Proceeding
The First International Conference
Technology on Biosciences and Social

"Industry based on Knowledges"

17th-19th November 2016, Convention Hall, Andalas University

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Animal Science Faculty of Andalas University
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Organized by:

Animal Science Faculty of Andalas University
and
Alumbi Center of Universiti Putra Malaysia

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Polymorphism Calpain-3 (CAPN3) Gene and Association with Carcass Traits and Meat Quality in Kampung Chicken

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Abstract

Meat quality is one of factor associated with consumers assessment especially tenderness. One of gene that control meat tenderness is calpain-3 (CAPN3) gene.This study was aimed to identify single nucleotide polymorphisms (SNPs) CAPN3 gene in breeds of chicken. The number of chickens were used 53 kampung chicken 3 month, 46 kampung chicken 6 month,6 strain cobb, 6 F1crossbreedkampung chickens with strain cobb, 5 merawang chicken, 5 sentul chickens, 5 nunukan chickens, and 6 pelung chicken. The methods that were used the extraction of DNA from blood samples, amplification using Polymerase Chain Reaction (PCR) machine and then the SNPs were detected by DNA sequencing. Association between genotype and carcass traits and meat quality in kampung chicken was analyzed with SAS program. The results showed that CAPN3 geneintron 9 in chicken observationwere detected 2 SNPs (g.12831C>A and g.12888T>C) with 5 genotypes.SNP g.12831C>A showed in kampung chickens and F1 kampung with strain cobb. SNP g.12888T>C showed in all breeds of chicken observation. The chi-square test in all SNPs was revealed in Hardy-Weinberg equilibrium.The CAPN3 gene were notsignificantly associated with carcass traits and meat quality (P>0.05). The conclusin this study, CAPN3 gene showed SNPs atg.12831C>A and g.12888T>C and the CAPN3 gene were notsignificantly associated with carcass traits and meat quality in kampung chickens.

Keywords : CAPN3 gene, SNP, kampung chicken, carcass, meat quality

Introduction

Improvements in growth and carcass yield are one of factor in success of chicken meat production. Selection for may have also led to changes in meat quality attributes, such as tenderness in particular [1].Meat quality traits are essential for the processing industry and end consumers [2].Meat quality were affected physical and chemical traits, age of animal, muscular and cooking methods [3]. Molecular technology approach has become a powerful method for identifying animals with particular genetic traits associated with the desired tenderness and the selection process can be done on young animals even before birth [4].

One of gene that control meat tenderness is calpain-3 (CAPN3) gene. Calpain gene is one that important to the quality of carcass and meat quality. Calpain gene is a gene whose function is to degrade protein muscle cells (myofibril) within the

muscle tissue and acts as the main enzyme in the process tenderness of meat [5]. Calpains have been reported to be involved in muscle growth and development. They are also regarded as proenzymes that are regulated by Ca2+ binding and autoproteolytic modification [6].

The CAPN3 gene consists of 24 exons located on chromosome 5 [7].The researchhave been reported that founded SNPs in the CAPN3 gene at position 11818T>A and 12814T>G on chickens in China[7] and SNPs g.15486C>T in commercial population [8]. Association genotype CAPN3 (SNP 12814T>G) were significantly with body weigth, carcass weigth, breast muscle weigth and leg muscle weigth. According to [7], needs be doing for identification of the gene SNP CAPN3 on local and kampung chicken Indonesia. In this study, we screened Single Nucleotide polymorphis (SNPs) of CAPN3 gene in native chickens in Indonesia using the method of DNA sequencing and associated with carcass traits and meat quality in kampung chicken.

2. Materials and Methods

The samples of chicken for polymorphisms of CAPN3 gene were used 53 chicken kampung with 3 months of age (27 cocks and 26 hens) from collection of field laboratory, faculty of animal science, bogor agricultural university , 46 chicken kampung with 6 months of age from Sukabumi, West Java, Broiler, 6 F1 kampung with broiler, 6 merawang, 5 sentul, 5 nunukan, and 6 pelung chicken. From each population were randomly sampled for collecting the blood. For carcass partial analysis were used chicken kampung with 3 months of age and chicken kampung with 6 months of age. For meat quality analysis was used chicken kampung with 6 months of age.

