Hematological Characteristics and Their Correlation Coefficients in Adult New Zealand White Rabbits

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ABSTRACT

The hematological profile of female rabbits and the changes of these parameters during a 3-month period was determined for the basis of clinical diagnosis in veterinary medicine. Hematological parameters were assessed by collecting blood samples from six sexual mature New Zealand White does. The complete blood counts (CBC) including average white blood cell counts (WBC, x10³/µl), erythrocyte counts (RBC, x10⁶/µl), hematocrit (PCV, %), mean corpuscular volume (MCV, fl), platelet or thrombocyte counts (PLT, x10⁴/µl), hemoglobin concentration (Hb, g/dL), mean corpuscular hemoglobin (MCH, pg), and mean corpusular hemoglobin concentration (MCHC, g/dL) were analyzed using an automatic cell counter. As the age increased, significant weekly fluctuation was observed in serum biochemical components including MCHC, alanine aminotransferase (ALT), lactic dehydrogenase (LDH), creatine phosphokinase (CPK), creatinine (CR), uric acid (UA), amylase, total protein (TP) and minerals including Ca, P, Mg, Fe, Na, and Cl (P<0.05). No significant weekly fluctuation changes were found in PCV, Hb, MCV, MCH, PLT, the differential counts of leukocytes, and some other biochemical components (P>0.05). Further analysis of partial correlation coefficients of these blood and serum characteristics were found highly significant within the pairs of RBC-PCV (r = 0.89), MCV-MCH (r = 0.87), MCH-MCHC (r = 0.73), Het-WBC (r = 0.54), MCH-Glob (r = 0.40), and MCHC-Glob (r = 0.47). Negative correlations within pairs of RBC-MCV (r = -0.49), RBC-MCH (r = -0.54), RBC-MCHC (r = -0.37), Het-Lym (r = -0.94), and Lym-WBC (r = -0.48) were also observed. γGT was also negatively correlated to MCV (r = -0.49), MCH (r = -0.57), and MCHC (r = -0.42). In addition, globulin (Glob) was also negatively correlated to the A/G ratio (r = -0.83) and positively correlated to TG (r = 0.61). These data would be helpful in determining the normal from the pathological hemograms in New Zealand White rabbits for routine clinical diagnosis. © JADM 2009. All rights reserved.

Keywords: CBC, Hemogram, partial correlation coefficient, rabbit, serum

INTRODUCTION

For routine diagnosis in veterinary medicine, well-established hemograms are extremely important for the quick and accurate diagnosis of animal diseases, especially for the local or native species. We have completed hematological parameters in horses for this purpose (Ju et al., 1993; Fan et al., 2002). Little attention has been paid to those species with a relatively short life span such as rabbits. However, New Zealand White rabbits have been intensively used for many purposes such as meat consumption, pets, and model animals for scientific research (Foote and Carney, 2000). Also, due to the rapid growth of biotechnology and limited funding availability, transgenic rabbits are also chosen as a bioreactor for production of valuable pharmaceutical proteins (Ju et al., 1991; van der Hout et al., 2000). Therefore, the well-being, normally functioning physiology, as well as the diagnostic basal values for normal versus genetically modified animals become important. One of the factors influencing hematological parameters of rabbits may be its genetics. Therefore, we first established the hematological profiles of the
animal with the same genetic background, i.e., mature, female New Zealand White rabbits, being one of the most commonly used lab species, for clinical diagnosis and other comparisons.

MATERIALS AND METHODS

Animals, feeds and management

Six sexually matured (16 weeks old) New Zealand White female rabbits were used in this study. Each animal was kept in an individual pen, fed 120-150 g/day of pelleted feeds (crude protein >16%, crude fat >3%, crude fiber <20%, ash <16%, Ca=0.85% and P=0.75%), and free accessed to water supply.

Animals were kept in an artificially controlled light-dark regime with 14 h lighting and 10 h in dark. The temperature was maintained constant around 20-25 °C in a ventilation animal room. The use of animals was approved by the Institutional Animal Care and Use Committee (IACUC) of National Chung Hsing University.

