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# GENETIC PARAMETERS OF UDDER, MASTITIS AND MILK TRAITS IN TWO DIFFERENT CLIMATIC AREAS USING ANIMAL MODEL ANALYSIS

## GENETICKÉ PARAMETRY VEMENE, MASTITIDY A VLASTNOSTÍ MLÉKA VE DVOU KLIMATICKY ODLIŠNÝCH OBLASTECH ZJIŠŤOVANÉ POMOCÍ ANIMAL MODELU

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**ABSTRACT:** Lactation records of 2 567 Holstein-Friesian (HF) cows in Egypt (subtropical area) and Hungary (rainy area) were used to determine the effect of drought on changes in genetic parameters. The traits studied were udder length and depth, teat length, attachment and placement and milk production traits (total milk, fat and protein yield). Variations in all studied traits due to random effects of sire groups were much higher in the rainy area than in the subtropical one. Changes of all genetic (co)variances with an advancing order of lactation were greater for subtropical animals. In subtropical area heritability estimates of udder traits for HF were higher than estimates for milk production and their differences were also larger than the corresponding estimates of cold rainy area. Low heritability estimate was computed for mastitis infection for Hungarian HF. Highest genetic and phenotypic correlations (-0.67 and -0.87, respectively) for mastitis with udder depth were recorded in subtropical area. Many details concerning genetic parameters which could be taken into consideration during planning and carrying out phenotypic and genetic selection to increase the total economic benefit of dairy farms were tabulated and discussed.

**Keywords:** udder conformation; mastitis; milk production; genetic parameters; subtropical vs. cold rainy weather

**ABSTRAKT:** Ke zjištění vlivu sucha na změnu genetických parametrů byly použity údaje o užitkovosti za laktaci u 2 567 dojníc holštýnsko-fríského plemene (HF) z Egypta (subtropické oblasti) a Maďarska (vlhké oblasti). Mezi sledované znaky patřila délka a hloubka vemene, délka, nasazení a umístění struku a dále znaky produkce mléka (celková dojivost, celková produkce tuku a proteinů). Kolísání všech sledovaných znaků bylo v důsledku náhodných efektů u skupin plemenků mnohem vyšší ve vlhké než v subtropické oblasti. U zvířat ze subtropické oblasti byly s narůstajícím pořadím laktace zaznamenány větší změny všech genetických (ko)variací. U plemene HF v subtropické oblasti byly odhady dědivosti znaků vemene vyšší než odhady mléčné produkce a rozdíly mezi nimi byly rovněž větší než příslušné odhady u zvířat ze studené vlhké oblasti. U maďarského HF byl odhad dědivosti pro infekci mastitidy nízký. Nejvyšší genetické a fenotypové korelace mezi výskytem mastitidy a hloubkou vemene (-0,67 a -0,87) byly zjištěny v subtropické oblasti. Četné údaje týkající se genetických parametrů, které by mohly přicházet v úvahu při plánování a provádění fenotypové a genetické selekce zaměřené na zvýšení celkového hospodářského zisku mléčných farem, jsme uspořádali do tabulek a předložili je k diskusi.

**Klíčová slova:** utváření vemene; mastitida; produkce mléka; genetické parametry; subtropické versus chladné vlhké počasí

### INTRODUCTION

Possibilities of improving mastitis resistance by indirect selection for udder and teat conformation traits seem to exist if an appropriate method for each environment is used. Estimates of genetic parameters for dairy cattle performance were investigated in different countries using different statistical methods, but all in the same climatic conditions. Most subtropical coun-

tries import a large part of their dairy cattle herds from rainy frosty countries. The young imported individuals were reared under suitable temperatures and sufficient feed for optimum physiological functions. Management of these individuals for production will be under hot and draught desert climates. Therefore, the change of genetic performance due to the previous circumstances has not been obvious until now. Evaluation of the individual breeding value and effect of drought on the

performance of dairy cattle is needed. Many investigations have been reported genetic parameters for udder traits and milk production and relationships between them. Thomas *et al.* (1984) in the USA concluded that selection against deep udders, especially low rear udder widely placed teats, rear teats too far back, and teats that are short and wide might help reduce the incidence of mastitis in a mastitis control program. Estimates of heritability of udder traits ranged from 0.09 to 0.14 (Monardes *et al.*, 1989). Relations of some teat and udder morphological characteristics to mastitis incidence (Bakken, 1981; Van Vleck and Horman, 1972) were studied in different USA states and European countries with approximately similar environmental conditions. Computations of genetic parameters in the Middle East as a semi drought region showed that  $h^2$  for mastitis was 0.10, genetic and phenotypic correlations of mastitis with milk and fat were 0.22, 0.66, -0.032, -0.025, respectively (Weller *et al.*, 1992). Seykora and McDaniel (1986) found that  $h^2$  of udder and teat conformation ranged from 0.1 to 0.56 and 0.33 to 0.48 for HF, reared in North Carolina.

The objectives of the present study are i) pointing out the effect of hot drought vs. cold rainy environmental conditions on changes in the genetic and phenotypic parameters of performance of some dairy cattle breeds, ii) creating a genetic cofactor that can be used as a basis for comparisons between exotic and settled animals of the same breed.

## MATERIAL AND METHODS

**Structure of the data:** A total of 2 567 Holstein-Friesian (HF) cows were reared and managed for production under conditions of two different climates (chilly and rainy weather in Hungary and subtropical or drought weather in Egypt). Total number of 8 518 complete lactation records was used in the present study: 2 507 records of 549 cows and 6 011 records of 2 018 cows from Egypt and Hungary, respectively. Data of Hungarian HF involved the first three parities and were collected from four farms of more than one district while the data of HF in Egypt involved five parities collected from three farms located east of Cairo. The studied traits are udder conformation or descriptive udder traits (udder depth and height, teat length, attachment and placement); milk production traits (total milk, fat, and protein yield), and mastitis. Three data sets were used for the statistical analysis of variance using three different statistical models. The first data set of Hungarian HF population included 82 paternal half-sib families and involved 20–32 cows per sire (model I), the second data set of HF in Egypt included 69 paternal half-sib families and involved 4–9 cows per sire (model II), and the pooled data of both populations was considered as the third data set (model III). Identification of all individuals in the 1st data set was known as sire and dam, but only sire was known for

the 2nd data set. The log (natural) transformation was adopted in udder traits and mastitis score for the analysis of variance.

**Statistical analysis:** Multitrait REML Animal Model was used for the statistical analysis of variance to generate estimates of genetic and phenotypic variances and covariances for all studied traits by using VCE – Variance Component Estimation – package that was developed by Groeneveld (1996). Three mathematical models were considered for analysis of variance as follows:

$$Y_{ijklm} = \mu + S_i + H_j + P_k + D_{li} + \varepsilon_{ijklm} \quad (I)$$

$$Y_{ijkl} = \mu + S_i + H_j + P_k + \varepsilon_{ijkl} \quad (II)$$

$$Y_{ijklm} = \mu + R_i + S_{ji} + H_{ki} + P_l + \varepsilon_{ijklm} \quad (III)$$

where:  $Y_{ijklm}$  – natural log-transformation of udder traits, mastitis or neutral observed values for milk production traits

$\mu$  – population mean

$S_i$  – random effect of the  $i$ -th sire

$H_j$  – fixed effect of  $j$ -th herd (farm, year and season of calving in Egypt and + the district in Hungary)

$P_k$  – fixed effect of  $k$ -th parity

$D_{li}$  – random effect of  $l$ -th dam within  $i$ -th sire in the model I only, in model III

$R_i$  – fixed effect of  $i$ -th weather condition ( $i = 1$  for drought area – Egypt,  $i = 2$  for rainy area – Hungary) in model III, sire and herd effects are nested within weather conditions

$\varepsilon_{ijkl(m)}$  – random residual

**Sire Climatic Cofactor (SCF).** Evaluation of genetic differences in the performance of dairy cattle populations in different climatic conditions (like in the present study, subtropical or drought conditions in Egypt and cold rainy conditions in Hungary) is needed to obtain a cofactor as a genetic basis for drawing true conclusions during comparison. Therefore the present study computed a new derivation called Sire Climatic Cofactor (SCF<sub>d</sub>) and heritability of difference ( $h^2_d$ ). SCF<sub>d</sub> was calculated as the absolute value of differences between the ratio of sire variance component in each environment relative to the pooled sire variance component as follows:

$$\sigma_{S_{Spot}}^2 = \sigma_{S_{Sub}}^2 + \sigma_{S_{Rainy}}^2$$

$$\sigma_{S_{Sub}}^2 / \sigma_{S_{Spot}}^2 = r_1$$

$$\sigma_{S_{Rainy}}^2 / \sigma_{S_{Spot}}^2 = r_2$$

$$|SCF_d| = r_1 - r_2$$

$h^2_d$  was calculated as the ratio between the absolute difference in sire variance components in each environment and the difference between their phenotypic variance components, then the heritability of differences was computed as follows:

$$\sigma_{S_{Sub}}^2 - \sigma_{S_{Rainy}}^2 = |\sigma_{S_d}^2|$$

$$\sigma_{P_{Sub}}^2 - \sigma_{P_{Rainy}}^2 = |\sigma_{P_d}^2|$$

$$h^2_d = (|\sigma_{S_d}^2| / |\sigma_{P_d}^2|) \cdot 4$$

Genetic and phenotypic correlations between the performance of the same trait in both climatic conditions were calculated from the following equations:

$$R_g = \frac{Cov_{S_{1i}, S_{2i}}}{\sigma_{S_{1i}} \cdot \sigma_{S_{2i}}}, \quad R_p = \frac{Cov_{P_{1i}, P_{2i}}}{\sigma_{P_{1i}} \cdot \sigma_{P_{2i}}}$$

where:  $R_g, R_p$  – genetic and phenotypic correlation coefficients between performance of the same trait in both areas due to common gene effect and general management for production (non-special management)

$Cov_{S_{1i}, S_{2i}}$  – additive genetic covariance between the sire effect in the 1st environment (rainy conditions) for the  $i$ -th trait and sire effect within the 2nd environment (subtropical area) for the same trait

$Cov_{P_{1i}, P_{2i}}$  – covariance between total phenotypic performance of the  $i$ -th trait in the 1st and 2nd environment

$\sigma_{S_{1i}}, \sigma_{S_{2i}}$  – square roots of additive genetic variance for the  $i$ -th trait in the 1st and 2nd environment

$\sigma_{P_{1i}}, \sigma_{P_{2i}}$  – square roots of total phenotypic variance for the  $i$ -th trait in the 1st and 2nd environment

## RESULTS AND DISCUSSION

Estimates of sire and random error variance components are presented in Table 1. However absolute estimates of sire and error variance components for udder descriptive traits were higher in subtropical area than in the rainy one. While the components of total phenotypic variance in rainy area were sire, dam, error variance component, in subtropical area they included only sire and error variance component.

Therefore when comparing the amount of additive variance for udder descriptive traits in subtropical area, it was found that it is smaller than the corresponding estimate in the Hungarian one. In contrast, absolute estimates of sire variance component for milk production in rainy conditions were higher and more significant than the corresponding estimates in subtropical area.

In general, from the pooled data analysis of variance using model III (combined analysis), estimates of sire and residual component were high with a low increase of significance for all studied traits in both areas. This indicates that differences due to the effect of climatic condition play an important role of changing genetic variation between different populations.

**Estimates of heritability:** Estimates of heritability for all studied traits that generated by model I and II are given in Table 2. In general, heritability of udder conformation traits was medium to high in both environments and large in its magnitude compared to that of milk traits. However, the latter estimates ranged from low to medium values (0.14–0.30) in Hungarian and (0.19–0.22) in Egyptian environmental conditions. Also heritability of udder traits was higher than that of teat traits in both areas and all descriptive udder traits in subtropical conditions were higher in their magnitude than that in the other one except for teat length. Maximum difference between heritabilities of udder traits in rainy conditions was 50% lower than the corresponding one in the subtropical conditions.

Low heritability estimates of mastitis were obtained in both environments. This may indicate that direct selection within both populations will not be effective in reducing the incidence rate of mastitis. In general, heritability of milk production was higher for Hungarian HF population. This is may be due to increasing environmental variations in the Egyptian population (Table 1). Considering milk traits the lowest estimates of heritability were obtained for fat yield especially in the rainy area while protein yield reached the maximum compared to other estimates. Heritability estimates of descriptive udder conformation largely agreed with those reported by Schaeffer (1983). Some estimates of heritability herein were in close agreement with those reported for US Holstein in different states with different climatic conditions (Schaeffer *et al.*, 1985; Smith *et al.*, 1985; Thompson *et al.*, 1981, 1983). Monardes *et al.* (1990) reported that different scoring systems,

Table 1. Tests of significance for the sire effect generated from model I, II, III for all studied traits

df	Sire	$\sigma_s^2$	$\sigma_e^2$	Sire	$\sigma_s^2$	$\sigma_e^2$	Sire	$\sigma_s^2$	$\sigma_e^2$
	81	rainy	model I	68	subtropical	model II	149	combined	model III
UD	***	46.69	36.69	***	69.17	473.34	***	127.18	642.39
UH	***	63.05	32.94	*	86.13	497.80	**	119.13	951.02
TP	**	83.10	61.18	*	122.47	877.29	*	209.12	1 424.30
TL	*	53.18	43.30	***	134.19	1543.19	***	245.30	2 358.30
TA	**	69.11	51.71	**	94.28	675.35	**	107.98	1 554.71
Mas	*	0.01	0.23	**	0.07	2.03	**	0.12	5.31
MY	**	73 151	953 127	**	60 537	1 093 112	**	97 548.40	2 114.22
FY	**	215.03	2 023.20	*	198	3 885	***	327.01	654.23
PY	**	289.11	1 953.15	**	173	2 951	***	247.32	432.05

$\sigma_s^2, \sigma_e^2$  – sire and error variance component, Df – degree of freedom, UD – udder depth, UH – udder height, TL – teat length, TA – teat attachment, TP – teat placement, Mas – mastitis, MY – milk yield, FY – fat yield, PY – protein yield

Table 2. Genetic parameters generated by using separate data set analysis in Egypt and Hungary

	Rainy			Subtropical		
	$h^2$	$r_g$	$r_p$	$h^2$	$r_g$	$r_p$
UD	0.45 ± 0.13	-0.43 ± 0.16	-0.23 ± 0.09	0.51 ± 0.21	-0.67 ± 0.11	-0.87 ± 0.12
UH	0.42 ± 0.11	0.51 ± 0.17	0.43 ± 0.18	0.59 ± 0.32	0.39 ± 0.21	0.37 ± 0.11
TP	0.35 ± 0.11	0.22 ± 0.01	0.17 ± 0.07	0.49 ± 0.11	0.23 ± 0.11	0.29 ± 0.13
TL	0.37 ± 0.09	0.24 ± 0.12	0.28 ± 0.11	0.32 ± 0.09	0.44 ± 0.09	0.65 ± 0.17
TA	0.35 ± 0.12	0.32 ± 0.09	0.42 ± 0.11	0.49 ± 0.13	0.45 ± 0.19	0.57 ± 0.12
Mas	0.09 ± 0.13	10.00 ± 0.00	10.00 ± 0.00	0.13 ± 0.03	10.00 ± 0.00	10.00 ± 0.00
MY	0.24 ± 0.07	0.41 ± 0.13	0.73 ± 0.20	0.21 ± 0.07	0.39 ± 0.19	0.59 ± 0.23
FY	0.14 ± 0.04	-0.39 ± 0.11	-0.43 ± 0.12	0.19 ± 0.05	-0.44 ± 0.18	-0.47 ± 0.18
PY	0.30 ± 0.11	0.52 ± 0.23	0.77 ± 0.29	0.22 ± 0.10	0.47 ± 0.18	0.61 ± 0.11

$h^2$  – heritability,  $r_g$ ,  $r_p$  – genetic and phenotypic correlation with mastitis

different populations, sampling size, and different definitions of each trait must be considered when making comparisons. However, the population size is one of the main items that is quite different between the two populations studied.

Heritability estimates of udder and teat conformations of the first three parities for subtropical and rainy conditions are shown in Fig. 1. Results in this figure document that differences between the udder estimates of heritability in rainy area were small compared with estimates in subtropical area. This may indicate that in rainy area all udder traits can play a similar role in selection index if they are considered for indirect improvement of milk production. In subtropical area  $h^2$  estimates of udder traits increased with advancing order of lactation except teat attachment in the 3rd parity. An obvious ascending trend of  $h^2$  for teat attachment and a descending trend for  $h^2$  of teat length was obtained in rainy area. On the other hand,  $h^2$  of teat length increased rapidly from 1st to 3rd parity in subtropical area compared with that in Hungary. These data may indicate different genetic constitution in different lactations and/or a sort of genetic x environment interaction. This may also indicate those selection strategies for a specific trait(s) that are convenient in some locations but need not be appropriate for others.

Estimates of heritability for milk production and mastitis are presented in Fig. 2. Most of heritability changing rates of milk production were presented in a linear trend from 1st to 3rd lactation in both environments, except protein yield which showed a reduction in the second parity in subtropical area while Hungar-

ian estimates of fat yield increased suddenly in the 2nd lactation (Fig. 2). Estimates of heritability of mastitis in both areas showed a moderate change from parity to parity. This may indicate that direct selection against mastitis could be done early in the animal production life.

**Correlations with mastitis:** Values of genetic and phenotypic correlations between mastitis and each of the descriptive udder traits and milk production traits are presented in Table 2. In general the values are moderate to high. All genetic correlations of udder traits with mastitis in subtropical conditions were higher than the corresponding estimates in rainy conditions, except for udder height. In both countries there were significant negative genetic correlations between udder height and mastitis with a higher value in subtropical conditions. This may be due to an increasing frequency of mastitis incidence and high frequency of udder length below the knee in subtropical area in comparison with rainy area. In addition, growth of mastitis bacteria and distribution or infection rate may be faster in the subtropical climate.

Correlations between udder traits (except udder depth) and mastitis were mostly positive. The same method and score categories were applied for scoring mastitis and udder traits in both populations, these positive correlations suggest that a better score was associated genetically with a decreasing order of conformation especially in subtropical conditions.

Genetic correlations between mastitis and milk production traits were mostly positive and the differences between correlation estimates of both environments are

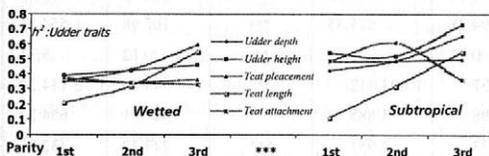


Fig. 1. Estimates of heritability for udder traits of the first three parities within rainy and subtropical areas

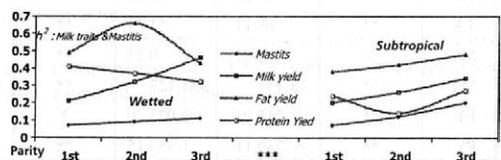


Fig. 2. Heritability estimates for milk production traits and mastitis in the first three parities

Table 3. Genetic correlations  $r_g$  between the first three (1, 2, 3) parities for all studied traits

	Rainy			Subtropical		
	$r_{g1,2}$	$r_{g1,3}$	$r_{g2,3}$	$r_{g1,2}$	$r_{g1,3}$	$r_{g2,3}$
UD	0.73 ± 0.11	0.61 ± 0.18	0.63 ± 0.12	0.42 ± 0.14	0.47 ± 0.11	0.41 ± 0.18
UH	0.69 ± 0.13	0.75 ± 0.17	0.62 ± 0.09	0.38 ± 0.11	0.41 ± 0.09	0.45 ± 0.11
TP	0.85 ± 0.19	0.81 ± 0.14	0.79 ± 0.17	0.83 ± 0.21	0.83 ± 0.19	0.82 ± 0.17
TL	0.86 ± 0.32	0.65 ± 0.18	0.62 ± 0.11	0.52 ± 0.20	0.67 ± 0.24	0.69 ± 0.24
TA	0.87 ± 0.40	0.79 ± 0.32	0.82 ± 0.28	0.42 ± 0.09	0.52 ± 0.11	0.50 ± 0.18
Mas	0.11 ± 0.10	0.09 ± 0.10	0.09 ± 0.11	0.22 ± 0.10	0.27 ± 0.11	0.11 ± 0.09
MY	0.53 ± 0.07	0.63 ± 0.14	0.73 ± 0.20	0.47 ± 0.07	0.47 ± 0.14	0.53 ± 0.09
FY	0.72 ± 0.14	0.54 ± 0.11	0.49 ± 0.10	0.54 ± 0.18	0.65 ± 0.17	0.69 ± 0.14
PY	0.57 ± 0.10	0.73 ± 0.20	0.82 ± 0.20	0.42 ± 0.19	0.51 ± 0.20	0.53 ± 0.15

not large and ranged from 0.04 to 0.05 (Table 3). On the other hand differences between phenotypic estimates were higher for rainy area. Fat yield showed significant negative genetic and phenotypic correlations with mastitis in both populations (Table 3). Therefore, a reduction in the incidence rate of mastitis by considering fat yield in selection index may impose a genetic restriction on increasing fat yield.

