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COLOUR VISION IN PIGS AND POULTRY*

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The organism’s perception of colours in the surrounding environment is not completely understood and the mechanism of colour perception has been explained by various scientific theories, which are not fully confirmed. Colour perception is best understood in humans, where visual effects can be expressed verbally. Studies on the biophysical and biochemical processes connected with eyesight function in animals are still at the development stage. However, behavioural studies are needed to gain a complete understanding, because the process itself has a mental dimension. Attempts are being made in this area to obtain a behavioural response to colour stimuli from the environment.

Given the findings of several colour vision specialists, it must be assumed that poultry and pigs not only perceive colour stimuli from the environment but are also affected by these stimuli in terms of behaviour, production results and some physiological indicators of the body.

Characteristics of vision

Vision is a combined analysis of amplitude, wavelength and the distribution of light sources (original and secondary, reflecting light sources). Thanks to the sense of sight, animals and humans can distinguish shapes, magnitudes, distances and colours. However, not all animals can distinguish colours, because the eyes are not equally developed in all vertebrates, with some differences possible even within the same species. Vision is classified into monochromatic, dichromatic, trichromatic

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and even tetrachromatic (Death, 1998; Fleishman et al., 1998), which enables ultraviolet light to be perceived (Lewis and Morris, 1998). Colour vision can be directly associated with the perception of movement (Derrington, 2000). It must be remembered, however, that vision is a parallel psychophysical process (Zeki and Bartels, 1998).

It is known that the sense of sight is less developed in mammals than in birds. In mammals, colour vision is oriented towards red and green, with certain shifts (10-30 nm) in individual species (Yokoyama and Radlwimmer, 1998). Evolutionary studies have shown that it was not until the development of the colour vision mechanism that some vertebrates could adopt a predatory lifestyle (Maximov, 2000).

It is worth recalling that colour vision in humans and primates is enabled by three types of cone (trichromatic vision) with different radiation absorption maxima. Thanks to the cone pigments — erythrolabe, chlorolabe and cyanolabe — they selectively capture quanta of red, green and blue light (Załucki, 1995). The image of other spectral colours is formed by a simultaneous stimulation of all cone types. These pigments allow vision in the range of 380 – 780 nm, with the highest efficiency at 550 – 560 nm. It is noteworthy that the UV band is not perceived by the human eye.

Domestic birds have very good eyesight and in good light, can see things that are far away. Birds have large and well-developed eye lobes in the brain, and proportionately, their eyes occupy more space in the head than in mammals. Unlike in mammals, birds’ eyes are not spherical and their shape varies according to species. The functional organization of a bird’s retina is generally the same as in all vertebrates. In nocturnal birds and in some mammals, the retina only contains rods. In diurnal birds, including poultry, cones are more numerous than rods (Załucki, 1995). Rods contain the visual pigment rhodopsin. The absorbance maximum of rhodopsin is 510 nm in hens and 497 nm in humans. The photosensitive pigment of the cone cells is called iodopsin. In hens, the absorbability of iodopsin peaks at 562 nm. In general, the full spectrum perceived by the avian eye ranges from 320 to 700 nm (Maddocks et al., 2002 b).

As early as 1921, Honigmann showed that the maximum sensitivity of the eye is 560 nm in hens and 580 nm in mature birds. Still earlier, Watson (1915) and Lashley (1916) accurately found that the visible spectrum in chickens ranges from 700 – 715 to 395 – 405 nm. Załucki (1995) holds that birds’ mechanism of colour distinction and ability to distinguish colours are probably the same as in humans, but not all birds can distinguish red from green. Osorio et al. (1999) stated that the mechanism of colour vision in poultry is based on 4 types of single cones, which allow them to perceive UV light. This is called tetrachromatic vision. According to their theory, colour perception is controlled by 3 different comparative mechanisms. The authors found the presence of a fifth cone type (double cone) enabling the detection of movement. These findings are supported by Bowmaker (1991) and Jones et al. (2001 a), who pointed to an additional mechanism of interpolation between primary colours. It is therefore concluded that in birds, colours are
categorized in a similar way as in humans. A different opinion was expressed by Lewis and Morris (2000), although they admit that human and avian eyes are most sensitive to a similar range of light (545–575 nm). However, avian eyes are more sensitive to the blue and red part of the spectrum. According to Nuboer et al. (1992), wavelength absorption in domestic poultry peaks at 525–575 nm and 350–375 nm. On the other hand, Bennett and Cuthill (1994) suggest that birds are more sensitive to UV waves than to the band seen by humans. For example, pigeons have five types of cone with a maximum wave absorption of 370 nm (Emmerton and Delius, 1980; Palacios and Varela, 1992).

Like most vertebrates, pigs are capable of dichromatic vision. The maximum absorption for two cone types is 439 nm and 556 nm (Neitz and Jacobs, 1989). This is indicative of ametropia and the adaptation to both nocturnal and diurnal activity. Pigs therefore have a limited ability to distinguish spectrally similar colours, e.g. green from blue. For contrast, it is worth noting the well-developed sense of hearing and smell in pigs; thus, their sense of sight is weaker. In addition, laterally located, small eyes with poor muscling do not allow for good zooming, although they ensure a relatively wide angle of vision.

The importance of colour

Many studies and experiments have been conducted to investigate the effect of colour and light type on production and physiological parameters in poultry, but the results are still inconclusive (Herbut and Pietras, 1993; Hulet et al., 1992). The first studies on the behaviour of birds with regard to colour vision were conducted by Hess in 1912, who illuminated grain sprinkled on a floor with six colours of the visible spectrum. He found that hens consumed grain illuminated by red, yellow and green light, but not grain illuminated by blue or violet light. According to Vehse and Ellendorff (2000), white light accelerates the sexual maturity of birds and blue light delays it. Lewis and Morris (1998) hold the view that in poultry rearing, light intensity is more important than light type or colour. Gwara et al. (1997) found an increased level of T3 in the blood serum of cockerels kept in red or yellow light. Gwara et al. (1999) reported a favourable effect of red or green light on the body weight, feed conversion, and health of broiler chickens.

Many studies have reported that light colour has no effect on sexual maturity or laying performance (Felts et al., 1990; Hulet et al., 1992). According to Wathes et al. (1982), a blue (425 nm), green (525 nm) or red light colour (610 nm) has no effect on the body weight or feed intake of broiler chickens. Likewise, Proudfoot and Hulan (1987) found no differences in the body weight, feed intake or mortality of chickens kept in a white or pink light (640–650 nm).

Herbut (1988) reported that white protective clothing, followed by blue and claret protective clothing, makes birds the most timid. According to the same author, the reaction to colour has an indirect effect on feed conversion and bird mortality. Lewis and Morris (2000) reported that the growth of hens and turkeys in red light is poorer than in blue or green light. This can result from the fact that birds kept in red light are more active and aggressive than birds kept in light at a lower
wavelength. It is thought that light wavelength has no effect on egg production, although some uncertainty about this remains.

For Jones et al. (2001 b), the perception of UVA light by poultry is an expression of their adaptation to natural lighting, which is not fully used under farm conditions. In their studies they found that the perception of UVA is strictly related to the reception of sexual signals (mating signals, sex differences and age differences) and the communication between birds, which may affect poultry welfare and productivity. Therefore, the use of additional UV lighting may improve production results not only through improvement of environmental hygiene, but also in other ways (Dobrzański and Czupa, 1992). Some authors believe that poultry behaviour depends largely on the length of the light waves perceived by birds (Maddocks et al., 2002 a, b). The latter study (Maddocks et al., 2002 b) showed that the perception of UV light plays a considerable role during the search for food and choice of a mating partner. Chickens and broilers respond to food colour by giving preference to colours from the longwave range of the spectrum (yellow to red). This can be attributed to the fact that these are more attractive than shortwave radiation widespread in nature (Fischer et al., 1975; Miklosi et al., 2002; Sahin and Forbes, 1998; Jones et al., 2001 b). This argumentation is confirmed by the preference for blue colour of the environment reported by Davis and Fischer (1978). During cold stress and noise stress, birds give preference to the more longwave range of the spectrum (green colour). In addition, poultry can learn to associate colours with other environmental stimuli (Kutln and Forbes, 1993). Stress influences egg colour by making it darker. Enriching cage colours reduces stress, which results in hens producing brighter eggs (Walker and Hughes, 1998).

Young broilers are strongly motivated to peck coloured objects (Jones and Carmichael, 1998). White and yellow colours are more attractive for broilers and layers than orange and blue. This may result from stronger reflection, i.e. greater brightness. Pecking itself is generally connected with the poultry house being an insufficiently varied environment. However, layers were observed to peck feathers more when there was increased contrast and a larger number of colours on the bedded floor (Savory and Mann, 1999). Turkeys exposed to additional intermittent lighting have fewer injuries, and perceive the lighting as an environmental enrichment (Sherwin et al., 1999). Vehse and Ellendorff (1999) report that blue light has a favourable effect on turkey growth during the first period of rearing.

According to Lewis and Morris (1998), it is impossible to conclusively determine the effect of wavelength and light intensity on poultry growth, feed intake, reproductive performance, mortality and behaviour. Death and Stone (1999) studied the social behaviour of laying hens by exposing them to white, red and blue light at intensities of 77 and 5.5 lx. They found that light intensity has no effect on the reaction of birds, while recognition of other birds from the same flock is easier in white light than in red light. The existing inconsistencies can be explained to a certain extent by the issue of light intensity. Praytino and Philips (1997) showed that light intensity has an effect on colour perception by broilers. According to these authors, blue light must be 3.2 to 2.6 times brighter for birds to
be equally well visible. This indicates a lack of differences in the acuteness of colour vision and the need to adjust light intensity during rearing. It is worth noting the findings of Roper and Marples (1997), who studied chickens’ preferences for food and water colour (red, green and black). They found that the choice of food and water colour is dependent on the mode of administration and location, because at certain times birds preferred black, and at other times they preferred red. Green was chosen as an intermediate colour.

The issue of light in relation to pigs has only been investigated in terms of the effect of photoperiod length on the reproductive parameters of sows and boars (Andersson et al., 1998; Mabry et al., 1983). An additional result of changes in photoperiod length was an increase in piglet suckling frequency and the rearing of a greater number of weaners. The photoperiod also has an effect on glycocorticoids and gonadotropic hormones in sows (Kraeling et al., 1983). However, it is difficult to confirm the effect of increasing the photoperiod length under production conditions (Matuszewska and Walczak, 2001). It is known from breeding practice that keeping fatteners in the dark shortens their fattening period and increases their fatness. It is hard to deny pigs the capacity to distinguish colours, but eyesight does not play the most important role as their other senses are well developed. In an impoverished farm environment, colour stimuli from the environment begin to stimulate pigs to an increasing extent. This often results in cannibalism. The various toys used in breeding in practice to add variety to the living environment of pigs are more attractive to pigs if they are red or yellow. This confirms the theory on mental sensitivity to colours that are rare in a natural environment.

**Conclusion**

Research methods and the use of digital colour readers (Villafuerte and Negro, 1998), electroretinographs (Jacobs et al., 1998) and laser extraction methods (Janknecht et al., 2001) have contributed a lot to our knowledge about the perception of colours by animals. The sensory aspect of colour perception by animals is relatively well understood. The function of mechanisms such as LUM (light reception), allowing for fast identification of movement, remains unstudied in animals (Stromeyer et al., 2000). Likewise, the role of variable wavelengths in the identification and perception of colours remains unexplained (Lomas et al., 1998). The initial results of studies on the colour centre of the cerebral cortex, using magnetic resonance, are promising (Bartels and Zeki, 2000). It seems that the time for wide-ranging studies on the effect of environmental stimuli on animals is still to come.

The present review of the literature shows that the issue of colour stimuli from the environment has been relatively well studied in poultry, but that there are very few studies on colour perception by pigs. This justifies efforts to explore the issue in greater depth.
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Widzenie barwne u świń i drobiu

STRESZCZENIE

Postrzeganie barw otaczającego organizm środowiska jest procesem nie do końca poznawanym. Nie wszystkie zwierzęta mogą rozróżnić barwy, gdyż oko nie jest jednakowo rozwinąte u wszystkich kręgowców, a nawet w obrębie tego samego gatunku mogą występować pewne różnice. Zmysł wzroku jest gorzej rozwinąty u ssaków niż u ptaków. Widzenie barwne u ssaków zorientowane jest przede wszystkim na barwy czerwone i zielone. Ptaki domowe mają wzrok bardzo dobry i widzą na dużą odległość, pod warunkiem jednak dobrego oświetlenia.

Wpływ długości fali i intensywności światła na wzrost, spożycie paszy, wskazniki reprodukcyjne, upadki i behawior drobiu nie został do tej pory jednoznacznie określony. Natomiast u świń, badano dotychczas wpływ światła jedynie pod kątem długości dnia świątelnego.

Przegląd piśmiennictwa wskazuje na niepełne rozpracowanie problemu wpływu barwnych bodźców otoczenia w odniesieniu do drobiu. Zauważa się również znikomy udział badań w postrzeganiu barw przez świń. Dowodzi to celowości podejmowania problematyki badawczej w tym zakresie.

Key words: pig, poultry, colour, stimuli, behaviour, productivity
GENETIC IMPROVEMENT OF REPRODUCTIVE TRAITS IN CHINCHILLAS

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Abstract
The aim of the study was to evaluate genetic variation in and the breeding value of reproductive traits in chinchillas kept in small populations. A 3-generational population of chinchillas was investigated. The (co)variance components and breeding values of animals were estimated using multitrait, repeatable animal models. A package of programs for analysis of multitrait mixed models, which uses the REML method, was used in the calculations. The results obtained indicate that the selection of animals for the reproductive herd should be based on BLUP estimates. There were highly significant rank correlations between the breeding value of offspring and parents (from 0.92 to 0.94). The selection criteria should include the body weight of young animals at 2 weeks of age. This trait is largely determined by the additive effect of animal. At the same time, high and positive genetic correlations between body weight, weight gains and number of litters reared may facilitate the improvement of reproductive traits in chinchilla.

Key words: chinchilla, breeding value, prolificacy

In chinchilla breeding, reproductive issues are still a problem. On Polish farms, the average number of young chinchillas per litter is approximately 2 (Socha and Wrona, 2000). The low prolificacy in relation to the number of maturing ovarian follicles (Sulik et al., 2001) and the high periparturient mortality are the most frequent causes of the divergence between genetic potential and breeding results (Sulik et al., 2001). The milk yield of mothers could be a particularly important issue in chinchilla breeding. Studies have shown that mortality of the young is related to litter size. This phenomenon may be associated with the inadequate milk yield of females (Sulik et al., 2001).

In improving the reproductive traits of animals, only the number of animals born and reared is usually used. However, it seems that this limited number of analysed traits provides an inadequate characterization of the female in terms of reproduction. Animal reproduction can be considered separately with regard to various parameters such as female and male fertility, litter size, maternal instinct,
mothers’ milk yield, and the embryonic and postnatal mortality of kits (Maciejowski and Jeżewska, 1993).

Selection of chinchillas is based solely on the productive value of animals, on the assumption that phenotypic classification reflects genotypic classification. This assumption may only be reliable with regard to highly heritable traits. Given that reproductive traits are characterized by low coefficients of heritability (Jeżewska et al., 2003; Kenttamies, 1996), the genetic improvement of fur animals based solely on their productive value is rather inefficient (Rozempolska-Rucińska et al., 2004). It is necessary to evaluate breeding value, which requires knowledge of variance components. This means that the additive variation of traits included in the breeding goal and the existing interrelations between these traits need to be determined. The use of BLUP estimates in the improvement of the chinchilla population may face problems connected with the size of the foundation herd on chinchilla farms. In Poland, the vast majority of farms have 50–100 chinchillas. The accuracy of breeding value estimation depends on the quantity and quality of information sources used in animal evaluation and on the population structure.

The aim of the present study was to evaluate genetic variation in and the breeding value of reproductive traits in chinchillas kept in a small population.

Material and methods

A 3-generational population of chinchillas was investigated. The foundation herd included 61 females of standard variety, which were kept in a polygamous system. Reproductive data were taken from females whelped in the years 2004–2005. On the farm, 67 litters from year-old mothers and 49 litters from two- and three-year-old mothers were obtained in the period studied. The following traits were analysed:

1) number of young chinchillas born in successive litters — number of live born and stillborn animals,
2) number of young reared in successive litters — number of young reared to 6 weeks of age,
3) body weight of kits at 1 week of age,
4) body weight of kits at 2 weeks of age,
5) weight gains between 1 and 2 weeks of age.

The body weight of young chinchillas and their weight gains were traits that indirectly characterized the milk yield of mothers and the body condition of their offspring.

The values of traits in the analysed population are shown in Table 1.

The parameters of litter size were analysed based on 116 whelpings, and the parameters of body weight and weight gains were analysed based on 241 kits.

Females were assigned to 4 groups depending on the month of whelping. There were 27 litters whelped in the winter season (December–February), 33 litters whelped in the spring season (March-May), 28 litters whelped in the summer
season (June-August), and 28 litters whelped in the autumn season (September-November). Females were assigned to 4 groups according to litter number. A total of 116, 76, 26 and 23 chinchillas were born in the first, second, third, and fourth litters, respectively.

Table 1. Average value of analysed traits in the chinchilla population studied

<table>
<thead>
<tr>
<th>Trait</th>
<th>x</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of young born per litter (LMU)</td>
<td>2.3</td>
<td>0.79</td>
</tr>
<tr>
<td>No. of young reared per litter (LMO)</td>
<td>1.9</td>
<td>0.90</td>
</tr>
<tr>
<td>Body weight of kits at 1 week of age (MC1)</td>
<td>129.78</td>
<td>54.13</td>
</tr>
<tr>
<td>Body weight of kits at 2 weeks of age (MC2)</td>
<td>165.0</td>
<td>75.03</td>
</tr>
<tr>
<td>Body weight gains (PR)</td>
<td>18.65</td>
<td>9.0</td>
</tr>
</tbody>
</table>

The covariance components and breeding values of animals were estimated using multivariate, repeatable animal models, whose factors are shown in Table 2. The significance of the effect of fixed environmental factors has previously been validated using multivariate analysis of variance and the least squares method (SAS, 2000).

Table 2. Comparison of factors in computational models for different traits

<table>
<thead>
<tr>
<th>Factor</th>
<th>Trait</th>
<th>Type</th>
<th>LMU</th>
<th>LMO</th>
<th>MC1</th>
<th>MC2</th>
<th>PR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter size</td>
<td>x</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Litter size</td>
<td></td>
<td>C</td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Litter number</td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Season of whelping</td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Year × season of whelping</td>
<td></td>
<td>F</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Additive effect of animal</td>
<td></td>
<td>A</td>
<td></td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Effect of specific environment</td>
<td></td>
<td>R</td>
<td>x</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

x — presence of factor in a model for the analysed trait.

Type of factor: A — random factor associated with relationship matrix, R — random factor, F — fixed, C — covariance variable (regression).

The pedigree included 257 animals and the base population was 95 animals. In the calculations, a package of programs for analysis of multivariate mixed models that use the REML method was used (Misztal, 1998).

The evaluation of the breeding value of different traits was followed by a scaling of BLUP estimates from −1 to 1. The estimates obtained for different traits were the basis for determining the total breeding value of each animal. The total breeding value was determined using 2 methods:

1. The sum of BLUP estimates for the number of litters born and reared — breeding value I (WH I);
2. The sum of BLUP estimates for the number of litters born and reared, body weight at 1 and 2 weeks of age, and weight gains — breeding value II (WH II).

Total productive value was determined analogously. In the first case, it was the average number of litters born and reared (WU I), and in the second case it was the average number of litters born and reared, average body weight of kits, and kits’ weight gains (WU II). The scores were standardized on a scale of –1 to 1.

Rank correlations were determined between:

1. Total breeding value of animals, obtained using the WH I and WH II method;
2. Total breeding value of offspring and parents, obtained using the WH I and WH II method;
3. Total productive value of animals, obtained using methods I and II (WU I and WU II);
4. Total productive value between mothers and daughters, obtained using the WU I and WU II method.

On the analysed farm, animals had optimum and uniform hygienic conditions throughout the year. The analysis did not account for females that showed some signs of disease.

Results

The genetic parameters of the analysed traits are given in Table 3. The body weight of the offspring was a trait indirectly characterizing the milk yield of females and the condition of kits.

<table>
<thead>
<tr>
<th>Trait^</th>
<th>h^2</th>
<th>r^2</th>
<th>Genetic correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MC1</td>
</tr>
<tr>
<td>MC1</td>
<td>0.41</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>MC2</td>
<td>0.36</td>
<td>0.37</td>
<td>0.80</td>
</tr>
<tr>
<td>PR</td>
<td>0.01</td>
<td>0.06</td>
<td>0.91</td>
</tr>
<tr>
<td>LMU</td>
<td>0.01</td>
<td>0.02</td>
<td>0.36</td>
</tr>
<tr>
<td>LMO</td>
<td>0.12</td>
<td>0.14</td>
<td>0.88</td>
</tr>
</tbody>
</table>

* for the designation of traits, see Table 1.

The estimates obtained are evidence that the phenotypic variation of kit body weight, observed in the population resulted largely from the additive effect of animal, i.e. the offspring’s father and mother. The coefficients of heritability and repeatability were similar at 0.41 and 0.42, and 0.36 and 0.37, respectively. Higher parameter values were observed for body weight at 1 week of age.

Body weight gain in kits between 1 and 2 weeks of age is a trait that can objectively characterize the milk yield of females. Genetic parameters for this trait

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proved very low: $h^2 = 0.01$ and $r^2 = 0.06$. Variation of the analysed trait was determined to a very limited extent by animal genotype. Analogous results were obtained for the number of young born: the coefficients of heritability and repeatability for this trait were only 0.01 and 0.02. Lower values were found for the number of kits reared, but in this case they show that this trait was genetically determined to only a limited extent.

In order to establish an appropriate breeding programme, knowledge of the relationships between the traits being improved is required.

In the present study, there were very high and positive correlations (0.91 and 0.97) between weight gains and body weight at 1 and 2 weeks of age, respectively (Table 3). These were higher than the correlations between body weight at 1 and 2 weeks of age (0.80). The correlation between the number of kits reared and body weight was 0.88. Likewise, very high correlations were found between this trait (LMO) and weight gains (0.95). The present results indicate that selection for increased body weight of offspring at 2 weeks of age should help to improve the other reproductive traits, in particular the milk yield of mothers and the related rearing performance of kits. In the present study, we also found positive correlations between the number of kits born and reared. However, the value of this parameter (0.48) confirms that the number of offspring born does not determine the number of offspring reared. This is particularly noticeable in this species, in which considerable kit mortality is observed during the first weeks of life.

In the present study, we also validated the selection system used on the farm. The only selection criterion for reproductive traits used on the majority of Polish farms, is performance of mothers or, additionally, performance of closely related animals (e.g. sisters). In our study we analysed rank correlations between the performance of mothers and of daughters (Table 4). The consistency of classifications was determined according to the adopted selection criterion, which was the number of kits born and reared in the first case, and additionally the milk yield of females in the second case. In neither case were there significant rank correlations. This indicates that the selection of animals for the reproductive herd based on the performance of their mothers is inappropriate. The phenotypic value of females cannot be the basic selection criterion for chinchilla reproductive traits. It was also found that the productive value of animals, determined using the two methods, varied markedly. Rank correlations between the productive value of females evaluated using litter size, or additionally milk yield, were only 0.08 and were not significant. Females occupied different positions in the ranking of productive value depending on the traits included as selection criteria.

The consistency of classifications of breeding values, estimated using the two methods, was 0.87.

The selection of animals for a breeding herd should be based on BLUP estimates. Highly significant rank correlations were found between the breeding value of offspring and parents (Table 4). These correlations ranged from 0.92 to 0.94 depending on the trait concerned.
Table 4. Rank correlations between some indicators

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Rank correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>WU I mothers and daughters</td>
<td>−0.54</td>
</tr>
<tr>
<td>WU II mothers and daughters</td>
<td>−0.15</td>
</tr>
<tr>
<td>WU I — WU II</td>
<td>0.08</td>
</tr>
<tr>
<td>WH I — WH II</td>
<td>0.87***</td>
</tr>
<tr>
<td>WH I parents and offspring</td>
<td>0.94***</td>
</tr>
<tr>
<td>WH II parents and offspring</td>
<td>0.92***</td>
</tr>
</tbody>
</table>

*** correlations significant at P ≤ 0.0001.

Discussion

Reproductive traits are characterized by low heritability. Differences between estimates obtained by different authors result mainly from the computational model used, the population analysed and the species of animals. The coefficient of heritability, estimated using the animal model, usually ranges from 0.01 to 0.15 (Lagerkvist et al., 1994; Rozempolska-Rucińska, 2004). The coefficients of heritability obtained in the present study were evidence of the small additive effect of animal on the number of kits born and reared. Analogous results were obtained by Jeżewska et al. (2003), who analysed genetic variability in a 9-generational population of chinchillas. The coefficients of heritability and repeatability were $h^2 = 0.01$, $r^2 = 0.1$ and $h^2 = 0.016$ and $r^2 = 0.09$ for litters born and reared, respectively. These values were markedly lower than those reported in a study by Socha (2002), in which the repeatability of the number of young chinchillas born and reared was 0.172 and 0.185, respectively. The results obtained in our study and in other studies confirm the lack of clear genetic variation, which can make it very difficult to achieve breeding progress.

The body weight of animals is a trait with medium or high heritability. The coefficient of heritability usually ranges from 0.12 to 0.5 (Lohi and Hansen, 1990; Lagerkvist et al., 1994). In the majority of the publications available, heritability of body weight is a parameter characterizing the additive effect of an animal on the variation of this trait. In these studies, the body weight of young animals was a trait that differentiated the mothers of animals, rather than the actual animals in which this trait was measured. The body weight of offspring was treated as another measurement of the same trait in a female (a repeatability model). The coefficient of heritability explained variation in the body weight of the offspring resulting from the additive effect of father and mother. In this case, the coefficient of heritability can be a parameter corresponding to the coefficient characterizing the additive effect of an animal’s mother. In the present study we found that an important source of variation in chinchilla body weight, in both the first and second week of age, was parents’ genotype. This result is confirmed by studies carried out with different species of animals, in which the additive effect of mother on the body weight of offspring was determined (Jeżewska et al., 2004; Rozempolska-Rucińska, 2004;
Hansen and Berg, 1997). Variation of this trait, resulting from the genetic effect of mother, often accounted for half the variation resulting from the additive effect of an animal. Analyses made in a population of mink showed that with regard to body size and conformation, the genetic effect of mother exceeded the additive effect of an animal (Rozempolska-Rucińska, 2004). Analogous results were obtained in another study, which analysed genetic variation for body size and conformation in chinchillas (Jeżewska et al., 2004). Berg (1993) reported that the effect of mother accounted for 10–40% of total variation in the body weight of the mink. The effect of mother persists mainly during the first weeks of kits’ life (Hansen and Berg, 1997; Hansen et al., 1992). This can explain the obtention of higher coefficients of heritability during the first week of chinchillas’ lives. This seems justified because the body weight of the offspring during this period is dependent on the mother’s milk yield only. In the second week of life, although female milk is still the basic food, young animals may consume small amounts of feed and thus the genetic effect of mother on the observed variation can decrease. The value of the estimate obtained is evidence that animals can be genetically improved to enhance offspring quality.

Studies with different species of fur animals have shown high correlations between the number of kits born and reared. The coefficient of genetic correlations usually ranges from 0.8 to 0.97 (Socha and Adamska, 2001; Filistowicz et al., 1999; Rozempolska-Rucińska, 2004). These values were therefore considerably higher than those found in the present study. Similar results to those we observed were obtained by Jeżewska et al. (2003). The correlations between the number of chinchillas born and reared was approximately 0.6. Lower values of this parameter noted among chinchillas show that the size of the litter born is not the main factor determining the number of chinchilla offspring reared. Large litters generally differ in terms of size and development, which leads to high mortality in young animals during rearing (Sulik et al., 2001). On the other hand, very high coefficients between body weight, weight gains and litter size can indicate that the main factor responsible for the number of offspring reared is the milk yield of a female. These conclusions are also confirmed by farm observations. Only in very rare cases (e.g. dystocia or congenital genetic defects) did young chinchillas die within several or a dozen hours of birth despite the fact that the mother’s milk yield was adequate. Of course, when speaking of abnormal lactation as the main factor determining the rearing of animals, environmental factors were not accounted for because the provision of adequate conditions to animals is the basis of production of any kind, and these conditions were fulfilled for the population studied.

Mothers’ milk yield is a trait that should be included among the parameters characterizing the reproduction of females. It seems that it is appropriate to determine the milk yield of mothers by controlling the body weight of their offspring. Studies with a population of goats found very high genetic correlations between litter body weight and mothers’ milk yield (Snowder et al., 2001). The milk yield of female chinchillas and the related weight gains of young chinchillas were largely determined by environmental factors. Farm observations showed that the appropriate level of milk yield and the development of sucklings were largely
dependent on environmental factors, of which the most important is the appropriate feeding of periparturient mothers. The value of the genetic parameters obtained confirms that the level of milk yield in females can be improved by providing animals with appropriate conditions.

However, genetic associations between traits also have to be considered. When carrying out selection for increased body weight of young at 2 weeks of age, a second trait — weight gains of young animals — can be concurrently improved. Thus we should improve the level of lactation in females. This is particularly important given that the body weight of young chinchillas was characterized by a markedly higher coefficient of heritability compared to weight gains. Selection for increased body weight of young at 2 weeks of age should also contribute to improvements in the other reproductive traits, in particular the number of offspring reared.

When estimating the productive and breeding value of females, the birth weight of offspring was not accounted for because selection for increased postparturient body weight is not recommended, as it may lead to difficult parturitions and thus to mortality among young and mothers. At the same time, body weight after birth cannot be used as an indicator of mothers’ milk yield.

Validation of the selection system used on fur farms has been the subject of studies conducted by different authors (Rozempolska-Rucińska et al., 2004; Filistowicz and Żuk, 1995; Filistowicz et al., 1999). Analyses carried out in a mink population indicate a lack of correlations between traits regarded as selection criteria and the breeding value of animals (Rozempolska-Rucińska et al., 2004). Rank correlations between the productive value of mothers and offspring were also very low, at approximately 0.1 (Rozempolska-Rucińska et al., 2005). The improvement of reproductive traits in mink based on phenotype proved inefficient. The breeding herd included a considerable percentage of animals with negative breeding value in relation to the base generation (Rozempolska-Rucińska et al., 2004). The need to make changes to selection methods has also been reported by other authors (Filistowicz and Żuk, 1995; Filistowicz et al., 1999).

In the present study, we found high consistency of chinchilla classifications (parents and offspring) in terms of breeding value. This should definitely facilitate the selection of animals for the breeding herd. Analyses performed in a mink population confirm that animal selection should be carried out on the basis of the total breeding value of parents. The highest consistency of classifications was obtained between the breeding value of offspring and the total breeding value of both parents. Rank correlations were approximately 0.9 (Rozempolska-Rucińska et al., 2005). This is particularly important for reproductive traits, because decisions regarding whether or not animals should remain in the herd must be made by the breeder when there is no information about the animals’ own performance. Danish studies (Lohi, 1993) indicate that an appropriate breeding programme and selection of animals based on genetic value have made it possible to increase the size of the mink litter born per mated female by an average of 0.1 kit per year. This result was much better than that obtained when selection in a population was based on animal phenotype only.
At the same time, the analyses show differences between estimates obtained using different models. Animals can occupy different places in the breeding rank depending on the selection criteria adopted. Considering the other results obtained in the present study, it seems that the evaluation of breeding value should account for the milk yield of mothers, determined by measurements of body weight and weight gains of offspring.

To sum up the analyses made, it is possible to estimate genetic variation in and the breeding value of animals kept on small and medium-sized farms. Evaluation of the efficiency of selection carried out based on BLUP estimates requires further analysis, once subsequent generations have been produced that were selected on the basis of genetic value. Considering the consistency of classifications, it seems, however, that selection based on the breeding value of parents should produce the expected genetic and productive progress in the reproductive traits of chinchillas. The existing system of selecting young animals for the selection herd is burdened with a considerable error: there are no correlations between the performance of females and the future performance of their daughters. The selection criteria should include the body weight of offspring at 2 weeks of age. This trait is largely determined by the additive effect of animal (parents), and the high and positive genetic correlations between body weight, weight gains and the number of litters reared can facilitate the improvement of reproductive traits in chinchillas. When carrying out selection for increased body weight of offspring at 2 weeks of age, it can be expected that the other reproductive traits (notably litter size and females’ milk yield) will improve through a correlated response.

References


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Genetyczne doskonalenie cech reprodukcyjnych szynszyli

STRESZCZENIE

Celem przeprowadzonych analiz była ocena genetycznej zmienności i wartości hodowlanej cech reprodukcyjnych zwierząt, utrzymywanych w małych populacjach szynszyli. Badaniami objęto 3-pokoleniową populację szynszyli. Szacunki komponentów (ko)wariancji i wartości hodowlanych zwierząt szacowano stosując wielocechowe, powtarzalnościowe modele osobnicze. W obliczeniach zastosowano pakiet programów do analizy wielocechowych modeli mieszanego wykorzystujący metodę REML. Uzyskane wyniki wskazują, że wybór zwierząt do stada reprodukcyjnego powinien opierać się o szacunki BLUP. Stwierdzono wysoko istotne korelacje rangowe pomiędzy wartością hodowaną potomstwa i rodziców (od 0,92 do 0,94). Kryterium selekcyjne powinno obejmować masę ciała młodych w 2. tygodniu życia. Cecha ta jest w znacznym stopniu uwarunkowana adytywnym wpływem osobnika. Jednocześnie wysokie, dodatnie korelacje genetyczne pomiędzy masą ciała, przyrostami i liczebnością odchowanych miotów mogą ułatwić doskonalenie cech reprodukcyjnych szynszyli.
UTILIZATION OF THE PROBE GENERATED BY CHROMOSOME MICRODISSECTION, FOR DETECTION OF EQUINE X CHROMOSOME ANEUPLOIDY*

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Abstract

Fluorescence in situ hybridization (FISH) plays an essential role in research and clinical diagnostics. The use of chromosome painting in farm animals is mainly limited by the fact that chromosome-specific probes are not commercially available for individual animal species. We report on a method for generation of fluorescence in situ hybridization from conventional microdissection of X chromosomes of the domestic horse. The described method was used on chromosome spreads originating from six mares with poor reproductive performance (infertility, lower fertility). In all the mares a low frequency of X chromosome mosaicism was identified. This application is of great importance because chromosome aberrations cause economical losses in horse breeding.

Key words: horse, microdissection, chromosome painting, clinical cytogenetics, X mosaicism

For several decades chromosomes were analysed by traditional karyotyping, which depends on the analysis of characteristic banding patterns. The major disadvantage of conventional cytogenetic banding methods is the limited resolution. The equine X chromosome is usually identified by the presence of an interstitial C-band on the Xq. Unfortunately, the diagnosis based on C-banding technique may not be conclusive in cases where chromosome spreads are not of good quality. The development of fluorescence in situ hybridization (FISH) plays an

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essential role in research and clinical diagnostics. Detection of equine X chromosome abnormalities using a whole X chromosome painting probe was described by Breen et al. (1997), Wieczorek et al. (2001), and Bugno et al. (2003, 2005). The diversity and resolution of FISH depends critically on the probe set used. The use of chromosome-specific probes in farm animals is mainly limited by the fact that chromosome-specific probes are not commercially available for individual animal species. Chromosome-specific DNA probes can be generated by flow sorting of whole chromosomes and subsequent universal amplification by degenerate oligonucleotide primed polymerase chain reaction (DOP — PCR) (Telenius et al., 1992).

An alternative to flow sorting is microdissection. The conventional microdissection approach uses fine extended glass needles for the collection of chromosomes or subchromosomal regions by GTG technique (Meltzer et al., 1992).

In the present work, we used conventional microdissection to generate a domestic horse whole X chromosome painting probe (WXCPP) that was then applied to the diagnosed X chromosome mosaicism.

Material and methods

Six mares with poor reproductive performance (infertility, lower fertility) were taken under cytogenetic analysis (Table 1). They were phenotypically normal with normal external genitalia.

