Associations between two gene markers and traits affecting Fresh and Dry-Cured Ham Processing Quality

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Summary and Implications

Two genetic markers were examined for their associations with fresh and dry-cured ham processing characteristics. The PRKAG3 gene marker denoted as RN199 had no affect on dry-cured ham processing characteristics. The CAST gene marker was a significant source (P<.05) variation for cured ham moisture content and tended to be a significant source (P<.10) of variation for yield, ham weight loss, salt and Minolta color change. The beneficial allele is likely different depending whether you are viewing the results from a processors or consumers perspective. The CAST 11 genotype appears to have beneficial affects for processing yield. This genotype would be preferred by processors as they would have more salable ham when compared to hams having CAST genotypes 12 or 22. However, if you are a consumer looking for a drier ham with a more traditional flavor, then the CAST genotype 22 is likely the most preferred. This demonstrates that the benefit of a particular CAST genotype that is most favorable can be dependent on which portion of the pork chain being discussed.

Introduction

The U.S. dry-cure ham (country-style) industry processed over 5.5 million hams annually 1998 (National Country Ham Association, personal communication). These hams had a retail value exceeding 200 million dollars. Inconsistent muscle quality can lead to variation yield and muscle color of dry-cured hams. It has been demonstrated that pork quality differences exist among pure breeds and company composite lines of seedstock (Goodwin and Burroughs, 1995). Previous research has indicated some breeds possess muscle quality traits that affect the characteristics of dry-cured Spanish Serrano hams (Oliver et al., 1993). Some other breeds or lines known to have a high frequency of the gene marker HAL 1843™ or the gene markers like rendement napole (RN^-) which will produce market hogs with undesirable muscle quality variation. This variation contributes to increased economic losses to the dry-cured ham industry through excessive water loss, poor processing characteristics and possibly increased spoilage. Additionally, these factors may play a role in consumers' acceptance of dry-cured ham, their cooking and eating experience, and their decision to buy country ham again.

Previous work has demonstrated that there are other genetic factors that influence meat quality. Ciobanu and co-workers (2002) reported a gene region affecting tenderness on chromosome 2 in the pig. They showed that the causative gene for these effects is called CAST and is thought to play a role in control the calpains which affect meat tenderization (Koohmarie, 1992). Additionally, another genetic region on chromosome 15 was found to be associated with the PRKAG3 gene marker RN199, and has been reported to explain 4-6% of ultimate pH variation in Berkshire x Yorkshire F2 pigs (Ciobanu, et al. 2001).

If these gene markers are found to influence the dry-cured processing properties and consumer acceptance, then selection could be utilized to increase the desirable marker in breeding populations. Additional profit could result from improved yield, decreased spoilage, etc. Additionally, improvement in the dry-cured processing and eating quality characteristics (salty flavor) has the potential to increase demand for pork through improved domestic and international acceptance of U.S. dry-cured products.

The objective of this study was to evaluate the effects of two gene molecular markers, RN199 and CAST, thought to impact fresh and dry-cured processing characteristics.

Materials and Methods

The National Swine Registry (NSR) arranged for breeders to deliver purebred Duroc market hogs on two marketing days to commercial harvesting facility (SouixPreme Packing Co., Sioux Center, IA). Pigs were harvested and after a 24-hour chill, the carcasses were broken into primal cuts. Hams were delivered to a commercial dry-cured ham processing facility (Clifty Farms Country Hams, Paris, TN) by refrigerated truck. Hams (17-20 lbs.) were processed on two test days (to evaluate day of slaughter effects) such that at least 60 hams were processed on the same day. The numbers of hams by type and test day are reported in Table 1.

Processing (salt mixture and procedures, curing time, curing temperature and humidity, etc.) followed normal commercial curing procedures (Clifty Farm Country Ham, Paris, TN). Fresh ham data collected included ham source, fresh weight, circumference, thickness, pH, temperature, lipid and moisture content of a muscle on the ham face. The objective color values recorded included Minolta Y (indicates percentage of light reflected based on 100%, higher values indicate more light reflected and hence a lighter color), Minolta L* (an index that is a lightness variable and more closely represents human sensitivity to color), and Hunter L (an additional lightness variable, higher values indicate more pale colors or lighter color).

