

## CHAPTER IV

### RESULTS AND DISCUSSION

#### 4.1 Carcass and Meat Quality Characteristics

The carcass and meat quality traits of both broiler and native Malaysian chickens as summarized in Table 4.1.

**Table 4.1:** Carcass and Meat Quality Characteristics of Native and Broiler Chickens

Traits	Broiler chicken	Native chicken
Slaughter weight (kg)	1.98±0.11 <sup>a</sup>	1.41±0.21 <sup>b</sup>
% Thaw loss	2.50±1.08 <sup>b</sup>	5.93±2.05 <sup>a</sup>
% Cook loss	21.70±1.49 <sup>a</sup>	22.33±1.51 <sup>a</sup>
Shear force (kg)	1.98±0.57 <sup>b</sup>	3.80±0.41 <sup>a</sup>

\* n=10, Analysis was done using one-way ANOVA, means in columns with different superscripts were significantly (P<0.05) different, while means in columns with the same superscript were not significantly (P>0.05) different.

##### 4.1.1 Slaughter Weight

The native Malaysian chickens are slow growing breed which weigh between 1.0-1.6 kg at 5 months. As showed in Table 4.1, the mean slaughter weight of the native chicken was significantly (P<0.05) lower than that of commercial broilers. The almost 2 kg weight recorded in broiler chicken may be due to the fact that broiler chickens are bred for their meat having an excellent feed conversion ratio and reaching market weight within 6-8 weeks of rearing ((Bramfeld et al., 2003; Beutler, 2007).

#### 4.1.2 Thaw and Cook Losses

The water loss of the two chicken types were measured by the percentage thaw and cook losses. As showed in Table 4.1 based on the results in Table 4.2 and Table 4.3, the percentage of thaw loss of native chicken was significantly ( $P < 0.05$ ) higher than those recorded for broiler chicken in term of breast meat. The result is similar to that obtained by Zhang et al. (2015) who found that chicken meat from Chinese native breeds had higher thaw loss percentages than commercial broilers. Their results have been correlated with the intramuscular fat (IMF) content that was found higher in Chinese commercial broilers breeds. In general meat containing high quantities of fat tends to have lower water loss rates and smaller shear force values (Zhang et al. 2015; Young, 2012). Debut et al. (2003) have also reported a higher drip loss and thaw loss in breast from slow-growing broilers as compared to the fast-growing broilers. However, in a study on Thai native and broiler chickens, Jaturasitha et al. (2008) found no significant difference in thawing loss between the studied populations.

Several researchers have discussed the effect of pH value on meat quality. The thaw loss occurs as a result of the reduction of pH value down to near iso-electric point, where there is no net charge on the proteins to bind to water molecules and also there is no enough space for water within the myofibrils due to increased affinity within myofibrils (Jaturasitha et al., 2008). The significant reduction of muscle pH in Malaysian native as the age increased might be due to the glycolytic process that produces higher lactic acids accumulation after bleeding (Lokman et al., 2015). In general the slow-growing chicken meat tends to have a longer time of rigor inset with lower ultimate pH compared to broilers resulting in lower water holding capacity (Fanatico et al., 2007; Castellini et al., 2008).

**Table 4.2:** Weight before and after thawing and thaw loss for broiler chicken

Sample	Carcass weight Kg	breast meat weight		Thawing Loss, %
		Before thawing, g	After Thawing, g	
1	2.0	298.2	291.9	2.11
2	2.1	239.0	233.2	2.43
3	1.8	241.9	234.4	3.10
4	2.0	281.1	276.0	1.81
5	2.1	242.9	236.8	2.51
6	2.0	262.2	249.2	4.96
7	1.8	192.4	185.4	3.64
8	1.9	220.1	216.3	1.73
9	2.0	276.1	272.4	1.34
10	2.1	250.4	247.0	1.36
Average	1.98	250.43	244.26	2.50

**Table 4.3:** Weight before and after thawing and also thaw loss for native chicken

Sample	Carcass weight Kg	breast meat weight		Thawing Loss, %
		Before thawing, g	After thawing, g	
1	1.0	69.6	65.00	6.61
2	1.4	92.4	88.20	4.55
3	1.5	94.9	92.00	3.06
4	1.1	86.1	82.00	4.76
5	1.5	119.4	116.30	2.60
6	1.3	101.1	95.30	5.74
7	1.6	104.8	95.30	9.06
8	1.6	116.2	106.80	8.09
9	1.5	99.2	92.10	7.16
10	1.6	124.1	114.60	7.66
Average	1.41	100.78	65.00	5.93

For cooking loss, Table 4.4 and Table 4.5 showed cook loss after boiling the samples in a water bath and reaching internal temperature of 75°C. In this study the percentage of cooking loss was not significantly different ( $p>0.05$ ) in both chicken genotypes

(Table 4.1), with slightly higher average value has been recorded for the native chicken samples as compared to the broiler samples.

