PE1.57 Influence of genetic line on lipid metabolism traits of rabbit muscle 364.00

<u>Cristina Zomeño</u> (1) crizose@posgrado.upv.es, Agustin Blasco(1), Pilar Hernandez (1) (1)universidad politecnica de valencia

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. Line A showed higher lipid content in LD than V and R at 9 weeks of age. At 13 weeks of age, line A had higher muscle lipid content than R and line V showed an intermediate value. Perirenal fat content was also influenced by genetic line, showing line A higher values. Intramuscular and perirenal fat content were positively correlated in line A and V, but no relationship was found in line R. Perirenal fat increased between 9 and 13 weeks in three lines, whereas lipid content of LD increased in line A and V, remainig stable in line R. 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. A line effect was observed for lipogenic activities. In LD, line A and V had higher G6PDH activity than line R. In SP, line R and V had lower G6PDH and ME than line A. An increase of G6PDH and ME activities with age was observed in SP. In SP muscle, a higher 3hydroxyacyl-CoA (HAD) activity was found in line R, while citrate synthase (CS) activity was higher in line R and A than in line V. Glycolitic activity (LDH) was higher in LD, whereas SP showed higher oxidative activity (HAD and CS). 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.

C. Zomeño is with the Institute for Animal Science and Technology, Universidad Politécnica de Valencia, 46022 Valencia, Spain (corresponding autor phone: +34 963879756; fax: +34 963877439; e-mail: crizose@posgrado.upv.es). A. Blasco is with the Institute for Animal Science and Technology, Universidad Politécnica de Valencia, 46022 Valencia, Spain (email: ablasco@dca.upv.es). P. Hernández is with the Institute for Animal Science and Technology, Universidad Politécnica de Valencia, 46022 Valencia, Spain (e-mail: phernan@dca.upv.es).

Index Terms— genetic variability, lipid content, muscle, rabbit

I.

INTRODUCTION

MUSCLE lipid content is one of the main factors that influence meat quality characteristics. Nutritional meat quality depends on the lipid content and the composition of the fatty acids. In addition, lipid content is a key determinant of sensory quality traits such as tenderness, juiciness and taste [20]. Fat content depends on a reciprocal between catabolic (oxidative balance and intracellular transport) and anabolic (lipogenesis) fatty acid fluxes [10]. Differences in the activity of lipogenic enzymes could result in a different fat carcass content [13, 22]. 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 [28]. There is an increasing interest in the improvement of meat quality as a result of the consumer demands. Comparison between lines of different genetic origins can be used to find major genes or to create new synthetic lines when having genetic variation for the traits of interest. Several studies have described genetic variability associated to carcass fat content between lines selected for different criteria in rabbit [23, 15]). In addition, differences between lines among fat content (inter and intramuscular) and fatty acid composition of hind leg meat have been studied [16]. Nevertheless, studies have not been focused these on intramuscular fat which is a main trait involved in the improvement of meat quality. The objective of this study is to compare rabbit lines of different genetic origin in intramuscular fat, perirenal fat content and the activity of some enzymes related to lipid metabolism. '

II. MATERIALS AND METHODS

A total of 60 animals from three synthetic lines (A, V and R) were used in this experiment. Line 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 -80°C until analysis. Total lipid content of the LD muscle was determined by ether extraction on Soxtec [2] and was expressed as g per 100 g of fresh tissue. Activities of lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH) [9], malic enzyme (ME) [19] and fatty acid synthase (FAS) [7] 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. [17]. One unit of lipolytic activity is defined as the amount of enzyme capable of hydrolysing 1µmol of substrate in 1 h at 37°C. The activity of the oxidative enzymes 3-hydroxyacyl-CoA (HAD) [5] and citrate synthase (CS) [27], and glycolitic enzyme lactate dehydrogenase (LDH) [6] 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 [24] statistical package.

