Some Haemato-Biochemical Values in White New Zealand **Rabbits**

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Abstract: Haematological and biochemical parameters are important and reliable media used to monitor, evaluate health and nutritional status of animals. This studywas conducted toestablish the normal rangesof haematological and somebiochemical parameters inboth male and female rabbits and to determine thegender effect on these values. Blood and urine samples from a total of 44 New Zealand white rabbits were investigated. The haematological parameters were determined in whole blood samples, while biochemical parameters were determined in serum and urine samples. Normal values of these parameters were determined and statistical comparisons between male and female rabbits were performed. Current study results revealed that haematologicalandbiochemicaldata were identicalin both male and female animals and statistically no significant differences were found for the parameters analysed except, RBCs count, haemoglobin concentration (Hb), haematocrit value (Hct), white blood cell (WBC) count, neutrophils %, lymphocytes %, monocytes%, basophils% and platelets count. It was concluded that defining the normal values of the given haematological and biochemical parameters in New Zealand white rabbits would be valuable for both researchers and clinicians as they may be considered as useful baseline values for domestic rabbit populations.

Keywords: New Zealand rabbit, Haematological, Biochemical, Normal parameters.

I. Introduction

Rabbits are being used as a valuable source of animal protein in rural communities, as well as for scientific research in academic institutions. The need to increase livestock production as a mean of alleviating the awesome shortage of animal protein is very vital to humanity [1]. The demand for protein of animal origin in some developing countries is greater than its supply. Therefore, the grave shortage in dietary animal protein in these countries demands extra effort to be directed to livestock that are prolific and have short gestation interval such as rabbit. Delicious high protein content, low calories, and fat content meat of domestic rabbit are extra reasons for the aching demands that encourage rearing of domestic rabbits, easier availability, high fertility, feeding and housing features make the New Zealand white rabbits to be widely used in laboratory works [2, 3]. Besides the use of the rabbits as a protein source, the increasing of rabbit popularity increased the demand for veterinary services for these animal. So hematological and biochemical reference values aiding in diagnosis and prognosis of rabbit disease became crucial [4]. Haematological parameter is a reliable mean for health and nutritional status evaluation [5]. Breed, gender, age, pregnancy, circadian rhythm, disease conditions, environmental, physical or nutritional stressful conditions are factors affecting haematological and biochemical parameters [3, 6, 7, 8, 9, 10]. All these conditions can be monitored through biochemical and haematological parameters examination that allow the detection of numerous metabolites and extra constituents of blood [6, 8]. The rabbits have become widely used for laboratory and domestic purposes, this ensures the value and the importance of studies on this animal species [10, 11, 12, 13]. Numerous studies had been done on the blood parameters of various domestic animals, nevertheless extra biochemical and haematological values of New Zealand white rabbits are still needed. These reference values are of a great importance in evaluation of rabbit health conditions and provides valuable information for researchers and clinicians [7, 8, 14, 15, 16]. So the present study was carried out to determine the normal values of some haematological, biochemical parameter in serum and urine of both male and female New Zealand white rabbits and to define the gender effect on these parameters.

II. Materials and Methods

2.1Experimental animals

Atotal of44 New Zealand white rabbits (22 males and 22 female); age 8-12 months and of 3.5 to 4.5 kg weight were used in the current study. Each rabbit was physically examined and unhealthy ones (feverish, with nasal or ocular discharge, diarrhoea, ear mites, atypical lung sounds, or large wounds) were excepted from the study. Before the beginning of the experiment, the experimental animals were treated against endo/ectoparasites and coccidiosis for one month and their average body weight (3.5-4.5 kg) was kept uniform as possible. The animals were individually kept in cages in appropriate laboratory conditions $(23\pm 2^{\circ}C, 12$ -h dark and light cycles). Commercial pellet (Purina[®] Rabbit ChowTM) contained a minimum of 86% dry matter, minimum 14% crude protein, maximum 16% cellulose, maximum 10% crude ash and minimum 2400 kcal/kg metabolic energy was given *ad libitum* to the rabbits. Drinking water was provided by automaticnipples. Feed and Environment adaptation period (2 weeks) were allowed to the experimental animals before samples collection. All procedures were performed consistent with the Physiological Society's guidelines for experimental animal's care.

