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Effects of Age and Season on Blood Parameters of Domesticated Giant Pandas

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Abstract: The giant panda (*Ailuropoda melanoleuca*) is one of the most endangered species of land mammals and has significant academic research value worldwide. The evaluation of hematological parameters in giant pandas is important for assessing the physiological status of animals. This study collected blood samples from 12 healthy giant pandas from September 2018 to August 2020 under non-anesthetic conditions. The samples were then analyzed for basic blood indices and blood rheology, and normal values for hematological parameters and blood rheology under non-anesthetic conditions in giant pandas in Beijing area were obtained. The study confirmed the influence of age and season on hematological and biochemical parameters in captive giant pandas: hematological morphology, blood rheology, and serum enzymes showed seasonal variations, while age affected the changes in serum enzymes, serum protein content, and serum metabolite content. There are significant differences in blood indicators among different age groups of giant pandas, indicating variations in the overall metabolic processes and differences among pandas of different age groups. Seasonal variations showed a regulatory effect on blood rheology parameters and a negative effect on blood viscosity. Further research should be conducted to investigate whether factors such as season and climate cause environmental stress in captive giant pandas. The results obtained in this study aim to better protect the stability of the giant panda population and provide references for the appropriate feeding and medical care of captive giant pandas.

Keywords: giant pandas; seasonal; age; blood indices; hemorheology

1. Implications

The giant panda is one of the most endangered species of all land mammals. Due to the limitation of population size and sample collection, reports on blood routine and blood biochemistry of giant pandas are very limited. And the effects of seasons on the physiology and health of giant pandas is even rarer. Understanding the impacts of meteorological factors on giant pandas is necessary for future. In this paper, we obtained the data of normal values and seasonal changes of hematological indexes of giant pandas, which can provide a reference for reasonable feeding and medical care of captive giant pandas.

2. Introduction

The giant panda (*Ailuropoda melanoleuca*) is one of the most endangered species of land mammals and has significant academic research value worldwide(Nie et al., 2015; Kang and Li, 2016; Wei et al., 2020). However, due to population size and sample collection limitations, there have been limited reports on the blood routine and blood biochemistry of giant pandas. Blood variables are crucial for understanding the impact of diseases on individuals and populations, as well as assessing the health status of individual animals(Maceda-Veiga et al., 2015). Some reports have shown that certain hematological variables in giant pandas are influenced by gender, age, physical condition, season, and region(Du et al., 2019; Huang et al., 2020; Kehoe et al., 2020; Shen et al., 2021). However, due to the unique characteristics of the giant panda population, there is still considerable controversy in the above-mentioned studies. There are some limitations in exploring the blood-related aspects of giant pandas. This study is a report on blood variable measurements in captive giant pandas obtained without anesthesia. It includes parameters such as morphology, hemorheology, serum enzymes, serum proteins, and serum metabolites of captive giant panda blood. The purpose of this study is to describe the age and seasonal changes in blood parameter variables in captive giant pandas in Beijing, China. The aim is to better protect the stability of this population and provide references for the rational feeding and medical care of captive giant pandas.

3. Material and methods

3.1. Animal Location

This study included an equal number of male and female giant pandas, ranging in age from 2 to 28 years old. All pandas were healthy, with no diseases, and had a good appetite and mental state. They were kept at the Beijing Zoo, located at geographic coordinates 39.9° N, 116.3° E. The basic information about the giant pandas can be found in Table 1, and the distribution of their clinical history characteristics can be found in Table 2. The types of bamboo fed to giant pandas in Beijing Zoo are mainly Phyllostachys propinqua and Pleioblastus amaru, supplemented by bamboo shoots and steamed corn-breads. The pandas' energy, carbohydrate and protein intake did not change significantly over the four seasons.

Table 1. Basic information about giant pandas in captivity.

Name	Sex	Age	Pedigree number	Birthday
Dadi	♂	28	394	1992.9.22
Jini	♀	27	403	1993.11.4
Gugu	♂	21	496	1999.9.25
Mengmeng	♀	14	652	2006.9.13
Fulu	♀	7	883	2013.8.8
Mengda	♂	7	894	2013.8.22
Menger	♂	7	895	2013.8.22
Diandian	♀	5	946	2015.6.20
Menglan	♂	5	954	2015.7.5
Fuxing	♂	3	1072	2017.6.25
Mengbao	♀	2	1122	2018.5.23
Mengyu	♀	2	1123	2018.5.23

Table 2. Characteristics of giant pandas.

