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# Effect of Naturally Changes in Photoperiod and Temperature on Hematology and Cytokines in Striped Hamsters

**Yan Wang[1], Xiao-Kai Hu[2], De-Li Xu[2,*]**

[1]Hospital, Qufu Normal University, Qufu, 273165, Shandong Province, China
[2]College of Life Sciences, Qufu Normal University, Qufu, 273165, Shandong Province, China.

***Correspondence:** De-Li Xu, College of Life Sciences, Qufu Normal University, 57 Jingxuan West Road, Qufu, 273165, People's Republic of China, Tel: +86-13280081296; E-mail: xudl1975@163.com; xudeli@mail.qfnu.edu.cn.

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## Abstract

Previous research has shown that immune responses change seasonally in striped hamsters (*Cricetulus barabensis*) in the field. Many ecological factors change with the season. Hematological parameters and the levels of cytokines were investigated seasonally in this species under a semi-natural environment. Twenty-nine male and 30 female hamsters were randomly assigned into the winter, spring, summer, and autumn groups, respectively. All hematological parameters detected demonstrated seasonal variation, and mean corpuscular hemoglobin (MCH), mean corpuscular Hb concentration (MCHC), intermediate granulocytes percent (MID%) were higher, but red blood cell distribution width (RDW) was lower in males than in females, other hematological parameters did not differ between sexes. IL-2, IL-4, TNF-α, and INF-γ all changed seasonally and differed between sexes. IL-2 was the highest in the winter, whereas INF-γ was the highest in the spring, IL-4 was higher in the spring and autumn, and TNF-α was higher in the spring, and they were all affected by the interaction of season and sex. In summary, different parts of the immune system showed distinct patterns of seasonal variations, which are mainly triggered by naturally seasonal changes in photoperiod and temperature.

**Keywords:** Cytokine; Hematology; Striped hamsters (*Cricetulus barabensis*).

## INTRODUCTION

The immune system protects animals from invading pathogens (i.e., bacteria, virus, fungi etc.), and hence it is crucial to their survival [1]. Seasonal variation in immune function is often observed in non-tropical animals [2,3]. The winter immunoenhancement hypothesis assumes that immune activity tends to be enhanced in the winter to offset the immunosuppressive effects of stressors such as cold temperature and food shortage occurring during this season [4]. This hypothesis is supported by some field research. For example, immune function is higher during the fall and winter compared to the spring and summer in cotton rats (*Sigmodon hispidus*) [5], red (*Clethrionomys rutilus*), and bank (*Clethrionomys glareolus*) voles, prairie voles (Microtus ochrogaster) [4], and Mongolian gerbils (*Meriones unguiculatus*) [6]. However, this hypothesis is against other field studies. For instance, spleen mass was heavier in the summer than in the winter in field voles (*Microtus agrestis*) [7], and lymphoid cells had a greater potential to synthesize the proinflammatory cytokines during the summer than winter in rhesus monkeys (*Macaca mulatta*) [8]. The reasons for these discrepancies among the above researches may lie in the different immunological parameters detected and different species used. Consequently, further research is required to investigate multiple immunological indices simultaneously in more species.

The health status of animals can be monitored by hematological profiles [9,10]. For example, red blood cells (RBC), hemoglobin (HGB), and hematocrit (PCV) are indicative of the animal metabolism via the circulatory system [11,12]. Moreover, white blood cells (WBC) including lymphocytes (LYMP), intermediate granulocytes (MID), and neutrophilic granulocytes (GRAN) are important for mounting immune responses against pathogens [13,14]. Cytokines are also crucial in regulating innate and adaptive immunity, activating inflammatory responses [15]. T helper (Th) type 1 cytokines such as INF-γ, IL-2, and TNF-α are primarily responsible for fighting for intracellular pathogens

[16,17]. The cytokines such as interleukin-4 (IL-4) typically drive immune responses towards antibody-mediated humoral responses and hence mainly control extracellular pathogens [15].

