

HEMATOLOGICAL AND BIOCHEMICAL DYNAMICS OF RABBITS AND GUINEA PIGS USED FOR SCIENTIFIC PURPOSES AT CANTACUZINO INSTITUTE, BUCHAREST

DINAMICA HEMATOLOGICĂ ȘI BIOCHIMICĂ A IEPURILOR ȘI COBAILOR UTILIZAȚI ÎN SCOPURI ȘTIINȚIFICE ÎN INSTITUTULUI CANTACUZINO, BUCUREȘTI

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ABSTRACT | REZUMAT

"Cantacuzino" National Medico-Military Institute for Research and Development (INCDMM Cantacuzino) is the main producer and provider of laboratory animals in Romania. Knowing the normal values of blood parameters for the species used in biomedical research is essential, this study aims to provide information on the evolution of clinical biochemistry and haematological parameters in time, by gender and age, for New Zealand White rabbits and Dunkin Hartley guinea pigs, held by the Animal Facility, animals which are used for scientific purposes. The tests results were correlated based on gender and age, following the numerical fluctuations of the analysed parameters and the statistically significant differences between them. Rabbits' results for haematological parameters showed statistically significant variations ($p < 0.05$) between life stages taken into account, but not between genders, while guinea pigs results showed statistically significant differences between the genders. The biochemical parameters highlighted statistically significant differences between life stages, both in rabbits and guinea pigs. The haematological and biochemical parameters showed numerical fluctuations specific to the developmental needs of the animal organism, for both species. These values tend to stabilize in the young-adult – adult period.

Keywords: haematological parameters, biochemical profile, reference range, rabbits, guinea pigs

Institutul Național de Cercetare-Dezvoltare Medico-Militară (INCDMM) Cantacuzino este cel mai mare producător și furnizor de animale de laborator din România, iar cunoașterea valorilor normale ale parametrilor sangvini aferenți fiecărei specii este esențială în cercetarea biomedicală. Acest studiu își propune să ofere valorile fiziologice normale, în dinamică, pe sexe și vârstă, ale parametrilor biochimici și hematologici pentru tulpinile de iepuri New Zealand White și cobai Dunkin Hartley din biobaza proprie, animale utilizate în scopuri științifice. Rezultatele analizelor au fost corelate pe sex și vârstă, urmărindu-se fluctuațiile numerice ale parametrilor analizați și diferențele semnificative statistic dintre acestea. Iepurii înregistrează, pentru parametrii hematologici, variații semnificative statistic ($p < 0,05$) între stadiile de viață luate în calcul, dar nu și între sexe, în timp ce cobaii prezintă diferențe semnificative statistic și între sexe. Parametrii biochimici analizați evidențiază în special diferențe semnificative statistic între stadiile de viață, atât la iepuri cât și la cobai. Parametrii hematologici și biochimici prezintă fluctuații numerice specifice nevoilor de dezvoltarea ale organismului animal, pentru ambele specii. Aceste valori au tendință de stabilizare în perioada tânăr-adult – adult.

Cuvinte cheie: parametri hematologici, profil biochimic, interval de referință, iepuri, cobai

The rabbits and guinea pigs are among the animal species most used worldwide in biomedical research. Due to their susceptibility to infections, of the similarity of their anatomy and immune system to those in humans, guinea pigs are used as an ideal model for infectious diseases studies (8). Rabbits are commonly used to test toxicity and safety use of substances such as drugs, chemicals and medical devices, in genetics

studies and infectious disease research (11).

The characterization of the biochemical and haematological profile of the animals originated from/held by our animal facility is important for reducing variability, because the values of blood parameters vary depending on species, sex, health, nutrition, accommodation conditions, environment condition, geographical position, etc. Benchmarking for biochemical and haematological parameters provide a better perspective into the overall health of animals and validates deviations from the expected ranges as clinically relevant when they are used as a human disease model (2).

This study aimed to determine the normal spec-

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trum values of biochemical and haematological parameters in time, by gender and age for New Zealand White rabbits and Dunkin Hartley guinea pigs born and raised in INCDMM Cantacuzino's Animal Facility, Ba-neasa Station, under standard conditions.

INCDMM Cantacuzino is the only provider of rabbits and guinea pigs used for scientific purposes in Romania, being authorized in this respect by the competent authority, Sanitary Veterinary and Food Safety Directorate (DSVSA) Bucharest. These values provided to our clients, researchers in the fields, certify the health status of the animals, help them to interpret appropriately the results of the tests, facilitate the reduction of the stress to which the animals are subjected when biological samples are collected at the beginning of the study, offering them the necessary condition for the reproducibility and repeatability of the multiple tests results, if necessary.

We chose our testing panel according to the recommended core clinical chemistry panel for safety and toxicity studies in laboratory animals (12). This core panel includes glucose, blood urea nitrogen, creatinine, total protein, albumin, globulin, calcium and total cholesterol. Additionally, we recommend at least two hepatocellular function tests, including alanine aminotransferase or aspartate aminotransferase and two hepatobiliary function tests, including alkaline phosphatase, gamma glutamyl-transferase or total bilirubin. There are authorities who recommend the additional inclusion of assays for triglycerides and phosphorus (1). The haematological panel covers the 24 parameters of a complete hemogram.

MATERIALS AND METHODS

Ethics Statement

This study was favourably evaluated by the Committee of Ethics of INCDMM Cantacuzino, authorized by the competent authority (authorization number 423/2018, issued by DSVSA Bucharest), and performed in compliance with the provisions of Directive 2010/63/EU on the protection of animals used for scientific purpose.

Animals

In this study, we used 40 New Zealand White rabbits and 28 Dunkin Hartley guinea pigs, number considered statistically relevant for the population from the INCDMM Cantacuzino's Animal Facility.

