and 5 mg/kg), progesterone (0.1 and 1 mg/kg) and vitamin C + progesterone effective doses (vitamin C 5 mg/kg + progesterone 0.1 mg/kg) administration improved the acquisition and retrieval in MS rats. Ethidium bromide induced focal demyelination by selectively damaging glial cells, which include oligodendrocytes (central nervous system myelin forming cells) and astrocytes (Spanevello *et al.,* 2009). Several studies have proposed that demyelinating insults occur in the central nervous system gray matter of MS patients. Hippocampal formation is known as one of the important gray matters which are reported to be affected by MS (Geurts *et al.,* 2007). Using this model, the study found that direct single injection of a 0.01% EB solution into the cornu ammonis (CA1) of hippocampal formation impaired hippocampal-dependent spatial learning and memory performances. Analyses of swimming velocity to reach the hidden platform revealed no differences between experimental groups, disproving any non-specific effects of EB microinjection on spatial acquisition and memory. These results demonstrated that the impairing effects of gliotoxin microinjection on spatial learning and memory were not due to any non-specific fluctuations in gross motor activity or motivational state. In this study progesterone in both doses significantly improved the spatial memory in comparison to MS or lesion group. In the field of progesterone effects on memory, there are different and sometimes paradoxical reports. May be some factors such as the model of administration, behavioural test kind, gender, age of animal, time of hormone treatment, and dose of hormone can induce different results. For example, it has been shown that progesterone alone or in combination with estrogen, improved scopolamine-induced impairment of working memory and reference memory as effectively as estrogen supplementation.

Estrogen and progesterone improved scopolamine-induced impairment of spatial memory (Tanabe *et al.,* 2004). In another study, it has been found that levels of progesterone appeared to be tied to verbal memory and global cognition among women who were in early post menopause, and the higher the levels of progesterone, the better the outcomes on tests of verbal memory and global cognition in these younger women (Henderson *et al.,* 2013). In another report, long-term treatment with estrogen or estrogen + progesterone (3 months, or 10 months after ovariectomy) significantly enhanced acquisition of the memory by aged animals after long-term loss of ovarian function in female Sprague-Dawley rats (Gibbs, 2000). These findings suggested that repeated treatment with estrogen and progesterone initiated within a specific period of time after the loss of ovarian function may be effective at preventing specific negative effects of hormone deprivation on brain aging and cognitive decline (Gibbs, 2000).

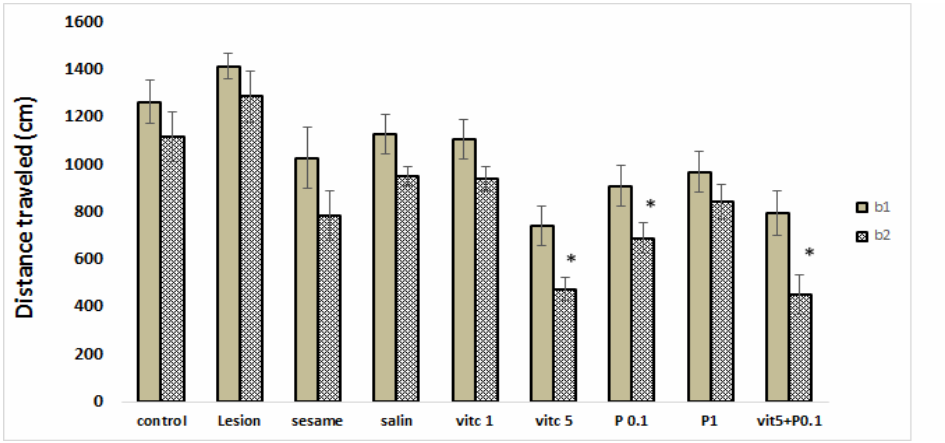

Figure 1: Effects of vitamin C and progesterone on the travelled distance to find hidden platform in two consecutive blocks (b1 and b2) in MS (or lesion) rats. Data represent means ± SEM (n=7),*p<0.05, significantly different when compared with the b1 same group

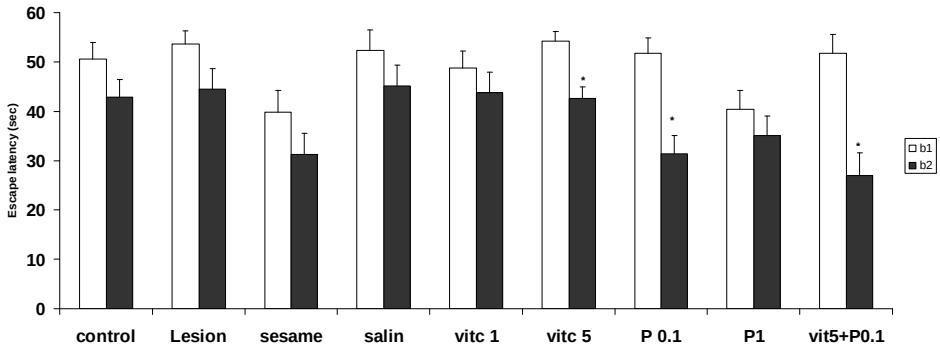

Figure 2: Effect of vitamin C and progesterone on the escape latency to find hidden platform in two consecutive blocks (b1 and b2). Data represent means ± SEM (n=7), *P<0.05, significantly different when compared with the b1 same group

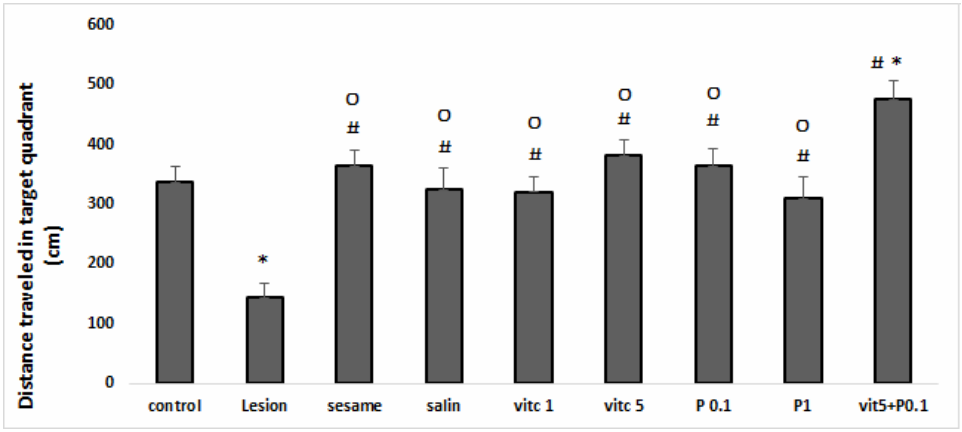

Figure 3: Effect of vitamin C and progesterone on the travelled distance in trial sessions of the Morris water maze test. Data represent means ± SEM (n=7), *p<0.05 significantly different when compared with the control group. # P<0.05 significantly different when compared with the lesion group, O p<0.05 significantly different when compared with the effective dose group

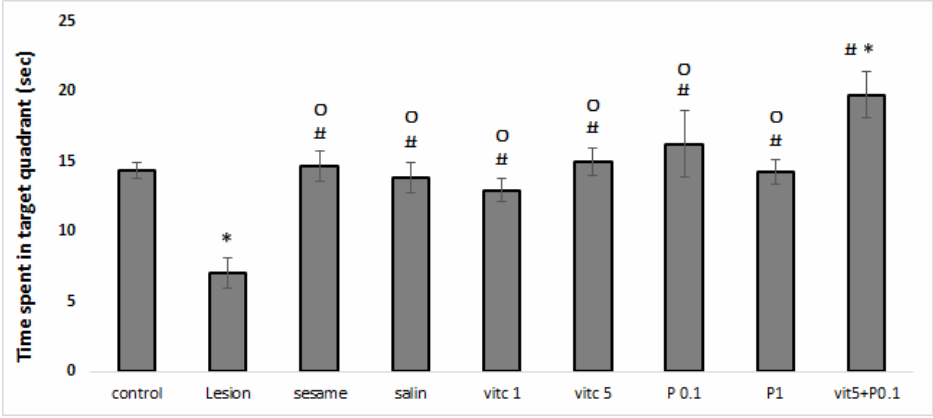

Figure 4: Effect of vitamin C and progesterone on the escape latency (or time spent in target quadrant) in trial sessions of the Morris water maze test. Data represent means ± SEM (n=7), *P<0.05 significantly different when compared with the control group, # p<0.05 significantly different when compared with the lesion group, O p<0.05 significantly different when compared with the effective dose group

It has been reported that acute progesterone treatment (subcutaneous injections of progesterone at 500 microgram) impaired spatial working memory in intact male and female rats. These results suggested that acute progesterone treatment interferes with spatial working memory consolidation, but not recognition (non-spatial) working memory. As such, the observed sexual incongruities in progesterone's effects on working memory suggested that progesterone-based hormone therapies have a negative impact on cognition (Sun *et al.,* 2010). In another study it has been reported progesterone supplementation reversed the cognitive enhancing effects of ovariectomy. This result suggested that whereas ovariectomy of the aged female rat enhanced learning and the ability to handle numerous items of spatial working memory information, progesterone was detrimental to this aspect of performance (Bimonte-Nelson *et al.,* 2004). Also El-Bakri *et al.* (2004) indicated that progesterone treatment in ovariectomized rats did not show significant learning compared to the vehicle treated groups in a Morris water maze task. It has been indicated that progesterone up-regulates the mRNA and protein expression of neuronal BDNF in the injured spinal cord and also BDNF protein in the normal tissue.

Concomitantly, steroid treatment also prevented the lesion-induced chromatolysis, supporting at the molecular and morphological levels the neuroprotective actions of progesterone (Gonaza *et al.,* 2004). A growing list of publications also gives evidence of the protective and trophic effects of progesterone. In the PNS, progesterone promotes myelination (Azcoitia *et al.,* 2003) and this stimulatory effect can be extended to the CNS. Indeed, progesterone stimulates myelination in organotypic slices cultures of 7-days-old (P7) rat and mouse cerebellum (Ghoumari *et al.,* 2003) and partially reverses toxin-induced demyelination in old male rats (Ibanez *et al.,* 2004). Progesterone also facilitated cognitive recovery and prevents neurodegeneration after cortical contusion (Stein, 2001). Finally, increased stability of BDNF protein and mRNA will result from the inhibition of oxidants and free radicals arising after spinal cord injury, since progesterone prevents injury-induced lipid peroxidation (Roof *et al.,* 1997) and exerts antioxidants effects in a murine model of spinal cord neurodegeneration (Gonzalez-Deniselle *et al.,* 2003). The mechanisms involved in the neuroprotective effects of progesterone are still not completely understood. However, it is known that the hormone has antioxidant properties (Roof *et al.,* 1997), regulates the expression of trophic factors such as brain-

derived neurotrophic factor (Gonzalez-Deniselle *et al.,* 2007), elicits the activation of intracellular signalling pathways involved in the promotion of cell survival (Singh, 2005), increases the expression of antiapoptotic molecules such as Bcl-2 and Bcl-XL, and reduces the expression of proapoptotic molecules such as Bax, Bad and caspase-3 (Yao *et al.,* 2005). Some of these effects of progesterone may be mediated by the activation of classical progestin receptors, which are widely expressed in the brain (Guerra-Araiza *et al.,* 2003). According to these reports, progesterone has different effects in memory including enhancement, no effect or decrease of memory formation. Taken together, these observations highlight the fact that progesterone is perhaps determinants of memory. In this study progesterone improved spatial memory in MS rats.

Also according to the results of study, intrahippocampal microinjection of vitamin C increased the distance travelled in target quadrant (increasing the spatial memory). Shahidi *et al.* (2008) indicated that both short-term and long-term supplementation with ascorbic acid had facilitatory effects on acquisition and retrieval processes of passive avoidance learning and memory in rats. It has been reported that, ascorbic acid could reduce the risk of dementia caused by aging, or prevent memory impairment due to the scopolamine (Parle and Dhingra, 2003) and homocyctein (Reis *et al.,* 2002). Also, it has been reported that local applications of ascorbic acid enhanced the response of neurons to dopamine and glutamate (Rebec and Pierce, 1994). Glutamate is a neurotransmitter which has a critical role in learning and memory processing. Therefore it is possible that part of the ascorbic acid effects may be due to its neurotransmitter modulator functions. Several studies have demonstrated that vitamin C is neuroprotective in adult animal models of hypoxic-ischemic injury (Wang, 2000), ascorbic acid injection is considered to inhibit necrotic cell death by suppression of calpain activation. In a study it has been showed that intraventricular vitamin C injection had a neuroprotective effect against hypoxic-ischemic brain injury in neonatal rats. Vitamin C reduced the percent brain damage, macroscopic brain injury and the number of necrotic cells. Vitamin C also inhibited calpain activation associated with necrotic cell death after hypoxic-ischemic in neonatal rat brain (Miura *et al.,* 2006). Also it has been reported that vitamin C restores acetyl cholinesterase activity that has an essential role in learning and memory (Ambali *et al.,* 2010).

Conclusion: The data from this study showed that vitamin C and progesterone were capable of protecting MS rats against spatial memory reduction. Although this was predictable based on antioxidant and protective effects of these substances but more experimental data, and in particular more information about the actions and effects of progesterone and vitamin C in multiple sclerosis are necessary for the development of more targeted and efficient steroid plus antioxidant treatments.

ACKNOWLEDGMENT

The presented data in this article is from the research protocol which was supported financially by the Research Institute for Fundamental Sciences (RIFS), University of Tabriz, Iran.

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PREVALENCE AND MANAGEMENT OF DIARRHOEA AMONG CHILDREN IN NSUKKA, NIGERIA

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ABSTRACT

Two months preliminary survey of the prevalence of diarrhoea among children (aged 5 – 10 years) was carried out in Nsukka area of Enugu State, Nigeria. Questionnaires were used to obtain information on the demographic details of the parents and children, prevalence, management and control of diarrhoea among children of this age group. These were distributed to parents of the children used for the study. Using Pearson's correlation coefficient, we found that diarrhoea among children of this age was neither correlated with residential location (r = -0.47, p = 0.652), occupation of the parent (r = 0.134, p = 0.194) nor sex of the child (r = 0.092, p = 0.377). However, the educational level of the parents was correlated with childhood diarrhoea (r = 0.346, p = 0.001). These results suggested that the children of less educated people were more prone to diarrhoeal infection when compared to children of the more educated counterparts.

Keywords: Prevalence, Management, Diarrhoea, Children, Nsukka

INTRODUCTION

Diarrhoea is derived from the Greek word 'diarroia' meaning 'flowing through'. World Health Organization (WHO) defined diarrhoea as having three or more loose or liquid stools per day, or having more stools than normal for any individual. Diarrhoea is common in children and usually accompanied by vomiting, abnormal increases in stool weight and liquidity. Hogue *et al*. (2000) suggested that increase in stool water excretion above 150 to 200 ml every 24-hour is an objective parameter for acute diarrhoea (a chronic form of the disease). In terms of pathology, diarrhoea often results from gastrointestinal infection caused by bacteria (Lastovica and Le Roux, 1993), virus, parasitic organisms and protozoa. The infection is spread through contaminated food or drinking water or from person to person as a result of poor hygiene. As noted above, chronic diarrhoea leads to fluid loss and may be life threatening, especially in young children, malnourished individuals, and immunocompromised patients. The most severe symptom in many patients is the urgency of defecation and faecal inconsistencies (Haslett *et al*., 1999). All over the world, diarrhoea is leading cause of child morbidity. In 2004, it was the third leading cause of death among populations of low-

income countries, and the second leading cause of death among children under five years of age responsible for 6.9% of the overall deaths and accounting for 1.5 million the deaths of children yearly. Unfortunately, 80% of these deaths were estimated to occur among those less than two years of age (Bryce *et al*., 2005; WHO, 2009; Fischer-Walker *et al*., 2012). Traveller's diarrhoea, a peculiar kind of diarrhoea that commonly affects travellers visiting developing countries, is an attack of usually abrupt and watery stool with abdominal cramps, anorexia and vomiting lasting for 2 – 5 days (Hogue *et al*., 2000). However, in 60 – 70% of affected patients, no organism is identified on examination. Antibiotics and cytotoxic drugs can also cause drug-induced form of diarrhoea.