Variables	Total	Sub-adult	Adult	Old
n	12	5	4	3
Age (Year)	10.67±9.12	2.33±0.47	6.2±0.98	22.50±5.59
Sex (Male/Female)	6/6	2/3	2/2	2/1
Weight (Kg)	94.58±8.59	90.63±9.01	96.8±7.6	96.17±8.99

3.2. Season and Climate

Beijing, China belongs to a temperate monsoon climate, characterized by hot and rainy summers, and cold and dry winters. This study lasted for 2 years, from mid-2018 to the end of June 2020. According to the regular climate patterns in Beijing, February to April is considered spring, May to August is summer, September to October is autumn, and November to January is winter.

3.3. Blood Sampling

A total of 12 giant pandas were sampled once a month in a non-anesthetized state, with fasting on the day of sampling. The sampling period was from mid-2018 to the end of August 2020. Blood was collected from the median vein in the front paw of the giant pandas, with a volume of 5ml, preserved in EDTA anticoagulant tubes, and blood-related tests were completed within 2 hours.

3.4. Sample Treatment

Blood cell counting was performed using manual microscopy, with professional laboratory personnel counting on a blood cell counting plate. Hemoglobin data was measured using a hemoglobin analyzer, where 20μl of whole blood was added to 5ml of diluted Drabkin's solution, mixed, and left for 5 minutes before measurement. Hematocrit was measured using the Wintrobe method, with a centrifuge speed of 12000r/min for 5 minutes, and readings taken using a counting tray. Blood biochemical parameters were measured using a Japanese FUJIFILM automatic biochemical analyzer and corresponding test plates.

Blood rheology was measured using the HT-100A blood rheology viscometer produced by Zibo Hengtuo Analytical Instruments Co., Ltd., with whole blood viscosity shear rates of 3S-, 30S-, and 200S-; plasma viscosity was measured after centrifugal separation. Other related parameters were calculated based on the above measurement results using relevant formulas.

3.5. Environmental Temperature and Body Temperature

Data from the four seasons of 2018-2020 were compared. Independent sample t-tests were conducted based on the monthly average temperature provided by the observatory. Due to multiple comparisons, Bonferroni adjustment was applied to the significance level. Giant panda's body temperature was measured using an infrared ear thermometer inserted directly into the ear.

3.6. Statistical Analysis

The collected data were analyzed using GraphPad Prism software for statistical analysis. The data were represented as mean ± standard error. Two-way analysis of variance (ANOVA) was used to analyze the data for the main effects: age and season. Duncan's multiple range test was used to confirm significant differences between groups. The significance of the differences was divided into two levels: $P < 0.01$ (A, B) and $P < 0.05$ (a, b).

4. Results

4.1. Sheltering environment and diet

Table 3 shows the outdoor living environment temperature and body temperature of giant pandas. The outdoor environment temperature in summer is significantly higher than in winter, with no significant difference between spring and autumn. The body temperature of giant pandas fluctuates in a regular pattern with the temperature of outdoor activities throughout the four seasons, increasing in summer and decreasing in winter. The indoor temperature is kept constant. In the experiment, giant pandas in a non-healthy state were not included.

Table 3. Outdoor living environment temperature and body temperature of giant pandas (Mean±SEM).

Variables	Spring	Summer	Autumn	Winter
Temperature (℃)	14.17±4.19	25.67±9.12 ^{A}	12.83±5.93	-0.5±1.5 ^{B}
Body temperature (℃)	36.6±0.3	37.2±0.4	36.5±0.5	35.8±0.5

^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$. ^{A,B} Values within a row with different superscripts differ significantly at $P < 0.01$.

4.2. Morphology of Blood in Captive Giant Pandas

Season and age both affect the morphological parameters of blood in giant pandas. Table 4 shows season significantly influences the levels of Hemoglobin ($P < 0.05$) and RBC ($P < 0.05$). Compared to other seasons, blood from giant pandas kept in winter exhibits higher levels of Hemoglobin, RBC, and Hematocrit. On the other hand, age significantly affects the levels of red blood cells, Hemoglobin, WBC, RBC ($P < 0.05$), as well as Hematocrit ($P < 0.001$). When compared to the blood of older and younger giant pandas, the characteristics of adult giant pandas' blood show higher values for these parameters.