Blood collection and treatments

Approximately 5 mL blood sample was collected from the ear veins or arteries of the does once a week for 13 consecutive weeks. Immediately after collection, each blood sample was separated into two parts. One part of the blood was completely mixed with anticoagulant (1% EDTA) for CBC analysis. The rest, without EDTA, was left standing at room temperature for 30-60 min for serum separation. A relative centrifugation force (RCF) of 1,800 g (3,000 rpm, 15 min) was used for serum separation. After centrifugation, the supernatant plasma/serum was aspirated carefully and transferred to Eppendorf tubes for storage. The serum was kept in the fridge (4 °C) overnight prior to analysis. Changes of biochemical components during the 13-week period are computed with MANOVA in the GLM. Partial correlation coefficients between two variables are computed with MANOVA in the GLM.

Analysis of blood cell and serum

The complete blood counts including erythrocyte or red blood cell counts (RBC, x10^6/μl), white blood cell counts (WBC, x10^3/μl), platelet counts (PLT, x10^4/μl), corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), mean hemoglobin concentration (Hgb, g/dL), mean corpuscular hemoglobin concentration (MCHC, g/dL), mean platelet volume (MPV, fl) and differential WBC counts were analyzed using an automatic cell counter (Sysmex F-800; Schalm et al., 1982; Ju et al., 1993).

Parameters for the biochemical assays including AST, ALT, LDH, CK, glucose, BUN, creatinine, cholesterol, etc., and mineral elements were examined by automatic analyzer (Ciba-Corning Express Plus Analyzer and 644 Na/K/Cl analyzer, Ciba-Corning Co., England) as described in our previous study (Ju et al., 1993).

Statistical analysis

The data were statistically analyzed using the general linear model (GLM) procedure of SAS (1988). The statistical analysis model is

\[ Y_{ij} = \mu + W_i + R_j + \varepsilon_{ij} \]

where \( Y_{ij} \) are dependent variable, overall mean, week effect, individual rabbit effect and random error, respectively. Differences between the means by week are measured and compared using the least square means. Extreme significance, high significance and significance are declared at \( P < 0.001 \), \( P < 0.01 \) and \( P < 0.05 \), respectively, unless specified otherwise.

Partial correlation coefficients between two variables are computed with MANOVA in the GLM.

RESULTS

Changes of biochemical components during the 13-week period

All the CBC parameters, except the MCHC, were not significantly different over the period. Biochemical parameters including ALT, glucose, BUN, γGT, Chol, TG, ALB, lipase, and total bilirubin (T-bil) had no significant changes during this period. All the mineral levels analyzed, except potassium (K), changed significantly over time. Only the biochemical components showing sig-

### Table 1. Significant changes of serum biochemical components of female New Zealand White rabbits over a 13-week period of blood collection.

<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>P levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT, U/L</td>
<td>18.4</td>
<td>19.6</td>
<td>12.2</td>
<td>18.9</td>
<td>17.4</td>
<td>22.4</td>
<td>26.5</td>
<td>24.6</td>
<td>27.2</td>
<td>29.5</td>
<td>28.8</td>
<td>27.8</td>
<td>35.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>LDH, U/L</td>
<td>250</td>
<td>276</td>
<td>147</td>
<td>162</td>
<td>204</td>
<td>166</td>
<td>256</td>
<td>127</td>
<td>241</td>
<td>202</td>
<td>181</td>
<td>198</td>
<td>185</td>
<td>0.0103</td>
</tr>
<tr>
<td>CPK, U/L</td>
<td>189</td>
<td>456</td>
<td>438</td>
<td>339</td>
<td>706</td>
<td>475</td>
<td>684</td>
<td>409</td>
<td>678</td>
<td>487</td>
<td>560</td>
<td>538</td>
<td>407</td>
<td>0.0051</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.78</td>
<td>0.73</td>
<td>1.44</td>
<td>1.47</td>
<td>1.53</td>
<td>1.45</td>
<td>1.52</td>
<td>1.43</td>
<td>1.47</td>
<td>1.52</td>
<td>1.60</td>
<td>1.55</td>
<td>1.56</td>
<td>0.0001</td>
</tr>
<tr>
<td>TP, g/dL</td>
<td>6.11</td>
<td>5.71</td>
<td>6.06</td>
<td>5.90</td>
<td>6.15</td>
<td>6.07</td>
<td>6.12</td>
<td>6.15</td>
<td>6.53</td>
<td>6.47</td>
<td>6.38</td>
<td>6.25</td>
<td>6.30</td>
<td>0.0474</td>
</tr>
<tr>
<td>Glob, g/dL</td>
<td>2.28</td>
<td>1.87</td>
<td>2.46</td>
<td>2.67</td>
<td>2.63</td>
<td>2.53</td>
<td>2.42</td>
<td>2.67</td>
<td>2.73</td>
<td>2.90</td>
<td>2.57</td>
<td>2.67</td>
<td>2.62</td>
<td>0.0002</td>
</tr>
<tr>
<td>UA, mg/dL</td>
<td>0.15</td>
<td>0.15</td>
<td>0.95</td>
<td>0.72</td>
<td>0.85</td>
<td>0.87</td>
<td>0.83</td>
<td>0.77</td>
<td>0.87</td>
<td>1.05</td>
<td>1.12</td>
<td>0.88</td>
<td>0.94</td>
<td>0.0001</td>
</tr>
<tr>
<td>Amylase, U/L</td>
<td>175</td>
<td>184</td>
<td>328</td>
<td>325</td>
<td>417</td>
<td>320</td>
<td>336</td>
<td>305</td>
<td>303</td>
<td>332</td>
<td>325</td>
<td>322</td>
<td>339</td>
<td>0.0001</td>
</tr>
<tr>
<td>A/G</td>
<td>1.75</td>
<td>2.11</td>
<td>1.47</td>
<td>1.28</td>
<td>1.36</td>
<td>1.43</td>
<td>1.47</td>
<td>1.35</td>
<td>1.43</td>
<td>1.24</td>
<td>1.49</td>
<td>1.36</td>
<td>1.43</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Significant changes over the 13-week period are presented in Table 1. ALT levels increased approximately starting from the fifth week and kept increasing up to the 13th week. On the contrary, LDH levels reduced from the second week and then recovered at the end of this period. The CPK values were, basically, increased from the 2nd week and peaked at the middle of the entire period. Other parameters including creatinine, total protein, globulin, uric acid, and amylase concentrations, all increased at the first week, but only the A/G ratios reduced after the onset of blood collection (Table 1).