According to CHRSPR (1998), diarrhoea is one of the top causes of childhood mortality in sub Sahara African and has been estimated to be responsible for 25 to 75% of all childhood illnesses in Africa. In addition, episodes of diarrhoea leads to about 14% of outpatient visits, 16% of hospital admissions and accounts for an average of 35 days of illness per year in children less than five years old. The report also stated that unlike the decline in mortality rates, diarrhoea incidence does not appear to have changed substantially over the last decade. In Nigeria, available reports indicate that more than 315,000 deaths of preschool age children are recorded annually as a result of diarrhoea disease (Ogbu *et al.*, 2008). A study "review of diarrhoeal disease cases admitted to a busy referral hospital in Ghana" (Baffoe-Bonnie *et al.*, 1998) indicated that children less than 5 years of age make up 84% of all child admissions and 56.5% of them being infants below one year. Approximately, one third of deaths among children less than 5 are caused by diarrhoea (Snyder and Merson, 1982). In terms of mechanism of infectivity inhibition of ion absorption, stimulation of ion secretion, retention of fluid in the intestinal lumen, and disorders of intestinal motility may cause diarrhoea. Retention of fluid in the bowel lumen may also occur precipitated by food intolerance associated with carbohydrate malabsorption, disaccharides deficiencies, lactulose therapy, poorly absorbable salts (magnesium sulphate, sodium phosphate and citrate, antacids), and ingestion of mannitol and sorbitol. Digestion and absorption of nutrients is a complex, highly coordinated and extremely efficient process; normally less than 5% of ingested carbohydrate, fat and protein are excreted in the faeces. Acute diarrhoea is extremely common and usually due to faecal-oral transmission of bacterial toxins, viruses, bacteria or protozoan organism. Infective diarrhoea is usually short-lived and patient with a history of diarrhoea lasting more than 10 days rarely have an infective cause (Haslett *et al*., 1999). The most common cause of chronic or relapsing diarrhoea is irritable bowel syndrome, which can present with increased frequency of defecation and loose, watery or pellet stool. Diarrhoea rarely occurs at night and is most severe before and after breakfast. The stool often contains mucus but never blood, and 24 hour stool volume is less than 200g. Chronic diarrhoea can be categorized as disease of the colon or small bowel, or mal-absorption (Haslett *et al.*, 1999). Contaminated food or water, early introduction of milk formula or solid food, poor personal hygiene, lack of maternal education and care, diseases and malnutrition, are the common means of acquiring diarrhoea. Transmission of diarrhoeal pathogens through human faeces is very common. Such pathogens from contaminated faeces excreted into the environment, often get onto people's hands or water bodies where they are consumed. Significant transmitters of diarrhoeal pathogens include flies and animals. Flies that have come in contact with faeces easily transmit pathogens to food and water as they perch around, while animals (especially in rural settings) also spread the pathogens by walking in faecal material and by frequenting domestic environments they make contacts with children, domestic materials and foods (Curtis *et al.*, 2000). This study attempts to establish a link between prevalence of diarrhoea and demographic variables among children in Nsukka, Nigeria.

MATERIALS AND METHODS

Description of Study Area: Nsukka (latitude $6^{o}51^{o}$N and longitude $7^{\circ}\ 27^{1}$E) (Figure 1) is a

town and Local Government Area (LGA) in Enugu State, south eastern Nigeria. The landmass and topography is characterised by hill and grasslands where rodents are widely distributed. Nsukka LGA has an area of 1,810km^2 and a population of 270,257 at the 2006 census (Okoye and Obiezue, 2008). Nsukka is a tropical rainforest region with sharply falling density population to average density.

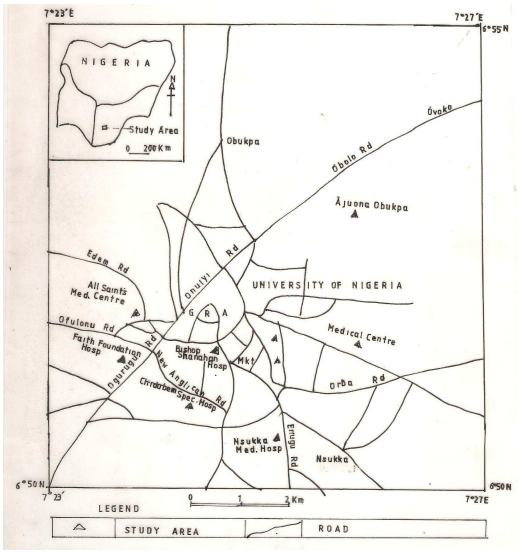

Figure 1: Map of Nsukka town showing locations of study area. Source: Topographic Map of Nigeria (2000)

The study area can be seen as underdeveloped or still developing, save for the university campus located within it. It is dotted by areas of farmlands close to residential areas, poor drainage systems leading to flooded roads and pools of stagnant waters, indiscriminate construction of houses and unplanned urbanization. The annual rainfall for Nsukka town varies from 986 mm to 2,098.2 mm (Oformata, 1978).

Data Collection: Information on the diarrhoea status of children was collected using structured questionnaire. The questionnaire was distributed to parents of the children used for the study. The questionnaire was made up of information on the demographic details of both the parents and the child. Also, the prevalence, management and control measures of diarrhoea were included in the questionnaire (Table 1).

Data Analysis: The questionnaires were analyzed using SPSS (Statistical Package for Social Sciences). Percentage frequencies were obtained for variables while correlation was used to ascertain relationship between variables and diarrhoea.

RESULTS

The prevalence of diarrhoea in Nsukka was higher, with 24.2% infections. Following it was Ajuona (7.4%), while University of Nigeria Nsukka, Bishop Shannahan Hospital, Onuiyi, Odim and Amaigbo had percentage of 6.3. Also, from the sampled population, the age bracket of 18 – 30 years had the highest prevalence (45.3%), while 40 and above had the lowest prevalence (25.3%). More female parents (68.4%) were sampled than the males (31.6%). Meanwhile, from the educational level of the parents, more of the parents sampled had university education (64.2%), followed by those that attended Senior Secondary School (SSS) (13.7%). Only 1.1% of the parents left the question unanswered. Civil servants however, recorded the highest prevalence (48.4%), while students and traders followed with 24.2% and 18.9% respectively. 4.2% of the parents did not respond to the question (Table 2).

On the other hand, 42.1% of the children at 5 years of age had the highest prevalence. Children at 10 years recorded 18.9% and then, 6 and 7 year – old – children, 15.8% and 13.7% respectively. More male children (53.7%) were sampled than the females (46.3%). The educational level of the child indicated that children in primary school recorded the highest prevalence (71.6%), while nursery school children recorded only 18.9% prevalence, but 1.1% of the children had no response (Table 3).

The questionnaire also revealed that 54.7% of the children had diarrhoea before 2011, while 31.6% had diarrhoea in 2011. On the other hand, 38.9% of the children had not diarrhoea before 2011 and 62.1% had not diarrhoea in 2011, while 1.1% of the sampled population left the question unanswered (Table 4). While the children suffered this ailment, 42.1% of the parents sampled visited the

Table 1: Data collection instrument for diarrhoea patients

Dear Respondent,

This interview is designed to find out your views on prevalence and management of diarrhoea in children aged 5 to 10 years. Your response will be strictly confidential.

A: Demographic Details of the Parent

1) Residential Area ……
2) Age (years): 18-30 [ ] 30-40 [ ] 40 and above [ ]
3) Sex: Male [ ] Female [ ]
4) Educational Level: Primary[ ] JSS [ ] SSS/O'Level [ ] A Level [ ] University [ ] Other (specify)……
5) Occupation: Trader [ ] Farmer [ ] Civil servant [ ] other (specify)……

B: Demographic Details of the Child

1) Age (years): 5 [ ] 6 [ ] 7 [ ] 8 [ ] 9 [ ] 10 [ ]
2) Sex: Male [ ] Female [ ]
3) Educational Level: Nursery School [ ] Primary [ ] Other [ ]

C: Prevalence and Management of Diarrhoea

1) Has your child had diarrhoea before? Yes [ ] No [ ] Can't say [ ]
2) Has he/she had diarrhoea this year? Yes [ ] No [ ] Can't say [ ]
3) Which of these healthcare providers did you see when your child suffered the ailment? Doctor [ ] Pharmacist [ ] Nurse [ ] Chemist [ ] Herbal home [ ] other (specify)……
4) Did the healthcare provider tell you about diarrhoea? Yes [ ] No [ ] Can't say [ ]

D: Control of Diarrhoea

5) Were you told that poor personal hygiene (e. g. not washing the hands after using the toilet, not washing vegetables or fruits before eating etc) can cause/trigger the transmission of diarrhoea? Yes [ ] No [ ] Can't say [ ]
6) Do you know that lack of good toilet facilities can cause/trigger the transmission of diarrhoea? Yes [ ] No [ ] Can't say [ ]
7) Do you know that lack of good drinking water can cause/trigger the transmission of diarrhoea? Yes [ ] No [ ] Can't say [ ]
8) Do you regularly honour appointment dates with the healthcare providers based on your child's health condition? Yes [ ] No [ ] Can't say [ ]
9) Do you give your child the medication prescribed by the healthcare providers? Yes [ ] No [ ] Can't say [ ]
10) If No, how then do you manage your child's health condition? ……..

Thank you.

hospital (doctor) for treatment. 32.6% of the question was not attempted either because their wards had not suffered the ailment or due to self medication. However, 9.5% met nurses, while 7.4% visited the pharmacist (Table 5).

Notwithstanding, most of the contacts were aware that poor personal hygiene, lack of good toilet facilities, lack of good drinking water etc can trigger/cause diarrhoea (Table 6).

The residential location was not correlated with diarrhoea. Occupation of the parent, age and sex do not also have any influence on the prevalence of childhood diarrhoea because they were not significantly correlated with diarrhoea. However, educational level of the parent was significantly correlated with diarrhoea ($r = 0.218$, $p = 0.034$) and ($r = 0.346$, $p = 0.001$) (Table 7).

DISCUSSION

It is widely recognized that diarrhoea is a major cause of child morbidity and mortality and it is affected by several socio-economic, environmental and behavioural factors, this has rarely been confirmed by longitudinal studies or clinical diagnosis. There are a number of studies on diarrhoeal morbidity based on cross-sectional surveys. However, the measurement of diarrhoea from such surveys is complicated and comparison across different background characteristics is difficult (Woldemicael, 1995). In Nigeria, the prevalence of diarrhoea was found to be highest among children 6 – 12 months of age, the period when most children are weaned (Olugbemiro *et al.,* 1994).

Table 2: Demographic details and percentage composition of the parents of children diagnosed for diarrhoea in Nsukka, Nigeria

Variables	Number	Percentage (%)
Location		
Nsukka	23	24.2
Ajuona	7	7.4
UNN	6	6.3
BSH	6	6.3
Onuiyi	6	6.3
Odim	6	6.3
Amaigbo	6	6.3
Age (Years)		
18 – 30	43	45.3
40 and Above	24	25.3
Sex of Parent		
Male	30	68.4
Female	65	31.6
Educational Level		
University	61	64.2
Senior Secondary School	13	13.7
No response	1	1.1
Occupation		
Civil servant	46	48.4
Student	23	24.2
Trader	18	18.9
No response	4	4.2

Table 3: Demographic details and percentage composition of children diagnosed for diarrhoea in Nsukka, Nigeria

Demographic details	Number	Percentage (%)
Age of the child (years)		
5	40	42.1
6	15	15.8
7	13	13.7
10	18	18.9
Sex of child		
Male	51	53.7
Female	44	46.3
Educational level		
Primary	68	71.6
Nursery	18	18.9
No response	1	1.1

It was also reported that at five years, children are still very much prone to this disease because the child has lost inborn immunity and it is exposed to different types of infections from eating food prepared by unclean water and from unhealthy environment (Woldemicael, 1995). Although, researches have not really been carried out on the stipulated age bracket (5 – 10 years), the reports generated in the course of this work suggested that diarrhoea was prevalent in the age bracket researched on. Children at 5 years showed the highest infection rate because they were still acclimatizing to the environmental conditions dirty floor, contaminated food and/or water etc which causes/triggers diarrhoea. Consequently, the educational level of the parents of the child is one of the determinants of diarrhoeal infection. This suggests that the children of less educated people are more prone to diarrhoeal infection than children of more educated individuals who must have read or heard much about the ailment. Though in this research work, residential area and age of the child has little or no correlation with diarrhoea, Woldemicael (1995) noted that the level of diarrhoeal morbidity are the age of the child, number of children in the house, type of floor material and place of residence.

Table 4: Prevalence of diarrhoea among children in Nsukka, Nigeria

Diarrhoeal cases/ Response	Number	Percentage (%)
	Diarrhoea before	
Yes	52	54.7
No	37	38.9
Can't say	6	6.3
No response	–	–
	Diarrhoea this year	
Yes	30	31.6
No	59	62.1
Can't say	5	5.3
No response	1	1.1

Table 5: Healthcare provider visited during childhood diarrhoea in Nsukka, Nigeria

Healthcare provider	Number	Percentage (%)
Doctor	40	42.1
Nurse	9	9.5
Pharmacist	7	7.4
No response	31	32.6

Table 6: Control measures of childhood diarrhoea in Nsukka, Nigeria

Control measures	Response	
	Yes	**No**
Poor personal hygiene	91.6	5.3
Lack of good toilet facilities	87.4	7.4
Lack of good drinking water	89.5	7.4

Table 7: Correlationship between diarrhoea and some demographic variables

Variables	r	*p*
	Diarrhoea before	
Residential location	-0.179	0.082
Educational level of parent	0.218	0.034
Occupation	0.123	0.235
Age of the child	0.022	0.834
Sex of the child	-0.127	0.219
	Diarrhoea this year	
Residential location	- 0.047	0.652
Educational level of parent	0.346	0.001
Occupation	0.134	0.194
Age of the child	0.022	0.851
Sex of the child	-0.092	0.377

Conclusion: Parents who visit the hospital should be educated on safe food and water precautions which are the mainstream of diarrhoea prevention. There must be co-operation between patient and medical practitioners for easy and correct physical examination, history and diagnosis. Rural residents should be sensitized on the behavioural effects of diarrhoea, and then be provided with the essential/basic amenities like pipe-borne water, which they mostly need to keep themselves, children and homes clean. Also, parents should teach their children to practice the act of regular washing of hands mostly after using the toilet, playing and before and after meal. This survey would help necessary authorities in formulating and implementing policies that would ensure a `diarrhoea-free society` in Nsukka, Nigeria, Africa and the world at large.

ACKNOWLEDGEMENT

We immensely acknowledge the critics of this study by members of the Parasitology and Public Health Research Group, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka.

