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IJAZAH: BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

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GENETIC DIVERSITY STUDY ON *Gallus gallus* var. THAILAND INDIGENOUS CHICKEN, TAIWAN INDIGENOUS CHICKEN AND BROILER BASED ON GHR-INTRON 2

LIM LEE GING

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

LIVESTOCK PRODUCTION PROGRAMME
SCHOOL OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2010

DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this dissertation has been previously or concurrently submitted for a degree at this or any other university.

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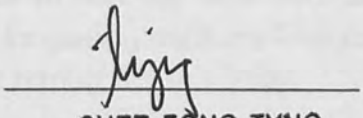

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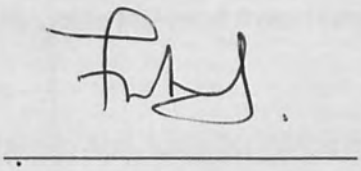

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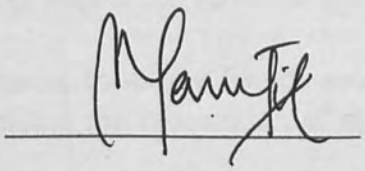

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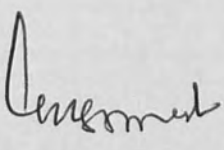

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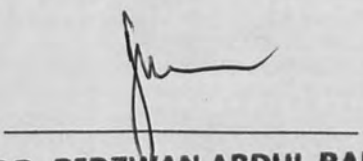

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ABSTRACT

It was known that growth of broiler is rapid than indigenous chicken. The growth rate (phenotype) is influenced by genotype and environment. The primary objective of the research was to study the diversity of growth related genes of *Gallus gallus* var. Thailand indigenous chicken, Taiwan indigenous chicken and broiler (Hubbard) based on GHR-Intron 2. First, blood samples of Thailand Indigenous Chicken, Taiwan Indigenous Chicken and broiler were collected. After that, DNA of blood of three different varieties was extracted. Then, PCR amplification was carried out by using GHR Intron-2. RFLP-PCR was followed after PCR amplification with the digestion of enzyme *Hind*III. The frequency of A_1/A_2 alleles in the overall population was 0.2115 (A_1) and 0.7885 (A_2) for GHR-Intron 2. The genotype frequency in overall population was 0.0447 (A_1A_1), 0.3336 (A_1A_2) and 0.6217 (A_2A_2). The chi square value (χ^2) for overall population is 42.98 with the probability 0.00. The observed heterozygosity and expected heterozygosity of overall population are 0.0909 and 0.3535, respectively. Low genetic diversity found with the genetic distance value of 0.0047 (Broiler/Thailand Indigenous Chicken), 0.0578 (Broiler/Taiwan Indigenous Chicken) and 0.0973 (Thailand/Taiwan Indigenous Chicken). The study found that indigenous chicken and broiler had no difference in GHR-Intron 2 gene.

KAJIAN KEPELBAGAIAN GENETIK TENTANG *Gallus gallus* var, AYAM KAMPUNG THAILAND, AYAM KAMPUNG TAIWAN DAN AYAM PEDAGING MENGGUNAKAN GHR-INTRON 2

ABSTRAK

Pertumbuhan ayam pedaging adalah lebih pesat daripada ayam kampung. Kadar pertumbuhan (fenotip) dipengaruhi oleh faktor genotip dan persekitaran. Objektif utama kajian ini adalah untuk mengkaji kepelbagaian pertumbuhan berkaitan gen-gen *Gallus gallus* var. ayam kampung Thailand, ayam kampung Taiwan dan ayam pedaging (Hubbard) menggunakan GHR-Intron 2. Mula-mula, sampel-sampel darah ayam pedaging, *Gallus gallus* var. ayam kampung Thailand, ayam kampung Taiwan diambil sampel. Darah ketiga-tiga ayam dijalankan ekstrasi DNA. Kemudian, PCR telah dijalankan dengan menggunakan GHR-Intron 2. RFLP-PCR dijalankan selepas PCR dengan penambahan enzim *Hind*III. Frekuensi bagi A_1/A_2 alel dalam keseluruhan populasi adalah 0.2115 (A_1) dan 0.7885 (A_2) untuk GHR-Intron 2. Frekuensi bagi genotip keseluruhan populasi adalah 0.0447 (A_1A_1), 0.3336 (A_1A_2) dan 0.6217 (A_2A_2). Nilai Khi kuasa dua (χ^2) untuk keseluruhan populasi adalah 42.98 dengan kebarangkalian 0.00. Diperhatikan keheterozigositi dan dijangka keheterozigositi keseluruhan populasi masing-masing adalah 0.0909 dan 0.3535. Didapati kepelbagaian genetik adalah rendah dengan genetik jarak yang bernilai 0.0047 (Ayam pedaging / ayam kampung Thailand), 0.0578 (Ayam pedaging / ayam kampung Taiwan) dan 0.0973 (Thailand / Ayam kampung Taiwan). Kajian mendapati bahawa gen GHR-Intron 2 tidak menunjukkan perbezaan antara ayam kampung dan ayam pedaging.

