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CAST (CALPASTATIN) GENE INCIDENCE IN AUSTRALIAN PIGS AND EFFECTS ON PORK QUALITY

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I. INTRODUCTION

To address current knowledge gaps on the incidence of meat quality genes in Australian commercial pigs, this study surveyed 726 pigs for CAST (calpastatin) gene polymorphisms and investigated the associations between CAST mutations and pork quality indicators. Calpastatin is a specific inhibitor of μ- and m-calpain proteases and plays a key role in post-mortem tenderisation of meat. CAST gene polymorphisms have been identified in two gene locations; CAST249 is associated with variations in tenderness with the allele 249Lys associated with higher tenderness, lower cooking loss, and lower Instron force relative to 249Arg [1]. CAST638 allele 638Arg is associated with higher moisture in cured ham vs 638Ser [2]. Given the close proximity of the genes, the effects of CAST249/638 haplotypes on objective pork quality were investigated.

II. MATERIALS AND METHODS

Samples for genetic analysis and meat quality measurement were collected from 726 pigs (from terminal and maternal lines of three Australian suppliers) at processing plants located in Queensland, New South Wales and Western Australia over 2023 and 2024. Samples were collected ~15 min post-exsanguination, by taking ear clip samples into TSU tubes (Kit -Tissue sampling unit pk 100, Neogen Australasia, Bundamba QLD, Australia). These samples were analysed at Neogen Australasia laboratories (Bundamba, QLD, 4304, Australia) using GeneSeek® Genomic Profiler Porcine 50K Array and added tests for gene traits (Neogen ref: GGP_Por_TRAITS_BDL-GAA). At one day post-slaughter, the left loin was collected from each carcase, deboned and then cut in half at the 9th rib. The half loin primals were vacuum packed and transported to The University of Melbourne meat research facility. At two days post-slaughter, the half loins from each animal were assigned to either 0 (fresh) or 12 (aged) days of ageing at 0 $\pm$ 2 ^{o}C. At day 0 and 12, the LTL (*longissimus lumborum et thoracis)* was excised, and meat quality was measured. Drip loss was measured using the EZ-Drip loss method [3] and was calculated as weight loss over 48 h at 0-2 °C, as a % of initial weight. A 55 ± 5 g block of LTL was cooked in a plastic pouch in a 75 °C water bath until the core temperature reached 71 °C, cooled, then removed from the bag, patted dry, and cook loss was calculated as % weight loss relative to the weight of uncooked meat. The cooked block of pork was fabricated into 6 samples, 1 cm^2 and ~5 cm long (length parallel to the direction of the muscle fibres). WBSF (Warner-Bratzler shear force) was measured on these samples using a V-shaped blade attached to a texture analyser (Lloyd Instruments Ltd., Largo, FL, USA).Texture profile analysis for hardness was measured 5 times on one 1 cm thick steak sample using the Lloyd Instruments LRX Materials Testing Machine (Lloyd Instruments Ltd, Hampshire, UK) with 0.63 mm diameter probe perpendicular to the muscle fibre direction, and the result was expressed as hardness (N). Mixed linear modelling was conducted in Genstat v22 (VSN International, Hemel Hempstead, UK). Meat quality outcomes for day 0 and day 12 were individually analysed. The fixed factors included the CAST249/638 variants and their interaction, and the random model included carcase number nested within kill date, nested within supplier.

III. RESULTS AND DISCUSSION

CAST249 ArgArg and CAST638 ArgArg were the most prevalent haplotype (36.1%), followed by CAST249 ArgLys and CAST638 ArgSer (28.6%), CAST249 ArgLys and CAST638 ArgArg (16.6%), (Table 1). The most favourable haplotype for eating quality CAST249 LysLys and CAST638 ArgArg [1] constituted 2.6% of the sample population. The WBSF of aged pork tended ($P < 0.10$) to be influenced by the interaction of CAST249 and CAST638 ($P = 0.064$) (Table 2). Aged pork that had CAST249 LysLys and CAST638 ArgArg had the lowest WBSF at day 12, whereas other aged pork had similar WBSF. Similarly, aged pork with CAST249 LysLys and CAST638 ArgArg had the lowest hardness compared to other aged samples. In contrast, aged pork that had both CAST249 LysLys and CAST638 SerSer had higher hardness at day 12. EZ-drip loss of fresh pork was also influenced by the interaction of CAST249 and CAST638 ($P < 0.05$). In fresh pork, samples that had CAST249 LysLys and CAST638 ArgArg had lower EZ drip

loss than the other genotypes. The cook loss of fresh pork tended to be influenced by the interaction of CAST249 and CAST638 (P = 0.058). Fresh pork with CAST249 LysLys and CAST638 ArgArg had the lowest cook loss, whereas the fresh pork of other haplotypes had similar cook loss. Overall, the results indicate that the combination of CAST249 LysLys and CAST638 ArgArg led to higher water-holding capacity in fresh pork.

Table 1 – Relative frequency of CAST249 and CAST638 haplotypes

		CAST249		
		ArgArg	ArgLys	LysLys
CAST638	ArgArg	36.1	16.6	2.6
	ArgSer	0	28.6	9.4
	SerSer	0	0	6.6

Table 2 – Effects of calpastatin genes CAST249 (ArgArg vs ArgLys vs LysLys) and CAST638 (ArgArg vs ArgSer vs SerSer) on Warner-Bratzler shear force (WBSF), Hardness, EZ Drip loss and Cook Loss. Standard error of differences (SED) is for the interaction of CAST249 and CAST638 variants.

			CAST249				P-values		
			ArgArg	ArgLys	LysLys	SED	CAST249	CAST638	C249 x C638
WBSF (N) day 1	CAST638	ArgArg	29.6	29.7	29.3	1.74	0.67	0.12	0.81
		ArgSer		28.7	27.6				
		SerSer			30.9				
WBSF (N) day 12	CAST638	ArgArg	20.2	20.8	18.1	0.97	0.81	0.21	0.064
		ArgSer		19.6	19.8				
		SerSer			20.6				
Hardness (N) day 1	CAST638	ArgArg	34.0	34.4	32.1	0.89	0.31	0.65	0.46
		ArgSer		34.6	34.3				
		SerSer			34.3				
Hardness (N) day 12	CAST638	ArgArg	33.2	33.9	31.4	0.96	0.53	0.19	0.084
		ArgSer		33.4	33.6				
		SerSer			34.9				
Cook Loss (%) day 1	CAST638	ArgArg	20.4	20.6	19.2	0.48	0.26	0.33	0.058
		ArgSer		28.7	27.6				
		SerSer			20.8				
Cook Loss (%) day 12	CAST638	ArgArg	21.8	21.6	20.9	0.49	0.56	0.48	0.55
		ArgSer		20.8	20.8				
		SerSer			21.9				
EZ Drip Loss (%) day 1	CAST638	ArgArg	4.28	4.665	3.22	0.344	0.35	0.95	0.002
		ArgSer		4.41	4.74				
		SerSer			4.35				
EZ Drip Loss (%) day 12	CAST638	ArgArg	1.26	1.24	1.02	0.223	0.73	0.43	0.48
		ArgSer		1.33	1.36				
		SerSer			1.50				

IV. CONCLUSION

The low frequency (2.6%) of the optimum eating quality haplotype CAST249 LysLys and CAST638 ArgArg indicates there is scope to improve eating quality through selection for eating quality genes in Australian commercial pork breeding programs. The analysis of effects of haplotypes on quality indicators demonstrates that after ageing, favourable haplotypes have reduced WBSF and hardness, increased water holding capacity and reduced cook loss.

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