Genetic Parameters for Growth, Warner Bratzler Shear Force And The Calpain System in Beef

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Introduction

Tenderness is the most crucial quality trait in beef related to consumer acceptance (Devitt, Wilton, Mandell et al. 2002). Inconsistent tenderness has been identified as the major cause of consumer dissatisfaction with beef worldwide (Koohmaraie, Kent, Shackelford et al. 2002). Even if beef tenderness may be improved by various efforts along the production chain, considerable residual variation in tenderness remains. In a review, Åby (2008) reported a mean heritability estimate for Warner-Bratzler shear force of 0.25, demonstrating that a substantial part of this variation is due to genetics. Evidence that the calpain proteolytic enzyme system plays a crucial role for beef tenderness variability has been reported by many workers (i.e. Koohmaraie 1996). However, studies of the genetic (co)variation of these enzymes and their relation to beef tenderness are few.

Material and methods

Animals. Data were recorded 2001-2006 on 765 performance tested dual purpose NRF (Norwegian Red) bulls sired by 47 NRF A.I. bull sires. The bulls were culled following the ordinary test from 90-330 days of age. The test regime is a part of the national breeding scheme for the NRF breed, which include daily gain 90-330 days of age (ADG), conformation and fertility traits. Approx. 90 % of the NRF population is included in the breeding programme through use of A.I. Additionally, NRF accounts for 90 % of the beef produced in Norway. Thus, the test bulls included in the study were genetically related to most of the contemporary commercial slaughter cattle. Bull performance from the test station was recorded in addition to both carcass and meat quality traits. A limited part of the study is presented here, only.

Carcass and meat quality traits. The bulls were transported (1 h) and slaughtered in batches (6/year) at a commercial abattoir 1-2 h after arrival. The bulls were stunned by a captive bolt pistol and immediately bled. The carcasses were electrically stimulated (low-volt; 90 V) approx. 20 min. *p.m.* A sample of *m.l.dorsi* (LD) muscle (10^{th} thoracic to 2^{nd} lumbar vertebrae) from each carcass was hot-boned at 1 h *p.m.*, vacuum-packed and kept at approx. 12°C for 10 h to avoid cold-shortening before ageing at 4°C for 7 days. For Warner-Bratzler shear force (WBSF) analyses, meat slices (3.5 cm thick) were vacuum-packed in

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polyethylene bags, heated in a water bath at 70.5°C for 50 min and chilled in iced water for 50 min. This procedure gives a core temperature of 70.5°C in the WBSF samples. The slices were cut into rectangular pieces of 1x1x3 cm along the fiber direction, which were sheared perpendicular to the fiber direction with a WB shear force device attached to an Instron Materials Testing Machine. The average maximum force (N/cm^2) for ten parallels per animal was used in the data analysis.

Surface colour (L*a*b; MINOLTA CR-200) was measured on a fresh sample of the LD muscle from the 13^{th} thoracic vertebrae 8 days *p.m.* following exposure to air for 1 h at 20°C. Afterwards, the sample was cut clean from epimysium and fat, homogenised and frozen at -20°C for later analysis (Soxlet) of intramuscular fat % (IMF) and water % (WT). Muscle pH were recorded 1, 6, 10 and 48 h *p.m.*, the latter is presented here, only.

During hot-boning of the LD muscles on-line, muscle samples was snap-frozen in liquid nitrogen for later analyses of μ- and m-calpain enzyme activity (Raser, Posner, Wang 1995). Due to technical problems in the last of three analysis periods, only preliminary μ-calpain (CALP) analytical results are presented here. The three analysis periods for CALP consisted each of 6-10 days with 8-10 samples analysed per day. Calpastatin (CSTAT) samples were extracted from the *m.l.dorsi* sample 24-31 h *p.m.* by the procedure of Shackelford, Koohmaraie, Cundiff et al. (1994). Calpastatin activity was determined by use of the BODIPY-FL labeled casein (Thompson, Saldana, Cong et al. 2000). Due to costs, samples for enzyme analyses were collected from a reduced number of animals. These were chosen at random but balanced within sire and slaughter batch (calpains, n=192; calpastatin, n=251). For μ-calpain, the present preliminary results is based on n=109 records. The relative relationship between μ-calpain and calpastatin activity (CA/CT) was calculated as well.

Statistical analyses. Data were initially analysed in SAS (SAS, 2004) to determine the fixed effects included in the final models. These varied slightly with the traits studied. The basic model included the fixed effect of slaughter batch, slaughter age and pen within batch. Additionally, for μ-calpain, the fixed effect of analysis period x day was included as well. Genetic parameters were estimated by DMU-AI (Version 6; Madsen and Jensen 2008). Due to the relatively low number of animals with observations, single-trait models for variance-, and multi-trait (2-3 traits) animal models for covariance estimation were used, respectively. The analysis included 765 animals with records and 22 487 animals in the pedigree file. For analyses involving ADG, 1048 selected bulls contemporary in test with the 765 bulls were included as well (n=1813 in total).