2.1 DNA extraction and Polymerase Chain Reaction (PCR)

DNA extraction from colection of chicken blood were modified [9]. Then the extraction of DNA samplesare taken by 0.5 - 1 mL plus primer with forward 5‘ TCT GGT AAG GCT GAG AAA CCC 3‘ anf reverse 5‘ AAG AAA CTG CCC TGC TTC ACT C 3‘ by 0.35-0.4 mL, 0.3 mL of dNTPs, 1 mL of MgCl2, 1.5 mL of 10 x buffer, 0:15 Taq Polymerase and 36-46 mL of destilation water. The all mixture incubated using PCR thermocycler machine. Amplification process begins with a denaturation step at 94°C for five minutes. The second phase consists of 35 cycles, each cycle consisting of denaturation process at 94^{o}C for 10 seconds, primer annealing at temperatures range from 60^{o}C for 20 seconds and extension of DNA at a temperature of 72^{o}C for 30 seconds. The next stage is the last extension at a temperature of 72^{o}C for five minutes. The results of the DNA amplification visualized by 1.5% agarose gel.

2.2 DNA Sequencing

DNA sequencing using a sequencer machine (ABI Prims 3100-Avant Genetic Analyzer) on the forward and reverse primer fragments through the 1st Base sequencing services company in Selangor, Malaysia. The results of sekeuncing analyzed with Bioedit program and MEGA6[10]

2.3 Statistical Analysis

Genotype and allele frequency following model [5] was used :

$$X_{ii} = \frac{n_{ii}}{N} \qquad X_i = \frac{2n_{ii} + \Sigma n_{ij}}{2N}$$

Where Xii is the genotype frequency, Xi is the allele frequency, N is the total of sample, nii is the total of sample with genotype ii, nij is thetotal of sample with genotype ij.

Observed and expected heterozogosity following model [11] was used :

$$H_0 = \sum_{i \neq j} \frac{n_{ij}}{N} \qquad H_e = 1 - \sum_{i=1}^{q} x_i^2$$

Where Ho is the observed proportion of heterozygotes, He is the expected proportion of heterozygotes, N is the total of sample, n_{ij} is total of sample with genotype ij, X_i^2 is the frequency of allele i, q is the total of allele.

Chi-square or Hardy-weinberg equilibrium model [12] was used :

$$\chi^2 = \frac{O-E^2}{E}$$

Where χ^2 is the Hardy-weinberg proportion, O is the observed frequency of genotype and E is the expected frequency of genotype.

Data were analyzed with GLM procedures of Statistics Analysis Sistem (SAS) Inst. Inc Cary NC, (USA).

3. Result and Discussion

3.1 PCR Amplification

PCR amplification of the gene CAPN3 has been successfully with length products 328 base pare (bp). The CAPN3 gene fragment targets were located in exon 9 and intron 9. Visualization of PCR amplification in CAPN3 gene presented in Figure 1.

3.2 DNA Sequencing

The CAPN3 polymorphisms detected by the PCR amplification were also conformed by DNA sequencing in all of observed breeds.Alignment sequences DNA of CAPN3 gene using Mega6 program presented at figure 2.

The result of alignment sequences DNA were founded 2 SNPs (g.12831C>A and g.12888T>C). SNP g.12831C>A were founded in kampung chicken with 3 months of age, kampung chicken with 6 months of ageand F1 crossbreed between kampung chicken with broiler strain cobb chicken. SNP g.12888T>C were founded in all of observed breeds.The research were reported that founded SNP in 12814T>G [7], but for this study not founded SNP 12814T>G.

3.3 Genotype and allele frequency of CAPN3 gene

Polymorphysms of genes CAPN3 based on genotype and allele frequency at the SNP g.12831C>A and g.12888T>C was showed in Table 1. Position g.12831C>A were analyzed of genotype frequency CAPN3 gene, the CC genotype was the higher frequency than CA genotype frequency. The value of CC genotype frequency was showed0.83 – 1.00 and CA genotype frequency was showed0.07 – 0.10. AA genotype frequency has no value because no founded individuals with genotype of AA. Based on the position g.12831C>A, the CC genotype was the highest frequency in all chickens were observed.

