CZECH ACADEMY OF AGRICULTURAL SCIENCES

Czech Journal of ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

3

VOLUME 48
PRAGUE 2003
ISSN 1212-1819

CZECH JOURNAL OF ANIMAL SCIENCE

An international journal published under the auspices of the Czech Academy of Agricultural Sciences and financed by the Ministry of Agriculture of the Czech Republic

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The journal is cited in the bibliographical journal Current Contents – Agriculture, Biology and Environmental Sciences and abstracted in Animal Breeding Abstracts. Abstracts from the journal are comprised in the databases: Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Envi-ronmental Sciences, Czech Agricultural Bibliography, Food Science and Technology Abstracts, Toxline Plus.

Periodicity: The journal is published monthly (12 issues per year). Volume 48 appearing in 2003.

Acceptance of manuscripts: Two copies of manuscript should be addressed to: Ing. Zdeňka Radošová, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic, tel.: +420 227 010 352, fax: +420 227 010 116, e-mail: edit@uzpi.cz.

Subscription information: Subscription orders can be entered only by calendar year (January–December) and should be sent to: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic. Subscription price for 2003 is 1176 CZK or 214 USD.

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MK ČR E 4886

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Preliminary mapping of QTLs affecting egg quality on chromosomes 1–5 in chickens

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ABSTRACT: In order to detect QTLs affecting egg quality traits, an experimental population was developed from two breeds: Green-legged Partridgenous (GlP), a native Polish breed, and a highly productive Rhode Island Red (RIR) stock. These breeds differ considerably in egg laying performance – the mean egg weight for GlP hens is 48.9 g and for RIR 59.4 g; during the first 100 days of laying GlP hens lay about 40 eggs while the RIR flock, selected over several generations for high laying performance, 81 eggs. QTL analysis was conducted concerning egg quality traits, based on a population of 519 birds (F_2). 23 microsatellite loci on chromosomes 1, 2, 3, 4 and 5 were chosen from the Roslin Institute database on the basis of their utility and location. Within the mapped population a linkage analysis was performed of 23 microsatellite markers. Through a linkage analysis twelve QTLs were identified, affecting 9 egg quality traits: egg weight at week 53 of life, albumen weight at week 53, albumen weight at week 33, yolk weight at week 33, yolk weight at week 53, shell weight at week 53, shell thickness at week 33, shell thickness at week 53, shell colour at week 53.

Keywords: QTL mapping; egg quality traits; microsatellites; chicken

One of the major goals of agricultural research is the identification of genes controlling the expression of economically important traits. Most of these traits display a wide variation in the expression of genes at distinct loci, referred to as quantitative trait loci (QTLs) (Cheng *et al.*, 1995). The current availability of highly polymorphic DNA markers in many species renders possible the elaboration of well saturated genetic maps and consequently, genetic dissection of complex quantitative traits (Vallejo *et al.*, 1998). Among the genetic markers which are currently employed, microsatellites have been found to be abundant, evenly distributed and highly polymorphic in all resource populations (Cheng *et al.*, 1995).

Recently, a large number of genetic markers that facilitate QTL analysis has been generated and mapped in experimental populations. The genetic linkage maps of chicken contain over 1 900 loci, out of which nearly 800 are highly polymorphic microsatellite markers (Groenen *et al.*, 2000). The development of high resolution genetic maps and the necessary powerful statistical methods have initiated QTL mapping experiments for a variety of traits. Studies aimed at QTLs in chickens were reported by Khatib (1994), who studied juvenile growth rate and by Vallejo *et al.* (1998), who detected QTLs affecting susceptibility to tumours induced by Marek's disease virus. Moreover, Van Kaam *et al.* (1998, 1999a,b) reported QTLs affecting growth, feed efficiency and carcass traits while Yonash *et al.* (2001) identified QTLs affecting antibody response and survival rate in meat-type chickens. Recently, QTLs affecting body weight were reported on chromosomes 1 and 2 (Tatsuda and Fujinaka, 2001).

The results of the whole genome scan aimed at detection and localisation of QTL affecting egg quality traits were described by Tuiskula-Haavisto *et al.* (2001). At 1% genomewise significance level, 14 chromosomal areas affecting egg quality were found and at 5% only 6 suggestive QTLs. The effect of the RIR allele was associated with high egg weight, body weight, feed intake and with late sexual maturity and lower number of eggs.

In order to detect QTLs affecting egg quality traits, a F_2 cross between lines was developed, derived from two genetically different breeds. The search for QTLs was performed on progeny means of the second generation.

MATERIAL AND METHODS

Experimental population

The mapped population consisted of 10 full-sib families, originating from a cross between two chicken breeds: Green-legged Partridgenous (GlP), a native Polish breed maintained as a conservative flock, and a highly productive stock of Rhode Island Red (RIR). On the basis of DNA fingerprinting analysis, according to the lowest band sharing coefficient, 10 GlP cocks and 10 RIR hens were chosen as parental generation F_0. These 10 F_0 couples were mated in order to obtain 10 families for generation F_1 (130). 519 birds of full-sib F_2 generation were obtained by crossing one brother and one sister from each of the 10 F_1 families. These 669 birds were used as the experimental population.

The birds were maintained in batteries, in individual cages and an individual egg-laying control was performed. During the laying period 20 egg quality traits were recorded (Table 1). The albumen quality was evaluated in Haugh units (log of albumen height corrected for egg weight). Egg shell strength is a major trait affecting egg stability so shell quality was evaluated as a direct measurement of shell strength (SS). The evaluated shell traits included also shell weight, shell density, shell thickness and shell colour. Egg quality traits from F_2 individuals were measured at week 33 and 53 of life.

DNA samples

Blood samples were collected into vacuum tubes containing EDTA and stored at –20°C. The DNA was extracted by standard methods. DNA concentration was determined spectrophotometrically and DNA was diluted to a final concentration of 0.1 μg/μl.

Microsatellite markers

Microsatellite loci were chosen from *MCW* (Crooijmans *et al.*, 1996; Groenen *et al.*, 1997), *LEI* (Gibbs *et al.*, 1997) and *ADL* markers (Cheng *et al.*, 1995). In total 23 informative markers were mapped. The microsatellite markers are available from the Roslin Institute database (http://www.thearkdb.org.).

Microsatellite repeat *MCW0041* is situated in the chicken *GGVAY Y*-gene of the chicken ovalbumin family, *MCW0051* in *GGCALBO4* chicken vitamin D-induced calbinding *D28K* gene, and *MCW0047* in high-mobility group protein 14 *A1* gene.

PCR conditions

The PCR was carried out in a volume of 7.5 μl comprising 100 ng of template DNA, 2.5 pmol

Table 1. List of recorded egg quality traits

Trait	Symbol	Trait	Symbol
Egg specific gravity at week 33 (g)	SG33	Egg specific gravity at week 53 (g)	SG53
Shell strength at week 33 (kg/cm2)	SS33	Shell strength at week 53 (kg/cm2)	SS53
Shell colour at week 33	SC33	Shell colour at week 53	SC53
Egg weight at week 33 (g)	EW33	Egg weight at week 53 (g)	EW53
Haugh units at week 33	HU33	Haugh units at week 53	HU53
Shell weight at week 33 (g)	SW33	Shell weight at week 53 (g)	SW53
Shell density at week 33	SD33	Shell density at week 53	SD53
Shell thickness at week 33 (μm)	ST33	Shell thickness at week 53 (μm)	ST53
Yolk weight at week 33 (g)	YW33	Yolk weight at week 53 (g)	YW53
Albumen weight at week 33 (g)	AW33	Albumen weight at week 53 (g)	AW53

of each primer, 100 μM of each dNTP, 0.5 unit of DNA polymerase, 10 mM tris- HCl, pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100.

One primer for each locus was labelled with fluorescein. The following amplification conditions were adopted: 5 min denaturation at 94°C followed by 25–37 cycles of denaturation at 94°C for 45 s, annealing at 48–68°C and extension at 72°C for 60 s. The PCR was performed using a PTC-200 Programmable Thermal Controller.

DNA genotyping

The fluorescent PCR products were separated on 6% denaturing polyacrylamide gels, using an Automated Laser Fluorescent (ALFexpress) DNA Sequencer. The PCR products were analysed after 5 min denaturation in a 50% formamide solution containing blue dextran. The results were visualised and the genotyping completed with Allele Links 1.01. After automated allele calling and binning within the Allele Links 1.01 software, individual genotypes were checked by a manual inspection before exporting the genotype database to Excel. Moreover, (potential) typing errors detected with the CRI-MAP program were re-checked and corrected. The data was extracted from Excel worksheets and put into a correct format for the CRI-MAP linkage analysis program. Linkage analysis was performed using CRI-MAP version 2.4 program (Green *et al.*, 1990).

Variance and covariance components were estimated using EM-REML with multitrait animal model (Misztal, 1999).

QTL analysis and significance thresholds

The mapping of QTLs was performed using the program QTL Cartographer vers. 1.13 (Basten *et al.*, 1999). The program uses linear regression, composite interval mapping (Zeng, 1993, 1994) methods to dissect the underlying genetics of the quantitative traits. Composite interval mapping combines interval mapping with multiple regression.

Significance thresholds were calculated using the permutation test (Churchill and Doerge, 1994). It is an empirical method that accounts for the structure of marker data and distribution of phenotypic data. Through the random shuffling of phenotypic observations and the corresponding weighting factors for these observations, any relation between QTL and marker genotypes is broken. The distribution under the null hypothesis of no QTL is constructed in this way. For each shuffle a test statistic was calculated and stored. The stored test statistics were sorted in a descending order – the threshold for each required significance level can be derived in this way. Genomewise thresholds were derived for each trait by permutating all markers simultaneously. It was repeated 500 times in order to construct the distribution under a null hypothesis (Table 2). Three levels of significance thresholds were derived: 1% genomewise significance thresholds, 5% genomewise significance thresholds and 10% genomewise significance thresholds.

RESULTS

Heterozygosity

The average number of different alleles for the markers in this population was 4.12 per locus. In total, over 65% of markers in the tested F_2 showed a high heterozygosity exceeding 60% (Jaszczak *et al.*, 2001).

Linkage analysis

A linkage analysis was performed for 23 microsatellite markers within the mapped population. Map distances between adjacent loci are given using the Kosambi scale in centimorgans (cM) (Table 3).

Heritabilities and genetic correlations

Table 4 shows correlations between the mean adjusted progeny trait values obtained for all birds. Shell strength (SS) proved to be highly correlated with shell density (SD) and shell thickness (ST). The correlation between albumen weight (AW) and egg weight (EW) was close to 1. High correlations were also observed between EW, SW and YW as well as between shell weight (SW) and SD, ST and AW.

The heritabilities estimated for these traits vary on average between 0.4 (egg weight, shell colour) and 0.2 for the remaining analysed traits.

Table 2. Threshold *P*-values for genomewise QTL analysis

Significance level	Traits	*P*-values	Traits	*P*-values	Traits	*P*-values	Traits	*P*-values
0.1	SG33	12.3851	SW33	12.4713	SG53	12.9502	SW53	13.2319
0.05		14.0008		14.6013		14.2942		14.6185
0.01		16.8038		19.6956		18.5063		18.2477
0.1	SS33	12.7509	SD33	12.9955	SS53	13.3255	SD53	13.2216
0.05		14.1597		15.0747		14.8999		15.3849
0.01		19.1018		19.9337		17.7742		18.3651
0.1	SC33	13.5141	ST33	12.5342	SC53	13.6760	ST53	12.6794
0.05		14.5763		13.8237		15.3230		14.3963
0.01		19.8742		16.8468		20.0591		16.7848
0.1	EW33	12.5833	YW33	12.0423	EW53	12.9066	YW53	13.1109
0.05		14.3098		13.2971		14.7752		15.3215
0.01		17.6079		17.2604		18.9988		21.7459
0.1	HU33	13.1197	AW33	12.6586	HU53	13.1153	AW53	13.3460
0.05		15.0394		13.9797		15.0501		14.8469
0.01		19.1495		18.0169		18.5606		17.8389

Significance thresholds

Genomewise significance thresholds are presented in Table 2. These 1%, 5%, and 10% thresholds were calculated by means of permutation over all linkage groups simultaneously in one analysis.

QTL analysis

A linkage analysis between genotypes and 20 egg quality traits, recorded for generation F_2, led to the identification of twelve QTLs. The effect of the found QTLs' area explained approximately from

Table 3. Linkage analysis of 23 microsatellite markers on chromosomes 1–5

Position	Chromosome 1	Chromosome 2	Chromosome 3	Chromosome 4	Chromosome 5
Locus 1	*MCW0018*	*MCW0063*	*MCW0127*	*MCW0167*	*MCW0081*
(cM)	0	0	0	0	0
Locus 2	*MCW0200*	*MCW0131*	*MCW0126*	*MCW0170*	*ADL0187*
(cM)	100	27.5	45.2	100	21.9
Locus 3	*MCW0068*	*MCW0051*	*LEI113*	*MCW0114*	*MCW0029*
(cM)	142.2	95.1	91.5	182	38.8
Locus 4	*MCW0283*	*MCW0056*	*MCW0040*	*MCW0047*	*MCW0032*
(cM)	185.8	132.6	134.2	241.5	57.7
Locus 5	*MCW0145*	*MCW0041*	*MCW0139*		
(cM)	267.7	188.3	223.8		

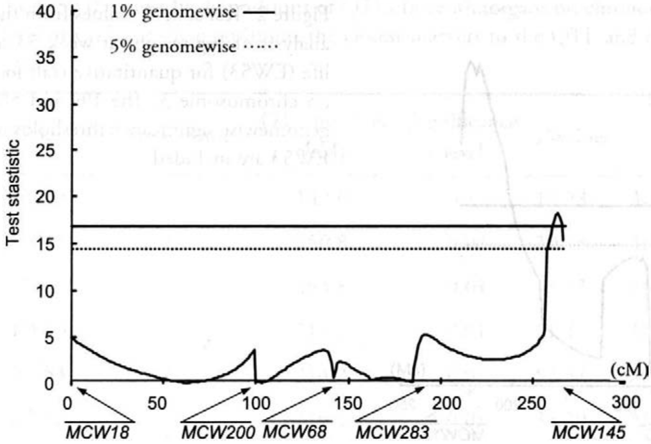

Figure 1. Test statistic values from the analysis of shell thickness at week 53 of life (ST53) for quantitative trait loci on chromosome 1. The 1% and 5% genomewise significance thresholds of ST53 are included

1.7% to 31.1% of the variance within the analysed traits (Table 5).

Two QTL regions were found on chromosome 1 for shell weight at week 53 (142 cM and 256 cM; SW53), and one region around 263 cM for shell thickness at week 53 (ST53, Figure 1). SW and ST proved to be highly correlated (Table 4).

Three QTLs were found on chromosome 3 linked with marker *MCW00139*: egg weight (EW53, Figure 2), yolk (YW53) and albumen weight at week 52 (AW53). A high correlation was observed between these traits.