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$$ADG = \frac{W_1 - W_2}{T_2 - T_1}$$; where

W_1 = weight after birth
W_2 = weight after period of growth stage
T_1 = birth time
T_2 = time after growth stage

2.2 Weight per day of age (WDA) 12

$$WDA = \frac{\text{animal's weight}}{\text{age in days}}$$

2.3 Allele Frequency 21

$$\text{Frequency(A)} = \frac{\text{allele A}}{\sum \text{allele}}$$

2.4 Band Sharing 21

$$BS = \frac{2(n_{sb})}{n_s + n_b}$$; where

n_{ab} = number of bands shared between samples a and b
n_a = total number of bands in samples a
n_b = total number of bands in samples b

Formula **Page**

2.5 Genetic Distance 21

GD = -In (I);where

$$I = \frac{1}{n}\sum \frac{[(2V_i^{(a)} \bullet V_i^{(b)})]}{[(V_i^{(a)})^2 + (V_i^{(b)})^2]}$$; where

i = 1 ... n; n=number of sample
I = genetic identity index for a pair of lines (a) and (b)
n = total number of bands scored in both lines
vi = frequency of i^{th} band

2.6 Proportion of polymorphic loci (P) 21

$$P = \frac{\text{number of polymorphic loci}}{\text{number of loci examined}}$$

2.7 Heterozygosity 22

$$H = 1 - \sum p_i^2 \text{ , } i=1,....n; \text{ where}$$

P_i =frequency of i^{th} allele in n sampled alleles
n = number of sample

2.8 Observed Heterozygosity 22

$$H_O = \frac{\text{number of heterozygotes at a locus}}{\text{total number of individuals}}$$

2.9 Expected Heterozygosity 22

$$H_E = 1 - \sum Pi^2$$

Formula **Page**

2.10 Polymorphic information content 23

$$PIC = 1 - (\sum_{i-1}^{n-1} p_i^2) - \sum^{n-1} \sum^{n} 2 p_i^2 \bullet pj^2$$

n= number of different alleles for the specific locus

pi^2 and pj^2 = the population frequencies of the i^{th} and j^{th} allele

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$Q_n = (A_{260})$ X dilution factor X 50; where

Q_n = Yield of DNA

A_{260} = Absorbance at 260 nm

3.2 Dilution factor

30

$$\text{Dilution factor} = \frac{\text{Volume used}}{\text{Total volume}}$$

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

nm	Nanometer
A	Absorbance
ADG	Average daily gain
AFLP	Amplified fragment length polymorphism
BE	Baier
BF	Beijing fatty
bp	Base pair
cGH	Chicken growth hormone
CH	Chahua
DG	Dagu
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FCR	Feed conversion rate
HG	Henan game
HWE	Hardy-Weinberg Equilibrium
GH	Growth hormone
GS	Gushi
IGF-I	Insulin-like growth factor I
JPHPT	Jabatan Pertanian Haiwan dan Perusahaan Ternak Sabah
KK	Kota Kinabalu
LS	Langshan
LY	Luyuan
$MgCl_2$	Magnesium chloride
MT	Metric tonne
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphism DNA
RFLP	Restriction fragment length polymorphism
SD	Standard deviation
SNP	Single-nucleotide Polymorphism
STSs	Sequence Tagged Sites
TBE	Tris-Borate-EDTA
TC	Tibetan
TS	Taihe Silkies
U	Unit
USA	United States of America
UV	Ultraviolet
WDA	Weight per day of age
XJ	Xianju
XS	Xiaoshan

