

Biochemical reference intervals for homing pigeons in China

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ABSTRACT As indicators of diseases, blood biochemical values play a crucial role in clinical practice and assessments of animals' health condition. The rising population of homing pigeons in China has prompted needs for reliable blood biochemical reference intervals. Therefore, the aim of this study was to establish biochemical reference intervals for homing pigeons. Heparinized whole blood samples obtained from 77 clinically healthy pigeons were analyzed by Zoetis

Abaxis VetScan VS2 with VetScan Avian/Reptilian Profile Plus Rotor. Reference intervals for pigeons were computed by Excel with Reference Value Advisor V2.1. The statistical analysis performed by SPSS program revealed correlations between biochemical analytes. Effects of sex and age and validity of published reference intervals were also discussed. The present results serve as a useful guide to broaden the scope of pigeon breeding industry.

Key words: avian, homing pigeon, biochemistry, reference interval

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INTRODUCTION

As one of the most popular and widespread birds in China, in accordance with economic use, pigeons can be divided into homing pigeon, meat pigeon, and show pigeon. Pigeon racing developed on the basis of pigeons' homing instinct. Pigeon enthusiasts are looking forward to forming a whole system including breeding, management, and training. Homing pigeons do have certain commercial value, but little is known about their health status. Then, how to prevent and treat diseases of pigeons has become an urgent issue to be solved clinically. Blood biochemical indexes are essential for assessment of organ system function, such as liver function and renal function. They also have predictive value in disease evolution and prognosis of avian patients. Previous studies focus on feeding management of pigeons, extending to infectious diseases and parasites. Few studies began to pay attention to influences on blood physiology of pigeons (Xia, et al., 2019). For a correct interpretation of these biochemical variables, reliable reference values for pigeons are required. However, because of region and time, currently available data are unable to meet

the current demand and require more practical biochemical reference intervals for pigeons.

Therefore, this study aimed to define reference ranges for biochemical analytes in clinically healthy homing pigeons in China, which will contribute to monitoring of health condition and diagnosis of disease.

MATERIALS AND METHODS

Pigeons

Seventy-seven homing pigeons were included in this study, which comprised young (<1 yr of age, 20), adult (1–5 yr old, 22 females and 26 males), and old (>5 yr of age, 3 females and 6 males) pigeons. Roughly assessment and selection of healthy pigeons were based on their behaviors and breeders' experience. Healthy pigeons appear active and alert; on the contrary, diseased pigeons always present slow response and loss of appetite. Further pre-examination included parasitic examination, rapid test strips, and pathological anatomy. Pigeons were fed a normal diet and appeared clinically normal at the time of sampling.

Blood Collection and Analysis

Blood samples were collected by venipuncture of the wing vein using 1-ml syringes and 25-gauge needles. Approximately 0.5 mL blood was sampled for each

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pigeon in lucid state and transferred into heparin-containing tubes immediately. In accordance with the manufacturer's recommendations, Zoetis Abaxis VetScan Avian/Reptilian Profile Plus Rotor was used to quantitatively measure the following variables: albumin (ALB), aspartate aminotransferase (AST), bile acids, total calcium (CA), creatine kinase (CK), glucose (GLU), potassium (K), sodium (NA), phosphorus (PHOS), total protein (TP), and uric acid (UA). In addition, globulin (GLOB) was a calculated value. Samples were analyzed within 1 h after collection and maintained at room temperature.

This study was carried out in accordance with the Guiding Principles for the Care and Use of Laboratory Animals of Zhejiang University. The protocol was approved by the Animals Care Ethics Committee of Zhejiang University (ZJU20160377).

Statistical Analysis

Outliers were examined by Tukey's tests, consulting the recommends of Clinical and Laboratory Standards Institute C28-A3 that retaining the values rather than deleting unless they were known to be aberrant observations (Horowitz GL, 2010).

Descriptive statistics including sample size, mean, median, SD, minimum, and maximum values, confidence intervals (CI), and biochemistry reference intervals for pigeons were computed in Microsoft Excel 2016 using Reference Value Advisor V2.1 by nonparametric methods (Geffre, et al., 2011), with the guidance of American Society for Veterinary Clinical Pathology reference interval guidelines (Friedrichs, et al., 2012). The CI of the limits were determined using a bootstrap method. Correlations between measured biochemical analyte estimates were evaluated by 2-tailed Pearson test using SPSS program, with statistical significance set at $P < 0.01$. Sexual and age-related differences for biochemical analytes were also analyzed by one-way ANOVA or Mann-Whitney U test, where appropriate. Moreover, validation of existing blood biochemical reference intervals in *Exotic Animal Formulary* for pigeons (*Columba livia*) (Carpenter, 2015) and reference ranges

of avian for VetScan VS2 was examined in accordance with the guidelines (Horowitz GL, 2010).

