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STUDIES ON THE EUROPEAN HARE XVIII.

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# Effect of Keeping European Hares in Transport Cages

#### [With 5 Tables & 3 Figs.]

Seventy eight hares were kept in transport cages for 30 days. This resulted in decrease of body weight, haematocrit, haemoglobin content, and L/N ratio, increase of *MCHC*, leucocyte number, and blood sedimentation rate, decreased running ability and a steady increase of mortality.

#### I. INTRODUCTION

The European hare, *Lepus europaeus* (Pallas, 1778) is the most important species of game in Poland. Consequently, it is subjected to many measures of game management including transfers for repopulating within this country and export abroad.

It is well known that keeping European hares in captivity is quite difficult and causes considerable mortality. Several studies pertaining to this problem were concerned mostly with breeding hares in specially designed cages (Hediger, 1948; Ocetkiewicz, 1961).

The export of European hares and repopulating in this country require keeping the hares in small transport cages for prolonged periods. High mortality during storing and transportation indicates deleterious effects of this practice. It is also unknown what influence the stay in transport cages may exert on the adaptive ability of hares in new environmental conditions after introduction.

The purpose of this work was to study the effects of keeping European hares in transport cages, on their mortality and on several physiologic indexes which may change when the animals get out of form. Simultaneously the effects of a tranquiliser were tested. Administration of a tranquiliser should reduce both physical and psychic stimuli of stay in a cage. The study was suggested by the Polish Hunting Union which also provided the animals.

#### II. MATERIALS AND METHODS

The studies were carried out between January 10 and March 4, 1966 at the Field Station of the Institute of Ecology, Polish Academy of Sciences at Dziekanów Leśny. Male European hares were used. They were from three Experimental Breeding Centers of the Polish Hunting Union: Gradów (Sochaczew county), Nowy Przybyszew (Białobrzegi county) and Rębowola (Grójec county). The hares were caught with nets and shipped to the Field Station in transport cages on the next day. They were shipped in groups of different size. The animals from Gradów were transported for 72 km by train and for 22 km by car. The hares from Przybyszów and Rębowola were brought by car from a distance of 92 and 77 km, respectively.

The hares were kept in the experiment for 30 days. Cages with the hares were placed in a three wall open shack in three layers divided into three chambers. Each chamber was 470 mm long, 220 mm wide and 370 mm high and was housing one hare.

The hares were divided into three groups depending on the diet. Group A (30 animals) was fed mangels (fodder beets) and meadow hay, group B (29 animals) was fed garden carrots and meadow hay. Group C (19 animals) differed from group B by receiving every third day intramuscular injection of Combelen <sup>1</sup>), 0.5 mg/kg.

The composition of hay was the following <sup>2</sup>): Agrostis vulgaris (W it h.) —  $11.9^{0}/_{0}$ , Dactilis glomerata (L.) —  $29.7^{0}/_{0}$ , Festuca pratensis (H u d s.) —  $15.7^{0}/_{0}$ , Lolium perenne (L.) —  $15.1^{0}/_{0}$ , Poa trivialis (L.) —  $7.5^{0}/_{0}$  Medicago sativa (L.) —  $7.5^{0}/_{0}$ , Trifolium pratense (L.) —  $5.2^{0}/_{0}$ , others —  $4.8^{0}/_{0}$  (generally:  $81.9^{0}/_{0}$  of grass and  $15.3^{0}/_{0}$  of Papilionaceae).

Both diets (carrot with hay and mangels with hay) are standard diets used in storing and transporting hares. The European hares in our experiment were fed *ad libitum* once daily, before noon. It was found that the average daily consumption of carrots or mangels was 670 g per animal; some hay was also eaten.

The following tests were done on the animals kept in the above described conditions:

The body weight was determined to the nearst 0.1 kg every third day beginning on the first day of confinement.

Blood was collected on the 1-st, 14-th, 21-st and 28th day from the marginal wein of the ear. The haemoglobin content was determined in the Sahli haemoglobin-meter using 0.1 n HCl. The volume of morphotic elements of blood was measured as haematocrit in glass tubes employing centrifugation for 20 min at 3500 to 4000 rpm. The rate of blood sedimentation was measured in Pancenkov micropipetes usin sodium citrate. The leukocytes were counted in Thom's chamber and the percentage of separate forms was calculated from the blood smears stained with the Pappenheim method.

