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Conference paper

THE INFLUENCE OF RYR1-GENOTYPE ON FERTILITY TRAITS OF BREEDING SOWS

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Abstract

Malignant typerthermis (MH) in swine is an economically important pharmacogenetic disease that affects calcium regulation in skeletal muscules and results in sudden, stress induced death and economic losses through reduction of viability and meat quality (Britt, 1987). Genetically susceptible pigs are characterized by muscular hypermetabolism and rigor due to an alteration in the gating pathway between closed and open states of the calcium release channel (RYR1) in skeletal musculature (Nelson et al., 1995).

Molecular genetic studies have indicated that MH of pigs is caused by a single amino acid substitution in the RYR1-calcium release channel (Fujii et al., 1991). On the basis of this point mutation in the ryanodine receptor 1 gene a molecular genetic test procedure has been developed which allows to discriminate homozygous stress resistant (NN), heterozygous (Nn) and homozygous stress susceptibility (nn) pigs (Fujii et al., 1991; Otsu et al., 1992; Brenig et al., 1992). The molecular genetic test gives more reliable results than the formerly used "halothane challenge test" (Eikelenboom et al., 1974). It is well established that the RYR1-genotype influences performance traits in pigs like lean yield and carcass quality (Wittmann et al., 1993; Pommier et al., 1993; Schmitten et al., 1993; Scholz et al., 1994; Rempel et al., 1995). Studies on the effect of the RYR1-genotype on fertility traits are however rare.

The objective of our study is to investigate the relationship between RYR1-genotypes and reproductive performance in sows.

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Introduction

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Materials and Methods

Animals

A total of 2483 litters from 565 sows of German Landrace dam line (GL) and 2289 litters from 847 sows of the breed Leicoma (Lc) were used to analyse the influence of RYR1-genotype on fertility traits. The breeding sows were kept in two farms (tab. 1).

Tab. 1. - FREQUENCIES OF RYR1-GENOTYPES (NN/Nn/nn) AND ALLELE FREQUENCY (N) OF THE BREEDING SOWS

	NN	Nn	nn	N
Farm A German Landrace n=565	0.50	0.49	0.01	0.745
Farm B Leicoma n=847	0.54	0.45	0.01	0.765

Fertility traits

The following traits were included in the analyses:
- TNB: number of piglets total born per litter,
- NBA: number of piglets born alive per litter,
- NBD: number of piglets born dead per litter,
- NR: number of piglets reared per litter,
- reason for culling,
- age of first service,
- farrowing-to-farrowing intervall and
- average daily gain.

RYRI Genotyping

DNA from 80 µl blood was used to amplily a fragment of 659 base pairs with appropriate primers (Otsu et al., 1992) using the polymerase chain reaction (PCR). Subsequently, the PCR product was digested with Cfo I and separated in a 2% agarose ethidium bromide gel. The gene variants of the RYR1 were typed under UV.

Statistical Analysis

The influence of the RYR1-genotype on fertility traits was analysed by a Generalized Linear Model procedure (SAS 6.08). The following statistical models were used to estimate the last squares means (LSM):

1. Model for the analysis of the gilt and second litter

$$Y_{i,j,k,l} = \mu + G_i + S_j + A_k + I_l + e_{k,j,k,l}$$

2. Model for the analysis of all litters

$$Y_{i,j,k,l} = \mu + G_i + S_j + L_k + B_l + e_{i,j,k,l}$$

With:

$Y_{i,j,k,l}$: the dependent variable
G_i : the main effect of the RYR1-genotype
A_k : the main effect of the age of first service
L_k : the main effect of the parity
B_l : the random effect of the mated boar (only boars with at least 10 litters)
μ : the overall mean
S_j : the main effect of the season (successive 2 monthly classes)
I_l : the covariate for the farrowing-to-farrowing intervall
$e_{i,j,k,l}$: the random error

All statistical models used were significant ($p<0,05$). Due to imbalance of the data structure the low number of homozygous stress susceptible sows have not been included in the analysis.