QTL for shell thickness at week 53 (ST53) was mapped on chromosome 4 (around 182 cM, Figure 3) and for albumen weight at week 33 – on the same chromosome (around 99 cM, Figure 4).

Shell colour at week 53 (SC53) showed a linkage at significance level $P = 0.05$ on chromosome 5.

Two suggestive linkages were identified at significance level $\alpha = 0.1$: yolk weight at week 33 (chromosome 1) and shell thickness at week 33 (chromosome 5).

Table 4. Correlations between mean adjusted progeny trait values of all animals

Trait	h^2	EW	SG	SS	SC	HU	SW	SD	ST	YW
EW	0.395									
SG	0.190	–0.172								
SS	0.175	–0.147	0.389							
SC	0.463	–0.053	–0.101	–0.017						
HU	0.267	0.245	0.307	0.155	–0.010					
SW	0.244	0.532	0.486	0.293	–0.359	0.256				
SD	0.200	0.065	0.730	0.491	–0.394	0.135	0.836			
ST	0.226	0.318	0.299	0.520	–0.104	0.355	0.553	0.673		
YW	0.138	0.651	–0.459	–0.292	–0.149	0.049	0.224	–0.162	–0.099	
AW	0.298	0.952	–0.083	–0.169	0.043	0.275	0.510	–0.017	0.300	0.423

EW = egg weight; SG = egg specific gravity; SS = shell strength; SC = shell colour; HU = haugh units; SW = shell weight; SD = shell density ST = shell thickness; YW = yolk weight; AW = albumen weight

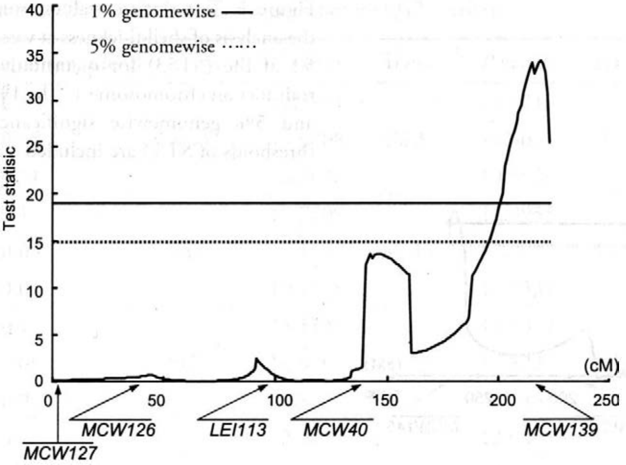

Figure 2. Test statistic values from the analysis of egg weight at week 53 of life (EW53) for quantitative trait loci on chromosome 3. The 1% and 5% genomewise significance thresholds of EW53 are included

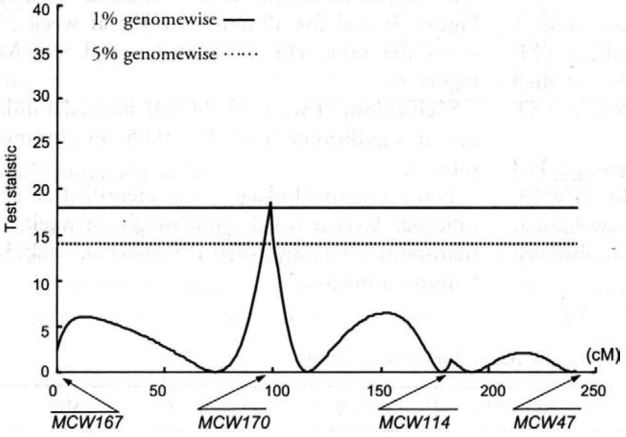

Figure 3. Test statistic values from the analysis of albumen weight at week 33 of life (AW33) for quantitative trait loci on chromosome 4. The 1% and 5% genomewise significance thresholds of AW33 are included

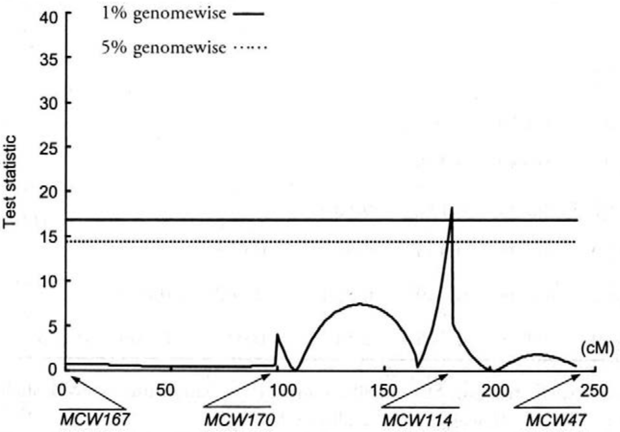

Figure 4. Test statistic values from the analysis of shell thickness at week 53 of life (ST53) for quantitative trait loci on chromosome 4. The 1% and 5% genomewise significance thresholds of ST53 are included

Table 5. The most likely positions of QTLs in centimorgans on chromosomes per traits. Genomewise significance level of QTLs at these positions, the closest markers to the QTL and this location and the explained variance of QTLs

Traits	Chromosome	QTL position (cM)	Significance level	*P*-values	Marker	Marker position	Explained variance (%)
YW33	1	142.0	0.1	12.28	*MCW0068*	142.2	1.7
SW53		256.8	0.01	30.18	*MCW0145*	267.7	31.1
ST53		263.8	0.01	18.13	*MCW0145*	267.7	21.9
YW53	3	213.2	0.01	21.57	*MCW0139*	223.8	26.2
AW53		214.2	0.01	53.57	*MCW0139*	223.8	25.3
EW53		219.2	0.01	34.20	*MCW0139*	223.8	20.1
AW33	4	099.0	0.01	18.55	*MCW0170*	100.0	3.3
ST53		182.0	0.01	18.21	*MCW0114*	182.0	7.5
ST33	5	0.0	0.1	12.89	*MCW0081*	0.0	2.2
SC53		56.8	0.05	16.57	*MCW0032*	57.7	7.4

YW33 = yolk weight at week 33; SW53 = shell weight at week 53; ST53 = shell thickness at week 53; YW53 = yolk weight at week 53; AW53 = albumen weight at week 53; EW53 = egg weight at week 53; AW33 = albumen weight at week 33; ST33 = shell thickness at week 33; SC53 = shell colour at week 53; *MCW* = microsatellite chicken wageningen

DISCUSSION

Egg quality traits

There are several factors affecting the detection power for QTL mapping. Traits-associated factors, such as the number and genome locations of genes affecting the traits, the distribution of the gene effects and interactions and trait heritability, are not controllable. Some factors including population size and type can be artificially manipulated to enhance the detection for QTL mapping. Theoretically, a large full-sib family that is fully informative, with all distinguishable parental alleles at all loci, is sufficient for QTL mapping (Zhu *et al.*, 2001).

In order to increase the probability of parents being heterozygous for the given QTLs, and thus enhance the detection power of QTL mapping, the population was produced by crossing two genetically different breeds (GlP and RIR). As proposed by Hillel (1997), DNA fingerprinting was used to select distantly related individuals for mating and thus to obtain highly informative offspring. Seventeen alleles were identified as specific for the GlP, and forty-two for the RIR chickens from the parental generation. The reference family was characterised by a high level of polymorphism at the examined microsatellite loci and high heterozygosity. Highly heterozygous individuals not only enhance the probability of QTL detection but also improve the accuracy of linkage maps and determination of linkage phases (Zhu *et al.*, 2001).

In our study the critical values for test statistics were determined for each trait on significance levels of $\alpha = 0.01$, $\alpha = 0.05$ and $\alpha = 0.1$ (Lander and Kruglyak, 1995). Twenty egg quality traits have been analysed. At the genomewise significance level of 1% nine sites were found while at 5% one chromosomal area.

The QTL for shell weight was found on chromosome 1 around 256 cM (SW53), and in a near position a significance linkage was found (around 263 cM) for shell thickness (ST53) (Figure 1). These results could be expected as the mentioned traits (SW and ST) are highly correlated. Similarly, for three highly correlated traits (egg weight, yolk and albumin weight) a significance linkage with marker *MCW00139* was estimated on chromosome 3.

The presented results lead to a conclusion that chromosomes 1, 3, 4, 5 could contain genes affecting chicken egg quality traits.

In our study the effect of the RIR alleles was associated with high yolk weight at week 33, shell weight at week 53, shell thickness at week 53, albumen weight at week 33, shell colour at week 53 and GlP alleles were favourable for yolk weight at week 53, albumen weight at week 53, egg weight at week 53, shell thickness at week 33.

Further analyses should include a larger number of markers in the examined chromosome regions so as to reach a final distance of around 20 cM between bracketing markers. Moreover, a comparison with the human genome map should be performed as it is essential for finding candidate genes, their sequence and mutations affecting egg quality traits.

Acknowledgements

We thank Krystyna Wicinska and Zofia Piechal for technical support.

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Received: 02–08–22
Accepted after corrections: 03–02–27

ABSTRAKT

Předběžné mapování QTL ovlivňujících kvalitu vajec na chromozomech 1–5 u slepic

K detekci QTL, které ovlivňují znaky kvality vajec, jsme vytvořili pokusnou populaci složenou ze dvou plemen: zelenonohé koroptví vlašky (GlP), domácího polského plemene, a vysoce produktivní červené rodajlendky (RIR). Tato plemena se značně odlišují svou užitkovostí – průměrná hmotnost vejce u GlP je 48,9 g a u RIR 59,4 g; během prvních 100 dní snášky nosnice GlP snesou kolem 40 vajec, zatímco nosnice RIR, které byly po několik generací šlechtěny na vysokou užitkovost, snesou 81 vajec. Analýzu QTL zaměřenou na znaky kvality vajec jsme provedli v rámci populace 519 jedinců (F_2). Z databáze Roslinova ústavu jsme na základě vhodnosti a polohy vybrali 23 lokusů mikrosatelitů na chromozomech 1, 2, 3, 4 a 5. V rámci mapované populace jsme provedli analýzu genetických vazeb u 23 mikrosatelitních markerů. Pomocí analýzy genetických vazeb bylo identifikováno 12 QTL, které ovlivňují těchto devět znaků kvality vajec: hmotnost vejce v 53. týdnu života, hmotnost bílku v 53. týdnu, hmotnost bílku ve 33. týdnu, hmotnost žloutku ve 33. týdnu, hmotnost žloutku v 53. týdnu, hmotnost skořápky v 53. týdnu, tloušťku skořápky ve 33. týdnu, tloušťku skořápky v 53. týdnu, barvu skořápky v 53. týdnu.

Klíčová slova: mapování QTL; znaky kvality vajec; mikrosatelity; slepice

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Effects of feeding on ammonium excretion and growth of the African catfish (*Clarias gariepinus*) fry

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ABSTRACT: The study was aimed at assessing the effects of different protein contents and feed rations on the growth and metabolic rate of African catfish (*Clarias gariepinus*) kept in a recirculation system. The fish were fed every day, between 8.00 and 20.00 hours, three feeds (A, B, and C) differing in their protein content (about 36, 40, and 46%, respectively). The daily feed ration, expressed by the amount of consumed protein, ranged from 2 to 4% of the stock weight. The highest growth rate (SGR = 5.21%) was typical of the fish receiving feed B at the 4% level while the lowest feed conversion ratio (FCR = 1.21) was recorded in the fish receiving the same feed, but administered at the 2% level. The latter treatment also showed the best protein and energy retention levels (32.33 and 27.23%, respectively). In all the treatments, an increase in the mean individual weight was accompanied by a drop in the rate of ammonia nitrogen release. The lowest values (0.0094–0.0071 mg/g fish weight/h) were found in the fish receiving feed C at the 2% level, the highest values (0.0337–0.0183 mg/g fish weight/h) being typical of the fish administered feed A at the 4% level. The maximum ammonia nitrogen release in all the experimental treatments was found to occur between 18.00 and 21.00 hours.

Keywords: ammonium excretion; African catfish; feeding level

The African catfish (*Clarias gariepinus*) is appreciated by consumers for the quality of its meat. In addition, the species shows a fast growth rate, tolerates adverse environmental conditions far better than the other fish do, and can use atmospheric air to breathe. The African catfish cultures in Europe were conducted in recirculation systems (Bovendeur *et al.*, 1987; Pruszyński and Adamek, 1995), in cages placed in power-station cooling water (Filipiak *et al.*, 1993), and even in ponds (Adamek and Sukop, 1995). The recirculation system cultures, independent of climatic variations, aided by nutritionally balanced diets, have proved most effective. Inappropriate feeds considerably affect the fish growth and can cause excessive pollution of water with fish metabolism products. For this reason, sound knowledge of the African catfish feeding requirements can be crucial for improving the economic effectiveness of its culture.

The present paper is an attempt to elucidate a relationship between the type and ration of feed *versus* growth and metabolic rates of the fish kept in a closed water system.

