Preliminary study of the effect of the IGF-II genotype on meat quality in pigs

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Introduction

During the last decades, pig breeding programs have been strongly focusing on selection for fast growth of lean meat. However, this is often accompanied with a negative effect on certain meat quality characteristics. Recently, Van Laere et al. (2003) identified the paternally expressed QTN (Q allele), affecting the percentage lean meat in pigs with apparently no negative effects on meat quality, as a mutation located in the regulatory sequence of the IGF-II gene (insulin-like growth factor II). Because of the mutation, an interaction with a repressor is probably abrogated and as a consequence, a threefold increase of IGF-II mRNA expression in skeletal muscle can be observed. These authors reported an effect on muscle growth, fat deposition and size of the heart, but no effect on birth weight. Jeon et al. (1999) found a large effect of the QTN on the lean meat content in the ham, heart weight and backfat thickness in a Wild boar/Large White intercross. A moderate effect on the reflectance value could be detected. Nezer et al. (1999) concluded that the IGF-II genotype causes an increase in carcass lean content at the expense of fat, but found no evidence for an effect on growth performance or meat quality measurements in a Large White/Piétrain intercross. Our research group found in a preliminary study that the IGF-II genotype was associated with a slightly lower muscle fibre cross sectional area (Van den Maagdenberg et al., 2005).
The aim of this study was to investigate the effect of the IGF-II paternal allele (Qpat/qpat) on bio-electrical impedance as a measure of carcass lean content and on meat quality parameters like pH and PQM 40 minutes and 24 hours post mortem, water holding capacity, colour and tenderness.

Material and methods

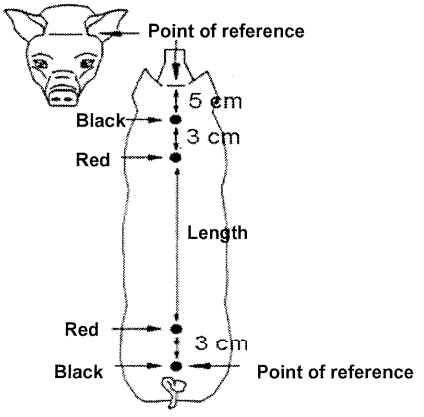
Animals: Animals (entire males) originated from Rattlerow-Seghers (Belgium) and were the progeny of five sires heterozygous for IGF-II (Qpat and qpat alleles). Because of the paternal imprinting of the IGF-II gene (Nezer et al., 1999), the genotype of the mother was not taken into consideration. The pigs tested negative for the halothane gene (NN). An equal number of Qpat and qpat animals (n=15) was included in the study. During the trial for practical reasons, the pigs were housed into two different types of pens with a similar area per pig, but the number of pigs per housing pen was different. The number of Qpat/qpat animals per pen type was 5/8 and 10/7. This factor was not taken into consideration.

Slaughtering: The pigs were slaughtered in groups of six animals, 3 animals per genotype, at two different slaughterhouses. Animals were slaughtered following electrical stunning at an age of 26 weeks (average 182 days – min/max: 174/192 days). After the bio-electrical impedance measurements, the left carcass side was cut while still warm and the weight of the *Longissimus (LD), Semimembranosus (SM) and Triceps brachii (TB), Psoas Major, Semitendinosus* and *Masseter* were recorded. Carcass weight at slaughter was on average 89.8 ± 15.3 kg. Carcass growth was calculated as carcass weight divided by age at slaughter.

Bio-electrical impedance measurements: Conventional carcass classification was not possible in this trial. Therefore, bio-electrical impedance measurements were done as an approximate estimate for the carcass lean content. A four-terminal impedance plethysmograph (Model BIA-101, RJL Systems Detroit, MI) was used to obtain resistance (Rs, Ω) and reactance (Xc, Ω) readings on the whole carcass immediately after slaughtering.

Lean tissue is a highly conductive substance composed mostly of water containing electrolytes whereas fat serves as an insulator that impedes the flow of an applied electrical current (Swatland, 1984).