Four trained evaluators subjectively scored the face of the hams for color, marbling and using the National Pork Producers Council (1999) standards.

After curing, the hams were cut using a band saw, and center cut slices approximately 3/8 inch in thickness were obtained. Using these slices, objective color evaluation occurred. Additionally, pH, moisture content and salt content of the cured center slices were evaluated. A muscle sample weighing approximately 100 grams of was obtained from the center cut slices and frozen for later DNA collection.

DNA was obtained from the muscle samples (Strauss, 1998) and multiple copies made using PCR. Porcine stress syndrome (Hal 1843™ genotype) (Fujii et al., 1991) and Napole (Milan et al., 2000) genotypes were determined following standard procedures. The genotype of each ham for the two markers of interest in this study, CAST and RN199, were determined following the methods outlined by Ciobanu et al. (2002) and Ciobanu et al. 2001, respectively.

Fixed model statistical analyses that included the effects of ham type, treatment day, packer, and molecular marker genotype were performed and a covariate for fresh ham weight was included when appropriate. The remaining residual effects are considered random error.

Results and Discussion

A total of 134 hams were evaluated for a variety of fresh and dry-cured processing quality characteristics. Of the 134 hams, DNA from only 125, 122, 127, and 127 of the original samples for the gene markers: HAL 1843™, rendement napole, CAST, and RN199, respectively, was amplified successfully. It appears that selection has occurred in the population against the negative allele for the HAL1843™ (n) and the rendement napole (RN^-) as indicated by the number of homozygous nn (0) HAL1843™ animals and the homozygous $RN^- RN^-$ (1) rendement napole samples.

The overall frequency for the N allele of the HAL1843™ gene marker was 0.976, while the frequency for the rn+ allele of the rendement napole gene was 0.94. Because the large effects on the traits of interest and the low frequency of RN^- and porcine stress syndrome carriers and homozygous positive animals, these animals were deleted from the data set to look only at the effects of the RN199 and CAST on meat quality. This resulted in 104 samples in the CAST data (62 with genotype 11, 33 with genotype 21, and 9 with 22 genotype) and 105 samples in the RN199 data (14 with genotype 11, 35 with genotype 21, and 55 with 22 genotype).

In this limited sample, CAST had no affect on any of the quality traits evaluated on fresh hams. However, there was one notable exception, CAST genotype was a significant (P<.05) source of variation for fresh ham temperature, which was measured in the center of the ham (Table 2). The average temperature for the CAST 11genotype was 1.98 $^-$C which was 0.43 $^-$C higher (P<.04) than the 12 and 0.69 $^-$C higher (P<.05) than the CAST 22 genotypes, respectively. Similarly, the RN199 gene marker had no detectable significant (P > 0.05) affects on the fresh quality traits evaluated in this study. However, the RN199 gene marker was trending toward being a significant source of variation for ham circumference (P<.10), ham weight (P<.14), and the objective measures of muscle color, Minolta L (P<.14), Hunter L (P<.13), and Minolta Y (P<.12). In all cases that were trending towards significance, the RN199 22 genotype had the superior score.

In this sample set, the dry-cured processing traits among the RN199 genotypes were not different (P>.10). More over, the only trait that was below a P-value of .22 was the percentage change in Minolta L values from the fresh ham evaluation to the end of processing evaluation (P<.13).

Several processing traits were significant (P<.05) or tended (P <.10) to be impacted by CAST genotype. Cured ham moisture content was significantly (P<.003) impacted by CAST genotype. The CAST 11 genotype cured moisture content was higher when compared to both the CAST 12 (P<.02) and 22 (<.01) genotypes respectively (Table 3). Similarly, CAST genotype trended towards being a significant source of variation for ham weight loss (P<.06) and yield (percentage of fresh ham weight lost during the curing process) (P<.10). Hams having CAST genotype 11 tended to have had a higher cured ham yield when compared to hams having CAST genotype 12 (Table 3). Similarly, hams having CAST genotype 11 tended to have lower weight loss when compared to the weight loss of hams having CAST genotype 12 (Table 3). There were no detectable differences between CAST genotypes 12 and 22 for these three traits. CAST genotype approached (P<.09) being a significant source of variation for cured salt content. Although not significantly (P<.10) different, hams having the CAST genotype 11 had lower percentage salt content in the after the curing process when compared to CAST genotype 12 and genotype 22. Additionally, the CAST genotype variation approached significance (P<.07) for Minolta L color change. It is difficult to interpret these results, but it appears that the curing process made the color of the ham more consistent when compared to the fresh ham objective measure of color. There were no other detectable differences in the cured quality parameters evaluated in this study among the CAST genotypes.

