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Reference Values for Some Physiological and Biochemical Parameters in Rats at Puberty

[1]Özgür Kaynar, [2]Armagan Hayirli, [3]Ahmet Yildiz, [4]Zafer Okumus, [5]Fikrullah Kisa,
[6]Emel Banu Büyükünal Bal, [7]Harun Polat and [8]Fuat Erdem
[1]Department of Biochemistry, [2]Department of Animal Nutrition and Nutritional Disorders,
[3]Department of Animal Husbandry, [4]Department of Surgery,
[5]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine,
Atatürk University, Erzurum 25700, Turkey
[6]Department of Biology, Faculty of Science and Letter, Sütçü Imam University,
Kahramanmaras, 46100, Turkey
[7]Department of Biochemistry, [8]Department of Hematology,
Faculty of Medicine, Atatürk University, Erzurum 25240, Turkey

Abstract: In this experiment, 18 female and 18 male Sprague Dawley rats were utilized to evaluate physiological and biochemical traits. Rats were fed to ad libitum consumption of the conventional diet and housed in cages separately by gender at density of 160 cm^2 per rat from 3 to 10 weeks of age. At the end of the experiment, body weights were measured for growth, blood samples were collected for metabolic profile and hemogram and then rats were euthansized for organ development. After log transformation of blood cell data, all response variables were subjected to t-test for gender comparison and normality and correlation tests for generation of reference values and determination of autocorrelations. Growth and organ development were greatly affected by gender. However, gender effect on weights of organs altered when weights were expressed in proportion to body weight. Differences in blood chemistry by gender were in agreements with those in weights of organs. White blood cell counts were not different. Erythrocyte and related hemogram variables differed by gender. In conclusion, variations and differences in biochemical and physiological traits by gender should be taken into consideration for future investigations coping with medical and nutritional issues.

Key words: Rat, reference value, organ, blood chemistry, hemogram

INTRODUCTION

Laboratory animals are frequently used in experimental studies to contribute scientific knowledge in various research areas. Results generated from laboratory animals are often extrapolated to address medical Knox *et al.*[1] and nutritional Even *et al.*[2]; Ferrell and Koong[3] issues. Provisions of a comfort area to allow normal postural and behavioral adjustment are important for the animal and the outcome of the animal experiments Les [4]. Several factors in the animal experimentation may cause deviations in response variables, which may broadly include husbandry practices (e.g., cage design, lightening program, air quality, bedding and furnishing and animal handling), feeding management (e.g., nutrient and physical composition of diet and amount and frequency of feeding and watering), animal factor (e.g., age, strain, health status, comfort-immune system and gender) and testing materials and sampling procedure (e.g., drugs, surgery and anesthesia). Among housing factors, cage intensity, for example, may influence well being and measurable response variables such as feed intake and growth Gamallo *et al.*[5] directly by limiting physical activity Rock *et al.* [6] or indirectly by increasing exposure to accumulated gaseous compounds Serrano[7]. Weights of liver, kidney and heart Muraoka *et al.*[8] Armario *et al.*[9] and consequently blood chemistry may also be affected in response to crowding. Hemogram variables may characterize stress and immune potency Peng *et al.*[11]; Salvin *et al.*[12] especially when animals are housed at high stocking density Yildiz *et al.*[12] or large population size Yildiz *et al.*[13]. In study of making

Corresponding Author: Armagan Hayirli, Department of Animal Nutrition and Nutritional Disorder, Faculty of Veterinary Medicine, Ataturk University, Ilica-Erzurm 25700, Turky

inferences from the animal experimentation, factors affecting animal welfare and comfort should therefore be excluded Baumans[14]; Lahti[15]; Monteiro *et al.*[16] Woolverton *et al.*[17]. Thus, elaboration of variability in response variables under physiological conditions is essential. This experiment was conducted to determine physiological and biochemical variables in rat at puberty grown under normal conditions and present variability in response variables to set future reference values.

MATERIALS AND METHODS

Animals, diet and management: Eighteen female and 18 male Sprague Dawley rats weighing an average of 47.6±4.1 and 45.1±3.6 g (mean±standard deviation) at three weeks of age were obtained from Atatürk University Experimental Animal Teaching and Research Center (ATADEM Breeding Facility). The protocol under this experiment was reviewed and approved by the Research Animal Ethic Committee of Atatürk University. Animals were confirmed to be free of several pathogens including *Salmonella* sp., *Shigellae* sp., *Leptospira* sp., *Streptobacillus moniliformis*, *Spirillum minus*, *Mycobacterium tuberculosis*, *Pastorella pseudotuberculosis* and *Sarcoptes scapiei*. From weaning (week 3) to 10 weeks of age, rats were housed in cages in the dimension of 24×40×20 cm (width×depth×height) to provide a 160 cm^2 surface area per rat (6 rats per cage). Caging was separate for females and males.