MATERIAL AND METHODS

The experiment was run for 28 days, the total period being divided into four 1-week long stages. The experiment involved the African catfish fry of 6.4 g individual weight, produced by the Institute of Ichthyobiology and Aquaculture (IIA), Polish Academy of Sciences, at Gołysz (Poland). The fish were randomly placed in 70dm^3 glass aquaria supplied with recirculating water (28.0 ± 0.4°C; pH 7.3–7.95) of the total volume about 1 m^3 (Figure 1). The water flow rate was set at 72 dm^3/s. Before the experiment, the fish adapted themselves to experimental conditions for 10 days. The experiment involved 9 treatments, each run in triplicate (Table 1). Each aquarium was stocked with 40 fry individuals. The fish received feeds manufactured

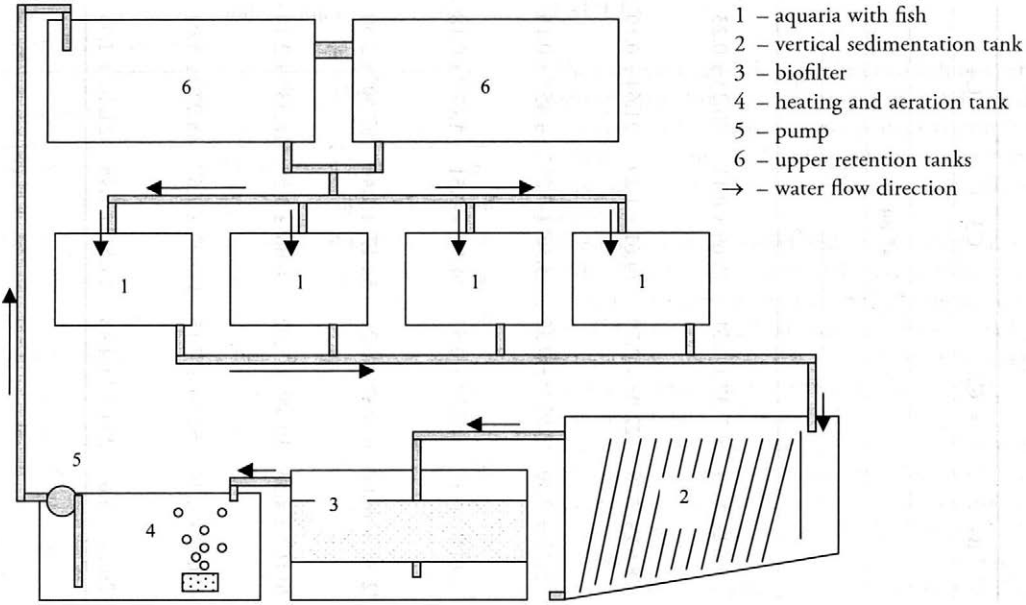

Figure 1. Experimental water recirculation system

in IIA (Table 2), offered at a ration calculated from the following formula:

$$D = \frac{M_o - d}{B} \tag{1}$$

where: D = daily feed ration (g)
M_o = stock weight (g)
d = daily protein level (%)
B = feed protein content (%)

The feed ration calculated in this way was divided into 4 portions and offered to the fish at 8.00; 12.00; 16.00; and 20.00 hours. The pellet diameter ranged from 1.0 to 3.2 mm and changed with fish growth. At 1-week intervals, the fish in all the aquaria were weighed after their metabolic rate was measured. Throughout the experiment, the following assays were made in the circulating water: dissolved oxygen content (a WTW oxymeter with an Oxi 320 probe), ammonia nitrogen (Nessler technique; assays made at 3-h intervals over a 24-h cycle), and nitrite nitrogen (with sulphanilic acid and α-naphthylamine) (Hermanowicz *et al.*, 1976). Water pH was measured with an Elmetron pH meter. The ammonia nitrogen excretion rate (A_w, mg N-NH_4/g fish weight/h) was calculated from

$$A_w = \frac{S_o - S_d}{M} \times p \tag{2}$$

where: S_o, S_d = ammonia nitrogen concentrations in water leaving the aquarium and coming to it, respectively (mg/dm^3)
M = weight of fish in an aquarium (g)
p = water flow rate (dm^3/h)

In order to prevent ammonia leakage to the atmosphere, pH = 7.36–7.95 was maintained. Besides this, the ammonia content in control aquarium (without fish) was tested and no ammonia leakage was observed at 72 dm^3/s flow.

Based on control weighings of fish and on the relevant chemical assays, the principal parameters describing the fish growth rate and feed assimilation (SGR, FCR, FR, ER and aNPU) were calculated using the formulae given by Filipiak and Trzebiatowski (1992). Chemical composition of feed and the whole (homogenised) body of fish were analysed according to Skulmowski (1974). The feed and fish gross energy contents were calculated using the following conversion factors given by Brody (1945): 5.65; 9.45; and 4.1 kcal/g (or 23.62; 39.5; and 17.14 kJ/g) for protein, fat,

Table 1. Scheme and results of the experiment (mean values ± SD)

Treatment	A2	A3	A4	B2	B3	B4	C2	C3	C4
Protein contents in diets (%)									
		35.95			40.06			45.84	
Daily protein ratio (% stock weight)									
	2	3	4	2	3	4	2	3	4
Initial mean individual weight (g)									
	6.55 ± 0.82	5.94 ± 0.39	6.04 ± 0.38	6.61 ± 0.60	6.35 ± 0.28	6.69 ± 1.04	7.37 ± 1.61	5.69 ± 0.11	6.23 ± 0.23
Final mean individual weigh (g)									
	20.66 ± 3.03	16.69 ± 1.80	16.82 ± 2.00	20.53 ± 2.06	23.20 ± 2.44	28.63 ± 2.78	22.07 ± 2.79	14.61 ± 1.29	21.64 ± 0.10
SGR (%)									
	4.09^{bcd} ± 0.32	3.68^{ab} ± 0.46	3.64^{ab} ± 0.21	4.04^{bcd} ± 0.04	4.62^{d} ± 0.49	5.21^{e} ± 0.30	3.95^{abc} ± 0.35	3.36^{a} ± 0.25	4.45^{cd} ± 0.15
FCR									
	1.73^{ab} ± 0.24	2.09^{bc} ± 0.42	2.54^{c} ± 0.32	1.21^{a} ± 0.04	2.02^{bc} ± 0.61	1.81^{ab} ± 0.37	1.72^{ab} ± 0.51	4.17^{d} ± 0.51	1.67^{ab} ± 0.18
Survival rate (%)									
	72.50^{c} ± 4.33	93.33^{d} ± 3.82	90.83^{d} ± 1.44	90.83^{d} ± 7.64	60.83^{ab} ± 3.82	72.50^{c} ± 10.00	65.83^{bc} ± 6.29	54.17^{a} ± 1.44	97.50^{d} ± 2.50
aNPU (%)									
	23.15^{c} ± 3.22	21.85^{bc} ± 4.28	16.27^{b} ± 1.97	32.33^{d} ± 0.93	19.89^{bc} ± 4.93	20.16^{bc} ± 4.33	19.48^{bc} ± 5.02	6.66^{a} ± 1.50	18.53^{bc} ± 2.14
FR (%)									
	41.32^{cd} ± 3.84	28.98^{ab} ± 4.48	20.93^{a} ± 2.20	41.96^{cd} ± 0.52	35.08^{bc} ± 6.31	42.08^{cd} ± 6.30	51.54^{e} ± 7.15	34.44^{bc} ± 2.67	48.97^{de} ± 5.02
ER (%)									
	22.78^{cd} ± 2.57	18.06^{bc} ± 3.18	13.72^{ab} ± 1.55	27.23^{d} ± 0.57	18.68^{bc} ± 4.01	20.21^{c} ± 3.67	23.01^{cd} ± 4.48	11.51^{a} ± 1.45	21.40^{c} ± 2.33

The results in columns denoted by the same letters are not significantly different ($P = 0.05$)

Table 2. Components and chemical composition of experimental diets (%)

Component	Diet A	Diet B	Diet C
Wheat flour	25.00	15.00	5.50
Fish meal	27.00	38.00	48.50
Fodder yeast	20.00	20.00	20.00
Soya flour	18.00	18.00	18.00
Vitamin mixture	0.50	0.50	0.50
Mineral mixture	1.50	1.50	1.50
Fish oil	8.00	7.00	6.00
Total	100	100	100
Dry matter	93.71	95.00	96.40
Crude protein	35.95	40.06	45.84
Crude fat	15.02	12.25	11.94
NFE	33.72	32.45	26.56
Ash	9.02	10.24	12.06
Gross energy (kcal/kg)	4 833	4 751	4 807

and carbohydrates, respectively. The recorded differences were tested for statistical significance by the analysis of variance (ANOVA) and LSD test, applied at the $P < 0.05$ significance level.

RESULTS

The survival rate of the experimental fish was satisfactory, apart from one treatment in which it did not exceed 60%. There was no evident reason for the mortality detected. Neither pathogenic bacteria nor parasites were detected on the skin and gills of dead fish.

The highest daily individual weight increment, i.e. the highest specific growth rate (SGR), was recorded in treatment B4, the lowest SGR in treatment C3. The lowest feed conversion ratio (FCR) was typical of treatment B2, the highest FCR being observed in treatment C3 (Table 1). The highest apparent net protein utilisation (aNPU) was produced by treatment B2, the lowest aNPU was a result of treatment C3. The highest fat retention (FR) was recorded in treatments C2 and C4 while treatments A4 and A3 were characterised by the lowest FR. It was clearly a result of the feed type used, whereby FR increased as the feed fat content decreased. The resultant energy retention (ER) values were distributed similarly like aNPU (Table 1).

The ammonia nitrogen release rate was found to change with the increase in both the feed protein level and the mean fish individual weight. The lowest rates were recorded at the first stage of the experiment, in treatments C2 and B2 (0.0094 and 0.018 mg N/g fish weight/h), while the highest rate (0.0337 mg N/g fish weight/h, i.e. three times the lowest rate) was observed in treatment A4.

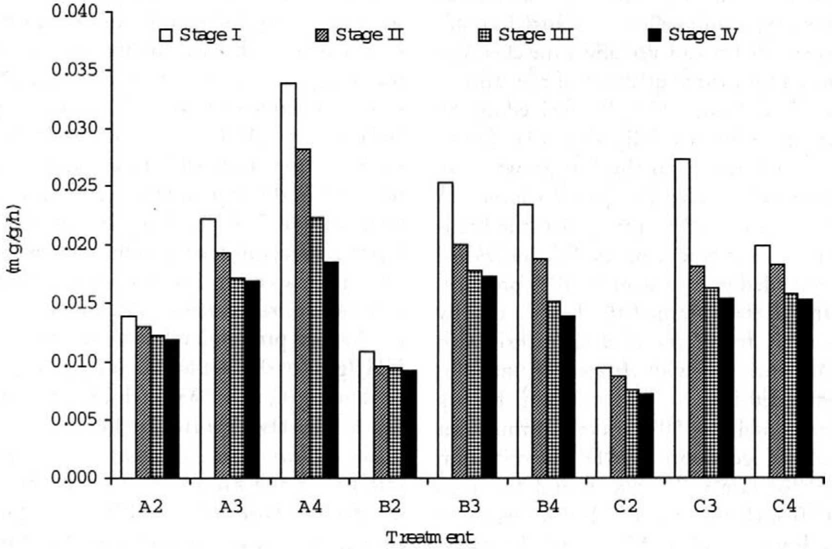

Figure 2. Changes in ammonia excretion during the experiment (mg/g/h)

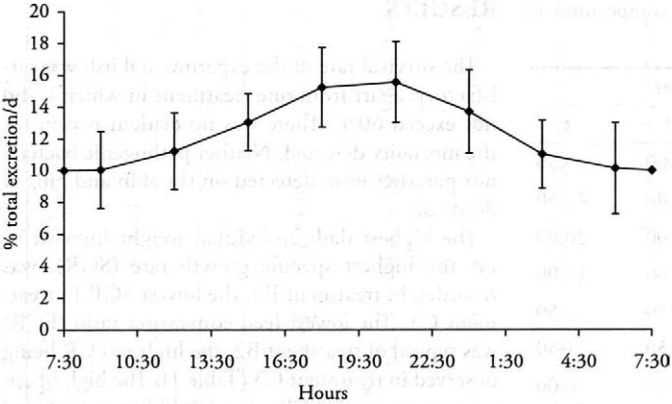

Figure 3. Daily changes in ammonia excretion (mean values, as % of total nitrogen excretion ± SD)

Although during the next stages of the experiment the pattern was similar, a tendency toward a reduction of ammonia nitrogen release was observed (Figure 2). Throughout the experiment, the dynamics of daily changes in ammonia nitrogen release was very similar in all the treatments. The A_w values were observed to steadily increase to the maximum between 18.00 and 21.00 hours, to decrease thereafter down to the original value (Figure 3).

DISCUSSION

The level and dynamics of fish metabolism can be affected by numerous factors, including the photoperiod (Britz and Pienaar, 1992) as well as the feed ration, type, and pellet size (Hossain *et al.*, 2000). The feed quality can also affect the chemical composition and nutritive qualities of the African catfish meat (Wedekind, 1995). While feeding the carp different feed rations, Filipiak (1991) found no significant differences in the fish growth rate when the rations had identical protein levels. In the experiment described in this paper, the highest daily per cent increment of the fish individual weight was recorded in treatment B (40% protein). Hogendoorn (1980) obtained the best results by feeding the early fry of the African catfish with a trout starter enriched with *Artemia* sp. nauplii. It is recommended that the fish weighing 1–10 g receive a feed containing 50% protein throughout the daily cycle (Hogendoorn, 1981). The optimum protein level for larger fish was found to range from 40 to 50% (Henken *et al.*, 1986; Degani *et al.*, 1989, Filipiak *et al.*, 1993). A similar range (37–40%) was also reported by Fagbenro (1992) for a related species, *Clarias isheriensis*. According to various authors the weight of feed offered to the African catfish of the size similar to that used in experiment I should range from 4 to 8% of the stock weight (Hogendoorn, 1981; Hogendoorn *et al.*, 1983), which – converted to the feed protein level – amounts to about 2–4% of the stock weight. In the described experiment, the lowest FCR (1.21) that incidentally accompanied the high growth rate, was obtained in treatment B2 involving the daily protein level equal to 2% of the stock weight.

E.g. Rychly (1980) and Buttle *et al.* (1995) reported that the rate of ammonium release by fish increased with dietary protein content. No such trend was found in the described experiment. On the other hand, the ammonium release rate was seen to clearly increase in those treatments that supplied the fish with higher protein levels, that means higher amounts of nitrogen. Such a relationship is in agreement with the results reported by Brunty *et al.* (1997) and Zakęś (1999). The ammonia nitrogen release rate of the African catfish, recorded in this experiment, was found to be in the range of 0.0071–0.0337 mg N/g/h. When analysing the effects of feed protein content on African catfish metabolism, Buttle *et al.* (1995) found the ammonia nitrogen release rate to range, depending on the feed protein level, from 0.6 to 1.2 μmol N-NH_4/g/h (or 0.0084 to 0.0168 mg N/g/h) in feeds containing 41 to 49% protein, respectively. Similar values were also reported by Jobling (1981) for the plaice (*Pleuronectes platessa*), by Guerin-Ancey (1976) for the European sea bass (*Dicentrarchus labrax*), by Porter *et al.* (1987) for the gilthead seabream (*Sparus aurata*), and by Thomas and Piedrahita (1998) for the white sturgeon (*Acipenser* *transmontanus*). The daily dynamics of changes in the ammonia nitrogen release rate, found in this study, reflected a gradual increase in the fish metabolic activity, starting about 1 h after the feed was offered. The maximum release was observed 10–12 h later, followed by a decrease. A similar history was reported by Zakęś (1999) and Zakęś and Karpiński (1999) for the zander (*Sander lucioperca*) as well as by Ming (1985) for the rainbow trout (*Oncorhynchus mykiss*). When studying *Lates calcarifer*, Almendras (1994) found the maximum $N\text{-}NH_4$ release rate as early as 3 h after the start of feeding.

CONCLUSION

The results obtained in this study allow to conclude that the African catfish metabolic rate, expressed by the rate of ammonia nitrogen release, is affected by the magnitude of the daily protein level to a larger extent than by the feed protein content. The best results, i.e. high growth rate, good feed conversion and low ammonia nitrogen release, were recorded when the fish were offered a feed containing about 40% of protein, the daily protein level being 2% of fish weight.