Serum mineral concentrations revealed similar trends as those of biochemical parameters. Calcium concentrations in serum reduced after the second week. Sodium and chloride concentrations also slightly reduced at the first week and then returned to the normal levels thereafter (Table 2). Other serum minerals including phosphorus, magnesium, and iron, all increased after the first week of blood collection (Table 2).

**Correlation coefficients (r) between hematological parameters**

No significant correlation existed between the majority (> 2/3) of the blood parameters. Only two parameters showing significant correlation coefficients (r) at different significant levels, i.e., P<0.05 (*), P<0.01 (**), and P<0.001 (** *), are selectively presented in Tables 3-8. Briefly, the greatest correlation coefficients existed between RBC and PCV (r=0.89; Table 3). RBC with Hb and Hb with PCV also highly correlated to each other with r = 0.79 and 0.81, respectively. Similarly, a close relationship is also observed between MCH and MCV (r = 0.87, Table 3) or MCHC (r = 0.73, Table 4). Total protein (TP) concentrations also exhibited good correlations with serum albumin (ALB, r = 0.57) and globulin (Glob, r = 0.61, Table 8). Many other significant correlation coefficients (positive and negative) obtained from these calculations are presented in those self-explanatory tables.

**DISCUSSION**

Several factors including age (Sato et al., 1978), feedstuffs (Kerr and Snow, 1982), and anesthesia (Steffey et al., 1980) have been known to affect the concentrations of blood cells in circulation, especially when animals are under stressful or exciting conditions. The peripheral concentrations of RBC and some related parameters, rise drastically during or immediately after

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### Table 2. Significant changes of serum mineral compositions of female New Zealand White rabbits over a 13-week period of blood collection.