The conventional pellet diet fed to rats was formulated to meet nutrient requirements for growing rats NRC[19]. The diet was consisted of 38.5% corn, 10.7% rye, 4.0% wheat bran, 35.0% soybean meal, 4.3% sunflower meal, 1.5% fish meal, 2.8% sunflower oil, 1.0% limestone, 0.35% salt, 0.25% vitamin-mineral premix, 0.15% methionine and 0.5% sodium bicarbonate. The diet contained 23.6% crude protein, 3.4% crude fiber, 5.7% fat and 7.5% ash.

Feed was offered *ad libitum* and water was always available via glass bottles with rubber nipples. Room temperature and humidity were 20-24°C and 58%. Rooms were set to 12L: 12D cycle during the experimental period CCAC[18]. Cages were cleaned twice weekly.

Sample collection and analytical procedure: After fasting for 24 h at the end of the experiment, body weights were measured and blood samples were taken from the heart. Blood samples were drained into additive-free vacutainers (BD vacutainer SST™, BD Vacutainer Systems Preanalytical Solutions, Belliver Industrial Estate, London, UK) for blood chemistry. Following separation of serum at 3000 g for 15 min at 20°C, aliquots were kept at -20°C until analyses for glucose, total protein, albumin, creatine, triglyceride, cholesterol, very low-density lipoprotein, alkaline phosphatase, Ca and P using spectrophotometric methods with commercial kits (DDS, Diasis Diagnostic Systems Co., Istanbul, Turkey).

Blood samples were also drained into vacutainers with K_3-EDTA for hemogram. Within one hour postsampling, whole blood samples were subjected to flow-cytochemistry (Coulter STKS™ Hematology Flow Cytometer, Beckman Coulter Co., Miami, FL, USA) for neutrophil, lymphocyte, monocyte, eosinphile, basophile, erythrocyte and platelet counts, hemoglobin concentration and hematocrit value. The ratio of neutrophil to lymphocyte was calculated as an indicator for stress-related parameter. Mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, mean platelet volume, plateletcrit and platelet distribution width were other hematological variables generated from hemogram.

Finally, rats were sedated by intraperitoneal injection of xylazine hydrochloride (5 mg kg^{-1}) (Rompun, Bayer, Istanbul, Turkey) and then anesthetized by 2% sevoflurane (Sevorane, Abbott Laboratories, Istanbul, Turkey). Euthanasia was performed by exsanguinations under anesthesia. Internal organs were excised, emptied (e.g., stomach), blotted and then weighed.

Statistics: Blood cell data were normalized by log-transformation before statistical analyses SAS[20]. After sorting the data by gender, the MEANS Procedure was employed to determine mean, standard deviation, standard error, variance and coefficient variation values and the UNIVARIATE Procedure was used to elucidate distribution (mode, skewness, kurtosis, range, median and percentiles). Differences between genders and 95% confidence interval (lower and upper limits) were attained using the Student's t-test. Moreover, correlations among response variables were generated using the CORR Procedure after pooling all data. Statistical inferences were made to be tendency, significant and highly significant when probability values were within 0.05-0.10 and 0.01-0.05 and were less than 0.001, respectively.

RESULTS AND DISCUSSION

Statistical approach: Data subjected to posteriori statistical analyses in the present experiment were complied from the control groups of previous experiments, which were published elsewhere Yildiz *et al.*[12]. Thus, the sample size can be considered as low for proposing reference values. However, the outcome of the present

study could be comparable to those generated from larger databases. In fact, utilization of a large number of animals in a single experiment under the current animal ethics issues and welfare concerns is unlikely Schweisthal *et al.*[21].

In consideration with sample size, descriptive statistics is a useful method to attain reference values Berndtson[22]. Outcomes of descriptive statistics are not limited those presented in the present study. For instance, standard deviation was not presented, because it can be estimated from variance, standard error and 95% confidence interval as long as sample size is known. It is imperative to report the variability indicators such as variance and coefficient variation. The nature of data distribution could be explicated by presenting the mean, median, mode, skewness and kurtosis values. Of these, median indicates the value in the middle; mode refers to value that is the most frequently repeated or observed; skewness refers to deviation from the normal distribution; and kurtosis indicates steepness of the normal distribution curve. Variability within the percentiles could also be important Neter *et al.*[23].