The striped hamster (*Cricetulus barabensis*) mainly lives in northern China, Russia, Mongolia, and Korea [18]. This species is granivorous, nocturnal, and feeds mainly on stems and leaves of plants during summer and forages crop seeds in winter [18,19]. The climate is characterized by warm and dry summers (the extreme maximum temperature is 42.6 °C) and cold winters (the extreme minimum temperature is below -20 °C). Therefore, this species must be confronted with great seasonal fluctuation in environmental conditions [20,21]. Our field research has shown that striped hamsters demonstrated seasonal variations in cellular and humoral immunity, hematology and cytokines [21,22]. As we know, many environmental factors such as photoperiod, temperature, food, predation, rain, vegetation and so on are associated with seasonal changes. In order to understand which environmental factors would influence seasonal changes in immune responses, hamsters were raised in a semi-natural environment and plenty of food and water were supplied. Hematological profiles and the levels of cytokines would be examined, and the winter immunoenhancement hypothesis was also to be tested.

## MATERIAL AND METHODS

### Animals and Experimental Design

All animal procedures were licensed under the guidelines of the Animal Care and Use Committee of Qufu Normal University. Adult male and female striped hamsters used in this study were captured from Jiuxian Mountain (35o46.275'N, 116o59.976'E) in Qufu of Shangdong province. These hamsters were housed individually in plastic cages (30cm×15cm×20cm) with sawdust as bedding under semi-natural conditions (inside the pavilion). Standard rat pellet chow (Beijing KeAo Feed Co., Beijing, China) and water were provided *ad libitum* throughout the experiment. Twenty-nine males and 30 females were randomly assigned into the winter, spring, summer, and autumn groups, respectively. Therefore, there were 8 groups: the winter male group (Winter-M, n=8), the winter female group (Winter-F, n=8), the spring male group (Spring-M, n=7), the spring female group (Spring-F, n=7), the summer male group (Summer-M, n=7), the summer female group (Summer-F, n=8), the autumn male group (Autumn-M, n=7) and the autumn female group (Autumn-F, n=7). One female hamster in the autumn group died on 26th April in 2015, and it was not included in the subsequent analysis. The experiment was carried out from 27th December in 2014 to 8th October in 2015, and the hamsters in the winter, spring, summer, and autumn groups were killed on January 4th, April 12th, July 10th, and October 8th in 2015, respectively.

### Hematological Analysis

After collecting blood samples from the retro-orbital sinus 10 days before hamsters were killed, 20 μL whole blood was diluted immediately in 4 mL diluent and red blood cells (RBC), hemoglobin concentration (HGB), white blood cells (WBC), lymphocytes (LYMP), intermediate granulocytes (MID) including eosinophil and basophil granulocytes, neutrophilic granulocytes (GRAN) and other hematological indices (Table S1) were counted in the Hematology Analyzer (Auto Counter 910EO+) [22]. The other blood samples were allowed to clot for 1 h and then were centrifuged at 4°C for 30 min at 4000 rpm. Sera were collected and stored in polypropylene microcentrifuge tubes at -80°C for later measurement of IgG titers.

### Serum Cytokine Assays

The levels of IL-2, IL-4, TNF-α, INF-γ were determined by hamster the ELISA kits of IL-2, IL-4, TNF-α, INF-γ (Shanghai Jiupin Biotechnology Co., Ltd, China), respectively. The ranges of IL-2, IL-4, TNF-α, INF-γ detected by these assays were 0.8-30 pg/mL, 4-200 pg/mL, 25-500 ng/L, and 50-1500 ng/L, respectively, when using a 10 μL sample (see manufacturer's instructions for hamster ELISA kits of IL-2, IL-4, TNF-α, INF-γ). The detailed procedures followed the manufacturer's instructions of the hamster ELISA kits of IL-2, IL-4, TNF-α, INF-γ. The assays for IL-2, IL-4, TNF-α, INF-γ were run in singles, and the intra- and inter-assay variability for IL-2, IL-4, TNF-α, INF-γ ELISA were all 9% and < 15%, respectively.

