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ANALYSIS OF GENETIC POLYMORPHISM OF BLOOD PROTEINS AND SELECTED MEAT QUALITY TRAITS IN RABBITS

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ABSTRACT

We analysed genetic polymorphism of proteins in blood, evaluated selected parameters of meat quality in broiler rabbits of the line M 91, and detected statistically the relations between genotypes of individual polymorphous systems and parameters of meat quality. Polymorphous systems were realized as two allele systems (esterase 1, haemoglobin, albumin, alkaline phosphatase, and serum esterase); only the system ceruloplasmin was with three alleles. We detected low genetic variability of polymorphous systems, in four systems prevailed homozygotes BB and in system serum esterase homozygotes AA. The highest genetic variability was in the system ceruloplasmin and the highest heterozygosis was in the system haemoglobin. In polymorphous systems allele B had higher frequency than allele A, only in serum esterase system allele A prevailed slightly over allele B. Line M 91 was selected for meat performance; it attains suitable parameters for meat quality.
Statistically significant higher content of proteins was detected from heterozygous genotypes AB in polymorphous systems alkaline phosphatase and esterase 1, and from homozygotes BB in the haemoglobin system. We confirmed statistically the influence of homozygotes BB in system ceruloplasmin, esterase 1 and serum esterase on meat colour. We found greater influence of allele B on protein content and meat colour in line M 91. We recommend use of the mentioned polymorphous systems in selection of meat lines of broiler rabbits.

Key words: rabbit, polymorphism, blood proteins, parameters of meat quality

INTRODUCTION

Systematic breeding work aimed at intensification of reproductive, growth, fattening and slaughter properties in rabbits resulted in creation of specialized broiler lines marked by high values of meat efficiency. Polymorphism of proteins in blood can also be used to control selection processes under particular conditions. Study of biochemical polymorphism is among others aimed at identification of correlations between polymorphic traits and efficiency. With statistically confirmed relation between polymorphic biochemical traits and performance traits, polymorphic traits can be used as markers in selection and breeding.

Study of biochemical polymorphism is aimed at characteristics of zoo-technological units in various biochemical traits, selection value of these traits, effectiveness of crossing parents with different polymorphic traits in inbred breeding, degree of heterozygosis, determination of correlation between polymorphic traits and efficiency (Rafay, 2001). Objective of works of many authors was to use polymorphic traits as a selection criterion that is used mainly in species with longer generation interval and of great economic importance (Kúbek and Bardún, 1990). In the last few years we found many pieces of evidence that there exist close relations between polymorphic traits and performance traits in farm animals (FA). It was revealed that already

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a number of marker or candidate marker genes which are supposed to influence the sensibility resistance to some diseases, to have relation to reproductive parameters in FA, qualitative and quantitative growth characteristics in FA as well as to qualitative characteristics of resulting products of animal production intended for consumption (Bulla, 1997).

In population of broiler rabbits were found correlations between genotypes of polymorphous systems of proteins and performance traits. On the basis of statistical evaluation of genotypes of individual polymorphous systems of blood proteins, Bežová et al. (2002) found significant influence of heterozygotes AB in the system esterase 3 on weight of litter in the population of Nitra rabbits, significant influence of homozygotes AA, BB in the system esterase 1 on litter weight in population of Zobor rabbits, and significant influence of genotypes AB, BB in the system haemoglobin on size at weaning in the population of New Zealand White rabbits. They confirmed further significant statistical differences between individual genotypes of polymorphous systems and slaughter properties in populations M 91 x P 91 and Cunistar. The greatest number of correlations with genotypes of polymorphous systems was found with weight at slaughter, weight of thigh, weight of foreleg and weight of skin.