The plethysmograph transmits a deep homogenous alternating current (800 µA; 50kHz) between the outer two transmitter electrodes (black) and measures the voltage drop between the inner two detector electrodes (red) (Swantek et al., 1992).
Measurements were made on the whole carcass by inserting needles to the specific positions as shown in the figure (according to Swantek et al., 1992) and attaching the electrodes to the needles.
For an alternating current, the impedance Z (Ω) is calculated as $\sqrt{(Rs^2 + Xc^2)}$ and the electrical volume EV (cm^2/Ω) as $Length^2/\sqrt{(Rs^2 + Xc^2)}$.



Determination of meat quality parameters: Meat quality measurements were done on the right carcass side. pH and conductivity (Pork Quality Meter, PQM) were measured in the right carcass side in the *Longissimus* between the 6th and the 7th rib and in the *Semimembranosus* at 40 minutes and 24 hours post mortem (pH_1, pH_u, PQM_u). The LD, SM and TB were removed 24 hours post mortem and sampled for further meat quality measurements. Water holding capacity was evaluated by measuring drip loss as the percentage weight loss after 48 hours at 4°C and by the filter paper method according to Kauffman et al. (1986). Meat colour was determined by measuring the CIE L* a* b* values with a HunterLab Spectrocolorimeter after 30 minutes of blooming. The CIE L* value gives an indication of the lightness (Black-White axis), CIE a* gives an indication of the redness (red-green spectrum) and CIE b* gives an indication of the yellowness (yellow-blue spectrum). Shear force was determined using a Lloyd TA 500 Texture Analyser equipped with a Warner-Bratzler shear on cooked (40 minutes at 70°C) cylindrical meat samples with a diameter of 1.27 cm taken parallel to the direction of the muscle fibres. The shear force value corresponds with the highest peak and the work (N.mm) is measured by calculating the area under the shear deformation curve. The Warner-Bratzler work can be divided into a part related with myofibrillar component (first peak) and one related with the connective tissue (rest of the curve's surface) (Claeys et al., 2000).

Statistics: The effect of the IGF-II genotype on carcass parameters was analysed with a univariate general linear model with IGF-II genotype (G) and sire (S) as fixed factors. Sire had a significant influence on carcass parameters and was therefore taken into consideration. The pH_1, pH_u, PQM_u and meat quality measurements were analysed separately per muscle with ANOVA for the effect of IGF-II genotype. Sire number did not have an effect on meat quality parameters and because of the rather low number of samples, sire was not taken into consideration for these variables. Effect of muscle on meat quality measurements was also analysed separately across genotypes with ANOVA. All data were analysed using SPSS 11.0 for windows.

Results and discussion

Growth and carcass parameters: Least square means, standard errors and levels of significance for carcass growth and carcass quality traits are shown in Table 1. No differences between the two genotypes were found for carcass weight and carcass growth.

The relative weight of the LD (based on carcass weight) and the relative weight of the sum of the muscles removed from the carcass immediately after slaughter were significantly higher in animals with the IGF-II genotype.

Although no significant difference was found for the bio-electrical impedance measurements, these data suggest a tendency for more lean meat in the Qpat animals. The impedance, inversely related with the carcass lean content is lower and the electrical volume, proportional with the lean meat content, is higher in Qpat compared to the qpat animals.
The growth and carcass parameters are in accordance with the findings of Jeon et al. (1999) and Nezer et al. (1999), namely the IGF-II genotype affects the percentage lean meat in pigs without an effect on growth performance.

