REFERENCE VALUES FOR THE HAEMATOLOGY PROFILE OF CONVENTIONAL GRADE OUTBRED ALBINO MICE (*Mus musculus*) IN NSUKKA, EASTERN NIGERIA

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ABSTRACT

This study established reference values for the haematology profile of the conventional grade out-bred albino mice (Mus musculus) in Nsukka, Eastern Nigeria. A total of 336 apparently healthy mice, made up of 168 males and 168 females, were used for the study. Mice of 4, 8, 12, 16, 20, 24, 30 and 40 weeks of age (eight age sets) were studied, and for each age set the haematology profile of 21 males and 21 females were assessed following standard manual procedures immediately upon blood sample collection from the orbital sinus. Results showed that there were significant ($p < 0.05$) age related variations in the erythrocyte counts (EC), haemoglobin concentrations (HbC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), total leukocyte counts (TLC) and absolute lymphocyte counts (ALC) of males and females and mean corpuscular haemoglobin of males only. There were significant differences ($p < 0.05$) between the males and females in their TLC at weeks 4 and 40 of age, ALC at week 24 of age and absolute neutrophil counts (ANC) at weeks 4, 20 and 24 of age. The absolute values obtained in this present study were for some parameters different from the reference values documented for mice in the temperate countries, but the trend of age related variations and differences between the sexes were nearly the same except for the ANC.

Keywords: Haematology, Reference values, Mice, *Mus musculus*, Nsukka, Nigeria

INTRODUCTION

Population reference values, formerly known as normal values, is defined as a set of values of a certain type of quantity obtainable from a group of apparently healthy individuals (reference population) corresponding to a spelt-out stated description with a specified criteria for inclusion and exclusion of individuals measured (Elvebach, 1973; Dybkaer and Grasbeck, 1973; Sunderland, 1975; Stockham and Scott, 2008). Population based reference values thus describes the variation of values that can be expected in healthy individuals or animals of a population from which samples are submitted to the laboratory, such that measured values outside the reference intervals (that serve as guideline) are unlikely to originate from a

healthy individual/animal (Solberg and Grasbeck, 1989; Solberg, 2008; Kjelgaard-Hansen and Jensen, 2010). The term "reference values" was chosen to replace the older term "normal values" on semantic and scientific grounds in order to overcome the conceptual problems and syleptic ambiguities that arose as a result of the use of the term "normal", taking into consideration the fact that absolute health does not exist (Amador, 1975; Sunderland, 1975; Lumsden, 2000).

The laboratory mouse (*Mus musculus*), family *Muridae*, is a common experimental animal in the biomedical sciences and psychology, and it is the most commonly used mammalian model organism (Austin *et al*, 2004; Foster *et al*., 2006; NJABR, 2011; Sellers and Ward, 2012). Animal models are necessary for biomedical research because it is impractical and unethical to use humans in most aspects of research on diseases. It is generally accepted that it would be wrong to deliberately expose humans to health risks in order to observe the course of disease processes (Gallagher, 2003; NJABR, 2011, Prieto *et al*., 2011). Among all model organisms, the mouse offers particular advantages for the study of human biology and diseases because the mouse is a mammal, and its development, body plan, physiology, behavior and diseases have much in common with those of humans; almost all mouse genes (99%) have homologs in humans (Austin *et al*., 2004; Foster *et al*., 2006; NJABR, 2011; Sellers and Ward, 2012). Further, mice are small, inexpensive, easily maintained, generally very docile if raised from birth with sufficient human contact and can reproduce quickly making it possible to observe several generations within a relatively short period of time (Foster *et al*., 2006). The mouse had played a prominent role as a model organism for the study of human diseases for more than a hundred years, and still serves as an important animal model for preclinical, pharmacological and toxicological evaluation of drugs and the investigation of various diseases and disease mechanisms. It had been shown that studies on mice provide clinical toxicity predictions that in many respects may be comparable or perhaps superior to predictions from dog or monkey studies (Goldsmith *et al*., 1975; Austin *et al*., 2004; Foster *et al*., 2006; Sellers and Ward 2012).

