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SEASONAL EFFECTS ON THE HEMATOLOGY AND BLOOD PLASMA PROTEINS OF TWO SPECIES OF MICE *MUS MUSCULUS DOMESTICUS* AND *M. SPRETUS* (RODENTIA: MURIDAE) FROM PORTUGAL

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ABSTRACT - Blood samples were taken from *Mus musculus domesticus* (Rutty, 1772) and *M. sprehts* (Lataste, 1883), live-trapped at one month intervals, from September 88 to July 89, in the district of Lisbon, Portugal. The seasonal hematological variations in the cornmensal species, *M. musculus domesticus*, were characterized by an increase in red blood cells, hernoglobin and hematocrit values in winter which reverse during summer. On the contrary, in *M. spretus* hematocrit values slightly change throughout the year. In both species the albumin/globulin ratio was low in spring and high in autumn. These results were analysed and discussed taking into 'account environmental factors and physiological conditions of mice.

***Key** words:* mice, hematology, plasma proteins, Portugal.

RIASSUNTO — *Effetti stagionali sull'ematologia e le proteine del plasma di* Mus musculus domesticus e M. spretus *(Rodentia: Muridae) in Portogallo* — I campioni di sangue provengono da individui di *Mus musculus domesticus* (Rutty, 1772) e *M. sprehis* (Lataste, 1883). Gli animali sono stati catturati vivi ogni mese, da settembre 88 a luglio 89, nei distretto di Lisbona, in Portogallo. Le variazioni stagionali ematologiche nella specie commensale, *M. m. domesticus*, sono caratterizzate da un incremento di cellule rosse del sangue, di emoglobina e dei valori di ematocrito in inverno che assumono un andamento opposto in estate. Al contrario, in *M. spretus* i valori di ematocrito non cambiano sensibilmente durante tutto l'anno. In entrambe le specie il rapporto albumina/globulina risulta basso in primavera e alto in autunno. Questi risultati sono stati analizzati e discussi tenendo conto dei fattori ambientali e le condizioni fisiologiche dei topi.

Parole chiave: topi, ernatologia, proteine del plasma, Portogallo.

INTRODUCTION

Seasonal variations in the blood chemistry and hematology may be related with some factors of the environment or with events in the life cycle of individual animals (e.g. Dobrowolska, 1975; Dobrowolska et al., 1983; Gromadzka-Ostrowska and Zalewska, 1984; Mihok and Schwartz, 1989).

In fact, regular changes in physiological attributes are nothing but adaptations directed at the maintenance of homeostasis **and** as so can be very useful as indicators of the status of populations and their habitat.

The present study is part of a larger one dealing with the biology of both the man-associated and wild mice, respectively the House Mouse (*Mus musculus domesticus* Rutty, **1772)** and the Algerian Mouse (*M. spretus* Lataste, **1883)** and was undertaken in order to identify the seasonal changes in blood parameters and plasma protein fractions of these mice during an annual cycle. *An* attempt was made to gain knowledge on the physiological responses of these mice to environmental changes as well as to such factors as sex and reproduction.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS:

Mice were live-trapped for three consecutive days and nights at approximately one month intervals from September **1988** to July **1989** near Lisbon, southern Portugal. Animals were kept at the same conditions of feeding and management for no longer than six hours before being analysed. Only healthy-appearing adults, judged by pelage, weight and teeth wear, of either sex, were used in this study.

Seasonal trends were tested by pooling together the monthly samples as follows: September, October, November - autumn **1988;** December, January, February - winter **1988/89;** March, April, May - spring **1989;** June, July - summer **1989** (Table 1).

BLOOD SAMPLING:

The blood was collected from the eye vein under light ether anaesthesia. Blood for hematological measures was run through a heparinised micro-haematocrit tube and used immediatly after sampling. A small quantity of blood was centrifuged and the plasma was drawn off and frozen until needed for further use.