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HAEMATOLOGICAL CHANGES IN THE BLOOD OF CULTURED *CLARIAS GARIEPINUS* STORED AT ROOM AND REFRIGERATOR TEMPERATURES

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ABSTRACT

This study investigated the artifactual changes in the haematological values of Clarias gariepinus blood stored at room (32°C) and refrigerator (4°C) temperatures. Blood samples were collected from 12 apparently healthy fish weighing between 0.8 and 1kg. Samples were divided into two parts immediately after collection and baseline haematological values determined. Haematological determinations were thereafter carried out at 6 hourly intervals up to the 36th hour and then at the 48th hour. Results showed significant reduction (p<0.05) in PCV and RBC values at the 48th hour from the baseline value at room temperature and no significant variation (p>0.05) at the refrigerator temperature. There were significant increases (p<0.05) in the MCV and MCHC values from the baseline values at the 48th hour and MCH value at 36th hour at room temperature while no significant variations (p>0.05) were observed at refrigerator temperature. There were significant reductions (p<0.05) in the WBC count at the 36th and 48th hours when compared with the baseline value at both room and refrigerator temperatures while HBC values did not vary significantly (p>0.05) in both temperatures all through the duration of the study. Reliable RBC count can be obtained up to the 12th hour; WBC and MCH up to the 30th hour; PCV, MCV and MCHC up to the 36th hour and HBC up to the 48th hour at room temperature while at refrigerator temperature, reliable values can be obtained up to the 48th hour in all the parameters determined except WBC count that can be obtained up to the 30th hour of storage.

Keywords: *Clarias gariepinus,* Blood, Artifactual changes, Haematology, Duration of storage, Storage temperature

INTRODUCTION

Changes in the haematological values are reflection of conditions in the body (Schalm *et al*., 1975; Fry and McGavin, 2007) due to diseases or toxicities (Van Vuren *et al*., 1994; Velisek *et al*., 2013; Gadhave *et al,*. 2014) and determination of these parameters can give valuable information regarding physiological alterations in the body (Benjamin, 1978; Coles, 1986). However, changes sometimes may occur due to poor handling or storage of samples leading to misdiagnosis of diseases (Schalm *et al*., 1975; Cohle *et al*., 1981; Meyer and Harvey, 1998; Wood *et al*., 1999; Butarrello, 2004). Delay in analysis of blood samples may be due to restricted access to laboratories, due to the distance between where samples were collected (farm in remote villages) and laboratories located in the cities; delay in collection when large samples are involved; power outage especially in the developing countries; lack of test reagents; test kits and equipment at the time samples are submitted and inability to

finish sample analysis as soon as possible because of too many samples especially with manual procedures.

Refrigeration had been recommended to keep human blood sample in stable state and reduce artifactual changes (Goosens, 1994; Al-Ismail *et al*., 1995; Wood *et al*., 1999; Butarrello, 2004). Blood samples stored in room and refrigerator temperatures showed significant variations in the stability of blood sampled from different species (Clarke *et al*., 2002; Bluel *et al*., 2002; Ihedioha and Onwubuche, 2007). Contrary to the reports in human and some farm animals, Clarke *et al*. (2002) reported that cell counts in equine blood are more stable at room temperature (20 – 25^{o}C) than at refrigerator temperature (4^{o}C). Bluel *et al*. (2002) reported that refrigeration had a stabilising effect on erythrocyte count but led to reduction in WBC count after 24 hours of storage in cattle. Ihedioha and Onwubuche (2007) using manual procedures also reported more pronounced artifactual changes in blood samples stored at room temperature (30^{o}C) than those stored at refrigerator temperature (4^{o}C) in cattle, pigs and goats. In another study on avian blood samples, there were significant increases in the packed cell volume (PCV) and mean corpuscular volume (MCV) values after 18 and 12 hour storage, respectively with significant reduction in mean corpuscular haemoglobin concentration (MCHC) and white blood cells (WBC) count after 12 and 18 hours storage respectively in blood samples stored at room temperature (Ihedioha *et al*., 2008). Clinically significant increase in buffy coat percentage had also been reported in cattle and chicken blood samples stored at 30^{o}C and 37^{o}C at hour 72 and in pig blood samples stored at 30^{o}C and 37^{o}C as from the 48th hour of storage onwards (Ihedioha and Aba, 2010).

Fishes are reared most times in locations far away from diagnostic laboratories and blood samples collected from such locations at emergencies are prone to artifactual changes due to time lag between sample collection and analysis. Artifactual changes due to duration of storage and storage temperature had been reported in blood of cultured *Heteroclarias* hybrid (Okorie-Kanu and Solomon, 2015) but no reports exist for *Clarias gariepinus*.

This study therefore investigated the effect of time of storage and storage temperature on the packed cell volume, haemoglobin concentration, red blood cell and white blood cell counts and mean corpuscular values of blood samples collected from cultured *Clarias gariepinus*. The result of this study shall guide researchers and field veterinarians in proper handling of blood samples for haematological determinations to ensure the reliability and usefulness of haematological results as tools for diagnosis, prognosis and assessment of efficacy of therapeutic interventions in this species.

MATERIALS AND METHODS

Catfish: A total of 12 apparently healthy fish (*Clarias gariepinus)* weighing between 0.80 and 1.00 kg with an average weight of 0.92 kg were used for the study. They were obtained from the Michael Okpara University of Agriculture, Umudike Fish Farm raised in a 15 x 15 m^2 indoor concrete ponds under strict standard cultural practices. They were fed by point-feeding method with standard fish feed (Coppens, Germany) and water changed every week.

Blood: The blood samples were collected in the morning hours and were immediately divided into two parts and baseline haematological values determined. One part was kept on the laboratory bench at room temperature ranging between 29^{o}C and 35^{o}C (average 32^{o}C) and the other part was kept under refrigerator temperature ranging between 3^{0}C and 6^{o}C (average 4^{o}C). Haematological determinations were subsequently carried out at six hourly intervals for the first 36 hours and then at the 48th hour.

Haematology: Blood samples were collected through the caudal vein and put into bottles treated with appropriate quantity of ethylene diamine tetracetic acid (EDTA) Haematological determinations were carried out immediately after collection following standard procedures.

Packed cell volume (PCV) was determined by the microhaematocrit method, while haemoglobin concentration (HBC) was determined by the cyanomethaemoglobin method (Kachmar, 1970; Schalm *et al.,* 1975; Coles, 1986). Red blood cell (RBC) and total white blood cell (WBC) counts were carried out by the haemocytometer method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Schalm *et al.,* 1975; Coles, 1986).

Data Analysis: Data from all the determinations were analyzed using Analysis of Variance (ANOVA) using SPSS 16.0 Statistical Package (SPSS 16.0 for Windows, SPSS Inc., Chicago, IL, USA). Different means were separated using the Least Significant Different (LSD) method and significant difference was accepted at $p<0.05$.

RESULTS

The mean PCV value recorded was significantly lower ($p<0.05$) at the 48th hour when compared with the baseline value at room temperature while there was no significant variation ($p>0.05$) at the refrigerator temperature all through the study (Table 1). The values recorded for haemoglobin concentration up to the 48th hour did not vary significantly ($p>0.05$) in both room and refrigerator temperatures (Table 1).

There was a significant reduction ($p<0.05$) in the RBC values recorded from 18th to 48th hour when compared with the baseline value at room temperature while there was a significant reduction ($p<0.05$) only at the 48th hour when compared with the baseline value at refrigerator temperature (Table 2).

Furthermore, there were significant reductions ($p<0.05$) in the WBC count recorded from the 36th hour at both room and refrigerator temperatures when compared with the baseline (Table 2).

The MCV values recorded at the 48th hour was significantly higher ($p<0.05$) when compared with the baseline value at room temperature but no significant variation ($p>0.05$) at refrigerator temperature all through the study (Table 3).

The MCH values recorded from the 36th hour was significantly higher ($p<0.05$) when compared with the baseline at room temperature while at refrigerator temperature, there was no significant variation ($p>0.05$) in the 48th hour period of the study (Table 3). The MCHC values recorded at the 48th hour was significantly higher ($p<0.05$) when compared with the baseline at room temperature while there was no significant variation ($p>0.05$) all through the study at the refrigerator temperature (Table 3).

DISCUSSION

The significant reduction in the PCV at the 48th hour is in agreement with the result observed in Heteroclarias hybrid (Okorie-Kanu and Solomon, 2015) and also may be due to lysis of RBCs as there was also significant reduction in total RBC count. The result is in contrast to the values reported for humans (Cohle *et al.* 1981; Wood *et al.,* 1999; Buttarello, 2004), horses (Clarke *et al.,* 2002), rat, bovine, caprine and porcine blood stored at room and refrigerator temperatures and avian blood samples stored at varying temperatures (Bluel *et al.,* 2002; Ihedioha and Ibeachu, 2005; Ihedioha and Onwubuche, 2007; Ihedioha *et al.,* 2008). This could be attributed to degenerative changes that resulted in widening of the pores on the surfaces of RBCs, permitting ingress of water into the cells (Jandl, 1965; Schalm *et al.,* 1975; Coles, 1986). This may be that the elastic limit of the fish RBCs is less compared to humans and other animals resulting in early cell lysis even though there was initial cell swelling.

The lysis of RBCs led to the significant reduction in the RBC values recorded far earlier than humans and many farm animals. The result followed the same trend with what was reported in avian blood samples although the reduction was not significant (Ihedioha *et al.,* 2008). The significant increases in the erythrocytic indices were due to the reduction in RBC and PCV values as the two parameters were the denominators in the calculation of the

Table 1: Packed cell volume and haemoglobin concentration of *Clarias gariepinus* kept at different temperatures for fourty eight hours

Hour	Packed cell volume		Haemoglobin concentration	
	Room	Refrigerator	Room	Refrigerator
0	27.20 ± 1.93	27.30 ± 1.83	10.97 ± 0.81	10.96 ± 0.68
6	26.40 ± 1.44	26.70 ± 1.77	10.86 ± 0.79	11.07 ± 0.71
12	26.20 ± 1.82	27.20 ± 2.01	10.55 ± 0.72	11.07 ± 0.74
18	24.50 ± 1.76	25.50 ± 1.62	10.76 ± 0.62	10.76 ± 0.58
24	25.42 ± 1.29	25.50 ± 1.71	10.43 ± 0.54	10.76 ± 0.50
30	25.42 ± 1.42	25.90 ± 1.82	10.35 ± 0.42	10.71 ± 0.52
36	22.80 ± 1.74	25.20 ± 1.93	11.07 ± 0.83	10.60 ± 0.58
48	21.20 ± 1.74[b]	24.80 ± 1.81	10.76 ± 0.76	10.81 ± 0.68

*Different superscripts in a column indicate significant difference between the time intervals ($p<0.05$)

Table 2: Red blood cell and white blood cell counts of *Clarias gariepinus* kept at different temperatures for fourty eight hours

Hour	Red blood cell counts		White blood cell count	
	Room	Refrigerator	Room	Refrigerator
0	2.17 ± 0.12[a]	2.15 ± 0.09	17.53 ± 0.87[a]	17.24 ± 0.82[a]
6	1.99 ± 0.05[a]	2.05 ± 0.08	16.32 ± 0.98[a]	16.77 ± 0.95[a]
12	1.97 ± 0.07[a]	2.03 ± 0.11	16.01 ± 0.90[a]	16.45 ± 0.87[a]
18	1.76 ± 0.11[b]	1.83 ± 0.17	14.47 ± 1.01[a]	15.34 ± 1.42[a]
24	1.82 ± 0.10[b]	2.09 ± 0.10	14.25 ± 1.42[a]	14.34 ± 0.98[a]
30	1.83 ± 0.04[b]	2.09 ± 0.08	14.59 ± 0.89[a]	15.26 ± 0.99[a]
36	1.52 ± 0.15[c]	2.06 ± 0.13	9.93 ± 1.18[b]	13.49 ± 1.63[b]
48	1.24 ± 0.20[d]	2.20 ± 0.25	6.82 ± 1.53[b]	10.28 ± 1.28[b]

*Different superscripts in a column indicate significant difference between the time intervals ($p<0.05$)

Table 3: Mean corpuscular volume (fl), mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration of *Clarias gariepinus* kept at different temperatures for fourty eight hours

Hours	Mean corpuscular volume		Mean corpuscular haemoglobin		Mean corpuscular haemoglobin concentration	
	Room	Refrigerator	Room	Refrigerator	Room	Refrigerator
0	124.65 ± 7.25[a]	126.74 ± 4.20	51.03 ± 4.59[a]	50.95 ± 2.35	42.25 ± 3.67[a]	40.41 ± 1.96
6	132.52 ± 6.56[a]	130.38 ± 6.16	54.63 ± 4.11[a]	54.04 ± 2.37	41.05 ± 1.89[a]	41.55 ± 2.05
12	133.52 ± 8.76[a]	133.42 ± 5.35	53.70 ± 3.96[a]	54.29 ± 2.19	40.41 ± 1.73[a]	41.13 ± 2.82
18	134.24 ± 5.14[a]	124.47 ± 2.67	65.90 ± 5.53[a]	51.11 ± 7.27	44.32 ± 2.56[a]	42.71 ± 2.56
24	134.17 ± 2.09[a]	121.80 ± 6.23	66.08 ± 4.59[a]	51.51 ± 1.67	44.31 ± 3.25[a]	43.33 ± 2.19
30	139.23 ± 7.10[a]	127.22 ± 4.31	57.56 ± 2.26[a]	51.33 ± 2.75	41.57 ± 1.80[a]	41.72 ± 2.53
36	139.14 ± 4.04[a]	123.12 ± 7.93	78.13 ± 10.39[b]	52.65 ± 5.04	49.34 ± 4.90[a]	43.03 ± 4.27
48	162.20 ± 10.56[b]	128.54 ± 9.10	87.42 ± 7.51[b]	61.06 ± 4.17	54.44 ± 5.05[b]	45.03 ± 1.97

*Different superscripts in a column indicate significant difference between the time intervals ($P < 0.05$)

mean corpuscular values (Thrall and Weiser, 2002). The lack of significant changes in the HBC values was in agreement with reports of previous studies in humans, cattle, goats, horses and pigs (Cohle *et al*., 1981; Goosens, 1984; Al-Ismail *et al.,* 1995; Wood *et al.,* 1999; Clarke *et al.,* 2002; Buttarello, 2004; Ihedioha and Onwubuche, 2007). This could be due to the fact that haemoglobin fractions released from the lysed RBCs are still available in the sample unlike when they are in circulation where they are degraded into their various components where bilirubin is conjugated and excreted and iron returned to the storage pool to be reused for haemoglobin production (Benjamin, 1978; Coles, 1986).

The reduction in the WBC counts recorded at both room and refrigerator temperatures are in agreement with the reports of Ihedioha *et al.* (2008) in chickens but contrasts with the findings in studies with blood of mammals including humans (Cohle *et al.,* 1981; Goosens, 1984; Al-Ismail *et al.,* 1995; Wood *et al.,* 1999; Clarke *et al.,* 2002; Buttarello, 2004; Ihedioha and Onwubuche, 2007). This may be because the WBC of birds and fishes are nucleated and therefore degenerate earlier making them unable to take up stains and therefore are not seen and counted (Campbell and Coles, 1986; Dein, 1986).

Conclusion: Based on the results of this study, it was concluded that reliable RBC count can be obtained up to the 12th hour; MCH up to the 30th hour; PCV, MCV and MCHC up to the 36th hour and HBC up to the 48th hour at room temperature while at refrigerator temperature, reliable values can be obtained up to the 48th hour in all the parameters determined. Also, reliable WBC count can be obtained up to the 30th hour in both room and refrigerator temperatures. The significant reduction in RBC as early as the 18th hour and significant reduction in WBC count in both room and refrigerator temperatures are worthy of note, therefore blood sample from this species must be analysed within 12 hours at room temperature and 30th hours for both room and refrigerator temperatures for the two parameters to be useful in disease diagnosis, prognosis and monitoring of therapeutic processes.

