ASSOCIATIONS BETWEEN EARLY DRIP LOSS MEASUREMENTS, GENE MARKERS, AND PURGE LOSS DEVELOPMENT OF CASE-READY PORK

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Introduction

Drip loss of fresh pork is an economically important meat quality characteristic as it affects both yield and quality of the end product. Case-ready fresh meat that shows high amounts of purge loss has an unattractive appearance for the consumer, which may lead to a reduction in sales at the retail store. Furthermore, high drip loss or low water-holding capacity reduces the yield of further processed products. The genetic make-up of a pig, the entire system of live animal production and handling through initial chilling, and finally storage and handling of the meat and case-ready products all play significant roles in influencing the amount of moisture that is lost by the end product (Andersen, 2000).

It is of economic interest to know whether drip loss of case-ready products can be predicted by earlier measurements of drip loss in order to improve the genetic make-up of a pig by using selective breeding techniques. Examples of accepted early drip loss measurements are the Honikel method (Honikel, 1987) and EZ-DripLoss method (Rasmussen and Andersson, 1996), which could be used in full- and half-sib-carcass analysis for selection purposes. In addition meat quality markers can be used to directly select live pigs with the best genetic make-up for reduction of drip loss (Dekkers et al., 2001, Knap et al. 2002, Plastow et al. 2004).

Objectives

The objectives of this paper were to study the associations between standardized early drip loss measurements and daily purge loss measurements of case-ready packaged pork during a one-week period. Furthermore, the effect of several genetic markers on drip loss was quantified.

Methodology

The experimental pigs were crossbreds from four lines reared at one commercial farm. Piétrain based sires were mated to crossbred dams originating from a three line cross of Large White, Landrace and Leicoma based lines. DNA analysis showed that 213 pigs were homozygous for the stress resistant allele of the ryanodine receptor gene (NN genotype) and 161 were of the heterozygous Nn genotype. There were 191 gilts and 183

barrows included in this study. All pigs were processed at a commercial abattoir in batches of about 60 pigs during 7 different slaughter days.

At 24h post-mortem case-ready meat samples were cut from the *M. longissimus dorsi* of all 374 pigs. Purge loss was measured on each of the 7 days ($CRM_{1–7}$) after packaging of these loin samples. At 24h post-mortem loin samples were also cut for the reference drip loss methods as described in Honikel (1987; HM method) and Rasmussen and Andersson (1996; EZ-DripLoss (EZ-DL) method). Drip loss was measured at 24 and 48 h after preparation for the HM samples and at 48h after cutting for the EZ-DL probes. Drip loss is expressed as percentage of the initial weight.

Drip loss data (HM and EZ-DL) of a previous trial conducted at the same slaughter house were added to the total data base (1155 pigs of similar genotypes) for the association analysis between the different drip loss measurements ($CRM_{1–7}$, HM, and EZ-DL) and genetic markers. The ryanodine receptor genotypes were determined according to *Fujii et al.* (1991; *RYR1; HAL1843™).* Genotypes were also obtained for markers in *PRKAG3* (Milan et al., 2000; Ciobanu et al., 2001) and *HMGA1* (Kim et al., 2004). The *PRKAG3* I199V mutation is located on chromosome 15 and for the population under study the genotype frequency was II=89, IV=534, and VV=496. The *HMGA1* locus is on chromosome 7 and the NaeI polymorphism used is in intron 5 of the gene. Only a few pigs showed the genotype 11 of marker *HMGA1* (n=18), whereas the genotypes 12 and 22 accounted for 355 and 709 animals.

Association between DNA markers and the amount of drip loss was tested by analysis of variance using the general linear model procedure (GLM) of SAS (SAS Institute Inc., Cary, NC, USA). The following model was used for the analysis ($Y_{ijklmnop}$):

$$Y_{ijklmnop} = \mu + L_i + F_{ij} + D_k + S_l + R_m + RYR1_n + M_o + e_{ijklmnop}$$

The model included the fixed effects line (L_i), farm within line (F_{ij}), slaughter day (D_k), sex (S_l), resting time (R_m), *RYR1* genotype ($RYR1_n$) and *PRKAG3* I199V or *HMGA1*, respectively (M_o). Furthermore, drip loss obtained with the Honikel method was corrected for the sample weight.

Results & Discussion

The HM method samples taken 24h post-mortem showed a drip loss of 1.8 and 3.1% after 24 h and 48 h storage, respectively. The EZ-DL method had a drip loss of 4.7% after 48 h storage. This is in the same range as was previously reported by Otto et al. (2004) but in contrast to Christensen (2003) who reported 1.2% more drip loss when using the HM method compared to EZ-DripLoss technique. The 24 h and 48 h HM drip loss measurements were highly correlated (0.98), as were the 48 h measurements of both the HM and EZ-DL methods (0.89).

Increasing display times of meat in retail shops require meat of consistently good quality. Therefore, the development of purge loss was examined during a one-week period. Mean drip loss % of the case-ready meat samples (CRM) increased substantially from 1.6% at 24 hours to 5.6% during 7 days of storage (Figure 1). High variation was obtained for all drip loss measurements resulting in coefficients of variation ranging from 35.2 and 70.4 for CRM at day 7 after sampling and HM after 24 h storage, respectively.