Blood is the major transport system of the body, and both input and output substances of almost all the body's metabolic processes and deviations from normal caused by disease pathogens, injuries, harmful substances, deprivation and stress are commonly reflected by changes in the blood picture (Ihedioha *et al*., 2004; Poiout-Belissent and McCartney, 2010). A comprehensive haematology, also known as completed blood count, is the foundation of the evaluation of the haematopoietic system response in preclinical and clinical trials, and is a basic requirement for the preclinical assessment of drugs and drug candidates for toxicity (Harrison *et al*., 1978; Reagan *et al*., 2010). The assessment of haematological parameters plays a critical role in diagnosis, prognosis and characterization of diseases and phenotypes in clinical and research situations (Everds, 2006; Forbes *et al*., 2009).

Amongst all other factors that affect the haematology profile, variations in the climatic and geographical location factors such as temperature, humidity, altitude and day length make it imperative that reference haematology values should be established for specific geographical locations (Coles, 1986; CLSI, 2008; Stockham and Scott, 2008; Kjelgaard-Hansen and Jensen, 2010). To date, the reference haematology values established for albino mice bred and raised in the temperate developed countries of Europe and America are being used by researchers in Nigeria. There are no reference haematology values for albino mice bred and raised in Nigeria in available literature. Yet massive amounts of preclinical pharmacology, toxicology and pathology studies utilizing albino mice bred and raised in Nigeria had been going on as evidenced by the large number of publications of such studies in numerous local and international journals in which reference values generated from temperate countries are referred to. The objective of this study was to establish reference values for the haematology profile of the conventional grade out-bred albino mice in Nsukka, Eastern Nigeria.

MATERIALS AND METHODS

The mice used for the study were the conventional grade UN-FERH:NS outbred strain of albino mice (*Mus musculus*) bred and raised at the Laboratory Animal Facility of the Foundation for Education and Research on Health (FERH), Nsukka Nigeria. This strain was adapted from the mouse colony bred and maintained at the Faculty of Veterinary Medicine Laboratory Animal House, University of Nigeria, Nsukka.

The study location (Nsukka) is situated within the derived savannah belt of Eastern Nigeria, with coordinates 6º 51′ 24″ north and 7º 23′ 45″ east and an average elevation of approximately 550 m (1,810 ft) above sea level with an ISO 3166 code of NG.EN.NS. Nsukka is an area of fairly high temperature with a yearly minimum and maximum of 23.6º C and 34.2º C, with a mean of 27.8º C (FMANR, 2011). The angle of the sun's ray over Nsukka is near vertical, and the difference between the longest and shortest days in the year is only 48 minutes (FMANR, 2011). Nsukka experiences two seasons – a rainy season from March to October and dry season from November to February, with a yearly average rainfall of 120.5 mm. The relative humidity in Nsukka is about 70% during the rainy season and about 20% during the dry season (FMANR, 2011).

A total of 336 apparently healthy albino mice were used for the study, made up of 168 males and 168 females. Eight age sets (mice of 4, 8, 12, 16, 20, 24, 30 and 40 weeks of age) were studied. For each age set, 21 males and 21 females were used for the study. Mice showing any signs of abnormality were excluded from the study. The mice included in the study group were kept in clean cages in a fly-proof animal house and were fed with mice chow (Grand Cereals Nigeria Limited, Jos, Nigeria) formulated to meet the nutritional requirements of mice (NRC, 1995) and provided with clean drinking water *ad libitum*. Guidelines for the humane use and handling of laboratory animals for research (NAS, 2011) were followed all through the study. Blood samples for haematology were obtained from the orbital sinus using the orbital bleeding technique (Bolliger and Everds, 2010). The blood samples were collected in the morning periods between 7.30 am and 9.30 am on each day of study. The study period was March to September, 2011. About 1 ml of blood was collected from each mouse, and the anticoagulant used was sodium ethylene diamine tetra acetic acid (EDTA). Each mouse was bled only once. All the haematological determinations were conducted immediately after blood sample collection, following standard procedures.

The packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002), while the haemoglobin concentration was determined following the cyanomethaemoglobin method (Higgins *et al.*, 2008). The erythrocyte and total leukocyte counts were conducted following the haemocytometer method, while the differential leukocyte counts were done on air-dried thin blood smears stained by the Leishman technique and enumerated by the meander method (Thrall and Weiser, 2002). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of the erythrocytes was calculated using the standard formulae (Thrall and Weiser, 2002).

