GROWTH PERFORMANCE AND CARCASS QUALITY OF DIFFERENT BREEDS AND DIETS WITH IDENTIFICATION OF POSSIBLE MOLECULAR PROTEIN BIOMARKER AS A STRESS INDICATOR IN SLAUGHTERED VILLAGE CHICKEN

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FACULTY OF SCIENCE
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GROWTH PERFORMANCE AND CARCASS QUALITY OF DIFFERENT BREEDS AND DIETS WITH IDENTIFICATION OF POSSIBLE MOLECULAR PROTEIN BIOMARKER AS A STRESS INDICATOR IN SLAUGHTERED VILLAGE CHICKEN

ABSTRACT

The development of village chicken industry in Malaysia is still slow despite of high demand from consumers. Factors that contribute to the slow progress are due to the feed management, breed selection and farm management. The objectives of this research were to evaluate the effect of different breeds and diets on the growth performance and carcass quality of village chicken. In addition, the effect of stress during slaughtering on possible molecular protein biomarker was identified. Two (2) strains of village chicken, which were purebred (PVC)(n=100) and crossbred (CVC)(n=300) were reared and fed on 2 types of diet, namely commercial finisher diet (CFD) and formulated finisher diet (FFD). In Experiment 1, the body weight and feed intake were measured weekly and carcass evaluation was done at 2 slaughter ages for each strain, which were (PVC: on Weeks 12 and16) and (CVC: on Weeks 8 and 12. In Experiment 2, the quality of crossbred village chickens were determined by using molecular approach to identify stress occurrence resulted from slaughtering by using 2 slaughtering methods, namely Non-stress slaughtering method (NSSM) and stress slaughtering method (SSM). Glutathione-S-transferases expression and activity was measured and observed using proteomic approached. Generally, the body weight for CFD fed chicken for both strains showed no significant differences with FFD. Male CFD was significantly heavier compared to male FFD fed chicken. However, in crossbred village chicken, female CFD and FFD showed no significant differences across the weeks in terms of body weight. PVC recorded high feed conversion ratio

(FCR) for feed, CFD (2.9) and FFD (5.2), whereas CVC recorded lower FCR for CFD (2.4) and FFD (2.6). It is suggested when fed with FFD, the feed conversion ratio for CVC would be better than for PVC, probably due to the former were well adapted and efficient utilisation of this formulated feed, thus resulting in faster growth and productive FCR. Interestingly, carcass evaluation showed that the meat to bone percentage for FFD fed chicken in both strains was higher compared to chicken fed on CFD, however no significant difference was detected in absolute meat quantity between CFD and FFD in both strains of village chicken. This is indicating that CFD develop more bone mass. In CVC, FFD fed chicken showed significantly higher in crude protein (84.5±1.1%) compared to CFD (78.4±2.5%), moreover, CFD recorded significantly higher in ash content (9.4±1.6%) compared to FFD fed chicken meat (5.3±0.4%). Besides, in PVC, FFD fed chickens showed significantly higher in crude fibre content (5.2±3.1%) compared to CFD (2.4±1.8%). Amino acid profile was analysed, where 12 amino acids showed significantly higher in FFD fed chicken's meats than CFD, which indicating high nutritive value of the meat. Generally, amino acid contents in the CVC given CFD and FFD were higher compared to amino acid in commercial broilers and other indigenous chicken. There were significant differences in GSTs activity between NSSM and SSM indicating SSM method could induced oxidative stress. In conclusion, FFD chicken produced higher nutritive value meat compared to CFD chicken as shown the data of proximate and amino acid. Although FFD result in higher FCR, but the cost of the feed was lower and producing high quality of village chicken.

Keywords: Village chicken, local feed sources, growth performance, Glutathione-S-transferases, slaughtering.

PRESTASI PERTUMBUHAN DAN KUALITAS KARKAS DARIPADA BAKA DAN DIET YANG BERBEZA DISAMPING IDENTIFIKASI KEMUNGKINAN BIOMARKER PROTEIN MOLEKULAR SEBAGAI INDIKATOR STRESS DALAM AYAM KAMPUNG

ABSTRAK

Kemajuan industri pengeluaran ayam kampung di Malaysia masih perlahan walaupun mendapat permintaan yang tinggi daripada pengguna. Faktor-faktor yang menyumbang kepada kemajuan yang perlahan ini adalah kerana pengurusan makanan, permilihan baka dan pengurusan ladang. Objektif penyelidikan ini adalah untuk menilai kesan baka dan diet yang berbeza terhadap prestasi pertumbuhan dan kualiti karkas ayam kampung. Di samping itu, kesan tekanan stress semasa proses penyembelihan terhadap ekspresi biomarker molekul protein juga dikenalpasti. Dua (2) jenis baka ayam kampung yang diternak iaitu ayam kampung tulen (PVC) (n = 100) dan kacukan (CVC) (n = 300) dan diberi makan 2 jenis diet, iaitu diet komersial pengakhiran (CFD) dan formulasi pengakhiran diet (FFD). Dalam Eksperimen 1, berat badan dan pengambilan makanan diukur secara mingguan dan penilaian karkas dilakukan pada 2 peringkat umur penyembelihan bagi setiap baka, iaitu (PVC: pada Minggu 12 dan 16) dan (CVC: pada minggu 8 dan 12). Dalam Eksperimen 2, kualiti ayam-ayam kampung kacukan ditentukan dengan menggunakan pendekatan molekul untuk mengenalpasti kejadian faktor stres terhadap kaedah-kaedah penyembelihan dengan menggunakan 2 jenis kaedah penyembelihan, iaitu kaedah penyembelihan bukan stres (NSSM) dan kaedah penyembelihan stres (SSM). Ekspresi dan aktiviti Glutathione-S-transferases diukur dan diperhatikan dengan menggunakan pendekatan proteomik. Secara amnya, berat badan ayam yang diberi makan CFD untuk kedua-dua jenis baka tidak menunjukkan perbezaan yang signifikan berbanding dengan ayam yang diberikan FFD. Ayam CFD