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Table 4. Morphology of Giant Pandas Blood (Mean±SEM).

		Hemoglobin (g/L)	**WBC (*10^9/L)**	**RBC (*10^12/L)**	**Hematocrit (L/L)**
Season	Spring（n=22）	138.87±4.50 [a]	8.17±0.49	7.82±0.38 [a]	0.44±0.01
	Summer（n=19）	125.84±4.50 [b]	8.54±0.64	6.83±0.28 [b]	0.43±0.01
	Autumn（n=13）	120.87±3.27 [b]	7.49±0.58	6.50±0.24 [b]	0.45±0.01
	Winter（n=13）	139.19±7.05 [a]	9.52±0.50	7.76±0.45 [a]	0.46±0.01
Age	Old（n=27）	127.50±4.92 [b]	7.32±0.38 [b]	7.29±0.34	0.39±0.01 [B]
	Adult（n=19）	136.47±5.18 [a]	9.74±0.59 [a]	7.68±0.44 [a]	0.45±0.01 [A]
	Sub-adult（n=27）	133.37±3.73 [ab]	8.57±0.47 [b]	7.04±0.24 [b]	0.45±0.01 [A]

[a,b] Values within a row with different superscripts differ significantly at $P < 0.05$. [A,B] Values within a row with different superscripts differ significantly at $P < 0.01$.

4.3. Hemorheology in Captive Giant Pandas

The age of giant pandas does not affect ($P > 0.05$) the hemorheological parameters of blood collection. However, the season does affect the hemorheological index parameters of giant pandas (Table 5).

Table 5. Hemorheology of Giant Pandas Blood (Mean±SEM).

		LBV（mPa·S)	MBV（mPa·S)	HBV（mPa·S)	PV（mPa·S)	ESR（mm/H）	K Value	Fibrinogen（g/L）	EAI	EDI	ERI
Season	Spring（n=22）	12.76±0.30	6.71±0.16	5.38±0.13	1.69±0.04 ^{aA}	15.04±0.15	59.17±1.37	3.30±0.08	7.63±0.23 ^{bAB}	0.83±0.01	4.96±0.18 ^{bB}
	Summer（n=19）	12.27±0.55	6.45±0.29	5.17±0.23	1.82±0.03 ^{bcAB}	15.32±0.24	56.71±2.55	3.41±0.13	6.73±0.23 ^{aA}	0.79±0.01	4.24±0.14 aA
	Autumn（n=13）	13.18±0.31	6.92±0.16	5.55±0.13	1.86±0.04 ^{cB}	15.13±0.24	60.16±2.08	3.61±0.12	7.14±0.22 ^{abAB}	0.80±0.02	4.51±0.18 ^{abAB}
	Winter（n=13）	13.55±0.34	7.12±0.17	5.71±0.14	1.74±0.03 ^{abAB}	14.81±0.14	62.80±1.59	3.51±0.07	7.82±0.21 ^{bB}	0.82±0.01	4.98±0.12 ^{bB}
Age	Old(n=27)	12.08±0.56	6.42±0.24	7.51±2.23	2.24±0.45	18.11±2.92	57.92±1.52	3.42±0.08	7.07±0.23	0.80±0.01	4.49±0.17
	Adult(n=19)	13.11±0.42	6.88±0.22	5.52±0.18	1.75±0.04	15.05±0.22	59.78±2.24	3.42±0.13	7.52±0.22	0.83±0.02	4.87±0.16
	Sub-adult(n=27)	13.07±0.32	6.86±0.17	5.51±0.14	1.76±0.03	15.00±0.13	60.76±1.55	3.48±0.082	7.44±0.20	0.81±0.01	4.73±0.13

Abbreviations: WBLSV = Whole Blood Low-shear Viscosity; WBMSV = Whole Blood Moderate-shear Viscosity; WBHSV = Whole Blood High-shear Viscosity; PV = plasma viscosity; ESR = Erythrocyte Sedimentation Rate; EAI = erythrocyte aggregation index; EDI = erythrocyte deformation index; ERI = Erythrocyte Rigidity Index. ^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$. ^{A,B} Values within a row with different superscripts differ significantly at $P < 0.01$.

