*Mwo*I and *Sma*I RFLPs polymorphisms of porcine obese gene and their association with carcass and meat characteristics of heavy pigs

Romina Floris[1], Bruno Stefanon[2], Alberto Pallavicini[1], Piero Susmel[2], Giorgio Graziosi[1]

[1]Dipartimento di Biologia. Università di Trieste, Italy
[2]Dipartimento di Scienze della Produzione Animale. Università di Udine, Italy

Corresponding author: Dr Romina Floris. Dipartimento di Biologia. Università di Trieste. Piazzale Valmaura 9, 34100 Trieste, Italy – Tel. +39 040 812237 – Fax: +39 040 810860 – Email: animorval@hotmail.com

Paper received November 17, 2003; accepted March 14, 2004

ABSTRACT

The obese gene encodes leptin, a 16-kDa protein involved in the regulation of fat deposition and energy consumption. Backfat is one of the peculiar characteristics of Italian ham, and represents a fundamental quality factor. Therefore, the obese gene can be considered as a candidate marker for determining economically important production traits such as backfat thickness, feed intake, and growth rate in swine. The aim was to investigate the relationship between obese gene polymorphisms and carcass and meat characteristics of pigs reared for ham production. In the present research, the analyses of three new RFLPs are reported. An *Mwo*I polymorphism occurs at nucleotide 1792, within the intron. Pigs heterozygous at this position have heavier thighs with a thinner layer of fat. Two *Sma*I polymorphisms occur at nucleotides 5018 and 5410 within the 3' UTR of the obese gene. Animals heterozygous at position 5410 have characteristics suitable for the production of San Daniele ham: lower backfat thickness and heavier thighs with a thinner fat layer, relative to other genotypes.

Key words: Leptin, Obese gene, RFLP, Ham, Pig

RIASSUNTO

*MWO*I E *SMA*I *RFLPs* ALL'INTERNO DEL GENE *OBESE* (LEPTINA) SUINO

Il gene obese *codifica per la leptina, una proteina di 16-kDa con funzione ormonale coinvolta nella regolazione del metabolismo lipidico e del consumo energetico. Il prosciutto di San Daniele è un prodotto nazionale tipico per il quale il grasso di copertura rappresenta un aspetto peculiare e caratteristico, un fattore fondamentale di qualità. Considerata l'importanza della leptina nella regolazione del metabolismo lipidico, il gene* obese *può essere considerato un gene candidato per la determinazione di caratteri economicamente importanti come lo spessore del grasso di copertura, l'assunzione di cibo e il tasso di crescita nei suini. Obiettivo della nostra ricerca è quello di individuare e studiare i polimorfismi del gene* obese *presenti nelle razze allevate in Italia in rapporto all'attitudine alla produzione di cosce per la trasformazione in prosciutto di San Daniele. In questo lavoro viene riportata l'analisi di 3 nuovi polimorfismi di tipo RFLP. Un polimorfismo identificato dall'enzima di restrizione* Mwo*I è presente in posizione 1792 all'interno dell'introne. Si è osservato che gli animali eterozigoti presentano cosce più pesanti con un minor spessore del grasso di copertura (P<0.05). Altri due polimorfismi evidenziati dalla restrizione con* Sma*I si trovano in posizione 5018 e 5410, all'estremità 3' del gene* obese*. Si è visto che gli animali eterozigoti in posizione 5410 possiedono caratteri più favorevoli alla trasformazione in prosciutto di San Daniele; infatti presentano un minor spessore sia del lardo dorsale che del grasso di copertura delle cosce, mentre il peso di quest'ultime è maggiore (P<0.01) rispetto agli altri genotipi evidenziati da SmaI. Lo scopo di individuare*

più polimorfismi associati a particolari caratteristiche della carne è quello di rendere più semplice ed efficiente la selezione degli animali adatti alla produzione di un prodotto di pregio. La caratterizzazione di polimorfismi propri del prosciutto di San Daniele permetterebbe, inoltre, di creare una "carta d'identità genetica" facile da verificare tramite un semplice esame di laboratorio.

