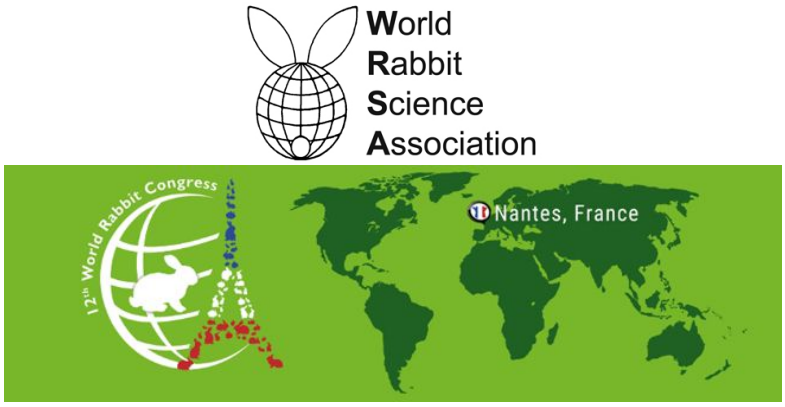


PROCEEDINGS OF THE 12th WORLD RABBIT CONGRESS

Nantes (France) - November 3-5, 2021
ISSN 2308-1910

Session
BIOLOGY and PHYSIOLOGY

Zubiri-Gaitán A., Martínez-Álvaro M., Ccalta R., Satué K., Blasco A., Hernández P.

CORRELATED RESPONSE TO SELECTION FOR INTRAMUSCULAR FAT ON THE LIVER FAT AND ITS FATTY ACID PROFILE

Full text of the communication
+
Slides of the oral presentation

How to cite this paper
Zubiri-Gaitán A., Martínez-Álvaro M., Ccalta R., Satué K., Blasco A., Hernández P., 2021. Correlated response to selection for intramuscular fat on the liver fat and its fatty acid profile. Proceedings 12th World Rabbit Congress - November 3-5 2021 - Nantes, France, Communication BP-35, 4 pp. + presentation

CORRELATED RESPONSE TO SELECTION FOR INTRAMUSCULAR FAT ON THE LIVER FAT AND ITS FATTY ACID PROFILE

Zubiri-Gaitán A.[1], Martínez-Álvaro M.[1], Ccalta R.[1], Satué K.[2], Blasco A.[1], Hernández, P.[1]*

[1]Institute for Animal Science and Technology, Universitat Politècnica de València, 46022, València, Spain
[2]Department of Animal Medicine and Surgery, Universidad Cardenal Herrera, 46113 València, Spain.
*Corresponding author: phernan@dca.upv.es

ABSTRACT

A divergent selection experiment for intramuscular fat content (IMF) in *longissimus thoracis et lumborum* (LTL) muscles was performed in rabbits during 10 generations at the Universitat Politècnica de València in order to unravel the mechanisms involved in fat deposition. The aim of the present experiment was to study the correlated response to selection on liver fat content and its fatty acid profile, as well as in plasma metabolites related to liver metabolism. A total of 190 rabbits from the 10th generation of selection were used to estimate the direct response to selection. A subsample of 54 animals was selected to study the correlated response to selection on the liver fat, its fatty acid profile and on plasma metabolites related to the liver metabolism. Direct and correlated responses to selection were estimated as the phenotypic differences between high-IMF and low-IMF lines. A relevant value of the difference (r) of 1/3 of the phenotypic standard deviation of the trait was proposed in order to estimate the probability of relevance P_r. Bayesian inference was used. The direct response to selection was 0.49 g/100 g of LTL muscle, which represents a 47% of the mean of the trait and 4.1 of its standard deviation. Higher liver percentage was found in high-IMF line, with relevant differences (P_r = 0.81). No evidence of difference between lines was found on their liver fat content (P_0 = 0.65). Nonetheless, its fatty acid profile was modified. Monounsaturated fatty acids were higher in high-IMF line, whereas polyunsaturated fatty acids were higher in low-IMF line with relevant differences (P_r = 0.90 in both cases). Higher plasma concentration of triglycerides and lower of alanine transaminase were found in the low-IMF line, with relevant differences (P_r = 0.97 and 0.92 respectively). Selection for IMF led to a correlated response on the liver percentage and on its fatty acid composition, indicating a genetic relationship between those traits. The higher concentration of triglycerides found in L suggests that differences in IMF at 9 wks of age may be due to a reduced capacity of lipid uptake from the plasma.

