ISSN 1644-0714
ISSN 2300-6145 (online)
DOI:10.21005/asp.2016.15.2.06
www.asp.zut.edu.pl

Acta Sci. Pol. Zootechnica 15(2) 2016, 59–76

GENETIC AND PHENOTYPIC CHARACTERISTICS OF POLISH KONIK HORSES MAINTAINED IN THE RESERVE AND STABLE SYSTEM IN CENTRAL-EASTERN POLAND

Michał Pluta[1]✉, Zbigniew Osiński[2], Angelika Cieśla[3], Ryszard Kolstrung[1]

[1]Departament of Horse Breeding and Use, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland
[2]Department of Hygiene of Animal Feedingstuffs, National Veterinary Research Institute, Partyzantow Avenue 57, 24-100 Pulawy, Poland
[3]Unit of Horse Breeding and Animal Assited Therapy, West Pomeranian University of Technology, Szczecin, Doktora Judyma 12, 71-466 Szczecin, Poland

Abstract. The aim of this study was to determine the impact of genetic diversity on the features like breeding, performance parameters and adaptation to living in natural habitat resulting from the particular degree of consanguinity to primitive horses of the Polish Konik breed. Selected features were also evaluated with the reference to chosen breeding lines as an indication of the effect of breeding progress during a long period of time. Additionally, the assessment of interaction between environmental and genetic factors was made. The study involved 257 Polish Konik horses (two herds), inhabiting in the breeding centre, where the conditions similar to natural for population of primitive horses originally inhabited Europe were provided. During the conducted study the statistically significant effect of the degree of consanguinity on chest circumference and the greatest dispersion of point-scale estimation ("bonitation") results of horses with higher inbreeding coefficient degree $F \geq 15\%$ was shown. We indicated that the value of $F = 15\%$ is in fact a limit, from which the negative impact of homozygosity on the traits associated with the phenotype of primitive horse breed, the Polish Konik, starts to increase. As shown in the analysis of breeding lines, in the long term to maintain the appropriate breed traits associated with the body structure increased heterozygisity is more privileged.

Key words: ancestral inbreeding, impact of homozygosity, primitive horses, Polish Konik horses

✉michal.pluta@up.lublin.pl

INTRODUCTION

Civilization changes which have taken place over the last several hundred years worldwide, particularly in Europe, have caused great transformations in the environment. The reduction or even extinction of species commonly found in the environment has occurred as the result of the demographic expansion of people. This was done by the reduction of the areas of the original habitat of many wild living animals and replacing them with agricultural crops and by the disturbance of fauna biodiversity by a huge increase in the number of the domesticated and farm species [Lunch and Lande 1993]. This concerned mainly large mammals from which domesticated animals and livestock originated [FAO 2007]. One of the families is equine, whose wild species became extinct in Europe [Vila et al. 2006]. Since 30s–40s of the 20th century the rapid mechanization of agriculture, industry and transport has taken place, resulting in further reduction of domesticated horses' population. The strenuous work, concerning the inhibition of the rate of that species extinction, is being continued, and attempts are being made to reconstruct some populations within biodiversity preservation. This includes primitive horse breeds of the closest genetic relationship with already extinct wild living equines of Europe and Asia [FAO 2007, Polak 2012, Maćkowski et al. 2015]. This kind of activity is also undertaken in Poland. Polish Konik horses were recovered at the beginning of 20th century thanks to professor Tadeusz Vetulanii. This is primitive horse breed with the phenotypic and genotypic characteristics closely related to its wild ancestor, the Tarpan horse, known as Eurasian wild horse [Jaworski 1997]. Polish Konik horses are considered a unique natural-breeding relict, which must be preserved as a specific reservoir of genetic resources for future breeding works. The aim of this study was to determine the impact of genetic variation on the breeding and performance parameters resulting from the particular degree of relatedness to Polish Konik horses. In this case, this kind of variation is the result of breeding works associated with recovery from certain, in this case, small number of individuals. The scientific characterization of primitive horses has been performed, in regard to the degree of inbreeding, with the influence of this parameter on breed characteristics and adaptation to living in natural habitat. Selected parameters were also evaluated with the reference to chosen breeding lines as an indication of the effect of breeding progress during a long period of time [Sargolzaei et al. 2006]. This characterization has served the authors to expand their knowledge of the effects of differentiated inbreeding of primitive horses that can be useful for the restoration of small populations of endangered species.

MATERIAL AND METHODS

Animals, environment, handling and management. The study involved 257 Polish Konik horses (two herds), inhabiting the breeding centre in the central – eastern Poland. First breeding herd, since its creation in 1982 until 2010, in Roztoczański National Park (RNP) in Zwierzyniec, has consisted of 163 animals, including 81 stallions and 82 mares. The herd is maintained in the reserve system, in a so-called "Ostoja". Inside the Park comprising a total area of 8483 ha, about 180 ha was isolated and devoted to horses, the area of diverse natural environment is enclosed with wooden fence [Wlizło and Szwed 2007]. In the "Ostoja" area horses meet their nutritional demands on their own, yet it is allowed to feed them with roughages (hay, straw) only during unfavourable conditions of the year.

The second herd under the study, established in 1996, is also kept in RNP in the so-called "Florianka". During its operation (since 1996 until 2010) there were 94 Polish Konik horses including 46 stallions and 48 mares [Pluta and Osiński 2014]. The breeding was carried out in the stable system in the mid-forest settlement, in town from which the name "Florianka" was adopted. Due to stable breeding, it is possible to maintain the following herd structure: four adult stallions among which it is possible to select an individual for breeding (mares from the stable and outside), about 14 adult mares and their offspring: foals, weaned foals, yearlings, and older youth. Both groups of horses are interrelated, since the stable herd appeared as a consequence of development of the reserved herd [Sasimowski and Kaproń 1984b]. Planned breeding operations were to bring the appropriate development of both groups. The catching of 2–2.5 year old colts and fillies took place in Roztoczański National Park in "Ostoja", then at certain periods horses were kept in stable breeding centre. This concerned 65 animals including 30 stallions and 35 mares.