Parole chiave: *Leptina, Gene* obese, RFLP, *Prosciutto, Maiale*

Introduction

San Daniele or Parma ham is a typical national product, covered by the D.O.P. mark and subjected to European regulations for products of protected origins. The main aims of ham producers are to improve meat quality, and to select and rear the most suitable pigs under defined conditions, to give a high quality product.

It is possible to improve thigh characteristics for ham production by supporting traditional selection, performed by breeders using mathematical–statistical methods, with molecular genetic tests that allow the most suitable animals to be identified by sampling the blood or hair of piglets.

Backfat is one of the peculiar characteristics of Italian ham, and represents a fundamental quality factor, together with intramuscular fat. Recent studies have indicated that backfat thickness can be correlated with some polymorphisms of the obese gene (Jiang and Gibson, 1999). The obese gene encodes leptin (Zhang *et al.*, 1994; Farooqi *et al.*, 1998), a 16-kDa protein with a hormonal function (Halaas *et al.*, 1997). It is secreted by adipocytes and its concentration in the blood correlates highly with the level of adipose tissue (Frederich *et al.*, 1995). Ramsay *et al.* (1998) reported that leptin mRNA expression is higher in fat pigs than in lean ones. Leptin binds to a specific receptor in the hypothalamus, which inhibits feed intake (Trayhurn *et al.*, 1998), thus interacting with body weight and energy balance (Halaas *et al.*, 1995). The association of leptin mRNA expression with fatness in pigs has been preliminarily investigated (Robeseert *et al.*, 1998).

Considering the role of leptin in the regulation of fat deposition and energy consumption, the obese gene can be considered a candidate marker for determining economically important production traits such as backfat thickness, feed intake, and growth rate in swine (Wu *et al.*, 2002). The obese gene in pigs has been sequenced and assigned to chromosome 18 (Bidwell *et al.*, 1997; Cepica *et al.*, 1999; Campbell *et al.*, 2001). However, these authors collected data in pigs reared for meat production, and therefore with different characteristics from those of heavy pigs, which are normally used for ham production.

In the present research, analyses of three new restriction fragment length polymorphisms (RFLPs) are reported, one within the intron and two in the 3' untranslated region (UTR) of the obese gene. The aim was to investigate the relationship between obese gene polymorphisms and carcass and meat characteristics of pigs reared for ham production.

Material and methods

Animals

Six populations (batches) of heavy pigs, reared for the production of Italian cured ham, were studied.

Two populations were crossbreeds of Large White x Landrace (LWxL1, LWxL2) and four were commercial hybrids (SCAAPAG1, SCAAPAG2, JSR, PIC). For each population, from 50 to 56 contemporary piglets (half female and half castrate males) were selected from each population, and their growth was recorded until the final slaughter weight (about 160 kg live weight). Only those individuals that reached the appropriate weight in the expected time were included in the analysis. The initial total live weights for each batch were recorded and the nutrition programme was established to ensure that the same commercial feeds were administered in equal amounts to the six batches. Animals were reared under standard conditions, according to the San Daniele Consortium protocol.

A complete description of rearing and feeling conditions of pigs used in the experiment is reported in a companion paper (Stefanon *et al.*, 2004)

Pigs with carcass weights within the range of 125–140 kg were selected after slaughtering. These and other culling reasons reduced the final number of pigs. Gender distribution within batches remained almost unchanged.

The weight of each carcass was recorded, and measurements of backfat thickness and the percentage of lean and fat cuts in the carcass were calculated using a Fat-O-Meter instrument. The thighs were then dissected and their weight and fat thickness recorded. Samples of both the vastus lateralis and biceps femoris were taken and stored immediately at –20 °C for DNA extraction. After 24 h, the pH was measured.

DNA analysis

DNA was extracted from frozen muscle samples using a phenol–chloroform/proteinase K method (Sambrook *et al.*, 1989), and its concentration was determined by spectrophotometry at 260 nm. The quality of the genomic DNA was assessed by electrophoresis on 1% agarose gels stained with ethidium bromide.