HEMATOLOGY:

Hemoglobin concentration (Hb) was determined by the cyano-methemoglobin method. Hematocrit value (Hct) was measured after three minutes centrifugation at **12,000** r.p.m. in a Hawksley microhematocrit centrifuge. The erythrocytes (RBC) as well as the total leucocytes (WBC) were counted in a Hycel Counter 222. Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)

Tab. **1 – Numbers** of **mice used in this study**

	AUTUMN 1988	WINTER 1988/89	SPRING 1989	SUMMER 1989	TOTAL
M. spretus	36	27	47	24	134
M. m. domesticus	8	5	48	20	81

as well as mean corpuscular volume (MCV) were estimated as referred to by Mitruka and Rawnsley (1981) and Jandl (1987).

PLASMA PROTEINS:

Plasma proteins were assayed by electrophoresis. The runs were carried out in a three-strip chamber on Sepraphore III cellulose polyacetate strips using a tris-barbital buffer (pH 8.8). Protein fractions were stained with amido black 10B and cleared in 90:10 v/v methanol-acetic acid after migration at 250 V, for 35 minutes. Quantitative interpretation was done with a DC-16 Gelman densitometer.

STATISTICS:

Seasonal differences were tested by one-way analysis of variance after Bartlett's test for analysis of the homogeneity of variances. When samples proved to be not-homogeneous, Games-Howell test was used instead (Sokal and Rohlf, 1981). Moreover, we have followed Tukey-Kramer method for pair-wise comparisons and Student t-test or t's test (in not-homogeneous samples) for comparisons between two samples. The null hypothesis was rejected at $p < 0.05$. Results are presented as means ± standard error.

RESULTS

ERYTHROCYTE PARAMETERS (Table 2):

Ail analysed parameters showed in both species changes with the time of year. Significant seasonal differences were mostly evident in spring and summer.

In *M. musculus domesticus* the majority of the females were captured during spring and summer. Thus, in this species seasonal variation in blood parameters will be mostly referred to the males.

i) Red blood cells count (RBC)

The mean red blood cells count varied in both males and females *M. spretus* from a mean low count of $6.4 \pm 0.17 \times 10^6$/ml and $6.6 \pm 0.14 \times 10^6$/ml, respectively, in spring to a mean high count of $7.4 \pm 0.34 \times 10^6$/ml and $7.5 \pm 0.39 \times 10^6$/ml in winter. However, these differences were significant only for females.

In males *M. musculus domesticus* a maximal mean value of RBC occurred in winter (7.5k0.37 x 10^6/ml). Slightly lower values were found during autumn and spring but the lowest mean count refers to the summer ($6.5 \pm 0.13 \times 10^6$/ml). The values in summer significantly differ from those in spring and winter ($p < 0.05$). Significant differences were also found between sexes in spring ($p < 0.05$).

ii) Hemoglobin (Hb)

The mean hemoglobin concentration in *M. spretus* decreased between winter and spring in both males (13.420.34 g/dl to 12.0 ± 0.24 g/dl) and

	M. spretus			*M.musculus domestìcus*		
	RED BLOOD CELL COUNT (10^6/ml)					
Autumn (A)	7.210.25 (5)	7.210.38 (10)	ns	7.4k0.46 (3)	-	-
Winter (W)	7.4k0.34 (14)	7.510.39 (9)	ns	7.510.37 (5)	-	-
Spring (Sp)	6.4k0.17 (18)	6.6±0.14 (28)	ns	7.2k0.16 (20)	6.7k0.12 (24)	*
Summer (Sm)	7.010.16 (10)	6.8±0.17 (11)	ns	6.5±0.13 (11)	6.9k0.21 (7)	ns
	G-Hns	F* (*W/Sp)		F** (*W/Sm, *Sp/Sm)	t's irs	
	HEMOGLOBIN (g/dl)					
Autumn (A)	13.521.01 (5)	11.6k0.51 (10)	ns	12.3k1.04 (3)	-	-
Winter (W)	13.410.34 (15)	13.8±0.61 (9)	ns	14.8k0.98 (5)	-	-
Spring (Sp)	12.0±0.24 (18)	12.710.28 (27)	**	14.0±0.22 (21)	13.310.21 (27)	*
Summer (Sm)	13.2±0.33 (11)	13.2k0.43 (13)	ns	12.9±0.22 (12)	13.1k0.32 (8)	ns
	F* (*W/Sp)	F*(A*/W)		G-Hns	t's ns	
	HEMATOCRIT (%)					
Autumn (A)	45.9±1.05 (14)	46.6±0.89 (22)	ns	48.5±2.03 (3)	49.0±3.05 (3)	ns
Winter (W)	47.4k0.96 (18)	47.5k1.36 (9)	ns	55.2±0.44 (4)	-	-
Spring (Sp)	45.3k0.82 (19)	48.1k0.80 (27)	*	52.0k0.62 (21)	49.6±0.71 (27)	*
Summer (Sm)	47.5k0.88 (10)	49.0±1.11 (13)	ns	49.210.73 (12)	50.8k0.91 (8)	ns
	Firs	Firs		F**	G-Hns	