Breeding lines. The analysis of biometric and performance parameters in relation to breeding lines was also the subject of the study. That was the basis for investigating the creation or preservation of certain features in longer period, associated with the degree of relatedness characteristic for particular line. During the first years of reserve breeding of Polish Konik in Roztoczański National Park horses belonged to 2 male lines: Wicek and Glejt I, and to 3 female lines – Traszka, Zaza and Urszulka [Sasimowski and Kaproń 1984a]. Then, to counteract the increasing affinities in the herd, the representative of Chochlik family (stallion Palasz) was introduced into reserve breeding and mares out of Tarpanka and Karolka families into the stable breeding. During the research time, horses from three male lines: Chochlik II (29 horses), Glejt I (39 horses), and Wicek I (55 horses) were subjected to the analysis. Out of female lines, mares of Traszka (48 horses), Urszulka (33 horses), Zaza (40 horses), Tarpanka (6 horses) and Karolka (4 horses) were studied.

Data analysis. Collected and analysed data is related to described herds, and involves the period since the moment of their creation (1982 – reserve group, 1996 – stable group) until 2010. The population of tested animals has been subjected to analysis in terms of degree of inbreeding of selected male and female breeding lines. As a measure of the degree of homozygosity the Wright's inbreeding coefficient was used, which is a function of the number and location of the common ancestors in a pedigree [Wright 1978, Sargolzaei et al. 2006]. It takes the value from 0 to 100%, and tells what part of the pairs of genes in an individual stock or population is homozygous above average. The inbreeding index (F) was calculated on the basis of documentation carried in the Maintenance Breeding Centre in Roztoczański National Park and in Polish Konik studbooks kept by the Polish Horse Breeders Association in Warsaw (1990–2010) with the use of OptiMate® software, which uses modified Henderson matrix method. In order to demonstrate the diversity of different breeding lines of horses of Roztoczański National Park, the following analyzed data: three basic biometric measurements, weight, point-scale estimation (the so-called "bonitation" scale) and the results obtained during field performance test (saddle and riding) in reference to degree of inbreeding F [Wright 1978]. The average results of three basic biometric measurements of Polish Konik expressed in centimetres were measured according to the recommendations of Komosa and Frąckowiak [2007]. They referred to the desired traits established as the breed standard, given by Jaworski and Wojciechowska [2013]. The data obtained from the examination of 4-year-old horses were used for the analysis of biometric and body mass measurements. These were:

- the height at the withers, measured with zoometric cane from the highest point of the withers perpendicularly to the ground, which for adult Polish Konik horse (mares and stallions) should be in range of 130 to 140 cm;
- circumference of the chest, measured with the tape from the end point of withers through sternum, along the girth line, minimum value for adult horses is 165 cm;
- circumference of the left fore cannon, measured at the thinnest part at the 1/3 of metacarpal, and for adult horses should be: mares-16.5 cm, stallions-17.5 cm.

Adult Polish Konik horses at the age of 4 were weighed on the weighbridge (500 kg) and their calculated average mass was compared with the standards given by Sasimowski et al. [1991], and were: mares 345–430 kg, stallions 300–420 kg. Mentioned parameters define the typical conformation of animals as well as their usefulness. The point-scale estimation ("bonitation") was also analysed, and involved the assessment of: type, forehead, trunk, forelimbs, hindlimbs, hooves, gaits and overall impression. The analysis was to confirm compliance with the standards of breed and the overall health of the animals.

The analysis of variance ANOVA was used for the statistical comparative analysis and evaluation of particular parameters of Polish Konik horses. The aim was to test the significance of the difference between means by the comparisons of variances. This was accomplished by analyzing the variance, by partitioning the total variance into the component that is due to true random error, i.e. within-group, and the components that are due to differences between means. These latter variance components are then tested for statistical significance and, if significant, we accept that the means in the population are different from each other. The significance of differences among many means of particular breeding parameters of horses originating in selected family groups was tested in terms of characteristic factors that were statistically grouping variables. If some of selected groups did not meet the requirements of the analysis, mostly due to small number of individuals, the mean value and the information that this group was not taken under consideration in the analysis was provided. When it was shown that the means of parameters evaluated for particular group of animals differed significantly from one another, it was concluded that the analysed factor affects the grouping variable. Subsequently, out of the total variance a part was distinguished for which the grouping variable is responsible, and it was compared to the rest of the variance with the use of Fisher test (Ft). The calculation was done with the critical value at the significance level $P \leq 0.05$. The analysis was performed using Statistica (version 10).

RESULTS

The results of the study of population of 257 Polish Konik horses – divided according to the place and way of maintenance and the degree of consanguinity – are presented in Table 1. Due to calculated inbreeding coefficient F, it was possible to arrange the individuals according to the value of the coefficient. Horses were divided into groups, whose limits were set according the generally accepted values [Balloux and Lugon-Moulin 2002]. Three groups of animals were obtained: low related individuals, coefficient $F < 5\%$, medium, $5\% \leq F < 15\%$, and highly related individuals, $15\% \leq F$.

The entire population of Polish Konik horses under study was characterized by the domination of the individuals belonging to group with average value of the F coefficient – 43.6% (112 horses). Highly related individuals stated 19.1% (49 horses). Horses with the average value of F coefficient dominated (41.1% "Ostoja", 47.9% "Florianka"), with slightly lower number of horses with lower values of F coefficient, indicating low degree of inbreeding (33.7% "Ostoja", 43.6% "Florianka"). The smallest group involved closely related horses (19.1%). In the herd from Florianka, where human has much greater impact on the mating of individuals, this the group involved 8.5%. The value was much lower than in

Table 1. Inbreeding structure of Polish Konik horses in reserve and sable breeding, and the values of sudied biometric and performance parameters

Tabela 1. Struktura koników polskich w hodowli rezerwatowej i stajennej oraz wielkości badanych parametrów biometrycznych i użytkowych