### Statistical Analysis

Data were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Prior to all statistical analyses, data were examined for normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. Group differences in the hematological indices and the levels of cytokines were analyzed by two-way (season and sex) ANOVA followed by Bonferroni post hoc tests. Specifically, General Linear Model Multivariate was selected, and the dependent variables were hematological parameters and the levels of cytokines, while the fixed factors (i.e., independent variables) are season and sex. If the parameters detected did not differ between sexes, the data of male and female hamsters were pooled together and then were analyzed

by one-way ANOVA followed by Tukey's post hoc tests. If the parameters detected differed between sexes, and/or were affected by the interaction of season ×sex, the data in each sex was analyzed by one-way ANOVA followed by Tukey's post hoc tests, respectively; the data in each season was also analyzed by the Independent-Samples T-Test, respectively, in order to clarify the differences between sexes. Results are presented as mean ± SE, and $P < 0.05$ was considered to be statistically significant.

## RESULTS

### Hematological Parameters

All hematological parameters detected demonstrated seasonal variation, and MCH, MCHC, MID% were higher, but RDW% was lower in males than in females, other hematological parameters did not differ between sexes (Table 1). All the hematological parameters except MCHC were not influenced by the interaction of season × sex (Table 1). Therefore, the data of male and female hematological profiles except MCH, MCHC, MID%, and RDW% were pooled together and then were analyzed by one-way ANOVA followed by Tukey's post hoc tests. Specifically, RBC, PCV, GRAN were higher in the winter and autumn than in the spring and summer (Table 2). In addition, HGB, GRAN%, PLT, MCV were the lowest in summer, while LYMF% was the lowest in winter, MPV, PDW was the highest in the autumn among the four seasons (Table 2). WBC, LYMF, MID were higher in the spring and autumn than in the winter and summer (Table 2).

### Cytokines

IL-2 titres was affected by the season ($F_{3,49}$=30.24, P<0.001), sex ($F_{1,49}$=5.445, P=0.024), and the interaction of season × sex ($F_{3,49}$=6.319, P=0.001). Similarly, IL-4 level was also affected by the season ($F_{3,49}$=54.57, P<0.001), sex ($F_{1,49}$=4.141, P=0.047), and the interaction of season × sex ($F_{3,49}$=7.327, P<0.001). In addition, TNF-α level was affected by the season ($F_{3,49}$=9.081, P<0.001), and the interaction of season × sex ($F_{3,49}$=6.730, P=0.001), but did not differ between sexes ($F_{1,49}$=2.237, P=0.141). INF-γ concentration was also influenced by the season ($F_{3,49}$=50.82, P<0.001), sex ($F_{1,49}$=18.01, P<0.001), and the interaction of season × sex ($F_{3,49}$=11.4, P<0.001). Therefore, the data of IL-2, IL-4 and INF-γ in male or female hamsters, and the pooled data of TNF-α in both sexes were analyzed by one-way ANOVA, respectively. TNF-α ($F_{3,54}$=7.25, P<0.001) was higher in spring than in winter in both sexes (Figure 1). As for males, IL-2 ($F_{3,25}$=18.18, P<0.001) was higher in winter and summer,