2.2Collection of samples

According to the described method, 24 h urine sample was collected between 9:00-11:00 a.m. from each rabbit through metabolic cages, using graduated glasses; The urine samples were centrifuged at 3000 rpm for 10 minutes then the obtained supernatant was kept at -20° C until assayed. Moreover, 5 ml of blood was obtained from each rabbit by marginal ear vein puncture using a 22-gauge sterile needle. For haematological analysis, the blood samples were expelled gradually into tubes containing K3-EDTA (Ethylene DiamineTetra acetic acid), the tubesweredirectly cappedand gentlymixedby frequent inversion. The samplesweredirectly transportedtothelaboratory within 3 hat4°C for further analysis. Whereas,for biochemical analysis; non anticoagulated blood samples were collected in sterile tube, centrifuged for 10 min at 3000 rpm; the serum was obtained and kept at -20° C until analysis

2.3 Haematologicaland BiochemicalStudies

Bloodsamples with K3-EDTA anticoagulant were used for the determination of haematological parameters: which include RBCs count, haemoglobin concentration (Hb), haematocrit value (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC) count, neutrophils %, lymphocytes %, monocytes %, eosinophils %, basophils %, platelets count). Anticoagulated blood samples Analyses were performed via Cell-DYN3500HaematologyAnalyser (Abbott DiagnosticDivision, SantaClaraCA). As statedbythemanufacturer'soperationmanual Suggested settings andadjustmentfor rabbit haematologywereapplied.

Serum contents of glucose, albumin, total protein (Tp), globulin, nitrite, calcium (Ca), chloride (Cl), phosphorus (P), potassium (K), sodium (Na), creatinine, total bilirubin (Tb), blood urea nitrogen (BUN), activities of γ -glutamyl transferase (GGT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT)were measured.

Also, urine contents of glucose, protein, creatinine, creatinine clearance, GGT, urea, potassium (K), sodium (Na), chloride (Cl), nitrite, were measured. Urine contents of glucose, protein, creatinine, urea and GGT were evaluated spectrophotometrically (Photometer 5010®-Boehringer Mannheim) following the procedures defined in the commercial test kits (Biolabo, Italy), while the urine and serum Cl⁻, Na⁺ and K⁺ analyses were done on an ion selective device (ISE®-Medica). Urine glucose, protein, creatinine, urea, γ -glutamyl transferase (GGT), Cl, nitrite, Na, and K levels were evaluated at the urine supernatant.

Nitrite levels of serum and urine samples were determined by Griess Reagent method at 540 nm on ELISA device (ELISA reader[®]-DAS) using nitric oxide kit (Griess Reagent System, Cat No: G2930[®]-Promega), as defined in commercial test kits. Creatinine clearance was calculated using the formula for creatinine clearance (Kaneko *et al.*, 1997).

2.4Statistical analysis

Comparison between female and male rabbits were done using independent sample t- test through SPSS software (Ver. 11.5) at a significant level P<0.05. and P<0.001. Other statistical parameter as Means \pm SEM, Maximum and minimum normal values were also determined.

III. Results

In the present study, the normal values of some haematological, biochemical parameter in serum and urine of both male and female New Zealand white rabbits were displayed. The arithmetic means, standard error of means, maximum and minimum values of blood parameters haematologically revealed very highly significant differences (P<0.001) regarding RBC count, Hb, Hct, monocytes, basophils and platelet count in males ($5.71\pm0.50, 14.82\pm0.90, 46.64\pm2.40, 0.50\pm0.30, 0.20\pm0.10$ and 482.80 ± 22.10 ; respectively) compared to females ($4.31\pm0.32, 10.45\pm0.45, 40.14\pm2.11, 1.00\pm0.24, 0.40\pm0.10$ and 577.30 ± 32.19 ; respectively). Likewise, in male a significant elevation (P<0.05) was observed regarding WBC count (13.90 ± 1.23) and lymphocytes % (68.60 ± 5.80) compared with females (10.44 ± 1.08 and 64.40 ± 3.84 ; respectively). But, there was a significant decrease(P<0.05) in neutrophils % in males (30.10 ± 3.40) versus females (34.50 ± 2.42).On the other hand, no significant variation for the other blood parameter measured as MCH, MCV, MCHC, eosinophils % (Table1). Also, no variation (P|>0.05) was reported in the serum(glucose, Tp, albumin, globulin, Ca, P, Na, K, Cl, creatinine, nitrite BUN, GGT, Tb, AST, ALT and ALP) and urine biochemical parameters (glucose, protein, creatinine, creatinine clearance, urea, nitrite, GGT, K, Na, Cl) among males and females (Table 2, 3).