jantan mempunyai berat badan yang signifikan berbanding dengan ayam jantan FFD. Walau bagaimanapun, untuk ayam kampung kacukan betina, CFD dan FFD tidak menunjukkan perbezaan yang signifikan pada minggu-minggu yang lain dari segi berat badan. Ayam PVC mencatatkan nisbah penukaran makanan (FCR) yang tinggi bagi pemberian makanan, CFD (2.9) dan FFD (5.2), manakala CVC mencatatkan FCR yang lebih rendah untuk CFD (2.4) dan FFD (2.6). Ini menunjukkan bahawa jika FFD diberikan, CVC akan merekodkan nisbah penukaran makanan yang lebih baik berbanding PVC, ini mungkin disebabkan oleh baka yang telah disesuaikan dengan baik dan penggunaan makanan yang dirumuskan yang efisien sehingga dapat menghasilkan pertumbuhan yang lebih cepat dan produktif dari segi FCR. Menariknya, penilaian pada daging menunjukkan bahawa peratusan daging kepada tulang untuk ayam yang diberi FFD untuk kedua-dua jenis baka adalah lebih tinggi berbanding dengan ayam yang diberi makan CFD, namun tiada perbezaan yang signifikan dikesan dalam kuantiti daging mutlak antara CFD dan FFD dalam kedua-dua jenis ayam kampung. Ini menunjukkan CFD membangunkan lebih banyak jisim tulang. Dalam CVC, ayam yang diberi FFD menunjukkan peningkatan yang lebih tinggi dalam protein kasar (84.5 ± 1.1%) berbanding dengan CFD (78.4 ± 2.5%). Selain itu, CFD mencatatkan lebih tinggi kandungan abu (9.4 ± 1.6%) berbanding daging ayam FFD 5.3 ± 0.4%). Selain itu, ayam-ayam yang diberikan PVC juga menunjukkan kandungan serat mentah yang lebih tinggi untuk ayam FFD (5.2 ± 3.1%) berbanding dengan CFD (2.4 ± 1.8%). Profil asid amino yang dianalisis menunjukkan 12 asid amino yang menunjukkan pernilaian yang lebih signifikan pada daging-daging ayam yang diberikan FFD berbanding CFD, yang menunjukkan nilai nutrisi yang baik pada daging. Secara amnya, kandungan asid amino dalam CVC yang diberikan CFD dan FFD adalah lebih tinggi berbanding dengan asid amino pada ayam pedaging komersil dan ayam asli yang lain. Terdapat perbezaan yang signifikan dalam aktiviti spesifik GSTs antara NSSM dan SSM. Ini menunjukkan bahawa metode SSM boleh mengakibatkan stress. Sebagai kesimpulan, ayam FFD

menghasilkan daging yang mempunyai nilai pemakanan nutrisi yang lebih tinggi berbanding ayam CFD seperti yang ditunjukkan dalam data asid proksim dan asid amino. Walaupun FFD menghasilkan FCR yang lebih tinggi, namun kos makanan adalah lebih rendah dan menghasilkan ayam kampung yang lebih berkualiti.

Kata kunci: Ayam kampung, bahan makanan tempatan, pertumbuhan tumbesaran, Glutathione-S-transferases, sembelihan.

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%	:	Percentage
°C	:	Degree celcius
µl	:	Microlitre
mg	:	Miligram
l	:	Litre
ml	:	Mililitre
mM	:	milimolar
g	:	Gramme
pH	:	Hydrogen potential
w/v	:	Weight/volume
V	:	Voltage
APS	:	Ammonium persulfate
CFD	:	Commercial finisher diet
CHAPS	:	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CP	:	Crude protein
CF	:	Crude fat
CMC	:	Coconut meal cake
DM	:	Dry matter
DTT	:	Dithiothreitol
EDTA	:	Ethylenediaminetetraacetic acid
FCR	:	Feed conversion ratio
FFD	:	Formulated finisher diet
GST	:	Glutathione-S-Transferases
GSH	:	Glutathione
HPLC	:	High performance liquid chromatography

Kcal	:	Kilocalories
kg	:	Kilogramme
ME	:	Metabolizable energy
NSSM	:	Non-stress slaughtering method
PKC	:	Palm kernel cake
PTU	:	Propylene Thiourea
PROTOX	:	Protein oxidation
SDS	:	Sodium dodecyl sulfate
SDS-PAGE	:	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SSM	:	Stress slaughtering method
UPLC	:	Ultra Performance Liquid Chromatography

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CHAPTER 1: INTRODUCTION

1.1 Background

Domestication in poultry had been carried out for centuries for eggs, meats and human food purposes through effective selection and conventional animal breeding. This practice has been further improved by incorporating modern technologies such as molecular assisted marker, reproductive technologies and good animal management practice. With continuous research and development (R & D) using advanced biotechnologies, it is the only way to meet the pressing demand of animal protein for human food, including poultry industry so that this commodity could be produced efficiently in quantity and quality in line with global inspiration for food security and safety for ever growing in the number of future human population that increase proportionally with food demand and world poverty.