**Genetic correlations between parities:** Genetic correlation coefficients between the first three parities of the same trait are given in Table 3. These correlations reflect the developing mechanism of the genetic makeup with an advancing order of lactation. Estimation of these parameters in two different climatic conditions explains the role of environmental effects on changes in the interactions between genetic makeup mechanism and the performance of the same traits from parity to parity. Results in Table 3 show that all correlation coefficients of udder and teat descriptive traits in rainy area were higher than the corresponding estimates in subtropical conditions except  $r_{g1,3} - r_{g2,3}$  for teat placement and length. This may indicate that under drought conditions changes from parity to parity had unfavorable effects on developing and increasing the power of genetic makeup efficiency. This could be a reason for weakness of the relationship between the performance of the same trait in subtropical conditions in comparison with rainy conditions. The same results obtained for milk production traits except  $r_{g1,3} - r_{g2,3}$  for fat yield in rainy area, which were lower than those in subtropical area. In general, most of genetic effects on milk production were appeared per parity mainly under rainy conditions. Estimates of genetic correlations between parities for milk production traits in the present study were lower than those reported by Banos and Shook (1990). These differences may be due to the use of different statistical methods, mathematical models and population size. Maijala and Hanna (1974) reported that low estimates of genetic correlations for milk yield between 1st and later parities indicate that milk yield may be considered a somewhat different trait in later parities than in the first. Estimates of genetic correlations between parities for udder and teat

descriptive traits did not agree with the previous conclusions because of the weak correlations obtained between different parities especially in rainy area. On the other hand, estimates of genetic correlations for mastitis between parities in subtropical area may indicate that genetic effects play an important role in mastitis incidence. Low genetic correlations for mastitis in different conditions may indicate the weakness of the genetic relationship between genetic makeup influence on the performance of this trait in different parities. This may also mean that different groups of genes were responsible in different lactations for susceptibility to this disease. Under subtropical conditions genetic correlation between parities for mastitis was higher. This might indicate a higher number of genes that participate in the influence on mastitis in subsequent lactations. Banos and Shook (1990) reported that a reduction in genetic correlations for milk production and mastitis between 1st and 2nd parity may be due to culling in the 1st parity that reduces correlations between sire evaluations.

**Sire Climatic Cofactor:** Results in Table 4 shows that estimates of sire climatic cofactor  $SCF_d$ , heritability of difference  $h_d^2$  and genetic and phenotypic correlations  $R_g, R_p$  between performance of the same traits in different climatic conditions. This parameter ( $SCF_d$ ) is aimed to determine the influence of different climatic conditions on changing genetic performance of dairy cattle, especially the cattle used in this study. This estimate was not reviewed earlier in literature. Therefore, considering the previous opinion will be nil. These estimates indicate that in the traits that show high  $SCF_d$  estimates their genetic performance was affected more by changing climatic conditions than in those with low  $SCF_d$ . The highest values of  $SCF_d$  were obtained for mastitis and protein yield, and the lowest for FY while the residual estimates were lower than 0.20 or a bit more (Table 4). Therefore mastitis, protein yield and teat length in the present study will be the most important traits that had a wide range of their inherited change between different countries.

Heritabilities of difference between weather conditions ( $h_d^2$ ) are presented in Table 4. The basis of com-

Table 4. Estimates of Sire Climatic Cofactor (SCF<sub>d</sub>), heritability of difference between weather conditions ( $h_d^2$ ) and genetic-phenotypic correlations ( $R_g$ ,  $R_p$ ) between the same traits in both environments

	$r_1$	$r_2$	SCF <sub>d</sub>	$ \sigma_{S^2}^2 $	$ \sigma_{P^2}^2 $	$h_d^2$	$R_g$	$R_p$
UD	0.37	0.54	0.18	69.17	436.65	0.55	0.43 ± 0.12	0.31 ± 0.11
UH	0.53	0.72	0.19	23.08	464.86	0.19	0.52 ± 0.12	0.29 ± 0.11
TP	0.40	0.59	0.19	39.37	816.11	0.18	0.33 ± 0.23	0.20 ± 0.11
TL	0.22	0.55	0.33	81.01	1 499.89	0.20	0.32 ± 0.19	0.22 ± 0.15
TA	0.64	0.87	0.23	25.17	623.64	0.16	0.32 ± 0.25	0.20 ± 0.13
Mas	0.08	0.58	0.50	0.06	1.80	0.13	0.11 ± 0.19	0.32 ± 0.20
MY	0.75	0.62	0.13	12 614	139 985.00	0.33	-0.25 ± 0.18	-0.45 ± 0.10
FY	0.66	0.61	0.05	17.03	1 861.80	0.04	-0.35 ± 0.19	-0.40 ± 0.10
PY	1.17	0.70	0.47	116.11	997.85	0.41	-0.39 ± 0.12	-0.32 ± 0.11

$$\sigma_{S_{pol}}^2 = \sigma_{S_{sub}}^2 + \sigma_{S_{wet}}^2, \sigma_{S_{sub}}^2 / \sigma_{S_{pol}}^2 = (r_1), \sigma_{S_{wet}}^2 / \sigma_{S_{pol}}^2 = (r_2), |SCF_d| = r_1 - r_2$$

$$\sigma_{S_{sub}}^2 - \sigma_{S_{wet}}^2 = |\sigma_{S^2}^2|, \sigma_{P_{sub}}^2 - \sigma_{P_{wet}}^2 = |\sigma_{P^2}^2|, h_d^2 = |\sigma_{S^2}^2| / |\sigma_{P^2}^2|$$

putation of this parameter is the ratio of the differences between additive genetic variances of each trait in both environments relative to the difference between total phenotypic variances of each trait. This parameter can also be taken as an indicator for the effect of weather conditions on changes in the genetic-phenotypic ratio for descriptive udder and milk production traits. The highest estimates of  $h_d^2$  were obtained for udder depth, protein yield and milk yield as 55%, 41% and 33%, respectively (Table 4). These results indicate that the proportions between genetic and phenotypic changes were very high in these traits which in turn means that the changes in additive genetic variance relative to the phenotypic variance. This may be partly due to variable rearing, management and climatic conditions. On the other hand, the lowest estimates of  $h_d^2$  were observed for fat yield and most of udder descriptive traits. This indicates that the differences between the estimates of general heritabilities for the previous traits between different climates will be within a narrow range.

Genetic and phenotypic correlations between the performance of the same trait under different climatic conditions are also given in Table 4. The scientific basis of these calculations is i) genetic correlations generated due to a common gene effect of the same trait within the same breed in different locations (weather conditions) of the world, ii) phenotypic correlations due to both common genetic and general management practices of production for all Holstein populations away from very specific management methods for optimizing total economic benefit.

The highest positive genetic correlations were presented for udder depth and height. On the other hand, phenotypic correlations were lower than estimates of genetic correlations. This partly may be due to the difference in specific management methods (i.e. very modern artificial insemination tools, special feed additives, modern processing for milking, and etc.). Negative genetic and phenotypic correlations were determined for all milk production traits but phenotypic

estimates were higher than the genetic ones except for fat yield. This indicates that the animals selected for high milk production in the rainy area when live within subtropical condition will not expected to arrive the same production level of their parents.

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# ESTIMATION OF BREEDING VALUE OF ENGLISH THOROUGHBRED SIRES IN THE CZECH REPUBLIC

## ODHAD PLEMENNÉ HODNOTY PLNOKREVNÝCH PLEMENÍKŮ V ČESKÉ REPUBLICE

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**ABSTRACT:** The breeding value of English Thoroughbred sires in the Czech Republic was estimated while using results from the period of ten years (1987–1996). The performance of stallions under study was evaluated using the general handicap (GH) and the index of the performance (IDP) of two and three-year-old horses. This analysis involves altogether 3 569 horses (51 48 results) – GH, 4 168 horses (6 530 results) – IDP. The horses under study represented progeny of 519 sires (GH), 583 sires (IDP). The performance was analysed with regard to the following effects: sex, age, year of start, number of starts, breeder and trainer. All these effects were highly significant ( $P < 0.001$ ) for GH (in all cases) as well as for IDP (in sex, age, number of starts, breeder and trainer). By involving the effects gradually, we were able to explain 47.44% and 37.59% of performance variability. Using the so-called animal model (AM), we tried to estimate the breeding value of thoroughbred stallions.

**Keywords:** English Thoroughbred; racing performance; estimation of breeding value; general handicap; index of performance

**ABSTRAKT:** Pro odhad plemenné hodnoty plnokrevných plemeníků v České republice byla vybrána perioda 10 dostihových sezon (1987–1996). Výkonost potomstva sledovaných plemeníků byla hodnocena generálním handicapem (GH) a výkonostním indexem (IDP) dvou- a tříletých koní. Sledování zahrnovalo 3 569 koní (5 148 hodnocených výkoností) pro kritérium GH a 4 168 koní (6 530 hodnocených výkoností) pro IDP. Hodnocení koně byli potomky 519 plemeníků (GH) a 583 plemeníků (IDP). Výkonost byla analyzována při zohlednění těchto efektů: pohlaví, věk, rok startu koně, počet startů, chovatel, trenér. Tyto efekty byly statisticky vysoce významné ( $P < 0,001$ ) pro kritérium GH ve všech případech a pro kritérium IDP u pohlaví, věku, počtu startů, chovatele a trenéra. Postupným zařazováním faktorů se nám podařilo vysvětlit 37,99 % (GH) a 26,41 % (IDP) proměnlivosti výkoností. Pomocí animal modelu (AM) jsme odhadli plemennou hodnotu plnokrevných plemeníků.

**Klíčová slova:** anglický plnokrevník; dostihová výkonost; odhad plemenné hodnoty; generální handicap; výkonostní index

### INTRODUCTION

The performance of thoroughbred horses results from a long-term selection for maximum gallop speed which is tested in races. Their results enable to evaluate trends in speed development and to estimate the breeding value of thoroughbred sires. Due to a low heritability of recorded race times and their high dependence on climatic, pedological and other factors, the performance of thoroughbred sires is most frequently specified on the basis of subsidies, prize money or on the basis of results of general handicaps (time form). According to authors of selection models, these performance characteristics have a medium coefficient of heritability. We have used both of them in our model of estimation of breeding value, and results of the estimation of

breeding value according to the criterion general handicap (GH) with an index of performance (IDP).

Selection of English Thoroughbred horses is implemented using a system of special performance tests. The structure and selection importance of these tests were characterised by Misař (cit. Dušek, 1999). Results of these tests are used for a comparison of inter-generation and intra-generation performances of thoroughbreds as well as for the estimation of breeding value of thoroughbred sires. Misař (1976) characterised basic principles of the estimation of breeding value of thoroughbred sires on the basis of these criteria.

In the second half of the 20th century, there were great efforts to estimate and define more precisely the coefficient of heritability of racing performance of thoroughbred sires. Oscag and Toth (1959) and Pern

(1970) tried to estimate the level of heritability on the basis of recorded racing times. Other authors (Artz, 1961; Bormann, 1964) used corrected racing results. In all cases a low level of heritability of racing performance was found out ( $h^2 = 0.04-0.24$ ).

Other authors (Dušek, 1968, 1973; Bormann, 1964; Schwark and Neisser, 1971) observed a medium to higher level of  $h^2$  when using the criterion of GH and/or time form ( $h^2 = 0.19-0.60$ ). Similar results were obtained by Pirri and Steel (1951) on the basis of an analysis of logarithmised sums of progeny prize money ( $h^2 = 0.60$ ), Watanabe (1974) when using progeny prize money ( $h^2 = 0.45$ ) and Foye *et al.* (1973) on the basis of tests of repeatability of average progeny prize money per 1 start ( $h^2 = 0.30$ ). These as well as other data were generalised by Langlois (1980). This author mentioned that the heritability of measured racing times was usually low ( $h^2 = 0.20$ ). When using criteria of GH and prize money, the values of  $h^2$  were 0.30 and 0.40, respectively. These data corresponded with values of  $h^2$  estimated later by Tolley *et al.* (1985) in time form ( $h^2 = 0.21$ ), prize money ( $h^2 = 0.26$ ) and GH ( $h^2 = 0.54$ ), by Gaffney and Cunningham (1988) in time form ( $h^2 = 0.36$ ) and by Chrzanowski and Koebecke (1993) in form of IDP ( $h^2 = 0.25$  and  $h^2 = 0.15$ ).

Performance criteria used for the estimation of coefficients of heritability of racing performance were used by the majority of authors for evaluating differences in their importance for breeding purposes. In the second half of the 20th century there was an increasing tendency to make the evaluation of thoroughbred sires more precise so that the most popular extensive method of evaluation was gradually replaced by others that were more perfect. Estes (cit. Vaňourek, 1967) published a method for estimation of the coefficient of breeding value on the basis of average progeny prize money in the ideal average sum of prize money in the racing season. Dušek (1968, 1977) mentioned possibilities of a further improvement of estimation of progeny performance evaluated on the basis of point rating of individual races, definition of optimum performance and/or conversion of measured racing results. Langlois (1975) used logarithmised values of IDP for the estimation of breeding value of thoroughbred stallions in France. Misař (1976) published a survey of performance criteria of thoroughbreds used for the evaluation of sires and suggested how to give it more precision on the basis of results of their progeny in races of the highest selection rank. Computers opened new possibilities how to improve estimations of breeding value of thoroughbred sires by means of more precise methods. Tavernier (1988) described advantages of multifactorial statistic method BLUP when estimating the breeding value of horses.

Some authors used GH for the estimation of breeding value by means of BLUP while others IDP. The first of these criteria was very often criticised (Dušek, 1968, 1973; Misař, 1976) due to a subjective effect of handicapper on the final evaluation of horses. Besides,

the GH method itself was modified in various countries in the 1970s and 1980s. Estimation of performance respected distance abilities of evaluated horses (Varola, 1980).

BLUP became the most frequent method of estimation of breeding value of horses. In spite of this fact some authors of breeding programmes of thoroughbreds used methods of variation statistics to analyse performance of progeny on the basis of average results and of positive and possibly also or negative deviations (Bodenstedt, 1982).

Other authors tried to estimate the influence of fixed and random factors on horse performance and, thus, to estimate the breeding value in a more precise way. Chrzanowski and Koebecke (1993) considered the effect of trainer, year of start and breeder when estimating the breeding value of Polish thoroughbreds on the basis of IDP. Silvestrelli *et al.* (1995) estimated the breeding value of stallions of special breeds including thoroughbreds on the basis of fixed effects of sex, age, year of start and year of birth for two performance criteria: GH and IDP. Jaitner *et al.* (1994) used fixed effects of race, sex, age and trainer for a similar estimation. Preisinger *et al.* (1990) characterised the genetic progress of thoroughbreds in Germany with regard to external effects, above all trainer.

A new developmental tendency in estimation of breeding value was mentioned by Ricard (1996). Other possible effects were characterised by Misař and Jiskrová (1994) on the basis of estimation of mental power (moral + will) on performance of horses. Misař *et al.* (1998) estimated the breeding value of thoroughbred sires in the Czech Republic by means of BLUP method with consideration of fixed and random effects.

Objectives of this project involved the following:

- (1) evaluation of some fixed (sex, age, year of start, number of starts) and some random effects (trainer, breeder) on racing performance of thoroughbreds;
- (2) estimation of breeding value of all horses by means of Animal Model on the basis of their performance within the period 1987–1996 when using the following criteria:
  - (a) general handicap (GH) of two and three-year-old horses
  - (b) individual performance index (IDP) calculated on the basis of prize money won by the same age categories;
- (3) comparison of the results and evaluation of their suitability for selection of breeding animals.

## MATERIAL AND METHODS

### Establishment of d-base

The d-base for the estimation of breeding value was created from results of two and three-year-old horses in racing seasons 1987–1996. When evaluating individual

horses, the following factors were taken into account: year of birth, origin, trainer's name and breeder's name. In the case of imported horses the breeder was characterised by an official suffix. Horses of foreign owners with incomplete identification data were eliminated (altogether 0.7% of all horses under study).

Data about the level of GH were excerpted from Racing Annual Books of corresponding racing seasons.

Individual indexes of horse performance (IDP) were calculated on the basis of the number of starts and total prize money using the formula

$$IDP = \frac{\sum X_{z,d}}{Pd,r,d}$$

where:  $X_{z,d}$  – sum of prize money for winning and placement of horse in the course of racing season

$Pd,r,d$  – proportion of total sum of prize money per horse (i.e. average prize money obtained by horse in races)

Using data on each of these two performance criteria a matrix of kinship and a set of data on performance were constructed. The set of performance data contained information necessary for the estimation of fixed and random effects on horse performance.

#### Methods of statistical analysis

Effects of fixed and random factors were calculated for both criteria under study using the GLM programme package SAS (SAS Institute Inc., 1988). Programme UNISTAT was used for the computation of additional statistical characteristics and correlations.

Evaluation of both d-bases was carried out by means of linear models of the SAS programme using the following equation:

$$y_{ijklm} = \mu + s_i + a_j + d_k + \beta(x_{ijklm}) + t_l + b_m + e_{ijklm}$$

where:  $y_{ijklm}$  – evaluated value (GH, IDP)

$\mu$  – general average

$s_i$  – fixed effect of  $i$ -th sex ( $i = 1, 2, 3$ )

$a_j$  – fixed effect of  $j$ -th age ( $j = 1, 2$ )

$d_k$  – fixed effect of  $k$ -th year of start ( $k = 1, \dots, 10$ )

$b(x_{ijklm})$  – regression of GH (IDP) on the number of starts

$t_l$  – random effect of  $l$ -th trainer ( $l = 1, \dots, 238$ )

$b_m$  – random effect of  $m$ -th breeder ( $m = 1, \dots, 307$ )

$e_{ijklm}$  – residual effect

Using gradual insertion of effects under study, it was tried to express the proportion of explained variability of GH values:

$$y_{ijklm} = \mu + s_i + e_{ijklm}$$

$$y_{ijklm} = \mu + s_i + a_j + e_{ijklm}$$

$$y_{ijklm} = \mu + s_i + a_j + d_k + e_{ijklm}$$

$$y_{ijklm} = \mu + s_i + a_j + d_k + \beta(x_{ijklm}) + e_{ijklm}$$

$$y_{ijklm} = \mu + s_i + a_j + d_k + \beta(x_{ijklm}) + t_l + e_{ijklm}$$

$$y_{ijklm} = \mu + s_i + a_j + d_k + \beta(x_{ijklm}) + t_l + b_m + e_{ijklm}$$

The coefficient of rank order correlation between GH and IDP was calculated using the Pearson's method. The relationship between estimated breeding values for GH and IDP was evaluated in the same way. The estimation of breeding value was carried out using the BLUP Animal Model method in program JAA 20 (Misztal, 1993).

## RESULTS AND DISCUSSION

### Characteristics of d-base according to performance criteria

The matrices of kinship and performance for IDP criterion were more numerous than those for GH (Table 1).

The observed differences were caused by a different method of performance estimation.

