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Genetic relationship of drip loss to further meat quality traits in purebred Piétrains

Abstract

Drip loss is an important quality criterion for the meat processing industry and also the consumer. Therefore this characteristic is discussed as a target trait for breeding schemes. In this study the EZ-DripLoss method was implemented in a routine testing procedure to determine drip loss regularly at 48 hours post mortem. As further meat quality traits conductivity and meat brightness were recorded at 24 hours post mortem. Additionally, pH value was measured at 45 minutes post mortem in the loin muscle and at 24 hours post mortem in the loin and in the ham. Reflectance was adopted from the FOM-protocol of the abattoir. For the estimation of the genetic parameters the determined percentage drip losses were logarithmicly transformed to get nearly normal distributed values. In total data of 2337 purebred Piétrains were analysed from which 782 were investigated on drip loss. Heritability of drip loss was 0.34±0,04 and decreased on 0.14±0,04 when the influence of the MHS-gene was corrected. Genetic correlation of drip loss to pH_{45} was r_g = -0.91±0.03, to pH_{24} (loin) r_g = -0.72±0.04, to pH_{24} (ham) r_g = -0.41±0.06, to conductivity r_g = 0.93±0.02, to meat brightness r_g = -0.87±0.03, and to reflectance r_g = 0.63±0.07. Considering MHS-genotype the corresponding correlations were r_g = -0.66±0.11, r_g = -0.72±0.09, r_g = -0.50±0.10, r_g = 0.74±0.09, r_g = -0.64±0.11, and r_g = 0.13±0.13.

Key Words: Piétrain, drip loss, meat quality, MHS-genotype

Zusammenfassung

Titel der Arbeit: **Genetische Beziehung von Tropfsaftverlust zu anderen Fleischqualitätsmerkmalen bei reinrassigen Piétrains**

Tropfsaftverlust ist ein bedeutendes Qualitätskriterium für die Fleisch verarbeitende Industrie und ebenfalls für den Verbraucher. Deshalb wird diese Eigenschaft als Zielmerkmal für Zuchtprogramme diskutiert. In dieser Untersuchung wurde die EZ-DripLoss Methode in die LPA-Routine mit einbezogen, um den Tropfsaftverlust 48 Stunden post mortem regelmäßig zu bestimmen. Als weitere Fleischqualitätsmerkmale wurden die Leitfähigkeit und die Fleischfarbe 24 Stunden post mortem erfasst. Außerdem wurde der pH-Wert 45 Minuten post mortem im Kotelett sowie 24 Stunden post mortem im Kotelett und im Schinken gemessen. Der Reflexionswert wurde dem FOM-Protokoll des Schlachthofs entnommen. Für die Schätzung der genetischen Parameter wurden die ermittelten, prozentualen Tropfsaftverluste logarithmiert, um annähernd normal verteilte Werte zu erhalten. Insgesamt wurden Daten von 2337 Piétrainschweinen analysiert, von denen 782 auf Tropfsaftverlust untersucht waren. Die Heritabilität für den Tropfsaftverlust betrug 0,34±0,04 und nahm auf 0,14±0,04 ab, als der Einfluss des MHS-Gens heraus gerechnet wurde. Die genetische Korrelation des Merkmals Tropfsaftverlust zum pH_{45} betrug r_g = -0,91±0,03, zum pH_{24} (Kotelett) r_g = -0,72±0,04, zum pH_{24} (Schinken) r_g = -0,41±0,06, zur Leitfähigkeit r_g = 0,93±0,02, zum Wert für die Fleischfarbe r_g = -0,87±0,03 und zum Reflexionswert r_g = 0,63±0.07. Bei Berücksichtigung des MHS-Genotyps waren die entsprechenden Korrelationen r_g = -0,66±0,11, r_g = -0,72±0,09, r_g = -0,50±0,10, r_g = 0,74±0,09, r_g = -0,64±0,11 und r_g = 0,13±0,13.