Conversely, Lokman et al., (2015) have showed significant differences in the cooking loss of breast muscles among Malaysian breeds. In their study, Red jungle fowl and Malaysian domestic (native) chickens had higher percentages of cooking loss than the broilers, with an increasing pattern as the age increased. Other research has shown that the denaturation of myosin head at low pH is responsible for higher shrinkage within the myofibre that causes water to be squeezed out from the muscle. However, according to Lokman et al., (2015) the collagen content is a prominent factor than the pH of the muscles in determining the cooking loss, and the increase in the content of collagen within the muscles with age is more associated with the increase in cooking loss. Other researchers have also related the results of drip loss, thaw loss and cooking loss to the genotypes.

**Table 4.4:** Weight of sample before and after cook and cook loss for broiler samples

Sample	Breast meat weight		Cooking loss, %
	Before cooking, g	After cooking, g	
1	289.9	220.6	23.90
2	231.9	184.0	20.66
3	232.7	183.1	21.31
4	273.6	209.9	23.28
5	234.9	182.0	22.52
6	245.1	189.3	22.77
7	184.2	144.5	21.55
8	215.3	171.5	20.34
9	270.7	219.4	18.95
10	245.9	193.8	21.19
Average	242.42	189.81	21.65

**Table 4.5:** Weight of sample before and after cook and cook loss for native samples

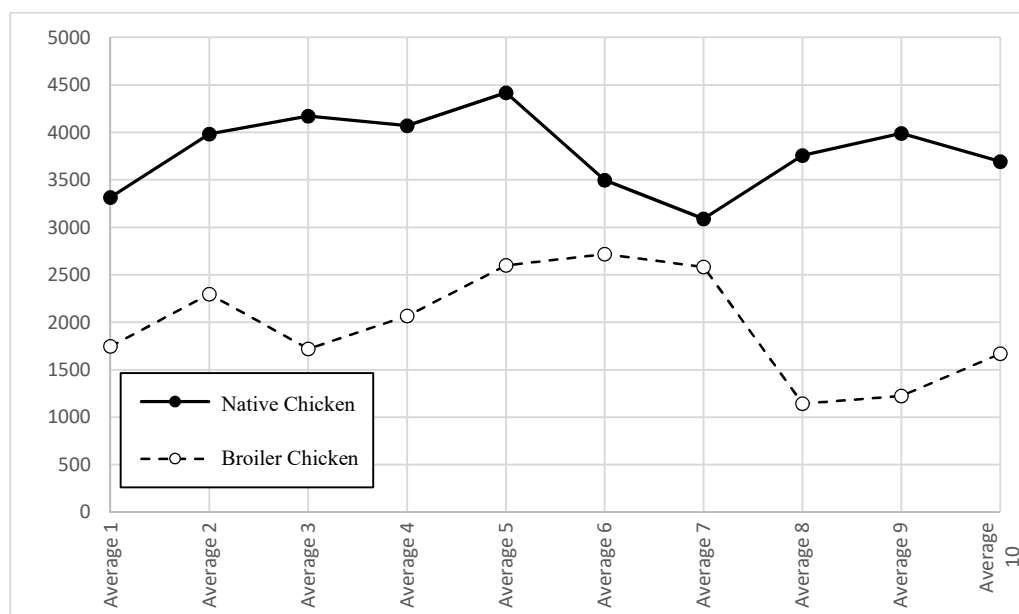
Sample	Breast meat weight		Cooking loss, %
	Before cooking, g	After cooking, g	
1	64.2	50.4	21.50
2	85.8	67.1	21.79
3	91.5	73.7	19.45
4	80.7	60.6	24.91
5	116	91.4	21.21
6	95.2	72.7	23.63
7	94.8	73.4	22.57
8	106.5	81.7	23.29
9	91.8	70.7	22.98
10	114.5	89.4	21.92
Average	94.10	73.11	22.33

### 4.1.3 Shear Force

The average WBSF value for the triplicate test of each sample was presented in Table 4.6 and Figure 4.1. Statistical analysis based on these results showed that the native chicken samples had significantly higher shear force values ( $P < 0.05$ ) than the broiler chicken samples.

**Table 4.6:** Averages WBSF values of the boiled native and broiler meat samples

Native chicken samples		Broiler chicken samples	
Sample	Average WBSF value, g	Sample	Average WBSF value, g
1	3314.739	1	1746.884
2	3983.342	2	2296.344
3	4170.756	3	1721.873
4	4069.642	4	2069.940
5	4418.237	5	2602.889
6	3497.601	6	2719.885
7	3089.132	7	2584.410
8	3756.848	8	1145.420
9	3988.936	9	1225.773
10	3693.618	10	1671.142



**Figure 4.1:** Average WBSF value for 20 samples

This finding is in agreement with Zhang et al. (2015), Lokman et al., (2015), Jeon et al. (2010), Jaturasitha et al. (2008), Wattanachant et al. (2004) and Intarachot et al. (1997). They reported that the toughness associated with native chickens meat may be related to the slaughter age and production systems. Slaughter age of birds has been implicated as a major determinant of tenderness of chicken meat (Sandercock et al., 2001; Anadon, 2002; Smith et al., 2002; Bianchi et al., 2006). According to Fletcher et al. (2000) and Gigaud et al. (2008) as slaughter age decrease, the flavour of meat decreases whereas tenderness and juiciness increase. Furthermore, Lokman et al., (2015) were related the high collagen contents in Malaysian native chicken meat as the age increase with the high shear force value and toughness of the meat.