Table 1: Least square means and standard errors for growth and carcass quality traits per IGF-II genotype (Qpat and qpat)

	Qpat			qpat			P	
	Mean	SE	N	Mean	SE	N	G	S
Age (days)	183.7	1.4	15	183.4	1.3	15		***
Slaughter weight (kg)	92.8	44.0	15	90.7	42.7	15		
Carcass growth (g/day)	503.5	21.6	15	493.2	21.0	15		
Z (Ω)	80.8	2.4	14	83.4	2.5	13		
EV (cm^2/Ω)	116.1	4.9	14	106.8	5.0	13		
% LD	2.83	0.06	14	2.63	0.06	14	*	***
% Muscle	6.13	0.10	14	5.72	0.10	14	*	***
Legend: *** = P < 0.005 ** = P < 0.01 * = P < 0.05 T = P < 0.1 G = IGF-II genotype – S = Sire								

pH and PQM: Means and standard errors for pH and PQM are shown in Table 2. Although the pigs are homozygous for the halothane gene (NN), one carcass (Qpat animal) was classified as a PSE (Pale, Soft and Exudative) carcass (pH_1 < 5.5 and L* value > 60). The mean pH_1 was lower (P < 0.1) for Qpat animals, but this was due to this one PSE carcass. No other significant effects of the IGF-II genotype could be detected for pH_1, pH_u and PQM_u.

Table 2: Ordinary means and standard errors for pH and PQM values per IGF-II genotype (Qpat and qpat) and muscle (LD and SM)

	Muscle	Qpat			qpat			P
		Mean	SE	N	Mean	SE	N	G
pH_1	LD	6.15	0,08	15	6.32	0,05	15	T
pH_u		5.57	0,02	15	5.55	0,02	15	
PQM_u		7.13	0,74	15	6.16	0,57	15	
pH_1	SM	6.26	0,10	13	6.40	0,06	12	
pH_u		5.65	0,05	15	5.66	0,05	15	
PQM_u		8.54	0,63	15	8.42	0,78	15	
Legend: *** = P < 0.005 ** = P < 0.01 * = P < 0.05 T = P < 0.1 G = IGF-II genotype								

Colour: No significant differences were found in the meat L* and b* values between the two IGF-II genotypes (Table 3), although for the three muscles, the L* values for the Qpat animals were always slightly lower, and therefore the meat was somewhat darker.

The a* values were always higher in the three muscles for the animals with the IGF-II genotype (for the LD and TB: $P < 0.1$). The meat of the Qpat animals has a slightly more red colour.

Significant differences were found between the muscle types. TB is a more oxidative muscle compared to the SM and LD (Laborde et al., 1985). Muscles vary in myoglobin content, the major pigment-containing compound in meat, based on the physiological role of the muscle. Although the LD and the SM are two muscles with an equivalent metabolism, the L*, a* and b* values were significant different ($P < 0.05$). The TB is an oxidative muscle and significant differences were found for L* and a* values ($P < 0.05$) compared with the two other muscles. The L* values in this study correspond with the L* values measured by Warner et al. (1993) for the three different muscles.

Table 3: Ordinary means and standard errors for colour parameters per IGF-II genotype (Qpat and qpat) and muscle (LD and SM)

		Qpat		qpat		P
		Mean	SE	Mean	SE	G
L* value	LD	54.35	0,86	54.96	0,86	
a* value		6.20	0,31	5.33	0,33	T
b* value		14.77	0,15	14.63	0,16	
L* value	SM	47.53	1,30	49.03	1,43	
a* value		8.36	0,68	8.09	0,87	
b* value		15.15	0,29	15.47	0,28	
L* value	TB	39.76	0,95	40.77	0,95	
a* value		12.19	0,36	11.25	0,39	T
b* value		14.68	0,31	14.64	0,35	

N = 15/15, 12/12, 13/10 for respectively LD, SM, TB and Qpat/qpat animals
Legend: *** = $P < 0.005$ ** = $P < 0.01$ * = $P < 0.05$ T = $P < 0.1$
G = IGF-II genotype

Shear force: No significant differences were found for the shear force values, the work and the work separated in a myofibrillar and collagen component between the two genotypes (Table 4) for the SM and the TB. For the LD, a tendency was found ($P < 0.1$) for a lower myofibrillar work and a lower first peak for animals with the IGF-II genotype. Although the differences were not significant, the shear force values were always lower for the Qpat animals in the three different muscles, suggesting that the IGF-II genotype could be associated with more tender meat. This has to be confirmed on a larger number of animals. No clear differences between the two genotypes for work related with the collagen content was found.

