

EFFECT OF GENOTYPE ON ESTIMATED INDEXES OF FATTY ACID METABOLISM IN RABBITS

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Abstract: To analyse the effect of rabbit genotype on fatty acid metabolism, 60 weaned rabbits (30 d old, sex ratio 1:1; 20 New Zealand White, 20 Leprino of Viterbo and 20 rabbits of a Local grey population) were reared in bicellular standard cages. At 80 d, 10 rabbits per group were selected and slaughtered. The muscle fatty acid composition and relative indexes of metabolism of the *longissimus lumborum* of these rabbits were determined. The New Zealand White and Leprino rabbits exhibited lower percentages of myristic acid and higher percentages of palmitic acid and palmitoleic acid, and the levels of monounsaturated fatty acids were significantly higher in these rabbits. The grey-coloured rabbits exhibited the highest values of total n-3 fatty acids. With respect to fatty acid metabolism, the New Zealand White and Leprino rabbits exhibited higher thioesterase and Δ^9 -desaturase indexes and lower elongase and Δ^5 - Δ^6 -desaturase indexes. The estimated Δ^5 - Δ^6 -desaturase activity was significantly higher in the Local grey rabbits, suggesting a genetic effect on the desaturase and elongase mechanisms responsible for the synthesis of long-chain n-3 fatty acids.

Key Words: rabbit, genotype, fatty acid, lipid metabolism.

INTRODUCTION

In recent years, the fatty acid composition of meat has been studied intensively in light of its implications for human health. Such research is also performed considering that meat represents the main source of long-chain n-3 polyunsaturated fatty acids (LCPn-3) for humans, because fish consumption, while extremely variable, is generally low in European countries (Welch *et al.*, 2002). Many factors are involved in the fatty acid composition of meat (see reviews by Wood and Enser, 1997 and Wood *et al.*, 2008), including both genetic and environmental factors. The animal species is the major source of variation in the fatty acid composition of meat; indeed, some studies have investigated differences in fatty acid composition among diverse breeds (Cameron, 1990; De Smet *et al.*, 2004). Nonetheless, there have been very few investigations of the ability of different genotypes to convert precursor linoleic (C18:2n-6, LA) and α -linolenic acids (C18:3n-3, ALA) into LCP. Fish and terrestrial species that evolved in conditions of LCPn-3 abundance have progressively lost their ability to convert ALA into docosahexaenoic acid (C22:6n-3, DHA). Recent findings (Costa Castro *et al.*, 2012) have shown that crucial gene families for LCP fatty acid synthesis [fatty acid desaturase (FADS) genes] over geological time scales have had gene duplications, losses and diversifications that were most likely linked to habitat-specific food chains in various environments.

As a result, fish and terrestrial animals have developed different abilities to convert ALA in LCPn-3, and the extent of conversion greatly depends on elongase and desaturase (Δ^5 - Δ^6) enzymes. In teleost fish, a main theory has been that species diverge according to the environment and trophic levels they occupy, with requirements for essential fatty acids being satisfied by fatty acid precursors in freshwater/diadromous species, whereas marine fish need LCPn-3. All fish exhibit Δ^6 -activity, which is required for the initial desaturation of LA and ALA, whereas Δ^5 -activity, which is necessary to desaturate C20:4n-3 to eicosapentaenoic acid (C20:5n-3, EPA), has only been detected in diadromous/freshwater fish. In terrestrial species, the main monogastric species (swine, poultry) exhibit a certain degree of conversion of ALA to

EPA, whereas the degree of DHA synthesis is generally very low (García, 2011). Dietary supplementation of ALA is not sufficient to maintain adequate tissue levels of LCPn-3 fatty acids (Barcelo-Coblijn and Murphy, 2009).

However, within a given species, this conversion is greatly affected by the feeding plan (the amounts of precursor and derivative, the n-3/n-6 ratio and the oxidative status), sex, hormonal status, intestinal biota and genetic strain (Alessandri *et al.*, 2011).