RESULTS

Descriptive statistics (mean, SD, minimum, maximum, 90% CI for lower and upper limit) and biochemical reference intervals for homing pigeons are shown in Table 1. All sampled pigeons appeared to be in good health. Seventy-seven blood samples were available for analysis. Outliers identified and removed were AST (1 outlier excluded), CK (2), and CA (9). Almost all bile acid values (73 of 77) fell below a lower limit of dynamic range of the rotor (35–200 $\mu\text{mol/L}$), which could not be calculated and was no longer discussed in this article.

Correlations Between Biochemical Analytes

Relatedness analyses revealed that most biochemical analytes were unrelated (Table 2). Statistically significant positive correlation ($P < 0.01$) was observed between AST and NA ($r = 0.429$), CK and K ($r = 0.372$), UA and TP ($r = 0.366$), UA and GLOB ($r = 0.430$), GLU and TP ($r = 0.330$), GLU and ALB ($r = 0.317$), CA and GLOB ($r = 0.449$), CA and K ($r = 0.346$), TP and ALB ($r = 0.759$), and TP and GLOB ($r = 0.578$). Statistically significant negative correlation ($P < 0.01$) was observed between GLU and PHOS ($r = -0.338$).

Effects of Sex and Age

The comparisons of blood biochemical analytes between male and female homing pigeons are presented in Table 3 and Figure 1. Significantly different analytes between male and female groups included higher AST, ALB, and NA, with lower UA and PHOS values in male than in female.

Table 4 lists biochemical analytes that determined as significantly different in age-related groups. Creatine kinase decreased significantly with age, whereas adult pigeons had statistically significant higher level in UA, GLU, TP, and ALB and lower level in PHOS and K

Table 1. Blood biochemical reference intervals for healthy homing pigeons.

Analyte	SI units	n	Mean	Median	SD	Min	Max	RI	90%CI for lower limit	90%CI for upper limit
AST	U/L	76	108.8	111.0	22.8	66	166	67.9 – 163.2	66.0 – 74.9	148.5 – 166.0
CK	U/L	75	378.4	302.0	195.7	151	888	153.9 – 872.7	151.0 – 187.0	787.3 – 888.0
UA	$\mu\text{mol/L}$	77	229.9	181.0	129.0	57	595	62.7 – 577.9	57.0 – 65.9	452.8 – 595.0
GLU	mmol/L	77	19.12	18.8	1.10	17.3	21.7	17.40 – 21.61	17.30 – 17.80	21.10 – 21.70
CA	mmol/L	68	2.522	2.515	0.155	2.19	3.14	2.248 – 2.973	2.190 – 2.328	2.790 – 3.140
PHOS	mmol/L	77	1.105	1.090	0.277	0.6	1.83	0.629 – 1.640	0.600 – 0.669	1.521 – 1.830
TP	g/L	77	31.6	31.0	3.8	20	40	23.8 – 40.0	20.0 – 26.9	38.1 – 40.0
ALB	g/L	77	29.5	30.0	2.6	20	35	23.8 – 35.0	20.0 – 25.0	33.1 – 35.0
GLOB	g/L	77	2.3	1.0	2.7	0	10	0.0 – 9.1	0.0 – 0.0	8.1 – 10.0
K	mmol/L	77	4.39	4.40	0.42	3.4	5.4	3.50 – 5.31	3.40 – 3.79	5.01 – 5.40
NA	mmol/L	77	148.2	149.0	3.9	135	155	137.9 – 155.0	135.0 – 141.9	154.0 – 155.0

Abbreviations: ALB, albumin; AST, aspartate aminotransferase; CA, total calcium; CK, creatine kinase; GLOB, globulin; GLU, glucose; K, potassium; NA, sodium; PHOS, phosphorus; RI, reference interval; TP, total protein; UA, uric acid.

Table 2. Estimates of correlations (r) between biochemical analytes (above diagonal) and their corresponding P values (below diagonal).