Data generated from the study were subjected to appropriate statistics using the statistical package for social sciences (SPSS) version 16 software package. Data on the sexes (males and females) were compared using student's t – test, while age related variations were subjected to one way analysis of variance (ANOVA). Variant means were further separated using the least significant difference (LSD). Significance was accepted at $p < 0.05$, and the results were presented as means ± standard deviation, along with the minimum and maximum values obtained.

RESULTS

The mean PCV of all the mice (both males and females) ranged from 41.07 ± 1.57 % recorded for the 4-week old females to a maximum of 46.29 ± 2.00 % recorded for the 24-week old females, and there were no significant ($p > 0.05$) variations in PCV across the age sets

(Table 1). There was no significant difference ($p > 0.05$) between the PCV of males and females for all the age sets studied (Table 1). The erythrocyte count and haemoglobin concentration of both the male and female mice significantly ($p < 0.05$) increased from their lowest values obtained for the 4-week old mice to a relatively stable value recorded for the 8-week old mice, and did not further vary significantly ($p > 0.05$) across the age sets up to 40 weeks of age (Table 1). There were no significant differences ($p > 0.05$) between the sexes (males and females) in their erythrocyte counts and haemoglobin concentration all through the age sets studied (Table 1).

The erythrocyte MCV of the male and female mice were highest in the 4-week old mice, and values significantly ($p < 0.05$) lower than that obtained for the 4-week old mice were recorded for male mice of 8, 12 and 40 weeks of age and female mice of 8 and 40 weeks of age (Table 2). There were no significant differences ($p > 0.05$) between the MCV of the males and females across the age sets studied (Table 2). The erythrocyte MCH of both the male and female mice was highest in the 4-week male and female mice (Table 2). The MCH of the 8, 12 and 40-week old male mice were significantly ($p < 0.05$) lower than that recorded for the 4-week olds, but that of male mice aged 16, 20 24 and 30 were not significantly ($p > 0.05$) different from that of other age sets (Table 2). There were no significant ($p > 0.05$) age-related variations in the MCH of the females all through the age sets studied (Table 2). The erythrocyte MCHC rose from its lowest value recorded for the 4-week old male and female mice up to the highest values obtained for the 24-week old male and female mice, which was significantly ($p < 0.05$) higher than the values recorded for the 4-week olds (Table 2). There were no significant ($p > 0.05$) differences between the MCHC of males and females all through the age sets studied (Table 2). The total leukocyte counts (TLC) of the male mice increased from the value obtained for the 4-week old mice up to a significantly ($p < 0.05$) higher value recorded for the 16, 20 and 24 old males, and later significantly ($p < 0.05$) decreased in the 40-week old males (Table 3). The TLC of the females rose from its lowest value obtained for the 4-week olds up to its highest value recorded for the 16-week old females, which was significantly ($p < 0.05$) higher than the values obtained for the younger age sets (Table 3). The TLC of female mice of 20, 24 and 30 weeks of age were not significantly ($p > 0.05$) different from that of other age sets, but that of the 40-week olds was significantly ($p < 0.05$) higher than that recorded for the 4, 8 and 12 week old females (Table 3). The TLC of the 4-week old males was significantly ($p < 0.05$) higher than that of their female age mates, but that of the 40-week old females was significantly ($p < 0.05$) higher than that of their male age mates (Table 3). There were no significant ($p > 0.05$) differences between the TLC of males and females for age sets between 8 and 30 weeks of age (Table 3).

There were no significant variations ($p > 0.05$) in the absolute lymphocyte counts (ALC) of 4, 8, 12 and 30 week old male mice, but that of the 16 and 24 week old male mice were significantly ($p < 0.05$) higher while that of the 40-week old males were significantly ($p < 0.05$) lower (Table 3). The ALC of the female mice rose from its lowest value recorded for the 4-week old females to a significantly ($p < 0.05$) higher value obtained for the 12, 16, 20, 24 and 30 week old females (Table 3). There were no significant ($p < 0.05$) differences between the ALC of the males and females all through the age sets studied, except for the 24-week old age set in which the ALC of the males was significantly ($p < 0.05$) higher than that of the females (Table 3). There were no significant variations ($p > 0.05$) in the absolute neutrophil counts (ANC) of all the age sets studied (both males and females), but the ANC of the 4, 20 and 24-week old male mice were significantly ($p < 0.05$) higher than those of their female age mates (Table 3). The absolute monocytes, eosinophil and basophil counts obtained for all the age sets of mice studied were relatively very low when compared to the absolute numbers of lymphocytes and neutrophils (Table 4).

