GENETIC VARIABILITY OF LIPOGENIC ACTIVITY AND LIPID COMPOSITION IN RABBIT ADIPOSE TISSUE

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***Abstract*— the effect of genetic line on perirenal fat content, fat composition and lipogenic enzyme activities was studied in rabbits. Forty eight rabbits from three synthetic lines (A, V and R) were used in this experiment. Half of the animals were slaughtered at 9 or 13 weeks of age. Activities of glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and fatty acid synthase (FAS) were measured. Live weight (LW), carcass weight (CW) and perirenal fat content (PF) increased with age. LW and CW were higher in line R. At 9 weeks of age there was no difference in PF between lines A and V, and line R showed a lower PF ($p<0.10$). A higher value of PF was found in line A at 13 weeks of age, with no differences between lines R and V. Perirenal fat showed higher activities of G6PDH and FAS and lower ME than it has been previously found in muscles. There was a decrease with age in FAS activity whereas G6PDH and ME activities were almost not affected by age. A higher lipogenic activity (G6PDH and ME) was found in rabbits from line A in agreement with its higher PF. At 9 weeks of age, the correlations between the PF and lipogenic enzyme activities were higher for G6PDH than for ME and no correlation was found with FAS. No differences between lines or ages were found for fatty acid composition. Our results showed between lines genetic variability in the activity of lipogenic enzymes in rabbit perirenal fat. These enzymes (especially G6PDH) could be relevant indicators of lipid deposition. Some evidence about the relative independence of metabolic capacity of intramuscular fat from other adipose tissues was found suggesting the possibility that intramuscular fat in rabbit could increase without an excessive increase of carcass adiposity.**

***Index Terms*—fat, genetic variability, lipogenic activity, rabbit.**

I. INTRODUCTION

Fat content is an important factor for carcass and meat quality. An increase in intramuscular fat content contribute to eating quality, however an excessive increase in carcass adiposity is a detrimental quality factor. Rabbit meat has low fat content (Hernández &Gondret 2008) and consequently, comparing with other species, it has a lower fatty feeling in the mouth and its juiciness is medium-low (Rødbotten, Kubberød, Lea & Ueland, 2004). Therefore, an increase in intramuscular fat content without excessive increase of carcass fat content would be desirable.

Lipogenic enzymes play an important role in fat deposition (Gondret, Mourot & Bonneau, 1997). Glucose-6-phosphate dehydrogenase and malic enzyme are involved in supplying NADH phosphate for fatty acid synthesis. Differences in the activity of these enzymes could lead to differences in fat content as it has been previously found in rabbits (Gondret et al., 1997), pigs (Mourot & Kouba, 1998) and cattle (Bonnet et al., 2007). Fatty acid synthase (FAS) catalyzes the last step in the fatty acid biosynthetic pathway and it could influence carcass fat deposition (Clarke, 1993).

Comparison between lines of different genetic origins can be used to find major genes or to find genetic variability useful for selection programs. In a previous study (Zomeño, Blasco & Hernández, 2009) we found a genetic line effect on intramuscular fat deposition and lipogenic and oxidative enzyme activity. A better understanding of mechanisms regulating intramuscular fat content and carcass fat content and their relationship is required for a better control of carcass and meat quality. The objective of this study is to compare rabbit lines of different genetic origin in perirenal fat content and the activity of lipogenic enzymes involving lipid metabolism.

II. MATERIALS AND METHODS

A. Animals

A total of 48 animals (24 animals per sex) from three synthetic rabbit lines A, V and R (16 animals per group) from experimental farm from Universidad Politécnica de Valencia (Spain) were selected and slaughtered at age of 9 and 13 weeks (24 animals per group). Line A has a New Zealand origin, line V is a blend of New Zealand and Californian origins and line R was formed by mixing commercial hybrids used as a terminal sires. Lines A and V were selected for litter size at weaning (4th week of age) for 37 and 34 generations, respectively. Line R was selected for growth rate between weaning and slaughter for 25 generations by individual selection.

