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POLYMORPHISM VS. EXPRESSION OF PROTEIN CODING GENES IN JAPANESE QUAIL

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ABSTRACT

The study covered 97 Japanese quails of various genotypes of the protein referred to as preactin (PAK). The birds of AA genotype had a single, intensively stained, fast–migrating band; the BB genotypes had a single, slow–migrating, also intensively stained band, whereas the AB birds had two bands. The protein concentration, established basing on the area and height of peaks, was significantly higher in the BB homozygotes than in the AA ones, which appeared also in the form of more intensive staining of the slow–migrating band than that of the

fast–migrating band of the AB heterozygotes. The differences, however, were insignificant. The protein level of heterozygotes in the sub–region, calculated as the sum of two bands, was more than 50% higher than in either of the homozygous forms. The differences, confirmed statistically, can demonstrate that the genotype determining the proteins influences the expression of the genes coding particular proteins.

Key words: polymorphism, proteins, gene expression

INTRODUCTION

The polymorphism of constitutional proteins, electrophoretically separated, may appear on the electrophoregrams in the form of different speed of band migration, different number of bands or varied intensity of stain. The polymorphism may be caused by physiological factors or genetic variability of the individuals. The methods of classical genetics, but also the methods molecular biology, at the DNA level of research, may serve to verify the polymorphism. The latter research methods [4, 9] demonstrated a relationship between the polymorphism of constitutional proteins and the polymorphism of the genes related to the biosynthesis of the proteins. DNA polymorphism, present in the regions responsible for the expression of αS_2 casein gene, had significant influence on the differences in milk protein composition and milk casein content [9]. A relationship was also observed between the polymorphism in the coding region of bovine kappa casein gene and the percent content of milk protein [4]. Various levels of proteins may thus be a result of uneven gene expressions, which depend on many factors [10].

Gene expressions can be studied at the level of mRNA, which is a product of gene transcription, or at the protein level, which is the final product of the expression [9].

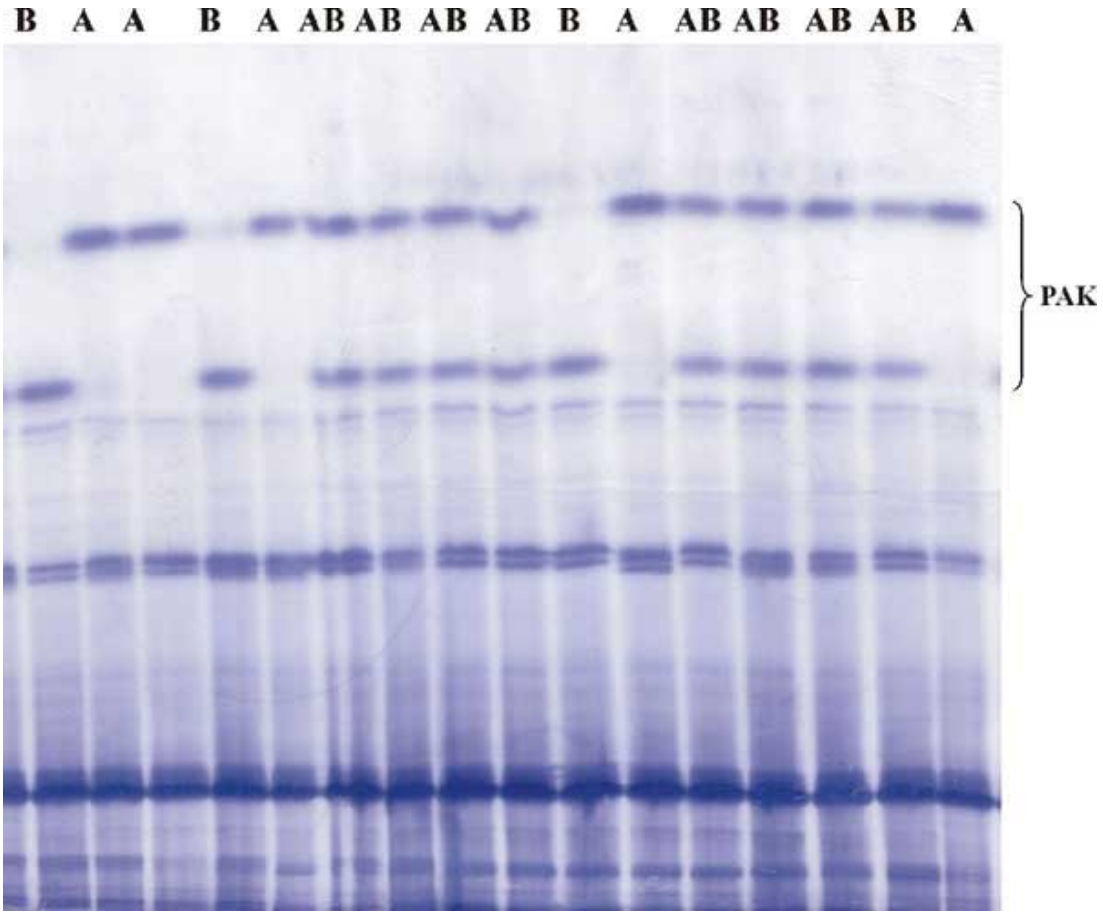
The aim of this study was to compare the expressions of the genes coding fast–migrating proteins of Japanese quail femoral muscle in relation to phenotypic forms of the proteins.