The frequency of alleles at position g.12831C>A was showedthat 0.92 - 0.10 for the allele C and 0.00 - 0.04 for the allele A. The frequency of alleles at position g.12831C>A included polimorphics in kampung chicken 3 months of age, kampung chicken 6 months of age and F1 crossbreed between kampung chicken with broiler strain cobb.But, The frequency of alleles included monomorphic broiler strain cobb, sentul, merawang, nunukan and pelung chicken.

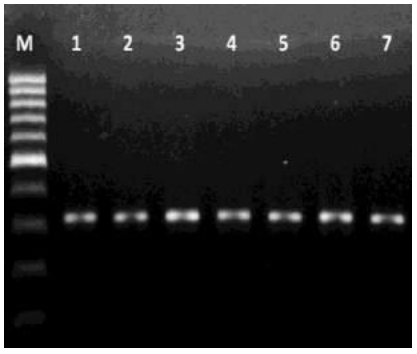

Fig 1. Results visualization amplification of CAPN3 gene in a 1.5% gel agarose. M: Marker 100 bp and 1-7

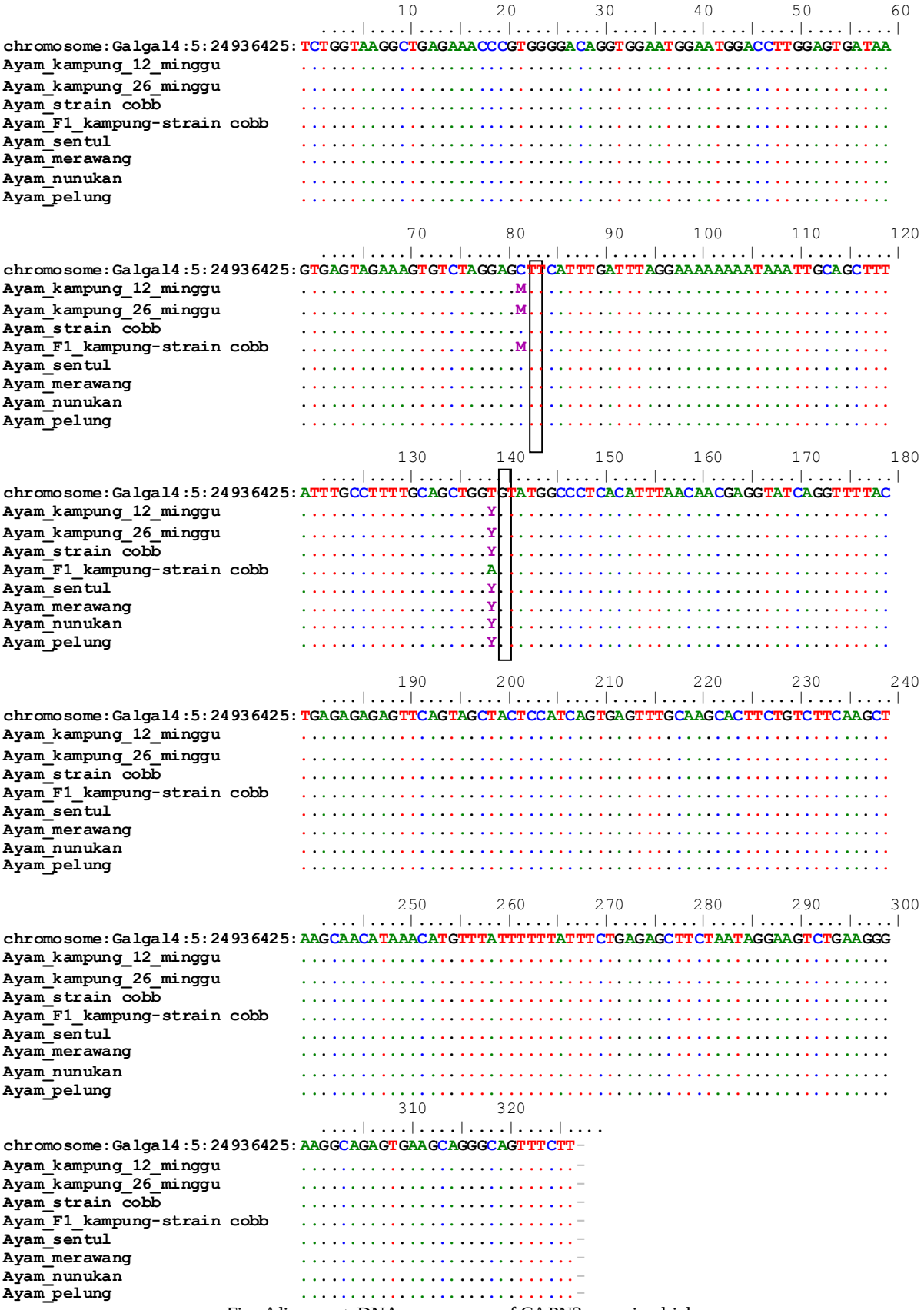

Fig. Alignment DNA sequences of CAPN3 gene in chicken

Table1. Genotype and allele frequency of CAPN3 gene in some breeds of chicken

Breeds	N	g.12831C>A					g.12888T>C				
		CC	CA	AA	C	A	TT	TC	CC	T	C
Kampung 3 months	53	0.91	0.09	-	0.95	0.05	0.60	0.29	0.11	0.75	0.25
Kampung 6 months	46	0.93	0.07		0.93	0.03	0.76	0.29	0.02	0.87	0.13
Broiler Strain cobb	6	1.00	-	-	1.00	0	0.50	0.33	0.17	0.67	0.33
F1 kampung – strain cobb	6	0.83	0.17	-	0.92	0.08	-	0.33	0.67	0.17	0.83
Sentul	5	1.00	-	-	1.00	0	0.80	0.20	-	0.9	0.1
Merawang	5	1.00	-	-	1.00	0	0.40	0.20	0.40	0.5	0.5
Nunukan	5	1.00	-	-	1.00	0	0.80	0.20	-	0.9	0.1
Pelung	6	1.00	-	-	1.00	0	0.67	0.33	-	0.83	0.17

Tabel 2. Observed heterozigosity, expected heterozigosity and Hardy–Weinberg equiblirium of CAPN3 gene in chicken