Results

As shown in table 2 the RYR1-genotype did not significantly affect ($p<0,05$) the considered traits (tab. 2).

Table 2. - LEAST-SQUARES-MEANS (LSM) AND STANDARD ERRORS (S.E.) OF FERTILITY TRAITS IN THE GILT AND SECOND LITTER FOR RYR1-GENOTYPES AND BREEDS

Traits	German Landrace			Leicoma		
	RYR1-genotypes		significance	RYR1-genotypes		significance
	NN	Nn	p	NN	Nn	p
	(n=558)			(n=844)		
gilt litter						
TNB	9.29	8.80		10.43	10.55	
	0.24	0.24	0.059	0.24	0.25	0.575
NBA	9.22	8.74		9.95	10.14	
	0.24	0.24	0.062	0.23	0.24	0.362
NBD	0.07	0.06		0.47	0.41	
	0.04	0.04	0.864	0.06	0.06	0.186
NR	7.96	7.80		9.57	9.42	
	0.18	0.18	0.404	0.14	0.15	0.236
	(n=498)			(n=592)		
second litter						
TNB	10.62	10.99		10.67	10.77	
	0.23	0.23	0.194	0.28	0.29	0.691
NBA	10.57	10.91		10.37	10.57	
	0.23	0.23	0.223	0.27	0.28	0.418
NBD	0.06	0.08		0.30	0.20	
	0.03	0.03	0.520	0.06	0.06	0.078
NR	9.00	9.07		9.68	9.63	
	0.17	0.17	0.732	0.14	0.15	0.725

The homozygous stress resistant (NN) sows of GL provide 0,49 piglets TNB and 0,48 piglets NBA more in the gilt litter than the compared heterozygous (Nn) sows. Nevertheless, both values are near to the level of significane. In contrast to these results, the heterozygous sows of the breed Leicoma yield 0,12 piglets TNB and 0,19 piglets NBA more in gilt litter than the compared NN-sows.

According to expectations, the influence of the season of the fertility traits is large whereas the age of first service does not significantly affect the analysed traits in the gilt litter. In the second litter the litter size is only insignificantly influenced by the RYR1-deffective allele "n". The farrowing-to-farrowing intervall and the age of first service do not significantly influence the considered fertility traits.

Nevertheless, differences between both stress stable genotypes seem to appear tendentiously. In the breed Lc the heterozygous sows yield 0,10 and 0,20 piglets TNB and NBA more in the second litter than the homozygous genotypes. Surprisingly, in the German Landrace the heterozygous sows give 0,37 and 0,34 piglets TNB and NBA more in second litter than homozygous sows. However, this relation changes in the following parties in favour of the homozygous genotypes (about 0,4 piglets more per litter, results are not represented in this paper). Nevertheless, in all cases studied the differences obtained are not statistically significant at a level of $p<0,05$.

On the basis of all parities (litter number 1 to 12), including the effect of the mated boar it could be shown (tab. 3) that the heterozygous RYR1-animals of the breed Leicoma are tendentiously superior whereas in the German Landrace both RYR1-genotypes (in contrast to the results from the gilt litter) have a similar litter size.

Table 3. - LEAST-SQUARES-MEANS (LSM) AND STANDARD ERRORS (S.E.) OF THE FERTILITY TRAITS FOR RYR1-GENOTYPES OF ALL LITTERS

	German Landrace (n=2094)			Leicoma (n=2110)		
Traits	RYR1-genotypes		significance	RYR1-genotypes		significance
	NN	Nn	p	NN	Nn	p
TNB	11.07	10.94		12.01	12.25	
	0.14	0.15	0.497	0.15	0.16	0.088
NBA	10.82	10.67		11.52	11.77	
	0.14	0.15	0.310	0.14	0.15	0.057
NBD	0.25	0.29		0.49	0.48	
	0.04	0.04	0.236	0.04	0.04	0.607
NR	8.89	8.88		9.01	8.98	
	0.10	0.11	0.912	0.09	0.09	0.730

The mated boar, the parity and season significantly influenced the female fertility.