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Seasonal conditions significantly affect plasma viscosity ($P < 0.05$), red blood cell aggregation index ($P < 0.05$), and red blood cell rigidity index ($P < 0.05$). Compared to spring (1.69±0.04 mPa·S ; $P < 0.05$), higher levels of plasma viscosity were observed in blood samples collected during summer (1.82±0.03 mPa·S ; $P < 0.05$) and autumn (1.86±0.04; $P < 0.05$) in giant pandas. Additionally, plasma viscosity measured in blood samples collected during autumn was significantly higher compared to winter (1.86±0.04 mPa·S , 1.74±0.03 mPa·S ; $P < 0.05$). Compared to summer (6.73±0.23), the red blood cell aggregation index significantly increased in spring (7.63±0.23; $P < 0.05$) and winter (7.82±0.21; $P < 0.05$). Red blood cell rigidity index was significantly elevated in spring (4.96±0.18; $P < 0.05$) and winter (4.98±0.12; $P < 0.05$) in giant pandas compared to summer (4.24±0.14).

4.4. Serum Enzyme Levels in Captive Giant Pandas

Both season and age have an effect on the serum enzyme parameters of giant pandas. Statistical analysis shows in Table 6 that seasonal changes significantly affect the serum enzymes of giant pandas: ALT ($P = 0.001$), CHE ($P < 0.001$), ALP ($P = 0.01$), and LPS ($P = 0.01$). Compared to summer, autumn, and winter, the spring season has higher levels of ALT, Cholinesterase, and LPS ($P < 0.01$). In contrast, the ALP levels in autumn are significantly lower than in other seasons ($P < 0.05$). Age also has an impact on the serum enzymes of giant pandas: ALT, AST, Cholinesterase, ALP, ADA, LD, α-HBDH ($P < 0.001$), and CK-MB ($P < 0.05$). The statistical analysis of the results also suggests a significant interactive effect of age on the seasonal variation of serum cholinesterase levels in giant pandas ($P < 0.05$).

Table 6. Serum Enzymes of Giant Pandas Blood (Mean±SEM).

		ALT (U/L)	AST(U/L)	Cholinesterase(U/L)	GGT(U/L)	ALP(U/L)	ADA(U/L)	Amylase(U/L)	Lipase(U/L)	CK(U/L)	CK-MB(U/L)	LD(U/L)	α-HBDH(U/L)
Season	Spring (n=22)	59.48±3.90 ^{b}	54.49±3.93	980.20±73.67 ^{b}	4.63±0.54	160.18±15.91 ^{b}	25.01±3.33	988.11±42.46	12.30±1.38 ^{b}	116.78±7.69	116.78±7.69	978.30±80.09	900.38±60.22
	Summer (n=19)	49.01±3.43 ^{ab}	53.55±4.16	745.42±60.24 ^{a}	4.48±0.49	123.47±17.39 ^{ab}	19.09±2.33	989.94±83.98	7.28±1.01 ^{a}	129.74±8.82	129.74±8.82	883.41±87.28	769.33±62.44
	Autumn (n=13)	43.98±3.59 ^{a}	50.17±2.57	799.82±36.21 ^{ab}	4.18±0.64	110.26±11.86 ^{a}	20.08±2.42	896.46±68.60	7.76±0.57 ^{a}	103.49±9.46	103.49±9.46	913.77±68.39	779.60±56.18
	Winter (n=13)	55.22±5.06 ^{ab}	55.07±5.23	875.84±105.35 ^{ab}	5.65±0.91	163.75±16.61 ^{b}	19.19±2.58	1167.40±122.34	10.98±1.88 ^{ab}	110.75±8.16	110.75±8.16	771.27±106.53	752.15±69.40
Age	Old(n=27)	44.83±1.49 ^{aA}	67.72±3.96 ^{cB}	735.79±52.13 ^{aA}	4.83±0.61	104.57±7.26 ^{aA}	27.22±2.88 ^{bB}	1072.70±40.76	756.74±55.51	115.91±7.84	183.10±13.07 ^{ab}	894.36±60.52 ^{aA}	756.74±55.51 ^{aA}
	Adult(n=19)	67.69±5.02 ^{bB}	55.90±3.89 ^{bB}	782.68±35.37 ^{aA}	3.18±0.31	106.71±8.26 ^{aA}	25.07±2.59 ^{bB}	1086.73±74.83	1042.02±37.00	102.98±7.24	149.73±9.97 ^{a}	1209.04±52.22 ^{bB}	1042.02±37.00 ^{bB}
	Sub-adult(n=27)	51.20±2.41 ^{aA}	41.75±1.69 ^{aA}	1094.67±39.53 ^{bB}	5.66±0.49	196.18±12.05 ^{bB}	14.50±1.25 ^{aA}	950.70±72.91	708.74±44.25	122.85±7.85	199.50±12.91 ^{b}	834.56±53.57 ^{aA}	708.74±44.25 ^{aA}