The results of this study correspond with those previously reported. Ciobanu and others (2002) reported that the CAST allele 1 is more beneficial for fresh pork quality traits when compared to allele 2. Additionally, they indicated that CAST allele 2 could be the beneficial allele for processed or cured pork traits. The results of the present study appear to follow these results especially when focusing on the dryness of the country style cured hams (Table 3). Although not significant (P>.05), the results mirror that of the cured salt content. Hams having CAST 22 genotype tended to have greater cured salt content. Hams

that are drier and possess a greater percentage of salt are preferred by some country-style cured pork product consumers. Drier muscle and greater salt content tend to contribute to their traditional flavor. However, the desirable CAST genotype might not be 22 if making selection decisions based upon processor quantities / qualities factors. CAST genotype tended be significant sources of variation for ham weight loss (P<.06) and cured ham yield (P<.10). CAST genotype 11 tended to have better values for these two traits when compared to genotypes 12 and / or 22. The impact of CAST genotype on fresh ham temperature is difficult to explain, but may be related to the ultimate pH or rate of pH decline in the ham muscles.

The affects of RN199 on the dried cured hams were unknown based upon the results of Ciobanu (2001). They reported that the RN199 QTL affects fresh quality traits. The present study found that RN199 had no detectable affect (P<.05) on any of the cured ham traits evaluated. However, the present study found no effects of RN199 on the fresh ham traits which are not in agreement with those reported by Ciobanu (2001). This may have resulted from the few numbers of hams examined.

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Table 1. Number of experimental (Duroc) and control (genetically undefined) hams by test day.

Processing Date	Duroc Hams	Undefined Hams	Total
1	30	30	60
2	34	40	74

Table 2. CAST and RN 199 effects on fresh ham traits.[1]

	CAST Genotype			CAST	RN199 Genotype			RN199
Trait	**11**	**12**	**22**	**P-Value**	**11**	**12**	**22**	**P-Value**
Circum, cm	65.4 ± 0.31	66.1 ± 0.38	66.0 ± 0.73	0.23	65.0 ± 0.58	65.3 ± 0.39	66.1 ± 0.31	0.10
Depth, cm	15.6 ± 0.18	15.6 ± 0.22	14.9 ± 0.42	0.31	15.6 ± 0.34	15.6 ± 0.23	15.5 ± 0.18	0.96
Temp, °C	1.98 ± 0.14[a]	2.41 ± 0.16[b]	2.67 ± 0.32[b]	0.04	2.51 ± 0.26	2.24 ± 0.18	2.09 ± 0.14	0.34
Wt., kg	8.67 ± 0.08	8.80 ± 0.10	8.60 ± 0.19	0.48	8.56 ± 0.15	8.64 ± 0.10	8.81 ± 0.08	0.14
Minolta L	50.5 ± 0.50	51.0 ± 0.61	48.8 ± 1.16	0.23	51.8 ± 0.91	50.9 ± 0.62	49.9 ± 0.49	0.14
Hunter L	43.5 ± 0.49	43.9 ± 0.59	41.7 ± 1.13	0.23	44.7 ± 0.89	43.9 ± 0.61	42.9 ± 0.47	0.13
Minolta Y	19.1 ± 0.43	19.4 ± 0.52	17.5 ± 0.99	0.24	20.1 ± 0.78	19.4 ± 0.53	18.5 ± 0.41	0.12
Color Score (1-6)	3.83 ± 0.09	3.76 ± 0.11	3.82 ± 0.20	0.87	3.70 ± 0.16	3.80 ± 0.11	3.83 ± 0.09	0.77
Marbling Score	2.40 ± 0.10	2.35 ± 0.13	2.00 ± 0.24	0.32	2.52 ± 0.19	2.38 ± 0.13	2.27 ± 0.10	0.48
Firmness Score	2.15 ± 0.05	2.13 ± 0.07	1.98 ± 0.13	0.44	2.18 ± 0.10	2.11 ± 0.07	2.12 ± 0.05	0.81
pH	5.94 ± 0.04	5.93 ± 0.05	6.00 ± 0.09	0.84	6.00 ± 0.08	5.91 ± 0.05	5.94 ± 0.04	0.57