In addition to percentile, maximal, minimal and median values were also presented in quantiles. There can be still outliers when only maximal and minimal values (range) are presented. Following the Student's t-test, indicating frequency of data within mean plus/minus three standard deviation (95% confidence interval lower and upper limits) provides the optimum range for a response variable. Response variables that are autocorrelated or have multicolinearity should be excluded for modeling studies Even *et al.*[2] Hayirli *et al.*[24] Although correlation does not reflect cause and effect relationship, it has a merit for better understanding of the nature of relationship Draper and Smith[25]. Neter *et al.*[23].

Growth and organ development: In both sexes, there was no case of death during the experimental period. From weaning to puberty, growth rate for females was slower than for males (2.7 vs. 3.3 g d^{-1}), which was reflected as lower body weight at the end of the experiment (178.2 vs. 206.5 g; Table 1). Heart, stomach and spleen weights and large intestine length were not different between genders. Weights of lungs, liver and kidneys and length of small intestine for males were greater than for females. Adrenal glands for females were 1.7-fold heavier than those for males. Differences in weights of organs may cause variability of feed intake, feed digestibility and utilization, as well as nutrient partitioning Ferrell and Koong[3] Also, gender effect on the weight of adrenal glands may explain differences in responsiveness to or tolerance the stressors that cause immune suppression. Nevertheless, basal metabolic rate of females and males can be interpreted better when organ weights are expressed as percentage of body weight Schweisthal *et al.*[21]. In this case, heart (0.42 vs. 0.38%), stomach (0.64 vs. 0.56%) and adrenal glands (0.02 vs. 0.01%) accounted for greater portion in females than in males and proportions of lungs (0.53%), liver (4.51%), kidneys (0.72%) and spleen (0.22%) did not differ. Expectedly, ovaries accounted for a small portion of body weight

Table 1: Gender differences in body weights and weights of internal organs of rats at age of puberty

							95% CI limits[1]				Quantiles				
Variable[2]	Sex[3]	LSM[4]	SE[5]	Var[6]	CV[7]	Mode	Lower	Upper	Skewness	Kurtosis	100% Max	75%	50% Median	25%	0% Min
Body	F	178.2	2.7	141.3	6.70	180.0	172.5	183.9	0.20	0.35	204.0	184.0	180.0	170.0	156.0
	M	206.5	3.7	253.3	7.70	196.0	198.9	214.2	0.35	-0.56	240.0	218.0	206.0	194.0	182.0
Heart	F	0.752	0.100	0.170	54.90	-	0.540	0.960	3.83	15.27	2.318	0.716	0.656	0.593	0.529
	M	0.788	0.019	0.007	10.80	-	0.750	0.830	2.09	6.46	1.071	0.826	0.774	0.730	0.682
Lungs*	F	0.968	0.028	0.015	12.50	-	0.910	1.030	0.46	-0.92	1.202	1.049	0.933	0.879	0.800
	M	1.067	0.039	0.029	15.80	-	0.990	1.150	1.11	0.85	1.482	1.145	1.041	0.935	0.864
Stomach	F	1.137	0.031	0.018	11.80	-	1.070	1.200	0.11	-0.79	1.397	1.247	1.157	1.003	0.903
	M	1.151	0.030	0.017	11.40	-	1.090	1.210	-1.26	1.19	1.309	1.235	1.185	1.092	0.826
Small	F	101.5	1.0	18.5	4.20	101.0	99.4	103.6	-0.41	0.47	110.0	104.0	102.0	99.5	93.0
intestine**	M	108.4	2.0	77.2	8.10	101.0	104.2	112.7	-0.07	-0.65	124.0	115.5	107.0	101.0	92.0
Large	F	18.32	0.58	6.34	13.70	19.00	17.10	19.53	0.51	0.25	24.00	19.00	18.00	16.00	14.00
intestine	M	18.87	0.47	4.27	11.00	18.00	17.87	19.86	-1.15	2.51	22.00	20.00	19.00	18.00	13.00
Liver*	F	8.35	0.18	0.59	9.20	-	7.98	8.72	-0.20	-0.44	9.84	8.87	8.47	7.59	6.82
	M	8.93	0.19	0.69	9.30	-	8.53	9.33	0.47	0.39	10.94	9.38	8.94	8.21	7.63
Kidneys**	F	1.300	0.023	0.010	7.70	1.259	1.250	1.350	0.58	-0.24	1.502	1.367	1.259	1.230	1.122
	M	1.480	0.032	0.020	9.50	-	1.410	1.550	-0.47	0.16	1.734	1.577	1.492	1.185	1.185
Spleen	F	0.423	0.031	0.018	31.50	-	0.360	0.490	2.73	7.12	0.860	0.400	0.380	0.358	0.334
	M	0.429	0.013	0.003	13.10	0.440	0.400	0.460	-0.30	-1.26	0.507	0.481	0.440	0.383	0.337
Adrenal	F	0.027	0.002	<0.000	27.10	0.023	0.020	0.030	-0.52	0.09	0.040	0.032	0.029	0.023	0.012
glands**	M	0.016	0.001	< 0.000	16.80	0.017	0.010	0.020	1.27	3.51	0.024	0.017	0.016	0.014	0.012
Ovaries/	F	0.110	0.004	< 0.000	15.40	0.097	0.100	0.120	0.11	-1.04	0.141	0.128	0.109	0.097	0.083
Testes**	M	2.617	0.035	0.023	5.80	-	2.540	2.690	-0.24	1.36	2.950	2.696	2.607	2.568	2.255