Intramuscular fat is one of the main quality attributes of meat as it plays a determinate role in providing flavour and juiciness that may extend to meat tenderness development (Young, 2012). Many studies have proposed that meat containing high

quantities of fat provides a resistant layer that diminishes the heat transfer throughout the muscle resulting in more tender meat (Young, 2012; Grandin, 2007). Other studies have related meat pH and its importance in determining meat quality and the ability of muscle proteins to bind water and the subsequent juiciness and tenderness of the meat (Sandercock et al. 2001).

On the other hand the type of production system affects the tenderness of chicken meat (Castellini et al., 2008; Fanatico et al., 2007; Lokman et al., 2015). Chickens reared under extensive system are known to have less tender meat than chickens reared under intensive system. This may be a consequence of their motor activity from one point to the other.

Therefore, the high shear force value recorded in this study for the native chicken may be as a result of the age of the chicken which was about 16-20 weeks before slaughter against the 6 weeks slaughter age of broiler chicken. It may also be as a result of the differences in the production system between chicken types. Most native chickens are reared in the extensive way as they are very rugged breed that can withstand adverse condition. Most commercial broiler chickens are reared in a semi-intensive system (deep litter system). Due to their high feed conversion ratio, they gain weight within a very limited time and are usually too heavy to move around thereby resulting in excess fat and more tender meat. In addition genotype has potential effects on meat tenderness and the results can also be related to the genotypic differences.

#### **4.2 DNA Extraction**

After the DNA samples were extracted using InnuPREP DNA Mini Kit, the eluted DNA samples were tested for its concentration and purity using NanoPhotometer P-

class (AYKA Ltd) at the ratio of absorbance at 260nm and 280nm. The A260/A280 ratio of absorbance for pure extraction of nucleic acid was ensured to be not more than 2.0. This is due to the fact where samples might be contaminated by RNA presence in the sample. The A260/A280 ratio was ensured not to be lower than 1.8, which indicates the presence of protein contaminants in the sample. The samples were analysed by PCR and electrophoresis. Table 4.7 showed the result of DNA extraction from twenty samples from two types of chicken tissue using Nanophotometer.

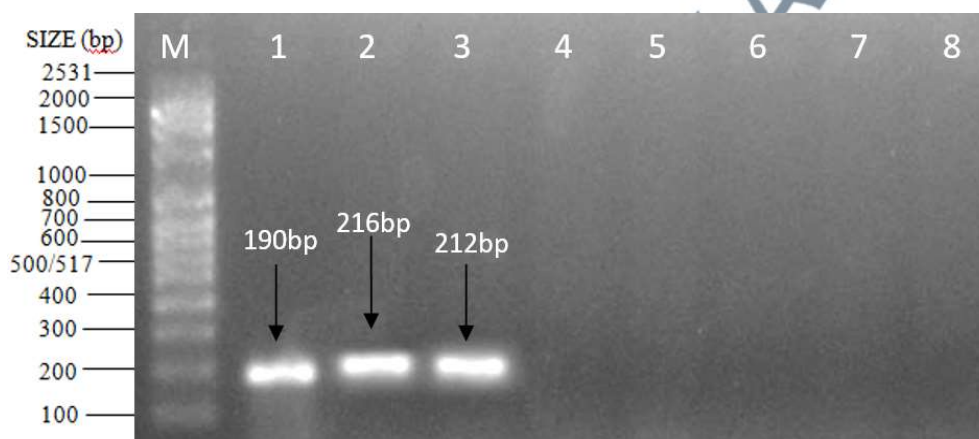
**Table 4.7:** Results of DNA extraction using nanophotometer

Native chicken samples			Broiler samples		
Sample	Concentration ng/μl	A260/A280	Sample	Concentration ng/μl	A260/A280
1	92.5	1.947	1	16.8	2.000
2	72.5	1.933	2	25.8	2.000
3	140	1.931	3	28.7	1.933
4	30.0	2.000	4	18.3	2.056
5	60.0	2.000	5	24.8	2.000
6	110	2.000	6	20.8	2.000
7	232	1.979	7	27.8	1.931
8	67.5	1.800	8	30.0	2.000
9	90.0	2.000	9	27.3	2.037
10	35.0	2.000	10	19.3	2.053

#### 4.3 Primer Specificity Analysis

Primer specificity test is important to define which primer would give more specific product at good intensity and clear DNA bands when run on the agarose gel during electrophoresis. PCR products were carried out with annealing temperature of 53°C were tested on 2% agarose gel, 70 Volt and 400 Ampere for 45 minutes. From the eight pairs, only the primers from previous studies (G3535A, C7198A, and G9950A) gave the most specific PCR products as indicated in Figure 4.2. These are the primers

of pairs 1, 2 and 3 which resulted in clear bands of 190, 216 and 212 bp respectively. However, the five primer pairs (4, 5, 6, 7 and 8) that had been designed according to the NCBI website were unable to provide PCR products. Amplification were tested several times using different temperature profiles and additives, however, PCR product were undetectable for the target regions.