As shown in table 4 the homozygous stress stable sows in the German Landrace exceed the heterozygous sows in the average daily gain and the age of first service and thus, they have a higher live weight at the age of first

service. This could be a physiological cause for their higher litter size in the gilt litter.

In contrast to the breed Leicoma there is also a difference of two days in the farrowing-to-farrowing intervall between the RYR1-genotypes in German Landrace.

Table 4. - MEANS AND STANDARD ERRORS (S.E.) OF THE AVERAGE DAILY GAIN AND OTHER FERTILITY TRAITS FOR RYR1-GENOTYPES

	German Landrace		Leicoma	
	RYR1-geotypes			
Traits	NN	Nn	NN	Nn
average daily gain				
n	253	247	370	299
mean (g)	527.8[a]	516.0[b]	539.5[a]	535.3[a]
s.e.	2.47	2.75	2.60	2.54
age of first service				
n	281	277	462	382
mean (days)	271.5[a]	263.0[b]	252.0[a]	251.3[a]
s.e.	1.72	1.45	0.86	0.81
farrowing-to-farrowing intervall				
n	1025	898	823	621
mean (days)	159.5[a]	157.4[b]	148.9[a]	149.7[a]
s.e.	0.50	0.46	0.24	0.40

[a,b] Means with different superscripts are significantly different ($p<0.05$; t-test).

Out of all litters two classes were subdivided with small (1-6 piglets NBA per litter) and large litter size (13 - 21 piglets NBA per litter). To test whether the homozygous or heterozygous sows gave more small or large litters by means of contingency tables the Chi-Square test procedure was employed.

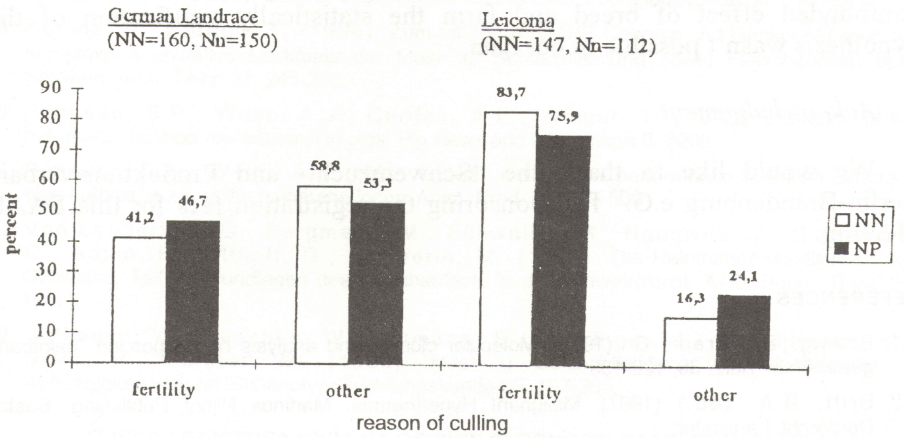
It could be shown that the homozygous sows of GL give significant more large (31% of all litters) and less small litters (9 %) than the heterozygous sows (27% and 11% respectively). In the breed Leicoma the difference is not significant.

Of further interest was whether there are relations between the RYR1-genotype and the reason of culling. For that purpose, all cullings were separated in two groups: 1. culled in relation to fertility (poor rearing performance, abortion, high returning rate) and 2. culled because of other reasons (diseases, exterior, putting down, skeletal deformation).

Within the homozygous sows in the GL nearly 18% less cullings are related to fertility (fig. 1) whereas the difference within the heterozygous sows is distinct lower (about 7%). In the breed Leicoma these relationship within the

RYR1-genotypes NN and Nn are reverse to GL which is in accordance with the results represented in the first part of the paper.