ACKNOWLEDGEMENT

We are grateful to the Pond supervisor, Mr. Candidus I. Obodagu for his assistance throughout the period of the study.

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HAEMATO-BIOCHEMICAL PROFILE OF APPARENTLY HEALTHY DOMESTIC TURKEYS (*Meleagris gallopavo*) IN NSUKKA, ENUGU STATE, NIGERIA

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ABSTRACT

Performing comprehensive health assessment on turkey populations enables one to determine their responses to physiological factors such as sex, breed and environmental stressors. The haemato-biochemical parameters of 58 apparently healthy domestic turkeys of varying breeds and sexes, raised by small scale turkey farmers in Nsukka metropolis, Enugu State, Nigeria and the physiological factors (sex and breed) that influence them were determined. All haemato-biochemical procedures were carried out following standard procedures. The mean for the haemato-biochemical parameters were as follows: packed cell volume 30.70 ± 0.63 %; red blood cell count 3.75 ± 0.22 x 10^6/µl; hemoglobin concentration 12.95 ± 0.62 g/dl; total leukocyte count 3.67 ± 0.09 x 10^3 /µl; heterophil 2.24 ± 10^3/µl; lymphocyte 1.36 ± 0.06 x 10^3/µl; monocyte 0.03 ± 0.00 x 10^3/µl; eosinophil 0.10 ± 0.02 x 10^3/µl; basophil 0.01 ± 0.00 x 10^3/µl; aspartate aminotransferase 73.99 ± 5.40 IU/L; alanine aminotransferase 11.00 ± 2.12 IU/L; alkaline phosphatase 165.19 ± 15.77 IU/L; total proteins 4.64 ± 0.17 g/dl; albumin 2.54 ± 0.12 g/dl; globulin 2.11 ± 0.12 g/dl; cholesterol 157.83 ± 12.81 mg/dl; creatinine 0.98 ± 0.11 mg/dl; uric acid 5.62 ± 0.45 mg/dl. There were no sex-related variations in all the studied haematological and biochemical parameters. It was also found that the local breed had significantly higher ($p<0.05$) monocyte numbers than the foreign breed (Nicholas large white). This study shall form a basis for establishing haemato-biochemical reference range of values for clinical and scientific uses by avian clinicians for turkey populations in Nsukka.

Keywords: Haematology, Biochemicals, Domestic turkeys, Nsukka metropolis

INTRODUCTION

Domestic turkey (*Meleagris gallopavo*) (Crawford, 1993) which originated from North America has been domesticated worldwide including Nigeria, and serves as an important source of animal protein (Nixey and Grey, 1985). It is reared for its tasty and high quality meat (high in protein and low in cholesterol) and for egg production in the rural and urban areas of Nigeria (Prabakaran, 2003). Currently, there are different breeds/varieties of turkeys which include white turkey (Nicholas large white, Beltsville small white, White Holland turkey), black turkey, (Narragansett - predominantly white with interspersed white, royal palm - predominantly white with few black feathers, slate - ash coloured/ashy blue and sometimes dotted with black, Bourbon red - predominantly black with white tail, bronze - shimmering green-bronze that appears metallic under sunlight, Czech wild white-braided turkey, Dindon Rouge des Ardennes of France and the Zargorje of Croatia). In Nigeria, our local breed

usually includes the black and lavender varieties.

Turkey production is one of the most important and probably most profitable form of poultry production as it can be the sole means of livelihood for backyard poultry farmers (Adene, 1990). The economic significance of turkey production varies considerably, although it has become increasingly specialized and integrated into a dynamic industry of national and international importance. The importance of turkey production in the national economy of developing countries and its role in improving the nutritional status and income of many small communities has been very significant.

However, production levels of turkeys in many African countries are far below desirable levels mainly due to the menace of infectious diseases and poor management (FAO, 1997). Important factors in the continued growth of the poultry industry in many countries include the efficiency of poultry in converting vegetable protein into animal protein, the attractiveness and acceptability of turkey meat and eggs to many people, their competitive cost, the perceived healthfulness of turkey meat in human diets, acceptability to all religions, and the relative ease with which new technologies can be transferred between countries (FAO, 1997).

In veterinary practice, the assessment of the haematology and biochemical profile is important as it is used to evaluate the physiological and pathological status of birds and animals (Carter, 1996; Yaqub *et al*., 2013). Evaluation of the haemato-biochemical parameters in birds is also important in ascertaining response to its internal and external environments (Sparling *et al*., 1999; Esonu *et al*., 2001). Haematological assessments are used as basis for diagnosis of avian diseases (Tibbo *et al*., 2004), monitoring recovery during treatment and assessment of the health status of a single bird or entire flock (Messer, 1995). Quantitative determination of a wide variety of substances (substrates, enzymes and hormones) in plasma or serum helps to assess the present functional status of the vital body organs especially the pancreas, heart, muscles, liver and the kidney. Therefore, an evaluation of the haematology and biochemical parameters is important in arriving at a definitive diagnosis, assessing the efficacy of instituted therapy, determine the toxicity of drugs and chemical substances and to make a prognosis (Coles, 1986; Stockham and Scott, 2008). Factors affecting haematological values include the age, species, breeds, sex, nutritional state and management, environmental factors such as temperature, humidity, altitude and day length, the type of anticoagulant used and sample handling (Sparling *et al*., 1999; Stockham and Scott, 2008).

It has been established that there are differences in the haematological and biochemical parameters of different breeds of turkeys and because of this every laboratory or clinic need to establish reference values for the turkey population in its environment (Coles, 1986; Stockham and Scott, 2008). Because of the scanty information on the haematological and biochemical profile of domestic turkeys, the need for sufficient information for diagnostic and management purposes and the increase in demand for turkey production in Nsukka, this present study therefore, evaluated the haematological and biochemical profile of domestic turkeys and determined the influence of sex and breed on these parameters.

MATERIALS AND METHODS

The study was carried out on 58 apparently healthy domestic turkeys of varying breed (local and Nicholas large white) and sexes for a period of 6 months. These turkeys were raised by backyard/small scale poultry farmers in Nsukka metropolis. Nsukka is situated within the derived savanna belt of the state between latitude 5° 50' and 7° 00' North and longitude 6° 50' and 7°54'. It is also located at the Northern part of Enugu State. History (vaccination, nutrition and management system) was also obtained from the farmers before blood samples were collected for haematology and biochemical analysis. The turkeys were all raised on free range system. These turkeys were bled through the jugular vein and 3 ml of blood was collected. Blood sample (1 ml) for haematology was collected into appropriately labeled sample

bottles containing ethylene diamine tetra acetic acid (EDTA) (1 mg/ml of blood). Blood samples were placed on ice for transport to the laboratory.

Blood Collection: Two (2) ml of blood was put into plain glass test tubes and allowed to clot at room temperature within one hour of collection. Serum was obtained by spinning the clotted blood at 3000 rpm for 10 minutes using a clinical table centrifuge (Ajmer, India). Both haematological and biochemical parameters were evaluated immediately upon sample collection. None of the samples were stored.

Haematology: The haematological determinations were carried out following standard procedures. The packed cell volume (PCV) was determined by the micro-haematocrit method (Thrall and Weiser, 2002), haemoglobin concentration (Hbc) was determined by the cyanomethemoglobin method (Higgins *et al.*, 2008). Red blood cell (RBC) counts and total leukocyte counts (TLC) were carried out by haemocytometer method (Thrall and Weiser, 2002), while blood smear made on clean glass slides for differential leukocyte count were stained following the Leishman technique and were enumerated by the battlement counting method (Thrall and Weiser, 2002). The mean corpuscular values; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Schalm *et al.*, 1975; Coles, 1986).

Serum Biochemistry: The serum biochemistry determinations were carried out using commercial test kits, Quimica Clinica Aplicada (QCA) test kits (QCA, Spain) and a digital colorimeter (Lab-tech, India). The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by the Reitman-Frankel method (Reitman and Frankel, 1957). The serum alkaline phosphatase (ALP) activity was determined by the phenolphthalein monophosphate method (Klein *et al.,* 1960; Babson *et al.,* 1966), while the serum total protein (TP) was determined by the direct Biuret method (Lubran, 1978) and the serum albumin was determined by the Bromocresol green method (Doumas *et al.*, 1971). The serum globulin was calculated as the difference between the serum TP and serum albumin (Colville, 2002). The serum uric acid was determined by the Uricase-POD method (Trinder, 1969), while the serum creatinine was determined by the modified Jaffe method (Blass *et al.,* 1974). The serum cholesterol was determined by the enzymatic colorimetric method (Allain *et al.,* 1974).

Data Analysis: Data generated from the study was subjected to descriptive statistics using SPSS 17.0. The differences between the haematological and biochemical parameters of the apparently healthy male and female turkeys were analyzed using students t – test, while the differences between the haematological and biochemical parameters of apparently healthy local and foreign breeds were also analyzed using students t-test. The data were presented as means and standard errors of means, and the minimum and maximum values for each parameter. Significant difference was accepted at $p<0.05$.

RESULTS

The values obtained for the haematological and serum biochemistry parameters of apparently healthy domestic turkeys, with their minimum and maximum values are presented in Tables 1 side by side with the reference values and ranges for these parameters as reported in literature. There were no significant sex-related differences ($p>0.05$) in all the haematological parameters though the mean red blood cell, total leukocyte, heterophil, monocyte, and eosinophil counts were numerically higher in males than in females (Table 2). The mean haemoglobin concentration and lymphocyte count were numerically higher in the females than in the males (Table 2). There were also no significant sex-related differences ($p>0.05$) in all the serum biochemical parameters but the mean total cholesterol and creatinine values were numerically higher in males than in females

(Table 2). The serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, albumin, globulin and uric acid values were numerically higher in females than in males (Table 2).

There were no significant breed differences ($p>0.05$) in all the haematological and serum biochemistry parameters, except in the monocyte count which was significantly ($p<0.05$) higher in the local breed than in the foreign breed (Table 3). The mean red blood cell, haemoglobin concentration, heterophil and eosinophil counts, serum aspartate aminotransferase, alkaline phosphatase, total protein, globulin, creatinine and uric acid values were lower (but not significant) in local breed than foreign breed (Tables 3). In foreign turkeys, the total leukocyte and lymphocyte counts, serum albumin and total cholesterol values were numerically lower but not significantly different ($p>0.05$) when compared to those of local turkeys.

DISCUSSION

The haematological and serum biochemistry parameters of domestic turkeys recorded in this study were within ranges as reported by Gylstroff (1983), LAVC (2009) and Ajaonuma *et al.* (2013), both in mean and reference range of values. However, the report of this study contradicts that of Bounous *et al.* (2000) who recorded higher values for total leucocyte, heterophil and lymphocyte counts. The not significant increase in PCV and RBC counts in males than females could be a function of high levels of testosterone in adult male birds and mammals (Sturkie, 1986), which stimulate erythropoiesis (Villers and Dunn, 1998). The present study also showed that there were no significant sex differences in RBC count, total and differential leukocyte counts and thus agreed with the report on a study on ring-necked pheasant (Schmidt *et al.,* 2007) and free-range helmeted guinea fowl (Nalubamba *et al.,* 2010).

In this study, it was established that heterophil is the most abundant white blood cell in turkeys. This is not in agreement with that of other avian species such as budgerigars (Harper and Lowe, 1998), helmeted guinea fowl (Nalubamba *et al.,* 2010), ducks (Okeudo *et al*., 2003), Muscovy ducks (Sulaiman *et al*., 2010) and Juvenile wild turkeys (Bounous *et al*., 2000) where lymphocyte was found to be the most abundant white blood cell. Ibrahim *et al.* (2012) reported no sex-related differences in serum TP, creatinine, albumin, globulin, ALP, AST and ALT. This was in agreement with the findings of this study. Also, the serum AST, ALT and ALP values obtained in this study for both male and female turkeys were much higher than reported values by Ibrahim *et al*. (2012). The serum creatinine value for female turkeys and serum globulin value for male turkeys were similar to that documented by Ibrahim *et al.* (2012). No significant sex variation in serum TP concentration was observed in local ducks (Oladele *et al*., 2001a) and pigeons (Oladele *et al*., 2001b). This finding was in agreement with the findings of this study where sex had no influence on the serum TP concentration. The high but not significant serum creatinine concentration in males could be attributed to increase muscle mass of male turkeys than females. The recorded increase but not significant uric acid concentration for adult female turkeys may be attributed to ovulatory activity of female birds (Ritchie *et al.*, 1994; Ibrahim *et al.,* 2012), and it has also been suggested that concentration of estrogen and protein diet may influence plasma uric acid concentration in chickens (Sturkie, 1961). The mean serum AST value obtained in this study for female turkeys were lower than that reported by Schmidt *et al*. (2010), while the serum TP, albumin and globulin values were similar to the report of Schmidt *et al.* (2010). It is suggested that laying season in female pigeons (Gayathri and Hedge, 2006) and wild turkeys (Martin *et al*., 1981) may lead to an increase in blood proteins induced by estrogen.

Table 1: Haematological and serum biochemical profiles of apparently healthy domestic turkeys compared to reference range of values in available literature

Parameters	This study[a]	LAVC (2009)	Mehner and Hartfiel (1983) (Turkey)	Bounous *et al.* (2000) (Wild turkey)	Ajuonuma *et al.* (2013) (Domestic turkey)
Haematology		**Haematological profile**			
Packed cell volume (%)	30.70 ± 0.63 (20.00 – 39.00)	-	36 – 41	31 – 42	31.75
Red blood cell count ($x10^6$/µl)	3.75 ± 0.22 (2.41 – 8.23)	-	2.3 – 2.8	-	2.03
Haemoglobin concentration (gldl)	12.95 ± 0.62 (10.42-15.20)	-	10.3 – 15.2	-	10.57
Total leukocyte count ($x10^3$/µl)	3.67 ± 0.09 (2.40 – 4.35)	-	2.35 – 2.68	10.4 – 46.5	-
Heterophil count ($\times10^3$ /µl)	2.24 ± 0.08 (1.33 – 3.00)	-	-	4.06 – 27.61	-
Lymphocyte count ($x10^3$/µl)	1.36 ± 0.06 (0.68 – 1.90)	-	-	4.22 – 34.27	-
Monocyte count ($x10^3$ /µl)	0.03 ± 0.00 (0.00 – 0.12)	-	-	0 – 4.0	-
Eosinophil count ($x10^3$/µl)	0.10 ± 0.02 (0.00 – 0.28)	-	-	0 – 0.4	-
Basophil count ($x10^3$/µl)	0.01 ± 0.00 (0.00 – 0.07)	-	-	0 – 2.2	-
Serum Biochemistry		**Serum biochemical profile**			
Aspartate aminotransferase (IU/l)	73.99 ± 5.40 (55.50 – 120.21)	100 - 400	-	255 – 499	117.50
Alanine aminotransferase (IU/l)	11.00 ± 2.12 (3.08 – 37. 99)	-	-	-	10.00
Alkaline phosphatase (IU/l)	165.19 ± 15.77 (108.95 – 370.20)	35 – 410	-	-	-
Total Proteins (g/dl)	4.64 ± 0.17 (3.04 – 6.21)	-	-	3.6 – 5.5	4.56
Albumin (g/dl)	2.54 ± 0.12 (1.26 – 3.90)	-	-	1.1 – 2.1	1.91
Globulin (g/dl)	2.11 ± 0.12 (1.12 – 3.14)	1.2 – 3.2	-	-	2.65
Total Cholesterol (mg/dl)	157.83 ± 12.81 (63.00 – 366.67)	139 -202	-	60 – 220	101.00
Creatinine (mg/dl)	0.98 ± 0.11 (0.50 – 2.00)	0.1 – 0.5	-	-	-
Uric acid (mg/dl)	5.62 ± 0.45 (2.50 – 466.67)	6	-	3 – 17	-