CHAPTER 1

INTRODUCTION

1.1 Introduction

Poultry farming constitutes a major livestock activity in Malaysia. From a backyard industry of the 1950s, the broiler and layer production has developed into a highly industrialized industry today. It has developed through four decades of rapid technological, genetic, management and structural changes, made possible by the liberal importation policy of the Government. Although the development of the industry has been tremendous, the production of indigenous chicken has been static with a standing population of 6.5 million (Ramlah, 1996) which is about 10% of the total population of indigenous chicken in Malaysia.

Over three-quarters of a million rural families in Peninsular Malaysia still keep indigenous chicken under backyard production in flocks of 15-20 birds of various ages. This practice of keeping indigenous chicken in this way is also widespread in South-East Asia (Aini, 1990). Information on the performance of these indigenous chickens under various farming systems could be beneficial to rural households by helping them to increase their income through greater productivity (Ramlah, 1996).

The terminology used to describe undomesticated chickens is "indigenous" or "native". According to the Webster's New World Encyclopedia (1992), indigenous refers to the people, animals or plants that are native to a region while native means existing naturally in a place. Indigenous is synonym to native.

Chicken comes from the Galliformes Orders. Indigenous chicken is known as *Gallus gallus* (Appleby *et al.*, 1992). The indigenous chickens are a crossbred between Red Jungle Fowl and mixed exotic domestic breed that are usually fed once or twice a day with variety of left over food such as rice or used coconut pulp. The indigenous chickens are free roaming and studies have showed that rearing indigenous chicken is cost effective since very little financial input is needed (Ramlah, 1996).

The Thai indigenous chicken is one of the oldest known breeds of domestic fowl. Indigenous chicken predominate in villages. Indigenous chicken play an important role as a food reserve for the households, they serve as an important source of protein (Theerachai, 2006). It has unique taste, tough, strong muscle, low fat contain, free of antibiotics, very popular among native consumers and the market price is two or three times higher than the commercial broiler (Wattanachant *et al.*, 2004). However, the growth rate of indigenous chicken is low. A study conducted in Northeast Thailand found that the indigenous chicken growth rate was only 9 g/bird/day (Theerachai, 2006).

The broiler chicken is known as *Gallus gallus domesticus*. Broiler is domesticated from the Red Jungle Fowl for cockfighting. They have been successfully domesticated because they form large flocks and have a hierarchical structure with males affiliated to female groups. They show promiscuous mating where males are dominant over females. The broiler industry began in USA in early 1950s. Special strains and hybrids had been developed in USA for meat production, selected for both rapid growth rate and an efficient food. Growth rate is so rapid that nowadays broiler chickens can grow from a 1-day-old weight 45 g to a body weight of 2200 g by 42-45 days of age (Appleby *et al.*, 1992).

Broilers are very similar as the ancient Jungle Fowl except it lies on muscle faster. Modern broilers are typically a third generation offspring (an F_2 hybrid) and are fed with high quality formulated diet to increase muscle growth hence increase the body weight.

Growth rate (phenotype) is influenced by both the genotype and environment. The environment factors include feeding management, housing system and stocking density. Mupeta *et al.* (unpublished paper) studied the performance of indigenous chickens in Zimbabwe and concluded that the growth performance of indigenous chicken is still slow under an improved feeding management system. Thus, the slow growth rate of indigenous chicken is influenced by genotype. Indigenous chicken is lack of genetic improvement compared to broiler.

1.2 Objective

The objective of this study was to study the diversity of growth related genes of *Gallus gallus* var. Thailand indigenous chicken, Taiwan indigenous chicken and broiler (Hubbard) based on GHR-Intron 2.

1.3 Justification

Indigenous chickens are always thought to be good in term of carcass composition compared to the broilers due to its low fat content (Ganabadi *et al.*, 2009). In addition, Wattanachart *et al.* (2004) stated that indigenous chicken is popular among the native consumers and has higher market value. However, indigenous chicken has slower growth rate than broiler chicken. The beneficial of this project was to provide more information in understanding about gene diversity among breeds contribute to growth gene and growth-related gene. This information is useful for breeder in the future development of breeding program, developed breeding program may improve the growth rate of indigenous chicken which has high market demand in Malaysia.