b. 2 Statistical significance of seasonal values in blood parameters of *Mus spretus* and *m. vmesticus*. Values are means ± SE with number of animals in parentheses. Symbols are as llows: G-H = Games-Howell test; F = one way ANOVA (followed by the results obt ain after Tukey-Kramer test); t = t-Student (or t's test); ns = not-significant; * = p< 0.0 ** = p<0.01; *** = p<0.001.

	MEAN CORPUSCULAR HEMOGOBIN (pg)					
Autumn (A)	19.4±1.45 (3)	16.6±1.77 (6)	ns	16.6±0.40 (3)	-	-
Winter (W)	18.610.66 (14)	18.6k0.47 (9)	(ns)	19.6k0.87 (5)	-	-
Spring (Sp)	18.9±0.29 (18)	19.510.46 (27)	(ns)	19.5±0.37 (20)	19.4±0.24 (23)	ns
Summer (Sm)	18.9k0.48 (10)	19.0k0.48 (11)	ns	20.120.33 (11)	19.110.75 (7)	ns
	G-Hns	G-Hns		F* (*A/W, **A/Sp, **A/Sm)	t's ns	
Autumn (A)	63.812.33 (5)	63.312.61 (10)	ns	64.3k1.59 (3)	-	-
Winter (W)	66.3k2.16 (14)	64.3k2.79 (9)	ns	70.6±3.49 (5)	-	-
Spring (Sp)	71.5±0.99 (18)	72.611.59 (27)	(ns)	72.4k1.15 (20)	73.6k1.33 (24)	ns
Summer (Sm)	67.8±1.86 (10)	71.3k1.60 (11)	ns	75.9±1.22 (11)	74.0k1.68 (7)	ns
	F* (*A/Sp)	F* (**A/Sp, *W/Sp)		F** (**A/Sm)	t ns	
	MEAN **CORPUSCULAR HEMOGLOBIN CONCENTRATION (g/dl RBC)**					
Autumn (A)	28.811.73 (5)	25.220.99 (19)	*	25.9±1.00 (3)	-	-
Winter (W)	27.9±0.62 (14)	29.1k0.94 (9)	ns	27.920.48 (5)	-	-
Spring (Sp)	26.510.30 (18)	26.6±0.29 (27)	ns	26.910.30 (21)	26.8±0.27 (27)	ns
Summer (Sm)	28.0±0.66 (10)	27.010.44 (13)	ns	26.310.42 (12)	25.8k0.86 (8)	ns
	G-Hns	G Hns		F ns	t ns	
	WHITE BLOOD CELL **COUNT** (10^3/ml)					
Autumn (A)	3.810.92 (5)	4.2±0.54 (10)	ns	4.2±0.71 (3)	-	-
Winter (W)	3.8k0.52 (14)	3.1±0.30 (8)	ns	4.5±0.77 (5)	-	-
Spring (Sp)	4.0M.40 (17)	4.0k0.30 (26)	ns	2.8±0.30 (21)	2.410.19 (26)	ns
Summer (Sm)	3.1M.34 (10)	2.810.37 (12)	ns	3.2±0.43 (11)	3.210.59 (7)	ns
	G-Hns	F ns		F ns	t's ns	

females (13.8±0.61 g/dl to 12.7±0.28 g/dl). However, these changes were significant only for the males ($p < 0.05$). In the females, a significant increase was found between the minimal value of 11.6±0.51 g/dl in autumn and the maximal value of 13.8±0.61 g/dl in winter. In males limit values were found in autumn (13.5±1.00 g/dl) and spring (12.0+0.24 g/dl).