Kiačková (2003) confirmed statistically significant differences between genotypes of polymorphous systems and slaughter properties in the population of California rabbits. She found significant statistical influence of BB and CC homozygotes on weight at birth, body weight and weight of thigh. Statistically significant higher weight of thigh was with heterozygous genotypes in polymorphous systems carbonic anhydrasis, alkaline phosphatase and albumins. In the population of Zobor rabbits heterozygous genotypes attained significantly higher values for weights of liver in polymorphous systems carbonic anhydrasis, alkaline phosphatase and serum esterase. Heterozygous genotypes had in the system ceruloplasmin higher body weight and in the system esterase 5 higher weight of thigh.

Use of rabbit's meat is substantiated in rational nutrition because of its high nutritional value. Rabbit's meat contains the highest content of proteins, more than beef and pork, and it is getting near to chicken meat. Low content of cholesterol is significant (25.1 mg); it is 2.4 times lower than in chicken meat and 2.4 times lower than in veal. Nutritive value of rabbit's meat is the highest (40.15 %). Meat of other farm animal species is of lower nutritive value (chicken meat 31.62 %, pork 27.11 %, veal 24.61 %, and beef 24.20 %). Chemical composition of muscular substances in rabbit as raw food material in human nutrition corresponds to principles of rational alimentation. Rabbit's meat is marked by low energetic content (420 to 680 kJ/100 g), average fat content (2.5 to 6 %) and relatively higher content of essential fatty acids.

Submitted work brings results of evaluation of polymorphic traits, evaluation of meat efficiency traits in broiler rabbits' line M 91 and assessment of relations between genotypes of polymorphous systems and meat quality traits.

MATERIALS AND METHOD

We used the meat line M 91 of broiler rabbits to analyse genetic polymorphism of proteins in blood. The line was bred in the Research Institute for Animal Production in Nitra with the aim to produce mothers for commercial crossing. It was created by multiple cross breeding and it is represented by albinotic animals with live weight of 4.2 – 4.5 kg, average dressing percentage of 59%; the animals attained live weight of 2.6 kg at the age of 84 days. Rabbits were kept in partially air conditioned hall, in iron cages with grates. They were fed with standard feed mixture KKCH-1.

We analysed the polymorphous systems haemoglobin and esterase 1 from the hemolysate of erythrocytes. We identified albumin, alkaline phosphatase, ceruloplasmin and serum esterase from the blood serum. Approximately 1 hour after killing we took samples from musculus longissimus dorsi; wrapped them with aluminium foil and stored them in refrigerator at 4°C for 24 hours. The samples were subsequently analysed for meat quality. Polymorphous systems of blood proteins were identified by the method of cooled horizontal electrophoresis in 12 % starch gel with tris-citrate borate buffer. To stain the polymorphous variants we used amidoblack, alphanaftylacetate for esterases and paraphenylen-diamin-dichloride for ceruloplasmin.

In meat samples from dorsal muscle we studied the following traits: water content (%), protein content (%), fat content (%), energy (kJ), pH, colour and water holding capacity (%). Value of pH_{24} was assessed by stab electrode of the portable pH meter RADELKIS OP-109. Water holding capacity was determined by press method as described by GRAV-HAMM modified on the apparatus (Hašek and Pálanská, 1976). These characteristics were expressed by content of "loosely" bound water. Colour was assessed by measurement of per cent of remission at 540 nm wave length in SPEKOL 11 equipped with remission extension piece R 45/0. Baking losses were assessed in slices of samples wrapped with aluminium foil, in drying kiln at 180° C. In baked samples, tenderness was assessed as shear force (kg), read on dynamometer of consistence-meter WARNER-BRATZLER. Content of total water in homogenized samples was assessed by drying in microwave drying kiln of the programme apparatus ULTRA-X. Total content of intramuscular fat, contents of total proteins (C) and ash were assessed by

standard method according to the standard STN 57 0185. Content of hydroxyproline, as a quantitative criterion of connective tissue proteins (V), was determined colorimetrically after acid hydrolysis in 6N HCL and subsequent oxidation by chloramine T. Index V/C was used to express nutritive value of proteins; it represents percentual proportion of connective tissue proteins (V) out of total proteins (C). This expression originates from the fact that collagen as connective tissue is characterized by lower content or total lack of essential amino acids and therefore decreases the nutritive value of muscle proteins. On the basis of identified rabbits' genotype of M 91 line we calculated frequencies of alleles conditioning the occurrence of variants and genotypic structure according to Hardy-Weinberg's equation (Kúbek and Bardún, 1998).

