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EFFECT OF GENETIC LINE ON LIPID CHARACTERISTICS OF RABBIT MUSCLE

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ABSTRACT

The effect of genetic line on intramuscular fat content, perirenal fat content and the activity of some enzymes related to lipid metabolism was studied. *Longissimus* (LD) and *Semimembranosus proprius* (SP) muscles were used in this experiment. A total of 60 animals from three lines (A, V and R) selected for different criteria were slaughtered at 9 and 13 weeks of age. Differences between lines were found for intramuscular fat (IMF) and perirenal fat content (PF), having line A higher values than V and R. IMF and PF content were positively correlated in line A and V, but no relationship was found in line R. PF increased with age in three lines, whereas IMF increased only in line A and V. SP muscle showed higher lipogenic activities (glucose-6-phosphate dehydrogenase (G6PDH) and fatty acid synthase (FAS)) than LD. A line effect was observed for lipogenic activities. Line A had higher lipogenic activity and line R showed lower activity. An increase of G6PDH and malic enzyme activities with age was observed. Glycolytic activity was higher in LD, whereas SP showed higher oxidative activity. An influence of genetic line was found for oxidative enzymes in SP, HAD and CS activity was higher in line R compared with A and V. The LDH activity increased with age in both muscles, while the HAD activity decreased in LD. Results from this study indicate that genetic line has an effect on intramuscular fat deposition and related characteristics that could lead to differences in meat quality.

Key words - genetic variability, lipid content, muscle, rabbits

INTRODUCTION

Muscle lipid content is one of the main factors influencing dietetic and sensory meat quality. Lipid deposition in muscle depends on a reciprocal balance between anabolic (lipogenesis) and catabolic (oxidative and intracellular transport) fatty acid fluxes. Glucose-6-phosphate

dehydrogenase and malic enzyme are involved in supplying NADPH for lipogenesis, and therefore differences in the activity of these enzymes could lead to differences in fat content (Gondret et al., 1997; Mourot and Kouba, 1999). The activity levels of oxidative muscle enzymes are also involved in muscle fat deposition. A depressed capacity for fatty acid oxidation may lead to an increase in lipid deposition (Astrup et al., 1997). An age-related relationship between muscle fat content and lipogenic and metabolic traits has been observed in rabbits (Gondret et al., 2004). However, there are no studies about the influence of genetic line on lipogenic and metabolic traits in rabbit muscle.

Muscle lipases and phospholipases contribute to the hydrolysis of the lipid fraction releasing free fatty acids and related compounds. Differences in the activity of these enzymes could lead to different concentration of flavour precursors and, consequently, differences in flavour meat (Toldrá and Flores, 1998).

Comparison between lines of different genetic origins could be used to find major genes or to create new synthetic lines when having genetic variation for the traits of interest. Differences between rabbit lines among fat content and fatty acid composition of hind leg meat have been studied (Hernández et al., 2008), although these studies have not been focused on intramuscular fat.

The objective of this study is to compare rabbit lines of different genetic origin in intramuscular fat and the activity of enzymes related to lipid metabolism.

MATERIALS AND METHODS

A total of 60 animals from three synthetic lines (A, V and R) were used in this experiment. Lines A and V were selected for litter size at weaning and line R for growth rate between weaning (4weeks) and slaughter (9weeks). Animals were reared at the experimental farm of the Universidad Politécnica de Valencia. From weaning to 9 weeks of age, rabbits were reared collectively and were fed *ad libitum* with a commercial diet (15.5% crude protein, 15.5% fiber, 3.1% fat). During the subsequent experimental period, rabbits were housed in individual cages and received a restricted feed with a diet formulated for adults (17% crude protein, 16.7% fiber, 3.2% fat). The amount of feed was 135 g per day and was distributed one time at day. Rabbits were slaughtered by electrical stunning and exanguination at 9 or 13 weeks of age. Perirenal fat and two muscles, *Longissimus* (LD) and *Semimembranosus proprius* (SP), were excised from the carcass. The samples were weighed, frozen in liquid nitrogen, vacuum-packed and stored at -80ºC until analysis.

Total lipid content of the LD muscle was determined by ether extraction on Soxtec (AOAC, 1990) and was expressed as g per 100 g of fresh tissue.

