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QUANTITATIVE ASPECTS OF COAT COLOR IN OLD KLADRUBER BLACK HORSES

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Original scientific paper

SUMMARY

The aim of this study was to evaluate potential factors influencing coat color intensity in black variety of Old Kladruber horse breed and to estimate heritability for this trait. A total number of 145 individuals (105 females, 40 males), aged from 1 to 24 years were included in the analysis. The measurement of coat color was performed by Minolta Spectrophotometer 2500d using CIE (L*a*b*) color system. Measurements were taken at 4 body parts (neck, shoulder, belly and back) on each horse. The GLM procedure (SAS/STAT® software) was used to examine the influence of effects of age, line, sex, body part and housing on recorded coat color characteristics. All the effects except line were found statistically significant (p<0.001) related to all three parameters. The estimated heritabilities using REMLF90 (Misztal, 2002) ranged between 0.14–0.37 by the parameter and the body part.

Key-words: Old Kladruber horse, black coat color, variability, heritability, Minolta Spectrophotometer

INTRODUCTION

Old Kladruber horse (OKH) is the only autochtone warmblood breed of Czech origin created on a basis of the old Spanish and Italian blood. This breed has been breeding on Czech territory continuously more than 400 years the General Studbook of the breed has been held since 1757. Nowadays, OKH is included in gene resources of the Czech Republic and the Studbook has been fully closed since 2002. The breed is kept in two coat colors – grey and black one. The breeder's aim is to keep the OKH in carriage (galakarosier) type usable for ceremony and representative purposes, driving event, dressage, baroque and leisure riding. The OKH population consists of 46 stallions and 501 mares, 23 of which are black stallions and 261 black mares. The black variety consists of 5 lines.

The horse coat color is generally taken as a qualitative trait with mendelian inheritance. There are about 10 known genes responsible for coat color in horses (Thiruvenkadan et al., 2008; Rieder, 2009; Adrian, 2013), three basic colors (chestnut, bay, black) are determined by loci AGOUTI (gene ASIP) and EXTENSION (gene MC1R) (Marklund et al., 1996; Rieder et al., 2001). Black coat color is determined by recessive homozygote genotype of AGOUTI locus and at least one dominant allele on EXTENSION locus (***aa E-***) (Sponenberg, 1996).

There are remarkable differences within color phenotypes which are not possible to explain by Mendelian inheritance, for example the differences in level of greying (Curik et al., 2013), the shades of chestnuts or bays (Toth et al., 2006). There are two different types of black coat color – non-fading (jet or raven black) is charcoal black with metallic or blueish shine and fading – black with color without shine, fading to reddish-brown tinge especially when exposed to the sunshine in summer months (Sponenberg, 1996). The genetic determination of these two types of black color is not known. Black OKH population exhibits both color types mentioned, but undesirable „fading black" in high proportion.

The aims of this paper were to get quantitative characteristics of black horse color, to determine factors influencing variability of black color regarding possible effects of line, sex, age, body part, stay on pasture and to estimate heritability for black coat color.

Ph.D. Barbora Hofmanová (hofmanova@af.czu.cz), Pavla Kohoutová, M. Eng., Assoc. Prof. Luboš Vostrý, Hana Vostrá Vydrová, M. Eng. Agr., Assoc. Prof. Ivan Majzlík - Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Kamýcká 129, 165 21 Prague 6, Czech Republic

MATERIAL AND METHODS

There were 145 OKH blacks (105 mares and 40 stallions) at the age of 1 – 24 years included into this study. The statistical evaluation was carried out for 5 groups of age (1-2 years, 3-4 years, 5-10 years, 11-15 years, 16 and more years). The black color parameters were measured using Minolta Spectrophotmeter 2500d. The parameters measured under the system CIE (Commision Internationale de l'Eclairage) consist of: L* - lightness (0=Black, 100=white), a* - redness (+60=red, -60=green), b* - yellowness (+60=yellow, -60=blue). The parameter L* is the most important in case of black color, substantial is also the parameter of axis red-green (a*) detecting possible fading to reddish-brown tinge of hairs. Measurement of parameters mentioned was carried out on every animal on four body parts – neck (place with no mane covering), shoulder, belly and back (thigh). The every value of parameter measured is an average of three consecutive measuring on the same spot. The analysis of effects influencing the intensity of black color was performed by SAS/STAT® software - general linear model (GLM), using the least square method (LSM).