It should be noted that ALA and LA are both substrates for Δ^6 -desaturase; accordingly, an excess of dietary n-6 reduces the availability of enzyme for n-3 desaturation. Even the LCP level in the tissues modulates the activities of Δ^9 - and Δ^6 -desaturase; high amounts of LCP downregulate the conversion of the precursors to their longer and more unsaturated metabolites. LCP fatty acids not only modify the desaturase activities but are also regulators of the expression of other genes involved in lipogenesis and lipid oxidation (Aktas and Halperin, 2004).

Where the effect of feeding on such enzyme activity has been assessed, the ALA elongation/desaturation ability of different genetic lines has been poorly investigated, although in chickens, the genotype seems to play an important role in this synthesis (Dal Bosco *et al.*, 2012).

Some authors (e.g., Vessby *et al.*, 2002) have reported that the estimated activities could be used as surrogates of the measure of the true desaturase/elongase activity, which is influenced not only by the amount of and relationship between the precursors but also by the levels of the derivatives in the tissues. Thus, the activities of Δ^9 - and Δ^6 -desaturase are downregulated by LCP fatty acids, and hence the degree of conversion of essential fatty acids to their longer and more unsaturated metabolites is lower.

To the best of our knowledge, no information is available regarding the differences among rabbit genotypes in terms of the activities of the Δ^5 - and Δ^6 -desaturase complex in the synthesis of LCPn-3. Thus, the aim of the present study was to assess several aspects of the fatty acid metabolism of different rabbit genotypes.

MATERIALS AND METHODS

Animals and housing

Sixty weaned (30 d old, sex ratio 1:1) rabbits of 3 different genotypes characterised by different selection pressures were used in this trial, specifically: 20 New Zealand Whites, 20 Leprino of Viterbo and 20 Grey-coloured local rabbits.

The New Zealand White strain was selected by the ANCI (Associazione Nazionale Coniglicoltori Italiani) genetic centre; the Leprino of Viterbo has been developed through 30 yr of research at the Experimental Centre for Rabbit Alternative Farms of the University of Tuscia. It is now officially recognised in the Italian Standard and protected by the provincial registry office. Grey-coloured local rabbits have been reared for more than 30 yr in several small-sized farms in central Italy (Tuscany) and for over 10 yr at the Pisa University Rabbit Station (Paci *et al.*, 2005). This population has the following characteristics: high rusticity, high fertility, medium litter size, adult live weight 4000 ± 100 g (male) and 3400 ± 200 g (female), weaning weight 890 ± 155 g (35 d), slaughter weight 2500 ± 300 g, and dressing percentage of approximately 60% (Lambertini *et al.*, 2006; D'Agata *et al.*, 2009).

The rabbits were housed in bicellular cages (17 rabbits/m²) under standard fattening conditions. All animals were reared according to EU Regulation 834/07 and Italian directives (Gazzetta Ufficiale, 1992) on animal welfare for experimental and other scientific purposes. They were housed in the same naturally lighted room, with temperatures ranging from 15 to 25 °C and relative humidity of 60 to 70%.

Live weights were recorded individually, while the feed intake was registered collectively every week. The average feed consumption of the group was used to calculate individual feed:gain ratios. The mortality was registered and the causes were ascertained.

Feeding

The rabbits were fed *ad libitum* a standard diet. No medical treatment was performed. The formulation of the feed and its chemical composition are reported in Table 1.

Table 1: Ingredients and chemical composition (%) of feed.

Ingredients	
Alfalfa hay	30.0
Corn meal	10.0
Soybean meal 48%	18.0
Barley	25.0
Wheat bran	12.0
Ca-phosphate	1.3
Vitamin mineral premix ¹	1.2
Molasses	1.0
Salt	0.7
Ca-Carbonate	0.7
DL-methionine	0.05
Chemical composition	
Dry matter	89.0
Crude protein	17.3
Ether extract	2.0
Ash	7.2
Crude Fibre	12.8
Neutral detergent fibre	24.0
Acid detergent fibre	14.1
Acid detergent lignin	2.9
Hemicellulose	9.9
Digestible Energy ² (MJ/kg)	10.5
Fatty acids (% total fatty acids)	
C16:0	19.5
C18:0	3.1
C18:1n-9	16.9
C18:2n-6	41.5
C18:3n-3	14.7