Table 1: The packed cell volume, erythrocyte counts and haemoglobin concentration of albino mice of varied ages and sexes

Age (weeks)	Packed cell volume (%)		Erythrocyte counts (10^6/µl)		Haemoglobin concentration (g/dl)	
	Males	Females	Males	Females	Males	Females
4	41.29±1.70 [38.00–43.00]	41.07±1.57 [39.50–43.50]	5.49±0.91 [a] [4.25–5.75]	6.63±1.56 [a] [4.30–7.80]	13.02±1.52 [a] [10.42–15.26]	12.94±0.63 [a] [12.28–13.77]
8	41.43±1.79 [39.00–44.00]	42.57±3.14 [38.00–47.00]	8.09±0.82 [b] [6.60–9.00]	8.18±1.13 [b] [6.90–9.80]	13.90±0.43 [a b] [13.58–14.52]	14.30±0.89 [b] [12.65–15.26]
12	43.50±1.44 [42.00–46.00]	44.29±1.68 [42.00–47.00]	8.44±0.80 [b] [6.95–9.65]	7.91±0.36 [b] [7.40–8.35]	14.38±0.73 [b] [13.40–15.26]	14.88±0.81 [b] [13.77–16.00]
16	43.50±2.72 [39.00–46.50]	45.36±1.80 [43.00–48.00]	7.94±1.24 [b] [6.15–9.40]	7.75±1.07 [b] [6.55–9.80]	14.64±0.89 [b] [12.79–15.63]	14.78±1.00 [b] [12.65–15.63]
20	45.07±1.90 [42.50–47.50]	44.00±3.12 [38.00–45.50]	7.89±1.33 [b] [6.05–9.35]	7.88±1.31 [b] [6.05–9.70]	14.96±1.12 [b] [13.58–16.56]	14.65±0.68 [b] [13.21–15.26]
24	43.64±0.94 [42.00–45.00]	46.29±2.00 [43.50–49.00]	7.72±1.29 [b] [6.15–9.62]	8.08±1.14 [b] [6.28–9.75]	15.36±0.72 [b] [14.28–16.06]	15.83±0.92 [b] [15.61–17.85]
30	42.40±2.80 [39.00–46.00]	44.20±1.48 [42.00–46.00]	8.06±0.80 [b] [7.25–8.68]	7.64±0.51 [b] [7.15–8.18]	15.17±1.25 [b] [14.28–16.28]	15.39±0.80 [b] [14.28–16.28]
40	42.17±1.61 [41.00–44.00]	42.00±2.55 [39.00–45.00]	8.30±0.69 [b] [7.55–9.60]	7.97±0.98 [b] [6.60–9.10]	14.14±0.81 [b] [13.58–15.07]	14.37±1.07 [b] [13.40–16.00]

Results are presented as means ± standard deviation, with the minimum and maximum values in parentheses. [a b] Different superscripts in a column indicate significant difference between the designated means across the ages ($p < 0.05$); No significant differences between the sexes ($p > 0.05$).

Table 2: The mean erythrocyte corpuscular values of albino mice of varied ages and sexes