[1]CI = confidence interval.[2]Except for intestines (cm), other variables are in gram. Gender effect = *0.05 <P = 0.10; **0.01 <p = 0.05; ***p = 0.001. [3]F = female; M = male. Sample size was 18 for each gender at 10 weeks of age.[4]LSM = least square means.[5]SE = standard error.[6]Var = variance. [7]CV = coefficient variation

(0.06%), whereas testes constituted a considerable portion of body weight (1.27%).

Correlations between body weight and weights organs and among organs may be helpful to propose strategies to enhance health status and body condition in animals and humans. Expectedly, body weight was positively correlated with organs that occupy relatively large mass in the body Table 2. This may reflect the functional capacity, but not necessarily the functional efficiency of organs, to support large body size. For instance, animals that have greater body weight had longer small intestine (r = 0.58), which could increase the capacity of nutrient absorption to support maintenance Gamallo *et al.*[5]. Restrepo and Armario[26]. Liver weight was also positively correlated with intestinal length (r = -0.39), which may reflect roles of these organs in nutrient absorption and nutrient partitioning. Corticosterone is the major hormone released from the adrenal glands in response to discomforting conditions in laboratory animals Del Pup and Palmes[27]; Squires[28] and chickens Gross and Siege[29]. Post *et al.*[30] and modulates leukocyte function Stark *et al.*[31]. Negative relationship between body weight and adrenal glands' weight (r = -0.37) could partially explain why small animals tend to be more excitable and responsive to stressors than heavy animals Brown and Grunberg[32]. Finally, in regard to welfare and immune status, negative correlation between weights of spleen and adrenal glands (r = -0.30)confirms their interactive roles, -even under non-stressing environment Peng *et al.*[10]. Rabin *et al.*[33] Squires[28]. Moreover, adrenal glands' weight was inversely correlated with lymphocyte count (r = -0.27), but not neutrophil count (data not shown). Weight of spleen was not correlated with hemogram variables (e.g., neutrophil count, lymphocyte count and their ratio).

Metabolic profile: Table 3 summarizes blood chemistry. Females had greater serum total protein and cholesterol concentrations than males. There were no differences in concentrations of serum creatine, glucose, triglyceride and very low-density lipoprotein between genders.

Table 2: Pearson's correlation coefficients (*r*) among body weight and internal organs[1]

Variables[2]	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Body weight (1)	-0.09	0.40**	0.24	0.58***	0.19	0.75***	0.77***	0.30	*-0.37**
Heart (2)		0.13	-0.17	0.06	-0.22	-0.15	-0.01	-0.06	-0.04
Lungs (3)			0.18	0.18	0.19	0.29*	0.26	0.38*	*-0.25
Stomach (4)				0.25	0.35**	0.22	0.19	0.29	*0.14
Small intestine (5)					0.58***	0.39**	0.39**	-0.04	-0.26
Large intestine (6)						0.29*	0.12	-0.05	-0.18
Liver (7)							0.65***	0.38**	-0.27
Kidneys (8)								9*0.27	-0.30*
Spleen (9)									0.06
Adrenal glands (10)									-

[1]*0.05 < p = 0.10; **0.01 <p = 0.05;***p = 0.001. N = 36.[2]Except for intestines (cm), other variables are in gram