Table 1. Reproductive performance and chromosome painting results of mosaic mares

<table>
<thead>
<tr>
<th>Mare name</th>
<th>Mare age (years)</th>
<th>Reproductive performance</th>
<th>Total number of spreads</th>
<th>Number and frequency of cells with a given karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>64,XX</td>
</tr>
<tr>
<td>Mizurii</td>
<td>4</td>
<td>infertile</td>
<td>322</td>
<td>298</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(92.5%)</td>
</tr>
<tr>
<td>Basia</td>
<td></td>
<td>infertile</td>
<td>106</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(93.4%)</td>
</tr>
<tr>
<td>Bianka</td>
<td></td>
<td>infertile</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(92.3%)</td>
</tr>
<tr>
<td>Alborada</td>
<td>9</td>
<td>infertile</td>
<td>253</td>
<td>243</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(96%)</td>
</tr>
<tr>
<td>Fama</td>
<td>7</td>
<td>1 foal</td>
<td>268</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(98.9%)</td>
</tr>
<tr>
<td>Garonna</td>
<td>5</td>
<td>infertile</td>
<td>122</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(98.4%)</td>
</tr>
</tbody>
</table>

Chromosome preparation and identification

Male metaphase chromosomes of the domestic horse were obtained from Pokeweed-stimulated lymphocyte cultures according to the standard protocols.
Chromosome preparations for microdissection and FISH experiments were stored at -80 and -20°C, respectively. GTG technique was used for the identification of X chromosomes (Wang and Fedoroff, 1974).

**Preparation of a horse whole X chromosome painting probe (WXCPP)**

An aliquot of the equine metaphase chromosome culture was spread onto a coverslip and stained with Giemsa. For each microdissection experiment eight X chromosome copies were dissected with glass microneedles controlled by a manipulator attached to an inverted microscope. The dissected chromosomes were transferred by breaking off the microneedle to a PCR tube containing collection drop solution.

The dissected DNA material was amplified in a DOP-PCR using degenerate oligonucleotide primes (5′— CCGACTCGAGN_{6}ATGTGG-3′) (DOP PCR; Tele- nius et al., 1992).

Aliquots of the amplified DNA material were labelled by DOP-PCR with biotin-16-dUTP for FISH experiment. The labelled PCR products were purified using Nick Columns according to the manufacturer’s protocol and co-precipitated with 5 µg salmon sperm DNA and 5 µg horse Cot-1 DNA.

**Fluorescence in situ hybridization**

The labelled probe was denatured at 70°C for 10 min. After RNase and pepsin digestion, target metaphase spreads were denatured in a hybridization solution containing 2×SSC and 70% formamide, at 70°C for 2.5 min. The probe was dropped onto the metaphase spread, covered, sealed with rubber cement and hybridized overnight in a moist chamber at 37°C. Post-hybridization washes were as follows: three times at 50% formamide in 2×SSC and three times in 2×SSC at 42°C. A biotin-labelled probe was detected by the avidin-FITC and anti-avidin system on propidium iodide-stained slides. Microscopic evaluation was performed under a fluorescence microscope equipped with a camera and Lucia software.

**Results**

The horse WXCPP was hybridized onto metaphase chromosomes of the six mares with poor reproductive performance (infertility, lower fertility). Screening with the direct PCR probes by FISH demonstrated that the products had a high specificity for whole X chromosome, since the probes gave a clear FISH-signal. Microscopic evaluation of the preparations revealed the presence of three cell lines in one mare (Mizurii) 63,X/64,XX/65,XXX. This status was identified by analysis of 322 chromosome spreads and the frequency of the particular cell lines was 6.8%, 92.5% and 0.7%, respectively (Figure 1). The mare diagnosed as a carrier of X chromosome aneuploidy in mosaic form had normal external reproductive organs and showed normal oestrus. In a seven-year-old Shetland pony mare (Fama) we diagnosed 64,XX/65,XXX mosaicism with a low frequency of the 65,XXX line.
This mare gave birth to one foal for five seasons, in the other four mares it was a mosaic of two cell lines — 63,X/64,XX. The percentage of the monosomic line in relation to heterosomes was in the 1.6–7.7% range (Table 1). The animals diagnosed with X chromosome monosomy in the form of a mosaic had normal reproductive organs and showed normal oestrus but were infertile.

Discussion

A review of cytogenetic studies in horses by Power (1990) indicates that among mares with abnormal complements of sex chromosomes a vast majority (142 cases) were carriers of 63,X karyotypes. Among mosaic mares the majority (62 cases) was characterized by the 63,X/64,XX karyotype, but in many cases (55) other types of mosaic or chimeric karyotypes with two or more cell lines, i.e. 64,XY/63,X; 64,XX/65,XXY; 64,XX/65,XXX; 63,X/64,XX/65,XXX; 63,X/64,XX/65,XXY etc., were identified.

The random inactivation of one of the X chromosomes in females with normal karyotype formula ensures an equal dosage of expressed X-linked genes in the somatic cells of both males and females (Lyon, 1961). However, genes localized in this region are present in 2 copies in both sexes and therefore genes with loci on the pseudoautosomal region of both X and Y chromosomes are exempt from inactivation (Goodfellow et al., 1983; Brown et al., 1997). Hence, the missing or extra active genes in the inactivated X chromosome affect growth and reproduction in horses with X chromosome aneuploidy (reviewed by Power, 1990).

Mares carrying X chromosome monosomy have normal external genital organs, but usually a hypoplastic uterus and underdeveloped ovaries. Such individuals show no oestrous cycle or oestrus is manifested weakly and irregular. In mares with X chromosome monosomy in mosaic form of 63,X/64,XX, there were few cases of pregnancy and offspring delivery (Halnan, 1985; Bugno et al., 2001; Wieczorek et al., 2001). It should be noted that these mares usually gave birth to one foal each, despite many breeding attempts and veterinary treatments. These cases can be attributed to the survival of single oogonia derived from the cell line 64,XX or, in exceptional cases, to the normal course of meiosis in X0 cells (Sysa et al., 1995).

The X trisomy in the horse was usually connected with infertility (for review see Power, 1990). Recently, several new cases have been reported, confirming the results obtained previously. Makinen et al. (1999) and Bugno et al. (2003) described a non-mosaic X trisomy in two infertile mares with very small ovaries each. An extensive cytogenetic study carried out by chromosome painting of an infertile mare revealed 63,X/64,XX/65,XXX mosaicism (6%/93%/1%) (Wieczorek et al., 2001). In this case infertility could be caused by the presence of a 63,X cell line. On the other hand, Breen et al. (1997) identified two cell lines 63,X (94%) and 65,XXX (6%) among 300 chromosome spreads of a filly, analysed by the painting approach. The authors observed on one occasion a Graffian follicle. In this case altered reproductive performance was probably mainly caused by the presence of the 63,X cell line.
The analysis of a large number of chromosome spreads seems to be very important since low-level mosaicism in lymphocyte cultures could show a different ratio in other tissues (Nazarenko et al., 1999). This means that the identification of a single cell line among 40 metaphase spreads in one tissue (i.e. blood) allows us to exclude mosaicism at the level of 8%, with 95% confidence. Therefore, a small number of the analysed spreads may cause misdiagnosis.

In the horse sex chromosome aneuploidies often occur in a mosaic form (Power, 1990). The non-mosaic X0 and XXX mares are infertile, whereas the mosaic aneuploidy carriers are sometimes subfertile.

Our study confirmed the thesis that the application of the chromosome painting technique is very useful in studies of sex chromosome aneuploidies in the horse. Thus, this approach is suggested for routine application because it is fast and makes it possible to analyse a large number of metaphase spreads, including those of poor quality (overlapping of chromosomes, very short chromosomes, etc).

References


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Sonda uzyskana poprzez mikrodysekcję chromosomów i jej zastosowanie do detekcji aneuploidii chromosomu X koni

STRESZCZENIE

Fluorescencyjna hybrydyzacja in situ (FISH) odgrywa znaczącą rolę w pracach badawczych i diagnostyce klinicznej. Zastosowanie sond malujących chromosomy u zwierząt gospodarskich jest znacznie ograniczone z powodu braku na rynku komercyjnych sond specyficznych dla chromosomów poszczególnych gatunków zwierząt. W pracy opisana została technika fluorescencyjnej hybrydyzacji in situ z zastosowaniem sony uzyskanej drogą mikrodysekcji chromosomów X konia domowego. Opisaną metodę zastosowano na płatkach metafazowych pochodzących od sześciu klaczy wykazujących zaburzenia reproductywne (bezpłodność, obniżona płodność). U wszystkich badanych klaczy zdiagnozowano mozaicyzm chromosomu X, z niską częstością komórek linii nieprawidłowej. Zastosowanie tych badań w praktyce ma istotne znaczenie, ponieważ występowanie aberracji w chromosomach powoduje ekonomiczne straty w hodowli koni.
EVALUATION OF SEVEN STR SYSTEMS IN FOUR HORSE BREEDS FOR PARENTAGE TESTING*

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Abstract
In this study, 7 microsatellite markers (CA457, COR058, COR070, COR072, COR089, COR096 and LEX073) were evaluated for parentage testing in Polish populations of Arabian, Hucul, Silesian and Thoroughbred horses. In the investigated loci, 12 new alleles were detected in the studied populations of horses. Except two markers with low polymorphism in two horse breeds, all of the investigated markers had a PIC value above 0.5. The combined probability of exclusion (PEc) for the 7 markers was equal to or greater than 99%, depending on the breed. During development of PCR multiplex conditions, redesigning primer sequences can improve the yield of PCR products much more efficiently than increasing the primer concentrations in the multiplex reaction.

Key words: horse, microsatellites, PCR multiplex, parentage control

For around 16 years, a number of Short Tandem Repeat (STR) loci have been efficiently used in parentage tests, forensic science, phylogenetic analysis and genome mapping projects in humans and a number of animal species. Microsatellites, evenly distributed in the mammalian genomes, form one of the largest groups of genetic markers. For example in the INRA horse genome database 1537 STR loci have been reported until now (http://dga.jouy.inra.fr/cgi-bin/lgbc/summary.operl?BASE=horse). The small size of microsatellite alleles and the polymorphic nature of short tandem repeats make them very useful in these studies. However, some problems still exist in the use of STR systems, which result in the time-consuming development of PCR multiplexes for a number of microsatellite loci, adenylation of PCR products by using Taq polymerase during amplification of microsatellites with 2 nucleotide repeats (Ginot et al., 1996) and the frequent

* This work was conducted as part of the NRIAP statutory activity, project no. 3119.1.
occurrence of null alleles cause mutations in the primer binding sites of amplified markers (Eggleston-Stott et al., 1997). These factors are the reason for frequent genotyping errors during analysis of a large number of samples. In this study we describe some of the above-mentioned problems in the case of seven STR systems applied to parentage testing in Polish populations of four horse breeds with different degrees of genetic variability.

Material and methods

The investigated breed panel included purebred Arabian horses (AR) (30 animals from the Michałów and Janów Podlaski studs), Hucul horses (Hc) (28 animals from the Odrzechowa stud), Silesian horses (Sl) (30 animals from the Książ and Strzegom studs) and Thoroughbred horses (TH) (31 individuals from the Żółkiewka, Skarżyce and Stubno studs). DNA from hair root samples was prepared using a modified version of the method of Kawasaki (1990) and that from blood using a WIZARD genomic DNA purification kit (PROMEGA). Genotyping was performed on a set of 9 microsatellite loci, including CA457 (Eggleston-Stott et al., 1999), COR058 (Ruth et al., 1999), COR070, COR072 (Tallmadge et al., 1999 a), COR089, COR096 (Tallmadge et al., 1999 b), LEX073 (Bailey et al., 2000), and two other microsatellites, which were ultimately excluded from the investigations because of the complex allelic pattern (HTG13) (Marklund et al., 1994) probably controlled by two loci and close linkage to the markers in the set (COR007 on ECA 17q13). All the markers were dinucleotide repeats and 3 of them were characterized by imperfect repeats (Table 1).

Table 1. Microsatellite markers evaluated in this study

<table>
<thead>
<tr>
<th>Marker</th>
<th>Type of tandem repeat</th>
<th>Chromosome location</th>
<th>Primer concentration in multiplex reaction (pmol/µl)</th>
<th>Allelic range reported earlier (bp)</th>
<th>New allelic variants reported in this study (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA457</td>
<td>(GT)$_{17}$</td>
<td>11</td>
<td>6</td>
<td>75 – 97</td>
<td>71$<em>{(Sl)}$, 73$</em>{(R, Sl)}^*$</td>
</tr>
<tr>
<td>COR058</td>
<td>(GT)$<em>{15}$ GA (GT)$</em>{2}$ G</td>
<td>12</td>
<td>0,3</td>
<td>206 – 228</td>
<td>230$<em>{(Hc, Th)}$, 232$</em>{(Hc)}$</td>
</tr>
<tr>
<td>COR070</td>
<td>(GA)$_{30}$</td>
<td>6</td>
<td>2</td>
<td>267 – 299</td>
<td></td>
</tr>
<tr>
<td>COR072</td>
<td>(GT)$_{14}$</td>
<td>17q13</td>
<td>0,5</td>
<td>135 – 155</td>
<td>157$<em>{(AR, Th)}$, 159$</em>{(Sl)}$</td>
</tr>
<tr>
<td>COR089</td>
<td>(CA)$_{20}$</td>
<td>4</td>
<td>0,3</td>
<td>272 – 290</td>
<td>292$<em>{(Hc)}$, 298$</em>{(Sl)}$, 305$_{(Hc)}$</td>
</tr>
<tr>
<td>COR096</td>
<td>GTGAGTGAGT (GT)$_{15}$</td>
<td>18</td>
<td>3</td>
<td>307 – 319</td>
<td></td>
</tr>
<tr>
<td>LEX073</td>
<td>(TC)$<em>{3}$ GC (TC)$</em>{2}$ TG (TC)$<em>{2}$ CC (TC)$</em>{12}$ + (CA)$<em>{7}$ TA (CA)$</em>{3}$ CC (CA)$<em>{2}$ CC (CA)$</em>{2}$ CC (CA)$_{15}$</td>
<td>19</td>
<td>4</td>
<td>234 – 264</td>
<td>268$<em>{(Hc, AR, Sl)}$, 270$</em>{(AR)}$, 274$_{(Sl)}$</td>
</tr>
</tbody>
</table>

* breed in parenthesis.
Microsatellite markers were selected from the literature according to their polymorphism (a minimum of 6 allelic variants reported) and the melting point of the primer sequences for different loci, which had to be similar. First, markers were separately amplified to choose the optimal annealing temperature of primers to be later combined in the multiplex reaction. Finally, the microsatellites were simultaneously amplified in one polymerase chain reaction (PCR) containing: 1.5 × PCR buffer (100 mM Tris-HCl, 500 mM KCl, pH 8.3), 1.75 mM MgCl₂, 0.2 mM dNTPs, 2.5 U Taq Gold polymerase (Applied Biosystems), 30 to 60 ng DNA (1 µl) and H₂O to the final volume of 15 µl. The primer concentrations are presented in Table 1. PCR amplification entailed initial denaturation (95°C, 10 min), 31 cycles of 30 s at 94°C, 1 min at 60°C and 1 min at 72°C, and a final extension step at 72°C for 60 min in a GeneAmp PCR System 9600 (Applied Biosystems). Fluorescently labelled PCR products were subjected to vertical electrophoresis in a 4% denaturing polyacrylamide gel on a Genetic Analyser (ABI PRISM 377). The allele sizes in base pairs (bp) were determined after processing of raw data using the software packages GENESCAN 2.0 and GENOTYPER 2.1 (Applied Biosystems).

Using TFPGA software, a test for the presence of Hardy-Weinberg equilibrium (H-W) was conducted, based on comparison between the expected and observed numbers of genotypes in three genotype classes (homozygotes for the most common allele, heterozygotes for the most common allele and all other genotypes) (Guo and Thompson, 1992). The calculation of allele frequency and allele number, observed (Ho) and expected heterozygosity (He) (Nei, 1978), polymorphism information content (PIC) (Botstein et al., 1980) and probability of exclusion (PE) of wrongly assigned parentage when both parental genotypes are known (Jamieson and Taylor, 1997) were performed using the CERVUS 2.0 program. Also, the frequency of null alleles was estimated for each marker locus, using an iterative algorithm based on the difference between the observed and expected frequency of homozygotes (Summers and Amos, 1997) (CERVUS 2.0 software).

### Results

The DNA profiles of the investigated microsatellite loci are presented in Figure 1. During the development of multiplex conditions for the set of investigated markers, primers for loci COR089 and COR096 had to be redesigned to improve the yield of PCR products. For locus CA457, +A PCR products were observed which often disturbed the proper determination of genotypes (Figure 2).

In the panel of four investigated horse breeds, the number of alleles detected varied from 3 for locus COR096 in the AR population to 10 in locus COR058 in TH horses (Table 2). Twelve new microsatellite variants were observed as follows: 71 and 73 bp at the locus CA457, 230 and 232 bp at COR058, 157 and 159 bp at COR072, 292 and 298 bp at COR089, 305 bp at COR096, and 268, 270 and 274 bp at LEX073, which are out of the allelic range reported earlier (Table 1).
* Markers COR007 and HTG13 were excluded from parentage analysis.

Figure 1. DNA profiles of the investigated panel of STR loci amplified in multiplex reaction

For markers COR096 and COR072 in the Hc population, CA457 in Sl and COR089 in TH, the population frequency of null alleles exceeded 0.05 (Table 1).

The assessment of genetic equilibrium in the investigated horse breeds revealed highly significant deviations from H-W proportions in locus LEX073 in the Hc population and in locus COR096 in Sl horses.

The degree of polymorphism of the investigated microsatellites varied according to locus and breed.
Table 2. Marker characteristics in the studied populations of horses (observed heterozygosity, Ho; expected heterozygosity, He; polymorphic information content, PIC; probability of exclusion, PE)

<table>
<thead>
<tr>
<th>Marker</th>
<th>Breed</th>
<th>Allele No.</th>
<th>Ho</th>
<th>He</th>
<th>PIC</th>
<th>PE</th>
<th>Null allele frequency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA457</td>
<td>AR</td>
<td>5</td>
<td>0.733</td>
<td>0.796</td>
<td>0.748</td>
<td>0.57</td>
<td>0.0318</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>6</td>
<td>0.714</td>
<td>0.742</td>
<td>0.68</td>
<td>0.485</td>
<td>0.0075</td>
</tr>
<tr>
<td></td>
<td>Sl</td>
<td>9</td>
<td>0.654</td>
<td>0.817</td>
<td>0.776</td>
<td>0.62</td>
<td>0.0647</td>
</tr>
<tr>
<td></td>
<td>TH</td>
<td>5</td>
<td>0.6</td>
<td>0.614</td>
<td>0.532</td>
<td>0.335</td>
<td>0.0042</td>
</tr>
<tr>
<td>COR058</td>
<td>AR</td>
<td>7</td>
<td>0.833</td>
<td>0.8</td>
<td>0.756</td>
<td>0.587</td>
<td>-0.0339</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>9</td>
<td>0.964</td>
<td>0.873</td>
<td>0.842</td>
<td>0.716</td>
<td>-0.0623</td>
</tr>
<tr>
<td></td>
<td>Sl</td>
<td>8</td>
<td>0.727</td>
<td>0.815</td>
<td>0.766</td>
<td>0.598</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TH</td>
<td>10</td>
<td>0.839</td>
<td>0.847</td>
<td>0.813</td>
<td>0.669</td>
<td>-0.0039</td>
</tr>
<tr>
<td>COR070</td>
<td>AR</td>
<td>7</td>
<td>0.8</td>
<td>0.838</td>
<td>0.8</td>
<td>0.648</td>
<td>0.0142</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>7</td>
<td>0.821</td>
<td>0.75</td>
<td>0.698</td>
<td>0.517</td>
<td>-0.0632</td>
</tr>
<tr>
<td></td>
<td>Sl</td>
<td>7</td>
<td>0.824</td>
<td>0.802</td>
<td>0.748</td>
<td>0.579</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TH</td>
<td>8</td>
<td>0.806</td>
<td>0.821</td>
<td>0.782</td>
<td>0.625</td>
<td>0.0037</td>
</tr>
<tr>
<td>COR072</td>
<td>AR</td>
<td>4</td>
<td>0.633</td>
<td>0.605</td>
<td>0.524</td>
<td>0.326</td>
<td>-0.0315</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>6</td>
<td>0.536</td>
<td>0.599</td>
<td>0.556</td>
<td>0.376</td>
<td>0.0726</td>
</tr>
<tr>
<td></td>
<td>Sl</td>
<td>7</td>
<td>0.84</td>
<td>0.814</td>
<td>0.768</td>
<td>0.603</td>
<td>-0.0241</td>
</tr>
<tr>
<td></td>
<td>TH</td>
<td>6</td>
<td>0.742</td>
<td>0.767</td>
<td>0.711</td>
<td>0.52</td>
<td>0.0116</td>
</tr>
<tr>
<td>COR089</td>
<td>AR</td>
<td>5</td>
<td>0.867</td>
<td>0.759</td>
<td>0.704</td>
<td>0.515</td>
<td>-0.0777</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>8</td>
<td>0.857</td>
<td>0.849</td>
<td>0.813</td>
<td>0.669</td>
<td>-0.0149</td>
</tr>
<tr>
<td></td>
<td>Sl</td>
<td>7</td>
<td>0.632</td>
<td>0.795</td>
<td>0.739</td>
<td>0.564</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TH</td>
<td>6</td>
<td>0.419</td>
<td>0.495</td>
<td>0.456</td>
<td>0.287</td>
<td>0.1129</td>
</tr>
<tr>
<td>COR096</td>
<td>AR</td>
<td>3</td>
<td>0.567</td>
<td>0.636</td>
<td>0.545</td>
<td>0.332</td>
<td>0.0495</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>5</td>
<td>0.643</td>
<td>0.741</td>
<td>0.678</td>
<td>0.479</td>
<td>0.0618</td>
</tr>
<tr>
<td></td>
<td>Sl</td>
<td>4</td>
<td>0.267</td>
<td>0.356</td>
<td>0.324</td>
<td>0.189</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TH</td>
<td>4</td>
<td>0.452</td>
<td>0.451</td>
<td>0.37</td>
<td>0.201</td>
<td>-0.003</td>
</tr>
<tr>
<td>LEX073</td>
<td>AR</td>
<td>8</td>
<td>0.667</td>
<td>0.702</td>
<td>0.658</td>
<td>0.479</td>
<td>0.0214</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>6</td>
<td>0.679</td>
<td>0.603</td>
<td>0.548</td>
<td>0.362</td>
<td>-0.1101</td>
</tr>
<tr>
<td></td>
<td>Sl</td>
<td>8</td>
<td>0.389</td>
<td>0.716</td>
<td>0.668</td>
<td>0.495</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TH</td>
<td>7</td>
<td>0.774</td>
<td>0.745</td>
<td>0.687</td>
<td>0.496</td>
<td>-0.024</td>
</tr>
</tbody>
</table>

* Possible presence of null allele if frequency is greater than 0.05.

The most polymorphic markers were: COR070 in AR horses (PIC = 0.8), COR058 in Hc (PIC = 0.842) and in TH horses (PIC = 0.813), and CA457 in Sl horses (PIC = 0.776). The lowest polymorphism was shown by the following markers: COR072 (PIC = 0.524) in AR, LEX073 (PIC = 0.548) in Hc, COR096 (PIC = 0.324) in Sl and COR096 (PIC = 0.37) in TH horses (Table 2).

The probability of exclusion calculated separately for each locus corresponds to the PIC values for investigated markers in different breeds.

With the use of 7 microsatellites, the combined probability of exclusion (PEc) was 99.1 for Sl horses, 99.4 for AR horses, 99.7 for Hc horses and 99.0 for TH horses.
Discussion

In general, adjustment of the primer concentration was the main factor in obtaining well-readable DNA profiles for amplified loci in PCR multiplex conditions. However, despite the large increase in the primer concentration in the PCR multiplex, a small yield of PCR products was observed for the two markers (COR089 and COR096) with the largest alleles. To obtain well-readable DNA profiles for these markers the primer sequences were enlarged, which effectively improved the annealing conditions and thus the yield of PCR products. Another problem was the non-specific addition of adenine through Taq polymerase to the 3’ end of the PCR products during the amplification of markers with smaller alleles, such as CA457. This confused proper allele designation for this locus. Even the increase in the time for the final extension step did not eliminate the occurrence of +A products. Instead, the microsatellites with the largest alleles, especially those with imperfect dinucleotide repeats (COR096), were less affected by the +A phenomenon (Figure 1). Treatment of adenylated PCR products with polymerase T4 (Ginot et al., 1996) in locus CA457 could produce a DNA profile free of +A products but further redesign of the primer sequences to change the annealing conditions for CA457 would be desirable.

Deviations from H-W proportions were observed only in single loci in Hc (LEX073) and Sl horses (COR096) and probably resulted from genetic subdivision of these populations or a type I error connected with the smaller sample size. The significantly higher frequency of null alleles points to the potential occurrence of this phenomenon in two loci in Hc horses (COR072 and COR096) and in one locus in Sl (CA457) and TH horses (COR089). It was not possible to detect null alleles at these loci because of the lack of complete families in the studied populations. The higher frequency of homozygotes than the expected frequency detected in locus CA457 in Sl horses is more likely to be the result of mistyping errors connected with the impact of +A products on correct allele designation. Also, the observed excess of homozygotes in locus COR089 in Thoroughbreds is consistent with the narrow genetic basis for this horse breed. Other loci with higher frequency of null alleles require further investigation to detect a lack of allele amplification.

In this study, the polymorphism of selected microsatellite markers was investigated in different horse populations. Because of the pure breeding scheme, AR and TH horses are characterized by lower genetic variability in relation to the crossbred population of Sl horses or Hc primitive horses (Ząbek et al., 2003; Hamanova et al., 2001). Because of the polymorphic nature of tandem repeats, most of the investigated markers in the studied horse populations are characterized by great heterozygosity and high PIC values. The most useful for genetic analyses is marker COR058, which has the highest heterozygosity and PIC values in Hc and TH populations. High heterozygosity for COR058 locus was also reported in a range of primitive and riding horse populations from Germany (Aberle et al., 2004). As is shown in this study, two markers are less useful in particular breeds for
genetic testing because of their low allele number (COR096 in AR horses) and low polymorphism (PIC < 0.5) (COR089 in TH horses and COR096 in TH and Sl horses).

The PEc for wrongly determined parentage with the use of 7 microsatellites was equal to or above 99%, making this panel useful for parentage testing in the studied populations of 4 horse breeds.

In conclusion, the 7 microsatellites investigated are sufficiently polymorphic to achieve the required level of exclusion (PEc) and can be used for parentage testing in the studied horse breeds. In the case of some microsatellite loci, the potential occurrence of null alleles has to be taken into account, and as a consequence, the primer sequences for these loci need to be redesigned.

References


STRESZCZENIE

Oceniono przydatność 7 sekwencji mikrosatelitarnych DNA do testów weryfikacji pochodzenia koni w polskich populacjach koni czystej krwi arabskiej, koni huculskich, śląskich i pełnej krwi angielskiej. W loci testowanych markerów zidentyfikowano 12 nowych alleli mikrosatelitarnych w badanych populacjach koni. Z wyjątkiem dwóch markerów wykazujących niski polimorfizm w dwóch rasach koni, testowane markery posiadały wartość PIC > 0.5. Łączne prawdopodobieństwo wykluczenia dla 7 markerów mikrosatelitarnych było równe bądź wyższe od 99% w zależności od rasy. Przy opracowywaniu warunków reakcji PCR multipleks zmiana sekwencji starterów znacznie lepiej wpływa na jakość produktu PCR, aniżeli zwiększanie stężenia starterów w multipleksie.
APPLICATION OF CHROMOSOME MICRODISSECTION AND CHROMOSOME PAINTING TECHNIQUES FOR RECIPROCAL TRANSLOCATIONS DIAGNOSIS IN PIGS*

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3 Swiss Federal Institute of Technology, Zürich, Switzerland
4 Faculty of Veterinary Medicine, University of Zürich, Switzerland

Abstract
Chromosome microdissection and chromosome painting methods were used to confirm the diagnosis of the reciprocal translocation trcp(7;13)(q13;q46) in pigs, which had previously been identified using high-resolution RBA- and GTG-banding techniques. The experiment showed the usefulness of painting probes obtained with an aid of chromosome microdissection for diagnosis of reciprocal translocations or other chromosomal rearrangements in pigs.

Key words: pig, chromosome microdissection, chromosome painting, reciprocal translocations

Reciprocal translocations, involving an exchange of chromosome fragments of two different pairs, are structural aberrations common in pigs (Gustavsson, 1990; Long, 1991; Ducos et al., 2002). Amongst these rearrangements described so far (above 90) there are four cases of different translocations identified in the pig population bred in Poland: trcp(7;13)(q13;q26), trcp(8;14)(p21;q25), trcp(1;5)(q21;q21), trcp(9;14)(q14;q23) (Danielak-Czech et al., 1994; Rejduch et al., 2003).

The majority of reciprocal translocations originate “de novo” as a result of chromosome breaks connected with sensitivity of the pig genome to environmental effects (Danielak-Czech and Słota, 2004). Expression of these aberrations is shown by decreased litter size caused by a post-zygotic selection of aneuploid embryos (developed from chromosomally unbalanced gametes as a result of disturbed

* This work was conducted as part of NRIAP statutory activity, projects no. 3210.1 and 3212.1.
chromosome pairing during gametogenesis) (Danielak-Czech et al., 1996, 1997; Long, 1988; Świtoniński and Stranzinger, 1998; Rejduch et al., 2003, 2006). On the other hand, boars affected by translocations in balanced form contribute to a rapid rate of these defects among populations. Carriers of translocations have normal external appearance, and males have normal semen parameters.

In many countries, karyotype defects such as reciprocal translocations are considered as a major breeding problem due to their highly adverse effect on pig reproduction. Data presented in the literature show that reciprocal translocations reduce fertility by 5 to 100%, but their individual effects depend on the morphology of the chromosomes involved, the size of translocated chromatid fragments, and the location of breakpoints (Gustavsson, 1990; Long, 1991; Danielak-Czech et al., 1997).

In order to predict breeding consequences and prevent by early diagnosis, translocations need to be characterized precisely not only using classical cytogenetic techniques but also by molecular methods. For better diagnosis, several cases of translocations in hypoprolific boars-candidates for reproduction have been recently studied using chromosome painting with chromosome-specific molecular probes derived from flow-sorted chromosomes (Pinton et al., 1998, 2000; Ducos et al., 2002). The whole pig chromosome painting probes obtained by the chromosome microdissection method have not been used for this purpose until now.

The aim of this paper was to apply the chromosome microdissection and chromosome painting techniques for diagnosis of reciprocal translocations in pigs. The experiment was carried out on the basis of the trcp(7;13)(q13;q46) which had been identified previously by high-resolution G- and R-banding techniques supplemented with meiotic chromosome studies (including synaptonemal complex analysis at the first meiotic division) (Danielak-Czech et al., 1994, 1997).

Material and methods

Metaphase chromosomes of the hypoprolific boar carrying the trcp(7;13)(q13;q46) were obtained from lymphocyte cultures according to the standard protocol (Arakaki and Sparkes, 1963) and banded by the GTG technique (Wang and Fedoroff, 1972). The painting probes from whole 7 and 13 pig chromosomes were prepared at the Swiss Federal Institute of Technology (ETH, Zürich) by way of chromosome microdissection (ten copies each) using glass microneedles controlled by a manipulator attached to an inverted microscope. The dissected DNA material was amplified and labelled by DOP-PCR (using degenerate oligonucleotide primers: 5’-CCGACTCGAGN_6 ATGTGG- 3’) with biotin-16-dUTP and digoxygenin-11-dUTP (Telenius et al., 1992).

The labelled PCR products were purified in Nick Columns (Amersham Biosciences AB, Uppsala, Sweden) according to the manufacturer’s protocol, co-precipitated with salmon sperm DNA, and used in FISH experiments (Pinkel et al., 1986). Briefly, the chromosomes on slides were denaturated in 70% formamide in 2×SSC for 2.5 min at 70°C. The probe was denatured at 70°C for 10 min. The
hybridization was carried out at 37°C, overnight. Post-hybridization washes were as follows: three times at 50% formamide in 2×SSC and three times in 2×SSC at 42°C.

The biotynylated probe was detected using avidin conjugated to FITC and amplified by goat biotynylated anti-avidin antibody. The digoxygenin-labelled probe was detected with goat anti-digoxygenin antibody conjugated to rhodamine (all detection products were from Roche).

The chromosomes were counterstained with DAPI, and the slides were mounted in an antifade solution. Image analysis was performed under a fluorescent microscope (Opton Axiophot) equipped with a CCD camera, using triple attenuation filters DAPI/FITC/Rhodamine and the computer image analysis system Lucia-FISH (Laboratory Imaging Ltd, Prague, Czech Republic).

**Results**

The chromosome painting analysis of G-banded rearranged 7 and 13 chromosomes with specific probes derived from chromosome microdissection proved the reciprocity and size of exchanged fragments as well as accurately ascertained breakpoint locations in the 7q13 and 13q46 bands. The painted rearranged chromosomes were well visible in each metaphase observed (even in preparations of poor quality) (Figure 1). Dual-colour painting confirmed the tcrp(7;13)(q13;q46) diagnosis carried out previously with the use of high-resolution RBA and GTG-banding techniques and meiotic chromosome analyses (Danielak-Czech et al., 1994, 1997). The boar carrying the translocation being studied had normal exterior, and parameters of its semen (including vitality) did not vary from generally accepted evaluation standards.

**Discussion**

The number of reciprocal translocations described in pigs has strongly increased recently and reached above 90 (all of them related to a marked decrease in fertility or total infertility) (Gustavsson, 1990; Long, 1991; Danielak-Czech et al., 1996; Ducos et al., 2002; Rejduch et al., 2003). This can be explained by the intensification of the cytogenetic control systems of hypoprolific boars on the one hand, and the improvement of the G and R chromosome banding techniques on the other.

In case of the translocations, which cannot be characterized accurately even with the use of high-resolution banding techniques (e.g. microrearrangements or pericentromeric region exchanges), it is very useful to apply molecular methods, particularly chromosome painting. Such advanced cytogenetic analyses are based on fluorescent *in situ* hybridization (FISH) with flow-sorted or microdissected whole chromosome probes and primed *in situ* DNA labelling (PRINS) with
centromere-specific probes (Pinkel et al., 1986; Rønne, 1990; Yerle et al., 1991; Telenius et al., 1992; Pinton et al., 1998, 2000; Ducos et al., 2002).

A number of dual-colour chromosome painting experiments with probes prepared from thirteen flow-sorted pig chromosomes (supplemented by pancentromeric probes) have recently been carried out in France and made it possible to determine the refined formula of fifteen translocations, initially ascertained on the basis of G- and R-bands. The chromosome painting analysis made it possible to improve the characterization of eight translocations and maintain the proposed hypothesis for the other ones (Pinton et al., 1998, 2000; Ducos et al., 2002).

Dual-colour painting presented in this paper confirmed also the previous identification of trcp(7;13)(q13;q46) with high resolution RBA- and GTG-banding techniques (Danielak-Czech et al., 1994, 1997), including such important details as size of exchanged chromosome fragments and location of breakpoints — factors determining individual effects of particular translocations on fertility. This translocation was proved to decrease mean litter size by 48% and cause significant financial losses related to the use of the boar-carrier in a commercial herd, estimated (on the basis of simulation account) to be about 8,000 US dollars for natural mating and about 162,000 US dollars for artificial insemination in the active Polish pig population (Danielak-Czech et al., 1996).

It is worth noting that our experiment was the first one concerning the application of whole chromosome painting probes derived from chromosome microdissection for reciprocal translocation diagnosis in hypoprolific pigs. We found that the quality of pig chromosome preparations (due to the distinct morphological differentiation of target chromosomes) is not a limiting factor of the microdissection and chromosome painting. Our studies showed that such methods could effectively supplement classical banding techniques in reciprocal translocation diagnosis in metaphase cells. However, the use of molecular methods for karyotype evaluation is still little developed in pigs because the commercial painting probes for this species are not available.

The experiments described in this paper suggest that chromosome microdissection can be a very helpful technique to obtain a panel of pig chromosome-specific painting probes. Such molecular probes can be used for currently developing “sperm-FISH” technique which enables an accurate estimation of the rate of chromosomally unbalanced spermatozoa in boar semen. It will allow predicting in vitro the potential effect of different reciprocal translocations and other chromosomal rearrangements in young boars before reproduction (Ducos et al., 2002; Pinton et al., 2004). Such analyses followed by early selection of boars carrying karyotype abnormalities would efficiently prevent genetic defects from spreading in a population and limit economic effects.