Age (weeks)	Mean corpuscular volume (fl)		Mean corpuscular haemoglobin (pg)		Mean corpuscular haemoglobin concentration (g/dl)	
	Males	Females	Males	Females	Males	Females
4	77.03±13.83[a] [63.08–98.82]	65.65±10.42[a] [52.32–93.02]	24.56±5.68[a] [18.12–30.83]	20.63±5.60 [16.75–29.02]	31.67±2.50[a] [27.42–35.49]	31.49±0.51[a] [30.70–32.03]
8	51.68±6.05[b] [45.56–55.70]	52.55±4.99[b] [43.88–57.89]	17.32±1.66[b] [15.37–20.58]	17.66±1.63 [15.73–19.35]	33.61±1.57[ab] [30.86–33.95]	33.62±0.93[ab] [32.47–34.63]
12	51.90±4.98[b] [44.56–61.15]	56.16±4.33[ab] [50.90–63.51]	17.19±2.09[b] [13.89–20.88]	18.78±1.21 [17.11–20.62]	33.06±1.24[ab] [31.16–34.63]	33.52±1.82[ab] [30.60–35.96]
16	56.03±9.65[ab] [45.88–68.70]	59.32±7.16[ab] [47.96–68.70]	18.87±3.34[ab] [15.05–23.90]	19.37±2.82 [15.24–22.15]	33.67±1.23[ab] [32.24–35.85]	32.58±1.81[ab] [29.08–35.05]
20	58.50±9.98[ab] [48.13–72.52]	56.89±7.75[ab] [43.81–67.67]	19.29±2.50[ab] [16.65–23.01]	18.97±2.68 [14.96–22.39]	33.21±2.15[ab] [30.52–36.00]	33.35±1.04[ab] [31.79–34.76]
24	58.06±9.70[ab] [43.66–70.73]	58.55±10.09[ab] [47.18–74.84]	20.30±2.83[ab] [15.77–23.70]	21.23±3.69 [17.85–27.71]	35.18±1.46[b] [32.83–36.50]	36.40±2.37[b] [31.86–38.67]
30	52.73±5.31[b] [46.71–60.69]	58.15±5.46[ab] [51.34–63.62]	18.89±2.34[ab] [16.30–22.46]	20.23±1.78 [17.46–22.21]	35.75±0.79[b] [34.90–37.00]	34.82±1.30[ab] [33.71–37.00]
40	50.44±4.19[b] [43.92–64.95]	53.56±9.20[b] [43.96–66.67]	16.92±2.66[b] [14.57–20.84]	18.33±3.29 [15.13–22.56]	33.52±0.64[ab] [32.04–34.75]	34.20±1.01[ab] [32.79–35.56]

Results are presented as means ± standard deviation, with the minimum and maximum values in parentheses. [a b] Different superscripts in a column indicate significant difference between the designated means across the ages ($p < 0.05$); No significant differences between the sexes ($p > 0.05$).

Table 3: The total leukocyte counts and absolute lymphocyte and neutrophil counts of albino mice of varied ages and sexes

Age (weeks)	Total leukocyte counts (10^3/µl) Males	Total leukocyte counts (10^3/µl) Females	Absolute lymphocyte counts (10^3/µl) Males	Absolute lymphocyte counts (10^3/µl) Females	Absolute neutrophil counts (10^3/µl) Males	Absolute neutrophil counts (10^3/µl) Females
4	*5.57±1.89 [a] [3.45–8.70]	*4.29±1.12 [a] [3.10–6.45]	3.34±1.04 [a] [2.23–4.96]	2.63±0.86 [a] [1.52–4.19]	*2.15±0.91 [1.14–3.74]	*1.55±0.38 [1.22–2.09]
8	6.04±1.49 [a] [4.20–8.30]	5.26±1.86 [a] [3.30–8.15]	4.03±0.95 [a] [2.52–5.23]	3.22±1.16 [a b] [2.08–5.46]	1.90±0.60 [1.24–2.82]	1.88±0.73 [1.22–3.34]
12	5.97±0.46 [a] [4.30–6.60]	5.38±1.03 [a] [3.35–6.40]	3.72±0.32 [a] [2.32–4.28]	3.43±0.66 [b] [2.21–4.46]	2.13±0.30 [1.50–2.66]	1.94±0.39 [1.12–2.64]
16	7.76±2.11 [b] [4.65–10.55]	7.69±2.67 [b] [4.85–11.80]	5.34±1.58 [b] [2.79–7.39]	4.67±1.54 [b] [3.29–7.04]	2.30±0.63 [1.35–3.09]	2.85±1.34 [1.31–5.07]
20	7.25±2.52 [b] [4.50–11.30]	5.76±1.68 [a b] [3.70–7.35]	4.98±1.81 [a b] [3.11–7.80]	4.14±1.11 [b] [2.59–5.29]	*2.20±0.72 [1.35–3.39]	*1.52±0.53 [0.97–2.32]
24	7.77±1.27 [b] [5.50–9.45]	5.74±1.81 [a b] [3.70–8.80]	*5.47±0.87 [b] [4.18–6.62]	*3.90±1.30 [b] [2.46–6.16]	*2.21±0.51 [1.32–2.82]	*1.71±0.56 [1.00–2.55]
30	5.97±1.52 [a] [3.75–7.80]	6.80±2.49 [a b] [4.05–9.90]	3.55±1.03 [a] [2.29–5.07]	4.17±1.45 [b] [2.79–6.02]	2.38±0.58 [1.43–2.90]	2.45±1.26 [1.21–4.16]
40	*4.83±0.75 [c] [3.20–5.15]	*7.00±1.63 [b] [4.20–8.70]	2.45±0.08 [c] [1.87–2.82]	3.43±1.37 [a b] [1.62–5.31]	2.33±0.80 [1.15–3.20]	3.53±1.66 [2.10–6.48]