Basic statistical characteristics were calculated while testing the qualitative properties of meat: arithmetic mean, standard deviation, standard mean error and coefficient of variation. Significance of differences between genotypes of polymorphous systems and parameters of quality was elaborated by means of the computer programme SAS 8.2.

RESULTS AND DISCUSSION

Genotypic structure and allele frequency of six polymorphous systems in rabbit line M 91 is given in table 1. Table 1 demonstrates that the studied systems were with two alleles except for the system ceruloplasmin, which was realized with three alleles.

We identified all three possible genotypic combinations with different frequency of occurrence in the system esterase 1 (Es 1). Homozygous genotypes BB (51.3 %) followed by AA (35.9 %) had the largest representation and heterozygotes AB (12.8 %) had the lowest representation. In the polymorphous system haemoglobin (Hb), the frequency of allele B (0.67) was proportionately higher than of allele A (0.33). Homozygous genotypes AA did not occur in the system haemoglobin (Hb) and heterozygotes AB prevailed highly (66.7 %). In the polymorphous system albumin (Alb), the frequency of allele B (0.76) was markedly higher than the frequency of allele A (0.24). Homozygotes BB (59 %) prevailed in the identified genotypes, proportion of heterozygotes AB was 33.3 % and homozygotes AA were the least (7.7 %). In the two allele system alkaline phosphatase (AF), allele B (0.76) exceeded allele A (0.24) with respect to the frequency. From table I we identified three genotypic combinations with the highest representation of homozygotes BB (61.5 %), the proportion of heterozygotes was 28.2 % and homozygotes AA (10.3 %) had the lowest representation. In the system ceruloplasmin (Cp) with three alleles, we identified five out of six possible genotypic combinations: AA, AB, BB, CC and BC. Heterozygous genotype AC was absent. Homozygotes BB (66.7 %) had the highest representation and the lowest ones were recorded by homozygotes CC (2.5 %) and AA (5.1 %). Proportion of heterozygotes AB was 15.4 % and BC 10.3 %, respectively. We found the highest frequency

Table 1: Genotypic structure and allele frequency of polymorphous systems in rabbit

Polymorphous system	Geno-types	Number of individuals			Frequency of alleles		
		e	t	%	p^A	q^B	r^C
Esterase 1 (E1)	AA	14	7	35,9	0,42	0,58	-
	AB	5	19	12,8			
	BB	20	13	51,3			
	n	39	39	100			
Haemoglobin (Hb)	AA	0	4	0	0,33	0,67	-
	AB	26	17	66,7			
	BB	13	18	33,3			
	n	39	39	100			
Albumin (Alb)	AA	3	2	7,7	0,24	0,76	-
	AB	13	14	33,3			
	BB	23	23	59,0			
	n	39	39	100			
Alkaline phosphatase (AF)	AA	4	2	10,3	0,24	0,76	-
	AB	11	14	28,2			
	BB	24	23	61,5			
	n	39	39	100			
Ceruloplasmin (Cp)	AA	2	0,7	5,1	0,13	0,79	0,08
	AB	6	8	15,4			
	BB	26	24	66,7			
	BC	4	5	10,3			
	CC	1	0,3	2,5			
	AC	0	1	0			
	n	39	39	100			
Serum esterase (E-ser.)	AA	17	13	43,6	0,59	0,41	-
	AB	12	19	30,8			
	BB	10	7	25,6			
	n	39	39	100			