Abbreviations: ALT = alanine transaminase; AST = aspartate aminotransferase; GGT = γ-glutamyl transpeptidase; ALP = alkaline phosphatase; ADA = adenosine deaminase; CK = creatinine kinase; LD = lactate dehydrogenase; α-HBDH = hydroxybutyrate dehydrogenase. ^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$. ^{A,B} Values within a row with different superscripts differ significantly at $P < 0.01$.

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4.5. Serum Protein in Captive Giant Pandas

Table 7 shows the age of giant pandas significantly affects ($P < 0.05$) the serum protein levels in their blood, with sub-adult pandas showing lower levels of total protein and globulin. Season does not have an effect on the serum protein levels of giant pandas ($P > 0.05$).

Table 7. Serum Proteins of Giant Pandas Blood (Mean±SEM).

		Total protein(g/L)	Albumin(g/L)	Globulin(g/L)
Season	Spring（n=22）	61.55±1.13	29.08±1.24	33.47±1.87
	Summer（n=19）	58.64±1.44	29.00±1.46	29.63±2.26
	Autumn（n=13）	64.04±2.48	26.27±2.07	36.66±3.52
	Winter（n=13）	61.44±1.78	30.90±1.35	30.68±2.38
Age	Old(n=27)	62.72±1.58 ^{a}	26.72±1.24	36.34±2.37 ^{b}
	Adult(n=19)	60.54±3.32 ^{a}	30.04±1.43	32.87±2.60 ^{ab}
	Sub-adult(n=27)	58.66±1.07 ^{b}	29.85±1.26	28.80±1.48 ^{a}

^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$. ^{A,B} Values within a row with different superscripts differ significantly at $P < 0.01$.

4.6. Serum Metabolites in Captive Giant Pandas

Age did not have a significant effect ($P > 0.05$) on the levels of serum metabolites collected from giant pandas, while season did not have an effect. (Table 8) The bile acids level in adult giant pandas (3.88±0.18 mmol/L) was significantly lower than in sub-adult pandas (4.59±0.20 mmol/L; $P < 0.05$) and old pandas (4.20±0.20 mmol/L; $P < 0.05$). The bile acid level in sub-adult pandas (51.35±4.78 μmol/L) was significantly higher than in adult pandas (35.50±3.89 μmol/L; $P < 0.05$). As for the serum uric acid level, old pandas (41.86±2.50 μmol/L) had significantly lower levels than adult pandas (59.56±3.48 μmol/L; $P < 0.001$) and sub-adult pandas (57.86±1.62; $P < 0.001$).

Table 8. Serum Metabolites of Giant Pandas Blood (Mean±SEM).

		Bile acids(mmol/L)	Triglycerides(mmol/L)	Bilirubin (total)(μmol/L)	Bile acids(μmol/L)	Glucose(mmol/L)	Creatinine(μmol/L)	Urea(mmol/L)	Uric acid(μmol/L)
Season	Spring（n=22）	4.20±0.23	1.53±0.16	0.58±0.05	40.51±4.18	3.88±0.17	93.53±5.87	4.61±0.40	56.78±2.55
	Summer（n=19）	4.67±0.25	1.64±0.20	0.54±0.07	54.54±5.64	3.63±0.16	114.18±7.64	4.66±0.41	49.88±2.78
	Autumn（n=13）	4.16±0.23	1.60±0.24	0.84±0.13	39.04±4.84	3.88±0.35	115.22±10.55	5.69±0.49	50.60±3.84
	Winter（n=13）	4.12±0.20	1.26±0.19	0.70±0.09	43.09±3.640	4.33±0.17	96.73±6.94	5.52±0.56	56.17±3.53
Age	Old(n=27)	$4.20±0.20^{ab}$	$1.78±0.16^{a}$	0.73±0.12	$42.36±2.96^{ab}$	3.46±0.12	115.14±7.56	5.32±0.43	$41.86±2.50^{aA}$
	Adult(n=19)	$3.88±0.18^{a}$	$0.97±0.06^{b}$	0.88±0.20	$35.50±3.89^{a}$	4.00±0.30	95.60±6.75	4.30±0.40	$59.56±3.48^{bB}$
	Sub-adult(n=27)	$4.59±0.20^{b}$	$1.69±0.17^{a}$	0.66±0.08	$51.35±4.78^{b}$	4.26±0.14	97.39±6.43	5.10±0.38	$57.86±1.62^{bB}$

