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Hematological, biochemical, and growth parameters of Sprague Dawley rat of the Scientific Research and High Technology Services Institute of Panama

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Abstract
The Sprague-Dawley rat is used as an experimental model in neuroscience research, among others, developed at the Scientific Research and High Technology Services Institute of Panama; being important to have reference values of some parameters such as: growth (weight-length), hematological and blood biochemical parameters. In this trial, from weaning to 8 weeks, the weight gains and growth in length of 10 females and 10 males were measured. At 8 weeks, some hematological parameters were measured, such as hemoglobin, hematocrit, white blood cells, and their differential cell count. Some biochemical blood parameters were also measured, such as glucose, cholesterol, GOT, and GPT. The sample size used was calculated considering the total population of the colony. The parameters measured were compared between sexes, finding significant differences in weight gain ($p=0.01$), height ($p=0.02$), hemoglobin ($p=0.03$), total leukocytes ($p=0.05$), monocytes ($p<0.001$), neutrophils ($p=0.02$), GPT ($p=0.02$) and GOT ($p=0.01$), where males presented higher values in weight, height, total leukocytes, monocyte, neutrophils, GPT and GOT, while females presented higher values in hemoglobin. The values found in this trial can be used as data bases for different studies allowing the reproducibility of the experimental results at regional and global scope. In addition to applying the R's of Reduction and Refinement of ethics in the use of laboratory animals.

Key words: Rats, growth, hematology, biochemistry, ethics, welfare.

Parámetros hematológicos, bioquímicos y de crecimiento en ratas del Bioterio del Instituto de Investigaciones Científicas y Servicios de Alta Tecnología de Panamá

Resumen. La rata Sprague-Dawley es usada como modelo experimental en investigaciones de neurociencia, entre otras, desarrolladas en el Instituto de Investigaciones Científica y Servicios de Alta Tecnología de Panamá; siendo importante contar con valores de referencia de algunos parámetros tales como: crecimiento (peso-talla), hematológicos y bioquímicos sanguíneos. Se midieron, en este ensayo, desde el destete hasta las 8 semanas, la ganancia de peso y el crecimiento en talla de 10 hembras y 10 machos. A las 8 semanas se midieron algunos parámetros hematológicos, como la hemoglobina, el hematocrito, los glóbulos blancos, y su recuento celular diferencial. Igualmente se midieron algunos parámetros bioquímicos sanguíneos como: la glucosa, el colesterol, la GOT y la GPT. El tamaño de muestra usado fue calculado considerando la población total de la colonia. Los parámetros medidos se compararon entre sexos, encontrándose diferencias significativas en el aumento de peso ($p=0,01$), talla ($p=0,02$), hemoglobina ($p=0,03$), leucocitos totales ($p=0,05$), monocitos ($p<0,001$), neutrófilos ($p=0,02$), GPT ($p=0,02$) y GOT ($p=0,01$), donde los machos presentaron valores más altos en peso, talla, leucocitos totales, monocitos, neutrófilos, GPT y GOT, mientras que las hembras presentaron valores más altos en hemoglobina. Los valores encontrados en el ensayo

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pueden ser utilizados como datos bases para diferentes estudios permitiendo la reproducibilidad de los resultados experimentales a nivel regional y mundial. Además de aplicar las erres de Reducción y Refinamiento de la ética en el uso de los animales de laboratorio.

Palabras clave: ratas, crecimiento, hematología, bioquímica, ética, bienestar.

INTRODUCTION

The use of rodents in the development of different models for biomedical research is due to the similarity that their DNA has with human DNA among other important characteristic (Zhao et al. 2004). Of the numerous rodent models developed, those using the Sprague Dawley (SD) rat are preferred for studies in neuroscience, nutrition, oncology, pharmacology, and others, due to the behavioral characteristics of this outbred line (Ku et al. 2016, Rybnikova et al. 2018, Gileta et al. 2022). In Panama, this animal model is widely used by different institutions. Among them is the Scientific Research and High Technology Services Institute of Panama (INDICASAT AIP), which supplies SD rats to different projects throughout the country.

