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Association of SNPs in AMY1A and AMY2A genes with chicken meat quality and clinical-chemical traits in chicken

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QTL studies can provide useful information with which to identify positional candidate genes and DNA sequence variations that can be used to develop genetic markers. In particular, the amylase in chicken serum may affect meat quality traits that are associated with improved starch digestion, which can be used as an indicator of the health status of a chicken. In this study, we investigated two amylase genes, AMY1A and AMY2A, because these genes are located in the QTL region affecting serum amylase levels. Association analyses based on a mixed linear model were performed to identify relationships between the SNPs present in these two genes and clinical–chemical and meat quality traits. As a result, we confirmed that these genes were not significantly associated with any clinical–chemical traits, indicating that these two genes are not directly involved in the amylase QTL effect. However, the AMY1A gene is significantly associated with the degree of yellow coloration (b*) in the thigh muscle, and the AMY2A gene is significantly associated with the water–holding capacity (WHC) of breast muscle. With further verifications, these genes could be used to improve the quality of these traits in chickens.

Key words: amylase, positional candidate gene, QTL, serum

INTRODUCTION

Poultry meat is favored by many people of different ethnic background, mainly because of its nutritional and health values. In addition, poultry meat contains high levels of protein and a low caloric concentration (Magdelaine et al., 2008). In fact, the global consumption of poultry meat is continuously increasing, particularly in developed countries, which consume more poultry meat than beef or pork. This trend has been increasing rapidly in recent years.

The development of livestock animals has previously focused on the improvement of growth traits, which reduced the animals' adaptability to changing environments. Thus, productivity was maintained or increased by controlling the environment with limited facilities (Hume et al., 2011; Scollan et al., 2011). However, the increase in global warming in recent years has led to rapid climate change, which will ultimately increase the cost of animal production by increasing the need for reinvestment in facilities, and by increasing the cost of controlling the environment in which livestock is raised. Thus, there will probably be a crisis within the livestock industry in the near future (Malik et al., 2015). Therefore, the development and improvement of breeds with greater environmental adaptability is required.

The Korean native chicken (KNC) has the disadvantage of growing more slowly than broiler chickens. However, its meat quality and robustness are unique, making it well adapted to all four seasons experienced in Korea (Jung *et al.*, 2011). In general, native breeds are more adaptable than commercial breeds. Thus, native breeds may have various characteristics that facilitate the maintenance of homeostasis. Therefore, identifying the causal factors of their robustness could help secure the future of the livestock industry (Calus *et al.*, 2013).

In the treatment and management of animals, including humans, serum clinical-chemical traits are considered to be very important, and are useful biomarkers for evaluating the health status of an individual (Yoo et al., 2012). Serum amylase plays an important role in the digestion and absorption of food, as an enzyme that facilitates the hydrolysis of starch into sugar. Amylase is found in three different isoforms: alpha, beta, and gamma. Alpha-amylase is a digestive enzyme produced by the salivary glands and pancreas (Akira et al., 1987). Therefore, alpha-amylase facilitates the digestion and absorption of metabolites vital to the maintenance of health, which can in turn support higher levels of adaptability to changing environments. Moreover, amylase is linked to meat quality, because it can influence the supply of nutritional factors by promoting digestion. In a previous study, two functional candidate genes (AMY1A and AMY2A) for amylase were identified in the QTL region in GGA8 (unpublished data). These two genes have been reported to be expressed in association with the secretion of digestive enzymes by the salivary glands and pancreas, in both humans and other animals. Therefore, this study focuses on the relationship between the two amylase genes (AMY1A and AMY2A), located in the QTL region, and both meat quality and serum clini-

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cal-chemical traits.

MATERIALS AND METHODS

Animals and measurement of phenotypes

A total of 88 parents (G₀) from five Korean native chicken lines, consisting 15 sires and 73 dams, were mated to generate 597 progeny (G1). A within-line mating design was used to establish the resource population used in this study. The animals were reared under the same feeding and control environment system and slaughtered using the same procedure and in the same environment, provided by the National Institute of Animal Science, Republic of Korea. Of these birds, 593 G₁ individuals were used to measure the traits investigated in this study, and were divided into 109 gray (G), 90 black (L), 135 red (R), 125 white (W), and 134 yellow (Y) lines, based on their plumage coloration. This study was performed according to recommendations described in "The Guide for the Care and Use of Laboratory Animals," published by the Institutional Animal Care and Use Committee of the National Institute of Animal Science (2012-C-037) in the Republic of Korea.

The blood samples were stored in a deep freezer at $-70^{\circ}\mathrm{C}$ until use. Serum samples were separated via centrifugation in EDTA blood tubes. Eight serum traits were measured in a previous KNC QTL study (unpublished data). The 26 meat quality–related traits of breast and thigh muscles were also measured in a previous meat quality study (Jung $et\ al.$, 2015).