Table 3: Gender differences in blood chemistry of rats at age of puberty

							95% CI limits[1]				Quantiles				
Variable[2]	Sex[3]	LSM[4]	SE[5]	Var[6]	CV[7]	Mode	Lower	Upper	Skewness	Kurtosis	100% Max	75%	50% Median	25%	0% Min
Total protein	*F	5.86	0.11	0.24	8.30	5.50	5.63	6.10	0.32	-0.68	6.80	6.30	5.90	5.50	5.10
	M	5.59	0.07	0.10	5.60	5.40	5.44	5.74	0.30	-0.99	6.20	5.80	5.50	5.30	5.10
Albumin**	F	3.39	0.04	0.03	5.40	3.60	3.31	3.48	-0.09	-1.15	3.70	3.60	3.40	3.20	3.10
	M	3.21	0.03	0.02	4.50	3.20	3.14	3.28	-0.20	-1.18	3.40	3.30	3.20	3.10	3.00
Creatine	F	0.442	0.035	0.024	34.80	0.300	0.370	0.520	1.55	3.11	0.900	0.500	0.400	0.300	0.300
	M	0.375	0.039	0.028	44.90	0.200	0.290	0.460	0.91	-0.16	0.720	0.400	0.300	0.200	0.200
Glucose	F	222.1	7.1	946.7	13.90	212.0	207.3	236.9	1.39	4.19	316.0	231.0	218.0	210.0	173.0
	M	207.0	6.5	814.2	13.80	-	193.3	220.8	0.53	0.13	269.0	226.0	204.0	189.0	157.0
Triglyceride	F	57.04	6.36	767.8	48.60	-	43.68	70.40	0.86	0.58	117.00	72.00	46.30	40.00	9.36
	M	49.69	5.05	485.2	44.30	-	39.08	60.31	-0.38	-0.83	82.40	63.50	58.30	26.20	5.30
Cholesterol**	F	57.87	1.36	35.1	10.20	54.70	55.01	60.72	0.66	1.05	72.50	60.40	57.20	54.70	47.60
	M	50.08	1.53	44.41	13.30	-	46.87	53.29	0.34	-0.90	63.90	55.50	47.90	44.20	40.80
Very low	F	17.21	1.18	26.36	29.80	16.10	14.73	19.68	-0.19	0.07	26.50	21.00	17.10	14.30	6.80
-density	M	16.45	0.71	9.46	18.70	12.50	14.96	17.93	0.20	-0.66	22.60	19.50	15.80	14.30	11.20
Calcium	F	11.44	0.06	0.06	2.20	11.20	11.32	11.56	0.56	-0.24	12.00	11.60	11.40	11.20	11.10
	M	11.39	0.08	0.14	3.20	11.20	11.22	11.57	1.31	1.79	12.40	11.60	11.30	11.10	11.00
Phosphorus	F	7.27	0.23	1.01	13.80	7.70	6.78	7.75	-0.10	-0.42	9.30	8.00	7.40	6.50	5.50
	M	7.48	0.24	1.13	14.20	6.60	6.97	8.00	0.10	-1.23	9.40	8.50	7.40	6.60	5.80
Alkaline	F	303.7	15.3	4423.3	21.90	-	271.7	335.8	-0.02	-1.37	407.0	372.0	305.0	248.0	205.0
Phosphatase	M	462.3	24.2	11149.8	22.80	428.0	411.4	513.2	0.20	-0.82	663.0	530.0	434.0	375.0	298.0

[1]CI = confidence interval.[2]Except for alkaline phosphates (U L^{-1}), other variables are in mg dL^{-1}. Gender effect = *0.05 < p = 0.10; **0.01 <p = 0.05;***p = 0.001.[3]F = female; M = male. Sample size was 18 for each gender at 10 weeks of age.[4]LSM = least square means.[5]SE = standard error.[6]Var = variance. [4]CV = coefficient variation

Table 4: Pearson's correlation coefficients (r) among blood metabolites and alkaline phosphatase level[1]

Variables[2]	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Total protein (1)	0.82***	0.31*	-0.08	0.30*	0.53***	0.22	0.18	-0.12	-0.20
Albumin (2)		0.26	-0.02	0.33**	0.39**	0.27*	0.02	-0.12	-0.23
Creatine (3)			0.18	-0.11	0.25**	-0.32**	-0.13	0.07	-0.10
Glucose (4)				0.05	-0.20	0.13	0.29*	0.08	-0.26
Triglyceride (5)					0.22	0.64***	0.20	-0.12	-0.40**
Cholesterol (6)						-0.09	0.21	-0.08	-0.40**
VLDL (7)							-0.13	-0.25	-0.41**
Calcium (8)								0.52***	-0.05
Phosphorus (9)									0.11
ALP (10)									-

[1]*0.05<p≤0.01; **0.10;<p≤0.05; **p≤0.001. N = 36.[2] Except for ALP (U L^{-1}), other variables are in mg dL^{-1}. ALP = alkaline phosphatase; VLDL = very low-density lipoprotein

Interestingly, despite lacking differences in concentrations of calcium and phosphorus, males had 1.52-fold greater alkaline phosphatase concentration than females. As can be seen, organ weights as proportional to body weight, but not organ masses, seem to be related to blood profile as gender effects diminished Table 1. It was reported that males had greater ALP activity and glucose and P concentrations and lower albumin and free fatty acid concentrations than females. There were also no differences in concentrations of Ca and cholesterol Tsuchiya *et al.*[34]. Uribe *et al.*[35]. Despite small sampling size, blood chemistry values were within physiological range as reported elsewhere Kahn and Line[36]. Petterino and Argentino-Storino[37].