In M. *musculus domesticus* the highest value of Hb was found in males in winter (14.8±0.98 g/dl). This value did not significantly differ from any other seasonal value.

Significant differences were found between sexes in spring for both species.

iii) Hematocrit (Hct)

In *M. spretus* the mean hematocrit presented the highest values in summer in both males (47.5±0.88 %) and females (49.0k1.11 %). The lowest values of 45.3±0.82 % and 46.6±0.89 % were found, respectively, in males in spring and in females in autumn. The mean hematocrit values for males and females were significantly different in spring ($p < 0.05$).

Between autumn and winter the mean hematocrit in males House Mice increased significantly (48.5±2.03 % to 55.2k0.44 %). Intermediate values were found in spring and summer.

Mean values for males and females of both species were significantly different in spring ($p < 0.05$).

iv) Mean corpuscular hernoglobin (MCH)

The mean corpuscular hemoglobin for males *M. spretus* increased from 18.6±0.66 pg in winter to reach the highest value of 19.421.45 pg in autumn. In the females the lowest value of 16.6k1.77 pg recorded in autumn increased to a maximum of 19.5±0.46 pg in spring.

In the males of the commensal species the mean corpuscular hemoglobin increased significantly between autumn and summer from 16.6±0.40 pg to 20.120.33 pg ($p < 0.01$).

v) Mean corpuscular volume (MCV)

In M. *spretus* the mean corpuscular volume in the males increased from a low value of 63.8±2.33 fl in autumn to reach a maximum of 71.5±0.99 fl in spring ($p < 0.05$). Similarly, in the females, this parameter significantly increased from a lowest value in autumn (63.3±2.61 fl) to a highest in spring (72.6±1.59 fl) ($p < 0.01$).

The mean corpuscular volume for males House Mice fluctuated significantly from a minimal value of 64.3±1.59 fl in autumn to a maximal value of 75.9± 1.22 fl in summer ($p < 0.01$).

vi) Mean corpuscular hemoglobin concentration (MCHC)

In the males of the free-living species the mean corpuscular hemoglobin concentration slightly increased between a minimal value in spring

Tab. 3 – Statistical significance of seasonal values in albumin/globulin ratio of *Mus spretus* and *M. m. domesticus*. Values are means ± SE with number of mice and albumin mean percentage/globulin mean percentage in parentheses. Symbols as in Table 2.

	Males	Females	t
	M. spretus		
Autumn (A)	**1.28f0.3 (14)** (55.44/44.56)	**1.15f0.5 (22)** **(52.63147.37)**	ns
Winter (W)	**1.15f0.4 (19)** (51.53/48.47)	**1.25f0.3 (9)** (54.96/45.04)	ns
Spring (Sp)	0.91±0.2 **(17)** (47.33/52.67)	**1.05f0.2 (25)** (50.43/49.47)	ns
Summer (Sm)	0.97±0.2 **(10)** (48.60/51.40)	**1.29f0.3** (13) (55.53/44.47)	ns
	G-H* (*A/Sp)	F ns	
	M. musculus domesticus		
Autumn (A)	**1.48±0.3 (6)** **(58.67142.63)**	**1.3310.2 (3)** (56.71/43.29)	ns
Winter (W)	**1.17f0.3** (3) (52.27/47.73)		
Spring (Sp)	**0.9210.3 (19)** **(4721152.79)**	**1.20f0.3 (27)** **(53.81146.19)**	***
Summer (Sm)	**1.08f0.3** (11) (52.52/48.48)	**1.16f0.4 (8)** (52.44/47.56)	ns
	F** (*A/Sp)	G-H ns	

(26.5±0.30 g/dl RBC) and a maximal value in autumn (29.8±1.73 g/dl RBC) ($p > 0.05$). In the females the lowest value was observed in autumn (25.2±0.99 g/dl RBC) and the highest value in winter (29.1±0.94 g/dl RBC) ($p > 0.05$). **A** significant difference between the sexes was found in autumn ($p < 0.05$).