Key words: divergent selection, intramuscular fat, liver fat, fatty acids, rabbit

INTRODUCTION

Intramuscular fat content (IMF) is one of the main parameters in meat quality, since it affects its juiciness, tenderness and flavor. A divergent selection experiment for IMF in *longissimus thoracis et lumborum* (LTL) muscle was developed in rabbits at the Universitat Politècnica de València with the scope to study this trait, its correlation with other relevant traits and to contribute to unravel the mechanisms of fat deposition.

Lipid deposition in muscle depend on a balance between lipogenic and catabolic fatty acid fluxes. In this aspect, the liver plays a very important role since it is an important lipogenic site in growing rabbits (Gondret et al., 1997). Previous studies in these lines have shown that the liver lipogenic activity was positively correlated with fat deposition in muscle (Martínez-Álvaro et al., 2018), supporting that, in rabbits, the liver lipogenic activity has a very important function in the development of the trait under study. Nonetheless, the relationship between IMF, liver fat and liver fatty acid profile has not been studied so far. In our divergent selection experiment we can compare

these relationships in rabbit lines that have the same genetic origin and environment and differ only in IMF and correlated traits.
The aim of the study was to study the correlated response to selection for intramuscular fat content in rabbits on the liver fat content and its fatty acid profile, as well as in plasma metabolites related to the liver metabolism.

MATERIALS AND METHODS

A divergent selection experiment for intramuscular fat content (IMF) was performed in rabbits during 10 generations at the Universitat Politècnica de València. The selection criterion was the average phenotypic value of IMF measured in two full sibs (one male and one female) of the candidate, at 9 weeks of age in LTL muscle. The complete selection procedure is described in Martínez-Álvaro et al. (2016).
One hundred animals from the high-IMF line and 90 from the low-IMF line from the 10th generation were used to estimate the direct response to selection. 82 of these rabbits were born after an embryo transfer procedure, since these lines were also involved in a different project. The correlated response to selection on liver fat and its fatty acid composition, and on plasma metabolites were estimated in a subsample of 54 animals (27 from each line), not included in the embryo transfer group.
Animals were slaughtered at 9 weeks of age by exsanguination prior electric stunning. Blood samples were collected at slaughter from the jugular vein in 1 ml lyophilized lithium heparin tubes and plasma was prepared by centrifugation at 3000 rpm for 10 min. Carcasses were chilled for 24 h at 4ºC. The liver weight was recorded and its percentage was estimated relative to the reference carcass weight, recorded following the norms of the World Rabbit Science Association (WRSA) (Blasco and Ouhayoun, 1993). LTL muscle was dissected, minced, lyophilized and scanned using near infrared spectroscopy (NIRS 5000, FOSS NIRSystems Inc., Hilleroed, Denmark). IMF was estimated as g IMF/100 g of fresh muscle applying the equations developed by Zomeño et al. (2012). Total lipid content of the liver were determined by ether extraction (Soxtec 2055, Tecator, Höganäs, Sweden) with a previous acid hydrolysis (Soxcap 2022, Tecator, Höganäs, Sweden) and the results were expressed as g fat/100 g of liver. To obtain the liver fatty acid profile, fatty acid methyl esters were prepared using the method described in O'Fallon et al. (2007) and analyzed by gas chromatography (FOCUS, Thermo, Milan, Italy). Plasma concentrations of albumin, alanine transaminase, glucose, cholesterol, triglycerides and bile acids, were determined by photometric methods in the laboratory of the veterinary clinical hospital of the Universidad CEU Cardenal Herrera.
Direct and correlated responses to selection were estimated as the phenotypic differences between high and low-IMF lines. The model included line, sex, transference (transferred or non-transferred) and parity order (2 levels: first parity or second and more) as fixed effects, and common litter random effect. The former was assumed to be normally distributed with mean $\mathbf{0}$ and variance $\mathbf{I}\sigma_c^2$, and uncorrelated with the residuals. Residuals were also assumed to be normally distributed and uncorrelated. Bounded flat priors were assumed for all fixed effects and variances. Bayesian inference was used by means of the program Rabbit (Institute for Animal Science and Technology, UPV, Valencia, Spain). The parameters obtained from the marginal posterior distributions of the phenotypic differences were: the median of the difference between high and low-IMF lines (D), the highest posterior density region at 95% ($HPD_{95\%}$) and the probability of the difference being greater than zero when $D > 0$ or lower than zero when $D < 0$ (P_0). Besides, a relevant value of the difference (r) was assumed to be 1/3 of the phenotypic standard deviation of the trait, and the probability of the difference being greater than r when $D > 0$ or lower than r when $D < 0$ was calculated (probability of relevance, P_r) (Blasco, 2017).