Poultry is one of the most important domesticated animal industries in the world due to their high demand for human consumption and also one of the major contributors of protein supply to human, that is contributing to more than 32% of the meat consumption around the globe. Furthermore, global per capita consumption of poultry meat is approximately 13.5 kg (International Poultry Council, 2011). In Malaysia today, the population of chicken in Malaysia is about 308.1 million chickens and in 2016 self-sufficiency ratio (SSR) for the poultry meat is 98.5% with per capita consumption (PCC) 50.1 kg/year (Department of Statistic Malaysia, 2016). From the large poultry industry, village chicken included at least 13% of the total poultry population in the country (Supramaniam, 1987), where it was assumed that there were about 30 million populations in year of 2016. One of the reasons for the village chicken preference was due to their taste and nutritive value comparatively with domestic broiler chickens, thus

causing high demand of the village chickens by the consumers and consequently hiking the price of this commodity (Oh, 1987; Liang *et al*., 1995).

Village chicken, *Gallus gallus domesticus,* was previously originated from Malaysia Red Jungle Fowl (*Ayam Hutan*), *Gallus gallus*, where the chicken underwent several uncontrollable cross-breeding with the chickens that had been brought from overseas into Malaysia. This phenomenon had caused the dilution of the pure village chicken blood lines originated from this country. In 2007, there were 8 million of village chickens been reared and 334 metric tonnes of village chickens were exported (Arif *et al*., 2010) with 57% higher in term of return rate value compared to commercial broilers (Saonoah, 2006). Nowadays, consumers are realising the danger to their health resulted from the usage of growth hormones in the poultry industry to maximize the growth performance with reduced the rearing period. As a result, consumers are looking for alternative animal protein sources in the market such as from the village chicken, which were believed to be healthier in term of low fat content and free from chemical contaminants, as well as locally preferred for its delicacy taste and naturally reared. In addition to their superior nutritive value for both the consumers and producers, it would provide as a new source of additional income, particularly for rural farmers.

Practically rural population of Malaysia rears village chicken for their livelihood and wellbeing. However, the number of farmers rearing village chicken in this country is very difficult to determine as there is no proper statistics recorded of the small rural farmers, who reared 10 to 30 village chickens per family. Typically, this village chickens are reared with low level of animal management practises with unplanned diets given to the birds daily, thus resulting in low productivity particularly growth rate and eggs production of village chicken (Azahan *et al.*,1980; Azahan, 1983; Jalaludin *et al.*, 1985; Oh, 1987; Ramlah & Shukor, 1987; Azahan *et al.*, 1990).

There are 2 major strains of village chicken categorised in this country, namely purebred village chicken and crossbred village chicken. Purebred chicken has been reported to have body weights of 1.1 and 1.5 kg over 4 months of age and produced about 100 eggs/year. Many farmers from rural areas reared village chickens by using scavenging system with minimum supervision from chick until adult, thus contributing to low performance of the village chickens. Domesticated village chickens that are under semi-intensive system grow better but it is costly due to the high commercial feed cost needed for this management system. Furthermore, different breeding objectives and approaches of the individual village chicken breeders, resulting in variation in live performance of the various strains exists, as do variation caused by feeding regime and sex as reported for various poultry such as the broiler (Azahan *et al*., 2007). The parameters such as body weight (BW), weight gain (WG), feed intake (FI), and carcass evaluation (CE) are the consideration that are important in poultry (Kingori *et al.,* 2003; Khawaja *et al*., 2007; Ahmed & Azahan, 2011; Azahan *et al.,* 2011). The chicken growth not only due to strains but also the diet formulation given. Azahan *et al*. (2011) in his research reported that proximately 11 MJ/kg of metabolisable energy given feed could induce chicken growth and subsequently gave economic advantage for the farmers.

The cost for commercial poultry feed are increasing tremendously in the recent years, affecting the farmers, especially village chicken's farmers due to the village chicken's traits that grow slower compared to commercial broiler, thus requiring more rearing time and increase feed consumption, as well as management cost. One of the solutions to reduce the feed cost is by using local feed sources that are well abundant and easily obtained from the market. Palm kernel cake (PKC) and milk-extracted coconut meat cake (CMC) are highly available in the country and relatively cheap. Usually PKC are being given to ruminant instead of chicken due to the chicken

digestive system, however it is still being considered to be used as energy feed for the chicken. High PKC content might be causing toxicity to the animals due the high fibre contents (Alimon, 2004), because poultry only could withstand below than 20% of PKC in their feed. The milk-extracted coconut meat cake usually thrown away in local market but surprisingly containing considerably high energy content, which is suitable to be used for grower phase chickens. However, due to their high moisture content which is about 47%, that could cause spoilage of the feed ingredients. Sun dried technique could be a solution, whereby it could be dried to maintain it quality. It is suggested that formulated diets consisting of local and commercial ingredients could be considered in order to reduce the feed cost and thus possibly recommended to the rural farmers to incorporate into their village chicken farm practises.