DNA extraction and genotype analysis

The genomic DNA was extracted using a manual extraction method, and the concentrations of the extracted gDNA samples were measured using a NanoDrop 2000C spectrophotometer (Thermo Scientific, USA). Stock DNA was diluted for amplification and stored at – 20°C until use. The AMY1A and AMY2A genes were selected as potential amylase trait—related genes in a QTL region identified during a previous study. The SNP variations were obtained via the filtering of KNC SNP annotation information, and PCR primers were designed for the amplification of the targeted SNP regions (Seo et al., 2014). Validations of SNPs were performed using a direct sequencing method, and the KASP assay was designed for SNP genotyping (Table 1). The allele

Table 1. SNP information for *AMY1A* and *AMY2A* genes

Gene (SNP) (location)	SNP ID	Amino acid Change	Mutation type	FAM	HEX
AMY1A (g.17T>C) (exon 1)	rs314355067	ATT >GTT (I[Ile] >V[Val])	missense	Т	С
AMY2A (g.4271C>T) (exon 10)	rs10728230	CGC >CAC (R[Arg] >H[His])	missense	С	Т

Table 2. The results of the association analysis of the SNPs in AMY1A and AMY2A genes with clinical-chemical traits

Gene	Trait	P-value	CC	CT	TT	Additive	Dominant
AMY1A	Glu	0.199	260.88±2.76	255.63±2.02	256.34±2.55	-2.97±2.29	2.26±1.95
	T_Pro	0.469	4.21 ± 0.07	4.18 ± 0.05	4.28 ± 0.06	-0.06 ± 0.05	-0.03 ± 0.05
	GPT	0.871	2.91 ± 0.04	2.92 ± 0.03	2.93 ± 0.04	0 ± 0.03	-0.01 ± 0.02
	Cre	0.294	-0.23 ± 0.03	-0.23 ± 0.02	-0.17 ± 0.02	-0.02 ± 0.02	-0.02 ± 0.02
	HDL_c	0.507	95.89±3.66	96.75±2.98	100.46±3.46	-1.42 ± 2.41	-2.28 ± 2.18
	T_Cho	0.291	135.12±4.12	133.71±3.18	140.31±3.85	-3.99 ± 3.04	-2.59 ± 2.69
	GOT	0.871	5.38 ± 0.03	5.37 ± 0.02	5.39 ± 0.03	-0.01 ± 0.03	0 ± 0.02
	Amy	0.923	5.28 ± 0.07	5.29 ± 0.04	5.26 ± 0.06	0.02 ± 0.06	0.01 ± 0.05
AMY2A	Glu	0.135	256.2±1.58	261.64±2.75	253.45±5.65	6.81±3.63	-1.37±2.97
	T_Pro	0.808	4.21 ± 0.04	4.21 ± 0.07	4.31 ± 0.14	-0.04 ± 0.09	0.04 ± 0.07
	GPT	0.594	2.93 ± 0.03	2.90 ± 0.04	2.85 ± 0.08	0 ± 0.05	-0.04 ± 0.04
	Cre	0.971	-0.21 ± 0.01	-0.22 ± 0.03	-0.23 ± 0.06	0 ± 0.04	0 ± 0.03
	HDL_c	0.576	98.54±2.75	97.36±3.59	91.81±6.43	2.18 ± 3.7	-3.36±3.2
	T_Cho	0.811	137.01±2.86	135.02±4.16	132.22±8.01	0.4 ± 4.83	-2.39 ± 4.11
	GOT	0.757	5.37 ± 0.02	5.41 ± 0.04	5.42 ± 0.08	0 ± 0.05	0.02 ± 0.04
	Amy	0.422	5.29 ± 0.03	5.21±0.07	5.41±0.15	-0.13±0.1	0.05 ± 0.08

Glu: Glucose (mg/dl); T_Pro: Total protein (g/dl); Cre: Creatinine (mg/dl); HDL-C: High-density lipoprotein cholesterol (mg/dl); T-Cho: Total cholesterol (mg/dl); GOT: Glutamic oxaloacetic transaminase (IU/L); Amy: Amylase (IU/L)

 $\textbf{Table 3.} \ \ \text{The association results for the SNPs in } \ \textit{AMY1A} \ \ \text{and } \ \textit{AMY2A} \ \ \text{genes with meat quality traits}$