¹ Added per kg: 11.000 U.I. vit. A; 2.000 U.I. vit. D₃; 2.5 mg vit. B₁; 4 mg vit. B₂; 1.25 mg vit. B₆; 0.01 mg vit. B₁₂; 25 mg vit. E; 0.06 mg biotine; 2.5 mg vit. K; 15 mg niacine; 0.30 mg folic ac.; 10 mg D-pantotenic ac.; 600 mg coline; 60 mg Mn; 3 mg Cu; 50 mg Fe; 15 mg Zn; 0.5 mg I; 0.5 mg Co; 50 mg lysine; 40 mg methionine.

² Estimated according to Maertens *et al.* (1984).

Slaughter traits and muscle sampling

At 85 d, 10 rabbits per group, with weights close to the average of the group ($\pm 10\%$), were selected and killed by cutting their carotid arteries and jugular veins after electrical stunning.

Handling and dissection of the refrigerated carcasses (24 h at 4 °C) were performed as proposed by Blasco and Ouhayoun (1996). The *longissimus lumborum* muscles (between the 1st and 7th lumbar vertebrae) were excised from the 2 sides, trimmed of all external fat and epimysial connective tissue and then packaged and frozen until analysed.

Analytical determinations

Analyses of the rabbits' diet were carried out in duplicate using AOAC (2000) methods. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to Mertens (2002), AOAC (2000) and Van Soest *et al.* (1991), respectively. Digestible energy was estimated according to Maertens *et al.* (1984).

Meat lipids (samples of approximately 5 g) were extracted in a homogeniser with 20 mL of 2:1 chloroform-methanol (Folch *et al.*, 1957), followed by filtration through a Whatman No. 1 filter paper. Fatty acid methyl esters (FAME) were prepared using a methanol and sulphuric acid solution (3%) and, after methylation at 80 °C for 60 min, and after 4 mL H₂O and 4 mL petroleum ether were added. The FAME were determined by a GC HRG MEGA 2 FISIONS gas-chromatograph apparatus equipped with a flame ionisation detector (FID) and FAME were separated by capillary column (D-B Wax AGILENT TECHNOLOGIES, 30 m in length, 0.250 mm in thickness and 0.25 μ m in thickness of the internal film). Helium was used as the carrier gas at a flow of 1 mL splitless. The oven temperature was programmed

at 130 °C and held for 1 min, then increased at a rate of 5 °C/min to a temperature of 180 °C, then maintained for 5 min, then increased 4 °C/min up to 230 °C, held for 40 min and up to 245 °C for 5 min. The injector and detector temperature were equal to 255 °C. The identification of individual FAME was based on a standard mixture (SUPELCO MIX FAME). The fatty acid percentages were calculated using Chrom-Card software. Some fatty acids were also expressed as g/100 g of muscle, assuming a direct relationship between peak area and weight of internal standard (C13:0).

Several indexes were used to estimate the desaturase and elongase activity of the muscle tissue. The activity of Δ^9 -desaturase and elongase was estimated by relating the percentage of the product to the percentage of the precursor (Okada *et al.*, 2005). The Δ^9 -desaturase index for C18:1, which is the main monounsaturated fatty acid in meat, was calculated as 100 times the ratio of oleic acid (C18:1) to the sum of the percentages of C18:1 and stearic acid (C18:0). The total Δ^9 -desaturase index (for both 16 and 18) was calculated as 100 times the ratio of the sum of the contents of palmitoleic acid (C16:1) and C18:1 to the sum of the contents of C16:1, palmitic acid (C16:0), C18:1, and C18:0. The elongase index was calculated as the ratio of the contents of C18:0 to C16:0, and the thioesterase index was calculated as the ratio of the contents of C16:0 to myristic acid (C14:0; Zhang *et al.*, 2007).