The animals were evenly grouped based on gender and dynamically monitored for 48 weeks. The breeding system was conventional, and the health monitoring was done according to the FELASA recommendations (5), all animals being negative for the specified pathogens. Throughout the study, the animals were kept under controlled conditions: artificial

light 12 of 24 hours, temperature of $18^{\circ}\text{C} \pm 2^{\circ}\text{C}$, relative humidity between 45%-65% and they received standard pellets food and water *ad libitum*. The study started at age of 10 weeks for rabbits and 9 weeks for guinea pigs. The animals were daily inspected for health status and weighed at each biological sample collection. The rabbits were kept individually in 713x716x115 mm stainless steel cages and the guinea pigs 7 per 612x435x216 mm polycarbonate cages (Tecniplast spa, Italia).

Sample collection

Blood sampling was harvest in aseptic conditions from the ear vein in rabbits and the saphenous vein in guinea pigs. For the haematological tests, the blood was sampled in EDTA pre-conditioned tubes, and for biochemistry, in lithium-heparin pre-conditioned tubes. The animals were kept on diet for 12-14 hours before sampling. Bleeding was done dynamically, for all the animals (40 rabbits and 28 guinea pigs) between 08:00-10:00 a.m., by the same veterinary technician, trained and experienced in blood collection. We chose to collect blood in 3 stages of animal development: youth after weaning (10 weeks in rabbits, 9 weeks in the guinea pig), young-adult (20 weeks in rabbits, 24 weeks in guinea pigs), and adult (48 weeks in rabbits, 36 and 48 weeks in guinea pigs). Values of haematological and biochemical parameters were investigated to determine subpopulation differences according to age and sex.

Laboratory analyses

The blood samples were processed with a ProCyt Dx Hematology Analyzer 5 Diff (IDEXX, Westbrook, Maine, USA), within 2 hours from collection. The hemogram includes the following parameters: Total number of erythrocytes - RBC, Haematocrit value - HCT, Haemoglobin concentration - HGB, Mean erythrocyte volume - MCV, Mean haemoglobin volume - MCH, Mean haemoglobin concentration of erythrocytes - MCHC, The degree of variation in size of the erythrocyte population - RDW, Reticulocyte percent - %RETIC, Reticulocyte count - RETIC, Total number of leukocytes - WBC, Heterophil percent - %HETERO, Neutrophil percent - %NEU, Lymphocyte percent - %LYM, Monocyte percent - %MONO, Eosinophil percent - %EOS, Basophil percent - %BASO, Heterophil count - HETERO, Neutrophil count - NEU, Lymphocyte count - LYM, Monocyte count - MONO, Eosinophil count - EOS, Basophil count - BASO, Total number of platelets - PLT, Mean platelet volume - MPV, Platelet distribution width - PDW, Plateletcrit value - PCT.

For the biochemistry tests, the blood samples on lithium-heparin were processed by centrifugation for 2 minutes at 12,000 RFC at the temperature of 4°C for obtaining plasma. The plasma was immediately tested

Table 1

Haematological parameters measured in youth after weaning New Zealand White rabbits

Parameter	10 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
RBC $10^{12}/L^{\#}$	5.22	0.76	3 - 6.14	5.26	0.37	4.81 - 6.11
HCT $\%^{\#}$	33.16	4.80	31 - 36.9	33.24	2.81	29.6 - 37.4
HGB g/dL[#]	10.72	1.49	9.8 - 12.7	10.70	0.77	9.7 - 12.2
MCV fL[#]	63.67	4.11	59.1 - 68.9	63.21	2.98	60.1 - 69.9
MCH pg	20.59	0.76	19.3 - 22.5	20.37	0.54	19.8 - 21.9
MCHC g/dL[#]	32.37	1.01	30.4 - 33.9	32.23	1.03	30.1 - 33.7
RDW $\%$	15.08	1.31	13.2 - 17.5	15.28	2.05	13.2 - 17.5
RETIC $\%^{\#}$	3.20	2.08	1.1 - 6.1	3.92	1.19	1.1 - 5.9
RETIC K/μL[#]	152.61	37.38	102.1 - 217.6	198.95	27.98	153.1 - 236.6
WBC $10^9/L^{\#}$	5.65	2.91	1.82 - 7.98	4.32	1.65	2 - 6.44
HETERO$\%^{\#}$	30.99	8.77	23 - 40.2	29.56	9.69	19 - 45.9
LYM $\%^{\#}$	51.29	11.94	36 - 71.4	52.56	12.19	30.1 - 66.7
MONO $\%^{\#}$	11.82	4.98	7.3 - 20.7	10.74	3.34	6.7 - 19.4
EOS $\%$	1.06	0.51	0.4 - 1.9	1.35	0.65	0.5 - 2.6
BASO $\%^{\#}$	5.31	1.75	2.3 - 7.6	5.72	1.09	3.6 - 7.5
HETERO $10^9/L$	2.03	1.83	0.23 - 7.97	1.28	0.75	0.67 - 2.97
LYM $10^9/L^{\#}$	2.55	0.95	1.3 - 4.95	2.26	0.96	0.8 - 3.6
MONO $10^9/L$	0.74	0.69	0.3 - 1.36	0.48	0.27	0.2 - 0.97
EOS $10^9/L^{\#}$	0.05	0.03	0.02 - 0.12	0.06	0.04	0.01 - 0.13
BASO $10^9/L$	0.28	0.13	0.4 - 0.54	0.24	0.10	0.3 - 0.48
PLT K/μL	215.67	90.13	101 - 418	220.80	74.20	170 - 428
MPV fL[*]	6.84	0.68	5.5 - 7.9	6.54	0.47	6.1 - 8.1
PDW fL[*]	6.49	0.71	5.1 - 7.4	5.98	0.22	5.7 - 6.4
PCT $\%$	0.18	0.15	0.05 - 0.29	0.13	0.06	0.09 - 0.27