Primers for the amplification of the porcine obese gene, based on available genomic sequences (GenBank U66254; Bidwell *et al.*, 1997) were designed using the PRIMER 3 web program.

The fragment-1 primer pair (forward 5'-GTTACCGGAATCCAGGGTCT-3'; reverse 5'-ACAGAGTCCCTCCTGGACAA-3') amplifies the intronic region from nucleotide 1728 to 1990 (264 bp, fragment 1), and the fragment-2 primers (forward 5'-TGAAAGCTCTGGGAGGTCTG-3'; reverse 5'-TGCCTGCAACATGTGAAAGT-3') amplify the 3' UTR from nucleotide 4958 to 5653 (696 bp).

PCR reactions for fragments 1 and 2 were performed in a 25-μl total volume containing 50 ng genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 μM each primer, 0.2 units *Taq* DNA polymerase (Roche) and 1 x the manufacturer's reaction buffer. The PCR profile was 95 °C for 3 min, 60 °C for 1 min, and 72 °C for 1 min, followed by 30 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 10 min.

Amplified fragments were digested with *Mwo*I (fragment 1) or *Sma*I (fragment 2) and separated electrophoretically on a 3% SeaKem (FMC) agarose gel stained with ethidium bromide. PCR products corresponding to each allele of the *Mwo*I and *Sma*I DNA polymorphisms were sequenced on an Applied Biosystems ABI PRISM 3700 automated DNA sequencer.

NIRS analysis

Subsamples of meat were analysed for dry matter, protein, lipid, and ash contents with a chemiometric method using near-infrared reflectance spectroscopy (NIRS) equipment (Foss NIRSystems 5000), with scanning from 1100 to 2498 nm, and reading every 2 nm. Calibration and data collection was carried out using ISI 2.00 software, version 3.11 (Intrasoft International).

Chemical analysis

A group of 76 samples was used to draw a calibration curve. Samples were weighed, lyophilized for 72 h, and, after re-equilibration with air, were weighed once more and homogenized in a blender. Intramuscular lipid percentage was calculated from petroleum extraction (40/60) in a "Randall" apparatus. Dry matter and ash percentages were determined according to Martinotti *et al.* (1987). Protein percentage was calculated by subtraction.

Data analysis

All variables were analysed by the general linear model (GLM), using of the SPSS statistical software package (1995). Normal distribution of data was assessed using the Kolmogorov-Smirnov (Lilliefors) test (SPSS, 1997). *Mwo*I and *Sma*I polymorphisms, batches, and sex, and their effects on carcass and meat composition, taken both singly and in combination, were considered to be fixed factors. Because of the different numbers of samples in the batches, the means were calculated according to the least squares procedure (LSMEANS). Analysis of variance of carcass weight of animals indicated a significant differences between batches (LWxL 132.6 and 127.6, SCAAPAG 139.0 and 133.8, JSR 131.5 and PIC 130.9).

Table 1. Frequencies of *Mwo*I polymorphism at nucleotide 1792 of the obese gene.

Commercial hybrid	N.	Allele A	Allele B	Homozygote A/A		Heterozygote A/B		Homozygote B/B		N.
LWxL1	51	0.01	0.99	0	(0%)	1	(2%)	50	(98%)	51
LWxL2	36	0.08	0.92	0	(0%)	6	(17%)	30	(83%)	36
SCAAPAG1	31	0.05	0.95	0	(0%)	3	(9%)	29	(91%)	32
SCAAPAG2	47	0.11	0.89	0	(0%)	10	(21%)	37	(79%)	47
JSR	34	0.06	0.94	0	(0%)	4	(11%)	31	(89%)	35
PIC	31	0.00	1.00	0	(0%)	0	(0%)	33	(100%)	33

Results and discussion

Mwo*I polymorphism at nucleotide 1792 of the obese gene*

*Mwo*I digestion of fragment 1 (264 bp) produced a consistent 28-bp band in each sample, plus two bands of 33 bp and 203 bp (allele A), or a unique band of 236 bp (allele B; Fig.1). Allele A showed a low allelic frequency, below 0.11 (Table 1).