*Results are presented as means ± standard deviation, with the minimum and maximum values in parentheses. [a b c] Different superscripts in a column indicate significant difference between the designated means across the ages ($p < 0.05$); * Asterisk superscript on the sexes indicates significant differences between them at the specified age ($p < 0.05$).*

Table 4: The absolute monocyte, eosinophil and basophil counts of albino mice of varied ages and sexes

Age (weeks)	Absolute monocyte counts (10^3/µl) Males	Females	Absolute eosinophil counts (10^3/µl) Males	Females	Absolute basophil counts (10^3/µl) Males	Females
4	0.03±0.03 [0.00–0.06]	0.07±0.04 [0.03–0.14]	0.02±0.04 [0.00–0.11]	0.02±0.04 [0.00–0.09]	0.01±0.02 [0.00–0.05]	0.01±0.02 [0.00–0.06]
8	0.03±0.06 [0.00–0.14]	0.06±0.07 [0.00–0.16]	0.04±0.05 [0.00–0.15]	0.06±0.07 [0.00–0.16]	0.00±0.00 [0.00–0.00]	0.01±0.02 [0.00–0.05]
12	0.06±0.05 [0.00–0.11]	0.04±0.03 [0.00–0.05]	0.05±0.05 [0.00–0.11]	0.04±0.03 [0.00–0.04]	0.01±0.02 [0.00–0.05]	0.00±0.00 [0.00–0.00]
16	0.09±0.08 [0.00–0.21]	0.13±0.09 [0.00–0.27]	0.04±0.09 [0.00–0.24]	0.04±0.08 [0.00–0.21]	0.00±0.00 [0.00–0.00]	0.00±0.00 [0.00–0.00]
20	0.05±0.07 [0.00–0.16]	0.03±0.06 [0.00–0.16]	0.02±0.03 [0.00–0.07]	0.06±0.08 [0.00–0.21]	0.00±0.00 [0.00–0.00]	0.00±0.00 [0.00–0.00]
24	0.08±0.05 [0.00–0.14]	0.05±0.03 [0.00–0.09]	0.02±0.04 [0.00–0.08]	0.04±0.08 [0.00–0.06]	0.01±0.03 [0.00–0.09]	0.02±0.03 [0.00–0.06]
30	0.02±0.02 [0.00–0.05]	0.06±0.09 [0.00–0.20]	0.03±0.02 [0.00–0.05]	0.08±0.04 [0.04–0.12]	0.01±0.03 [0.00–0.07]	0.04±0.04 [0.00–0.10]
40	0.03±0.05 [0.00–0.09]	0.04±0.06 [0.00–0.14]	0.02±0.02 [0.00–0.04]	0.01±0.02 [0.00–0.06]	0.00±0.00 [0.00–0.00]	0.00±0.00 [0.00–0.00]

Results are presented as means ± standard deviation, with the minimum and maximum values in parentheses. No significant differences between the sexes and also between the varied ages ($p > 0.05$).

The absolute monocytes, eosinophil and basophil counts did not significantly ($p > 0.05$) vary across the age sets studied for both males and females, and there were no significant differences ($p > 0.05$) between the sexes (males and females) all through the age sets studied (Table 4).