Groups divided according to Wright's coefficcient (F) Grupa wydzielona w zależności współczynnika Wrighta (F)	I F < 5%	II 5% ≤ F < 15%	III F ≥ 15%
Number of Polish Konik horses Liczba koni rasy Konik polski	96 (37.4%)	112 (43.6%)	49 (19.1%)
Herd in reserve breeding in "Ostoja" Stado w hodowli rezerwatowej „Ostoja"	55 (33.7%)	67 (41.1%)	41 (25.2%)
Herd in stable breeding in "Florianka" Stado w hodowli Stajennej „Florianka"	41 (43.6%)	45 (47.9%)	8 (8.5%)
Height at the withers, cm ±SE Wysokość w kłębie, cm ±SE	135.1 ±3.6	134.2 ±3.3	135.1±3.8
*) Chest circumference, cm ±SE *) Obwód klatki piersiowej, cm ±SE	169.8 ±7.0	167.3 ±8.5	165.8 ±6.8
Cannon circumference, cm ±SE Obwód nadpęcia, cm ±SE	18.1 ±0.9	18.1 ±0.8	18.0 ±1.2
Body mass, kg ±SE Masa ciała, kg ±SE	360.5 ±22.7	356.7 ±16.5	367.9 ±13.9
**) "Bonitation", pts ±SE **) Ocena bonitacyjna, pkt ±SE	78.9 ±1.5	79.1 ±1.6	78.4 ±1.5
Field under-saddle performace test, pts ±SE Polowa próba wierzchowa, pkt ±SE	37 ±1.0	34 ±2.1	31 ±5.7
Field driving performance test, pts ±SE Polowa próba zaprzęgowa, pkt ±SE	36 ±2.0	35 ±2.2	36 ±1.4
Triming evaluation, pts ±SE Ocena przy rozczyszczaniu, pkt ±SE	4.2 ±0.9	3.8 ±0.9	3.6 ±0.9

*) Chest circumference. ANOVA Current effect: Ft = 4.30, P = 0.040 for groups I + II and III
**) Confirmation. ANOVA Current effect: Ft = 4.17, P = 0.043 for groups I + II and III
*) Obwód klatki piersiowej. ANOVA Obecny efekt: Ft = 4,30, P = 0,040 dla grupy łącznie I + II i III
**) Ocena bonitacyjna. ANOVA Obecny efekt: Ft = 4,17, P = 0,043 dla grupy łącznie I + II i III

case of herd from "Ostoja" (25.2% – 41 horses), where individuals mate in a manner similar to the natural, maintaining relations in the herds characteristic to wild living primitive horses.

Using ANOVA for the analysis of biometric and performance parameters of Polish Konik horses in relation to the increase of homozygosity of individuals, in most cases it did not indicate any statistically significant differences between values of the parameters and the diversification of the degree of inbreedings (Table 1). This also concerns the field under-saddle performance test which the mean decreases with the increase of homozygosity, but simultaneously increases the distribution of the parameter expressed with standard error, that did not allow to demonstrate the statistical significance. The increase in diversity in the results of field under-saddle performance test proves the change in the homogeneity of

selected horse groups in terms of this parameter with the increase of inbreeding level.

In case of assessment of horses' chest circumference parameter, no significant differences among three groups with differential Wright's coefficient were shown. The critical values in ANOVA, in this case were below threshold of significance ($Ft = 2.2$, $P = 0.093$). However, the data analysis in which two groups of horses with low and medium inbreeding levels were combined and compared to parameters of the third group with higher inbreeding coefficient F, showed the statistical significance $Ft = 4.30$, $P = 0.040$ of the degree of relatedness on the chest circumference (Table 1). It can be concluded that only for individuals with certain degree of relatedness this parameter changes (deteriorates). The inbreeding limit value from which the parameter connected with decrease of chest circumference begins to deteriorate was identified as $F = 15\%$. Similar relationship occurred during the analysis of the "bonitation". With the use of ANOVA, the association ($Ft = 4.17$, $P = 0.043$) of deterioration of the "bonitation" and the value $F \geq 15\%$ was shown.

The breeding lines. Population of Polish Konik horses from Roztocze was subjected to the analysis in terms of degree of inbreeding in relation to male and female breeding lines. Table 2 presents inbreeding structure for particular breeding lines in the studied time period. Stallions of Chochlik line were characterized by the dominance of number of individuals with the lowest degree of inbreeding (20 horses which is 69.0% for $F < 5\%$). For horses of Glejt I line, the dominance of middle values of inbreeding is characteristic (56.4% for $15\% > F \geq 5\%$). Horses of Wicek line have the highest percentage of animals classified as the third group with the highest values of inbreeding (17 horses – 30.9% for $F \geq 15\%$). At the same time, there is a uniform distribution of individuals belonging to the groups with a low and middle value of F coefficient, it is almost 60% of animals of this breeding line. There is no such diversification in the number of individuals in all three groups distinguished on the basis of inbreeding coefficient as it was observed in previously analyzed lines: Chochlik and Glej I. Performed analyses of mare breeding families involved three representative groups belonging to sufficiently numerous ones in Polish breeding [Jaworski 1997]: Traszka, Urszulka and Zaza. There was too small number of representatives of lines: Tarpanka and Karolka, to obtain reliable results of the analysis (Table 2). Families: Urszulka and Zaza were characterized by the dominance of middle inbreeding values, and the population with such value includes more than half of the animals of these lines. The structure of the inbreeding is similar to male line Glejt I. The line Traszka consists of individuals with low and middle inbreeding values. The percentage of horses with low inbreeding values was the highest among three analyzed female lines and was 39.6% (19 horses), but not predominant as in male Chochlik

line. Some individuals from lines Tarpanka and Karolka were also subjected to the analysis, however due to small number, the obtained results could not be used as a characteristic of particular breeding line. The results of analysed parameters: biometric measurements, body mass and point-scale estimation ("bonitation") of particular breeding lines were referred to the inbreeding structure of individuals, descended from the same ancestors and are presented in Figure 1. The results obtained determine the relationship between the structure of inbreeding (Table 2) of analyzed breeding lines and parameters achieved by individuals belonging to particular breeding line. Regularities arising out of the comparison of the two groups of results can confirm or reject in the further analysis the thesis of negative impact of homozygosity on the further development of the primitive breed – Polish Konik.