Gene	Trait	P-value	CC	CT	TT	Additive	Dominant
Bi Bi Bi Bi	Br_WHC	0.751	63.46±0.90	64.09±0.66	63.65±0.83	0.53±0.73	-0.09±0.6
	Br_H2O	0.912	4.29±1.0E-03	4.29±7.9E-04	4.29 ± 0.01	0±0	0 ± 0
	Br_cProtein	0.437	24.43 ± 0.04	24.44±0.03	24.37±0.04	0.03 ± 0.04	0.03 ± 0.0
	Br_cFat	0.226	0.81 ± 0.01	0.83 ± 0.01	0.82 ± 0.01	0.01 ± 0.01	0 ± 0
	Br_cAsh	0.331	1.16 ± 0.01	1.18 ± 0.01	1.18 ± 0.01	0±0	0 ± 0
	Br_Collagen	0.449	1.94 ± 0.04	1.94 ± 0.03	2.01 ± 0.03	-0.03 ± 0.03	-0.03 ± 0.0
	Leg_WHC	0.754	61.68±0.58	62.14±0.41	61.85±0.53	0.37 ± 0.52	-0.08 ± 0.4
	Leg_H2O	0.338	74.89 ± 0.11	74.99 ± 0.08	75.14±0.11	-0.02 ± 0.09	-0.12 ± 0.0
	Leg_cProtein	0.946	21.93±0.12	21.89±0.08	21.92±0.11	-0.03 ± 0.1	0±0.0
	Leg_cFat	0.977	1.11±0.02	1.11 ± 0.01	1.11 ± 0.02	0 ± 0.02	0±0.0
	Leg_cAsh	0.202	1.09 ± 0.01	1.11±4.5E-03	1.11 ± 0.01	0±0	0 ± 0
	Leg_Collagen	0.125	2.03 ± 0.03	2.01 ± 0.02	2.10 ± 0.03	-0.04 ± 0.02	-0.03 ± 0.0
	Br_Cooking loss	0.862	20.6±0.25	20.68±0.18	20.81±0.23	-0.02 ± 0.23	-0.1 ± 0.1
	Br_pH1	0.763	1.81±0.01	1.81 ± 0.01	1.81 ± 0.01	0 ± 0.01	0±0.0
	Br_pH2	0.683	1.75±0.01	1.75±0.01	1.76±0.01	0±0	0±0
	Br_Delta_pH	0.468	0.36 ± 0.03	0.37 ± 0.02	0.32 ± 0.03	0.02 ± 0.02	0.01±0.0
	Br_L*	0.532	59.62±0.44	59.63±0.31	60.20±0.41	-0.28 ± 0.38	-0.28±0.3
	Br_a*	0.482	2.00±0.02	1.97±0.01	1.95±0.02	0±0.02	0.02±0.
	Br_b*	0.058	21.58±0.18	21.19±0.14	21.21±0.17	-0.2 ± 0.12	0.18±0.
	Leg_Cooking loss	0.427	30.21±0.48	29.59±0.35	30.00±0.44	-0.51 ± 0.41	0.1±0.
	Leg_pH1	0.767	6.42±0.04	6.45±0.03	6.44±0.04	0.02±0.03	-0.01±0.
	Leg_pH2	0.170	6.13±0.05	6.10±0.04	6.23±0.05	-0.08 ± 0.05	-0.05±0.
	Leg_Delta_pH	0.702	0.27±0.02	0.25±0.01	0.25±0.02	-0.01±0.02	0.01±0.
	Leg_L*	0.195	49.24±0.39	48.49±0.28	48.39±0.36	-0.32±0.34	0.42±0.
	Leg_a*	0.601	13.70±0.18	13.52±0.13	13.65±0.16	-0.15±0.15	0.02±0.
	Leg_b*	0.012	20.68±0.15	20.16±0.11	20.17±0.13	-0.26±0.13	0.25±0.
AMY2A	Br_WHC	4.2E-04	63.72±0.55	65.26±0.90	58.12±1.81	-4.33±1.14	2.79±0.9
AMY2A	Br_WHC Br_H2O	4.2E-04 0.239	63.72±0.55 4.28±0	65.26±0.90 4.29±1.1E-03	58.12±1.81 4.29±2.2E–03	-4.33±1.14 0±0	2.79±0.9 0±0
AMY2A							0±0
AMY2A	Br_H2O	0.239	4.28 ± 0	4.29±1.1E-03	4.29±2.2E-03	0 ± 0	0±0 -0.01±0.
AMY2A	Br_H2O Br_cProtein	0.239 0.945	4.28±0 24.42±0.02	4.29±1.1E-03 24.42±0.04	4.29±2.2E-03 24.38±0.10	0±0 0.01±0.06	0±0 -0.01±0. 0±0.
AMY2A	Br_H2O Br_cProtein Br_cFat	0.239 0.945 0.732	4.28±0 24.42±0.02 0.82±0	4.29±1.1E-03 24.42±0.04 0.83±0.01	4.29±2.2E-03 24.38±0.10 0.81±0.02	0±0 0.01±0.06 0.01±0.01	0±0 -0.01±0. 0±0. -0.01±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh	0.239 0.945 0.732 0.515	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02	0±0 0.01±0.06 0.01±0.01 0.01±0.01 0.07±0.06	0±0 -0.01±0. 0±0. -0.01±0. -0.04±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC	0.239 0.945 0.732 0.515 0.458	4.28±0 24.42±0.02 0.82±0 1.17±0	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27	0±0 0.01±0.06 0.01±0.01 0.01±0.01 0.07±0.06 0.22±0.84	0±0 -0.01±0. 0±0. -0.01±0. -0.04±0. -0.8±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen	0.239 0.945 0.732 0.515 0.458 0.442	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25	0±0 0.01±0.06 0.01±0.01 0.01±0.01 0.07±0.06 0.22±0.84 -0.13±0.15	0±0 -0.01±0. 0±0. -0.01±0. -0.04±0. -0.8±0. 0.16±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27	0±0 0.01±0.06 0.01±0.01 0.01±0.01 0.07±0.06 0.22±0.84 -0.13±0.15 0.13±0.17	0 ± 0 -0.01 ± 0 . 0 ± 0 . -0.01 ± 0 . -0.04 ± 0 . -0.8 ± 0 . 0.16 ± 0 .