**Figure 4.2:** Bands produced on gel by using all designed primers and primers from previous studies. M for DNA marker 100 bp (Bioline). The primer pairs 1, 2, and 3 matched the 190bp, 216bp, and 212bp of the product size respectively and gave the best and intense bands, at the 200bp position. The primer pairs 4, 5, 6, 7 and 8 were unable to provide PCR products.

After sending the unpurified PCR products for sequencing, the veracity of the DNA sequences for the three loci SNPs markers (G3535A, C7198A, and G9950A) of CAPN1 gene were carried out using NCBI/BLAST. This step is to identify how much the nucleotide sequences of the CAPN1 gene were conserved against chicken genome of *Gallus gallus* isolated from Red Jungle fowl (Figure 4.3).

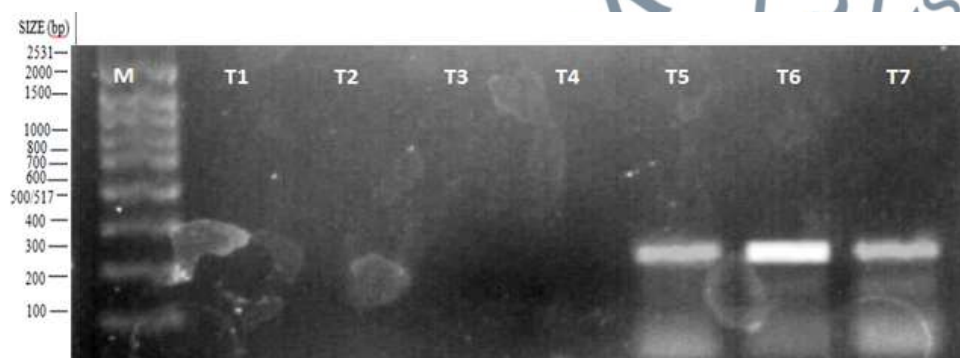
<b>G3535A</b>				
Score	Expect	Identities	Gaps	Strand
259 bits(140)	1e-67	150/154(97%)	3/154(1%)	Plus/Minus
<b>Features:</b> <a href="#">calpain-1 catalytic subunit isoform X1</a> <a href="#">calpain-1 catalytic subunit</a>				
Query 8	CTTTGA-ATGG-AGCAGTGACCTTCA-GAAGCTGGTGAAAGGCCATGCCTATTCTGTGCAC			64
Sbjct 30412997	CTTTGATATGGAAGCAGTGACCTTCAAGAAGCTGGTGAAAGGCCATGCCTATTCTGTGCAC			30412938
Query 65	CGCCTTCAAAGATGTGAGTTGGGTACATAAAGACTGTAATAGAGCTAAAATGCCGTGGCCT			124
Sbjct 30412937	CGCCTTCAAAGATGTGAGTTGGGTACATAAAGACTGTAATAGAGCTAAAATGCCGTGGCCT			30412878
Query 125	GTGGTGTGGGTTTGACTTCTGCATTTGATGGCT		158	
Sbjct 30412877	GTGGTGTGGGTTTGACTTCTGCATTTGATGGCT		30412844	
<b>C7198A</b>				
Score	Expect	Identities	Gaps	Strand
294 bits(159)	4e-78	162/163(99%)	1/163(0%)	Plus/Minus
<b>Features:</b> <a href="#">calpain-1 catalytic subunit isoform X1</a> <a href="#">calpain-1 catalytic subunit</a>				
Query 21	CAGAC-AAGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACC			79
Sbjct 30409320	CAGACAAAGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACC			30409261
Query 80	TGATGGATGTATCCTCTTGGCATTGTGGGCAGGGGCTGGCGTTCTGGCGCTCGGCCCTGG			139
Sbjct 30409260	TGATGGATGTATCCTCTTGGCATTGTGGGCAGGGGCTGGCGTTCTGGCGCTCGGCCCTGG			30409201
Query 140	CAGCCAGCACACTTCTCCGTGGGAAC TAGGGCTCGGCTCTCTA		182	
Sbjct 30409200	CAGCCAGCACACTTCTCCGTGGGAAC TAGGGCTCGGCTCTCTA		30409158	
<b>G9950A</b>				
Score	Expect	Identities	Gaps	Strand
311 bits(168)	4e-83	171/172(99%)	1/172(0%)	Plus/Minus
<b>Features:</b> <a href="#">3970 bp at 5' side: uncharacterized protein LOC421441</a> <a href="#">645 bp at 3' side: calpain-1 catalytic subunit isoform X1</a>				
Query 12	TTTCTGCGTGGCCTTCA-GCTCTCCCTCACACCCCTTACTCTCTGCCCCCTCTGTCCCA			70
Sbjct 30406690	TTTCTGCGTGGCCTTCAAGCTCTCCCTCACACCCCTTACTCTCTGCCCCCTCTGTCCCA			30406631
Query 71	CCATAGGGCCAAAGCCTATCATTAAAGCACTCCCTGCCACGTGCCACTATAACACAGCC			130
Sbjct 30406630	CCATAGGGCCAAAGCCTATCATTAAAGCACTCCCTGCCACGTGCCACTATAACACAGCC			30406571
Query 131	CATCGTGCTCCTCTCATCTATGCACGCACAGCAGTACCACTACACCCCTTTC		182	
Sbjct 30406570	CATCGTGCTCCTCTCATCTATGCACGCACAGCAGTACCACTACACCCCTTTC		30406519	

**Figure 4.3:** Comparison of the 3 loci SNPs in CAPN1 gene with *Gallus gallus* isolated from breed Red Jungle fowl, within chromosome 3.