To evaluate the activity of both Δ^5 -desaturase and Δ^6 -desaturase, the enzymes catalysing the formation of LCPn-6 and LCPn-3 starting from the precursors LA and ALA, the following equation was used (Dal Bosco *et al.*, 2012): Δ^5 - Δ^6 -desaturase = $[(C20:2n-6 + C20:4n-6 + EPA + C22:5n-3 + DHA) / (LA + ALA + C20:2n-6 + C20:4n-6 + EPA + C22:5n-3 + DHA)] \times 100$.

Statistical analysis

A linear model (STATA, 2005, procedure GLM) was used to evaluate the effect of genetic strain. Difference between genotypes were assessed by ANOVA test with a Bonferroni multiple t-test. Differences with at least a $P < 0.05$ value were considered statistically significant. Non-parametric tests were performed on the mortality rate using the CATMOD procedure, and significance was evaluated by χ^2 values.

RESULTS AND DISCUSSION

The results shown in Table 2 clearly demonstrate the different growing rates of the 3 genotypes. As expected, the NZW rabbits reached the highest slaughter weights, followed by the grey-coloured rabbits and the Leprino rabbits. Other productive traits followed this trend, confirming the effect of genetic selection on the improvement in meat production.

The fatty acid profile (%) of the *longissimus lumborum* muscle is presented in Table 3. The major fatty acids in all groups were palmitic, oleic and LA. The NZW and Leprino rabbits had lower levels of myristic acid and higher levels of palmitic acid and palmitoleic acid. The levels of monounsaturated fatty acids were also significantly higher in these rabbits. The grey-coloured rabbits had the highest values of total n-3 fatty acids. In general, the fatty acid profile observed in this trial did not differ from those obtained in our previous studies (Dal Bosco *et al.*, 2004; Dal Bosco *et al.*, 2007; Mugnai *et al.*, 2008; D'Agata *et al.*, 2009).

The lipid content showed significant differences, with higher values in the NZW rabbits (Table 4), most likely due to the difference in genetic selection for growth. This finding is consistent with the findings of other researchers (Pla *et al.*,

Table 2: Effect of genotype on post-weaning performance of rabbits.

	New Zealand White	Leprino of Viterbo	Grey-coloured rabbits	Pooled standard error	χ^2
Initial weight (g)	990	855	918	85	
Final weight (g)	2750 ^c	2220 ^a	2400 ^b	155	
Weight gain (g/d)	31.9 ^b	24.8 ^a	27.2 ^{ab}	5.2	
Feed intake (g/d)	138.3 ^b	128.1 ^a	125.6 ^a	9.5	
Feed:gain ratio	4.33 ^a	5.16 ^b	4.61 ^b	0.3	
Mortality rate (%)	5	5	0		2

No.: 20/genotype.

^{a,c} Means in the same row with the same superscript do not differ significantly ($P < 0.05$).

Table 3: Effect of genotype on fatty acid profile (% of total fatty acids) of *longissimus lumborum* muscle.

	New Zealand White	Leprino of Viterbo	Grey coloured rabbits	Pooled standard error
C14:0	3.02 ^a	3.06 ^a	4.63 ^b	0.59
C16:0	30.26 ^b	29.57 ^{ab}	27.15 ^a	1.76
C18:0	5.94	6.11	7.07	1.20
Others SFA	2.56 ^a	2.11 ^a	3.57 ^b	0.45
Σ SFA	41.79 ^a	40.85 ^a	44.42 ^b	2.84
C16:1n-7	6.21 ^b	6.16 ^b	4.61 ^a	0.96
C18:1n-9	22.51 ^b	22.04 ^b	20.74 ^a	2.51
C20:1n-9	0.17	0.25	0.20	0.09
Others MUFA	0.33 ^a	0.58 ^b	0.33 ^a	0.09
Σ MUFA	29.35 ^b	28.83 ^b	25.88 ^a	2.08
C18:2n-6	21.19	22.45	20.88	1.93
C20:4n-6	3.33	3.39	3.92	0.79
Others n-6	0.77	0.71	0.69	0.20
Σ n-6	25.29	26.55	25.5	1.79
C18:3n-3	1.76	1.86	1.90	0.55
C20:5n-3	0.30	0.31	0.27	0.05
C21:5n-3	0.23	0.18	0.24	0.08
C22:5n-3	0.42 ^a	0.45 ^a	0.72 ^b	0.22
C22:6n-3	0.16 ^a	0.23 ^b	0.25 ^b	0.07
Others n-3	0.70	0.74	0.82	0.16
Σ n-3	3.57 ^a	3.77 ^a	4.20 ^b	0.35
Σ PUFA	28.86	30.32	29.70	2.28

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid. No.: 10/genotype.

