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ENVIRONMENTAL ENRICHMENT IN BREEDING AND MAINTENANCE OF LABORATORY MICE (*Mus musculus*)

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ABSTRACT

Enrichment of environmental conditions of laboratory mice model in most *in-vivo* studies may be attributed to quantity and quality of data output. Despite reports on welfare of *Mus musculus* (albino mice), there is dearth of studies in comparing standard breeding of this specie in enriched group (EG) and Un-enriched group (UG). The study examined productivity by breeding *Mus musculus* under standard laboratory condition, using comparison of this specie in proper (enriched) versus poor (un-enriched) condition. Six male and female mice (20 to 25g) were immaculately housed, fed, grouped into 2 (EG and UG) and bred using 2 phases of trio system (one male and two females) for 12 weeks (84 days). Results inferred production of more litters in EG (18/19) than UG, (12/14), respectively and more females' production than males in both groups for both phases. The number of cannibalised litters was more in UG (5 litters were cannibalised with 71.4 to 91.7 % survival rate) compared to EG (1 litter was cannibalised with 94.7 to 100% survival rate), during both phases. A significant ($p < 0.05$) difference was observed in volume of water consumed and average weight of mice in EG, when compared to UG. However, no significant ($p > 0.05$) difference observed in feed consumption of the two groups. The temperature readings (33.53±0.16 / 34.89 ±0.21) of clinical and mercury in glass thermometers of both groups revealed no significant difference. Thus, enriched group of laboratory mice appears to be more productive than un-enriched group during breeding period.

Keywords: Cannibalism, enrichment, husbandry, litters, *mice*.

INTRODUCTION

Environmental enrichment in housing mice especially in a certain caging condition with good welfares in laboratories can be a major key determinant in accurate and reliable research results in *in-vivo* studies (Clarkson et al. 2020). Many studies had proven that barren, restrictive and socially deprived housing conditions (enrichment) interfere with development and functioning of brain, behavioural activities (Pham et al. 2005, Garner, 2005, Arakawa, 2018). Many authors suggest that the use of enrichment improve animal welfare, however some other authors inferred that application of various enrichment items might interfere with animal health adversely (Lutz and Novak, 2005, Abou-Ismail et al. 2010, Clarkson et al. 2020). Enrichments can also affect comparability of scientific results, as even seemingly minor alterations can be a great void on experimental outcomes and interpretation (Toth, 2015). Maintenance, sustenance and animal welfare had been a legal issue as requested and directed by European Union in 2010/63/EU statement (EU, 2010). In lieu to EU report there is need for proper ethic and practice when using animal model. Currently, scientists are more concerned whether or not environmental enrichment would introduce more variability in experimental results than the breeding and maintenance of mice. Thus, our research question is "What is the role of simple enrichment in breeding and maintenance of mice model (*Mus musculus)?"* and our null hypothesis (H_0) is "Simple enrichment can significantly enhance breeding and maintenance of mice model (*Mus musculus)"*. Hence, this study

objectively compare and showed the significance of enrichment and un-enriched environment on study mice, most especially on breeding pattern.

MATERIALS AND METHODS

Animal Handling

The mice were bred and maintained in accordance with the ethical standards of the European and German Animal Welfare legislation, declaration principles set out by Helsinki and the National Institutes of Health guidelines for care and use of animals in research. The experiment was carefully structured and conducted to align with the 3R (replacement, reduction and refinement) principles of animal welfare. All protocols were approved by the local ethics committee of the linkages, research and advancement in our University at Olabisi Onabanjo University, Ago-Iwoye, Ogun-State (regulation CEE 86/609).

Twelve (12) conventional grade outbred strains (BALB/cJ) of *Mus musculus*(6–8 weeks old, weighing 20 –25g) were procured from Institute for Advance Medical Research and Technology (IMRAT) at the University College Hospital (UCH) Ibadan. Animals were arranged in a clean open-top cages ensuring a specific-pathogen-free facility in accordance with the Federation of European Laboratory Animal Science Associations recommendations (Mähler et al. 2014). The mice were acclimatized for two weeks prior the commencement of the study in a quiet standard animal house of the Department of Zoology and Environmental Biology, Olabisi Onabanjo University, Ogun State, Nigeria.