Total protein concentrations were positively correlated with concentrations of albumin (r = 0.82), creatine (r = 0.31), triglyceride (r = 0.30) and cholesterol (r = 0.53) Table 4. There were similar relationships between albumin and lipid metabolites. Inverse correlations between concentrations of creatine and very low-density lipoprotein (r = -0.32) and between concentrations of very low-density lipoprotein and alkaline phosphatase (r = -0.41) could be linked to the etiology of obesity (hyperlipidemia), aging and bone disorders (e.g., osteoporosis). Positive correlation between triglyceride and cholesterol concentrations was expected (r = 0.64). Alkaline phosphotase plays role in calcium and phosphorus homeostasis. However, this study did not confirm any correlation.

Hemogram: As can be seen, lacking differences between genders and low number of leukocytes confirmed that animals were infection-free Table 5. In general, the number of neutrophil, monocyte and eosinophil increases in the case of bacterial and viral infections and parasitic infestations, respectively Deniz[38]. Although males had 1.31-fold higher lymphocyte count than females, the ratio of neutrophil to lymphocyte, a stress indicator, did not differ Gross and Siegel[28]. Post *et al.*[29]. suggesting that animals were housed in comfortable and stress-free environment and that males and females had different blastogenic capacity despite no differences in spleen weights as gram and percentage of body weight Stark *et al.*,[30] Greater erythrocyte count, hemoglobin concentration and hematocrit value for males than for females could be related to greater weights of organs (e.g., lung) Table 1 and hence greater hematopoetic capacity. Significantly greater platelet count for females than for males (1.14-fold) may not necessarily implicate any abnormality because it may even vary in response to the way of bleeding.In addition to quantitative evaluation of blood cellsAroch *et al.*[39], their qualitative characteristics can also contribute to the medicinal issues Petterino and Argentino-Storino[37]. Mean corpuscular volume did not differ between males and females. It indicates the average erythrocyte volume and used for diagnosis of anemias (e.g., microcytic, macrocytic and normocytic). Females hadgreater mean corpuscular hemoglobin value than males, but values were still within physiological range. It refers to the average of of hemoglobin/the erythrocyte count) and is used amount of hemoglobin in erythrocytes (the amount for differentiating anemias (e.g., normochromic, hypopochromic and hyperchromic). There was also significant difference between genders when hemoglobin concentration was expressed per unit erythrocyte (mean corpuscular hemoglobin concentration).This parameter (hemoglobin count/hematocrit value) is used as a part of standard in reporting hemogram. It is remarkably high in spherocytosis. It yields hypochromic in microcytic anemia or normochromic in macrocytic anemia because of larger cell size, though the hemoglobin amount or mean corpuscular hemoglobin is high, the concentration remains normal. Under normal condition, erythrocytes are in a standard size. Red cell distribution width (standard deviation of erythrocyte volume/average cell volume) is an important parameter to diagnose certain diseases in which erythrocyte size varies. Deviation in erythrocyte size for males was greater than for females, but both were still with in