In both sets of animals under study horses of Czech origin predominated (87.7 and 86.9%, resp.). Their ratio to imported horses changed in dependence on year, age and performance criterion. In the first year of

Table 1. Characteristics of entry data for the estimation of breeding value according to performance criteria

Data	Number	Proportion in total number (%)	Number	Proportion in total number (%)
	GH		IDP	
Number of horses in the matrix of kinship	5 990	–	6 813	–
Number of evaluated performances	5 148	–	6 530	–
Number of horses	3 569	100	4 168	100
Of them: born and reared in the CR	3 131	87.7	3 623	86.9
imported	438	12.3	545	13.1
Number of sires	519	100	583	100
Of them: sires of horses born and reared in the CR	211	40.7	217	37.2
sires of imported horses	308	59.3	366	62.8
Average number of evaluated horses born and reared in the CR per 1 sire	15*	–	17*	–
Average number of evaluated imported horses per 1 sire	1.5*	–	1.5*	–

\* These values were rounded off

evaluation, imports of two and three-year-old horses represented 8.5 and 10.3% for GH and 8.2 and 11% for IDP, respectively. In 1988–1990, the proportion of two-year-old imported horses decreased to 1.7% (GH) and 1.6% (IDP) in 1988 and that of three-year-old horses to 4% (GH) and 5% (IDP) in 1989. The reason for this decrease in numbers of imported animals resulted from economic destabilisation of a part of former owners of racing horses.

After 1991, the proportion of imported horses increased in both age categories and for two-year-old horses the maximum was reached in 1993 (16.9% for GH and 17.1% for IDP, resp.). The corresponding figures for three-year-old horses were 24.5% for GH and 25.4% for IDP, resp.). This fact influenced the spectrum of evaluated sires classified according to the numbers of evaluated progeny (Table 2).

Table 2 indicates a relatively high variability of the population under study. A high number of sires with only 1 evaluated racing horse was caused by the fact that owners preferred imported horses to Czech animals and documents also a low intensity of selection.

Table 2. Characteristics of sires according to the numbers of horses evaluated on the basis of individual performance criteria

Number of evaluated animals	Number of sires	
	GH	IDP
1	230	255
2–6	127	146
7–14	36	36
15 and more	68	75
Total number of sires	461	512

Table 3. Correlations of performance data for IDP and GH

Compared age categories	Characteristics of recorded data		
	correlation coefficient	95% range of correlation coefficient	significance
GH and IDP for two-year-old horses	0.6796	0.6530 – 0.7040	+++
GH and IDP for three-year-old horses	0.5796	0.5590 – 0.6024	+++

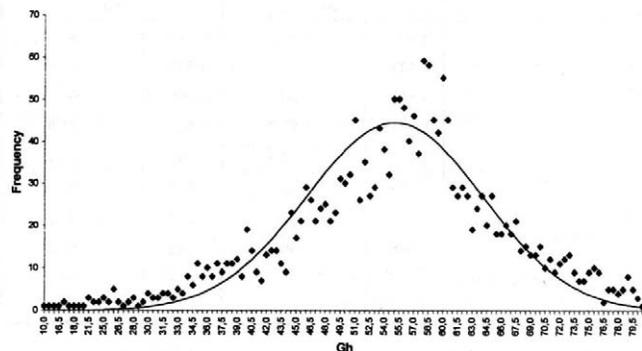


Fig. 1. Distribution of performance frequencies – GH for two-year-old horses

### Characteristics of performance criteria and their stability

The spread and frequency of distribution for GH and IDP are different due to the application of two different methods of estimation. In the seasons under study, GH ranged from 10 to 90 kg and its distribution corresponded with Gauss curve both for two and three-year-old horses (Figs. 1 and 2).

As compared with this distribution, performance evaluated on the basis of IDP showed a narrower range and lognormal distribution (Figs. 3 and 4).

In spite of different methods of performance estimation their correlation was significant. This is documented by correlations of the characteristics used (Table 3).

In spite of significant correlations existing between both performance characteristics it was found out that they developed in a different way within the period under study.

While the performance characterised by IDP was relatively stable, the other one (GH) showed a gradually decreasing trend. At present, however, we have no reliable explanation for this fact. In our understanding it is possible to take into account possible subjective factors occurring during the process of handicapping of racing horses. A relative stability of IDP calculated without subjective effects is in contrast with a decreasing level of GH.

### Characteristics of the extent of effects of fixed and random factors under study on the performance of horses

Significance of effects of fixed and random factors according to both criteria is documented in Table 4.

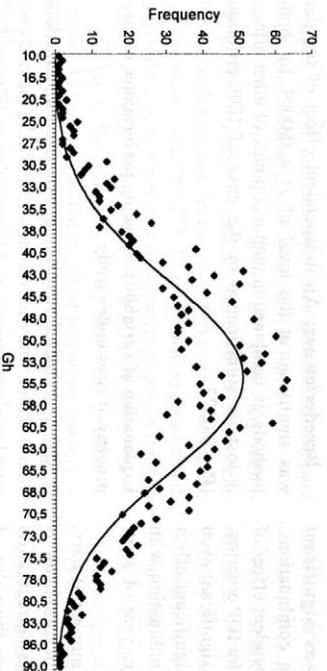


Fig. 2. Distribution of performance frequencies – GH for three-year-old horses

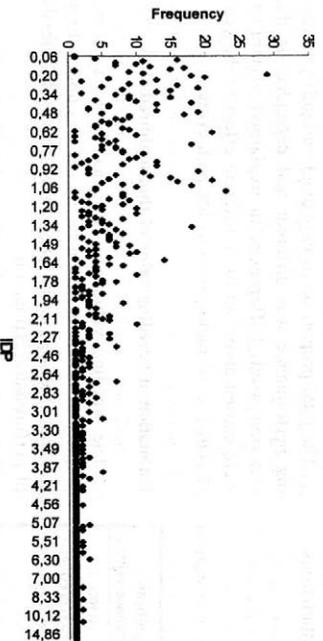


Fig. 3. Distribution of performance frequencies – IDP for two-year-old horses

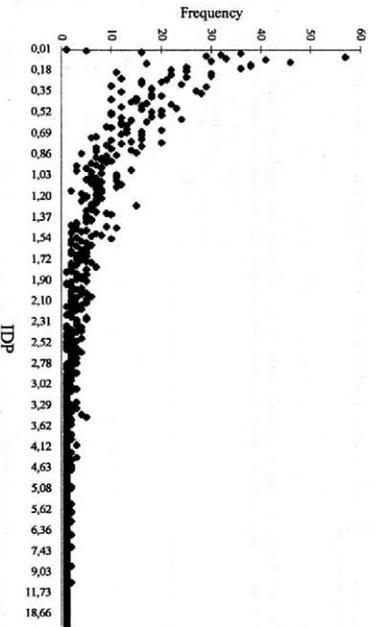


Fig. 4. Distribution of performance frequencies – IDP for three-year-old horses

This table characterises effects of factors under study on horse performance. With the exception of the year of start, the statistical significance of effects of both fixed and random factors under study was very similar for both criteria used. The only difference consisted in a non-significant effect of the year of start on the criterion of IDP. This could be caused by a relative stability of IDP.

Within the framework of independent and combined evaluation of factors under study it was possible to draw the following conclusions:

**Sex:** The performance of stallions was significantly better than that of mares and geldings ( $P < 0.0001$  for

GH and  $P = 0.0021$  and  $0.0009$  for IDP). Mares were better than geldings ( $P = 0.0001$  for GH and  $P = 0.0117$  for IDP, resp.). For combinations of sex with age and of age with year of start the effect of sex was not significant.

**Age:** The effect of age was significant. In combinations with sex, year and year of start this significance was lost but appeared again if other fixed and random factors were added ( $P < 0.0001$  for age, sex, year of start and number of starts).

**Year of start (tracing season):** Significance of the effect of the year of start differed in dependence on the criterion of performance. In the case of GH the effect

of racing season (year of start) was significant ( $P < 0.0001$ ) both individually and in combinations. On the other hand, no significant independent effect of this factor on performance was observed for IDP criterion. The effect of the year of start was not significant even in combination with other effects. A significant effect of this factor was observed only in combination with all fixed and random factors (sex, age, year of start, trainer, breeder;  $P = 0.0029$ ).

**Number of starts:** The effect of the number of starts was significant both independently and in combinations at the level of  $P < 0.0001$ . When analysing regression of performance on the number of starts the value of correlation coefficient was  $b_{yx} = 1.62$ .

**Trainer:** Significance of the effect of trainer was  $P < 0.0001$  both independently and in combinations.

Table 4. Statistical significance of effects of factors under study on racing performance of horses

Factor under study	The level of statistical significance according to individual performance criteria	
	GH	IDP
Sex	$P < 0.0001$	$P < 0.0001$
Age	$P < 0.0001$	$P = 0.0004$
Year of start	$P < 0.0001$	$P = 0.1140$
Number of starts	$P < 0.0001$	$P < 0.0001$
Trainer	$P < 0.0001$	$P < 0.0001$
Breeder/owner	$P < 0.0001$	$P < 0.0001$

**Breeder/owner:** An independent effect of breeder was significant at the level of  $P < 0.0001$  for both methods. In combination with a complex of other effects it lost its significance in the case of IDP criterion ( $P = 0.0572$ ).

#### Explanation of variability in racing performance of the progeny of sires under study

Using a gradual involvement of fixed and random effects under study according to the GLM methods we could explain proportions of variability in GH and IDP values. A comparison of explained proportions of variability is presented in Table 5.

The total proportion of explained variability in racing performance was different and depended on the criterion used. Differences in explained variability were caused above all by a different effect of the year of start (i.e. of racing season) for both criteria.

#### Estimation of breeding value of thoroughbred sires

The breeding value was estimated for all sires the progeny of which started in racing seasons under study and was evaluated using one of the described methods of performance estimation.

Sires with a low number of starting offspring formed the most numerous group in both d-bases. Unfortunately, the estimation of breeding value on the basis of a low number of progeny shows a low degree of

Table 5. A survey of proportions of explained variability ( $r^2$ ) for performance criteria under study with regard to gradually involved effects

Rank	Effect	Proportion of explained variability ( $r^2$ )	
		GH	IDP
1	sex	1.23	0.03
2	sex + age (according to breeders)	1.24	0.03
3	sex + age + year of start	8.70	0.03
4	sex + age + year of start + number of starts (according to breeders)	19.42	20.31
5	sex + age + year of start + number of starts (including breeders)	37.97	20.11
6	sex + age + year of start + number of starts + trainer (including breeders, according to trainers)	47.44	24.89
7	sex + age + year of start + number of starts + trainer	37.99	26.41

Table 6. Results of Pearson's method of calculation of correlations between GH and IDP performance values and estimates of breeding value when using GH and IDP criteria (GH BV and IDP BV)

Compared age categories	Characteristics of recorded data			
	correlation coefficient	95% range of correlation coefficient		significance
GH BV and GH of two-year-old horses	0.5818	0.5521	0.6100	++++
GH BV and GH of three-year-old horses	0.6230	0.6010	0.6439	++++
IDP BV and IDP of two-year-old horses	0.6616	0.6363	0.6855	++++
IDP BV and IDP of three-year-old horses	0.7868	0.7731	0.7998	++++
GH BV and IDP BV	0.5558	0.5380	0.5732	++++

reliability. For that reason it was decided to compare only sires with at least 7 evaluated offspring.

For GH criterion altogether 104 sires were identified. The estimated breeding value ranged from relatively high negative figures (-71.69) to positive figures of the same order (81.27). These figures express breeding values of the progeny of a concrete sire in relation to the average of the whole set of horses under study.

As far as the criterion of IDP was concerned, altogether 111 sires with the required minimum number of offspring were evaluated. Their breeding values ranged from -73.4 to 127.11.

From the viewpoint of final position, a part of estimates of breeding value on the basis of GH and IDP showed a great similarity (16.3% and 15.3% for GH and IDP, resp.). The major part of other estimates were more different but in spite of this fact the evaluated sires occurred in the same, either positive or negative, range. A relatively small part of estimates (7.7% and 7.2% for GH and IDP, resp.) showed to be markedly different (one evaluation in the positive and the other in the negative range). For that reason the interrelationship of estimated values was compared using Pearson's correlation method. Results of this comparison are presented in Table 6.

Regardless of this degree of correlation between these two figures the aforementioned differences in estimates of breeding value suggest that either one or neither of the used criteria represents a reliable characteristic of racing performance. Neither GH nor IDP reflects distance abilities of thoroughbreds. For that reason it was decided to take into account also distance capabilities of the progeny of evaluated thoroughbreds in the following part of our research. This methodological principle is being tested at present.

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# ANALÝZA FAKTORŮ PŮSOBÍCÍCH NA VÝKONNOST PLNOKREVNÝCH KONÍ

## ANALYSIS OF FACTORS INFLUENCING THE PERFORMANCE OF ENGLISH THOROUGHBRED HORSES IN THE CZECH REPUBLIC

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**ABSTRACT:** Significance of important factors influencing one of the main parameters of performance in the English Thoroughbred, i.e. general handicap (GH), was determined. GH data from flat races of Thoroughbred horses in the Czechoslovak Republic in 1968–1993 and in the Czech Republic in 1994–1996 were used for analyses. Evaluations were carried out separately for each age category: GHs of two-year, three-year and four-year horses. Two linear models with mixed effects were used. Model 1 comprised these factors: year of birth (racing season), sex, father, mother's GH and mother's age. All factors were fixed except the factor father, it was considered as random. The factor father was omitted from model 2, the other effects were the same as in model 1. An analysis to determine the significance of differences between effects within factors was made by help of model 1. The factors father and mother's GH were significant in all age categories, the factor mother's age was insignificant in all categories, and the factors year of birth and sex were significant in two age categories every time. A variation-covariance analysis was made by means of model 2 when means were estimated by the least-squares method (LSM) for the year of birth, sex, mother's GH and mother's age. GH values ranged from 55 to 64 kg in all three age categories between 1976 and 1988, but there was a steep fall of GH values beginning in 1989 and 1990 and lasting to the end of observations in 1993; it was likely to be caused by a different handicapper. It was confirmed by the highly significant factor mother's GH that this information should be taken into account to estimate breeding value. Mother's age was insignificant in all age categories in model 1 while in model 2 it was significant in two-year horses only. A conclusion can be drawn on the basis of model 1 with higher informative capacity that no adjustment to the factor mother's age is necessary, that means it need not be applied to estimate and/or predict breeding value.

**Keywords:** English Thoroughbred; performance; general handicap

**ABSTRAKT:** Byla stanovena významnost důležitých faktorů, které působí na jeden z hlavních ukazatelů výkonnosti anglických plnokrevníků, tj. generální handicap (GH). Pro analýzu bylo využito údajů o GH z rovinových dostihů plnokrevných koní, působících v Československé republice v letech 1968 až 1993 a v České republice v letech 1994 až 1996. Hodnocení bylo provedeno samostatně pro každou věkovou kategorii, tedy GH dvouletých, tříletých a čtyřletých koní. Bylo použito dvou lineárních modelů se smíšenými efekty. V modelu 1 byly zahrnuty tyto faktory: rok narození (sezona konání dostihu), pohlaví, otec, GH matky a věk matky. S výjimkou faktoru otce byly všechny faktory fixní, faktor otce byl uvažován jako náhodný. V modelu 2 byl faktor vypuštěn, ostatní efekty byly stejné jako v modelu 1. Modelem 1 byla provedena analýza, která byla východiskem pro stanovení významnosti rozdílů efektů uvnitř faktorů. Faktor otce a GH matky byly u všech věkových kategorií významné, faktor věku matky u všech kategorií nevýznamný a faktor roku narození a pohlaví byly významné vždy u dvou věkových kategorií. Modelem 2 byla provedena variančně-kovarianční analýza s odhadem průměrů pomocí metody nejmenších čtverců (LSM) pro roky narození a pohlaví. U všech tří věkových kategorií se zhruba od roku 1976 až do 1988 udržoval GH v rozpětí 55 až 64 kg, avšak počínaje roky 1989 a 1990 až do konce sledování v roce 1993 došlo k prudkému poklesu hodnot GH, což bylo s největší pravděpodobností způsobeno změnou handicapera. Vysoce významný faktor GH matky jen potvrdil, že je nezbytné tuto informaci při odhadu plemenné hodnoty jedince zohlednit. V modelu 1 byl u všech věkových kategorií věk matky nevýznamný, zatímco v modelu 2 byl významný pouze u dvouletých koní. Vycházejí z výsledku modelu 1, který má vyšší vypovídací schopnost, můžeme formulovat závěr, že není zapotřebí korekce na faktor věku matky, a tudíž není třeba jej ani zohledňovat při odhadu, resp. predikci plemenné hodnoty.

**Klíčová slova:** anglický plnokrevník; výkonnost; generální handicap

Anglický plnokrevník je vysoce prošlechtěné plemeno koní, jehož základní užitkovou vlastností je rychlost a vytrvalost ve cvalu. Charakteristickým znakem struktury populace je relativně malý počet potomků na jednoho plemeníka. Vzhledem k tomu, že jedinec získává polovinu dědičného základu od otce a polovinu od matky, a dále vzhledem k faktu, že na otce je vyvíjen dostatečný tlak, je třeba věnovat zvýšenou pozornost samičí části populace, a to jak odhadu plemenné hodnoty klisen, tak i selekci v úseku matek. Je třeba zdůraznit skutečnost, že v našich podmínkách je selekční tlak u klisen nedostatečný. Do chovu jsou totiž zařazovány i ty klisny, jejichž výkonnost se pohybuje hluboko pod průměrem populace. Za jedno z hlavních selekčních kritérií je u anglických plnokrevníků považován generální handicap (GH).

GH vykazuje střední hodnoty koeficientu dědivosti ( $h^2$ ) a je proto vhodným selekčním kritériem. Schulze-Schleppinghoff *et al.* (1987) odhadli pro ukazatele výkonnosti dvou-, tří-, resp. čtyřletých koní  $h^2$  o velikosti 0,22, 0,48 a 0,52. Pro GH uvádí Dušek (1981) pro tříleté koně  $h^2 = 0,52$  a dále Dušek (1965) pro klisny 0,45 a pro hřebce 0,25. Každý výkonnostní ukazatel je ovlivňován souborem vnitřních a vnějších prostředí efektů. Kvantifikaci těchto efektů je možno provést pomocí lineárních modelů s fixními nebo smíšenými efekty (Jakubec, 1990). Ukazuje se, že zejména rok narození, rok startu, věk jedince, pohlaví a trenér mají vliv na ukazatele výkonnosti anglického plnokrevníka (Schulze-Schleppinghoff *et al.*, 1987; Jaitner *et al.*, 1994; Preisinger *et al.*, 1989; Barrey *et al.*, 1997). Duruttya (1996) se zaměřil na analýzu věku rodičů sledovaného jedince. Jestliže byly zkoumány jednotlivé konkrétní starty, objevily se v analýzách další faktory, jako konkrétní dostih, nesená hmotnost, jezdec, závodistiště, měsíc startu (Oki *et al.*, 1994a, b, 1995), distance dostihu (Williamson *et al.*, 1996, 1998).

Cílem práce bylo stanovení významnosti důležitých faktorů, které působí na hlavní ukazatel výkonnosti anglických plnokrevníků, tj. generální handicap (GH), a vyvození závěrů pro využití odhadů těchto faktorů pro šlechtění v současné populaci anglických plnokrevníků v ČR.

## MATERIÁL A METODY

Jako podkladová data s údaji o GH byly využity výsledky rovinových dostihů plnokrevných koní působících v Československé republice v letech 1968 až 1993 a v České republice v letech 1994 až 1996. Sledování koně absolvovali své starty v letech 1968 až 1996, tj. narodili se v letech 1966 až 1994. Do souboru byli zařazeni jedinci, o nichž byly známy tyto údaje: jméno jedince, rok narození, pohlaví, jméno matky, rok narození matky, GH matky ve věku 2, 3 a 4 let (minimálně jeden z nich), jméno otce, jméno otce matky, vlastní

výkonnost daná generálním handicapem koně ve věku 2, 3 a 4 let (opět minimálně jeden z nich). Hodnocení bylo provedeno samostatně pro každou věkovou kategorii, tedy GH dvouletých, tříletých a čtyřletých koní. Údaje o GH otce nebyly zařazeny, neboť plemeníci absolvovali svou kariéru v různých státech, a proto handicapy nejsou vzájemně srovnatelné. Hlavními zdroji dat byla Plemenná kniha A1/1 chovaných v ČR, XIII. svazek. Výsledky plemenitby v chovu A1/1 za rok 1993 až 1995 a Dostihové ročenky z let 1987 až 1996.

Analýza byla provedena pomocí metody nejmenších čtverců s použitím postupu GLM (SAS, 1998).