III. RESULTS AND DISCUSSION

Table 1 shows lipid content of LD muscle of three rabbit lines. Line A showed higher lipid content than V and R at 9 weeks of age. At 13 weeks of age, line A had higher muscle lipid content than R and line V showed an intermediate value. 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 [25]) to 0.64 [26]. 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. The different relationship between intramuscular and perirenal fat content found in lines A, V and R is in agreement with this observation. 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. [13, 11]. 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. [12] 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 than A and V lines. Probably, this fact is related to an increase of appetite in lines selected for increased growth rate [8]. 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 [1]. Some line effect was observed for lipogenic activities. In LD, line A and V had higher G6PDH activity than line R. In SP, line R and V had lower G6PDH and ME than line A. 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 et al. [21, 22] 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. [11] 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 [3]. In pork meat, several works have shown differences between genetic types in their lipolytic activities [4, 14]. Table 3 shows catabolic activities of LD and SP muscle. The greatest glycolitic 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 glycolitic (type IIa and IIb) muscle [1]. An influence of genetic line was observed for metabolic 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 in LD. Gondret et al. [11] found a decrease in oxidative activity in Longissimus lumborum muscle of rabbits between the age of 10 and 20 weeks.

IV. CONCLUSION

Results from this study indicate that genetic line has an effect on intramuscular fat deposition and related characteristics. This effect could lead to differences in meat quality 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.

ACKNOWLEDGEMENT

This work was supported by Spanish MEC project no. AGL2006-10172.

REFERENCES

[1] Alasnier, C., Rémignon, H. & Gandemer, G. (1996). Lipid characteristics associated with oxidative and glycolytic fibres in rabbit muscles. Meat Science, 43, 213-224.

[2] AOAC. (1990). Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, Virginia.

[3] Ariño, B., Hernández, P. & Blasco, A. (2003). Efecto de la selección por velocidad de crecimiento sobre la actividad

de enzimas proteolíticos y lipolíticos de la carne de conejo. Información Técnica Económica Agraria, 24, 253-255.

[4] Armero, E., Barbosa, J.A., Toldrá, F., Baselga, M. & Pla, M. (1999). Effects of the terminal sire type and sex on pork muscle cathepsins (B, B+L and H), cysteine proteinase inhibitors and lipolytic enzyme activities. Meat Science, 51, 185-189.

[5] Bass, A., Brdiczka, D., Eyer, P., Hofer, S. & Pette, D. (1969). Metabolic differentiation of distinct muscle types at the level of enzymatic organisation. European Journal of Biochemistry, 10, 198-206.

[6] Bergmeyer, H. U., & Bernt. E. (1974). Lactate deshydrogenase: UV-essay with pyruvate and NADH. In: Methods of enzymatic analysis. (pp. 574-579). New-York, USA, Academic Press.

[7] Chang, H. C., Seidman, I., Teebor, G. & Lane, D. M. (1967). Liver acetyl CoA carboxylase and fatty acid synthase: relative activities in the normal state and in hereditary obesity. Biochemical and Biophysical Research Communications, 28, 682-686.

[8] Feki, S., Baselga, M., Blas, E., Cervera, C., & Gómez, E.A. (1996). Comparison of growth and feed efficiency among rabbit lines selected for different objectives. Livestock Production Science, 45, 87-92.

[9] Ficht, W. M., Hill, R. & Chaikoff, I. L. (1959). The effect of fructose feeding on glycolytic enzyme activities of the normal rat liver. Journal of Biological Chemistry, 234, 1048-1051.

[10] Gerbens, F. (2004). Genetic Control of Intramuscular Fat Accretion. In Muscle Development of Livestock Animals: physiology, genetics, and meat quality (pp. 343-356). CABI Publishing.

[11] Gondret, F., Hocquette, J.F. & Herpin, P. (2004). Age-related relationship between muscle fat content and metabolic traits in growing rabbits. Reproduction Nutrition Development, 44, 1-16.

[12] Gondret, F., Lebas, F. & Bonneau, M. (2000). Restricted feed intake during fattening reduces intramuscular lipid deposition without modifying fiber characteristics in rabbits. Nutrient metabolism. The Journal of Nutrition. 228-233.