A large variation was shown in the development of case-ready purge loss. The 25% worst purge loss samples had an average drip loss of 8.4% drip after 7 days of storage. The best 25% of the case-ready samples showed a purge loss of only 3.5% at day 7 (Figure 1). The 25% undesirable drip loss samples had especially high amounts of drip within the first days of the observation period. Correlations between the $CRM_{1–7}$ purge loss measurements and the HM or EZ-DL drip loss % were in the range of 0.82 to 0.90 (Table 1). This indicates that the earlier drip loss measurements carried out at the slaughterhouse could be highly informative of predicting the purge loss found in case-ready packaged pork after several days of storage at a retail store.

The use of marker-assisted selection in animal breeding is of particular interest with meat quality traits. Improvement of meat quality is difficult using conventional selection methods, since most traits of interest can only be measured after slaughter and, therefore, only information of relatives can be used for selection (Dekkers et al., 2001). The effects of the *RYR1* gene on meat quality are well known and the drip loss results in this study were as expected. Heterozygous carriers of the recessive stress susceptible allele (Nn) and homozygous stress resistant (NN) animals showed 5.8% and 3.7% drip loss with the EZ-DL method, respectively (Table 2). The *PRKAG3* I199V and *HMGA1* markers were also found to significantly influence drip loss % as measured with the EZ-DL method. The difference between the extreme genotypes within these markers was 1.0% and 1.1% drip loss, respectively (Table 2). Better meat quality of carriers of the II genotype of *PRKAG3* I199V in terms of higher loin pH was also observed by Ciobanu et al. (2001). The analysis across five commercial lines resulted in loin pH values of 5.78, 5.74 and 5.71 for genotypes II, IV and VV, respectively. Low frequencies of the *HMGA1* 11 genotype caused a high standard error. Therefore, more research has to be done to verify the effects of this particular marker. Overall the results indicate the usefulness of marker-assisted selection for reduction of drip loss in pigs.

Conclusions

Early measurements of drip loss using both the Honikel and EZ-DripLoss methods are adequate to predict purge loss in case-ready packaged pork over a one-week period. The EZ-DripLoss method is a standardised easy-to-perform method and is therefore recommended for use in selection programs of breeding animals. Finally, marker-assisted selection is shown to be a powerful tool for reducing drip loss of case-ready meat, which can be used for early selection of breeding animals.

References

Andersen, H. J. (2000). What is pork quality? In *Quality of meat and fat in pigs as affected by genetics and nutrition.* EAAP publication No. 100, 15–26. Zurich, Switzerland.

Ciobanu, D., Bastiaansen, J., Malek, M., Helm, J., Woollard, J., Plastow, G., & Rothschild, M. (2001). Evidence for new alleles in the protein kinase adenosine monophospate-activated, γ_3- subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. *Genetics*, 159(3), 1151–1162.

Christensen, L. B. (2003). Drip loss sampling in porcine *m. longissimus dorsi*. *Meat Science*, *63*(4), 469–477.

Dekkers, J. C. M., Rothschild, M. F., & Malek, M. M. (2001). Potential and application of marker assisted selection for meat quality. In *Second international virtual conference on pork quality*, November, 05 to December, 06 Via internet (http://www.conferencia.uncnet.br/pork/seg/pal/anais01p2_deckkers_en.pdf).

Fujii, J., Otsu, K., Zorzato, F., De Leon, S., Khanna, V.K., Weiler, J.E., O'Brien, P.J., & MacLennan, D.H. (1991). Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, *253, 448–451.*

Honikel, K. O. (1987). The water binding of meat. *Fleischwirtschaft, 67*(9), 1098–1102.

Kim, K. S., Thomsen, H., Bastiaansen, J., Nguyen, N. T., Dekkers, J. C. M., Plastow, G. S., & Rothschild, M. F. (2004) Investigation of obesity candidate genes on porcine fat deposition quantitative trait loci regions. *Obesity Research*, 12(12), 1981–94.

Knap, P.W., Sosnicki, A.A., Klont, R.E. and Lacoste, A. (2002). Simultaneous Improvement of Meat Quality and Growth and Carcass Traits in Pigs. In *Proceedings of the 7th World Congress on Genetics Applied to Livestock Production 31*, (pp.339–346) INRA, Castanet-Tolosan, France.

Milan, D., Jeon J.-T., Looft, C., Amarger, V., Robic, A., Thelander, M., Rogel-Gaillard, C., Paul, S., Iannuccelli, N., Rask, L., Ronne, H., Lundström, K., Reinsch, N., Gellin, J., Kalm, E., Le Roy, P., Chardon, P. & Andersson, L. (2000). A mutation in *PRKAG3* associated with excess glycogen content in pig skeletal muscle. *Science* 288(5469), 1248–1251.

Otto, G., Roehe, R., Looft, H., Thoelking, L., & Kalm, E. (2004). Comparison of different methods for determination of drip loss and their relationships to meat quality and carcass characteristics in pigs. *Meat Science*, 68(3), 401–409.