Bylo použito lineárního modelu se smíšenými efekty:

$$Y_{ijkl} = \mu + a_i + b_j + \gamma_k + d \times D_{ijkl} + g \times G_{ijkl} + e_{ijkl} \quad (1)$$

- kde:  $Y_{ijkl}$  – pozorovaná hodnota GH  
 $\mu$  – populační průměr  
 $a_i$  – fixní efekt roku narození  
 $b_j$  – fixní efekt pohlaví  
 $\gamma_k$  – náhodný efekt  $k$ -tého otce  
 $d$  – parciální regresní koeficient pro věk matky při porodu  
 $D_{ijkl}$  – věk matky při porodu  
 $g$  – parciální regresní koeficient pro hodnotu GH matky získanou v tomtéž věku, jako ji získal potomek  
 $G_{ijkl}$  – hodnota GH matky (ve věku potomka)  
 $e_{ijkl}$  – reziduální chyba

Pomocí tohoto modelu bylo hodnoceno ve věku dvou let 943 jedinců, ve věku tří let 2 094 jedinců a ve věku čtyř let pouze 160 jedinců. Skupiny podle pohlaví byly dvě (hřebce a valaši, klisny). Samostatná kategorie valachů nebyla vytvořena z důvodu jejich malé četnosti a obtížného získávání údajů o datu kastrace.

Alternativně byl použit lineární model s fixními efekty (bez faktoru otce):

$$Y_{ijk} = \mu + a_i + b_j + d \times D_{ijk} + g \times G_{ijk} + e_{ijk} \quad (2)$$

- kde:  $Y_{ijk}$  – pozorovaná hodnota GH  
 $\mu$  – populační průměr  
 $a_i$  – fixní efekt roku narození  
 $b_j$  – fixní efekt pohlaví  
 $d$  – parciální regresní koeficient pro věk matky při porodu  
 $D_{ijk}$  – věk matky při porodu  
 $g$  – parciální regresní koeficient pro hodnotu GH matky získanou v tomtéž věku, jako ji získal potomek  
 $G_{ijk}$  – hodnota GH matky (ve věku potomka)  
 $e_{ijk}$  – reziduální chyba

## VÝSLEDKY

Do modelu 1 byl kromě faktorů prostředí zařazen i faktor otce. Vzhledem k tomu, že vlivem tohoto faktoru byl soubor dat značně nevyvážený, nebylo možné odhadnout efekty uvnitř faktorů prostředí a otce. Z toho důvodu byl alternativně použit model 2, ve kterém byl faktor otce vypuštěn. V tab. 1 jsou uvedeny základní statistické charakteristiky celého souboru.

### Model 1

V tab. 2 je uvedena významnost rozdílů efektů uvnitř faktorů pro věkové kategorie dvou-, tří- a čtyřletých

tých jedinců. 943 jedinců kategorie dvouletých koní bylo potomky 152 otců, tj. v průměru připadlo na jednoho plemeníka pouze 6,2 potomků. Pro tuto kategorii činil průměr GH  $55,49 \pm 9,20$  kg (tj.  $\bar{x} \pm 1$  směrodatná odchylka, kde přes 99 % jedinců má GH v rozpětí hodnot průměru  $\bar{x} \pm 3$  s). Efekty roku narození GH matky byly vysoce významné, efekt otce byl významný, efekty pohlaví jedince a věk matky při porodu byly nevýznamné. Variční koeficient pro tuto kategorii byl 16,59 %. Z 094 tříletých jedinců bylo potomky 182 otců, počet potomků na plemeníka se zvýšil na 11,5. Průměr GH dosáhl hodnoty  $54,74 \pm 11,24$  kg, variční koeficient 20,53 %. Věk matky v době porodu nebyl významný, zatímco ostatní faktory byly vysoce významné.

Vlivem chybějících údajů o výkonnosti čtyřletých koní nebo jejich matky bylo k dispozici pouze 160 koní, kteří byli potomky 68 otců, proto je jejich využití pro analýzu výkonnosti málo vhodné. Počet potomků na jednoho plemeníka byl velmi nízký (2,35). Průměr GH byl u této kategorie nejvyšší ( $56,79 \pm 10,29$  kg), variční koeficient dosáhl hodnoty 18,13 %. Faktor pohlaví byl vysoce významný, faktory otce a GH matek byly významné, avšak rok narození a věk matky byly nevýznamné.

## Model 2

V tab. 3 je uvedena významnost faktorů pro věkové kategorie dvou-, tří- a čtyřletých jedinců. Změna modelu (vypuštění faktoru otce) neměla téměř žádný vliv na změnu významnosti faktorů. Rozdíl spočíval v tom, že ve dvou případech se v různých věkových kategoriích nevýznamný faktor v modelu 1 změnil na faktor významný v modelu 2 (věk matky u dvouleté kategorie a rok narození u kategorie čtyřletých koní). Navíc došlo ke změně u faktoru pohlaví z významnosti v modelu 1 na vysokou významnost v modelu 2. Průměry, směrodatné odchylky a variční koeficienty GH jsou u všech věkových kategorií v obou modelech v podstatě stejné.

Z údajů uvedených v tab. 2 a 3 lze učinit závěr, že největší proměnlivost vykazoval GH u tříletých koní. I když se jedná pouze o fenotypový parametr, je zde určitý náznak, že existuje i vyšší genetická proměnlivost této vlastnosti výkonnosti. Znamená to, že u tříletých koní je GH vhodným selekčním kritériem. Na druhé straně by to ovšem znamenalo, že při výběru této vlastnosti jako selekčního kritéria dochází k prodloužení generačního intervalu oproti selekci podle GH u dvouletých koní. Vzhledem k použití plnokrevných klisen k chovu až po třetím roce věku a k zákazu používání biotechnologických metod v plnokrevném chovu není toto prodloužení generačního intervalu závažné.

Při použití modelu 2 však bylo možné pro jednotlivé efekty uvnitř faktoru rok a pohlaví odhadnout průměry (LSM) a standardní chyby těchto průměrů (SE) metodou nejmenších čtverců (SE je zde pouze chybou od-

Tab. 1. Průměry ( $\bar{x}$ ), standardní odchylky ( $s$ ) a variační koeficienty ( $v$ ) celého souboru – Means ( $\bar{x}$ ), standard deviations ( $s$ ) and coefficients of variance ( $v$ ) of the whole set

GH	Dvouletí <sup>1</sup>		Tříletí <sup>2</sup>		Čtyřletí <sup>3</sup>	
	model 1	model 2	model 1	model 2	model 1	model 2
$\bar{x}$ (kg)	55,49	55,49	55,49	55,75	55,75	56,79
$s$ (kg)	9,20	9,41	11,24	11,55	10,29	11,50
$v$ (%)	16,59	16,95	20,53	21,10	18,13	20,25

<sup>1</sup>two-year, <sup>2</sup>three-year, <sup>3</sup>four-year horses

Tab. 2. Významnost faktorů pro věkové kategorie dvou-, tří- a čtyřletých jedinců (model 1) – Significance of factors for the age categories of two-, three- and four-year horses (model 1)

Faktor <sup>1</sup>	Dvouletí <sup>7</sup>	Tříletí <sup>8</sup>	Čtyřletí <sup>9</sup>
Rok narození <sup>2</sup>	**	**	
Pohlaví <sup>3</sup>		**	*
Otec <sup>4</sup>	*	**	*
GH matky <sup>5</sup>	**	**	*
Věk matky <sup>6</sup>			

\*  $P < 0,05$ ; \*\*  $P < 0,01$

<sup>1</sup>factor, <sup>2</sup>year of birth, <sup>3</sup>sex, <sup>4</sup>father, <sup>5</sup>mother's GH, <sup>6</sup>mother's age, <sup>7</sup>two-year, <sup>8</sup>three-year, <sup>9</sup>four-year horses

Tab. 3. Významnost faktorů pro věkové kategorie dvou-, tří- a čtyřletých jedinců (model 2) – Significance of factors for the age categories of two-, three- and four-year horses (model 2)

Faktor <sup>1</sup>	Dvouletí <sup>6</sup>	Tříletí <sup>7</sup>	Čtyřletí <sup>8</sup>
Rok narození <sup>2</sup>	**	**	*
Pohlaví <sup>3</sup>		**	**
GH matky <sup>4</sup>	**	**	*
Věk matky <sup>5</sup>	*		

\*  $P < 0,05$ ; \*\*  $P < 0,01$

<sup>1</sup>factor, <sup>2</sup>year of birth, <sup>3</sup>sex, <sup>4</sup>mother's GH, <sup>5</sup>mother's age, <sup>6</sup>two-year, <sup>7</sup>three-year, <sup>8</sup>four-year horses

Tab. 4. Obecné průměry ( $\mu$ ) GH, získané metodou nejmenších čtverců, pro věkové kategorie dvouletých, tříletých a čtyřletých koní – General means ( $\mu$ ) of GH calculated by the least-squares method for the age categories of two-, three- and four-year horses

Kategorie <sup>1</sup>	Dvouletí <sup>2</sup>	Tříletí <sup>3</sup>	Čtyřletí <sup>4</sup>
GH (v kg)	59,35	58,83	58,86

<sup>1</sup>category, <sup>2</sup>two-year, <sup>3</sup>three-year, <sup>4</sup>four-year horses

hadu a není totožná se směrodatnou odchylkou průměru výběrového souboru).

Zajímavé jsou i obecné průměry GH ( $\mu$ ), které byly odhadnuty pro jednotlivé věkové kategorie pomocí metody nejmenších čtverců (tab. 4). Z této tabulky vyplývá, že obecný průměr pro všechny věkové kategorie je téměř stejný. V modelu 2 byl průměr každého efektu uvnitř faktoru vyjádřen (pro konkrétní faktor  $a_1$ ) jako  $\mu + a_1$ .

Tab. 5. Průměry (LSM) a standardní chyby (SE) GH pro jednotlivé roky narození (s uvedením četnosti jedinců) u kategorie dvouletých koní – Means (LSM) and standard errors (SE) of GH for the years of birth (with frequency of individuals) in the category of two-year horses

Rok narození <sup>1</sup>	Četnost <sup>2</sup> (n)	LSM (kg)	SE (kg)
1971	3	56,89	5,47
1972	2	71,08	6,68
1973	2	72,93	6,71
1974	1	61,88	9,42
1975	3	59,45	5,46
1976	4	64,31	4,72
1977	7	62,98	3,59
1978	4	59,16	4,72
1979	10	60,46	2,99
1980	8	60,17	3,35
1981	11	63,52	2,86
1982	18	62,79	2,24
1983	11	64,01	2,86
1984	19	62,87	2,18
1985	68	60,93	1,14
1986	84	57,90	1,03
1987	85	58,40	1,02
1988	107	58,60	0,91
1989	123	56,33	0,85
1990	91	54,10	0,99
1991	70	56,94	1,13
1992	77	48,13	1,08
1993	68	47,76	1,14
1994	67	42,89	1,15

<sup>1</sup>year of birth, <sup>2</sup>frequency

Dále byla provedena analýza faktoru roku narození, pohlaví, GH a věku matky.

#### Faktor roku narození

Rok narození určuje sezonu, která v našem případě představuje souhrn vlivu podmínek prostředí v konkrétním roce a také vliv osoby handicapera. Tak např. pro dvouleté koně odpovídá sezona, ve které byl kůň příslušným GH oceněn, roku narození plus 2.

V tab. 5 jsou uvedeny průměry (LSM) a standardní chyby (SE) GH pro jednotlivé roky narození (s uvedením četnosti jedinců) u kategorie dvouletých koní. Hodnoty GH do roku 1978 nemají dostatečnou vypovídací schopnost pro nízký počet jedinců v jednotlivých letech. S tím také souvisejí vysoké standardní chyby průměrů v tomto období. Od roku 1979 až do roku 1985 se hodnoty GH držely nad hranicí 60 kg. Od roku 1986 došlo k trvalému poklesu, který byl nejvýraznější po roce 1991. Průměr GH pro poslední ze sledovaných roků činil pouze 42,89 kg. Jednou z příčin

Tab. 6. Průměry (LSM) a standardní chyby (SE) GH pro jednotlivé roky narození (s uvedením četnosti jedinců) u kategorie tříletých koní – Means (LSM) and standard errors (SE) of GH for the years of birth (with frequency of individuals) in the category of three-year horses

Rok narození <sup>1</sup>	Četnost <sup>2</sup> (n)	LSM (kg)	SE (kg)
1968	1	67,91	11,56
1970	3	61,30	6,68
1971	5	66,01	5,19
1972	4	62,08	5,80
1973	5	64,12	5,20
1974	1	75,39	11,56
1975	7	56,35	4,38
1976	11	64,28	3,49
1977	19	60,60	2,66
1978	18	63,21	2,73
1979	22	60,63	2,47
1980	24	63,27	2,37
1981	29	60,58	2,16
1982	44	63,40	1,76
1983	35	62,85	1,96
1984	140	61,05	0,98
1985	179	59,62	0,87
1986	190	59,00	0,84
1987	216	55,98	0,79
1988	221	55,08	0,78
1989	226	53,23	0,77
1990	206	51,44	0,81
1991	190	50,79	0,84
1992	149	48,27	0,95
1993	148	45,23	0,95

<sup>1</sup>year of birth, <sup>2</sup>frequency

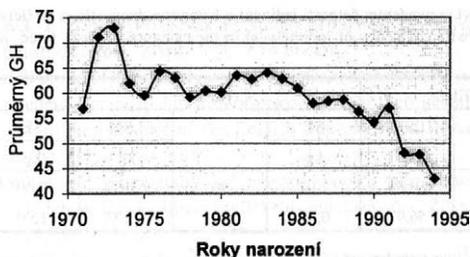
poklesu průměru GH po roce 1985 byla změna v osobě handicapera.

Pro větší názornost je kolísání LSM hodnot GH dvouletých koní zobrazeno na obr. 1.

V tab. 6 jsou uvedeny průměry (LSM) a standardní chyby (SE) GH pro jednotlivé roky narození (s uvedením četnosti jedinců) u kategorie tříletých koní.

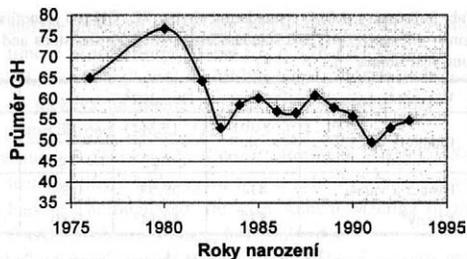
Obdobně jako u dvouletých koní byly počty koní uvnitř roků od roku 1968 do roku 1975 nízké, a proto nelze efekt jednotlivých let analyzovat. V intervalu let 1976 až 1984 se pohybovaly GH mírně nad 60 kg. V roce 1985 klesla poprvé hodnota GH pod 60 kg (59,62 kg), a ještě v roce 1986 se udržela na hodnotě 59 kg. Od roku 1987 se hodnoty GH neustále snižovaly. Od roku 1990 došlo k prudkému poklesu až na hodnotu 45,23 kg, které bylo dosaženo v roce 1993. Velikost standardních chyb je v přímé závislosti na četnosti jedinců uvnitř roků. Průběh průměru GH v časové řadě je uveden na obr. 2.

V tab. 7 jsou uvedeny průměry (LSM) a standardní chyby (SE) GH pro jednotlivé roky narození (s uvede-



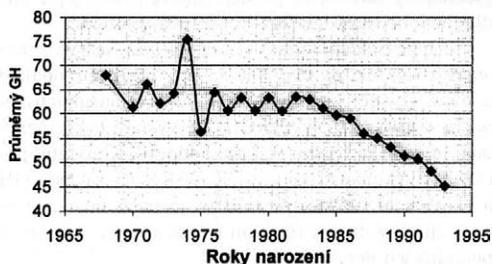
Obr. 1. Změny průměru GH (v kg) v čase u dvouletých koní – Time variations of GH means (in kg) in two-year horses

x-axis – years of birth, y-axis – GH mean



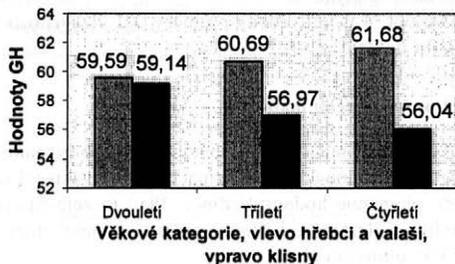
Obr. 3. Změny průměru GH (v kg) v čase u čtyřletých koní – Time variations of GH means (in kg) in four-year horses

x-axis – years of birth, y-axis – GH mean



Obr. 2. Změny průměru GH (v kg) v čase u tříletých koní – Time variations of GH means (in kg) in three-year horses

x-axis – years of birth, y-axis – GH mean



Obr. 4. Průměrný GH v závislosti na věku a pohlaví sledovaných koní – GH means in relation to the age and sex of horses

x-axis – age categories, left: studs and geldings, right: mares, y-axis – GH values

ním četnosti jedinců) u kategorie čtyřletých koní. Hodnoty pro roky 1976, 1980 a 1982 nejsou pro nízký počet sledovaných jedinců a malou průkaznost uvedeny.

I když byl GH sledován u čtyřletých koní od roku 1976, o vlastním sledování lze hovořit teprve od roku 1983, kdy byla zjištěna průměrná hodnota GH 53,06 kg u 9 jedinců. V letech 1984 až 1989 kolísaly průměrné hodnoty GH v jednotlivých letech v rozpětí 56,66 až 60,87 kg. Avšak od roku 1990 do roku 1993 byl zaznamenán výrazný pokles hodnot GH. Průběh průměru GH v časové řadě je uveden na obr. 3.

Všechny tři věkové kategorie vykazují při hodnocení GH v jednotlivých letech stejnou tendenci, a sice, že počínaje roky 1989 a 1990 až do konce sledování v roce 1993 došlo k prudkému poklesu hodnot GH, což bylo nepochoybně způsobeno mimo jiné změnou handicapera. Ke změnám v osobě handicapera ve sledovaném období skutečně došlo, což lze doložit údaji zveřejňovanými pravidelně jako součást Dostihových ročenek.

#### Faktor pohlaví

V tab. 8 jsou uvedeny průměry (LSM) a standardní chyby (SE) GH pro jednotlivá pohlaví (s uvedením četnosti jedinců) u kategorie dvou-, tří- a čtyřletých koní.

Tab. 7. Průměry (LSM) a standardní chyby (SE) GH pro jednotlivé roky narození (s uvedením četnosti jedinců) u kategorie čtyřletých koní – Means (LSM) and standard errors (SE) of GH for the years of birth (with frequency of individuals) in the category of four-year horses

Rok narození <sup>1</sup>	Četnost <sup>2</sup> (n)	LSM (kg)	SE (kg)
1983	9	53,06	3,85
1984	8	58,55	4,07
1985	13	60,27	3,20
1986	16	57,00	2,91
1987	15	56,66	3,01
1988	19	60,87	2,65
1989	22	58,00	2,51
1990	15	55,84	2,99
1991	18	49,64	2,73
1992	20	52,97	2,67
1993	2	54,80	8,27

<sup>1</sup>year of birth, <sup>2</sup>frequency

Rozdíl v hodnotě GH mezi pohlavími byl v případě dvouletých koní nevýznamný, což je patrné z téměř stejných hodnot LSM.

Tab. 8. Průměry (LSM) a standardní chyby (SE) GH pro jednotlivá pohlaví (s uvedením četnosti jedinců) u kategorie dvou-, tří- a čtyřletých koní – Means (LSM) and standard errors (SE) for the studs and mares (with frequency of individuals) in the category of two-, three- and four-year horses

Věková kategorie <sup>1</sup>	Dvouletí <sup>5</sup>			Tříletí <sup>6</sup>			Čtyřletí <sup>7</sup>		
	n	LSM	SE	n	LSM	SE	n	LSM	SE
		kg	kg		kg	kg		kg	
Hřebci a valaši <sup>3</sup>	411	59,59	0,94	922	60,69	1,02	90	61,68	2,00
Klisny <sup>4</sup>	532	59,14	0,80	1 172	56,97	0,97	70	56,03	1,90

<sup>1</sup>age category, <sup>2</sup>sex, <sup>3</sup>studs and geldings, <sup>4</sup>mares, <sup>5</sup>two-year, <sup>6</sup>three-year, <sup>7</sup>four-year horses

Pro tříleté a čtyřleté koně byl rozdíl mezi pohlavími vysoce významný – pohyboval se od 3,72 kg u tříletých koní do 5,65 kg u čtyřletých koní ve prospěch hřebců a valachů.