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Received: 02–04–02
Accepted after corrections: 03–03–03

ABSTRAKT

Vliv výživy na exkreci amoniaku a růst plůdku sumečka afrického (*Clarias gariepinus*)

Studie je zaměřena na zjištění účinku různého obsahu proteinů a tuků v krmné dávce na růst a rychlost metabolismu u sumečka afrického *(Clarias gariepinus)* chovaného v recirkulačním systému. Ryby byly denně krmeny mezi 8,00–20,00 h třemi krmivy (A, B a C) s obsahem různého podílu proteinů (36, 40 a 46 %). Denní krmná dávka představovala spotřebu proteinů v rozpětí 2–4 % hmotnosti obsádky. Vysoká rychlost růstu (SGR = 5,21 %) byla charakteristická pro ryby krmené krmivem B při 4% úrovni krmné dávky, ale nejnižší konverze krmiva (FCR = 1,21) bylo docíleno při použití stejného krmiva, ale při denní krmné dávce 2 %. U druhé z variant byla také zjištěna nejvyšší retence proteinů a energie (32, 33 a 27,23 %). U všech variant při zvýšení průměrné individuální hmotnosti bylo průvodním jevem snížení úrovně produkce amoniakálního dusíku vtažené k biomase ryb. Nejnižší úroveň (0,0094–0,0071 mg/g/h) byla zjištěna u ryb krmených krmivem C při 2% hladině krmné dávky, nejvyšší úroveň (0,0337–0,0183 mg/g/h) byla typická pro pro krmení krmivem A při 4% krmné dávce. Maximální množství produkovaného amoniakálního dusíku u všech variant bylo zjištěno mezi 18. až 21. hodinou.

Klíčová slova: exkrece amoniaku; sumeček africký; úroveň krmné dávky

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Influence of growth rate in two growth periods on intramuscular connective tissue and palatability traits of beef

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ABSTRACT: The study is concerned with the effect of daily gain in two growth periods on collagen and meat characteristics in 45 Brown bulls. Average daily gain 90 days before slaughter was 1 066 g/day and from birth to slaughter 1 088 g/day. Daily gain in the last 90 days before slaughter affected the values of shear force ($P \leq 0.05$) and sensory evaluation of meat tenderness ($P = 0.08$) and juiciness ($P = 0.05$). Daily gain from birth to slaughter affected collagen characteristics ($P \leq 0.05$), shear force values ($P \leq 0.05$) and sensory evaluation of meat juiciness ($P = 0.08$), yet the effect on meat tenderness was not as explicit ($P = 0.16$) as the effect of daily gain in the last 90 days before slaughter. The influence of growth rate on meat tenderness depends mainly on changes in the muscle protein turnover. Presumably, the differences in growth rate in this study were a consequence of the lower rate of muscle protein degradation which also resulted in lower post mortem degradation rate and lower meat tenderness.

Keywords: bulls; growth rate; connective tissue; meat characteristics

Sensory traits of meat, such as colour, juiciness and tenderness, are the most important traits of meat quality for the consumer (Varnam and Sutherland, 1995). All mentioned traits depend on the quantity and solubility of intramuscular collagen and myofibril fragmentation (Sorensen, 1981; Bailey and Light, 1989). Collagen concentration does not change significantly during growth until slaughter (Cross *et al.*, 1973; Pearson and Young, 1989), but collagen solubility decreases with increasing animal age (Gerrard *et al.*, 1987; Bailey and Light, 1989). Collagen concentration and solubility differ between different breeds (Cross *et al.*, 1973; Sorensen, 1981; Šubrt and Mikšik, 2002) and also depend on growth intensity in the last fattening period (Etherington, 1987; Micol *et al.*, 1992). Live weight gain represents a difference between tissue synthesis and degradation. So the growth rate can be improved by increasing tissue synthesis, decreasing tissue degradation, or by both of them. Altering the rate of tissue degradation *in vivo* can also change the rate of protein degradation *post mortem* and meat tenderness in this way.

The aim of the paper was to test the effect of daily gain in different growth periods on collagen and meat characteristics in Brown bulls.

MATERIAL AND METHODS

Forty-five Brown bulls from a progeny testing station, offsprings of 7 different sires, were included in the analysis. Calves were collected at the testing station at approximately 100 kg live weight. They were fed a mixture of maize and grass silage *ad libitum* and 2.5 kg of concentrates. Bulls were weighed monthly. Daily gain 90 days before slaughter was calculated by interpolation of the two nearest weighings. Bulls were slaughtered at the same degree of fatness that was subjectively estimated over the back, tail head, pins, hooks, ribs and brisket. 24 hours after slaughter pH (pH_{24}) and colour measurements were recorded in a cross-section of the *m. longissimus pars lumborum et thoracis* between the 7th and 8th rib. Colour was measured with Minolta CR 300 chroma meter presented as CIE (Commission Internationale de l'Eclairage) L*, a* and b* values. *M. longissimus pars lumborum et thoracis* samples for chemical, sensory and collagen analyses were taken between the 8th and 13th rib and frozen at –20°C and for collagen at –70°C prior to analysis. Half-carcasses were then dissected into lean meat, fat, bones and tendons. The values of shear force were recorded with Instron. Colorimetric analysis for hydroxyproline was used to determine the collagen concentration (Bergman and Loxely, 1963 adapted by Matissek *et al.*, 1992). Collagen concentration was calculated assuming that collagen weighed 8 times the measured hydroxyproline weight. Collagen solubility was determined after 1 h extraction at 77°C in ringer solution (Hill, 1966).

Statistical analysis was performed by GLM procedure (SAS, 1989). To test the influence of growth rate in different growth periods, two different models were used. Besides the fixed effect of sire and carcass fat as a covariable, daily gain 90 days before slaughter (model 1) or daily gain from birth to slaughter (model 2) were included in the models.

RESULTS AND DISCUSSION

Bulls were slaughtered at average age of 498 days and average live weight of 583 kg (Table 1). Daily gain from birth to slaughter was on average 1 088 g/day and in the last three months before slaughter 1 066 g/day.

Carcasses had on average almost 14% fat. From the meat characteristics, intramuscular fat showed the greatest variability, followed by soluble collagen content. The values of shear force exhibited similar

Table 1. Basic statistics for growth rate in different periods, age and live weight at slaughter and meat quality characteristics in the *longissimus pars lumborum et thoracis* muscle

	Average	SD	Min	Max
Age at slaughter (days)	498	38	406	559
Live weight at slaughter (kg)	583	40	510	658
Daily gain (g/day)				
from birth to slaughter (g/day)	1 088	86	883	1 256
90 days before slaughter (g/day)	1 066	188	626	1 454
Carcass fat (%)	13.7	2.0	9.5	17.4
M. longissimus pars lumborum et thoracis				
Soluble collagen (mg/g)	0.80	0.25	0.28	1.32
Insoluble collagen (mg/g)	4.92	0.76	3.44	6.75
Total collagen (mg/g)	5.72	0.94	3.85	7.88
Soluble collagen (%)	15.06	3.82	6.94	23.07
Intramuscular fat (%)	4.13	1.72	1.39	10.40
Tenderness⁺	5.1	1.2	1.8	6.8
Juiciness⁺	5.8	0.5	5	6.7
Aroma⁺	5.8	0.4	4.8	6.3
Shear force – transverse (N)	81.3	21.3	43.8	126.2
Shear force – longitudinal (N)	45.2	8.9	27.2	61.9
pH_{24}	5.67	0.17	5.49	6.25
CIE L*	38.6	2.9	31.4	44.7
a*	21.0	2.7	14.7	27.2
b*	11.5	1.7	6.7	14.8

⁺subjective evaluation, 1 is the worst and 7 is the best note; L* = lightness, a* = redness, b* = yellowness variability like subjectively evaluated meat tenderness. The latter was much higher than the variability of meat juiciness and aroma.

In Table 2 analysis of variance for model 1 with daily gain in the last 90 days before slaughter is presented. Fixed effect of sire significantly ($P < 0.05$) affected intramuscular fat, pH_{24} and meat colour. P-values for sire effect on collagen characteristics were between 0.05 and 0.10. Carcass fat percentage, included as a covariable in the model, also affected intramuscular fat and meat colour. Daily gain in the last 90 days before slaughter affected the values of shear force ($P \leq 0.05$), sensory evaluation of meat tenderness ($P = 0.08$) and juiciness ($P = 0.05$).

In model 2 with daily gain from birth to slaughter (Table 3), sire as fixed effect and carcass fat percentage as a covariable also had a similar effect on intramuscular fat, pH_{24}, meat colour and collagen characteristics. Daily gain from birth to slaughter affected quantitative collagen characteristics ($P \leq 0.05$), shear force values ($P \leq 0.05$) and sensory evaluation of meat juiciness ($P = 0.08$), yet the effect on meat tenderness was not as explicit ($P = 0.16$) as the effect of daily gain in the last 90 days before slaughter in the previous model.

The highest coefficients of determination for collagen characteristics were attained when daily gain from birth to slaughter was included in the model. From 31 to 37% of variability in some collagen characteristics could be explained by the model with daily gain from birth to slaughter. So, if daily gain from birth to slaughter was included in the model, another 2 to 9% of variability could be explained. The same was true of the shear force values. Much less variability of sensory traits could be explained; the highest r^2 was calculated for meat tenderness. The highest coefficients of determination were attained when daily gain in the last 90 days before slaughter was included in the model. The differences between the two models were smaller than for collagen characteristics and shear force values. Another 2 to 4% of variability could be explained when daily gain in the last 90 days was included in the model. We concluded on the basis of these results that of the two analysed models, the model with growth rate from birth to slaughter as a covariable is more suitable for the analysis of meat traits.

In Figure 1 the relationship between daily gain from birth to slaughter and soluble collagen content is presented. Regression coefficient was 0.001 (Table 3), so with increased daily gain, the content

Tab. 2. Analysis of variance for meat traits (model 1)

	Model r^2	Level of significance, P-values			Regression coefficient for daily gain 90 days before slaughter ± SEE
		sire	carcass fat content	daily gain 90 day before slaughter	
Soluble collagen (mg/g)	0.28	0.10	0.28	0.40	0.000179 ± 0.000212
Insoluble collagen (mg/g)	0.30	0.06	0.34	0.29	0.000673 ± 0.000633
Total collagen (mg/g)	0.28	0.08	0.29	0.28	0.000783 ± 0.000783
Soluble collagen (%)	0.29	0.06	0.37	0.71	0.001194 ± 0.003186
Intramuscular fat (%)	0.50	0.01	0.01	0.82	−0.000279 ± 0.001200
Tenderness⁺	0.23	0.33	0.77	0.08	−0.001834 ± 0.001027
Juiciness⁺	0.20	0.63	0.65	0.05	−0.000835 ± 0.000411
Aroma⁺	0.10	0.91	0.94	0.19	−0.000468 ± 0.000354
Shear force – transverse (N)	0.29	0.12	0.86	0.05	0.035309 ± 0.017764
Shear force – longitudinal (N)	0.27	0.34	0.64	0.04	0.015849 ± 0.007536
pH_{24}	0.55	0.00	0.61	0.88	−0.000021 ± 0.000133
CIE L*	0.45	0.00	0.09	0.77	−0.000628 ± 0.002129
a*	0.40	0.02	0.02	0.59	0.001123 ± 0.002105
b*	0.48	0.00	0.01	0.14	−0.001876 ± 0.001229

⁺subjective evaluation, 1 is the worst and 7 is the best note; L* = lightness, a* = redness, b* = yellowness of soluble collagen also increased. The same is also true of insoluble collagen content (Figure 2) where the regression coefficient was 0.003192 (Table 3).

Higher daily gain from birth to slaughter resulted in higher values of shear force measured transversely (b = 0.095, Table 3, Figure 3) and longitudinally (b = 0.051, Table 3, Figure 4). Regression coefficients for the values of transverse and longitudinal shear force in the model with daily gain in the last 90 days before slaughter were much lower (b = 0.035 and b = 0.16, Table 2).

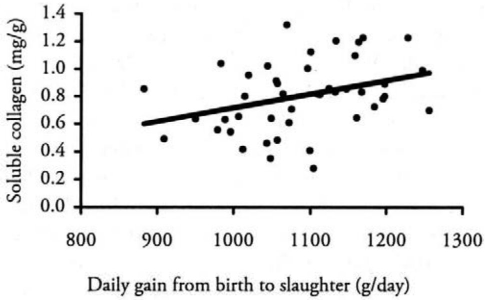

Figure 1. Relationship between daily gain from birth to slaughter and soluble collagen content

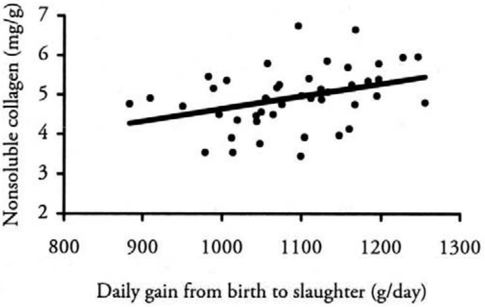

Figure 2. Relationship between daily gain from birth to slaughter and insoluble collagen content

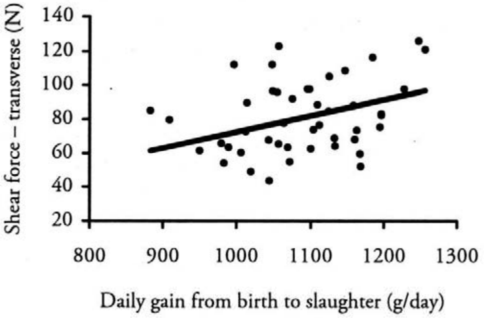

Figure 3. Relationship between daily gain from birth to slaughter and the value of transverse shear force

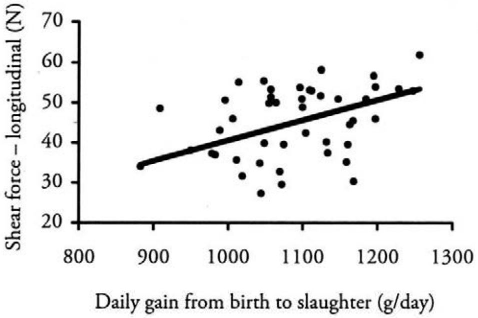

Figure 4. Relationship between daily gain from birth to slaughter and the value of longitudinal shear force

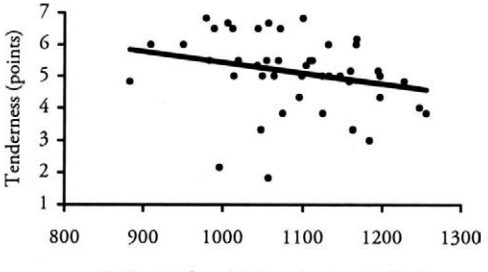

Figure 5. Relationship between daily gain from birth to slaughter and meat tenderness

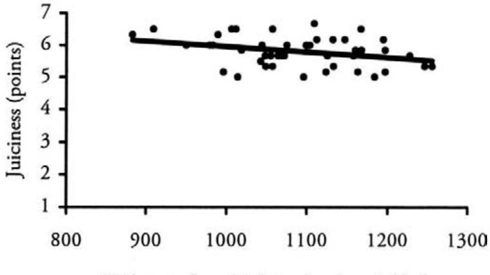

Figure 6. Relationship between daily gain from birth to slaughter and meat juiciness

Regression coefficients for sensory traits were all negative, so with increased daily gain from birth to slaughter subjectively evaluated meat tenderness, juiciness and aroma decreased. The regression coefficient for tenderness was –0.003412 and for juiciness –0.001719 (Table 3), but none of the regression coefficients was statistically significant (*P*-value for tenderness was 0.16 and for juiciness 0.08).