Table 5: Gender differences in hemogram of rats at age of puberty

							95% CI limits[1]				Quantiles				
Variable[2]	Sex[3]	LSM[4]	SE[5]	Var[6]	CV[7]	Mode	Lower	Upper	Skewness	Kurtosis	100%Max	75%	50% Median	25%	0% Min
NEU	F	0.284	0.078	0.115	119.50	0.040	0.120	0.450	1.59	1.42	1.120	0.460	0.120	0.060	0 . 0 3 0
	M	0.539	0.165	0.517	133.40	0.070	0.190	0.890	1.75	1.98	2.330	0.600	0.190	0.070	0.030
LYM***	F	4.698	0.329	2.053	30.50	3.320	4.010	5.390	0.78	0.15	8.060	5.570	4.410	3.540	2.530
	M	6.171	0.356	2.404	25.10	-	5.420	6.920	0.16	-0.33	9.070	7.020	5.850	5.340	3.410
NEU:LYM	F	0.066	0.020	0.008	134.40	0.009	0.020	0.110	1.77	1.69	0.273	0.068	0.024	0.015	0.009
	M	0.085	0.025	0.011	125.20	0.106	0.030	0.140	1.70	2.05	0.356	0.106	0.044	0.012	0.005
MON	F	0.015	0.006	0.001	175.20	0.000	0.000	0.030	2.25	5.05	0.100	0.010	0.000	0.000	0.000
	M	0.011	0.002	< 0.000	79.20	0.010	0.010	0.020	0.34	-0.46	0.030	0.020	0.010	0.000	0.000
EOS	F	0.013	0.003	< 0.000	95.00	0.010	0.010	0.020	1.04	0.49	0.040	0.020	0.010	0.000	0.000
	M	0.010	0.003	< 0.000	115.50	0.010	0.000	0.020	2.42	7.95	0.050	0.010	0.010	0.000	0.000
BAS	F	0.206	0.075	0.107	158.50	0.000	0.050	0.360	1.51	1.10	1.040	0.380	0.010	0.000	0.000
	M	0.306	0.138	0.360	195.90	0.000	0.020	0.600	2.51	6.61	2.310	0.260	0.020	0.000	0.000
RBC***	F	7.286	0.088	0.146	5.20	-	7.100	7.470	-0.47	-0.66	7.814	7.569	7.357	7.028	6.570
	M	7.842	0.069	0.092	3.90	-	7.700	7.990	0.09	-0.88	8.335	8.093	7.778	7.660	7.337
PLT***	F	904.2	20.6	8036.2	9.90	-	861.0	947.4	0.70	1.68	1141.0	971.9	873.3	851.8	727.6
	M	790.7	22.9	9943.5	12.60	-	742.6	838.7	-0.34	-0.48	953.2	879.8	795.8	729.7	606.5
Hb*	F	13.77	0.18	0.63	5.80	-	13.38	14.15	0.04	-1.12	15.09	14.39	13.67	13.04	12.43
	M	14.16	0.14	0.38	4.40	13.76	13.86	14.45	-0.16	-0.69	15.13	14.74	14.13	13.76	12.95
HCT***	F	37.94	0.54	5.64	6.30	-	36.80	39.09	0.38	-0.62	42.52	40.04	37.30	36.01	34.11
	M	40.61	0.41	3.25	4.40	43.06	39.74	41.48	-0.35	-0.41	43.06	41.76	40.57	39.48	36.93
MCV	F	52.00	0.25	1.22	2.10	51.30	51.47	52.53	0.55	0.01	54.40	52.60	51.90	51.30	50.10
	M	51.72	0.27	1.41	2.30	50.90	51.15	52.29	0.26	0.24	54.20	52.30	51.70	50.90	49.60
MCH***	65	18.89	0.09	0.15	2.00	-	18.71	19.08	0.66	0.37	19.80	19.13	18.84	18.61	18.27
	66	18.05	0.08	0.13	2.00	17.77	17.88	18.22	-0.30	-0.81	18.59	18.37	18.03	17.78	17.34
MCHC***	67	36.29	0.11	0.25	1.40	36.93	36.05	36.54	-0.38	-0.67	37.00	36.67	36.25	36.01	35.30
	68	34.87	0.18	0.60	2.20	-	34.49	35.24	0.26	-1.23	36.07	35.69	34.54	34.34	33.61
RDW**	69	14.52	0.23	0.98	6.80	-	14.04	14.99	-0.05	-0.86	16.12	15.18	14.59	13.66	12.79
	70	16.79	0.97	17.79	25.10	-	14.76	18.82	3.99	16.65	33.73	16.47	15.85	14.98	14.74
MPV**	73	5.23	0.05	0.04	3.90	5.06	5.13	5.32	0.31	-0.86	5.60	5.41	5.21	5.06	4.91
	74	5.41	0.05	0.05	4.00	5.31	5.30	5.51	0.49	0.62	5.89	5.52	5.42	5.30	5.02
PCT***	75	0.474	0.012	0.003	11.10	-	0.450	0.500	0.11	1.25	0.599	0.507	0.487	0.437	0.357
	76	0.427	0.011	0.002	11.40	-	0.400	0.450	-0.66	-0.35	0.496	0.470	0.428	0.394	0.328
PDW	77	15.67	0.07	0.08	1.80	15.65	15.53	15.81	-0.51	1.17	16.19	15.88	15.65	15.55	14.96
	78	15.68	0.09	0.14	2.40	15.78	15.50	15.86	1.52	3.51	16.81	15.86	15.64	15.43	15.20

[1]CI = confidence interval.[2]NEU = neutrophil ($x10^3 \mu L^{-1}$); LYM = lymphocyte ($x10^3 \mu L^{-1}$); MON =monocyte ($x10^3 \mu L^{-1}$); EOS = eosinophil ($x10^3 \mu L^{-1}$); BAS = basophile ($x10^3 \mu L^{-1}$); RBC = red blood cells (erythrocytes) ($x10^6 \mu L^{-1}$); PLT = platelets ($x103\mu^{-1}$); Hb = hemoglobin (g dL^{-1}); HCT = hematocrit (%); MCV = mean corpuscular volume (fL); MCH = mean corpuscular hemoglobin (pg); MCHC = mean corpuscular hemoglobin concentration (g dL^{-1}); RDW = red cell distribution width (%); MPV = mean platelet volume (fL); PCT = plateletcrit (%); PDW = platelet distribution width (%). Gender effect = *0.05 < p = 0.10; **0.01 < p = 0.05; ***p = 0.001.[3]F = female; M = male. Sample size was 18 for each gender at 10 weeks of age.[4]LSM = least square means.[4]SE = standard error.[4]Var = variance.[4]CV = coefficient variation