Na obr. 4 jsou uvedeny průměry GH v závislosti na věku a pohlaví.

#### Faktor GH a věku matky

Faktor GH matky byl zjištěn jako vysoce významný, a proto je žádoucí tuto informaci vzít v úvahu při odhadu plemenné hodnoty jedince. Platí to zejména pro predikce plemenné hodnoty jedince pomocí metody BLUP-animal modelu.

Věk matky při porodu byl významný pouze v modelu 2 u dvouletých koní. V modelu 1 byl u všech věkových kategorií nevýznamný. Při hodnocení tohoto faktoru se budeme opírat o model 1.

#### DISKUSE

Důležitý je dopad odhadu uvedených efektů na korekci výkonnosti jedinců. Takto korigované údaje jsou východiskem pro odhad plemenné hodnoty. Před využitím metody BLUP pro odhad plemenné hodnoty byl odhad plemenné hodnoty prováděn ve dvou krocích. V prvním kroku byla provedena korekce fenotypových hodnot na systematické prostředí efekty a v druhém kroku byly odhadnuty plemenné hodnoty. V současné době se při využívání metody BLUP-animal modelu provádí korekce fenotypových hodnot a odhad plemenné hodnoty najednou.

Otázku významu pohlaví u dvouletků analyzoval ve své práci Bodó (1997). Využil GH ve věku dvou let k posouzení ranosti koní. Prokázal, že klisny se rychleji přibližují svému životnímu maximu. Tyto výsledky jsou ve shodě s naším poznatkem, že klisny jsou ve výkonnosti ranější než hřebci. Ve sledovaném souboru je mezi hřebci a valachy na straně jedné a klisnami na straně druhé vysoce významná diference mezi LSM pro GH u všech věkových kategorií ve prospěch hřebců. U klisen GH s věkem klesá, naopak u samčího pohlaví dochází k vzestupu hodnot GH.

Všeobecně klesající hodnoty GH klisen, resp. růst diference ve prospěch hřebců v závislosti na věku může být vysvětlen kvalitou dostihů, kterých se klisny

zúčastňují. Odliv nadprůměrných klisen do chovu je nepochybně jednou z příčin výrazného poklesu průměru GH u klisen již po třetím roce věku. Hřebci jsou do plemenitby zařazováni později, teprve poté, co prokáží dlouhodobou výkonnost a konstituční tvrdost.

Schulze-Schleppinghoff *et al.* (1987) ve své studii analyzovali faktory podobné našim, tj. sezónu, pohlaví a otce. Uvádějí průměr GAG (Generalausgleichsgewicht – německá obdoba GH) ve výši 75,6 kg. Tento údaj je vyšší než námi získané hodnoty. Konkrétní porovnání výkonnosti koní z ČR a SRN na základě GH a GAG není možné, protože se jedná o údaje, které byly zjištěny dvěma různými handicapery a v různých podmínkách dostihů.

Nejvyšší četnost pozorování byla v kategorii tříletých koní (2 094 jedinců). Je všeobecná tendence nechat koně běhat ve 3 letech, tedy ve věku klasického ročníku.

Faktor GH matky byl vysoce významný, což potvrdilo očekávání. Z toho plyne, že použití metody regrese matka–dcera pro odhad koeficientu dědivosti má své opodstatnění (Dušek, 1965, 1985). Vyšší dosahované hodnoty koeficientu heritability u klisen oproti hřebcům dokládají i Williamson *et al.* (1996).

Trenér ani chovatel nebyli v naší studii zohledněni. Důvodem byla především struktura námi sledovaného souboru koní. Při požadavku na existenci GH matek by zařazení dalších efektů, které mohou nabývat mnoha hodnot, téměř znemožnilo odhad výše jakýchkoli efektů uvnitř faktorů. Vycházíme z faktu, že v této práci byla primární snaha zmapovat vliv základních faktorů prostředí v současné populaci A1/1 na území ČR.

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# EFFECT OF RESTRICTED FOOD INTAKE AND BLOOD COLLECTION ON PLASMA LEVELS OF HORMONES, GROWTH FACTORS AND RELATED SUBSTANCES IN FEMALE DOMESTIC NUTRIA (*MYOCASTOR COYPUS*)

VPLYV REŠTRIKCIE VÝŽIVY A ODBERU KRVI NA HLADINU HORMÓNŇOV, RASTOVÝCH FAKTOROV A S NIMI SPOJENÝCH LÁTKŇV V PLAZME DOMÁCICH NUTRIÍ (*MYOCASTOR COYPUS*)

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**ABSTRACT:** The aim of this study was to identify a number of hormones and growth factors in the blood of female domestic nutria (*Myocastor coypus*) and to examine the effects of short term dietary energy restriction and blood collection on their concentrations. Samples of blood plasma were obtained daily from sexually mature animals kept under conditions of normal and restricted (1/2 of standard ration) feeding. Samples were collected for 2 days before and 5 days during the controlled feeding period. Concentrations of insulin-like growth factor I (IGF-I), IGF binding protein 3 (IGFBP-3), triiodothyronine (T3), thyroxine (T4), progesterone (P), estradiol (E), cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) were measured using RIA/IRMA. It was observed that food restriction induced a significant decrease in plasma P, significant increases in plasma IGF-I, T4 and cAMP, but had no effect on IGFBP-3, E, T3 or cGMP concentrations. The chronic blood collection regime resulted in significant changes in most of the assayed substances except IGF-I. These observations indicate (1) that domestic nutria secrete IGF-I, IGFBP-3, P, E, T3, T4, cAMP and cGMP, (2) that blood collection can affect plasma IGFBP-3, T3, T4, P, E, cAMP and cGMP level, and (3) that nutritional status influences the concentration of IGF-I, P, T4 and cAMP. These substances therefore have the potential to be mediators of the effects of altered nutrition on physiological processes in this species.

**Keywords:** coypu; insulin-like growth factor I (IGF-I); insulin-like growth factor binding protein-3 (IGFBP-3); thyroxine (T4); triiodothyronine (T3); progesterone; estradiol; cyclic adenosine monophosphate (cAMP); cyclic guanosine monophosphate (cGMP)

**ABSTRAKT:** Cieľom nášho štúdia bolo identifikovať prítomnosť hormónov a rastových faktorov v krvi samíc domácich nutrií (*Myocastor coypus*) a preskúmať vplyvy krátkodobej diéty a odberu krvi na ich koncentrácie. Vzorky krvi sa odoberali každý deň od pohlavne dospelých zvierat, ktoré držali v podmienkach normálnej a obmedzenej výživy (1/2 štandardnej krmnej dávky). Vzorky sa odoberali dva dni pred začatím experimentu a päť dní počas experimentu s obmedzenou výživou. Koncentrácie inzulínu podobného rastového faktoru typu I (IGF-I), väzbového proteínu pre IGF typu 3 (IGFBP-3), triiodotyronínu (T3), thyroxínu (T4), progesterónu (P), estradiolu (E), cyklického adenosín monofosfátu (cAMP) a cyklického guanozín monofosfátu (cGMP) sme merali pomocou RIA/IRMA. Pozorovali sme, že reštrikcia výživy signifikantne znižovala hladinu P, zvyšovala IGF-I, T4 a cAMP, neovplyvňovala však koncentrácie IGFBP-3, E, T3 a cGMP v plazme. Chronický odber krvi podstatne menil koncentráciu väčšiny z analyzovaných látok okrem IGF-I. Tieto pozorovania svedčia o tom, že (1) domáce nutrie vylučujú IGF-I, IGFBP-3, P, E, T3, T4, cAMP a cGMP, (2) odber krvi môže ovplyvňovať hladinu IGFBP-3, T3, T4, P, E, cAMP a cGMP, a (3) že podmienky výživy ovplyvňujú koncentrácie IGF-I, P, T4 a cAMP. Tým pádom tieto látky môžu byť potenciálnymi sprostredkovateľmi efektov výživy na fyziologické procesy u tohto druhu zvierat.

**Kľúčové slová:** nutria; inzulínu podobný rastový faktor typu I (IGF-I); väzbový proteín pre inzulínu podobný rastový faktor typu 3 (IGFBP-3); thyroxín (T4); triiodotyronín (T3); progesterón; estradiol; cyklický adenosín monofosfát (cAMP); cyklický guanozín monofosfát (cGMP)

## INTRODUCTION

Domestic nutria or coypu, river otter (*Myocastor coypus*) is a species of rodent widely used for fur production. Despite being well known, the endocrinology of this species has not been studied to any significant extent. There are a few reports on the presence of testosterone (Jelínek *et al.*, 1981), LH (Schally *et al.*, 1969) and progesterone (Rowlands and Heap, 1966; Heap and Illingworth, 1974; Jakubicka *et al.*, 1988, 1989; Barta and Jakubicka, 1991) in nutria blood plasma. Non-steroid hormones, growth factors, growth factor binding proteins and intracellular messengers have not previously been identified in this species.

Nutria are recorded as having oestrous cycles of somewhat variable length (5–28 days according to Hayssen *et al.*, 1993 and 16–35 days according to Szeleszczuk and Jarosz, 1993) and may be induced ovulators. Fertility rate in this species is highly variable (Barta and Jakubicka, 1991, Hayssen *et al.*, 1993). Food intake in nutria as in other species, is a decisive factor in the regulation of reproduction, growth, moult, stress and viability (Skřivan, 1976; Berestov, 1985) but the mechanisms by which nutrition influences reproduction, metabolism and other biological processes have not been investigated. Generally in mammals hormones, growth factors and related substances (binding proteins, receptors and intracellular messengers) play a key role in regulation of the processes mentioned above. In particular, changes in energy intake directly affect the production of growth hormone (GH), insulin-like growth factors (IGF-I and IGF-II), IGF binding proteins (IGFBP-1, 2, 3, 4), thyroxine (T<sub>4</sub>), but in most cases not triiodothyronine, T<sub>3</sub>), LH, corticosterone, P, growth, ovulation and pregnancy (rat: Straus and Takemoto, 1991; Straus, 1994; Gautsch *et al.*, 1998; Higashi *et al.*, 1998; sheep: Hodkinson *et al.*, 1987; Schillo, 1992; Hua *et al.*, 1993; Wester *et al.*, 1995; Carpenter *et al.*, 1997; Gatford *et al.*, 1997; O'Callaghan *et al.*, 1998; pig: Cox *et al.*, 1987; Cosgrove *et al.*, 1992; Simmen *et al.*, 1998; cow: Brier *et al.*, 1986, 1988; Spicer *et al.*, 1992; Schillo, 1992; Hayden *et al.*, 1993; Richards *et al.*, 1995; Wester *et al.*, 1995; Gerrits *et al.*, 1998). Furthermore, food restriction stimulates the metabolism of IGF-I (rat: Thissen *et al.*, 1992; sheep: Hodkinson *et al.*, 1987) and reduces the IGF-I and IGFBP-3 response to GH treatment (cow: Breier *et al.*, 1988; sheep: Gatford *et al.*, 1997).

Some effects of feeding on growth, metabolism, hormone production and reproductive processes can be mimicked by injections of GH, IGF-I or their analogues (sheep: Spicer and Echternkamp, 1995; pigs: Nitray *et al.*, 1992; Spicer and Echternkamp, 1995; Simmen *et al.*, 1998, cow: Schemm *et al.*, 1990; Spicer and Echternkamp, 1995), although in pigs an inhibitory effect of the long R-3 IGF-I analogue on growth and plasma GH and IGFBP-3 levels has also been reported (Dunaiski *et al.*, 1997). This suggests that in these species nutrition regulates physiological processes via the

GH/IGF-I/IGFBP system and that direct stimulatory and inhibitory feedback mechanisms are involved. The GH/IGF-I/IGFBP system can be controlled by thyroid hormones: there is a strong positive correlation between growth rate and the levels of IGF-I, IGFBP-2 and thyroid hormone in porcine blood (Nikolic *et al.*, 1998), and thyroid hormones are able to stimulate rat GH and IGF-I release (Näntö-Salonen *et al.*, 1993). On the other hand, IGF-I can suppress thyroid hormones receptors and its mRNA, and metabolic response of rat liver to thyroid hormones (Pellizas *et al.*, 1998). Some effects of hormones and growth factors can be mediated by intracellular messengers including cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (Hillier, 1991; Sirotkin, 1996), but the interrelationships between these cyclic nucleotides and nutrition do not appear to have been studied. In nutria the possible hormonal mechanisms by which nutrition can influence various physiological processes are completely unknown.

The aim of our study was to identify some of the sex steroid hormones, thyroid hormones, growth factor and related substances in the blood of female domestic nutria and to determine the influence of blood collection and food restriction on the plasma concentrations of these substances.

## MATERIALS AND METHODS

Sexually mature (8 months of age) healthy female domestic nutria of a standard variety, with a body weight of  $4.1 \pm 0.1$  kg, were kept 4 animals per cage provided with pools, in a hall at the experimental farm of the Research Institute of Animal Production, Nitra. The animals were fed with a standard granulated rabbit food mixture (Complete Rabbit Food, PD Cataj, Slovak Republic) which containing 50% barley, 15% maize and 10% ground pea meal plus vitamins, minerals and amino acids but was devoid of coccidiostats or growth stimulators. The energy content of this food was 8 MJ/kg. Control animals received the standard fixed dose of food recommended for the animals of this age (100 g/animal; Mertin *et al.*, 1994) at 8.00 a.m. and 2.00 p.m., whilst experimental animals received 1/2 of this ration (50 g/animal, twice a day). Blood samples were collected daily just prior to the morning feed, starting 2 days before and continuing for the duration of the experiment (5 days). Four ml of blood were obtained from each animal at the *ventriculus sinister cordis*, using a 5 ml syringe with a 0.9 x 400 mm needle and puncturing between the 3rd and 4th ribs under halothane anaesthesia (Narcotan, Léčiva, Prague, Czech Republic). Blood was collected into glass tubes containing 0.1 ml of 4% sodium citrate. Plasma was separated by centrifugation for 10 min at 4 °C and 300 x g, and then frozen at -18 °C until analysis.

Concentrations of substances in 25–100 µl of plasma were determined in duplicate without extraction

except for insulin-like growth factor I (IGF-I), which was extracted from the samples as noted below. Insulin-like growth factor binding protein 3 (IGFBP-3), triiodothyronine (T3), thyroxine (T4), progesterone (P) and oestradiol (E) were assayed using commercial RIA/IRMA kits from DSL (Webster, Texas, USA). The RIA kits for cAMP and cGMP assays were from Immunotech (Marseilles, France). IGF-I was measured according to the method of Furlanetto *et al.* (1977) with our modifications (Makarevich and Sirotkin, 1998) using IGF-I from Calbiochem-Novabiochem AG (Lucerne, Switzerland) as standard and anti-IGF-I antiserum (dilution 1 : 5 000) from Peninsula Laboratories, Inc. (Belmont, USA). All the assays were previously validated for nutria plasma. The characteristics of these assays are presented in Table 1.

Each experimental group comprised 8 animals. Differences from controls (animals kept at normal feeding) were determined by ANOVA followed by Duncan's multiple range test using the Statgrafics computer program. Differences between the groups with  $P < 0.05$  were considered as significant.

## RESULTS

Samples of plasma from domestic nutria contained significant amounts of IGF-I, IGFBP-3, T3, T4, P, E and cGMP (Fig. 1a–g) and only a barely detectable amount of cAMP (not shown). The majority of blood samples collected from either experimental or control animals on the first day of the experiment contained significantly more IGFBP-3, T3, T4 and cGMP and lower amounts of P, E, and cAMP than samples collected on subsequent days. No substantial changes in plasma levels of IGF-I were observed during the experiment.

IGF-I levels in experimental animals were higher than in controls on days 1 and 2 of food restriction but subsequently returned to control levels (Fig. 1a). Some significant differences in IGFBP-3 content between the groups occurred immediately before food restriction but these were lost once restricted feeding had begun (Fig. 1b). Significant reductions in P and E concentrations occurred after 4 and 5 days of treatment, respectively (Fig. 1c–d). There were significant differences in T3 levels between the groups prior to the experiment; these subsequently disappeared although on day 2 of food restriction the experimental animals had significantly higher T3 levels than controls. Because of the pre-treatment differences in T3, the influence of diet on this parameter was discounted (Fig. 1e). Nutria on the first day of food restriction had significantly higher plasma T4 levels than control animals but these differences gradually disappeared (days 2–5 of treatment; Fig. 1f). The cAMP levels in both the control and the treated group before and up to the last day of the experiment were below the limit of sensitivity of the assay; on the 5th day of food restriction the cAMP level

Table 1. Characteristics of immunoassays

Assay	Specificity (cross-reactivity of antiserum)	Sensitivity	Coefficient of variation (%)	
			intra-assay	inter-assay
Progesterone	< 0.001% to cortisol, corticosterone, androstenediol, pregnenolone, estradiol, testosterone	0.12 ng/ml	< 8.0	< 13.1
Estradiol	< 0.01% to DHEA, progesterone, cortisol, androstosterone, testosterone, corticosterone, cortisone	6.5 pg/ml	< 9.4	< 19.2
IGF-I	< 0.02% to IGF-II < 0.001 to insulin, oxytocin, somatostatin, amylin	0.14 ng/ml	< 3.4	< 8.2
IGFBP-3	< 0.03% to IGFBP-1, IGFBP-2, IGFBP-4, IGFBP-5, IGFBP-6, IGF-I, IGF-II, oxytocin	0.5 ng/ml	< 3.9	< 1.9
T3	< 0.003 to T4 < 1.76 to triiodothyraetic acid < 0.002 to diiodothyronine, monoiodothyronine, diiodotyrosine	0.43 ng/ml	< 6.5	< 6.0
T4	< 0.091 to T3 < 1.13 to triiodothyraetic acid < 0.003 to diiodothyronine, monoiodothyronine, diiodotyrosine	40 ng/ml	< 5.1	< 7.4
Cyclic AMP	< 0.02% to succinyl cGMP < 0.04% to cGMP < 0.7% to ATP	0.2 nM	< 11.0	< 16.0
Cyclic GMP	< 0.03% to succinyl cAMP < 0.001% to cAMP	10 pM	< 4.0	< 5.0

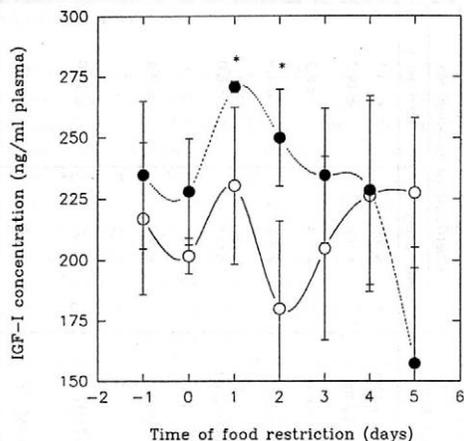


Fig. 1a. Effect of food restriction on plasma concentrations of IGF-I level in female domestic nutria

White circles – normal feeding (control), black circles – restricted feeding

Values are means  $\pm$  S.E.M

\* significant ( $P < 0.05$ ) difference compared with control

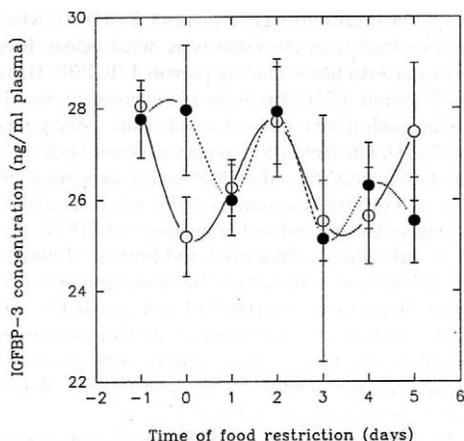


Fig. 1b. Effect of food restriction on plasma concentrations of IGFBP-3 level in female domestic nutria

White circles – normal feeding (control), black circles – restricted feeding

Values are means  $\pm$  S.E.M

\* significant ( $P < 0.05$ ) difference compared with control

in treated animals rose to  $1.7 \pm 0.5$  nM, whilst in controls it remained at undetectable levels (not shown). No marked differences in cGMP level were observed between the groups (Fig. 1g).