* - $p < 0.05$ between sex-specific values

- $p < 0.05$ between age-specific values

with a VetTest Chemistry Analyser (IDEXX, Westbrook, Maine, USA), in dry system, focusing on: Glucose - GLU, Creatinine - CREA, Urea - UREA, Inorganic phosphate - PHOS, Calcium - CA, Total protein - TP, Albumin - ALB, Globulin - GLOB, Alanine aminotransferase - ALT, Alkaline phosphatase - ALKP, Total bilirubin - TBIL, Cholesterol - CHOL and Amylase - AMYL in guinea pigs and on: GLU, CREA, Uric Acid - URIC, ALT, Aspartate aminotransferase - AST, Gamma-glutamyltransferase - GGT, CHOL and Triglycerides - TRIG in rabbits. The equipment meets the qualification requirements, the methods used are validated and the results obtained fulfill/satisfy the criteria of reproducibility and accuracy.

Statistical analysis

The data results were statistically analysed using ANOVA two-factor with replication, expressed as average, standard deviation, minimum and maximum values, and values of $p < 0.05$ considered significant from a statistical point of view between genders and life stages. The tests were performed with Microsoft Excel 2010, software.

RESULTS AND DISCUSSIONS

Haematology

The results of the haematological parameters for rabbits and guinea pigs are presented in Tables 1–7 as

Table 2

Haematological parameters measured in young-adult New Zealand White rabbits

Parameter	20 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
RBC $10^{12}/L^{\#}$	5.50	0.34	5.01 - 6.26	5.38	0.15	4.80 - 6.51
HCT $\%^{\#}$	34.19	1.72	31.6 - 38.8	34.27	2.09	29.8 - 38.7
HGB g/dL[#]	11.45	0.50	10.5 - 12.4	11.34	0.82	9.7 - 13.1
MCV fL[#]	62.20	2.16	60.1 - 65.3	62.66	2.33	59.4 - 66.4
MCH pg	20.83	0.67	19.3 - 21.7	20.71	0.73	19.8 - 22.2
MCHC g/dL[#]	33.48	0.71	32.1 - 34.8	33.08	0.86	31.7 - 34.2
RDW $\%$	16.28	1.36	14.8 - 17.8	15.97	0.90	14.7 - 17.4
RETIC $\%^{\#}$	3.31	1.10	1.5 - 5.4	2.52	1.02	1.2 - 3.8
RETIC K/μL[#]	162.95	47.15	84.3 - 243.2	135.70	51.86	55.9 - 199.7
WBC $10^9/L^{\#}$	7.04	2.47	4.44 - 9	7.30	1.65	5.54 - 9.68
HETERO$\%^{\#}$	26.19	6.52	18.6 - 34.2	30.36	10.11	17.2 - 45.9
LYM $\%^{\#}$	61.68	7.32	44.6 - 75.9	54.80	12.46	36.2 - 72.9
MONO $\%^{\#}$	7.10	1.47	4.9 - 9.2	8.60	1.91	6.7 - 11
EOS $\%$	1.07	0.60	0.4 - 2.4	1.51	0.52	0.7 - 2.5
BASO $\%^{\#}$	4.49	1.43	2.7 - 6.9	4.73	1.35	3.6 - 7.1
HETERO $10^9/L$	1.89	1.07	0.99 - 4.34	2.30	1.10	0.87 - 3.94
LYM $10^9/L^{\#}$	4.28	1.40	2.72 - 6.93	3.89	0.95	2.95 - 4.91
MONO $10^9/L$	0.49	0.19	0.3 - 0.94	0.65	0.30	0.33 - 1.45
EOS $10^9/L^{\#}$	0.07	0.05	0.02 - 0.17	0.11	0.05	0.06 - 0.22
BASO $10^9/L$	0.30	0.09	0.16 - 0.45	0.34	0.12	0.17 - 0.5
PLT K/μL	274.47	56.84	199 - 393	260.07	84.02	167 - 447
MPV fL[*]	6.61	0.41	6.1 - 7.3	6.49	0.25	6.1 - 6.9
PDW fL[*]	6.41	0.60	5.7 - 7.4	6.19	0.25	5.6 - 6.6
PCT $\%$	0.18	0.04	0.13 - 0.25	0.17	0.07	0.11 - 0.35

* - $p < 0.05$ between sex-specific values

- $p < 0.05$ between age-specific values

an average, standard deviation, minimum, and maximum values. In the case of rabbits, statistically significant variations ($p < 0.05$) of haematological parameters were found between the life stages (youth at weaning - young-adult - adult) but not between the genders. Exceptions are MPV and PDW in which we observed statistically significant variations between the genders. With the growth and development of animals, the parameters RBC, HCT, HGB, MCHC, LYM, EOS, MONO, and PLT register increased values, values of the parameters MCV, HETERO, MONO, BASO, MPV, and PDW tended to decrease while RDW, RETIC, and WBC increased until young-adult stage - 20 weeks after which decreased slightly.

In guinea pigs, the values of haematological parameters were influenced by gender and age ($p < 0.05$ for most parameters). Fluctuations were observed for RBC, HCT, HGB, MCV, MCH, RETIC, WBC, HETERO, LYM, and MONO with a tendency to stabilize in young-adult. Instead, the parameters RDW, MCHC, BASO, EOS, PLT, MPV, PDW, and PCT registered consecutive increases with the growth and development of the animal organism.