Regression coefficients for meat tenderness and juiciness from the model with daily gain in the last 90 days before slaughter were as lower as the absolute values (–0.001834 and –0.000835, Table 2) with lower SEE and also with lower *P*-values ($P = 0.08$ and $P = 0.05$, Table 2).

It was reported that a higher growth rate before slaughter could result in more tender meat (Fishell *et al.*, 1985; Thomson *et al.*, 1999; Vestergaard *et al.*, 2000). A general increase in total nutrient supply stimulates both muscle protein synthesis and degradation at the intake above maintenance (Lobley, 1998). Thomson *et al.* (1999) reported that the increased growth rate was accompanied by higher µ-calpain activities. µ-calpain should be a key protease that is responsible for meat tenderisation *post mortem* (Koohmaraie, 1996, Dransfield and Siosnicki, 1999). So the higher rate of muscle protein degradation likely resulted in the higher post-slaughter rate of muscle protein degradation and better meat tenderness.

On the other hand, Sinclair *et al.* (2001) could find no consistent effects of growth rate on beef tenderness. As reported, Calkins *et al.* (1987) did not find any differences in endogenous muscle enzymes and tenderness of beef from bulls fed *ad libitum* on maintenance and sub-maintenance diet. Oddy *et al.* (1998) reported different responses to increased feed intake in different cattle lines. Cattle of the low-growth rate line responded to increased feed intake by higher rates of muscle protein synthesis and degradation while cattle of the high-growth rate line tended to increase the muscle protein gain through a reduction in muscle protein synthesis and degradation. Similar results were reported for the weaning weight of divergently selected lambs (Oddy *et al.*, 1995).

Selection for higher growth rate can also change tissue metabolism. In cattle divergent selection for

Table 3. Analysis of variance for meat traits (model 2)

	Model r^2	Level of significance, *P*-values			Regression coefficient for daily gain from birth to slaughter ± SEE
		sire	carcass fat content	daily gain from birth to slaughter	
Soluble collagen (mg/g)	0.35	0.10	0.18	0.04	0.001000 ± 0.000462
Insoluble collagen (mg/g)	0.37	0.07	0.22	0.03	0.003192 ± 0.001375
Total collagen (mg/g)	0.37	0.10	0.17	0.02	0.004192 ± 0.001687
Soluble collagen (%)	0.31	0.05	0.31	0.25	0.008325 ± 0.007190
Intramuscular fat (%)	0.51	<0.00	0.01	0.50	0.001848 ± 0.002737
Tenderness⁺	0.21	0.44	0.92	0.16	–0.003412 ± 0.002390
Juiciness⁺	0.18	0.80	0.83	0.08	–0.001719 ± 0.000954
Aroma⁺	0.06	0.90	0.85	0.52	–0.000526 ± 0.000826
Shear force – transverse (N)	0.32	0.11	0.64	0.02	0.095235 ± 0.039877
Shear force – longitudinal (N)	0.35	0.14	0.40	<0.00	0.051065 ± 0.016217
pH_{24}	0.55	<0.00	0.60	0.86	–0.000053 ± 0.000306
CIE L*	0.45	<0.00	0.10	0.78	–0.001376 ± 0.004884
a*	0.47	0.01	0.01	0.03	0.010348 ± 0.004530
b*	0.45	<0.00	0.01	0.63	0.001419 ± 0.002899

⁺subjective evaluation, 1 is the worst and 7 is the best note; L*= lightness, a* = redness, b* = yellowness daily gain to 1 year of age resulted in higher rates of muscle protein turnover in the low-growth line than in the high-growth line (Oddy *et al.* 1998). Fractional degradation rates of muscle proteins were also lower in the fast-growth line than in the slow-growth line of divergently selected chickens for growth rate (Tesseraud *et al.*, 2000). Tomas *et al.* (1991) also noted the lowest fractional degradation rates of muscle proteins in chickens selected for the efficiency of food conversion. Higher muscle protein accretion in mice selected for a high amount of carcass protein compared to mice selected for high body weight seems to be due rather to a lower rate of muscle protein breakdown than to an increased rate of muscle protein synthesis (Schadereit *et al.*, 1997).

The influence of growth rate on meat tenderness seems to depend mainly on changes in muscle protein turnover. If higher growth rate is related to higher rates of muscle protein synthesis and degradation, increased meat tenderness can be expected and vice versa. If higher growth rate is related to a lower rate of muscle protein degradation, decreased meat tenderness can be expected.

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Received: 02–07–30
Accepted after corrections: 03–02–20

ABSTRAKT

Vliv rychlosti růstu ve dvou růstových obdobích na intramuskulární pojivovou tkáň a chuťové vlastnosti hovězího masa

Tato studie se zabývá vlivem denního hmotnostního přírůstku ve dvou růstových obdobích na vlastnosti kolagenu a masa u 45 býků hnědého skotu. Průměrný denní přírůstek 90 dní před porážkou činil 1 066 g/den a v období od narození do porážky 1 088 g/den. Denní přírůstek v posledních 90 dnech před porážkou ovlivňoval hodnoty střižné síly ($P \leq 0{,}05$) a senzorické hodnocení křehkosti ($P = 0{,}08$) a šťavnatosti masa ($P = 0{,}05$). Denní přírůstek v období od narození do porážky ovlivňoval vlastnosti kolagenu ($P \leq 0{,}05$), hodnoty střižné síly ($P \leq 0{,}05$) a senzorické hodnocení šťavnatosti masa ($P = 0{,}08$), ale vliv na křehkost masa nebyl tak jednoznačný ($P = 0{,}16$) jako vliv denního přírůstku za posledních 90 dnů před porážkou. Vliv rychlosti růstu na křehkost masa závisí hlavně na změnách v metabolismu svalových proteinů. Lze předpokládat, že rozdíly v rychlosti růstu v této studii byly důsledkem nižšího stupně degradace svalových proteinů, který rovněž vedl i ke sníženému odbourání proteinů v postmortálním období a nižší křehkosti masa.

Klíčová slova: býci; rychlost růstu; pojivová tkáň; vlastnosti masa

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Evaluation of Slovene local pig breed Krškopolje for carcass and meat quality

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ABSTRACT: The study is the first evaluation of the carcass and meat quality of Slovene local pig breed Krškopolje in comparison with the crosses of Krškopolje and a modern breed. Subjects were 29 pigs; 17 pigs were purebred Krškopolje pigs (KK), 12 pigs were the offspring of Krškopolje sow and modern breed male (LN55). Only a limited effect on carcass traits was obtained by crossing KK to a modern breed. Carcass length was longer (103.9 vs. 100.8 cm, $P < 0.01$), ham was heavier (14.5 vs. 14.1 kg, $P < 0.05$) and less fat (26 vs. 30 mm, $P < 0.05$) in LN55 × KK than in KK pigs. Pure KK pigs had lower pH_1 (pH 1 hour *post mortem*) (5.84 vs. 6.17, $P < 0.01$) but higher ultimate pH (pH_{24}) (5.60 vs. 5.51, $P < 0.05$) than LN55 × KK pigs. More intramuscular fat in the *longissimus dorsi* muscle (3.0 vs. 2.1%, $P < 0.05$) and darker ham colour (4.2 vs. 3.5, $P < 0.01$) were found for KK than for LN55 × KK pigs. No significant difference in the eating quality of roasted loin was observed, however KK pigs tended to have less tender meat that LN55 × KK crosses (5.4 vs. 5.7, $P < 0.10$). When processed into dry hams, a lower ham weight loss at salting was observed for KK than for LN55 × KK pigs (2.9 vs. 3.5%, $P < 0.01$). Dry hams of KK pigs had more salt (7.9 vs. 7.6, $P < 0.10$) and consequently lower non-protein nitrogen content (18.7 vs. 21.3, $P < 0.001$); dry-ham slices of KK pigs had darker colour (5.2 vs. 4.9, $P < 0.05$ for *biceps femoris* muscle; 5.8 vs. 5.5, $P < 0.01$ for *semimembranosus* muscle), better aroma (5.7 vs. 5.4, $P < 0.05$) and overall impression (5.8 vs. 5.4, $P < 0.01$) compared to dry hams of LN55 × KK pigs. The limited improvement of carcass quality in KK crosses, low pH_1, and slightly less tender meat of pure KK pigs is likely to be related to the higher incidence of *RYR1* gene mutation found in pure KK pigs (70% *RYR1-Nn* and 18% *RYR1-nn*) than in LN55 × KK crosses (17% *RYR1-Nn*).

Keywords: carcass traits; meat quality, pig; local breed; *RYR1*

One of the reasons for the increased interest in local pig breeds is a better quality of their meat compared to modern breeds (Dunn, 1996; Santos e Silva *et al.*, 2000; Labroue *et al.*, 2000). According to the literature, pigs of local breeds present poorer growth performance, they are fatter and less conformed than pigs of modern breeds (Legault *et al.*, 1996; Warriss *et al.*, 1996; Santos e Silva *et al.*, 2000; Labroue *et al.*, 2000). As for the quality of meat or meat products, the above-mentioned studies report an advantage of local pig breeds in relation to pigs of modern breeds. In Slovenia, the Krškopolje or the Blackbelted pig is the only local breed of pigs. Due to a very small herd size it is endangered by extinction. More than thirty years ago few studies dealt with production characteristics of this breed (Ferjan, 1969; Eiselt, 1971) but later on all professional and research work on this local breed was stopped. The interest in this breed of pigs was revived in the past ten years (Šalehar, 1994) and today the Krškopolje breed is included in the national preservation program for autochthonous breeds. The breed is adapted to poor breeding conditions and is reputed for good meat

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Supported by the Ministry of Education, Science and Sport, and the Chamber of Agriculture and Forestry of Slovenia.

quality (Šalehar, 1994). The reputation of good meat quality has not been objectively assessed so far. Therefore the present study was aimed at the first evaluation of meat quality in Slovene local pig breed Krškopolje in comparison with crosses with Krškopolje raised in the same environment.

MATERIAL AND METHODS

Material

The experiment was organized by the Chamber of Agriculture and Forestry of Dolenjska region and conducted on a small family farm. Subjects were 17 pigs (9 females, 8 castrates) of local Krškopolje breed (KK), the offspring of 5 sows and two boars, and 12 pigs (7 females, 5 castrates) were the offspring of three Krškopolje sows and three landrace boars (landrace male line – LN55). Due to a very small herd size of KK breed (at the time the nucleus comprised 30 sows and 3 boars), the pigs did not start the experiment at the same time, but were housed in three series. They were raised in the same stable and received two diets during the fattening; from 25 to 60 kg they were fed a diet containing 12.7 MJ of metabolizable energy, 16% crude protein (8.8 g/kg lysine), from 60 kg the pigs received a diet which consisted of feed containing 11.7 MJ of metabolizable energy, 19.5% crude protein (12 g/kg lysine) and maize grain silage at a 50:50 ratio. Pigs were fed twice daily. All pigs were slaughtered on the same day, according to routine procedure (fasting, 2 hours resting, electrically stunned, 1.5 A, 200 V).

Carcass measurements

After slaughter, carcass weight and lean meat content were recorded by the authorized service. A day after slaughter weights of ham (including the last lumbar vertebrae and shank), loin (from the 5th thoracic vertebrae to the cranial edge of the last lumbar vertebrae), shoulder (including shank) and belly were recorded. Loin eye area and fat area were measured and belly leanness was evaluated (1– only fat to 7 – only meat) at the level of the last rib. Subcutaneous fat thickness was measured at the level of the last rib 8 cm laterally to the split-line, and on the trimmed leg below *ossis caput femoris*. Carcass length was taken as a distance from *atlas* to *os pubis*. All measurements were performed on the left half of the carcass.

One hour post mortem pH (pH_1) was measured directly in the *longissimus dorsi* (LD) muscle at the level of the last rib using a pH meter equipped with InLab 427 electrode (Mettler Toledo GmbH Analytical, 8603 Schwerzenbach, Switzerland). Ultimate pH (pH_{24}), meat colour (Japanese scale from 1 = very pale to 6 = very dark as proposed by Nakai *et al.*, 1975), L value (Minolta Chromameter CR300) and water holding capacity as imbibing time (time necessary for 1 cm^2 of Schleicher-Schell 589^1 filter paper put on a fresh cut to become wet) were assessed on the *longissimus dorsi* muscle at the level of the last rib. Losses of juice at thawing and cooking were calculated in relation to the initial sample weight. Ham colour was scored from 1 (pale) to 5 (dark). Minolta L value was measured on *gluteus medius* and *gluteus profundus* muscles. Lipids were extracted from 10g samples of *longissimus dorsi* muscle (at the level of the last rib) according to Folch *et al.* (1957).

DNA test for *RYR1* gene mutation

A modified DNA test of Fujii *et al.* (1991) was used to detect a point mutation on the *RYR1* gene (cytosine on position 1843 is replaced by thymine; the allele with the mutation is denoted by *n*). DNA isolation was carried out from muscle samples according to Zabavnik-Piano and Uršič (1996). The pigs with wild *RYR1* gene (without mutation on both alleles) are denoted as *RYR1-NN*, heterozygous (mutation on one allele) pigs as *RYR1-Nn* and recessive homozygous (mutation on both alleles) pigs as *RYR1-nn*.

Sensory analysis

Samples of *longissimus dorsi muscle* were taken cranially from the last rib, vacuum-packed and stored at –20°C. Prior to analysis, the samples were thawed overnight at 4°C and roasted in an oven to a core temperature of 71.6 ± 0.2°C (mean ± se). Six panelists scored aroma, juiciness, tenderness on a 1 to 7 point scale (increasing intensity of perception). The resistance to shear force (N) was determined on cooked meat using an Instron Universal Testing Machine, model 1111 (Instron, Canton, MA), equipped with a 10-mm cutting blade (Φ = 1 mm, 60° angle between the sides). Cross-head speed was 5 cm/min and penetration depth 9.6 mm.

Dry ham processing

A day after slaughter, hams were trimmed for dry cured ham processing and put to salting for 2 weeks (at 2 to 4°C) followed by a resting period of 9 weeks at 4 to 6°C and 70 to 85% relative humidity. Then hams were dried at 14 to 20°C and 60 to 80% relative humidity for 42 weeks. After drying, hams were covered with a mixture of fat, flour and spices to prevent further desiccation and left to ripen for 5 additional weeks. Ham weight losses were recorded during the processing and calculated in relation to the trimmed leg weight prior to processing.