Direct sequencing of the fragment 1 PCR product revealed a C/G point mutation at position 1792, which abolishes the *Mwo*I restriction site, creating a new restriction site for the *Mnl*I enzyme.

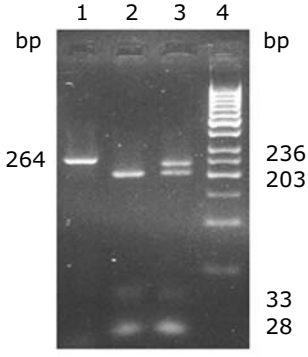
Analysis of the *Mwo*I polymorphism associated with meat characteristics is reported in Table 2. Only two genotypes were considered because genotype A/A did not occur. Data recorded at the slaughterhouse included carcass weight, backfat thickness, and percentage of lean and fat in the carcass, as well as ham weight and fat thickness.

Table 2. Effect of *Mwo*I-polymorphism at the position 1792 on the meat characteristics (least square means).

Traits		Heterozygote A/B	Homozygote B/B	Effects				
				Mwo I – polymorphism	Sex	*Mwo* I – polymorphism x Sex	Weight (covariated)	RMSE
Live weight	kg	135.4	132.5	ns	ns	ns	-	7.38
Lean percentage	%	46.8	47.8	ns	***	*	ns	4.00
Backfat thickness	mm	26.4	26.0	ns	***	*	ns	4.84
Back muscle	"	53.9	54.8	ns	***	ns	***	6.78
Ham weight	kg	13.7	13.5	*	ns	ns	ns	1.00
Ham fat	mm	27.7	29.1	*	***	ns	ns	5.72
Biceps femoris:								
Dry matter	%	27.3	27.2	ns	ns	ns	***	0.44
Ash content	"	1.22	1.22	ns	ns	ns	***	0.02
Lipid	"	2.07	2.01	ns	***	ns	***	0.81
Protein	"	24.00	23.96	ns	ns	ns	***	0.47
Vastus lateralis:								
Dry matter	%	27.5	27.6	ns	***	ns	ns	0.56
Ash content	"	1.20	1.21	ns	ns	ns	ns	0.04
Lipid	"	3.33	3.20	ns	***	ns	ns	0.91
Protein	"	23.03	23.17	ns	***	ns	ns	0.64

*ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001*

Figure 1. *Mwo*I polymorphism in the porcine leptin gene.



Lane 1: unrestricted amplification product;
lane 2: homozygote A/A;
lane 3: heterozygote A/B;
lane 4: 50-bp ladder.

Furthermore, the chemical composition of the vastus lateralis and biceps femoris muscles was evaluated.

All data were analysed as a function of the *Mwo*I polymorphism, sex, and covariate weight readings.

Heterozygote A/B pigs had heavier thighs with a thinner fat layer ($P < 0.05$; Table 2). It is possible that this mutation affects mRNA stability or translation efficiency, resulting in specific biological effects. An intron not only coordinates correct splicing, but can also influence gene expression by interacting with transcription factors (Finkbeiner, 2001). The *Mwo*I polymorphism might influence the interaction of transcription factors by altering the binding site. The reported polymorphism may also act as a molecular marker linked to a specific locus that controls backfat thickness.

There are different opinions on the influence of the obese gene intron on the regulation of the gene itself.

Kennes *et al.* (2001) found that an A/T point mutation at position 2845 was associated with feed intake and growth rate ($P < 0.0078$) in two different populations selected for higher and lower backfat thickness. These data suggest that these polymorphisms influence such traits.

According to Jiang and Gibson (1999), the *Taq*I polymorphism at position 1112 does not influence meat characteristics. This result was probably affected by the low allelic frequency of the mutation within the pig population. According to the authors, the low allelic frequency is due to selection and indicates that the mutation is unfavourable.