RESULTS AND DISCUSSION

Descriptive statistics and the differences between lines for every trait are summarized in table 1. The divergent selection experiment was successful, showing a direct response of 0.49 g/100 g of muscle, that represents 47% of the mean or 4.1 standard deviations of the trait. This is the only selection

experiment for IMF performed in rabbits; in other species they are scarce (Sapp et al. (2002) in cattle, Zhao et al. (2007) in chickens and Schwab et al. (2009) in pigs), all showing high response to selection.

Table 1: Descriptive statistics and differences between lines for: intramuscular fat in *longissimus thoracis et lumborum* muscle (n=190), liver weight, liver percentage, liver fat, liver fatty acid profile, and plasma metabolites (n=54)

Trait	Mean	SD[1]	D[2]	$HPD_{95\%}$[3]		P_0[4]	r[5]	P_r[6]
Intramuscular fat (g/100 g muscle)	1.05	0.12	0.49	0.45	0.53	1.00	0.04	1.00
Liver weight (g)	60.3	10.9	3.85	-3.30	10.95	0.86	3.63	0.52
Liver percentage (%)	7.3	1.09	0.66	-0.05	1.30	0.97	0.36	0.81
Liver fat (g/100 g liver)	3.83	0.41	-0.05	-0.28	0.22	0.65	0.14	0.25
Fatty acids (%)	**Mean**	**SD[1]**	**D[2]**	**$HPD_{95\%}$[3]**		**P_0[4]**	**r[5]**	**P_r[6]**
Myristic Acid (C14:0)	0.48	0.17	0.05	-0.07	0.16	0.84	0.06	0.49
Palmitic Acid (C16:0)	20.17	2.06	0.42	-0.86	1.78	0.74	0.69	0.35
Heptadecanoic Acid (C17:0)	0.92	0.16	-0.14	-0.25	0.04	0.99	0.05	0.95
Stearic Acid (C18:0)	19.09	1.38	0.10	-0.85	0.99	0.59	0.46	0.20
Saturated fatty acids (SFA)	**41.06**	**1.27**	**0.44**	**-0.32**	**1.24**	**0.86**	**0.42**	**0.51**
Palmitoleic Acid (C16:1)	0.66	0.28	0.23	0.03	0.40	0.99	0.09	0.92
Oleic acid (C18:1n9c)	13.63	2.13	1.73	0.33	3.32	0.99	0.71	0.91
Vaccenic acid (18:1n-7)	1.69	0.23	0.14	-0.01	0.29	0.97	0.08	0.79
Monounsaturated fatty acids (MUFA)	**16.70**	**2.67**	**2.11**	**0.03**	**3.86**	**0.98**	**0.89**	**0.91**
Linolenic Acid (C18:3n3)	0.72	0.13	-0.11	-0.19	0.01	0.99	0.04	0.92
Arachidonic Acid (C20:4n6)	8.17	1.01	0.10	-0.67	0.79	0.61	0.34	0.25
Linoleic Acid (C18:2n6c)	29.10	2.33	-2.31	-3.99	0.76	1.00	0.78	0.97
Polyunsaturated fatty acids (PUFA)	**42.26**	**3.21**	**-2.48**	**-4.74**	**0.21**	**0.98**	**1.07**	**0.90**
Plasma metabolites	**Mean**	**SD[1]**	**D[2]**	**$HPD_{95\%}$[3]**		**P_0[4]**	**r[5]**	**P_r[6]**
Glucose (mg/dl)	115.00	34.10	-1.87	-23.86	21.92	0.56	11.37	0.20
Cholesterol (mg/dl)	48.06	16.42	-3.95	-16.67	8.38	0.74	5.467	0.40
Triglycerides (mg/dl)	82.10	32.00	-34.04	-56.55	-11.19	1.00	10.67	0.97
Bile acids (mol/ml)	15.60	7.33	-2.10	-7.15	2.61	0.81	2.44	0.44
Albumin (g/dl)	3.09	0.62	0.29	-0.17	0.74	0.89	0.21	0.65
Alanine transaminase (IU/l)	11.64	3.16	2.60	0.46	4.90	0.99	1.05	0.92