Clinical biochemistry

The results of the biochemical parameters for rabbits and guinea pigs are presented in Tables 8–14 as an average, standard deviation, minimum, and maxi-

Table 3

Haematological parameters measured in adults New Zealand White rabbits

Parameter	48 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
RBC $10^{12}/L^{\#}$	5.83	0.57	5.06 - 7.14	5.65	0.39	5.1 - 6.57
HCT $\%^{\#}$	35.65	3.04	34.1 - 39.2	34.78	2.25	31.7 - 37.6
HGB g/dL[#]	12.02	1.06	11.3 - 14.5	11.75	0.64	10.9 - 13.1
MCV fL[#]	61.32	2.84	55.7 - 65.5	61.61	1.53	59.1 - 63.3
MCH pg	20.67	1.01	18.7 - 22.4	20.82	0.57	19.9 - 21.7
MCHC g/dL[#]	33.72	0.63	32.8 - 34.5	33.79	0.69	32.8 - 35.2
RDW $\%$	16.09	2.74	13.4 - 18.5	14.73	0.99	13 - 16.2
RETIC $\%^{\#}$	2.27	0.89	1.5 - 4.9	2.22	0.56	1.4 - 3.6
RETIC K/μL[#]	129.73	41.66	83.4 - 145.4	122.94	28.03	83 - 169.7
WBC $10^9/L^{\#}$	6.89	1.43	5.95 - 9.46	7.56	2.25	4.92 - 9.79
HETERO$\%^{\#}$	23.79	5.39	18 - 32.8	23.85	9.18	13.4 - 40.2
LYM $\%^{\#}$	64.20	6.78	54 - 77.1	63.47	9.61	46.6 - 75.6
MONO $\%^{\#}$	6.32	1.79	4.8 - 11.3	6.98	1.08	5 - 8.5
EOS $\%$	1.46	0.57	0.6 - 2.4	1.37	0.49	0.8 - 2.6
BASO $\%^{\#}$	4.23	1.10	2.4 - 6.9	4.33	0.81	3.6 - 6.5
HETERO $10^9/L$	1.63	0.50	1.01 - 2.77	1.91	1.20	0.66 - 4.06
LYM $10^9/L^{\#}$	4.43	1.07	2.33 - 5.74	4.69	1.15	2.86 - 6.07
MONO$10^9/L$	0.44	0.19	0.3 - 1.07	0.53	0.20	0.3 - 0.94
EOS $10^9/L^{\#}$	0.10	0.03	0.04 - 0.14	0.10	0.05	0.04 - 0.25
BASO $10^9/L$	0.29	0.08	0.2 - 0.49	0.33	0.12	0.19 - 0.57
PLT K/μL	254.07	58.95	185 - 386	250.47	71.94	144 - 388
MPV fL[*]	6.59	0.32	6.2 - 7.4	6.29	0.27	5.7 - 6.8
PDW fL[*]	6.38	0.45	5.9 - 7.7	5.90	0.42	5.1 - 6.8
PCT $\%$	0.17	0.04	0.14 - 0.27	0.16	0.05	0.12 - 0.25

* - $p < 0.05$ between sex-specific values

- $p < 0.05$ between age-specific values

imum values. In rabbits, except GGT, the parameters GLU, CREA, ALT, AST, CHOL, and TRIG show statistically significant variation ($p < 0.05$) between the analysed life stages: youth after weaning - young-adult - adult. These variations, in the sense of the increase for CREA, ALT, AST respectively, the decrease for GLU, CHOL, and TRIG were present in both genders. For AST and TRIG values, there is statistically significant variation between the genders. GGT values increased up to 20 weeks after which they decreased. In the case of guinea pigs, statistically significant differences were observed between the genders for the parameters GLU, ALT, UREA, PHOS, and ALB while, for the parameters CREA, ALT, PHOS, ALB, CHOL, TP, and GLOB

were differences associated with age stages. Throughout the period 9-48 weeks, the ALT and PHOS values increased while the ALB values decreased. The GLU, CHOL, TP, CA, and AMYL values increased until young-adult stage then they decreased with a tendency to stabilize during adulthood. The increased values of AMYL, during the all-life stages were noticeable in females, while in males these variations were less accentuated. The GLOB values were relatively constant in males throughout the study period, unlike females who presented transient variation reaching the peak on week 36 of the study. The CREA, UREA, and ALKP showed a decrease in values followed by an increase with age. These fluctuations were similar in both gen-

Table 4

Haematological parameters measured in youth after weaning Dunkin Hartley guinea pigs