Activities of lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH) (Ficht et al., 1959), malic enzyme (ME) (Hsu and Lardy, 1969) and fatty acid synthase (FAS) (Chang et al.,

1967) were measured on LD and SP muscle. Enzyme activities were expressed in nmol of NADPH produced (G6PDH, ME) or oxidized (FAS) per min and per g of fresh tissue.

Acid lipase, acid phospholipase and neutral lipase were assayed on LD muscle according to the method described by Hernández et al. (1999). One unit of lipolytic activity is defined as the amount of enzyme capable of hydrolysing 1µmol of substrate in 1 h at 37ºC.

The activity of the oxidative enzymes 3-hydroxyacyl-CoA dehydrogenase (HAD) (Bass et al., 1969) and citrate synthase (CS) (Srere, 1969), and glycolytic enzyme lactate dehydrogenase (LDH) (Bergmeyer and Bernt, 1974) were determined on LD and SP muscle. Enzyme activities were expressed as µmol of NADH (HAD, LDH) or mercaptide ion (CS) released per minute per min and per g of fresh tissue.

The statistical model used included line (A, V, R), age (9 weeks, 13 weeks) and sex (M, F) as fixed effects. Least square analysis was carried out and correlations were estimated. Data were analysed using the SAS (2004) statistical package.

RESULTS AND DISCUSSION

Table 1 shows lipid content of LD muscle of three rabbit lines. Differences between lines were found for intramuscular fat content, having line A higher values than V and R. Perirenal fat content was also influenced by genetic line, showing line A higher values (26.2 g) than V (19.3 g) and R (21.9 g). Intramuscular and perirenal fat content were positively correlated in line A (r=0.56) and V (r=0.70); however, no relationship was found in line R (r=0.06). A positive genetic correlation between fat content of the carcass and intramuscular fat has been reported in pigs. Genetic correlations among intramuscular fat and backfat thickness had a wide range from 0.04 (reviewed by Sellier (1998)) to 0.64 (Solanes et al., 2009). Thus, intramuscular fat is partially independent of the overall lipid content of the carcass. Besides, different lines with different genetic composition may lead to different genetic relationship between intramuscular fat and backfat thickness.

Perirenal fat increased between 9 and 13 weeks in the three lines, whereas lipid content of LD increased in line A and V but in line R remained stable. An increase of lipid content in *Longissimus lumborum* muscle with age was observed by Gondret et al. (2004). Nevertheless, in our results lipid content did not increase with age in line R. The reason for this discrepancy could be the feed restriction received from 9 to 13 weeks of age. Gondret et al. (2000) reported that feed restriction during fattening affects intramuscular lipid deposition in rabbits. Although feed restriction affected the three lines, it had more impact on lipid characteristics of line R which had a higher growth rate (Feki et al., 1996).

The SP muscle showed higher activities of glucose-6-phosphate dehydrogenase (G6PDH) and fatty acid synthase (FAS) than LD, while malic enzyme (ME) activity was higher in LD (Table 2). The enhanced lipogenic capacity of SP is related to a high lipid content and oxidative metabolism of this muscle (Alasnier et al., 1996). A genetic line effect was observed for

lipogenic enzymes activities in both muscles, although the pattern often differed between muscles. In SP muscle, differences between lines were found for G6PDH and ME activity. Line A had higher G6PDH and ME activity than R and V lines. In LD, line A and V had higher G6PDH activity than line R. These results are related to the higher muscle lipid content and perirenal fat content of line A rabbits. This association between fat content and lipogenic activity was also observed by Mourot and Kouba (1998; 1999) in pig breeds. G6PDH and ME activities increased with age in SP. An increase in muscle lipogenic activity has been previously observed by Gondret et al. (2004) between the age of 10 and 20 weeks.

Lipolytic activities showed small differences between lines. Line A had higher neutral lipase (2.85 U/g) than lines V (2.47 U/g) and R (2.59 U/g). No differences between lines were found for acid lipase and acid phospholipase. In rabbit meat, no differences have been found between the same lines for lipolytic enzymes activities in leg meat (Ariño et al., 2003). In pork meat, several works have shown differences between genetic types in their lipolytic activities (Armero et al., 1999).