**Table 1:** Seasonal changes of hematological parameters in striped hamsters.

| | Winter | | Spring | | Summer | | Autumn | | Statistical summary | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female | Season | | Sex | | Season×Sex | |
| **Sample size** | 8 | 8 | 7 | 7 | 7 | 8 | 7 | 6 | $F_{3,50}$ | *P* | $F_{1,50}$ | *P* | $F_{3,50}$ | *P* |
| RBC ($10^{12}$/L) | 9.4±0.6 | 9.2±0.4 | 8.8±0.1 | 8.3±0.5 | 7.5±0.2 | 7.1±0.2 | 9.9±0.2 | 10.0±0.4 | 18.5 | <0.001 | 1.01 | 0.32 | 0.24 | 0.87 |
| PCV (%) | 43.9±2.5 | 41.8±1.8 | 37.2±0.7 | 34.9±1.8 | 31.7±0.8 | 29.9±0.5 | 42.0±1.1 | 43.7±1.5 | 29.4 | <0.001 | 1.05 | 0.31 | 0.69 | 0.56 |
| MCV (fl) | 47.8±0.9 | 45.9±0.7 | 42.2±0.6 | 42.2±0.5 | 42.1±0.3 | 42.1±0.9 | 42.4±0.5 | 44.3±1.0 | 20.0 | <0.001 | 0.00 | 0.97 | 2.25 | 0.09 |
| RDW% | 13.2±0.4 | 13.1±0.3 | 12.2±0.2 | 12.9±0.2 | 11.7±0.1 | 12.7±0.4 | 11.5±0.1 | 11.7±0.2 | 11.9 | <0.001 | 5.58 | 0.02 | 1.99 | 0.13 |
| HGB (g/L) | 202±13 | 192±11 | 190±3 | 175±11 | 139±4 | 132±3 | 188±6 | 178±3 | 22.4 | <0.001 | 3.36 | 0.07 | 0.10 | 0.96 |
| MCH (pg) | 22.4±0.6 | 21.5±0.4 | 21.6±0.2 | 21.0±0.3 | 18.4±0.3 | 18.5±0.3 | 19.4±0.7 | 17.7±0.4 | 32.3 | <0.001 | 5.50 | 0.02 | 1.29 | 0.29 |
| MCHC (g/L) | 477±11 | 471±4 | 513±5 | 499±7 | 438±4 | 440±2 | 465±16 | 402±11 | 29.4 | <0.001 | 10.7 | <0.001 | 5.40 | 0.003 |
| PLT ($10^9$/L) | 393±20 | 422±25 | 432±22 | 405±40 | 328±7 | 342±18 | 341±20 | 364±22 | 5.3 | 0.003 | 0.30 | 0.59 | 0.50 | 0.69 |
| PCT% | 0.28±0.02 | 0.30±0.02 | 0.29±0.01 | 0.28±0.03 | 0.22±0.02 | 0.24±0.01 | 0.26±0.02 | 0.27±0.02 | 5.5 | 0.003 | 0.58 | 0.45 | 0.27 | 0.85 |
| MPV (fl) | 6.1±0.1 | 6.1±0.1 | 5.8±0.1 | 6.1±0.1 | 5.8±0.1 | 6.0±0.1 | 6.5±0.1 | 6.3±0.1 | 8.8 | <0.001 | 0.20 | 0.66 | 1.27 | 0.30 |
| PDW (fl) | 10.1±0.2 | 10.0±0.3 | 9.6±0.1 | 10.2±0.2 | 9.7±0.3 | 10.1±0.2 | 11.0±0.2 | 10.7±0.2 | 7.9 | <0.001 | 1.02 | 0.32 | 2.01 | 0.12 |
| WBC ($10^9$/L) | 4.3±0.5 | 3.5±0.4 | 7.0±0.6 | 7.9±1.4 | 3.8±0.5 | 3.1±0.2 | 6.9±0.6 | 7.3±0.4 | 22.0 | <0.001 | 0.01 | 0.93 | 0.83 | 0.49 |
| LYMF ($10^9$/L) | 3.1±0.4 | 2.4±0.3 | 5.8±0.5 | 6.4±1.0 | 3.2±0.4 | 2.8±0.2 | 5.1±0.3 | 6.2±0.4 | 25.1 | <0.001 | 0.17 | 0.68 | 1.48 | 0.23 |
| LYMF% | 70.8±3.5 | 68.7±3.9 | 81.8±1.4 | 81.4±1.8 | 84.9±1.2 | 89.4±0.9 | 75.0±3.8 | 84.7±1.2 | 16.3 | <0.001 | 2.49 | 0.12 | 1.94 | 0.14 |
| MID ($10^9$/L) | 0.36±0.05 | 0.29±0.04 | 0.86±0.08 | 0.96±0.25 | 0.41±0.06 | 0.30±0.03 | 0.89±0.14 | 0.72±0.11 | 14.2 | <0.001 | 0.65 | 0.42 | 0.51 | 0.68 |
| MID% | 7.7±0.6 | 7.1±0.6 | 11.9±1.0 | 10.6±1.2 | 10.3±0.5 | 7.8±0.8 | 11.7±1.0 | 9.4±1.0 | 8.4 | <0.001 | 7.84 | 0.01 | 0.58 | 0.63 |
| GRAN ($10^9$/L) | 0.88±0.17 | 0.84±0.18 | 0.39±0.04 | 0.57±0.12 | 0.11±0.03 | 0.04±0.02 | 0.99±0.34 | 0.40±0.04 | 9.5 | <0.001 | 1.31 | 0.26 | 1.95 | 0.13 |
| GRAN% | 21.5±3.0 | 24.2±3.4 | 6.4±0.5 | 8.0±0.8 | 4.8±0.9 | 2.8±0.3 | 13.3±2.9 | 5.9±0.4 | 34.0 | <0.001 | 0.71 | 0.40 | 2.24 | 0.09 |

Values are significantly different at *P*<0.05, determined by a two-way ANOVA and Bonferroni post-hoc tests.