Table 2. Degree of inbreeding of Polish Konik horses of male and female breeding lines in Roztoczański National Park

Tabela 2. Stopień inbredu koników polskich w zależności od męskiej i żeńskiej linii hodowlanej w Roztoczańskim Parku Narodowym

Groups divided according to Wright's coefficcient (*F*) Grupa wydzielona w zależności współczynnika Wrighta (*F*)		I	II	III	Mean inbreeding value of given line Średnia inbredu dla danej linii
		$F < 5\%$	$5\% \leq F < 15\%$	$F \geq 15\%$	
	Chochlik	20 (69.0%)	7 (24.1%)	2 (6.9%)	5.4
A	Glejt I	11 (28.2%)	22 (56.4%)	6 (15.4%)	8.8
	Wicek	22 (40.0%)	16 (29.1%)	17 (30.9%)	9.1
	Traszka	19 (39.6%)	19 (39.6%)	10 (20.8%)	9.3
	Urszulka	8 (24.2%)	17 (51.5%)	8 (24.2%)	11.4
B	Zaza	8 (20.0%)	26 (65.0%)	6 (15.0%)	10.2
	Tarpanka [1)]	3 (50.0%)	3 (50.0%)	–	6.6
	Karolka [1)]	3 (75.0%)	1 (25.0%)	–	4.2

A – Number of horses subjected to the study of male breeding lines.
A – Liczba koni objęta badaniem z męskich linii hodowlanych.
B – Number of horses subjected to the study of female breeding lines
B – Liczba koni objęta badaniem z żeńskich linii hodowlanych
[1)] Small number of horses, that does not allow to use the results as reliable reference characterizing particular breeding line.
[1)] Mała liczba badanych koni, która nie pozwala na wiarygodne odniesienie ich, jako charakteryzujących daną linię hodowlaną.

In conducted studies the comparison of parameters achieved by individuals from male breeding line versus female was made, for horses from two herds jointly (Fig. 2). This comparison, referring to the mean values of the morphological traits (biometric and body mass) and the evaluation of the point-scale estimation ("bonitation") was necessary to minimize the influence of natural differences between parameters achieved by stallions and mares, on the final conclusions.

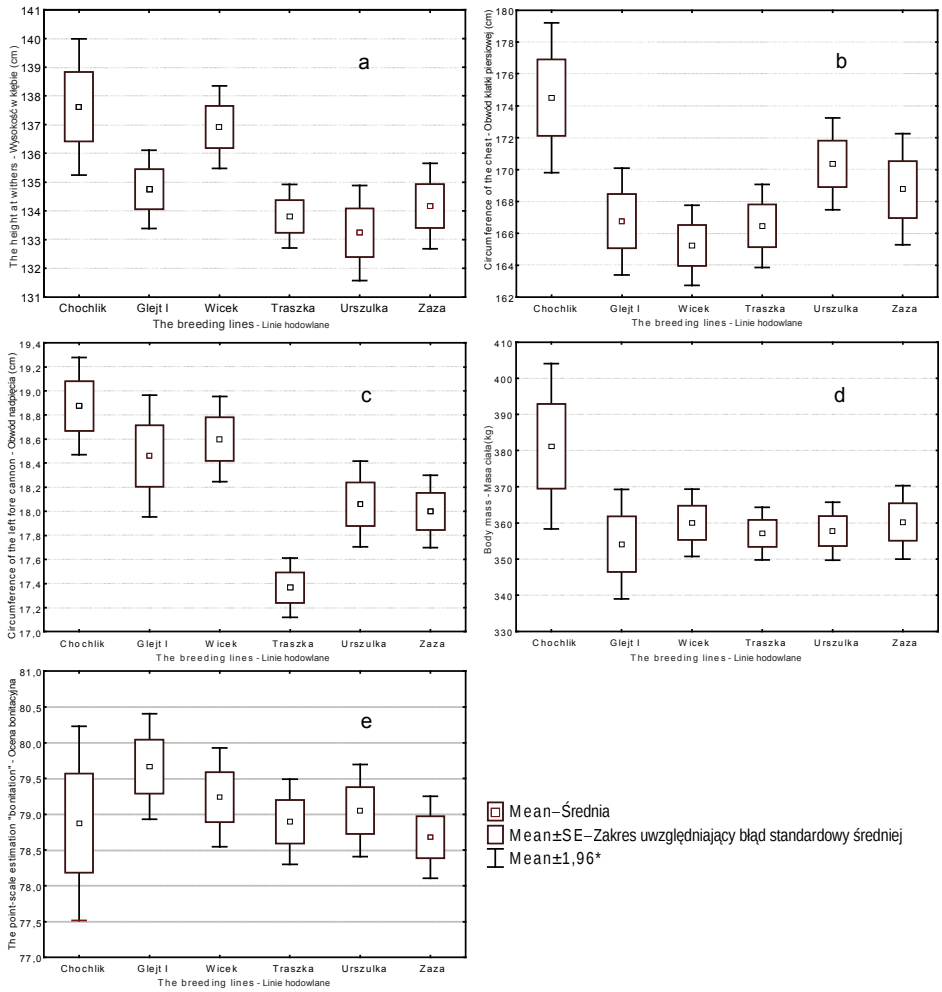

Fig. 1. Comparison of parameters of Polish Konik horses in relation to breeding lines

Rys. 1. Porównanie osiąganych parametrów koni rasy konik polski w zależności od linii hodowlanej

ANOVA results show the statistical significance of differences of group means in relation to the parameter of evaluation that is the height at withers (Fig. 1a) of Polish Konik horses of particular breeding lines ($Ft = 4.50$, $P = 0.0009$). The greater diversification of this parameter was observed ($Ft = 16.96$, $P = 0.00007$) with the division on male and female lines (Fig. 2a). It can be concluded that this parameter is strongly related to sex and less of inbreeding. Analyzing the male lines, the relationship between the favourable impact of inbreeding on the discussed trait, characteristic for Chochlik and Wicek and less favourable for Glejt

I, can be noted (Fig. 1a). The means of measurements of the height at the withers were higher and proportional to the number of individuals in the group with the lowest degree of inbreeding in the case of male lines (Table 2).