#### 4.4 Gradient PCR

Gradient PCR was carried out to identify the most suitable annealing temperature which was 53.1°C for the primers from previous studies (G3535A, C7198A, and G9950A). Seven tubes of PCR reaction [included the specific region C7198A (216bp), DNA template isolated from native chicken sample number 7, PCR Master Mix, and

Nuclease free water] were placed on seven different PCR wells with different annealing temperature selected. The seven wells chosen are ranging from higher, optimum and lower annealing temperatures so that the actual temperature that provides better results can be detected. Bands produced from different annealing temperature were presented in Figure 4.4, where M indicated the DNA marker (100 bp) and T1, T2, T3 and T4 were on wells 1, 2, 3, and 4 with different annealing temperatures of 58.0, 57.5, 56.8, and 55.7°C respectively. The test showed that T6 had the optimum annealing temperature of 53.1°C.

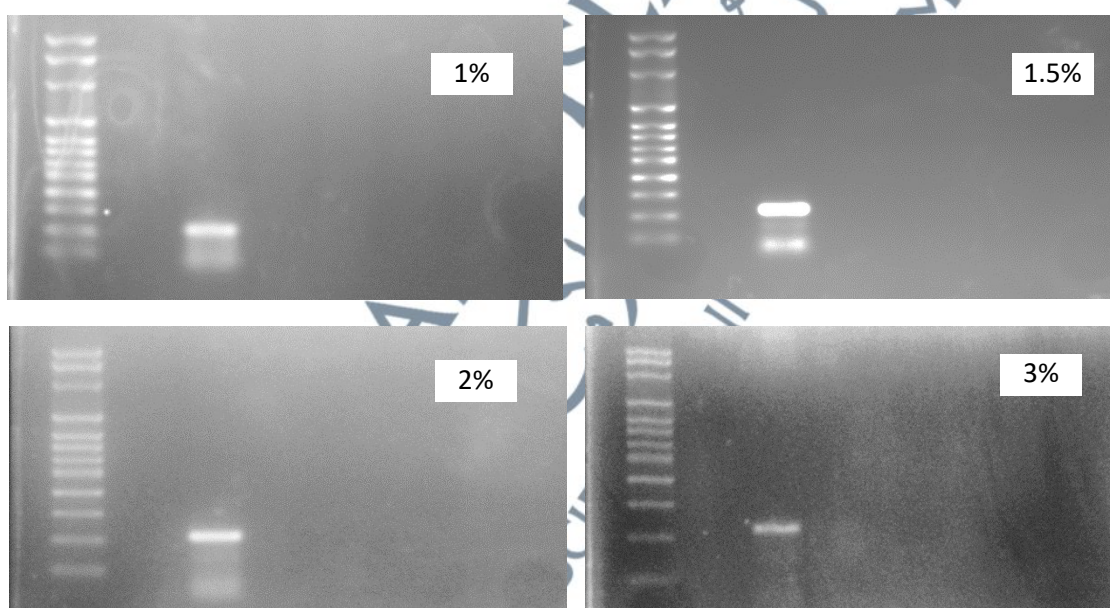


**Figure 4.4:** Bands produced from different annealing temperature. M indicates the DNA marker (100 bp). T1, T2, T3 and T4 were on wells 1, 2, 3 and 4 with different annealing temperatures of 58.0, 57.5, 56.8, and 55.7°C respectively. T5 was higher than the optimum annealing temperature on well 5 with annealing temperature of 54.3°C, while T6 was the optimum annealing temperature which was 53.1°C. Lastly, T7 was lower than the optimum temperature, which was 52.4°C set on well 7.

#### 4.5 Effect of Gel Concentration on DNA Band Migration

The efficiency of nucleic acid separation (migration) can be increased in the non-denaturing agarose gel by adjusting the concentration of agarose. Higher concentrations of agarose facilitate separation of small DNA fragments with size in the range of 0.1-0.3kb, while low agarose concentrations allow resolution of much