Significant differences were found between the three muscle types for shear force values, work and work associated with the myofibrillar component of the meat. The SM has the highest shear force values compared to the LD and the TB. The TB is a more tender muscle than the LD according to the shear force values and the values for work. This is in accordance with the tenderness ratings of Wheeler et al. (2000) and the shear force evaluation of 40 bovine muscles of Belew et al. (2003).

For the three different muscles, no difference in the work related with the collagen component was found. The collagen work is well correlated with the collagen content (Claeys et al., 2000). According to Wheeler et al. (2000), the collagen content decreases from the TB to the SM to the LD. Although the TB contains more visible collagen in the muscle (epimysium and perimysium), these collagen plates are avoided when measuring shear force, so the values

given in Table 4 are related to the collagen between the fibre bundles (endomysium) and can explain the contradictory results with Wheeler et al. (2000).

Table 4: Ordinary means and standard errors for shear force parameters per IGF-II genotype (Qpat and qpat) and muscle (LD and SM)

		Qpat		qpat		P
		Mean	SE	Mean	SE	G
Shear force (N)	LD	28.18	0,99	30.38	0,96	
First peak (N)		27.14	0,93	29.64	0,89	T
Work (N.mm)		248.9	8,4	265.1	9,9	
Myofibrillar work (N.mm)		141.2	5,5	157.9	6,1	T
Collagen work (N.mm)		107.8	5,0	108.0	5,9	
Shear force (N)	SM	34.74	2,28	36.11	1,66	
First peak (N)		33.73	2,10	34.52	1,74	
Work (N.mm)		299.6	15,9	288.7	13,5	
Myofibrillar work (N.mm)		187.0	15,0	190.3	12,0	
Collagen work (N.mm)		111.8	9,1	98.5	6,2	
Shear force (N)	TB	27.98	1,06	28.44	1,23	
First peak (N)		27.34	1,10	28.12	1,11	
Work (N.mm)		234.9	10,3	242.4	11,5	
Myofibrillar work (N.mm)		129.9	5,9	135.1	6,2	
Collagen work (N.mm)		105.3	5,8	107.3	6,4	

N = 15/15, 12/12, 13/10 for respectively LD, SM, TB and Qpat/qpat animals
Legend: *** = P < 0.005 ** = P < 0.01 * = P < 0.05 T = P < 0.1
G = IGF-II genotype

Water holding capacity: No significant differences were found for the % drip loss and the filter paper method as measures for water holding capacity between the two genotypes (Table 5).

Table 5: Ordinary means and standard errors for water holding capacity measurements per IGF-II genotype (Qpat and qpat) and muscle (LD and SM)

		Qpat		qpat		P
		Mean	SE	Mean	SE	G
Drip loss (%)	LD	5.56	0,53	5.78	0,74	
Filter paper method (mg)		69.58	6,30	67.21	8,95	
Drip loss (%)	SM	2.91	0,34	3.33	0,44	
Filter paper method (mg)		58.67	5,83	56.71	4,92	
Drip loss (%)	TB	0.73	0,07	0.79	0,10	
Filter paper method (mg)		27.80	3,18	28.66	4,99	

N = 15/15, 12/12, 13/10 for respectively LD, SM, TB and Qpat/qpat animals
Legend: *** = P < 0.005 ** = P < 0.01 * = P < 0.05 T = P < 0.1
G = IGF-II genotype

In contrast with the halothane genotype where the muscle fibre area is enlarged with negative consequences for drip loss (Essén-Gustavsson et al., 1992, Pedersen et al., 2001), the IGF-II genotype seems to have no effect on muscle fibre area (Van den Maagdenberg et al., 2005). Muscle type has a significant influence on water holding capacity with TB as the muscle with the highest water holding capacity. In contrast with the results of Warner et al. (1993), the water holding capacity of the SM is higher than the LD, but this is in accordance with the somewhat higher final pH in the SM compared to the LD (Table 2).