Schlüsselwörter: Piétrain, Tropfsaftverlust, Fleischqualität, MHS-Genotyp

1. Introduction

OFFER and KNIGHT (1988) described drip as a reddish fluid mainly consisting of water and proteins. It can be expelled from cut surfaces of muscles or pieces of meat without any mechanical force other than gravity. A general definition does not exist, because the height of drip loss depends on the conditions under which it is measured.

The percentage of drip loss is affected by several ante- and post-mortem factors. When the amount of ATP (adenosine triphosphate) undergoes a certain level, no calcium (Ca^{2+}) is transported back into the endoplasmatic reticulum and an actomyosin-complex is built (KÜCHENMEISTER and KUHN, 2003). The complex is responsible for a non-reversible association between actin and myosin filaments. In regular this is the case at an ATP concentration of 1 µm/g and a pH value of 5.9. If this process is influenced by unfavourable pre-slaughter conditions (e.g. transport, other environment) or wrong treatment post-mortem (e.g. cooling) the meat quality can be disturbed in terms of pH value, colour, water-holding capacity and tenderness (BINKE, 2003).
In the past the assessment of drip loss was done by several methods. For example the filterpaper-press method of GRAU and HAMM (1953) and the bag method of HONIKEL (1987) are widespread and internationally accepted methods. A variety of different methods can be found in Table 1.

Table 1
Different methods of measurement drip loss and water-holding capacity of meat (adopted from OTTO, 2005) (Verschiedene Messmethoden für den Tropfsaftverlust und Wasserbindungsvermögen von Fleisch, entnommen aus OTTO, 2005)

Method	**Reference**
Filterpaper-press method	Grau and Hamm, 1953
Loose bound water	Beutling, 1969
Capillary volumeter	Hofmann, 1975
Tray method	Lundström and Malmfors, 1985
Filterpaper method	Kauffman et al., 1986
Bag method	Honikel, 1987
Centrifugation methods	Honikel and Hamm, 1994
EZ-DripLoss method	Rasmussen and Andersson, 1996
Absorptive material	Walukonis et al., 2002

The listed methods for measuring drip loss differ in terms of applied force or sample size. Tray method, bag method and EZ-DripLoss method are gravimetric methods, whereas the other methods use centrifugal or other external pressure. A main reason using force to determine drip is probably the smaller duration for analysing the samples with a higher throughput at the same time. In contrast to other methods which only need a few minutes, gravimetric methods are normally conducted for at least 24 hours. Concerning sample size, the bag method is carried out with meat pieces of 100 g whereas the EZ-DripLoss uses approximately 10 g, and a centrifugation method needs only 3-4 g. According to RASMUSSEN and ANDERSSON (1996) additional advantages of the EZ-DripLoss method are an easier handling at abattoir conditions, an easier performing in a reproducible way and higher sensitivity.
The increased market share of case-ready meat (kitchen-ready, consumer ready) led to new discussion on improvement of meat quality. Therefore in the recent past in Germany the EZ-DripLoss method became more popular, because drip is a direct measurable and visible meat quality trait in contrast to e.g. pH and conductivity which are not suitable for judgement by the consumer. But information of the genetic foundation of the EZ-DripLoss method is rare. The aim of this study was to investigate the genetic relationship of drip loss, measured at 48 hours post mortem by the EZ-DripLoss method, to meat quality traits which are routinely recorded in German performance test stations in purebred Piétrains.

2. Material and methods

Routine testing procedure

All 2337 Piétrains were fattened from January 2001 to June 2005 in the performance test station Achterwehr. Afterwards the pigs were slaughtered in a commercial abattoir. The carcass assessment was done according to the German performance test directives on the left carcass half (ALZ, 2000). Besides the carcass composition traits, the following meat quality traits were measured: in the *musculus longissimus dorsi* pH value 45 minutes and electrical conductivity 24 hours post mortem as single measurements, pH value and meat brightness (Opto Star apparatus) 24 hours post mortem at the cut between 13^{th} and 14^{th} rib in the *musculus longissimus dorsi* as an average of three and two measurements, respectively. Also as an average of two values the pH in the *musculus semimembranosus* of the ham was measured at 24 hours post mortem. Reflectance was ascertained by the Fat-O-Meater apparatus and was taken from the grading protocol.