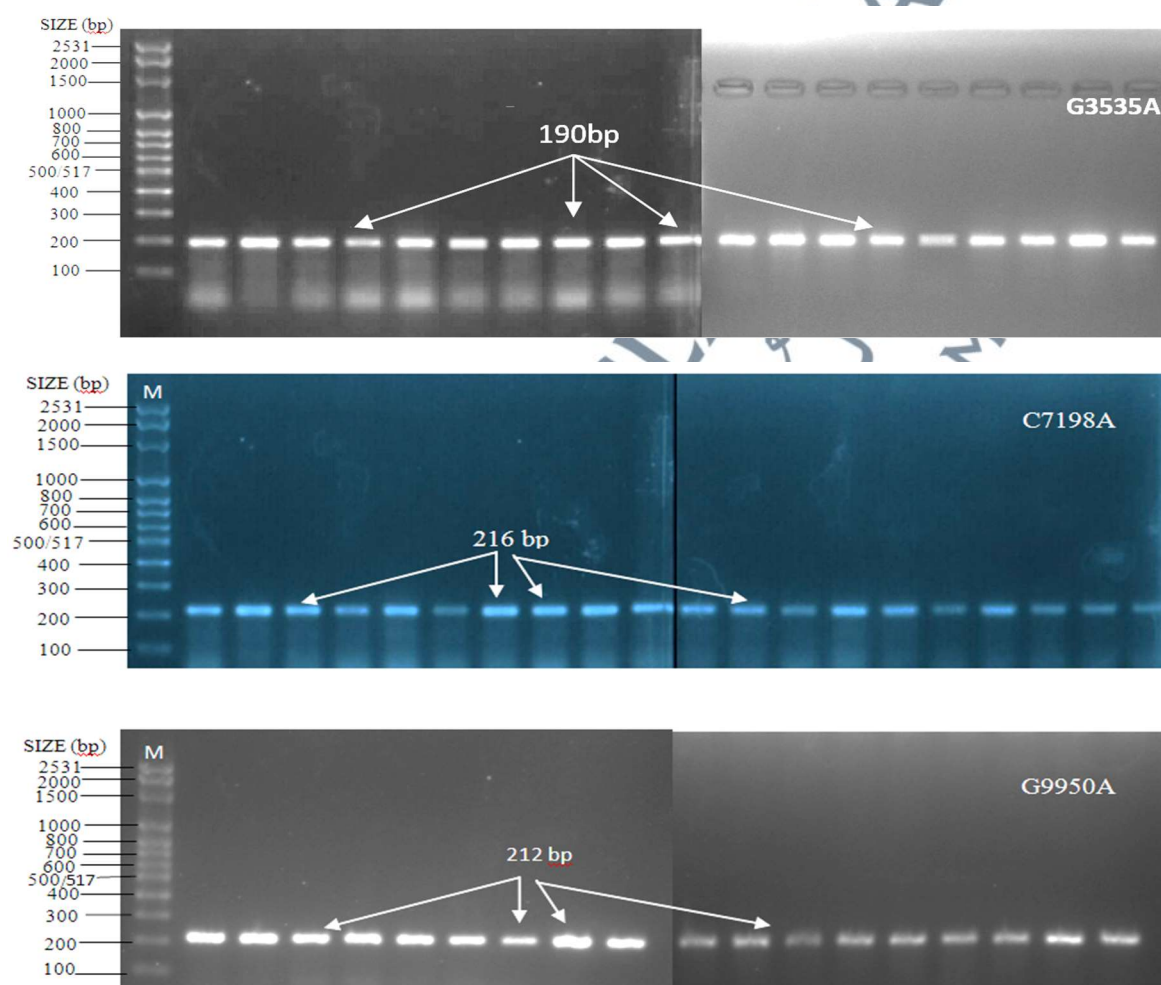
larger DNAs. In this study, gel concentration is tested as a parameter to increase gel resolution and clarity of the amplicons band by increasing the concentration of agarose. PCR amplicon of the CAPN1 gene in the specific region [C7198A with a product size (216bp)], and HyperLadder 100bp were tested on all the gels of different agarose concentrations: 1, 1.5, 2 and 3% (w/v) with genome isolated from commercial chicken sample number 8 (Figure 4.5). Separation and clarity of DNA band in agarose gel was shown to be significantly enhanced when the concentration of gel was increased from 1% to 1.5% (w/v). The trend continues when a 2% agarose gel was used for electrophoresis of the amplicons. In 3% agarose gel the 100bp ladder separation was not agreed.



**Figure 4.5:** Migration of the same PCR product for different concentrations of agarose, all of which were electrophoresed at the same voltage (70 v) and for identical times (45 minutes). The DNA marker or ladder should be separated to a degree that allows for the useful determination of the sizes of sample bands. DNA fragments of 216 bp are separated on 1%, 1.5%, 2%, and 3% agarose gel along with a 200bp DNA ladder. The DNA fragments are much better resolved in 2% gel, as it is easier to differentiate the PCR product and the DNA ladder at 200bp.