	AST	CK	UA	GLU	CA	PHOS	TP	ALB	GLOB	K	NA
AST		0.183	-0.019	0.182	-0.016	-0.158	0.124	0.161	0.048	0.062	0.429
CK	0.142		-0.291	-0.169	0.110	0.282	-0.149	-0.109	-0.066	0.372	-0.019
UA	0.878	0.018		-0.036	0.093	-0.018	0.366	0.105	0.430	-0.032	-0.210
GLU	0.143	0.176	0.773		-0.079	-0.338	0.330	0.317	0.081	-0.299	0.297
CA	0.898	0.378	0.460	0.530		0.259	0.307	0.077	0.449	0.346	0.263
PHOS	0.321	0.022	0.889	0.006	0.036		-0.141	-0.276	0.161	0.182	-0.282
TP	0.204	0.233	0.003	0.007	0.012	0.260		0.759	0.578	-0.262	0.126
ALB	0.195	0.384	0.400	0.010	0.537	0.025	< 0.001		-0.065	-0.245	0.150
GLOB	0.704	0.597	< 0.001	0.516	< 0.001	0.197	< 0.001	0.606		-0.056	0.044
K	0.622	0.002	0.797	0.015	0.004	0.143	0.033	0.047	0.654		0.131
NA	< 0.001	0.878	0.090	0.015	0.033	0.022	0.313	0.230	0.728	0.294	

Bold numbers indicate significant ($P < 0.01$) correlations between the 2 variables.

Abbreviations: ALB, albumin; AST, aspartate aminotransferase; CA, total calcium; CK, creatine kinase; GLOB, globulin; GLU, glucose; K, potassium; NA, sodium; PHOS, phosphorus; TP, total protein; UA, uric acid.

than subadults. In addition, biochemical values of old pigeons fell in between them, except for CK. Figure 2 shows the comparison in age-related groups described previously.

Validity of Existing Intervals

Published biochemical reference intervals in *Exotic Animal Formulary* for pigeons (*C. livia*) and reference ranges of VetScan VS2 for avian were tested for validation. Table 5 revealed their applicability to homing pigeons demographic in this study. Of 11, 8 existing reference intervals in *Exotic Animal Formulary* were not suitable, whereas most values lay within the reference ranges of VetScan VS2 for avian.

DISCUSSION

Biochemical parameters are often used to assess health status of avian patients, accelerating the process of diagnosis and treatment. Selected clinical analytes have been determined in terms of their means and SD without respective reference ranges (Chao, et al., 2015; Xia, et al., 2019), which are more fundamental.

Abaxis Vetscan VS2 possesses several unique features and advantages, such as simple operation, automatic quality control, compact size, rapid results, and wide range of applications. Otherwise, it offers reliable values by using a relatively small sample size (Greenacre, et al., 2008). Taking these into account, extensive studies on

reference ranges have been performed with Vetscan VS2, especially for avian species (Hamilton, et al., 2016; Board, et al., 2018). Therefore, this study adopted a Vetscan analyzer to establish the biochemical reference intervals.

Compared with the published references, values of CK, UA, and GLU were found to lie within the reference ranges described in *Exotic Animal Formulary*, other analytes fell out of the ranges. This could be interpreted as differences in distribution and population characteristics. It should be noted that most values lay within the reference ranges of VetScan VS2 for avian, but the ranges were too wide to assure their accuracy and specificity. Moreover, its ranges were carried out on the basis of 305 avian samples including cockatoos, cockatiels, conures, amazons, macaws, African greys, and so on. Therefore, it can be concluded that there are no reference intervals available for homing pigeons in China with mixed sex and age.

There were statistically significant correlations between the biochemical parameters but not strong: most values showed low correlation. The correlation coefficients between TP and ALB and between TP and GLOB were medium to high, which is undoubted.

Aspartate aminotransferase concentrations were significantly higher in male pigeons than in females possibly owing to a higher physical activity level and larger body mass (Scope, et al., 2005). Females showed higher UA concentrations than males, as previously reported in osprey, could be related to higher food need during female nestlings (Muriel, et al., 2013). The

Table 3. Comparison of biochemical values that were significant different ($P < 0.05$) between male and female pigeons.

