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REPORT

Identification of genetic markers associated with laying production in ostriches (*Struthio camelus*) – a preliminary study

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The aim of the study was a search for genetic markers associated with laying production in African Black ostriches *(Struthio camelus)* based on DNA fingerprinting (DFPs) method. Although analysis of the DFPs failed to distinguish bands specific for the highest or lowest parameters of laying performance, the present results did not allow for deduction about the potential lack of linkage between minisatellite DNA markers and QTLs of laying. A need for further research in this field is concluded.

KEY WORDS: genetic markers / laying production / ostrich

Since the ostriches provide valuable meat, skin, feather and eggs [Horbańczuk *et al*. 1998, Sales and Horbańczuk 1998, Cooper and Horbańczuk 2002] a growing interest in ostrich breeding and farming worldwide, including Poland [Cooper *et al*.

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2007, Horbańczuk *et al*. 2008] is observed. However, one of the principal reasons for the hindered development of this new poultry branch is low reproduction rate of African ostriches [Horbańczuk *et al*. 2001, Rybnik *et al*. 2007]. Clearly, there must be programmes in place that aim at obtaining birds with higher productivity since annual housing costs of ostrich breeders are still high. It is more profitable to keep one hen which produces 60 eggs per season than two with a productivity of 30 eggs [Horbańczuk 2002]. In order to accelerate progress in laying production, genetic evaluations are essential at the stage of selecting parents of subsequent generations. The use of traditional breeding work is very limited because of a relatively short period of ostrich husbandry in Europe (from only the beginning of the 1990s) and a relatively small average flock size [Horbańczuk 2002]. However, due to the development of molecular methods in the last decade (e.g. mini- and microsatellite sequences) new opportunities for genetic improvement of ostrich flocks have emerged [Horbańczuk *et al*. 2007, Kawka *et al*. 2007]. Considering a high level of allelic diversity, sequences of minisatellite DNA can be useful as a source of informative genetic markers linked to quantitative traits *loci*. Findings of those markers facilitate a broader utilization of variability of economically important traits.

The aim of the current investigation was to examine whether characteristic DNA fingerprinting bands associated with the level of laying production may exist in ostriches.

Material and methods

The material for the study consisted of twenty African Black ostrich hens in their third year of laying, kept according to European Union regulations, as described by Horbańczuk [2002], and recorded in a single year of production. The breeders used in this study descended from cocks and hens maintained in reproduction pairs unrelated from the grandparental generation. Two groups of hens were chosen, consisting of 10 birds, which were arranged according to a total egg production of up to 30 eggs (group I – low productivity), and a minimum of 60 eggs (group II – high productivity), respectively.

Genomic DNA samples were isolated from feathers**,** which were incubated overnight at 56°C with proteinase K [Taberlet and Bouvet 1991]. DNA was purified by two phenol/chloroform/isoamyl-alcohol extractions**.** Because DNA fingerprints can be obtained only from the undegraded DNA, each sample was examined by a spectrophotometer (Spectrophotometer DU-68 Beckman) and electrophoresis (GNA-200 Pharmacia). DNA samples of 10 µg were digested with *Hinf*I restriction enzyme for 16h. DNA fragments were subsequently separated by electrophoresis in 0.8% agarose gel for 48 h and visualized by staining with ethidium bromide. Then, DNA fragments were transferred overnight onto standard Hybond-Npf nylon filters in 20 x SSC buffer using the standard capillary method. The filters were then pre-hybridized for 40 min. at 50°C, thereafter being hybridized to probe 33.15. (NICE-Cellmark

Diagnostic), respectively [Jeffreys *et al*. 1985] for 30 min. in the same temperature. The chemiluminescent signal was induced using a Lumi-Phose® 530 solution (Cellmark Diagnostics, Germantown, MD). The DFP analysis included only bands representing fragments larger than 2 kb. For control samples all paths on the luminogramme were standarized according to the paths on the NICE DNA size standard. Banding patterns were compared between lines to classify shared and non-shared bands. The band patterns were analyzed using visual inspection and the software DNA-ProScan. The results were derived from two inspecting persons. The bands emanating were accepted as similar for both paths compared if: 1) the difference in migration between the two bands did exceed 0.5 mm [Hau *et al*. 1997] and 2) the intensity of one band was not more than double that of the other.