## DISCUSSION

This is the first report to identify a growth factor (IGF-I), growth factor binding protein (IGFBP-3), thyroid hormones (T3, T4), estrogen (E) and cyclic nucleotide (cAMP, cGMP) in nutria. Our data on P generally confirms that of other authors (Rowlands and Heap, 1966; Heap and Illingworth, 1974; Jakubicka *et al.*, 1988, 1989; Barta and Jakubicka, 1991) although the levels reported by those authors were slightly lower than measured in our experiments. In general, the concentrations of substances which we detected in nutria plasma are comparable to those observed previously in other rodents (rat: Straus and Takemoto, 1991; Thissen *et al.*, 1992; Nanto-Salonen *et al.*, 1993; Straus, 1994; Higashi *et al.*, 1998; mice: Hillier, 1991; Hayssen *et al.*, 1993; guinea pig: Heap and Illingworth, 1974; hare: Hayssen *et al.*, 1993). It was evident from our experiment that chronic sample collection can itself influence the plasma concentrations of most of the assayed substances. These changes may be indicative of a stress response by the animals to repeated immobilisation, anaesthesia or bleeding. Further endocrine studies are needed to understand the significance of these changes.

Our observations of food restriction-induced decreases in plasma P, T4, but not T3 levels in nutria are consistent with the observations of other authors on

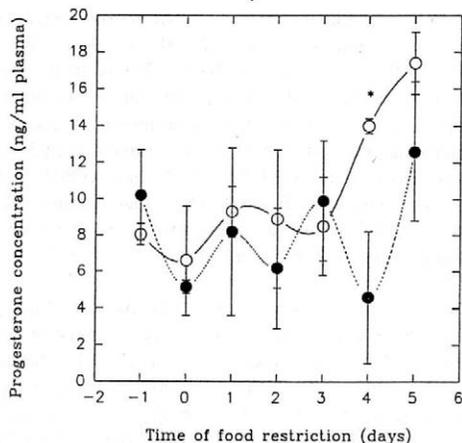


Fig. 1c. Effect of food restriction on plasma concentrations of progesterone level in female domestic nutria

White circles – normal feeding (control), black circles – restricted feeding

Values are means  $\pm$  S.E.M

\* significant ( $P < 0.05$ ) difference compared with control

sheep (Wester *et al.*, 1995) and cattle (Hayden *et al.*, 1993; Richards *et al.*, 1995; Gerrits *et al.*, 1998), although O'Collaghan *et al.* (1998) observed fasting-induced increase in P level in sheep ovarian follicular fluid. Since P and T4 are known regulators of reproduction (Hillier, 1991), of protein and fat metabolism and of thermoregulation (Hayden *et al.*, 1993; Straus,

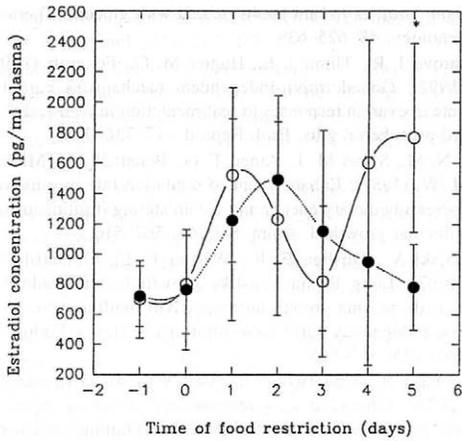


Fig. 1d. Effect of food restriction on plasma concentrations of estradiol level in female domestic nutria

White circles – normal feeding (control), black circles – restricted feeding  
 Values are means  $\pm$  S.E.M  
 \* significant ( $P < 0.05$ ) difference compared with control

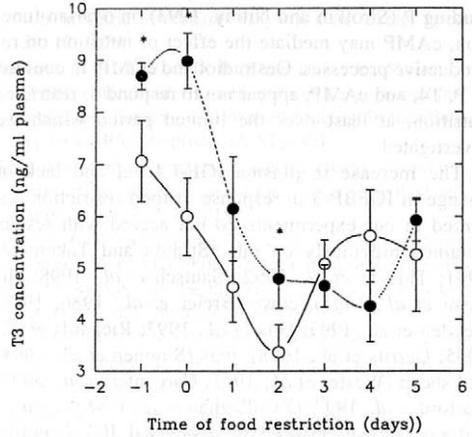


Fig. 1e. Effect of food restriction on plasma concentrations of triiodothyronine level in female domestic nutria

White circles – normal feeding (control), black circles – restricted feeding  
 Values are means  $\pm$  S.E.M  
 \* significant ( $P < 0.05$ ) difference compared with control

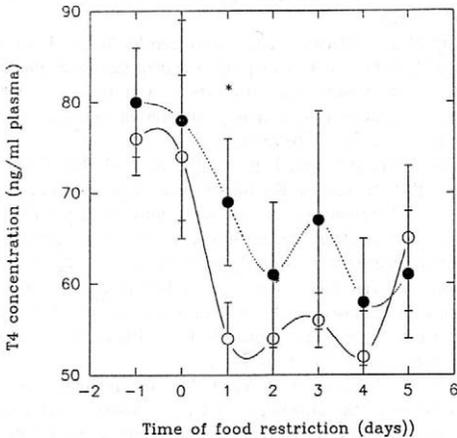


Fig. 1f. Effect of food restriction on plasma concentrations of thyroxine level in female domestic nutria

White circles – normal feeding (control), black circles – restricted feeding  
 Values are means  $\pm$  S.E.M  
 \* significant ( $P < 0.05$ ) difference compared with control

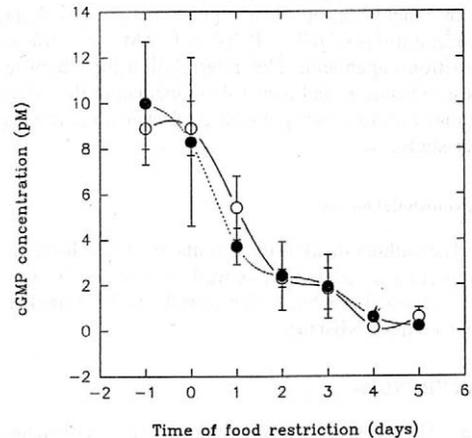


Fig. 1g. Effect of food restriction on plasma concentrations of cGMP level in female domestic nutria

White circles – normal feeding (control), black circles – restricted feeding  
 Values are means  $\pm$  S.E.M  
 \* significant ( $P < 0.05$ ) difference compared with control

1994; Wester *et al.*, 1995; Pellizas *et al.*, 1998), the present data suggest that P and T4 may be possible mediators of altered nutritional status on these processes in nutria. We did not monitor the reproductive status of our experimental animals but, due to the diet-induced depression of P secretion, it remains possible that adequate nutrition is a requirement for optimal re-

productive activity. Our observation of some fasting-induced increase of cAMP level suggests that this cyclic nucleotide can be also involved in the response to reduced energy intake. Since cAMP can be a second messenger involved in the action of gonadotropins (Hillier, 1991), growth hormone (Nitray *et al.*, 1992), nonapeptide (Sirotkin, 1996) and steroid hormones in-

cluding P (Sirotkin and Nitray, 1993) on ovarian function, cAMP may mediate the effect of nutrition on reproductive processes. Oestradiol and cGMP, in contrast to P, T4, and cAMP, appear not to respond to restricted nutrition, at least over the limited period which we investigated.

The increase in plasma IGF-I level and lack of change in IGFBP-3 in response to food restriction observed in our experiments do not accord with results obtained previously on rats (Strauss and Takemoto, 1991; Thissen *et al.*, 1992; Gautsch *et al.*, 1998; Higashi *et al.*, 1998), cows (Breier *et al.*, 1986, 1988; Heyden *et al.*, 1993; Hua *et al.*, 1993; Richards *et al.*, 1995; Gerrits *et al.*, 1998), pigs (Simmen *et al.*, 1997) and sheep (Wester *et al.*, 1995; Carpenter *et al.*, 1997; Gatford *et al.*, 1997; O'Collaghan *et al.*, 1998) in which reduced intake resulted in decreased IGF-I and/or IGFBP production. This suggests that nutria may exhibit an unusual interrelationship between IGF-I, IGFBP-3 and nutrition or possibly a biphasic response of IGF-I and IGFBP-3. We observed a small, albeit insignificant reduction in both IGF-I and IGFBP-3 levels in the diet-restricted group when compared with controls at the end of experiment; it is possible that continuation of the treatment for a longer period may have produced a greater depression in concentrations.

In conclusion, our data suggest that the circulating concentrations of IGF-I, P, T4 and cAMP in nutria are nutrition-dependent. The interrelationships between these substances and their role in mediating the effects of nutrition on other physiological events require further study.

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# AN EVALUATION OF LYSOZYME USABILITY IN TURKEY IMPROVEMENT

## HODNOCENÍ VYUŽITELNOSTI LYSOZYMU PŘI ŠLECHTĚNÍ KRŮT

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**ABSTRACT:** Aiming at an improvement of natural resistance, a selection of turkeys for high and low activity of lysozyme in serum was undertaken. The presented results are the preliminary evaluation of the first three generations ( $G_0$ ,  $G_1$ ,  $G_2$ ). The selected lines differed significantly in lysozyme activity, while no performance variations between the particular lines were found. A considerable influence of selection was observed in the birds livability in the first weeks of life. The mortality of poults after parents with a high lysozyme activity was nearly twice lower than that of poults after parents with a low activity.

**Keywords:** turkey; selection; lysozyme activity

**ABSTRAKT:** Prováděli jsme selekci krůt na vysokou a nízkou aktivitu lysozymu s cílem zvýšit jejich přirozenou odolnost. Předkládané výsledky jsou předběžným hodnocením prvních tří generací ( $G_0$ ,  $G_1$ ,  $G_2$ ). V aktivitě lysozymů existovaly mezi šlechtěnými liniemi významné rozdíly, zatímco v uživatelnosti nebyly mezi jednotlivými liniemi zjištěny žádné změny. Zaznamenali jsme značný vliv selekce na životaschopnost jedinců v prvních týdnech života. Mortalita krůtat po rodičích s vysokou lysozymovou aktivitou byla téměř dvakrát nižší než u krůtat s nízkou aktivitou.

**Klíčová slova:** krůty; selekce; lysozymová aktivita

### INTRODUCTION

Both in the population of breeders and in commodity flocks, most of the economic loss is caused by contagious diseases. Although modern organization of production ensures poultry protection by its isolation from infectious agents and general application of preventive vaccination, these measures take the edge off natural selection and may cause an increase in genetic susceptibility to diseases. The selection aimed at increasing natural immunity improves physiological abilities of the organism, at the same time improving vaccination effectiveness. Although the progress in one generation may be small, it is inherited and cumulated in subsequent generations. At the same time, instead of a direct exposure of birds to the action of pathogens, an indirect improvement through a selection of appropriately chosen markers is preferable.

Lysozyme (E.C. 3.2.1.17) – an enzyme that disrupts peptidoglycans of bacterial cell wall is an important factor of non-specific antibacterial immunity (Jollés, Jollés, 1984; Kowalska, Rzedzicki, 1988; Seyfert, 1999). It also exhibits antiviral anticarcinogenic activity, although the mechanisms of this effect have not

been fully known yet (Jollés, Jollés, 1984). Lysozyme heritability, estimated in turkey population (Prusinowska, Jankowski, 1997), showed a possibility to carry an effective selection aimed at an increase in the content of this enzyme in blood serum.

The results presented are an evaluation of the first three generations of turkeys selected for a high or low lysozyme activity in blood serum.

### MATERIAL AND METHODS

The material consisted of turkeys from a maternal strain J-44. A measurement of lysozyme activity in the initial population ( $G_0$   $n = 750$ ) was the basis for estimating the heritability coefficient (Prusinowska, Jankowski, 1997) and classifying the birds into groups of a high (H) and low (L) enzyme activity. A two-directional selection was initiated based on lysozyme activity in blood serum in 14-week-old birds. In subsequent generations ( $G_1$   $n = 290$ ) ( $G_2$   $n = 129$ ), 4 toms and 22 hens were selected from each line to become parents of the next generation. The selected hens were weighed prior to being placed in cages (at the age of 30 weeks)

and after completing a 24-week egg-laying period, i.e. at the age of 56 weeks. During the egg-laying period, the following parameters were determined for each bird: age of reproductive maturity (laying of the first egg), number of eggs, days of egg-laying, and some hatching indices, viz. fertility of eggs and hatching of normal poults from fertile and set eggs. At the time of the analysis of the results, the reproductive traits evaluation data for the G<sub>2</sub> generation females were not available yet.

The offspring of selected parents were weighed at the age of 14 weeks. At the same time measurements of the breast width were performed and the livability of poults from the time of hatching was established.

It should be stressed that during the experiment there was a change of the flock location (generation G<sub>2</sub>) due to reasons that were beyond the authors' control.

Lysozyme activity in blood serum was determined using the turbidimetric method according to Metzger (1970). Suspension of lyophilised cells of *Micrococcus lysodeikticus* in 0.067 phosphate buffer with pH = 6.8 in 0.1% NaCl was used as the substrate. Enzyme activity was expressed as the percentage of bacteria lysis.

The statistical analysis included:

- characteristic traits of the experimental material on the basis of arithmetic means ( $\bar{x}$ ) and coefficient of variation ( $v$ )
- significance of differences between the means for lines in the analysed traits (analysis of variance in the non-orthogonal one-factor system).

## RESULTS AND DISCUSSION

Mean values and measures of lysozyme activity variability in the successive turkey generations are presented in Table 1. The turkeys of the initial generation (G<sub>0</sub>) were characterized by a high variation of lysozyme activity ( $v > 40\%$ ), while average enzyme activity in toms and hens was at an equal level and amounted to 21.2% of lysis and 21.8% of lysis. High lysozyme variability in blood serum of turkeys of different breeds was observed by Sotirov *et al.* (1997). In

generation G<sub>1</sub>, selected lines H and L differed significantly ( $P < 0.01$ ). In accordance with the methodological assumptions, the birds of line H had a higher lysozyme activity than the turkeys of line L. At the same time, lower activity variability was observed in both lines (from 25.8% to 27.3%) compared with the initial generation. In generation G<sub>2</sub> there was a considerable decrease in lysozyme activity; in addition, the difference between selected lines H and L was lower ( $P < 0.05$ ). A decline in activity probably resulted from the influence of non-genetic factors caused by a transfer of turkeys to a farm with much better raising conditions. The lower lysozyme activity may testify to over intensification of pathogenic factors in this environment.

The carried out divergent selection for high and low lysozyme activity did not cause a significant variation of the values of the traits evaluated in the 14th week of life in the offspring (Table 2). Average body weight and breast width in the birds of lines H and L were at a similar level. Irrespective of the line membership, the toms in generations G<sub>0</sub>, G<sub>1</sub>, G<sub>2</sub> had body weight which was higher than that of hens by over 2 kg. This is in agreement with the results of previous studies concerning turkeys of strain J-44 (Jankowski *et al.*, 1996).

While in generations G<sub>0</sub> and G<sub>1</sub> analysing the values of traits estimated during the reproduction period of

Table 1. Lysozyme activity (% of lysis) in blood serum in successive turkey generations

Sex	Statistics	Generation					
		G <sub>0</sub>	G <sub>1</sub>		G <sub>2</sub>		
			H	L	H	L	
Males	$\bar{x}$	21.8	24.7 <sup>A</sup>	19.4 <sup>B</sup>	13.2 <sup>a</sup>	11.1 <sup>b</sup>	
	$v$	43.12	25.91	27.32	36.36	20.00	
Females	$\bar{x}$	21.2	23.2 <sup>A</sup>	18.9 <sup>B</sup>	12.8 <sup>a</sup>	11.6 <sup>b</sup>	
	$v$	42.45	25.85	26.94	30.45	36.81	

A, B – means with different superscripts within the generation and sex differ significantly ( $P < 0.01$ )

a, b – means with different superscripts within the generation and sex differ significantly ( $P < 0.05$ )

Table 2. Body weight and breast width of turkeys at the age of 14 weeks

Trait	Sex	Statistics	Generation					
			G <sub>0</sub>	G <sub>1</sub>		G <sub>2</sub>		
				H	L	H	L	
Body weight (kg)	♂	$\bar{x}$	7.4	7.6	7.6	6.8	6.7	
		$v$	9.45	9.23	9.54	4.49	5.22	
	♀	$\bar{x}$	5.0	5.2	5.3	5.1	5.0	
		$v$	8.12	13.08	10.57	6.53	8.82	
Breast width (cm)	♂	$\bar{x}$	8.1	8.2	8.3	9.9	9.9	
		$v$	6.55	5.8	9.15	5.39	5.41	
	♀	$\bar{x}$	8.0	7.6	7.5	9.3	9.4	
		$v$	6.51	8.47	9.77	6.66	5.36	

Table 3. Body weight and the values of reproductive traits of turkey hens

Trait	Statistics	Generation			
		G <sub>0</sub>		G <sub>1</sub>	
		H	L	H	L
Body weight at the age: (kg)					
- of 30 weeks	$\bar{x}$	7.8	7.9	7.8	7.9
	$v$	10.74	9.90	13.79	11.91
- of 56 weeks	$\bar{x}$	9.1	9.4	9.3	8.8
	$v$	8.54	11.17	14.19	12.99
Reproduction maturity (days)	$\bar{x}$	241.3	244.2	230.3	228.3
	$v$	3.09	2.87	4.01	3.76
Number of eggs	$\bar{x}$	87.1	98.8	93.6	96.2
	$v$	23.84	19.98	19.67	14.64
Number of laying days	$\bar{x}$	131.3	142.4	139.1	144.5
	$v$	24.69	14.81	14.41	8.28
Fertility of eggs (%)	$\bar{x}$	90.8	95.2	88.9	93.7
	$v$	20.70	9.53	20.75	12.9
Hatching from fertile eggs (%)	$\bar{x}$	78.8	81.4	69.6	65.5
	$v$	18.38	17.45	23.65	30.21
Hatching from set eggs (%)	$\bar{x}$	71.9	77.7	61.4	61.5
	$v$	28.06	19.96	32.09	33.87

Table 4. Turkey mortality (%) in the rearing period

Period	Generation				
	G <sub>0</sub>	G <sub>1</sub>		G <sub>2</sub>	
		H	L	H	L
0 to 2 weeks	1.90	0.79	1.12	0.28	0.68
0 to 4 weeks	2.12	1.59	2.79	0.28	0.90
0 to 14 weeks	4.39	3.97	6.15	3.33	5.00

hens (Table 3), no significant differences between the selected lines H and L were found, either. In both generations, the hens of low lysozyme activity were characterized by a little higher egg yield. The results indicate indirectly that selection should not significantly differentiate the values of important performance traits. Selection experiments aimed at increasing immunity did not provide unequivocal information concerning the relation between the immunity coefficients and productive traits. The results of some authors indicate a lack of significant interrelations (Flock, 1995; van der Zijpp, 1983). However, in hens selected for an increase in the humoral response, a negative correlation with the body weight was found (Sieguel *et al.*, 1982; Paramentier *et al.*, 1996). In earlier studies (Prusinowska, Jankowski, 1996), a negative correlation between lysozyme activity in serum and the number of eggs and poults hatchability was observed in the hens of strain J-44, but it was found out only in one test. In the other three tests carried out on different dates, the coefficients of correlation between these traits were low and statistically insignificant.

A positive influence of selection was observed while analysing the livability of birds in the first 14 weeks of life (Table 4). The mortality of poults after parents with high lysozyme activity (H) was almost twice lower than that of poults descending from line L. Although the differences between lines H and L turned out to be only close to the statistically significant ones, the fact that they were recorded already after the first two selections indicates the advisability of continuing this experiment.

Preliminary evaluation of the effects of the turkey divergent selection indicates the usability of lysozyme activity as a selection criterion.