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.17 -0.01 ± 0.03	0 ± 0 -0.01 ± 0 . 0 ± 0 . -0.01 ± 0 . -0.04 ± 0 . -0.8 ± 0 . 0 ± 0 . -0.03 ± 0 .
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.15 0.13 ± 0.17 -0.01 ± 0.03 0.01 ± 0	0 ± 0 -0.01 ± 0 . 0 ± 0 . -0.01 ± 0 . -0.04 ± 0 . -0.8 ± 0 . 0.16 ± 0 . 0 ± 0 . -0.03 ± 0 . 0 ± 0
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01 2.08±0.03	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07	0±0 0.01±0.06 0.01±0.01 0.01±0.01 0.07±0.06 0.22±0.84 -0.13±0.15 0.13±0.17 -0.01±0.03 0.01±0 0.06±0.04	0±0 -0.01±0. 0±00.01±00.04±00.8±0. 0.16±0. 0±00.03±0. 0±00.01±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01 2.08±0.03 20.66±0.28	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.15 0.13 ± 0.17 -0.01 ± 0.03 0.01 ± 0 0.06 ± 0.04 -0.05 ± 0.4	0±0 -0.01±0. 0±00.01±00.04±00.8±0. 0.16±0. 0±00.03±0. 0±0 -0.01±0. 0.02±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss Br_pH1	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14 1.81±0	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01 2.08±0.03 20.66±0.28 1.81±0.01	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.15 0.13 ± 0.17 -0.01 ± 0.03 0.01 ± 0 0.06 ± 0.04 -0.05 ± 0.4 0 ± 0.01	0±0 -0.01±0. 0±00.01±00.04±00.8±0. 0.16±0. 0±00.03±0. 0±00.01±0. 0.02±0. 0±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14 1.81±0 1.75±0	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01 2.08±0.03 20.66±0.28 1.81±0.01 1.75±0.01	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.15 0.13 ± 0.17 -0.01 ± 0.03 0.01 ± 0 0.06 ± 0.04 -0.05 ± 0.4 0 ± 0.01 -0.01 ± 0.01	0±0 -0.01±0. 0±00.04±00.8±0. 0±00.03±0. 0±00.01±0. 0±0. 0.02±0. 0±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2 Br_Delta_pH	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556 0.548	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14 1.81±0 1.75±0 0.34±0.02	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01 2.08±0.03 20.66±0.28 1.81±0.01 1.75±0.01 0.38±0.03	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02 0.33±0.07	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.15 0.13 ± 0.17 -0.01 ± 0.03 0.01 ± 0 0.06 ± 0.04 -0.05 ± 0.4 0 ± 0.01 -0.01 ± 0.01 0.04 ± 0.04	0±0 -0.01±0. 0±00.04±00.8±0. 0±00.03±0. 0±00.01±0. 0.02±0. 0±0. 0±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2 Br_Delta_pH Br_L*	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556 0.548	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14 1.81±0 1.75±0 0.34±0.02 59.69±0.26	$4.29\pm1.1E-03$ 24.42 ± 0.04 0.83 ± 0.01 1.18 ± 0.01 1.99 ± 0.04 61.60 ± 0.61 75.02 ± 0.12 22.02 ± 0.13 1.06 ± 0.02 1.11 ± 0.01 2.08 ± 0.03 20.66 ± 0.28 1.81 ± 0.01 1.75 ± 0.01 0.38 ± 0.03 60.08 ± 0.46	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02 0.33±0.07 60.69±0.94	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.15 0.13 ± 0.17 -0.01 ± 0.03 0.01 ± 0 0.06 ± 0.04 -0.05 ± 0.4 0 ± 0.01 -0.01 ± 0.01 0.04 ± 0.04 -0.11 ± 0.01	0±0 -0.01±0. 0±00.04±00.8±0. 0.16±0. 0±00.03±0. 0±0. 0.02±0. 0±0. 0.01±0. 0.01±0. 0.049±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2 Br_Delta_pH Br_L* Br_a*	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556 0.548 0.548 0.928	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14 1.81±0 1.75±0 0.34±0.02 59.69±0.26 1.97±0.01	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01 2.08±0.03 20.66±0.28 1.81±0.01 1.75±0.01 0.38±0.03 60.08±0.46 1.96±0.02	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02 0.33±0.07 60.69±0.94 1.96±0.05	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.15 0.13 ± 0.17 -0.01 ± 0.03 0.01 ± 0 0.06 ± 0.04 -0.05 ± 0.4 0 ± 0.01 -0.01 ± 0.01 0.04 ± 0.04 -0.1 ± 0.61 0 ± 0.03	0±0 -0.01±0. 