Uribe *et al.*[35] reported that male and female rats had different leukocyte count and hemoglobin concentration. These may be attributed to differences in predisposition to stress and immune potency. As shown in Table 6, there were strong correlations among variables that were calculated from other variables, reflecting autocorrelation, such as between neutrophil count and ratio of neutrophil to lymphocyte (r = 0.94) and between erythrocyte count, hemoglobin concentration, hematocrit value, platelet count and their calculated characteristics. Evaluation of red cell distribution size in combination with mean corpuscular volume may be useful to differentiate etiology of hematopoetic diseases. Mean platelet volume (the average size erythrocyte count, hemoglobin concentration and hematocrit value for males than for females could be linked to greater heart and lung weights. Hemogram parameters for male and female rats are reported to be similar under nonstressing conditions Leonard and Ruben,[40]; Robel *et al.* [41] Animal performance, blood metabolites and responsiveness to stressors vary by gender Hurst *et al.*,[42]. In association with behavioral differences under stressing conditions, gender effect on hemogram may be more likely Weisse *et al.*,[43]. Wolford *et al.*,[44]. Mean platelet volume (ratio of the platelet volume to total blood voplume) for males was less than for females. These reflect platelet production apacity by bone marrow. However, platelet distribution width did not differ between genders, which may increase in the case of anisocytosis.

In conclusion, this study was carried out to quantify physiological and biochemical variables in rats at age of puberty that were raised under optimum environmental conditions. There were distinct differences in growth and organ development and hemogram parameters and variable differences in blood chemistry between females and males. In addition to considering these differences, descriptive

Table 6: Pearson's correlation coefficients (*r*) among hemogram variables[1]

Variables[2]	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
Neutrophil, x10^3 µL^{-1} (1)	0.21	0.94***	-0.03	-0.10	-0.22	0.10	0.08	0.10	0.14	0.12	-0.03	-0.13	0.55***	0.18	0.14	-0.08
Lymphocyte, x10^3 µL^{-1} (2)		<0.00	0.23	0.33**	-0.18	0.50***	-0.24	0.43***	0.51***	0.15	-0.24-	0.39**	0.08	0.44	-0.11	-0.08
Neutrophil: Lymphocyte(3)			-0.10	-0.14	-0.23	< 0.00	0.20	0.04	0.04	0.11	0.07	-0.01	0.51***	0.08	0.23	-0.04
Monocyte, x10^3 µL^{-1} (4)				0.60***	-0.11	0.21	-0.04	0.33**	0.29*	0.27*	0.16	-0.05	-0.14	0.24	0.04	-0.07
Eosinophil, x10^3 µL^{-1} (5)					0.02	0.07	0.17	0.18	< 0.00	-0.17	0.16	0.32**	-0.03	0.22	0.26*	-0.14
Basophile, x10^3 µL^{-1} (6)						0.08	0.12	0.05	-0.04	-0.30*	-0.05	0.18	-0.04	0.03	0.14	0.06
RBC, x10^6 µL^{-1} (7)							-0.21	0.86***	0.94***	0.03	-0.47***	-0.54***	0.10	0.39**	-0.10	0.03
Platelets, x10^3 µL^{-1} (8)								-0.02	-0.28*	-0.23	0.38**	0.62***	0.06	-0.34**	0.95***	-0.26*
Hemoglobin, g dL^{-1} (9)									0.90***	0.31**	0.06	-0.19	-0.12	0.36**	0.10	-0.08
Hematocrit, % (10)										0.38**	-0.26*	-0.59***	-0.06	0.45***	-0.15	0.07
MCV, fL (11)											0.49***	-0.26	-0.44***	0.25	-0.16	0.13
MCH, pg (12)												0.71***	-0.40**	-0.14	0.37**	-0.17
MCHC, g dL^{-1} (13)													-0.08	-0.36**	0.54***	-0.29*
RDW, % (14)														-0.07	0.04	-0.15
MPV, fL (15)															-0.03	0.07
Plateletcrit, % (16)																-0.26*

[1]*0.05 PDW, % (17)<p = 0.10; **0.01<p = 0.05; ***p = 0.001. N = 36.[2]RBC = red blood cells (erythrocytes); MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; MPV = mean platelet

statistics on response variables presented in this experiment may be invaluable for comparing the treatments and interpreting the outcome of the animal experimentations effects in future studies.

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