While ad libitum feeding on commercial or self-mixed complete feeds is the norm for the modern commercial broiler, the feeding systems practiced for village chickens vary widely, ranging from full feeding on complete commercial feeds to the more popular practiced feeding restrictions of commercial broiler feeds with or without supplementations. With such a varied nutritional regime variation in live performance (Saleh *et al.,* 2005; Azlina & Azahan, 2011), and possibly characteristics of carcass as well, would be expected to occur between different feeding regimes. In the past, numerous studies have been carried out on growth responses and carcass yield of broilers fed restrictively. These included works on early restriction during the starter phase (Mohebodini *et al.,* 2009; Azarnik *et al.,* 2010), restriction during grower-finisher phase (Boostani *et al.,* 2010), as well as restrictions during the starter and finisher phases (Urdaneta-Ricorn & Lesson, 2002). Benefits, detrimental effects as well as lack of response to such treatments have been recorded. With indigenous chickens such as the crossbred village chicken, apparently no such work has been reported. Azahan. (2011) has improved village chicken by monitoring their growth phases. The maximum

growths of the chicken were 8th to 9th weeks and the male will be physically superior to female during the 8th week. Body weight is probably the most important economic factor in the in poultry. Another factor of economic importance to the broiler industry is carcass characteristics, specifically carcass dressed yield and fat content. Indigenous chicken and jungle fowl are always thought to be good in term of carcass composition compared to the broilers due to its low-fat content. However, there is not much quantitative information available to support the statement. Due to reason that village chicken is smaller in size, the requirement for feed in these chickens is lower compare to the commercial chicken. Furthermore, the village chicken rearing can be considering as low input, low output production in agricultural sector (Pedersen, 2000).

Other important aspect to be considered in poultry industry is the slaughtering method, especially when it is being done in the high Muslim population countries such as Malaysia which emphasise halalness and wholesomeness. Incorrect slaughtering method could result in meat spoilage and decrease the shelf-life of poultry meat durability when frozen. In mass production of chicken, easy and fast technique of slaughtering method is essential, where it is usually being done using machines. In Islam, chickens slaughtering is done by cutting the carotid arteries, jugular veins, oesophagus and trachea, where it must be done by Muslims (Riaz & Chaudry, 2003). However, sometimes as it is being conducted in mass production, some of tracts are not cut well. Incorrect slaughtered chicken such as failure to cut carotid arteries and jugular veins could result in oxidative stress in chicken, resulting in respiratory complication, thus increasing body temperature and expression of an enzyme known as Glutathione (GSH). The usage of proteomics method to identify correct slaughtered chicken could be an advantage to the industry as well as to the consumers. As GSH directly co-existing with Glutathione-S-Transferases (GSTs) enzyme, it is an advantage and possible method to identify slaughtered chicken that had been slaughtered correctly

based on oxidative stress present in the chicken. Glutathione-S-Transferases (GSTs) are enzymes from dimeric multifunctional enzymes family. The GSTs are found in many living creatures including human, animals, plants, and bacteria. GSTs involve in process of detoxification and a few chemical activations. Furthermore, it is also functioning in increasing the activity of glutathione (GSH) by catalysing the nucleophilic attack of GSH (Eaton & Bammler, 1999). During pre-slaughtered condition of chicken, chicken will undergo oxidative stress where this could affect the quality of meat. It is known that GSTs could counter the oxidative stress by acting as a peroxidase. The GSH-dependent peroxidase reaction uses toxic organic hyperoxides and also prevents oxidative damage (Moons, 2005). Moreover, the GSTs also deactivate certain secondary metabolites such as quinones, unsaturated aldehyde, epoxides, and hydroperoxides that formed resulted from oxidative stress (Hayes *et al.,* 2005). Due to many functions and benefits gained from the GSTs, there were lot studies has been made to investigate this enzyme capabilities especially for its role in physiologically preventing or protecting against chronic diseases that were caused from damaging of oxidative tissue. This is possible due to the present of quinone metabolites inside the GSTs, which is suggesting the GSTs are beneficial for curing diseases. In previous study, GSTs was said to interact with other proteins, where protein-protein interaction occurs. It was being proposed that c-Jun, N-terminal Kinase (JNK) formed a complex with Pi-GST as subunit. Theoretically, during oxidative stress the JNK been activated due to the dissociation of class Pi GST and under non-stressed conditions, the JNK would bind with class Pi GST which would help in blocking the signal along the stress kinase pathway (Sherrat & Hayes, 2001).

This study attempted to examine the growth performance of village chicken using 2 types of strains, which were purebred and crossbred village chicken when given 2 types of diet, commercial finisher diet (CFD) and formulated finisher diet (FFD), as

well as to examine the carcass performance in the village chicken. Furthermore, preliminary study also conducted to investigate the quality of village chicken that were slaughtered using 2 types of slaughtering method, Non-stress slaughtering method (NSSM) and Stress slaughtering method (SSM) by evaluating the expression of Glutathione-s-transferases and their activities in the 2 treatments.