The progenitors of the SD rats of the INDICASAT AIP animal facility were obtained in 2018 from an international supplier. Since 2018, this colony has remained a close colony, currently breeding the 8th generation in non-inbred conditions, which can compromise the genetic variability expected in non-inbred lines (Benavides and Guénet 2003). Furthermore, using reference values reported by international studies or comparing with the ones reported by the supplier could lead to inaccurate conclusions, because of the differences in housing and handling conditions that can cause phenotypic changes in the colony, including variations in the values of physiological, hematological, and biochemical parameters (Kelada et al. 2012). Studying physiological parameters such as weight (g) and length (cm) can evaluate these animal´s development; hematological parameters such as hemoglobin (Hb), hematocrit (Hct), total white blood cell (WBC), and differential white blood cell count: neutrophils (Neut), basophils (Baso); lymphocytes (Lym); monocytes (Mono) and eosinophils (Eos); and biochemical parameters, such as glucose (Glu), cholesterol (CL) and transaminases: glutamic pyruvic aminotransferase (GPT) and glutamic oxaloacetic aminotransferase (GOT) belong to the group of basic parameters used as reference values in the animals colonies (Enesco and Leblond 1962, Harris et al. 1998, Gross 2009, Delwatta et al. 2018).

The objective of this study was to establish values for selected hematological, biochemical, and physiological parameters for the colony of Sprague Dawley rats produced in the INDICASAT AIP, in order to be used as reference for the different studies carried out in the institution.

MATERIALS AND METHODS

This research was approved by the Institutional Animal Care and Use Committee (IACUC) INDICASAT AIP, code CICUA-19-011.

Animals and housing. Ten female and ten male Sprague Dawley (SD) rats were randomly selected from all offspring born of the different couples in the foundation nucleus during the ongoing eighth generation acquired from the supplier. The sample was calculated considering what was reported in a similar study (Delwatta et al. 2018), due to the size of the colony at the INDICASAT AIP animal facility. Each of the 20 rats was considered an experimental unit. After weaning, the animals were housed in 3 groups: 2 groups of 4 animals per cage and 1 group of 2 animals, which were identified by cards and kept in the same rack during the study. None of them were mated during the study. The animals were temporarily identified by marking their tails. These animals were kept in "Specific Pathogen Free" (SPF) conditions, inside ventilated racks (Tecniplast, Blue Line, 129H (Figure 1), in standardized conditions. The cages were prepared with bedding (Bed-o'Cobs 1/4") and different types of enrichment, such as PVC or cardboard tubes, as well as paper towels. The microbiological status of the colony is verified every six months. The room was maintained at a temperature of 68.4°F (20.2°C) and a relative humidity of 56.7%. The photoperiod was 12 hours of light and 12 hours of darkness; noise did not exceed 85 dB. Food (DietLab 5001) and filtered water were sterilized by autoclaving and offered *ad libitum*.

Figure 1. Ventilated racks where animals were housed during the research at INDICASAT AIP.

Experimental design. Completely random design (CRD).

Procedures. At PD8, physical assessment of growth, weight (g), with an OHAUS Model VIIP6 scale, precision: 0.001 kg/0.002 lb; and length (cm), using a conventional ruler, with a maximum length of 30 cm, was started until the eighth week. These measurements were taken at 7:00 a.m. once a week. In the eighth week, intracardiac blood extraction was performed with the thorax cavity close and under anesthesia with Isoflurane, USP (TERRELL®) (Weiss et al. 2000, Kumar at al. 2017), in an anesthetic chamber, to collect an adequate volume of blood. The hemoglobin (Hb), glutamic oxaloacetic aminotransferase (GOT), and cholesterol (CL) were analyzed using the Semi-automatic analyzer BTS-350 Biosystems; the hematocrit was analyzed using the LW Scientific Zip-IQ PCV Digital 24 Place Hematocrit Centrifuge. The total white blood cell (WBC) count was analyzed using a Motic BA310E Microscope and a Neubauer Chamber (Hausser 1492 Bright-line, 0.1mm deep), and a Turk solution was used to prepare the sample. The differential WBC count was assessed using a blood smear and Giemsa stain at 10%. The Abaxis VetScan VS2 was used to measure glucose (Glu) and glutamic pyruvic aminotransferase (GPT) (Figure 2). The equipment used to process each parameter was calibrated by the providers to process rat and mouse blood samples, using the reference values reported by the supplier. Euthanasia was performed after blood extraction by anesthetic overdose (the animals did not wake up from it) (Leary et al. 2013).

Figure 2. Experimental Laboratory where samples were processed at INDICASAT AIP.