From weaning rabbits were reared collectively and were fed *ad libitum* with a commercial diet formulated for growing rabbits (15.5% DM crude protein, 15.5% crude fibre, 3.1% DM fat). During the subsequent experimental

period, rabbits were housed in individual cages and received a restricted feed with a diet formulated for adults (17% DM crude protein, 16.7% crude fibre, 3.2% DM fat). The amount of feed was 135 g per day and was distributed one time at day. Animals were slaughtered at 9 or 13 weeks of age at the abattoir next to the farm, thus they did not suffer stress due to transport. Rabbits were weighted and slaughtered by electrical stunning and exsanguinations. Immediately after the slaughter the perineral fat was excised from the carcass, weighted, frozen in liquid nitrogen, vacuum packed and stored at -80°C until analysis.

B. Measurements of Lipogenic Enzyme Activities

Activities of glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME), NADPH-producing enzymes for lipogenesis, and fatty acid synthase (FAS), controlling a key step of fatty acid synthesis, were measured on rabbit adipose tissue. Briefly, an extract of adipose tissue was prepared by weighting a 1g of adipose tissue and homogenized in 2.5 ml of ice-cold 0.25 M sucrose (D-saccharose) solution containing 1mM dithiothreitol and 1mM EDTA. Homogenates were centrifuged at 10000 x g for 1h at 4ºC, and supernatants were collected for enzyme assays. The activities of G6PDH, ME and FAS were assessed at 37ºC using a spectrophotometer (Model UV-1601, Shimadzu Co., Tokyo, Japan) at 340 nm according to the methods described by Ficht, Hill, & Chaikoff (1959), Hsu and Lardy (1969) and Chang, Seidman, Teebor, & Lane (1967), respectively. Enzyme activities were expressed in nanomoles of NADPH produced (G6PDH, ME) or oxidized (FAS) per minute and per gram of fresh tissue.

C. Fatty Acid Analyses

Fatty acid methyl esters (Fame) of perirenal fat were prepared as previously described O´Fallon, Busboom, Nelson & Gaskins (2007). Fame were analyzed in a Focus Gas Chromatograph (Thermo, Milan, Italy) equipped with a split/splitless injector and a flame ionization detector. The separation of methyl esters was performed in a fused silica capillary column SPTM 2560 (Supelco, PA, USA) (100 m x 0.25 mm x 0.2 µm film thickness). The carrier gas was Helium at a linear velocity of 20 cm/sec. The samples were injected with a split ratio of 1/100. The initial oven temperature was set at 140 ºC held for 5 min and increased to 240 at 4 ºC/min and finally maintained at that temperature for 30 min. Both detector and injector temperatures were set at 260 ºC. The individual fatty acids were identified by comparing their retention times with standards of fatty acid methyl esters supplied by Supelco (PA, USA) and quantified by using C21:0 as internal standard.

D. Statistical analysis

Data were analyzed using a model including Line (A, R, V), age (9 and 13 weeks of age) and sex as fixed effects. Data analysis was carried out applying the General Linear Models (PROC GLM) method from SAS (Statistical Analysis Software; SAS Inst., Inc., Cary, NC) package. Correlations were computed applying the CORR procedure of SAS (SAS Inst., Inc., Cary, NC).

III. RESULTS AND DISCUSSION

Table 1 shows means and standard errors of live weight, carcass weight and perirenal fat content of three rabbit lines (A, V and R) and two ages (9 and 13 weeks). Rabbits were slaughtered at 9 or 13 weeks of age in order to obtain light and heavy carcasses, respectively. Light carcasses are generally consumed in Spain and heavy carcasses are generally consumed in France and Italy. Live weight, carcass weight and perirenal fat content changed with age increasing from 9 to 13 weeks of age. This is in agreement with previous results reported by Hernández, Aliaga, Pla, & Blasco (2004).