IV. Discussion

The current study was performed to determine the reference standards of some haematological, serum and urine biochemical parameters for both female and male white New Zealand rabbit species and to examine the gender effect on these factors. Mainly, the blood parameter is a dependable and essential mean used to evaluate and monitor nutritional and health status of animals [5]. The haematological examination provides the chance to examine numerous metabolites and other constituents that aid in detecting the stress conditions, which could be nutritional, environmental or physical[6]. Moreover, the blood parameters mostly offer data on inflammation, necrosis, numerous infections of visceral organs and stress factors[2, 8, 17]. Identifying biochemical and haematological reference values of rabbits provides valuable data for investigators in their studies, and clinicians for regarding the health condition of animals in clinical practice [7, 8, 10, 15, 16]. Breed, gender, age, feeding, disorders, stress, circadian rhythm, pregnancy and environmental conditions are factors that influence the blood parameters [3, 7, 8, 9, 10]. Additionally, WBCs count seldom elevated due to infection in rabbit but it is more related to blood collection methods and various stress factors [8, 15, 18]. Rabbits kept alone possess higher WBCs count than those fed in groups [12].

In the study presented here, some of haematological parameters (RBC, Hb, Hct, MCHC, WBC, PLT) of the white New Zealand rabbits were within the normal range values previously recorded by [2,10,16,18,19, 20], Moreover the results were consistent with [10] who proved that the blood parameters showed significant increase in RBCs count, Hct, Hb, WBCs count and Lymphocytes %, and significantly lower neutrophils, monocytes %, basophils and platelets counts value among males than females. The increase in male erythrocytesattributed to the androgenic effect that increased erythropoietin production [21].

Regarding the biochemical parameters of serum, defining these parameters provides an important information about visceral organs damage in rabbits, particularly for the liver and the kidneys[2,8, 10, 18]. Total protein, Glucose, creatinine, albumin, BUN, P, Ca, K, Na and Cl levels for the white New Zealand rabbits determined in the current study were within the reference values range reported in previous studies [8,10, 15, 22, 23]. The significantly higher protein and albumin levels among males may be ascribed to the androgens anabolic effect. Though, the other biochemical parameters of serum showed no significant difference among female and male rabbits.

Earlier studies reported that the elevated blood glucose levels among rabbits are generally due to various stress factors [8, 14, 18]. Moreover, blood glucose levels were reported to be lesser than the serum or plasma glucose levels [11]. The serum glucose levels determined in the current study were higher than those reported in some studies [2,18] and lower than that of [15]. Hypothetically, this variance may be caused by the variations in some factors as blood collection methods, housing conditions and stress. However, [24] and [10], under the same condition of this study, showed no significant difference for serum glucose levels between female and male animals. Concerning Tb values, both male and female rabbits showed a normal ranges of values matches those previously described by previous investigators[8, 10, 14]. They were higher than that of [20] and [22]. Serum GGT activity was higher than those found in former studies [8, 11, 20, 22, 23]. AST activity was close to the reported levels in some preceding studies [10, 20], but lower than the observed levels in others [8, 15, 23]. ALT activity was lesser than the reported values in some prior studies [8, 15, 18, 20, 22, 23]. ALP activity was close to the reported levels by other researchers [8, 14], but lesser than the levels obtained by others [15, 25]. The activity of serum ALP originates from both bone and liver varied according to the age, where younger individuals showed a higher serum ALP levels, this is because of the rapid bone growth [26]. The decreased serum ALP activity in the existing study might be accredited to the differences in growth period and age of the rabbits. This study revealed no significant variation in the serum nitrite levels among males and females. This finding agreed with the result of [10]. However, [27] reported a higher nitrite level of 8.93±1.09 µmol/L.