0±00.04±00.04±0. 0±0. 0±00.03±0. 0±00.01±0. 0±0. 0±0. 0.01±0. 0±0. 0±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_CAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2 Br_Delta_pH Br_L* Br_a* Br_b*	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556 0.548 0.548 0.928 0.882	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14 1.81±0 1.75±0 0.34±0.02 59.69±0.26 1.97±0.01 21.3±0.14	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01 2.08±0.03 20.66±0.28 1.81±0.01 1.75±0.01 0.38±0.03 60.08±0.46 1.96±0.02 21.34±0.18	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02 0.33±0.07 60.69±0.94 1.96±0.05 21.18±0.34	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.15 0.13 ± 0.17 -0.01 ± 0.03 0.01 ± 0 0.06 ± 0.04 -0.05 ± 0.4 0 ± 0.01 -0.01 ± 0.01 0.04 ± 0.04 -0.12 ± 0.01 0.04 ± 0.04 -0.101	0±0 -0.01±0. 0±00.04±00.08±0. 0.16±0. 0±00.03±0. 0±0. 0.02±0. 0±0. 0.01±0. 0.049±0. 0±00.06±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_CAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2 Br_Delta_pH Br_L* Br_a* Br_b* Leg_Cooking loss	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556 0.548 0.548 0.928 0.882 0.725	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14 1.81±0 1.75±0 0.34±0.02 59.69±0.26 1.97±0.01 21.3±0.14 29.9±0.31	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01 2.08±0.03 20.66±0.28 1.81±0.01 1.75±0.01 0.38±0.03 60.08±0.46 1.96±0.02 21.34±0.18 29.54±0.52	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02 0.33±0.07 60.69±0.94 1.96±0.05 21.18±0.34 30.24±1.07	0 ± 0 0.01 ± 0.06 0.01 ± 0.01 0.01 ± 0.01 0.07 ± 0.06 0.22 ± 0.84 -0.13 ± 0.15 0.13 ± 0.17 -0.01 ± 0.03 0.01 ± 0 0.06 ± 0.04 -0.05 ± 0.4 0 ± 0.01 -0.01 ± 0.01 0.04 ± 0.04 -0.11 ± 0.01 0.04 ± 0.04 -0.11 ± 0.01 0.09 ± 0.19 -0.53 ± 0.67	0±0 -0.01±0. 0±00.04±00.08±0. 0±00.03±0. 0±00.01±0. 0±0. 0±0. 0.01±0. 0±0. 0.01±0. 0±0. 0
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2 Br_Delta_pH Br_L* Br_a* Br_b* Leg_Cooking loss Leg_PH1	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556 0.548 0.548 0.928 0.882 0.725 0.499	4.28 ± 0 24.42 ± 0.02 0.82 ± 0 1.17 ± 0 1.95 ± 0.02 62.17 ± 0.32 74.98 ± 0.08 21.89 ± 0.07 1.12 ± 0.01 1.1 ± 0 2.03 ± 0.02 20.69 ± 0.14 1.81 ± 0 1.75 ± 0 0.34 ± 0.02 59.69 ± 0.26 1.97 ± 0.01 21.3 ± 0.14 29.9 ± 0.31 6.44 ± 0.02	4.29±1.1E-03 24.42±0.04 0.83±0.01 1.18±0.01 1.99±0.04 61.60±0.61 75.02±0.12 22.02±0.13 1.06±0.02 1.11±0.01 2.08±0.03 20.66±0.28 1.81±0.01 1.75±0.01 0.38±0.03 60.08±0.46 1.96±0.02 21.34±0.18 29.54±0.52 6.43±0.04	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02 0.33±0.07 60.69±0.94 1.96±0.05 21.18±0.34 30.24±1.07 6.55±0.09	0±0 0.01±0.06 0.01±0.01 0.01±0.01 0.07±0.06 0.22±0.84 -0.13±0.15 0.13±0.17 -0.01±0.03 0.01±0 0.06±0.04 -0.05±0.4 0±0.01 -0.01±0.01 0.04±0.04 -0.1±0.61 0±0.03 0.09±0.19 -0.53±0.67 -0.05±0.06	0±0 -0.01±0. 0±00.04±00.8±0. 0.16±0. 0±00.03±0. 0±0. 0.02±0. 0±0. 0.01±0. 0±0. 0.49±0. 0.17±0. 