**Table 2:** Seasonal changes of hematological parameters (data pooled from both sexes) in striped hamsters.

| | Winter | Spring | Summer | Autumn | Statistical summary | |
|---|---|---|---|---|---|---|
| Sample size | 16 | 14 | 15 | 13 | $F_{3,91}$ | *P* |
| RBC ($10^{12}$/L) | 9.3±0.3$^{a}$ | 8.6±0.2$^{bc}$ | 7.3±0.2$^{c}$ | 10.0±0.2$^{a}$ | 19.6 | <0.001 |
| PCV (%) | 42.9±1.5$^{a}$ | 36.1±1.0$^{b}$ | 30.7±0.5$^{c}$ | 42.8±0.9$^{a}$ | 29.9 | <0.001 |
| MCV (fl) | 46.8±0.6$^{a}$ | 42.2±0.4$^{b}$ | 42.1±0.5$^{c}$ | 43.3±0.6$^{b}$ | 19.1 | <0.001 |
| HGB (g/L) | 197±8$^{a}$ | 183±6$^{a}$ | 135±2$^{b}$ | 184±4$^{a}$ | 22.9 | <0.001 |
| PLT ($10^{9}$/L) | 408±16$^{a}$ | 418±22$^{a}$ | 336±16$^{b}$ | 352±15$^{ab}$ | 5.6 | 0.002 |
| PCT% | 0.29±0.01$^{a}$ | 0.29±0.01$^{a}$ | 0.23±0.01$^{b}$ | 0.26±0.01$^{ab}$ | 5.6 | 0.002 |
| MPV (fl) | 6.1±0.1$^{b}$ | 6.0±0.1$^{b}$ | 5.9±0.1$^{b}$ | 6.4±0.1$^{a}$ | 8.9 | <0.001 |
| PDW (fl) | 10.0±0.2$^{b}$ | 9.9±0.1$^{b}$ | 9.9±0.2$^{b}$ | 10.9±0.2$^{a}$ | 7.6 | <0.001 |
| WBC ($10^{9}$/L) | 3.89±0.33$^{b}$ | 7.46±0.72$^{a}$ | 3.41±0.25$^{b}$ | 7.11±0.35$^{a}$ | 22.8 | <0.001 |
| LYMF ($10^{9}$/L) | 2.71±0.26$^{b}$ | 6.08±0.57$^{a}$ | 2.98±0.22$^{b}$ | 5.61±0.27$^{a}$ | 24.8 | <0.001 |
| LYMF% | 69.8±2.6$^{c}$ | 81.6±1.1$^{ab}$ | 87.3±0.9$^{a}$ | 79.5±2.5$^{b}$ | 15.5 | <0.001 |
| MID ($10^{9}$/L) | 0.33±0.03$^{b}$ | 0.91±0.13$^{a}$ | 0.35±0.04$^{b}$ | 0.81±0.09$^{a}$ | 15.0 | <0.001 |
| GRAN ($10^{9}$/L) | 0.86±0.12$^{a}$ | 0.48±0.07$^{ab}$ | 0.07±0.02$^{b}$ | 0.72±0.19$^{a}$ | 9.3 | <0.001 |
| GRAN% | 22.9±2.2$^{a}$ | 7.2±0.5$^{bc}$ | 3.7±0.5$^{c}$ | 9.9±1.9$^{b}$ | 32.2 | <0.001 |

Data are pooled from the male and female hamsters and are expressed as mean ± SE.

Values for a specific parameter that share different superscripts are significantly different at *P*<0.05, determined by one way ANOVA and Tukey's post-hoc tests.