In *M. musculus domesticus* the MCHC fluctuated in the males from a low value of 25.9±1.00 g/dl RBC in autumn to a high value of 27.9±0.48 g/dl RBC in winter. The values for males and females did not differ significantly in spring and summer ($p > 0.05$).

TOTAL LEUCOCYTE COUNT (WBC) (Table 2)

No significant changes in total leucocyte count were found in *M. spretus* between sexes at any sampling time. In males this count decreased from 4.0±0.40 x 10^3/ml in spring to 3.1±0.34 x 10^3/mi in summer; in the females minimal and maximal counts were found in summer (2.8±0.37 x 10^3/ml) and autumn (4.2±0.54 x 10^3/ml), respectively.

In *M. musculus domesticus* the most evident variation was the decrease in males from 4.5±0.77 x 10^3/ml in winter to 2.8±0.30 x 10^3/ml in spring.

PLASMA PROTEINS (A/G) (Table 3)

Table 3 shows the albumidglobulin ratios for *M. spretus* and *M. musculus domesticus* throughout the year, as well as their statistical significance.

In *M. spretus,* for both sexes, the decrease in the relative albumin level (decrease albumidglobulin ratio) in spring was the most relevant fact to be recorded, High values of albumin were found in the males in autumn and in the females in summer.

On the contrary, in *M. musculus domesticus* the high relative content in albumin (high albumin/globulin ratio) found in both sexes in autumn was the more conspicuous change recorded.

In either of the species seasonal differences in albumidglobulin ratio were only significant for the males between spring and autumn ($p < 0.05$).

Between sexes significant differences in A/G value were only observed in the commensal species in spring ($p < 0.001$).

DISCUSSION

Previous studies in other small mammals characterized the seasonal variations in their hematological values by an increased RBC, Hb and Hct in winter which reverse during summer. It was suggested that this variation may be related with environmental acclimatization. The winter low ambient temperatures requiring **a** higher metabolic rate for body temperature regulation could be a stimulus for erythropoiesis which would be of great advantage in oxygen transport and delivery to the tissues (Sealander, 1964; MacLean and Lee, 1973; Berry and Jakobson, 1975 and references therein). Similar responses were also reported in several species of small mammals at altitude, where the atmospheric oxygen pressure is low (e.g. Sealander, 1964).

This pattern is followed by *M. musculus domesticus* and partly by *M. spretus.* In this species the high RBC and Hb values found in winter decrease during spring and summer. The red blood cells decrease in Algerian mice in spring, (in association with a decrease in hernoglobin concentration and a sligth increase in MCH and MCV) may be partly related with their reproductive condition (e.g. Sealander, 1964 and 1966; Perez- Suarez et al., 1990).

In fact, sexual activity in *Mus spretus* occurs during spring-summer. Reproduction almost ceases in late November or early December and does not resume until March. In *M. musculus domesticus* high numbers of pregnant or lactating females as well as high numbers of active males can be referred to throughout the year (Mathias and Mira, in press).

During summer the very high temperatures of the environment and the related shortage of water (an average summer temperature of 23.6 °C with

a maximum of 32.0 °C and a total precipitation of 113.6 mm) may account for the slight increase in erythrocyte parameters observed in *M. spretus* from spring to summer, reflecting a decrease in plasma volume.

Similarly, the slight spring to summer increase in albumin/globulin ratio, found in both species, although more evident in the free-living one, can be partly correlated with high ambient temperatures and water shortage. In fact, this higher A/G ratio is due to an increase in the relative albumin content which, being a protein with a low molecular weight, may control the osmotic pressure of plasma preventing water losses to the outside (Gras, **1967).** High relative albumin content was also found in both species in autumn. This may also testify to the existence of a greater protein reserve, as it could be the case of mice preparing for wintering.

On the other hand, low A/G values have been related with an increase in thyroid activity and general metabolism associated with the onset of reproduction (e.g. Dobrowolska, **1975)** which may explain the relative increase of globulins (lower albumin/globulin ratio) reported in Algerian mice during spring. However, the continuous sexual activity of the commensal species makes it difficult to justify the spring decreasing A/G ratio, also found in the males of this species. Therefore, other factors, besides reproduction, should also be considered in the regulation of globulin levels (e.g. Dobrowolska, **1975;** Gromadzka-Ostrowska and Zalewska, **1984 & 1985).**

The iack of sexual dimorphism in hematological values has been referred to several species of small mammals (e.g. Sealander, **1964;** Wolk, **1974;** Pérez-Suarez et al., **1990).** This is also true €or *M. spretus* and *M. musculus domesticus* for all the analysed parameters and for all seasons except for the spring values of hemoglobin and hematocrit.