1.2 Statement of Problems

Village chicken industry has been developing very quickly in this country due to the demand from consumers here. The population of village chicken in country only comprise of 13% from the total poultry population in Malaysia has causing lack of supply of the commodity for the consumers. Furthermore, due to many constraints in breeding plan of the village chicken, many farmers turn to another livestock industry. One of major factor of constraint in village chicken farming is feed nutritional value and feed cost. The relationship between feed cost and the nutritional value are strongly correlated to the nutritional contents such as crude protein, fat, fibre, ash and metabolisable energy, which had been used to evaluate the feed cost. Generally, as crude protein in the feed increase, the cost of the feed also will be also increased. Crude protein sources such as soya bean meal, fish meal, corn and many more are expensive due to their sources, which are been imported from other countries. The usage of the feedstuff in the production of commercial feed has caused the price for the commercial feed to increase significantly, and the price was being estimated to keep increase due to the import export current situation. Thus, it is timely to conduct research to locate new local sources of feedstuff that could replace the ingredients in the feed for the poultry production, especially in village chicken production. However, many formulated diets that were processed in the local market does not increase the village chicken body

weight as good as commercial feed due to many reasons such as digestibility and palatability of the feed when given to the village chicken.

Besides, the quality of poultry is also important before it could be sold to be used for human consumption. It is believed that, the quality of meat in chicken could be affected by the oxidative stress that was induced during preslaughter and slaughtering process, affecting the quality of meat in term of shelf-life of frozen-thawed chicken, taste and nutritional content. There is limited number of research activities that had been done to study the effect of oxidative stress to meat in molecular level, especially in local village chicken. Thus, it is important for researchers from countries that produce and consume chicken in large amount to understand and study the problematic issues that may affect our poultry industry as well as consumers health and economic value. These research statements of problems are as follows:

a) Could the cheap local feed ingredients (Coconut Meal Cake and Palm Kernel Cake) have potential to replace the expensive commercial ingredients?

b) Would the commercial diet give better performance compared to formulated diet?

c) Could Non-Stress Slaughtering Method (NSSM) and Stress Slaughtering Method (SSM) be determined using proteomic approach?

d) Could oxidative stress in poultry during slaughtering be measured by using enzyme activity of GST?

1.3 Justification

The main aim of this experiment was to identify suitable sources of feed to replace commercial ingredients that are currently used in the making of commercial diet for poultry. The problematic factor of using commercial feed is due to its cost, which is very high and the consistency of the price through years as it is been control by outside market. This situation is burdening farmers, especially small farmers from rural area as well as village chicken farmers, which is known that village chickens grow slower and require more feed compared to commercial broilers. Various formulated diets had been formulated before, but most of them gave negative outcomes as chicken weights were much lower compared to chicken that had been fed with commercial diets. Thus, this research aimed to improve the formulation diet by using local feed sources and intensive system management to increase the productivity of the village chicken as well as to evaluate the quality of the meat in term of human nutritional content. The usage of local feed could be beneficial feed materials for village chicken farmers, especially for farmers from rural areas as it is not only cheap but also could easily available and accessible. However, proper ration and formulation need to be done to ensure the nutritive composition within the diet reach the minimum requirement for village chicken for growth and maintenance, especially in term of crude protein and metabolizable energy.

Furthermore, for economic reason, this research could be used to identify the profitable margin and cost of production using commercial and formulation diets. This research could provide useful information for small and big farmers, thus could increase the productivity for village chicken industry in the future. The demand for village chicken in Asean region including Malaysia is relatively high due to their high nutritive values and preferable taste to the local community, thus contributing significantly to the socio-economic prospect and positive development of village chicken industry in the

country. There were about 30 million of village chickens in the country at the year of 2016, which account for 13% of total poultry production. This small production has effect in supply-demand of village chicken significantly, whereby low supply and high demand has created positive condition for the growth of village chicken. However, this economic potential of village chicken has been hampered due to high feed cost due to import of feed ingredients such as corn and soya bean to feed the poultry particularly the commercial broilers as well as the village chicken. Consequently, many farmers had given up the livestock entrepreneurship, while some others attempted to overcome feed cost problem by incorporating local feed sources such as PKC and coconut meal cake.

In addition, this research was conducted to obtain the preliminary results to evaluate the possibility of using molecular proteomic approach to identify specific enzymes such as the expression of Glutathione-s-transferases (GSTs) enzyme in village chicken liver using 2 different slaughtering methods and to relate this enzyme to stress condition during slaughtering which might have direct or indirect effect on the meat quality of the village chicken carcass. This experiment was done to evaluate the probability of slaughtering method may cause chicken to undergo oxidative stress. This preliminary experiment would open new window of opportunity to study the quality of meat after slaughtering that could be related to oxidative stress. Molecularly speaking, the identification of GST enzyme ('good enzyme' which acts as a counter enzyme for oxidative stress and it interacts with xenobiotics compound to reduce stress in the living organism) would be a good indicator of oxidative stress, whereby the increase in GST activity would catalyse the activity of Glutathione (GSH), thus preventing the stress of the organisms. The application of the enzyme such as GST for detecting oxidative stress in slaughtered chicken is a novel approach whereby no definitive previous studies were conducted in this area of research. Therefore, it is justifiable and timely that this research was carried out using proteomic approach to identify specific protein

compounds such as GST that could be related to oxidative stress in animals such as stress during slaughtering of village chicken. The justifications for the current research to be conducted are as follows:

a) Improving feed formulation using local feed sources for the efficient rearing of village chicken under intensive system.

b) Producing high quality of village chicken meat for the safety and health of consumers.

c) Identify the stress related proteins such as GST as an indicator of stress during slaughtering of village chicken that may affect the quality of the carcass.

d) Improving socio-economic wellbeing of the rural community through efficient village chicken farming.

e) After establishment of a molecular method as detector for stress identification during slaughtering of village chicken, this information could be extended to be applied in identification of Halal poultry meat including village chicken.