Breeds	N	g.12831C>A			g.12888T>C		
		Ho	He	$\chi 2$	Ho	He	$\chi 2$
Kampung 3 months	53	0.09	0.10	Ns	0.29	0.38	ns
Kampung 6 months	46	0.07	0.13	Ns	0.22	0.23	ns
Broiler Strain cobb	6	-	-	-	0.33	0.44	ns
F1 Kampung – broiler strain cobb	6	0.17	0.15	Ns	0.33	0.28	ns
Sentul	5	-	-	-	0.20	0.18	ns
Merawang	5	-	-	-	0.20	0.50	ns
Nunukan	5	-	-	-	0.20	0.18	ns
Pelung	6	-	-	-	0.33	0.28	ns

ns : not significant ($\chi 2$ test < $\chi 2$ table)

Table 3. Association SNP of CAPN3 gene with carcass traits in hens kampung chicken 3 months of age

Parameter	g.12831C>A		g.12888T>C		
	CC (26)	CA (1)	TT (18)	TC (7)	CC (2)
Live weigth (g)	663.46 ± 62.10	622	662.00 ± 44.94	659.86 ± 102.54	668.50 ± 23.33
Carcass (g)	396.69 ± 86.44	375	406.89 ± 98.62	382.00 ± 46.92	345.50 ± 10.61
Breast (g)	99.38 ± 16.69	113	102.11 ± 17.21	98.86 ± 15.64	83.50 ± 0.71
Thigh (g)	69.54 ± 8.42	68	69.33 ± 7.72	71.43 ± 10.05	64.00 ± 8.49
Drum (g)	65.85 ± 9.10	63	66.44 ± 8.26	66.2 ± 11.31	57.50 ± 2.12
Wing (g)	62.54 ± 7.88	65	62.33 ± 7.02	64.86 ± 10.22	57.50 ± 2.12
Back (g)	96.92 ± 13.74	102	97.94 ± 14.75	99.14 ± 5.93	82.50 ± 19.09
Breast muscle (g)	59.62 ± 10.13	54	60.94 ± 10.04	57.86 ± 10.6	51.00 ± 1.41
Thigh muscle	40.23 ± 5.63	43	40.11 ± 5.17	42.00 ± 6.88	36.50 ± 3.54
Drum muscle (g)	36.85 ± 5.57	37	36.78 ± 5.12	38.29 ± 6.80	32.50 ± 0.71