$$\mathbf{Y_{ijklm} = \mu + AGE_i + LINE_j + SEX_k + STABLE_l + PART_m + e_{ijklm}}$$

$\mathbf{Y_{ijklm}}$ – parameter value (L*, a *, b*), μ – overal mean, $\mathbf{AGE_i}$ – fixed effect of the age group (i = 1, 2, 3, …5), $\mathbf{LINE_j}$ – fixed effect of the line (j = 1– Favory - Generalissimus, 2 - Sacramoso, 3 – Siglavi Pakra, 4 - Solo, 5 - Romke), $\mathbf{SEX_k}$ – fixed effect of the sex (k = 1 - male, 2 - female), $\mathbf{STABLE_l}$ – fixed effect of the housing (l = 1 – stable, 2 – outside), $\mathbf{PART_m}$ – fixed effect of the body part (m = 1 - neck, 2 - shoulder, 3 - belly, 4 - back), $\mathbf{e_{ijklm}}$ – residual error

The variance components and coefficients of heritability were estimated by REML method using the programme REMFL90 (Misztal, 2002). The same fixed effects (except the line) as in GLM analysis were included, the effect of an individual horse and the one of a residual error were considered as random effects. The dependent variables are parameters of black color measured on defined body parts and average value of all four parts on every horse. The pedigree of every horse under the study consists of five ancestor generation.

RESULTS AND DISCUSSION

The observed L*, a* and b* values ranged between 16.24–31.18, 0.72–7.87 and -0.25–13.68, with mean values (± standard deviation) 21.92 ± 2.33, 3.29 ± 1.20 and 3.70 ± 2.01 respectively. All effects except line were found out statistically significant (p<0.001) related to all three parameters. The differences between stallions and mares or horses kept in stable vs. horses kept on pasture („tabun") are presented in Table 1. Even though breeding mares are usually kept separately from stallions, there were a number of young horses of both sexes kept under the same conditions (permanent stay on pasture), thus we evaluated sex and stable as the separate effects.

Table 1. Effects of the gender and housing system on L*, a*, b* values

	L*		a*		b*	
	MEAN	SD	MEAN	SD	MEAN	SD
Male	20.48	0.16	2.64	0.09	2.61	0.15
Female	21.06	0.12	3.43	0.07	3.84	0.11
Stable	20.06	0.16	2.81	0.09	2.75	0.15
Outside	21.49	0.13	3.26	0.07	3.71	0.12

The mares showed significantly higher values of all parameters studied, since they are of lighter color with higher fading to reddish-brown tinge in hairs compared to stallions. Also, it was confirmed that black horses on pasture, permanently in summer, show lighter color with reddish-brown tinge. These conclusions are in an agreement with common knowledge on changes of black color influenced by sunlight. Our results are corresponding with conclusions of Stachurska et al. (2004) studying differences in „blue duns" diluted color derived from black.

The differences in parameters under the study in connection with the age are presented in Figure 1. The influence of age on parameter L* was not proved (p = 0.22). The values of L* showed substantial differences depending on the age in papers dealing with grey horses (Majzlik et al. 2010, Curik et al., 2013, Hofmanová et al., 2015). The color changes with the age are not remarkable in non-greying horses, but the difference in color especially between foals and mature horses were published (Sponenberg, 1996). Parameters a* and b* showed similar changes. The youngest foals showed the highest values, decreasing with the age to minimum at the age of 11–15 years. Higher values were noticed again in the oldest category may be due to small number of old horses. Higher values of parameters a* and b* (more „red" and „yellow" color) in foals comparing adult horses were presented by Stachurska et al. (2004) in blue duns.