References

Chromosome microdissection in diagnosis of reciprocal translocations in pigs

Accepted for printing 26 X 2006
Zastosowanie technik mikrodysekcji chromosomów i malowania chromosomów do diagnozy translokacji wzajemnych u świń

STRESZCZENIE

Przy wykorzystaniu metod mikrodysekcji chromosomów i malowania chromosomów potwierdzono diagnozę translokacji wzajemnej trcp(7;13)(q13;q46) u świń, przypadku zidentyfikowanego wcześniej wysoko rozdzielczymi technikami prażkowymi RBA i GTG. Opisany eksperyment wykazał przydatność sond malujących uzyskanych przy pomocy mikrodysekcji chromosomów do diagnozy translokacji wzajemnych lub innych reanżacji chromosomowych u świń.
COMPARISON OF THE G-BANDED KARYOTYPE OF THE FALLOW DEER (DAMA DAMA) AND SHEEP (OVIS ARIES)*

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Abstract
Karyotypes of the fallow deer (Dama dama) (2n = 68) and sheep (Ovis aries) (2n = 54) were compared based on G-banded chromosomes at the 450-band level. The common G-banded karyotype showed 28 pairs of fallow deer autosomes and heterosomes that were analogous to sheep chromosomes or sheep chromosome p and q arms. The analogy of the G-banding pattern in sheep and fallow deer suggests conservatism in the linear arrangement of genetic material on the chromosomes of these species, confirming that comparative cytogenetics can be a useful tool in gene mapping.

Key words: fallow deer (Dama dama), sheep (Ovis aries), comparative cytogenetics, G-banding

Comparative studies of the genomes of different animal species are based mainly on the phenomenon of genetic conservatism. This concerns chromosome banding patterns (Iannuzzi et al., 1990; Hayes et al., 1991; Ansari et al., 1999; Słota et al., 2001), nucleotide sequences (e.g. microsatellite sequences) (Edwards et al., 2000) and groups of linked or syntenic genes that often have the same relationships even in taxonomically distant species (Di Berardino et al., 2004; Kozubska-Sobocińska et al., 2005).

Comparison of karyotypes after differential staining of chromosomes using the GTG, RBA, RBG and QFQ techniques reveals conservatism at chromosome banding level (Słota et al., 2001). The identification of analogous chromosomes or fragments of them in the karyotypes of different animal species that are most often compared within systematic units provides further evidence that evolutionary relatedness is paralleled by karyotypic similarity (Iannuzzi and Di Meo, 1995).

* This work was conducted as part of NRIAP statutory activity, project no. 3209.1.
The conservative nature of some chromosomes in relation to syntenic groups makes it possible to use several molecular probes obtained from one species of animal to map the chromosomes of another species, using the FISH technique (Kozubska-Sobocińska et al., 2005).

The aim of the study was to compare the karyotype of the fallow deer (*Dama dama*) and sheep (*Ovis aries*) based on G-banding patterns obtained on GTG-stained metaphase chromosomes.

**Material and methods**

Comparison was made of the members of two families representing the suborder *Ruminantia*: fallow deer (*Dama dama*) of the *Cervidae* family (3 animals) and sheep (*Ovis aries*) of the *Bovidae* family (15 animals).

The analysed animals originated from the Experimental Station of the National Research Institute of Animal Production in Pawłowice (Romanov sheep) and from private farms (fallow deer).

Samples of blood extracted from the jugular vein into probes containing lithium heparin as an anticoagulant were investigated.

Metaphase chromosome preparations obtained after routine *in vitro* lymphocyte culture were analysed. The GTG differential staining technique was used for accurate identification of the chromosome pairs (Wang and Fedoroff, 1972).

The sheep karyotype was arranged based on the G-banding standard developed by Ansari et al. (1999). Because there is no international standard for the G-banding pattern of *Dama dama*, the karyogram for this species was arranged based on chromosome morphology, chromosome size and G-band homology (Świtoński et al., 2006). A comparative karyogram was then made by comparing the G-band patterns on the chromosomes of each species. The sheep karyogram was assumed to be the basic karyogram due to the existing international standard for this species. Fallow deer chromosomes from individual pairs were compared with sheep chromosomes and analogies were identified between them.

**Results**

Analysis of fallow deer and ovine metaphase chromosomes, routinely stained with 10% Giemsa solution, showed that the karyotype was normal in all the animals analysed and made it possible to compare these species in terms of chromosome number and morphology and fundamental number of autosomal arms (NF) (Table 1).

Based on the G-banding patterns obtained on fallow deer chromosomes following differential GTG staining, pairs of homologous chromosomes were juxtaposed and arranged into a karyogram with regard to morphological type, chromosome size and transverse band system (Figure 1).
Table 1. Comparison of *Ovis aries* and *Dama dama* karyotypes

<table>
<thead>
<tr>
<th>Species</th>
<th><em>Ovis aries</em> sheep</th>
<th><em>Dama dama</em> fallow deer</th>
</tr>
</thead>
<tbody>
<tr>
<td>2n</td>
<td>54,XX 54,XY</td>
<td>68,XX 68,XY</td>
</tr>
<tr>
<td>NF autosomes</td>
<td>58</td>
<td>68</td>
</tr>
<tr>
<td>Number of pairs of metacentric autosomes</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Number of pairs of acrocentric autosomes</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td>X heterosome</td>
<td>acrocentric</td>
<td>acrocentric</td>
</tr>
<tr>
<td>Y heterosome</td>
<td>metacentric</td>
<td>metacentric</td>
</tr>
</tbody>
</table>

Figure 1. G-banded karyotype of the fallow deer (*Dama dama*)
Comparison of GTG-stained ovine and fallow deer chromosomes showed complete conformity of G-banding patterns for 28 pairs of fallow deer autosomes (pairs of metacentrics and 27 acrocentric pairs) and heterosomes, with no homologous or homeologic chromosomes found in the sheep karyotype for fallow deer autosome pairs 18, 19, 29, 30 or 32 (Figure 2).
Discussion

Genetic conservatism concerning chromosome banding patterns has most often been analysed in species of the same family (Słota et al., 2003). The first comparative study in the Bovidae family showed band homology on the chromosomes of cattle, sheep and goats (Evans et al., 1973). These findings were confirmed by Iannuzzi and Di Meo (1995), who identified autosome pairs with a homologous pattern of G- and R-bands in these three species. These authors also performed detailed analyses of the X heterosome in cattle, water buffaloes, sheep and goats, and based on the analogies identified, suggested possible rearrangements of this chromosome in the evolutionary process (Iannuzzi and Di Meo, 1995).

Comparison of GTG-stained, haploid sets of sheep (2n = 54) and aoudad (Ammotragus lervia) chromosomes (2n = 58) revealed complete chromosome homology in the karyotypes of both species and indicated that centric fusions of autosomes led to evolutionary rearrangements (Słota et al., 2001).

Comparison was also made of the G-banding patterns of the X heterosome in sheep, goats and aoudads (Ammotragus lervia), showing full homology of the banding pattern in the acrocentric X heterosome of these three species (Słota et al., 2001).

Differences in karyotypes within Bovidae, due to different types of chromosome rearrangements, support the hypothesis that there was a common ancestor with the initial 2n = 60 karyotype (Wurster and Benirschke, 1968). It is assumed that in the course of evolution, the number of chromosomes was reduced as a result of Robertsonian translocations of acrocentric chromosomes. These suggestions are confirmed by studies of polymorphic forms of karyotype in Ovis sheep living in the wild, in which different diploid numbers of chromosomes were observed: 2n = 58 (O. vignei), 2n = 56 (O. ammon), 2n = 54 (O. aries, O. canadensis, O. dalli, O. musimon, O. orientalis), and 2n = 52 (O. nivicola) (Bruere et al., 1972; Bunch and Nadler, 1980).

The evolution of karyotypes of Bovidae species by way of centric fusions was corroborated by Hayes et al. (1991). According to these authors, despite different diploid numbers characteristic of Bovidae species (cattle and goats 2n = 60; sheep 2n = 54), the banding pattern obtained on chromosomes after RBA staining made it possible in most cases to arrange pairs of homologous chromosomes for the animal species compared. Only slight differences were found in the R-bands of chromosome pair 9 and X and Y heterosomes. This difference, diagnosed by the authors as paracentric inversion of a short fragment within the q arms of chromosome 9 in goats and cattle in relation to chromosome pair 9 in sheep, is further proof of the intrachromosomal evolutionary rearrangements in Bovidae.

The first karyotype patterns of the Bovidae species (cattle, sheep and goats), determined at an international conference in Reading (Ford et al., 1980), have been verified several times (Long, 1985; Ansari et al., 1999; Di Berardino et al., 2001), but there are no international banding standards for Cervidae species.

Karyotype studies of Cervidae animals (elk, roe deer, red deer, sika deer and fallow deer) living in the wild, conducted by Gustavsson and Sundt (1968),
concerned routinely stained metaphase chromosomes, which were classified according to size and morphology. For the *Dama dama* species, the 68,XY or 68,XX karyotype as well as the number of arms of autosomal chromosomes (68) were determined. In addition, one pair of long metacentric chromosomes and 32 pairs of acrocentrics were identified among the autosomes. For sex chromosomes, X was identified as the acrocentric chromosome and Y as a small submetacentric.

In the next attempts at determining the karyotype of fallow deer, the differential staining techniques used were GTG, with 350 G-bands obtained on metaphase chromosomes (Rubini et al., 1990) and RBA, with 527 bands obtained on prometaphase chromosomes (Lioi et al., 1994). In our analyses, the GTG-stained karyotype of fallow deer, used for comparison with G-banding patterns on the metaphase chromosomes of sheep, revealed 450 bands and helped to pinpoint homologous chromosomes in the species compared, indicating that a level of 450 bands is sufficient for comparative studies.

Cytogenetic comparative studies enable chromosome markers to be identified even in species representing different families, as exemplified by the pairs of homologous chromosomes identified in sheep and fallow deer. These analogies could be used in evolutionary studies as well as for diagnosing chromosomal changes in wild-living species whose karyotypes are much less known than the karyotypes of farm animals.

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Comparison of G-bands on fallow deer and sheep chromosomes


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Analiza porównawcza wzorów prążków G w kariotypie daniela (Dama dama) i owcy domowej (Ovis aries)

STRESZCZENIE

Porównano układ prążków G w kariotypie daniela (Dama dama) (2n = 68) i owcy (Ovis aries) (2n = 54), po zastosowaniu barwienia GTG z rozdzielczością 450 prążków. Zestawienie kariotypowych wzorów prążkowych wykazało analogię 28 par autosomów oraz pary heterosomów daniela w porównaniu z owcymi chromosomami lub ich ramionami p i q. Analogie wzorów prążków G u owcy i daniela sugerują konserwatywizm w linearnym uporządkowaniu materiału genetycznego w chromosomach tych gatunków, co potwierdza, że porównania cytogenetyczne mogą być przydatnym narzędziem w mapowaniu genów.
ADJUSTING THE METHOD OF MEAT-AND-BONE MEAL IDENTIFICATION IN FEED MIXTURES TO SPECIES IDENTIFICATION OF BLOOD PLASMA AND FOOD PRODUCTS

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Abstract

Oral ingestion is one of the transmission routes of prion diseases. In cattle, the sources of infection are animal meals containing pathogenic protein, while in humans, these are beef or beef products. Meals fed to animals and marketed processed food products must be inspected for the presence of undesirable components. The method for the identification of meat-and-bone meals in feed mixtures was developed several years ago and is routinely used in analyses. Market demand suggests that this method of analysing blood meal and food products should be modified. Plasma samples from porcine blood, analysed at the Department of Animal Immuno- and Cytogenetics of the National Research Institute of Animal Production, as well as commercial samples of pâté, jelly, sausage, brawn and minced meat, were studied. A method for the identification of porcine components based on mtDNA was used in the study. The analytical cycle included the isolation of DNA from the samples, amplification of the isolated DNA in a PCR reaction, agarose gel electrophoresis and analysis of the results of electrophoretic separation. DNA was isolated using Wizard kit and silica with casein. The PCR reaction between porcine starters and DNA, isolated using plasma silica gave a product of 212 bp, characteristic of the porcine component. Similarly, the use of the Wizard isolation kit for food products produced positive results. The present study shows that the methods developed can be used for routine control of commercial food products to determine whether they contain illegal components.

Key words: porcine DNA, prion diseases, mtDNA

Prion diseases are a major epidemiological threat to both cattle and humans. The pathogenic factor for this disease are prions present in the bodies of diseased animals. Prions can enter the bodies of other animals through animal meals, and the bodies of people eating beef or beef products. Prevention of prion diseases includes monitoring animal meals and food products. An mtDNA (mitochondrial DNA) marker is used for the species identification of animals. It is widely used in this kind of test due to its large variation among vertebrates (Saccone and Sbisa, 1994; Wolstenholme, 1992) and the presence of many million copies in every cell (Michaels et al., 1996).
A recent method that uses mtDNA to identify meat-and-bone meals enables analysis of meat-and-bone meals processed at 133°C and 3 Ba for 20 min (Natonek et al., 2004). In practice, many meals are produced from blood plasma or feathers rather than from whole animals. In addition, food products are increasingly subjected to expert analysis. During the production process, each of these products is subjected to different thermal and pressure treatments, which necessitates an individual approach to analysing every type of sample.

It is therefore appropriate to adjust the methods of meat-and-bone meal identification to identify the animal component in blood plasma and in food products. The study was carried out to analyse the porcine component declared by food producers.

**Material and methods**

Porcine blood plasma and samples of food products (pâté, jelly, jelly beans, sausage, brawn and minced meat) were analysed. The negative control was a feed mixture without meat-and-bone meal, and the positive control was porcine DNA.

The analytical process included the isolation of DNA from the analysed samples, amplification of the isolated mtDNA in a PCR reaction, agarose gel electrophoresis and analysis of the results of electrophoretic separation. DNA was isolated from blood plasma using silica, which is a standard method employed for feed mixtures. It uses a guanidine thiocyanate (GuSCN) buffer, a suspension of silicon dioxide (SiO₂) and alpha-casein. The method is based on the property of silicon, which binds DNA in the presence of GuSCN (Boom et al., 1999; Tartaglia et al., 1998). Alpha-casein binds degraded DNA and is eluted with DNA in the final stage of isolation (Boom et al., 1990). DNA from food products was isolated using a Wizard kit in accordance with the manufacturer’s protocol.

Isolated DNA was amplified in a PCR reaction using porcine starters (Lahiff et al., 2001) and a thermal programme (Natonek et al., 2004). The concentration of particular components in the mixture was as follows: 10× Buffer — 1×; dNTPmix — 0.2 mM; AmpliTaq Gold polymerase — 1 U; gelatin — 0.028%; MgCl₂ — 1.50 mM; each primer — 0.24 µM.

The PCR reaction product was separated during horizontal electrophoresis in 3% agarose gel and identified by comparison with an X 174 DNA/Hae III marker (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 bp). The meal obtained from pigs in feed mixtures was identified on the basis of a DNA product of 212 bp.

**Results**

The results of the porcine component identification in the analysed samples are given in the figures below. Figure 1, which presents the result of electrophoresis, shows that a product of 212 bp was obtained from a PCR reaction between porcine
starters and DNA isolated from jelly beans (1), pâté (2), jelly (3), sausage (4), brawn (5) and minced meat (6). Likewise, the amplification of DNA isolated from blood plasma (Figure 2, lane 1) gave a product characteristic of the porcine component.

Figure 1. Gel image obtained after electrophoresis: 1) jelly beans, 2) pâté, 3) jelly, 4) sausage, 5) brawn, 6) minced meat, 7) positive control, 8) negative control, 9) PCR control, M) marker

Figure 2. Gel image obtained after electrophoresis: 1) blood plasma, 2) negative control, 3) positive control, 4) PCR control, M) marker

Discussion

The literature describes several methods for the isolation of DNA from animal components. GuSCN and silica are conventionally used for animal meals and were used in the present study to analyse blood plasma. This technique, although a modified method of DNA isolation from hard tissues, performs well in the species identification of blood plasma. Many studies have also used Chelex-100
(Bio-Rad), which allows for identification of material heated to 80°C (Wang et al., 2000) and the BACC-3 Amersham kit, which enables DNA to be isolated from samples processed at 130°C (Frezza et al., 2003). For raw material, a method using SDS has often been applied (Sambrook et al., 1989). The species identity of the food product samples analysed in the present study was successfully determined using the Wizard kit. This technique enabled us to isolate DNA from raw and processed meat. Isolated DNA can later be analysed for the presence of components from other species (not stated by the producer), making it possible to eliminate them. The use of mtDNA enables identification from both raw and processed tissues. The present results show the effectiveness of the animal component identification method in a wide range of samples differing in the type of material from which it was made and in the processing method. The development of this method is valuable for the prevention of prion diseases.

References


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Dostosowanie metody identyfikacji maćzek mięsno-kostnych w mieszankach paszowych do analizy gatunkowości plazmy z krwi oraz przetworów spożywczych

STRESZCZENIE

Jednym ze sposobów przenoszenia chorób prionowych jest droga pokarmowa. W przypadku bydła źródłem zakażenia są maćki zwierzęce mogące zawierać chorobotwórcze białko, a w przypadku ludzi wołowina lub jej przetwory. Skarmianie zwierzę maćzkami oraz dopuszczenie do sprzedaży przetworów spożywczych wymaga ich kontroli na obecność niepożądanych komponentów. Metoda identyfikacji maćek mięsno-kostnych w mieszankach paszowych została opracowana kilka lat temu i jest rutynowo wykorzystywana do analiz. Potrzeby rynku sugerują zmodyfikowanie i dostosowanie tej metody do analizy maćki z krwi i przetworów spożywczych. Materiałem badawczym były próbki plazmy z krwi wieprzowej będącej przedmiotem ekspertyz w Dziele Immuno- i Cytogenetyki Zwierząt Instytutu Zootechniki oraz komercyjne próbki galaretki spożywowej, pasztetu, kiełbasy, salcesonu, mięsa mielonego.

W badaniach stosowano metodę identyfikacji komponentów wieprzowych na podstawie mtDNA. Cykl analityczny obejmował izolację DNA zawartego w badanych próbkach, amplifikację wyizolowanego DNA w reakcji PCR, elektroforezę w żelu agarowym oraz analizę wyników rozdziału elektroforetycznego. Do badań wykorzystano izolację DNA Wizardem oraz krzemionką z kazeiną. Reakcja PCR między starterami wieprzowymi a DNA, wyizolowanym za pomocą krzemionki z plazmy z krwi, dała produkt o długości 212pz, charakterystyczny dla komponentu wieprzowego. Podobnie pozytywne rezultaty dało zastosowanie do przetworów spożywczych izolacji zestawem Wizard. Z przeprowadzonych badań wynika, że opracowane metody mogą być wykorzystane do rutynowej kontroli komercyjnych przetworów spożywczych w kierunku zakazanych komponentów.
ANTIGENIC MARKERS OF SHEEP PLASMA GLOBULINS IN PREDICTING RESISTANCE TO MAMMARY GLAND INFECTIONS*

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Abstract
The aim of the study was to determine the usefulness of sheep plasma globulin epitopes for predicting udder resistance to infections. A total of 253 and 262 Polish Merino ewes, reared in two NRIAP Experimental Stations, were investigated. Udder health was tested three times during lactation using field methods; palpation, measurement of the electric resistance of milk and Bovi-Vet Indicator Paper Test were done. These provisional tests were validated based on laboratory determinations of the somatic cell count (SCC) per cm³ and the presence of pathogenic microorganisms in milk. The flocks analysed in 2004 (Koluda Wielka) and 2005 (Pawłowice) were ultimately divided into groups of 71 and 74 ewes with diagnosed mastitis (mastitis⁺) and groups of 182 and 188 mastitis-free ewes (mastitis⁻). The blood of all ewes was analysed for the presence of six antigenic markers of beta-globulins and IgM immunoglobulins (A11, A12, A13, A14, A15, A16), using the double immunodiffusion test on agar gel. Analysis of statistical relationships (χ² and success frequency tests) showed significant correlations between good udder health and the presence of A11, A12, A13 and A16 epitopes. The results obtained indicate that at least two of the epitopes used (A11 and A13) can be regarded as indicators for resistance of Polish Merino sheep to mammary gland infections.

Key words: sheep, plasma, globulins, epitopes, mastitis

The growth and rearing of lambs depends on the milk yield of ewes, which is determined to a large extent by udder health. In Poland, an average of 20% ewes suffer from mastitis, a disease that can affect as many as 60% of the animals in a flock (Charon et al., 1996; Świderek et al., 1999). Mastitis can vary in form and intensity, but even its subclinical form adversely affects milk quality. Problems with diagnosis and the limited action of veterinary drugs have motivated breeders to find and apply breeding methods that use genetic variation in resistance to mastitis (Charon, 1994).

* This study was conducted as part of the research project no. 3 P06D 009 24, financed by the State Committee for Scientific Research.
Detecting and exploring the indicators of mastitis resistance that can be used as selection criteria in sheep breeding seems to be of key importance (Bergonier et al., 2003). The possibility of using antigenic markers of plasma globulins for this purpose was indicated in the 1990s, when researchers used epitopes of alpha-, beta- and immunoglobulins that were identified during this period (Skiba and Węgrzyn, 1993; Charon, 1994; Charon et al., 1996). From that time on, six new protein markers of different sheep plasma fractions were obtained at the Department of Animal Immuno- and Cytogenetics of the National Research Institute of Animal Production.

The aim of the present study was to determine the usefulness of newly detected epitopes of plasma globulins for predicting the resistance of sheep to udder infections.

**Material and methods**

Polish Merino ewes, used in sheep farms of the National Research Institute of Animal Production in 2004 and 2005, were investigated. A flock of 253 sheep was studied at the Experimental Station in Kołuda Wielka in the first year, and a flock of 262 sheep at the Experimental Station in Pawłowice was studied in the second year. During lactation, milk samples were taken from the sheep three times: at ∼56, 86 and 116 days in 2004, and at ∼30, 60 and 90 days of milk production period in 2005. Sheep blood was sampled once (253 samples in Kołuda Wielka Station and 262 samples in Pawłowice Station).

Ewe udder health (each udder half separately) was tested during milk sampling by palpation and by measurement of the electric resistance of milk (using a Dramiński electronic mastitis detector), and verified using Bovi-Vet Indicator Paper (Pakulski and Osikowski, 1997). The threshold value for healthy udders was assumed to be 330 electric resistance units (ERU) of milk from each udder half (Świderek et al., 1999). Lower values or differences of more than 50 ERU between the milk of the right and left udder half resulted in a ewe being included in the group with suspected mastitis. The indicator paper test was used to verify ∼10% of the milk samples. Based on the obtained observations, ewes were included either in the group of animals with suspected clinical/subclinical mastitis or in the group of mastitis-free animals.

Laboratory tests of milk samples (468 samples in 2004 and 499 samples in 2005) were performed to verify this preliminary segregation of animals.

The milk from animals with suspected mastitis was analysed for the somatic cell count (SCC) in 1 cm³ using flow cytometry (Fossomatic). The presence of pathogenic microorganisms was determined using the plate method (following Instruction No. 48 of the Ministry of Agriculture, Department of Veterinary Services of 22 September 1978, concerning the routine identification of pathogenic microorganisms in the udder) and further identified with API biochemical tests (Malinowski et al., 2003). The milk of ewes from the group of mastitis-free animals
was also subjected to control analyses at a laboratory. Based on the overall results of field tests and laboratory determinations, the analysed flock of ewes was divided into a group with diagnosed mastitis (mastitis\(^+\)) and a mastitis-free group (mastitis\(^-\)).

The blood of all sheep was analysed for the presence of six newly detected antigenic markers of plasma globulins. Five immune sera containing six different alloantibodies were used for the determinations. These detect epitopes associated with proteins of mainly beta-globulin fractions with differential molecular weight and immunoglobulin IgM. Particular antigenic markers, identified with immune sera nos. 5814, 5822, 5756, 2948 and 5759, were provisionally designated as A11, A12, A13, A14, A15 and A16 (antibodies of serum no. 5759 detected two different epitopes). Natural and immune sera were analysed in the double immunodiffusion test on agar gel (TPI) according to Ouchterlony (1953). The occurrence of a precipitation line indicated the presence of the analysed marker, and thus the marker-carrying globulins (Skiba et al., 2000).

The presence and significance of statistical differences between particular epitopes in ewe blood and the susceptibility (or resistance) of these animals to mammary gland infections were determined. For this purpose, the chi-square tests \(\chi^2\) and the success frequency validation tests were performed.

**Results**

**Field evaluation of udder health**

Reduced electric resistance of milk (below 330 ERU) was detected in 72 sheep in 2004 (flock in Kołuda Wielka). In 25 animals, this concerned the milk from both udder halves, and in 47 animals the milk from one udder half only. In ten other ewes, the electric resistance of milk did not drop below the threshold value, with differences in the measurement results between the right and the left half (above 50 ERU). A total of 82 ewes were therefore included in the group with suspected mastitis. Udder palpation in these ewes showed lesions (udder indurations and lumps) in the udder of 19 ewes.

In the next year of the study (2005, flock in Pawłowice), 79 ewes (30% of the flock) were included in the group of sheep with suspected mastitis. Reduced electric resistance of milk from both udder halves was noted in 29 sheep, and in the milk from one udder half in the other 43 sheep. The remaining 7 ewes were included in this group based on differences in the electric resistance of milk from different udder halves. Palpation showed the presence of udder indurations and lumps in \(\sim24\%\) of the sheep from the group with suspected mastitis.

**Laboratory analysis of milk**

The results of SCC tests and the presence of pathogenic microorganisms (etiological factors of mastitis) in the milk in the year 2004 and 2005 are shown in Table 1.
Table 1. Somatic cell count (SCC) and the presence of pathogenic microorganisms in the milk of Polish Merino ewes with suspected mastitis in 2004 and 2005

<table>
<thead>
<tr>
<th>Milk samples</th>
<th>Sampling period</th>
<th>Milk samples with SCC determined per cm³ milk (thous.)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 500</td>
<td>500 – 1000</td>
</tr>
<tr>
<td>A — day 56</td>
<td>70</td>
<td>38.89</td>
<td>10</td>
</tr>
<tr>
<td>B of milking</td>
<td>10</td>
<td>14.28</td>
<td>8</td>
</tr>
<tr>
<td>A — day 86</td>
<td>44</td>
<td>26.19</td>
<td>8</td>
</tr>
<tr>
<td>B of milking</td>
<td>18</td>
<td>40.91</td>
<td>7</td>
</tr>
<tr>
<td>A — day 116</td>
<td>34</td>
<td>28.33</td>
<td>5</td>
</tr>
<tr>
<td>B of milking</td>
<td>18</td>
<td>52.94</td>
<td>4</td>
</tr>
<tr>
<td>A Total</td>
<td>148</td>
<td>31.63</td>
<td>23</td>
</tr>
<tr>
<td>B</td>
<td>46</td>
<td>31.08</td>
<td>20</td>
</tr>
</tbody>
</table>

2004 (flock in Kołuda Wielka)

2005 (flock in Pawłowice)

Milk samples with SCC below 500,000 in 1 cm³ accounted for 31.63% of the samples in 2004 and for 39.68% of the samples from ewes with suspected mastitis in 2005. Almost 30% of these samples (31.08% in Kołuda Wielka and 28.28% in Pawłowice) were infected with pathogenic microorganisms. In the flock investigated in 2004, 4.91% of all the samples obtained contained 500,000 – 1,000,000 somatic cells per cm³, but as much as 86.96% of them contained pathogens. In Pawłowice (2005), the respective figures were 18.44% and 60.89%. Even a higher percentage of infected milk samples occurred in the 297 samples with SCC exceeding 1,000,000 per cm³ (as much as 96.63% in 2004 and 89% in 2005).

Microorganisms were detected in 353 and 298 milk samples (75.43% and 59.72%), which were collected from ewes with suspected mastitis in 2004 and 2005. The number of milk samples infected with particular pathogens in those years is shown in Figure 1. The bacteria most often isolated from milk in Kołuda Wielka were streptococci, with *Streptococcus uberis* detected in the largest number (107) of milk samples. Staphylococci dominated in the milk in Pawłowice, with *Sta-
Phylococcus aureus detected in almost 25% of the infected samples. Escherichia coli were numerous (in 2004), as were Candida yeast-like fungi. Smaller numbers of pathogens such as Pseudomonas aeruginosa and Corynebacterium pyogenes were detected.

In the milk of 11 ewes from the group with suspected mastitis, singled out from all the animals based on field tests in 2004, as well as in the milk of 5 sheep from the analogous group in 2005, none of the samples was found to contain an elevated SCC (≥500,000) or pathogenic microorganisms. Therefore, these ewes were excluded from the group of sheep with detected mastitis.

**Presence of protein epitopes in sheep sera**

The determinations of antigenic markers A11, A12, A13, A14, A15 and A16 in blood sera of 253 ewes from Koluda Wielka (2004), and in blood sera of 262 ewes from Pawłówice (2005), are given in column IV of Table 2. The frequency of these
epitopes in the examined populations and in designated groups of sheep are presented in Table 3. Marker A15 was the most common and was detected in the blood of ~94% animals. Markers A16, A13 and A12 occurred in 84–85%, 83% and 79% of the examined ewes, respectively. The A11 epitope was detected in the serum of half the flock, while the A14 determinant was only present in several animals examined (6 in 2004 and 7 in 2005). The frequency of A11, A12, A13, A15 and A16 epitopes in the group of sheep free from mastitis was higher than the

Table 2. Statistical relationships and their significance between the presence of antigenic markers of globulins in blood and udder health in the ewes analysed in 2004 and 2005

<table>
<thead>
<tr>
<th>Markers in blood: present (+) or absent (−)</th>
<th>Udder health</th>
<th>Total number of animals</th>
<th>Statistical relationships and their significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mastitis+</td>
<td>mastitis−</td>
<td>χ² s.s. = 1</td>
</tr>
<tr>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
</tr>
<tr>
<td>2004 (flock in Kołuda Wielka)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A11+</td>
<td>25</td>
<td>99</td>
<td>124</td>
</tr>
<tr>
<td>A11−</td>
<td>46</td>
<td>83</td>
<td>129</td>
</tr>
<tr>
<td>A12+</td>
<td>51</td>
<td>150</td>
<td>201</td>
</tr>
<tr>
<td>A12−</td>
<td>20</td>
<td>32</td>
<td>52</td>
</tr>
<tr>
<td>A13+</td>
<td>42</td>
<td>169</td>
<td>211</td>
</tr>
<tr>
<td>A13−</td>
<td>29</td>
<td>13</td>
<td>42</td>
</tr>
<tr>
<td>A14+</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>A14−</td>
<td>68</td>
<td>179</td>
<td>247</td>
</tr>
<tr>
<td>A15+</td>
<td>65</td>
<td>173</td>
<td>238</td>
</tr>
<tr>
<td>A15−</td>
<td>6</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>A16+</td>
<td>55</td>
<td>159</td>
<td>214</td>
</tr>
<tr>
<td>A16−</td>
<td>16</td>
<td>23</td>
<td>39</td>
</tr>
<tr>
<td>2005 (flock in Pawłowice)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A11+</td>
<td>27</td>
<td>104</td>
<td>131</td>
</tr>
<tr>
<td>A11−</td>
<td>47</td>
<td>84</td>
<td>131</td>
</tr>
<tr>
<td>A12+</td>
<td>51</td>
<td>156</td>
<td>207</td>
</tr>
<tr>
<td>A12−</td>
<td>23</td>
<td>32</td>
<td>55</td>
</tr>
<tr>
<td>A13+</td>
<td>44</td>
<td>174</td>
<td>218</td>
</tr>
<tr>
<td>A13−</td>
<td>30</td>
<td>14</td>
<td>44</td>
</tr>
<tr>
<td>A14+</td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>A14−</td>
<td>71</td>
<td>184</td>
<td>255</td>
</tr>
<tr>
<td>A15+</td>
<td>67</td>
<td>179</td>
<td>246</td>
</tr>
<tr>
<td>A15−</td>
<td>7</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>A16+</td>
<td>57</td>
<td>165</td>
<td>222</td>
</tr>
<tr>
<td>A16−</td>
<td>17</td>
<td>23</td>
<td>40</td>
</tr>
</tbody>
</table>

* 0.01 < P ≤ 0.05.
** P ≤ 0.01.

Significance of differences: a — with 5% error, b — with 1% error, c — with 0.1% error.
frequency in the group of ewes infected, both in the flock in Kołuda Wielka and in the flock in Pawłowice. The highest difference occurred for epitope A13 (0.59 in the mastitis+ group vs. 0.93 in the mastitis− group), and the lowest for marker A15 (0.92 and 0.95 in 2004 and 0.91 and 0.95 in 2005, respectively). The frequency of the A14 marker in the group of diseased sheep was slightly higher in both flocks than its frequency in the mastitis− group (0.04 and 0.02).

Table 3. Frequency of globulin epitopes in the analysed populations of Polish Merino — total in flock and in groups of sheep differing in udder health

<table>
<thead>
<tr>
<th>Antigenic marker</th>
<th>Frequency of epitope total in flock</th>
<th>Mastitis+ group</th>
<th>Mastitis− group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2004¹</td>
<td>2005²</td>
<td>2004¹</td>
</tr>
<tr>
<td>A11</td>
<td>0.49</td>
<td>0.50</td>
<td>0.35</td>
</tr>
<tr>
<td>A12</td>
<td>0.79</td>
<td>0.79</td>
<td>0.72</td>
</tr>
<tr>
<td>A13</td>
<td>0.83</td>
<td>0.83</td>
<td>0.59</td>
</tr>
<tr>
<td>A14</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>A15</td>
<td>0.94</td>
<td>0.94</td>
<td>0.92</td>
</tr>
<tr>
<td>A16</td>
<td>0.84</td>
<td>0.85</td>
<td>0.77</td>
</tr>
</tbody>
</table>

no. of sheep 253 262 71 74 182 188

¹ flock in Kołuda Wielka.
² flock in Pawłowice.

Statistical calculations

Statistical correlations concerning the presence of epitopes in blood in 2004 were analysed in a group of 71 ewes (87% of ewes with suspected mastitis), in which different degrees of mastitis were diagnosed (mastitis+ — column II of Table 2), and in a group of 182 mastitis-free ewes (mastitis− — column III of Table 2). In 2005, 74 and 188 sheep, respectively, were included in these groups. The results of the statistical tests are shown in columns V (chi-square) and VI (success frequency test) of the same table. In both flocks, highly significant correlations (P ≤ 0.01) were found between udder health and the presence of A11 and A13 epitopes in sheep blood. In addition, in 2005 there were statistically significant relationships (0.01 < P ≤ 0.05) between udder health and the presence of the other two markers (A12 and A16).

Discussion

Both the field test of udder health and the laboratory milk analyses were aimed to assign the animals to groups of sheep differing in the health status of the mammary gland. Determination of the somatic cell count in milk and its microbiological analyses are standard methods for determining the condition of the mammary gland in ruminants (Charon et al., 1996; Malinowski, 2001; Pengov,
(2001) and in this study they were used to validate the results of the field tests. The high percentage of ewes with suspected mastitis in which the incidence of mastitis was confirmed based on milk analyses (86.59% in Kołuda Wielka and 93.67% in Pawłowice), are indicative that straightforward milk resistance measurements are efficient in preliminary identification of infected udders.

In both investigated populations, as the somatic cell count in milk increased, the number of samples infected with pathogenic microorganisms also increased. This tendency persisted on all three dates of milk sampling. The microflora detected in milk was found to contain microorganisms considered as pathogenic agents of mastitis (Vishinsky and Markusfeld-Nir, 1995; Charon et al., 1996; Pakulski at al., 2000; Malinowski at al., 2003). In the first year of the study (Kołuda Wielka), the most frequently isolated microorganism was *Streptococcus uberis*, which is included in the group of environmental pathogens. In Pawłowice, *Staphylococcus aureus*, considered by specialists in the field as one of the major etiological factors of mastitis (Tietze et al., 1999; Pakulski et al., 2000; Pengov, 2001), was dominant.

Comparison of the frequency of A11, A12, A13 and A16 (very similar in both analysed flocks) in the mastitis+ and mastitis− groups, and in the whole population of the ewes suggests positive correlations between the presence of these markers in sera and the favourable health status of the udder. For other markers, small differences in frequency were observed between the groups of sheep with different udder condition (0.02 for A14 in both 2004 and 2005, and 0.04 in 2004 and 0.03 in 2005 for A15), which indicates that there is no association between the presence of these epitopes in serum and the mammary gland condition.