Position g.12888T>C were analyzed of genotype frequency CAPN3 gene, the TT genotype was the higher frequency than TC and CC genotype frequency in kampung chicken 3 months of age, kampung chicken 6 months of age, broiler strain cobb, sentul, merawang, nunukan andpelung chicken. But, CC genotype in F1 crossbreed between kampung chicken with broiler strain cobb The value of CC genotype was the higher frequency than TC genotype. The value of TT genotype frequency was showed 0.40 – 0.80. The value of TC genotype frequency was showed 0.20 – 0.33. The value of CC genotype frequency was showed 0.02 – 0.67. Allele frequency at position g.12888T>C was showed0.17 - 0.90 for the T allele and 0.10 - 0.83 for C allele. In all breeds of chicken included polymorphic because the allele frequency less than 0.99 [13].

3.4 Observed heterozigosity, expected heterosity and Hardy-Weinberg in CAPN3 gene

Values of observed heterozygosity, expected heterozigosity and Hardy Weinberg equilibrium CAPN3 genes was showed in Table 2. Observed heterozygosity included the position g.12831C>A CAPN3 gene in kampung chicken 3 months of age and 6 months was higher than expected heterozygosity. SNP g.12831C>A in F1 crossbreed between kampung chicken with broiler strain cobb strain showedobserved heterozygosity higher than expected heterozygosity.

CAPN3 gene position g.12888T>C, observed heterozygosity was higher than expected heterozygosity in kampung chicken with 3 months of age, kampung chicken with 6 months of age, broiler strain cobb, merawang chicken. But, expected heterozygosity was higher than observed heterozygosity in F1 crossbreed kampung chicken with broiler strain cobb, sentul, nunukan and pelung chicken.A population was a high genetic diversity if it has a value of heterozygosity more than 0.50. If value of expected heterozigosity was higher than observed heterozigosity in different populations indicatedhigh random mating[14]. Chi-square test at SNP g.12831C>A was showed in hardy-weinberg equiblirium in kampung chicken with 3 months of age, kampung chicken with 6 months of age and F1 crossbreed kampung chicken with broiler strain cobb. Chi-square test at SNP g.12888T>C was showed in hardy-weinberg equiblirium in all breeds of chicken.

3.5 Association CAPN3 gene with carcass and meat quality in kampung chicken

SNP genotypes were associated with an carcass traits in chicken kampung with 3 months of age and 6 month of age were summarized in Table 3, 4 and 5.

Analisys association CAPN3 gene were not significantly ($p>0.05$) with carcass traits in hens kampung chicken 3 months of age, cocks kampung chicken 3 months of age and cocks kampung chicken 6 months of age. The reason for this contradictory because be due to type of mutation in SNP g.12831C>A and g.12888T>C.Type of mutation in SNP g.12831C>A is transversions and Type of mutation in SNP g.12831C>A is transitions. The result can be caused slow growth in kampung chicken in each genotype SNP. So, the average of carcass traits were not different significantly.

3.6 Association of CAPN3 SNP with meat qualityin kampung chicken

The result of association analysis by using the GLM between the CAPN3 gene polymorphism with meat quaity in kampung chicken were showed in Table 6. Result of association the all SNPs of CAPN3 gene not signicificant were detected for meat quality (pH, cooking loss, water holding capacity, tenderness and fat) in kampung chicken.