Defining the urine biochemical parameters provides important information on renal functions and electrolyte disturbance in rabbits. However, limited data describing normal biochemical parameters of rabbit urinewas available. This fact makes it to be one of the current study aims to find out reference biochemical urine values for both sex in white New Zealand rabbits.Earlier studies [8,10,18, 28] found that trace amounts of protein and glucose in rabbit urine is normal. [29] showed that healthy rabbits urine contains 0.15-0.52 mg/kg protein a day. The increases urine glucose and protein levels indicated impaired renal functions while, transient increase was exercise and stress induced [18]. Urine GGT rise is an earlier indicator and one of the markers considered for renal functions impairment [8, 10, 28].

While conducting this study reference ranges values for urine parameters such as protein, glucose, creatinine, urea, GGT, nitrite, Na, K, Cl and creatinine clearance in rabbits were rare. In other available literature, no statistical difference in the urine parameters like protein, glucose, creatinine, urea, GGT, nitrite, Na, K, Cl and creatinine clearance was found between female and male white New Zealand rabbits. Hence, describing the normal values of these parameters in both female and male rabbits in the current study is remarkable.

The observed variation in the present study result compared with some earlier studies may be related to the different approaches that were used to calculate the respective variables. and the manual approach that relied more on the technician/researcher to conduct the tests Narrower ranges suggest precise estimates and of greater value for monitoring the health status. Instead, narrower standard deviation indicates that these differences in biochemical and haematological values could be caused by nutritional, environmental factors and hormonal among each experimental animal group. Therefore, a specific analysis and comparison between different units has to be planned and performed for better understanding the usefulness of haematology and clinical chemistry for assessing the health and welfare of rabbits. So more investigation is needed.

V.Conclusion

In conclusion, the recorded results can provide a set of normal values of haematological parameters, serum and urine biochemical parameters that can be helpful for both researchers and clinicians and may therefore be considered useful baseline values for domestic rabbit populations.

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Blood parameters	Male (No.=22)		Female (No.=22)	
	Mean \pm SEM	Range	Mean \pm SEM	Range
RBC (×10 ⁶ /mm ³)	$5.71 \pm 0.50^{***}$	4.11-7.20	4.31 ± 0.32	3.22-5.98
Hb (g/dL)	$14.82 \pm 0.90^{***}$	10.45-16.81	10.45 ± 0.45	7.57-13.45
Hct (%)	$46.64 \pm 2.40^{***}$	38.41-51.50	40.14 ± 2.11	34.68- 47.54
MCV (fl)	67.52 ± 3.40	58.42-72.8	62.50 ± 1.22	55.57-66.89
MCH (pg)	23.71 ± 1.00	19.45-27.83	22.11 ± 1.01	18.55-25.52
MCHC (%)	33.44 ± 0.81	28.54-36.29	30.24 ± 0.33	25.52-34.61
WBC (×10 ³ /mm ³)	13.90± 1.23*	9.32-15.84	10.44 ± 1.08	8.45-13.34
Neutrophils (%)	$30.10 \pm 3.40^{*}$	26.57-34.69	34.50 ± 2.42	25.81-36.55
Lymphocytes (%)	$68.60 \pm 5.80^{*}$	59.55-73.53	64.40 ± 3.84	56.46- 68.57
Monocytes (%)	$0.50 \pm 0.30^{***}$	0.50- 0.90	1.00 ± 0.24	0.50- 1.20
Eosinophils (%)	0.60± 0.20	0.40- 0.90	0.60 ± 0.15	0.40- 0.90
Basophils (%)	$0.20 \pm 0.10^{***}$	0.00-0.30	0.40 ± 0.10	0.00-0.30
PLT (×10 ³ /mm ³)	482.80± 22.10***	95.00-1079.00	577.30 ± 32.19	79.00-1167.00

Table 1: Normal values of some haematological parameters in white New Zealand rabbit's seru

* Significant at PO< 0.05 and *** highly significant at P < 0.001

Abbreviation.RBCs: red blood cells count, Hb:haemoglobin, HCT: haematocrit, MCHC:mean corpuscular haemoglobin concentration, MCV: mean corpuscular volume, MCH:mean corpuscular haemoglobin, WBCs:white blood cell count, PLT: platelets count, SEM: standard error of the mean.

Table 2: Normal values of some biochemical parameters in white New Zealand rabbit's serum.

Biochemical	Male (No.=22)		Female (No.=22)	
parameters	Mean \pm SEM	Range	Mean ± SEM	Range
Glucose (mmol/L)	8.32 ± 0.31	3.22-11.01	6.97 ± 0.40	2.70-9.45
Tp (g/dl)	7.10 ± 0.50	4.19-10.65	6.20 ± 0.30	2.42-9.13
Albumin (g/dl)	3.60 ± 0.30	2.11-3.98	3.10 ± 0.31	2.01-3.81
Globulin (g/dl)	2.80 ± 0.40	1.94-3.10	2.40 ± 0.35	1.50-2.95
Nitrite (µM)	5.62 ± 0.57	0.96-11.39	5.59 ± 0.49	1.50-13.54
Ca (mmol/L)	3.35 ± 0.08	2.01-4.01	3.40 ± 0.21	2.55-4.43
P (mmol/L)	1.37 ± 0.06	1.01-1.96	1.25 ± 0.04	1.07-1.85
Na (mmol/L)	148.31 ± 0.84	136.63-151.45	145.45 ± 0.75	132.56-149.81
K (mmol/L)	4.91 ± 0.06	3.23-5.78	4.08 ± 0.04	3.03-5.12
Cl (mmol/L)	115.50 ± 0.72	100.09-119.56	114.6 ± 0.85	101.43-118.37
Creatinine (mmol/L)	0.10 ± 0.00	0.01-0.15	0.11 ± 0.00	0.01-0.15
BUN (mmol/L)	11.31 ± 0.54	7.23-16.65	11.70 ± 0.44	6.54-15.98
Tb (µmol/L)	3.60 ± 0.15	1.1-4.98	3.20 ± 0.13	0.86-5.16
GGT (U/L)	16.78 ± 1.87	10.56-24.68	14.63 ± 1.13	8.39-21.53
AST (U/L)	10.98 ± 1.01	6.14-18.57	10.12 ± 0.89	7.34-17.52
ALT (U/L)	7.80 ± 0.32	5.56-10.44	7.20 ± 0.12	4.79-9.93
ALP (U/L)	18.04 ± 1.02	13.29-27.74	17.11 ± 0.86	12.63-24.89

Abbreviation. Tp: total protein, BUN: urea nitrogen, Tb: total bilirubin, GGT: γ-glutamyltransferase, AST:aspartate aminotransferase, ALT: alanineaminotransferase, ALP: alkaline phosphatase,

SEM:Standard error of the mean.

Table 3: Normal values for some biochemica	l parameters in New Ze	aland white rabbits' urine.
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Biochemical parameters	Male (No.=22)		Female (No.=22)	
	Mean \pm SEM	Range	Mean \pm SEM	Range
Glucose (mmol/L)	0.41 ± 0.08	0.02-1.09	0.39 ± 0.07	0.00-0.97
Protein (g/L)	4.01 ± 0.60	0.00-12.87	4.23 ± 0.95	0.00-14.7
Creatinine (mmol/L)	12.38 ± 2.24	1.71-22.27	14.43 ± 1.65	5.36-30.88
Creatinine Clearance (mL/min kg)	1.57 ± 0.29	0.12-7.52	1.26 ± 0.14	0.46-3.11
Urea (g/24h)	1836 ± 208	362-1804	1365 ± 157	280-3472
GGT (U/L)	28.34 ± 2.16	13.68-56.39	32.78 ± 2.83	16.83-64.72
Nitrite (µM)	7.47 ± 0.54	3.98-16.78	6.96 ± 0.35	2.48-13.27
Na (mmol/L)	62.12 ± 3.98	37.11-112.92	55.36 ± 4.72	30.44-107.37
K (mmol/L)	219.45 ± 11.76	36.67-354.23	189.15 ± 17.63	32.56-338.92
Cl (mmol/L)	194.63 ± 12.69	25.58-350.52	164.96 ± 16.93	22.58-319.67

Abbreviation.GGT: γ-glutamyl transferase

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