Dry ham analysis

A 10-cm thick slice was cut off, perpendicularly to the medial part of the femur for sensory and chemical analysis. Two slices per sample were served to each of six panelists, experienced in dry ham analysis. Slice leanness was evaluated as the fat to meat ratio (1 – only fat to 4 – half fat, half meat to 7 – only meat). Slice marbling was evaluated as the quantity of visible intramuscular fat. Aroma was scored for the whole slice, whereas colour intensity, texture (from very pasty to very hard), residual stroma and saltiness were assessed separately for *biceps femoris* and *semimembranosus* muscles. All sensory traits were noted on a 7 point scale with the intensity of perception increasing from 1 to 7. From the remaining sample *biceps femoris* and *semimembranosus* muscles were separated and homogenized together. Water content, non-protein nitrogen and salt content were determined as described previously (Čandek-Potokar *et al.*, 2002).

Statistics

Data were analyzed by SAS (SAS Inst. Inc. Cary, NC) using GLM procedure. The model comprised the effects of genotype and sex (genotype × sex interactions were insignificant); for carcass and fresh meat quality traits a regression on carcass weight, whereas for dry ham traits a regression on a ham weight was included. When a significant genotype effect was detected, the least squares means were compared at the 5% probability level.

RESULTS AND DISCUSSION

Incidence of *RYR1* gene mutation (Table 1)

At the start of the experiment we had no knowledge of *RYR1* gene status in the herd of KK breed. As low pH_1 was observed in pure KK pigs, we decided to perform a DNA test for the presence of *RYR1* gene mutation. A high incidence of *RYR1* gene mutation (70% *RYR1-Nn* and 18% *RYR1-nn*) was found in pure KK pigs. On the other hand, only two carriers (*RYR1-Nn*) were found in LN55 × KK crosses, where LN55 males were mutation free (*RYR1-NN*). There is little information on the *RYR1* gene status of local breeds in the literature, but the presence of *RYR1* gene mutation has also been reported for a Portuguese local breed Bisara (Santos e Silva *et al.*, 2000).

Table 1. Frequency of *RYR1* gene mutation in experimental pigs

Number of pigs	KK	LN55 × KK
RYR1-NN	2	10
RYR1-Nn	12	2
RYR1-nn	3	0
Total	17	12

KK = Krškopolje; LN55 = Landrace male line

Carcass traits (Table 2)

LN55 × KK pigs were slightly younger ($P < 0.10$) and lighter (not significant) at slaughter than KK pigs, so the results are compared for the same carcass weight (97.1 kg). LN55 × KK crosses were only slightly ($P < 0.10$) leaner than pure KK pigs. Weights of noble cuts, loin, shoulder and belly were similar, whereas heavier and leaner hams were found for LN55 × KK crosses compared to pure KK pigs. Previous studies on various local breeds (Legault *et al.*, 1996; Santos e Silva *et al.*, 2000; Labroue *et al.*, 2000) reported that local pig breeds presented fatter and less conformed carcasses. Crossing local and modern breeds was shown to improve growth performance and muscularity (Legault *et al.*, 1996).

Table 2. Growth and carcass traits[1] (mean ± se) of two pig genotypes

	Genotype		
	KK	LN55 × KK	Prob.
Number of pigs	17	12	
Age at slaughter (days)	244.7 ± 5.7	226.8 ± 6.9	†
Carcass weight (kg)	98.0 ± 3.1	95.4 ± 3.8	ns
Lean meat content (%)	46.2 ± 0.6	47.9 ± 0.7	†
Carcass length (cm)	100.8 ± 0.7	103.9 ± 0.8	**
Fat thickness at the last rib (mm)	29 ± 1	27 ± 1	ns
Loin eye area (cm^2)	41.7 ± 1.2	44.4 ± 1.5	ns
Loin eye fat area (cm^2)	31.9 ± 1.2	31.3 ± 1.3	ns
Loin (kg)	6.8 ± 0.1	6.9 ± 0.2	ns
Shoulder (kg)	7.3 ± 0.1	7.2 ± 0.1	ns
Shoulder (meat + bone) (kg)	5.2 ± 0.1	5.2 ± 0.1	ns
Belly (kg)	10.2 ± 0.1	10.3 ± 0.1	ns
Belly leanness (1–7)	3.6 ± 0.2	3.7 ± 0.2	ns
Ham (kg)	14.1 ± 0.2	14.5 ± 0.2	*
Trimmed ham (kg)	8.7 ± 0.1	9.1 ± 0.1	*
Ham fat thickness (mm)[2]	30 ± 1	26 ± 1	*

ns = $P > 0.10$; $^{\dagger}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$

[1] regression on the carcass weight included in the model; [2] measured on trimmed ham below *ossis caput femoris*

KK = Krškopolje; LN55 = Landrace male line

However, in our study only a minor improvement of carcass quality was observed for pigs crossed to modern breed. In our opinion, this result could be attributed to the presence of the *RYR1* gene mutation at a high frequency in the pure KK pigs, which is known to increase muscularity (Aalhus *et al.*, 1991; Guéblez *et al.*, 1995; Leach *et al.*, 1996).

Fresh meat quality (Table 3)

Pure KK pigs had lower pH_1 than LN55 × KK pigs due to the incidence of halothane gene, but a higher pH_{24} was found in KK than in LN55 × KK pigs. Labroue *et al.* (2000) also reported lower muscle pH_1 (*longissimus dorsi*) and higher muscle pH_{24} (*semimembranosus*) for local breeds in comparison with Large White, however no indication was given on the stress susceptibility gene status of their local breeds. On the contrary, Warriss *et al.* (1996) reported higher muscle pH_1 for local breeds. A tendency for better water holding capacity (higher imbibing time) was observed in KK pigs, which can be related to their higher pH_{24}. The study of Labroue *et al.* (2000) reported a lower imbibing time for local breeds whereas, on the contrary, Warriss *et al.* (1996) and Santos e Silva *et al.* (2000) reported better water holding capacity for the meat of local breeds. Pure KK pigs had higher intramuscular fat content and marbling of LD muscle than LN55 × KK pigs. A higher intramuscular fat content was generally reported for local breeds (Simon *et al.*, 1996; Labroue *et al.*, 2000; Santos e Silva *et al.*, 2000). Lower Minolta L values of ham muscles and higher colour scores for ham were found for KK compared to LN55 × KK pigs. Colour variation between the genotypes could partly be related to age differences, since myoglobin content increases with age and weight (Monin and Ouali, 1991).

Table 3. Fresh meat quality traits[1] (mean ± se) of two pig genotypes

	Genotype		
	KK	LN55 × KK	Prob.
Number of pigs	17	12	
pH_1[2]	5.84 ± 0.07	6.17 ± 0.08	**
pH_{24}[2]	5.60 ± 0.03	5.51 ± 0.03	*
Imbibing time (sec)[2]	38 ± 9	11 ± 10	†
Marbling (1–7)[2]	3.2 ± 0.2	2.8 ± 0.3	ns
Intramuscular fat (%)[2]	3.0 ± 0.2	2.1 ± 0.3	*
Colour (1–6)[2]	3.7 ± 0.1	3.7 ± 0.1	ns
Minolta L			
M. longissimus dorsi	54.1 ± 1.1	53.9 ± 1.3	ns
M. gluteus medius	49.0 ± 1.4	52.8 ± 1.5	*
M. gluteus profundus	39.2 ± 0.9	41.3 ± 1.0	†
Ham appearance (1–7)	6.1 ± 0.2	5.5 ± 0.2	†
Ham colour intensity (1–5)	4.2 ± 0.2	3.5 ± 0.2	**

ns = $P > 0.10$; †$P < 0.10$; *$P < 0.05$; **$P < 0.01$

[1] regression on the carcass weight included in the mode; [2] *longissimus dorsi* muscle

KK = Krškopolje; LN55 = Landrace male line

Eating quality of meat (Table 4)

Eating quality of roasted loin was similar for KK and LN55 × KK pigs. However, a tendency ($P < 0.10$) of lower meat tenderness was found in KK pigs compared to LN55 × KK pigs. Contrary to our results, the study of Labroue *et al.* (2000) reported more tender meat for local pig breeds. The favourable breed effect is likely to be lost in pure KK pigs, due to low pH_1 which was shown to affect tenderness adversely (Fernandez *et al.*, 1994).

Table 4. Eating quality[1] and shear force resistance[1] (mean ± se) of *longissimus dorsi* muscle of two pig genotypes

	Genotype		
	KK	LN55 × KK	Prob.
Number of pigs	17	12	
Tenderness (1–7)	5.4 ± 0.1	5.7 ± 0.1	†
Juiciness (1–7)	4.9 ± 0.1	5.1 ± 0.1	ns
Aroma (1–7)	5.2 ± 0.1	5.3 ± 0.1	ns
Instron shear force (N)			
Perpendicular to fibres	58.4 ± 1.9	57.9 ± 2.2	ns
Parallel to fibres	46.1 ± 1.5	44.2 ± 1.7	ns

ns = $P > 0.10$; †$P < 0.10$

[1] regression on the carcass weight included in the model

KK = Krškopolje; LN55 = Landrace male line

Table 5. Dry ham processing losses[1] and chemical traits[1] (mean ± se) of two pig genotypes

	Genotype		
	KK	LN55 × KK	Prob.
Number of hams	17	12	
Processing weight loss (%)			
Salting	2.9 ± 0.2	3.5 ± 0.2	**
Resting	13.9 ± 0.2	13.9 ± 0.2	ns
Drying	14.5 ± 0.2	14.9 ± 0.2	ns
Overall	31.3 ± 0.5	32.3 ± 0.5	ns
Chemical traits[2]			
Water content (%)	52.1 ± 0.3	53.1 ± 0.4	*
Non-protein nitrogen (%)	18.7 ± 0.5	21.3 ± 0.5	***
Salt (%)	7.9 ± 0.1	7.6 ± 0.2	†

ns = $P > 0.10$; $^{\dagger}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

[1] regression on the ham weight included in the model; [2] determined on a homogenised mixture of *m. semimembranosus* and *m. biceps femoris*

KK = Krškopolje; LN55 = Landrace male line

Table 6. Dry ham sensory traits[1] (mean ± se) of two pig genotypes

	Genotype		
	KK	LN55 × KK	Prob.
Number of hams	17	12	
Slice leanness (1–7)	2.4 ± 0.1	2.8 ± 0.1	*
Slice marbling (1–7)	4.5 ± 0.3	3.4 ± 0.3	*
Aroma (1–7)	5.7 ± 0.1	5.4 ± 0.1	*
Colour intensity (1–7)			
M. biceps femoris	5.2 ± 0.1	4.9 ± 0.1	*
M. semimembranosus	5.8 ± 0.1	5.5 ± 0.1	**
Texture (1–4–7)[2]			
M. biceps femoris	3.4 ± 0.0	3.3 ± 0.1	ns
M. semimembranosus	4.3 ± 0.1	4.3 ± 0.1	ns
Residual stroma (1–7)			
M. biceps femoris	2.3 ± 0.1	2.6 ± 0.1	†
M. semimembranosus	1.5 ± 0.0	1.5 ± 0.0	ns
Saltiness (1–7)			
M. biceps femoris	4.9 ± 0.1	4.9 ± 0.1	ns
M. semimembranosus	4.4 ± 0.1	4.3 ± 0.1	ns
Overall impression (1–7)	5.8 ± 0.1	5.4 ± 0.1	**

ns = $P > 0.10$; $^{\dagger}P < 0.10$; $^{*}P < 0.05$; $^{**}P < 0.01$

[1] regression on the ham weight included in the model; [2] from very pasty (1) to very hard (7)

KK = Krškopolje; LN55 = Landrace male line

Dry ham processing loss and chemical traits (Table 5)

A lower ham processing loss at salting was found for KK than LN55 × KK pigs, whereas during the other stages of processing as well as overall losses were not significantly different between KK and LN55 × KK pigs. This result can be related to literature data since ham processing losses are lower in heavier and/or fatter hams (review of Russo and Nanni Costa, 1995). Moreover, lower losses at salting could partly result also from higher salt intake observed for hams of KK pigs. Lower non-protein nitrogen content found for hams from KK pigs can also be related to their higher salt content.

Dry ham quality traits (Table 6)

In accordance with the observed ham traits, leaner and less marbled dry-ham slices were found for LN55 × KK pigs. Sensory analysis of dry hams revealed some advantages of KK pigs regarding colour and aroma. As a consequence, overall impression was also better for hams of KK compared to LN55 × KK pigs. On the other hand, no differences between the two groups was observed for dry ham texture and saltiness. A study of Simon *et al.* (1997) on Gascon and Limousin breeds also reported some advantages regarding the dry-ham quality of local breeds. They reported lower processing losses, more intensive colour and flavour but also a beneficial effect of the local breed Gascon on the texture of dry hams which we did not observe.

CONCLUSIONS

In the present study, the expected improvement of carcass quality in pigs crossed to a modern breed was likely lost due to the high incidence of the *RYR1* gene mutation in Krškopolje breed. For the same reason, due to the low pH_1, meat eating quality, especially tenderness could be deteriorated in KK pigs. Despite of this, the results of this study demonstrated some advantages (intramuscular fat, dry ham quality) of Krškopolje breed for meat quality. As in the present study the effect of *RYR1* gene mutation is comprised within Krškopolje breed, it would be interesting to make further studies with a balanced sample of three *RYR1* genotypes within the breed, to distinguish between the two effects. High incidence of the *RYR1* gene mutation determined for Krškopolje breed presents a major problem for future work on the preservation of this breed.

Acknowledgements

We appreciate the co-operation of the meat company MIP d.d., Nova Gorica, and pig farmer Franc Pribožič, and the technical assistance of I. Karnel and M. Kač.