^{a,b} Values within a row with different superscripts differ significantly at $P < 0.05$. ^{A,B} Values within a row with different superscripts differ significantly at $P < 0.01$.

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5. Discussion

This article provides a reference for the reasonable feeding and medical care of pandas in zoos by studying the age and seasonal changes in blood indicators of pandas under conditions of captive feeding in the northern region. It helps to develop a rational feeding strategy for pandas in the future. Based on the parameters collected in this study, if factors such as age and season are not considered, there are significant differences compared to the reference range of the pandas in Beijing Zoo. The main differences are reflected in: an increase in hemoglobin, red blood cell count, white blood cell count, and hematocrit; a decrease in blood sugar, total protein, albumin, globulin, triglycerides, uric acid, creatinine, total bile acid, alanine aminotransferase, aspartate aminotransferase, creatine kinase, cholinesterase, gamma-glutamyl transpeptidase, and amylase; an increase in urea nitrogen, total cholesterol, lactate dehydrogenase, alkaline phosphatase, adenosine dehydrogenase, and alpha-hydroxy acid dehydrogenase. Analyzing the reasons, although the existing reference range in Beijing Zoo includes a total of 29 healthy pandas, it also includes samples collected after anesthesia, and the influence of stress on blood indicators cannot be ruled out. Therefore, the reference range cannot be regarded as the average level of the tested animals. The blood samples measured in this study were obtained from captive pandas without anesthesia, which can enrich and improve the rigor of the reference range for pandas in Beijing Zoo and correct the previous reference standards for panda blood indicators. It provides reference suggestions for medical care and helps to develop a reasonable feeding strategy for pandas in zoos in the future.

Specific lifestyles can lead to welfare issues in various wild animals kept in captivity, including poor health, repetitive stereotypical behavior, and breeding difficulties(Clubb and Mason, 2003). Analyzing hematological and biochemical parameters is an important part of assessing the physiological, nutritional, and pathological conditions of animals. Determining the levels of biochemical blood parameters is important for evaluating the physiological status of animal organisms, as it enables the diagnosis of various diseases affecting internal organs. The reports by Deng and Luo et al. indicate that age significantly affects various hematological and biochemical indicators(Deng et al., 2019),(Luo et al., 2017). In this study, age influenced hematological parameters, hemorheology, serum enzymes, serum proteins, and serum metabolites. It was found that the elderly group of giant pandas had the lowest levels of Glucose, Hematocrit, Hemoglobin, WBC, highest level of Globulin, lowest level of Urea, highest level of AST, CRE, and BUN, which may be related to the aging state of the giant pandas in Beijing Zoo or perhaps chronic stress. However, based on the analysis of ALT, CK, Creatinine and Urea, all animal groups in the experiment showed normal values for these parameters; therefore, although the values of the elderly group of pandas showed significant changes, the animals were not in a diseased state. The alkaline phosphatase level in the sub-adult group was significantly higher than in the adult and elderly groups, which may be due to the fact that alkaline phosphatase can reflect bone formation and osteoblast metabolic activity, thus leading to higher levels in young animals. In clinical practice, we currently only know that the differences need to be treated accordingly, which can help clinical practitioners make correct and objective assessments when managing health(Peng et al., 2021).