After acclimatization under a laboratory condition (33 ± 2°C (91.4 ± 2°F); 65 ± 5% Relative Humidity; 12/12 hours light and dark cycle) for two weeks, mice (6) were randomly selected and divided into 2 groups (n = 3), comprising enriched group (EG) and un-enriched group (UG). Aluminium based Individual ventilation caging system (IVC) of 30 cm Length × 27cm Width ×15 cm Height was used in housing the mice in a well uncontaminated conducive environment. Opaque cages filled with autoclaved clean wood shavings beddings, feeding sections and automated water systems were used throughout the breeding period (84 days). Cages were routinely cleaned twice a (Burn and Mason, 2008). Environmental temperature (33 ± 2°C) was maintained with 100% fresh air and uninterrupted power supply (Kathleen et al. 2009). Clinical and Mercury thermometers were used to monitor the internal temperature in the two groups throughout the breeding period.

Study design

Four (4) clean plastic bottles were opened at both ends as enrichment materials to serve as plastic tunnel for mice to cuddle in and out, move through as tunnel or burrow (Garner, 2005). These four opened plastic bottles were provided for the enriched group in the absence of litters. Two (2) additional enrichment materials were further provided after three weeks when litters were present in the cages (Rattazzi et al. 2016). The enrichment materials are routinely cleaned and replaced throughout the study (84 days).

Trio-breeding system of one male and two females was adapted for the EG and UG with two phases of breeding; first phase ran for 6 weeks and the second phase ran for another 6 weeks (Carpenter et al. 2020). After the first phase of conception, the trio system was distorted by carefully removing the two males (gently lifted through their tails) separating each sex into a separate cage to control indiscriminate mating. The breeding system was divided into two phases; the first 6 weeks represented the first phase while the second six (6) weeks covered the second breeding phase of the study. Weaning of litters in the two groups at each breeding phase was carried out after 21-24 days.

Pregnancy was detected with the appearance of plugs from virginal smear prepared 24 hours after mating (Ochiogu et al. 2006). Confirmatory pregnancy detection was subsequently verified with the recorded body weights (Grant, 2006). Three weeks after production of litters, the litters in each group were gently lifted out of the cage and carefully placed on their backs in a new cage to identify the anus and urethra for anogenital distance to determine sex (Szenczi et al. 2013).

Mice feed constituents comprising of carbohydrate, protein, vitamins and minerals were mixed thoroughly and pelletized into 6mm at Fasolab Animal and Aquaculture Feeds, Ijebu-

Igbo, Ogun state, Nigeria. Mice were fed well-preparedstandard laboratory pelleted rodent chow (g) with clean water (ml) *ad-libitum* over the period of 84 days (12 weeks). Pelletized feed and water were quantified daily by weighing with the aid of high precision weighing scale (Camry ISO 9001, Model EK5055). The volume of water consumed was also measured with 500 ml measuring cylinder and recorded daily (Castelhano-Carlos et al. 2014).

Data analysis

Statistical Package for social Science (SPSS) IBM Corp (version 20.0., 2011 for windows) was used for inferential (body weights, feed and water consumption) and descriptive (Sex frequency (%) of weaned litters and body weights of pregnant mice) statistical analyses of all the data recorded. Student's t-test was employed to compare the data generated from enriched and un-enriched groups. All the readings were analysed as means and standard errors of means (means ± SEM) for both groups. $P < 0.05$ was considered significant.

RESULTS

Productivity of mice (Mus musculus), cannibalism and survival rate

Enriched group (EG) produced more litters than un-enriched group (UG) during the two phases of breeding within 84 days. During the first breeding phase (42 days) 18 litters were produced in enriched group (EG) while un-enriched group (UG) recorded12 litters. The second breeding phase (42 days) resulted in production of 19 litters in EG where UG was able to produce 14 litters (Table 1).

There was cannibalism and low survival rate in UG compared to EG during the two phases of breeding within 84 days**.** During the first phase no litter was cannibalized (100% survival rate) in EG, where 18 litters were produced and weaned after 42 days. However, on the other side in UG, 1 litter was cannibalized (91.7% survival rate), 11 litters were weaned out of the 12 produced litters. Furthermore, during the second breeding phase EG had 1 cannibalized litter (94.7% survival rate) out of the 19 produced litters and in UG, 4 litters were cannibalized (71.4% survival rate) out of the 14 litters produced (Table 1).