Recording drip loss

Drip loss was determined since January 2004 by the EZ-DripLoss method (RASMUSSEN and ANDERSSON, 1996). At 24 hours after slaughter two 10 g pieces of loin eye were put out on the caudal side of the cut between $13^{th}/14^{th}$ rib with a circular knife. Two meat samples were taken, because OTTO (2005) found in a preliminary study a higher drip loss in the ventral part of the *musculus longissimus dorsi* than in the dorsal. The samples were placed in drip loss containers (KABE Labortechnik, Nümbrecht-Elsenroth) and weighed. Afterwards they were stored in a chill room at 6 °C. The funnel-like shape of the container made it possible that the drip could flow away. 24 hours later the containers were weighed again without the piece of meat. From both weights the weight of the empty container was subtracted, and from the resulting fresh meat and drip weights drip loss was calculated as a percentage of the initial weight. In total, samples of 782 animals were collected.

Logarithmic transformation

The determined drip loss percentages showed a log normal distribution. Therefore, the percentages were transformed by the natural logarithm to get nearly normal distributed values which were presupposed for the further statistical analysis. The measurements of electrical conductivity were transformed in the same way. In this place it should be remarked that the pH value is also a logarithmic number.

Statistical analysis

The statistical models were developed with the MIXED procedure from the SAS® package (SAS, 2000). Estimation of the genetic parameters was carried out with the programme VCE, version 4.2.5 (NEUMAIER and GROENEVELD, 1998), in two multivariate runs, with and without considering MHS-genotype. The following mixed animal model was applied:

$$y_{ijklmn} = \mu + H_i + D_j + MHS_k + li_l + an_m + e_{ijklmn} \quad ,$$

where y_{ijklmn} is the individual observation for the considered trait, μ is the fixed effect of the overall mean, H_i is the fixed effect of the herd ($i = 1\ldots12$), D_j is the fixed effect of the slaughter day ($j = 1...213$), MHS_k is the fixed effect of the MHS-genotype ($k =$

NN, NP or PP), li_l is the random environmental effect of each litter (l = 1...1190 with and l = 1…1271 without considering MHS-genotype), an_m is the breeding value of each animal (m = 1...2185 with and m = 1…2337 without considering MHS-genotype), and e_{ijklmn} is the residual.

3. Results and discussion

Table 2 shows the descriptive statistical parameters of the analysed data. The means not point to unsatisfying meat quality like PSE (pale, soft, exudative) caused by the different MHS-genotypes, especially PP. But wide ranges in all traits indicate that the P-allele is still present in the population.

Table 2
Number of records, means, standard deviations, minima and maxima of the analysed data, n = 2337 (Anzahl Beobachtungen, Mittelwerte, Standardabweichungen, Minima und Maxima des untersuchten Datenmaterials, n = 2337)

Trait	Joint	Time[1)]	N	Mean	Std.	Min.	Max.
drip loss	loin	48 h	782	4.10	3.21	.21	16.51
pH	loin	45 Min.	2326	6.16	.37	5.30	6.90
pH	loin	24 h	2317	5.46	.12	5.20	6.18
pH	ham	24 h	2314	5.60	.14	5.21	6.20
conductivity	loin	24 h	2320	5.11	2.35	2.13	12.92
meat brightness	loin	24 h	2328	64.3	9.6	30	89
reflectance	loin	45 Min.	2282	25.7	8.6	15	80

[1)] time post mortem (Messzeitpunkt post mortem)

Least squares means in Table 3 for the MHS-genotypes show for almost all investigated traits clear significant differences. Middling drip loss percentages in homozygous stress resistant pigs (NN) are at 2.14 and increase in heterozygous stress resistant pigs (NP) to 4.13 and even to 10.32 percent in stress susceptible pigs (PP).