These conjectures were confirmed by the results of statistical analyses. They showed statistical relationships between the presence of four antigenic markers (A11, A12, A13 and A16) in blood and the good udder condition of the analysed animals. The highly significant correlation between two epitopes (A11 and A13) and udder health was shown in 2004 in Kołuda Wielka and confirmed in 2005 in Pawłowice. For the other markers (A12 and A16), the $\chi^2$ values calculated in 2004 were close to the theoretical $\chi^2$ value ($P \leq 0.05$), which at that time could only suggest a relationship between the presence of each marker in blood and the favourable udder status of Polish Merino. The significance of these correlations was proved statistically in 2005.

The best health status of the mammary gland is associated with the presence of epitopes A11 and A13. The associations of this type were only studied in one breed of sheep, i.e. Żelaźnieńska (Charon, 1994; Charon et al., 1996). It was detected during that time that favourable udder health was highly significantly correlated with the presence of two antigenic determinants of beta-globulins (A2 epitope, connected with molecules with molecular weight of ~160 kDa and NS1 marker, carried by macromolecules with a molecular weight of over 200 kDa) and one marker of immunoglobulins (GB1 epitope, a marker of IgG antibody heavy chains). According to the authors of this study, two of the described markers might be good selection indicators for resistance of sheep to mastitis. In Holstein cattle, differences were found in the mastitis sensitivity of cows with different markers of IgG2
immunoglobulins (Kelm et al., 1997). Significantly higher resistance to infection was shown by cows with IgG2a allotypes in relation to those with IgG2b allotypes. The above studies with sheep and cattle showed the usefulness of epitopes, carried by the molecules of proteins of the same fractions as the globulins used in the present study.

The results obtained suggest that at least two newly identified markers of serum globulins (out of the six used in this study), provisionally designated as A11 and A13, can be considered as indicators of the insensitivity of Polish Merino ewes to udder infections. It also appears that continuing the studies in this breed might finally determine the usefulness of the next two epitopes (A12 and A16) in this respect. The possibility of using antigenic markers in breeding practice should be viewed while taking into account the complexity of mastitis resistance, which is a disease of complex etiology. The possible use of the identified epitopes of serum globulins in ewes selection for improved udder health requires further studies in other sheep breeds.

It is concluded that resistance of Polish Merino ewes to udder infections is related to the presence in sheep of globulins carrying antigenic markers A11, A12, A13 and A16. The best udder health is correlated with the presence of A11 and A13 determinants. The detected relationships suggest the possibility of considering these epitopes as indicators of the insensitivity of Polish Merino ewes to udder infections.

Acknowledgements

The author thanks Dr Kazimierz Korman for his technical assistance in organizing and performing the study at the Experimental Station of the National Research Institute of Animal Production in Kołuda Wielka, Dr Ireneusz Dymarski, Director of the Experimental Station of the National Research Institute of Animal Production in Pawłowice, for making this study possible in his facility, and Marek Grycz MSc for generous help in organizing work at the sheep farm in Kociugi in the second year of the study.

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PIOTR KRZYŚCIN

Antygenowe markery globulin osocza krwi owiec w prognozowaniu odporności na zakażenia gruczołu mlekowego

STRESZCZENIE

Celem badań było określenie przydatności epitopów globulin osocza krwi u owiec w prognozowaniu odporności na zakażenia gruczołu mlekowego. Badaniami objęto 253 oraz 262 maciorki merynowska polska, utrzymywane w dwóch Zakładach Doświadczalnych Instytutu Zootechniki. Trzykrotnie w okresie laktacji oceniano stan zdrowotny wymion metodami terenowymi; wykonywano badanie palpacyjne, pomiar oporności elektrycznej mleka i test bibuł wskazówkowych. Te wstępna ocenę weryfikowano na podstawie oznaczeń laboratoryjnych w mleku: liczby komórek somatycznych (l.k.s.) oraz obecności mikroorganizmów patogennych. Ostatecznie stada, badane w 2004 roku w Kołudzie Wielkiej oraz w 2005 w Pawłowicach, podzielono na grupy 71 i 74 owiec ze stwierdzonym stanem zapalnym wymienia (mastitis+) oraz grupy 182 i 188 zwierząt wolnych od tego schorzenia (mastitis−). Krew wszystkich maciordek przebadano na obecność sześciu markero- w antygenowych beta-globulin i immunoglobulin IgM (A11, A12, A13, A14, A15, A16), stosując test podwójnej immunodyfuzji w żelu agarowym. Analiza zależności statystycznych (testy χ² i frekwencji sukcesu) wykazała istnienie istotnych powiązań pomiędzy korzystnym stanem zdrowotnym wymienia a obecnością epitopów A11, A12, A13 i A16. Uzyskane wyniki wskazują na możliwość uznania co najmniej dwóch z użytych epitopów (A11 i A13) za wskaźniki odporności owiec rasy merynowska polska na zakażenia gruczołu mlekowego.
IS THERE A RELATIONSHIP BETWEEN COAT COLOUR, AGE AND RACING PERFORMANCE IN HORSES?

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Abstract

The aim of the study was to investigate the hypothesis that racing scores are influenced by the combined effects of the horse’s age and coat colour. A total of 11,567 starts of 974 Thoroughbred horses and 732 Purebred Arabian horses that ran in flat races were considered. Scores from two-year-olds and three-year-olds of eumelanic, phaeomelanic and grey colour classified according to genotypes on the basis of the MC1R and Grey loci were compared. The number of starts, logarithm of earnings, logarithm of earnings per start and general handicap were scored. The results do not support the hypothesis. In the study, no combined effect of the horse’s age and basic colour on racing performance was found: both two-year-old and three-year-old bay and black horses achieved similar racing scores compared to chestnuts and a relationship was not found for grey and non-grey colours. The coat colour of a foal does not help to predict racing performance in Thoroughbreds or Purebred Arabians at the age of two or three years.

Key words: horse, racing performance, coat colour

Horse breeders and users would be interested in knowing whether the performance of horses differs according to their colour. This would make the early assessment of foals more accurate. It is possible for such a relationship to exist since the genes producing coat colour can be localized on the same parts of chromosomes as the polygenes responsible for horse performance. A linkage would be particularly likely if QTLs were involved in performance (Edwards et al., 1987). A pleiotropy of colour or performance genes may also exist (Falconer, 1981).

Skorkowski (1976), on the basis of an analysis performed on Thoroughbred mares, stated that “speed over short distances up to 2000 metres is as characteristic of chestnuts as endurance over distances greater than 2000 metres is of bays. The chestnut two-year-olds won more, on average, than bays; on the other hand, three-year-old bays won more than chestnuts.” Skorkowski associated the chestnut colour with early maturity, speed, and lower resistance, and the bay colour with late
maturity, endurance and higher resistance. These findings are discussed in the world literature, by Galizzi Vecchiotti (1986) and Sponenberg (2003), among others. The difference in the results of chestnuts over short distances and bays over longer distances is difficult to examine since currently, there are few races over distances greater than 2000 metres. There have been some studies on the possible relationship between horse’s colour and racing performance. An analysis carried out on winners of five English classic races in 1777–1938 found no differences related to coat colour (reported by Galizzi Vecchiotti, 1986). A similar result based on the racing scores of Thoroughbreds at the age of three years was obtained by Dušek (1980). Likewise, the latest investigation of racing scores in differently coloured horses led to the conclusion that basic (bay, chestnut and black) and grey (intermingled with white) coat colours do not have an important relationship to racing performance (Stachurska et al., 2006). A very weak relationship may result from the brightness of the hair and the activity of the MC1R locus. None of these analyses, however, consider the possibility of a relationship between coat colour and age, and no studies have documented or rejected Skorkowski’s findings.

The aim of this study was to examine the hypothesis that racing scores are influenced by the combined effect of the horse’s age and coat colour.

**Material and methods**

The study examined 11,567 starts of 974 Thoroughbred horses and 732 Purebred Arabian horses that ran in flat races at the Warsaw-Służewiec course over three years, in the first and second seasons of their career: Thoroughbreds as two-year-olds and three-year-olds and Arabians as three-year-olds and four-year-olds. The following indices were scored to measure the horses’ performance: the number of starts (Starts), the logarithm of earnings (Log), the logarithm of earnings per start (Log/start), and the general handicap (GH). The earnings (in zloty) were converted into the logarithm because of the skew distribution. The horses were of basic and grey colours. Although shades of bay and chestnut colours are identified in racehorses, in the present study they were not considered since their inheritance mechanisms are not fully documented (Stachurska et al., 2002). The basic colours are controlled by the ASIP and MC1R loci (Adalsteinsson, 1974), which are located on ECA22 and ECA3, respectively (Marklund et al., 1996, Rieder et al., 2001). The grey pattern is produced by the Grey locus (Salisbury, 1941) found on ECA25 (Henner et al., 2002, Swinburne et al., 2002). The horses were classified according to age and genotypes in two loci controlling coat colour:

— MC1R locus, i.e. bay and black (E-) horses of eumelanin colours (eumelanin mixed with phaeomelanin in bays, eumelanin only in blacks) versus chestnut (ee) horses of phaeomelanin colour,
— Grey locus, i.e. grey (G-) horses versus non-grey (gg) horses.

The significance of differences between the horse groups was estimated by analysis of variance:
\[ Y_{ijkl} = \mu + CA_i + G_j + B_k + e_{ijkl} \]

where: \( Y_{ijkl} \) is the logarithm of the trait value, \( \mu \) — the overall mean, \( CA_i \) — the colour combined with the age (\( i = 4 \), as specified above), \( G_j \) — the gender (\( j = 3: \) stallions, mares, geldings), \( B_k \) — the breeder (\( k = 3: \) Polish state studs, Polish private breeders, foreign breeders), and \( e_{ijkl} \) — the random error.

To focus on the case studied by Skorkowski (1976), scores from mares of both breeds of solely bay and chestnut colours were compared according to colour combined with age, and taking the breeder effect into account. The results of all the analyses are presented as Least Square Means (LSM) and Standard Errors (SE).

Results

There were no differences in the indices of Thoroughbreds and Arabians of eumelanic and phaeomelanic colours at the same age (Table 1). All of the important differences were associated exclusively with age: older horses of both breeds started more frequently, and gained a higher General Handicap, and three-year-old Thoroughbreds achieved a greater log of earnings than two-year-olds. Following the findings of Skorkowski (1976) and taking into account solely bay and chestnut mares, the result is similar (Table 2). In this case, there are fewer differences related to age due to the smaller number of individuals.

Comparison of grey and non-grey horses showed that there is no considerable difference in the racing scores of horses of the same age (Table 3). Instead, older horses of both colours ran more often and non-grey Thoroughbreds at the age of three years obtained a greater General Handicap than at the age of two years.

The logarithm of earnings per start did not show any important differences in the examined groups.

Discussion

The study does not show any influence of coat colour on horses’ racing performance, whereas an effect of age on the number of starts and General Handicap, and to some extent on the logarithm of earnings, is evident. In contrast to the findings of Skorkowski (1976), two-year-old chestnut mares do not win more races than bays and three-year-old bays do not win more races than chestnuts. However, it should be mentioned that a weak effect may appear in homozygous eumelanic (EE) horses since the dominance of the E gene in heterozygotes might be not complete. This may also be the case with the G locus: GG horses could differ slightly from Gg horses.

The present investigation was performed using the current statistical methods and on a considerably larger body of data than that used in the Skorkowski study under discussion (1976). Nowadays, it is known that the E allele can produce bay or
Table 1. Racing scores from horses of eumelanic (bay and black) and phaeomelanic (chestnut) colours at different age

<table>
<thead>
<tr>
<th>Colour</th>
<th>Age (years)</th>
<th>N</th>
<th>Starts</th>
<th>Log</th>
<th>Log/start</th>
<th>N_GH</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LSM</td>
<td>SE</td>
<td>LSM</td>
<td>SE</td>
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<tr>
<td>Thoroughbreds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eumelanic</td>
<td>2</td>
<td>477</td>
<td>2.81 A</td>
<td>0.3</td>
<td></td>
<td>6.15 Aa</td>
<td>0.4</td>
</tr>
<tr>
<td>Phaeomelanic</td>
<td>2</td>
<td>126</td>
<td>2.80 C</td>
<td>0.4</td>
<td></td>
<td>5.70 BC</td>
<td>0.5</td>
</tr>
<tr>
<td>Eumelanic</td>
<td>3</td>
<td>463</td>
<td>6.07 A</td>
<td>0.3</td>
<td></td>
<td>6.83 AB</td>
<td>0.4</td>
</tr>
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<td>Phaeomelanic</td>
<td>3</td>
<td>133</td>
<td>5.66 B</td>
<td>0.4</td>
<td></td>
<td>6.84 Ca</td>
<td>0.4</td>
</tr>
<tr>
<td>Purebred Arabians</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eumelanic</td>
<td>3</td>
<td>212</td>
<td>4.89 A</td>
<td>0.4</td>
<td></td>
<td>6.61</td>
<td>0.5</td>
</tr>
<tr>
<td>Phaeomelanic</td>
<td>3</td>
<td>73</td>
<td>4.50 C</td>
<td>0.5</td>
<td></td>
<td>6.05</td>
<td>0.6</td>
</tr>
<tr>
<td>Eumelanic</td>
<td>4</td>
<td>109</td>
<td>6.92 A</td>
<td>0.5</td>
<td></td>
<td>6.84</td>
<td>0.5</td>
</tr>
<tr>
<td>Phaeomelanic</td>
<td>4</td>
<td>23</td>
<td>6.46 B</td>
<td>0.7</td>
<td></td>
<td>6.58</td>
<td>0.7</td>
</tr>
</tbody>
</table>

N — number of horses; N_GH — number of horses with General Handicap assigned.

A-D, a — differences between LSMs in columns marked with the same letters within one breed significant at: capitals P<0.01, small letters P<0.05.

Table 2. Racing scores from bay and chestnut mares at different age

<table>
<thead>
<tr>
<th>Colour</th>
<th>Age (years)</th>
<th>N</th>
<th>Starts</th>
<th>Log</th>
<th>Log/start</th>
<th>N_GH</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LSM</td>
<td>SE</td>
<td>LSM</td>
<td>SE</td>
</tr>
<tr>
<td>Thoroughbreds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bay</td>
<td>2</td>
<td>244</td>
<td>3.35 A</td>
<td>0.2</td>
<td></td>
<td>6.98 a</td>
<td>0.3</td>
</tr>
<tr>
<td>Chestnut</td>
<td>2</td>
<td>62</td>
<td>3.58 C</td>
<td>0.3</td>
<td></td>
<td>7.25</td>
<td>0.4</td>
</tr>
<tr>
<td>Bay</td>
<td>3</td>
<td>209</td>
<td>7.04 A</td>
<td>0.2</td>
<td></td>
<td>7.59 a</td>
<td>0.3</td>
</tr>
<tr>
<td>Chestnut</td>
<td>3</td>
<td>68</td>
<td>6.47 B</td>
<td>0.3</td>
<td></td>
<td>7.36</td>
<td>0.4</td>
</tr>
<tr>
<td>Purebred Arabians</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bay</td>
<td>3</td>
<td>89</td>
<td>4.90 A</td>
<td>0.3</td>
<td></td>
<td>6.56</td>
<td>0.4</td>
</tr>
<tr>
<td>Chestnut</td>
<td>3</td>
<td>30</td>
<td>4.79 B</td>
<td>0.4</td>
<td></td>
<td>6.31</td>
<td>0.6</td>
</tr>
<tr>
<td>Bay</td>
<td>4</td>
<td>34</td>
<td>6.41 A</td>
<td>0.4</td>
<td></td>
<td>7.23</td>
<td>0.5</td>
</tr>
<tr>
<td>Chestnut</td>
<td>4</td>
<td>10</td>
<td>5.38 B</td>
<td>0.8</td>
<td></td>
<td>5.44</td>
<td>1.0</td>
</tr>
</tbody>
</table>

N — number of horses; N_GH — number of horses with General Handicap assigned.

A-D, a — differences between LSMs in columns marked with the same letters within one breed significant at: capitals P<0.01, small letters P<0.05.
Table 3. Racing scores from grey and non-grey horses at different age

<table>
<thead>
<tr>
<th>Colour</th>
<th>Age (years)</th>
<th>N</th>
<th>Starts</th>
<th>Log</th>
<th>Log/start</th>
<th>N_{GH}</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LSM</td>
<td>SE</td>
<td>LSM</td>
<td>SE</td>
<td></td>
</tr>
<tr>
<td>Thoroughbreds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grey</td>
<td>2</td>
<td>28</td>
<td>3.12</td>
<td>AB</td>
<td>0.5</td>
<td>6.54</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-grey</td>
<td>2</td>
<td>603</td>
<td>2.75</td>
<td>CD</td>
<td>0.3</td>
<td>6.10</td>
<td>0.4</td>
</tr>
<tr>
<td>Grey</td>
<td>3</td>
<td>33</td>
<td>5.79</td>
<td>AC</td>
<td>0.5</td>
<td>6.35</td>
<td>0.6</td>
</tr>
<tr>
<td>Non-grey</td>
<td>3</td>
<td>596</td>
<td>5.93</td>
<td>BD</td>
<td>0.3</td>
<td>6.87</td>
<td>0.3</td>
</tr>
</tbody>
</table>

| Purebred Arabians |            |     |        |      |           |        |    |
| Grey       | 3           | 317 | 5.49   | AB   | 0.4       | 6.48   | 0.5 |
| Non-grey   | 3           | 293 | 5.16   | CD   | 0.4       | 6.64   | 0.5 |
| Grey       | 4           | 159 | 7.41   | AC   | 0.4       | 6.93   | 0.5 |
| Non-grey   | 4           | 135 | 7.12   | BD   | 0.4       | 6.95   | 0.5 |

N — number of horses; \(N_{GH}\) — number of horses with General Handicap assigned.

A-D, a — differences between LSMs in columns marked with the same letters within one breed significant at \(P<0.01\).
black colours, depending on the ASIP locus. Hence, in the present investigation bays with blacks could have been classified together. However, to examine the hypothesis more accurately, exclusively bay mares were also considered. Since the MC1R and ASIP loci are assigned to autosomes, gender could have been regarded only as a factor in the statistical model, whereas groups of different sexes and colours or a sex-colour interaction were not distinguished. The result in Thoroughbreds was also confirmed in Arabians. All of these aspects of the study guarantee reliable results.

Overall, the answer to the question investigated is negative: racing scores from horses of eumelanic and phaeomelanic colour and of the same age are similar, and the results for bay and chestnut mares are also similar. In conclusion, the results do not support Skorkowski’s hypothesis. In the study, no combined effect of the horse’s age and basic colour on racing performance was found, and nor was such a relationship found for grey and non-grey colours. The coat colour of a foal does not help to predict racing performance in Thoroughbreds or Purebred Arabians at the age of two or three years.

References


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ANNA STACHURSKA, MIROSŁAW PIĘTA

Czy istnieje związek między maścią konia i jego wiekiem a dzelnością wyścigową?

STRESZCZENIE

Celem pracy było zweryfikowanie hipotezy na temat wpływu połączonego efektu wieku konia i maści na wyniki wyścigowe. Przeanalizowano rezultaty 11 567 startów w wyścigach płaskich 974 koni pełnej krwi angielskiej i 732 koni czystej krwi arabskiej. Porównano wyniki dwuletnich i trzyletnich maści eumelanicznej, feomelanicznej i siwej sklasyfikowanych według genotypu w loci MC1R i G. Rozpatrywano liczbę startów, logarytm sumy wygranych, logarytm sumy wygranych na liczbę startów i generalny handicap. Rezultaty badań nie potwierdzają hipotezy. Nie stwierdzono połączonego wpływu wieku konia i maści podstawowej na dzelność wyścigową. Zarówno dwuletki, jak i trzyletki maści gniadnej i karej osiągnęły podobne wyniki wyścigowe do koni kasztanowatych. Nie znaleziono również takiego związku z maścią siwą i nie siwą. Rodzaj maści źrebięcia nie ułatwia przewidywania dzelności wyścigowej koni pełnej krwi angielskiej i czystej krwi arabskiej ani w wieku dwu ani trzech lat.
Modern dairy cattle production requires new tools for fast, reliable and more effective breeding and parentage verification at herd scale. In this paper, we present a chip containing 32 SNPs (Single Nucleotide Polymorphisms) for determining two genetic diseases: Bovine Leukocyte Adhesion Deficiency (BLAD) and Complex Vertebral Malformations (CVM). Three QTL genes: CSN3 (kappa-casein), DGAT1 (acylCoA:diacylglycerol acyltransferase) and GHR (growth hormone receptor), together with other SNPs form a panel useful in marker-assisted selection and veterinary control. SNPs were genotyped using the PCR-APEX (Arrayed Primer Extension) technique. Special attention was paid to evaluation of the 32-SNPs chips as an alternative approach to parentage and identity control. Based on the allele frequency estimations, for a sample of 40 of Polish Holstein-Friesian cattle, the probability that a randomly chosen candidate parent would be excluded from paternity or maternity was estimated to be 99.6% when the genotypes of both parents were known and 93.7% when the genotype of only one parent of a calf was available. For the presented marker set, the probability of identity was 10–12, which enables animals and their products to be conclusively distinguished among millions of individuals. Further improvements for upcoming chip versions were also considered.

Key words: Holstein cattle, SNP microarray, paternity control, genetic disease carriers

In modern animal breeding, different approaches for more effective selection and higher breeding gain are in demand. On a farm scale, selection programmes created by breeders may require information on genetic defects, QTL markers and confirmation of paternity. This specific information comes through different routes (veterinary services and breeding organizations).
Identity and parentage control of livestock is a particularly indispensable element of every efficient breeding programme. An accurate pedigree analysis is necessary in selecting the best animals to be the parents of future generations, evaluating their breeding value, and maintaining animal food safety and quality. Traditionally, parentage testing in cattle has been performed using blood group typing, but in the 1990s molecular tests based on short tandem repeat (STR, microsatellites) polymorphism were gradually introduced, and are now recommended in many countries. Numerous studies have shown the usefulness of microsatellite sequences for identification and parentage verification in cattle (Glowatzki-Mullis et al., 1995; Usha et al., 1995; Heyen et al., 1997; Peelman et al., 1998; Radko et al., 2004). More recently, single nucleotide polymorphism (SNP) has been proposed as an alternative approach (Fries and Durstewitz, 2001). The main advantages of SNPs over STRs are: (1) genetic stability; (2) suitability for standardized representation of genotyping results as a digital DNA signature (Fries and Durstewitz, 2001; Werner et al., 2004); (3) no need for a specific typing platform; and (4) amenability to automated high throughput genotyping systems (Vignal et al., 2002). Two separate sets of highly informative SNP markers with sufficient power for use in individual identification and kinship analysis in beef and dairy cattle populations have been described (Heaton et al., 2002; Werner et al., 2004). Recently, the usefulness of SNP markers to verify sample tracking in a commercial slaughter facility during beef processing has been shown (Heaton et al., 2005).

Our group has developed and validated an oligonucleotide microarray consisting of 77 SNPs addressed to study the genetic background of the protein content of cow’s milk (Kaminski et al., 2005). The aim of the current report is to compose a 32 SNP chip and to evaluate it as a potential source of useful information in practical selection, veterinary control, parentage control and identity verification in Holstein dairy cattle.

Material and methods

Forty individuals (25 cows and 15 bulls) of the Polish Holstein-Friesian breed were included in the analysis. The animals were unrelated and chosen from several herds and insemination centres. 1 ml of blood or one commercial straw of semen was used to extract genomic DNA according to the manufacturer’s instructions (MasterPure DNA Purification Kit, Epicentre).

The chip content and SNP genotyping procedure have been described in detail previously (Kaminski et al., 2005). Briefly, gene fragments containing SNPs were PCR-amplified from genomic DNA, then purified, fragmented and utilized as a template using the APEX (Arrayed Primer Extension) microarray technique. Each APEX process consists of a sequencing reaction primed by an oligonucleotide anchored with its 5’end to a glass slide and terminating one nucleotide before the polymorphic site. The extension with one fluorescently labelled dideoxynucleotide
complementary to the variant base reveals the polymorphism. Reading the incorporated fluorescence identifies the base in the target sequence. APEX primers were spotted onto 24 × 60 mm aminosilane plus phenylene diisothiocyanate-coated microarray slides (Asper Biotec) using a ChipWriter Pro spotter (Virtek Vision Corp.). After APEX reaction, the signals were acquired by a custom-built total internal reflection fluorescence (TIRF)-based charge coupled device (CCD) detector. Images were processed using Image Pro Plus software. SNP genotypes were identified using Genorama 4.0 and PicDB software (Kurg et al., 2000). Each SNP was represented on the chip in duplicate for both strands, thus generating 4 times more information on each SNP. The current study was conducted with a modified version of an SNP chip supplemented by a few additional SNPs (Table 1). The sequences of PCR and APEX primers for analysis of these additional SNPs are available on request.

Based on the frequency of detected alleles, the suitability of the 32-SNP chip for parentage control and individual verification was evaluated utilizing the probability of exclusion (PE) and probability of identity (PI) parameters. Exclusion probability was defined as the probability that an alleged parent is excluded as a potential parent, assuming that the alleged parent is a random individual. The exclusion probability for each SNP marker was calculated for two cases: 1. (PE1), genotypes are known for an alleged parent, a confirmed parent and an offspring and 2. (PE2), genotypes are known for an alleged parent and an offspring but the genotype of a confirmed parent is unknown, according to formulas described by Jamieson and Taylor (1997).

\[
PE_1 = \sum_{i}^{n} p_i (1-p_i)^2 - \sum_{i \geq j=1}^{n} (p_i p_j)^2 [4 - 3(p_i + p_j)]
\]

\[
PE_2 = \sum_{i}^{n} p_i^2 (1-p_i)^2 + \sum_{i \geq j=1}^{n} 2p_i p_j (1-p_i - p_j)^2
\]

where: \(p_i, p_j\) — are the frequencies of the i-th and j-th allele and \(n\) — is the number of alleles.

The overall probability of exclusion for all markers included in the system is given by the equation:

\[
PE = 1 - (1 - PE \text{ for locus 1}) (1 - PE \text{ for locus 2})...(1 - PE \text{ for locus } n)
\]

The probability of identity was defined as the estimated probability that two unrelated individuals selected at random would possess identical multilocus genotypes and was calculated for individual and combined SNP markers according to Waits et al. (2001):
\[ PI = \prod_{i=1}^{r} \left( \sum_{j=1}^{n_i} p_i^4 + 4 \sum_{j=1}^{n_j} p_i^2 p_j^2 \right) \]

where: \( p_i, p_j \) — are the frequencies of the i-th and j-th allele, \( n \) — is the number of alleles, and \( r \) — is the number of markers.

**Results**

Using optimized PCR-APEX technology, 1280 individual genotypes (40 animals × 32 SNPs) were recorded. Genotypes were identified by reading filtered signal intensity data evaluated statistically using specialized software. Of the 77 SNP markers analysed by MilkProtChip for the purposes of milk protein yield and content survey (Kaminski et al., 2005), 24 SNPs were selected and supplemented by 8 new SNPs as appropriate to dispute falsely reported pedigree records and for cattle identification. The selection criteria were based on the frequency of the minor SNP allele (greater than 0.1) and the chromosome map position of each SNP (SNPs on different chromosomes or not closely linked were preferred).

Table 1. Allele frequencies, probabilities of exclusion and probabilities of identity of 32 SNPs identified in the analysed individuals of Polish Holstein cattle

<table>
<thead>
<tr>
<th>Locus</th>
<th>SNP No.</th>
<th>GenBank Acc. No.</th>
<th>SNP allele1/position2 allele2</th>
<th>Frequency of allele2</th>
<th>PE1</th>
<th>PE2</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSN2</td>
<td>4</td>
<td>M55158</td>
<td>C8101A</td>
<td>0.49</td>
<td>0.187</td>
<td>0.125</td>
<td>0.375</td>
</tr>
<tr>
<td>CSN3</td>
<td>8</td>
<td>X14908</td>
<td>A5345C</td>
<td>0.16</td>
<td>0.116</td>
<td>0.036</td>
<td>0.571</td>
</tr>
<tr>
<td>LGB</td>
<td>11</td>
<td>X14710</td>
<td>G1740C</td>
<td>0.48</td>
<td>0.187</td>
<td>0.125</td>
<td>0.375</td>
</tr>
<tr>
<td>LGB</td>
<td>16</td>
<td>X14710</td>
<td>A5233G</td>
<td>0.13</td>
<td>0.100</td>
<td>0.026</td>
<td>0.624</td>
</tr>
<tr>
<td>LALBA</td>
<td>18</td>
<td>U63109</td>
<td>A263G</td>
<td>0.43</td>
<td>0.185</td>
<td>0.120</td>
<td>0.380</td>
</tr>
<tr>
<td>PRL</td>
<td>21</td>
<td>X16641</td>
<td>T1286C</td>
<td>0.28</td>
<td>0.161</td>
<td>0.081</td>
<td>0.437</td>
</tr>
<tr>
<td>LTF</td>
<td>39</td>
<td>AY036584</td>
<td>C216G</td>
<td>0.27</td>
<td>0.158</td>
<td>0.078</td>
<td>0.445</td>
</tr>
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<td>ITGB2</td>
<td>45</td>
<td>M81233</td>
<td>C880T</td>
<td>0.19</td>
<td>0.130</td>
<td>0.047</td>
<td>0.526</td>
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<tr>
<td>GH</td>
<td>46</td>
<td>M57764</td>
<td>C303T</td>
<td>0.20</td>
<td>0.134</td>
<td>0.051</td>
<td>0.514</td>
</tr>
<tr>
<td>IGF I</td>
<td>48</td>
<td>AF210383</td>
<td>C1407T</td>
<td>0.42</td>
<td>0.184</td>
<td>0.119</td>
<td>0.382</td>
</tr>
<tr>
<td>IGF I</td>
<td>49</td>
<td>AF210386</td>
<td>C270T</td>
<td>0.21</td>
<td>0.138</td>
<td>0.055</td>
<td>0.501</td>
</tr>
<tr>
<td>BUT</td>
<td>50</td>
<td>Z93323</td>
<td>C2281A</td>
<td>0.39</td>
<td>0.181</td>
<td>0.113</td>
<td>0.388</td>
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<td>POU1F1</td>
<td>52</td>
<td>Y15995</td>
<td>G1256A</td>
<td>0.28</td>
<td>0.161</td>
<td>0.081</td>
<td>0.437</td>
</tr>
<tr>
<td>DGAT1*</td>
<td>54</td>
<td>AJ318490</td>
<td>G10433A</td>
<td>0.41</td>
<td>0.183</td>
<td>0.117</td>
<td>0.383</td>
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<td>OBESE</td>
<td>58</td>
<td>AJ236854</td>
<td>T305C</td>
<td>0.43</td>
<td>0.185</td>
<td>0.120</td>
<td>0.380</td>
</tr>
<tr>
<td>COXPI</td>
<td>59</td>
<td>Y14076</td>
<td>T217C</td>
<td>0.14</td>
<td>0.106</td>
<td>0.029</td>
<td>0.605</td>
</tr>
<tr>
<td>COXPI</td>
<td>60</td>
<td>Y14076</td>
<td>A436G</td>
<td>0.32</td>
<td>0.170</td>
<td>0.095</td>
<td>0.414</td>
</tr>
<tr>
<td>IL8</td>
<td>61</td>
<td>AF061521</td>
<td>A1227G</td>
<td>0.46</td>
<td>0.187</td>
<td>0.123</td>
<td>0.377</td>
</tr>
<tr>
<td>APOE</td>
<td>67</td>
<td>X64839</td>
<td>G159C</td>
<td>0.37</td>
<td>0.179</td>
<td>0.109</td>
<td>0.394</td>
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<tr>
<td>NOS2</td>
<td>68</td>
<td>AF333248</td>
<td>A90C</td>
<td>0.18</td>
<td>0.126</td>
<td>0.044</td>
<td>0.540</td>
</tr>
<tr>
<td>NOS2</td>
<td>69</td>
<td>AF333248</td>
<td>G5494C</td>
<td>0.50</td>
<td>0.188</td>
<td>0.125</td>
<td>0.375</td>
</tr>
</tbody>
</table>
Based on the allele frequency estimates performed for a random sample of the economically most important and common dairy breed, the suitability of chosen SNPs for parentage and identity control was evaluated (Table 1). The combined probability of exclusion in a bull-dam-calf combination for the whole set of SNPs was 99.6%. In the event of there being no genotype information from a confirmed parent, the probability of detecting a falsely alleged parent was 93.7%. The theoretical probability of identity was calculated as $8.93 \times 10^{-12}$. The likelihood of there being a coincidental genotype match between 2 animals with the same 32 SNP genotypes was approximately 1 in 112 billion.

### Discussion

In the world of animal production, methods to identify superior animals and their products are of great importance for accurate selection, animal health and food safety. In this work we attempted to construct a mini-chip containing SNPs of practical value for breeders. The chip presented in this paper contains SNPs determining two genetic diseases (BLAD — Bovine Leukocyte Adhesion Deficiency, and CVM — Complex Vertebral Malformations), which are required to be identified in Holstein sires in most countries to avoid the spreading of undesired alleles which cause losses in health and reproduction. Additionally, 3 QTL genes — CSN3 (kappa-casein), DGAT1 (acylCoA:diacylglycerol acyltransferase) and GHR (growth hormone receptor) — were included in the 32-SNP panel and can be used in marker-assisted selection to identify animals carrying desirable genotypes. Also,
the genotypes of a handful of SNPs in candidate genes were obtained to be eventually used in cow selection. We recently showed that some of these (e.g. LTF, lactoferrin) are associated with milk performance traits (Kamiński et al., 2006).

The presented mini-chip was also evaluated as a potential tool for parentage control in dairy cattle. Commercial tests such as “Stock Marks for Cattle Parentage Verification Bovine II” (Applied Biosystems) are now available and utilized for parentage testing in many countries. The alternative approach for cattle parentage and identity analysis based on SNP (Fries and Durstewitz, 2001) requires the development of a highly informative set of markers with easily scored alleles. Such sets of SNP markers with discrimination ability comparable to STR markers have been developed (Heaton et al., 2002; Werner et al., 2004).

The probability of exclusion calculated for the 32-SNP chip (0.996) was higher than that achieved for serological systems reported by Holm and Bendixen (1996) and Radko et al. (2004) (0.98 and 0.84, respectively). In Holstein cattle, similar PE values were obtained for six and seven STR marker sets (Glowazki-Mullis et al., 1995; Peelman et al., 1998) (0.992 and 0.997, respectively). An eleven STR marker system, e.g. Stock Marks for Cattle Parentage Verification Bovine II, ensures higher PE values (0.999) for Holstein cattle (Heyen et al., 1997). Higher PE (0.999) and PI (1.92×10^{-13}, 8.59×10^{-15}) values for beef cattle and the Holstein breed were also achieved by the SNP marker sets described by Heaton et al. (2002) and Werner et al. (2004). These sets comprise an equal or greater number of SNPs (32 and 37, respectively).

Paternity or maternity exclusion in the absence of the other parent genotype is desirable because it may reduce the cost of genotyping by nearly 50%. Moreover, as calves are bought and sold, new owners may not have access to complete sire-dam-calf genotype information, yet need to investigate the accuracy of the paternity or maternity claim (Heaton et al., 2002).

It is noted that, at the current stage, the collection of SNPs is less informative for parentage and identity testing than those selected by US and German groups; however, our strategy is to construct an assay not for single, but rather multipurpose, applications. There is a kind of contradiction in the dual application of SNPs: those linked to or involved in selection should not be used for paternity control because after several generations, one allele may be disappearing from the population, decreasing its value in paternity/maternity control. Therefore, the content of the chip should be dynamically redesigned by adding new SNPs if the frequency of minor alleles (in SNPs developed so far) decreases too much. The lower information content of SNP markers, compared to highly polymorphic STR markers, can be easily compensated by using greater numbers of markers, scored simultaneously in an automated high throughput genotyping system. One can simulate that fifty SNPs with an average minor allele frequency of 20% will yield an exclusion power of 0.999 and a probability of identity of 3.98×10^{-15} (data not shown).

Prospectively, the chip should mostly consist of non-coding SNPs with balanced frequency which guarantee very reliable parentage control and some functional
SNPs useful in breeding and veterinary control. Obviously, further efforts are necessary to validate the chip in Holstein cattle and PCR multiplexing in order to reduce the overall cost of single analysis.

The mini-chip described offers an alternative that is cheaper than separated selection analysis and parentage testing, and may therefore be of interest to some categories of breeders utilizing their own selection criteria independent of larger selection programmes.

In conclusion, the results point the way for the construction of a standardized set of single nucleotide polymorphism markers as a new tool for selection, veterinary control, identity and parentage analysis. The current version of the 32-SNP chip represents a promising foundation for a future updated chip version.