This study found seasonal variations in hematological parameters, hemorheology, and serum enzymes. The seasonal changes in serum enzymes may be related to the spring estrus of giant pandas. Meanwhile, hematological parameters, such as HGB and RBC, were higher in winter. Plasma viscosity ($P < 0.05$), red blood cell aggregation index ($P < 0.05$), and red blood cell rigidity index ($P < 0.05$) showed significant seasonal variations. These findings may indicate a regulatory role of seasonal changes in blood viscosity in giant pandas. Clinical studies have found that many diseases are accompanied by abnormalities in various hemorheological indicators. Some of these abnormal indicators often appear earlier than symptoms or signs, and they often lag behind when symptoms or signs disappear during recovery(Müller and Musikić, 1987; Koenig and Ernst, 1992; Tikhomirova et al., 2016). Blood viscosity is mainly determined by red blood cell hematocrit, plasma viscosity, red blood cell aggregation, and red blood cell deformability(Müller and Musikić, 1987; Baskurt and Meiselman, 2003). Similarly, it has been shown that the effective viscosity of circulating blood

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increases as plasma osmotic pressure increases with age(Hahr, 2019). There is a significant difference in red blood cell aggregation index between summer and winter. Therefore, the main reason for increased whole blood viscosity may be due to red blood cell aggregation. Studies have shown that ambient temperature (climate) has an effect on both blood viscosity and plasma viscosity in animals(Halikas and Bowers, 1973; Chapman et al., 1982; Fröhlich et al., 1997; Windberger et al., 2018). When the temperature decreases, blood flow slows down, blood viscosity increases, and red blood cell aggregation is enhanced(Eckmann et al., 2000; Cinar et al., 2001; Lim et al., 2010). This is because during cold weather, animals need to consume a large amount of oxygen to generate heat to maintain relatively stable body temperature. Inhaling cold air can also increase pulmonary artery pressure, leading to impaired gas exchange and tissue hypoxia, resulting in increased red blood cells (Giesbrecht et al., 1993; Muller et al., 2011; Gaustad et al., 2021). This may explain the observed seasonal variations in blood parameters in pandas in this study. Additionally, the activity rhythm of many animals is significantly influenced by seasonal changes, and activity levels are directly related to temperature. When activity levels increase, the surface area of red blood cells relatively increases, which facilitates the release of oxygen from red blood cells and the removal of carbon dioxide from tissues, accelerating gas exchange rate. This also reduces blood viscosity and increases blood flow velocity. However, currently there is a lack of seasonal research on behavioral patterns or activity levels of giant pandas, and further related research is needed.

A successful ex-situ conservation project has required us to not only save an animal species but also research the appropriate thermal environment for individual animal survival in order to develop corresponding conservation strategies(Keulartz, 2023). The construction of a comfortable and reasonable animal house is one of the important conditions for the captive survival and health of giant pandas, and the control of the indoor environmental quality is directly related to the health of the giant pandas. Some scholars have paid attention to the need to set up regulating measures to reduce heat stress in giant pandas during high temperatures in summer(Zhang et al., 2018). However, this study shows that even though giant pandas are believed to tolerate cold environments, hemorheology is often a forgotten factor in vascular health. The risks of changes in hemorheology in giant pandas during the winter (cold) period and their clinical relevance to vascular diseases should still be considered when housing and feeding them. The future research directions can include the following aspects: Exploring the impact of different ages and seasons on the metabolic activity of captive giant pandas: Further studying the activity changes and differences in the overall metabolic processes of giant pandas in different age groups to understand their physiological and metabolic needs at different stages, and to find suitable feeding and management methods for giant pandas of different age groups. At the same time, continuing to explore the effects of factors such as seasons and climate on giant pandas, and whether these factors may lead to environmental stress, thus affecting the health and survival status of giant pandas. In-depth study of the relationship between blood viscosity and the health condition of giant pandas: Further analyze the negative effects of blood viscosity on giant pandas and explore the relationship between blood viscosity and the health condition of giant pandas. By monitoring and analyzing the changes in blood viscosity of giant pandas over a long period of time, it can help to identify the risk of giant pandas getting sick early and take corresponding treatment and preventive measures. Study the effects of environmental factors on the physiology and behavior of giant pandas: Further understand the effects of environmental factors such as temperature, humidity, and lighting on the physiology and behavior of giant pandas. By controlling and improving the environment of giant pandas, a more suitable captive environment can be provided.

6. Conclusions

In the research, it was confirmed that age and season have an impact on the hematological and biochemical parameters of captive giant pandas. There are significant differences in blood indicators among different age groups of giant pandas, indicating variations in the overall metabolic processes and differences among pandas of different age groups. Seasonal changes are shown to have a regulatory effect on blood viscosity, and further research is needed to determine if factors such as season and climate can cause environmental stress in captive giant pandas. This is because the analysis of animal blood indicates a negative effect on blood viscosity.

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