1.4 Application

The usage of formulated diets in poultry industry is important, especially for local farmers due to its cost and availability. Commercial diets are more expensive compared to formulated diet due to their imported ingredients such as corn and soya bean meal. Furthermore, the commercial diet price cannot be maintained throughout the years due to their imported feed status. The usage of formulated diets by farmers could be a beneficial solution for the problems and could attract new farmers to participate into the poultry industry, especially in village chicken farming. It is an advantage for village chicken farmers because village chicken was known for their organic taste, thus giving formulated diet could induce their organic characteristics. The availability of the feedstuff for formulated diets is easy due to their ingredients, which come from local

sources such as palm kernel cake and coconut meal cake. Furthermore, the usage of formulated diets could be a good assurance for consumers, who going to questioning about the ingredients of feedstuff in the formulated diets, thus could also increase the consumers trust to the farmers and also to the village chicken industry.

The application of detecting of oxidative stress in slaughtered village could be used by government to detect the standard operating procedure (SOP) and the quality assurance of poultry production in slaughtered house. This new method or SOP could ensure the production of high quality poultry before it is being distribute to vendors or export to other countries. The consumption of high quality chicken would be a health benefit to the consumers and could increase country poultry production as well as increase the economy of the country.

1.5 Objectives

The objectives of this research were:

a) To evaluate the growth and carcass performances of purebred and crossbred village chicken fed with commercial finisher diet (CFD) and formulated finisher diet (FFD)

b) To determine the meat quality of purebred and crossbred village chicken fed with commercial finisher diet (CFD) and formulated finisher diet (FFD).

d) To evaluate the expression and activity of Glutathione-S-Transferases (GSTs) in poultry liver subjected with difference slaughtering method.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The population of chicken in Malaysia is about 308.1 million birds. In 2016 self-sufficiency ratio (SSR) for the poultry meat was 98.5% with per capita consumption (PCC) 50.1 kg/year (Department of Statistic Malaysia, 2016), thus showing that the chicken industry in Malaysia is developing very rapidly. The demand for village chickens is increasing every day due to their taste and nutritive value compared to broiler chickens, thus causing high demand of the village chickens as well as increasing its price tremendously (Liang *et al*., 1995). It was known that village chicken (*Ayam Kampung*), *Gallus domesticus* was previously originated from Malaysia Red Jungle Fowl (*Ayam Hutan*), where the chicken undergoes several uncontrollable cross breeding. In 2007, there was 8 million of village chicken been reared and 334 tonnes of village chicken were exported (Arif *et al*., 2010) and it was assumed in 2016 there were 30 millions of village chicken, where account for 13% from overall poultry population in Malaysia (Supramaniam, 1987). Demand for organic chicken is high especially during celebration holiday, thus many of the village chicken was crossbreed with fast growth broiler to gain more suitable breed that has higher body weight and could lay more eggs compared to pure breed of village chicken. However, this phenomenon of crossing of the breeds might cause the pure line of village chicken to be extinct. Thus, keeping the lineage of pure breeds of village chicken is important for genetic diversity. The village chicken also been claimed have unique taste of the meat which had been highly demanded by the local people. Furthermore, due to their scavenge characteristic for their feed, they are more naturally grown, less fat and have high immune response to the environmental condition or diseases.

Table 2.1: Comparison between village chicken and commercial broilers, (Adapted from Ganabadi *et al.,* 2009; Alders and Pym, 2009)

Strains / Parameters	Village chicken	Commercial broilers
Infrastructure	Scavenge Intensive Semi-intensive Cost – low	Intensive ventilated room Cost – high
Work force	Low	High
Diet	Scavenge diet Mixed ration Cost – low	Formulated commercial diet Cost – high
Production	Slow growth Low production	Fast growth High production
Disease control	Newcastle disease vaccination	Vast vaccination for many diseases and bacteria as well as parasitic prevention
Fat	Low	High

Table 2.2: Significant finding in village chicken research

Year	Researcher	Significant finding
1977	March & Hansen	fat deposition in chicken is influenced by age, gender, and strains.
1984	Hamid & Syukor	Only 1% of farmers reared village chicken intensively.
1987	Supramaniam	Stated that the population of village chicken in Malaysia account for 13% from total poultry.
1994	Azahan	Purebred village chicken recorded weight 1.1–1.5 kg at 4 months of age and lay eggs about 100 eggs/year.
1994	Azahan	Malaysian purebred village chicken morphological characterization.