It is noteworthy that the suggested collection of SNPs could also be used for research on evolutionary studies, evaluating the genetic distances between wild and domestic cattle breeds, investigation into the domestic history of bovine species, and the development of so-called “traceability tests”.

References


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Mikropłytka SNPs o różnorakich zastosowaniach w hodowli bydła holsyńsko-fryzyjskiego

STRESZCZENIE

Nowoczesna produkcja bydła mlecznego wymaga nowych narzędzi do szybkiej, wiarygodnej i bardziej efektywnej hodowli oraz weryfikacji pochodzenia w skali stada. W pracy prezentujemy chip zawierający 32 polimorfizmy typu SNP (Single Nucleotide Polymorphisms) umożliwiające określenie dóch chorób genetycznych (BLAD — Bovine Leukocyte Adhesion Deficiency, CVM — Complex Vertebral Malformations), trzy geny QTL (Quantitative Trait Loci): CSN3 (kappa-casein), DGAT1 (acylCoA:diacylglycerol acyltransferase) and GHR (growth hormone receptor), które razem z pozostałymi SNPs tworzą panel użyteczny w selekcji wspomaganej markerami i kontroli weterynaryjnej. Genotypy SNPs zostały określone techniką PCR-APEX (Arrayed Primer Extension). Specjalną uwagę zwrócono na ocenę 32 SNPs jako alternatywnej metody kontroli pochodzenia i identyczności zwierząt. Na podstawie szacowanych frekwencji alleli, dla próby 40 sztuk bydła polskiego holsyńsko-fryzyjskiego, prawdopodobieństwo, że losowo wybrany rodzic będzie wykluczony zostało oszacowane na poziomie 99,6% w przypadku, gdy genotypy obu rodziców są znane i na poziomie 93,7% wówczas, gdy genotypy tylko jednego z rodziców ciecia są dostępne. Zaprezentowany zestaw markerów osiągnął prawdopodobieństwo identyczności rzędu $10^{-12}$, co pozwala na wyraźną dyskryminację zwierząt i ich produktów spośród milionów osobników. Omówiono dalsze udoskonalenia dla kolejnej wersji chipu.
CORRELATIONS BETWEEN BACKFAT THICKNESS AND LOIN EYE AREA MEASUREMENTS AND FATNESS AND MUSCLING OF CUTS IN LINE 990 × PIETRAIN PIGS

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Abstract
Data from a detailed dissection of 38 crossbred gilts (line 990 × Pietrain) were studied. Analysis included backfat thickness measurements at five sites (over shoulder, on the back and at sacrum points I, II and III), loin eye area measurements, percentage of subcutaneous fat with intermuscular fat and percentage of meat in neck, shoulder, ham, leg, belly and ribs. The relationships obtained between backfat thickness and loin eye area measurements show that selection can be carried out for fatness and muscling of some carcass cuts, particularly the most valuable cuts such as neck, ham and loin. Better results should be expected from selection based on backfat thickness measurements.

Key words: pigs, correlations, backfat thickness, loin eye area, fatness and muscling of cuts

One of the breeding goals is to increase meat yield at the expense of fatness. This can be achieved thanks to negative relationships between these traits. Many studies have shown high negative correlations between backfat thickness and carcass meatiness (Cameron, 1990; Smith et al., 1992; Szyndler-Nędza and Różycki, 2004; Mucha and Różycki, 2004). In addition, high and positive correlations were found between loin eye area measurements and carcass meat percentage. Therefore, mean backfat thickness from 5 measurements and loin eye area were among the traits included in the selection index in postmortem evaluation at pig testing stations.

The Pietrain breed, which is widely used in crossbreeding schemes, is characterized by the best meatiness in Poland. This breed has also been used to improve the slaughter traits of line 990 pigs. It seems appropriate to find the proportions of fat and meat in the carcass cuts of pigs with improved meatiness, as well as to analyse the relationships between some linear measurements and fatness and muscling of half-carcass cuts.
The aim of the present study was to estimate correlations between backfat thickness and loin eye area measurements and fat and meat percentage in the carcass cuts of line 990 × Pietrain pigs.

Material and methods

Data from a detailed dissection of 38 crossbred gilts (line 990 × Pietrain) were analysed. Animals were kept and fed in accordance with the methods applied in pig testing stations to 210 days of age (Różycki, 1996).

Following slaughter and 24-hour cooling, right half-carcasses were measured for loin eye area and for backfat thickness at 5 sites (over shoulder, on the back and at sacrum points I, II and III). The half-carcasses were then cut into neck, shoulder, ham, leg, belly and ribs. The carcass cuts obtained were subjected to further detailed dissection to separate subcutaneous fat, intermuscular fat and meat. The results obtained were used to calculate the total percentage of subcutaneous and intermuscular fat and the total and individual percentage of meat in carcass cuts.

Arithmetic means, standard deviations (SD) and estimation errors (SE) were estimated for fatness and muscling traits. Correlations between linear measurements and fat and meat percentage in carcass and carcass cuts were estimated based on simple correlation coefficients. The correlations were calculated using STATGRAPHICS Plus 6.0.

Results

Average body weight of the slaughtered gilts was 111.6 kg (SD = 6.76) and average half-carcass weight after cooling was 45.2 kg (SD = 3.40).

Table 1 gives mean values for postmortem measurements of backfat thickness and loin eye area and fat and meat percentage in different cuts. Backfat was thickest over shoulder (3.24 cm) and thinnest at sacrum point II (1.47 cm). All the measurements of backfat thickness were characterized by a similar standard deviation ranging from SD = 0.36 to SD = 0.43. Loin eye area was 56.22 cm and standard deviation for this trait was SD = 7.31.

Mean fat percentage in all the cuts was 22.91%. In terms of subcutaneous and intermuscular fat together, belly (39.12%) had the highest fatness and shoulder (17.94%) and ham (17.64%) were the lowest in fat. Only subcutaneous fat was determined in ribs (9.50%). Meat percentage in all the cuts was 60.70%. The highest meatiness was characteristic of ham (72.36%), followed by leg (50.51%) and belly (50.54%). The highest standard deviations of the analysed traits were found for belly.

The coefficients of correlation between backfat thickness and loin eye area measurements and percentage of fat and meat in particular cuts are shown in Table 2. Backfat thickness measurements were positively correlated to fat percen-
Correlations between backfat thickness and loin eye area in pigs

Correlations between backfat thickness and meatiness of carcass cuts. The highest correlations were estimated between backfat measurements and fatness of loin (r = 0.469 – 0.787) and neck (r = 0.435 – 0.780), and the lowest between backfat thickness and fatness of ribs (r = 0.136 – 0.338) and leg (r = 0.224 – 0.442). In addition, higher correlations were found for backfat thickness measurements over shoulder and at sacrum points I and III than for the other two measurements. The highest correlations between loin eye area and fatness of carcass cuts were found for ham, belly, shoulder and ribs (–0.363, –0.358, –0.356 and –0.322, respectively).

Table 1. Mean values of backfat thickness and loin eye area measurements and fat and meat percentage in different carcass cuts

<table>
<thead>
<tr>
<th>Traits x ¯S D S E</th>
<th>(\bar{x})</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>111.6</td>
<td>6.75</td>
<td>1.10</td>
</tr>
<tr>
<td>Half-carcass weight after cooling (kg)</td>
<td>45.2</td>
<td>3.40</td>
<td>0.55</td>
</tr>
<tr>
<td>Backfat thickness:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>over shoulder (cm)</td>
<td>3.24</td>
<td>0.43</td>
<td>0.07</td>
</tr>
<tr>
<td>on the back (cm)</td>
<td>2.11</td>
<td>0.36</td>
<td>0.05</td>
</tr>
<tr>
<td>at sacrum point I (cm)</td>
<td>2.04</td>
<td>0.37</td>
<td>0.06</td>
</tr>
<tr>
<td>at sacrum point II (cm)</td>
<td>1.47</td>
<td>0.43</td>
<td>0.07</td>
</tr>
<tr>
<td>at sacrum point III (cm)</td>
<td>1.63</td>
<td>0.41</td>
<td>0.07</td>
</tr>
<tr>
<td>Mean backfat thickness from 5 measurements (cm)</td>
<td>2.10</td>
<td>0.31</td>
<td>0.05</td>
</tr>
<tr>
<td>Loin eye area (cm²)</td>
<td>56.22</td>
<td>7.31</td>
<td>1.19</td>
</tr>
<tr>
<td>Fat percentage (subcutaneous + intermuscular fat)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>neck</td>
<td>25.89</td>
<td>4.54</td>
<td>0.74</td>
</tr>
<tr>
<td>shoulder</td>
<td>17.94</td>
<td>2.61</td>
<td>0.42</td>
</tr>
<tr>
<td>ham</td>
<td>17.64</td>
<td>2.45</td>
<td>0.40</td>
</tr>
<tr>
<td>leg</td>
<td>18.40</td>
<td>3.38</td>
<td>0.55</td>
</tr>
<tr>
<td>loin</td>
<td>23.38</td>
<td>4.51</td>
<td>0.73</td>
</tr>
<tr>
<td>belly</td>
<td>39.12</td>
<td>6.38</td>
<td>1.04</td>
</tr>
<tr>
<td>ribs*</td>
<td>9.50</td>
<td>2.72</td>
<td>0.44</td>
</tr>
<tr>
<td>total</td>
<td>22.91</td>
<td>3.35</td>
<td>0.54</td>
</tr>
<tr>
<td>Meat percentage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>neck</td>
<td>53.46</td>
<td>4.46</td>
<td>0.72</td>
</tr>
<tr>
<td>shoulder</td>
<td>62.57</td>
<td>4.00</td>
<td>0.65</td>
</tr>
<tr>
<td>ham</td>
<td>72.36</td>
<td>2.43</td>
<td>0.36</td>
</tr>
<tr>
<td>leg</td>
<td>50.51</td>
<td>2.61</td>
<td>0.42</td>
</tr>
<tr>
<td>loin</td>
<td>56.83</td>
<td>4.07</td>
<td>0.66</td>
</tr>
<tr>
<td>belly</td>
<td>50.54</td>
<td>6.32</td>
<td>1.02</td>
</tr>
<tr>
<td>ribs</td>
<td>52.37</td>
<td>5.43</td>
<td>0.88</td>
</tr>
<tr>
<td>total</td>
<td>60.70</td>
<td>3.40</td>
<td>0.55</td>
</tr>
</tbody>
</table>

* Intermuscular fat only.

Similar to fatness of cuts, the highest correlations for meat percentage of cuts were found between backfat thickness and meatiness of loin (r = –0.336 to –0.711), neck (r = –0.487 to –0.684) and ham (r = –0.360 to –0.653). Loin eye area showed the highest correlation with the meat percentage of shoulder, loin, belly and ham (0.501, 0.405, 0.348 and 0.311, respectively).
Table 2. Correlations between backfat thickness and loin eye area measurements and fat and meat percentage in different carcass cuts

<table>
<thead>
<tr>
<th>Item</th>
<th>Backfat thickness</th>
<th>Loin eye area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>over shoulder</td>
<td>on the back</td>
</tr>
<tr>
<td>Fat percentage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>neck</td>
<td>0.636</td>
<td>0.485</td>
</tr>
<tr>
<td>shoulder</td>
<td>0.452</td>
<td>0.408</td>
</tr>
<tr>
<td>ham</td>
<td>0.484</td>
<td>0.317</td>
</tr>
<tr>
<td>leg</td>
<td>0.227</td>
<td>0.442</td>
</tr>
<tr>
<td>loin</td>
<td>0.710</td>
<td>0.588</td>
</tr>
<tr>
<td>belly</td>
<td>0.596</td>
<td>0.251</td>
</tr>
<tr>
<td>ribs*</td>
<td>0.338</td>
<td>0.245</td>
</tr>
<tr>
<td>total</td>
<td>0.612</td>
<td>0.378</td>
</tr>
</tbody>
</table>

| Meat percentage| neck | -0.622 | -0.493 | -0.684 | -0.578 | -0.487 | -0.746 | -0.060 |
|                | shoulder | -0.261 | -0.065 | -0.357 | 0.037 | -0.365 | -0.261 | 0.501 |
|                | ham | -0.455 | -0.360 | -0.653 | -0.366 | -0.639 | -0.642 | 0.311 |
|                | leg | -0.073 | -0.064 | -0.012 | 0.175 | -0.166 | -0.034 | 0.250 |
|                | loin | -0.630 | -0.352 | -0.711 | -0.336 | -0.558 | -0.673 | 0.405 |
|                | belly | -0.500 | -0.458 | -0.487 | -0.307 | -0.430 | -0.566 | 0.348 |
|                | ribs | -0.356 | -0.075 | -0.498 | -0.362 | -0.353 | -0.433 | -0.039 |
|                | total | -0.538 | -0.322 | -0.672 | -0.301 | -0.646 | -0.645 | 0.338 |

* Intermuscular fat only.

Discussion

Backfat measured behind the last rib was thinner than in the rostral or caudal part of the carcass. This is consistent with the findings of Hulsegge et al. (1999), who reported that backfat measured behind the last rib (6 cm off the midline) was increasingly thick in the rostral direction. However, it is difficult to compare these results with those of other authors because of different body weights on the day of measurement and different breeds studied.

The results obtained for percentage of fat and meat in carcass cuts are slightly different from those reported by Orzechowska and Eckert (2002) for Polish Large White and Polish Landrace pigs slaughtered at 86 and 100 kg body weight. The pigs studied were characterized by higher muscling and lower fatness of carcass cuts (by 60.70% and 22.91%, respectively). In the study of the above authors, meat percentage in primal cuts was 56.38% and 55.42%, and fat percentage (subcutaneous and intermuscular fat together) was 27.36% and 27.1%. In our study, meat percentage was highest in ham (68.45% and 69.61%) and lowest in belly (42.56% and 42.15%). In a study by Bąk et al. (2003), ham meatiness was 73.89% and ham fatness was 14.29%.
For fatteners slaughtered between 80 and 120 kg of body weight, Winiarski et al. (2004) estimated the meat percentage in belly, loin, leg and shoulder to be 48.81%, 52.69%, 66.70% and 62.38%, respectively, with a total meat percentage in the dissected cuts of 59.15%. These findings are slightly different from those found in the present study because different half-carcass dissection methods were used. In the study of the above authors, dissection was carried out in accordance with the method of Walstra and Merkus, and we used the method applied in pig testing stations.

In the present study, the highest correlations between backfat thickness measurements and fat percentage in carcass cuts were estimated for loin and neck. The highest correlations between backfat measurements and meatiness were estimated for the above cuts and ham. The highest relationships between loin eye area and fatness of carcass cuts were found for ham, belly, shoulder and ribs, and between loin eye area and meat percentage for shoulder, loin, belly and ham.

For backfat thickness from 5 measurements, Orzechowska (2000) found significantly high phenotypic correlations with the weight of subcutaneous fat, ham, neck, shoulder and leg (from $r_p = 0.527$ to $r_p = 0.256$), as well as with the weight of total loin and belly fat ($r_p = 0.696$ and $r_p = 0.331$). The highest relationships of this parameter with the weight of meat were obtained for ham, neck, loin and shoulder (from $r_p = -0.275$ to $r_p = -0.211$). Loin eye area showed significant correlations with the weight of loin fat ($r_p = -0.289$) and subcutaneous fat of ham ($r_p = -0.283$) and with the weight of meat from all the cuts except the ribs ($r_p = 0.578$ and $r_p = 0.266$).

Higher coefficients of correlation between loin eye height and meat percentage in belly, loin, leg and shoulder (from $r = 0.567$ to $r = 0.611$) were obtained by Winiarski et al. (2004). Lower phenotypic relationships between ham meatiness and average backfat thickness and loin eye area ($r_p = -0.36$ and $r_p = -0.08$) were estimated by Hicks et al. (1998), who found relatively high genetic correlations between these traits ($r_G = -0.65$ and $r_G = 0.37$, respectively).

The relationships obtained in the present study for backfat thickness and loin eye area measurements show that selection can be carried out for fatness and muscling of some carcass cuts, particularly the most valuable cuts such as neck, ham and loin. Better results should be expected for selection based on backfat thickness measurements.

References


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Korelacje między pomiarami grubości słoniny i powierzchni oka półędwicy a otluszczeniem i umiśnieniem wyrębów u świń linii 990 × Pietrain

STRESZCZENIE

Materiał do badań stanowiły dane ze szczegółowej dysekcji 38 loszek linii 990 × Pietrain. W badaniach uwzględniono pomiary grubości słoniny w 5 punktach: nad łopatką, na grzbiecie, nad krzyżem I, II, III i powierzchni oka połędwicy oraz procentową zawartość tłuszczu podskórnego łącznie z mięśniami mięśniowymi i mięsa w karkówce, łopatce, szynce golonce, boczku i żeberkach.

Uzyskane w badaniach zależności między pomiarami grubości słoniny i powierzchni oka połędwicy wskazują na możliwość prowadzenia prac selekcyjnych w zakresie otluszczenia i umiśnienia niektórych wyrębów, zwłaszcza karkówki, szynki, połędwicy, czyli najcenniejszych wyrębów tuszy. Lepszych efektów można oczekiwać prowadząc selekcję na podstawie pomiarów grubości słoniny.
CHANGES IN BOAR BACKFAT AND LOIN MUSCLE THICKNESS AS RELATED TO BODY WEIGHT AND CARCASS MEAT PERCENTAGE

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Abstract

The aim of the study was to determine the effects of increasing body weight and carcass meat percentage in young boars tested for backfat and loin muscle thickness. As the body weight increased (from 95 to 125 kg), so did the thickness of backfat ($P \leq 0.05$) and loin muscle ($P \leq 0.01$) measured on live animals and postmortem. In the weight range analysed, carcass meat percentage, determined based on detailed dissection, did not change. As carcass meat percentage increased, the thickness of the longissimus dorsi muscle measured on live animals and postmortem increased, and backfat thickness decreased.

Key words: boars, body weight, meat percentage, backfat and muscle thickness

In live evaluation of young boars and gilts, growth rate and muscling are determined based on live ultrasound measurements of backfat thickness and loin muscle thickness. In Poland, boars and gilts aged 150–210 days are subjected to live evaluation (Eckert and Szyndler-Nędza, 2005). Such a wide age range is mainly due to organizational reasons. It is aimed to minimize the frequency of testers visiting pig houses and thus to reduce the costs. This procedure causes backfat and muscle thickness to be determined at different body weights.

Authors performed one-time live measurements of backfat thickness in gilts (Standal, 1973) at specific body weights and ages, or multiple measurements throughout the lives of gilts (Mucha and Różyczki, 2005). These authors showed that backfat deposition in gilts is linearly correlated with age and body weight. In studies on growth rate, Metz et al. (1985) and Buczyński and Marciniak (1988) showed that barrows are characterized by daily gains that are 60–70 g higher than those of gilts. Similar conclusions were drawn by Walstra (1986), who showed that the coefficients of growth estimated for particular tissues in boar carcasses were higher than the coefficients of growth in gilts and castrates, by 0.06% for muscles and bones and by 0.2% for skin.
The aim of the study was to determine changes in backfat and muscle thickness in young boars subjected to live evaluation as a result of increased body weight and carcass meat percentage.

**Material and methods**

When selecting the study material we aimed to include animals whose muscling and body weight would reflect the situation in practical breeding. In practice, the animals evaluated differed in body weight and muscling. The study involved 144 boars of different breeds. Boars were divided according to body weight into three groups: 95–100.9 (36 animals), 101–110.9 (52 animals) and 111–125 kg (56 animals). In terms of carcass meat percentage, boars were divided into four groups: 45–50.9 (37 animals), 51–55.9 (47 animals), 56–60.0 (37 animals) and above 60% (23 animals) on a EUROP scale. Animals were chosen according to body weight and carcass meat percentage, which were determined based on live measurements of backfat and muscle thickness using a Piglog 105 ultrasound device. On the day of slaughter, the thickness of backfat and *longissimus dorsi* muscle was measured on live animals at the following points:

- P1 — along a vertical line proximal to the elbow joint (around the 6th-7th thoracic vertebrae), 3 cm off the mid-line — backfat thickness (mm),
- P2 — behind the last rib, at the juncture of the thoracic and lumbar vertebrae, 3 cm off the mid-line — backfat thickness (mm),
- P3 — along a vertical line proximal to the elbow joint around the penultimate thoracic vertebra, 3 cm off the mid-line — backfat thickness (mm),
- P4 — behind the last rib, at the juncture of the thoracic and lumbar vertebrae, 8 cm off the mid-line — backfat thickness (mm),
- P4M — muscle thickness at point P4 (mm).

Animals were then slaughtered and after 24-hour cooling of carcasses at 4°C, the following postmortem measurements were taken on right half-carcasses:

- backfat thickness (cm):
  - over shoulder (cm)
  - on the back (cm)
  - over sacrum I, II, III (cm)
- backfat thickness at point C1 (cm),
- loin eye width and height at the section behind the last rib (cm),
- loin eye area (cm²).

Right half-carcasses were then divided into basic cuts, which were subjected to detailed dissection into meat, fat, bone and skin tissue.

Differences between the mean values of the traits for particular body weight groups and carcass meat percentage classes were determined using two-way analysis of variance with interaction. The analysis was performed using SAS software.
Results

This study determined the effect of body weight and dissected carcass meat percentage on live and postmortem measurements of backfat and longissimus dorsi muscle thickness.

Analysis of changes in backfat and muscle thickness measured live, depending on body weight (Table 1), revealed few significant differences between particular groups. Statistically significant differences occurred between the lightest animals (95 – 100.9 kg body weight) and the heaviest animals (111 – 125 kg body weight). As the body weight increased, backfat and muscle thickness was observed to increase at the measurement points. The differences were significant at $P \leq 0.05$ for backfat thickness measured live at points P2, P3 and P4, and at $P \leq 0.01$ for muscle thickness at point P4M.

A similar situation occurred when differences between half-carcass measurements were estimated (Table 1). Statistically significant differences ($P \leq 0.05$) were only found for backfat thickness on the back, at sacrum III and at point C1 between the lightest and heaviest animals. The increase in body weight resulted in a highly significant ($P \leq 0.01$) increase in carcass meat and fat weight and in an increase in loin eye height, width and area. In terms of loin muscle measurements, highly significant differences ($P \leq 0.01$) were found between most of the body weight groups investigated. These relationships followed a different pattern for carcass meat percentage. As the body weight increased, carcass meat percentage remained at a similar level.

Changes in backfat thickness and loin muscle thickness, measured live and postmortem, were analysed depending on carcass meat percentage determined based on detailed dissection. It was shown that as carcass meat percentage increased, longissimus dorsi muscle thickness increased and backfat thickness decreased. Differences between particular groups were highly significant ($P \leq 0.01$) for most of the traits analysed.

The results showed significant interactions between body weight and carcass meat percentage. They were determined for weight of meat and loin eye area ($P \leq 0.05$). No significant interactions between body weight and meat percentage were found for the other traits.

Discussion

The present study showed that the body weight of the boars on the day of live measurement has a significant effect on backfat thickness measured live and postmortem and a highly significant effect on loin muscle thickness. As the body weight increased, so did backfat and muscle thickness measured at points, as well as loin eye area. Increased body weight also resulted in an increased weight of meat and fat in carcass. These relationships were different for carcass meat and fat percentage. As the body weight of the boars increased from 95 to 125 kg, carcass meat and fat percentage did not change significantly.
Table 1. Mean live traits and postmortem measurements of boar groups differing in body weight and carcass meat percentage*

<table>
<thead>
<tr>
<th>Item</th>
<th>Live traits and measurements</th>
<th>Postmortem traits and measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight (kg) (m)</td>
<td>Carcass meat percentage (%) (p)</td>
</tr>
<tr>
<td></td>
<td>95–100.     101–110.9     111–125</td>
<td>45–50.9.     51–55.9.     56–60.0.</td>
</tr>
<tr>
<td>P1 (mm)</td>
<td>17.8        19.1          19.2</td>
<td>24.0 ABC       21.6 ADE       15.9 BDa</td>
</tr>
<tr>
<td>P2 (mm)</td>
<td>11.1a       12.1          12.7a</td>
<td>15.7 ABC       13.7A</td>
</tr>
<tr>
<td>P3 (mm)</td>
<td>13.9 a       15.1          15.9a</td>
<td>17.8 AB        17.5 CD</td>
</tr>
<tr>
<td>P4 (mm)</td>
<td>11.1 a       12.2          12.5 a</td>
<td>16.5 ABC       13.1 ADE</td>
</tr>
<tr>
<td>Muscle thickness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4M (mm)</td>
<td>46.2 A       47.3          49.0 A</td>
<td>44.2 ABC       46.7 ADE</td>
</tr>
<tr>
<td>Meat weight (kg)</td>
<td>19.6 AB      22.8 AC       25.6 BC</td>
<td>19.5 BC        21.7 ADE</td>
</tr>
<tr>
<td>Fat weight (kg)</td>
<td>6.7 AB       8.1 AC        9.0 BC</td>
<td>11.6 ABC       8.7 ADE</td>
</tr>
<tr>
<td>Meat percentage (%)</td>
<td>55.0         54.6          55.3</td>
<td>46.9 ABC       52.9 ADE</td>
</tr>
<tr>
<td>Fat percentage (%)</td>
<td>18.6         19.7          19.4</td>
<td>28.1 ABC       21.2 ADE</td>
</tr>
<tr>
<td>Backfat thickness over shoulder (mm)</td>
<td>22.7         24.9          25.3</td>
<td>29.6 AB        28.5 CD</td>
</tr>
<tr>
<td>Backfat thickness on the back (mm)</td>
<td>8.7 a         10.1          10.8 a</td>
<td>13.6 ABC       10.8 ADE</td>
</tr>
<tr>
<td>Backfat thickness at sacrum I (mm)</td>
<td>11.6         12.9          13.0</td>
<td>19.6 ABC       14.7 ADE</td>
</tr>
<tr>
<td>Backfat thickness at sacrum II (mm)</td>
<td>7.5           8.5           10.9</td>
<td>15.2 ABC       11.2 ADE</td>
</tr>
<tr>
<td>Backfat thickness at sacrum III (mm)</td>
<td>11.4 a        13.1          13.7 a</td>
<td>19.9 ABC       16.1 ADE</td>
</tr>
<tr>
<td>Backfat thickness at C1 (mm)</td>
<td>9.5 a         9.6 b          11.6 ab</td>
<td>15.8 ABC       11.5 ADE</td>
</tr>
<tr>
<td>Loin eye width (mm)</td>
<td>97.3 A        100.0 B        104.6 AB</td>
<td>93.6 AB        95.8 CD</td>
</tr>
<tr>
<td>Loin eye height (mm)</td>
<td>63.2 aA        66.5 a          68.4 A</td>
<td>60.4 ABC       65.3 AD</td>
</tr>
<tr>
<td>Loin eye area (cm²)</td>
<td>45.7 AB        49.9 AC          54.4 BC</td>
<td>41.7 ABC       46.4 ADE</td>
</tr>
</tbody>
</table>

* Statistical differences were determined separately for groups differing in body weight and separately for groups differing in carcass meat percentage.

Means with statistical differences bear the same capital letters for P≤0.01 and small letters for P≤0.05.
The results obtained conform with the findings of Mroczek et al. (1996), Milewska and Grudniewska (1999), Smith et al. (1992) and Klimas (2002), who found fattening to 95 kg body weight to be the most economical, while body weights beyond this value reduced meatiness and increased backfat thickness, resulting in poorer dressing percentage of these animals. The study of Paschma et al. (1989) showed that the loin eye area of boars and gilts increased along with increased body weight. For animals weighing 85 kg, loin eye area was 31.2 cm² in boars and 32.1 cm² in gilts. For animals weighing 115 kg, loin eye area was higher, i.e. 36.9 cm² in boars and 41.99 cm² in gilts. Similar results were obtained by Smith et al. (1992), who determined loin eye area to be 30.0 cm² in animals weighing 91 kg and 36.6 cm² in animals weighing 118 kg. Mroczek et al. (1996) were the only authors to find that as body weight increased, loin eye height decreased.

The second factor that had a highly significant effect on the traits studied was carcass meat percentage. As expected, carcass meat percentage was inversely proportional to backfat thickness and fat weight, and directly proportional to muscle thickness and weight of meat. As carcass meat percentage increased, so did the thickness of longissimus dorsi muscle and the total weight of meat in carcass. These results conform with the studies of Borzuta et al. (1995) concerning the meat and fat content of carcass and half-carcass of fatteners in particular meatiness classes.

It is concluded from the study that the increased body weight of the boars caused a highly significant increase in muscle thickness measured live and postmortem and a significant increase in backfat thickness. In the weight range studied, carcass meat percentage determined based on detailed dissection did not change. As the carcass meat percentage increased, the thickness of longissimus dorsi muscle increased on live animals and postmortem, and backfat thickness decreased.

References


Klimas R. (2002). Methods and measures for improvement of biological and farming qualities of pigs bred in Lithuania. Lithuanian Veterinary Academy, Biomedical sciences, zootechny (13B) Kaunas.


Zmiany grubości słoniny i mięśnia polędwicy knurów w zależności od masy ciała i procentowej zawartości mięsa w tuszy

STRESZCZENIE

Celem pracy było określenie wpływu zwiększania się masy ciała oraz procentowej zawartości mięsa w tuszy młodych knurów ocenianych przyżyciowo pod względem grubości słoniny i mięśnia polędwicy.

Stwierdzono, że wraz ze wzrostem masy ciała (od 95 kg do 125 kg) wzrasta mierzona przyżyciowo i poubojowo grubość słoniny (P ≤ 0,05) i mięśnia polędwicy (P ≤ 0,01). W analizowanym przedziale wagowym procentowa zawartość mięsa w tuszy knurów, określona na podstawie dysekcji szczegółowej, nie uległa zmianie. Wraz ze wzrostem procentowej zawartości mięsa w tuszy wzrastała — mierzona przyżyciowo i poubojowo — grubość mięśnia najdłuższego grzbietu oraz zmniejszała się grubość słoniny.
PORK MEAT QUALITY IN LINE 990 AND 890 PIGS COMPARED WITH DANISH CROSSBRED PIGS [(L × Y) × Du]

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Abstract

The meat quality of pigs derived from synthetic lines 990, 890¹[(♀ Line 900 × Pi) × (♂ Line 990 × Pi)] and 890²[(♀ Line 900 × ♂ Pi)] in comparison with the hybrids of Danish pigs [(L × Y) × Du] was slightly worse only with regard to traits such as brightness of colour (L*), tenderness and juiciness. In addition, a certain amount of variability was observed in meat quality within the analysed synthetic lines. Slightly more favourable values of the assessed meat quality traits were found in Line 990 and 890(²) pigs, which was presumably due to a considerably lower frequency of the Pietrain genes in these genotypes. The meat of pigs derived from the 890(¹) line showed a significantly higher proportion of defective meat (44.44%) in comparison with the remaining genotypes of synthetic lines (990 — 18.75% and 890(²) — 11.32%).

Key words: pigs, genotype, meat quality

Meat quality depends primarily on genetic and environmental factors and interactions that occur between them (Kortz et al., 1994; Kortz, 2001; Rak et al., 1994; Villé et al., 1997; Wood et al., 1998; Wajda 1994; Koćwin-Podsiadła et al., 1999; Łyczynski et al., 2002). Many researchers claim that the improvement of the breeding stock of domestic breeds by mating sows with imported boars resulted in the enhancement of musculature and reduction of fatness in mass-produced Polish porkers. This led to an increase in the slaughter value of carcasses paralleled by a deterioration in meat quality (Koćwin-Podsiadła et al., 1994; Koćwin-Podsiadla, 1999; Pospiech et al., 1998; Łyczynski et al., 2002; 2004). These researchers reported the occurrence of defects in the meat of Polish pigs at the level of about 30%, with acid meat quoted as the most common meat defect.
The aim of the study was to compare meat quality of pigs from the synthetic line 990 and two 890 lines: 1[{♀ (Line 990 × Pi) × (♂ Line 990 × Pi)}] and 2[{♀ Line 990 × ♂ Pi}] with hybrids of Danish pigs as a control group [{♀ (L × Y) × ♂ Du}] and to determine the level of normal quality meat (RFN) and defective meat of PSE and ASE type.

Material and methods

A total of 164 carcasses of four animal genotypes (Line 990 — 32, Line 890(1) — 36, Line 890(2) — 53, crosses of Danish pigs: Landrace × Yorkshire × Duroc [{♀ L × Y) × ♂ Du} — 43], maintained at controlled environmental conditions, were investigated. When the animals reached the mean final body weight of approximately 105 kg, they were slaughtered in accordance with standard technological procedures employed in meat processing plants. In addition, the meat obtained was classified into the following categories: normal quality meat (RFN), watery meat of PSE type and acid meat of ASE type. Meat quality was assessed in the longissimus lumborum muscle on the basis of measurements of its acidity (pH45' and pH24h), electrical conductivity (EC90' and EC24h), colour brightness (L*), its chemical composition (water, fat, protein), technological parameters (thermal drip and water holding capacity) and on the basis of the meat consumer parameters (tenderness and juiciness). Meat acidity (pH45' and pH24h) was determined using a Handylab 2 apparatus (SCHOTT GERÄTE), electrical conductivity (EC90' and EC24h) by an LF-STAR apparatus (Matthäus) and colour brightness by a Minolta spectrophotometer. Water content was determined using the dryer method, fat by the Soxhlet method and protein by the Kjeldahl method, whereas thermal drip was estimated employing the method of Janicki and Walczak and water holding capacity using the Grau and Hamm method. The sensory evaluation of the examined meat (tenderness and juiciness) was performed on a 10-point scale developed by Baryłko-Pikielna, while methods developed by Borzuta and Pospiech were employed to carry out meat classification. All these measurements were carried out on the left half-carcasses. All the results obtained were subjected to a single-factor analysis of variance using the STATISTICA 6.0 program.

Results

Table 1 presents meat quality parameters of the assessed genotypes of porkers. The most favourable meat quality was found in Danish crosses and pigs from Lines 990 and 890(2). The highest level of intramuscular fat was determined in the meat of pigs of Line 990 (2.17%) and in Danish crosses (2.10%). The highest initial meat reaction (pH45') was recorded in the meat of Danish crosses and Line 890(3). The lowest electrical conductivity was observed in the meat of Danish pigs and the meat derived from Line 890(2). The darkest meat colour was found in Danish pigs...
Table 1. Comparison of pork meat quality traits of different genotypes

<table>
<thead>
<tr>
<th>Analysed parameters</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Line 990 (Control)</td>
</tr>
<tr>
<td>pH45</td>
<td>x</td>
</tr>
<tr>
<td>pH24h</td>
<td>6.14 A 0.54</td>
</tr>
<tr>
<td>EC99</td>
<td>5.40 A 0.08</td>
</tr>
<tr>
<td>EC24h</td>
<td>6.40 Ab 5.98</td>
</tr>
<tr>
<td>L*</td>
<td>6.44 Ab 3.11</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>74.11 a 1.06</td>
</tr>
<tr>
<td>Intramuscular fat content (%)</td>
<td>2.17 a 1.12</td>
</tr>
<tr>
<td>Total protein content (%)</td>
<td>22.88 A 1.02</td>
</tr>
<tr>
<td>Thermal drip (%)</td>
<td>27.15 A 2.97</td>
</tr>
<tr>
<td>WHC (%)</td>
<td>39.76 B 3.27</td>
</tr>
<tr>
<td>Tenderness (points)</td>
<td>5.79 AB 1.18</td>
</tr>
<tr>
<td>Juiciness (points)</td>
<td>5.07 Ab 1.27</td>
</tr>
</tbody>
</table>

a, b, c — means marked with different small letters differ statistically significantly at P ≤ 0.05.
A, B, C — means marked with different capital letters differ statistically significantly at P ≤ 0.01.

(1)Line 890: [♀ (Line 990 × Pi) × (♂ Line 990 × Pi)], (2)Line 890: (♀ Line 990 × ♂ Pi).
(L* = 53.72) and the determined difference was statistically significant in comparison with the other genotypes analysed. The lowest level of water was determined in the meat of pigs from Line 990 and it was presumably associated with the highest level of intramuscular fat. The fat level depended significantly on the genotype (P ≤ 0.05); the highest level of fat was found in the meat of pigs from line 990 and Danish crosses in comparison with the meat derived from pigs of line 890(2). Genotype failed to exert any significant influence on the level of protein in meat and its thermal drip. The highest water holding capacity was found in the meat of Danish crosses and Line 890(2) and the determined differences between these genotypes and Lines 990 and 890(1) were statistically significant. Meat derived from Danish pigs and crosses of Line 890(1) was characterized by the most favourable sensory assessment. Table 2 presents the level of normal quality meat (RFN) as well as meat of watery (PSE) and acid (ASE) types. No defective meat was found in the meat derived from Danish crosses. The highest proportions of defective meat were determined in the meat of pigs from Line 890(1), i.e. pigs with a higher frequency of Pietrain genes.