Table 1: Number of litters, cannibalized litters and percentage survival rates in EG and UG for the two breeding phases (84 days).

Group	Numbers of Litters	Number of litters cannibalized	Survival Rate (%)
First Phase Breeding (42 days)			
Enriched (EG)	18	-	100
Un-enriched (UG)	12	01	91.7
Second Phase Breeding (42 days)			
Enriched (EG)	19	01	94.7
Un-enriched (UG)	14	04	71.4

Sex frequency

Anogenital distance in male litters was about 2 to 3cm more than that of female litters (1 to 2 cm). The percentages of female of weaned litters were more than male in both EG and UG during the two phases of breeding. (Figure 1).

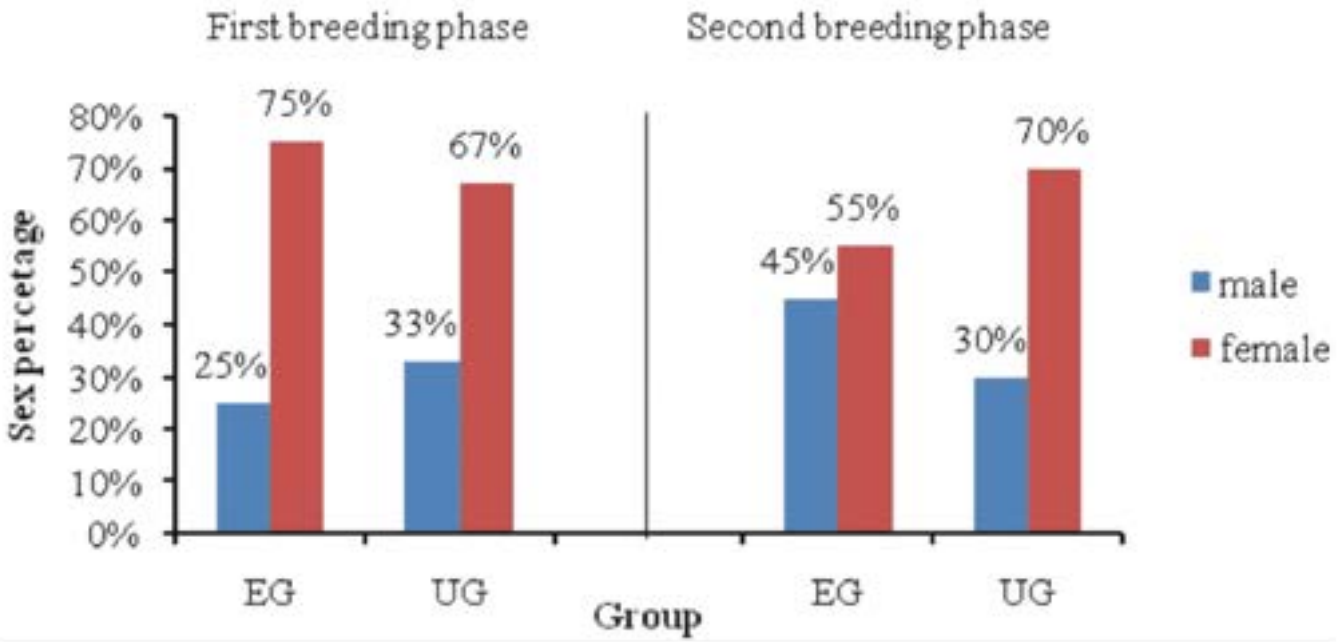

Figure 1: Sex frequency (%) of weaned litters in enriched and un-enriched group during the two phases of breeding.

General body weights and feeding

Generally, there was significant increase (p<0.05) in average body weights (g) of EG when compared with UG. There was no significant difference (p>0.05) in quantity of feed (g) consumed in EG and UG during the breeding. However, there was significant difference (p<0.05) in volume of water intake (ml) in UG with higher water consumption as compared to EG within the space of 84 days. See table 2 below for means, SEM and corresponding P-values.