Plastow G.S., D. Carrión, M. Gil, J.A. García-Regueiro, M. Font i Furnols, M. Gispert, M.A. Oliver, M.D. Guàrdia, M. Hortós, M.A. Rius, I. Díaz, A. Valero, A. Sosnicki, R. Klont, S. Dornan, J.M. Wilkinson, G. Evans, C Sargent, G. Davey, D. Connolly, B. Houeix, C.M. Maltin, H.E. Hayes, V.Anandavijayan, A. Foury, N. Geverink, M. Cairns[d], R.E. Tilley[e], P. Mormède[f] and S.C. Blott[a] (2004). Quality Pork Genes and Meat Production. In *Proceedings 50th International Congress of Meat Science and Technology*, Helsinki (pp. 1), 8–13 August 2004 Helsinki, Finland.

Rasmussen, A. J., & Andersson, M. (1996). New method for determination of drip loss in pork muscles. In *Proceedings 42nd International Congress of Meat Science and Technology*, Lillehammer (pp. 286–287), 1–6 September 1996, Lillehammer, Norway.

Tables and Figures

Table 1: Residual[a] correlations[b] between sample drip loss measurements[c] after 24 or 48 h storage and daily measurements of case-ready pork drip loss[d]

	Drip loss % of case-ready pork at day						
	1	2	3	4	5	6	7
HM_{24}	0.86	0.88	0.87	0.86	0.85	0.85	0.85
HM_{48}	0.82	0.87	0.87	0.88	0.87	0.87	0.87
$EZ\text{-}DL_{48}$	0.87	0.90	0.89	0.89	0.88	0.88	0.87

[a] Residual correlations after adjustment for the effects slaughter day, resting time, sex, *RYR1* genotype and additionally for sample weight when using bag $method_{48}$.

[b] Significance of all estimated regression coefficients was $P<0.001$.

[c] Abbreviations: HM_{24}: Drip loss measured using Honikel method from 24 to 48 h post-mortem; HM_{48}: Drip loss measured using Honikel method from 24 to 72 h post-mortem; $EZ\text{-}DL_{48}$: Drip loss measured using EZ-DripLoss method from 24 to 72 h post-mortem.

[d] Sampling took place at 24 h post-mortem

Table 2: Least squares means (standard errors) of several genetic marker genotypes for drip loss by using EZ-DripLoss method at 48 h after sampling

Marker	Marker genotype			P[a]
	11	12	22	
RYR1[b]	.	5.8 (0.13)a[c]	3.7 (0.13)b	***
PRKAG3 I199V[d]	3.9 (0.24)a	4.7 (0.13)b	4.9 (0.13)b	***
HMGA1	3.6 (0.50)a	4.9 (0.15)b	4.7 (0.13)ab	*

[a] * $P < 0.05$; *** $P < 0.001$.

[b] *RYR1*, HAL1843™ – allele 1 is allele T or amino acid Cys, allele 2 is C or Arg.

[c] Within rows, values with the same letter are not significantly different.

[d] Genotypes of *PRKAG3* I199V: 11=II; 12 = IV; 22=VV.

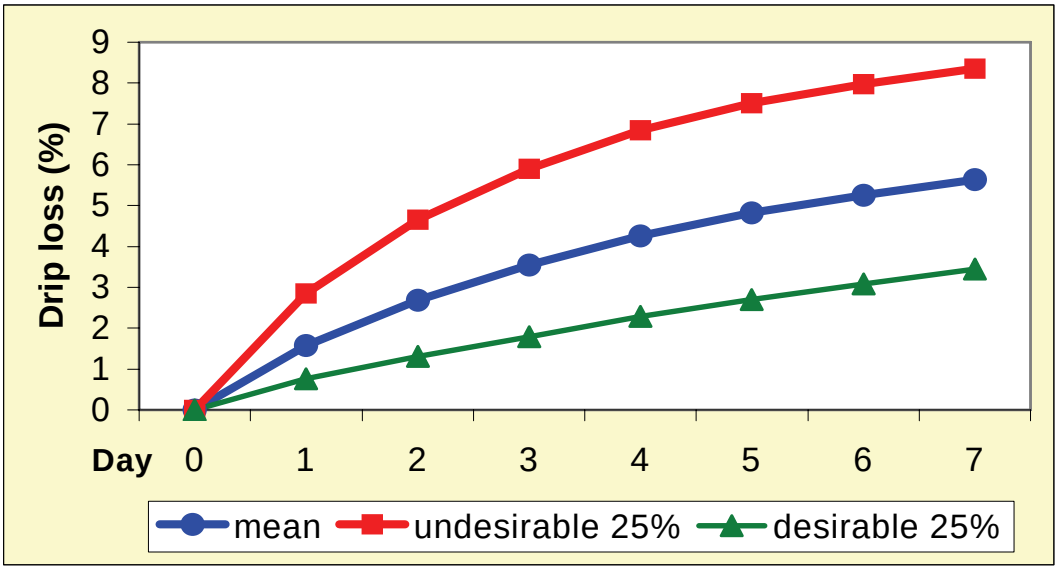

Figure 1: Development of purge loss in case-ready pork within a week (n=374)