The difference was statistically significant on the probability level $P = 0.04$ between Chochlik and Glejt I lines. For mares and their lines these indicators were aligned for this parameter (Fig. 1a). Analyzing the chest circumferences the statistically significant difference for his parameter was not shown in rela-

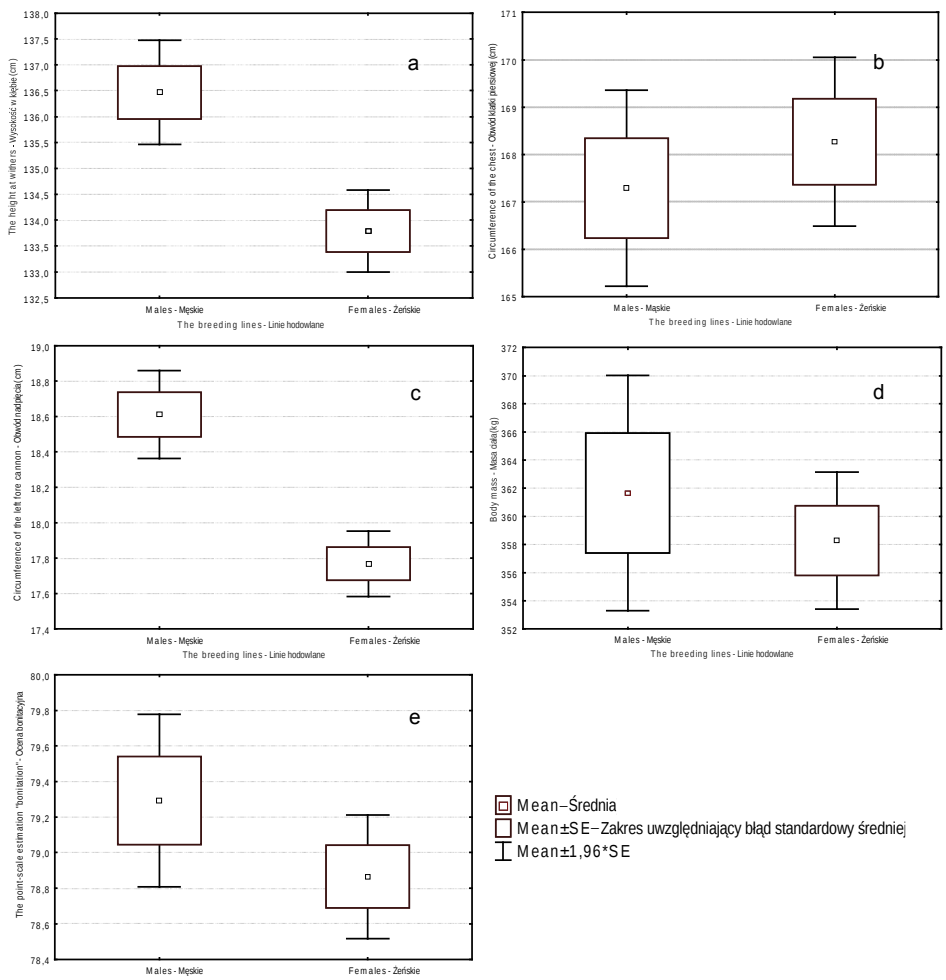

Fig. 2. Breeding parameters of Polish Konik horses of male and female lines

Rys. 2. Osiągane parametry hodowlane koni rasy konik polski dla linii męskich i żeńskich

tion to the sex. The ANOVA value for the stallions and female comparison was Ft = 0.48 P = 0.49. When comparing individual lines and inbreeding structure the influence was significant (Ft = 2.93, P = 0.0162). The highest values were achieved by stallions from the Chochlik line, and lower by mares from Urszulka and Zaza families. However, a clear regularity in comparison of this trait with the inbreeding structure cannot be seen. Analyzing much higher values of the chest circumference for stallions from Chochlik line in relation to the rest of the horses it was concluded that they correspond to vast majority of individuals classified to the group with the lowest inbreeding coefficient (69.0%). At the same time male line Wicek I, with large percentage (40.0%) of individuals in group with F $<$ 5%, but also with the greatest proportion of individuals (20.8%) in group F $\geq$ 15%, in relation to other breeding lines, was characterized by the lowest values of the analyzed parameter.

The situation was different in case of the female lines. Higher, more favourable values were achieved by horses from the lines in which the inbreeding structure was symmetrical, and most individuals belonged to the second group. In case of cannon circumference measurements, very strong influence of sex on this parameter was demonstrated (Fig. 1c). ANOVA values were Ft = 29.76 and P $<$ 0.00001. The effect associated with the structure of inbreeding was Ft = 9.07 at P $<$ 0.00001. The results of both analyses were associated with distinctively low values of cannon circumference for mares from Traszka family in comparison to the horses from other lines. Higher values of cannon circumference were also observed for stallions from Chochlik line. The other lines did not show significant diversity of the parameter values, with exclusion of sex impact. Thus, the influence on the increase of cannon circumference in male lines can be associated with the population structure where there is the prevalence of individuals with inbreeding coefficient F $<$ 5%. At the same time results achieved by mares from Traszka line can indicate other factors, not related to higher homozygosity. Another parameter analyzed in relation to relatedness structure was body mass. The impact of the sex was not observed (Ft = 0.53, P = 0.47). The occurrence of individuals with higher body mass was observed only for horses of Chochlik line (Fig. 1d). Considering all the breeding lines, statistically significant difference of the parameter was not observed (Ft = 1.56, P = 0.182). The same situation was in case of analysis of the assessment by point-scale estimation ("bonitation") (Ft = 0.79, P = 0.56), where only for horses of Chochlik line the higher distribution of the body morphology score was observed (Fig. 1e). This means that significantly more individuals of this family got lower score than representatives of other breeding lines.

