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EFFECTS OF DIVERGENT SELECTION FOR HIND LEG MUSCLE VOLUME ON ITS LIPID PEROXIDE AND GLUTATHIONE REDOX STATUS, AND FATTY ACID COMPOSITION IN GROWING RABBITS

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Abstract: Pannon White bucks were selected divergently using CT method by the volume of the hind leg muscle. Animals showed the highest and lowest muscle volumes were selected as minus and plus-selected variants. The male progenies of the minus and plus-selected parents were slaughtered as first generation which was selected again by CT method and the male progenies of the parents were slaughtered. Results in the first and second generation suggest that selection, as a genetic effect did not affect the rate of lipid peroxidation, as was measured by malondialdehyde content and glutathione redox status, as was measured by the reduced glutathione content and glutathione peroxidase activity of the hind leg muscle. However, there were some differences in the fatty acid composition. Significant ($P<0.05$) difference was found in palmitoleic acid content which was higher in the minus as compared to the plus variants in the second generation, in eicosadienoic acid which was higher in the first as compared to the second generation of minus variants, and total monounsaturated fatty acids which was higher in the minus as compared to the plus variants in the second generation. It means that selection for higher hind leg volume would not causes marked in changes in the rabbit meat quality as measured by lipid peroxide and glutathione status as well as fatty acid composition.

Key Words: malondialdehyde, glutathione, glutathione peroxidase, fatty acids, rabbit meat.

INTRODUCTION

Dressing out percentage is one of the most important traits in rabbit breeding. The heritability of rabbit carcass traits is moderately high (Lukefahr *et al*., 1996, Nagy *et al*., 2006). Therefore selection for hind leg muscle volume using a non-invasive (e.g. computer tomography, CT) method may lead to genetic improvements in dressing out percentage (Szendrő *et al*., 2008, Gyovai *et al*., 2008). A genetic variation in glutathione peroxidase (GSHPx) activity has previously been suspected in rabbits (Mézes *et al*., 1994), and for the correlation of GSHPx activity and production traits, such as body weight and weight gain (Mézes *et al.,* 1994). Glutathione peroxidase activity has been proposed as a possible selection criterion

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in rabbit breeding, as slightly negative phenotypic correlation has been found between dressing out percentage and the GSHPx activity of red blood cell haemolysate in Pannon white rabbits (Virág *et al*., 1996).

A higher rate of lipid peroxidation and higher malondialdehyde content was found in the thigh muscle homogenate in plus variant progenies using CT-based selection for greater hind leg muscle volume (Mézes *et al*., 2006). It was also found that selection for higher growth rate (Ramírez *et al*., 2005, Hernandez *et al*., 2008) or for litter size at weaning (Hernandez *et al*., 2008) affects the fatty acid composition of leg meat and the perirenal fat of rabbits.

The purpose of the present study was to investigate the effects of CT-based divergent selection for hind leg volume in first and second generation Pannon White rabbit progenies on the possible genetic differences in the lipid peroxide and glutathione redox status, and fatty acid composition of hind leg meat.

MATERIALS AND METHODS

Experimental animals

Using CT, Pannon White bucks were selected divergently for hind leg muscle volume (Szendrő *et al*., 2008). In the first and second stages of selection, animals (of both sexes) with the highest and lowest muscle volume were selected as minus (M) and plus-selected (P) variants. The progenies of the minus (M) and plus-selected (P) parents (M×M and P×P) were randomly slaughtered as first generation specimens. The first generation was selected again using CT and the progenies of the parents (MM×MM and PP×PP) were randomly slaughtered. Experimental slaughtering of the progenies (bucks only) was carried out at 70 d of age after 24 h of fasting (n=15 in each group). Hind leg samples were taken immediately after dissection, following the procedure described by Blasco and Ouhayoun (1996). The whole (deboned) right hind leg was ground up using a mincing machine (Retsch, Grindomix, Haan, Germany) and samples of leg muscle were stored at −70°C until they were analysed.

Biochemical methods

The lipid peroxidation rate was evaluated by measuring malondialdehyde (MDA) in muscle 1:9 homogenates (homogenised in physiological saline: 0.9 % w/v NaCl) using the method described by Mihara *et al*. (1980). The reduced glutathione (GSH) content of the 10,000 g supernatant fraction of leg muscle homogenate was determined using the method described by Sedlak and Lindsay (1968), while the activity of glutathione peroxidase (GSHPx) was measured using the method described by Lawrence and Burk (1978). Enzyme activity was expressed in units (U=1 nmol). GSH oxidation per min at 25 °C was calculated for the protein concentration determined in the tissue homogenate of the 10,000 g supernatant fraction using Folin-Ciocalteu phenol reagents (Lowry *et al*., 1951).