- $P > 0.05$; + $P < 0.05$; ++$P < 0.01$

e - experimental number of genotypes; t - theoretical number of genotypes

of allele B (0.79) in the above mentioned system with three alleles. We also found three possible genotypic combinations in the system serum esterase (E-ser.), which suggests the existence of two alleles in the line M 91. In this system, allele A prevailed in frequency (0.59) over allele B (0.41). Out of all the genotypic combinations, the homozygotes AA (43.6 %) had the highest representation while heterozygotes AB were 30.8 % and homozygotes BB 25.6 %. Our results on polymorphism of the locus haemoglobin differ from results of Oravcova, Durcova (1992), who reported a monomorphous system in populations of New Zealand white, Zobor and Nitra rabbits and in Big Chinchilla (Rafay et al., 2001) confirmed the two allele locus of haemoglobin in populations of Nitra, New Zealand white and Zobor rabbits in line with our results. They found high genetic variability expressed with high number of heterozygous individuals in this system. Bežová et al. (2003) also identified two-allele locus of haemoglobin in populations of Hyla, Hyplus, Cunistar and M 91/P 91 but they found small genetic variability in this polymorphous system.

Results obtained in the sphere of genetic polymorphism of esterase 1 correspond with results of Oravcová and Durčová (1992), who found the influence of two co-dominant alleles for the systems Es 1, Es 2, Es 3 and Es 5. We identified three genotypic combinations in the system serum esterase, which suggests the existence of 2 alleles. Bežová et al. (2003) confirmed the two-allele system of serum esterase in rabbit populations Hyplus and Cunistar, but they identified also the third allele C in genotypes CC and AC in population of M 91 x P 91. We found markedly higher frequency of allele B in the studied polymorphous blood systems (Es 1, Hb, AF, ALB, Cp) compared to the above mentioned authors for other rabbit lines. We suppose that it is a consequence of selection of individuals for meat efficiency in line M 91.

Statistical characteristics of selected parameters of meat quality in line M 91 are given in table 2. Content of total water, which was assessed by drying (%) in homogenized samples in microwave drying kiln, reached the values from 69 % to 77 %, on an average 72.92 %. The studied set of individuals had low variability (v=3.03 %) in content of water.

Table 2: Statistical characteristics of meat quality parameters

Individual	water (%)	protein (%)	fat (%)	energy (kJ)	pH	colour	loosely bound water (%)
n	26	26	26	26	26	26	26
means	72,92	22,27	3,31	488,65	5,54	24,41	34,15
min	69	19	2,90	474	5,20	21,20	30,80
max	77	26	3,80	501	5,80	26,70	38,40
s	2,21	1,89	0,24	6,70	0,19	1,25	1,74
$s_{\bar{x}}$	0,43	0,37	0,05	1,31	0,04	0,25	0,34
v (%)	3,03	8,48	7,15	1,37	3,39	5,13	5,11

Index VIC was used to express nutritive value of protein content; it represents percentual proportion of connective tissue proteins (V) out of total proteins (C). Average content of proteins was 22.27 %. The span of maximum and minimum values varied from 19 % to 26 %. Degree of variability of the given parameter was low but it was the highest out of the evaluated traits (v = 8.48 %). The reached value of protein content corresponds with results of a number of authors. Doležal and Bureš (2004) found that proteins account for approximately 18 – 23 % of muscle.

Total content of intramuscular fat as well as content of total proteins (C) and ash was determined by the standard method after the standard STN 57 0185. Total content of fat in meat samples reached an average value of 3.31 %. Approximately equal content (4 %) was reported by Halaj (2002), around 2 – 5 % by Sekerka et al. (1994), and 2.5 - 9 % by Doležal and Bureš (2004). Degree of variability of this parameter was low (v = 7.15 %) in the set of M 91 individuals.