Table 2: Average body weights, feed and water consumption in EG and UG for 84 days

Group	Body weight (g)	Feed (g)	Water (ml)
Enriched (EG)	33.76 ± 0.43	30.49 ± 1.52	56.78 ± 2.60
Un-enriched (UG)	29.14 ± 0.51	31.73 ± 1.50	64.66 ± 2.90
***P*-values**	0.00	0.33	0 .01

All the data analysed as means and standard errors of means (means ± SEM) for both groups. P< 0.05 was considered significant.

Body weights (pregnant mice)

The mean body weights (g) of pregnant female mice of EG were found to be significantly higher (P<0.05) than that of UG pregnant female mice, which was observed for 7 days after pregnancy confirmation until week 6 where there was significant decrease in weight within the 6th to 7th week (see Figure 2).

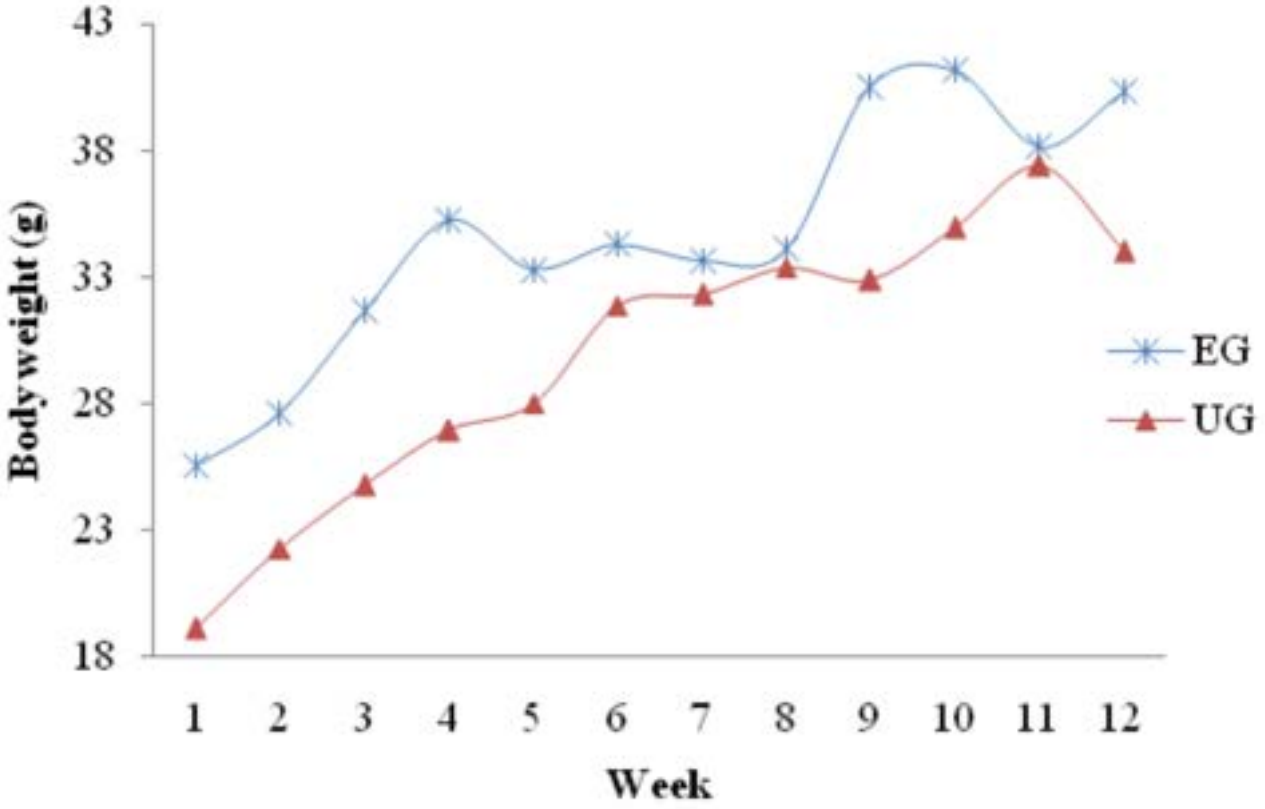

Figure 2: Average body weight of pregnant mice in EG and UG for 12 weeks

Temperature monitoring

Clinical and mercury thermometers paired wise comparison show no significant difference in mean temperature readings of the in EG and UG for 84 days (see Figure 3).