Parameter	9 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
RBC $10^{12}/L^{*#}$	4.67	0.33	3.99 - 5.33	4.52	0.38	3.97 - 5.23
HCT %^{*#}	35.31	2.73	32.2 - 41	36.66	2.21	34.5 - 40.4
HGB g/dL^{*#}	11.51	0.63	10.8 - 13	11.34	0.83	10.1 - 12.7
MCV fL	73.50	2.41	68.7 - 77.7	72.63	3.70	66.8 - 79.8
MCH pg^{*#}	24.47	0.76	22.9 - 25.8	25.53	0.93	24.3 - 27.3
MCHC g/dL	33.02	0.52	32.8 - 34.5	33.88	1.70	31.9 - 38.7
RDW %^{*#}	13.57	0.57	12.8 - 14.7	13.94	0.71	12.9 - 15.2
RETIC %^{*#}	1.46	0.59	0.7 - 3.1	1.79	0.63	0.4 - 2.8
RETIC K/$\mu L^{*#}$	63.52	16.07	53 - 96.6	71.81	11.95	46.8 - 90.1
WBC $10^9/L$	6.99	2.54	4.06 - 13.25	7.10	1.32	5.26 - 9.87
HETERO%^{*#}	43.71	5.11	31.8 - 50.2	55.41	10.57	44.5 - 78.2
LYM %^{*#}	39.79	8.28	21 - 50.6	38.77	5.72	26.5 - 46.5
MONO %^{*#}	11.06	3.53	6.4 - 17.7	12.18	2.84	8.9 - 16.5
EOS %^{*#}	1.65	0.68	0.3 - 3.1	1.56	0.44	0.9 - 2.1
BASO %[#]	0.57	0.73	0.2 - 2.93	0.63	0.40	0.1 - 1.2
HETERO $10^9/L^{*#}$	3.18	1.18	2.19 - 5.69	3.37	0.74	2.11 - 4.44
LYM $10^9/L^{*#}$	2.63	1.09	0.84 - 5.12	2.68	1.10	1.12 - 4.44
MONO $10^9/L^{*#}$	0.85	0.46	0.41 - 2.02	0.91	0.48	0.23 - 1.85
EOS $10^9/L^{*#}$	0.13	0.06	0.04 - 0.24	0.19	0.27	0.01 - 0.25
BASO $10^9/L^{*#}$	0.02	0.02	0.01 - 0.06	0.05	0.03	0.01 - 0.09
PLT K/$\mu L^{*#}$	418.07	42.34	366 - 480	469.36	40.55	397 - 524
MPV fL^{*#}	7.16	0.44	6.4 - 8.2	7.39	0.59	6.2 - 8.5
PDW fL^{*#}	7.05	0.72	6.2 - 8.8	7.25	0.61	6.2 - 8.4
PCT %^{*#}	0.31	0.06	0.25 - 0.42	0.37	0.16	0.15 - 0.48

* - $p < 0.05$ between sex-specific values

- $p < 0.05$ between age-specific values

ders during the entire period of the study.

Discerning the differences between females and males is important when interpreting clinical data. For this reason, we considered it appropriate that, in addition to the age-specific reference range, we investigate the differences associated with sex for both haematological and biochemical parameters. Haematological parameters offer information about inflammation, necrosis, infections, and stress factors (6). Compared to adults, 10 weeks rabbits had a lower HGB values, but the MCV and MCH values were higher than those from adults. Male rabbits had a higher RBC values and a slightly increased concentration of HGB compared to female, results supported by the results

obtained by Moore et al. (7). Unlike Jeklova et al. (3), we found that for the rabbits held by our animal facility, there are significant statistically differences between youth after weaning, young-adult, and adult for the majority of the haematological parameters.

The WBC and PLT values increased by the age, males having higher values than females for WBC but lower for PLT.

Female guinea pigs had a higher number of RBC, WBC, and PLT than males. The number of reticulocytes grew constantly throughout the period 9–48 weeks. The 36–48 weeks interval represented the period in which most of the haematological parameters reach their peaks and stabilize.

Table 5
Haematological parameters measured in young-adult Dunkin Hartley guinea pigs

Parameter	24 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
RBC $10^{12}/L^{*#}$	4.51	0.46	3.54 - 5.21	4.66	0.54	3.91 - 5.45
HCT %^{*#}	33.67	4.21	23.5 - 39.5	35.24	1.69	32.6 - 37.5
HGB g/dL^{*#}	11.58	1.30	8.3 - 13.4	13.64	2.13	11.9 - 19.4
MCV fL	74.48	3.37	66.4 - 78.1	74.87	2.77	69.2 - 79.3
MCH pg^{*#}	25.64	0.84	23.4 - 26.3	27.20	1.49	25 - 30.1
MCHC g/dL	34.44	0.97	32.9 - 35.6	35.59	2.38	29.9 - 38.9
RDW %^{*#}	13.77	1.87	12.2 - 17.7	14.28	1.43	12.1 - 16.9
RETIC %^{*#}	1.92	1.01	0.7 - 4.6	1.88	0.67	0.8 - 3.6
RETIC K/$\mu L^{*#}$	86.75	18.81	62.3 - 116.8	95.91	14.35	67.8 - 121.9
WBC $10^9/L$	7.50	4.29	3.22 - 13.17	7.94	2.27	4.55 - 13.01
HETERO%^{*#}	35.26	12.32	23.5 - 45.1	45.00	9.35	33.7 - 52.3
LYM %^{*#}	56.41	12.75	41 - 80.3	61.97	10.91	45.9 - 82.4
MONO %^{*#}	10.44	3.27	5.6 - 18.6	11.17	1.67	8.5 - 14.5
EOS %^{*#}	1.39	0.69	0.3 - 2.7	0.86	0.29	0.3 - 1.3
BASO %[#]	1.09	1.78	0.2 - 2.1	0.61	0.31	0.1 - 1.1
HETERO $10^9/L^{*#}$	2.25	1.38	0.49 - 4.49	1.65	0.70	0.87 - 2.67
LYM $10^9/L^{*#}$	3.89	2.09	1.16 - 6.99	4.25	1.51	1.65 - 6.56
MONO $10^9/L^{*#}$	0.71	0.48	0.38 - 1.98	0.77	0.17	0.55 - 1.12
EOS $10^9/L^{*#}$	0.13	0.10	0.03 - 0.35	0.16	0.13	0.01 - 0.43
BASO $10^9/L^{*#}$	0.11	0.17	0.01 - 0.47	0.17	0.12	0.02 - 0.43
PLT K/$\mu L^{*#}$	419.07	66.28	333 - 533	496.07	55.64	398 - 589
MPV fL^{*#}	7.54	0.90	6.8 - 9.4	8.22	0.86	6.8 - 10.2
PDW fL^{*#}	7.49	1.20	6.4 - 10.2	7.90	0.89	6.9 - 9.3
PCT %^{*#}	0.30	0.15	0.12 - 0.65	0.69	0.19	0.34 - 0.98