[a]*Mean ± SE, with minimum and maximum values in bracket, n = 58*

Table 2: Sex differences in the haematological and serum biochemical profiles of apparently healthy domestic turkeys

Parameters	Males (n = 19)	Females (n = 39)
Haematology	**Haematological profile**	
Packed cell volume (%)	31.08 ± 1.03 (25.00 – 39.00)	30.50 ± 0.80 (20.00 – 37.00)
Red blood cell count (x10^6/µl)	4.04 ± 0.53 (2.41 – 8.23)	3.56 ± 0.14 (2.95 ± 5.35)
Haemoglobin concentration (g/dl)	11.10 ± 0.68 (10.42 – 11.78)	12.95 ± 0.62 (10.42 – 15.20)
Total leukocyte count (x10^3/µl)	3.75 ± 0.14 (2.60 – 4.10)	3.62 ± 0.12 (2.40 – 4.35)
Heterophil count (×10^3/µl)	2.37 ± 0.15 (1.33 – 3.00)	2.15 ± 0.08 (1.47 – 2.65)
Lymphocyte count (x10^3/µl)	1.24 ± 0.11 (0.68 – 1.89)	1.45 ± 0.07 (1.02 – 1.90)
Monocyte count (x10^3 /µl)	0.03 ± 0.01 (0.00 – 0.11)	0.02 ± 0.00 (0.00 – 0.12)
Eosinophil count (x10^3/µl)	0.12 ± 0.03 (0.00 – 0.28)	0.09 ± 0.02 (0.00 – 0.25)
Basophil count (x10^3/µl)	0.003 ± 0.003 (0.00 – 0.03)	0.009 ± 0.005 (0.00 –0.07)
Serum biochemistry	**Serum biochemical profile**	
Aspartate aminotransferase (IU/l)	68.68 ± 3.98 (55.50 – 98.09)	83.79 ± 6.60 (55.50 – 120.21)
Alanine aminotransferase (IU/l)	8.53 ± 1.64 (3.24 – 20.38)	13.02 ± 3.59 (3.08 – 37.99)
Alkaline phosphatase (IU/l)	152.87 ± 19.03 (108.95 -320.00)	175.46 ± 24.54 (110.45 – 370.20)
Total Protein (g/dl)	4.30 ± 0.27 (3.04 – 6.21)	4.87 ± 0.02 (3.07 – 6.01)
Albumin (g/dl)	2.41 ± 0.17 (1.64 – 3.25)	2.63 ± 0.16 (1.26 – 3.90)
Globulin (g/dl)	1.90 ± 0.19 (1.12 - 3.92)	2.25 ± 0.15 (1.30 – 3.14)
Total Cholesterol (mg/dl)	185.72 ± 25.44 (112.50 – 366.67)	141.09 ± 12.49 (63.08 – 222.20)
Creatinine (mg/dl)	1.06 ± 0.19 (0.50 – 2.00)	0.92 ± 0.13 (0.50 - 2.00)
Uric acid (mg/dl)	5.48 ± 0.63 (2.50 – 10.00)	5.74 ± 0.65 (2.50 – 10.00)

Mean ± SE, with minimum and maximum values in bracket, n = 58

Table 3: Breed differences in the haematological and serum biochemical profiles of apparently healthy domestic turkeys

Haematological parameters	Local (n = 41)	Foreign (Nicholas large white) (n = 17)
Haematology	**Haematological profile**	
Packed cell volume (%)	30.78 ± 0.57 (23.00 – 37.00)	30.20 ± 3.12 (20.00 -39.00)
Red blood cell count (10^6/µl)	3.73 ± 0.24 (2.41 – 8.23)	3.92 ± 0.09 (3.83 – 4.01)
Haemoglobin concentration (g/dl)	12.54 ± 0.89 (10.42 – 14.39)	13.50 ± 0.91 (8.42 – 10.78)
Total leukocyte count (10^3/µl)	3.71 ± 0.08 (2.60 – 4.35)	3.19 ± 0.79 (2.40 – 3.98)
Heterophil count (10^3/µl)	2.22 ± 0.08 (1.33 – 3.00)	2.47 ± 0.08 (2.39 – 2.55)
Lymphocyte count (10^3/µl)	1.37 ± 0.07 (0.68 – 1.90)	1.31 ± 0.16 (1.15 – 1.47)
Monocyte count * (10^3/µl)	0.03 ± 0.00 (0.00 – 0.12)	0.00 ± 0.00 (0.00 – 0.00)
Eosinophil count (10^3/µl)	0.09 ± 0.02 (0.00 – 0.07)	0.20 ± 0.08 (0.12 – 0.28)
Basophil count (10^3/µl)	0.01±0.00 (0.00 – 0.07)	0.00 ± 0.00 (0.00 – 0.00)
Serum Biochemistry	**Serum biochemical profile**	
Aspartate aminotransferase (IU/l)	76.81 ± 5.24 (55.50 – 120.21)	77.72 ± 6.31 (63.37 – 92.12)
Alanine aminotransferase (IU/l)	11.34 ± 2.57 (3.08 – 37.99)	11.34 ± 2.57 (3.45 – 18.23)
Alkaline phosphatase (IU/l)	157.07 ± 15.13 (108.95 – 333.23)	201.75 ± 6.46 (129.21 – 370.20)
Total protein (g/dl)	4.60 ± 0.20 (3.04 – 6.21)	4.90 ± 0.35 (4.21 – 5.64)
Albumin (g/dl)	2.55 ± 0.14 (1.26 – 3.90)	2.45 ± 0.13 (2.12 – 2.76)
Globulin (g/dl)	2.04 ± 0.13 (1.12 – 3.14)	2.45 ± 0.26 (1.92 – 2.89)
Total Cholesterol (mg/dl)	165.14 ± 13.78 (87.50 – 366.67)	121.25 ± 31.67 (63.08 – 208.28)
Creatinine (mg/dl)	0.92 ± 0.11 (0.50 – 2.00)	1.50 ± 0.50 (1.00 – 2.00)
Uric acid (mg/dl)	5.48 ± 0.45 (2.50 – 10.00)	7.37 ± 2.38 (5.00 – 9.75)

*Mean ± SE, with minimum and maximum values in bracket, n = 58, *Asterisk superscript on any parameter indicates significant difference between the local and foreign breeds, p<0.05*

The values for the haemato-biochemical parameters of the local and foreign breeds recorded in this study were the same (except for the monocyte number) and this could be attributed to the fact that foreign turkeys acclimatized in our locality as turkeys are known to have the ability to acclimatize in various types of climate (Isidahomen *et al*., 2013). Previous studies in Indian turkeys (Pandian *et al.,* 2012) and other breeds of turkeys (Isidahomen *et al*., 2013) revealed that PCV values vary among breeds of turkeys and thus contrasts with the

findings of this study where there were no breed differences in PCV values. Significant differences in erythrocytic indices, total leukocyte, heterophil, lymphocyte, basophil and eosinophil numbers documented by Isidahomen *et al*. (2013) contrast with the findings of this study except in the monocyte numbers but agreed with that of Oke *et al.* (2007) in red blood cell number and haemoglobin concentration. The finding of a significantly higher monocyte number and its biological significance in local than Nicholas large white is not understood. The findings of this study disagreed with the work of Isidahomen *et al*. (2013) who found significant breed differences in serum concentrations of TP, albumin, cholesterol and creatinine, and agreed with that of El-Safty *et al*. (2006). The serum creatinine levels of Nicholas large white were numerically higher but not significant when compared to the local breed. This may be attributed to the high amount of phosphocreatine (a precursor of creatinine) found in muscle cells of foreign turkey when compared to that in the local turkeys. As a result of this, the muscle mass of the Nicholas large white is larger than that of local turkeys (Stockham and Scott, 2008).

Conclusion: Based on the results of this study, it was concluded that no statistical sex differences were recorded for all haemato-biochemical parameters of apparently healthy adult turkeys studied. It was also found that there were no statistical breed differences in all the haemato-biochemical parameters except for the monocyte numbers that were significantly higher in local than foreign breeds. Therefore, it is suggested that large sample size for male turkeys and Nicholas large white breed of turkeys be used for further studies. However, the present study will guide future use of this reference range of values for diagnostic and management purposes by veterinarians and avian specialists.

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CARCASS AND ECONOMIC VALUE OF RABBITS RAISED ON RIPE GMELINA FRUIT PULP BASED DIETS

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ABSTRACT

Carcass and economic indices of rabbits fed dietary ripe Gmelina fruit (RGFP) pulp were assessed. Sixty rabbits, aged six weeks (New Zealand White x Chinchilla cross) of mixed sexes (25 males and 35 females) were shared into five groups of 12 animals each and balanced on sex and initial weight and randomly allotted to the five experimental diets. Maize, the only energy source of diet I was replaced with 25, 50, 75 and 100% RGFP in diets II, III, IV and V, respectively in the 16 weeks trial. Carcass characteristics were determined pre-chill. Completely Randomized Design, one-way analysis of variance was used and significant ($p<0.05$) means were separated using least significant difference. The cost of feed (₦/kg), cost of feed consumed (₦/animal), cost of feed per weight gain (₦/kg weight gain), final weight, fasted weight, percentage weight loss, dressed weight and dressing percentage ranged from 55.07 – 28.21, 407.07 – 213.55 and 307.68 – 207.34, 1900 – 1605g, 1884.90 – 1589.78g, 15.23 – 15.09%, 1206.42 – 1017.46g and 64.01 – 63.97%, respectively. As a percentage of the dressed carcass, the shoulder, loin, rack, thigh, head, tail, belly fat, lung, kidney, liver and heart weights (g) ranged from 33.53 – 33.47, 8.97 – 8.74, 6.28 – 6.18, 35.29 – 35.16, 5.20 – 5.13, 3.29 – 3.19, 3.30 – 2.89, 0.32 – 0.30, 1.50 – 1.47, 2.80 – 2.77 and 0.18 – 0.15, respectively. Diet I had the highest cost of feed, cost of feed consumed, cost of feed per kilogramme weight gain, pre-slaughter weight and percentage belly fat, while diet V had the least values of the above parameters except percentage belly fat and dressed weight. Also, the values for these indices decreased as dietary RGFP increased. Though undesirable, the meat of control rabbits was the fattiest. Dietary inclusion of RGFP did not affect percentage weight loss, shoulder, loin, thigh, head, tail, lung, kidney, liver, heart and dressing percentage. For optimal returns on investment, RGFP may not replace above 75% of maize in rabbit diets.

Keywords: Rabbit, New Zealand white x Chinchilla cross, *Gmelina,* Fruit pulp, Carcass, Economic value

INTRODUCTION

The search for novel feedstuffs to broaden options available to livestock farmers in their quest for cost minimization and profit maximization in livestock farming enterprises is a continuous process. High nutritive value and assurances of safety of feed for livestock does not guarantee the adoption and use of novel feedstuffs by farmers. Economic returns as indicated by cost benefit indices and impact of the feed on the target products like carcass

quality, milk yield and egg production determine to a greater extent farmer decision to adopt feed innovation (Barnard, 1969). Ripe *Gmelina* fruit pulp (RGFP) meal has been under consideration as a novel energy source (Annongu and Foluronso, 2003; Amata, 2012). It has been reported to be good for feeding livestock with a little nutritive boost (Ingweye and Okon, 2012; Amata, 2012). Dietary evaluation with rats has recommended 25% replacement for maize (Ingweye and Okon, 2013; Ingweye and Kalio, 2013). Evaluation of its economic value when fed to rabbits as well as the carcass characteristics of the rabbit meat is yet to be carried out. Hence, the present study was designed to find out the carcass and economic worth of RGFP based diets on rabbits to recommend same for adoption by farmers.

MATERIALS AND METHODS

Study Location: The experiment was carried out at the Rabbit Unit, Faculty of Agriculture Teaching and Research Farm, University of Calabar. Calabar is located at latitude 04.57^{o}N and longitude 08.20^{o}E.

Feed Ingredient and Processing: The *Gmelina* plant was identified by a plant taxonomist at the Botany Department, University of Calabar. The ripe *Gmelina* fruits were obtained from the Nigeria Newsprint and Paper Manufacturing Company *Gmelina* plantation in Akamkpa Local Government Area, Cross River State. The maize, palm oil, groundnut oil, soybeans and vitamin/mineral premix were bought from markets in Calabar.

The fruits were depulped by removing the hard woody seed inside the fruit. The mucilage, juice and pericarp together formed the pulp. The pulp was sun dried to a constant weight, milled, packed in jute bags and stored in a cool dry place at room temperature for use in compounding the diets. All dietary ingredients were subjected to proximate analysis (AOAC, 2005).

Experimental Animals: Sixty rabbits (25 males and 35 females) were obtained from a reputable rabbit farmer in Calabar metropolis at the age of six weeks (42 ± 3 days). The rabbits were of the New Zealand White x Chinchilla cross.

Housing: The rabbits were housed individually in cages measuring 76 x 62 x 42 cm, elevated at 90cm above the ground (Akinfala *et al*., 2003). Well ventilated wire mesh (2.5 x 2.5 x 1.25 cm gauge) and hard wood were used to construct the cages. Each cage had two concrete bowls; one for holding concentrate feed and the other for drinking water.

Experimental Diets and Feeding: The five diets (Table 1) were iso-nitrogenous and iso-caloric. Diet 1 was the control with maize as the sole energy source (0% level of replacement). The maize was then replaced with 25, 50, 75 and 100% RGFP in diets II, III, IV and V, respectively. The diets were subjected to proximate analysis (AOAC, 2005) to ensure that the calculated and analyzed proximate values were similar. The diets had 17% crude protein and 2500 kcal (ME)/kg (Aduku, 1993). The rabbits were fed and watered *ad libitum* throughout the study period.

Medication: The cages were cleaned with Izal solution (disinfectant) each day. A dewormer (Albendazole) was administered to the rabbits before the start of the growth trials at the rate of 2.5 mg/kg body weight. As the situation demands, broad spectrum antibiotics were administered on the rabbits to keep them in a healthy active state. Coccidiosis was treated with a coccidiostat while mange was treated with ivermectin injection.

Performance Characteristics: Feed intake, body weight gain and feed conversion ratio (FCR) were measured and calculated. animals were daily offered fresh concentrate feed, water and forage with one half provided in the morning (07:30 hours) and the other half in the evening (16.00 hours). The leftover feed at the end of each day was subtracted from the feed supplied the previous day to obtain the daily feed intake. The feed conversion ratio was calculated by dividing the mean feed intake per treatment by the mean body weight gain at a

point in time. Feed intake and body weight were recorded before serving fresh feed and water in the morning. The feed cost/kg weight gain was calculated by multiplying feed cost/kg by feed conversion ratio. The animals were individually weighed at the start of the trial and thereafter after every seven days. On the last day of the experiment, the final weight was taken. Weekly weight gain in grammes was calculated by subtracting the current week weight from the preceding week's weight.