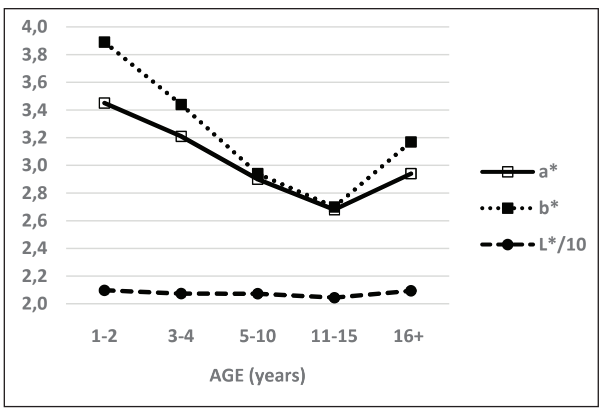

Figure 1. Effect of the age on L*, a*, b* values

The differences between body parts measured on horse reached statistical significance for all parameters under the study. The values measured on the neck and back were lower compared to values measured on the shoulder and the belly (Figure 2). This result is in agreement with practical knowledge – reddish fading in blacks is expressed primarily on belly region. The question is why on this body part, because the back is much more exposed to the sun's rays (there was no equipment covering measured place on the back parts of horses used). Despite the assumption of breeders observation that horses from Romke line (line founder stallion Romke of Friesian breed) are showed darker color, this study did not prove the line influence in any parameter ($p=0.06$, 0.71 and 0.63 for L*, a*, b* respectively). The explanation is, perhaps, in rotational mating planes in lines used with the result, that every line has some genetic contribution of other black lines.

Figure 2. Effect of the body part on L*, a*, b* values

Estimates of heritability for total color (average value of data measured on all four parts for every horse) and color of four body parts under the study are presented in Table 2. Standard errors of heritability estimate from REMLF90 were not available. We expect them to be high due to the low number of horses. Since the population of Old Kladruber blacks in quite limited, repeated measurements could be an option to assure larger number of observations. The lowest value for total color showed parameter L* ($h^2=0.11$), whereas the highest value was estimated for parameter a* ($h^2=0.18$). Heritabilities of body parts measured ranged between 0.14–0.32. The lowest heritabilities showed all parameters of neck color whereas the highest were found out for the back. The parameters a* and b* reached higher values compared to L* which could show the possible multifactorial inheritance of reddish tinge in hairs. Toth et al. (2006) published comparable heritabilities for L*, within-color class heritabilities for parameters a* and b* being negligible in this study. The parameter L* is an important criterion of greying in grey horses, as its value is corresponding to total melanin content in hairs (Toth et al., 2006). Heritability for greying in OKH greys were estimated as $h^2=0.52$ (Majzlik et al., 2010, Hofmanová et al., 2015) and comparable values for Lipizzaners were estimated by Curik et al. (2013).

Table 2. Genetic and residual variances and heritability estimates

Parameter	**L***			**a***			**b***		
Body part	**V(G)**	**V(R)**	**h^2**	**V(G)**	**V(R)**	**h^2**	**V(G)**	**V(R)**	**h^2**
Neck	0.44	2.59	**0.14**	0.08	0.44	**0.16**	0.20	0.79	**0.20**
Shoulder	0.62	2.68	**0.19**	0.31	0.72	**0.30**	0.88	2.43	0.27
Belly	0.49	2.78	**0.15**	0.22	0.97	**0.18**	0.73	2.84	**0.20**
Back	0.58	1.44	**0.29**	0.23	0.48	**0.32**	0.45	0.78	**0.37**
Average	0.18	1.51	**0.11**	0.11	0.52	**0.18**	0.22	1.34	**0.14**

The differences in genetic determination of fading and non-fading black color are not known yet. The different phenotype is perhaps under the influence of modifying genes having only a minor effect, which could be cumulative. The black color could be theoretically influenced by genotype on EXTENSION locus. We can assume that the most blacks in Kladruby population are homozygous dominant (***EE***) at this locus, but several cases of chestnut foals born in this population proves presence of heterozygous genotype (***Ee***). There is a possibility that presence of recessive allele ***e***, known as a „red factor", could influence the occurrence of fading to reddish-brown tinge in hairs. Rieder et al. (2001) states the influence of EXTENSION locus in bays – bays with lighter shade in hairs were heterozygous (***Ee***), whereas dark bays were homozygous (***EE***).

The presence of polygenic component within black coat color could be solved by genomics in the future. However, the precondition of successful analyses like this is to have available unbiased information on horse color phenotype. This paper and previous papers (Toth et al., 2006; Majzlik et al., 2010; Curik et al., 2013, Hofmanová et al., 2015) dealing with horse color showed that the Minolta Spectrophotometer could be an appropriate equipment for such studies.

CONCLUSION

This paper is the first study dealing with objective evaluation of coat color in black Old Kladruber horse. There are several similar studies in greys or other coat colors in other breeds unlike in blacks with not known such study. The results of this paper confirmed some generally known facts on black color, especially bleaching of hairs by sunlight during the stay of horses on pasture in summer. The influence of the age was proven particularly only – for parameters a* and b*. The results of this study did not show any differences in color between lines. Heritabilities estimated were in range 0.14-0.37 depending on parameter and body part measured.

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