0.05±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2 Br_Delta_pH Br_L* Br_a* Br_b* Leg_Cooking loss Leg_pH1 Leg_pH2	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556 0.548 0.548 0.928 0.882 0.725 0.499 0.765	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14 1.81±0 1.75±0 0.34±0.02 59.69±0.26 1.97±0.01 21.3±0.14 29.9±0.31 6.44±0.02 6.14±0.03	$4.29\pm1.1E-03$ 24.42 ± 0.04 0.83 ± 0.01 1.18 ± 0.01 1.99 ± 0.04 61.60 ± 0.61 75.02 ± 0.12 22.02 ± 0.13 1.06 ± 0.02 1.11 ± 0.01 2.08 ± 0.03 20.66 ± 0.28 1.81 ± 0.01 1.75 ± 0.01 0.38 ± 0.03 60.08 ± 0.46 1.96 ± 0.02 21.34 ± 0.18 29.54 ± 0.52 6.43 ± 0.04 6.19 ± 0.06	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02 0.33±0.07 60.69±0.94 1.96±0.05 21.18±0.34 30.24±1.07 6.55±0.09 6.15±0.13	0±0 0.01±0.06 0.01±0.01 0.01±0.01 0.07±0.06 0.22±0.84 -0.13±0.15 0.13±0.17 -0.01±0.03 0.01±0 0.06±0.04 -0.05±0.4 0±0.01 -0.01±0.01 0.04±0.04 -0.1±0.61 0±0.03 0.09±0.19 -0.53±0.67 -0.05±0.06 0.04±0.08	0±0 -0.01±0. 0±00.04±00.8±0. 0.16±0. 0±00.03±0. 0±0. 0.01±0. 0.02±0. 0±0. 0.01±0. 0±0. 0.15±0. 0±0. 0.49±0. 0.17±0. 0.05±0. 0.05±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2 Br_Delta_pH Br_L* Br_a* Br_b* Leg_Cooking loss Leg_pH1 Leg_pH2 Leg_pH2 Leg_pH2 Leg_pH2 Leg_Delta_pH	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556 0.548 0.548 0.548 0.928 0.882 0.725 0.499 0.765 0.239	4.28 ± 0 24.42 ± 0.02 0.82 ± 0 1.17 ± 0 1.95 ± 0.02 62.17 ± 0.32 74.98 ± 0.08 21.89 ± 0.07 1.12 ± 0.01 1.1 ± 0 2.03 ± 0.02 20.69 ± 0.14 1.81 ± 0 1.75 ± 0 0.34 ± 0.02 59.69 ± 0.26 1.97 ± 0.01 21.3 ± 0.14 29.9 ± 0.31 6.44 ± 0.02 6.14 ± 0.03 0.25 ± 0.01	$4.29\pm1.1E-03$ 24.42 ± 0.04 0.83 ± 0.01 1.18 ± 0.01 1.99 ± 0.04 61.60 ± 0.61 75.02 ± 0.12 22.02 ± 0.13 1.06 ± 0.02 1.11 ± 0.01 2.08 ± 0.03 20.66 ± 0.28 1.81 ± 0.01 1.75 ± 0.01 0.38 ± 0.03 60.08 ± 0.46 1.96 ± 0.02 21.34 ± 0.18 29.54 ± 0.52 6.43 ± 0.04 6.19 ± 0.06 0.27 ± 0.02	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02 0.33±0.07 60.69±0.94 1.96±0.05 21.18±0.34 30.24±1.07 6.55±0.09 6.15±0.13 0.35±0.05	0±0 0.01±0.06 0.01±0.01 0.01±0.01 0.07±0.06 0.22±0.84 -0.13±0.17 -0.01±0.03 0.01±0 0.06±0.04 -0.05±0.4 0±0.01 -0.01±0.03 0.09±0.19 -0.53±0.67 -0.05±0.06 0.04±0.08 -0.02±0.03	0±0 -0.01±0. 0±00.01±00.04±00.8±0. 0.16±0. 0±00.03±0. 0±0. 0.01±0. 0.02±0. 0±0. 0.49±0. 0±00.06±0. 0.17±0. 0±0. 0.05±0.
AMY2A	Br_H2O Br_cProtein Br_cFat Br_cAsh Br_Collagen Leg_WHC Leg_H2O Leg_cProtein Leg_cFat Leg_cAsh Leg_Collagen Br_Cooking loss Br_pH1 Br_pH2 Br_Delta_pH Br_L* Br_a* Br_b* Leg_Cooking loss Leg_pH1 Leg_pH2	0.239 0.945 0.732 0.515 0.458 0.442 0.418 0.642 0.117 0.531 0.338 0.991 0.912 0.556 0.548 0.548 0.928 0.882 0.725 0.499 0.765	4.28±0 24.42±0.02 0.82±0 1.17±0 1.95±0.02 62.17±0.32 74.98±0.08 21.89±0.07 1.12±0.01 1.1±0 2.03±0.02 20.69±0.14 1.81±0 1.75±0 0.34±0.02 59.69±0.26 1.97±0.01 21.3±0.14 29.9±0.31 6.44±0.02 6.14±0.03	$4.29\pm1.1E-03$ 24.42 ± 0.04 0.83 ± 0.01 1.18 ± 0.01 1.99 ± 0.04 61.60 ± 0.61 75.02 ± 0.12 22.02 ± 0.13 1.06 ± 0.02 1.11 ± 0.01 2.08 ± 0.03 20.66 ± 0.28 1.81 ± 0.01 1.75 ± 0.01 0.38 ± 0.03 60.08 ± 0.46 1.96 ± 0.02 21.34 ± 0.18 29.54 ± 0.52 6.43 ± 0.04 6.19 ± 0.06	4.29±2.2E-03 24.38±0.10 0.81±0.02 1.15±0.02 1.87±0.09 60.57±1.27 75.32±0.25 21.88±0.27 1.04±0.05 1.10±0.01 2.00±0.07 20.74±0.60 1.82±0.03 1.77±0.02 0.33±0.07 60.69±0.94 1.96±0.05 21.18±0.34 30.24±1.07 6.55±0.09 6.15±0.13	0±0 0.01±0.06 0.01±0.01 0.01±0.01 0.07±0.06 0.22±0.84 -0.13±0.15 0.13±0.17 -0.01±0.03 0.01±0 0.06±0.04 -0.05±0.4 0±0.01 -0.01±0.01 0.04±0.04 -0.1±0.61 0±0.03 0.09±0.19 -0.53±0.67 -0.05±0.06 0.04±0.08	0±0 -0.01±0. 0±00.04±00.8±0. 0.16±0. 0±00.03±0. 0±0. 0.01±0. 0.02±0. 0±0. 0.01±0. 0±0. 0.15±0. 0±0. 0.49±0. 0.17±0. 0.05±0. 0.05±0.