Carcass Evaluation: Three animals per treatment (i.e. two females and one male) weighing close to the group average were selected and used for the carcass evaluation. Prior to slaughtering, the animals were starved of feed for 16 hours but offered water. They were then stunned and bled by hanging the carcasses on rails upside down for proper bleeding. The carcasses were defurred by burning off the fur over open fire. The carcasses were then scraped and washed with cold water.

Evisceration was done by first cutting the head between the zygomatic arches and the atlas. A cut was made down the breast plate through the abdomen to the pelvis. The anus was cut round and retracted together with the trachea and esophagus. The esophagus was cut round at the distal end to separate it from the diaphragm. The lungs and the trachea were removed intact and weighed while the gastro intestinal tract was removed intact and weighed. The breast, shank and flank were removed by cutting from the face of the leg parallel to the vertebral axis, cutting through the fore shank, breast and shank. The shoulder was removed by cutting across between the fifth and sixth thoracic vertebrae while the leg was separated by cutting between the last lumbar and the first sacral bone or at the tip of the ilium. The ribs and loin were separated by cutting after the 13^{th} rib through the lumbar vertebrae. Carcass weight, relative cut-up parts and organ weights were determined pre-chill. The relative weights were calculated by expressing the weights of the cut parts and organs as percentage of the dressed carcass weight. Each primal cut was weighed and its proportion relative to the carcass was determined. The procedures were carried out as reported by Ukah *et al*. (2006).

Experimental Design: Sixty rabbits, aged six weeks (New Zealand White x Chinchilla cross) of mixed sexes (25 males and 35 females) were shared into five groups of 12 animals each and balanced on sex and initial weight. The groups were randomly allotted to the five experimental diets. Diet I had maize as the only energy source. The maize was replaced with 25, 50, 75 and 100% RGFP in diets II, III, IV and V, respectively. The diets were supplemented with *Calopogonuim muconoides* leaves as fed in the 16 weeks trial.

Data Analysis: Completely Randomized Design, one-way analysis of variance was used and significant ($p<0.05$) means were separated using least significant difference using SAS statistical package.

RESULTS AND DISCUSSION

Carcass Indices of Rabbits Raised on Ripe Gmelina Fruit Pulp Based Diets: The carcass indices of rabbits fed ripe *Gmelina* fruit pulp (RGFP) based diets indicated that the final weight, fasted weight, percentage weight loss, dressed weight and dressing percentage ranged from 1900 – 1605g, 1884.90 – 1589.78g, 15.23 – 15.09%, 1206.42 – 1017.46g and 64.01 – 63.97%, respectively (Table 2). Diet I had the highest ($p<0.05$) fasted or pre-slaughter weight, while diet V had the lowest ($p<0.05$). The highest ($p<0.05$) fasted weight was not different from that recorded for diets II, III and IV implying that administration of treatment reduced ($p<0.05$) the fasted weight when RGFP completely replaced maize. As dietary RGFP level increased, the pre-slaughter weight decreased meaning that overnight fasting must have led to dehydration and breakdown of body reserves which affected live weight of diet V. Compared to reported values of Idowu *et al*. (2006), the values obtained for the present study were higher. It could be due to the carry over effect from the higher final live weight in the present study.

Table 1: Composition of diets for experimental rabbits

Ingredients	Diets				
	I	II	III	IV	V
Maize	44.27	32.20	22.14	11.07	-
RGFP	-	14.26	25.29	37.86	50.38
Soybeans	20.73	18.54	17.57	16.07	14.62
Shrimp waste	2.00	2.00	2.00	2.00	2.00
Wheat offal	27.00	27.00	27.00	27.00	27.00
Palm oil	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Vitamin premix	0.50	0.50	0.50	0.50	0.50
Common salt	0.50	0.50	0.50	0.50	0.50
Total	**100**	**100**	**100**	**100**	**100**
Calculated					
Crude protein	17.00	16.87	16.97	17.00	17.00
Crude fibre	5.27	6.15	6.83	7.27	8.45
Ether extract	9.99	11.07	12.00	13.00	14.01
ME (kcal/kg)	2,518	2,516	2,510	2,500	2,497
Analyzed					
Crude protein	17.11	17.00	17.20	17.00	16.98
Crude fibre	5.56	6.11	7.01	7.42	8.60
Ether extract	10.00	10.59	12.30	13.01	14.20
ME (kcal/kg)	2,505	2,500	2,500	2,498	2,498

Table 2: Carcass indices of rabbits raised on gmelina fruit pulp based diets

Parameter	Diets				
	I	**II**	**III**	**IV**	**V**
Final weight (g)	1900.00±55.00^{a}	1899.86±55.11^{a}	1890.95±56.00^{a}	1800.20±57.12^{a}	1605.00±58.28^{b}
Fasted weight (g)	1884.90±56.01^{a}	1884.74±56.00^{a}	1875.86±55.88^{a}	1784.97±55.98^{a}	1589.78±57.01^{b}
Weight loss (%)	15.10±0.02^{NS}	15.12±0.011^{NS}	15.09±0.03^{NS}	15.23±0.01^{NS}	15.22±0.00^{NS}
Dressed weight (g)	1206.15±36.02^{a}	1206.42±35.59^{a}	1199.99±36.00^{a}	1142.02±35.99^{a}	1017.46±36.02^{b}
Dressing percentage	63.99±6.99^{NS}	64.01±6.98^{NS}	63.97±7.00^{NS}	63.98±7.02^{NS}	64.00±7.03^{NS}
Carcass quality [% dressed carcass]					
Shoulder	33.48±0.02^{NS}	33.49±0.01^{NS}	33.53±0.03^{NS}	33.47±0.00^{NS}	33.48±0.01^{NS}
Loin	8.76±0.04^{NS}	8.97±0.00^{NS}	8.74±0.01^{NS}	8.80±0.03^{NS}	8.92±0.01^{NS}
Rack	6.19±0.00^{b}	6.18±0.00^{b}	6.27±0.01^{a}	6.28±0.02^{a}	6.21±0.03^{ab}
Thigh	35.16±0.02^{NS}	35.19±0.00^{NS}	35.29±0.02^{NS}	35.29±0.01^{NS}	35.22±0.03^{NS}
Head	5.14±0.00^{NS}	5.13±0.00^{NS}	5.19±0.01^{NS}	5.20±0.00^{NS}	5.18±0.00^{NS}
Tail	3.22±0.02^{NS}	3.23±0.02^{NS}	3.23±0.02^{NS}	3.29±0.01^{NS}	3.19±0.03^{NS}
Belly fat	3.30±0.08^{a}	3.00±0.07^{b}	2.89±0.09^{b}	2.99±0.08^{b}	3.00±0.08^{b}
Lung	0.31±0.00^{NS}	0.30±0.00^{NS}	0.32±0.00^{NS}	0.31±0.00^{NS}	0.32±0.00^{NS}
Kidney	1.49±0.00^{NS}	1.48±0.00^{NS}	1.47±0.00^{NS}	1.49±0.00^{NS}	1.50±0.01^{NS}
Liver	2.79±0.00^{NS}	2.79±0.00^{NS}	2.79±0.00^{NS}	2.77±0.00^{NS}	2.80±0.00^{NS}
Heart	0.16±0.01^{NS}	0.15±0.01^{NS}	0.18±0.01^{NS}	0.16±0.01^{NS}	0.18±0.01^{NS}

^{NS} Not significantly different (p>0.05); $^{a-d}$ means in the same row with different superscripts are significantly different (p<0.05)

Table 3: Economic indices of rabbits raised on ripe gmelina fruit pulp based diets

Parameter	Diets				
	I	**II**	**III**	**IV**	**V**
Cost of feed/kg (₦)	55.07±5.17^{a}	50.11±5.00^{b}	47.87±4.99^{b}	33.25±4.98^{c}	28.21±5.10^{d}
Cost of feed consumed/animal (₦)	407.07±41.20^{a}	407.39±41.00^{a}	387.27±40.99^{b}	256.69±41.12^{c}	213.55±41.22^{d}
Cost of feed/kg weight gain (₦)	307.42±23.31^{a}	307.68±23.50^{a}	294.40±22.99^{b}	209.81±23.00^{c}	207.34±23.23^{c}
Savings on cost of feeding (%)	0.00	-0.08±0.00^{c}	4.24±0.01^{b}	31.75±4.01^{a}	32.55±3.99^{a}

^{NS} Not significantly different (p>0.05); $^{a-d}$ means in the same row with different superscripts are significantly different (p<0.05)

The percentage weight loss was not affected (p>0.05) by RGFP dietary inclusion. Diet II had the highest (p<0.05) dressed weight, while that of diet V was the least (p<0.05). However, diet II dressed weight was similar (p>0.05) to values obtained for diets I, III and IV. The trend showed decreasing dressed weight as dietary RGFP level increased depicting a carryover effect from the fasted weight and that administration of treatment did not affect (p>0.05) the by-products of carcass dressing like blood and intestine. The dressing percentage values were unaffected (p>0.05) by dietary inclusion of RGFP. Similar results were obtained when rats were fed with increasing levels of RGFP (Ingweye and Okon, 2013).

As a percentage of the dressed carcass, the shoulder, loin, rack, thigh, head, tail, belly fat, lung, kidney, liver and heart weights (g) ranged from 33.53 – 33.47, 8.97 – 8.74, 6.28 – 6.18, 35.29 – 35.16, 5.20 – 5.13, 3.29 – 3.19, 3.30 – 2.89, 0.32 – 0.30, 1.50 – 1.47, 2.80 – 2.77 and 0.18 – 0.15, respectively. The percentage shoulder, loin, thigh, head, tail, lung, kidney, liver and heart showed no significant (p>0.05) difference among the treatment means. The percentage weight of rack at diet IV was the highest (p<0.05) though not different (p>0.05) from values obtained for diets III and V. The least (p<0.05) percentage rack weight was obtained for diet II which was not different (p>0.05) from the values obtained for diets I and V. The trend shows an increasing percentage rack weight as the level of RGFP in the diet increased though it could not be traced to a particular reason.

Diet I had the largest (p<0.05) percentage belly fat while that of diet III was the smallest (p<0.05). However, the least (p<0.05) percentage belly fat was not different (p>0.05) from figures obtained for diets IV, V and II. The trend shows that the control rabbits accumulated the most (p<0.05) fat than any other group. This could be due to the complete use of maize in this diet and poor forage intake by this group. The net impact will be a negative influence on meat quality as the meat becomes too fatty and extracellular fat so accumulated is not desirable in carcasses.

Economic Indices of Rabbits Raised on Ripe Gmelina Fruit Pulp Based Diets: The economics of production of rabbits fed graded levels of RGFP indicated that the cost of feed (₦/kg), cost of feed consumed (₦/animal) and cost of feed per weight gain (₦/kg weight gain) ranged from 55.07 to 28.21, 407.07 to 213.55 and 307.68 to 207.34, respectively (Table 3). The control diet (diet I) was the most expensive (p<0.05), while diet V was the cheapest (p<0.05). Feed cost decreased as RGFP level increased. Ingweye and Okon (2013) had indicated similar reduction in feed cost when increased level of RGFP was incorporated into diets fed to rats.

This implied that inclusion of RGFP in the diet reduced (p<0.05) the cost of feed and could potentially reduce the cost of rabbits feeding. The cost of feed was higher than that reported for cocoa pod husk as feed for rabbits (Adejinmi *et al*., 2007). This could be due to the cost of processing RGFP which was higher than that of cocoa pod husk. Diet II had the highest (p<0.05) cost of feed consumed while diet V was the least (p<0.05). The highest (p<0.05) value was similar (p>0.05) to that of the diet I. The cost of feed consumed rose as the level of RGFP in the diet decreased implying that inclusion of RGFP in the diets reduced (p<0.05) the cost of feeding rabbits.

Diet I had the highest (p<0.05) cost of feed per kilogramme weight gain while diet V had the least (p<0.05). The highest (p>0.05) cost of gain was not different (p>0.05) from the cost of gain obtained for diet II while the least cost of gain was also similar (p>0.05) that recorded for diet III. As RGFP level in the diet increased, the cost of feed per kilogramme weight gain decreased implying that it was cheaper to produce rabbit meat by feeding the animals with diets V and IV but more expensive to produce the same quantity of rabbit meat feeding the animals on diets I and II.

Conclusion: The study assessed the carcass and economic indices of rabbits fed diets incorporating ripe gmelina fruit. Diet I had the highest cost of feed, cost of feed consumed, cost of feed per kilogramme weight gain, pre-slaughter weight and percentage belly fat while

diet V had the least except percentage belly fat where diet III was the least and dressed weight where diet II was the highest. Also, the values for these indices and dressed weight decreased as the level of RGFP increased. Though undesirable, the meat of control rabbits was the fattiest. The percentage weight loss, shoulder; loin, thigh, head, tail, lung, kidney, liver, heart and dressing percentage were not affected by dietary inclusion of RGFP. Therefore, for optimal returns on investment, RGFP may replace up to 75% of maize in rabbit diets.

ACKNOWLEDGEMENT

The authors are grateful to Science and Technology Education Post-Basic (STEP-B) Nigeria for financing this project.

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BEHAVIOURAL AND BIOCHEMICAL RESPONSES OF JUVENILE CATFISH (*CLARIAS GARIEPINUS*) EXPOSED TO GRADED CONCENTRATIONS OF CASSAVA WASTE WATER

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ABSTRACT

The behavioural and serum liver enzyme responses of juvenile catfish (Clarias gariepinus) were evaluated for 72 hours. Thirty-six (36) healthy fishes with standard weight, 20 ± 1.52 g and standard length, 18.25 ± 0.50 cm were used for the experiment in non-renewable bioassay system. The test fish exhibited stressful behavioural changes such as erratic swimming, vertical swimming, gasping, and body discolouration. The 24 and 48-hours LC50 were determined to be 96.937 and 9.765 mg/ml respectively. Increased serum aspartate amino transferase and alanine transferase concentrations were recorded on the final day of the experiment (p<0.05).

Keywords: Behavioural, Biochemical, Catfish, Cassava wastewater

INTRODUCTION

Cassava (*Manihot esculenta* crantz) is primarily grown for its starch-containing tuberous roots, which are the major source of dietary energy for more than 500 million people in the tropics (Lynam, 1993). The ability of cassava to grow and produce relatively well in marginal environment under low management levels makes it an attractive crop for poor resource farmers (Bencini, 1991). Cassava is generally considered to have a high content of dietary fibre, magnesium, sodium, riboflavin, thiamine, nicotinic acid and citrate (Bradbury and Holloway, 1988). It also contains cyanide which is extremely toxic to humans and animals. The roots have high moisture content (60 – 65%), making transportation from rural areas difficult and expensive.

To forestall spoilage following harvest and also due to its bulky nature, cassava is usually traded in some processed form. Processing reduces moisture content and converts it to a more durable and stable product, which makes it more transportable (IITA, 1990; Ugwu, 1996). Although, over the years, cassava has been processed into a number of domestic and industrial products, cyanide toxicity arising from cassava is still being reported. There is therefore a need to process it to reduce the cyanide content to safe levels (Eggleston *et al.,* 1992).

Cassava effluents generated during cassava processing are usually neither treated nor properly disposed in Nigeria.

The concentration profile of the chemical component of cassava effluent is in the order of sodium > potassium > magnesium, and iron (Adejumo and Ola, 2010). The occurrence of these chemicals and cyanide in the aquatic ecosystem may be beneficial or toxic. The hydrocyanic acid content of cassava tubers is removed by either washing, exposure to air, heating or pressing with the aid of processing equipment and technology (IITA, 2005). There are two important biological wastes derived from cassava processing which are the cassava peels and the liquid squeezed out of the fermented parenchyma mash (Oboh, 2006). The polluting potential of an effluent is measured by the amount of oxygen needed to oxidize the organic matter, the chemical oxygen demand (COD) and the amount of oxygen necessary to stabilize the organic matter by microorganisms and enzymes i.e. the biochemical oxygen demand (BOD) (Adejumo and Ola, 2010).