^{a,b} Means in the same row with the same superscript do not differ significantly ($P < 0.05$).

1998; Gašperlin *et al.*, 2006; Polak *et al.*, 2006). The absolute fatty acid content levels observed suggest that the higher lipid content observed in the NZW rabbits strongly affected the levels of all fatty acids, mainly in comparison to the grey rabbits, which had the leanest meat. As already noted, the differences in the fatty acid profiles of the meat can be attributed to genetic and epigenetic differences or to causal interactions between genes and their products, which brings the phenotype, which could affect lipid metabolism and fatty acid deposition, into play (Kitajka *et al.*, 2002).

Indeed, some estimated indexes of fatty acid metabolism showed that the animals selected for productive performance differed from the local strain: the NZW and Leprino rabbits had higher thioesterase, Δ^9 -desaturase and lower elongase and Δ^5 - Δ^6 -desaturase indexes.

In fatty acid synthesis, thioesterase is responsible for terminating the reaction and releasing the newly synthesised fatty acid. The ratio of palmitic to myristic acid was used to reflect the selective division of thioesterase on the C14-acyl-acyl carrier protein or C16-acyl-acyl carrier protein. The higher thioesterase index observed in our trial is related to less cleavage of the C14-acyl-acyl carrier protein. The Δ^9 -desaturase is specific for the major MUFA of rabbit lipids and catalyses the conversion of palmitic and stearic to palmitoleic and oleic acid (Jeffcoat, 1979). As noted above, the species is the major source of variation in the fatty acid composition of meat of livestock animals, so the comparison of results obtained in this study with those of other animals could be useful to better understand the mechanisms underlying these metabolic processes. In beef, some authors have suggested that different concentrations of palmitoleic acid between breeds could be attributed to increased Δ^9 -desaturase activity (Sturdivant *et al.*, 1992; Laborde *et al.*, 2001). The results of the present study confirm those obtained in our previous chicken study, in which this enzymatic complex was always lower in pure breeds than in commercial hybrids (Dal Bosco *et al.*, 2012). Malau-Aduli *et al.* (1998) observed that Jersey cattle had greater indices of Δ^9 -desaturase in C16 fatty acids and elongase and less Δ^9 -desaturase in C18 fatty acids than Limousine cattle. Moreover, Limousine meat was observed to have more arachidonic acid than the meat of Jersey cows, suggesting that genetics affect Δ^5 -desaturase activity (responsible for the conversion of C20:3 to C20:4) or lower elongase activity as a result of the relative accumulation of C18:3n-6 in the Jersey breed.

Table 4: Effect of genotype on the absolute content of fatty acids and indexes of lipid metabolism of *longissimus lumborum* muscle.

	New Zealand White	Leprino of Viterbo	Grey coloured rabbits	Pooled standard error
Lipid content (g/100 g muscle)	2.0 ^b	1.6 ^a	1.4 ^a	0.3
Σ SFA (mg/100 g muscle)	699.9 ^b	547.4 ^a	520.8 ^a	68.5
Σ MUFA (mg/100 g muscle)	491.6 ^b	386.3 ^{ab}	303.4 ^a	52.4
Σ PUFA (mg/100 g muscle)	483.4 ^b	406.3 ^{ab}	348.2 ^a	59.7
C18:2n-6 (mg/100 g muscle)	354.9 ^b	300.8 ^{ab}	244.8 ^a	26.5
C18:3n-3 (mg/100 g muscle)	29.4 ^b	24.9 ^a	22.3 ^a	5.8
C20:5n-3 (mg/100 g muscle)	5.3 ^c	4.0 ^{ab}	3.2 ^a	1.2
C22:6n-3 (mg/100 g muscle)	2.7	3.0	2.9	1.8
PUFA/SFA	0.69	0.74	0.67	0.12
Elongase ¹	0.20	0.21	0.24	0.4
Thioesterase ²	10.0 ^b	9.4 ^b	6.3 ^a	1.2
Δ ⁹ -desaturase (16) ³	17.0 ^b	17.6 ^b	13.6 ^a	1.9
Δ ⁹ -desaturase (18) ⁴	79.1 ^b	78.3 ^b	74.6 ^a	4.0
Δ ⁹ -desaturase (16+18) ⁵	44.2 ^b	44.7 ^b	41.1 ^a	2.1
Δ ⁵ - Δ ⁶ -desaturase ⁶	17.8 ^a	17.3 ^a	20.5 ^b	1.4