Table 2. Level of normal meat (RFN) and defective meat (PSE, ASE) depending on pig genotype

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No of carcasses</th>
<th>Type of meat</th>
<th></th>
<th></th>
<th></th>
<th>total (PSE + ASE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RFN</td>
<td>PSE</td>
<td>ASE</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n  %</td>
<td>n %</td>
<td>n  %</td>
<td>n %</td>
<td></td>
</tr>
<tr>
<td>Line 990</td>
<td>32</td>
<td>26 81.25</td>
<td>6 18.75</td>
<td></td>
<td></td>
<td>6 18.75</td>
</tr>
<tr>
<td>Line 890(1)</td>
<td>36</td>
<td>20 55.56</td>
<td>3 8.33</td>
<td>13 36.11</td>
<td>16 44.44</td>
<td></td>
</tr>
<tr>
<td>Line 890(2)</td>
<td>53</td>
<td>47 88.68</td>
<td>4 7.55</td>
<td>2 3.77</td>
<td>6 11.32</td>
<td></td>
</tr>
<tr>
<td>[(L × Y) × Du]</td>
<td>43</td>
<td>43 100.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>164</td>
<td>136 82.93</td>
<td>13 7.93</td>
<td>15 9.15</td>
<td>28 17.07</td>
<td></td>
</tr>
</tbody>
</table>

(1)Line 890: [♀ (Line 990 × Pi) × ♂ Line 990 × Pi]. (2)Line 890: (♀ Line 990 × ♂ Pi).

Discussion

Meat quality depends, among others, on the genotypes of porkers. Koćwin-Podsiadła et al. (1994) maintain that the crossing of sows of Polish breeds using Pietrain boars improves the slaughter value of carcasses but, at the same time, lowers meat quality. Rak et al. (1994) reported that the utilization of Pietrain boars for crossing resulted in an increase in the proportion of meat in carcass accompanied by a simultaneous decrease in fatness. Łyczynski et al. (2002) showed a statistically significant effect of the genotype of porkers on the slaughter parameters and quality of the meat obtained. For meat derived from pigs of the synthetic lines 990 and 890(1,2), they reported similar values of meat quality traits to those presented in our paper. This refers mainly to the meat reaction (pH45'), which is also confirmed by research results of Kamyczek (1999), Tyra and Kamyczek.
(1999), and Różycki (1999; 2002). The values of electrical conductivity (EC90' and EC24h), colour brightness (L*), chemical composition as well as technological parameters and selected sensory traits of the pork meat of the synthetic lines 990, 890(1) and 890(2) were similar to those obtained by Łyczynski et al. (2002). Czyżak-Runowska et al. (2005) reported that meat quality parameters of pigs from lines 990 and 890 depend on the sex of porkers. They reported more favourable meat quality parameters in gilts and hogs derived from late castration than in hogs castrated traditionally at the age of 3 days. Łyczynski et al. (2004) reported that the level of intramuscular fat depended on the genotype of pigs but it did not exert a significant effect on the quality of the meat obtained. Pigs from the synthetic lines 990 and 890 can be used domestically for the production of meat fatteners, both on the maternal and paternal sides.

References


Porównanie jakości mięsa wieprzowego świń linii 990 i 890 z mięsem mieszańców świń duńskich 

\[(L \times Y) \times Du\]

STRESZCZENIE

Jakość mięsa świń syntetycznych linii 990, 890(1) — \[\bigcirc (\text{Line 990} \times \text{Pi}) \times (\bigcirc \text{Line 990} \times \text{Pi})\] i 890(2) — \((\bigcirc \text{Line 990} \times \bigcirc \text{Pi})\), w porównaniu z mięsem mieszańców świń duńskich \[(L \times Y) \times Du\], była nieco gorsza w przypadku takich cech jak: jasność barwy (L*) oraz kruchość i soczystość. Ponadto obserwowano pewne zróżnicowanie jakości mięsa w obrębie analizowanych linii syntetycznych. Nieco korzystniejsze wartości ocenianych cech jakości stwierdzono w przypadku mięsa świń linii 990 i 890(2), co przypuszczalnie było spowodowane znacznie niższą frekwencją genów świń rasy Pietrain. Mięso świń linii 890(1) było w znacznie większym stopniu wadliwe (44.44%) niż mięso świń pozostałych genotypów linii syntetycznych (990 — 18.75% i 890(2) — 11.32%).
EVALUATION OF MEAT AND MILK FROM SHEEP OF DIFFERENT BREEDS AND THEIR CROSSES, KEPT UNDER ECOLOGICAL CONDITIONS*

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²Department of Technology, Ecology and Economics of Animal Production, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

Abstract
This study was aimed at determining the quality of products (milk and meat) from sheep of different breeds and their crosses, kept under ecological conditions. Milk collected from 287 Polish Mountain Sheep (PMS), Bergschaf (BF) and Weisse Alpenschaf (WAS) ewes and F₁ crosses (BF × PMS and WAS × PMS), as well as meat from the 140 derived purebred rams (PMS, WAS and BF), F₁ crosses (BF × PMS and WAS × PMS) and R₁ crosses [BF × (BF × PMS) and WAS × (WAS × PMS)] were investigated, with 20 animals per group. Sheep’s milk and lamb meat were evaluated for their components, physicochemical properties, and suitability for processing. The milk of WAS and BF ewes was characterized by the highest level of basic nutrients, providing high-yield dairy material. The crossing of PMS ewes with alpine rams created F₁ crosses characterized by favourable milk parameters. The meat of rams from all the experimental groups was characterized by desirable levels of the analysed parameters, particularly the ultimate pH [lowest in WAS — 5.54 and highest in BF × (BF × PMS) — 5.69]. No unfavourable signs of accelerated or slowed glycolysis in meat (DFD, PSE) were found.

Key words: sheep, meat, milk, ecological conditions, physicochemical properties

Ecological production is an interesting alternative for Polish farms. Based on the principles of sustainable development, it should guarantee the highest quality and biosafety of products for the consumer. Recent years have seen increased public interest in these products, resulting directly from growing consumer awareness and the increased importance attached to the quality of products consumed (Herbut and Walczak, 2003).

Sheep are the only species of farm animals to have avoided the intensification of production and are therefore particularly suited to ecological farming. Sheep’s milk

* This work was conducted as part of the NRIAP statutory activity, project no 4212.3.
(and particularly sheep’s milk products) and lamb meat obtained from organic farms could be attractive products on the increasingly demanding Polish and EU markets. Ecological production, coupled with the grading-up of native sheep breeds (such as Polish Mountain Sheep) through mating with breeds of sheep characterized by better productive and reproductive traits, should be viewed as one of the tools for improving the profitability of sheep production in Poland.

The aim of the present study was to determine the quality of products (milk and meat) from sheep of different breeds and their crossbreds, kept under ecological conditions.

**Material and methods**

The experiment was carried out at the Sheep Research and Implementation Farm in Piorunka, which is being converted to ecological production. Milk collected from 287 ewes representing the following groups was investigated: Bergschaf (BF; 77 animals), Weisse Alpenschaf (WAS; 46 animals), Polish Mountain Sheep (PMS; 68 animals), and F1 BF × PMS (50 animals) and WAS × PMS crosses (46 animals). All the ewes were between their 1st and 3rd lactations. They were kept in uniform environmental conditions and their feeding during milking was based on pasture. Lambs were reared with mothers to 70 days of age, after which time they were weaned and mothers were used for milking. For each breed separately, samples of bulk milk (from morning and evening milking) were taken five times per season to perform physicochemical analyses of solids, protein, casein, fat, lactose, calcium, and renneting time (Milkoscan). Bundz cheese was produced from the milk obtained and the yield was determined.

From the offspring obtained from all the experimental ewes, 20 rams were randomly selected from each experimental group. Until weaning at approx. 70 days of age, in addition to mother’s milk the lambs also received meadow hay and CJ concentrate mixture at 0.25 kg/day/animal, which contained 5.33 MJ net energy and 190 g crude protein per kg. After weaning, the rams were fed according to standards with hay, ensiled hay, ground maize, wheat bran and barley bran. From the beginning of the grazing season (mid-May, approx. 4 months of age), the rams were grazed in a rotational system and additionally given ground barley and wheat at approx. 0.25 kg/day/animal. Rams were subjected to experimental slaughter at the age of 200 days. The chemical composition and physical properties of meat (*longissimus dorsi* muscle) were determined for water, protein and fat content, pH at 24 h after cooling (Radelkis device), pigment content (Hornsey’s method), colour lightness (Momcolor colourimeter), water holding capacity (Grau-Hamm’s method) and thermal loss (loss of meat juices during cooking to an internal sample temperature of 85°C; *semimembranosus* muscle).

The results were analysed statistically using the ANOVA/MANOVA procedure of the multifactorial analysis of variance in the Statistica for Windows packet.
Results

The effects of breed on milk composition, some physical milk parameters, and cheese yield were evaluated in this experiment. For all the parameters analysed, highly significant differences were found according to breed (Table 1). The solids, protein and fat content of milk was the highest in the milk of WAS sheep (21.43, 6.92 and 9.02%, respectively) and the lowest in the milk of PMS sheep (18.25, 5.99 and 6.94%, respectively) \( (P \leq 0.01) \). The milk of the mothers of crossbreds did not differ in this respect from the milk obtained from BF sheep. The most casein was found in WAS milk (approx. 5.5%), and the least casein in the crossbreds sired by WAS rams (4.5%) \( (P \leq 0.01) \). The density of milk from PMS ewes (1.033 g/l) was the lowest of all the breeds (Table 1). The calcium content of the milk of the analysed breeds was similar and ranged from 1.078 in BF to 1.226 g/l in WAS animals. Breed had no effect on the lactose or ash content of milk. The amounts of these components were at a medium level (4.4 and 0.97%, respectively). The average renneting time was 303 s, with no statistically significant differences between the experimental groups. Bundz production was the lowest from the milk of BF × PMS (21.75%) and significantly higher when produced from the milk of PMS and PMS × WAS sheep. The cheese yield from the milk of alpine sheep was significantly higher than that from the milk of sheep from the other groups \( (P \leq 0.01) \) (Table 1).

The chemical composition of the rams’ meat is shown in Table 2. The water content of the longissimus dorsi muscle ranged from 76.58% in PMS animals to 77.13% in F1 crosses with 50% WAS blood, with no significant differences (Table 2).

The lowest dry matter content of 22.87% was found in the longissimus dorsi muscle of F1 crosses obtained by mating PMS ewes to WAS rams. The highest dry matter content (23.42%) was found in PMS sheep.

The fat percentage ranged from 1.82 in the crosses obtained by mating PMS ewes with BF rams to 2.23 in the group of F1 rams with 50% WAS blood.

The highest protein content was found in the meat of BF × PMS rams (20.39%) and the lowest in the meat of WAS × PMS rams (19.78%), with no significant differences between the groups (Table 2).

The highest pigment content was found in the muscle of PMS rams (116.17 ppm) (Table 3). This differed highly significantly compared to the average values for all the other experimental groups. The lowest pigment content was characteristic of the meat of WAS rams (84.18 ppm), with significant differences in relation to PMS, BF, F1 BF × PMS and R1 WAS × (WAS × PMS) animals. The pigment content of BF meat and of the meat of F1 and R1 crosses with BF blood assumed similar values (98.57, 96.64 and 93.05 ppm, respectively). These were close to those obtained in F1 and R1 crosses with 50 and 75% WAS blood (90.48 and 100.33 ppm, respectively) \( (P > 0.05) \) (Table 3).

The greatest colour lightness was found in the longissimus dorsi muscle of R1 BF × (BF × PMS) crosses (14.06%), and a slightly lower value in R1
Table 1. Physicochemical parameters of sheep’s milk (n = 20 animals per group)

<table>
<thead>
<tr>
<th>Trait</th>
<th>PMS</th>
<th>BF</th>
<th>BF × PMS</th>
<th>WAS</th>
<th>WAS × PMS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} )</td>
<td>SD</td>
<td>( \bar{x} )</td>
<td>SD</td>
<td>( \bar{x} )</td>
</tr>
<tr>
<td>Solids (%)</td>
<td>18.25 C</td>
<td>0.87</td>
<td>19.45 B</td>
<td>0.74</td>
<td>19.83 B</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>6.94 C</td>
<td>0.71</td>
<td>7.69 B</td>
<td>0.44</td>
<td>7.98 B</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>5.99 B</td>
<td>0.36</td>
<td>6.47 AB</td>
<td>0.72</td>
<td>6.16 AB</td>
</tr>
<tr>
<td>Casein (%)</td>
<td>4.79 AB</td>
<td>0.30</td>
<td>5.15 A</td>
<td>0.59</td>
<td>4.74 AB</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>4.34</td>
<td>0.59</td>
<td>4.33</td>
<td>0.51</td>
<td>4.73</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.985</td>
<td>0.002</td>
<td>0.962</td>
<td>0.001</td>
<td>0.968</td>
</tr>
<tr>
<td>Calcium (g/l)</td>
<td>1.092</td>
<td>0.16</td>
<td>1.078</td>
<td>0.15</td>
<td>1.191</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>1.0331 B</td>
<td>0.002</td>
<td>1.0352 A</td>
<td>0.003</td>
<td>1.0362 A</td>
</tr>
<tr>
<td>Renneting time (s)</td>
<td>306.0</td>
<td>19.7</td>
<td>307.5</td>
<td>20.5</td>
<td>305.5</td>
</tr>
<tr>
<td>Bundz yield (%)</td>
<td>22.03 B</td>
<td>0.76</td>
<td>23.25 A</td>
<td>1.01</td>
<td>21.75 C</td>
</tr>
</tbody>
</table>

a, b, c — the same letter symbol following arithmetic means designates qualification to a uniform group based on post-hoc analysis at \( \alpha = 0.05 \).

A, B, C — the same letter symbol following arithmetic means designates qualification to a uniform group based on post-hoc analysis at \( \alpha = 0.01 \).
**Table 2. Chemical composition of lamb meat (%) (n = 20 animals per group)**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Experimental groups</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMS</td>
<td>BF</td>
<td>BF × PMS</td>
<td>BF (BF × PMS)</td>
<td>WAS</td>
<td>WAS × PMS</td>
<td>WAS (WAS × PMS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(\bar{x})</td>
<td>SD</td>
</tr>
<tr>
<td>Water</td>
<td>76.58</td>
<td>1.33</td>
<td>77.04</td>
<td>1.20</td>
<td>76.68</td>
<td>0.69</td>
<td>76.59</td>
<td>0.63</td>
<td>77.06</td>
<td>0.95</td>
</tr>
<tr>
<td>Dry matter</td>
<td>23.42</td>
<td>1.33</td>
<td>22.96</td>
<td>1.20</td>
<td>23.32</td>
<td>0.69</td>
<td>23.41</td>
<td>0.63</td>
<td>22.94</td>
<td>0.93</td>
</tr>
<tr>
<td>Fat</td>
<td>1.84</td>
<td>0.19</td>
<td>1.83</td>
<td>0.42</td>
<td>1.82</td>
<td>0.50</td>
<td>2.16</td>
<td>0.52</td>
<td>1.98</td>
<td>0.63</td>
</tr>
<tr>
<td>Protein</td>
<td>20.32</td>
<td>0.95</td>
<td>20.30</td>
<td>0.89</td>
<td>20.39</td>
<td>0.89</td>
<td>19.81</td>
<td>0.85</td>
<td>19.95</td>
<td>0.73</td>
</tr>
</tbody>
</table>

**Table 3. Physical properties of lamb meat (n = 20 animals per group)**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Experimental groups</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PMS</td>
<td>BF</td>
<td>BF × PMS</td>
<td>BF (BF × PMS)</td>
<td>WAS</td>
<td>WAS × PMS</td>
<td>WAS (WAS × PMS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(\bar{x})</td>
<td>SD</td>
<td>(\bar{x})</td>
<td>SD</td>
</tr>
<tr>
<td>Pigment content (ppm)</td>
<td>116.17 c</td>
<td>21.05</td>
<td>98.57 b</td>
<td>23.94</td>
<td>96.64 b</td>
<td>15.72</td>
<td>93.05 ab</td>
<td>13.68</td>
<td>84.18 a</td>
<td>11.40</td>
</tr>
<tr>
<td>Colour lightness (%)</td>
<td>11.50 a</td>
<td>0.98</td>
<td>12.01 ab</td>
<td>1.51</td>
<td>12.11 ab</td>
<td>1.22</td>
<td>14.06 d</td>
<td>1.87</td>
<td>13.01 cd</td>
<td>2.57</td>
</tr>
<tr>
<td>Water holding capacity (%)</td>
<td>26.07 b</td>
<td>2.79</td>
<td>24.95 b</td>
<td>4.22</td>
<td>25.42 b</td>
<td>3.05</td>
<td>19.11 a</td>
<td>1.84</td>
<td>24.78 b</td>
<td>4.27</td>
</tr>
<tr>
<td>Thermal loss (%)</td>
<td>41.85 b</td>
<td>2.72</td>
<td>42.06 b</td>
<td>3.29</td>
<td>42.39 b</td>
<td>3.27</td>
<td>37.62 a</td>
<td>5.82</td>
<td>43.28 b</td>
<td>2.55</td>
</tr>
<tr>
<td>pH</td>
<td>5.57 a</td>
<td>0.15</td>
<td>5.63 b</td>
<td>0.16</td>
<td>5.55 a</td>
<td>0.09</td>
<td>5.69 b</td>
<td>0.09</td>
<td>5.54 a</td>
<td>0.08</td>
</tr>
</tbody>
</table>

For explanations see Table 1.
WAS × (WAS × PMS) rams (13.67%), with statistically significant differences between these means and those for other experimental groups except WAS rams (13.01%). The darkest longissimus dorsi muscle occurred in PMS animals (11.50%), and the lightness value was significantly lower than that measured in BF × (BF × PMS), WAS, WAS × PMS and WAS × (WAS × PMS) animals (Table 3).

The water holding capacity ranged from 19.11% in the meat of BF × (BF × PMS) rams to 26.07% in PMS rams (P ≤ 0.05). A slightly higher water holding capacity compared with that obtained in BF × (BF × PMS) animals was characteristic of the meat of WAS × (WAS × PMS) animals (20.10%), with statistically significant differences in relation to the other experimental groups (Table 3).

The results for thermal loss were similar to those for water holding capacity. Low values were found for the semimembranosus muscle in two crossbred groups: R1 WAS × (WAS × PMS) — 36.41% and BF × (BF × PMS) — 37.62%. The thermal loss of meat in all of the other experimental groups ranged from 41.31% (WAS × PMS) to 43.28% (WAS) and differed significantly from the values obtained in BF × (BF × PMS) and WAS × (WAS × PMS) rams (Table 3).

The lowest pH measured 24 h postmortem was characteristic of the meat of WAS rams (5.54), followed by BF × PMS (5.55) and PMS animals (5.57). In these three groups, these measurements were significantly lower than those obtained in all the other rams. The highest pH (5.69) was measured in the meat of R1 BF × (BF × PMS) animals (Table 3).

Discussion

Like milk yield, milk composition is determined by several factors, of which breed is responsible for great variation in milk composition.

In terms of composition, the milk of the WAS sheep differed substantially from that obtained from the ewes of the other breeds. The solids content (21.43%) is considered high, given that the usual value for sheep’s milk ranges from 16 to 20% (Alichanidis and Polychroniadou, 1997). The use of BF and WAS rams for crossbreeding had a beneficial effect on the quality of the milk produced by the offspring. The solids content in the milk of the crossbreds was high (BF × PMS — 19.83, WAS × PMS — 19.98%) and differed significantly from that found in PMS animals (18.25%). This value was similar to that obtained by Drożdż (2000).

Most fat was contained in the milk of WAS ewes, whereas the milk of PMS ewes had over 2% less fat. Alichanidis and Polychroniadou (1997) reported considerable differences in milk fat content between European and Asian sheep used for milk production: 5.33% in Nadjii sheep, 7.3% in the typically dairy breeds Lacaune and Friesian, and 9% in Vlahico sheep. The milk protein content in these sheep ranged from 4.7 to 6.3%. The crude protein content of the analysed milk ranged from 5.9% in PMS to 6.9% in WAS animals, results which are similar to the findings of Jordan and Boylan (1995).
Sheep’s milk is an excellent material for processing, as determined by a high casein content (4.5% on average), which accounts for approx. 70 to 80% of total milk proteins. Cow’s and goat’s milk contains around half as much casein (Alichanidis and Polychroniadou, 1997). The casein content (4.51 – 5.48%) was higher than that reported by Pirisi et al. (1999) for the milk of Sarda dairy ewes (4.06 – 4.40%).

Breed had no significant effect on lactose, the level of which was relatively low and averaged 4.5%. This value was lower than that reported by Jordan and Boylan (1995) (4.9%) as is characteristic of this parameter in the milk of sheep of different breeds.

The ash content during the milk period averaged 0.97% and was similar to that reported by Hadjipanayiotou (1995). Alichanidis and Polychroniadou (1997) found marked differences in milk ash content among Greek sheep of dairy breeds.

Among the mineral components determined in the analysed material was the level of calcium, which in the form of soluble salts (phosphates) is determinant for the milk renneting process. The analysed milk did not differ in the level of calcium, which averaged 1.14 g/l. The concentration of this element was relatively low compared to the analogous value characterizing this milk parameter in other sheep breeds (Pellegrini et al., 1994).

From the technological point of view, the suitability of milk for processing is determined not only by its composition but also by some of its physicochemical traits such as density, acidity, and renneting time. Milk density is directly associated with changes in its casein-calcium complex. Milk with a lower level of calcium ions and casein usually has lower density, and thus provides poorer conditions for renneting and the formation of a normal clot.

The milk of PMS ewes was characterized by lower density, resulting from the low level of the majority of the analysed components.

The efficiency of milk processing into cheese depends not only on the type of product but also on the composition of the milk from which the cheese is produced. Sheep’s milk differs from goat’s and cow’s milk in terms of chemical composition and some physical properties, as well as in cheese yield. The almost two-fold higher cheese yield obtained from sheep’s milk is due to its high protein and casein content, which makes this milk particularly suitable for processing (Alichanidis and Polychroniadou, 1997). Technologically speaking, such a high casein content improves the coagulation properties of milk, which results in an increased yield of the final product.

The yield of bundz cheese obtained in the present study, with manufacturing according to a formula similar to that used by Bonczar et al. (2001), was higher than the yield obtained by these authors. This was due to the fact that the milk of the analysed breeds was richer in basic nutrients than the milk used by Bonczar et al. The yield of the products obtained depended on the levels of milk components, and thus on the breed of the ewes. Studies concerning the effect of crossing heavy milking breeds on the composition of milk from the offspring have produced conflicting results. Berger (1998) observed that protein content decreased in the
milk of crossbreds derived from East Friesian sheep, whereas Drożdż (2000) failed to observe this pattern.

The crossbreeding scheme used had no significant effect on improving this parameter in the crossbreds, as reflected in cheese yield, which was similar in WAS × PMS compared to PMS sheep and even lower in BF × PMS than in PMS sheep.

Evaluation of meat quality is important for the producer and consumer alike. Lamb meat produced for export and for the domestic market should be lean and easily digestible. It has been shown that body weight, as well as carcass muscling and fatness, should not be the only criteria for assessing the eating quality of lamb meat (Barwick, 1980).

No significant differences were found between the experimental rams in the basic chemical composition of their meat as expressed by the water, fat and protein content. Studies investigating the effect of breed and crossbreeding have shown that protein content is either stable (Niedziółka et al., 2000) or varies (Lipeczk et al., 2000; Roborzyński et al., 2000).

Genetic factors responsible for overfatness of lamb meat vary according to breed, crossbreeding scheme and the age of lambs at slaughter. The highest protein content (BF × PMS — fat content 1.82%, protein content 20.39%) was found in the muscles that were lowest in fat. The highest fat content coupled with the lowest protein content was found in the meat of F1 WAS × PMS animals (2.23 and 19.78%, respectively).

The protein content of the meat of PMS, BF and F1 BF × PMS rams, found in the present study, was higher than that reported by Ciuruś et al. (1996) in an experiment with the meat of two-breed crosses (Friesian rams × PMS) and three-breed crosses [Friesian rams × (Blackheaded meat rams × PMS)] (20.10 and 19.99%, respectively). The protein content of the meat of BF × (BF × PMS), WAS and WAS × PMS rams was lower than the values obtained in our study.

The relatively low level of intramuscular fat concurs with the findings of Roborzyński et al. (2000), although the results reported by Drożdż and Ciuruś (1996) show that lamb meat was more overfat than in our study.

Meat pH, particularly pH measured 24 h postmortem, is a very good and objective indicator of the maturity of meat and its suitability for processing. The mean pH values of the analysed meat ranged from 5.54 in the group of purebred WAS rams to 5.69 in the group of BF × (BF × PMS) animals, and despite statistically significant differences they were typical of meat with normal properties (5.3 – 5.8) (Hofmann, 1987). These results are similar to the values obtained by Freudenreich et al. (1985).

It was found that in R1 BF × (BF × PMS) and WAS × (WAS × PMS) rams, greater acidity was paralleled by slightly lower water holding capacity and lower thermal loss of meat. Krełowska-Kułas et al. (1995) also reported that the meat of F2 crosses [Blackheaded meat rams × (Friesian rams × PMS)] has low water holding capacity.

The structure of muscle tissue is primarily dependent on pH value and has the greatest impact on meat colour. Light colour is associated with meat with low pH,
while dark colour is correlated to high pH values. A high pH value causes changes in muscle structure and makes oxygen permeation difficult, thus increasing myoglobin content. As a result of these changes, meat is darker in colour. Colour is therefore determined by muscle pigments (pigment content) and, to a lesser degree, by the general impression of meat colour (colour lightness). This relationship was observed in the meat of WAS × (WAS × PMS) lambs, in which pigment content was also relatively high (100.33 ppm) with a comparably high pH (5.66).

The lightest colour was found in the meat of rams from the groups BF × (BF × PMS) and WAS × (WAS × PMS) (14.06 and 13.67%, respectively), and the differences in relation to the other groups were significant except for WAS animals. Considerable differences in the colour of meat from PMS rams and F₁ Suffolks × PMS animals were also reported by Drożdż and Ciuruś (1996). It is supposed that the lighter meat colour in the group of R₁ BF × (BF × PMS) rams resulted from the higher intramuscular fat content of this meat.

The analysed meat of rams from different groups differed significantly in water holding capacity under pressure and in thermal loss, but the values obtained were in the desirable range (25 and 40%, respectively). The meat of R₁ crossbreds was characterized by significantly lower water holding capacity and thus by poorer suitability for processing.

Based on the physicochemical evaluation results, it is concluded that the meat of rams from all the experimental groups had a desirable level of the analysed parameters, particularly ultimate pH value. As a consequence, no unfavourable signs of accelerated or slowed glycolysis (DFD — dark, firm, dry; PSE — pale, soft, exudative) were found in the meat.

The desirable levels of the physicochemical parameters of the analysed products are evidence of the good quality of the meat and milk obtained from the experimental sheep of all the breeds and their crossbreds. These products, obtained under ecological conditions, are characterized by high suitability for processing.

References

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Ocena mięsa i mleka pochodzącego od owiec różnych ras i ich mieszańców, utrzymywanych w warunkach ekologicznych

STRESZCZENIE

Przeprowadzone badania miały na celu określenie jakości produktów owczarskich — mięsa i mleka — pochodzących od owiec różnych ras i ich mieszańców, utrzymywanych w warunkach ekologicznych.

Materiał doświadczalny stanowiło mleko pobrane od 287 maciorek nastupujących ras: polska owca górskia (pog), Bergschaf (BF), Weisse Alpenschaf (WAS) i mieszańców F₁ (BF × pog i WAS × pog) oraz mięso pozyskane od 140 pochodzących po nich tryczków czysto rasowych (pog, WAS i BF), mieszkańców F₁ (BF × pog i WAS × pog) i R₁ [BF × (BF × pog) i WAS × (WAS × pog)] — 20 szt. w każdej grupie. Przeprowadzono ocenę składu i właściwości fizykochemicznych mleka owczego i mięsa jagnięcatego. Określono stopień ich przydatności do celów przetworczych.

Mleko maciorek rasy Weisse Alpenschaf i Bergschaf wykazałoby się najwyższą zawartością podstawowych składników, stanowiąc wysoko wydajny surowiec mleczarski. Krzyżowanie maciorek polskiej owcy górskiej z tryckami ras alpejskich prowadzi do uzyskania mieszkańców F₁, odznaczających się korzystnymi parametrami użytkowości mlecznej.

CONCENTRATION OF SOME MINERALS IN THE SERUM, WHOLE BLOOD AND HAIR OF JUVENILE RACCOON DOGS WITH FUR DEFECTS BEFORE AND AFTER INJECTION OF SEDIMINE

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Abstract
The effect of Sedimine on supplementing the deficiency of trace elements in raccoon dogs with various fur defects raised on the Velour farm in the Kirov area (Russia) was investigated. Sedimine is an injectable solution containing 16–20 mg/ml of iron, 5.5–7.5 mg/ml of iodine and 0.07–0.09 mg/ml of stabilized selenium. Four groups of animals were established, at the age of 2 months. Each group contained 8 males and 8 female raccoon dogs with various fur defects. The animals evaluated for fur quality were given scores of 1 to 3 out of 5. Sedimine was injected at amounts of 1 ml (group I), 2 ml (group II) and 3 ml (group III), with group 4 serving as the control group. The preparation was injected intramuscularly in the hip area. Using atomic absorption spectrophotometry (AAS), the concentration of minerals (Cu, Mn, J, Zn, Se, Fe, Pb, Hg and As) in serum, whole blood and hair was determined before Sedimine injection and 14 days after a single injection of Sedimine. Sedimine had a positive effect on mineral metabolism in juvenile raccoon dogs as it increased the concentration of the analysed trace elements in serum, whole blood and hair. It is believed that the preparation will at the same time eliminate various fur defects in these animals.

Key words: fur coat defects, mineral elements, raccoon dogs

Farmed animals very often have various fur defects, such as split shear hair, curly and underdeveloped guard hair, white down and other defects, which markedly reduce the commodity quality of their fur. The reasons are still poorly understood, but it is possible that the occurrence of many fur defects is related to the effect of minerals on the body (Lohi et al., 1991; Mertin et al., 2005).

It has been scientifically proven that an inadequate supply of trace and major elements greatly changes fur fibre properties, with reductions in elasticity and solidity, loss of curliness, and breakability (Mertin et al., 1991; 1992). Cobalt deficiency inhibits, and copper accelerates, the moulting of fur animals. According
to Prasad (1979), a pathological loss of hair results from a deficiency of manganese, zinc, copper, cobalt or iodine. Supplementation of feeds with the missing elements not only prevents the occurrence of such conditions, but also eliminates disease symptoms.

There are numerous studies examining the levels of minerals in the blood, fur and internal organs of fur animals according to age, physiological condition and breed (Bialkowski and Saba, 1987; Jensen and Lohi, 1988; Mertin et al., 1991; 2002; Niedbała et al., 1999). However, few studies have explored the concentration of trace elements in various pathologies, including fur defects, in these animals.

Our study investigated the effect of Sedimine on supplementing the deficiency of trace elements in raccoon dogs with various fur defects.

**Material and methods**

The study was carried out from June to December 2005 on juvenile raccoon dogs raised on the Velour farm in the area of Kirov, Russia.

The experimental animals were selected based on the analogue principle according to age, sex, weight and origin. All the animals received balanced diets with proportions of food and nutrients accepted on the farm. The caloric content of the diets during the experiment was 500 kcal per animal (2100 MJ/animal), with protein accounting for 35%, fat for 35%, and carbohydrates for 30% of the energy. Four groups of animals were established, at the age of 2 months. Each group contained 8 male and 8 female raccoon dogs with various fur defects. During the estimation of fur quality, animals were given the scores of 1 to 3 out of a possible 5. Sedimine was injected at amounts of 1 ml (group I), 2 ml (group II) and 3 ml (group III), with group 4 serving as the control group. The preparation was injected intramuscularly in the hip area.

Sedimine (A-BIO, Moscow, Russia) is a complex preparation of sterile, nonvolatile liquid for injections which is brown in colour, and which contains the following active substances: 16 – 20 mg/ml of iron, 5.5 – 7.5 mg/ml of iodine and 0.07 – 0.09 mg/ml of stabilized selenium. Sedimine has been used in cattle, sheep and pigs, but never in fur animals.

Blood for analysis was taken in the morning (before feeding) from the saphenous vein from an animal in each group.

A small amount of blood was collected and immediately stabilized with heparin to prevent clotting, and whole blood was obtained. The remaining blood was centrifuged for 10 – 15 minutes at 3000 rev/min to obtain blood serum. Prior to analysis, serum and whole blood were stored for less than 2 days at 0°C.

Hair samples were taken from all the animals from the same area of the tail by cutting a clump of hair 0.5 cm from the skin.

During the experiment, the concentrations of minerals (Cu, Mn, J, Zn, Se, Fe, Pb, Hg and As) were determined in blood serum, whole blood and hair using atomic absorption spectrophotometry (AAS) before Sedimine injection and 14 days after a single injection of Sedimine.
The data obtained were analysed using analysis of variation by way of Statistica 6.0 software. The mean, maximum and minimum values of traits and the error were defined. The significance of differences in the arithmetic means was estimated using Student’s t-test (at a 0.05 significance level).

Results

The dynamics of concentration of some minerals in the blood serum, whole blood and hair of juvenile raccoon dogs with various fur defects before Sedimine injection is analysed below. The data on the quantity of trace elements in blood serum before and after Sedimine injection are presented in Table 1.

Before the experiment, the serum concentration of copper was at a standard level (11.52 mg%). After Sedimine injection, the concentration of copper increased significantly (P < 0.05) in the experimental animals to an average of 13 mg% in comparison with the control animals, with the greatest increase observed in the animals from group 3 (13.66 mg%).

Even greater fluctuations in the experimental compared to control raccoon dogs were found for the concentration of zinc. While prior to the experiment, the serum concentration of zinc was standard at 13.82 mg%, at two weeks of the experiment it ranged from 14.85 to 16.76 mg%.

The concentration of arsenic was 1.91 mg% greater in animals with fur defects before the experiment than in the animals from group 1 after Sedimine injection.

However, the level of mercury in the animals from group 1 increased by 31% (P < 0.05) in comparison with the initial data (0.36 vs 0.52 ng%). The mercury concentration reached standard levels (0.24 ng%) in the animals from group 2 and decreased to 0.15 ng% in the animals from group 3.

In the whole blood and serum of the animals tested, a significant increase in the concentration of copper was observed after Sedimine injection (Table 2).

During the study, the concentration of selenium did not change in the control animals (0.55 mg%) but increased to 0.66 and 0.61 mg% in the animals from groups 1 and 2, respectively (P < 0.05).

Before the experiment, the level of manganese in the whole blood of raccoon dogs was significantly lower (0.90 mg%) than 14 days after a single injection of Sedimine, when it increased to 0.96 – 0.98 mg%.

Alongside copper and selenium, there were changes in the concentration of arsenic during the experiment. The level of arsenic was the lowest (7.82 mg%) in the animals from group 2, with a statistically non-significant difference in relation to the other groups.

The single injection of Sedimine caused a shift in the concentration of trace elements in hair (Table 3). Sedimine increased the quantity of copper by an average of 38% (P < 0.05), with the greatest increase in the concentration of copper in hair observed in the animals from group 3.
### Table 1. The level of trace elements in blood serum of young raccoon dogs with fur defects before and after Sedimine injection

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>Concentration of trace elements in animals before the experiment — w</th>
<th>Concentration of elements in experimental and control animals 2 weeks after Sedimine injection</th>
<th>control — z</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>group 1 (1 ml Sedimine) — x</td>
<td>group 2 (2 ml Sedimine) — o</td>
<td>group 3 (3 ml Sedimine) — y</td>
</tr>
<tr>
<td>Cu (mg%)</td>
<td>11.52±0.51 wy</td>
<td>12.15±0.40 xz</td>
<td>13.66±0.58 yz</td>
</tr>
<tr>
<td>Mn (mg%)</td>
<td>0.56±0.03</td>
<td>0.57±0.20</td>
<td>0.60±0.02</td>
</tr>
<tr>
<td>Se (mg%)</td>
<td>0.08±0.01</td>
<td>0.07±0.01</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>I (mg%)</td>
<td>0.23±0.01</td>
<td>0.24±0.01</td>
<td>0.25±0.01</td>
</tr>
<tr>
<td>Zn (mg%)</td>
<td>13.82±0.67 wy</td>
<td>14.95±0.76 xy</td>
<td>16.76±0.48 yz</td>
</tr>
<tr>
<td>As (mg%)</td>
<td>5.01±0.42 wx</td>
<td>3.10±0.50</td>
<td>4.14±0.30</td>
</tr>
<tr>
<td>Pb (mg%)</td>
<td>0.04±0.01</td>
<td>0.03±0.01</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>Hg (ng%)</td>
<td>0.36±0.06 wy</td>
<td>0.52±0.03 xoyz</td>
<td>0.15±0.06</td>
</tr>
</tbody>
</table>

Note: letters w x o y z denote the level of significance between groups, \( P < 0.05 \).