CHAPTER 2

LITERATURE REVIEW

2.1 Chicken

Poultry are domestic birds such as chickens, turkeys, ducks and geese. Chicken is domestic fowl that are domesticated for meat and eggs by early farmers in China, Europe, Egypt and the America. Chicken were domesticated from the South-East Asian jungle fowl, *Gallus gallus* and then raised in the East, as well as the West. Varieties which are suitable for eating are Dorkings, Australorps, Brahmas and Cornish. Good egg-laying breeds of chicken are Leg-horns, Minorcas and Anconas. Those which are useful for both purposes are Orpingtons, Rhode Island Reds, Wyan dotes, Plymouth Rocks and Jersey White Giants (Webster's New World Encyclopedia, 1992).

Chicken belongs to the Kingdom of Metazoa. It is categorized into the Order of Galliformes and Family of Phasianidae. The Genus of chicken is *Gallus* and the species is *gallus* (WikiAnswer, 2009).

The chicken has been a valuable "model organism" for genetic studies because it is important as a major commodity for animal agriculture. Sequence analysis of selected gene clusters has shown that the chicken genome provides a very informative comparison to mouse and human genomes to aid in the annotation of exons and conserved regulatory domains (Margulies and Green, 2003). The chicken genome (haploid) exists as approximately 1.2 X 10^9 base pairs of DNA organized into 38 autosomes and two sex chromosomes, Z and W (Dodgson, 2003).

Most farm poultry are hybrids and they are selectively crossbred for certain characteristics including feathers and down. Chicken is an endothermic vertebrate which evolved from reptiles. Chicken is almost completely covered with feathers making them different from other vertebrates. Feathers do not uniformly cover the body but rather grow in rows producing feather tracts in specific areas over the body. Feathers can have many colours and colour patterns. It can be the result of genetic differences and the presence of gonadotrophic hormones. Different species of chicken contain different kind of comb such as single, rose, pea, strawberry, silkis, cushion, buttercup and V-shaped (Figure 2.1). Most chicken's body is covered with a thin skin with the exception of the uropygial gland.

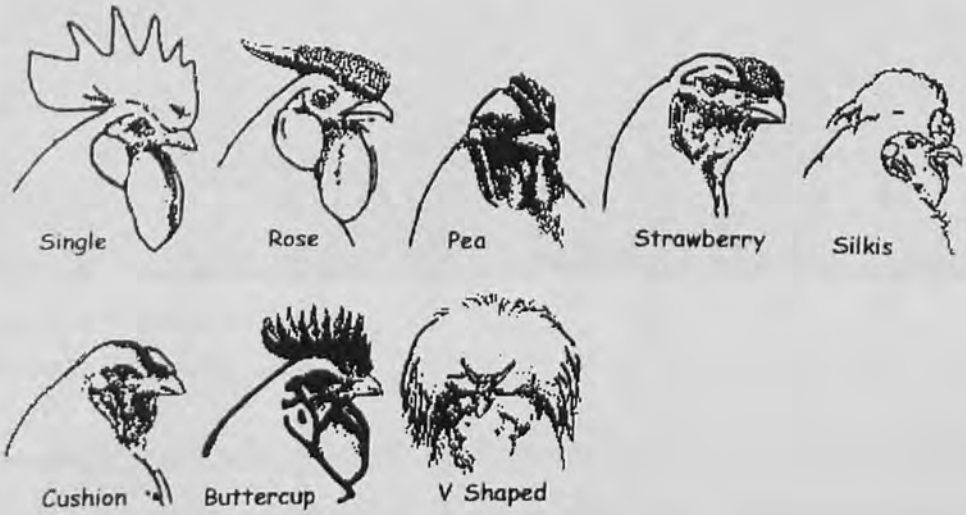

Figure 2.1 Different species of chicken have different kind of comb
Source: Animal Corner, 2009.