[1]Row means within trait and genotype with different superscripts differ (P<.05).

Table 3. CAST and RN199 effects on dry-cured ham traits.[1]

	CAST Genotype			CAST	RN199 Genotype			RN199
Trait	**11**	**12**	**22**	**P-Value**	**11**	**12**	**22**	**P-Value**
Final Wt., Kg	7.12 ± 0.08	7.15 ± 0.09	7.09 ± 0.18	0.95	7.02 ± 0.14	7.06 ± 0.10	7.20 ± 0.07	0.32
Cut Wt., kg	6.85 ± 0.08	6.83 ± 0.09	6.70 ± 0.18	0.74	6.71 ± 0.14	6.79 ± 0.10	6.89 ± 0.07	0.43
Fat, %	3.87 ± 0.20	4.26 ± 0.25	4.27 ± 0.47	0.39	4.44 ± 0.37	4.22 ± 0.25	3.84 ± 0.20	0.24
Minolta L	51.8 ± 0.88	50.4 ± 1.46	51.9 ± 1.54	0.25	51.7 ± 1.09	50.5 ± 0.74	51.7 ± 0.58	0.38
Minolta Change,%	3.36 ± 1.49	-0.87 ± 1.82	6.69 ± 3.48	0.07	0.40 ± 2.78	-0.19 ± 1.88	3.97 ± 1.48	0.13
Hunter L	44.5 ± 0.35	44.3 ± 0.43	44.0 ± 0.83	0.79	44.6 ± 0.66	44.1 ± 0.44	44.5 ± 0.35	0.75
Hunter L Change	3.31 ± 1.37	1.27 ± 1.68	5.88 ± 3.20	0.38	0.53 ± 2.53	1.39 ± 1.72	4.27 ± 1.35	0.22
Minolta Y	20.0 ± 0.33	19.7 ± 0.41	19.9 ± 0.78	0.80	20.0 ± 0.62	19.7 ± 0.42	20.0 ± 0.33	0.82
Minolta Y Change	8.24 ± 2.91	3.34 ± 3.55	15.6 ± 6.79	0.24	2.03 ± 5.40	4.38 ± 3.65	10.2 ± 2.88	0.23
Yield,%	78.9 ± 0.42	77.6 ± 0.52	77.9 ± 0.99	0.10	78.3 ± 0.80	78.8 ± 0.54	78.1 ± 0.42	0.55
Moisture, %	75.9 ± 0.25	75.2 ± 0.32	75.8 ± 0.60	0.20	75.2 ± 0.48	75.5 ± 0.33	75.8 ± 0.25	0.44
Wt. Loss, kg	4.02 ± 0.09	4.33 ± 0.11	4.19 ± 0.20	0.06	4.08 ± 0.16	4.02 ± 0.11	4.24 ± 0.09	0.22
Cured Moisture, %	63.4 ± 0.33[a]	62.2 ± 0.40[b]	61.0 ± 0.76[b]	0.0037	63.1 ± 0.64	62.9 ± 0.43	62.5 ± 0.34	0.50
Salt, %	4.16 ± 0.11	4.42 ± 0.13	4.63 ± 0.25	0.09	4.00 ± 0.20	4.28 ± 0.13	4.39 ± 0.11	0.23
Cured pH	6.29 ± 0.02	6.30 ± 0.03	6.40 ± 0.06	0.21	6.32 ± 0.05	6.29 ± 0.03	6.31 ± 0.02	0.82

[1]Row means within trait and genotype with different superscripts differ (P<.05).