* - $p < 0.05$ between sex-specific values

- $p < 0.05$ between age-specific values

Analysis of plasma biochemical parameters provides important information in visceral organs damage in rabbit, especially for liver and kidney (4). In New Zealand White rabbits, the AST, GGT, CHOL, and TRIG were higher in males than females, while for GLU, CREA, and ALT, females registered higher values. All the results presented the same tendency regardless the gender. The AST and ALT activity was close to the level reported by Evans (1). Compared to the values reported by Ozkan (9), the GLU plasma values determined into this study were higher, for the CREA were the same and for the GGT were lower. The determined CHOL registers higher values and TRIG lower than

those reported by Suckow (11). Female guinea pigs showed higher levels of CREA, ALT, UREA, PHOS, CHOL, CA, TP, ALB, and ALKP than males, and lower levels of GLU. AMYL and GLOB highlighted similar values for both genders. In guinea pigs, the serum values of GLU, TP, and ALB were slightly lower than the one reported by Genzer (2). Bone growth in young animals produced elevated ALP level, reason for which we observed a decrease of serum level in this parameter by age (11). The concentration level of CA, ALT, and PHOS determined in this study were like the ones reported by Rabe (10), while the CREA level was higher and the GLOB level lower.

Table 6

Haematological parameters measured in 36 weeks adults Dunkin Hartley guinea pigs

Parameter	36 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
RBC $10^{12}/L^{*#}$	4.58	0.52	3.22 - 5.34	5.10	0.74	3.89 - 6.32
HCT %^{*#}	34.42	4.22	25.7 - 39.7	45.88	6.05	33.4 - 55.4
HGB g/dL^{*#}	12.62	0.81	10.8 - 13.9	13.69	0.95	11.7 - 14.9
MCV fL	76.35	2.64	69.3 - 79.6	78.95	2.17	73.2 - 82.1
MCH pg^{*#}	26.96	1.33	24.7 - 29.8	30.16	1.55	28.7 - 34.2
MCHC g/dL	33.97	2.48	25.8 - 35.9	36.17	3.12	31.4 - 40.9
RDW %^{*#}	14.58	1.16	12.9 - 17.1	15.98	0.84	15.3 - 17.2
RETIC %^{*#}	2.02	0.49	1.4 - 2.8	3.08	0.63	2.3 - 4.1
RETIC K/$\mu L^{*#}$	95.97	16.90	66.8 - 119.7	122.71	9.49	109.4 - 143.2
WBC $10^9/L$	6.64	3.33	2.45 - 11.98	6.82	3.20	3.98 - 11.44
HETERO%^{*#}	37.89	11.23	21.8 - 66.9	41.08	5.07	29.5 - 48.3
LYM %^{*#}	60.79	10.27	45.9 - 77.2	71.42	10.67	49.8 - 87.8
MONO %^{*#}	9.70	2.23	4.9 - 12.8	12.58	1.47	10.6 - 16.5
EOS %^{*#}	0.99	0.28	0.4 - 1.7	0.86	0.30	0.3 - 1.2
BASO %[#]	1.57	1.03	0.5 - 4.5	1.62	0.99	0.3 - 3.4
HETERO $10^9/L^{#}$	2.32	1.38	0.49 - 4.74	2.30	1.11	0.87 - 4.87
LYM $10^9/L^{#}$	3.78	1.07	2.23 - 5.44	3.78	0.97	1.87 - 5.32
MONO $10^9/L^{#}$	0.64	0.22	0.19 - 1.02	1.06	0.35	0.65 - 1.67
EOS $10^9/L^{*#}$	0.17	0.06	0.05 - 0.28	0.24	0.14	0.07 - 0.45
BASO $10^9/L^{*#}$	0.08	0.05	0.03 - 0.18	0.11	0.07	0.01 - 0.23
PLT K/$\mu L^{*#}$	424.93	70.47	322 - 538	492.79	53.59	419 - 654
MPV fL^{*#}	7.10	1.45	4.6 - 8.8	8.10	1.07	6.5 - 9.5
PDW fL^{*#}	7.53	1.20	5.8 - 9.5	8.61	0.67	6.9 - 9.4
PCT %^{*#}	0.59	0.19	0.32 - 0.88	0.76	0.13	0.55 - 0.94

* - p<0.05 between sex-specific values

- p<0.05 between age-specific values

CONCLUSIONS

For both rabbits and guinea pigs, regardless the gender, the parameters of interest showed modification according to postnatal development, which reach the maximum level in young-adult period and after week 20-22 they tend to stabilize. Hence the need for this study to provide our customers, researchers, with a database that allows them to reduce variability in research and reduce the number of procedures performed on animals used for scientific purposes, thus contributing to the implementation of 3R's principles in experimentation on animals. We consider that this data

can also be used as a normal reference range for pets' rabbits and guinea pigs.