The total lipids were extracted from the tissue samples using the method proposed by Folch *et al*. (1957), and saponified and methylated using boron trifluoride as the methylating agent (Morrison and Smith, 1964). The fatty acid methyl ester composition was analysed using a gas chromatography system (Shimadzu 2010, Kyoto, Japan) equipped with an SP-2380 type fused silica capillary column (30 m×0.25 mm i.d., 0.25 μm film, Supelco Inc., Bellefonte, USA). Helium was used as the carrier gas. The split ratio was 50:1. The injection port temperature was 270ºC and the detector temperature was 300ºC. The oven temperature was set at 80ºC for 5 min. The temperature was subsequently increased by 2.5ºC/min to 205ºC and maintained for 5 min, and then increased by 10ºC/min to 250ºC and maintained for a further 5 min. The fatty acids were identified by comparing retention times with recognized external standards (Qualimix FA: Cat.No. 4-7057; Supelco Inc., Bellefonte, USA) and presented as weight percentages of determineded fatty acid methyl esters.

Table 1: Changes in the lipid peroxide and glutathione redox state of hind leg meat as an effect of selection (mean±SD).

	GSH	GSHPx	MDA
P×P	1.30±0.11	1.65±0.15	6.03±1.54
PP×PP	1.31±0.20	1.68±0.27	6.07±2.66
M×M	1.33±0.28	1.81±0.42	5.72±1.61
MM×MM	1.36±0.28	1.79±0.29	5.49±1.25

GSH: reduced glutathione (mmol/g muscle), GSHPx: glutathione peroxidase (U/g 10,000 g supernatant fraction protein content of muscle homogenate), MDA: malon-dialdehyde (mmol/g muscle), P×P: progenies of plus variant parents, PP×PP: progenies of plus variant grand-parents and parents, M×M: progenies of minus variant parents, MM×MM: progenies of minus variant grandparents and parents.

Statistical analysis

Statistical evaluation of the data was performed using the paired LSD test (Statsoft Inc., 1993).

RESULTS AND DISCUSSION

The malondialdehyde content of the hind leg meat homogenate did not show significant differences between the plus and minus variants of the first and second generation (Table 1), contrary to our earlier findings in the first generation (Balogh *et al*., 2007). This suggests that current nutrient supply and specific environmental factors may also affect the lipid peroxidation rate in muscles, as well as genetic factors (Gray *et al*., 1996, Lopez-Bote *et al*., 1998). The biological antioxidant defence system, particularly the parameters measured for the glutathione redox system, reduced glutathione content and glutathione peroxidase activity, were also unaffected by the selection process (Table 1). This means that selection did not seem to affect the synthesis and/or oxidation of reduced glutathione and, furthermore, had no effect on glutathione peroxidase activity. These results are also different from our previous results (Mézes *et al*., 2006) in the first divergent selection generation, where significant differences were found in the glutathione peroxidase activity of hind leg meat homogenates. This difference supports our previous proposal that, as well as the lipid peroxidation rate, the amount and/or activity of the glutathione redox system varied, depending on nutritional factors, such as methionine and/or cysteine supply for glutathione biosynthesis (Wang *et al*., 1997) and selenium supply for glutathione-peroxidase (Toyoda *et al*., 1989) biosynthesis, and environmental factors. The fatty acid composition of hind leg meat depends on the fatty acid composition of the diet (Maertens *et al*., 2008) but, according to the results of the present study, it differed significantly only for a number of individual fatty acids (Table 2) in the divergent selected lines which were investigated. In the second CT-based divergent selection generation the percentage of palmitoleic acid (C16:1 n-7) was significantly lower in the plus variant progenies compared to the minus variants. This significantly lower percentage of the above mentioned monounsaturated fatty acid in the plus variant progenies also has a significant effect on the proportion of monounsaturated fatty acids. There were no significant differences for the individual polyunsaturated fatty acids in either the first or the second generation. These results are similar to those of Ramírez *et al*. (2005), who describes similar changes in the fatty acid composition of rabbit hind leg meat after selection for higher growth rate. Also the total SFA and total PUFA percentages showed no differences between the plus and minus variant groups in both generations. The n-6/n-3 fatty acid ratio was relatively high, due to the high proportion of linoleic acid (C18:2n-6c), and our results were higher than the results (5.39, 11.6, 11.47 and 10.67) published by Maertens *et al*. (2008), Dalle Zotte (2002) and Ramírez *et al*. (2005). However, the n-6/n-3 ratio

Table 2: Means and standard deviation of fatty acid content in hind leg meat (g/100 g detemined fatty acid) depending on selection.