Table 3 shows catabolic activities of LD and SP muscles. The greatest glycolytic activity, LDH, was found in LD. Conversely, SP showed the highest oxidative activity, HAD and CS. These observations are in agreement with the different fibre type composition of both muscles, SP is a slow-twitch oxidative (type I) muscle and LD is a fast-twitch glycolytic (type IIa and IIb) muscle (Alasnier et al., 1996). Differences between lines were observed for oxidative enzymes in SP muscle. A higher HAD activity was found in line R, while CS activity was higher in line R and A than in line V. The LDH activity increased with age in both muscles, while the HAD activity decreased only in LD. Gondret et al. (2004) found a decrease in oxidative activity in *Longissimus lumborum* muscle of rabbits between the age of 10 and 20 weeks.

CONCLUSIONS

Results from this study indicate that genetic line has an effect on intramuscular fat deposition and the activity of enzymes related to lipid metabolism. This effect could lead to differences in meat quality characteristics related to intramuscular fat. The genetic variability found in lipid metabolism between lines suggests the possibility of finding this variability within line, allowing the improvement of intramuscular fat content by selection**.**

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TABLES

Table 1. Least square means and standard errors of lipid content of *Longissimus* muscle (g/100 g fresh tissue) of three rabbit lines.

Line	A	V	R
9 weeks	0.867 ± 0.045^{aA}	0.610 ± 0.053^{bA}	0.671 ± 0.038^{b}
13 weeks	1.267 ± 0.059^{aB}	0.827 ± 0.056^{bB}	0.652 ± 0.038^{c}

[abc] Within rows, the means with different superscripts differ significantly ($p<0.05$). [AB] Within columns, the means with different superscripts differ significantly ($p<0.05$).

Table 2. Least square means and standard errors of lipogenic enzyme activities in *Longissimus* (LD) and *Semimembranosus proprius* (SP) muscles of three rabbit lines.

Muscle	Enzyme	A	V	R
LD	**G6PDH**	68 ± 4^{a}	67 ± 4^{a}	55 ± 4^{b}
	ME	519 ± 29	547 ± 28	494 ± 29
	FAS	12.9 ± 1.5	9.9 ± 1.6	12.2 ± 1.5
SP	**G6PDH**	500 ± 25^{a}	373 ± 26^{b}	386 ± 25^{b}
	ME	305 ± 21^{a}	238 ± 21^{b}	237 ± 21^{b}
	FAS	108.7 ± 8.0	90.7 ± 8.0	101.2 ± 8.0

[ab] Within rows, the means with different superscripts differ significantly ($p<0.05$). G6PDH, glucose-6-phosphate dehydrogenase (nmol of NADPH produced/min.g fresh tissue); EM, malic enzyme (nmol of NADPH produced/min.g fresh tissue); FAS, fatty acid synthase (nmol of NADPH oxidized/min.g fresh tissue).

Table 3. Least square means and standard errors of catabolic enzyme activities in *Longissimus* (LD) and *Semimembranosus proprius* (SP) muscles of three rabbit lines.

Muscle	Enzyme	A	V	R
LD	**LDH**	800 ± 42	770 ± 42	768 ± 42
	HAD	1.72 ± 0.14	1.64 ± 0.14	1.52 ± 0.15
	CS	2.79 ± 0.21	2.76 ± 0.21	2.37 ± 0.21
SP	**LDH**	21.1 ± 2.3	21.8 ± 2.3	21.6 ± 2.3
	HAD	2.05 ± 0.14^{b}	2.05 ± 0.14^{b}	2.55 ± 0.14^{a}
	CS	5.80 ± 0.19^{a}	5.10 ± 0.19^{b}	6.02 ± 0.19^{a}

[ab] Within rows, the means with different superscripts differ significantly ($p<0.05$). LDH, lactate dehydrogenase (µmol of NADH released per min and per g of fresh tissue); HAD, 3-hydroxyacyl-CoA dehydrogenase (µmol of NADH released per min and per g of fresh tissue); CS, citrate synthase (µmol of mercaptide ion released per min and per g of fresh tissue)

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