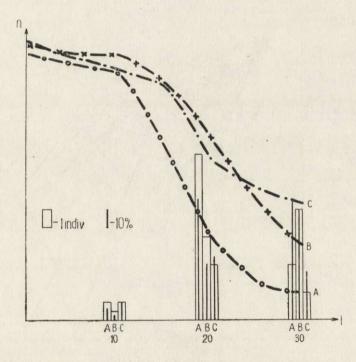
Urine. The daily urine excretion was measured and a standard analysis of urine was made on day 4, day 14 and day 29. To obtain the urine European hares were placed in single cages equipped with metal mesh floor for 24 hours. Beneath the floor were plastic gutters and the urine was collected into a glass container. These cages were of the same size as the single chamber in a transport cage. The standard analysis of urine consisted of: measuring specyfic gravity with the

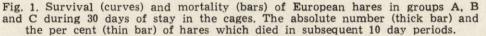
<sup>&</sup>lt;sup>1</sup>) Combelen-Bayer: N-3'-Dimethylaminopropyl/-3-propionyl-phenothiazin. Investigation on the effects of Combelen was carried out by L. Zaniewski and will be published separately.

<sup>2)</sup> I wish to thank Dr. Z. Wójcik for botanical analysis of the hay.

urometer, determing the reaction (pH) with litmus paper, testing for the presence of sugar and protein and determing composition of the precipitate. Sugar was measured with the Glukotest (Chemical Factory »Organica«). This method is not precise as the glukotest detects both glucose and other reducing substances. The protein was detected with the ring test using concentrated nitric acid. Precipitate was checked for the presence of blood morphotic elements, epithelial cells and inorganic substances.

Running performance was studied in a revolving wooden drum (circumference 3768 mm). The hare was moving along the metal mesh installed on the circumference of the drum thus making it turn. The number of revolutions per 5 min was registered by a counter. The running performance of each hare





was studied only once, after different periods of confinement in the cage. The running performance test undoubtedly introduces additional stimuli and therefore the number of these determinations was reduced to a minimum.

## III. RESULTS

1. Mortality. The mortality of European hares kept in cages was high and differed in various experimental groups (Fig. 1). The highest mortality occurred in group A where 90% of the animals died. In group

B the mortality was 84% and in group C — 58%. Only the difference between groups A and C was statistically significant.

In individual groups the mortality was unevenly distributed over the 30 day period of an experiment. The mortality between the first and tenth day of keeping was low in all groups. In group A mortality increased after ten days and stayed high for the rest of the experiment.

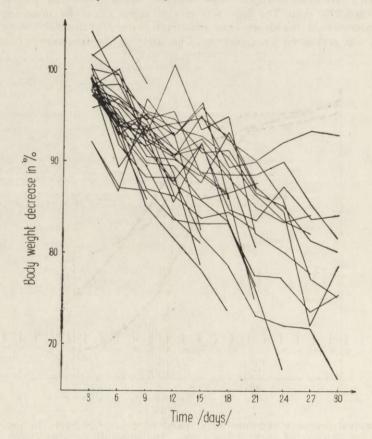


Fig 2. Per cent changes of body weight of hares in group B (Initial weight =  $100^{0}/_{0}$ ).

In group B mortality was the highest between the twentieth and thirtieth day of keeping the animals in cages. In group C mortality increased between the fifteenth and twentieth day.

2. Body weight. In all groups the body weight was decreasing during the experimental period. No correlation was found between the initial weight and survival time in the transport cage. Consequently, the initial body weight of each hare was considered 100% and the chan-

ges of body weight (of individuals and of whole groups) were expressed in per cent of the original values. The changes of body weight in group B are given in Fig. 2 as an example. The regression of the per cent changes of body weight was computed for every group and the slopes

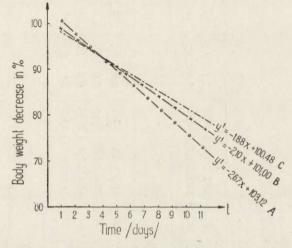


Fig. 3. Regression of the decrease of body weight on time spent in the cage in individual groups.