Our results are consistent with the fact that the more uniform habitat of the commensal species throughout the year does not seem to account for very different seasonal changes in hematology and plasma proteins of the two species analysed. *An* interaction of several factors, such as temperature, light, water and food availability, or sexual activity must be considered in the definition of the patterns found. However, we may suggest that some aspects of the environment which may act similarly on both species, e.g. photoperiod via pineal gland estimulation, should have a more important regulating role than others which affect them differentially, e.g. food availability.

REFERENCES

BERRY, R.J. & M.E. JAKOBSON. 1975. Adaptation and adaptability in wild-living mice (*Mus musculus*). J. Zool., Lond., 176: 391-402.

DOBROWOLSKA, A. 1975. Electrophoretic pattern of proteins and glycoproteins in the blood serum of common voles in relation to the season and physiological activity. Acta theriol., 20: 209-224.

DOBROWOLSKA, A., JABLONSKA, E., PATRZYKONT, A. & E, CHABROS. 1983. Variability of transferrin in field mouse from urban and suburban populations. Acta theriol., 28: 235-242.

GRAS, J. 1967. Proteinas plasmáticas. Fisicoquimica, fisiopatologia y clinica de las proteinas extracelulares, 3 ed., Ed. Jims, Barcelona, 494 pp.

GROMADZKA-OSTROWSKA, J. & B. ZALEWSKA. 1984. Changes in protein fractions level in blood plasma of female chinchilas during pregnancy and lactation. Acta theriol., 29: 243-250.

GROMADZKA-OSTROWSKA, J. & B. ZALEWSKA. 1985. Seasonal fluctuations in plasma protein fraction levels of chinchillas (*Chinchilla laniger*, M.). Comp. Biochem. Physiol., 80A: 215-224.

JANDL, J.A. 1987. Blood. Textbook of Hematology. Ed.Little, Brown and Company, Berton/Toronto, 1214 pp.

MACLEAN, G.S. & A.K. 1973. Effects of season, temperature and activity on some blood parameters of feral house mice (*Mus musculus*). J. Mammal., 5 4 660-667.

MATHIAS, M.L. & A. MIRA. (in press). Population structure and patterns of reproduction in commensal and free-living populations of mice (*Mus musculus domesticus* and *M. spretus*: Rodentia, Muridae).

MIHOK, S. & B. SCHWARTZ. 1989. Anemia at onset of winter in the meadow vole (*Microtus pennsylvanicus*). Comp. Biochem. Physiol., 94A 289-304.

MITRUKA, B.M. & H.M. RAWNSLEY. 1981. Clinical biochemical hematological reference values in normal experimental animals and normal humans, 2nd ed., Masson Pubi., New York, 387 pp.

PEREZ-SUAREZ, G., AREVALO, F., LOPEZ-CABALLERO, E. & P. LOPEZ-LUNA. 1990. Seasonal variations in hematological values and heart weight in two small mammals, a mouse: *Apodemus sylvaticus*, and a vole: *Pitymys duodecimcostatus*. Acta theriol., 35: 33-40.

SEALANDER, J.A. 1964. The influence of body size, season, sex, age and other factors upon some blood parameters in small mammals. J. Mammal., 45: 598-616.

SEALANDER. J.A. 1966. Seasonal variation in hemoglobin and hematocrit values in the northern red backed mouse *Clethrionomys rutilus awsoni* (Merriam) in interior Alaska. Can. J. Zool., 44: 213-224.

SOKAL, R. & F. ROHLF. 1981. Biometry. Principles and practice of statistics in biological research, 2nd ed., W.F. Freeman and Co., San Francisco, 859 pp.

WOLK, E. 1974. Variations in the hematological parameters of shrews. Acta theriol., 19 315-346.

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