[1]SD: standard deviation of the trait; [2]D: median of the marginal posterior distribution of the difference between high and low IMF lines; [3]$HPD_{95\%}$: highest posterior density region al 95% of probability [4]P_0: probability of the difference being greater than zero when D > 0 or lower than zero when D < 0; [5]r: relevant value, proposed as 1/3 of the standard deviation of the trait; [6]P_r: probability of relevance (probability of the difference being greater than r when D > 0 or lower than r when D < 0); [7]SFA: C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0; [8]MUFA: C14:1 + C16:1 + C17:1 + C18:1n9t + C18:1n9c+ C18:1n7 + C20:1 + C22:1n9; [9]PUFA: C18:2n6c + C18:3n6 + C18:3n3 + C20:2 + C20:3n6 + C203n3 + C20:4n6 + C20:5n3 + C22:4n6 + C22:5n3 + C22:6n3.

Liver weight and percentage were greater in the high-IMF line (D = 3.85 g and 0.66% respectively), but this difference was only relevant for liver percentage (P_r= 0.81). The greater liver size in high-IMF line suggests a greater lipogenic activity, as this tissue is an important site of lipogenic activity in growing rabbits (Gondret et al., 1997). The former relationship has already been observed in a previous study performed in the 8th generation of selection, in which larger livers with higher liver lipogenic activity were found in high-IMF line (Martínez-Álvaro et al., 2018). No correlated response to selection was found on the liver fat (P_0=0.65), although its fatty acid profile was modified. The main individual fatty acids are shown in table 1, along with the saturated (SFA) monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids groups. The high-IMF line showed higher

percentage of MUFA and lower of PUFA with relevant differences (P_r= 0.90 for both), though there were no evidence of relevant differences in SFA percentage (P_r= 0.51). The correlated response to selection on the fatty acid composition of the LTL muscle was analyzed by Martínez-Álvaro et al. (2017) in the 8th generation of selection with similar results to those shown in the liver, though differences were greater in muscle (D = 9.20 for MUFA and D = -10.3 for PUFA) compared to liver (D = 2.11 for MUFA and D = -2.48 for PUFA).

In plasma metabolites, higher concentration of triglycerides was found in low-IMF line, which could be caused by a lower uptake of this metabolite by muscles and other fat depots. The concentration of alanine transaminase (ALT) was also modified by selection, being higher in the high-IMF line. The relationship between ALT and fat deposition is not clear, but this result was also observed in the 8th generation (Martínez-Álvaro et al., 2018). No relevant differences were found in glucose, cholesterol, bile acids and albumin. The concentrations of the plasma metabolites detected were inside normal limits, indicating a good health status of the animals.

CONCLUSIONS

From these results we can conclude that a correlated response to selection for intramuscular fat content was found on the liver percentage and on its fatty acid composition, indicating a genetic relationship between those traits. Larger liver size has been proved to be related to higher liver lipogenic activity, and this liver activity plays an important role in the fat deposition of the lines, although no evidence of difference between lines was found on their liver fat content.

REFERENCES

Blasco, A. 2017. Bayesian Data Analysis for Animal Scientists. Springer International Publishing, Cham.

Blasco, A., and J. Ouhayoun. 1993. Harmonization of criteria and terminology in rabbit meat research. Revised proposal. World Rabbit Sci. 4:93–99. doi:10.4995/wrs.1996.278.

Gondret, F., J. Mourot, and M. Bonneau. 1997. Developmental changes in lipogenic enzymes in muscle compared to liver and extramuscular adipose tissues in the rabbit (Oryctolagus cuniculus). Comp. Biochem. Physiol. - B Biochem. Mol. Biol. 117:259–265. doi:10.1016/S0305-0491(97)00049-7.

Martínez-Álvaro, M., A. Blasco, and P. Hernández. 2017. Effect of selection for intramuscular fat on the fatty acid composition of rabbit meat. Animal. 1–7. doi:10.1017/S1751731117003494.

Martínez-Álvaro, M., P. Hernández, and A. Blasco. 2016. Divergent selection on intramuscular fat in rabbits: Responses to selection and genetic parameters. J. Anim. Sci. 94:4993–5003. doi:10.2527/jas.2016-0590.