Fish and other aquatic life are killed by cyanide concentrations in the microgram per liter range (Adeyemo, 2005). Cassava peels are normally discharged as wastes and allowed to rot in the open with a small portion used as animal feed, thus resulting in health and environmental hazards. Also, the waste water that comes with grinding and pressing is indiscriminately disposed. Cassava is also soaked in water bodies (whether lentic or lotic); such could have enormous adverse impact on aquatic life. This necessitated the present research.

MATERIALS AND METHODS

The Test Organisms: One hundred and twenty *Clarias gariepinus* juveniles with standard weight and standard length, 20 ± 1.52 g and 18.25 ± 0.50 cm, were purchased from Otuocha River, Otuocha, Anambra State, Nigeria. The fish were transported in improvised plastic containers of 25 litre capacity, which were partially cut open at about 15 cm below the handle to the Fisheries and Hydrobiology Research Unit, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. Seven catfish each were placed in 12 bowels containing freshwater. The bowels were covered mesh net of size 0.8 mm. The fishes were acclimatized at room temperature for 14 days in which the fishes were not fed in the first three days for them to digest all food materials in their stomach in which they would have acquired from the wild but were fed once a day in other day with commercial fish feed. Mortality during acclimatization was 3%. The fish were not fed for the period of the experiment.

Cassava Wastewater: Fresh cassava tubers (*Manihot utilissima*) (average total weight, 50.2 ± 2.05 kg) were bought from a farm in Eha-Alumona, Nsukka, Enugu State. Identification of the cassava was done at the taxonomic unit of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The tubers were first washed to remove sand and other debris. After, the tubers were reweighed to get a final weight of 41.5 kg. The tubers were then soaked in an air tight plastic container of about 175 litres, with 47 litre of water without peeling. This was to mimic the soaking pattern used in water bodies around Nigeria. Soaking lasted for five days. The waste water was removed after five days, stirred and sieved to remove debris. The resulting solution was poured into a 25 litre container and stored at 4ºC to avoid further microbial degradation. Also, the container was filled to the brim and was properly covered in order to minimize the volatilization of some constituents which might cause a reduction in the toxicity of the effluent. This is in accordance with the instructions of EPA (2002).

Lethal Toxicity Test: Forty fishes were divided into four experimental groups, one control and three treatments, each with three replicates. The treatments received 10 mg/ml, 20 mg/ml and 30 mg/ml of the waste water. The experiment was monitored for 72 hours while behavioural, morphological, and mortality responses were recorded.

Physico-Chemical Analysis: Dissolved oxygen (DO), alkalinity, PH, hardness, free CO2, biochemical oxygen demand (BOD), phosphate, hydrogen cyanide (HCN), chemical oxygen

demand (COD) were determined using the protocols described by Bhatia (2009).

Liver Enzymes Analysis: Two (2) ml of venous blood was collected from each fish and transferred to sample bottles to allow coagulation. Plasma was obtained by centrifugation for 15 minutes at 3,000 rpm and separated into plain bottles for analysis. Randox enzymatic kit (Randox Laboratories, United Kingdom) was employed for the *in vitro* determination of the activity of alanine transaminase (ALT) and aspartate transaminase (AST) in plasma, using the colorimetric method of Reitman and Frankel. Randox enzymatic kit (Randox Laboratories, UK) was also used for the *in vitro* determination of ALP activity in plasma, according to the colorimetric method of Englehardt.

Data Analysis: Data collected from the study were subjected to analysis of variance. Significant means were separated using Fisher's Least Significant Difference (F-LSD) at p = 0.05.

RESULTS

Behavioural Responses of the Test Organisms to the Toxicity of the Cassava Wastewater: During the lethal toxicity test, *Clarias gariepinus* showed marked distress (Table 1). This was recorded as erratic swimming, gasping of breath, weak movements and continuous surfacing. Distress in behavioural responses increased with increase in concentration of the waste water.

Table 1: Behavioural response of *Clarias gariepinus* exposed to graded concentrations of cassava wastewater

Concentrations (mg/ml)	Behavioural Responses Erratic swimming	Gasping
0	-	-
10	+	+
20	++	++
30	+++	++
	Vertical swimming	**Body discolouration**
0	-	-
10	+	-
20	++	+
30	+++	+++

The fish got weaker as the experiment progressed, with vertical swimming, ventral surface turned upward and finally, death. The control fish exhibited normal behavioural responses and zero mortality.

Lethal Concentration: The LC_{50} of the cassava waste water at 24 and 48 hours were 96.937 and 9.765 mg/ml, respectively (Table 2). The LC_{50} for the 24 and 48 hours were deduced from the probit plots (Figures 1 and 2).

Table 2: Mortality of *Clarias gariepinus* exposed to different concentrations of cassava wastewater

Concentration (mg/ml)	Duration (hours) 24	48	72
0	0	0	0
10	0	0	0
20	0	4	0
30	1	4	0

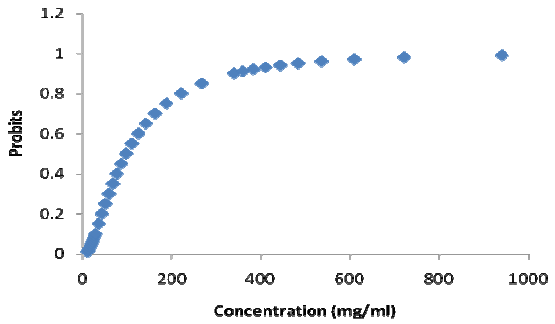

Figure 1: 24 hours LC_{50} of cassava wastewater

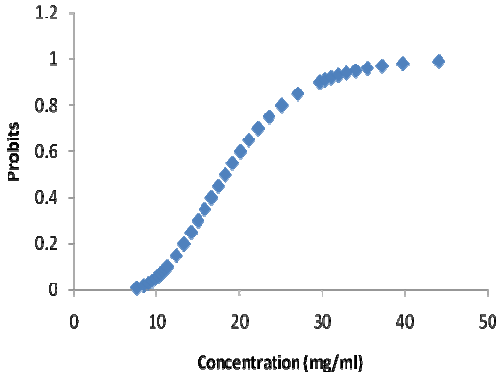

Figure 2: 48 hours LC_{50} cassava wastewater

Physicochemical Parameters of Test Cassava Wastewater: The pH, CO_2 and water hardness were significantly increased at the end of the study ($p<0.05$), whereas the biochemical oxygen demand and phosphorus content decreased significantly.

Table 3: Physicochemical parameters of the test cassava wastewater

Parameters	Initial	Final	P-value
pH	6.20 ± 0.06	6.58 ± 0.09*	0.00
HCN (mg/l)	0.09 ± 0.00	0.09 ± 0.00	0.88
Phosphorus (mg/l)	0.71 ± 0.00*	0.05 ± 0.00	0.00
BOD (mg/l)	8.23 ± 0.29*	7.12 ± 0.16	0.00
DO (mg/l)	3.70 ± 0.03	3.51 ± 0.10	0.06
CO2 (mg/lCO_3)	27.61 ± 5.04	183.33 ± 37.18*	0.01
Hardness (mg/l $CaCO_3$)	61.92 ± 6.90	97.93 ± 10.51*	0.01
Alkalinity(mg/l $CaCO_3$)	41.54 ± 4.20	107.53 ± 4.26*	0.00

Mean values with asterisk as superscript are significant (p<0.05)

Table 4: Effect of cassava wastewater on the liver enzyme concentration of *Clarias gariepinus*

Liver enzymes	Initial	Final	P-value
AST	11.50 ± 2.01a	66.58 ± 11.96b	0.00
ALT	31.58 ± 1.19a	63.75 ± 11.21b	0.01
ALP	19.00 ± 0.77	24.00 ± 4.20	0.21

There were changes in other physicochemical parameters, but such were not significant (p>0.05) (Table 3).

The Effect of Cassava Wastewater on the Liver Enzymes of *Clarias gariepinus*: The final value of the AST concentration was significantly (p<0.05) higher than the initial value of AST concentration (Table 4). Similar to the values of AST, the variation between the initial and the final ALT concentration was significant (p<0.05). Though, a variation existed in the ALP values, the final values of the ALP concentration was not significant when compared to the initial (p>0.05).

DISCUSSION

Agricultural practices and industrialization, all geared towards food production are ways of alleviating food scarcity and poverty. These practices have led to water and other forms of pollution thus, creating health hazards to living organisms including man. The annual cassava production statistics in Africa stands at about 84 million tonnes with Nigeria leading with a total production of 30 million tonnes, Tanzania 5.7 million tonnes, and Madagascar 2.4 million (Adeyemo, 2005). This upsurge in production in Nigeria has led to creation of cassava processing units where various cassava products are produced and waters are discharged into the environment and waters bodies.

Reduced distressful behavioural responses and mortality present in the lower concentrations used in the current study suggest that the test fish can tolerate low doses of the waste water. The behavioural responses of the fishes exposed to the cassava effluent may be attributed to the decreased in dissolved oxygen concentration resulting from the cassava effluent. The mortalities recorded in the course of this study were an indication of the toxicity of the waste water in the fish.

The increase in the dissolved CO_2 content on the final day of the experiment was an indication of heightened respiratory activities. This observation is supported by the fact that the biological oxygen demand level decreased significantly at the final day of the experiment. This is also an indication of elevated oxygen uptake. Water hardness and alkalinity also rose appreciably at the end of the study. Metallic cations such as Ca^{2+}, $Mg^{2+,}$ Na^{+}, K^{+} etc play vital roles in cellular functions. They regulate muscle contraction, generation of nerve impulses, and activation of enzymes during protein production and transport etc. The continuous addition of cations could result in uncoordinated muscular contractions, and when this occurs in vital organs, paralysis and even death could result. of the treatment water recorded no significant increase or decrease between the initial and final value. This suggests that fermentation may not appreciably reduce the cyanide content with time. Though, the extent of reduction may depend on the

quantity of cassava soaked in a particular volume of water. This tends to suggest that depending on organisms' susceptibility, cassava waste water may still be toxic due to its cyanide content irrespective of fermentation time.

The dissolved oxygen content did not change significantly at the end of the experiment. This is not surprising as the study took place in an open, shallow bowls in which oxygen dissolution could have been relatively stable for the duration of the experiment.

The 24 and 48 hours LC_{50} of the cassava waste water (96.939 and 9.765 mg/ml) showed that the toxicity increased maximally with fermentation time. This is also not surprising as the essence of fermentation is to get rid of toxic chemicals from the cassava that will be consumed as food. No wonder the mortality of the test fish increased to the extent that all had died by the 72^{nd} hour. Gintaras (2010) reported that the 96-hr LC_{50} values obtained from the toxicity test of Nickel on some freshwater fish; rainbow trout, three-spinned stickleback, roach, perch and dace ranged from 19.3 to 61.2 mg Ni/l.

The present study recorded significant increases in both the aspartate amino transferase alanine transferase concentrations on the final day of the study. The dose-dependent response could be why all fish in the 30 mg/ml concentration died before the completion of experiment. This could be as a result of adverse effects on some vital organs such as the liver, operculum, and gills etc. However, the alkaline phosphatase (ALP) concentration in the present study decreased when compared with the 0 mg/ml concentration. This reduction is also in line with the report of Olaniyi *et al.* (2013) in *C. gariepinus* exposed to graded levels of cassava mill effluent. They reported increase in the AST and ALT concentrations. They attributed such to hepatic cellular damage in the test fish. Also recorded was heightened leucocytosis which was associated with physiological response to maintain the health of the fish. Das *et al.* (2004) reported increase in the AST and ALT of Indian major carps exposed to nitrate toxicity and suggested that the elevation of the transferase could be as a result of the use of the α-amino groups in the tricarboxylic acid cycle of keto acids to augment energy production. Sepici-Dincel *et al.* (2009) observed that the increase in activities of AST and ALT in the muscle and liver of the common carp exposed to 10 mg/l of cyfluthrin may be due to a disturbance in the Kreb's cycle.

Iwama and Ackermen (1994) observed similar result in rainbow trout exposed to graded concentrations of MS-222 and clove oil.

The right amount of all twenty amino acids is important for protein build-up needed for growth and repair of damaged tissues (Kaslaw, 2013). However, eight of these cannot be produced by the body and therefore, must be generated from our diet. Keto acids of the Krebs cycle can be converted to amino acid by the addition of an amine group, produced by the breakdown of mother amino acid. Such transamination occur continuously in the body depending on its physiological status and, passable be is driven by the particular protein needed by the body of which the building block is in short supply.

Phosphatases remove phosphate groups from molecules. This removal of the phosphate group from a molecule within a cell makes it unable to leave the cell. For instance, the removal of the phosphate group from glucose makes it impermeable and even unable to be used for energy production during glycolysis. The response of plasma alkaline phosphatase during the experiment was mostly lower compared to the baseline but, the treatments had alkaline phosphatase concentrations similar to the control group.

Measurement of blood alkaline phosphatase is used to access the existence of biliary dysfunction. Since the mucosal cells lining the bile system of the liver are the sources of alkaline phosphatase, the free flow of bile through the liver and down into the biliary tract and gall bladder are responsible for maintaining the right homeostasis of this enzyme in the blood (EPA, 2002). The significant reduction in the plasma concentration of this enzyme recorded in the present study showed protection, rather than toxic impacts on the bones, the liver and associated billary system. Also, the production and secretion of bile is

central to the effective digestion and utilization of fat; the result of this study suggested that biliary function was not impaired during the study period.

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EFFECT OF DIFFERENT MECHANICAL VIBRATION ON BLOOD PARAMETERS OF ONE DAY OLD BROILER CHICKEN

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ABSTRACT

Mechanical vibrations are congenital to any system of transportation. In poultry industry, the higher or lower intensity of this agent during the transportation of eggs and chickens can increase the production losses and decrease the efficiency of the system as a whole. This study was carried out to investigate the effects of mechanical vibration (MV) generated by mechanized equipment on eggs, chickens mortality and levels of some blood biochemicals (triglyceride, glucose, cholesterol, calcium, phosphorus, urea) were analyzed in one days chickens. The data showed that the glucose and urea concentrations in blood of one day old chicks and their mortality increased with increasing vibration levels but calcium, cholesterol and triglyceride levels decreased. Levels of vibration seemed not to affect all of parameters. Mechanical vibrations may be responsible for the incidence of cracks, in addition to promoting the agitation of internal constituents (yolk and albumen), which could potentially compromise the quality of birth and hatchlings. The mechanical vibration are related to stress, discomfort and depreciation of the welfare of the birds, changed in glucose and corticosteroids levels and thus affect other factors as well as the quality of the meat.

Keywords: Glucose, Cholesterol, Triglyceride, Calcium, Phosphorus, Urea, Mortality, Broiler chick, Mechanical vibration

INTRODUCTION

Higher meat consumption necessitates increased number of broiler farms. Due to the development of the industry and livestock technology, traditional small-scale facilities have changed to large-scale mechanized units. Egg transport is an essential component in current system integration of poultry production; it is responsible for loading of fertilized eggs from stock plants to hatcheries, day-old chicks from hatcheries to farms and disposal poultry for slaughterhouses. However, this process still receives little attention and many aspects related to it may impose stress on the eggs and day old chicks, resulting in a higher incidence of productive losses. The vibration studies, affecting livestock productivity directly or indirectly, have been conducted (Graul *et al.,* 1976; Warriors *et al.,* 1997; Stephen *et al.,* 1998; Yun, 1998; Baek *et al.,* 2002; Lee *et al.,* 2003; Lee *et al.,* 2004; Campo *et al.,* 2005; Kim *et al.,* 2012). All the results referenced above, were related to the effects of noise and vibration generated in poultry industry. Studies on the effects of mechanical vibration on egg incubation and productivity of broilers production are limited.