MATERIALS AND METHODS

The material for the study comprised 97 femoral muscles of Japanese quails. The polymorphism of the femoral muscle proteins was determined by means of thin–layer, horizontal polyacrylamide (paa) gel electrophoresis, carried out according to the methodology by Gahne *et al.* [2], modified by Gluchowski *et al.* [30].

Figure 1 presents a fragment of electrophoregrams of the separated proteins. Quantitative analysis covered the protein, denoted as preactin (PAK), which migrates at the speed of blood serum prealbumin (Pa–M) and which demonstrates the variability of phenotypic form. This protein was chosen because its phenotypic form can be easily established and its concentration is high in comparison with the proteins of other regions. The paa gel preactin concentration was determined through analysing the previously scanned electrophoregrams by means of Image Master™ 1–D software (Pharmacia).

Figure 1. Electrophoregram of femoral muscle proteins in Japanese quail



Protein concentration for each bird was expressed with three measurements of optical density of bands on electrophoregrams: maximum and average peak height (POD, AOD) and peak area (OD × mm). The means from two repetitions for each of the three protein concentration indicators were calculated. Basing on the GLM procedure with Duncan test (SAS), the mean values of the protein indicators were compared for each of its phenotypic forms.

RESULTS AND DISCUSSION

The femoral muscle protein of the quails, denoted as preactin, which migrates with the speed of blood serum prealbumins, similarly as Pa–2 protein reported by Tanabe *et al.* [8] depending on genotype, occurred in its three phenotypic forms (Figure 1). The AA genotype quails had a single, intensively stained, fast–migrating band, the birds of BB genotype – a single, slow–migrating, also intensively stained band, whereas the AB genotypes had two, fast– and slow–migrating, less intensively stained bands.

The preactin molecules were monomers and were coded by two co–dominating alleles A and B, which was expressed with the fact that the heterozygotes were the mixtures of the homozygote phenotypes, and probably no post–translation changes took place in the molecules. Intensity of band staining was higher in homozygotes than in heterozygotes. The differences had been presented by Brodacki [1] and explained with the fact that in homozygotes, transcription of identical mRNA takes place on the matrices of both alleles. On the matrices, during the process of translation, the protein molecules are formed that are characterised by the same migration speed, which is demonstrated with the single, intensively

stained band. In heterozygotes, on the other hand, different mRNA is reflected on each of the DNA matrices, and two bands appear, differing as to the speed of migration and being less intensively stained than in the homozygotes.