Br: breast muscle; Leg: thigh muscle; cFat: crude fat content (%); cAsh: crude ash content (%); H2O: crude moisture (%); cProtein: crude Protein; L*: CIE lightness value; a*: CIE redness value; b*: CIE yellowness; WHC; water-holding capacity (%); pH 1: after slaughter 15 min pH; pH 2: ultimate pH

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discrimination PCR assay was designed using fluorescent dye (FAM and HEX), and the modification of an allele–specific primer pair was carried out using Bio–Rad real–time PCR (Bio–Rad, USA). Confirmed genotype information was arranged for further analysis using the Microsoft Excel software program.

Parentage and association analysis

The parentage analysis was performed to confirm the genotype validation, via the inheritance checking function of the CRI–MAP program. The normal distribution and descriptive statistics of meat quality and clinical chemical traits were obtained using MINITAB® version 14 (MINITAB Inc., USA). We used a mixed linear model to conduct SNP association analyses of the genes and traits, while simultaneously considering the pedigree structure of the KNC resource population:

$$Y_{ijklm} = \mu + S_i + B_j + L_k + G_l + b_1 CW_{ijklm} + A_{ijklm} + \varepsilon_{ijklm}$$
(MODEL 1)

where, $Y_{\mbox{\tiny liklmn}}$ represents the measured phenotypic data; μ represents the general mean, S_i represents the fixed effect of the ith sex, B_i represents the fixed effect of the jth batch (two levels), L, represents the fixed effect of the k^{th} line (five levels), G_1 represents the fixed effect of the lth genotype (three levels) of AMY1A or AMY2A, b₁ represents a regression coefficient, CW_{iiklm} represents the covariate for the carcass weight, $A_{\mbox{\tiny ijklm}}$ is the random additive polygenic effect and $\varepsilon_{\scriptscriptstyle ijklm}$ is the random residual effect. The mean and variance of the random additive polygenic effects can be defined as: $A \sim N(0, \mathbf{A} \sigma_0^2)$, where A is based on the relationship matrix computed using the nuclear families examined in this study, and σ_a^2 represents the additive polygenic variance. The mean and variance of the residual random effect of birds can be defined as: $\varepsilon \sim N(0, \mathbf{I} \sigma_a^2)$, where \mathbf{I} represents the identity matrix and σ_a^2 represents the residual variance.