The allelic frequency of the mutation at the *Mwo*I polymorphic site is also low. It has been proposed that animals with this polymorphism might be less suitable for the production of thighs with the characteristics required for San Daniele ham. However, the data reported here indicate that only pigs with this marker produce the heavier ham with the low ham-fat thickness that is required by the San Daniele Consortium.

Table 2 indicates that A/B animals are, on average, heavier and have a lower percentage of lean cuts, thicker backfat, and thinner back muscle. These parameters are the opposite of those pertaining to the pigs' thighs, which suggests the hypothesis that pre-emptive selection of young animals leads to a preference for pigs without this haplotype, with leaner carcasses but fatter thighs.

This hypothesis could be confirmed by widening the analysis to include a group of non-selected animals, and comparing the pigs' genotypes with the quality of the final product.

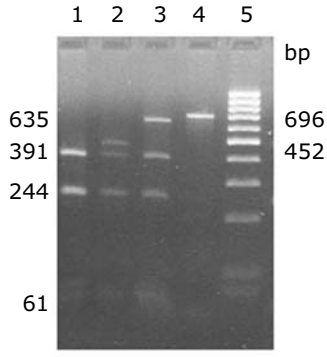
A significant interaction with sex was observed for the last data ($P < 0.001$). A complete analysis of the Least Square Means of the sex is reported in a companion paper (Stefanon *et al.*, 2004).

On farms, male pigs are castrated, and therefore tend to become excessively fat. Consequently, the sex of the animal influences all variables that correlate with lipidic content. Further investigation may elucidate this influence.

Sma*I polymorphisms at the 3' UTR of the obese gene*

Amplified fragment 2 (696 bp) has two *Sma*I restriction sites, with three different patterns. Three alleles were identified: allele C produces three bands of 61 bp, 244 bp, and 391 bp; allele D two bands of 244 bp and 452 bp; and allele E two bands of 61 bp and 635 bp (Fig. 2). The population analysed showed no homozygotes for the D and E alleles. Allelic frequencies are given in Table 3.

Figure 2. *Sma*I polymorphism in the porcine leptin gene.



Lane 1: homozygote C/C;
lane 2: heterozygote C/D;
lane 3: heterozygote C/E;
lane 4: unrestricted amplification product;
lane 5: 100-bp ladder.

Direct sequencing of the fragment 2 PCR product showed two point mutations: the first a C/T substitution at position 5018, which eliminates the first restriction site for *Sma*I (allele D); the second is a G/A substitution at position 5410, which causes the deletion of the second site (allele E).

Table 4 reports the effects of the *Sma*I polymorphisms at positions 5018 and 5410 on meat characteristics.

Analysis showed a significantly different thigh weights ($P < 0.01$) among these genotypes, although total carcass weight seemed to have a strong influence as well ($P < 0.001$). The percentages of dry matter ($P < 0.01$) and ash content ($P < 0.05$) in the vastus lateralis muscle were also significantly different in these pigs: ash content is higher in C/E animals, which indicates a lower water content. Generally, compared with both heterozygote C/D and homozygote C/C animals, heterozygote C/E pigs display more suitable characteristics for the production of San Daniele ham: lower backfat thickness and heavier thighs with a thinner fat layer.

C/D heterozygote individuals for SmaI at position 5018 are less suitable for the production of San Daniele quality ham because the thickness of the thigh fat layer is greater in these animals.

The *Sma*I polymorphism at position 5410 lies within the 3' UTR of the gene, which determines the half-life of the mRNA (Pesole *et al.*, 2001). This mutation could lie within an important site for mRNA regulation and influence thigh characteristics by fine-tuning leptin synthesis. A search of the "UTRScan" database neither confirmed nor rejected this hypothesis. On the other hand, this polymorphism could be a marker for the actual mutation that influences the translation or half-life of leptin mRNA.

Conclusions

Detection of a whole set of polymorphisms associated with particular meat characteristics would allow selection of the most suitable animals for the production of the prized San Daniele hams. The aim is to develop a reliable and objective instrument with which to optimise rearing proto-

Table 3. Frequencies of *Sma*I polymorphisms at the 3' UTR of the obese gene.