DISCUSSION

Composition of genes inherited from parents is responsible for the appropriate set and feature values. Some authors pointed out that certain features and congenital abnormalities are caused by recessive genes, mostly manifesting themselves when closely related individuals are cross-mating [Wolc and Balińska 2010]. If the breeding is carried out in an uncontrolled manner, inbreeding depression can occur [Madsen et al. 1996, Jackowski et al. 2004]. Maintaining the breed in good health and performance condition requires mating properly selected breeding material, monitored in terms of value of inbreeding coefficient F [Górecka and Jezierski 1997, Polak 2012, Maćkowski et al. 2015]. This allows to avoid meeting the same alleles, which can be a source of immunocompromised individuals or congenital abnormalities. Additionally, it generates higher polymorphisms in the population, and allows increase of the population size, when compared to the groups with greater homozygosity. This results in a greater resistance to change of the frequency of alleles, opposing accidental genetic drift [Wright 1978, Madsen et al. 1996]. However, as reported by some authors, in naturally closed populations or population mating with a special aim, this leads to a fixation of certain characteristics, which in some cases is not a negative phenomenon [Pikuła and Nogaj 2004, Avdi and Banos 2008]. Jezierski and Jaworski [1999] in their study reported that in the population of Polish Konik horses maintained in reserve system in another region than that of our study (town Popielno), there is a kind of behavioural mechanism of reducing the formation of increased inbreeding. Other authors have noted [Provine 2004] that greater genetic diversity prevents the fixation of alleles, which is privileged under the influence of local conditions and allows the population to adapt to the specific environment (e.g. National parks, geographically limited areas e.g. islands, lakes, stable breeding) and speciation processes are hampered. Most of domesticated breeds were created in this manner [Bjørnstad et al. 2000, Polak 2012]. Humans mated the individuals due to some positive performance features, preferring certain characteristics. With a view to the Mendel's laws, it can be indicated that in small and closed population the self-purification of recessive genes occurs. Currently, Polish Konik horses maintained in reserve breeding "Ostoja" in Zwierzyniec and the stable breeding in Florianka (Table 1) are characterized by a predominance of mid-related individuals (inbreeding between 5% and 15%), followed by individuals with low F coefficient (less than 5%). Horses with a high degree of consanguinity (inbreeding above 15%), are in particular the individuals from reserve breeding herd "Ostoja" in Zwierzyniec (25.2%) compared to 8.5% in "Florianka". This can lead to a frequent disclosure of the undesirable recessive genes. A negative consequence of such increased homozygosity can be the presence of inbreeding depression, which may result in a decrease of fertility, reduced vitality, resistance to disease and phenotype delicacy (a reduction in

size and mass, weaker skeleton), increased susceptibility to adverse environmental conditions and a general weakening of psychological resistance [Madsen et al. 1996, Sargolzaei et al. 2006]. These are very unfavourable factors especially in reserve breeding, where environmental conditions may vary depending on the season. During the conducted study the statistically significant effect of the degree of consanguinity on chest circumference for horses with higher inbreeding degree $F \geq 15\%$ was shown. In Polish Konik horses with higher homozygosity the decrease in chest circumference may occur. Such a relationship has already been noted in some works, such as for Haflinger breed in Italy [Gandini et al. 1992]. The decrease in chest size in reference to the breed standard, suggests a reduction in lung capacity and worse parameters associated with respiration. In a similar manner the relationship between the evaluation of horses' point-scale estimation ("bonitation") parameters and higher value of inbreeding coefficient was indentified. Since the evaluation is associated with the conformation and horses' morphology, it can be suspected that for higher values of homozygosity, these features will reveal and can have the negative impact on the particular characteristics of the breed. Taking into account the results, this can indicate that the value of $F = 15\%$ is in fact a limit, from which the negative impact of homozygosity on the traits associated with the phenotype of primitive horse breed, that is the Polish Konik starts to increase.

In the case of evaluation of field under-saddle and driving performance test the ANOVA did not show that statistically significant means of the parameter were decreasing with the increase of homozygosity. However, proportional increase of the diversification expressed metrically via standard error in terms of field performance test, showed the presence of individuals with extremely different evaluation results (Table 1). Taking into consideration that the field tests are influenced by many factors: stamina, heart rate, assessment of the horse behaviour during saddle, getting on the horse, movement in walk and trot on 80 m distance, it can reflect the occurrence of phenomena associated with inbreeding depression.

Studied population of Polish Konik horses was evaluated in terms of the degree of inbreeding in selected male and female lines (Table 2). The evaluation allowed to detect the relationships connected with long term impact of the degree of inbreeding. It could have been done, because it was possible to determine the relatedness structure of individuals derived from a few common ancestors, and then separated into breeding lines - groups. Such groups were analyzed for parameters that were defining the relevant characteristics of the particular lines depicted in Figure 1. Analyzing the data based on the thesis drawn by geneticists, it should be expected that in some lines there will be the favourable development or maintenance of relevant characteristics [Sargolzaei et al. 2006]. Where the structure is unfavourable it was possible to indentify a set of characteristics on which the