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Received: 02–08–26
Accepted after corrections: 03–03–06

ABSTRAKT

Hodnocení slovinského místního plemene prasat Krškopolje z hlediska kvality jatečného trupu a masa

Práce je prvním zhodnocením kvality jatečných těl a kvality masa u slovinského místního plemene prasat Krškopolje ve srovnání s kříženci plemene Krškopolje a moderního plemene. V pokusu bylo sledováno 29 ks zvířat; 17 ks byla čistokrevná prasata plemene Krškopolje (KK), 12 ks byli kříženci plemene Krškopolje s kancem moderního plemene (LN55). Křížení KK s moderním plemenem mělo pouze omezený vliv na znaky jatečného těla. Kříženci LN55 × KK měli delší jatečný trup (103,9 cm, resp.100,8 cm; $P < 0,01$), těžší kýtu (14,5 kg, resp. 14,1 kg; $P < 0,05$) a nižší výšku tuku (26 mm, resp. 30 mm; $P < 0,05$) než prasata KK. Ve srovnání s prasaty LN55 × KK měla čistokrevná prasata KK nižší hodnotu pH_1 (5,84, resp. 6,17; $P < 0,01$), ale konečná hodnota pH_{24} byla vyšší (5,60, resp. 5,51; $P < 0,05$). U prasat KK byl zjištěn vyšší obsah intramuskulárního tuku ve svalu *m. longissimus dorsi* (3,0 %, resp. 2,1 %; $P < 0,05$) a tmavší barva kýty (4,2, resp. 3,5; $P < 0,01$) než u prasat LN55 × KK. Nebyl zjištěn rozdíl v křehkosti pečeně, avšak u prasat KK byla zaznamenaná tendence k méně křehkému masu než u kříženců LN55 × KK (5,4, resp. 5,7; $P < 0,10$). Při zpracování na sušenou šunku byla zjištěna nižší ztráta hmotnosti u prasat KK ve srovnání s prasaty LN55 × KK (2,9 %, resp. 3,5 %; $P < 0,01$). Sušené šunky prasat KK měly vyšší obsah soli (7,9, resp. 7,6; $P < 0,01$) a v důsledku toho nižší obsah nebílkovinného dusíku (18,7, resp. 21,3; $P < 0,0001$); plátky sušené šunky prasat KK měly tmavší barvu (5,2, resp. 4,9, $P < 0,05$ pro *m. biceps femoris*; 5,8, resp. 5,5, $P < 0,01$ pro *m. semimembranosus*), příznivější vůni (5,7, resp. 5,4; $P < 0,05$) a celkové hodnocení (5,8, resp. 5,4; $P < 0,01$) ve srovnání se sušenými šunkami prasat LN55 × KK. Omezené zlepšení kvality jatečného trupu, nízká hodnota pH_1 a méně křehké maso čistokrevných prasat KK pravděpodobně souvisí s vyšší incidencí mutace genu *RYR1* (70 % *RYR1-Nn* a 18 % *RYR1-nn*), která byla zjištěna u prasat KK ve srovnání s kříženci LN55 × KK (17 % *RYR1-Nn*).

Klíčová slova: prase; místní plemeno; znaky jatečného trupu; kvalita masa; *RYR1*

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Effect of *RYR1* and *ESR* genotypes on the fertility of sows of Large White breed in elite herds

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ABSTRACT: The effect of *RYR1* and *ESR* genotypes on litter size was studied in sows of Large White breed in two elite herds. The genotypes were detected by the PCR-RFLP method. In herd A in the groups of parity 1 and parity 1–6 significant differences ($P \leq 0.01$) between *RYR1* genotypes in the number of all born piglets in favour of sows with *CT* genotypes were determined. In herd B the performance of sows *CC* was insignificantly better in this parameter. In herd A significant ($P \leq 0.05$, $P \leq 0.01$) differences between the genotypes *ESR* in the groups of parity 1–6 and 2–6 were demonstrated in the number of all born, alive born and weaned piglets. The best performance was achieved by sows with *DD* genotypes. In herd B there were significant ($P \leq 0.05$) differences between the genotypes in the group of parity 2–6 and in the group of parity 1–6 at the significance level ($P \leq 0.07$) in the number of all born, alive born and weaned piglets, but in favour of sows with *CC* genotypes. The correlations between litter size and parity and year of sow birth were significant ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$).

Keywords: pig; litter size; ryanodine receptor – *RYR1*; estrogen receptor – *ESR*

Steroid hormones and their receptors play a key role in the reproduction process of sows. Among others, they involve estrogens that stimulate protein synthesis on a cellular level, they significantly influence proliferation and differentiation of vaginal epithelium, oocyte ripening and quality and proliferation of uterine endometrium (Sundarrajan *et al.*, 1999). The effect of estrogen is realized through an estrogen receptor (*ESR*). Three polymorphisms are currently known at *ESR* gene. Rothschild *et al.* (1991) described point mutations at *ESR* gene in three Chinese pig breeds using endonuclease *Pvu*II; this polymorphic system is designated as *ESR2* (alleles *C, D*). The other two polymorphisms (*ESR1* and *ESR3*) were identified by Drögemüller *et al.* (1997). Significant differences in litter size between *ESR* genotypes in sows of Large White breed were reported by several authors (Rothschild *et al.*, 1995; Rothschild, 1996; Short *et al.*, 1997). Depuydt *et al.* (1999) studied associations of *ESR* genotypes with reproduction traits of hybrid sows but they did not confirm the assumed effect of *ESR* genotypes on fertility. Possible effects of *ESR* genotypes on the reproduction traits of sows (Meishan × Landrace SL93 PIC) were studied in one of the papers of van Rens *et al.* (2000). The effect of *ESR* gene on the length of estral cycle and estrus was not demonstrated, the sows of homozygous genotypes did not exhibit any differences in the secretion profiles of luteinizing hormone, estrogen and progesterone. Although allele *D* is associated with litter size (Rothschild, 1996), no differences in the number of corpora lutea or in the number of vital 35/36 day embryos were established. The above findings indicate that litter size depends on the fetus fitness. Significant differences were found in the size of placenta: the embryos of sows with *DD* genotype had larger placentas than the embryos of sows with *CC* genotype. It can be connected with different fetus mortality and tendency of embryonic mortality timing (van Rens *et al.*, 2000).

Supported by the Ministry of Agriculture of the Czech Republic (Project QD 1039) and Ministry of Education, Youth and Sports of the Czech Republic (CEZ: MSM 122200004).

Ryanodine receptor gene of the sarcoplasmatic reticulum of muscular cells (*RYR1*) is one of the most frequently studied genetic markers in pigs. It conditions the syndrome of malignant hyperthermia (MHS) that is a part of reduced adaptability designated as porcine stress syndrome (PSS). The mutation of that gene was identified by Fujii *et al.* (1991). *RYR1* gene in pigs occurs in the form of two different alleles: "normal", unmutated, dominant one designated *C* and "mutated" one designated *T*. Generally better reproduction traits in sows resistant to stress were reported by Reiner *et al.* (1993) in German White and German Landrace breeds. Similar conclusions were drawn by Dvořák (1994), who evaluated the reproduction performance of sows of Landrace breed.

The objective of the paper was to estimate genotype and allele frequencies of *RYR1* and *ESR* genes in Large White sows and to study associations with reproduction traits of sows.

MATERIAL AND METHODS

Sows of the Large White breed came from two elite herds. Reproduction performance of the sows of both herds was very good.

The PCR-RFLP method was used to determine the genotypes of estrogen receptor gene (*ESR*) from blood samples collected to EDTA. Investigations were aimed at *ESR2* polymorphism with alleles *C* and *D* published by Rothschild *et al.* (1991). The method of *ESR2* genotype detection was based on Short's *et al.* (1997) paper while small modifications were made. Detection of genotypes of *RYR1* gene was carried out according to Brenig and Brem (1992).

Several model equations with fixed effects were used; they were divided into three groups according to the parity included in the analysis (parity 1, parity 1–6, parity 2–6), parity as a factor not being included in the equations for parity 1.

$$y_{ijklm} = \mu + RYR1_i + ESR_j + \text{parity}_k + \text{year}_l + e_{ijklm}$$

where: y_{ijklm} = observation
μ = population mean
$RYR1_i$ = i-th *RYR1* genotype ($i = 1, 2, 3$)
ESR_j = j-th *ESR* genotype ($j = 1, 2, 3$)
parity_k = k-th parity ($k = 1, 2, 3, 4, 5, 6$)
year_l = l-th year of sow birth ($l = 1, 2, 3, 4, 5, 6, 7, 8$)
e_{ijklm} = residual effects

RESULTS AND DISCUSSION

Table 1 shows the distribution of genotypes and alleles of *RYR1* and *ESR* genes in sows from herds A and B. The frequencies of *T* allele (*RYR1*) were 0.025 in herd A and 0.045 in herd B. The frequency of allele *C* at *ESR* was similar in both herds (0.653 in herd A and 0.726 in herd B).

The average values of reproduction traits according to the groups of *RYR1* genotypes are shown in Table 2 for herd A and in Table 3 for herd B. In the groups of parity 1 and parity 1–6 of sows from herd A significant differences ($P \leq 0.01$) between the groups of *RYR1* genotypes were determined in the number of all born piglets. The reproduction performance of heterozygous sows was better in all parameters under study. On the contrary, in herd B dominantly homozygous sows *CC* had insignificantly better reproduction traits.

Tables 4 and 5 show the effect of the groups of genotypes of *ESR* gene on the reproduction performance of sows in herd A and B, respectively. In herd A significant differences ($P \leq 0.05$, $P \leq 0.01$) were determined between the genotypes in the groups of parity 1–6 and 2–6 in the number of all born, alive born and weaned piglets, respectively. Sows with *DD* genotypes achieved the best performance. A comparable level of reproduction for the particular groups of genotypes was recorded in the group of parity 1. In herd B there were significant differences ($P \leq 0.05$) between the genotypes in the group of parity 2–6 and at the significance level ($P \leq 0.07$) in the group of parity 1–6, in favor of sows with *CC* genotypes. No statistically significant differences were determined in the group of parity 1.

Significant correlations ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$) were determined between litter size and parity and year of sow birth, respectively. In hybrid combinations of Western breeds with the Chinese breed Meishan allele *D* in *ESR* locus is associated with additive effect of 1.15 piglet in parity 1 and 0.5 piglet in parity 2 and subsequent parities (Rothschild *et al.*, 1995). Short *et al.* (1997) expressed the effect of this allele in LW breed by the value 0.4 piglet in parity 1 and 0.3 piglet in subsequent parities. The effect of allele *D* in the herds in our study was rather disputable. The results of our statistical evaluation indicated significant differences between the groups of *ESR* genotypes in litter size for both herds while the effect of particular alleles of *ESR* was opposite. It was apparently caused by the effect of one of the external environmental

Table 1. Frequencies of *RYR1* and *ESR* genotypes (%) and alleles in two herds of sows of Large White breed

Herd	*n*	*RYR1*					*n*	*ESR*				
		CC	*CT*	*TT*	*C*	*T*		*CC*	*CD*	*DD*	*C*	*D*
A	178	94.94	5.06	–	0.975	0.025	137	39.42	51.82	8.76	0.653	0.346
B	144	90.97	9.03	–	0.954	0.045	82	53.66	37.80	8.54	0.726	0.274

Table 2. Effect of *RYR1* genotypes on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets (LSM ± SE) in herd A

RYR1 genotype	Parity 1 (*n* = 124)			Parity 1–6 (*n* = 575)			Parity 2–6 (*n* = 450)		
	TNB	NBA	NW	TNB	NBA	NW	TNB	NBA	NW
CC	10.84 ± 0.33^{A}	10.62 ± 0.32	10.11 ± 0.23	11.81 ± 0.16^{A}	11.40 ± 0.15	10.67 ± 0.11	12.00 ± 0.18	11.57 ± 0.16	10.78 ± 0.13
CT	12.72 ± 0.70^{A}	11.86 ± 0.68	10.69 ± 0.49	12.72 ± 0.34^{A}	11.85 ± 0.31	10.96 ± 0.24	12.72 ± 0.39	11.86 ± 0.36	11.01 ± 0.27

The values in columns marked with index: $^{A}P \leq 0.01$ are statistically different
n = number of litters

Table 3. Effect of *RYR1* genotypes on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets (LSM ± SE) in herd B

RYR1 genotype	Parity 1 (*n* = 74)			Parity 1–6 (*n* = 351)			Parity 2–6 (*n* = 276)		
	TNB	NBA	NW	TNB	NBA	NW	TNB	NBA	NW
CC	12.47 ± 0.52	11.75 ± 0.50	11.22 ± 0.38	12.94 ± 0.25	12.30 ± 0.22	11.54 ± 0.17	12.65 ± 0.36	12.15 ± 0.32	11.47 ± 0.24
CT	12.41 ± 0.89	12.03 ± 0.87	11.00 ± 0.65	12.71 ± 0.43	12.06 ± 0.38	11.12 ± 0.30	12.34 ± 0.54	11.73 ± 0.48	10.94 ± 0.37

n = number of litters

Table 4. Effect of *ESR* genotypes on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets (LSM ± SE) in herd A

ESR genotype	Parity 1 (n = 124)			Parity 1–6 (n = 575)			Parity 2–6 (n = 450)		
	TNB	NBA	NW	TNB	NBA	NW	TNB	NBA	NW
CC	11.77 ± 0.48	11.06 ± 0.46	10.44 ± 0.33	11.88 ± 0.23^{ab}	11.23 ± 0.21^{ab}	10.57 ± 0.16^{a}	11.87 ± 0.27^{Aa}	11.26 ± 0.24^{Aa}	10.58 ± 0.19^{ab}
CD	11.60 ± 0.42	11.14 ± 0.41	10.44 ± 0.29	12.30 ± 0.20^{a}	11.66 ± 0.19^{a}	10.80 ± 0.14	12.46 ± 0.23^{A}	11.78 ± 0.21^{A}	10.87 ± 0.16^{a}
DD	11.98± 0.71	11.51 ± 0.69	10.33 ± 0.49	12.61 ± 0.36^{b}	11.98 ± 0.33^{b}	11.06 ± 0.25^{a}	12.75 ± 0.41^{a}	12.10 ± 0.37^{a}	11.24 ± 0.29^{b}

The values in columns marked with index: $^{a,b}P \leq 0.05$, $^{A}P \leq 0.01$ are statistically different

n = number of litters

Table 5. Effect of *ESR* genotypes on total number of born (TNB), number of born alive (NBA) and number of weaned (NW) piglets (LSM ± SE) in herd B

ESR genotype	Parity 1 (n = 74)			Parity 1–6 (n = 351)			Parity 2–6 (n = 276)		
	TNB	NBA	NW	TNB	NBA	NW	TNB	NBA	NW
CC	12.23 ± 0.56	11.73 ± 0.54	10.83 ± 0.41	13.26 ± 0.27	12.55 ± 0.24	11.58 ± 0.19	13.08 ± 0.39^{ab}	12.45 ± 0.34^{a}	11.58 ± 0.26^{a}
CD	12.53 ± 0.62	12.08 ± 0.61	11.19 ± 0.46	12.83 ± 0.30	12.24 ± 0.27	11.38 ± 0.21	12.45 ± 0.43^{a}	11.95 ± 0.37	11.22 ± 0.29
DD	12.54 ± 1.17	11.88 ± 1.15	11.31 ± 0.86	12.39 ± 0.51	11.75 ± 0.45	11.02 ± 0.34	11.94 ± 0.61^{b}	11.42 ± 0.54^{a}	10.77 ± 0.41^{a}

The values in columns marked with index: $^{a,b}P \leq 0.05$ are statistically different

n = number of litters

factors as it was documented by different performance in both herds.