Table 2.2, continued

1999	Ramlah	The usage of local feed supplementation Intensive feed supplement such as rice bran, corn, coconut cake, wheat, soy waste and tapioca were could promote growth.
2004	Alimon	The usage of palm kernel cake in poultry could be utilised efficiently due to palatability and
2004	Wan Zahari & Alimon	Toxicity recorded at 20% of PKC used in feed for broilers.
2005	Moons	GSTs could counter the oxidative stress by acting as peroxidase
2005	Hayes *et al*	cytosolic GSTs was involved in oxidative stress protection by activating the antioxidant response element in cGST gene promoter
2010	Moorthy & Viswanathan	Recorded crude protein at 22.75% and 1552 kcal/kg in coconut meal.
2010	Arif *et al*	In 2007 there were 8 million of village chicken reared in Malaysia.
2011	Azahan	Crossbred village chicken recorded weight 1.5 kg in 2 months.
2014	Farouk *et al*	Reported that mass production tends for missed slaughtered after period of time running

Table 2.2, continued

2015	Estevez	The negative effect of oxidation to chicken including low feed intake, poor performance, disease susceptibility, formation of toxic compound and rancidity. endogenous enzyme such as glutathione peroxidase can counter the stress cause by oxidation of lipid and protein
2016	Francis *et al.* Khawaja *et al.*	Slow growth of purebred village chicken is due to the genetics factor
2017	Pushpakumara *et al.*	High level PKC could reduce the body weight of chicken

2.2 Strain of Village Chicken

Domesticated poultry has been existed since thousands of years, where research from archaeological evidence indicated that poultry was first domesticated in China since 8,000 years ago (Alders, 2004). Village chicken usually could be located at rural area, where most of them were reared using free range system to allow the village chicken to scavenge for food. However, due to enormous demand of village chicken for consumption in Malaysia, it is irrelevant to rear the chickens using scavenging system due to management planning, uncontrollable growth and diseases. Thus, the village chickens were domesticated either by semi-intensive or intensive system. In Malaysia, there are 2 prominent strains of village chicken that are been widely marketed, which are Purebred Village Chicken and Crossbred Village Chicken. Purebred village chicken in Malaysia could be also known as indigenous chicken or native village chicken, which is the descendant from wild south-east Asian Red Jungle Fowl or also known as *Ayam*

Hutan in Malaysia that had been also domesticated (Ramlah, 1996 ; Moiseyeva *et al*., 2003). Moreover, there are many varieties of strains of village chickens in Malaysia due to uncontrollable breeding from the past. It is believed that the descendant of Malaysia village chicken is *Gallus bankiva*. The commercial broilers strain is also known as *Gallus domesticus*. Meanwhile, the Jungle Fowl is known as *Gallus gallus* and then been domesticated by crossing with fast growth broilers resulting in production of crossbred village chicken strain or known as *Gallus gallus domesticus* (Ganabadi *et al*., 2009).

Purebred village chicken has smaller size and produce fewer eggs compared to domestic chicken. Azahan (1994), reported that the village chicken has body weight between 1.1–1.5 kg at 4 months of age and lay eggs about 100 eggs/year. Purebred village chicken market age is 16 weeks where the chickens would reach their average body weight approximately 1.26 kg. Indigenous chicken or purebred village chicken in Malaysia has few varieties such as black-red and red variety. Black-red variety village chicken has long and upright neck with red double wattle. Furthermore, the Black-Red variety male chickens also have rose or pea type comb and also have glossy black tail feathers with greenish red wing feathers. This Black-red variety has a pair of relatively long legs with hock joint. Red variety of village chicken is reddish in colour with single large comb. These varieties also have red double wattle but have relatively short neck and legs. There is no feather with whitish yellow shanks. Male from this variety have plumage of red over the entire body. Azahan (1994) was conducting research with these two varieties of village chicken and discovered that these two could accommodate well in intensive rearing system with low mortality rates. The low mortality of these strains might be due to their high resistance to local environment and diseases. However, still disease such as Newcastle Disease still the main problems of village chicken rearing (Cumming *et al.,* 1991; Spradbrow P. B. 1991.). The wild Red Jungle Fowl is known as

the ancestor of the Malaysia village chicken through crossbreeding where it produces smaller size fowl. The lineage of the wild Red Jungle Fowl is believed to be a good lineage for improvement of meat quality and also for disease resistance. Nowadays, the indigenous chickens have undergone hybridization with domestic chicken to produce marketable chicken which could produce more meat and eggs without compromising the good taste of village chicken.

Crossbred village chicken is the result from control breeding between purebred village chicken with domestic commercial broiler, where it resulted in almost similar morphological appearance as the purebred village chicken. However, crossbred village chicken is more promising strains for domestication due to its advantage in growth performance compared to purebred village chicken. Crossbred village chicken could grow faster and heavier compared to purebred village chicken, where they could reach approximately 1.5 kg in 2 months (Azahan, 2011). Furthermore, due to its lineage that originated from local village chickens, the resistance to the disease also good for the crossbred village chicken.

2.3 Farming System of Village Chicken

Village chicken usually are reared by traditional system or free-range system, where people keeping their chickens at loose to scavenge their own feed at their backyard. In Malaysia, there were 82.1% of village chicken farmers in 1984 that practice free-range system (Hamid & Shukor, 1987; Aini, 1990). Although the system beneficial to reduce feed cost, but it resulted in slow growth and higher mortality of the village chicken. Furthermore, the system is not suitable and not efficient for those people who are involve in large scale of village chicken farming. This is because village chicken produced low in term of meat production and eggs in the scavenging system. Furthermore, scavenge chickens usually ate almost anything, thus susceptible to

diseases and infection of contagious diseases such as Newcastle Disease (ND), Infectious Bronchitis (IB), Coccidiostat and many more. Thus, control and systematic system were endorsed which are semi-intensive and intensive system. In 1984, there were 15.4% of village chicken farmers practice semi-intensive system, whereas only 1% of farmers practicing intensive system for village chicken farming (Hamid & Shukor, 1987). However, the performances of village chicken in Malaysia in term of eggs and growths are low, generally, whether reared using semi-intensive or intensive system and this was due to the lack of planning breeding and nutritive supply for village chicken in the feed (Ramlah, 1996).