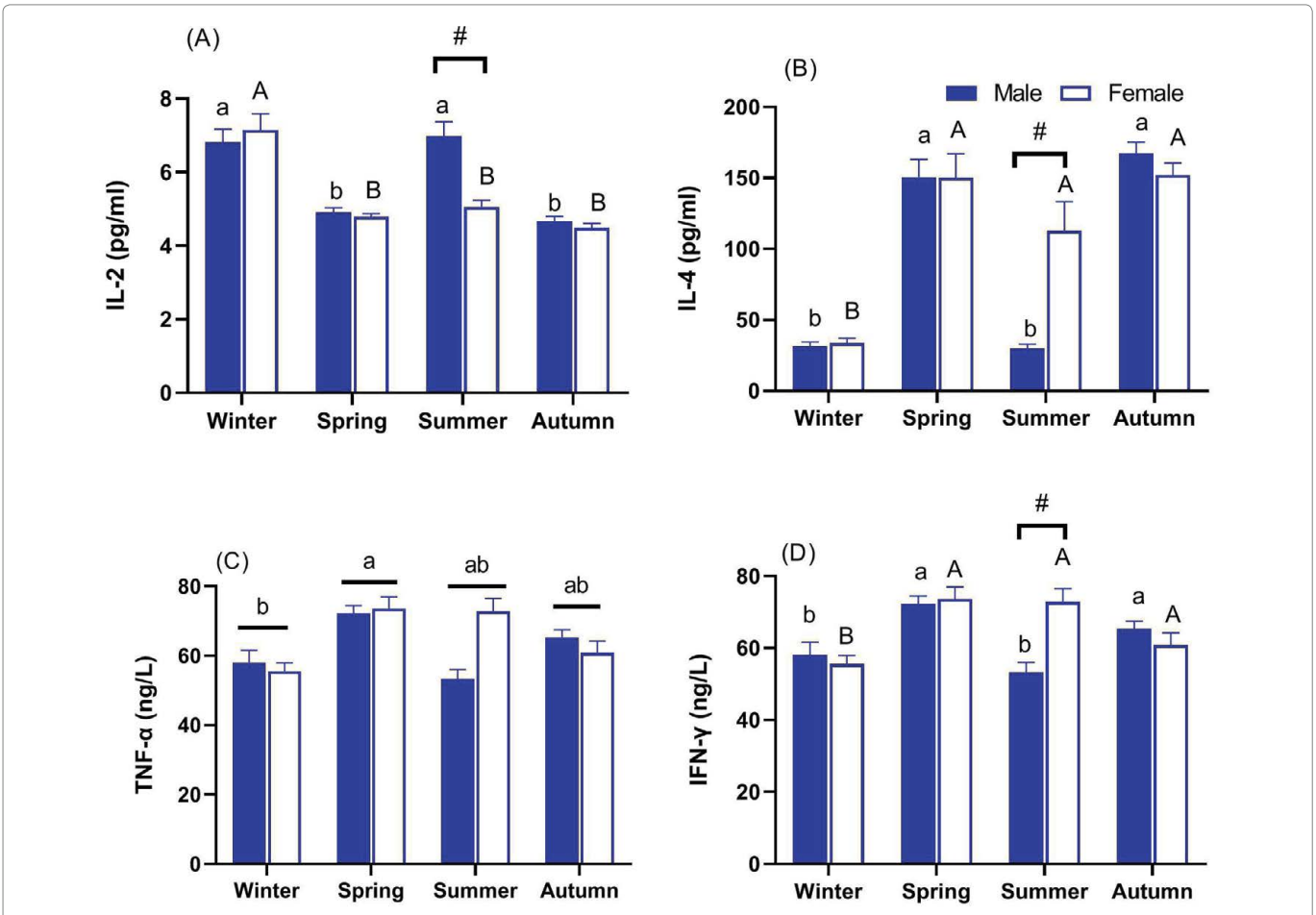


**Figure 1:** Seasonal changes of the titres of IL-2 (A), IL-4 (B), TNF-α (C) and IFN-γ (D) in striped hamsters. Different letters above the column indicate significant differences among seasons at *P*<0.05, and the pound (#) above the column indicate significant differences between sexes in a certain season at *P*<0.05.