Commercial hybrid	N.	Allele C	Allele D	Allele E	Homozygote C/C		Heterozygote C/D		Homozygote C/E		N.
LWxL1	51	0.92	0.08	0.00	42	(82.3%)	9	(17.7%)	0	(0%)	51
LWxL2	36	0.75	0.18	0.07	20	(55.5%)	11	(30.5%)	5	(14%)	36
SCAAPAG1	31	0.92	0.03	0.05	26	(83.9%)	2	(6.45%)	3	(9.65%)	31
SCAAPAG2	47	0.83	0.07	0.10	31	(65.9%)	7	(14.9%)	9	(19.2%)	47
JSR	34	0.94	0.02	0.04	30	(88.24%)	1	(2.94%)	3	(8.82%)	34
PIC	31	0.97	0.03	0.00	29	(93.55%)	2	(6.45%)	0	(0%)	31

Table 4. Effect of SmaI-polymorphisms at the 3' UTR of the obese gene on the meat characteristics (least square means).

Traits		Homozygote C/C	Heterozygote C/D	Heterozygote C/E	Effects				
					SmaI – polymorphism	Sex	*SmaI* - polymorphism x Sex	Weight (covariated)	RMSE
Live weight	kg	132.2	132.8	134.8	ns	ns	ns	-	46.97
Lean percentage	%	48.2	47.4	49.3	ns	**	ns	ns	17.70
Backfat thickness	mm	25.6	26.4	24.2	ns	**	ns	ns	24.19
Back muscle	"	55.6	55.3	57.7	ns	**	ns	**	44.90
Ham weight	kg	13.4^{B}	13.5^{B}	14.3^{A}	**	na	na	***	0.93
Ham fat	mm	27.6	29.7	27.2	ns	ns	ns	ns	34.35
Average pH		5.67	5.66	5.65	ns	ns	ns	ns	$2.74x10^{-2}$
Biceps femoris:									
Dry matter	%	25.58	25.43	25.47	ns	ns	ns	***	0.62
Ash content	"	1.17	1.17	1.17	ns	ns	ns	*	$2.38x10^{-4}$
Lipid	"	2.07	1.83	1.96	ns	ns	ns	*	0.85
Protein	"	22.35	22.42	22.34	ns	ns	ns	ns	0.72
Vastus lateralis:									
Dry matter	%	25.69^{B}	26.30^{A}	25.68^{B}	**	*	ns	ns	0.90
Ash content	"	1.16^{ab}	1.15^{b}	1.17^{a}	*	ns	ns	ns	$4.26x10^{-4}$
Lipid	"	3.14	3.33	2.70	ns	**	ns	ns	1.06
Protein	"	21.25	21.81	21.85	ns	ns	ns	ns	2.81

*ns = not significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001.*
A, B on the same row denote means significantly different for P<0.01
a, b on the same row denote means significantly different for P<0.05

cols. Furthermore, it would be useful to determine the polymorphisms associated with San Daniele ham production, to create a genetic fingerprint detectable with a simple experiment.

We thank Martini Alimentari S.N.C., Castiglione di Ravenna, Italy, for their assistance and cooperation.

This research was supported by Regione Friuli Venezia Giulia L.R. 3/98, 1999/2000 and MURST project 2001/2002.

REFERENCES

BIDWELL, C.A., JI, S., FRANK, G.R., CORNELIUS, S.G., WILLIS, G.M., SPURLOCK, M.E., 1997. Cloning and Expression of the Porcine Obese Gene. Anim. Biotechnol. 8:191-206.

CAMPBELL, E.M.G., FAHRENKRUG, S.C., VALLET, J.L., SMITH, T.P.L., ROHRER, G.A., 2001. An updated linkage and comparative map of porcine chromosome 18. Anim. Genet. 32:375-379.

CEPICA, S., YERLE, M., STRATIL, A., SCHROFFEL, J., REDL, B., 1999. Regional localization of porcine MYOD1, MYF5, LEP, UCP3 and LCN1 genes. Anim. Genet. 30: 476-478.