increased homozygosity has a negative impact. On the basis of settled relatedness structure described with Wright's coefficient, horses of Chochlik family, due to prevalence of individuals with a low degree of relatedness, should be the least genetically impacted by the increase of homozygosity. This relationship was confirmed in this study. It should be also stated that this line is not the most numerous or not most quickly developing as families: Wicek and Glejt I [Jaworski 1997]. Most of the evaluated parameters: the height at the withers, chest and cannon circumference, body mass of horses of this line were significantly higher than others (Fig. 1a–d). In the case of the female lines, the families characterized by the highest homozygosity were: Urszulka and Zaza, due to the middle-values of inbreeding. The individuals (with middle-values of inbreeding) comprised more than half of the animals in these lines (Table 2). It can be stated that due to negative effects caused by the increase of homozygosity, female lines Urszulka and Zaza will be similar to each other and horses of these groups will have similar parameters, which may be affected by inbreeding depression. A group of horses of Traszka line should have less risk of transmitting such negative effects, but not as minimized as a group of horses from the male Chochlik line. In this case, the results confirmed that the parameters characterizing the families Urszulka and Zaza will be similar, but they are better than the parameters achieved by the horses of Traszka line. In particular, such a link was observed for the evaluation of chest and cannon circumferences (Fig. 1b, c). Comparing the results of all means of zoometric parameters and body mass for Polish Konik horses bred in Roztoczański Polish National Park (Table 1, Figs. 1, 2), with the standards typical for this breed [Jaworski and Wojciechowska 2013], it should be emphasized that they correspond to the optimal requirements and are within the specified breeding limits. However, it should be noted that the differences between means of zoometric values for horses from Zwierzyniec breeding and others, for example from Popielno or other Polish regions indicate the positive effect of carrying out of, in a sense, isolated breeding [Kownacki 1995, Jackowski et al. 2004, Komosa and Frąckowiak 2007]. Such in-breed variability of traits, and genetic diversity, prevents the increase in inbreeding of Polish Konik population [Wolc and Balińska 2010]. The best confirmation results were achieved by horses of male breeding line Glejt I. The worst were achieved by: Zaza, Traszka, Chochlik (Fig. 1e). Analyzing the charts of mean values with marked standard error intervals (Fig. 1) which were obtained in ANOVA, it can be noticed that in the group of horses of Chochlik line there is the greatest dispersion of point-scale estimation ("bonitation") results. This shows the greatest diversity of evaluations for this group. Taking into account also the fact that this line is characterized by the largest majority of individuals with low inbreeding values, it can be stated that high homozygosity does not strongly affect traits evaluated during point-scale estimation ("bonitation"). This is also confirmed by the results of

point-scale estimation ("bonitation") of horses of female breeding line Traszka. It means that, the heritage of unfavourable traits connected with high homozygosity is more (or strongly) related to other characteristics, than these evaluated during conformation – type, structure and body proportions, movement.

CONCLUSIONS

In summary, studies of Polish Konik horses confirmed the negative effect of the increased level of inbreeding to only some analyzed parameters, however this is typical for individuals with higher values of Wright's coefficient. It has been identified that the limit value expressed with Wright coefficient is F = 15%. As shown in the analysis of breeding lines, heterozygosity is more privileged in the long term in order to maintain the appropriate breed traits associated with the body structure increased.

In the studied breeding this condition is possible to be fulfilled, regarding the following principles: planned mating of the least related individuals from own breeding, monitoring of the inbreeding occurrence and, if possible co-operation with other major breeding centres. This cooperation should focus on sharing and exchanging of the mainly male genetic material with regard to the individual breeding lines. Appropriate strategies should be adopted both for the reserve and stable breeding of Polish Konik horses.

REFERENCES

Avdi, M., Banos, G. (2008). Genetic diversity and inbreeding in the Greek skyros horse. Livest. Sci., 114, 362–365.

Balloux, F., Lugon-Moulin, N. (2002). The estimation of population differentiation with microsatellite markers. Mol. Ecol., 11, 155–165.

Bjørnstad, G., Gunby, E., Røed, K.H. (2000). Genetic structure of Norwegian horse breeds., J. Anim. Breed. Genet. 117, 307–317.

FAO (2007). The state of the world's. Animal genetic resources for food and agriculture. Commission on genetic resources for food and agriculture food and agriculture organization of the united nations, Rome, 2007, 512.

Gandini, G.C., Bagnato, A., Miglior, F., Pagnacco, G. (1992). Inbreeding in the Italian Haflinger horse., J. Anim. Breed Genet., 109, 433–443.

Górecka, A., Jezierski, T. (1997). Analiza wskaźników użytkowości rozpłodowej klaczy konika polskiego w latach 1956–1995 [An analysis of the reproduction performance of mares of the Polish primitive horse between 1956–1995]. Pr. Mater. Zootech., 51, 21–29 [in Polish].

Jackowski, M., Czaja, J., Długosz, B., Łuszczyński, J., Pieszka, M. (2004). Ocena inbredu i spokrewnienia ze sobą ogierów i klaczy huculskich hodowanych w wybranych rejonach Polski [The estimation of inbreeding and relationship of stallions and mares

of the Hucul horses bred at some regions of Poland]. Zesz. Nauk. Prz. Hod., 72(5), 45–48 [in Polish].

Jaworski, Z. 1997. Tablice genealogiczne koników polskich [Genealogical Tables of the Polish Primitive Horse]. Popielno [in Polish].

Jaworski, Z., Wojciechowska, P. (2013). Nowy Program hodowli zachowawczej koników polskich [New program of Polish Konik horses preservation breeding]. Hod. Jeź., PZHK, 2(37), 36–38 [in Polish].

Jezierski, T., Jaworski, Z. (1999). Czy istnieje spontaniczny mechanizm redukujący inbred w hodowli rezerwatowej koników polskich? [Does a spontaneous mechanism reducing inbreeding in free-roaming harems of Konik horses exist?] Mat. Symp. Międz. Aktualne kierunki hodowli i użytkowania koni w Europie. Kraków, 17–19 września 1999, 112–118 [in Polish].

Komosa, M., Frąckowiak, H. (2007). Zróżnicowanie morfologiczne koników polskich – – analizy wielowymiarowe [Morphological differentiation of Konik horses – multivariate analyses]. Acta Sci. Pol., Zootechnica, 6(4), 45–58 [in Polish].

Kownacki, M. (1995). Pochodzenie, historia i hodowla koników polskich [The origin, history and breeding of the primitive Polish horse]. Mat. konf. nauk.: ‚Biologia i hodowla zachowawcza konika polskiego", 18–19 maja Popielno, 5–12 [in Polish].

Księga Stadna Koni Rasy Konik Polski [Studbook of Origin of Konik Polski Breed]. PZHK Warszawa, Tom VI (1990), VII (1994), VIII (2001), IX (2010) [in Polish].

Lunch, M., Lande, R. (1993). Evolution and extinction in response to environmental change. [In:], P.M. Kareiva, J.G. Kingsolver, R.B. Huey (Eds.), Biotic Interactions and Global Change. Sinauer Associates, Sunderland, M.A., 234–250.