Fig. 1. - COMPARISON OF CULLINGS BECAUSE OF FERTILITY AND OTHER REASONS FOR RYR1-GENOTYPES



Discussion

Previous investigations on the basis of the halothane challenge test showed significant effects of the so called halothane gene on fertility of breeding sows (reviewed in Von Lengerken et al., 1988). Therefore, Hal$^-$ sows produced between 0,1 and 1.1 more piglets per litter than Hal$^+$ sows. These differences seemed to be more distinct in sire (Carden et al., 1985; Lampo et al., 1985) than in dam lines (Simpson et al., 1985). There were also some doubt whether there is any advantage in the homozygous or heterozygous Hal$^-$ sows with respect to their fertility due to the difficulty in determining the halothane-genotype of the pigs correctly.

Investigations by means of the molecular genetic test procedure (Fujii et al., 1991) proved that homozygous stress susceptible sows showed a decline in fertility (Falkenberg et al., 1993; Reiner et al., 1993; Hörügel et al., 1995). The differences between sows homozygous (NN) and heterozygous (Nn) for the RYR1-gene were not significant. However, the homozygous RYR1-genotypes seemed to be superior in litter size.

Our results confirm that between homozygous stress resistant (NN) and heterozygous (Nn) RYR1-genotypes no significant differences occur regarding fertility traits. However, the higher live weight of homozygous GL sows at the age of first service helps to explain the higher reproductivity in the gilt litter.

Furthermore, the tendentiously superiority of NN-sows in the German Landrace could support the hypothesis of an intermediary gene effect between

the alleles N and n or incomplete dominance of the defective allele as suggested by Simpson et al. (1989) for the appearance of PSE meat and by Schmitten et al. (1993) for stress susceptibility.

The contrary tendencies of RYR1-effect in the breed Leicoma could indicate interactions between breed and RYR1-genotype. Due to the confounded effect of breed and farm the statistically verification of that hypothesis wasn't possible in our data.

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REFERENCES

1. Brenig, B., Brem, G. (1992): Molecular cloning and analysis of the porcine "halothane" gene. Arch.-Tierz. 35, 129-135
2. Britt, B.A. (ed.) (1987): Malignant Hyperthermia. Martinus Nijhof Publishing, Boston, Dordrecht, Lancaster
3. Carden, A.E., Hill, W.H., Webb, A.J. (1985): The effects of halothane sesceptibility on some economically improtant traits in pigs. 1. Litter productivity. Anim. Prod. 40, 351-358.
4. Eikelenboom, G., Minkema, D. (1974): Prediction of pale, soft, exudative muscle with a non-lethal test for halothane induced porcine malignant hyperthermia syndrome. Neth. J. Vet. Sci. 99, 412-426.
5. Faleknberg, H., Matthes, W., Schwerin, M. (1993): MHS- und CK- Tests bei Schweinen, daren Bezeiehungen zueinander sowie zu weiteren Leistungsmerkmalen. Arch. Tierz. 36, 175-188.
6. Fujji, J., Otsu, K., Zorzato, F., De Leon, S. Khanna, V.K., Weiler, J., O'Brien, PIJ., Maclennan, D.H. (1991): Identification of a mutation in the porcine ryanodine receptor that is associated with malignant hyperthermia. Science 253, 448-451
7. Hörügel, K., Bergfeld, U., Eckert, S, Hallfarth, G. (1995): Bedeutung und Anwendung des MHS-Genetests beim Schwein, 2. Mitteilung: Auswirkungen einer Selektion nach dem MHS-Satus in Mutterrassen. Infodienst 2, Tierproduktion, 93-95
8. Lampo, P., Nauwynck, W., Bouquat, Y., Van Zeveren, A. (1985): Effects of stress susceptibility on some reproductive traits in Belgian Landrace pigs. Livest. Prod. Sci. 13, 279-287.
9. Nelson, T.E., Lin, M. (1995): Abnormal Function of Porcine Malignant Hyperthermia Calcium Release Channel in the Absence and Presence of Halothane. Cell Physiol Biochem 5, 10-22.
10. Otsu, K., Phillips, M.S., Khanna, V.K., De Leon, S., Mac Lennan, D.H. (1992): Refinement of Diagnostic Assays for a Probable Causal Mutation for Porcine and Human Malignant Hyperthermia. Genomics 13, 835-837.
11. Pommier, S.A., Houde, A. (1993): Effect of the Genotype for Malignant Hyperthermia as Determined by a Restrictioni Endonuclease Assay on the Quality Characteristics of Commerciai Pork Loins. J. Anim. Sci. 71, 420-425.