Statistics. Statistics were analyzed using IBM SPSS V.24 and a statistically significant difference was considered for all analyses using a significance level of $p<0.05$. The Kolmogorov-Smirnov test was used to determine the normality of the data. The test indicated that the distribution of length and weight of males and females was not normal; therefore, the non-parametric analysis used was the Kruskall-Wallis test. For the analysis of differences in the measurements of hematological and biochemical parameters, the Mann-Whitney U test was used. In this study, all selected animals were included in the analysis.

RESULTS AND DISCUSSION

The continuous growth tendency in weight and length of the study animals during the 8 weeks of measurements are presented in Figures 1a and 1b. Both showed significant differences with $p=0.01$ and $p=0.02$, respectively. A decrease in weight was observed between weeks 7 and 8 in males; this was not observed in females (Figure 1a).

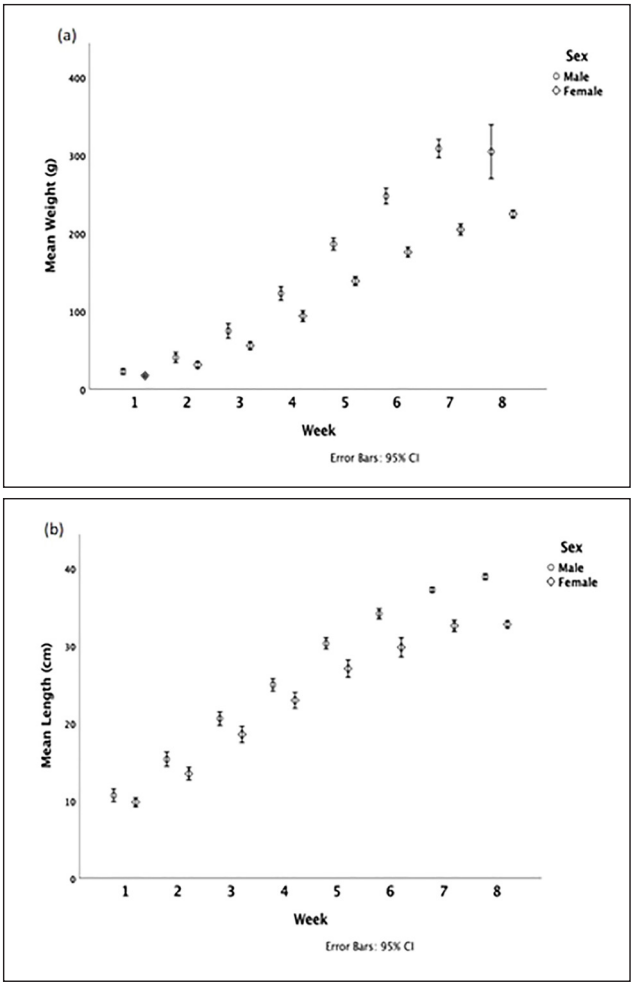

Figure 3. (a)Weight and (b) length of female and male SD rats of the INDICASAT AIP.

The reference values for weight and length of the INDICASAT-AIP Sprague Dawley rats from weeks 1 to 8 are presented in Table 1 and 2 with their respective Standard deviation per week.

Table 1. Reference values for the weight of the SD rats of the INDICASAT AIP animal facility.

Age (weeks)	Body Weight (g)			
	Male		Female	
	Mean	SD	Mean	SD
1	22.6	±5.19	17.4	±2.22
2	40.6	±9.35	31.2	±5.75
3	74.8	±13.16	55.9	±7.03
4	122.9	±11.98	93.9	±9.68
5	186.3	±10.95	138.8	±7.94
6	247.9	±14.07	175.9	±8.90
7	309.0*	±16.43	204.9	±10.13
8	304.9	±48.36	224.9	±6.8

*Weighing performed at a different time of the day

Table 2. Reference values for the length of the SD rats of the INDICASAT AIP animal facility.

Age (weeks)	Body length (cm)			
	Male		Female	
	Mean	SD	Mean	SD
1	10.71	± 1.12	9.83	± 0.79
2	15.38	± 1.30	13.51	± 1.13
3	20.64	± 1.21	18.59	± 1.47
4	25.00	± 1.11	23.00	± 1.43
5	30.34	± 1.01	27.09	± 1.54
6	34.19	± 0.92	29.84	± 1.71
7	37.27	± 0.46	32.62	± 1.04
8	38.96	± 0.56	32.81	± 0.66

These results evaluated during the first 56 days of growth of male and female SD rats, collect the data of the first, second and part of the third phase of growth which has been described previously (Enesco and Leblond 1962); during the first (0-17 days) and second phase (17-48 days) there is a cell proliferation and growth in most of the organs in the young rats which is reflected in the weight of the animal (Enesco and Leblond 1962). This coincides with the results of this study, since in both females and males, the greatest weight gain occurred between the third and fourth week (21-28 days).