Table 3
LS-Means (LSM), Standard Errors (SE) and Error Probabilities (F-Test) for the effects of the MHS-genotype (NN, NP, PP) on the recorded meat quality traits (LS-Mittelwerte (LSM), Standardfehler (SE) und Irrtumswahrscheinlichkeiten (F-Test) für die Effekte des MHS-Genotyps (NN, NP, PP) auf die untersuchten Fleischqualitätsmerkmale)

Trait	Joint	Time[1)]	NN LSM (SE)	NP LSM (SE)	PP LSM (SE)	F-Test
drip loss	loin	48 h	2,14^a	4,13^b	10,32^c	***
pH	loin	45 Min.	6,39^a	6,11^b	5,56^c	***
pH	loin	24 h	5,52^a	5,45^b	5,42^c	***
pH	ham	24 h	5,62^a	5,60^b	5,58^b	***
conductivity	loin	24 h	3,96^a	5,00^b	9,40^c	***
meat brightness	loin	24 h	70,2^a	63,8^b	51,3^c	***
reflectance	loin	45 Min.	23,3^a	24,0^a	39,5^b	***

[1)] time post mortem (Messzeitpunkt post mortem); *** Error probability < .001; LS-Means with unequal superscripts are significant different with the Bonferroni test (LS-Mittelwerte mit unterschiedlichen Buchstaben zeigen signifikante Unterschiede mit dem Bonferroni-Test)

This is in disagreement with the results of THOLEN et al. (2005). Using also EZ-DripLoss method they did not find such clear differences between the extreme genotypes NN and PP in Piétrains of North-Rhine-Westphalia. The rise of drip loss in the distinct genotypes agrees with the least squares means of the other meat quality traits. Thus a significant higher pH_{45} value can be observed in stress resistant animals as compared to stress susceptible. At the same time also the value for meat brightness decreases and conductivity as well as reflectance value increase. The differences of the

pH_{24} values are less expressed but present. Clear differences between the genotypes are also reported by HANSET et al. (1995), SELLIER (1998), WITTMANN et al. (1999), and THOLEN et al. (2005) for pH value up to 60 minutes after slaughter while differences of pH_{24} values in the loin are stated as small. Corresponding means for conductivity and meat brightness are found by THOLEN et al. (2005), but drip loss of the PP-animals was evidently lower. In a comparison of ante mortem biopsies with meat quality traits recorded after slaughter LAHUCKY et al. (1997) calculated similar differences between the genotypes for drip loss, initial pH, and reflectance. KRIETER and THOLEN (2001) stated that the differences between NN- and PP-animals of pH_{45} up to an amount of four phenotypic standard deviations are possible. Further detailed literature is reviewed by SELLIER (1998).

Table 4
Phenotypic correlations between the recorded meat quality traits (Phänotypische Korrelationen zwischen den erfassten Fleischqualitätsparametern)

Trait	Joint	Time[2)]		2	3	4	5	6	7
drip loss[1)]	loin	48 h	1	-.67***	-.51***	-.13***	.68***	-.63***	.41***
pH	loin	45 Min.	2		.33***	.08***	-.68***	.58***	-.50***
pH	loin	24 h	3			.53***	-.22***	.56***	-.13***
pH	ham	24 h	4				-.04***	.26***	-.05*
conductivity[1)]	loin	24 h	5					-.50***	.53***
meat brightness	loin	24 h	6						-.54***
reflectance	loin	45 Min.	7						

[1)] logarithmic value (logarithmierter Wert); [2)] time post mortem (Messzeitpunkt post mortem); * Error probabililty < .05;
*** Error probability < .001

Phenotypic correlations in Table 4 between drip loss and the routinely recorded meat quality traits range from $r_p = |.41|$ to $|.68|$ with the exception of pH_{24} (ham) and show the expected direction. The computed phenotypic correlations confirm the relationships between the traits ascertained shortly before in the least squares means of the MHS-genotypes in Table 3. On a slightly lower level are the correlations estimated by THOLEN et al. (2005) within the NN-, NP-, and PP-animals, but in the case of pH_{24} (loin) they tend to zero.