The energetic value ranged from 474 kJ to 501 kJ, on an average 488.65 kJ. This parameter of quality had the lowest variability (v = 1.37 %). On an average, the studied population is marked by pH 5.54, spanning from 5.2 to 5.8. We can consider the pH value of meat in rabbit line M 91 to be of low variability (v = 3.39 %). Colour was assessed by measuring the per cent of remission at 540 nm wave length in Spekol. Individuals of M 91 line differed markedly from the average value 24.21; the lowest value of per cent of remission was 21.2 and the highest one 26.7. We detected low degree of variability in meat colour (v = 5.13 %). The term “loosely” bound water describes water holding capacity; it is assessed by pressing method. From table II, we see that average value of “loosely” bound water is 34.15 %, minimum value being 30.8 % and maximum value being 38.4 %. Degree of variability for the given trait was low (v=5.11 %). Obtained results are comparable with results detected by Pálanská et al. (1982), who reported values of water holding capacity in dorsal muscle to be 38.31 % and in thigh muscle 35.07 %.

Results given in table 3 statistically confirmed the differences between genotypes of polymorphous systems in blood proteins and parameters of meat quality. In the content of proteins we confirmed statistically significant differences between genotypes in three polymorphous systems of alkaline phosphatase, esterase 1 and haemoglobin. We found significant difference between genotypes AB and BB in the mentioned polymorphous systems. Statistically significant higher content of proteins was found in heterozygous genotypes AB in

Table 3: Significance of differences between genotypes of polymorphous systems and selected meat quality parameters

Parameter	Polymorphous system	Genotype	n	mean	Significance of differences		
					AA:AB	AA:BB	AB:BB
Content of protein	Alkaline Phosphatase	AA	1	19,00			
		AB	10	23,30	-	-	+
		BB	14	21.71			
	Esterase 1	AA	5	22,40			
		AB	3	24,33	-	-	+
		BB	17	21,82			
	Haemoglobin	AB	16	21,67	-	-	+
		BB	9	23,22			
Colour of meat	Esterase 1	AA	5	23,14			
		AB	3	23,63	-	++	+
		BB	17	24,91			
	Serum esterase	AA	8	23,53			
		AB	7	24,49	-	++	-
		BB	10	25,05			
	Ceruloplasmin	AA	1	23,40			BC:BB
		AB	3	24,23	-	-	+
		BB	16	24,91			
		BC	4	23,15			
		CC	1	22,80			
pH	Albumin	AA	3	5,67			
		AB	7	5,39	++	-	+
		BB	15	5,58			

-P > 0.05; +P < 0.05; ++P < 0.01

polymorphous systems alkaline phosphatase and esterase 1 and in homozygotes BB of the polymorphous system haemoglobin. From the above mentioned data it can be said that the influence of allele B on content of proteins in rabbit meat line M 91 is higher. In meat colour, we found statistical differences between genotypes of polymorphous systems esterase 1, serum esterase and ceruloplasmin. Homozygotes BB had the highest average value of meat colour in the system esterase; they differed highly significantly from homozygotes AA and significantly from heterozygotes AB. In polymorphous system esterase we detected highly significant difference between homozygotes BB and AA in meat colour. Difference between heterozygotes AB and BB was not significant. We identified five genotypes out of six possible genotypes in three-allele system of ceruloplasmin; out of which the most represented genotype is BB with the best average value of the given characteristics (24.91), heterozygotes AB also had suitable value of colour (24.23). We confirmed significant difference between combinations BB – BC. We confirmed totally the influence of homozygotes BB in three polymorphous systems (ceruloplasmin, esterase 1 and serum esterase) on average value of meat colour, i.e. allele B has larger influence on meat colour. We found statistical differences between genotypes of polymorphous system albumin and pH parameter of meat. Highly significant differences were between genotypes AA with the highest pH value (5.67) and AB with the lowest pH value (5.39). Significant difference was also recorded between the combinations of genotypes AB – BB. We did not notice statistically significant differences in the rest of evaluated polymorphous systems and with further parameters of meat quality.

It was not possible to compare the obtained results of association in polymorphous systems including the parameters of meat quality in rabbits with other sources of literature because we found no similar works. Relations between genotypes of polymorphous blood systems with selected reproduction characteristics detected by Rafay et al. (2001) in the population of Nitra, Zobor and New Zealand white rabbits, and Kiačková (2003) with selected slaughter characteristics in the population of Californian rabbits.

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