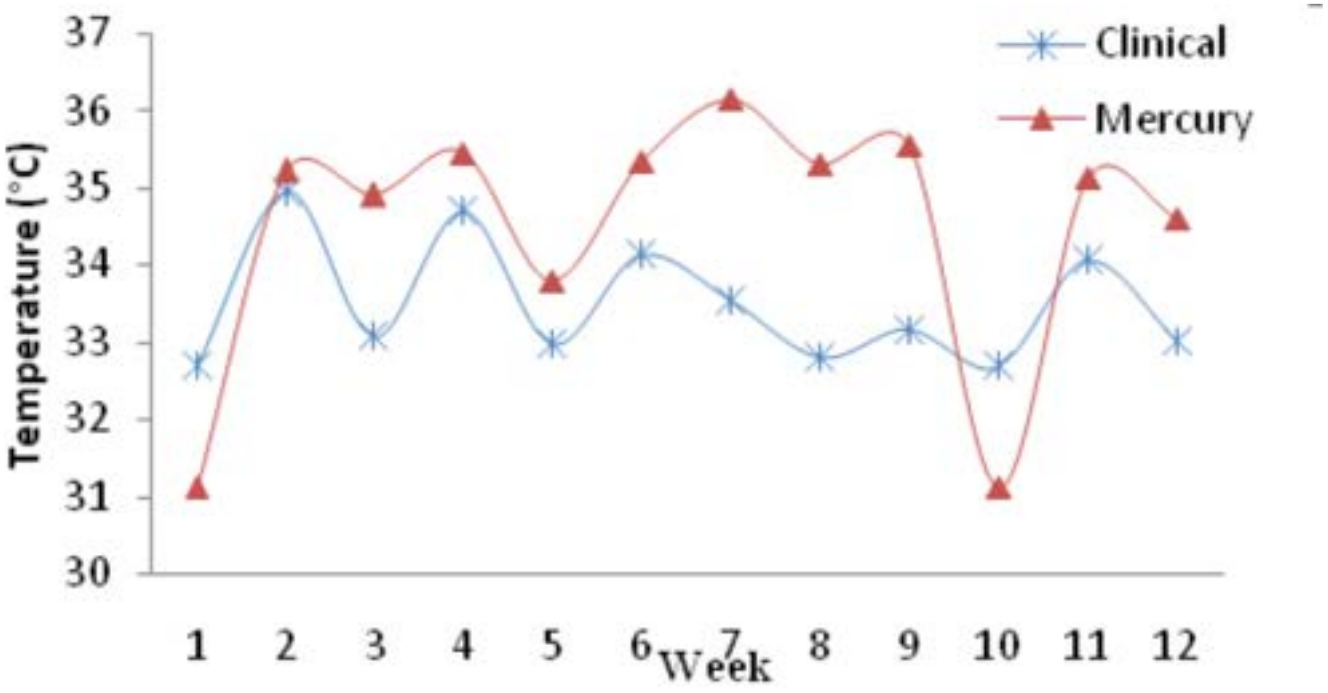

Figure 3: Clinical and Mercury temperature readings of bred laboratory mice for the two breeding phases (84 days).

DISCUSSION

Reasonable research outcome is dependent on standard animal housing and welfare which involves a simple and meaningful environmental enrichment (Salisu et al. 2021). However, effect of enrichment on rodent and animal model had been an interesting subject, as many researchers suggest that enrichment have both health and scientific implications (Van Loo et al. 2002; Restivo et al. 2005).According to EU prescription, provision of species-tailored enrichment to enhance research animal welfare is physiologically and ethically appropriate (EU, 2010). Provision of an enriched environment help meets the demands of the reputable research via high-quality and analytically reliant data.