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Table 7

Haematological parameters measured in 48 weeks adults Dunkin Hartley guinea pigs

Parameter	48 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
RBC 10¹²/L^{*#}	4.67	0.65	3.22 - 5.87	5.31	0.85	3.67 - 6.76
HCT %^{*#}	36.20	2.56	30.2 - 38.9	43.92	5.34	35.9 - 54.7
HGB g/dL^{*#}	11.70	1.21	9.8 - 13.2	14.61	2.00	10.9 - 17.8
MCV fL	74.77	3.93	67.5 - 78.9	77.14	3.62	68.5 - 81.4
MCH pg^{*#}	25.76	0.78	24.8 - 27.3	29.29	1.95	26.9 - 33.2
MCHC g/dL	35.31	1.60	34.1 - 38.4	37.38	1.67	33.9 - 40.2
RDW %^{*#}	14.69	1.33	13.1 - 17.6	18.69	2.51	14.3 - 23.6
RETIC %^{*#}	2.05	0.55	1.3 - 2.6	3.43	0.96	1.5 - 5.4
RETIC K/μL^{*#}	88.96	14.94	65.7 - 110.1	96.15	16.26	68.9 - 117.3
WBC 10⁹/L	6.67	2.09	3.87 - 11.1	8.32	1.08	6.54 - 9.87
HETERO%^{*#}	49.09	11.77	27.9 - 66.1	59.31	8.82	46.7 - 72.6
LYM %^{*#}	56.03	9.96	41.3 - 75.3	62.79	8.66	48.9 - 79.8
MONO %^{*#}	11.79	2.32	9.4 - 15.7	14.86	3.33	9.3 - 18.9
EOS %^{*#}	1.78	0.30	1.2 - 2.2	1.62	0.47	0.9 - 2.3
BASO %[#]	1.69	0.95	0.5 - 2.9	1.98	0.85	0.9 - 2.8
HETERO 10⁹/L[#]	2.67	0.94	0.87 - 4.23	3.66	0.79	2.4 - 4.76
LYM 10⁹/L[#]	3.93	1.80	1.86 - 6.87	3.81	1.45	2.15 - 6.54
MONO 10⁹/L[#]	1.26	0.76	0.51 - 1.87	1.39	0.52	0.54 - 1.95
EOS 10⁹/L^{*#}	0.24	0.23	0.06 - 0.81	0.66	0.55	0.07 - 1.21
BASO 10⁹/L^{*#}	0.54	0.36	0.02 - 0.99	1.48	0.48	0.76 - 1.98
PLT K/μL^{*#}	434.50	59.16	352 - 538	577.50	79.75	488 - 679
MPV fL^{*#}	7.99	0.66	6.8 - 9.1	8.59	0.93	6.7 - 9.8
PDW fL^{*#}	7.77	1.08	6.4 - 9.2	9.61	1.01	8.7 - 11.6
PCT %^{*#}	0.56	0.21	0.18 - 0.87	0.87	0.18	0.59 - 1.23

* - p<0.05 between sex-specific values

- p<0.05 between age-specific values

Table 8

Biochemical parameters measured in youth after weaning New Zealand White rabbits

Parameter	10 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
GLU # mmol/L	8.14	0.46	7.4 - 9.19	8.33	0.71	7.23 - 9.55
CREA # μmol/L	54.73	16.43	35.00 - 79.00	47.60	14.34	26.00 - 70.00
ALT U/L[#]	36.47	16.78	22.00 - 62.00	59.60	24.23	37.00 - 95.00
AST U/L^{*#}	21.93	14.81	12.00 - 55.00	17.80	5.35	12.00 - 34.00
GGT U/L	3.30	1.64	1.00 - 6.00	3.70	1.83	2.00 - 6.00
CHOL # mmol/L	3.16	1.36	2.07 - 6.38	2.64	1.13	1.69 - 5.82
TRIG ^{*#} mmol/L	1.04	0.59	0.58 - 2.26	0.72	0.29	0.33 - 1.48

* - p<0.05 between sex-specific values

- p<0.05 between age-specific values

Table 9

Biochemical parameters measured in young-adult New Zealand White rabbits

Parameter	20 weeks					
	Male			Female		
	Mean ±	SD	Range	Mean ±	SD	Range
GLU # mmol/L	8.19	0.64	7.36 - 9.19	8.21	0.43	7.2 - 8.89
CREA # μmol/L	90.33	5.57	86 - 103	94.00	14.39	66 - 120
ALT U/L#	91.43	25.01	62 - 132	111.87	22.22	64 - 137
AST U/L*#	31.90	5.87	22.00 - 39.00	24.13	5.46	14.00 - 32.00
GGT U/L	4.20	1.69	2.0 - 7.0	3.90	0.88	3.0 - 5.0
CHOL # mmol/L	2.09	0.49	1.24 - 3.09	1.55	0.27	1.07 - 1.94
TRIG *# mmol/L	0.65	0.15	0.45 - 0.87	0.39	0.06	0.3 - 0.52

* - p<0.05 between sex-specific values

- p<0.05 between age-specific values

Table 10

Biochemical parameters measured in adults New Zealand White rabbits

Parameter	48 weeks					
	Male			Female		
	Mean ±	SD	Range	Mean ±	SD	Range
GLU # mmol/L	7.73	0.52	7.37 - 8.28	7.80	0.50	7.2 - 8.58
CREA # μmol/L	110.47	12.30	90 - 130	128.47	9.72	110 - 144
ALT U/L#	102.93	25.41	73 - 142	81.73	29.21	42 - 130
AST U/L*#	30.53	9.76	12.00 - 46.00	31.60	4.39	28 - 43
GGT U/L	3.10	1.52	1.0 - 5.0	2.80	0.79	1.0 - 4.0
CHOL # mmol/L	0.89	0.53	0.5 - 1.94	1.75	0.68	0.69 - 2.95
TRIG *# mmol/L	0.53	0.24	0.2 - 0.8	0.63	0.25	0.25 - 1.03

* - p<0.05 between sex-specific values

- p<0.05 between age-specific values

Table 11

Biochemical parameters measured in youth after weaning Dunkin Hartley guinea pigs

