

## HAEMATOLOGICAL PARAMETERS OF JAPANESE QUAIL (*Coturnix coturnix japonica*) KEPT IN CAGES UNDER NORMAL CONDITIONS AND EXPOSED TO LONG-TIME EXPERIMENTAL HYPODYNAMY

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### Abstract

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The aim of the study was to compare the basic haematological parameters in peripheral blood of captive Japanese quail employed as model organisms for research in cosmic biology. In the first experimental series, red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration, and erythrocytic indices, i. e. mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) of 207 clinically healthy birds of both sexes was studied. The quail were kept in groups in cages under standard laboratory conditions. In the second series, 55 female individuals exposed to experimental hypodynamy (birds suspended in a special device) for up to 90 days were used. A total of 262 blood samples were analyzed for haematological parameters, and 5629 haematological data were evaluated using variation statistics.

Analysis of the first series made possible to establish a range of physiological values of RBC and WBC counts with significant ( $P < 0.05$ ) sex differences in RBC counts. In general, the blood of these birds showed a high oxygenating capacity and a remarkable lymphocytic character.

Haematological parameters of quail females exposed to artificial hypodynamy for 60 days remained unaffected. However, by the end of this exposure lasting for 90 days, a mild decrease occurred in some indices. It can be concluded that Japanese quail have shown an excellent adaptation ability and can serve as a suitable model organism for the study of cosmic physiology.

*Japanese quail, cosmic biology, haematology, hypodynamy*

Efficient use of Japanese quail (*Coturnix coturnix japonica*) as a model organism for simulated and real cosmic flight experiment requires a detailed study of its physiological functions, including the respiratory, circulatory and immune ones. In this type of original studies, however, even more detailed data are required than those commonly available in literature (Sturkie 1986; Campbell 1989 a. o.). Haematological data on Japanese quail are, to our knowledge, still rather fragmentary. The first more systematical approach was that of Atwal (1964) who studied its ontogeny. Similar basic information is provided in studies by Nirmalan and Robinson (1971). In papers by Morgan (1980) and others, again only selected haematological data are presented.

Therefore in the first part of our study, detailed haematological parameters were determined in a population of Japanese quail bred at the Institute of Biochemistry and Genetics of the Slovak Academy of Sciences. In the second part, the same parameters were studied under condition of simulated hypodynamy. Such information is of paramount importance for any later comparison with data obtained from birds flown under real microgravity conditions during a space flight.

### Materials and Methods

A population of Japanese quail bred for generations at the Institute of Biochemistry and Genetics of the Slovak Academy of Sciences was studied. A total of 25 blood samples were collected from birds of line 02, and 182 samples were from birds of line 07. Quail of both sexes, aged 4-5 months, i.e. sexually mature, were included in the first series of experiment. They were reared in groups in cages, fed a commercial feed mixture for growing turkey (KT) with extra vitamin a mineral supplements Roboran H and Plastin at a rate of 5 and 10 g, respectively, per 1 kg of feed mixture. Water was provided ad libitum. The lighting regime was 14:10 h L:D, the temperature was 20-22 °C.

The second series of the experiment was carried out using 55 female birds suspended in a special device „jacket“ that served to ensure their partial hypodynamic. The haematological parameters were determined using 55 clinically healthy female Japanese quail suspended each in a special device designed to render them hypodynamic. The birds wore the jackets for 90 days and they were placed in cages and reared as the previous group. They were blood sampled after 35, 64 and 87 days of hypodynamic, and a total of 93 blood samples were processed with 1720 haematological parameters determined.

Blood samples were taken invariably between 9.00 and 10.00 h from groups of 25 birds from September till November. The blood was collected after a gentle incision with a fine scalpel of the vena basilica dx. seu sin. onto a clean watchglass (3-4 drops), filled into micropipettes and distributed into the dilution media, i.e. Drabkin solution and Natt-Herrick solution. For haematocrit, microhaematocrit capillaries were used and the values were read with aid of the commercial Czech made equipment SOMET after 18 000 r.p.m. for 2 min, and two blood smears were made of each sample. Haemoglobin (Hb) was determined using the cyanmethaemoglobin method, RBC and WBC were counted in Bürker's chamber. From these basic data, erythrocytic indices, i.e. mean corpuscular volume (MCV), mean corpuscular haemoglobin (MHC), and mean corpuscular haemoglobin concentration (MCHC) were calculated. Differential leukocyte count was made using blood smears. Finally, the absolute leukocyte numbers were established.