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# FISH COMMUNITIES IN THE DRAINAGE AREA OF THE OSOBLAHA RIVER AND EFFECT OF THE 1997 FLOOD

## RYBÍ SPOLEČENSTVA V POVODÍ ŘÍČKY OSOBLAHY S ASPEKTEM POVODNĚ V ČERVENCI 1997

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**ABSTRACT:** The upper part of the drainage area of the Osoblaha river (43%) lies in the territory of the Czech Republic. The river empties into the Odra river in the territory of Poland. Ichthyological investigations of the fish communities inhabiting the river network of the Osoblaha river in the territory of the Czech Republic have revealed the occurrence of one lamprey species (*Lampetra planeri*) and 17 species of fishes. The upper reaches of the Osoblaha and its tributaries are populated by a community of the trout type (*Salmo trutta m. fario*, *Phoxinus phoxinus*, *Cottus poecilopus* and, in places, also *Thymallus thymallus* and *Barbatula barbatula*). In the lower part of the stream occur, besides *S. trutta m. fario* and *P. phoxinus*, also species of the barbel community, such as *Leuciscus cephalus*, *L. leuciscus*, *Barbus barbus*, *Chondrostoma nasus* and *Gobio gobio*. The species composition beyond the headwater region is significantly influenced by the presence of minor reservoirs and fishponds in the drainage area of the Osoblaha. From these reservoirs such species as *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Tinca tinca*, *Pseudorasbora parva*, *Carassius auratus* and *Perca fluviatilis* get into the streams, sometimes in large numbers. The species diversity index  $H'$  varied in the range 1.570–1.844, being considerably lower only in sections with high abundance of a single species (*S. trutta m. fario* or *R. rutilus*),  $H' = 0.084–0.471$ . However, the species diversity index cannot serve to assess the anthropic influence on the native species composition of the fish community of a stream. In the section in r. km 7.9 of the rivulet Osoblaha, the status of its fish community was assessed shortly before and after the July 1997 flood. After the flood discharges, which exceeded the level of so-called Q-100 year water, no marked changes were ascertained in the fish community of the section under study. The Osoblaha and its tributaries are subject to fishery management and the fish are caught by angling only. The fishery management intensity is low.

**Keywords:** Osoblaha river; ichthyofauna; species diversity; abundance; biomass; flood; fishery management

**ABSTRAKT:** Byl proveden ichtyologický výzkum povodí říčky Osoblahy na území České republiky. Osoblaha se vlévá do Odry na území Polska, z celkové plochy povodí 1 008 km<sup>2</sup> se na území ČR nachází 43 %. Jedná se o horní část povodí, které zahrnuje převážnou část tzv. Osoblažského výběžku. V průběhu ichtyologického průzkumu v letech 1997 a 1998 byl na 9 lokalitách v povodí Osoblahy zjištěn výskyt jednoho druhu mihulovců (mihule potoční) a 17 druhů ryb. Převážná část toku Osoblahy má rybí osídlení pstruhového charakteru, s převahou pstruha obecného a početným výskytem střevele potoční. Vranka pruhoploutvá se vyskytuje pouze v horní pramenné části, stejně jako mihule potoční. Výskyt lipana podhorního ve střední části je důsledkem vysazování jeho násad. V dolní části má rybí osídlení přechodný ráz k parmovému typu, kdy vedle prvků pstruhového společenstva se vyskytují jelec tloušť, jelec proudník, hrouzek obecný a mřenka mramorovaná, ojediněle pak ostroretka stěhovavá a parma obecná. Byl zjištěn výrazný vliv rybníků na druhovou skladbu rybního osídlení. Druhy plotice obecná, perlník ostrobříhý, lín obecný, střevlíčka východní, karas stříbřitý a okoun říční se dostávají do toku, některé i ve značném počtu, při vypouštění rybníků nebo při jejich vyplavení (povodeň v červenci 1997). V úseku Bohušov (ř. km 7,9 Osoblahy) byl vyhodnocen vliv povodně v červenci 1997 na rybní osídlení. Povodňový průtok tam 8. července 1997 dosáhl 137,9 % tzv. stoleté vody ( $Q_{-100} = 103 \text{ m}^3 \cdot \text{s}^{-1}$ ). Původní rybní osídlení se v průběhu povodně zásadně nezměnilo. Po povodni se tam objevily mihule potoční a střevele potoční, které byly splaveny z výše ležících úseků. Specifické osídlení má největší přítok Osoblahy potok Prudník, který ústí do Osoblahy těsně před státní hranicí. Jeho větší část leží na území Polska a na území ČR se nachází 5,5 km dolního úseku. Rybní osídlení tohoto toku bylo v minulosti devastováno průmyslovým a komunálním znečištěním z území Polska. V současnosti se v potoce Prudník nad ústím do Osoblahy vyskytují druhy, které tam migrují z toku Osoblahy, a dále pak druhy, které se tam dostávají z rybníků v jeho povodí. Hodnoty indexu diverzity ve zkoumaných úsecích toků v povodí Osoblahy byly poměrně vyrovnané (1,570–1,844) a pouze v lokalitách s vysokou počet-

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ností jednoho druhu dosahoval tento index nízkých hodnot (0,084–0,471). Index diversity nepostihuje případnou antropogenní zátěž původní druhové skladby rybního osídlení vodního toku. Toky v povodí Osoblahy na území ČR jsou obhospodařovány s malou intenzitou. Z původních druhů je loven jelec tloušť a pstruh obecný. Prameně úseky jsou využívány jako odchovné potoky pro produkci násady pstruha obecného. Do jednotlivých úseků Osoblahy, kde je prováděn lov na udici, jsou vysazovány omezeně násady pstruha obecného.

**Klíčová slova:** Osoblaha; ichtyofauna; druhové složení; početnost; biomasa; povodeň; rybářské hospodaření

## INTRODUCTION

In the territory of the Czech Republic, the drainage area of the Osoblaha river comprises the territory of the so-called Osoblaha projection. The river flows to Poland where it empties into the Odra river as its left tributary. Our investigations of the fish communities of whole river systems were stimulated, above all, by the „biodiversity“ phenomenon and related aspects. As regards fishes and fish communities in the study area, no concrete reports are available except for a short note by Kempný (1951). The present contribution should fill in the blank area in the hydrological network of the Czech Republic as regards data on the fish communities in the Osoblaha river.

## MATERIAL AND METHODS

The Osoblaha river is a left tributary to the Odra river into which it empties near Krapkowice in Poland.

The total length of the stream is 58 km, its drainage area is 1 008 km<sup>2</sup>, of which 34.7 km and 433 km<sup>2</sup> respectively lie in the territory of the Czech Republic. The rivulet, called the Petrovice Brook in its upper part, springs at 715 m above sea level and leaves the territory of the Czech Republic at 205 m a.s.l. Woodland covers about 45% of mainly the upper part of the drainage area, whereas its lower part comprises the Osoblažská nížina Lowland with arable land. The hydrological network of the Osoblaha consists of minor brooks. Of importance is only its tributary, the Prudník brook, the drainage area of which lies mostly in the Polish territory. The Prudník empties into the Osoblaha about 800 m in front of the Polish border and its waters were still heavily polluted in recent years. The annual mean water discharge in the Osoblaha in the border profile attains 2.08 m<sup>3</sup>.s<sup>-1</sup>, of which 1.17 m<sup>3</sup>.s<sup>-1</sup> comes from the Prudník.

The investigations of the fish community were implemented in 7 localities in the drainage area of the

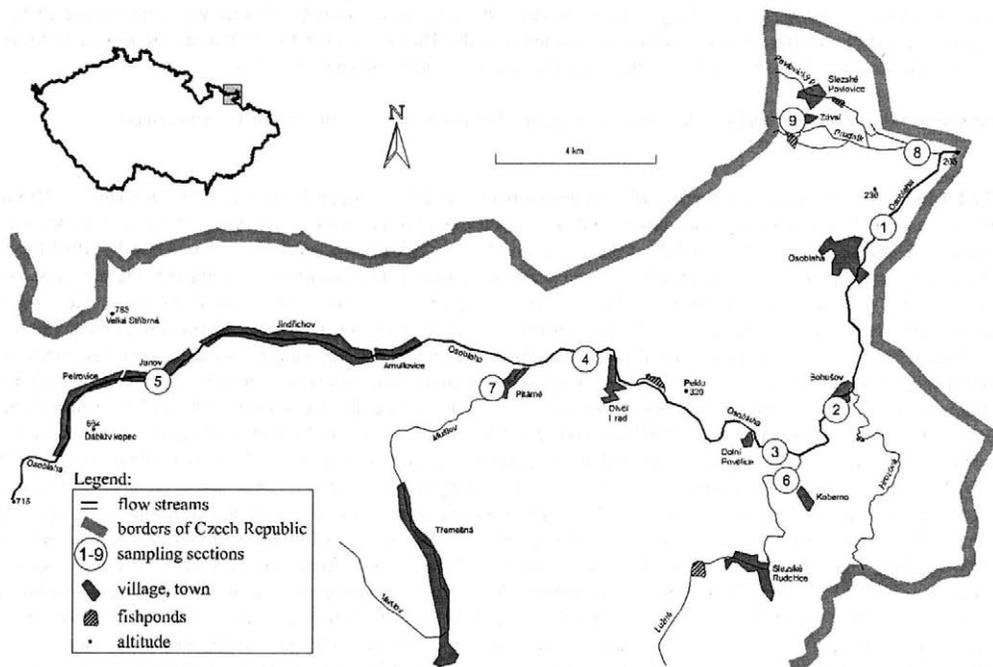


Fig. 1. Map showing the drainage area of the Osoblaha Rivulet and the sections investigated: 1 – Osoblaha, r. km 2.9; 2, 2a – Osoblaha, r. km 7.9; 3 – Osoblaha, r. km 11.5; 4 – Osoblaha, r. km 17.7; 5 – Osoblaha r. km 29.0; 6 – Lužná, r. km 0.7; 7 – Mušlov, r. km 1.5; 8 – Prudník, r. km 0.4; 9 – Prudník, r. km 3.9.

Osoblaha itself and in 2 localities on the stream of the Prudník. The location of the study sections is shown in Fig. 1.

Description of the sections examined:

1. Osoblaha (1), r. km 2.9, N-50°16'.51"/E-17°43'.32", fished on 16 July 1998, section 180 m long, stream 7.5 m wide. The stream is flanked with levees and streaming sections alternate.
2. Osoblaha (2, 2a), r. km 7.9 (limnigraph at Bohušovice), fished on 8 June 1997 before and on 15 August after the flood. Section 155 m long, stream width 10.0 m. Bed modified, one of its banks is formed by a perpendicular wall, the other river section lies in the village.
3. Osoblaha (3), r. km 11.5, N-50°14'.09"/E-17°41'.09", 16 July 1998, section 140 m long, stream width 7.5 m, the stream is provided with a levee, bed canalized, bank reinforced with stones, pools alternate with streaming sections.
4. Osoblaha (4), r. km 17.7, 30 July 1998, section 120 x 8.0 m, original unmodified stream with gravelly bottom and streaming water.
5. Osoblaha (5), r. km 29, 30 July 1998, section 90 x 4.0 m, a torrential section with rugged stony bottom, partly modified after the flood, its right bank reinforced. Occurrence of *Lampetra planeri*.
6. Lužná (6), r. km 0.7, 30 July 1998, right tributary to the Osoblaha in r. km 10.1. Section 90.0 x 1.5 m, bottom not rugged, with fine silt, several rather deep pools. Several fishponds are situated upstream in the drainage area.
7. Mušlov (7), r. km 1.5, right tributary to the Osoblaha in r. km 21.0. Fished on 30 July 1998. A section with modified bed in the village of Pitárné, torrential sections alternate with calm ones, bottom not very rugged. Section 90 x 2.0 m.
8. Prudník (8), r. km 0.30, N-50°17'.56"/E-17°44'.36", fished on 16 July 1998, section 125 m long, stream width 5.8 m. The stream is provided with levees, canalized, banks reinforced with stones, streams alternate with pools, bottom not rugged, shelters in banks.
9. Prudník (9), r. km 3.90, N-50°18'.14"/E-17°41'.46", fished on 16 July 1998, section 140 m long, stream width 7.50 m. Bed unmodified, stream meandering, shallow streaming sections alternate with deeper pools. Bottom gravelly and sandy.

The ichthyological investigations in the Osoblaha drainage area were implemented in 1997 and 1998. In electrofishing, we used a pulsating direct current (A 0.6–2.4, V 160–220), produced by a ZB 6 assembly made in the Czech Republic. Each section investigated was fished twice, the interval between any two consecutive fishings was 1.5–2 hours. In each fishing sample the number and weight of fish of each species were determined. The numbers and biomass of each species were estimated using a modified programme according to Seber and LeCren (1967). After all necessary data (body length, number, body weight) had been obtained

the fish were released. The river kilometers of the Osoblaha are measured from the border (r. km = 0.0), of the remaining streams from their mouth into the Osoblaha in the upstream direction. The indexes of the species diversity and equitability were calculated according to Shannon and Weaver (1963) and Sheldon (1969).

## RESULTS

### Occurrence of species

Investigations implemented in 9 localities on the Osoblaha rivulet have revealed the occurrence of the lamprey, *Lampetra planeri* (localities nos. 2, 4, 5) and of 17 species of fishes (for a list, see Table 1). The occurrence of *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Tinca tinca*, *Pseudorasbora parva*, *Carassius auratus* and *Perca fluviatilis* is due to the occurrence of fishponds and small reservoirs in the Osoblaha drainage area. The occurrence of these species, which are not indigenous or characteristic of the upper reaches of the Osoblaha and its tributaries, significantly affects the species composition and, in some sections, even the abundance and biomass of the fish community. Except for its lower reaches, the stream of the Osoblaha and its tributaries are inhabited by a community showing a salmonid character, with *Salmo trutta* m. *fario* and *Phoxinus phoxinus* predominating. The occurrence of *Cottus poecilopus*, another characteristic species of the salmonid community, is limited to the headwater sections of the Osoblaha (loc. 5). The lower section of the Osoblaha is inhabited by *Thymallus thymallus*, this fact is probably the result of stocking. The lower reaches of the Osoblaha (loc. nos 1, 2, 3) already harbour elements of the barbel community (*Leuciscus leuciscus*, *L. cephalus*, *Barbus barbus*, or even *Chondrostoma nasus*), similarly like Prudník (loc. no. 8). The species composition of the fish community of the left tributary, the Prudník (loc. nos. 8 and 9), is not clear-cut as the stream was heavily polluted still before 1990 from sources lying in the territory of Poland. Besides the elements of the barbel community (migrating there from the lower reaches of the Osoblaha), the stream contains species of fishpond origin. The occurrence of *Lota lota* and *Chondrostoma nasus* was ascertained in its lower part above the mouth into Osoblaha (loc. no. 8) ; for the occurrence of other species, see Table 1. Of the species ascertained, *Lampetra planeri* is protected as a „critically endangered“ species in the sense of Regulation No. 395/1992, and *Phoxinus phoxinus*, *Lota lota* and *Cottus poecilopus* as „endangered“ species.

### Abundance and biomass

The values of total abundance in the individual localities examined attain fairly high values of 9–20 thousand ind.ha<sup>-1</sup> (except in section 9 of the Prudník),

see Tables 1 and 2. The high value of total abundance is usually accounted for by only several species or, in some of the localities, by a single one. The highest values were produced by brown trout in the stream of the Osoblaha in r. km 27.7, viz., 9 195 ind.ha<sup>-1</sup>, in r. km 29.3 it was 13 195 ind.ha<sup>-1</sup> and in the lower section of the Mušlov brook as much as 17 247 ind.ha<sup>-1</sup>. An overwhelming part of this high abundance consisted of yearlings, which indicates successful spawning in the autumn 1997. We ascertained a high abundance of minnow in the Lužná stream, amounting to 8 033 ind.ha<sup>-1</sup>, see Tables 1 and 2 for details.

The values of the species diversity index (H') in the individual sections are considerably high, varying within the range 1.570–1.844 and being low (0.084–0.471) only in localities showing a high abundance and dominance of a single species (4, 5 and 7, see Table 2). The rather high value of species diversity (1.844) in the section in r. km 3.9 of the Prudník (locality 9), in which the fish community shows signs of heavy devastation (in total 2 635 ind. and 60.4 kg per hectare) as well as of the influence of fishponds (5 out of the 9 species originated from the fishponds) indicates that

the species diversity index should be evaluated very carefully. Especially with regard to the originality of the species composition of the fish communities in streams, the species diversity index may mask even a major change, and it does not signalize the consequences of the anthropic influence on the native species composition.

The total biomass varied from 59 to 520 kg.ha<sup>-1</sup>, its amount being determined, above all, by the biomass of the larger individuals (Tables 1 and 2). Usually, the numbers and weight were the highest in narrow streams, indicating a connection of these values with the bank line, which is distinctly higher in small streams than in the wide ones in relation to the area of water surface.

#### Extreme flood in July 1997

Profile Bohušov – Osoblaha (2, 2a)

In that section we were able to evaluate the influence of extreme flood discharges that occurred in July 1997 on its fish community. Directly in that section

Tab. 1. The variety of species of fish living in various sections of the Osoblaha river catchment area (Osoblaha 1–5, Lužná – 6, Mušlov – 7, Prudník – 8, 9) according to the dominant fish type

Species	Stream – No. of section									
	1	2	2a	3	4	5	6	7	8	9
<i>Lampetra planeri</i>	–	–	++	++	+	–	–	–	–	–
<i>Salmo trutta m. fario</i>	Sd	D	D	Ed	Spd	Spd	R	Spd	Sr	–
<i>Thymallus thymallus</i>	–	Sr	Sr	Sd	–	–	–	–	–	–
<i>Rutilus rutilus</i>	D	Spd	Ed	Spd	Sr	–	Spd	–	Ed	Ed
<i>Leuciscus leuciscus</i>	R	R	Sr	Sr	–	–	–	–	–	–
<i>Leuciscus cephalus</i>	Ed	Spd	Ed	Ed	–	–	D	–	Ed	R
<i>Phoxinus phoxinus</i>	Ed	–	Ed	Ed	Sd	–	Ed	D	Sr	Ed
<i>Scardinius erythrophthalmus</i>	–	–	–	–	–	–	–	–	Sr	D
<i>Tinca tinca</i>	Sr	Sr	–	–	–	–	–	–	Sr	Sd
<i>Chondrostoma nasus</i>	R	–	–	–	–	–	–	–	Sr	–
<i>Gobio gobio</i>	Spd	Ed	Ed	–	Sr	–	Sd	–	Spd	Ed
<i>Pseudorasbora parva</i>	R	–	Sr	–	–	–	Sr	–	–	–
<i>Barbus barbus</i>	Sr	–	–	–	–	–	–	–	–	–
<i>Carassius auratus</i>	Sr	R	Sr	Sr	–	–	Sd	–	D	R
<i>Barbatula barbatula</i>	Ed	Sd	Ed	Sd	D	–	D	Sd	D	D
<i>Lota lota</i>	–	–	–	–	–	–	–	–	Sr	–
<i>Perca fluviatilis</i>	Sd	Sd	Sr	D	–	–	Sd	–	Sd	Ed
<i>Cottus poecilopus</i>	–	–	–	–	–	R	–	–	–	–
Total N – species	15	10	11+1	9	5+1	2+1	9	2	12	9
Total abundance (n.ha <sup>-1</sup> )	9 158	3 433	3 586	9 133	10 291	13 417	16 624	20 200	9 371	2 635
Total biomass (kg.ha <sup>-1</sup> )	244.6	192.1	150.2	519.8	57.5	143.9	405.7	641.8	488.6	60.4
Index of species diversity H'	1.820	1.570	1.782	1.682	0.448	0.084	1.610	0.471	1.620	1.844
Equitability E	0.672	0.682	0.743	0.766	0.278	0.122	0.733	0.428	0.652	0.839

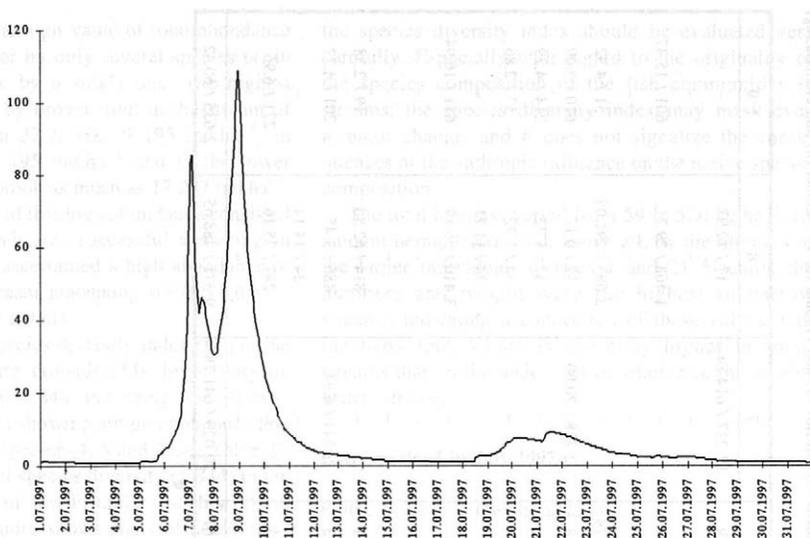
Sr – subrecedent (0–1%), R – recedent (1–2%), Sd – subdominant (2–5%), D – dominant (5–10%), Ed – eudominant (10–30%), Spd – superdominant (30–100%)