Analyte	Units	Male			Female		
		n	Mean ± SD	RI	n	Mean ± SD	RI
AST	U/L	31	119.58 ± 23.56	110.94 – 128.22	25	97.40 ± 21.83**	88.39 – 106.41
UA	µmol/L	32	212.69 ± 109.52	173.20 – 252.17	25	320.16 ± 126.89**	267.78 – 372.54
PHOS	mmol/L	32	0.94 ± 0.23	0.86 – 1.02	25	1.19 ± 0.28**	1.08 – 1.31
ALB	g/L	32	30.84 ± 2.13	30.08 – 31.61	25	28.96 ± 2.95*	27.74 – 30.18
NA	mmol/L	32	149.97 ± 4.00	148.53 – 151.41	25	146.04 ± 3.60**	144.55 – 147.53

* $P < 0.05$, ** $P < 0.01$.

Abbreviations: ALB, albumin; AST, aspartate aminotransferase; NA, sodium; PHOS, phosphorus; RI, reference interval; UA, uric acid.

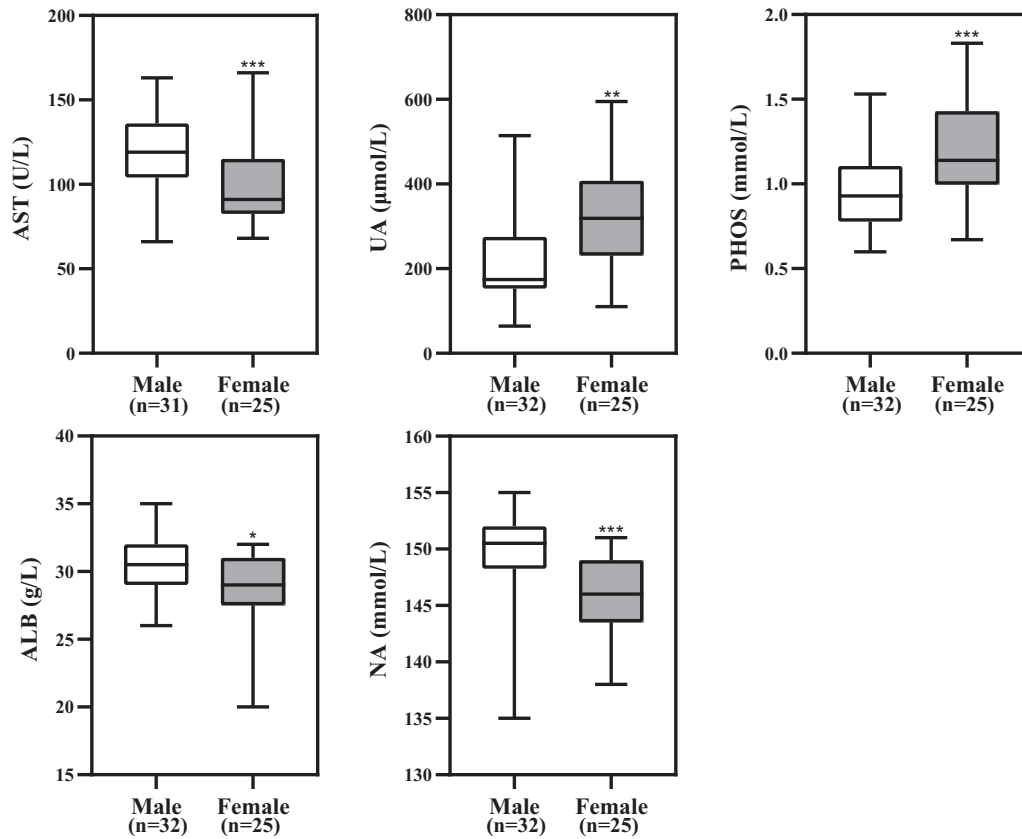


Figure 1. Box and whiskers diagram for the biochemical analytes that showed significant differences between male and female pigeons. The box represents the upper and lower quartiles and the center line represents the median. The whiskers below and above the box indicate the minimum and maximum range. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviations: ALB, albumin; AST, aspartate aminotransferase; NA, sodium; PHOS, phosphorus; UA, uric acid.

electrolytes involve in water homeostasis, whose levels might depend on diets. Consequently, the overall variation is limited.