#### 4.6 Polymerase Chain Reaction (DNA Amplification)

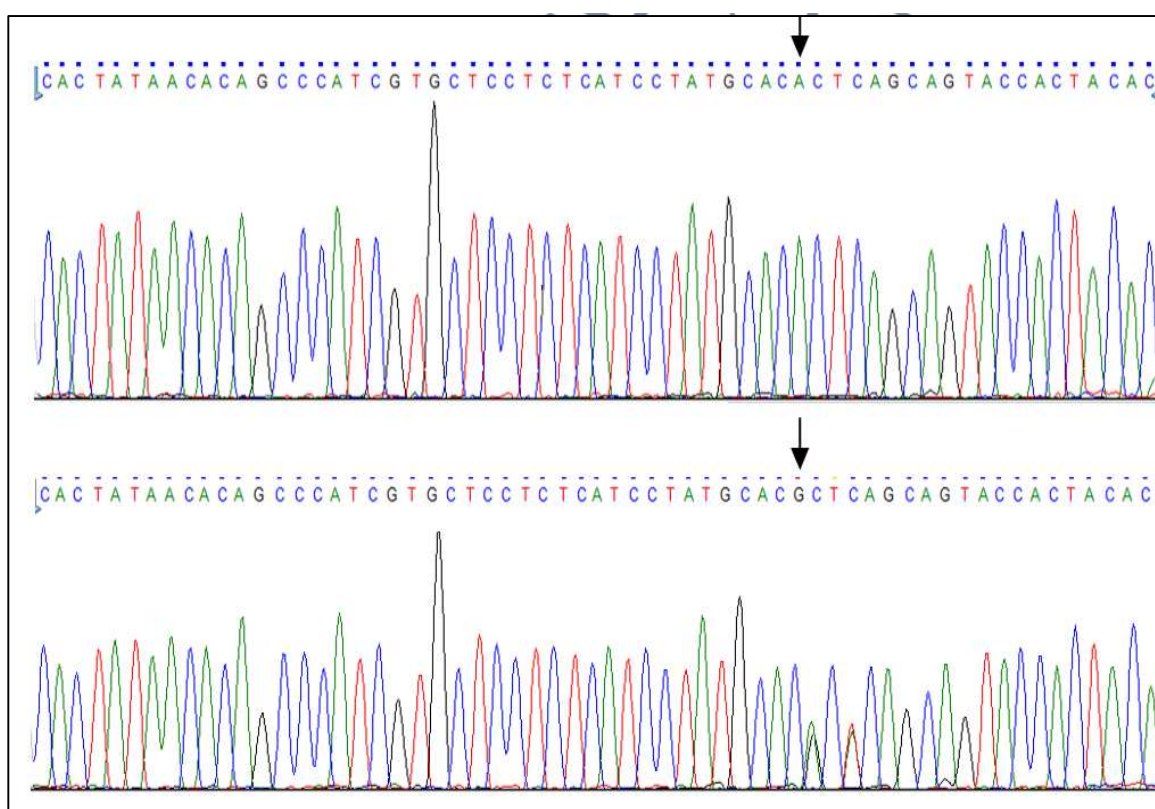
The PCR method was used for the detection of the three specific regions with the SNPs in the CAPN1 gene. The target fragments of the gene was amplified and denatured as described in section 3.3.4 in all breast samples, and was analysed by agarose gel electrophoresis 2% as showed in Figure 4.6.



**Figure 4.6:** Bands produced for all samples of both broiler and native chickens. M for DNA marker (100 bp). The bands sizes for the regions in the CAPN1 gene G3535A, C7198A, and G9950A were 190bp, 216bp, and 212bp respectively. Samples are amplified at annealing temperature 53°C. PCR products were checked on a 2% agarose gel stained with ethidium bromide. The loaded DNA samples were electrophoresed at 70 v, 400 A for 45 minutes. The gel was exposed to UV light.

#### 4.7 Gene and genotype frequencies

The CAPN1 polymorphisms were analysed using multiple sequence alignment for both chicken populations. The nucleotide sequences of the loci marker G9950A SNP in the CAPN1 gene are presented in Appendix I. Part of the results is shown in Figure 4.7. The alignments of sequences of the SNP markers (Appendix II, III, and IV) were carried out using multiple alignment sequence web site. The mutations in nucleotide sequences of the SNP markers are shown in Figure 4.8. Accordingly the observed number of alleles and their frequencies per SNP were determined and statistically analysed as presented in Table 4.8.



**Figure 4.7:** Mutation in nucleotide sequence in G9950A SNP in CAPN1 gene.

**Figure 4.8:** The alignment of sequences shows the mutation in nucleotide sequence of the markers G3535A, C7198A, and G9950A SNPs in the CAPN1 gene, using multiple alignment sequence software. For loci G3535A the allele G was less frequent than allele A in both types of chicken. In the loci C7198A the allele C was more polymorphic than the allele A in the native chickens. For CAPN1 G9950A, the allele G was dominant in both genotypes while the allele A was lost in the broilers and much less frequent than the allele G in the native chickens.

G3535A	
1_COMM	GTGAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
2_COMM	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
3_COMM	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
4_COMM	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
5_COMM	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
6_COMM	GTGAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
7_COMM	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
8_COMM	GTGAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
9_COMM	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
10_COMM	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
1_NAT	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
2_NAT	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
3_NAT	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
4_NAT	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
5_NAT	GTGAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
6_NAT	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
7_NAT	GTGAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
8_NAT	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
9_NAT	GTGAAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT
10_NAT	GTGAAAGGCCATGCCTATTCTGTCACCGCCTTCAAAGATGTGAGTTGGGTACATAAGACT