<table>
<thead>
<tr>
<th>Week</th>
<th>Ca (mg/dL)</th>
<th>P (mg/dL)</th>
<th>Mg (mg/dL)</th>
<th>Fe (mg/dL)</th>
<th>Na (mmol/L)</th>
<th>Cl (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16.2</td>
<td>2.15</td>
<td>0.97</td>
<td>133</td>
<td>141</td>
<td>109</td>
</tr>
<tr>
<td>1</td>
<td>16.5</td>
<td>1.88</td>
<td>1.38</td>
<td>161</td>
<td>139</td>
<td>109</td>
</tr>
<tr>
<td>2</td>
<td>12.1</td>
<td>3.80</td>
<td>2.25</td>
<td>139</td>
<td>140</td>
<td>110</td>
</tr>
<tr>
<td>3</td>
<td>12.1</td>
<td>3.72</td>
<td>2.38</td>
<td>160</td>
<td>142</td>
<td>109</td>
</tr>
<tr>
<td>4</td>
<td>12.4</td>
<td>4.07</td>
<td>2.45</td>
<td>171</td>
<td>142</td>
<td>110</td>
</tr>
<tr>
<td>5</td>
<td>12.5</td>
<td>4.27</td>
<td>2.52</td>
<td>169</td>
<td>138</td>
<td>108</td>
</tr>
<tr>
<td>6</td>
<td>12.2</td>
<td>3.50</td>
<td>2.15</td>
<td>172</td>
<td>139</td>
<td>109</td>
</tr>
<tr>
<td>7</td>
<td>12.8</td>
<td>4.05</td>
<td>2.40</td>
<td>184</td>
<td>141</td>
<td>110</td>
</tr>
<tr>
<td>8</td>
<td>12.5</td>
<td>4.23</td>
<td>2.30</td>
<td>177</td>
<td>138</td>
<td>110</td>
</tr>
<tr>
<td>9</td>
<td>12.6</td>
<td>4.07</td>
<td>2.58</td>
<td>172</td>
<td>143</td>
<td>115</td>
</tr>
<tr>
<td>10</td>
<td>12.4</td>
<td>4.32</td>
<td>2.37</td>
<td>178</td>
<td>143</td>
<td>111</td>
</tr>
<tr>
<td>11</td>
<td>12.5</td>
<td>4.43</td>
<td>2.24</td>
<td>180</td>
<td>142</td>
<td>111</td>
</tr>
<tr>
<td>12</td>
<td>12.4</td>
<td>3.95</td>
<td>2.43</td>
<td>164</td>
<td>143</td>
<td>111</td>
</tr>
</tbody>
</table>

**Table 3. Partial correlation coefficients (r) of red blood cell numbers (RBC), packed cell volume (PCV), and mean corpuscular cell volume (MCV) with other hematological parameters.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RBC</th>
<th>PCV</th>
<th>MCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.89</td>
<td>-0.49</td>
<td>-0.54</td>
</tr>
<tr>
<td>Ca</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>P</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Mg</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>P level</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>


* : P<0.05. ** : P<0.01. *** : P<0.001.
Changes of biochemical compositions

Most of the biochemical parameters investigated showed constant values but, some selected biochemical components of the serum exhibited prominent changes during this period of study (Tables 1 and 2).

AST and ALT: These two plasma enzymes are widely used indicators for clinical diagnosis of hepatic functions in humans. Liver damages and exercise of sufficient intensity will result in an elevation of the concentrations of these and related enzymes (Critz, 1966; Henley et al. 1960; Griffiths, 1966)

It is of value for studying their relationship between resting plasma enzymes and physical fitness in man (Hunter and Critz, 1971). The above information may also be applicable to rabbit system, because some plasma enzymes, such as ALT, are detectable for diagnosis of hepatic functions in small animals and primates. The livers of larger species including horses, cattle, and sheep contain only insignificant amounts of ALT (Cornelius, 1989).

In this study, the ALT, but not the AST levels, significantly increased from 18.4 U/L at the beginning to 28.35 U/L at the end of the sampling period (Table 1). Generally, species with the highest tissue levels of GOT and glutamic-pyruvic transaminase (GPT) have the highest plasma levels (Zimmerman et al., 1968). Unfortunately, no tissue levels of enzymatic activity were assayed in this study.

LDH: Marked increases in plasma or serum lactic dehydrogenase activity have been reported in untrained rats subjected to different types and intensity of exercise (Altland et al., 1968). The rise in LDH activity after exercise is due to the release of LDH from the tissues into the circulatory system (Doty et al., 1971). Interestingly, the LDH levels of these rabbits exhibited no changes in the first 2 weeks and then significantly decreased in later days (Table 1). Although the reason is not clear, it is possible that variation in the activity of this enzyme might reflect different degrees of physical stress of animals during blood collection. The influences of animal ages might be involved, which requires more study.

CK or CPK: The disturbance of CPK system has been observed in muscular damage and diseases of many organs. Increased levels of CPK activity in the blood can serve as a marker of skeletal muscle disease (Shivers and Atkinson, 1984), myocardial infarction (Nagai et al., 1983; Weinberger et al., 1989), or exercise-induced muscular damages (Ebbeling and Clarkson, 1983; Weinberger et al., 1989). In our study, blood levels of CPK in the rabbits were lower in the first week of blood collection, but increased to 2-3 folds from the second week onwards (Table 1), which might reflect the muscular damage caused by increase muscular activity during animal handling. Unexpectedly, the consecutive blood collection program resulted in delay or prolong of CPK levels up to one week, which was longer than that of post-exercise responses in humans. Given that rabbits are sensitive and easily stressed species, their blood CPK levels might not be a good indicator for measuring their well-being unless a better or stress-less handling technique is available.