12. Reiner, G., Hecht, W., Herzog, A., Dzapo, V. (1993): Die Reproduktionsleistung von Sauen mit unterschiedlichem MHS-Status. Dtsch. tierärztl. Wschr. 100, 144-146.

13. Rempel, W.E., Ming Yu Lu, Mickelson, J.R., Louis, C.F. (1995): The effect of skeletal muscle ryanodine receptor genotype on pig performance and carcass quality traits. Anim. Science 60, 249-257.

14. Schmitten, F. (1993): Schweinefleish in der Zuchtpraxis. Züchtungskunde 65, 455-467.

15. Schloz, A., Hardge, T. (1994): Zum Einfluß von MHS-Genotyp und Genkonstruktion auf komplexe quantitative Merkmale der Mast- u. Schlachtleistung sowie Fleischqualität beim Schwein. Arch. Tierz. 37, 245-256.

16. Simpson, S.P., Webb, A.J., Carden, A.E., Wilmut, T. (1985): Influence of the halothane gene on reproduction in pigs. Pig News and Information 6, 2266.

17. Simpson, S.P., Webb, A.J. (1989): Growth and carcass performance of British Landrace pigs heterozygous at the halothane locus. Anim. Prod. 49, 503-509

18. Von Lengerken, G., Bergmann, M., Schwalbe, M., Haugwitz, T., Schlenker, G., Adam, H., Olthoff, D., Schwerin, M. (1988): Die Halothanempfindlichkeit des Schweins, Teil 1: Grundlagen des Halothantests in der schweinzucht. ADL, Berlin, Band 26, Heft 2

19. Wittmann, W., Peschke, W., Littmann, E., Behringer, J., Birkenmaier, St., Dovc, P., Förster, M. (1993): Mast- u. Schlachtleistung von DL-Kastraten in Abhängigkeit vom MHS-Genotyp. Züchtungskunde 65, 197-205.

UTJECAJ GENOTIPA RYR1 NA OSIBINE PLODNOSTI RASPLODNIH KRMAČA

Sažetak

Maligna hipertermija (MH) u svinja ekonomsi je važna farmakogenetička bolest što djeluje na reguliranje kalcija u mišićama kralježnice i završava iznenadnom smrću izazvanom stresom, te utječe na ekonomske gubitke smanjenjem sposobnosti za održavanjem na životu i kakvoće mesa. Genetički osjetljive svinje karakterizira hipermetabolizam mišića i krutost (rigor) zbog promjene u prolazu između zatvorenog i otvorenog položaja kanala za oslobađanje kalcija (RYR1) u muskulaturi kralježnice.

Molekularna genetička istraživanja pokazuju da MH svinja prouzročava jedan jedini aminokiselinski nadomjestak u kanalu za oslobađanje kalcija RYR1. Na osnovi ove promjene u genu ryanodine receptoru 1 razvijen je postupak molekularnog genetičkog testiranja što omogućuje razlikovanje homozigotnih svinja otpornih na stres, (NN), heterozigotnih (Nn) i homozigotnih osjetljivih na stres (nn). Molekularno genetičko testiranje daje pouzdanije rezultate od ranije primjenjivanog "halothane challenge test-a". Utvrđeno je da genotip RYR1 utječe na osobine performance svinja kao što su prinos mršavog mesa i kvaliteta polovica. Proučavanja o djelovanju genotipa RYR1 na osobine plodnosti, međutim, su rijetka.

Cilj ovog rada je istražiti odnos između genotipova RYR1 i reproduktivne performance krmača.

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