The 3rd edition, Guide for the Care and Use of Agricultural Animals in Research and Testing outlined 4 best categorical means of assessing animal wellbeing of animals. These welfare indicators include behaviour patterns, pathologic and immunologic traits, physiologic and biochemical properties, and reproductive and productive performance of animal. Moreover, it can be concluded that reproductive performance is one of the best indicators of animal wellbeing (FASS, 2010). In agriculture, animal wellbeing is primitively associated with reproductive performance and production; whereas proper housing and animals' welfare are main means of maximize reproduction (Allen et al. 2016).

Previously, we had investigated standard breeding of *Rattus norvegicus* (Albino rat) via demonstrating, comparing reproductive performance and general welfare of rats in enriched and un-enriched environment. Currently, we are testing the same hypothesis, by breeding *Mus musculus* (mice) under enriched and un-enriched conditions.

Deductively, the two breeding phases (6 weeks each) described production of more litters and higher percentage of survival (94.7 to 100%) in EG compared to UG and where cannibalism and low survival rate of (71.4 to 91.7%) was found. This may be attributed to frequent interaction of mice in EG with enrichment material which ameliorate aggressive and antagonistic behaviour (Abou-Ismail *et al.* 2010) in EG. Additionally, high cannibalism and also low survival rate in UG might have triggered by negative mood (bad emotions) experienced by mice (Clarkson et al. 2020) due to lack of enrichment material. While on the contrary, it has been suggested that enrichment caging system is an 'analogous' approach (Lutz and Novak, 2005). Un-enriched animals may be more aggressive and restive due to lack of enriched materials during breeding.

Lately, we observed significant difference in general body weight and quantity of water consumed as ($P< 0.05$) in both parameters (Table 2). EG had significant increase in body weight when compared to UG, this may be attributed to enrichment and animal welfare according to Wirz et al. (2015) and Morano et al. (2019) who reported significant change in body weight of female mice. Furthermore, the observed significantly increase in weights of animals in EG as compared to UG, may be as a result of more pregnancies occurrence in EG as opposed to UG. However, a significant increase ($P < 0.05$) in water consumption was observed in UG as compared to EG in our study. This was not in accordance with Gurfein et al. (2012) who reported no significant difference in water consumption and also, contrary to water intake according to Van de Weerd et al.1997. Although, water intake had a great magnitude in mice welfare, no significant effect was seen owing to enrichment or non-enrichment. On the other hand, our previous findings in *Rattus norvegicus* Salisu et al. (2021) delineated that feed parameter was robust towards the influence of enrichment materials, as there was no improvement. Hence, suggest no significant effect ($p > 0.05$) on feed consumption (EG & UG) in corroboration with Gurfein et al. (2012) and Wirz et al. (2015).

Figure 2, depicts high sex frequency of weaned female mice to male mice (EG, 75/25%) and (UG, 67/33%) during the first phase, while (EG, 55/45%) and (UG, 30/70%) during the second phase. The result implies production of female mice than male mice in both groups and phases. This supports our earlier finding in *Rattus norvegicus* (Albino rat). However, this may or not be attributed to enrichment because random recombination of sex chromosomes determines the sex of an offspring during reproduction in genetics (Mackiewicz et al. 2018).

Although, a comprehensive investigation of standard ambient temperature for mice husbandry based on varying environmental zones is still debatable (Kolbe et al. 2022). Nonetheless, a study has shown that mice could be housed at significantly higher temperatures than the commonly recommended ambient temperature without compromising quality embryo production (Helppi et al. 2015). An average ambient temperature of 34^0C was recorded in both groups (EG & UG) during the two breeding phases from two different thermometers. Collectively, there was no significant difference ($p > 0.05$) between Clinical and Mercury thermometer readings throughout the 12 weeks study as shown in figure 3. This is accordance with recommendation that enrichment that is biologically safe and may be used to improve animal welfare without diminishing experimental outcome or altering of comparability to previous data collected under barren housing conditions (Bayne, 2018).

CONCLUSION

In conclusion, an enriched caging system ranging from novel to advance was preferable to un-enriched system. This study had been able to deduce that reproductive performances, less cannibalism and better well-being of species specific tailored standard breeding in *Mus musculus* (mice) can be achieved by enrichment system. Hence, our hypothesis (H_o) is not rejected. However, enrichment does not determine sex, and feeding habits as well as the temperature readings from clinical and mercury thermometers in mice and rodent generally.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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