Standard deviations (S.D.), standard error of the mean (S.E.M.) and coefficient of variation were calculated, and Student's t-test was used to assess the significance of differences.

### Results and Discussion

Erythrocytic indices of adult Japanese quail were characterized by a relatively high haematocrit, higher haemoglobin concentration and MCV (Table 1). Their haemogram revealed a marked lymphocytic character. In males, a higher haemoglobin concentration, higher haematocrit, and higher RBC was found along with larger MCV.

These findings seem to correspond with the neurohumoral and metabolic differences of males and females found also in other avian species (Gayathri et al. 1992; Klein 1974; Lazar et al. 1990; Sova 1971; Sova et al. 1981; Szép et al. 1976). The erythrocytic indices found in our experiment differ from those of Atwal et al. (1974) who found considerably higher values, also from those of Nirmalan and Robinson (1971).

Table 1  
Indices of the hemoglobin and erythrocytes of adult Japanese Quail under normal breeding conditions

Parameter	$\bar{x}$	n	min	max	$S_{\bar{x}1}$	$S_{\bar{x}2}$	$V_{\bar{x}}$	P
Hb ♂	136.45	104	78.86	179.10	16.10	1.58	11.80	++
Hb ♀	117.56	95	80.89	185.64	20.66	2.12	17.58	1.53.10 <sup>-8</sup>
Ery ♂	3.62	108	1.78	4.94	0.58	0.056	16.13	++
Ery ♀	3.27	96	1.06	4.34	0.50	0.051	15.39	7.45.10 <sup>-6</sup>
Hc ♂	0.49	122	0.29	0.58	0.05	0.005	10.20	++
Hc ♀	0.43	106	0.34	0.56	0.07	0.007	16.28	3.44.10 <sup>-8</sup>
MCHC ♂	28.36	117	22.72	46.25	3.62	0.34	12.76	
MCHC ♀	27.65	104	21.07	42.53	3.80	0.36	13.74	5.16.10 <sup>-1</sup>
MCH ♂	38.59	104	28.54	67.59	6.99	0.69	18.12	
MCH ♀	37.18	95	25.27	104.62	10.79	1.11	29.01	2.73.10 <sup>-1</sup>
MCV ♂	138.09	120	79.19	235.96	24.25	2.22	17.56	
MCV ♀	136.12	106	88.24	433.96	37.77	3.67	27.75	4.23.10 <sup>-1</sup>

+ P ≤ 0,05

++ P ≥ 0,01

Similarly, differences were found when comparing the haemoglobin and haematocrit values.

The differential white blood cell count revealed a higher percentage of heterophilic granulocytes and monocytes in females (Tables 2, 3), similar to data by Atwal et al.

Table 2  
Indices of the white blood component of Japanese Quail under normal breeding conditions.  
Number of leucocytes and leucogram

Parameter	$\bar{x}$	n	min	max	$S_{\bar{x}_1}$	$S_{\bar{x}_2}$	$V_{\bar{x}}$	P
Leu ♂	23.01	108	3.5	52.0	8.42	0.81	36.59	0.61.10 <sup>-1</sup>
Leu ♀	23.21	96	7.0	47.5	8.45	0.86	36.37	
Ne-rods ♂	1.43	108	0.30	4.55	0.86	0.085	56.73	8.11.10 <sup>-3</sup> ++
Ne-rods ♀	1.77	94	0.29	4.55	0.92	0.095	51.05	
Ne-seg ♂	34.55	108	13.42	67.99	10.12	0.97	29.32	6.31.10 <sup>-3</sup> ++
Ne-seg ♀	38.50	94	20.31	71.93	10.49	1.08	27.24	
Eo ♂	1.27	108	0.29	3.58	0.77	0.077	56.44	1.47.10 <sup>-1</sup>
Eo ♀	1.44	94	0.32	4.50	0.84	0.089	55.38	
Bas ♂	0.42	108	0.28	2.89	0.48	0.057	72.33	1.49.10 <sup>-2</sup> +
Bas ♀	0.59	94	0.26	2.48	0.47	0.054	64.81	
Lym ♂	59.01	108	27.95	83.02	10.54	1.01	17.86	3.76.10 <sup>-2</sup> +
Lym ♀	55.83	94	30.00	78.29	10.98	1.13	19.66	
Mono ♂	2.59	108	0.33	7.21	1.20	0.12	46.41	6.89.10 <sup>-3</sup> ++
Mono ♀	2.14	94	0.32	6.28	1.14	0.12	52.33	