Some of the poultry breeds were the basis of industrial poultry breeding (Van Kaam *et al.*, 1999) and this has lead to a big development of poultry industry. Since World War II, the development of battery-produced eggs and the intensive breeding of broiler have roused a public outcry against "factory" methods of farming (Figure 2.2). The birds are often kept constantly in small cages, have their beaks and claws removed to prevent them from pecking their neighbours and are given feed containing growth hormone and antibacterial drugs which eventually make their way up to the food chain of humans (Webster's New World Encyclopedia, 1992).

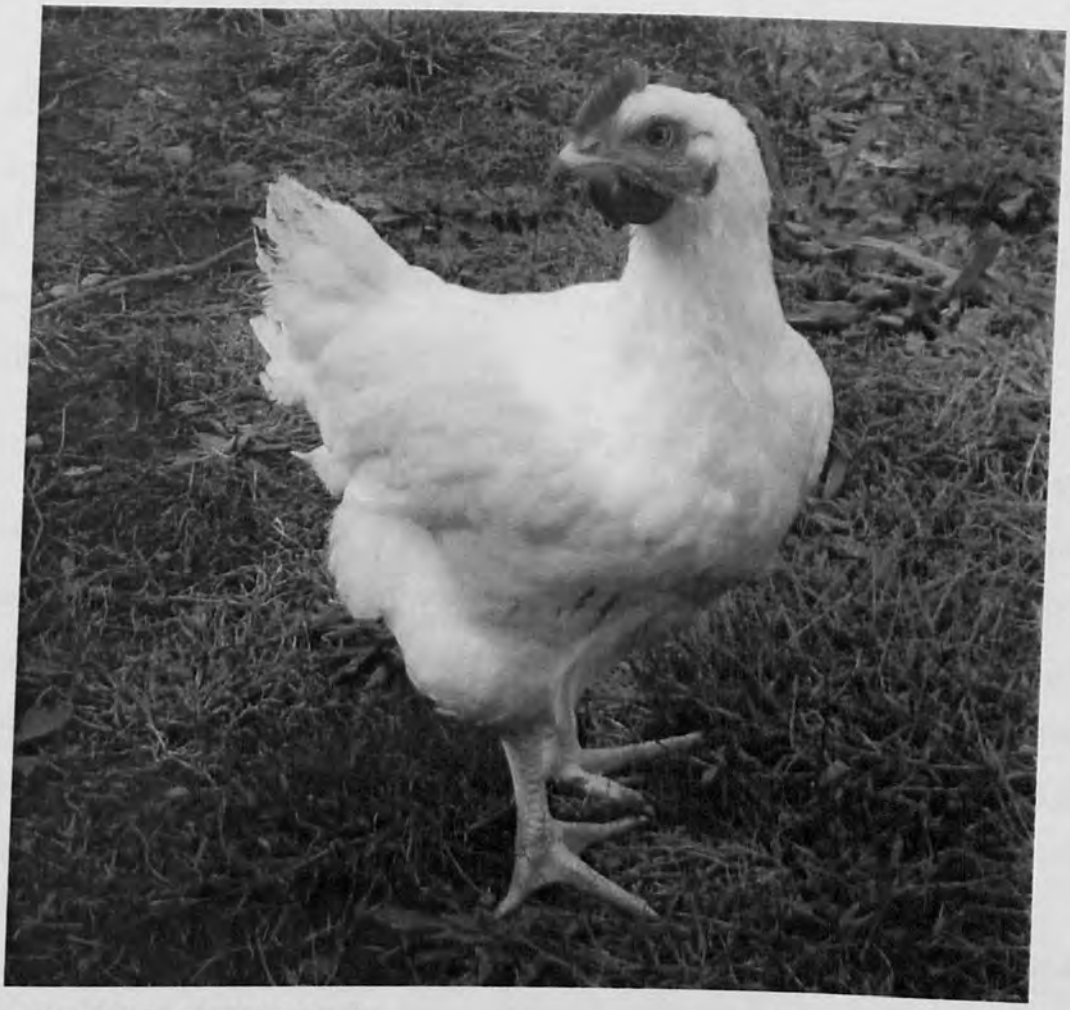

Figure 2.2 Broiler (Hubbard)
Source: JBFarming, 2008

Consumption of poultry meat and eggs is increasing steadily. It has moved from 8.6 kg per person in 1993 to 11.9 kg per person in 2003. It has gained approaching 40%. The biggest percentage gain was recorded in Asia where average consumption rose from 4.5 kg in 1993 to 7.3 kg in 2003. Hence, total poultry meat consumption in Asia in 2003 at 27.7 million tones was almost 13 million tones higher than 10 years earlier which achieve increase meat of 86% (Executive Guide to World Poultry Trends, 2003).