### Table 2. The level of trace elements in the whole blood of young raccoon dogs with fur defects before and after Sedimine injection

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>Concentration of trace elements in animals with fur defects before the experiment — w</th>
<th>Concentration of trace elements in experimental and control animals 2 weeks after Sedimine injection</th>
<th>control — z</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>group 1 (1 ml Sedimine) — x</td>
<td>group 2 (2 ml Sedimine) — o</td>
<td>group 3 (3 ml Sedimine) — y</td>
</tr>
<tr>
<td>Cu (mg%)</td>
<td>14.89±0.40 wy</td>
<td>16.13±0.59</td>
<td>16.90±0.39 yz</td>
</tr>
<tr>
<td>Mn (mg%)</td>
<td>0.90±0.01 wxy</td>
<td>0.96±0.02</td>
<td>0.97±0.02</td>
</tr>
<tr>
<td>Se (mg%)</td>
<td>0.59±0.01 wxo</td>
<td>0.66±0.02 xyz</td>
<td>0.57±0.03</td>
</tr>
<tr>
<td>I (mg%)</td>
<td>0.08±0.01</td>
<td>0.09±0.01</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Zn (mg%)</td>
<td>35.86±1.32</td>
<td>38.19±1.88</td>
<td>36.48±1.03</td>
</tr>
<tr>
<td>As (mg%)</td>
<td>9.68±0.60 wo</td>
<td>10.75±0.66 xo</td>
<td>9.78±0.51</td>
</tr>
<tr>
<td>Pb (mg%)</td>
<td>0.09±0.01</td>
<td>0.09±0.01</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>Hg (ng%)</td>
<td>0.98±0.30</td>
<td>0.73±0.19</td>
<td>0.80±0.18</td>
</tr>
</tbody>
</table>

Note: letters w x o y z denote the level of significance between groups, \( P < 0.05 \); y between groups, \( P < 0.05 \).
Table 3. The level of trace elements in the hair of young raccoon dogs with fur defects before and after Sedimine injection

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>Concentration of trace elements in animals with fur defects before the experiment — w</th>
<th>Concentration of trace elements in experimental and control animals 2 weeks after Sedimine injection</th>
<th>group 1 (1 ml Sedimine) — x</th>
<th>group 2 (2 ml Sedimine) — o</th>
<th>group 3 (3 ml Sedimine) — y</th>
<th>control — z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu (mg%)</td>
<td>0.27±0.01 wxoy</td>
<td>0.38±0.02 xoyz</td>
<td>0.46±0.01 oz</td>
<td>0.47±0.02 yz</td>
<td>0.29±0.02</td>
<td></td>
</tr>
<tr>
<td>Mn (mg%)</td>
<td>0.14±0.01 wo</td>
<td>0.17±0.01</td>
<td>0.19±0.01 oyz</td>
<td>0.16±0.01</td>
<td>0.15±0.01</td>
<td></td>
</tr>
<tr>
<td>Se (mg%)</td>
<td>0.02±0.01</td>
<td>0.03±0.01</td>
<td>0.03±0.01 oyz</td>
<td>0.03±0.01</td>
<td>0.02±0.01</td>
<td></td>
</tr>
<tr>
<td>I (mg%)</td>
<td>0.19±0.01</td>
<td>0.20±0.01</td>
<td>0.21±0.01 oyz</td>
<td>0.21±0.01</td>
<td>0.19±0.01</td>
<td></td>
</tr>
<tr>
<td>Zn (mg%)</td>
<td>2.64±0.02 wxy</td>
<td>2.82±0.06</td>
<td>2.67±0.05</td>
<td>2.72±0.03</td>
<td>2.70±0.04</td>
<td></td>
</tr>
<tr>
<td>As (mg%)</td>
<td>32.04±5.60</td>
<td>18.50±2.51 xz</td>
<td>24.88±2.13</td>
<td>24.77±2.31</td>
<td>27.51±1.69</td>
<td></td>
</tr>
<tr>
<td>Pb (mg%)</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
<td>0.01±0.01</td>
<td></td>
</tr>
<tr>
<td>Hg (ng%)</td>
<td>3.23±0.29</td>
<td>2.79±0.11</td>
<td>2.93±0.13</td>
<td>2.87±0.12</td>
<td>2.78±0.07</td>
<td></td>
</tr>
</tbody>
</table>

Note: letters w x o y z denote the level of significance between groups, P<0.05.
Similar changes were observed for manganese. After injection of Sedimine, the concentration of manganese increased significantly in the experimental animals and remained at the pre-study level in the control animals.

The greatest increase in the concentration of zinc in hair was observed in the animals that received 2 ml of Sedimine.

The level of arsenic after injection of Sedimine decreased significantly in the raccoon dogs from group 1 (from 32.04 to 18.50 mg%), with a statistically non-significant difference compared to the animals from the other groups. In addition to the internal changes that occurred in the experimental animals, in comparison with the control group there were marked changes in fur quality in animals from groups 1, 2 and 3.

Fourteen days after the Sedimine injection, fur quality was observed to improve by an average of 2 points in 35% of the experimental animals. In the animals with ruffled fur, hair matting stopped and hair knotting disappeared. Those animals that had thin layers of fur developed long hair all over the body. The best results were obtained for raccoon dogs from groups 2 and 3.

**Discussion**

Our study showed that a disturbed mineral metabolism can be one of the causes of various fur defects in farmed animals.

It is known that supplementing animals with trace elements alters the quantity of not only the trace elements studied, but also other trace elements (Mustonen et al., 2001).

A single injection of Sedimine resulted in significant changes in the levels of practically all of the trace elements studied (copper, manganese, selenium, zinc, arsenic and mercury) in blood serum, whole blood and hair.

The concentration of copper in the experimental animals increased in blood serum, whole blood and hair, although in the control animals the concentration of copper remained at virtually pre-experiment level. The increase was probably related to improvements in the absorbability and assimilation of copper by the bodies of the raccoon dogs. Similar changes were observed for zinc, manganese and selenium.

It is also interesting to note the changes in the serum concentration of mercury in the animals from different groups. In the animals from group 1 injected with a single dose of Sedimine, the concentration of this element increased by 31% (P<0.05) in comparison with the initial data (from 0.36 to 0.52 ng%). In the animals from group 2, the quantity of mercury remained at a standard level (0.24 ng%) and in the raccoon dogs from group 3 it decreased to 0.15 ng%.

It is concluded that the medical product Sedimine, which has never been used in fur animals, has a positive effect on the mineral metabolism of juvenile raccoon dogs as it increased the concentration of the analysed trace elements in blood serum, whole blood and hair and thus eliminated fur defects in the tested animals. Sedimine can therefore be recommended as a medical and preventive remedy.
Mineral concentration in raccoon dogs with fur defects

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OLGA E. EVENKO, NADIEZDA A. SUNTSOVA, VICTOR Z. GAZIZOV, BOGUSŁAW BARABASZ

Konzentracja niektórych składników mineralnych w surowicy, w pełnej krwi i we włosach u młodych jenotów z defektami okrywy włosowej przed i po użyciu preparatu Sedimin

STRESZCZENIE

Badano wpływ preparatu Sedimin na uzupełnienie niedoborów mikroelementów przy różnych wadach okrywy włosowej u jenotów utrzymywanych na fermie „Welur” w rejonie Kirowskim (Rosja). Sedimin — to roztwór przeznaczony do iniekcji, zawierający 16–20 mg/ml żelaza, 5,5–7,5 mg/ml jodu i 0,07–0,09 mg/ml stabilizowanego selenu. Do celów badawczych utworzono 4 grupy młodych jenotów, w wieku 2 miesięcy; każda grupa liczyła 8 samców i 8 samic z różnymi defektami okrywy włosowej. Przy ocenie pokroju za jakość okrywy zwierzęta te otrzymały od 1 do 3 punktów z 5 możliwych. Zwierzętom I grupy podano 1 ml preparatu Sedimin, w II grupie — 2 ml, w III — 3 ml, a IV była grupą kontrolną. Preparat podawano poprzez iniekcję, domieszano w okolicy biodra. W trakcie eksperymentu, za pomocą spektrofotometru atomowo-absorpcyjnego (AAS), określono koncentrację

References


składników mineralnych (Cu, Mn, J, Zn, Se, Fe, Pb, Hg, As) w surowicy, w pełnej krwi oraz we włosach — w okresie przed podaniem preparatu i po 14 dniach od jednorazowego podania.

Stwierdzono korzystny wpływ preparatu Sedimin na przemianę składników mineralnych u młodych jenotów. Powodował on wzrost koncentracji badanych mikroelementów w surowicy, w pełnej krwi oraz we włosach. Można sądzić, że preparat ten będzie równocześnie zapobiegał występowaniu licznych defektów okrywy włosowej u tych zwierząt.
EFFECT OF TYPE OF FEED AND BREED OF CATTLE ON PRODUCTIVE INDICATORS AND THE CHEMICAL COMPOSITION OF BEEF*

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¹Department of Animal Nutrition and Feed Science, ²Department of Animal Genetics and Breeding, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

Abstract

Two experiments were carried out with fattening bulls at an initial weight of approx. 440 kg. In experiment D1, Limousin animals were assigned to two feeding groups (6 animals per group) analogous in terms of age (approx. 14 months of age) and differing in the type of silage fed. In experiment D2, the three analogous breed groups included Limousin (L), Hereford (H) and Simmental (S) bulls (6 animals per group). In experiment D1, the basic bulky feed was maize silage (group KK) or wilted meadow grass silage (group KT). In experiment D2, both silages were fed at a 50:50 weight ratio and supplemented with meadow hay (1 kg/day) and concentrate (1 – 1.2 kg/100 kg body weight), which had the same feed components in both experiments. The following parameters were determined during the study: body weight; the chemical composition and fatty acid profile of feeds; the chemical composition and levels of fatty acids and total cholesterol in samples of meat from the longissimus dorsi (LD) muscle; and dressing percentage. It was found that Limousin bulls receiving meadow grass silage had a lower level of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in the LD muscle, a higher level of polyunsaturated fatty acids (PUFA) (especially n-3 C 18:3) and a narrower n-6:n-3 PUFA ratio than animals fed maize silage. Similar values were found in both groups for carcass meat content, dressing percentage and total cholesterol content. Within breed groups, Simmental and Limousin bulls were characterized by a higher fattening and slaughter value, higher levels of n-3 and n-6 PUFA, and a lower level of SFA (C 14:0 and C 16:0) than Hereford bulls. Simmental bulls had a lower MUFA level and a wider n-6:n-3 PUFA ratio.

Key words: beef cattle, feeding, breeds, fatty acid profile, chemical composition of meat, slaughter value

Beef continues to play a major role in the global food balance of highly developed countries and its elimination from the modern human diet seems unlikely (Pisulewski et al., 2001). In recent years food has come to be viewed not only as

* This work was conducted as part of NRIAP statutory activity, project no. 4208.1.
a source of nutrients but also in terms of its impact on human health (Belisle et al., 1998). The increasing consumer demand for high-quality beef has seen breeders adopting appropriate breeds for fattening and technologies for rearing and feeding young slaughter cattle (Spears, 1996). The current feeding methods for fattened animals are aimed at modifying not only the fat-to-meat ratio (French et al., 2000; Yang et al., 2002), but above all the percentage and mutual proportions of essential unsaturated fatty acids (EUFA) in bovine carcasses (Duckett et al., 1993; Enser et al., 1999; French et al., 2003). Previous studies on improving the dietetic value of beef by nutrition have used mostly dietary supplements of different vegetable fats (Skjervold, 1993; Strzetelski et al., 2001). There are no conclusive data in the available literature concerning the possibility of improving the dietetic properties of beef by using diets differing in the type of bulky feed or by choosing appropriate breeds of fattened bulls.

The aim of the present study was to determine the degree to which the type of silage fed during the finishing period and the breed of bulls affect the chemical composition and health-promoting properties of the beef obtained.

**Material and methods**

**Experimental design and animal feeding and management**

Two feeding trials were carried out during the finishing period. Experiment D1 involved 12 Limousin bulls at an initial body weight of approximately 440 kg, and experiment D2 involved 18 Limousin (L), Hereford (H) and Simmental (S) bulls weighing 451, 436 and 463.2 kg, respectively. In experiment D1, which lasted 69 or 76 days according to the group, animals were assigned to two feeding groups (6 animals per group) that were analogous in terms of age (approx. 14 months), and in the 70-day experiment, D2, bulls were allocated to three breed groups (L, W and S; 6 animals per group) that were analogous in terms of age. The bulls used in both experiments were reared during calfhood with foster mothers for 6–8 months and then fed indoors with farm-produced feeds (containing silage, hay and concentrate) to obtain weight gains of approximately 1000 g per day.

In the first experiment, the basic bulky feed was maize silage (group KK) or wilted meadow grass silage (group KT). In the second experiment, both silages were fed at a 50:50 weight ratio to ensure refusals. These feeds were supplemented with a limited amount of meadow hay (1 kg/day) and concentrate (1 – 1.2 kg/100 kg body weight), which in both experiments contained the same feed components (ground barley — 42%, ground wheat — 20%, ground triticale — 17%, rapeseed meal — 15%, soybean meal — 2%, mineral-vitamin mixture — 4%).

Bulls were fed individually and their feed intake was monitored by daily weighing of the feed offered and of refusals. The mean daily feed intake was determined by dividing the total feed intake by the number of experimental days. The energy and protein value of the feeds and the percentage of concentrate were determined in accordance with feeding standards (IZ-INRA, 2001), assuming
weight gains of 1300 g/day for Limousin and Simmental bulls and 1200 g/day for Hereford bulls. Wet roughages and concentrates were given twice daily, whereas meadow hay was given once daily after evening feeding. During the experiment, animals were kept in tethered stalls equipped with automatic drinkers and trough partitions, with separate containers for concentrates.

**Measurements, analyses and calculations**

During the experiment, the initial and final body weight of the animals, the chemical composition of the feeds and samples of meat from the *longissimus dorsi* (LD) muscle, and the fatty acid profile of feed and meat samples were determined. The basic chemical composition of the feeds was determined according to standard procedures (AOAC, 1990), and the NDF and ADF fibre fractions in silages and hay were determined according to the Goering and Van Soest method (1970). Analyses of volatile fatty acids (VFA) in silages and determinations of the profile of higher fatty acids in the feed samples were carried out using a Varian 3400 gas chromatograph with a CP-WAX 58 column, 25 m × 0.53 mm × 1.0 micron (FID detection, 260°C; range 11, helium as a carrier gas, 6 ml/min., injector temperature 200°C), using an 8200 CX autosampler. The analysis time was 17 min when the level of VFA in silages was determined, and 65.4 min when the profile of higher fatty acids was determined in feeds. In both cases, the sample injection was 1.0 µl. Lactic acid in silages was determined using high-performance liquid chromatography following centrifugation of water filtrates with 24% metaphosphoric acid, using a Shimadzu chromatograph and a Lichrocart column (Superspher RP 18, 250). A UV 210 nm detector was used for the analyses. The flow rate of the eluent (1 l H₂O + 100 µ concentrated H₂SO₄) was 1 ml/min. The sample injection was 20 µl, and the analysis time was 21 min. The pH value of the silages was determined using an Elwro N 5170 potentiometer.

After the end of fattening, the animals were transported to a slaughterhouse in which they were subjected to control slaughter following a 24-h feed withdrawal. Carcass analysis and dissection of 5 valuable cuts into meat, fat and bones were performed using the procedure developed at the National Research Institute of Animal Production (IZ, 2004). The basic chemical composition of the meat samples from LD muscle was determined using the standard method (AOAC, 1990), and the total cholesterol content was determined colourimetrically using a colour reaction with FeCl₂ solution. The fatty acid profile in the LD muscle was determined by gas chromatography, using the same chromatograph and procedures as for the determination of the profile of higher fatty acids in the feed samples. The analysis time was 66.2 min and the sample injection was 2.0 µl. All the chemical analyses and determinations were performed at the Main Laboratory of the National Research Institute of Animal Production in Aleksandrowice, in accordance with standard procedures.

Statistical analysis of the results obtained was performed using the ANOVA procedure of one-way analysis of variance in the SAS (1999/2001) statistical packet. The significance of differences between the breed groups was determined using Duncan’s multiple range test.
Results

The nutrient content of the silages and meadow hay and their nutritive value (Table 1) corresponded to values characteristic of medium-quality feeds. The daily intake of dry matter, energy (UFV) and protein (PDI) by the bulls (Table 2) was similar to the IZ-INRA (2001) feeding requirements for early-, medium- and late-maturing beef bulls, with daily weight gains of 1200–1400 g/day.

Table 1. Chemical composition (%) and nutritive value of the feeds

<table>
<thead>
<tr>
<th>Item</th>
<th>maize silage</th>
<th>grass silage</th>
<th>meadow hay</th>
<th>concentrate mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>29.2</td>
<td>37.5</td>
<td>85.24</td>
<td>87.70</td>
</tr>
<tr>
<td>Crude ash</td>
<td>1.31</td>
<td>4.16</td>
<td>7.87</td>
<td>6.95</td>
</tr>
<tr>
<td>Crude protein</td>
<td>2.51</td>
<td>4.67</td>
<td>7.76</td>
<td>14.80</td>
</tr>
<tr>
<td>Crude fat</td>
<td>1.16</td>
<td>1.47</td>
<td>1.52</td>
<td>2.41</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>5.79</td>
<td>11.01</td>
<td>27.13</td>
<td>5.58</td>
</tr>
<tr>
<td>N-free extractives</td>
<td>18.33</td>
<td>18.19</td>
<td>40.96</td>
<td>56.06</td>
</tr>
<tr>
<td>ADF</td>
<td>8.73</td>
<td>14.34</td>
<td>29.34</td>
<td></td>
</tr>
<tr>
<td>NDF</td>
<td>12.15</td>
<td>19.92</td>
<td>47.50</td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>2.21</td>
<td>1.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.41</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butyric acid</td>
<td>0.04</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>3.67</td>
<td>4.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Content in 1 kg feed:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UFV</td>
<td>0.24</td>
<td>0.27</td>
<td>0.56</td>
<td>0.92</td>
</tr>
<tr>
<td>PDIN (g)</td>
<td>15.2</td>
<td>27.5</td>
<td>48.0</td>
<td>101.0</td>
</tr>
<tr>
<td>PDIE (g)</td>
<td>19.1</td>
<td>22.8</td>
<td>57.0</td>
<td>98.1</td>
</tr>
<tr>
<td>P (g)</td>
<td>0.49</td>
<td>1.78</td>
<td>2.62</td>
<td>4.60</td>
</tr>
<tr>
<td>Ca (g)</td>
<td>0.79</td>
<td>3.66</td>
<td>5.39</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Table 2. Mean daily intake of feeds and nutrients by fattening bulls

| Feeds                | Experiment 1* | | | | |
|----------------------|---------------|---|---|---|
|                      | L             | KT | L | H | S  |
|                      | KK (n = 6)    | KT (n = 6) | (n = 6) | (n = 6) | (n = 6) |
| Maize silage (kg)    | 17.3          | 13.8 | 10.2 | 9.5 | 11.2 |
| Meadow grass silage (kg) | 1.0          | 1.0 | 1.0 | 1.0 | 1.0 |
| Meadow hay (kg)      | 4.97          | 4.89 | 4.93 | 4.73 | 4.95 |
| Concentrate mixture (kg) | 10.26      | 10.31 | 11.26 | 10.74 | 11.83 |
| Intake of nutrients: |               |     |     |     |     |
| dry matter (kg)      | 10.26         | 10.31 | 11.26 | 10.74 | 11.83 |
| UFV                  | 9.28          | 8.78 | 9.77 | 9.33 | 10.23 |
| crude protein (G)    | 1247.4        | 1445.8 | 1452.0 | 1388.7 | 1511.6 |
| PDIN (g)             | 813.0         | 921.4 | 928.0 | 889.5 | 965.0 |
| PDIE (g)             | 875.0         | 851.3 | 924.6 | 884.3 | 961.7 |

* Feeding groups: KK — maize silage; KT — meadow grass silage.
** Breed groups: L — Limousin; H — Hereford; S — Simmental.
Table 3. Body weight and daily weight gains, carcass analysis, chemical composition of the *longissimus dorsi* (LD) muscle and the level of total cholesterol in *LD* muscle

<table>
<thead>
<tr>
<th>Item</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>KK (n = 6)</td>
<td>KT (n = 6)</td>
<td></td>
<td>L (n = 6)</td>
<td>H (n = 6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>s.e.m.</td>
<td>CV</td>
<td></td>
<td>s.e.m.</td>
<td>CV</td>
</tr>
<tr>
<td>Initial body weight (kg)</td>
<td>440.5</td>
<td>433.3</td>
<td>12.14</td>
<td>451.0</td>
<td>436.0</td>
<td>463.2</td>
</tr>
<tr>
<td>Final body weight (kg)</td>
<td>536.3</td>
<td>525.3</td>
<td>22.04</td>
<td>541.0</td>
<td>514.7</td>
<td>556.0</td>
</tr>
<tr>
<td>Days of feeding</td>
<td>69</td>
<td>76</td>
<td></td>
<td>70</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Daily weight gain (g)</td>
<td>1388.7</td>
<td>1210.2</td>
<td>163.14</td>
<td>1285.7</td>
<td>1148.3</td>
<td>1328.6</td>
</tr>
<tr>
<td>Carcass weight (kg)</td>
<td>341.1</td>
<td>329.4</td>
<td>21.35</td>
<td>338.3</td>
<td>273.6</td>
<td>314.9</td>
</tr>
<tr>
<td>Dressing percentage</td>
<td>63.6</td>
<td>62.7</td>
<td>1.79</td>
<td>62.53</td>
<td>53.16</td>
<td>56.65</td>
</tr>
<tr>
<td>Carcass meat percentage</td>
<td>78.8</td>
<td>77.9</td>
<td>1.26</td>
<td>79.68</td>
<td>74.75</td>
<td>77.29</td>
</tr>
<tr>
<td>Chemical composition of meat (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dry matter</td>
<td>22.31</td>
<td>24.02</td>
<td>0.63</td>
<td>24.34</td>
<td>24.36</td>
<td>23.33</td>
</tr>
<tr>
<td>crude ash</td>
<td>1.12</td>
<td>1.1</td>
<td>0.02</td>
<td>1.104</td>
<td>1.083</td>
<td>1.073</td>
</tr>
<tr>
<td>crude protein</td>
<td>22.48</td>
<td>22.32</td>
<td>0.43</td>
<td>22.43</td>
<td>21.75</td>
<td>22.14</td>
</tr>
<tr>
<td>crude fibre</td>
<td>1.73</td>
<td>1.23</td>
<td>0.24</td>
<td>1.48</td>
<td>1.97</td>
<td>1.03</td>
</tr>
<tr>
<td>Total cholesterol (mg/100 g)</td>
<td>44.344</td>
<td>42.18</td>
<td>2.14</td>
<td>43.26</td>
<td>42.71</td>
<td>42.50</td>
</tr>
</tbody>
</table>

a, b, c ≤ 0.05; A, B, C ≤ 0.01.
For explanations see Table 2.
In both experiments, no statistically significant differences (P > 0.05) were found between the feeding and breed groups in daily weight gains or in the total protein and cholesterol content of LD meat samples (Table 3). No significant differences were found between the feeding groups (experiment 1) in the initial or final weight of animals, carcass analysis or chemical composition of the meat except for crude fat, the amount of which in the samples taken from KT animals was significantly (P ≤ 0.05) lower than in KK animals. Analysis of the growth of animals within breed groups (experiment 2) showed that at the start of control fattening at 14 months of age, Simmental bulls achieved a significantly (P ≤ 0.01) higher body weight than Hereford bulls, and a non-significantly (P > 0.05) higher body weight than Limousin animals. Compared to Hereford bulls, Simmental and Limousin bulls achieved higher daily weight gains (non-significant due to the high individual variation of this trait — CV 12.06) and thus a significantly (P ≤ 0.05 or P ≤ 0.01) higher final body weight. Statistically significant (P ≤ 0.01) differences were also found between the breed groups in carcass weight, dressing percentage, and the meat content of the five prime cuts, and in the crude protein content of the LD muscle. Statistically significant differences (P ≤ 0.05 or P ≤ 0.01) were also found between Simmental bulls and Limousin and Hereford bulls in the percentage of dry matter and crude ash in the meat samples analysed.

Table 4. Profile of fatty acids in the samples of the feed offered (% total acids)

<table>
<thead>
<tr>
<th>Item</th>
<th>Feeds</th>
<th>maize silage (n = 3)</th>
<th>grass silage (n = 3)</th>
<th>meadow hay (n = 3)</th>
<th>concentrate mixture (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA (total)</td>
<td></td>
<td>12.46</td>
<td>19.74</td>
<td>27.70</td>
<td>11.39</td>
</tr>
<tr>
<td>C 14:0</td>
<td></td>
<td>0.17</td>
<td>0.31</td>
<td>0.69</td>
<td>0.21</td>
</tr>
<tr>
<td>C 16:0</td>
<td></td>
<td>8.38</td>
<td>16.20</td>
<td>19.87</td>
<td>9.89</td>
</tr>
<tr>
<td>C 18:0</td>
<td></td>
<td>1.56</td>
<td>1.42</td>
<td>2.68</td>
<td>1.12</td>
</tr>
<tr>
<td>UFA (total)</td>
<td></td>
<td>87.54</td>
<td>80.26</td>
<td>72.29</td>
<td>88.61</td>
</tr>
<tr>
<td>C 18:2 n-6</td>
<td></td>
<td>66.83</td>
<td>30.38</td>
<td>31.49</td>
<td>65.44</td>
</tr>
<tr>
<td>C 18:3 n-6</td>
<td></td>
<td>0.035</td>
<td>0.084</td>
<td>0.121</td>
<td>0.014</td>
</tr>
<tr>
<td>C 18:3 n-3</td>
<td></td>
<td>3.89</td>
<td>45.88</td>
<td>34.68</td>
<td>4.68</td>
</tr>
<tr>
<td>MUFA (total)</td>
<td></td>
<td>16.41</td>
<td>3.70</td>
<td>5.80</td>
<td>17.60</td>
</tr>
<tr>
<td>PUFA (total)</td>
<td></td>
<td>71.14</td>
<td>76.56</td>
<td>66.49</td>
<td>71.00</td>
</tr>
<tr>
<td>PUFA n-6</td>
<td></td>
<td>66.86</td>
<td>30.47</td>
<td>31.61</td>
<td>75.46</td>
</tr>
<tr>
<td>PUFA n-3</td>
<td></td>
<td>3.89</td>
<td>45.88</td>
<td>34.68</td>
<td>4.68</td>
</tr>
<tr>
<td>PUFA n-6:n-3</td>
<td></td>
<td>17.21</td>
<td>0.66</td>
<td>0.91</td>
<td>13.99</td>
</tr>
<tr>
<td>CLA (sum of isomers)</td>
<td></td>
<td>0.388</td>
<td>0.216</td>
<td>0.208</td>
<td>0.870</td>
</tr>
</tbody>
</table>

Comparison of the results obtained for the fatty acid profile of feed samples (Table 4) showed that maize silage and concentrate contained much higher levels of MUFA and n-6 PUFA acids than meadow grass silage or hay (with similar values for the sum of PUFA). Meadow grass silage and hay were found to contain several-fold more n-3 PUFA and had a considerably lower n-6:n-3 PUFA ratio than
Table 5. Level of fatty acids in the intramuscular fat of the LD muscle of fattened bulls (% total acids)

<table>
<thead>
<tr>
<th>Item</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KK (n = 6)</td>
<td>KT (n = 6)</td>
</tr>
<tr>
<td>SFA (total)</td>
<td>40.50</td>
<td>37.06</td>
</tr>
<tr>
<td>C 14:0</td>
<td>1.06</td>
<td>0.94</td>
</tr>
<tr>
<td>C 16:0</td>
<td>20.01</td>
<td>18.68</td>
</tr>
<tr>
<td>C 18:0</td>
<td>19.05</td>
<td>18.70</td>
</tr>
<tr>
<td>UFA (total)</td>
<td>58.41</td>
<td>61.22</td>
</tr>
<tr>
<td>C 18:2 n-6</td>
<td>17.41</td>
<td>21.45</td>
</tr>
<tr>
<td>C 18:3 n-6</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>C 18:3 n-3</td>
<td>1.24 A</td>
<td>2.22 B</td>
</tr>
<tr>
<td>MUFA (total)</td>
<td>35.09</td>
<td>31.25</td>
</tr>
<tr>
<td>PUFA (total)</td>
<td>23.84</td>
<td>29.47</td>
</tr>
<tr>
<td>C 20:5 n-3</td>
<td>0.39</td>
<td>0.55</td>
</tr>
<tr>
<td>C 22:6 n-3</td>
<td>0.13</td>
<td>0.17</td>
</tr>
<tr>
<td>PUFA n-6</td>
<td>20.44</td>
<td>26.07</td>
</tr>
<tr>
<td>PUFA n-3</td>
<td>2.88</td>
<td>3.90</td>
</tr>
<tr>
<td>PUFA n-6/n-3</td>
<td>7.10</td>
<td>6.58</td>
</tr>
<tr>
<td>CLA (sum of isomers)</td>
<td>0.562</td>
<td>0.563</td>
</tr>
</tbody>
</table>

a, b, c ≤ 0.05; A, B, C ≤ 0.01.

For explanations see Table 2.
maize silage or concentrate. Compared to bulky feeds (silages and meadow hay), concentrate was characterized by a much higher level of the sum of conjugated linoleic acid (CLA) isomers.

The fatty acid content of LD meat samples is presented in Table 5. The data shown for the feeding groups indicate that the type of silage fed did not result in statistically significant differences (P > 0.05) in the fatty acid profile except for α-linolenic acid (C 18:3 n-3), which was significantly more abundant (P ≤ 0.01) in animals fed meadow grass silage (group KT) than in animals receiving maize silage (group KK). In the KT group, there were more favourable trends than in the KK group for the levels of the other polyunsaturated fatty acids (n-6 PUFA and n-3 PUFA, including C 20:5 n-3 and C 22:6 n-3), and for the n-6:n-3 PUFA ratio. Between the breed groups, statistically significant differences were not found for the level of stearic acid (C 18:0) and the sum of CLA isomers. For the other types of SFA and UFA acids and the sum of SFA, MUFA, UFA and PUFA acids, the differences found between the breed groups proved statistically significant. Compared to Hereford bulls, Simmental and Limousin bulls were characterized by a significantly higher (P ≤ 0.05 or P ≤ 0.01) n-3 PUFA concentration (including C 18:3 n-3, C 20:5 n-3 and C 22:6 n-3) and a lower SFA content (C 14:0 and C 16:0). In addition, Simmental bulls had a significantly higher (P ≤ 0.05 or P ≤ 0.01) concentration of C 18:2 n-6 and C 18:3 n-6 fatty acids compared to the other breeds. Hereford and Limousin bulls had a significantly higher (P ≤ 0.05 or P ≤ 0.01) sum of MUFA acids and a narrower n-6:n-3 PUFA ratio than Simmental bulls.

Discussion

In both experiments, traits characterizing the dressing percentage of bulls assumed slightly lower values than those reported by Oprządek et al. (2002) for the intensive TMR feeding of bulls of different beef breeds. The differences observed between the feeding groups of Limousin bulls in the crude fat content of LD muscle show that the type of silage fed and energy density of the diet had an effect on the degree of intramuscular fat deposition in the muscle tissues of the fattened animals.

The differences shown between the breed groups in the crude fat content of LD muscle probably resulted from the genetically conditioned propensity of the animals of a particular breed for different degrees of fat and protein deposition in muscle tissue at the same age. Nevertheless, these differences between the analysed breeds were within the minimum range recommended by Wichłacz et al. (1998) for the intramuscular fat content of the muscle tissue of young fattening cattle.

The results obtained in the KT and KK feeding groups for the fatty acid profile of LD meat samples are evidence that the health-promoting properties of beef can be nutritionally modified, e.g. by feeding rations differing in the type of silage fed. This is confirmed by the higher values obtained in the group fed meadow grass silage (compared to the group fed maize silage) for fatty acids (n-3 PUFA) desirable in the human diet (Pisulewski et al., 2001) and the lower values obtained for the
sum of SFA (C 14:0 and C 16:0) and total cholesterol content. Likewise, O’Sullivan et al. (2002) showed that feeding beef bulls with grass silage compared to maize silage increased the proportion of PUFA (C 18:3 n-3 and C 18:2 n-6) and α-tocopherol antioxidants in intramuscular fat. These authors also found that feeding grass silage to fattened cattle had a favourable impact on meat colour stability and reduced lipid oxidation.

The higher n-3 PUFA content of LD muscle observed in the bulls fed grass silage compared to the bulls fed maize silage probably resulted from the higher level of C 18:3 n-3 acid, which is the main fatty acid in meadow grass silages and the precursor of C 20:5 n-3 and C 22:6 n-3 acids (Marmer et al., 1984). Studies by Dymnicka et al. (2005) involving Charolais, Limousin and Hereford bulls also showed that animals fed grass silage supplemented with hay and concentrate had a lower concentration of medium-chain SFA and a higher concentration of health-promoting PUFA compared to animals fed maize silage.

The fatty acid profile of the LD meat samples for different bull breed groups demonstrated breed differences in the concentration of some types of health-promoting fatty acids. This is indicated by the more favourable samples of meat from Simmental and Limousin bulls compared to Hereford bulls in terms of PUFA (n-3 PUFA and n-6 PUFA) and SFA content (C 14:0 and C 16:0). However, Hereford and Limousin bulls were characterized by a higher proportion of MUFA and a more favourable n-6:n-3 PUFA ratio in LD muscles than Simmental bulls. Likewise, Dymnicka et al. (2005), who studied the levels of health-promoting fatty acids in the intramuscular fat (LD muscle and semitendinosus muscle) of beef bulls (Hereford, Limousin and Charolais) fed the same types of bulky feed and concentrates, observed that Hereford animals were characterized by a higher level of SFA and a lower level of PUFA (including C 20:5 n-3 and C 22:6 n-3) in their muscles. However, Hereford bulls had a more favourable n-6:n-3 PUFA ratio and a higher CLA concentration in both muscle types than the other breeds.

The high coefficients of heritability (h² = 0.57) estimated by Malau-Aduli et al. (1998) for MUFA and the breed differences shown in the present study and by other authors (Zembayashi and Nishimura, 1996) in the profile of some health-promoting PUFA acids are evidence of prospects for the genetic improvement of these intramuscular fat traits. These suggestions are corroborated by the results of other studies (Studivant et al., 1992; Zembayashi and Nishimura, 1996), which have shown that compared to other beef breeds, Wagyu cattle are genetically predisposed to the production of meat with a greater proportion of MUFA and PUFA, a wider MUFA:SFA ratio and better marbling in intramuscular fat (Boylston et al., 1995; May et al., 1993). Analogous studies performed with breeds of cattle raised in Europe (Laborde et al., 2001) have shown that compared to Red Angus cattle, the meat obtained from Simmental cattle is characterized by a greater proportion of MUFA acids in the LD muscle and a higher activity of the Δ-9 desaturase enzyme that is involved in the conversion of vaccenic acid (C 18:1 n-7) into CLA. The same studies also revealed differences in the total concentration of SFA and MUFA between F1 crossbreds derived from the mating of Hereford cows to Aberdeen
Angus, Belgian Blue, Hereford and Limousin bulls, especially between Hereford × Wagyu and the other crossbreds.

Like our study, other studies (Taylor and Smith, 1990) have shown that genetic (breed) or nutritional factors do not contribute to statistically significant differences in the total cholesterol content of beef.