Table 4. Association SNP of CAPN3 gene with carcass traits in cocks s kampung chicken 3 months of age

Parameter	g.12831C>A		g.12888T>C		
	CC (22)	CA (4)	TT (14)	TC (8)	CC (4)
Live weigth (g)	766.5 ± 113.53	753.00 ± 145.85	748.93 ± 95.20	748.63 ± 112.63	850.25 ± 176.69
Carcass (g)	447.55 ± 91.19	447.50 ± 91.08	428.36 ± 78.24	459.13 ± 85.70	491.50 ± 134.84
Breast (g)	113.32± 25.19	114.25 ± 22.23	108.50 ± 23.92	115.88 ± 20.24	126.00 ± 34.06
Thigh (g)	83.86 ± 15.94	82.75 ± 21.85	80.86 ± 12.34	83.63± 17.85	93.75 ± 26.09
Drum (g)	81.14 ± 15.18	81.25 ± 17.75	76.64 ± 11.54	83.63 ± 14.93	92.00 ± 23.73
Sayap (g)	73.77 ± 11.62	73.75 ± 8.06	72.50 ± 9.05	74.75 ± 9.94	76.25 ± 20.16
Back (g)	112.55 ± 24.17	108.00 ± 27.12	107.50 ± 22.57	112.00 ± 24.64	126.75 ± 28.96
Breast muscle (g)	65.95± 16.29	65.25 ± 16.76	60.93 ± 14.22	68.25 ± 15.11	78.25 ± 19.92
Thigh muscle (g)	48.09 ± 12.92	48.00 ± 11.91	45.64 ± 11.61	49.63 ± 12.53	53.50 ± 16.90
Drum muscle (g)	44.91 ± 8.54	39.75 ± 5.74	42.79 ± 7.55	45.5 ± 9.20	46.00 ± 10.55

.

Table 5. Association SNP of CAPN3 gene with carcass traits in hens kampung chicken 3 months of age

Parameter	g.12831C>A		g.12888T>C		
	CC (43)	CA (3)	TT (36)	TC (9)	CC (1)
Live weigth (g)	1626.09 ± 113.49	1532.67 ± 60.86	1642.06 ± 115.13	1542.00 ± 59.24	1525
Carcass (g)	1038.65 ± 100.78	972.00 ± 33.78	1054.67 ± 99.57	962.00 ± 56.52	952
Breast (g)	263.98 ± 26.69	252.67 ± 4.04	267.75 ± 26.92	246.11 ± 13.72	255
Thigh (g)	203.72 ± 25.95	185.33 ± 13.20	208.19 ± 25.21	181.44 ± 15.74	188
Drum (g)	190.40 ± 22.96	168.67 ± 3.21	193.64 ± 22.81	172.44 ± 14.47	170
Wing (g)	131.53 ± 11.34	124.00 ± 9.17	132.31 ± 11.57	127.00 ± 9.70	122
Back (g)	256.98 ± 38.60	257.33 ± 17.79	262.56 ± 34.50	236.89 ± 45.31	238
Breast muscle (g)	185.00 ± 25.41	179.67 ± 13.58	186.97 ± 26.33	177.33 ± 16.98	167
Thigh muscle (g)	149.67 ± 24.76	133.00 ± 18.52	153.92 ± 24.37	130.56 ± 14.12	119
Drum muscle (g)	125.67 ± 19.12	116.33 ± 9.29	128.36 ± 19.31	111.67 ± 9.38	127

Table 6. Association of CAPN3 gene with meat quality in kampung chicken

Parameter	g.12831C>A		g.12888T>C		
	CC (43)	CA (3)	TT (35)	TC (10)	CC (1)
pH	5.44 ± 0.27	5.48 ± 0.11	5.45 ± 0.22	5.42 ± 0.36	5.61
Cooking Loss (%)	48.41 ± 2.21	46.38 ± 1.09	48.10 ± 2.31	48.83 ± 1.82	45.15
Water Holding Capacity (%)	29.94 ± 2.18	29.01 ± 0.57	29.82 ± 2.33	30.32 ± 1.13	28.40
Tenderness ($kgcm^{-2}$)	2.91 ± 0.70	3.08 ± 0.50	2.93 ± 0.73	2.93 ± 0.60	2.90
Fat (%)	0.83 ± 0.60	0.41 ± 0.25	0.82 ± 0.62	0.78 ± 0.54	0.15

Conclusion

The gene of CAPN3 founded 2 SNPs in intron 9 (g.12831C>A and g.12888T>C). SNP g.12831C>A is polymorphism in kampung chicken and F1 kampung chicken with strain cobb with CC and CA genotype. SNP g.12888T>C is polymophism in all breed of chickens with TT, TC and CC genotype. Gene CAPN3 no significantly associated with carcass traits and meat quality in kampung chicken.

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