As described above the heritabilities and genetic correlations were calculated with two different models. In Table 5 the genetic correlations and Table 6 the variance components with the corresponding c^2-effects and heritabilities are represented, whereas the first row of each trait comprises the results of the model without MHS-genotype as a fixed effect.

The highest genetic correlations to drip loss are estimated for pH_{45}, conductivity and meat brightness ($r_g = |.87|$ to $|.93|$), if the MHS-genotype is not considered. When the influence the MHS-gene is corrected, the correlation coefficients decrease to values from $r_g = |.64|$ to $|.74|$. In general, it can be stated that the correlations between the pH_{24} values (loin and ham) and the remaining traits do not change so extremely between the applied models. In the most cases differences of $|.00|$ to $|.20|$ are observed, while the other pair traits show changes up to $|.50|$. This is due to the fact that the MHS-gene does not affect ultimate pH (SELLIER, 1998). In this context the correlation between drip loss and pH_{24} (loin) is of special interest; although ultimate pH is not influenced by the MHS-gene a correlation of $r_g = -.72$ can be computed and a correlation close to zero to conductivity. Thus, it can be concluded that this relationship is based on other genes than MHS. Therefore, a reduction of drip loss should be also achieved by an

indirect selection on conductivity and pH_{24} (loin).
Average genetic correlations between drip loss and pH_1, ultimate pH, and reflectance are reported by SELLIER (1998) with r_g = -.27, r_g = -.71, and r_g = .49, respectively. With the exception of the correlation between drip loss and pH_1 these values are in good accordance to the results in table 5. Widespread correlations between pH, conductivity and meat brightness are estimated by THOLEN et al. (2005) within the NN-, NP-, and PP-animals.

Table 5
Genetic correlations between the recorded meat quality traits without considering fixed effect of MHS-genotype in the first row and with in the second (Genetische Korrelationen zwischen den erfassten Fleischqualitätsparameter ohne Berücksichtigung des fixen Effektes MHS-Genotyp in der ersten Zeile und mit in der zweiten)

Trait	**Joint**	**Time[2)]**		**2**	**3**	**4**	**5**	**6**	**7**
drip loss[1)]	loin	48 h	1	-.91 (.03) -.66 (.11)	-.72 (.04) -.72 (.09)	-.41 (.06) -.50 (.10)	.93 (.02) .74 (.09)	-.87 (.03) -.64 (.11)	.63 (.07) .13 (.13)
pH	loin	45 Min.	2		.52 (.05) .49 (.09)	.19 (.06) .10 (.11)	-.92 (.02) -.57 (.11)	.76 (.04) .32 (.12)	-.75 (.04) -.22 (.11)
pH	loin	24 h	3			.77 (.05) .84 (.05)	-.43 (.07) -.14 (.12)	.86 (.03) .91 (.04)	-.19 (.09) -.05 (.11)
pH	ham	24 h	4				-.15 (.08) -.03 (.12)	.63 (.06) .86 (.07)	-.21 (.08) -.13 (.12)
conductivity[1)]	loin	24 h	5					-.67 (.06) -.20 (.12)	.74 (.05) .55 (.12)
meat brightness	loin	24 h	6						-.56 (.07) -.24 (.13)
reflectance	loin	45 Min.	7						

[1)] logarithmic value (logarithmierter Wert); [2)] time post mortem (Messzeitpunkt post mortem)