[13] Gondret, F., Mourot, J. & Bonneau, M. (1997). Developmental changes in lipogenic enzymes in muscle compared to liver and extramuscular adipose tissues in the rabbit (Oryctolagus cuniculus). Comparative biochemistry and physiology, 117B, 259-265.

[14] Hernández, P., Aliaga, S., Pla, M. & Blasco, A. (2004). The effect for growth rate and slaughter age on carcass composition and meat quality traits in rabbits. Journal of Animal Science, 82, 3138-3143.

[15] Hernández, P., Ariño, B., Grimal, A., Blasco, A. (2006). Comparison of carcass and meat characteristics of three rabbit lines selected for litter size or growth rate. Meat Science, 73, 645-650.

[16] Hernández, P., Cesari, V., Blasco, A. (2008). Effect of genetic rabbit lines on lipid content, lypolitic activities and

fatty acid composition of hind leg meat and perirenal fat. Meat Science, 78, 485-491.

[17] Hernández, P., Navarro, J. L., & Toldrá, F. (1999). Effect of frozen storage on lipids and lypolitic activities in the longissimus dorsi muscle of pig. Zeitschrift für Lebensmittel-Untersuchung und-Forschung, 208, 110–115.

[18] Hernández, P., Zomeño, L., Ariño, B. & Blasco, A. (2004). Antioxidant, lipolytic and proteolytic enzyme activities in pork meat from different genotypes. Meat Science, 66, 525-529.

[19] Hsu, R. Y. & Lardy, H. A. (1969). Malic enzyme. Methods in enzymolgy. (pp. 230-235). New-York, USA, Academic Press.

[20] Lawrie, R.A., & Leward, D.A. (2007). Lawrie's Meat Science. Woodhead press. Cambridge, UK

[21] Mourot, J. & Kouba, M. (1998). Lipogenic enzyme activities in muscles of growing Large White and Meishan pigs. Livestock Production Science, 55, 127-133.

[22] Mourot, J. & Kouba, M. (1999). Development of intra and intermuscular adipose tissue in growing Large White

and Meishan pigs. Reproduction Nutrition Development, 39, 125-132.

[23] Pla, M., Guerrero, L., Guardia, D., Oliver, M. A., Blasco, A. (1998). Carcass characteristics and meat quality of rabbit lines selected for different objectives: I. Between lines comparison. Livestock Production Science, 54, 115-123.

[24] SAS (2004) SAS System (Version 9.1). Cary, NC, USA: SAS Institute, Inc.

[25] Sellier, P. (1998). Genetics of meat and carcass traits.In: Rothschild, M.F. and Ruvisky, A. The Genetics of the Pig. (pp. 463-510) CAB International, Walliford, UK.

[26] Solanes, F.X., Reixach, J., Tor, M., Tibau, J. & Estany, J. (2009). Genetic correlations and expected response for intramuscular fat content in a Duroc pig line. Livestock Science, 123, 63-69.

[27] Srere, P. A. (1969). Citrate Synthase. In: Methods in enzymology. (pp. 3-5). New-York, USA, Academic Press.

[28] Toldrá F. & Flores, M. (1998). The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. Critical Reviews in Food Science and Nutrition 38, 331–352.

Table 1. Least square means and standard errors of lipid content in Longissimus muscle of 9 and 13 weeks rabbits.

Age	Α	V	R
9 weeks	0.867 ± 0.048^{aA}	0.610 ± 0.053^{bA}	0.671 ± 0.038^{b}
13 weeks	1.267 ± 0.059^{aB}	0.827 ± 0.056^{bB}	$0.652 \pm 0.038^{\circ}$

^{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) muscle of three rabbit line.

Muscle	Enzyme	Α	\mathbf{V}	R
LD	G6PDH	68 ± 4^{a}	67 ± 4^{a}	55 ± 4^{b}
	EM	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}
	EM	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).

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

Músculo	Enzima	Α	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).