FAROOQI, S., RAU H., WHITEHEAD, J., O'RAHILLY, S., 1998. Obese gene mutations and human obesity. Proc. Nutr. Soc. 57: 471-475.

FINKBEINER, S., 2001. New roles for introns: sites of combinatorial regulation of Ca2+- and cyclic

AMP-dependent gene transcription. Sci. STKE 94: PE1.

FREDERICH, R.C., LOLLMANN, B., HAMANN, A., NAPOLITANO-ROSEN, A., KAHN, B.B., LOWELL, B.B., FLIER, J.S., 1995. Expression of obese mRNA and its encoded protein in rodents. Impact of nutrition and obesity. J. Clin. Invest. 96:1658-1663.

HALAAS, J.L., GAJIWALA, K.S., MAFFEI, M., COHEN, S.L., CHAIT, B.T., RABINOWITZ, D., LALLONE, R.L., BURLEY, S.K., FRIEDMAN, J.M., 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 269:543-546.

HALAAS, J.L., BOOZER, C., BLAIR-WEST, J., FIDAHUSEIN, N., DENTON, D.A., FRIEDMAN, J.M., 1997. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc. Natl. Acad. Sci. USA. 94:8878-8883.

JIANG, Z.H., GIBSON, J.P., 1999. Genetic polymorphisms in the leptin gene and their association with fatness in four pig breeds. Mamm. Genome. 10:191-193.

KENNES, Y. M., MURPHY, B.D., POTHIER, F. PALIN, M.-F., 2001. Characterization of swine leptin (LEP) polymorphisms and their association with production traits. Anim. Genet. 32:215-218.

MARTINETTI, F., ANTONGIOVANNI, M., RIZZI, L., SANTI, E., BITTANTE, G., 1987. Metodi di analisi per la valutazione degli alimenti d'impiego zootecnico. Quaderni metodologici n. 8. IPRA-C.N.R., Roma, Italy.

PESOLE, G., MIGNONE, F., GISSI, C., GRILLO, G., LICCIULLI, F., LIUNI, S., 2001. Structural and functional features of eukaryotic mRNA untranslated regions. Gene. 276:73-81.

RAMSAY, T.G., YAN, X., MORRISON, C., 1998. The obesity gene in swine: sequence and expression of porcine leptin. J. Anim. Sci. 76:484-490.

ROBERT, C., PALIN, M.F., COULOMBE, N., ROBERGE, C., SILVERSIDES, F.G., BENKEL, B.F., MCKAY, R.M., PELLETIER, G., 1998. Backfat thickness in pigs is positively associated with leptin mRNA levels. Can. J. Anim. Sci. 78:473-482

SAMBROOK, J., FRITSCH, E.F., MANIATIS, T., 1989. Molecular cloning. 2nd ed. Cold spring Harbor Laboratory, New York, USA.

STEFANON, B., FLORIS, R., BRAGLIA, S., DAVOLI, R., FONTANESI, L., DALL'OLIO, S., GRAZIOSI, G., SUSMEL, P., RUSSO, V., 2004. A new approach in association study of single nucleotide polymorphism of genes for carcass and meat quality traits in commercial pigs. Ital. J. Anim. Sci. 3:177-189.

TRAYHURN, P., DUNCAN, J.S., HOGGARD, N., RAYNER, D.V., 1998. Regulation of leptin production: a dominant role for the sympathetic nervous system? Proc. Nutr. Soc. 57:413-419.

WU, X.L., LEE, C., JIANG, J., PENG,Y.L., YAN, H.F., YANG. S.L., XIAO, B.N., LIU, X.C., SHI, Q.S., 2002. Quantitative trait loci mapping for porcine backfat thickness. Asian Australas. J. Anim. Sci. 15:932-937.

ZHANG, Y., PROENCA, R., MAFFEI, M., BARONE, M., LEOPOLD, L., FRIEDMAN, J.M., 1994. Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425-432.