IL-4 ($F_{3,25}$=101.10, P<0.001) was higher in spring and autumn, INF-γ ($F_{3,25}$=80.58, P<0.001) was higher in spring and autumn than other seasons, respectively. As for females, IL-2 ($F_{3,25}$=20.36, P<0.001) was the highest in winter, while IL-4 ($F_{3,25}$=14.95, P<0.001) and INF-γ ($F_{3,25}$=20.10, P<0.001) were the lowest in winter among the four seasons. If the data of IL-2, IL-4, INF-γ in each season were analyzed by Independent-Samples T Test (Sex), respectively. In summer, IL-2 titres was higher in male than in female hamsters (t=4.777, df=13, P<0.001), while the levels of IL-4 (t=-3.771, df=13, P=0.002), INF-γ (t=-5.402, df=13, P<0.001) were all higher in female than in male hamsters. In other seasons, there were no differences between sexes in the levels of IL-2 (winter: t=-0.591, df=14, P=0.564; spring: t=0.870, df=12, P=0.401; autumn: t=0.893, df=11, P=0.391), IL-4 (winter: t=-0.461, df=14, P=0.652; spring: t=0.029, df=12, P=0.977; autumn: t=1.313, df=11, P=0.216) and INF-γ (winter: t=-0.329, df=14, P=0.747; t=-0.406, df=12, P=0.692; autumn: t=-0.994, df=11, P=0.341).

## DISCUSSION

In the present study, hematological parameters, the titers of Th1 cytokines (i.e., IL-2, TNF-α, INF-γ) and Th2 cytokine IL-4 all showed seasonal variations in hamsters, but the patterns of their seasonal changes were different.

### Seasonal Changes in Hematological Parameters

Regardless of sexes, RBC and PCV were higher in the fall and winter, or HGB was higher in the winter, spring, and autumn in hamsters, being consistent with our previous findings in male hamsters [22]. These results implied the increase of the oxygen-carrying capacity and the oxygen affinity of the blood during these seasons, which might be consistent with the augment of food intake and thermogenesis capacity across many species including the striped hamster during winter [20]. Oxygen consumption increases significantly at 5°C compared with warmer temperatures which fit very nicely with the increase in the need to transport more oxygen [24]. Our results also agreed with other studies in which RBC and HGB in frogs [25,26], HGB and PCV in Indian goats [27], or RBC in cold water swimmers [28] all increased in winter or winter-like conditions. However, our results disagreed with other research in which RBC and PCV were the lowest in cold months in wild chub (*Leuciscus cephalus*) [29], PCV was lower in winter than in summer in sea bass (*Dicentrarchus labrax*) [30], or HGB and PCV in northern red-backed mouse (*Clethrionomys rutilus*) were relatively stable throughout the year [31]. The differences in the species used, age, or the experimental protocols may be responsible for the discrepancies in different research [27,32-34]. PLT and PCT% were higher in the spring and winter than in summer, implying the increase of the coagulation ability during these seasons in hamsters. These results were consistent with our previous research to a degree in which PLT and PCT% were the highest in the spring during the four seasons [22].

WBC, LYMP, and MID, regardless of sexes, were higher in the spring and autumn than in the winter and summer in hamsters, which did not support the winter immunoenhancement hypothesis [4]. However, GRAN was higher in the autumn and winter than the spring and summer, which supported this hypothesis. Higher WBC, LYMP, MID and GRAN indicated the increase of the ability to fight pathogens during these seasons [35,36]. These results were inconsistent with our previous study in which WBC was higher in winter than in summer, MID, GRAN was the highest in winter, and LYMF was the highest in the spring among the four seasons [22]. These results also disagreed with other research in which WBC increased in cold water swimmers [28], or in wild brushtail possums in winter [37], or no seasonal variation in WBC in dairy cows [33] and normal individuals [38], or LYMP was higher in the summer than in the winter in Dhofari goats [39].