Parameter	9 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
GLU mmol/L*	8.56	1.03	6.37 - 10.37	8.86	0.77	7.56 - 10.11
CREA μmol/L#	26.64	8.72	13.00 - 38.00	26.43	3.16	21.00 - 32.00
ALT U/L*#	86.07	12.92	64.00 - 108.00	85.21	10.84	59.00 - 99.00
UREA mmol/L*	6.90	1.25	5.30 - 9.20	8.34	0.84	7.10 - 9.80
PHOS mmol/L*#	1.70	0.30	1.23 - 2.23	1.78	0.25	1.44 - 2.20
CHOL mmol/L#	0.77	0.37	0.23 - 1.44	0.82	0.21	0.34 - 1.06
CA mmol/L	2.59	0.39	1.97 - 3.01	2.58	0.29	2.05 - 2.98
TP g/L#	46.36	4.24	39.00 - 54.00	47.43	2.56	43.00 - 51.00
ALB g/L*#	20.29	3.52	15.00 - 27.00	23.64	4.27	16.00 - 29.00
GLOB g/L#	27.86	4.38	22.00 - 35.00	27.21	4.64	20.00 - 34.00
ALKP U/L	208.79	57.22	142.0 - 298.0	232.29	52.62	156.0 - 301.0
AMYL U/L	900.57	125.07	732.0 - 1087.0	884.36	98.61	659.0 - 1003.0

* - p<0.05 between sex-specific values

- p<0.05 between age-specific values

Table 12

Biochemical parameters measured in young-adult Dunkin Hartley guinea pigs

Parameter	24 weeks					
	Male			Female		
	Mean $\pm$	SD	Range	Mean $\pm$	SD	Range
GLU mmol/L*	8.99	1.26	6.58 - 10.45	9.40	0.94	8.11 - 11.3
CREA μ mol/L#	23.64	8.72	13.00 - 39.00	23.21	5.94	11.00 - 33.00
ALT U/L*#	95.50	15.42	66.00 - 118.00	99.29	12.29	86.00 - 121.00
UREA mmol/L*	6.56	1.71	4.10 - 9.60	7.59	1.82	4.50 - 9.80
PHOS mmol/L*#	1.82	0.22	1.38 - 2.07	2.13	0.45	1.66 - 2.98
CHOL mmol/L#	0.94	0.29	0.36 - 1.46	0.93	0.19	0.54 - 1.21
CA mmol/L	2.61	0.21	2.06 - 2.85	2.65	0.42	1.76 - 3.11
TP g/L#	46.21	5.41	37.00 - 58.00	46.86	7.03	37.00 - 57.00
ALB g/L*#	18.71	2.23	16.00 - 25.00	23.50	4.18	15.00 - 29.00
GLOB g/L#	27.43	3.86	20.00 - 34.00	26.36	4.52	19.00 - 34.00
ALKP U/L	185.29	78.28	81.00 - 303.00	190.93	75.13	92.00 - 302.0
AMYL U/L	900.07	141.40	690.0 - 1099.0	918.57	116.14	745.0 - 1111.0

* - $p < 0.05$ between sex-specific values# - $p < 0.05$ between age-specific values

Table 13

Biochemical parameters measured in 36 weeks adults Dunkin Hartley guinea pigs

Parameter	36 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
GLU mmol/L*	8.82	1.33	6.90 - 11.10	9.92	0.70	8.76 - 11.09
CREA μ mol/L#	22.59	6.95	13.20 - 33.00	24.89	3.99	18.09 - 30.05
ALT U/L*#	96.21	11.31	76.00 - 123.00	99.71	6.72	87.00 - 111.00
UREA mmol/L*	6.64	1.55	4.50 - 9.50	7.35	1.53	4.30 - 9.20
PHOS mmol/L*#	1.82	0.18	1.44 - 2.09	1.96	0.33	1.65 - 2.98
CHOL mmol/L#	0.57	0.29	0.21 - 1.36	0.82	0.25	0.37 - 1.11
CA mmol/L	2.51	0.31	1.99 - 2.89	2.52	0.38	1.87 - 3.11
TP g/L#	49.21	4.66	44.00 - 58.00	51.00	3.35	45.00 - 56.00
ALB g/L*#	19.14	2.77	16.00 - 25.00	20.07	4.07	15.00 - 28.00
GLOB g/L#	27.79	4.02	21.00 - 34.00	33.29	4.98	23.00 - 41.00
ALKP U/L	205.79	68.33	99.0 - 290.0	208.57	56.39	111.0 - 296.0
AMYL U/L	907.71	130.97	701.0 - 1098.0	908.71	102.70	721.0 - 1011.0

* - $p < 0.05$ between sex-specific values# - $p < 0.05$ between age-specific values

Table 14

Biochemical parameters measured in 48 weeks adults Dunkin Hartley guinea pigs

Parameter	48 weeks					
	Male			Female		
	Mean	SD	Range	Mean	SD	Range
GLU mmol/L*	8.81	1.07	6.90 - 10.77	9.57	0.61	7.89 - 10.25
CREA μ mol/L#	26.14	5.02	17.00 - 32.00	30.50	5.16	19.00 - 39.00
ALT U/L*#	96.36	11.95	82.00 - 121.00	105.64	17.60	79.00 - 134.00
UREA mmol/L*	6.99	1.81	4.23 - 9.40	8.06	1.34	5.76 - 10.00
PHOS mmol/L*#	1.85	0.16	1.56 - 2.11	1.98	0.25	1.45 - 2.32
CHOL mmol/L#	0.71	0.21	0.32 - 1.11	0.81	0.19	0.46 - 1.21
CA mmol/L	2.57	0.32	2.01 - 2.98	2.69	0.28	2.11 - 3.09
TP g/L#	46.00	5.20	36.00 - 56.00	47.29	4.48	39.00 - 56.00
ALB g/L*#	17.64	1.95	13.00 - 21.00	18.79	5.91	11.00 - 32.00
GLOB g/L#	27.79	3.19	21.00 - 32.00	26.86	3.68	21.00 - 33.00
ALKP U/L	182.36	45.77	99.00 - 267.00	192.86	43.78	139.0 - 298.0
AMYL U/L	895.29	92.35	690.0 - 1009.0	882.21	85.78	730.0 - 1023.0

* - $p < 0.05$ between sex-specific values# - $p < 0.05$ between age-specific values

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