Martínez-Álvaro, M., Y. Paucar, K. Satué, A. Blasco, and P. Hernández. 2018. Liver metabolism traits in two rabbit lines divergently selected for intramuscular fat. Animal. 12:1217–1223. doi:10.1017/S1751731117002695.

O'Fallon, J. V, J. R. Busboom, M. L. Nelson, and C. T. Gaskins. 2007. A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 85:1511–1521. doi:10.2527/jas.2006-491.

Sapp, R. L., J. K. Bertrand, T. D. Pringle, and D. E. Wilson. 2002. Effects of selection for ultrasound intramuscular fat percentage in Angus bulls on carcass traits of progeny. J. Anim. Sci. 80:2017–2022.

Schwab, C. R., T. J. Baas, K. J. Stalder, and D. Nettleton. 2009. Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. J. Anim. Sci. 87:2774–2780. doi:10.2527/jas.2008-1335.

Zhao, G. P., J. L. Chen, M. Q. Zheng, J. Wen, and Y. Zhang. 2007. Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. Poult. Sci. 86:2309–2314. doi:10.3382/ps.2007-00013.

Zomeño, C., V. Juste, and P. Hernández. 2012. Application of NIRS for predicting fatty acids in intramuscular fat of rabbit. Meat Sci. 91:155–159. doi:10.1016/j.meatsci.2012.01.009.

Correlated response to selection for intramuscular fat on the liver fat and its fatty acid profile

Zubiri-Gaitán, A., Martínez-Álvaro, M., Ccalta, R., Satué, K., Blasco, A., Hernández, P.

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

INTRAMUSCULAR FAT (IMF)