Stress could have considerable effect on animal behavior. Stress decrease growth and development. It has negative effect on embryo development. Stress impairs haemostasis (Fuchs

et al., 2001). At any stage of pregnancy, stress could affect embryo development, especially the brain development (Nishio *et al.,* 2005). Hypothalamo-hypophysis axis is disordered in embryo by stress (Reul *et al.,* 2000). In stress both hormone ACTH and cortisol increase in blood. Mechanical vibration is one type of stress. The effects of stress during transportation and other mechanical vibrations have been studied during egg production (Carter *et al.,* 1970). Shaking of the eggs should be reduced to a minimum because it has detrimental effect on egg quality (Walker *et al.,* 1972). Important damage during transportation occurs in egg shell and internal egg quality which could affect the egg characteristics. Transportation stress is one of the reasons recently noticed to affect eggs hatching, the development poultry industry, food consumption pattern and thus the Iranian economy. The negative effects of mechanical vibration on incubation are widely known. It is a common and years old belief that poor transportation of eggs may seriously decrease their hatchability (Saint-Hilaire, 1836; Dareste, 1877; Landauer and Baumann, 1943). Saint-Hilaire produced malformed chick embryos by subjecting eggs to various environmental conditions, including physical trauma and toxins (Saint-Hilaire, 1836).

There are data on the effects of vibration on egg development. Potter and Bassett (2001) studied the effects of different levels of vibration on egg hatchability, by examining the effects of transportation-induced jarring on embryo development and hatchability, and concluded that jarring did not increase physical abnormalities or defects in the development of hatched chicks.

Interpretation effect of transportation is difficult because the location of the pallet and the eggs in the truck has a large effect on egg characteristics. Effect of transportation with the help of vibrating table (electro-dynamic shaker) was investigated (Berardinelli *et al.,* 2003). This idea guided the use of a device for modeling the transport effects on egg hatchability and blood parameters of one day chicken broilers in small scale farms. The aim of this experiment was to investigate the effects of different vibration frequencies and levels on egg hatchability and blood parameters of one day chicken broilers.

MATERIAL AND METHODS

Vibration Simulator: The vibration simulator device was designed in Agriculture College of Lorestan University, Iran (Figure 1). This equipment has vertical movement (jarring simulator) and the vibrations calibrated for different frequencies. The simulator is a vibration machine with a two dimensional vibration plate moved by a motor.

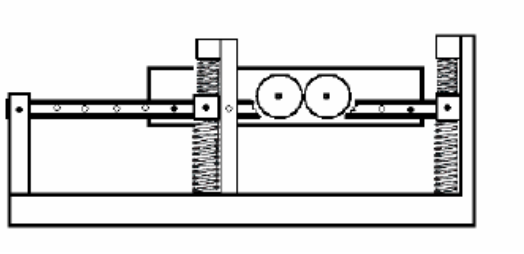

Figure 1: Vibration simulation device

The machine can be set to different levels of vibration between 6 – 15 Hz. The vibration simulator was provided with fixed amplitudes and frequencies covering the range measured on trucks. Similar device have been described by Guillou and O'Brien (1969), Ogut *et al.* (1999) and Vursavus and Ozguven (2004). The vibration simulator consist of a table of soft springs and attached to it an actuating system that include adjustable weights on two counter rotating shafts (counterweights) revolving in opposite directions and about the gravity center of the table and its load, providing vertical vibration only. Counterweights were powered by an electric motor (3.0 kW and 3,000 rpm).

The speed of the electric motor was adjusted by means of a speed control unit (inverter), which had a 4.0 kW power. The magnitude and angular velocity of the rotating masses can be changed. Because the frequency of the vibration simulator table is directly related to the rotation number of the counterweights, the frequency of the table was obtained based on the number of revolutions of the electric motor. Therefore, the speed of the electric motor was measured by means of the speed control unit and the number of the revolutions of electric motor measured in revolution per minute (rpm) was divided by 60 seconds and the frequency of the vibration simulator table was obtained in Hz. The acceleration of the vibration simulator table was directly measured using an

Table 1: Effect of different frequency of vibration on blood parameters and age mortality

Parameter	Frequency 1 [5 Hz]	Frequency 2 [7.5 Hz]	Frequency 3 [10 Hz]	Frequency 4 [12.5 Hz]
Urea (mg/dl)	25.83 ± 7.40^{a}	26.05 ± 5.93^{b}	30.30 ± 10.20^{c}	34.60 ± 4.79^{d}
Glucose (mmol/l)	153.70 ± 34.20^{c}	161.36 ± 9.80^{d}	129.94 ± 53.10^{a}	139.16 ± 29.50^{b}
Triglyceride (mmol/l)	429.83 ± 7.80^{d}	404.00 ± 90.00^{c}	386.15 ± 18.40^{b}	357.88 ± 9.70^{a}
Cholesterol (mmol/l)	84.88 ± 50.40^{c}	51.79 ± 42.80^{b}	52.72 ± 26.10^{b}	49.73 ± 31.00^{a}
Calcium (mmol/l)	7.98 ± 1.20^{d}	6.50 ± 0.40^{a}	6.85 ± 0.80^{cd}	6.73 ± 0.90^{b}
Phosphorus (mg/dl)	5.46 ± 1.20^{c}	5.47 ± 0.90^{c}	5.29 ± 1.20^{b}	4.46 ± 0.80^{a}
Age morality (days)	15.59 ± 3.90^{d}	11.03 ± 7.70^{c}	9.10 ± 7.00^{b}	7.58 ± 6.90^{a}

Means with similar superscripts on a row are not significantly different and means with different superscripts on a row are significantly different

Table 2: Effect of different levels of vibration on blood parameters and age mortality

Parameter	Level 1 [48 cm]	Level 2 [78 cm]	Level 3 [115 cm]
Urea (mg/dl)	27.33 ± 4.74^{a}	30.50 ± 5.80^{a}	29.88 ± 9.20^{a}
Glucose (mmol/l)	159.32 ± 27.70^{a}	179.30 ± 27.70^{a}	161.23 ± 39.52^{a}
Triglyceride (mmol/l)	34.03 ± 14.46^{a}	71.66 ± 11.02^{a}	73.67 ± 8.11^{a}
Cholesterol (mmol/l)	409.04 ± 51.68^{a}	388.08 ± 25.70^{a}	386.28 ± 35.19^{a}
Calcium (mmol/l)	7.50 ± 0.60^{a}	6.50 ± 0.70^{a}	6.95 ± 1.04^{a}
Phosphorus (mg/dl)	5.75 ± 1.70^{a}	4.93 ± 0.58^{a}	4.80 ± 1.30^{a}
Age morality (days)	10.60 ± 6.50^{a}	10.01 ± 6.20^{a}	11.84 ± 6.40^{a}

Means with similar superscripts on a row are not significantly different and means with different superscripts on a row are significantly different

acceleration measurement device and a piezoelectric accelerometer. Treated eggs were placed on three trays (the same type of fiber trays used in the field for transportation) then placed onto the simulator. Four thousand, three hundred and twenty (4320) fertilized eggs from Dorbar Industry (Iran – Brujerd) were allocated in three levels 48, 78 and 115 cm. Frequency of experimental treatments were 5, 7.5, 10 and 12.5 Hz with three replications each. Each replicate had 360 eggs. After fixed time of vibration, fertilized eggs were disinfected and incubated into setter for a period of 18 days. From the 18th day, eggs were transferred to hatching unit (incubator BKF, USA) and left until the 21 day. The number of hatched eggs was calculated. From the un-hatched eggs in each replicate, two un-hatched eggs were selected and broken. Age of the fetus was estimated. After hatching two chickens were selected randomly and the blood serum sampled with the help of centrifuge at 6500 rpm for 15 minutes. Serums were assayed for glucose, cholesterol, triglyceride, urea, calcium and phosphorus.

Data Analysis: Data collected were analyzed for their central tendencies, variances and significant difference ($p<0.05$) using SAS 9.2 software.

RESULTS

Eggs treated with different vibrations had no congenital defects. High-frequency vibration produced statistically significant changes such as (i) decrease of triglyceride, phosphorus, calcium and cholesterol level in blood and (ii) increase of urea, glucose level in blood in the day old chick (Table 1).

In Treatment 3 and 4, the treated eggs had a lower hatchability. A higher level of dead embryos and cracks/shell problems was observed between treatment 1 and 4 significantly ($p<0.05$). In trial 4, the treated eggs had a lower hatchability, higher level of dead embryos, more cracks/shell. In the treatment 1 which received 5 Hz, significantly low mortality was observed when compared with other treatments, while between treatments 2, 3 and 4 were not significantly different.

The frequency had significant ($p<0.05$) effect on glucose level. Glucose concentration in treatment 5 Hz was significantly higher than those in treatment 12.5 Hz. In this experiment, different levels of vibration had no effect on whole parameters (Table 2). Variations of different parameters were shown in Figures 2 and 3.

DISCUSSION

Vibration stress is a known kind of stress in poultry industry. The objective of the present study was to investigate the effects of different vibration frequencies and levels on egg hatchability and blood parameters of one day chicken broilers. Factors associated with chicken embryo mortality and blood parameters were vibration frequency and level related.

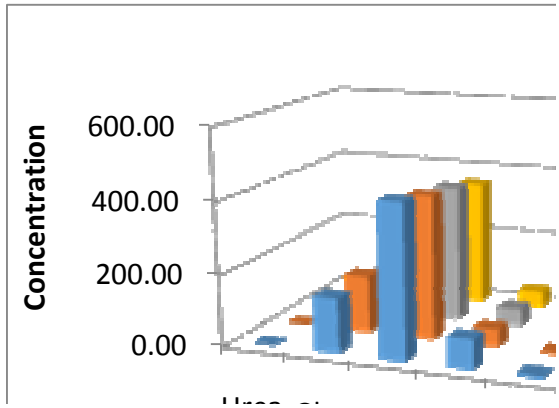

Figure 2: Different frequency on blood parameters, urea, glucose, triglyceride, cholesterol, calcium, phosphorous and age mortality

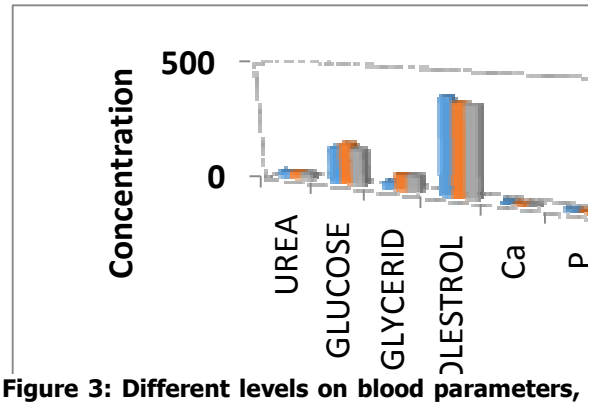

Figure 3: Different levels on blood parameters, urea, glucose, triglyceride, cholesterol, calcium, phosphorous and age mortality

As the magnitude of the frequency increased, mortality increased. The use of different vibration levels on eggs had no effect on all the experimental parameters.

Blood glucose is a sensitive, reliable indicator of environmental stress in chicken. Blood glucose was decreased in F4 as compared to control (F1). Glucose concentration was elevated by cadmium stress on chicken (Abdo and Abdulla, 2013). Cadmium induced hyperglycemia with decreased in liver glycogen in catfish *Heteropneustes fossilis* (Sastry and Subhadra, 1985). Soengas *et al.* (1996) reported that hyperglycemia that occurred in Atlantic salmon (*Salmo salar*) after toxicity with cadmium may be due to changes in liver carbohydrate metabolism (activation of liver glycogenolysis and glycolysis) as well as increased levels of plasma glucose.

Bone is a dynamic tissue influenced by physiological, nutritional and physical factors such as mechanical and physical activities (Rath *et al.,* 2014). It is formed and destroyed continually under the control of hormones and physical factors. This constant activity allows the modeling process, i.e., modification of the bone architecture to meet physical stresses (Stevens and Lowe, 1992).

Most bones have a large marrow cavity in the center; this may contain yellow marrow, which is mostly fat, or red marrow, the connective tissue in which red and some white blood cells are made (Villee *et al.,* 1989). No significant different between all treatments was observed.

The calcium : phosphorus ratio decreased significantly as compared to control. In their study clearly indicated that, there is an altered calcium and phosphorous metabolism. As calcium and phosphorous are important constituent of bone, ultimately, bone metabolism is altered, as reported by many workers (Oelzner *et al.,* 1998; Walwadkar *et al.,* 2006). Negative correlation between calcium/phosphorus ratio and lipid peroxide ($r = -0.76$) suggested that the generation of reactive oxygen species in excess may be particularly important in the bone resorption that occurs in association with inflammatory diseases (Garrett *et al.,* 1990). Decrease in calcium level may contribute to a negative calcium balance and acceleration of immunomodulatory effects (Kroger *et al.,* 1993; Oelzner *et al.,* 1998). Significant positive

correlations between vitamin E and calcium (r = + 0.67), suggested that vitamin E may also be an important for immunity of an organism along with calcium (Kroger *et al.,* 1993).

The effects of mechanical vibration on serum triglyceride concentrations in incubated eggs are depicted in Figure 2. Serum triglyceride concentrations in incubated eggs were significantly lower in treatment F4 ($p<0.05$) than control (F1). These findings are consistent with the reported of Moraes *et al.* (2003) in broiler chicks subjected to thermal stress. Applying of high vibration may exert an important effect on triglyceride absorption. Compared to the control group, chicks received 12.5 Hz had significantly lower concentrations of blood cholesterol. Lowering of cholesterol levels may be mediated by the stimulation of hepatic cholesterol -7-hydroxylase activity (Babu and Srinivasan, 1997; Asai *et al.,* 1999).

As a measure of renal function status, serum uric acid, urea and creatinine levels are often regarded as reliable markers (Oelzner *et al.,* 1998; Walwadkar *et al.,* 2006). Urea is the detoxification product of the ammonia derived from deamination of amino acids, thus urea considered to be the end product of protein catabolism (Kroger *et al.,* 1993).

The catabolism of the purines (adenine and guanine) produces uric acid using xanthine oxidase. Thus, increased in the serum concentrations of these components is indicative of renal injury simply because the kidneys excrete them. Mortality of embryos was increased significantly at 12.5 Hz in treatment 4. It is possible to verify that the mechanical vibration in poultry production produces negative effects.

Conclusion: The result of the current study confirmed that mechanical vibrations resulted in stress that increased production losses especially hatchability. However, the transport process is inevitable, there is no way not to carry eggs or birds and it is hard to control the quality of roads and trucks. Thus, strategies should be employed that reduces vibration based on new scientific investigations that address engineering concepts for the development of new vehicles; boxes, trucks and hatching trays with reduced vibrations. High vibration is harmful to the developing chicken embryo.

ACKNOWLEDGMENTS

The authors would like to thank Lorestan University for financial support of this study. Likewise we are thankful to all the various Departments in Lorestan University for use of equipments, reagents laboratory space and other technical supports.

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ANIMAL RESEARCH INTERNATIONAL

Volume 12 Number 1, April 2015

Global Impact Factor: 0.562

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Published by Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria