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NORMAL VALUES OF HAEMATOLOGICAL AND SOME BIOCHEMICAL PARAMETERS IN SERUM AND URINE OF NEW ZEALAND WHITE RABBITS

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Abstract: The purpose of the present study was to define the normal haematologic values and some biochemical parameters in serum and in urine in both male and female New Zealand white rabbits and to determine the effect of gender on these parameters. Blood and urine samples from a total of 40 New Zealand white rabbits were investigated. The haematologic parameters were determined in whole blood samples, while serum and urine (urine protein, glucose, creatinine, urea, GGT, nitrite, Na, K, Cl, creatinine clearance) biochemical parameters were determined in serum and urine samples. Normal values of these parameters were determined and statistical comparisons between male and female animals performed. No statistically significant differences were found between male and female animals for the parameters analysed except HCT, HGB, granulocyte %, L/M and serum K concentration. As a result, it was judged that defining the normal values of given haematological factors and serum and urine biochemical parameters in this study in New Zealand white rabbits would be helpful for both clinicians and researchers.

Key Words: biochemical parameters, haematological parameters, normal values, serum, urine, New Zealand rabbit.

INTRODUCTION

The New Zealand white is one of the most popular breeds of rabbit in the world. New Zealand whites are widely used in laboratory studies because of their high fertility, easier availability, feeding and housing features (Yu *et al.*, 1979; Jurcik *et al.*, 2007; Jeklova *et al.*, 2009). They have recently begun to be kept by individuals as domestic animals due to features such as their body structure, white fur or cute and likeable behaviour.

As disorders have considerable effects on blood parameters in rabbits, it is important to analyse blood and urine in rabbits as well as in other animal species. The rabbits may mask the symptoms of the diseases or manifest some complex clinical signs. Hence, some important findings may be achieved using haematological and biochemical parameters (Lepitzki and Woolf, 1991; Melillo, 2007). When assessing rabbit diseases, knowledge of the reference values of haematologic and biochemical parameters is helpful in the evaluation of rabbit health situations and provides important information for clinicians (Lepitzki and Woolf, 1991; Silva *et al.*, 2005; Chineke *et al.*, 2006; Melillo, 2007; Archetti *et al.*, 2008). However, haematological and biochemical parameters analysed are influenced by many factors such as breed, age, gender, feeding, environmental conditions, disorders, stress, pregnancy and cardiac rhythm (Chineke *et al.*, 2006; Melillo, 2007; Jeklova *et al.*, 2009; Abdel-Azeem *et al*., 2010).

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The fact that rabbits have become widely used for domestic and laboratory purposes ensures the studies on rabbits (Yu *et al.*, 1979; Hewitt *et al.*, 1989; Fuentes and Newgren, 2008; Zhao *et al*., 2010). Nevertheless, the lack of reference values for biochemical parameters in rabbits leads to problems for many clinicians and constrains many researchers, who need to study their own reference values.

The aim of the present study was to define the normal values of some haematologic and biochemical parameters in serum and urine in both male and female New Zealand white rabbits and determine the effect of gender on these parameters.

MATERIALS AND METHODS

Animals

The samples analysed were taken from 40 (24 male and 16 female) New Zealand white rabbits [8-10 (n=22) and 10-12 mo of age (n=18), weighing 2.5 to 4.3 kg]. The animals were housed in single rabbit cages in suitable laboratory conditions (24±2°C, 12-h light and dark cycles). One month before the study, the animals were treated against endo- and ecto-parasites and coccidiosis. The animals were allowed to adapt to the feed and environment for 15 d before collection of the samples. The rabbits were given *ad libitum* commercial pelleted rabbit food (Purina® Rabbit Chow™) and drinking water. Commercial pelleted food contained a minimum of 88% dry matter, minimum 15% crude protein, maximum 17% cellulose, maximum 10% crude ash and minimum 2300 kcal/kg metabolic energy.

Collection of blood and urine samples

Blood and urine samples were collected in the morning between 8:00-10:00 a.m. according to the method described. The blood sample, obtained by marginal ear vein puncture, was drawn into tubes using a 22 gauge sterile needle. The blood sample for haematological analysis was expelled gradually into tubes containing K_3-EDTA; the sample for biochemical analysis was collected in sterile tubes without anticoagulant. Twenty-four hour urine samples were collected in a graduated glass from rabbits in metabolic cages. Urine samples were centrifuged and urine supernatant stored at –20°C until assayed.

Analysis of haematologic and biochemical parameters

Hematocrit value (HCT), whole blood haemoglobin concentration (HGB), white blood cell count (WBC), mean erythrocyte haemoglobin concentration (MCHC), granulocyte count, granulocyte %, lymphocyte+monocyte count (L+M), lymphocyte/monocyte % (L/M%) and thrombocyte count (PLT) analyses of blood samples collected into test tubes with anticoagulant were performed on veterinary haematology device (QBCvetautoreader®-Idexx). Blood samples for haematological analyses were delivered to the laboratory within 2 h of collection and promptly assayed.

For biochemical analyses, blood samples collected into test tubes without anticoagulant were centrifuged (Rotofix 32®-Hettich) at 3000 rpm for 10 min; the serum was collected and kept at –20°C until analysis. Urine protein, glucose, creatinine, urea, γ-glutamyl transferase (GGT), nitrite, Na, K and Cl levels were measured from the supernatant obtained by the centrifugation of urine samples from the rabbits at 3000 rpm for 10 min.

Serum contents of total protein (Tp), albumin, glucose, creatinine, urea nitrogen (BUN), direct bilirubin (Db), total bilirubin (Tb), calcium (Ca) and phosphorus (P) and activities of GGT, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured. Urine contents of protein, glucose, creatinine, urea and GGT were measured spectrophotometrically (Photometer 5010®-Boehringer Mannheim) following the procedures defined in the commercial test kits (Biolabo, France), while the serum and urine Na^+, K^+ and Cl^- analyses were done on an ion selective device (ISE®-Medica). Creatinine clearance was calculated using the formula for creatinine clearance (Kaneko *et al.*, 1997). 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.

Statistical analysis

Statistical analyses were performed with SPSS 11.5 software. Arithmetic mean, standard error of means and minimum and maximum values of normal values were determined. The independent simple t test was used for the comparison between male and female animals. The significance level at $P<0.05$ was accepted.

RESULTS

The minimum and maximum values, arithmetic means and standard error of means of haematologic parameters (HCT, HGB, WBC, MCHC, granulocyte, granulocyte %, L+M, L/M%, PLT) and serum (Tp, albumin, glucose, creatinine, BUN, Db, Tb, GGT, AST, ALT, ALP, nitrite, Ca, P, Na, K, Cl) and urine (protein, glucose, creatinine, urea, GGT, nitrite, Na, K, Cl and creatinine clearance) biochemical parameters and statistical comparisons between male and female animals are presented in Tables 1, 2 and 3.

As shown in Table 1, the statistical comparison between male and female animals revealed a statistical difference for HCT ($P<0.001$), HGB ($P<0.001$), granulocyte % ($P<0.05$) and L+M

Table 1: Normal values for some haematological parameters in New Zealand rabbits.

	Male (No.=24)		Female (No.=16)	
	Mean±SEM	Range	Mean±SEM	Range
HCT (%)	48.91±0.83[b]	41.70-57.00	42.18±1.09[a]	33.10-47.70
HGB (g/L)	175.2±3.5[b]	147.0-208.0	148.1±5.4[a]	108.0-175.0
WBC ($\times10^9$/L)	11.40±0.61	5.90-18.30	10.44±1.05	5.80-20.10
MCHC (g/L)	357.9±2.3	317.0-368.0	349.7±4.6	315.0-368.0
Granulocyte ($\times10^9$/L)	6.04±0.45	1.70-12.10	6.61±0.85	1.70-14.70
Granulocyte % (%)	51.91±2.20[a]	27.00-73.00	61.87±3.58[b]	24.00-79.00
L+M ($\times10^9$/L)	5.37±0.30[b]	2.90-8.90	4.15±0.46[a]	1.40-8.20
L/M % (%)	48.08±2.20	27.00-73.00	38.12±3.58	21.00-76.00
PLT ($\times10^9$/L)	496.3±36.5	135.0-1005.0	589.4±72.0	62.0-1188.0

HCT, hematocrit value; HGB, whole blood haemoglobin concentration; WBC, white blood cell count; MCHC, mean erythrocyte haemoglobin concentration; L+M, lymphocyte+monocyte count; L/M%, lymphocyte/monocyte ratio; PLT, thrombocyte count.
SEM: Standard error of the mean.
[a, b] Means in the same row not sharing superscript are significantly different at $P<0.05$.

Table 2: Normal values for some biochemical parameters in New Zealand rabbit serum.

	Male (No.=24)		Female (No.=16)	
	Mean±SEM	Range	Mean±SEM	Range
Tp (g/L)	69.9±4.0	45.0-122.0	62.5±2.2	49.0-79.0
Albumin (g/L)	33.4±0.8	27.0-43.0	29.8±0.9	23.0-35.0
Glucose (mmol/L)	7.22±0.31	3.83-10.77	6.58±0.27	4.94-8.32
Creatinine (mmol/L)	0.10±0.00	0.06-0.14	0.11±0.00	0.06-0.14
BUN (mmol/L)	11.34±0.47	7.35-17.63	11.52±0.72	6.35-15.99
Db (µmol/L)	1.37±0.01	0.85-1.71	1.37±0.01	0.85-0.71
Tb (µmol/L)	3.42±0.17	0.71-5.13	3.25±0.34	1.71-5.13
GGT (U/L)	17.79±1.22	9.00-31.00	15.31±0.92	6.00-22.00
AST (U/L)	10.58±0.97	6.00-20.00	10.12±0.97	7.00-19.00
ALT (U/L)	7.20±0.19	6.00-9.00	7.00±0.27	5.00-8.00
ALP (U/L)	17.08±0.93	12.00-26.00	16.18±0.88	13.00-26.00
Nitrite (µM)	5.91±0.79	0.43-14.45	5.95±0.96	0.92-16.84
Ca (mmol/L)	3.01±0.09	2.44-3.72	3.02±0.11	2.50-3.72
P (mmol/L)	1.31±0.04	1.09-1.68	1.25±0.03	1.09-1.68
Na (mmol/L)	142.1±0.6	137.2-148.3	141.6±0.5	139.3-145.7
K (mmol/L)	4.71±0.08[b]	3.38-5.56	4.07±0.13[a]	3.02-4.67
Cl (mmol/L)	111.1±0.9	102.2-116.9	112.1±0.8	104.9-116.4

Tp, total protein; BUN, urea nitrogen; Db, direct bilirubin; Tb, total bilirubin; GGT, γ-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.
SEM: Standard error of the mean.
[a,b] Means in the same row not sharing superscript are significantly different at $P<0.05$.

($P<0.05$) among haematologic parameters. There was no statistically significant difference for other haematologic parameters between male and female animals.

As presented in Table 2 and 3, no statistically significant difference was found for serum and urine biochemical parameters analysed between male and female animals, except for serum K concentration ($P<0.001$).

Table 3: Normal values for some biochemical parameters in New Zealand rabbits urine and creatinine clearance values.

	Male (No.=24)		Female (No.=16)	
	Mean±SEM	Range	Mean±SEM	Range
Protein (g/L)	4.7±0.7	0.0-14	4.8±0.9	0.0-16
Glucose (mmol/L)	0.31±0.05	0.00-1.11	0.38±0.06	0.11-0.78
Creatinine (mmol/L)	11.18±1.19	1.41-24.40	12.88±1.82	4.24-29.88
Urea (g/24h)	1941±233	200-4160	1366±199	300-3020
GGT (U/L)	31.75±3.14	14.00-78.00	34.18±2.70	18.00-60.00
Nitrite (µM)	8.00±0.81	3.12-19.36	7.53±0.77	2.39-11.74
Na (mmol/L)	68.08±4.21	40.20-128.70	58.94±5.93	32.10-112.70
K (mmol/L)	216.5±16.4	31.6-339.1	197.6±24.2	35.7-339.2
Cl (mmol/L)	181.2±14.6	29.8-334.5	153.0±19.8	27.0-283.2
Creatinine Clearance (mL/min kg)	1.67±0.28	0.15-6.61	1.24±0.15	0.59-3.09

GGT, γ-glutamyl transferase.
SEM: Standard error of the mean.

DISCUSSION

This study was conducted in New Zealand white rabbits to define the normal values of haematological parameters and serum and urine biochemical parameters and determine the effect of gender on these factors.

Knowledge about the reference values of haematological and biochemical parameters of rabbits provides useful information for researchers in their studies and for veterinary physicians regarding the health situations of animals in clinical practice (Lepitzki and Woolf, 1991; Silva *et al.*, 2005; Chineke *et al.*, 2006; Melillo, 2007; Archetti *et al.*, 2008). Haematological parameters generally provide information on inflammation, necrosis, various infections of visceral organs and the presence of stress factors (Jurcik *et al.*, 2007; Melillo, 2007; Betancourt-Alonso *et al*., 2011).

It has been reported that red cells blood count and HCT values are influenced by stress, age, gender, season and genus in rabbits (Melillo, 2007; Jenkins, 2008); HCT value under 30% and the decrease in HCT parallel to HGB are evaluated as anaemia (Jenkins, 2008). Furthermore, it has been denoted that WBC count rise in rabbits rarely indicates an infection; it generally varies due to various stress factors and blood collection methods (Silva *et al.*, 2005; Melillo, 2007; Jenkins, 2008). In one study (Fuentes and Newgren, 2008), it was reported that WBC values of the rabbits kept alone were higher than in rabbits fed together in groups.

In this study, some haematological parameters (HCT, HGB, MCHC, WBC, PLT) presented in Table 1 were in the range of normal values defined for these parameters by previous studies (Hewitt *et al.*, 1989; Cetin *et al.*, 2004; Tavares *et al.*, 2004; Jurcik *et al.*, 2007; Archetti *et al.*, 2008; Jenkins, 2008) in rabbits. When haematological parameters of male and female rabbits were compared, HCT, HGB and L+M values of male animals were significantly higher and granulocyte % was significantly lower than that of females. No other statistically significant difference was determined between male and female animals for the remaining haematological parameters. Similarly, in a performed study (Jacobson *et al.*, 1978), HCT value was reported to be higher in male rabbits than female rabbits.

The analysis of serum biochemical parameters provides important information about visceral organ damage in rabbits, particularly for the liver and the kidneys (Jurcik *et al.*, 2007; Melillo, 2007; Jenkins, 2008).

Total protein, albumin, glucose, creatinine, BUN, Ca, P, Na, K and Cl levels determined in this study and presented in Table 2 were within the range of reference values reported for rabbits in previous studies (Yu *et al.*, 1979; Hewitt *et al.*, 1989; Lepitzki and Woolf, 1991; Yazar *et al.*, 2004; Silva *et al.*, 2005; Elmas *et al.*, 2006; Melillo, 2007). No statistically significant difference for any parameters, except serum K concentration, was determined in this study between male and female rabbits.

It has been reported that elevated glucose levels in rabbits are generally due to various stress factors (Lepitzki and Woolf, 1991; Melillo, 2007; Jenkins, 2008). Additionally, the whole blood glucose levels were reported to be lower than the serum or plasma glucose levels (Hewitt *et al.*, 1989). The serum glucose levels determined in this study were higher than in some other studies (Jurcik *et al.*, 2007; Jenkins, 2008) and lower than in another (Silva *et al.*, 2005). It is hypothesised that this difference may be due to the variations in some factors such as stress, blood collection methods and housing conditions. However, in a study (Yamada *et al.*, 2004) with similar conditions to our work, no statistically significant difference for serum glucose levels was determined between male and female animals.

As shown in Table 2, Tb values determined in both male and female rabbits were in normal ranges reported in some previous studies (Lepitzki and Woolf, 1991; Melillo, 2007), but higher than Db and Tb values found in some other studies (Tavares *et al.*, 2004; Yazar *et al.*, 2004). Serum GGT activity was higher than was found in other studies (Yu *et al.*, 1979; Hewitt *et al.*, 1989; Tavares *et al.*, 2004; Yazar *et al.*, 2004; Elmas *et al.*, 2006; Melillo, 2007). AST activity was near to the levels reported in some previous studies (Yu *et al.*, 1979; Tavares *et al.*, 2004) and lower than the levels observed in some others (Lepitzki and Woolf, 1991; Silva *et al.*, 2005; Elmas *et al.*, 2006; Melillo, 2007). ALT activity was lower than the values reported in some previous studies (Lepitzki and Woolf, 1991; Tavares *et al.*, 2004; Yazar *et al.*, 2004; Silva *et al.*, 2005; Elmas *et al.*, 2006; Melillo, 2007; Jenkins, 2008). ALP activity was near to the levels in some previous studies (Hewitt *et al.*, 1989; Lepitzki and Woolf, 1991; Melillo, 2007) and lower than the levels in some others (Yu *et al.*, 1979; Wicher *et al.*, 1984; Silva *et al.*, 2005). It has been reported that serum ALP activity originates from both liver and bone and varies by age, and in young individuals serum ALP levels are higher because of rapid bone growth (Kaneko *et al.*, 1997). It was thought that lower serum ALP activity in the present study might be due to the differences in age and growth period of the animals.

In a study with rabbits (Haghjooyjavanmard *et al.*, 2009), serum nitrite concentrations in the control group were reported to be 8.93±1.09 μmol/L. In the present study, serum nitrite levels of both male (5.91±0.79 μM) and female (5.95±0.96 μM) animals were lower, and no statistically significant difference in serum nitrite levels was found between male and female animals.

Urine analysis provides important information on renal functions and electrolyte imbalance in rabbits. Normal urine of rabbits is rich in minerals and concentrated. For this reason, it should be centrifuged or filtrated for biochemical analyses. Furthermore, it has been reported that urine should be collected from rabbits in the morning and analysed immediately (Melillo, 2007). The literature search performed revealed no comprehensive study about normal biochemical parameters of rabbit urine.

Normally, trace amounts of protein and glucose can be found in rabbit urine (Melillo, 2007; Jenkins, 2008; Özkan, 2009). Türkmen *et al.* (1997) determined 0.15-0.52 mg/kg day protein in the urine of healthy rabbits. It has been reported that an increase in urine protein and glucose levels indicated impaired renal functions and a transient increase was due to exercise and stress (Jenkins, 2008). Urine GGT rise is an earlier indicator and one of the markers considered for impaired renal functions (Melillo, 2007; Özkan, 2009).

In the literature search, no information on the reference ranges of urine parameters such as protein, glucose, creatinine, urea, GGT, nitrite, Na, K, Cl and creatinine clearance was found. Hence, describing the normal values of these parameters in both male and female rabbits in this study is remarkable. Additionally, as shown in Table 3, no statistical difference in the parameters of urine protein, glucose, creatinine, urea, GGT, nitrite, Na, K, Cl and creatinine clearance was found between male and female New Zealand white rabbits.

In conclusion, it was judged that defining the normal values of given haematological parameters, serum and especially urine biochemical parameters in the present study in New Zealand white rabbits would be helpful for both clinicians and researchers.

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