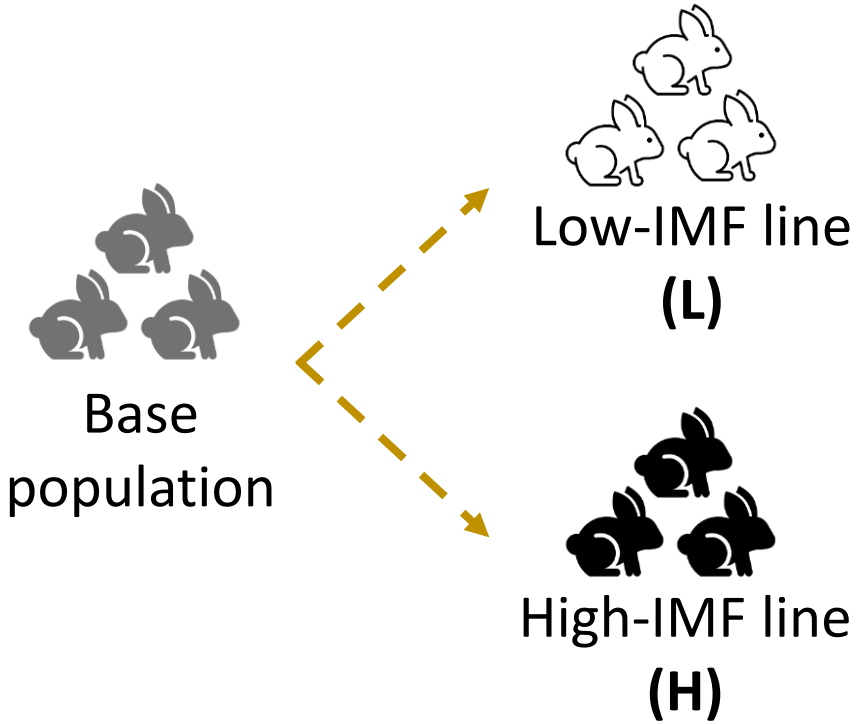
Meat quality

Juiciness

Tenderness

Flavor

Divergent selection for **IMF** in *Longissimus thoracis et lumborum (LTL)*



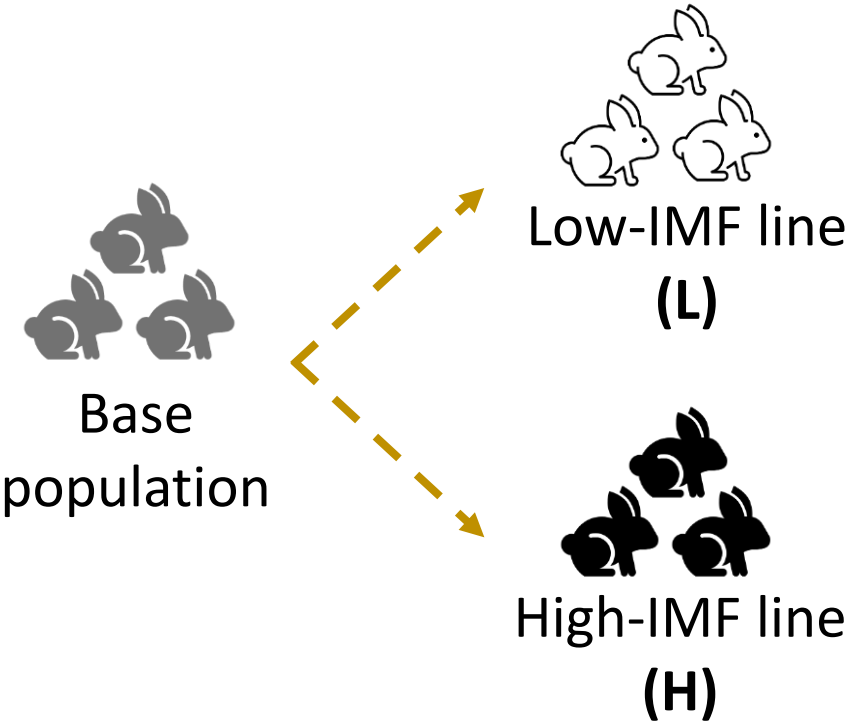
Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

INTRODUCTION

- Contemporaneous
- Same environmental conditions
- Same diet

GENETIC COMPOSITION

Divergent selection for **IMF** in *Longissimus thoracis et lumborum (LTL)*



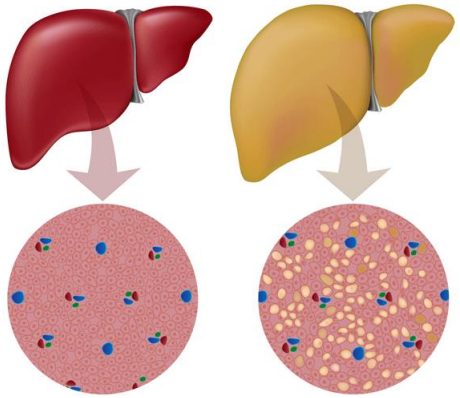
Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

INTRODUCTION

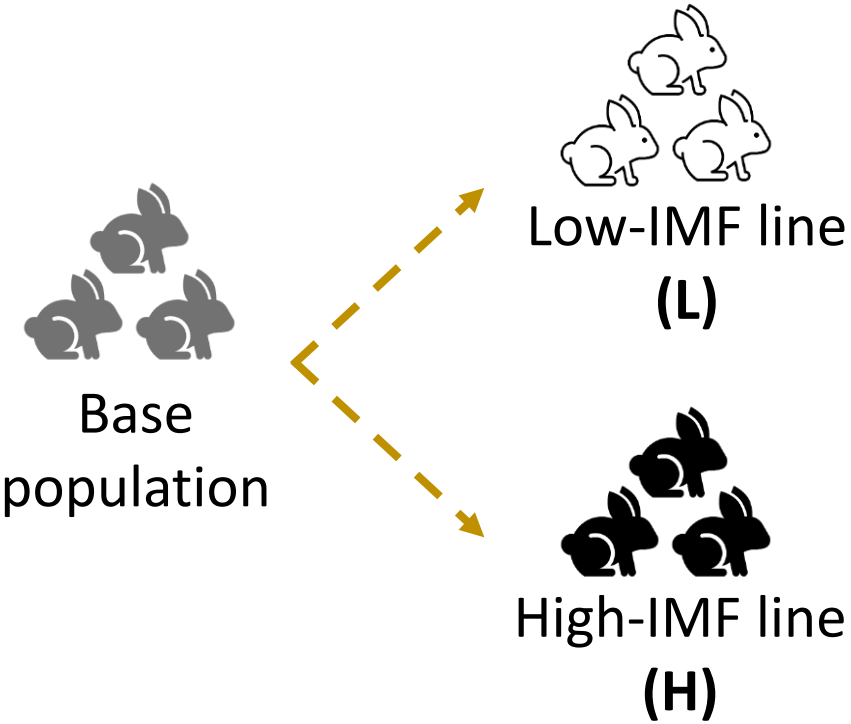
Lipogenic site in growing rabbits

LIVER

Liver lipid content?