Maćkowski, M., Mucha, S., Cholewiński, G., Cieslak, J. (2015). Genetic diversity in Hucul and Polish primitive horse breeds. Arch. Anim. Breed., 58, 23–31.

Madsen, T., Stille, B., Shine, R. (1996). Inbreeding depression in an isolated population of adders *Vipera berus*. Biol. Conserv., 75, 113–118.

Pikuła, R., Nogaj, A. (2004). Charakterystyka markerów genetycznych populacji koni w zależności od ich przynależności do tzw. linii sportowych [Characteristics of genetic markers of horses' population, depending on their sport line status]. Zesz. Nauk. Prz. Hod. 72(5), 39–43 [in Polish].

Pluta, M., Osiński, Z. (2014). Variability of heart rate in primitive horses and their relatives as an indicator of stress level, behavioural conduct towards humans and adaptation to living in wild., Bull. Vet. Inst. Pulawy, 58, 495–501.

Polak, G. (2012). Problemy związane z prowadzeniem programów ochrony zasobów genetycznych koni w typie sokólskim i sztumskim w latach 2008–2011 [Problems in the implementation of genetic resources conservation programmes for Sokólski and Sztumski horses in 2008–2011]. Rocz. Nauk. Zootech., 39(1), 47–59 [in Polish].

Provine, W.B. (2004). Ernst Mayr: Genetics and speciation. Genetics, 167(3), 1041–1046.

Sargolzaei, M., Iwaisaki, H., Colleau, J.J. (2006). CFC: A tool for monitoring genetic diversity. Proc. 8th World Congr. Genet. Appl. Livest. Prod., CD-ROM Communication 27–28. Belo Horizonte, Brazil, Aug. 13–18.

Sasimowski, E., Kaproń, M. (1984a). Badania nad klimatyzacją, hodowlą i zachowaniem się koników polskich w Roztoczańskim Parku Narodowym. Część III. Projekt założeń hodowlanych [Studies on Acclimatization, Breeding and Behaviour of the Polish

Konik in the Roztocze National Park. Part III. A Project of Breeding Plans]. Ann. UMCS, Sectio EE, Vol. II, 25, 231–242 [in Polish].

Sasimowski, E., Kaproń, M. (1984b). Badania nad klimatyzacją, hodowlą i zachowaniem się koników polskich w Roztoczańskim Parku Narodowym. Część I. Zootechniczna charakterystyka wyjściowej grupy koników [Studies on Acclimatization, Breeding and Behaviour of the Polish Konik in the Roztocze National Park. Part I. Zootechnical Characteristics of the Initial Group of Koniks]. Ann. UMCS, Sectio EE, Vol. II, 23, 197–213 [in Polish].

Sasimowski, E., Pietrzak, S., Kolstrung, R., Jaworski, Z., Słomiany, J., Pluta, M. (1991). Współczynniki do określania masy ciała koników polskich na podstawie pomiarów [The Coefficients Used to Determine the Body Mass of Polish Koniks on the Basis of the Measurements]. Ann. UMCS, Sectio EE, R.B. Vol. IX, 19, 143–149 [in Polish].

Vila, C., Leonard, J.A., Beja-Pereira, A. (2006). Genetic documentation of horse and donkey domestication. California, USA, University of California Press, 342–353.

Wlizło, B., Szwed, W. (2007). Roślinność Ostoi Konika Polskiego w Roztoczańskim Parku Narodowym [Vegetation of Polish Konik Horse reserve system in Roztoczański National Park]. Pr. Kat. Przyrodniczych Podstaw Leśnictwa AR Poznań, nr 2 [in Polish].

Wolc, A., Balińska, K. (2010). Inbreeding effects on exterior traits in Polish konik horses. Arch. Tierzucht, 53, 1–8.

Wright, S. (1978). Evolution and the Genetics of Population, Variability Within and Among Natural Populations. The University of Chicago Press, Chicago.

CHARAKTERYSTYKA GENETYCZNA I FENOTYPOWA KONIKÓW POLSKICH UTRZYMYWANYCH W SYSTEMIE REZERWATOWYM I STAJENNYM W ŚRODKOWO-WSCHODNIEJ POLSCE

Streszczenie. W pracy określono wpływ zróżnicowania genetycznego na właściwości adaptacyjne koników polskich do życia w naturalnym środowisku podobnie jak konie prymitywne. Wybrane właściwości zostały ocenione w odniesieniu do wybranych linii hodowlanych jako wskaźnik postępu hodowlanego podczas długiego okresu. Dodatkowo, wykonano ocenę interakcji między środowiskowymi i genetycznymi czynnikami. Badaniami objęto 257 koników polskich (dwa stada) żyjące w ośrodku hodowlanym, w którym zostały zapewnione warunki zbliżone do naturalnych dla populacji koni prymitywnych pierwotnie zamieszkujących Europę. Podczas przeprowadzonych badań wykazano statystycznie istotne zależności pomiędzy stopniem inbredowania, a osiąganym przez koniki obwodem klatki piersiowej oraz większym zróżnicowaniem wyników w skali bonitacji dla poszczególnych osobników o wyższym niż 15% współczynniku inbredu. Wskazano, że wartość F = 15% jest w istocie wartością graniczną, powyżej której uwidacznia się negatywny wpływ homozygotyczności na cechy związane z fenotypem rasy prymitywnego konika polskiego. Jak pokazała analiza linii hodowlanych, w dłuższym czasie dla utrzymania odpowiednich cech rasy związanych z budową ciała, korzystniejsza jest struktura jak najbardziej heterozygotyczna.

Słowa kluczowe: rodowy chów wsobny, wpływ homozygotyczności, konie prymitywne, konik polski

Accepted for print: 03.06.2016

For citation: Pluta, M., Osiński, Z., Cieśla, A., Kolstrung, R. (2016). Genetic and phenotypic characteristics of Polish Konik horses maintained in the reserve and stable system in Central-Eastern Poland. Acta Sci. Pol. Zootechnica, 15(2), 59–76.
DOI: 10.21005/asp.2016.15.2.06.