Conclusion

Selection for fast growth or lean meat is often accompanied with a negative effect on certain meat quality characteristics. Major genes like the halothane gene and the RN^- gene have negative effects on important pork quality attributes. This preliminary study suggests that the IGF-II genotype has no negative effect on meat quality. However, an extended trial is necessary to have a more profound view on the effect of the IGF-II genotype on meat quality.

Acknowledgement

This research is financially supported by the Institute for the Promotion of Innovation through Science and Technology, Brussels. Stefaan Lescouhier, Tom Luyten, Mark Seynaeve and Erwin Turtelboom are acknowledged for their skilled assistance.

References

Belew J.B., Brooks J.C., McKenna D.R. & Savell J.W. (2003). Warner-Bratzler shear evaluations of 40 bovine muscles. Meat Science, 64, 507 - 512.

Claeys, E., De Smet, S., Balcaen, A. & Demeyer, D. (2000). Analyse du profil de la mesure de force de cisaillement. Compte rendu des VIII journées des sciences du muscle et technologies de la viande, 237 – 240.

Essén-Gustavsson B., Karlström K. & Lundström K. (1992). Muscle fibre characteristics and metabolic response at slaughter in pigs of different halothane genotypes and their relation to meat quality. Meat Science, 31, 1 - 11.

Jeon J.T., Carlborg Ö., Törnsten A., Giuffra E., Amarger V., Chardon P., Andersson-Eklund L., Andersson K., Hansson I., Lundström K. & Andersson L. (1999). A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. Nature Genetics, 21, 157 - 158.

Kauffman R.G., Eikelenboom G., Van der Wal P.G., Merkus G. & Zaar M. (1986). The use of the filter paper to estimate drip loss of porcine musculature. Meat science, 18, 191 - 200.

Laborde D., Talmant A. & Monin G. (1985). Activités enzymatiques métaboliques et contractiles de 30 muscles du Porc. Relations avec le pH ultime atteint après la mort. Reproduction Nutrition Dévelopment, 25, 619 - 628.

Nezer C., Moreau L., Brouwers B., Coppieters W., Detilleux J., Hanset R., Karim L., Kvasz A., Leroy P. & Georges M. (1999). An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. Nature Genetics, 21, 155 - 156.

Pedersen P.H., Oksbjerg N., Karlsson A.H., Busk H., Bendixen E. & Henckel P. (2001). A within litter comparison of muscle fibre characteristics and growth of halothane carrier and halothane free crossbreed pigs. Livestock Production Science, 73, 15 - 24.

Swantek P.M., Crenshaw J.D., Marchello M.J. & Lukaski H.C. (1992). Bioelectrical impedance: a nondestructive method to determine fat-free mass of live market swine and pork carcasses. Journal of Animal Science, 70, 169 -177.

Swatland H.J. (1981). Electrical capacitance measurements on intact carcasses. Journal of Animal Science, 53, 666 - 669.

Van den Maagdenberg K., Stinckens A., Claeys E., Buys N. & De Smet S. (2005). Preliminary study of the effect of the IGF-II genotype in the pig on muscle fibre cross sectional area and the relationship with meat quality. Proceedings of the 30th meeting of the dutch speaking nutrition researcher, 1, 27 - 28.

Van Laere A., Nguyen M., Braunschweig M., Nezer C., Collette C, Moreau L., Archibald A.L., Haley C., Buys N., Tally M, Andersson G., Georges M. & Andersson L. (2003). A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature, 425, 832 - 836.

Warner R.D., Kauffman R.G. & Russell R.L. (1993). Quality attributes of major porcine muscles: A comparison with the Longissimus Lumborum. Meat Science, 33, 359 - 372.

Wheeler T.L., Shackelford S.D. & Koohmaraie M. (2000). Variation in proteolysis, sarcomere length, collagen content and tenderness among major pork muscles. Journal of Animal Science, 78, 958 - 965.