In general, organ function degeneration and metabolism slowing of old pigeons explained the value

variation in this group. Notable differences mainly existed between young and adult pigeons. Significant age-related decrease was found in CK, similar to what has been reported (Peruffo, et al., 2016). It is known that CK concentrations are related to muscle activity

Table 4. Comparison of biochemical analytes that showed significant difference ($P < 0.05$) among young, adult and old pigeons.

Analyte	Units	Age group	n	Mean \pm SD	RI
CK	U/L	Young	18	562.72 \pm 204.95	460.80 – 664.64
		Adult	48	334.40 \pm 160.23	287.87 – 380.92
		old	9	244.78 \pm 77.03	185.57 – 303.99
UA	µmol/L	Young	20	131.05 \pm 58.45	103.69 – 158.41
		Adult	48	273.06 \pm 132.51	234.58 – 311.54
		old	9	189.22 \pm 72.26	133.68 – 244.76
GLU	mmol/L	Young	20	18.49 \pm 0.84	18.10 – 18.88
		Adult	48	19.43 \pm 1.13	19.10 – 19.76
		old	9	18.87 \pm 0.76	18.28 – 19.45
PHOS	mmol/L	Young	20	1.26 \pm 0.20	1.16 – 1.35
		Adult	48	1.05 \pm 0.29	0.96 – 1.13
		old	9	1.08 \pm 0.27	0.88 – 1.29
TP	g/L	Young	20	28.45 \pm 1.61	27.70 – 29.20
		Adult	48	33.25 \pm 3.42	32.26 – 34.24
		old	9	30.00 \pm 4.12	26.83 – 33.17
ALB	g/L	Young	20	28.05 \pm 1.91	27.16 – 28.94
		Adult	48	30.44 \pm 2.38	29.75 – 31.13
		old	9	27.78 \pm 3.15	25.35 – 30.20
K	mmol/L	Young	20	4.62 \pm 0.32	4.47 – 4.77
		Adult	48	4.28 \pm 0.44	4.15 – 4.41
		old	9	4.47 \pm 0.34	4.20 – 4.73

Abbreviations: ALB, albumin; CK, creatine kinase; GLU, glucose; K, potassium; PHOS, phosphorus; RI, reference interval; TP, total protein; UA, uric acid.