+ P ≤ 0,05

++ P ≥ 0,01

Table 3  
Absolute numbers of the individual leucocyte types of Japanese Quail under normal breeding conditions

Parameter	$\bar{x}$	n	min	max	$S_{\bar{x}_1}$	$S_{\bar{x}_2}$
Ne-rods ♂	0.329	108	0.009	1.047	0.17	0.016
Ne-rods ♀	0.411	94	0.067	1.056	0.32	0.033
Ne-segm ♂	7.949	108	3.087	15.644	2.23	0.215
Ne-segm ♀	8.936	94	4.714	16.695	2.43	0.251
Eo ♂	0.292	108	0.067	0.824	0.19	0.018
Eo ♀	0.334	94	0.074	1.044	0.19	0.020
Bas ♂	0.097	108	0.064	0.665	0.12	0.012
Bas ♀	0.137	94	0.060	0.576	0.13	0.013
Lymph ♂	13.58	108	6.431	19.103	2.41	0.323
Lymph ♀	12.96	94	6.963	18.171	2.54	0.262
Mono ♂	0.596	108	0.076	1.659	0.29	0.028
Mono ♀	0.495	94	0.074	1.458	0.27	0.028

(1964), however with no significant differences between the sexes. These authors as well as Nirmalan and Robinson (1971) found lower WBC in mature Japanese quail cocks. In our study, lymphocytes and heterophilic granulocytes were predominant similar to findings of the above mentioned authors, as well as their sex distribution. On the other hand, Atwal et al. (1964) found heterophilic granulocytes as a predominant white blood cell type, similar to Nirmalan and Robinson (1971) and Ernst (1971) in *Colinus virginianus*. Eosinophilic granulocytes were less numerous in our material than in that of the above-mentioned writers. Interestingly, a striking vacuolization was detected in the cytoplasm of more than one half eosinophilic granulocytes.

Table 4 The influence of artificial hypodynamy in days

Days of hypodynamy	Mean $\pm$ s	Hematocrit value	Hemoglobin	Erythrocytes	MCV	MCH	MCHC	Leucocytes	Basophils	Eosinophils	Neutrophils rods	Neutrophils segment
		l/l <sup>-1</sup>	g/l <sup>-1</sup>	T.l <sup>-1</sup>	fl	pg	g.l <sup>-1</sup>	G.l <sup>-1</sup>	%	%	%	%
0	X	0.45	116.49	3.87	121.39	32.08	25.79	16.79	0.52	3.50	0.67	24.35
	s	0.03	16.58	0.64	18.17	4.67	2.7	3.6	0.49	1.60	0.71	6.09
35	X	0.51	131.48	4.14	127.87	32.96	26.05	27.46	0.60	3.51	0.48	19.25
	s	0.04	19.03	1.19	24.30	5.98	3.41	7.9	0.66	1.55	0.57	6.74
64	X	0.46	127.75	4.61	97.20	26.81	27.61	12.70	0.80	4.02	0.55	30.28
	s	0.05	17.58	1.46	16.15	5.09	2.87	4.88	0.68	1.92	0.63	9.75
87	X	0.45	117.71	4.59	101.26	26.23	25.68	9.81	0.33	2.52	0.50	27.84
	s	0.03	12.13	0.87	15.28	4.59	2.64	1.96	0.46	1.41	0.86	7.10

Haematological parameters of female birds exposed to hypodynamy for 90 days revealed that up to day 60, RBC, MCH and Hb concentration tended to rise to the upper limit of the physiological range (Table 4). In the last 30 days of hypodynamy a reversed trend was observed, i.e. a decrease to the lower limit of the range established in the first series of our experiment. The WBC showed a similar shift as soon as after 30 days of hypodynamy, i.e. a rise and fall, however, within the physiological range. No further differences were detected in the course of the study.

Overall, the study indicates that the Japanese quail possesses an excellent adaptation capacity as far as its haematological parameters are concerned for requirements of space flights, and seems to be a suitable species for long-time exposure to cosmic life conditions.