Figures 1a and 1b clearly show the trend of the males gaining more weight and length than females. Figure 1a presents a decrease in weight gain of the males between week 7 and week 8, this may be due to the difference in the weighting schedule since the weight can be different throughout the day (Kawamura et al. 2020).

The growth observed had the same tendency as that reported by the commercial company that supplies these animals (Charles River Laboratories 2011) and the difference between sexes that has been reported by other studies (Pahl 1969, Slob and Bosch 1975).

When comparing the values obtained in the hematological parameters between sexes, significant differences was observed in the values of hemoglobin ($p=0.03$), total white blood cells ($p=0.05$), monocytes ($p<0.001$), and neutrophils ($p=0.02$). Concerning biochemical parameters, significant differences were observed in the values of GOT ($p=0.01$) and GPT ($p=0.02$) (Table 3).

Table 3. Hematological and biochemical reference values for the SD rats of the INDICASAT AIP animal facility.

Hematological and Biochemical selected parameters	Age: 8 Weeks			
	Male		**Female**	
	Mean	**SD**	**Mean**	**SD**
*Hemoglobin (g dL^{-1})	17.07	± 2.12	19.49	± 2.19
Hematocrit (%)	47.20	± 3.40	45.15	± 1.16
Total White Blood Cell (Cell / mm^3)	4961.25	± 1221.19	3925.00	± 974.43
*Monocytes (Cell / $mm^{3)}$	189.31	± 95.26	37.39	± 38.70
Lymphocytes (Cell / $mm^{3)}$	4418.66	± 1163.72	3684.24	± 935.66
*Neutrophils(Cell / $mm^{3)}$	304.12	± 188.48	136.25	± 82.57
Eosinophils (Cell / $mm^{3)}$	24.04	± 33.94	60.63	± 96.85
Basophils (Cell / $mm^{3)}$	20.00	± 37.50	6.49	± 13.75
Cholesterol (mg dL^{-1})	395.45	± 191.71	328.30	± 47.53
Glucose (mg dL^{-1})	136.80	± 21.79	148.80	± 17.80
*Glutamic oxaloacetic transaminase (GOT) (U/I)	48.90	± 12.34	33.30	± 9.91
*Glutamic pyruvic transaminase (GPT) (U/I)	71.10	± 37.82	47.50	± 6.59

*$p \leq 0.05$

There are some causes during the procedures such as hemolysis and the used of isoflurane that can alter hemoglobin values (Katoh et al. 1992, Campbell 2012). However, these findings were in agreement with those reported between sexes by other authors (Bush 1992, Lillie et al. 1996, Maeda et al. 2000, Kohn and Clifford 2002, León Goñi et al. 2011, He et al. 2017). The mean difference between females and males in relation to white blood cell count has been reported in other studies (Kohn and Clifford 2002, Schnell et al. 2002, Delwatta et al. 2018), which may be a result of the different ages and different routes used for sampling, as it had been reported before to affect blood parameters (Schnell et al. 2002).

The values of neutrophils and monocytes were higher in males than in females, presenting significant differences. Higher neutrophil values have been reported previously (Charles River Laboratories 2011, Stöppeler et al. 2013). However, higher neutrophil values have been reported in females (Delwatta et al. 2018), which do not match our results. In addition, higher monocyte values in males have been reported before (Kohn and Clifford 2002, Schnell et al. 2002, Delwatta et al. 2018).

Regarding the values of biochemical parameters, significant differences were found in GOT and GPT, with the mean being higher in males than in females. Higher values in GOT and GPT were in agreement with higher values reported in males by other studies and vendors (Han et al. 2010, Charles River Laboratories 2011, He et al. 2017). It is considered that GOT and GPT values may be altered by the type of anesthetic used (Soubhia et al. 2011, Campbell 2012).

CONCLUSIONS

The characterization carried out in this essay serves as a database not only for the institute's researchers who use this animal model, but also for all those who, through this publication, can use them as a basis for their studies, not only to verify but also to improve research analysis which consequently with lead to better science. But we agreed that further research with a higher sample must be needed.

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