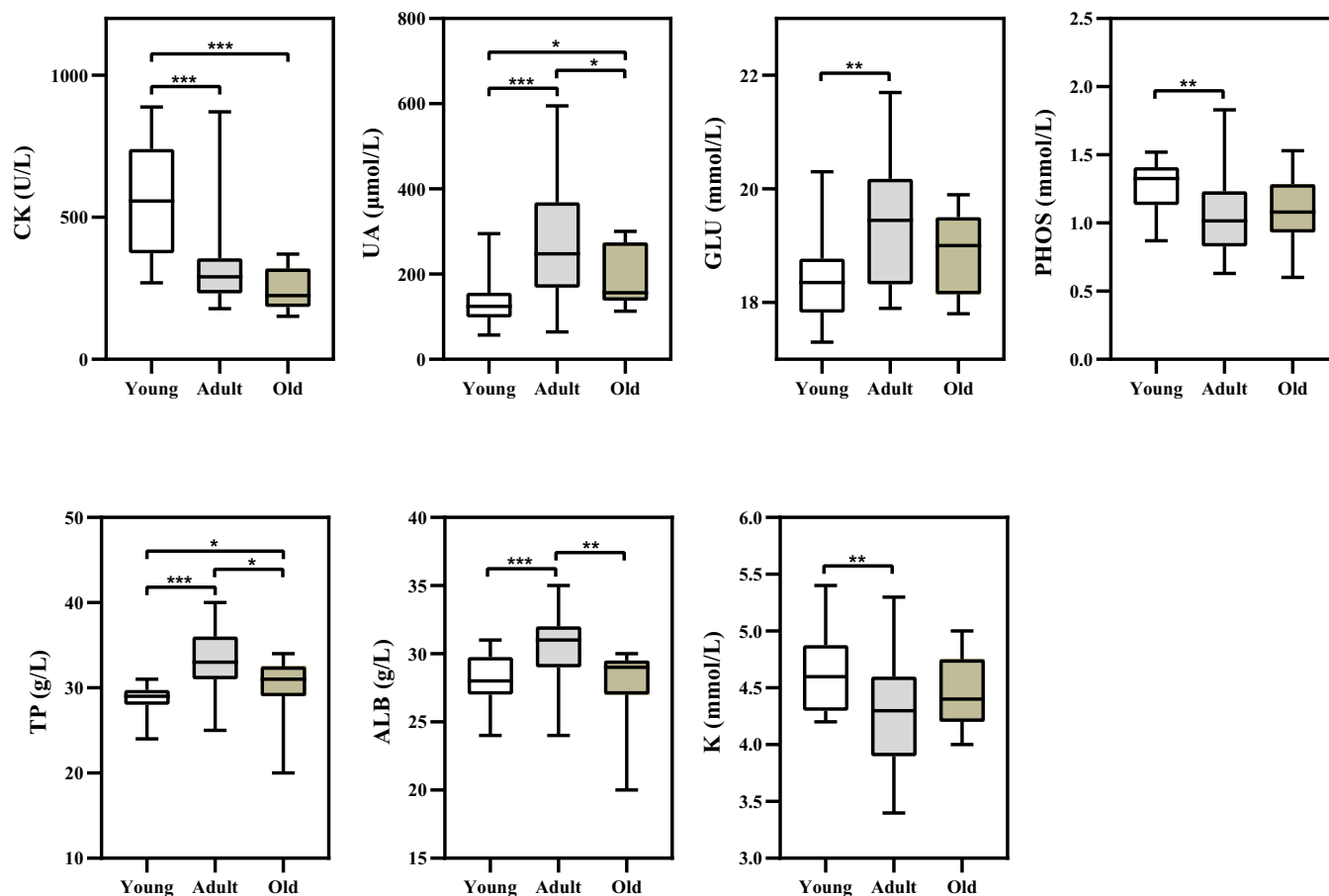


Figure 2. Box and whiskers diagram for the biochemical analytes that showed significant differences among pigeons of age groups. The box represents the upper and lower quartiles and the center line represents the median. The whiskers below and above the box indicate the minimum and maximum range. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Abbreviations: ALB, albumin; CK, creatine kinase; GLU, glucose; K, potassium; PHOS, phosphorus; TP, total protein; UA, uric acid.

and stress of birds. Blood GLU levels varied apparently between adult and young pigeons. Adult pigeons had significantly higher TP than young pigeons, which has been observed in other avian species (Ammersbach, et al., 2015). The higher PHOS concentrations in young pigeons are consistent with previous study on other avian species (Peruffo, et al., 2016). This can be interpreted as increased intestinal uptake and declined renal

excretion, which may promote bone growth (Jones, et al., 2014). Likewise, K concentrations decrease in the adults could be regarded as an age-related phenomenon. Similar findings in previous studies held that it possibly relate to rising physical activity before fledging (Muriel, et al., 2013). Different levels in TP and UA between young and adult pigeons may result from different nutritional status rather than age difference (Villegas, et al., 2004; Barbara, et al., 2017).

Table 5. Validation study of existing blood biochemical reference intervals for pigeons.

Variable	SI units	RI ¹	Validation	RI ²	Validation
AST	U/L	45 – 123	No	107 – 481	No
CK	U/L	110 – 480	Yes	69 – 524	Yes
UA	μmol/L	149 – 768	Yes	149 – 791	Yes
GLU	mmol/L	12.9 – 20.5	Yes	12.4 – 21.6	Yes
CA	mmol/L	1.9 – 2.6	No	1.95 – 2.78	Yes
PHOS	mmol/L	0.58 – 1.32	No	0.39 – 2.36	Yes
TP	g/L	21 – 33	No	21 – 47	Yes
ALB	g/L	13 – 22	No	17 – 33	Yes
GLOB	g/L	6 – 12	No		
K	mmol/L	3.9 – 4.7	No	3.0 – 5.7	Yes
NA	mmol/L	141 – 149	No	137 – 151	No

Abbreviations: ALB, albumin; AST, aspartate aminotransferase; CA, total calcium; CK, creatine kinase; GLOB, globulin; GLU, glucose; K, potassium; NA, sodium; PHOS, phosphorus; RI, reference interval; TP, total protein; UA, uric acid.

¹Exotic Animal Formulary for pigeon (*Columba livia*).

²VetScan VS2 for avian.

In conclusion, this study proposed more reliable and recent blood biochemical reference intervals for pigeons in China, which may serve as a critical baseline information for health status assessment and disease diagnosis. These reference intervals should be interpreted with caution for clinical decision making, as potential factors may lead to variations.

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