\* according to Lusk *et al.* (1998)

Tab. 2. Composition of species, abundance and fish biomass examined in locations along the Osoblaha river catchment area (1, 3, 4, 5), Lužná (6), Mušlov (7) and Prudník (8, 9). Number (*n*) and biomass (kg) are converted to 1 ha of water surface area

Species	1	3	4	5	6	7	8	9
<i>Salmo trutta m. fario</i>	301 / 8.87	1 598 / 128.33	9 195 / 45.84	13 195 / 142.77	296 / 49.79	17 247 / 614.84	17 / 12.97	–
<i>Thymallus thymallus</i>	–	244 / 4.29	–	–	–	–	–	–
<i>Rutilus rutilus</i>	645 / 17.39	2 883 / 60.00	83 / 8.33	–	8 033 / 79.01	–	1 468 / 29.21	601 / 15.37
<i>Leuciscus leuciscus</i>	93 / 7.23	19 / 0.95	–	–	–	–	–	–
<i>Leuciscus cephalus</i>	1 258 / 86.29	2 462 / 278.86	–	–	1 235 / 155.74	–	2 683 / 291.05	51 / 4.32
<i>Phoxinus phoxinus</i>	1 422 / 4.81	1 050 / 7.94	379 / 1.58	–	2 904 / 10.58	2 228 / 20.00	52 / 0.17	610 / 3.09
<i>Scardinius erythrophthalmus</i>	59 / 2.22	–	–	–	–	–	52 / 1.72	214 / 4.29
<i>Tinca tinca</i>	15 / 2.22	–	–	–	–	–	34 / 1.72	57 / 2.86
<i>Chondrostoma nasus</i>	161 / 46.26	–	–	–	–	–	34 / 11.21	–
<i>Gobio gobio</i>	3 755 / 44.34	–	42 / 1.67	–	605 / 8.23	–	3 410 / 36.19	611 / 16.94
<i>Pseudorasbora parva</i>	93 / 0.30	–	–	–	148 / 0.74	–	–	–
<i>Barbus barbus</i>	15 / 2.81	–	–	–	–	–	–	–
<i>Carassius auratus</i>	67 / 6.35	57 / 8.00	–	–	889 / 47.41	–	814 / 62.56	19 / 2.38
<i>Barbatula barbatula</i>	1 067 / 8.87	233 / 2.29	529 / 1.67	–	1 896 / 20.83	544 / 6.94	486 / 3.45	201 / 4.22
<i>Lota lota</i>	7 / 3.26	–	–	–	–	–	34 / 14.14	–
<i>Perca fluviatilis</i>	200 / 3.42	586 / 29.17	–	–	617 / 33.33	–	287 / 25.16	271 / 6.96
<i>Cottus poecilopus</i>	–	–	–	222 / 1.11	–	–	–	–
Total	9 158 / 244.61	9 133 / 519.82	10 291 / 59.09	13 417 / 143.88	16 624 / 405.67	20 020 / 641.78	9 371 / 488.65	2 635 / 60.42

Fig. 2. Daily water discharges in the Osoblaha river, station Bohušov (r. km 7.9), July 1997



there is a limnigraph which recorded the water discharge in the Osoblaha in the course of the flood. In that profile the annual mean water discharge attains  $0.78 \text{ m}^3 \cdot \text{s}^{-1}$ . The following values have been calculated for the N-year water discharges:  $Q-1 = 24 \text{ m}^3 \cdot \text{s}^{-1}$ ,  $Q-10 = 53 \text{ m}^3 \cdot \text{s}^{-1}$ ,  $Q-50 = 89 \text{ m}^3 \cdot \text{s}^{-1}$ ,  $Q-100 = 103 \text{ m}^3 \cdot \text{s}^{-1}$ .

In that section the extreme flood discharges occurred in July 1997 and, when culminating, they exceeded the level of the so-called "water of the century", i.e.  $Q-100$ . Intense rainfall was the decisive factor for the development of the extreme flood. The flood discharge began setting in already on 7 July and the maximum discharge was recorded on the very next day, 8 July. At the mid-day hours of that day the water discharge amounted to  $142 \text{ m}^3 \cdot \text{s}^{-1}$ , i.e. by 37.9% more than the  $Q-100$ . On the next day, 9 July, the water discharge dropped abruptly to about  $20 \text{ m}^3 \cdot \text{s}^{-1}$ . Another flood wave, considerably lower than the first one, occurred on 20–25 July, again due to increased rainfall, with the maximum discharges amounting to about  $20 \text{ m}^3 \cdot \text{s}^{-1}$ , see Fig. 2.

Quantitative electrofishings in that section were carried out prior to the flood on 8 June 1997, and the same section was fished again after the flood on 15 August 1997. The bed of the section is slightly curved, canalized, one of its sides is provided with a supporting wall, the other one has no such firm fortification. The flood water discharges did not appreciably alter the bed. Marked devastations of the bed occurred only close below the limit of the section investigated. The concrete values of species composition, abundance and biomass of the fish community are given in Table 3. The occurrence of *Pseudorasbora parva*, *Tinca tinca*, *Carassius auratus* and *Perca fluviatilis* was caused by their emigration from the minor pools in the drainage area, either when they were drawn out or during their being flooded. For this reason it is not commented here. After the flood the occurrence was ascertained of the

Tab. 3. Fish stock in section 2 (= 2a, Bohušov) of the Osoblaha river catchment area before – BF (June 8, 1997) and after – AF (August 15, 1997) the flood. The abundance (n) and biomass (kg) per 1 hectare of water surface area

Species	BF (n)	BF (kg)	AF (n)	AF (kg)
<i>Salmo trutta m. fario</i>	333	17.12	231	25.04
<i>Thymallus thymallus</i>	21	5.29	17	3.50
<i>Rutilus rutilus</i>	1 308	40.00	927	25.45
<i>Leuciscus leuciscus</i>	63	8.75	25	1.92
<i>Leuciscus cephalus</i>	1 741	78.53	976	67.52
<i>Phoxinus phoxinus</i>	–	–	417	2.08
<i>Tinca tinca</i>	21	3.33	–	–
<i>Gobio gobio</i>	440	14.06	469	16.48
<i>Pseudorasbora parva</i>	–	–	8	0.01
<i>Carassius auratus</i>	83	10.67	17	2.33
<i>Barbatula barbatula</i>	94	2.41	491	7.32
<i>Perca fluviatilis</i>	188	10.00	8	1.00
Total	4 292	190.16	3 586	152.65

lamprey, *Lampetra planeri*, which did not occur in the stream before the flood. Also, *Phoxinus phoxinus* was drifted down from sections lying higher upstream, similarly like *Barbatula barbatula* was. A significant drop in numbers was ascertained in *Salmo trutta*, *Rutilus rutilus*, *Leuciscus leuciscus* and *L. cephalus*, even though they had retained their previous significant representation.

Among the results obtained in our investigations on the upper reaches of the Osoblaha in the subsequent year (1998), it is worthwhile to note the high numbers of *S. trutta* yearlings resulting from the spawning in the autumn 1997. In this point one may assume a certain effect of the July 1997 flood. The flood water discharges cleaned the stream and also renewed bottom

materials which offered optimum conditions for the deposition of brown trout eggs. Likewise, the numerous occurrence of species not typical of streams of a trout character (*R. rutilus*, *P. fluviatilis*, *C. auratus*) in some of the localities may be connected with the flood discharges in July 1997 which flooded the reservoirs in the drainage area of the Osoblaha.

### Fishery management

All streams in the Osoblaha drainage area are parts of the fishing grounds. The stream of the Osoblaha from the Polish border up to r. km 12.0, including its tributaries, is the fishing ground Osoblaha no. 1 (E.n. 471 107), non-trout water, and from r. km 12 of the Osoblaha incl. its tributaries, the fishing ground Osoblaha no. 2 (E.n. 473 074), trout water. The lower reaches of the brook Prudník in the territory of the Czech Republic are a separate fishing ground Prudník no. 1 (E.n. 471 118), non-trout water. All these three fishing grounds are managed by the Anglers Organization in Krnov. It can be concluded from the data on catches (in 1993–1997) that the fishing intensity is not high. Of the species indigenous to the stream of the Osoblaha, the records show an annual catch of *L. cephalus* ranging from 90 to 120 individuals, of *S. trutta* 60–240 ind., and occasional catches of *T. thymallus*. Every year the stream of the Osoblaha is stocked with 1 200 *S. trutta* yearlings. No catches have been registered in the Prudník brook. The drainage area of the Osoblaha comprises minor reservoirs which are stocked with *Cyprinus carpio*, *Esox lucius*, *C. auratus*, *P. fluviatilis* and several other species, all of which are also fished in the reservoirs. The minor tributaries and the headwater area of the Osoblaha are utilized for rearing *S. trutta* stocking, which are used in the lower downstream lying parts of the Osoblaha.

### DISCUSSION

The only published data on fishes in the streams in the drainage area of the Osoblaha rivulet are contained in a report on investigations carried out in 1950 (Kempný, 1950). In the Osoblaha brook (or, more precisely, in the right tributary, the Mušlov) in the village of Třemešná the occurrence of brown trout, brook minnow, common gudgeon, and stone loach was ascertained. The same species have also been reported from the Petrovice Brook at Jindřichov (or, more precisely, the Osoblaha in r. kms 25–26).

The species diversity of the fish community inhabiting the lower part of the Osoblaha drainage area (sections 1, 2, 3, 6, 8 and 9) is significantly influenced by fish species which constitute the community in small reservoirs and fishponds lying in the Osoblaha drainage area. These species get into the streams when they are

drained or during high water discharges, such as during the July 1997 flood. The important influence of fishponds on the occurrence of some fish species in the streams in the Českomoravská vrchovina Highlands was already pointed out by Dyk (1957). Pivnička (1998) observed a significant correlation between the number of fishponds and species diversity of fish communities in small streams in the Křivoklátsko Protected Landscape Area.

A high abundance of brown trout, attaining 10 000 ind.ha<sup>-1</sup> and over (localities 4, 5, 7, see Table 1) is chiefly due to the enormous occurrence of yearlings which show no strong requirements for more extensive territories. In the above localities the average extent of the territory is 0.5–1.0 m<sup>2</sup> per individual. The high spawning success or subsequent successful hatching of the laid eggs could also appear due to the effect of the July 1997 flood which provided a clean gravelly and sandy bottom substrate. The hitherto known values of abundance of brown trout with a predominance of yearlings, observed in similar trout streams in the Czech Republic, are usually lower. In the Lušová brook in the Beskydy Mts. the maximum abundance of brown trout attained 6 094 ind.ha<sup>-1</sup> (Lusk and Zdražil, 1969). A high abundance (10 800 ind.ha<sup>-1</sup>) was ascertained in the upper part of the Chvojnice brook, which was utilized for rearing brown trout yearling (Lusk, 1973).

The effect of the flood on the fish community in r. km 7.9 (locality Bohušov) confirms that even extreme water discharges will cause no marked changes in the species composition or in the quantitative indices (abundance, biomass) of the indigenous riverine fish species. Similar conclusions have also been drawn for the sections examined in the upper part of the river Tichá Orlice in which the local fish community of the *Salmo* – *Thymallus* type was not fundamentally altered by the flood discharges in July 1997, exceeding the Q-100 level (Lusk *et al.*, 1998a, b). The overall evaluation of the July 1997 floods and their incidental effects on the fish communities in streams has been confirmed by our observations on the Osoblaha in the profile at Bohušov (Lusk *et al.*, 1998a, b). In particular, larger (older) individuals of brown trout show a high resistance to extreme water discharges (Lusk, 1979). Extreme floods considerably affect those species which, by their biology, are connected with the bottom materials (*C. gobio*, *B. barbatula*, lamprey larvae, etc.) and also the eggs and larvae of salmonids (Lusk *et al.*, 1998a, b; Čihař, 1976; Onodera and Ueno 1961, etc.). According to the observations of Matthews (1986) as well as our unpublished data from the stream of the Bečva river, the extent of the change caused by a flood to a fish community depends upon the physical characteristics of the bed, which means, whether the bed has been canalized or is natural, whether or not the water can leave it and flood the abutting floodplain, etc.

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# SHORT COMMUNICATION

## A RARE PCR-RFLP WITHIN INTRON 2 OF THE PORCINE *TSHB* GENE

### VZÁCNÝ PCR-RFLP V INTRONU 2 *TSHB* GENU PRASETE

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**ABSTRACT:** Based on cDNA sequence of the porcine *TSHB* (thyroid stimulating hormone, beta subunit) gene a fragment encompassing a part of exon 2 and exon 3 and intervening intron was amplified using polymerase chain reaction (PCR). The PCR fragment was sequenced and the sequence was deposited in EMBL/GenBank/DBJ databases (AJ131849). Of investigated pig breeds (Landrace, Large White, Duroc, Hampshire, Czech Meat Pig, Piétrain, and Meishan) a *MnII* PCR-RFLP was observed only in 2 of 11 Meishan pigs (frequencies  $A = 0.09$ ,  $B = 0.91$ ). *TSHB* was mapped by linkage analysis on chromosome 4 as follows: *S0073* – 3.3 – *ATPIA1* – 12.9 – *EAL* – 3.2 – *NGFB* – 2.3 – *TSHB* – 11.1 – *SW2435* (distances given in Kosambi cM).

**Keywords:** pig; *TSHB*; polymorphism; DNA sequence; linkage mapping

**ABSTRAKT:** Pomocí polymerázové řetězové reakce (PCR) byl amplifikován fragment genu pro beta podjednotku thyrotrpního hormonu (*TSHB*), zahrnující část exonu 2 a exonu 3 včetně vmezeřeného intronu. PCR fragment byl sekvenován a sekvence byla uložena do databázi EMBL/GenBank/DBJ (AJ131849). Z vyšetřovaných plemen prasat (landrace, bílé ušlechtilé, duroc, hampshire, české výrazně masné, piétrain a meishan) byl zjištěn *MnII* PCR-RFLP pouze u dvou z 11 zvířat plemene meishan (frekvence:  $A = 0,09$ ;  $B = 0,91$ ). Pomocí vazbové analýzy byl *TSHB* mapován na chromozomu 4 takto: *S0073* – 3.3 – *ATPIA1* – 12.9 – *EAL* – 3.2 – *NGFB* – 2.3 – *TSHB* – 11.1 – *SW2435* (vzdálenosti udány v Kosambi cM).

**Klíčová slova:** prase; *TSHB*; polymorfismus; DNA sekvence; vazbové mapování

Finding of polymorphism of Type I genes (coding sequences), which can easily be tested in farm animal species, is needed for identification of economically important loci (ETLs) including quantitative trait loci (QTLs) by means of positional candidate gene approach (Womack, 1997).

Thyroid stimulating hormone consists of the alpha subunit (encoded by the *TSHA* gene) shared with other glycopeptide hormones (CG, LH, and FSH) and the beta subunit (encoded by the *TSHB* gene). In the pig the *TSHB* gene was physically mapped to the chromosome region 4q22-q24 (Kojima *et al.*, 1996). Linkage analysis (Archibald *et al.*, 1995) performed with the

use of *TaqI* RFLP (Couperwhite *et al.*, 1992) placed *TSHB* to the proximity of QTL for growth (Knott *et al.*, 1998).

Based on cDNA sequence of the porcine *TSHB* gene (EMBL/GenBank accession number U39816) which was compared for exon-intron structure with DNA sequence (EMBL/GenBank accession number M25164) of exons 2 and 3 of the human *TSHB* (Tatsumi *et al.*, 1988) primers were designed (forward: 5'- TGT CGA AAG GAA AGA GTG TGC T - 3', reverse: 5' - GTA GGA GAA ATA AGG AGT AAC - 3') to amplify a fragment encompassing a part of exon 2 and exon 3 and intervening intron.

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Fig. 1. Separation of the *MnII*-digested PCR products in 4% NuSieve Agarose 3:1 (FMC BioProducts Europe, Vallensbæk Strand, Denmark)



Polymerase chain reaction (PCR) was carried out in 50 µl reactions using 100 ng porcine genomic DNA, 40 pM each primer, 200 µM each dNTP, 1.5 mM MgCl<sub>2</sub> and 1.5 U *Taq* polymerase in standard PCR buffer. After an initial 95 °C (2 min) the PCR was carried out at 95 °C (30 s), 58 °C (30 s) and 72 °C (40 s), the last extension 5 min) for 35 cycles. Digestion of 15 µl of each PCR product was performed with 5 U of restriction enzyme *MnII* at 35 °C overnight.

To verify the PCR product and search for breed differences in the fragments PCR products, one from Meishan and one from Piétrain, were ligated to plasmid pUC18 using SureClone Ligation Kit (Pharmacia Biotech, Uppsala, Sweden), transformed into *Escherichia coli* DH5α and sequenced on an ALFexpress Sequencing System (Pharmacia Biotech). Sequencing of the cloned PCR fragments confirmed their identity within amplified parts of the porcine cDNA (EMBL/GenBank accession number U39816) corresponding to exon 2 and exon 3 of the human *TSHB* gene (EMBL/GenBank accession number M25164). A complete sequence of intervening intron 2 was also determined. The sequence was deposited in EMBL/GenBank/DDJB databases (accession number AJ131849). The intron has 442 nt and its boundaries conform to the GT/AG rule. Comparison of the Meishan and Piétrain sequences revealed difference at position 492 of our sequence (C in Meishan, T in Piétrain) which was, however, not convenient for use as a genetic marker due to the fact that nucleotides at this position are not a part of a recognition site for any of known restriction enzymes.

To search for polymorphism the PCR products of individuals belonging to different breeds were digested with 20 restriction endonucleases (*AluI*, *Alw26I*, *AsuI*, *AvaI*, *AvaII*, *BclI*, *BseDI*, *BspLI*, *BsuRI*, *Csp6I*, *DdeI*, *FokI*, *HinI*, *HinII*, *MboII*, *MnII*, *MvaI*, *SacI*, *Sau3A*, and *TaqI*). Biallelic polymorphism was found with *MnII* (Fig. 1). The enzyme theoretically cuts the PCR prod-

uct (EMBL/GenBank AJ131849) to yield fragments 205, 174, 124, 111, 94, 86, 37 and 18 bp. Restriction site (RES) at position 409 was found to be polymorphic. Absence of RES gives fragment 205 bp (allele *TSHB A*). When RES is present, 205-bp fragment is cut to 111 and 94-bp fragments (allele *TSHB B*). Codominant inheritance was documented in Meishan x Piétrain three-generation pedigree prepared at Hohenheim University for QTL (quantitative trait locus) analysis on growth, carcass and meat quality traits (Geldermann *et al.*, 1999). The polymorphism was observed only in 2 out of 11 Meishan pigs (allele frequency A = 0.09 and B = 0.91), one of them was founder boar of the Hohenheim Meishan x Piétrain pedigree, and in one Meishan x White composite sow of USDA-MARC backcross pedigree (Rohrer *et al.*, 1994). In Landrace (*n* = 21), Large White (*n* = 34), Duroc (*n* = 20), Hampshire (*n* = 23), Czech Meat Pig (*n* = 15) only allele B was observed. Multipoint linkage analysis was performed in Hohenheim Meishan x Piétrain pedigree (Geldermann *et al.*, 1999) using CRI-MAP software package, version 2.4 (Green *et al.*, 1990). *TSHB* mapped on SSC4 as follows: *S0073* - 3.3 - *ATPIA1* - 12.9 - *EAL* - 3.2 - *NGFB* - 2.3 - *TSHB* - 11.1 - *SW2435* (distances given in Kosambi cM).

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