The improvement in poultry performance for meat during the last three-quarters of the 20th century has been tremendous. The days to achieve 1.5 kg live weight decreased from 120 days in 1925 to 33 days in 1998 (Decuypere *et al.*, 2003).

Since the early 1950s, poultry breeding has focused on increasing profitability with little regard for the effect on the skeletal, respiratory or cardiovascular systems or the well-being of the bird. In broiler, the keen competition between commercial breeding companies coupled with the high heritability of growth has resulted in a dramatic increase in the growth potential of broilers over the last 40 years (Whitehead *et al.*, 2003).

In the 1980s, genetic breeders worked to reduce fatness by direct selection against abdominal fat. They also work on weight gain: feed ratio through indirect selection. Geneticists have proposed new lines of birds selected for increased yield of breast or thigh muscle. It is due to the increasing requirement of consumers for processed poultry products and correspondingly less for whole ready-to-roast carcasses. All these selections have been carried out primarily to reduce breeding costs by improving production efficiency.

Many studies are done with the target to make a chicken to reach market weight in a shorter period of time just like the broilers and neglect the importance of the meat quality. Besides that, they emphasize more on high quality feeds which are being produced to optimize the growth rate. Genetic selections together with high quality diet have increased the broilers' body weight. However, excessive energy intake leads to increased fat deposition in the body (Moravej *et al.*, 2006). Kamran *et al.* (2008) reported that broilers fed with commercial diet containing high crude protein can affect the meat composition especially the fat content.

The broiler is known high in fat content meanwhile the indigenous chicken is low in fat content and is leanness. Therefore, the indigenous chicken is popular among consumers (Mogesse, 2007). However, indigenous chicken has slow growth rate.

Review from Kyvsgaard *et al.* (1999) indicated there is possibility to use poultry production for poverty eradication. Small ruminants and poultry have reproductive advantages over cattle and other livestock due to their short reproductive cycle

(Kondombo, 2005). Improvement in the poultry productivity can improve protein nutrition and increase the income levels of the rural population.

2.2 Indigenous Chicken

There are many indigenous chicken breeds. Different countries have different indigenous breeds. In China, Ji *et al.* (2005) had used 12 Chinese indigenous chicken breeds in the study; Luyuan (LY), Gushi (GS), Tibetan (TC), Baier (BE), Xianju (XJ), Chahua (CH), Dagu (DG), Beijing Fatty (BF), Langshan (LS), Henan Game (HG), Taihe Silkies (TS) and Xiaoshan (XS). Indigenous chickens are widely distributed in the rural areas of tropical countries and they are kept by the majority of the rural poor (Pedersen *et al.*, 2000).

In year 2007, Malaysia has produced almost one million indigenous chickens which put Malaysia at number 15 on world production (Table 2.1). Indigenous chicken meat production in Malaysia was 992142 Metric Tonne (MT) which ranked at number five in year 2007 in the list of various food products (Table 2.2).

2.2.1 Thailand Indigenous Chicken

The Thai indigenous chicken is one of the oldest known breeds of domestic fowl (Figure 2.2). There are two main Thai indigenous chicken; *Shamos* and *Batong*. *Shamos* originated from Malayan and have been widely used and bred for cock fighting. *Batong* is an indigenous chicken in the southern parts of Thailand. Its ancestry is associated with the *Langshan* breed and it was introduced to the area by Chinese migrants (Theerachai, 2006).

Thailand indigenous chicken can also be classified into two strains; *Ooh* and *Chae*. *Ooh* chickens are a heavy strain with large body. They are more of a meat type and economically important than other strains. The female is generally black. The male *Ooh* chicken is large and makes a good fighting cock. The *Chae* chickens are smaller and lighter in weight. These chickens are raised mainly as pets and are insignificant economically (Theerachai, 2006).

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