Table 1.

Phy	vsiologic cl			olood in groups . ys (4) of stay in		after 1 (1), 14 (2),
dn	Deter-	_	Hb conc.	Haematocrit	MCHC	Blood sedimen-

Group	Deter- mina- tion	n	Hb conc. (g/100 ml)	Haematocrit %	MCHC	Blood sedimen- tation test after 2 hours
A	1 2 3	29 20 7	$\begin{array}{c} 16.0 \pm 0.26 \\ 13.7 \pm 0.47 \\ 12.8 \pm 2.34 \end{array}$	$57 \pm 1.10 \\ 47 \pm 1.50 \\ 46 \pm 0.57$	$\begin{array}{c} 28.1 \pm 0.50 \\ 30.1 \pm 1.48 \\ 31.1 \pm 1.08 \end{array}$	$\begin{array}{c} 1.87 \pm 0.20 \\ 22.30 \pm 3.60 \\ 24.00 \pm 3.20 \end{array}$
в	1 2 3	30 26 17	$\begin{array}{c} 16.0 \pm 0.15 \\ 15.1 \pm 0.26 \\ 13.5 \pm 1.04 \end{array}$	$57 \pm 1.21 \\ 50 \pm 1.32 \\ 46 \pm 1.06$	$27.9 \pm 0.28 \\ 30.7 \pm 1.20 \\ 30.1 \pm 1.36$	$\begin{array}{c} 1.85 \pm 0.27 \\ 13.67 \pm 0.81 \\ 15.26 \pm 4.20 \end{array}$
с	1 2 3 4	21 16 9 8	$\begin{array}{c} 15.9 \pm 0.27 \\ 14.2 \pm 0.45 \\ 12.4 \pm 0.73 \\ 13.1 \pm 0.38 \end{array}$	$59 \pm 0.76 \\ 49 \pm 1.50 \\ 48 \pm 2.90 \\ 48 \pm 0.92$	$\begin{array}{c} 26.9 \pm 0.45 \\ 29.1 \pm 0.78 \\ 26.8 \pm 1.38 \\ 27.5 \pm 0.82 \end{array}$	$\begin{array}{c} 3.00 \pm 0.50 \\ 19.00 \pm 0.80 \\ 13.33 \pm 4.43 \\ 8.30 \pm 1.80 \end{array}$

were compared (Fig. 3). The relation of body weight and the time of keeping was linear in each group but the slopes were significantly different. The regression coefficient was the smallest in group C and largest in group A.

Table 2.

Number of leucocytes in 1 mm<sup>3</sup> of the blood of the hare as well as percent and absolute value of separate form in the 1th

F	- 1	reactory tes	Neutrophils	nils	Fosinophils	STIC	sundosed	en	Trympnocytes	cy (co	MUDIOCYLES		Lymph.
	per	r c. mm	26	c. mm	96	c. mm	%	c. mm	96	c. mm	96	c. mm	Neutr.
						G R	OUP	A					4
	28 37	$3793 \pm 264$	$62.8\pm1.3$	2382	$1.0 \pm 0.3$	38	$1.5\pm0.3$	57	$33.2 \pm 2.5$	1259	$1.2 \pm 0.3$	46	$0.59 \pm 0.07$
	19 87	8747 ± 590	$81.4 \pm 1.5$	7120	$0.4\pm0.2$	35	$2.2 \pm 0.6$	192	$15.1 \pm 1.3$	1321	$0.9 \pm 0.2$	79	$0.19\pm0.05$
						G R	OUP	В					
	24 36	$3692\pm660$	$55.6\pm4.1$	2053	$1.4 \pm 0.3$	52	$0.8\pm0.3$	30	$41.3\pm2.8$	1525	$0.9\pm0.2$	32	$0.99 \pm 0.11$
10.000	16 92	$9269 \pm 630$	$73.9\pm2.5$	.6850	$0.4\pm0.2$	37	$1.2 \pm 0.4$	111	$23.8 \pm 3.9$	2206	$0.6 \pm 0.3$	38	$0.40 \pm 0.10$
	10 129	$10 \ 12940 \pm 6090$	$82.2\pm2.3$	10638	$0.9\pm4.6$	116	$0.5\pm0.2$	65	$15.8\pm2.4$	2044	$0.6\pm0.2$	78	$0.20\pm0.04$
	5 79	$7900\pm1130$	$66.8 \pm 4.1$	5277	$0.6\pm0.3$	47	$1.0 \pm 0.2$	64	$31.2\pm6.3$	2465	$0.6\pm0.3$	47	$0.49 \pm 0.10$
			T.			GR	OUP	U				-	
	17 34	$3458 \pm 1970$	$58.6 \pm 4.2$	2026	$0.2 \pm 0.1$	7	$1.1 \pm 0.3$	38	33.8 ± 4.1	1342	$1.2\pm0.3$	41	0.91 ± 0.19
	16 111	$11162 \pm 1410$	$\textbf{79.5} \pm \textbf{4.8}$	8874	· 1.3 ± 0.5	145	$0.1 \pm 0.1$	11	$18.5\pm1.9$	2065	$0.6\pm0.2$	67	$0.22\pm0.05$
	9 79	$7978 \pm 1930$	$75.5\pm4.4$	6023	$0.2 \pm 0.1$	16	$0.4\pm0.2$	32	$23.1 \pm 4.3$	1843	$0.7 \pm 0.3$	56	$0.35\pm0.05$
	5 92	$9240\pm2120$	$69.2\pm7.2$	6394	$3.4 \pm 3.0$	314	0.6 ± 0.5	55	$25.7 \pm 3.5$	2375	$1.1 \pm 0.7$	102	$0.44 \pm 0.14$