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid. No.: 10/genotype.

^{a,b} Means in the same row with the same superscript do not differ significantly ($P < 0.05$).

¹: Calculated as 18:0/16:0;

²: Calculated as 16:0/14:0;

³: Calculated as $100 \times [16:1n-9 / (16:1n-9 + 16:0)]$;

⁴: Calculated as $100 \times [18:1n-9 / (18:1n-9 + 18:0)]$;

⁵: Calculated as $100 \times [(16:1n-9 + 18:1n-9) / (16:1n-9 + 16:0 + 18:1n-9 + 18:0)]$;

⁶: Calculated as $[(C20:2n-6 + C20:4n-6 + EPA + C22:5n-3 + C22:6n-3) / (C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3 + C22:6n-3)] \times 100$

In the present study, the estimated Δ⁵- plus Δ⁶-desaturase activity was significantly higher in the grey-coloured rabbits, suggesting a genetic effect on the mechanisms of desaturase and elongase responsible for the synthesis of LCP fatty acids.

The rate-limiting step in the enzymatic pathways of LCP biosynthesis is thought to be Δ⁶-desaturase (Yamazaki *et al.*, 1992). The commonly accepted pathway for the synthesis of DHA requires that the C22:5n-3 precursors be elongated to C24:5n-3, followed by Δ⁶-desaturation and then β-oxidation to obtain the final products. The unique β-oxidation step occurs in the peroxisomes via a multifunctional enzyme (Sprecher, 2000).

It is widely known that n-6 and n-3 fatty acids compete for the same Δ⁶-desaturase, but the affinity of the Δ⁶-desaturase is greater for n-3 than for n-6 fatty acids. Thus, the conversion of n-6 fatty acids to LCP metabolites is decreased by increasing the intake of n-3 fatty acids (Lands, 1992). Nevertheless, the above-mentioned preference of Δ⁶-desaturase for n-3 is neutralised by the fact that standard diets have high contents of n-6 fatty acid, especially LA, which is typically present at higher levels than ALA.

Our results agree with those of Sirri *et al.* (2010), who observed a higher Δ⁵- Δ⁶-desaturase index in slow-growing chickens, which were more efficient than medium- and fast-growing birds in long-chain fatty acid synthesis. In the same study, polyunsaturated fatty acids gradually decreased in slow- to medium- and fast-growing birds: the breast meat of slow-growing chickens was found to contain approximately 2 and 3 times more LCPn-3 and arachidonic acid, respectively, than the breast meat of medium- and fast-growing strains. The authors attributed this difference to differences in Δ⁵- Δ⁶-desaturase activity, which were 54.0, 34.4, and 23.6 for slow-, medium- and fast-growing birds, respectively.

Currently, the knowledge on enzyme activities and expression of genes involved in fatty acid synthesis and metabolism is too limited to fully explain the role of genetic variability in the fatty acid composition of animal meat. Hence,

biochemical and molecular genetic studies in this field should be encouraged to unravel the mechanisms underlying differences between genotypes in the metabolism and incorporation of specific fatty acids.

In conclusion, because the rabbits used in this trial were reared under the same management conditions, our results suggest a genetic basis for the differences observed in the meat fatty acid composition. Further studies are needed to confirm our hypothesis through the direct measurement of enzymatic activity and gene expression of the above-mentioned complex in liver mitochondria.

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