Divergent selection for **IMF** in *Longissimus thoracis et lumborum (LTL)*



Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

OBJECTIVE

Effect of selection in:

- liver **lipids**
- liver **fatty acid** composition
- **plasma metabolites** related to liver metabolism

Metabolism of the **intramuscular fat** deposition

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

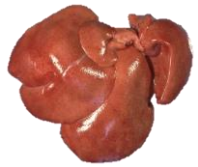
METHODOLOGY

Sampling

Low-IMF line **(27)**

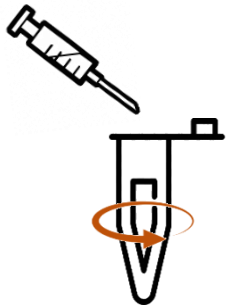
High-IMF line **(27)**

10th generation



SOXTEC → **Total lipids** (mg lipids/100mg tissue)

Gas chromatography → **Fatty acid composition** (mg FA/100mg tissue)



Plasma

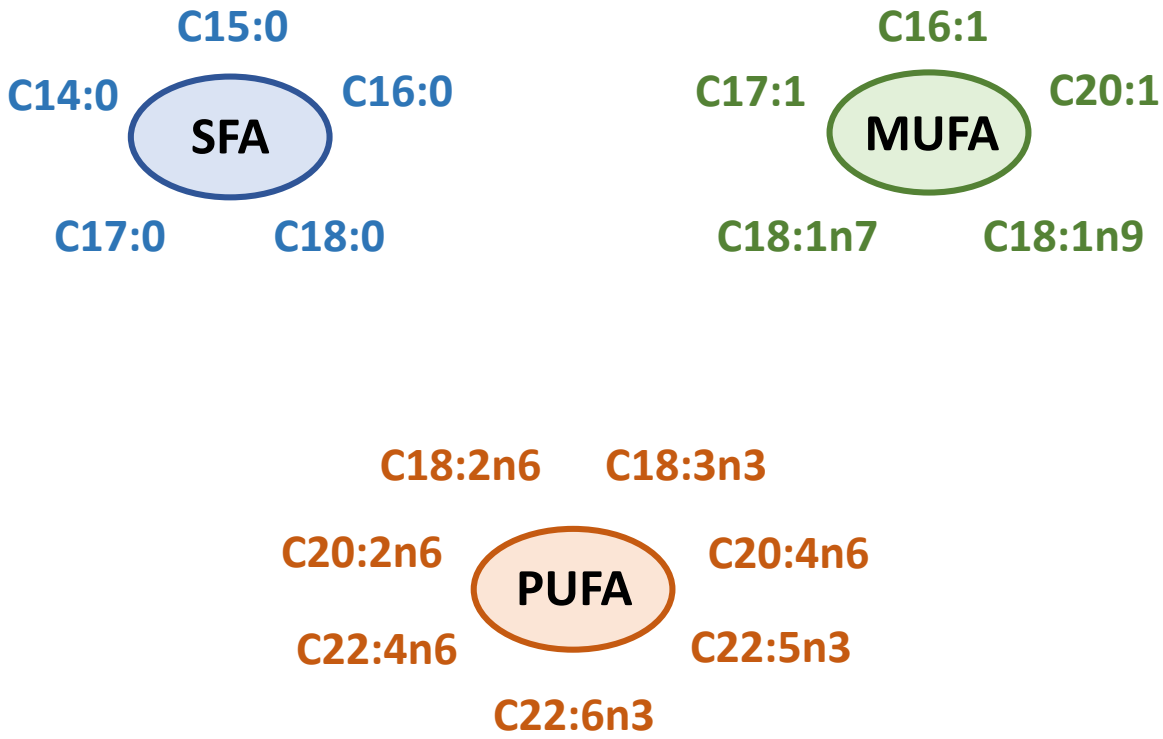
Photometric methods

Plasma metabolites

Albumin	Cholesterol
Bile acids	Glucose
Triglycerides	ALT

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

Fatty acids



$$\frac{\frac{\text{mg FA}}{100\text{ mg tissue}}}{\frac{\text{mg lipids}}{100\text{ mg tissue}}} \Rightarrow \frac{\text{mg FA}}{\text{mg lipids}}$$

Absolute values

Not compositional

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

Statistical analysis

Fixed effects:

- line
- sex
- parity order

$N(0, I\sigma^2_e)$

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Wc} + \boldsymbol{e}$$

Common litter random effect $N(0, I\sigma^2_c)$

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

Statistical analysis

Fixed effects:
- line
- sex
- parity order

N (0, $I\sigma^2_e$)

$$y = Xb + Wc + e$$

Common litter random effect N (0, $I\sigma^2_c$)