The significance levels of the fixed effects in **MODEL 1** were calculated using the Wald procedure implemented in ASReml–R. P–values of less than 0.05 were regarded as statistically significant. To predict genotypic values and their standard error in each SNP genotype, the *predict* command in ASReml–R was used (Gilmour *et al.*, 1995). In order to compute the additive and dominance coefficients of each SNP found in AMY1A or AMY2A, G_1 in **MODEL 1** was replaced by the additive variable X_{add} (coded as -1, 1 for the two homozygotes, 0 for heterozygote), and dominance variable $X_{dominance}$ (coded as 0 for two homozygotes, 1 for heterozygote). Trait values were then regressed onto each coefficient at each SNP marker of AMY1A and AMY2A, respectively.

RESULTS AND DISCUSSION

The positional candidate genes, AMY1A and AMY2A, were located in the amylase–associated QTL region in GGA8, and an association study of the clinical–chemical and meat quality traits of the KNC resource population was performed. As a result, a significant association was

found between AMYIA and the b* value of the thigh muscle. A significant additive association with yellowness was found, in that the animals had the highest value of the CC genotype, at 20.69 ± 0.16 . Animals had the lowest value of TT genotype, at 20.18 ± 0.14 . In addition, a significant correlation was found between the AMY2A gene and the water-holding capacity (WHC) of breast muscle (Table 3). Although we identified the meat quality traits with which the AMY1A and AMY2A genes were associated, our results indicate that there is no significant association between the candidate genes and the amylase levels in the serum (Table 2).

In the GGA8 QTL region, four genes related to amylase production (AMY1A, AMY2A, AMYP (a pseudo gene), and LOC768251) were identified using the NCBI database (http://www.ncbi.nlm.nih.gov/). The number of amylase-related genes in chickens is very small, compared to the size of the amylase-related gene family in humans and mice (Benkel et al., 1997; Perry et al., 2007). This may be because of differences between the amylase gene families of birds and mammals. In addition, this may be because there have simply not been many functional validation studies on the chicken amylase genes, because these genes have primarily been identified by comparative genomics studies using human and mouse data. Supporting this analysis, the amylase-related significant QTL region (0~15 M bp) in chickens was found to contain a large number of genes of unidentified function.

The AMY2A gene was most strongly associated with the magnitude of the WHC of the breast muscle. Animals of the TT genotype had the lowest WHC compared to animals of the CT and CC genotypes. Further, the crude fat content of the thigh muscle has an additive significant association with genotype. The relationship between meat quality and amylase can be ascribed to the digestion of food and the absorption of metabolites into the body. Thus, amylases can affect meat quality. We propose two reasons for the significant association between the SNP in the AMY2A gene and WHC.

First, it is possible that the AMY2A gene is directly involved in determining the WHC. The second possibility is that the SNP in the AMY2A gene either is very closely linked to or experiences linkage disequilibrium (LD) from the nearby gene significantly associated with WHC. Supporting the second possibility is the fact that the significant amylase-related QTL region contains a large number of genes of unknown function. This region contained various candidate genes for collagen synthesis, such as proline-rich coiled-coil 2C (PRRC2C), collagen type XI alpha 1 (COL11A1), and proteoglycan 4 (PRG4). The proline of the PRRC2C gene is hydro-proline, which is a non-essential amino acid and accounts for two-thirds of collagen biosynthesis in conjunction with a glutamic acid. COL11A1 is involved in the functioning of the growth plate and generates the agglomerated collagen in cartilage. The PRG4 gene serves to either protect both cellular and fiber components involved in tissue regeneration and the prevention of cartilage destruction. Furthermore, the fact that there are many hydroxyl groups included in these molecules because of the incorporation of large amounts of water should be considered. Therefore, there is a possibility that the gene(s) located in the vicinity of the *AMY2A* gene can affect the WHC of breast muscle.

In conclusion, this study showed that the AMY1A and AMY2A genes, located in the QTL region, are associated with serum and meat quality traits. Even though no significant associations were found between the two amylase genes and any serum clinical chemical traits, including those associated with amylase, AMY1A and AMY2A genes were found to be significantly correlated with the yellowness (b*) of the thigh muscle and the WHC of the breast muscle, respectively. These associations must be verified in other populations before incorporating their use into efforts to improve the quality traits of chicken breeds.

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