Results and discussion

Estimates of genetic parameters for ADG and the meat quality traits studied are shown in table 1. The h^2 for ADG corresponded to the estimate from earlier work (0.30) with NRF test bulls (Aass 1996), and to the parameter used in the current NRF breeding scheme. Heritability estimates for WBSF have been reported in a wide range from 0.11 to 0.50 (Renand 1988; Shackelford, Koohmaraie, Cundiff et al. 1994; Devitt, Wilton, Mandell et al. 2002) with our estimate (0.23) in the intermediate range. The h^2 for IMF (0.71) was higher than most literature estimates (0.26-0.88, mean 0.46; review, Åby 2008), while the h^2 for

colour (L, a, b) and pH48 corresponded reasonably well to those reported by Aass (1996) of 0.27, 0.17, 0.08 and 0.10, respectively.

In overall, the genetic correlations were intermediate to high, while the corresponding phenotypic ones were low. The s.e. of the r_g estimates involving pH48 were all high (0.52-1.00), and should thus be considered carefully. The genetic correlations between ADG and meat quality were generally low. The exception is the r_g (s.e. 0.26) with L-lightness, which corresponded with the estimate of 0.49 reported by Aass (1996) and indicate that selection for ADG gives a correlated response in beef with lighter colour. Higher genetic relationships were observed between WBSF and the other traits. Thus, tough beef was genetically connected with lighter meat with reduced colour intensity, higher water content and lowered IMF %. Correspondingly, tender beef was associated with higher IMF % and more intense colour. These changes in meat quality may be related to an alteration in the proportion of red oxidative muscle fibers (Type IA and Type IIA) relative to the proportion of the white, glycolytic Type IIB muscle fibers. Such changes, as a response to selection for high growth rate and/or meat %, have been reported for several species by numerous authors (review; Åby 2008).

Table 1: Genetic parameters for ADG and the meat quality traits studied[α]

Traits	n	ADG	WBSF	IMF	WT	L	a	b	pH48
ADG	1813	**0.31**	-0.08	-0.17	0.14	0.48	0.14	0.25	-0.88
WBSF	674	0.04	**0.23**	-0.69	0.23	0.23	-0.60	-0.46	0.58
IMF	719	-0.04	-0.23	**0.71**	-0.68	0.31	0.42	0.50	-0.20
WT	719	0.02	0.21	-0.58	**0.51**	-0.51	0.35	-0.11	-0.49
L	603	0.13	-0.08	-0.04	0.14	**0.25**	-0.52	0.10	n.c.
a	603	-0.02	-0.10	0.16	-0.08	0.20	**0.24**	0.76	-0.54
b	603	0.04	-0.25	0.12	-0.05	0.17	0.85	**0.22**	n.c.
pH48	707	-0.08	-0.25	0.01	0.14	-0.15	-0.32	-0.38	**0.02**

[α]Heritabilities on the diagonal, phenotypic and genetic correlations below and above the diagonal, respectively.
n.c: not converged, s.e. r_g: 0.10-1.00

The genetic parameters for ADG, WBSF and the proteolytic muscle enzymes are presented in Table 2. Due to the low number of observations, the results should be considered carefully. The h^2 estimate for CA/CT was higher than those of CALP (0.17) and CSTAT (0.05). While no heritability estimates have been found in literature for CALP or CA/CT, h^2 for CSTAT have been reported in a wide range from 0.07 (Riley, Chase, Hammond et al. 2003) to 0.65 (Shackelford, Koohmaraie, Cundiff et al. 1994).

The genetic correlation between CALP and WBSF (-0.61) suggest a logic relationship between tender beef and high μ-calpain activity. This was supported by the reverse r_g of 0.41 between CSTAT and WBSF, similar to the estimate of 0.50 reported by Shackelford, Koohmaraie, Cundiff et al. (1994), but lower than reported (0.73) by Riley, Chase, Hammond et al. (2003). The negative genetic correlation between CALP and CSTAT was in good agreement with the r_g between enzymes and WBSF. For CA/CT, the multi-trait covariance estimation did not converge within the set number of iterations (n=200) and the

Table 2: Genetic parameters for ADG, WBSF and proteolytic muscle enzymes [α]

Traits	n	ADG	WBSF	CALP*	CSTAT	CA/CT*
ADG	1813	**0.31**	-0.08	-0.72	0.38	n.c.
WBSF	674	0.04	**0.23**	-0.61	0.41	n.c.
CALP*	109	-0.03	-0.05	**0.17**	-0.88	-
CSTAT	251	0.09	0.22	0.08	**0.05**	-
CA/CT*	109	-0.04	-0.59	-	-	**0.28**

[α]Heritabilities on the diagonal, phenotypic and genetic correlations below and above the diagonal, respectively.
*preliminary estimates, reduced sample (n=109). n.c: not converged

default convergence criterion. The genetic relationship between ADG and CALP and CSTAT, respectively, suggest that high genetic growth capacity is associated with reduced μ-calpain and increased calpastatin activity level.

Conclusion

These results support evidence from other studies that the calpain proteolytic enzyme system plays an important role for beef tenderness, and that this is related to genetic variability in enzyme activity in muscles *p.m.* Additionally, a high, favourable, genetic relationship was observed between WBSF and IMF. The importance of IMF for tender beef has been widely discussed. Our findings suggest that IMF may have a positive influence on beef tenderness, which act independent of the contributions to tender beef by myofibrillar ruptures caused by the proteolytic enzymes. Furthermore, the results indicate that high genetic growth capacity is associated with reduced μ-calpain and increased calpastatin activity level.

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