Bayesian inference

Marginal posterior distribution of phenotypic **differences**

Difference (D): median of the distribution

Probability of D>0 / D<0 (P_0)

Highest posterior density interval 95% **($HPD_{95\%}$)**

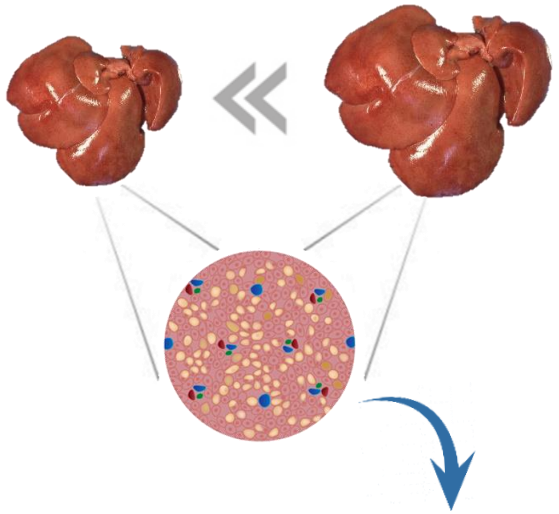
Relevant value: 1/3 of the SD of the trait

Probability of relevance (P_r)

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

Liver lipid

Low-IMF High-IMF



Differences in size and lipid metabolism previously reported

	D	$HPD_{95\%}$	P_0	r	P_r
Liver lipids	**-0.04**	**[-0.03 0.02]**	**0.62**	**-0.26**	**0.04**

No differences in **liver lipid** content between lines

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

Liver fatty acid profile

MUFA

Higher in **high-IMF**

	D	$HPD_{95\%}$	P_0	r	P_r
MUFA	1.46	[0.09 3.05]	0.97	0.67	0.86

PUFA

Higher in **low-IMF**

	D	$HPD_{95\%}$	P_0	r	P_r
PUFA	-1.46	[-3.72 0.84]	0.9	-1.22	0.59

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

Liver fatty acid profile

MUFA

Higher in high-IMF

SFA

No differences

PUFA

Higher in low-IMF

	D	$HPD_{95\%}$	P_0	r	P_r
SFA	0.69	[-1.02 2.48]	0.77	0.92	0.4

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

Liver fatty acid profile

MUFA

Higher in high-IMF

SFA

No differences

PUFA

Higher in low-IMF

Odd-chain SFA **(OCFA)**

C15:0 C17:0

Higher in **low-IMF**

	D	$HPD_{95\%}$	P_0	r	P_r
C15:0	-0.04	[-0.06 -0.01]	1	-0.01	0.98
C17:0	-0.09	[-0.17 -0.02]	0.99	-0.04	0.92

Microbiome metabolism

Propionate

Propionyl-CoA: primer for FA synthesis

Inhibitor of *de novo* lipogenesis in liver

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

Plasma metabolites

Trait	D	$HPD_{95\%}$	P_0	r	P_r
Bile acids (mol/ml)	-2.13	[-7.19 2.51]	0.8	-2.37	0.46
Albumin (g/dl)	0.3	[-0.17 0.74]	0.9	0.2	0.67
ALT (UI/l)	**2.58**	**[0.38 4.71]**	**0.99**	**1.02**	**0.92**
Cholesterol (mg/dl)	-3.85	[-16.43 7.98]	0.74	-5.31	0.41
Triglycerides (mg/dl)	**-34**	**[-56.11 -9.82]**	**1**	**-1.18**	**0.98**
Glucose (mg/dl)	0.82	[-22 20.4]	0.53	9.74	0.19

ALT higher in the **high-IMF** line

Higher amino acid metabolism in **high-IMF** line

Triglycerides higher in the **low-IMF** line

Reduced uptake in muscle of **low-IMF** line

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es

CONCLUSIONS

No differences in the **liver fat** content

Higher **MUFA** and lower **PUFA** in the high-IMF line

Higher **OCFA** in the low-IMF line ⟹ importance of the **microbiome**

Lower **triglycerides** uptake in the low-IMF line

Higher **amino acid metabolism** in the hig-IMF line

Thank you for your attention

Agostina Zubiri Gaitán, PhD. student
agzugai1@posgrado.upv.es