	M×M	P×P	MM×MM	PP×PP
C10:0 (capric)	0.04±0.02	0.05±0.01	0.04±0.01	0.05±0.02
C12:0 (lauric)	0.06±0.03	0.08±0.05	0.07±0.02	0.09±0.05
C14:0 (myristic)	1.69±0.54	1.80±0.64	1.43±0.36	1.70±0.84
C14:1n-5cis (myristoleic)	0.18±0.06	0.19±0.07	0.16±0.05	0.14±0.03
C15:0 (pentadecanoic)	0.49±0.12	0.57±0.12	0.47±0.07	0.58±0.19
C16:0 (palmitic)	27.46±6.63	27.01±3.88	24.79±4.79	25.18±4.87
C16:1n-7cis (palmitoleic)	2.25±0.69^{b}	2.06±0.84^{ab}	2.24±0.78^{b}	1.07±0.26^{a}
C17:0 (heptadecanoic)	0.72±0.13	0.79±0.12	0.73±0.08	0.79±0.20
C18:0 (stearic)	9.00±1.14	9.01±0.48	8.39±0.71	8.80±1.22
C18:1n-9cis (oleic)	25.38±3.35^{b}	24.38±2.96^{ab}	25.28±2.95^{ab}	20.97±1.68^{a}
C18:2n-6cis (linoleic)	25.90±9.01	27.32±4.30	29.71±6.81	33.62±3.09
C18:3n-6cis (γ-linolenic)	0.09±0.02	0.06±0.03	0.09±0.03	0.10±0.03
C18:3n-3cis (α-linolenic)	1.47±0.70	1.48±0.38	1.79±0.73	2.33±0.62
C20:0 (arachidic)	0.15±0.02	0.17±0.11	0.17±0.01	0.15±0.02
C20:1n-9cis (eicosenoic)	0.39±0.07	0.30±0.09	0.27±0.12	0.25±0.12
C20:2n-6cis (eicosadienoic)	0.48±0.21^{b}	0.47±0.12^{ab}	0.23±0.16^{a}	0.46±0.15^{ab}
C20:3n-3cis (eicosatrienoic)	0.51±0.31	0.45±0.27	0.39±0.21	0.44±0.34
C20:3n-6cis (eicosatrienoic)	0.11±0.04	0.07±0.01	n.d.	0.07±0.01
C20:4n-6cis (arachidonic)	3.70±2.15	3.82±2.91	3.88±1.70	3.32±2.42
Total SFA	39.60±8.50	39.45±4.81	35.95±5.62	37.29±5.80
Total MUFA	28.20±3.82^{b}	26.93±3.59^{ab}	27.95±3.61^{b}	22.43±1.74^{a}
Total PUFA n-3	1.98±0.97	1.93±0.45	2.78±0.75	2.77±0.55
Total PUFA n-6	30.28±11.26	31.69±6.98	33.91±7.93	37.52±5.15
Total PUFA	32.20±12.22	33.62±7.37	36.10±8.59	40.28±5.43
SFA/PUFA	1.66±1.49	1.24±0.42	1.08±0.45	0.95±0.25
n-6/n-3	17.11±5.02	16.57±1.90	15.93±2.66	13.90±3.02

P×P: progenies of plus variant parents, PP×PP: progenies of plus variant grandparents and parents, M×M: progenies of minus variant parents, MM×MM: progenies of minus variant grandparents and parents, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n.d.: not detected

^{a,b} Different superscript letters in the same row means significant difference at $P<0.05$.

decreased slightly, but not significantly, as an effect of selection, although there were no differences between the minus and plus variants in the two generations.

In conclusion, divergent selection for hind leg muscle volume did not affect the lipid peroxide and glutathione redox system of leg meat, but caused some changes in its fatty acid composition. These changes were significant in the case of palmitoleic acid only. This could indicate that selection for higher hind leg volume seems not affect rabbit meat quality.

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