C7198A	
1 comm	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
2 comm	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
3 comm	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
4 comm	AGATCTGAAGACGGATGGATTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
5 comm	AGATCTGAAGACGGATGGATTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
6 comm	AGATCTGAAGACGGATGGATTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
7 comm	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
8 comm	AGATCTGAAGACGGATGGATTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
9 comm	AGATCTGAAGACGGATGGATTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
10 comm	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
1 NAT	AGATCTGAAGACGGATGGATTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
2 NAT	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
3 NAT	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
4 NAT	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
5 NAT	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
6 NAT	AGATCTGAAGACGGATGGATTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
7 NAT	AGATCTGAAGACGGATGGATTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
8 NAT	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
9 NAT	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA
10 NAT	AGATCTGAAGACGGATGGCTTCAGTCTGGACTCCTGCCGCAACATGGTCAACCTGATGGA

G9950A	
1_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
2_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
3_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCACAGCAGTACCACTACAC
4_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCACAGCAGTACCACTACAC
5_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCACAGCAGTACCACTACAC
6_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
7_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCACAGCAGTACCACTACAC
8_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
9_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
10_COMM_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
1_NAT_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
2_NAT_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACACTCAGCAGTACCACTACAC
3_NAT_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCACAGCAGTACCACTACAC
4_NAT_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
5_NAT_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
6_NAT_F	ACTATAACACAGCCCATCTTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
7_NAT_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
8_NAT_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC
9_NAT_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACACTCAGCAGTACCACTACAC
10_NAT_F	ACTATAACACAGCCCATCGTGCTCCTCTCATCCTATGCACGCTCAGCAGTACCACTACAC

**Table 4.8:** Allelic frequencies of the 3 sites in the two chicken populations

Breed	N	G3535A		C7198A		G9950A	
		G	A	C	A	G	A
Broiler	10	0.30	0.70	0.50	0.50	1.00	0.00
Native	10	0.30	0.70	0.70	0.30	0.80	0.20
<i>P*</i>		0.908		0.361		0.136	

\*Means the probability.

According to Table 4.8, the allelic frequency in the marker G3535A was similar between chicken groups where the allele A was more frequent than the allele G. In the marker C7198A the allele C was observed polymorphic in both breeds and was more frequent than the allele A in the native chickens. For G9950A, the allele G was dominant in both genotypes while the allele A was lost in the broilers and much less frequent than the allele G in the native chickens. From these results there were no significant ( $P > 0.05$ ) differences in allele frequency between the two chicken groups.

Additionally, the allele frequencies obtained for the Malaysian populations differ significantly from those determined in other populations studied by Zhang et al., (2007a, b), Zhang et al. (2008) and Shun et al. (2015). Therefore, a much larger sample size is needed to see any homozygous mutant for the SNPs. Also the individuals of the native chickens were randomly selected from the local market and that could reduce the chance to differentiate their genotypic group.

#### 4.8 Polymorphism Association Analysis

One of the main objectives of this study is to evaluate the association of SNPs with meat quality traits and the effect of genotypic differences between breeds. Previous studies have reported significant association between G3535A polymorphism in the CAPN1 gene and quality tenderness traits among different chicken genotypes (Zhang et al., 2007a,b, 2008) (Shun et al., 2015). However, in this study according to the results of multiple sequence comparison, the polymorphism G3535A was not significantly ( $P>0.05$ ) associated with the differences in meat quality traits between Malaysian chickens. For the polymorphisms C7198A, and G9950A, our results confirm recent work that showed no significant associations ( $P>0.05$ ) between these SNPs and breeds and their meat quality traits (Shun et al., 2015). This finding is in disagreement with the documented specific association of G9950A SNP with meat quality traits in Chinese chicken populations (Zhang et al., 2007a, b), as they found a significant association with meat quality traits in this region.

A preliminary association study has been performed to determine whether the polymorphisms affected meat tenderness traits in the two chickens' types. A general linear model (GLM) was used to estimate the association between G9950A SNP and meat tenderness. The model is described as follows:

$$Y_{ij} = \mu + B_i + G_j + e_{ij}$$

where  $Y_{ij}$  is an observation on the traits,  $\mu$  is the overall population mean,  $B_i$  is the effect of breed,  $G_j$  is the effect of genotype, and  $e_{ij}$  is the residual error. Multiple comparisons were analysed with the least square means (LSM), and if an effect of a trait had a P value > 0.05 that means there was no significant association with the polymorphism. Based on the result summarized in Table 4.9 there were no significant association was found between this SNP and meat quality traits in chicken populations ( $p > 0.05$ ).

**Table 4.9:** Association analysis of G9950A polymorphisms in the CAPN1 gene with meat tenderness and water-holding capacity traits.

Chicken Type	N	Allele frequency (SNP 9950G>A)		Meat tenderness (kg)	Thawing loss (%)	Cooking loss (g)
		G	A			
Native	10	0.80	0.2	3.80±0.41	5.93±2.05	22.33±1.51
Broiler	10	1.00	0	1.98±0.57	2.50±1.08	21.70±1.49
<i>P-value</i>				0.140	0.328	0.703
<i>R<sup>2</sup> %*</i>				11.72	5.32	0.83

\* means the correlation coefficient

Previous research has confirmed other factors that alter the phenotypic data, such as differences in age and other gene-environmental factors (Lokman et al., 2015). The native Malaysian chickens are slow growing breed and so the toughness associated with native chickens meat might be related to slaughter age and production systems. So it will be interesting to further investigate the differences in meat tenderness traits between the two groups, it related to different genotype-environmental factors.