Results and discussion

Analyses of the representative DNA fingerprinting band patterns failed to distinguish bands specific for the high and low performance groups of layers (Photo 1). Nonetheless, the results of the present study did not allow for conclusion about the potential lack of linkage of alleles represented by hybridization bands with specific *loci* of genes engaged in expression of laying traits. Similar investigations concerning the identification of minisatellite DNA markers for quantitative trait *loci* in geese reported seven bands associated with low and five with high laying performance [Zawadzka *et al*. 2001].

Our study was a preliminary one and the results did not entitle us to deductively speculate about the potential linkage between alleles represented by specific observed hybridization bands and *loci* of genes thereof coding for the control of egg laying production. In order to obtain a much complete picture of the linkage, segregation and location of QTL in the genome, it is necessary to isolate and use probes specific for individual *loci*, corresponding to the identified bands [Kuhnlein *et al*. 1991].

The basic problem in applying the fingerprinting technique for descriptions of populations and, particularly, concluding on associations and linkages between minisatellite alleles and genes of quantitative trait, lies in the impossibility of assigning individual bands to the corresponding *loci*, and thus determining the genotype [Bruford *et al*. 1992]. Another difficulty is the limited number of samples analyzed simultaneously, caused by the lack of precise methods of comparing the results from numerous electrophoretic separations [Burke *et al*. 1991].

Further investigations are required, in which another combination of probe/enzyme and especially the application of highly polymorphic microsatellites should be applied, since the microsatellite analysis provides more detailed information and is widely used in linkage mapping of farm animals.

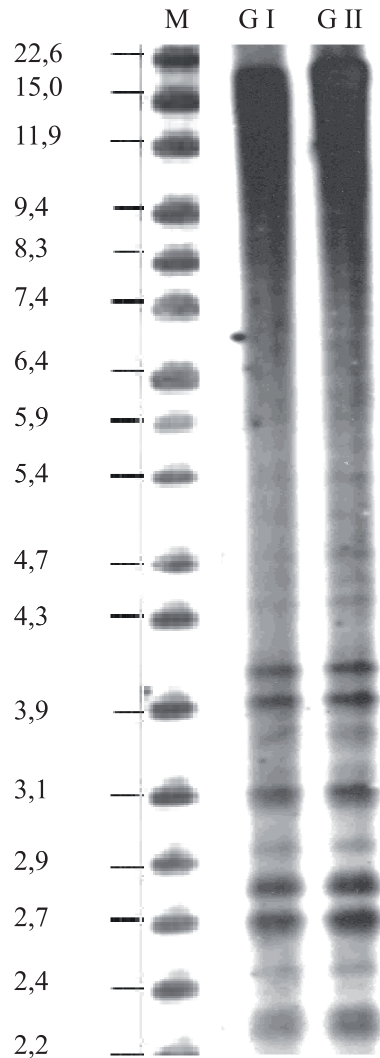

Photo 1. An example of DNA fingerprinting patterns obtained from DNA mixes of 10 ostriches from high (G I) and low (G II) laying rate groups of layers by digestion with *Hinf* I enzyme and hybrydization with 33.15 probe. M – the size standard was derived from digestion of λDNA with *Hind* III.

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Identyfikacja markerów genetycznych związanych z nieśnością u strusi – badania wstępne

Streszczenie

Celem badań była identyfikacja markerów genetycznych związanych z nieśnością u strusi czarnoszyich metodą DNA-fingerprinting (DFP). Aczkolwiek analiza DFP nie wykazała prążków różniących grupy ptaków o najwyższej i najniższej wartości badanej cechy, to jednak nie przesądza to o braku zależności między minisatelitarnymi markerami DNA a QTL. Wskazane są dalsze badania w tym zakresie.