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Received: 02–06–10
Accepted after corrections: 03–03–26

ABSTRAKT

Vliv genotypů *RYR1* a *ESR* na plodnost prasnic plemene bílé ušlechtilé ve vybraných šlechtitelských chovech

Byl sledován vliv genotypů *RYR1* a *ESR* na velikost vrhu prasnic plemene bílé ušlechtilé ve dvou šlechtitelských chovech. Detekce genotypů byla provedena metodou PCR-RFLP. V chovu A byly nalezeny průkazné rozdíly ($P \leq 0.01$) ve skupinách 1. vrhů a 1.–6. vrhů mezi skupinami genotypů *RYR1* v počtu všech narozených selat ve prospěch prasnic s genotypy *CT*. V chovu B měly v tomto parametru neprůkazně lepší užitkovost prasnice *CC*. V chovu A byly nalezeny průkazné rozdíly ($P \leq 0.05$, $P \leq 0.01$) mezi genotypy *ESR* ve skupinách 1.–6. a 2.–6. vrhů pro počet všech narozených, živě narozených i odstavených selat. Nejlepší užitkovosti dosahovaly prasnice s *DD* genotypy. V chovu B byly zjištěny průkazné rozdíly ($P \leq 0.05$) mezi genotypy ve skupině 2.–6. vrhů a ve skupině 1.–6. vrhů na hranici průkaznosti ($P \leq 0.07$) pro počet všech narozených, živě narozených i odstavených selat, ovšem naopak ve prospěch prasnic s genotypy *CC*. Dále byly zjištěny průkazné ($P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$) vztahy mezi velikostí vrhu a pořadím vrhu a rokem narození prasnice.

Klíčová slova: prase; velikost vrhu; ryanodinový receptor – *RYR1*; estrogenový receptor – *ESR*

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Heavy metal residues in tissues of wild boar (*Sus scrofa*) and red fox (*Vulpes vulpes*) in the Central Zemplin region of the Slovak Republic

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ABSTRACT: Samples of liver, kidneys and muscles of wild boars and red foxes were collected from an emission-contaminated area of the Central Zemplín region in 1998/1999 to evaluate the concentrations of heavy metals (Hg, Pb, Cd, Cr) and As. Percentual proportions of above-limit samples from the tissues of wild boars were as follows: Hg – liver 53.3%, kidneys 73.3%, muscles 13.3%; Cd – liver 20%, kidneys 26.6%, muscles 13.3%; Cr – muscles 6.6%; As – muscles 6.6%. The percentage of above-limit samples from red foxes were as follows: Hg – liver 44.4%, kidneys 66.6%, and muscles 5.5%; Cr – liver 22.2%, kidneys 27.3% and muscles 33.3%; As – muscles 22.2%. The presented results document that the occurrence of heavy metals in the tissues of wild animals is an urgent problem.

Keywords: heavy metals; wild boar; red fox; parenchymatous organs; muscles

Besides the compounds of cadmium and lead, mercury and arsenic compounds are among the most important and most frequent contaminants of the environment. The level of contamination of the environment with heavy metals and arsenic is a serious factor influencing its quality, and is of considerable health concern. Increased concentrations of these pollutants in individual components of the ecosystem (water, soil, plants, etc.) pose a risk of damage to physiological functions in living organisms. The chronic detrimental effect of heavy metals and arsenic on animals is demonstrated latently without clinical symptoms (immunopathological changes, teratogenicity, carcinogenicity, etc).

As most wild animals are components of the food chain, there is a need to trace heavy metal concentrations in the tissues of animals not only with respect to immunopathological changes and intoxications but also from the viewpoint of potential health hazard to humans. Metal toxicity depends on their chemical composition, animal species, dose, and time of exposure of animal organisms to heavy metals (Underwood, 1977; Bencko *et al.*, 1984; Páv and Márová, 1988).

Since their launching the operations of three key factories (Chemko Strážske, Bukóza Vranov nad Topľou and Vojany electric power plant) in the Central Zemplín region in Slovakia have generated the emissions of toxic substances to the air that resulted in a subsequent air-pollution load of this territory.

The aim of this study was to evaluate the concentrations of residues of selected metals in samples from parenchymatous organs and muscles of wild boars and red foxes in the area with air-pollution stress.

MATERIAL AND METHODS

In the period 1998–1999 samples of liver, kidneys and muscles of wild boars and red foxes were collected during hunts in the northern part of the Pozdišovský Ridge in the Central Zemplín region with air-pollution stress. Concentrations of Hg, Pb, Cd, Cr and As were determined in the tissues collected from 15 wild boars and 18 red foxes. Tissue samples obtained from 5 wild boars in a

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This study was supported by Grant Agency of the Slovak Republic – VEGA (Grant No. 2/1153/21).

more distant area in the Slovenský raj Mts. served as controls.

Samples from kidney (100 g), liver and *musculus semimebranosus* (both 200 g) of wild boars were collected from each animal directly at the shooting site. Complete liver and kidneys as well as 100 g of muscles (*m. semimebranosus*) of red foxes were taken. None of the samples was contaminated with bullets.

Mercury contents were quantified in animal tissues using a TMA 254 atomic absorption spectrometer (Tesla Holešovice). Samples for evaluation of other elements (Pb, Cd, Cr, As) were analysed by AAS method in a closed system using the spectrometer Varian 20ADQ after preceding mechanical homogenisation and following mineralisation. Concentrations of the above elements were expressed in mg/kg of freshly analysed tissue. The obtained data were compared with hygienic limits laid down in the Appendix Contaminants in Food to the Food Code of the Slovak Republic (1996).

The results were statistically evaluated by Student's *t*-test. The differences between wild boars and red foxes were considered significant at the levels of $P < 0.05$ and $P < 0.01$.

RESULTS

In the majority of cases the tissues of red foxes and wild boars were loaded by high concentrations of heavy metal residues throughout the two monitored hunting seasons in 1998–1999 (Table 1). Most samples with above-limit Hg concentrations from wild boar tissues came from kidneys (11 samples – 73.3%) and the maximum Hg concentration in this tissue reached a 16 times higher value (1.6 mg/kg) than the hygienic limit. A lower number of above-limit samples was obtained from liver (8 samples – 53.3%) with the maximum value of Hg concentration 0.48 mg/kg. Two muscle samples exceeded the hygienic limit of Hg concentration.

Similar results concerning Hg concentrations were found in red fox tissues. The highest percentual proportion of above-limit samples was observed in kidneys (12 samples – 66.6%) with the maximum Hg concentration of 1.43 mg/kg. Hg concentrations of 8 samples from liver (44.4%) and 1 sample from muscles reached higher values than the hygienic limit.

The values of Pb concentrations did not exceed the hygienic limits in any tissue from both species of the animals.

Samples with above-limit Cd concentrations came only from wild boar tissues. 26.6% of kidney samples (with maximum 2.68 mg/kg), 20% of liver samples (with maximum 0.94 mg/kg) and 13.3% of muscle samples (with maximum 0.17 mg/kg) were higher than hygienic limits.

On the other hand, samples with above-limit Cr concentrations came particularly from red fox tissues. 27.3% of kidney samples (with maximum 0.52 mg/kg), 22.2% of liver samples (with maximum 0.58 mg/kg) and 33.3% of muscle samples (with maximum 0.62 mg/kg) were higher than hygienic limits. In one sample from the muscles of wild boar the above-limit Cr concentration with maximum of 1.8 mg/kg was determined.

The hygienic limit of As concentration was exceeded in one sample from wild boar muscles (with maximum 0.31 mg/kg) and in 4 samples from red fox muscles (with maximum 0.9 mg/kg).

Five wild boars were examined in the control locality – the Slovenský raj Mts. The samples of wild boars from the control locality were not examined for the presence of Cr residues. The maximum permissible values in the control locality were exceeded for Cd in two samples of muscles (0.3 and 0.15 mg/kg).

DISCUSSION

Anthropogenic activities, mainly industry, agriculture and transport are sources of the occurrence of heavy metals in the environment. Hronec (1996) did not confirm the presence of Hg by chemical analyses of emissions from the factories Bukóza Vranov nad Topľou and Chemko Strážske, but he found 27% of above-limit samples in Cr, 6% in As, 2% in Pb and 0.3% in Cd. These factories in the studied locality were defined as sources generating gaseous and solid emissions into the air. Besides these main emission sources in the northern part of the Central Zemplín region, more distant sources (e. g. Vojany electric power plant) also probably pollute markedly the investigated area. According to Lodenius and Tulisalo (1984), emissions from some types of factories can contaminate their more distant surroundings up to 20–100 kilometres.

The animals examined in our study (wild boar, red fox) are positioned on the top of the local food

Table 1. The occurrence of metal residues in the tissues of wild boars and red foxes in a region with air-pollution load (mg/kg)

		Wild boars			Red foxes		
		liver	kidneys	muscles	liver	kidneys	muscles
Hg	$\bar{x}$	0.24	0.52	0.018	0.22	0.63	0.013
	± SD	0.2	0.42	0.021	0.23	0.51	0.019
	min	0.01	0.05	0.008	0.03	0.06	0.006
	max	0.48	1.6	0.07	0.62	1.43	0.09
	number of above-limit samples	8	11	2	8	12	1
	% of above-limit samples	53.3	73.3	13.3	44.4	66.6	5.5
	Hygienic limit	0.1	0.1	0.03	0.1	0.1	0.03
Pb	$\bar{x}$	0.24**	0.39	0.17	0.35**	0.38	0.14
	± SD	0.1	0.21	0.17	0.12	0.17	0.13
	min	0.06	0.14	0.04	0.26	0.26	0.02
	max	0.43	0.85	0.4	0.65	0.84	0.4
	Number of above-limit samples	0	0	0	0	0	0
	% of above-limit samples	0	0	0	0	0	0
	Hygienic limit	1	1	0.4	1	1	0.4
Cd	$\bar{x}$	0.28	0.56	0.04	0.21	0.25	0.03
	± SD	0.23	0.69	0.04	0.12	0.08	0.02
	min	0.12	0.14	0.02	0.12	0.2	0.02
	max	0.94	2.68	0.17	0.38	0.48	0.07
	Number of above-limit samples	3	4	2	0	0	0
	% of above-limit samples	20	26.6	13.3	0	0	0
	Hygienic limit	0.5	1	0.1	0.5	1	0.1
Cr	$\bar{x}$	0.15*	0.19*	0.29	0.26*	0.29*	0.33
	± SD	0.12	0.11	0.42	0.19	0.16	0.2
	min	0.02	0.09	0.1	0.12	0.14	0.18
	max	0.49	0.48	1.8	0.58	0.52	0.62
	Number of above-limit samples	0	0	1	4	5	6
	% of above-limit samples	0	0	6.6	22.2	27.3	33.3
	Hygienic limit	0.5	0.5	0.5	0.5	0.5	0.5
As	$\bar{x}$	0.21	0.17	0.07	0.38	0.3	0.17
	± SD	0.36	0.22	0.1	0.38	0.28	0.2
	min	0.01	0.01	0.01	0.08	0.1	0.09
	max	0.9	0.82	0.31	0.9	0.8	0.9
	Number of above-limit samples	0	0	1	0	0	4
	% of above-limit samples	0	0	6.6	0	0	22.2
	Hygienic limit	1	1	0.2	1	1	0.2
	Number of examined samples	15	15	15	18	18	18

$^{*}P < 0.05$; $^{**}P < 0.01$

pyramid, their feed consists of voles, hares, ducks, seeds, forest fruits, etc. Gastrointestinal and respiratory systems are considered the most frequent and the most important entrance gateways of noxious substances into the living organism. Metal substances reach their predilected tissues where they can accumulate and consequently might influence health and fitness of animals (Páv and Márová, 1988; Keogh and Siegers, 1996; Nieminen and Lemasters, 1996; Bukovjan, 1997).

In our study the values of Hg and Cd concentrations in liver (53.3% and 20% of samples, respectively), kidneys (73.3% and 26.6% of samples, respectively) and muscles (both 13.3% of samples) from wild boars were higher than hygienic limits. The hygienic limits of Cr and As concentrations were exceeded in one sample of wild boar muscles.

On the contrary, in comparison with the situation in the Czech Republic the percentage of above-limit muscle samples of wild boars is lower in our observations. According to the SVA of the CR (2002), the hygienic limit of Hg concentration in wild boar muscles was exceeded in almost all samples (97.8%). The percentual proportion of above-limit values of As and Cd concentrations was also higher than in our study (53.3% and 46.7%, respectively). Up to 40% of samples contained above-limit Pb concentrations whereas in our investigations no muscle sample exceeding the hygienic limit of Pb concentration was detected.

In our study the reference values of Cd were exceeded in the muscles of wild boars in the control locality of the Slovenský raj Mts. The maximum Cd concentration in the muscles also exceeded the maximum concentration of this metal recorded in muscles of wild boars from the monitored polluted area. On the contrary, red fox tissues contained above-limit concentrations of Hg and Cr (liver – 44.4% and 22.2%, respectively; kidneys – 66.6% and 27.3%, respectively; muscles – 5.5% and 22.2%, respectively). The above-limit values of As concentration were observed in 22.2% of red foxes muscles.

The presented results document that heavy metal emissions are an urgent problem because most species of wild animals are components of the food chain of humans. Even though in the monitored area the heavy metal concentrations showed a decrease to a certain extent over the last years, probably as a result of restricted use of chemicals in agriculture and limitation of emission sources. Regular monitoring of heavy metal concentrations in the tissues of wild and domestic animals from air-pollution areas is therefore highly needed in the nearest future. Furthermore, in the muscles of control animals from the Slovenský raj National Park (lying outside the area concerned), concentrations of Cd exceeding the maximum permissible values were also found out. Considering this fact, with respect to the transmission of emissions to long distances pollution surveys should not be focused solely on the territory with air-pollution load.

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Received: 02–11–13
Accepted after corrections: 03–03–27

ABSTRAKT

Reziduá ťažkých kovov v tkanivách diviakov (*Sus scrofa*) a líšok hrdzavých (*Vulpes vulpes*) v Strednozemplínskom regióne Slovenskej republiky

V imisne zaťaženej oblasti Strednozemplínskeho regiónu boli v rozmedzí rokov 1998/1999 odoberané vzorky pečene, obličiek a svaloviny svine divej a líšky hrdzavej na stanovenie obsahu ťažkých kovov (Hg, Pb, Cd, Cr) a As v uvedených orgánoch. Percentuálne zastúpenie nadlimitných vzoriek tkanív svine divej bolo zaznamenané nasledovne: Hg – pečeň 53,3 %, obličky 73,3 %, svalovina 13,3 %; Cd – pečeň 20 %, obličky 26,6 %, svalovina 13,3 %; Cr – svalovina 6,6 %; As – svalovina 6,6 %. Percento nadlimitných vzoriek tkanív líšky hrdzavej bolo zachytené nasledovne: Hg – pečeň 44,4 %, obličky 66,6 % a svalovina 5,5 %; Cr – pečeň 22,2 %, obličky 27,3 % a svalovina 33,3 %; As – svalovina 22,2 %. Prezentované výsledky poukazujú na skutočnosť, že problém imisií ťažkých kovov u voľne žijúcej zveri je vysoko aktuálny.

Kľúčové slová: ťažké kovy; sviňa divá; líška hrdzavá; pečeň; obličky; svalovina

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