DISCUSSION

The overall minimum and maximum PCV recorded for the mice used in this study (38.0 – 49.0 %) lies within the minimum and maximum values of 37.6 – 51.0 % reported for various strains of mice (Bolliger and Everds, 2010). The relatively lower mean PCV recorded for the 4-week old mice was mainly due to their lower erythrocyte counts when compared to other age sets. The finding of relatively lower PCV at a younger age which increases as the mice grows into adulthood is similar to what obtains in rats, dogs and cats, the reverse of which occurs in cattle, sheep and goats (Raskin and Wardrop, 2010). The later decrease in mean PCV at an older age (30 and 40 weeks of age) is in agreement with the reports of Bolliger and Everds (2010), and was attributed to plasma volume expansion as the mice aged, rather than lowered erythrocyte mass. The lack of significant variation in mean PCV across the ages and between the sexes as recorded in this study makes it possible for a researcher assessing for PCV to use any age set of mice to conduct his/her studies without worrying about age and sex differences.

The trend of increase in erythrocyte counts and haemoglobin concentration from a lower level at a younger age (4 weeks) to higher levels recorded for adults in this study is in agreement with that reported by Moore (2000) and Bolliger and Everds (2010) for various strains of mice. The minimum and maximum erythrocyte counts (10^6/µl) recorded in this present study (4.25 – 9.80) is relatively lower than the 7.00 – 11.00 reported by Bolliger and Everds (2010) and Charles River (2012) for mouse strains in North America, but the minimum and maximum HbC (g/dl) recorded in this present study (10.42 – 17.50) was not far different from the 10.00 – 17.00 g/dl reported for North American mouse strains by Bolliger and Everds (2010). The lack of significant difference in erythrocyte count between the males and females as recorded in this study was however not in agreement with the reports of Bolliger and Everds (2010) of slightly lower erythrocyte counts in males than in females.

The relatively higher MCV and MCH recorded at a younger age (4 weeks) and the progressive decrease as the mice grew into young adults (8 – 12 weeks of age) and the reverse trend recorded for the MCHC is in agreement with the patterns reported by Bolliger and Everds (2010). The lack of significant difference between the sexes (males and females) in their MCV. MCH and MCHC was also in agreement with reports by Bolliger and Everds (2010) and Charles River (2012). It is however worthy of note that the MCV, MCH and MCHC recorded in this present study were relatively higher than those reported for North American mouse strains/colonies by Bolliger and Everds (2010) and Charles River (2012).

The minimum and maximum total leukocyte counts (10^3/µl) recorded for mice used in this study (3.10 – 11.80) were relatively higher than those reported by Bolliger and Everds (2010) for mice strains in North America (2.0 – 10.0), but were lower than that reported by Charles River (2012). The pattern of increase in TLC from a lower level in the 4-week old mice to significantly higher levels at adulthood as recorded in this study was in agreement with reports by Bolliger and Everds (2010). The significant differences recorded between males and females at week 4 of age were not reported for North American strains but the differences between the sexes at week 40 of age were in agreement with the reports of Bolliger and Everds (2010).

The predominance of lymphocytes followed by neutrophils and the negligible absolute numbers of monocytes, eosinophils and basophils recorded in the differential leukocyte count of this study is in agreement with reports on North American mice strains (Bolliger and Everds, 2010; Charles River, 2012). The very low absolute numbers of monocytes, eosinophils and basophils is a common finding in the blood of all animals, but

the predominance of lymphocytes over neutrophils recorded in this study has been reported to also occur in rats, guinea pigs, cattle, sheep and goats, while in dogs and cats, neutrophils predominate (Raskin and Wardrop, 2010). The age-related trend of increase in the ALC from a lower level at week 4 of age to a higher level at adulthood and later decrease at weeks 30 and 40 of age is also in agreement with the reports of Bolliger and Everds (2010). This trend could be correlated with the immunological functions of lymphocytes and the age-related changes in immunocompetence which is usually lowest/weakest in the very young and aged when compared to adults (MacKinney, 1978; Ihedioha, 2004; Stockham and Scott, 2008). The absence of significant age-related variations in the ANC of the mice used in this study is in contrast to the reports of increase in ANC from young age to adulthood in North American mice strains (Bolliger and Everds, 2010). However, the significantly higher ANC recorded for the male mice at weeks 4, 20 and 24 of age concur with the reports of Bolliger and Everds (2010) that ANC are higher in male than in female mice. The lack of significant variations with age and differences between the sexes in the very low absolute numbers of monocytes, eosinophils and basophils is in agreement with the reports of Bolliger and Everds (2010) and Charles River (2012).

ACKNOWLEDGEMENTS

The authors are grateful to the Foundation for Education and Research on Health, Nsukka for their support of the study.

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