#### Keeping European hares in transport cages

3. Physiologic characteristics of the blood. For every group the following mean values were computed: haemoglobin content, haematocrit, *MCHC* (mean cell haemoglobin concentration), leucocytes number and rate of blood cells sedimentation. All determinations were made after 1, 14 and 21 days of stay in the cage (Table 1, 2). The results obtained after 28 days are given only for group C as there were very few animals in the remaining two groups. The values of individual characteristics measured after 1 and after 14 days of stay were not correlated. Therefore the significance of differences was calculated using the Students t test for comparing the means of two independent samples.

Within every group the haemoglobin content and the haematocrit were increasing while the *MCHC* and the number of leucocytes/mm<sup>3</sup> were decreasing. Changes were also observed in percentage of neutrophils and limphocytes and the ratio of L/N. All differences were statistically significant (Table 3). There were no significant differences between the groups of day 1, day 14, and day 21, exept one case. Significant

Choun	A			B					
Group				I	nde	x			
Determination	I-II	ш-ш	I-III	I-II	II-III	I-III	I-II	II-III	I-III
Hb	+	-	+	+	_	+	+	-	+
Haematocrit	+	-	+	+	-	+	+	_	+
MCHC	-	-	+	+	-		.+	-	_
Leucocyte/mm <sup>3</sup>	+	_	+	+	_	+	+	_	+

Table 3.

The significance of differences between physiologic blood characteristics in subsequent determinations. + statistically significant difference, — not significant difference.

differences were found in percentage of limphocyte and L/N ratio between group A and B during first (day 1) and second (on day 14) determinations.

4. Urine. The quantitative analysis of urine revealed increase of mean daily urine excretion in groups B and C with concomitant decrease of specific gravity. In group A the daily output and specific gravity did not change (Table 4). Qualitative studies did not reveal any directional changes depending on the time of stay in the cage or on the diet. The general characteristics of the urine of European hare was based on

numerous determinations made between day 3 and day 5 (Table 5). There were little variation and the urine was always alkaline.

Table 4.

		1st determ	ination	1 :	2nd determination			3rd determinati		
Group	n	Daily output (ml)	Specific gravity	n	Mean daily output	Mean specific gravity	n	Mean daily output	Mean specific gravity	
A	28	228	1.025	21	228	1.025	3	188	1.022	
в	30	180	1.026	27	282	1.022	13	335	1.016	
С	20	128	1.035	16	338	1.020	9	345	1.016	