### Table 4. Partial correlation coefficients (r) of hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) with each of the other hematological parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hb</th>
<th>MCH</th>
<th>MCHC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBC</td>
<td>PCV</td>
<td>MCH</td>
</tr>
<tr>
<td>r</td>
<td>0.74</td>
<td>0.81</td>
<td>0.73</td>
</tr>
<tr>
<td>P level</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

RBC: red blood cells or erythrocytes.  
PCV: packed cell volume.  
MVC: mean corpuscular volume.  
MCHC: mean corpuscular hemoglobin.  
γ-GT: gamma-glutamyl transferase.  
P: phosphorus.  
Glob: globulin.  
A/G: albumin/globulin ratio.  
CPK: creatine phosphokinase.  
Ca: calcium.  
P: phosphorus.  
PLT: platelet.  
Mg: magnesium.

* : P<0.05.  
**: P<0.01.  
*** : P<0.001.  
**** : P<0.0001.
Blood urea nitrogen (BUN) and creatinine (CR): The concentration of BUN is a direct indication of the renal or kidney function. Elevation of BUN levels could adversely affect many cellular processes, such as Na⁺-K⁺ ATPase, Na-K⁺ Cl⁻ cotransporter (Lim et al., 1995), activity of inducible nitric oxide synthase (NOS, Moeslinger et al., 1999), and other enzymatic activities (Rajagopalan et al., 1963). Normal values of CR are 0.5-1.2 mg/dL in humans, 1.0-2.7 mg/dL in pigs, and 1.2-1.9 mg/dL in horses, and sheep (Kaneko, 1989; Tietz, 1976) and rabbits.

Total proteins (TP), albumins and globulins: Total protein concentrations of biological fluids, such as plasma, lymph, and follicular fluids, are important in studies of vascular endothelial or cell membrane permeability. Albumin is the major osmotic proteins, but the concentration of non-albumin (globulin and fibrinogen) also influences osmotic pressure of body fluids (Peterson and Tate, 1993; Ahlqvist, 2004). No significant changes were found in albumin concentration, but significant variations of globulin and TP were observed during the sampling period. The major factors causing this variation could be ages of the animals.

Mineral elements: Some major mineral components of serum significantly changed over time with unknown reasons. (Table 2). The trends for Ca concentration decreased from the third week but for P, Mg, and Fe concentrations increased from the second week throughout the period of blood collection over time. Potassium has been known as one of the major intracellular cations and sodium is the major extracellular component in somatic cells. In human blood cells, 105 mmol/L (4106 mg/L) of potassium normally exists, which is approximately 23 times higher than that in extracellular fluids (Tietz, 1976). Consistent changes of these minerals in the serum could be indicative for a stressful condition or a sudden physiologic alteration to the animals.

Significant correlation coefficients exist between parameters

Most of the parameters show no significant relationships between one and the other (data not shown). However, in addition to the known closely related ones, some other parameters also show significant to highly significant correlation in this study (Table 3). As expected, between some parameters such as RBC-PCV, RBC-Hb, MCV-MCHC, MCH-MCHC, ALT-AST, AST-LDH, TP-ALB, and TP-Glob have extremely high (P<0.001) correlation coefficients ranging from 0.53 to 0.89. These information confirmed that a similar trend existed between New Zealand White does and other species from other studies. In contrast, some unexplainable negative correlations such as PCV with Fe (Table 3), MCV with Ca, P, or Mg (Table 3), Ca with MCH or MCHC (Table 4), PLT with LYMH, and CHOL with Ca or Mg and positive correlations including CR-CHOL, TG-Ca, and ALB-lipase were found. In addition, an in vitro analysis showed that total bilirubin may significantly stimulate amylase release in a concentration and time-dependent manner (Hirohata et al., 2002). However, we did not find a significant relationship between these two parameters (data not shown).

We have conducted an adequate analysis on the changes of major hematological and biochemical parameters in adult female rabbits over a 3-month period. Correlation coefficients between some of these blood components and mineral elements are also firstly reported in this study. Although the precise basis for some observations may not be available currently, these data are of value in interpreting clinical diseases and for future study in New Zealand White rabbits.

Acknowledgments

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