Table 1. Means and standard errors of live weight, carcass weight and perirenal fat content in three rabbit lines at 9 and 13 weeks of age

		A	**V**	**R**
Live weight (g)	9 weeks	1861 ± 43.8^{bB}	1875 ± 51.8^{bB}	2401 ± 36.7^{aB}
	13 weeks	2846 ± 51.8^{bA}	2642 ± 51.8^{cA}	3277 ± 36.7^{aA}
Carcass weight (g)	9 weeks	1075 ± 26.1^{bB}	1114 ± 30.8^{bB}	1373 ± 21.8^{aB}
	13 weeks	1792 ± 30.8^{bA}	1693 ± 30.8^{cA}	2005 ± 21.8^{aA}
Perirenal fat content (g)	9 weeks	15.2 ± 1.8^{aB}	10.4 ± 2.3^{aB}	14.9 ± 1.5^{aB}
	13 weeks	37.2 ± 2.2^{aA}	30.6 ± 2.3^{bA}	28.8 ± 1.5^{bA}

^{abc} means with different superscripts in the same row differ significantly ($p<0.05$)
^{AB} means with different superscripts in the same column for each variable differ significantly ($p<0.05$).

At 9 weeks of age, LW and CW were higher in line R, than in lines A and V, with no differences between lines A and V. Live weight at 13 weeks of age was higher in line R and lower in line V, with intermediate values in line A. The same results were found for carcass weight. The high size line selected for higher growth rate (line R) is used as

terminal sires because they have a better feed conversion ratio (Feki, Baselga, Blas, Cervera, & Gómez, 1996).

At 9 weeks of age there was no difference in perirenal fat content between lines. Although, line R showed a lower perirenal fat amount when a level of significance of $p<0.10$ was considered. A higher value of perirenal fat content was found in line A at 13 weeks of age, with no differences between lines R and V. Similar results were obtained when perirenal fat was referred as percentage of the carcass weight. Rabbit carcasses have a low fat percentage compared with other farm animals (Hernández &Gondret 2008), thus the higher dissectible fat found at 13 weeks of age does not represent problem in markets were heavy carcasses are preferred. No sex effect was found for these traits.

Table 2 shows enzyme activities (G6PDH, ME and FAS) in the rabbit perirenal adipose tissue at 9 and 13 weeks of age in three rabbit lines. Rabbit perirenal fat tissue displays higher activities of G6PDH and FAS than it has been found in muscles (Zomeño et al., 2009) according to the high capacity of perirenal fat for the lipid synthesis. However, ME activity was lower in perirenal fat than in muscle (520 and 260 nmol/min per g of tissue for *Semimembranosus propious* and *Longissimus*, respectively; Zomeño et al., 2009). Whatever the age or line, ME activity was lower than G6PDH activity in rabbit perirenal adipose tissue, as was previously reported in rabbit (Gondret et al., 1997).

There was an age effect in the FAS activity whereas G6PDH and ME activities were almost not affected. The activity of FAS decreased from 9 to 13 weeks of age in lines V and R ($p<0.05$) and in line A ($p<0.10$). G6PDH activity decreased from 9 to 13 weeks of age in line R with no differences between ages for lines A and V. There was no age effect in the activity of malic enzyme ($p>0.05$). However, at 13 weeks of age, a decrease of activity in line R was observed when a level of significance of $p<0.10$ was considered. Bonnet et al. (2007) in cattle pointed out that an inhibition of lipogenic activity could be a consequence of an increase of adipocyte hypertrophy.

The age related decrease in the lipogenic activity of perirrenal fat was not observed in intramuscular fat (Zomeño et al., 2009). In our previous research, an increase from 9 to 13 wk of age of lipogenic activity in *Semimembranosus propious* muscle and no differences with age in *Longissimus* muscle were found. This different behavior of fat depots could be related to a later development of intramuscular fat compared with perirenal fat. The relative independence of metabolic capacity of intramuscular fat from other adipose tissues suggests the possibility that intramuscular fat in rabbit could increase without an excessive increase of carcass adiposity. Zomeño, Blasco & Hernández (2010) pointed out that part of the genetic variation of intramuscular fat is independent of the genetic variation in overall lipid content of the carcass since the correlations between perirenal fat and intramuscular fat content of *Longissimus* muscle were 0.57 and 070 for lines A and V whereas line R showed no relationship between these traits ($r=0.06$).