### Cytokines

In the present study, Th1 cytokines (i.e., IL-2, TNF-α, INF-γ) and Th2 cytokine IL-4 all demonstrated different seasonal variations in striped hamsters. IL-2 is essential for the proliferation and differentiation of T cells [40], and we found that IL-2 was the highest in the winter in hamsters, implying its enhancing role in cellular immune responses. This result of IL-2 agreed with that cellular immunity was the highest in winter in hamsters. This result was incompatible with other studies in which IL-2 expression was higher in summer than in winter in rhesus monkeys [8]. TNF-α, a well-known pro-inflammatory cytokine [41], was higher in the spring than in the winter in hamsters, suggesting the inflammatory response was higher during spring. This result disagreed with normal individuals whose TNF-α level was the lowest in the fall [38], and goats whose TNF-α level was lower in the summer and winter than in other seasons [42]. INF-γ was the highest in the spring among the four seasons, indicating its higher pro-inflammatory activities against viral and intracellular bacterial infections during spring [43]. This result was inconsistent with other study in which INF-γ was significantly higher in summer than in winter in rhesus monkeys [8]. Th2 cytokines, most notably

IL-4, mainly control extracellular pathogens and typically drive immune responses towards antibody-mediated humoral responses [15]. Moreover, we found that IL-2 titers were higher in males than in female hamsters in summer, whereas the levels of IL-4, TNF-α, and INF-γ were all higher in females than in male hamsters during this season, and still there were no differences of IL-2, IL-4, TNF-α and INF-γ between sexes in other seasons, suggesting that season and sex had different influence on the cytokines.

## Summary

In summary, RBC and PCV were higher in the fall and winter, or HGB was higher in the winter, spring, and autumn in hamsters, suggesting the increase of the oxygen-carrying capacity of the blood, which was an adaptive strategy for the augment of metabolism and thermogenesis capacity in these seasons. IL-2 was the highest in the winter among the four seasons, which supported the winter immunoenhancement hypothesis. WBC, LYMF, MID were higher in the spring and autumn compared with other seasons, which did not support this hypothesis. This hypothesis was also not supported by the results of IL-4, TNF-α, INF-γ which showed different seasonal changes. Further research is required to examine the seasonal patterns of different immunological indices in more wild species and the mechanism of seasonal variation in hematological profiles, the levels of cytokines.

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**Table S1:** Hematological parameters measured [22,23].

| Parameter | Abbreviation | Meaning |
|---|---|---|
| Red blood cells ($10^{12}$/L) | RBC | Erythrocyte concentration in plasma. Measures oxygen-transport capacity. |
| Red blood cell specific volume (Packed cell volume or hematocrit) (%) | PCV or HCT | Volumetric proportion of erythrocytes in plasma. Oxygen-transport capacity. |
| Erythrocyte mean corpuscular volume (fl) | MCV | Mean volume of erythrocytes, calculated from HCT and RBC [(PCV/RBC)100]). Inversely related with oxygen-transport capacity in small mammals. |
| Red blood cell distribution width (%) | RDW | The distribution range of red blood cells. |
| Hemoglobin concentration (g/L) | HGB | Total hemoglobin concentration in plasma. Oxygen-transport capacity. |
| Mean corpuscular hemoglobin (pg) | MCH | Mean hemoglobin concentration. Oxygen-transport capacity. |
| Mean corpuscular Hb concentration (g/L) | MCHC | Obtained from the ratio HGB/PCV. Oxygen-transport capacity. |
| Blood platelet count ($10^9$/L) | PLT | Total platelet counts. Coagulation ability |
| Plateletocrit (%) | PCT | Volumetric proportion of platelet in plasma. |
| Mean platelet volume (fl) | MPV | Mean volume of platelet, calculated from PCT and PLC |
| Platelet distribution width (fl) | PDW | The distribution range of platelet. |
| White blood cells ($10^9$/L) | WBC | Total number of leukocytes, indicative of immune activity (increased during infection) |
| Lymphocytes | LYMF | Leukocyte type associated with specific immune response and viral infection. Its levels are reduced during inflammation. |
| Lymphocyte percent□%□ | LYMF% | Percent of lymphocytes among total number of leukocytes. |
| Intermediate granulocytes (eosinophil and basophil granulocytes) | MID | Leukocyte type associated with parasitic infection. |
| Middle leucocyte percent (%) | MID % | Percent of MID among total number of leukocytes. |
| Neutrophil granulocytes | GRAN | The most abundant leukocyte type. Levels increase during inflammation. |
| Neutrophil granulocytes percent (%) | GRAN % | Percent of GRAN among total number of leukocytes. |