### Hematologické parametry japonských křepelek (*Coturnix coturnix japonica*) chovaných v klecích za normálních podmínek a vystavených dlouhodobé experimentální hypodynamii

Cílem práce bylo porovnat základní hematologické parametry v periferní krvi japonských křepelek, používaných jako modelové zvíře ve výzkumu kosmické biologie. V první sérii pokusů byly sledovány počty erytrocytů, leukocytů, koncentrace hemoglobinu a erytrocytární indexy, (střední objem erytrocytů, hemoglobin erytrocytů a střední barevná koncentrace) u 207 klinicky zdravých křepelek obou pohlaví. Křepečky byly chovány ve skupinách v klecích za standardních laboratorních podmínek. Ve druhé sérii bylo použito 55 samic vystavených experimentální hypodynamii (zavěšení v speciálním zařízení) po dobu 90 dnů. Celkem byla analyzována krev 262 jedinců a 5629 hematologických údajů bylo vyhodnoceno.

Analýza první série umožnila stanovit rozmezí fyziologických hodnot pro počty erytrocytů a leukocytů s rozdíly v počtech červených krvinek. Krev těchto jedinců měla vyšší oxygenační kapacitu a pozoruhodný lymfocytární charakter.

### Гематологические параметры японских перепелок (*Coturnix coturnix japonica*), содержащихся в клетках с нормальными условиями и под влиянием длительной гиподинамии

Цель работы сводилась к сопоставлению основных гематологических параметров в периферической крови японских перепелок, используемых в качестве модели в исследованиях космической биологии. В ходе первой партии опытов исследовали на 207 клинически здоровых перепелках обоих полов численность эритроцитов, лейкоцитов,

## on the of hematological parameters in Japanese quail

small	Lymphocytes		Monocytes	Basophils	Eosinophils	Neutrophils, rods	Neutrophils, segment	small	Lymphocytes		Monocytes
	large	both							large	both	
%	%	%	%	G.1 <sup>-1</sup>	G.1 <sup>-1</sup>	G.1 <sup>-1</sup>	G.1 <sup>-1</sup>	G.1 <sup>-1</sup>	G.1 <sup>-1</sup>	G.1 <sup>-1</sup>	G.1 <sup>-1</sup>
66.87	2.78	69.65	1.21	0.09	0.62	0.12	4.17	11.07	0.47	11.54	0.22
7.95	1.57	7.82	1.28	0.08	0.38	0.14	1.57	2.12	0.29	2.19	0.24
70.36	4.44	74.81	1.32	0.16	0.89	0.13	5.15	19.36	1.15	20.79	0.32
7.36	2.36	7.50	0.98	0.20	0.33	0.14	1.88	6.78	0.58	6.87	0.22
58.99	3.92	62.91	1.41	0.09	0.47	0.08	3.88	7.50	0.50	8.01	0.15
10.15	2.55	10.23	1.55	0.10	0.18	0.10	2.11	3.51	0.39	3.71	0.14
65.57	2.29	67.80	0.92	0.03	0.24	0.04	2.76	6.40	0.23	6.63	0.09
8.55	1.36	8.43	0.95	0.04	0.14	0.07	1.03	1.29	0.15	1.35	0.09

концентрацию гемоглобина и эритроцитарные индексы, средний объем эритроцитов. Перепелки содержались группами в клетках в стандартных лабораторных условиях. Во второй серии опытов использовали 55 самок, подверженных экспериментальной гиподинамии (подвешенных в специальном устройстве) в течение 90 суток. В итоге проводили анализ крови 262 особей и оценивали 5629 гематологических данных.

Анализ первой серии способствовал определению пределов физиологических величин численности эритроцитов и лейкоцитов с расхождением в численности красных кровяных телец. Кровь упомянутых особей отличалась большей оксигеназной емкостью и четко выраженным лимфоцитарным характером.

Гематологические параметры самок, подвергаемых в течение 60 суток гиподинамии, остались неизменными, однако в конце эксперимента, после 90 суточной гиподинамии, наблюдали незначительное понижение некоторых индексов. Из результатов исследования вытекает, что японские перепелки отличаются исключительной адаптивной способностью и могут стать подходящей экспериментальной моделью для изучения космической физиологии.

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