The dependence of drip loss, pH_{45}, conductivity, meat brightness, and reflectance on the MHS-gene is clearly shown by the variance components in Table 6. In all these traits a significant reduction of the variance components can be observed when the MHS-genotype is considered as fixed effect in the model, while the variances of pH_{24} traits change not or only marginal. The decrease in variances also leads to lower h^2-values. Especially distinct differences are ascertained for drip loss and pH_{45}. The heritabilities decrease from h^2 = .34 and h^2 = .36, respectively to h^2 = .14 in both cases, emphasising the importance of the MHS-gene for the expression of these characteristics and suggesting that a consequent stress sanitation substantially contributes to a better meat quality. Constant h^2-values in pH_{24} measurements support that these traits are not affected by the MHS-gene. These results are in agreement with SELLIER (1998) who reviewed average heritabilities of drip loss with h^2 = .16, of initial pH with h^2 = .16, of ultimate pH with h^2 = .21, and of meat colour with h^2 = .28.
Appending from the statistical model a respectable proportion of .12 and .18, respectively of the phenotypic variance of drip loss is attributed to common litter effects. Only the pH_{24} (ham) shows comparable proportions. In the performance test station Achterwehr always two fullsibs per pen are fattened and slaughtered at the same time, often on the same day. A not exactly differentiation of litter and slaughter day effect maybe cause higher c^2-effects. In a preliminary multiple regression analysis of the investigated traits for drip loss the greatest influence of the slaughter day conditions could be proved. Dominance perhaps also plays a role, as it can be deduced for drip loss from the least squares means presented in Table 3.

Table 6
Variance components, corresponding c^2-effects (c^2), heritabilities (h^2), and standard errors (SE) of the analysed meat quality traits without considering fixed effect of MHS-genotype in the first row and with in the second (Geschätzte Varianzkomponenten, die entsprechenden Wurfumwelteffekte (c^2), Heritabilitäten (h^2) und Standardfehler (SE) der untersuchten Fleischqualitätsmerkmale ohne Berücksichtigung des fixen Effektes MHS-Genotyp in der ersten Zeile und mit in der zweiten)

Trait	**Joint**	**Time[2)]**	σ_A^2 [3)]	σ_C^2 [4)]	σ_E^2 [5)]	**c^2 (SE)**	**h^2 (SE)**
drip loss[1)]	loin	48 h	.176	.064	.285	.12 (.02)	.34 (.04)
			.043	.055	.212	.18 (.03)	.14 (.04)
pH	loin	45 Min.	.044	.010	.070	.08 (.02)	.36 (.04)
			.008	.004	.045	.07 (.02)	.14 (.03)
pH	loin	24 h	.002	.001	.008	.06 (.01)	.21 (.03)
			.002	.001	.007	.08 (.02)	.20 (.03)
pH	ham	24 h	.002	.003	.010	.17 (.02)	.15 (.02)
			.003	.003	.010	.18 (.02)	.17 (.03)
conductivity[1)]	loin	24 h	.039	.007	.096	.05 (.01)	.27 (.03)
			.012	.003	.061	.04 (.02)	.16 (.03)
meat brightness	loin	24 h	17.929	8.022	52.013	.10 (.02)	.23 (.03)
			7.855	3.144	35.877	.07 (.02)	.17 (.03)
reflectance	loin	45 Min.	10.415	2.929	50.069	.05 (.01)	.16 (.03)
			5.139	1.851	29.895	.05 (.02)	.14 (.03)

[1)] logarithmic value (logarithmierter Wert); [2)] time post mortem (Messzeitpunkt post mortem); [3)] σ_A^2 = additive-genetic variance (additiv-genetische Varianz); [4)] σ_C^2 = variance of the common litter environment (Varianz der gemeinsamen Wurfumwelt); [5)] σ_E^2 = residual variance (Restvarianz)

Conclusion

The MHS-gene is of great importance for the amount of drip in the Piétrain breed. Therefore, a quick and consequent elimination of the P-allele makes a considerable contribution to the reduction of drip loss. Furthermore, sufficient genetic correlations of the direct meat quality trait drip loss to the more indirect meat quality parameters pH_{24} (loin) and conductivity of the routine testing scheme could be proved. Thus, it is possible to select on drip loss without a direct recording of this characteristic. Of special interest is the fact that conductivity and pH_{24} (loin) are not close related. Additionally, pH_{24} (loin) is not affected by the MHS-gene, so the relationship to drip loss probably due to other genes. Therefore, under the current cost and labour conditions and the situation of a stagnating stress sanitation it is not necessary to record drip loss directly. When the P-allele is removed from the Piétrain population a direct measurement is to prefer because of decreasing relationships to the other meat quality parameters.

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