In conclusion, the intensive feeding of Limousin bulls during the finishing period with rations containing wilted meadow grass silage, hay and concentrate has a favourable effect, compared to feeding maize silage with the same supplements of hay and concentrate, on the profile of health-promoting PUFA, the $n$-$6$:$n$-$3$ PUFA ratio and the total cholesterol and crude fat content of LD muscle. Within the breed groups studied, Simmental and Limousin bulls were characterized by higher fattening and slaughter value than Hereford bulls. They also had higher $n$-$3$ PUFA, and lower medium-chain SFA levels in LD intramuscular fat. Compared to Simmental bulls, Hereford and Limousin animals had a higher MUFA content and a narrower $n$-$6$:$n$-$3$ PUFA ratio that was also closer to the recommended ratio.

References


Effect of type of feed and cattle breed on beef properties

KRYSZTOF BILIK, ZENON CHOROSZY, BOGUMIŁA CHOROSZY, MAGDALENA ŁOPUSZAŃSKA-RUSEK

Wpływ rodzaju skarmianej paszy i rasy bydła na właściwości produkcyjne i skład chemiczny wołowiny

STRESZCZENIE

Przeprowadzono dwa doświadczenia na buhajkach opasowych ras mięsnych o początkowej masie ciała około 440 kg. W doświadczeniu D1 zwierzęta rasy Limousine przydzielono do dwóch analogicznych pod względem wieku (około 14 miesięcy życia) grup żywieniowych (po 6 szt.), różniących się rodzajem skarmianej kiszonki, a w doświadczeniu D2 do trzech analogicznych grup rasowych: Limousine — L, Hereford — H i Simmental — S (po 6 szt.). W doświadczeniu D1 podstawową paszę objętościową stanowiła kiszonka z kukurydzy (grupa KK) lub kiszonka z podsuszonej runi łączowej (KT), a w D2 obie kiszonki w stosunku wagowym 50 : 50. Kiszony uzupełniano sianem łączowym

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(1 kg/dzień) i mieszanką treścią (1 – 1,2 kg/100 kg masy ciała), o tym samym w obu doświadczeniach składzie komponentów paszowych. Podczas badań określono masę ciała, skład chemiczny pasz i profil kwasów tłuszczowych w skarmianych paszach oraz skład chemiczny, zawartość kwasów tłuszczowych i cholesterolu całkowitego w próbkach mięsa z mięśnia najdłuższego grzbietu (M. longissimus) i wydajność rzeźną tusz.

W mięśniu najdłuższym grzebietu buhajków rasy Limousine żywionych kiszona z trawy łąkowej stwierdzono niższą zawartość kwasów tłuszczowych SFA i MUFA, a wyższą PUFA (zwłaszcza C 18:3 n-3) oraz węższy stosunek PUFA n-6/n-3, niż u zwierząt żywionych kiszonką z kukurydzy. Pod względem zawartości mięsa w tuszy i wydajności rzeźnej oraz zawartości cholesterolu całkowitego w obu grupach uzyskano zbliżone wartości. W obrębie grup rasowych buhajki rasy Simmental i Limousine charakteryzowały się wyższą wartością opasową i rzeźną oraz wyższą zawartością PUFA n-3 i n-6, zaś niższą niż zwierzęta rasy Hereford zawartością SFA (C 14:0 i C 16:0). Buhajki rasy simentalskiej miały natomiast niższą niż buhajki pozostałych ras zawartość kwasów tłuszczowych MUFA oraz szerszy stosunek PUFA n6/n-3.
ANALYSIS OF AIR MOVEMENT BETWEEN BROILER FARM BUILDINGS IN CALM WIND CONDITIONS

Piotr Herbut, Wacław Bieda

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Abstract

This study presents the results of an analysis of air movement, generated by transverse ventilation, between the buildings of a large commercial broiler farm. In calm wind conditions, air movement near the ground, generated by the ventilation system, was transverse to the buildings. This resulted in the movement of exhaust air from the ventilation outlet of one broiler house to the ventilation inlet of an adjacent broiler house. Measurements showed that the distribution and range of air flow was affected by fan housing. Recommendations are given for more efficient ventilation of the space between farm buildings.

Key words: large commercial poultry farm, ventilation, calm wind, air movement

A very large volume of ventilation air is necessary for efficient production of broiler chickens. In winter, a typical broiler house with a stocking density of some 18,000 chickens uses over 18 million m$^3$ of air during a 6-week production cycle. In summer, when the air requirement is 10-fold greater, this volume increases to over 180 million m$^3$.

Air discharged from a broiler house is a bioaerosol containing many kinds of contaminants such as dusts, gases, odours and microorganisms (Dobrzański et al., 1986; Herbut, 1998; Yoder and Van Wicklen, 1988; Wathes and Charles, 1994). The space between poultry farm buildings is filled by CO$_2$, NH$_3$, H$_2$S, CH$_4$, N$_2$O and trace gases such as aldehydes, amines, aromatic hydrocarbons and organic acids, which can have toxic, irritant or even carcinogenic effects (Kuczyński, 2002; Tymczyna and Chmielowiec-Korzeniowska, 2002). Compared to other livestock buildings, poultry houses are populated by the largest number of microorganisms. Due to their low mass, microorganisms float in the air and thus can be transmitted aerogenically not only inside the broiler house, but also between adjacent farm buildings, where they move according to the movement of air masses near the ground (Krzysztofik, 1992).
Observations of air movement between poultry farm buildings, made during long-term on-site experiments by the Department of Rural Building of the Agricultural University in Kraków, have shown that harmful gases, dusts and microorganisms are also transferred between adjacent poultry houses in calm wind conditions (Bieda and Herbut, 2002; 2004). This phenomenon is considered unacceptable in light of the current zoosanitary requirements for large commercial poultry farms (Dobrzanski et al., 1986; Herbut, 1998).

The aim of the present study was to determine the effect of transverse mechanical ventilation of a broiler house on air movement between adjacent buildings under simulated calm wind conditions.

**Material and methods**

On-site tests were carried out in October 2004 at a large commercial broiler farm in the town of Ujazd, Małopolska province. The farm had 10 broiler houses, each with a stocking density of approximately 18,000 chickens.

Broiler houses were located in two rows, with longitudinal axes facing east-west, as in Figure 1.
The broiler houses were one-storey buildings without cellars or windows and with gable roofs. The buildings were 90.0 m in length, 12.0 m in width and 5.0 m in total height, with roof slopes of 18°. The distance between the adjacent buildings was 25 m.

The ventilation system of each house had a capacity of approximately 100,000 m³h⁻¹ and contained 20 axial exhaust fans, which were positioned in the northern wall, as well as an inlet gap in the southern wall. The axes of the fan opening and of the inlet gap were 1.5 m above the building floor (Figure 2).

During the on-site tests, the distribution of air movement rates was measured in 6 series next to outlet fans and the inlet gap using portable cup anemometers (Conrad-Electronic).

Measurements of the distribution of exhaust air movement rates were performed for two variants: next to the housed fan with shutters, and next to the unhoused fan with shutters.

To eliminate the effect of air in the space between the two broiler houses, a “calm zone”, covered by 2.5 m-high construction foil, was created. Smoke
candles of different colours and indicator bands were used for the observations of air movement. During the measurements, the ventilation systems of the broiler houses separated by a measurement field (broiler houses no. I and II) operated at the maximum capacity of approximately 100,000 m$^3$h$^{-1}$ each.

**Results**

The distribution of air flow rates in the axis of the housed exhaust fan is shown in Figure 3a. It was characterized by a fairly narrow and rapid flow of exhaust air, which was first directed diagonally downwards and then deflected from the field and directed diagonally upwards. At a distance of 6 m from the fan, this stream had a speed of approximately 2 m·s$^{-1}$.

The distribution of the stream of exhaust air discharged by the unhoused fan was funnel-shaped, with the highest speed on the edges (Figure 3b). The reach of the 2 m·s$^{-1}$ air flow was approximately 3 m.

The external distribution of the air flow sucked into the broiler house had a reach of approximately 1.5 m, and the speed at the ventilation inlet was 2 mm·s$^{-1}$.

Figure 3. Distribution of ventilation air flow rate:

a — next to housed fan, b — next to unhoused fan
Observations of the movement of indicator smokes in the near-ground layer of the “calm zone”, created between the broiler houses, showed that initially the smoke moved rapidly in a narrow stream from the ventilation outlet of broiler house II towards the ventilation inlet of broiler house I. Some 8 m away from the fan, the trail of smoke began to gradually widen sideways and upwards, and at the same time lost speed. The smoke also thinned and next to the ventilation inlet, the smoke was barely visible (Figure 4).

Figure 4. Air movement and movement of indicator smokes in the “calm zone”, generated by the ventilation system of the broiler house

Discussion

The theoretical possibility of air being exchanged between adjacent livestock buildings has been discussed by only a few publications in the field of animal hygiene (Tombarkiewicz et al., 2000). Our own observations of the space between adjacent broiler houses in calm wind conditions, based on the misting-up of the space between adjacent broiler houses in calm wind conditions resulting from the condensation of water vapour from exhaust air, confirmed that contaminated air is recirculated between adjacent buildings (Bieda and Herbut, 2004).

Calm wind periods are particularly dangerous to aerosanitary conditions in large commercial poultry farms, especially when hen houses are equipped with a transverse ventilation system in which the ventilation outlets of one building are opposite the ventilation inlets of another building.

The results of our experiment confirmed the conjecture that in calm wind conditions, this ventilation system will suck in the contaminated air discharged from an adjacent hen house. When several hen houses are placed in a row, in unfavourable conditions the air can be transferred from one hen house to another, causing air quality to deteriorate further in each successive building.

At present, to limit the risk of recirculation of contaminated air, transverse ventilation systems can be replaced with systems where the ventilation outlet is in the roof ridge. In this system, contaminated air is directed over hen houses, where air velocity and turbulence is greater than near the ground (Krause, 1987), which would help to reduce the proportion of contaminations and remove them from the farm. Another solution is to change the layout of wall ventilation inlets and outlets.
so as to prevent the exchange of air between hen houses. This can be achieved by alternately positioning buildings in the “clean” zone (from which air will be drawn into the poultry house) and the “dirty” zone (into which contaminated air will be discharged from the poultry house). In this case, it is necessary to locate the longitudinal axes of the poultry houses along the prevailing direction of the wind, to ensure better ventilation of the space between adjacent hen houses (Bieda and Herbut, 2004).

It is expected that this problem will be solved in the future when it becomes compulsory to purify the air discharged from poultry houses, or maintain “safe” distances between poultry houses (Schirz, 1988; Richner and Schmidlin, 1995).

References


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PIOTR HERBUT, WACIAW BIEDA

Analiza ruchu powietrza pomiędzy budynkami fermy brojlerów w warunkach ciszy wiatrowej

STRESZCZENIE

Przedstawiono wyniki analizy badań ruchu powietrza pomiędzy budynkami wielkotowarowej fermy brojlerów powodowanego działaniem wentylacji poprzecznej. Podczas ciszy wiatrowej ruch powietrza w przygruntowej warstwie wywołany działaniem systemu wentylacyjnego miał kierunek poprzeczny w stosunku do budynków, a konsekwencją tego było przemieszczanie się zanieczyszczonego powietrza od wywiewu jednej brojlerni do nawiewu brojlerni sąsiedniej. Pomiary wykazały, że na rozkład prędkości i zasięg strumienia powietrza wywiewanego miały wpływ obudowy wentylatorów. Podano zalecenia dotyczące efektywniejszego przewietrzania przestrzeni między budynkami fermowymi.
EFFECT OF BROILER-HOUSE STOCKING DENSITY ON TEMPERATURE OF BEDDING AND THERMAL CONDITIONS IN THE LIVING AREA OF BIRDS

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Abstract

Temperature of bedding, temperature of air and stocking density, expressed as body weight of broiler chickens per m² production area, were measured in real operating conditions in a typical broiler house during 2 winter and 2 summer production cycles. Analysis of the measurement results showed that the thermal conditions in the living area of the birds were unfavourable during both the winter and summer periods. In the initial stage of rearing, the sensible temperature ($T_o$) was considerably lower than the required air temperature ($T_w$), while in the final stage of rearing, $T_o$ was considerably higher than $T_w$. The course of changes in bedding temperature ($\theta$) was clearly affected by stocking density (SD), or, more strictly speaking, by the stream of heat radiated by birds into bedding. During the last two weeks of rearing, stocking density should not exceed 30 kg·m⁻². Bedding temperature should be controlled to ensure optimum thermal conditions in the living area of broiler chickens during the two initial and two final weeks of rearing.

Key words: stocking density, bedding temperature, thermal conditions, broiler house

Research findings (Kaiser and van den Weghe, 1997; Bieda and Koźbial, 1999; Bieda et al., 2003; Radoń, 2004) show that the exchange of heat between birds’ bodies and the air and the medium composed of bedding, floor and the ground below is a highly complex process. The function of bedding varies according to the stage of the production cycle. In the first stage, it provides insulation by separating chickens from the cold floor. In the second stage, it emits a stream of heat produced as a result of fermentation (biothermal) processes. These processes make a considerable contribution to thermal conditions in the living area of broiler chickens.

Bedding temperature is one of the factors affecting thermal conditions in the living area of broiler chickens. To keep chickens in thermal balance in relation to the surroundings and thus in relation to the bedding, in each phase of rearing, the temperature of bedding should be close to the required air temperature in the living area of the chickens ($T_w$), which ranges from 31 to 33°C at the start of rearing and
from 18 to 20°C at the end of rearing. It is estimated that a 2°C difference between the air temperature and bedding temperature increases or decreases the sensible temperature of the air in the living area of chickens by 1°C. In production practice, the evaluation of thermal conditions in the living area of broilers is limited to the measurement of air temperature at bird head level, and less often to the measurement of two parameters — temperature and relative air humidity. It is widely believed that the measurement of these parameters is sufficient to control the broiler house climate. In practice, the sensible temperature \( T_o \) in the living area of chickens — which is influenced, among other things, by the temperature of the surface with which chickens are in direct contact — remains out of control. The results of bedding temperature measurements made in large commercial broiler facilities show that in the initial and final stages of rearing, thermal conditions in the living area of birds are inadequate, which results in poor weight gains by chickens, as well as other problems (Dobrzański and Rudzik, 1998; Nawalany et al., 2004; 2005).

Based on previous research findings, it is safe to assume that the following factors have the greatest effect on the pattern of bedding temperature \( \theta_s \):
- air temperature \( T_i \),
- floor temperature \( \theta_p \) and the temperature of the ground below \( \theta_g \),
- thermal and insulation value of bedding,
- stage of rearing,
- fermentation processes taking place in bedding.

The latter factor, shown to be the principal cause of heat production in the bedding, had previously not been associated with the heat radiated by sitting birds into bedding.

To determine birds’ contribution to thermal conditions in the living area of broilers, we attempted to prove the hypothesis that the pattern of bedding temperature in the second half of a production cycle is dependent mainly on stocking density (SD), expressed as the body weight of chickens per unit of production area.

**Material and methods**

The experiment was carried out in a broiler house with a production area of 1001 m² on a poultry farm in Ujazd (Małopolska region). The broiler house had warm air heating produced by water heaters and radiators placed in the sidewalls, as well as mechanical ventilation with transverse air flow. Several days before birds were introduced into the poultry house, a 10–12 cm layer of litter made from long barley and wheat straw was bedded.

Bedding temperature was measured using 10 PT-100 sensors, accurate to within ±0.15°C. The sensors were placed in the middle of the bedding layer in a measurement area of approx. 18 m², which covered the central and walled part of the production facility (Figure 1). The temperature of the internal air was measured...
using a PT-100 sensor placed 0.5 m above the bedding. Bedding temperature was measured at 15-minute intervals and the results were saved in the memory of a multichannel recorder.

In addition, stocking density (number of chickens introduced into the poultry house, mortality, number of chickens sold) was monitored continuously and the weight gains of broiler chickens were recorded every week. This enabled the stocking density (SD) pattern to be determined in kg·m⁻².

In the analysis, we used measurement data covering 4 production cycles in the years 2004–2005, including 2 winter cycles and 2 summer cycles.

Figure 1. Pattern of mean daily bedding temperature \( (\theta_s) \), internal air temperature \( (T_i) \) and stocking density \( (SD) \) in the winter production cycle (24 December to 8 February 2005)

Results

The measurement results were shown graphically as daily and hourly means (Figures 2–5).

In the two successive winter production cycles (24 December 2004 to 8 February 2005 and 25 February to 18 April 2005), the mean for the whole measurement area of bedding temperature \( (\theta_s) \) equalled air temperature \( (T_i) \) at the level of 29°C at 13 and 20 days of rearing, respectively. By that time, the bedding temperature was lower than the air temperature in the facility. The length of the period in which mean bedding temperature \( \theta_s \) was equal to, or ≤2°C greater or lower than, air temperature \( T_i \), and thus did not alter sensible temperature \( T_o = T_i \), was similar in both winter cycles (10 and 12 days, respectively).
From the time when $\theta_s$ and $T_i$ became equal, $\theta_s$ in bedding increased steadily to reach approx. $33^\circ$C at 39 days of rearing. On that day, in both cycles analysed, the first lot of chickens was sold and thus stocking density decreased by $4.4$ kg m$^{-2}$ (from 36.4 kg m$^{-2}$ to 32.0 kg m$^{-2}$) and by $4.3$ kg m$^{-2}$ (from 39.4 kg m$^{-2}$ to 35.1 kg m$^{-2}$). The decrease in SD was paralleled by a decrease in bedding
temperature by 3.0 and 2.5°C. After the first lot of chickens was sold, $\theta_s$ started to increase slowly (by approx. 1.5°C), and after the second lot was sold and SD decreased to 33.0 kg·m$^{-2}$, bedding temperature showed a downward tendency.

**Figure 4.** Pattern of mean daily bedding temperature ($\theta_s$), internal air temperature ($T_i$) and stocking density (SD) in the summer production cycle (6 July to 23 August 2005)

**Figure 5.** Pattern of hourly bedding temperature ($\theta$), internal air temperature ($T_i$) sensible temperatures ($T_{\text{omax}}$) and ($T_{\text{omin}}$) stocking density (SD) in the last 2 weeks of the winter production cycle (26 January to 8 February 2005)
Analysis of thermal conditions in the living area of chickens based on the difference between $\theta_s$ and $T_i$ shows that during the first 8–14 days of rearing, bedding temperature in the winter production cycles was lower than air temperature by as much as 13°C, indicating that $T_o$ in the living area of chickens was approx. 6–7°C lower than $T_i$. During the last 2 weeks of rearing, $\theta_s$ was higher than air temperature by as much as 12°C, meaning that $T_o$ was 6°C higher than $T_i$ and exceeded the required air temperature $T_w$ by 910°C.

The pattern of mean bedding temperature during the summer production cycles was similar to that during the winter period. The initial 18–21 days of rearing were characterized by $\theta_s < T_i$, and the period of neutral temperature $\theta_s \pm 2^\circ C = T_i$ was 10–12 days long, i.e. exactly the same as during the winter production cycles, while the other days of rearing were characterized by $\theta_s > T_i$.

The maximum mean bedding temperature $\theta_s = 34.5–35.5^\circ C$ was approx. 2 K higher than that prevailing during winter cycles and was obtained at a stocking density of 35.0–36.5 kg·m$^{-2}$, i.e. 1.5–2.0 kg·m$^{-2}$ less than in winter. After the first lot of chickens was sold, stocking density decreased by 3.4 and 7.0 kg·m$^{-2}$, respectively, and bedding temperature decreased by 2.0 and 2.7°C, respectively, after which time stocking density began to gradually increase. The change of the $\theta_s$ trend to a downward one began as late as at SD = 30 kg·m$^{-2}$.

With the highest mean temperature $\theta_s$, sensible temperature in the living area of chickens was approx. 28°C and was higher than the required air temperature by approx. 8°C.
The exchange of heat that takes place on the surface of bedding is a complex issue. It is due to the presence of birds, which occupy a certain portion of bedding according to stocking density, stage of rearing, time of day, etc. On the surface of the bedding occupied by sitting birds, heat is exchanged with birds’ bodies and with internal air on the remaining part of the surface. Calculation of the heat flow between the bird and its surroundings, for both the area occupied by birds and the vacant area, would be possible if we knew the temporal and spatial distribution of the surface index that determines the proportion of the total area of the production facility occupied by sitting birds. It would then be possible to use a relationship determined by Elfadil et al. (2000) between body weight and body area of broiler chickens. However, the literature provides varying data on the magnitude of sensible heat emissions by broiler chickens (Herbut et al., 1992; Podgórska and Lebiedowicz, 1995; Pedersen and Thomsen, 2000). Theoretical calculations by Radoń (2004), based on the measurement of bedding temperature patterns in real operating conditions (Bieda et al., 2003; Nawalany et al., 2004) show that in the final stage of rearing broiler chickens, total heat emission from bedding is 60–70 W m⁻². However, the flow of heat radiated to the bedding by sitting birds, which contributes to the fermentation process, has not been conclusively determined and requires further study, including of the temporal and spatial distribution of the proportion of the production facility occupied by sitting birds.

Thus, the contribution of individual factors to the fermentation process taking place in bedding under real production conditions cannot be conclusively determined due to a lack of data or considerable data differences, among other things.

The increment in the body weight of broiler chickens during the production cycle, which takes place at a gradually decreasing air temperature indoors, is paralleled by an increase in bedding temperature. Thus it can be said that the fermentation process in bedding is stimulated by sitting chickens, which dissipate a certain portion of sensible heat to the bedding. The decrease in stocking density to 30–33 kg m⁻², following the sale of successive lots of chickens resulted in a reduced magnitude of heat flow and thus in decreased θₛ. In the winter production cycles analysed, a decrease in stocking density of 4.1–4.4 kg m⁻² reduced bedding temperature by 2.0–2.5°C. After the first lot of chickens was sold (e.g. in the February–April 2005 production cycle), the increment in the body weight of birds resulted over 5 days in a repeat increase of θₛ by approx. 1.5°C. A significant decrease in mean bedding temperature to θₛ = 4°C took place as late as when stocking density was 30 kg m⁻², which is evidence of the inhibited flow of heat from fermented bedding, deprived of the supply of sufficient heat from birds.

More details about the pattern of bedding temperature in the bedding during the last two weeks of rearing are shown in the figures illustrating the course of Tᵢ, with bedding temperatures from all 10 measurement points and maximum and minimum Tₒ determined according to:
To inhibit the increase in bedding temperature, which has a negative effect on thermal conditions in the living area of birds, it is necessary to reduce the flow
of heat from the birds that occurs when stocking density decreases to below 30 kg m\(^{-2}\).

Another efficient way of decreasing bedding temperature, especially in the cold seasons — through a decrease in ground temperature under the floor and thus an increase in the flow of heat from the bedding to the ground — is to place an earth-tube heat exchanger beneath the production facility to heat the broiler house (Bieda et al., 2001).

In the final period of rearing, to reduce the risk of thermal stress in a broiler house, it seems appropriate to adopt bedding temperature control in production practice.

References


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Przeprowadzono pomiary temperatury w ściółce i temperatury powietrza przy uwzględnieniu obsady wyrażonej masą ciała brojlerów kurzych na 1 m² hali produkcyjnej, w rzeczywistych warunkach eksploatacyjnych typowej brojlerni w okresie 2 zimowych i 2 letnich cykli produkcyjnych.

Analiza wyników pozwoliła ocenić warunki termiczne w strefie przebywania ptaków jako niekorzystne, zarówno w okresie zimowym, jak i letnim. W początkowej fazie odchowu temperatura odczuwalna — $T_0$ była znacznie niższa od wymaganej temperatury powietrza — $T_w$, natomiast w końcowej fazie odchowu $T_0$ była znacznie wyższa od $T_w$. Na temperaturę ściółki — $\theta$, wyraźny wpływ miała obsada — SD, a ścisłej strumień ciepła, który ptaki oddawały do ściółki. W dwóch ostatnich tygodniach odchowu obsada ptaków nie powinna przekraczać 30 kg·m⁻².

W celu kształtowania optymalnych warunków termicznych w strefie bytowej brojlerów kurzych, podczas 2 początkowych i 2 końcowych tygodni odchowu, należy wprowadzić kontrolę temperatury ściółki.
EFFECT OF BAYCOX COCCIDIOSTAT ON COCCIDIA INFECTION IN PIGEONS

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²Pomeranian Medical University, Department of Infectious Diseases and Hepatology, Arkońska 4, 71-455 Szczecin, Poland

Abstract
A total of 617 pigeons were studied in 2000–2003. The prevalence and intensity of infestation with coccidia were determined using the Willis-Schlaaf and McMaster methods. The composition of the coccidia in the animals studied was determined based on the morphological traits of oocysts and sporulation time. Eimeria labbeana, E. Columbarum and E. columbae were isolated in pigeons during the study. The prevalence of coccidia invasion ranged from 73.85% to 97.50%. Baycox was given at a dose of 20 mg/kg body weight. After Baycox administration, the intensity of invasion decreased considerably to 50 oocysts per g of faeces. Baycox proved a highly efficient coccidiostat against pigeon coccidiosis.

Key words: pigeons, coccidiosis, Baycox

Coccidiosis is a chronic, parasitic disease of different animal species (Foronda et al., 2004; Balicka-Ramisz et al., 2000; Pilarczyk et al., 1999). It is induced by Eimeria protozoa, which live mainly in the epithelium of the small intestine.

In pigeons in Europe, coccidiosis is induced mainly by Eimeria labbeana, E. columbarum and E. columbae, the first being the most pathogenic.

In most cases, coccidiosis in pigeons runs a subclinical course. Birds excrete oocysts in faeces and usually show no clinical symptoms of the disease. As a result, coccidian invasions are often disregarded by breeders and veterinarians. In this situation it is essential that the correct diagnosis be made, as this forms the basis for preventive action and treatment (Vercruysse, 1990).

The clinical form of pigeon coccidiosis involves intestinal disorders and watery, mucous diarrhoea. Excreta are watery with sporadic traces of blood. Birds are debilitated, emaciated and apathetic (Kaleta and Bolte, 2000; Coussement et al., 1988; Mayer, 1954; Vercruysse, 1990; Wages, 1987).

Until now, sulphadimidines, coccidiovit and clazuril have been used to prevent coccidiosis in pigeons (Coussement et al., 1988; Vercruysse, 1990; Maes et al., 1988).
Of the marketed coccidiostats used with success in other animal species (e.g. poultry, sheep, rabbits and calves), Baycox deserves particular attention (Balicka-Ramisz, 1998; Pilarczyk et al., 1999).

The aim of the study was to determine the effect of Baycox on the intensity of coccidian invasion in pigeons.

Material and methods

The study was carried out in 2000–2003 in eight dovecotes in the area of Western Pomerania, Poland. A total of 617 ornamental and racing pigeons were studied. No infections or other parasitic infections were found in the birds during the experiment.

The effect of Baycox on the course of coccidia invasion was investigated in 192 pigeons. Baycox was used in dovecotes where the parasitological examination of faeces showed a high intensity of coccidia invasion. Birds from the experimental group were given Baycox (Bayer) in drinking water at a dose of 20 mg/kg body weight. The active ingredient in Baycox is toltrazuril.

The prevalence and intensity of infestation were determined based on coproscopic studies using two methods: the Willis-Schlaaf and Mc-Master’s methods (Ziomko et al., 1995). The species composition of coccidia was determined using Pellerdi’s system (1974). The study was complemented with oocyst culture performed in a moist chamber at 24–26°C. A 2.5% water extract of potassium dichromate (K₂Cr₂O₇) was used as an antimoulding agent.

Results

Three coccidia species were isolated during the study from the pigeons’ faecal samples (Table 1). The data in Table 1 show that *E. labbeana* was the most common species in the pigeons. The results indicate that monospecific infestations occurred in 70.20% of the pigeons and trispecific infestations in 0.90% of the birds (Table 2).

<table>
<thead>
<tr>
<th>Dovecote</th>
<th>Prevalence of coccidia species (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. labbeana</em></td>
</tr>
<tr>
<td>A</td>
<td>89</td>
</tr>
<tr>
<td>B</td>
<td>97</td>
</tr>
<tr>
<td>C</td>
<td>92</td>
</tr>
<tr>
<td>D</td>
<td>84</td>
</tr>
<tr>
<td>E</td>
<td>75</td>
</tr>
<tr>
<td>F</td>
<td>93</td>
</tr>
<tr>
<td>G</td>
<td>85</td>
</tr>
<tr>
<td>H</td>
<td>74</td>
</tr>
</tbody>
</table>
Table 2. Incidence of mono- and multispecific invasions in pigeons (%)

<table>
<thead>
<tr>
<th>Type of invasion</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monospecific</td>
<td>70.20</td>
</tr>
<tr>
<td>Bispecific</td>
<td>28.90</td>
</tr>
<tr>
<td>Trispecific</td>
<td>0.90</td>
</tr>
</tbody>
</table>

The prevalence of mixed coccidia invasion (Table 3) was found to range from 73.85% to 97.50%, and the intensity of invasion from 0 to 22 300 oocysts per g of faeces.

Table 3. Average prevalence and intensity of *Eimeria* invasions in the pigeons studied

<table>
<thead>
<tr>
<th>Dovecote</th>
<th>No. of analysed pigeons</th>
<th>No. of infected pigeons</th>
<th>Prevalence (%)</th>
<th>Oocysts per gram (x̄)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>65</td>
<td>48</td>
<td>73.85</td>
<td>1050 (0 – 19500)</td>
</tr>
<tr>
<td>B</td>
<td>85</td>
<td>72</td>
<td>84.71</td>
<td>1750 (0 – 12300)</td>
</tr>
<tr>
<td>C</td>
<td>96</td>
<td>81</td>
<td>84.34</td>
<td>1520 (0 – 2950)</td>
</tr>
<tr>
<td>D</td>
<td>80</td>
<td>60</td>
<td>75.00</td>
<td>2450 (0 – 13000)</td>
</tr>
<tr>
<td>E</td>
<td>92</td>
<td>79</td>
<td>85.87</td>
<td>1620 (0 – 21000)</td>
</tr>
<tr>
<td>F</td>
<td>109</td>
<td>93</td>
<td>85.32</td>
<td>2150 (0 – 12300)</td>
</tr>
<tr>
<td>G</td>
<td>40</td>
<td>39</td>
<td>97.50</td>
<td>2170 (0 – 22300)</td>
</tr>
<tr>
<td>H</td>
<td>50</td>
<td>40</td>
<td>80.00</td>
<td>1120 (0 – 9300)</td>
</tr>
<tr>
<td>Total</td>
<td>617</td>
<td>512</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OPG — oocysts per gram.

Table 4. Results of Baycox treatment of coccidiosis in pigeons

<table>
<thead>
<tr>
<th>No. of birds</th>
<th>Average intensity of invasion (oocysts per gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before treatment</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>192</td>
<td>2257</td>
</tr>
</tbody>
</table>

The effect of the coccidiostat on the intensity of coccidia invasion in pigeons is shown in Table 4. After use of the coccidiostat, the intensity of invasion decreased considerably, as evidenced by the single oocytes in the analysed faecal samples.
The study showed that giving Baycox to *Eimeria* sp.-infested pigeons at a dose of 20 mg/kg body weight radically reduced the faecal excretion of oocysts. 14 days after the end of treatment, a control analysis of the faeces was performed and the intensity of *Eimeria* parasites was determined (Table 4).

**Discussion**

In the studies carried out in Europe, the prevalence of *Eimeria* invasions was high, ranging from 50 to 100% (Dovč et al., 2004; Gaweł et al., 1994 a; b; Piasecki 2006; Mayer, 1954). In our study, the average prevalence of *Eimeria* infection in pigeons was similar to that reported by other authors. Piasecki (2006) found 64.1% of urban pigeons in the city of Wrocław to be infested with *Eimeria* protozoa. Gaweł et al. (1994 b) reported that *Eimeria* infestations are more common in ornamental pigeons than in racing pigeons.

Our study showed that giving *Eimeria* sp.-infested pigeons Baycox at a dose of 20 mg/kg body weight drastically reduced the faecal excretion of oocysts. Van Reeth and Vercruysse (1993) and Vercruysse (1990) reported that a single dose of Baycox given at 20 mg/kg body weight is effective against experimental coccidiosis in pigeons. Vercruysse (1990) found a 97% reduction in oocysts 14 days after Baycox administration. No negative effect of the preparation on the health of treated pigeons was found.

Szeleszczuk (1996) reported that the results of Polish studies indicate that Baycox is highly suitable in the treatment of pigeon coccidiosis. This author believes that in practice, complete healing of coccidiosis is not possible without breaking the chain of infection. The results of our study and those of other authors confirm the high efficiency of Baycox in controlling pigeon coccidiosis. Studies by Szeleszczuk (1995) confirmed that Baycox is effective and safe for pigeons. Giving Baycox at a dose of 7.5 mg per pigeon eliminated the faecal excretion of oocysts as soon as 72 hours post-dose. With very intense invasions, only a small number of coccidia were found administering this dose.

The principal aim of coccidiosis prevention in pigeons is to break the chain of infection by destroying oocysts in the dovecote. At the end of treatment, the dovecote and its accessories should be carefully cleaned using an agent that kills coccidian oocysts (e.g. Oo-cide).

The coccidiostat Baycox is highly effective against *Eimeria* protozoa and is useful in coccidiosis prevention and control in pigeons.

**References**


Baycox and intensity of coccidia invasion in pigeons


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Wpływ zastosowania preparatu Baycox na intensywność inwazji kokcydiów u gołębi

STRESZCZENIE

Badania przeprowadzono w latach 2000–2003 na 617 gołębiach. Ekstensywność i intensywność zarażenia kokcydiom określono metodami: Willis-Schlaafa i McMastera, natomiast skład kokcydiów u badanych zwierząt określono na podstawie cech morfologicznych oocyst oraz czasu sporulacji.
W wyniku przeprowadzonych badań u gołębi wyizolowano Eimeria labbeana, E. columbarum i Eimeria colombae. Ekstensywność inwazji kokcydiów utrzymywała się na poziomie od 73,85% do 97,50%. Baycox podano w dawce 20 mg/kg masy ciała.

Po zastosowaniu Baycoxu intensywność inwazji zmalała w znaczącym stopniu do 50 oocyst w 1 g kału. Preparat Baycox okazał się bardzo skutecznym kokcydiostatykiem przeciwko kokcydiozie u gołębi.
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3. The “Annals of Animal Science” cover the following range of topics: genetics and farm animal breeding; the biology, physiology and reproduction of animals; animal nutrition and feedstuffs; environment, hygiene and animal production technology; economics and the organization of animal production. The assignment of a paper to a given section should be proposed by the author(s), but the final decision rests with the Editors.

4. Papers are printed in English with a Polish summary.

5. Papers to be published should not exceed 16 manuscript pages (size A4) including tables, figures, photographs, etc. and a summary.

6. Papers are reviewed by two reviewers who are research workers specializing in the relevant field. One unfavourable review means that the paper will not be published. The costs of printing are covered by the authors or by the institutions from which the papers were sent, according to current rates of paper preparation and printing. Authors will receive 25 offprints of their paper free of charge.

II. Submission of Manuscripts

1. Manuscripts for publication are submitted to the Editor-in-Chief by research workers or the heads of research institutions where the studies were carried out, who take responsibility for their content, scientific value and the preparation of the text.

2. Manuscripts should be submitted in triplicate to: The Editors of “Annals of Animal Science”, National Research Institute of Animal Production, Sarego 2, 31-047 Kraków, Poland, tel. (+48) 12 422-73-33, fax (+48) 12 422-80-65, e-mail: annals@izoo.krakow.pl on diskettes, and meet the following requirements:
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   Justification: full
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The ENTER key should only be used to start a new paragraph.

3. Attached to the manuscript should be the Manuscript Submission Form as appended at the end of this Instruction.

III. Layout of the Text

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¹Department of Zoology, Jagiellonian University, św. Anny 12, 30-017 Kraków, Poland
²Department of Immuno- and Cytogenetics, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland
³Experimental Station of the National Research Institute of Animal Production, 39-331 Chorzów, Poland

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2. Manuscripts should be organized in the following order:
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4. Tables should present the most important data. The column on the left should list the parameters studied, while the columns in the middle and on the right should contain the results for individual experimental factors. Tables should contain numerical data, which are the mean values for a set of observations or measurements, replications and their statistical interpretation (e.g standard error, coefficient of variation). Tables numbered consecutively in Arabic numerals should be submitted on separate sheets. The titles of the tables should be brief. Each column should have a heading. Columns and lines should be spaced. No vertical lines are allowed. Horizontal lines can only be used for strictly justified purposes.

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