Table 1 presents the mean values of preactin concentration indicators in relation to the genotype of Japanese quails. The protein concentration, expressed with the results of POD, AOD and OD × mm measurements, was significantly higher in the BB homozygotes than in the AA ones. In the heterozygotes, on the other hand, the slow–migrating band (II), determined by the B gene, was characterised by higher protein level than the fast–migrating band (I). The differences, however, were statistically insignificant. In general, significantly lower protein concentration in both the fast– and slow–migrating band, in comparison with either AA or BB homozygotes, was observed for AB heterozygotes. The heterozygote level of preactin, calculated as the sum of both bands levels, was significantly higher than the level in either of the homozygous forms.

Table 1. Mean values of femoral muscle preactin concentration indicators in Japanese quail in relation to genotype

Indicator	Band	Preactin genotype					
		AA		AB		BB	
		n = 17		n = 49		n = 31	
		$\bar{x}$	V	$\bar{x}$	V	$\bar{x}$	V
POD	I	0.700^{B}	7.00	0.570^{A}	14.7	0.0	0.0
	II	0.0	0.0	0.590^{A}	15.6	0.750^{C}	5.47
	Total	0.700^{A}	7.00	1.160^{B}	16.8	0.750^{C}	5.47
AOD	I	0.298^{A}	18.5	0.280^{A}	22.5	0.0	0.0
	II	0.0	0.0	0.314^{A}	25.8	0.394^{C}	8.6
	Total	0.298^{A}	18.5	0.594^{B}	18.8	0.394^{C}	8.6
OD × mm	I	1.559^{B}	12.2	1.290^{A}	18.1	0.0	0.0
	II	0.0	0.0	1.390^{A}	24.2	1.900^{C}	18.4
	Total	1.559^{B}	12.2	2.680^{D}	26.1	1.900^{C}	18.4

$^{A, B, C}$ – differences significant at $p \leq 0.01$.

Significant differences between the means were demonstrated irrespectively of the indicator used for the studied trait, however, the magnitude of the differences expressed in percentage was not identical. The mean preactin content in the individuals of AA genotype was by 32.2, 99.3 and 71.9% lower than in AB individuals, respectively for POD, AOD and OD × mm. It should be noted, however, that the coefficients of variability for the studied indicators ranged from 5.5%, for POD, to 26.1%, for OD × mm. Basing on the studies carried out, it is difficult to univocally state, which of the three applied indicators best describes the differences between the studied groups.

The differences in preactin concentration between the individuals of different genotypes may demonstrate an effect of genotype on the expression of the genes that code the particular protein. The differences confirm the results obtained by other authors that gene expression – showing directly as the level of constitutional proteins, or indirectly, as the level of

performance traits – depends on the genotypes or a combination of genotypes from different loci. Relationships were demonstrated [10] between various polymorphic types of growth hormone gene and cattle meat performance traits. Our results correspond to the results of the studies [5], where it was demonstrated that iron percent saturation of pigeon blood serum transferrin depended on the genetic variant of the protein.

The knowledge on the sequence structure, as well as on the regulatory mechanisms of protein gene expression, can be used in animal transgenesis. Sutrave *et al.* [7] demonstrated that transgenic mice, equipped with a hen gene that code a protein of muscle cells, acquired hypertrophy of skeletal muscles and had lover fat content in the body. The same gene, with MSV virus promoter, was used to create transgenic pigs [6], which may demonstrate that an alteration of promoting sequences results in different expression of genes.

CONCLUSIONS

1. Higher concentration of preactin was observed in homozygotes BB than in homozygotes AA.
2. The level of preactin was significantly higher in heterozygotes than in either form of homozygotes.
3. The gene expression, measured with preactin concentration in femoral muscles of Japanese quails, depended on the genotype of the protein.

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