Our findings showed an influence of genetic line on lipogenic activity. At 9 weeks of age, the G6PDH activity was higher in line A and R than in line V. At 13 weeks of age, the activity of G6PDH was higher in line A, with no differences between lines V and R. A line effect was also found in ME activity. At 9 week of age, line V showed the lower activity with no differences between lines A and R. The ME activity at 13 weeks of age was higher in line A than in lines V and R, with no differences between them. No line effect was found in FAS activity. Differences in the activities of these enzymes should be related to differences in fat content. Indeed, the higher lipogenic activity (G6PDH and ME activities) in rabbits from line A was associated with a higher perirenal fat content of this line. Our previous work, also found a genetic line effect for lipogenic enzymes activities in muscle, having line A higher G6PDH and ME activity than V and R lines (Zomeño et al., 2009). Likewise, breed comparisons in pigs (Mourot and Kouba, 1998) and cattle (Bonnet et al., 2007) showed a higher lipogenic activity in breeds with high fat levels.

Table 2. Means and standard errors of the activities[1] of lipogenic enzymes in perirenal adipose tissue of rabbits of 9 and 13 weeks of age in three different rabbit lines.

Enzyme	Age	A	V	R
G6PDH	9 weeks	840.1 ± 66.3^{aA}	587.8 ± 62.7^{bA}	846.9 ± 70.0^{aA}
	13 weeks	725.0 ± 75.4^{aA}	507.9 ± 70.0^{bA}	$509.3 \pm 70,0^{bB}$
ME	9 weeks	94.6 ± 10.2^{a}	63.8 ± 9.6^{b}	93.6 ± 10.7^{a}
	13 weeks	115.2 ± 11.5^{a}	60.5 ± 10.8^{b}	63.1 ± 10.7^{b}
FAS	9 weeks	425.6 ± 29.6	418.9 ± 28.0^{A}	409.5 ± 31.3^{A}
	13 weeks	337.0 ± 33.6	291.5 ± 31.3^{B}	275.3 ± 31.3^{B}

^{ab} means with the different superscripts in the same row differ significantly ($p<0.05$). ^{AB} means with the different superscripts in the same column for each variable differ significantly ($p<0.05$)

[1]Activities of glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and fatty acid synthase (FAS) are expressed in nmol/min per g of tissue.

At 9 weeks of age, the correlations between the perirenal fat content and lipogenic enzyme activities were higher for G6PDH than for the ME and no correlation was found with FAS (table 3). No correlations were found at 13 weeks of age.

Regarding to the fatty acid composition, no differences between lines or ages were found in the saturated, monounsaturated and polyunsaturated fatty acid percentages. The average fatty acid percentages were 38.6, 32.1 and 29.4% for saturated, monounsaturated and polyunsaturated fatty acids, respectively. The n6/n3 polyunsaturated fatty

acid ratio was not affected by genetic line but, a small decrease was observed with the increase of animal age (7.2 and 6.9 at 9 and 13 weeks of age).

Table 3. Correlation coefficients between perirenal fat content and lipogenic enzyme activities of rabbits at 9 weeks of age in three different rabbit lines.

	G6PDH	ME	FAS
Perirenal fat content	0.71***	0.55**	0.17

** Significant correlation at p<0.01 or *** p<0.001.Glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME) and fatty acid synthase (FAS) activities.

IV. CONCLUSION

Our results showed genetic variability in the activity of lipogenic enzymes in rabbit perirenal fat content. These enzymes, especially G6PDH, and ME in a lower degree, could be relevant indicators of lipid deposition. Some evidence about the relative independence of metabolic capacity of intramuscular fat from other adipose tissues was found suggesting the possibility that intramuscular fat in rabbit could increase without an excessive increase of carcass adiposity.

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