Class	n	Class	n
Daily amount of u	rine (ml)	Specific grav	vity
0-200	55	1.011-1.020	22
201-400	14	1.021 - 1.030	25
401-600	7	1.031 - 1.040	18
		1.141-1.050	11
Sugar %		Protein <sup>0</sup> / <sub>0</sub>	0
• 0	13	0	61
trace	14	trace	5
0.25 - 0.49	27	0.033	7
0.50 - 0.99	13	0.132	2
1.00 - 1.49	. 4	0.298	1
1.50-1.99	-		1
2.00 - 2.49	5		

5. Running performance. The running performance was compared in two groups of European hares; the first group was tested after 3 days in the cage and the second after 14 days in the cage. The mean number of revolutions of the drum per 5 min. was 101.5 (standard deviation S.D. =  $\pm$  37.8) in the first group and 56.6 (S.D. =  $\pm$  40.8) in the second group. The difference was statistically significant (t = 4.045).

Table F

The dai content

From the number of revolutions the mean distance run by the tested animal during one minute was computed. It was 76.5 m/min in the first group and 42.6 m/min for the second.

# IV. DISCUSSION

The general physiologic state of European hares kept in transport cages is rapidly deteriorating. This is indicates by the changes of the studied characteristics. The haemoglobin content and haematocrit drop down while MCHC increases. The increase of MCHC indicates that the number of erythrocytes decreases faster than the haemoglobin level. Increase of the neutrophils number may be treated as reaction of the hares' organism on the rapid change of external conditions (cageing, artificial feeding. Cf. also Pujman, 1948, 1955). The decrease of the respiratory function of haemoglobin together with the increase of the leucocyte number and blood sedimentation rate may be caused by some deficiences in the diet and by the deteriorating health of the animals.

Interpretation of the results of the urine analysis is excidingly difficult as no data are available on the composition of European hare urine. The increase of daily urine excretion may indicate excessive water intake or disturbances in the water metabolism.

The sharp decrease of physical shape of the hares is strongly indicated by the constant decrease of body weight. The running performance decreased by half already after 14 days of stay in the cage. Simultaneously high mortality was observed especially after 10 days of stay.

The comparison of the groups fed with two diets presently employed for storing and transporting hares indicates that the diet composed of carrots and hay may be slightly better; there was a little less decrease in body weight and the mortality was somewhat lower.

Administration of Combelen yielded promising results in spite of the small number of animals tested.

The present results indicate that prolonged stay in cages presently employed for transportation has an adverse effect on the general physiologic state of the animals. This may result in increased mortality after introduction due to increased susceptibility to diseases and predation (decreased running performance). It appears that the period of keeping hares in transport cages should be reduced to a necessary minimum. Studies on improving the diet and the cage would be indicated also.

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#### WPŁYW PRZETRZYMYWANIA ZAJĘCY W KLATKACH TRANSPORTOWYCH

#### Streszczenie

Trzy grupy zajęcy liczące 30, 29 i 19 osobników przetrzymywano przez 30 dni w klatkach transportowych. Zastosowano dwie standartowe diety (buraki pastewne + siano łąkowe i marchew ogrodowa + siano łąkowe). W trzeciej grupie trzymanej na drugiej diecie podawano dodatkowo Combelen 0,5 mg/kg co trzeci dzień w postaci injekcji domięśniowych.

Przeprowadzono badania: zmian ciężaru ciała, wskaźników fizjologicznych krwi (hematokryt, Hb, liczba leukocytów, OB), ilości dobowej i analizy podstawowej moczu oraz kondycji biegowej.

Wyniki badań wskazują na szybkie tempo pogarszania się ogólnego stanu fizjologicznego przetrzymywanych zajęcy wszystkich grup. Obniżają się wartości Hb i hematokrytu, zwiększa się *MCHC*, liczba leukocytów i OB (Tabela 1, 2), następuje ostry spadek ciężaru ciała (Fig. 2 i 3). Wydolność biegowa zajęcy obniża się o połowę po 14 dniach przetrzymywania. Równocześnie obserwuje się wysoką śmiertelność nasilającą się po 10 dniach (Fig. 1). Z dwóch zastosowanych diet nieco lepsze wyniki (mniejszy spadek ciężaru ciała, mniejsza śmiertelność) daje dieta skomponowana z marchwi ogrodowej i siana łąkowego. Podawanie Combelenu może okazać się pozytywnym zabiegiem w procesie przetrzymywania.