2.3.1 Intensive system

Nowadays, there are many village chicken farmers already practicing intensive system, where the number of farmers who practising it was increasing tremendously resulted from promotion and education made by the government. Usually, intensive system is applied to obtain higher chicken body weight and eggs by 24 hours monitoring the chicken, where the lights, feeds, water, temperature, litter, and mating schedule were systematically planned. By using this system, the village chicken would be only focusing on growth and reproducing with limitation in movement, planned diet and health monitoring. However, intensive system is costly in term of money and energy. In term of money, farmers need to buy feed for starter diet and finisher diet, so that the chicken growth could be optimized. When we compare with free-range system, the need for feed is lesser because the chicken scavenges themselves for feed such as waste of rice, vegetable, grains, worms, insects, and many more. Intensive feed supplement such as rice bran, corn, coconut cake, wheat, soy waste and tapioca were mixed to produce diet that were more nutritious and could promote growth with supplementation of vitamins and antibiotics (Ramlah, 1999). Moreover, intensive systems have limiting

space for the chicken, thus require for removal of the chicken litter regularly to ensure cleanliness and preventing contagious diseases. However, this requires man power that was costly and management. Village chicken that were reared intensively usually vaccinated to prevent diseases. Usage of the vaccine such as Newcastle Disease Virus (NDIB), Fowl Pox Vaccine, Infectious Bursal Disease (IBD) and many more also costly as well as require man power.

Despite that, the usage of intensive system still could be an economical advantage as it could reduce mortality and also reduce rearing period, thus reducing also the maintenance cost for the chicken. Previous studies showed the profit of using intensive system was much higher compared when using scavenging system in term of egg production and profit (Sazzad, 1992). Despite that, chicken that been reared intensively recorded significantly higher in production of crude fat content in the leg meat and significantly lower in crude protein content in the breast meat. Furthermore, previous study also recorded lower chewiness and shear force of chicken in intensive system compared in free range system (Cheng *et al.,* 2008).

2.3.2 Semi-intensive system

Semi-intensive system is the most popular village chicken rearing system compared to the others among local farmers in Malaysia. Semi-intensive system is where the chicken is let loose for a certain period of time and also been kept into housing especially during the night. According to Mangesha (2012), semi-intensive system is when the chickens are reared surround fence and being fed with routine feeding regime where it could be monitor regularly. With this system farmers, not only could minimize the cost of feed and housing but also could minimizing the possibility of the village chicken from being infected by any contagious diseases. The benefit of the system is that it could provide a natural ecosystem as well as providing proper management and care through the indoor

housing for the chicken, thus could increase the growth performance and ensure good management of animal practises been done. However, although this system is low cost, the meat and eggs production tends to be low compared to intensive system based on previous research. The main cost for the semi-intensive system is the feed which the feed influence to the production rate of the village chicken in term of meat and eggs production (Wantasen *et al*., 2014).

Despite that, semi-intensive system still offers a good condition for village chicken comparatively with free-range system. This is because diseases prevention and routine vaccination still could be done to the village chicken in the system. Diseases such as Newcastle Diseases, Fowl pox, Marek disease and Infectious Bronchitis could be monitored from this system as chickens were kept in pen during night (Aini, 2013). Ramlah, (1996) indicated that the eggs and growth performance of using semi-intensive system for village chicken production was still low in term of their performances.

2.3.3 Extensive system

Local people in rural area usually reared village chicken extensively or free-range system by letting the chicken to scavenge for their feed at their backyard. This system is economically saved due to no cost for feed, management and housing. However, the growth performance is still low and recorded high mortality rate due to low management and disease preventive measures to the chicken. The chicken usually had been fed with leftover feed such as corn, grain, rice, soybean and many more without proper feeding management and diet planned. Researches also showed that majority of village chicken or 83% of local chickens were reared extensively around the world such as in Bangladesh, where 70% of chickens were reared extensively to scavenge for food (Morges *et al*., 2010). Usually in extensive system, chickens are freely scavenged for their food without housing. One of the system disadvantages is that chickens are

inevitable to disease such as Newcastle Disease and Avian Disease that are spread by contact with other wild bird. However, the constant movement of the chickens to new feeding area also could lower the chances of getting infected by pathogens that were formed due to their litter accumulation. Furthermore, in this system it is not economically effective in term of land area and labour.

Furthermore, the problem in this system also due to the lack of monitoring by the farmers, which causing mixing of many breeds of chicken in one area, thus making it more difficult to monitor chicken performance and characterise the chicken variation (Oh, 1987; Aini, 1990). However, certain countries such as Kenya and Bangladesh still reared their chicken extensively, which account at least 50% (Kingori *et al.,* 2010; Morges *et al*., 2010). This is due to the advantage of the village chicken that can withstand tropical environment and good scavengers (Barua & Yoshimura, 1997).

2.4 Village Chicken Growth Performance

Growth is a change that occurs in an individual that influence physiological aspect toward positive improvement. Growth performance could be determined by evaluating aspects such as live body weight, feed intake, body weight gained and feed conversion ratio (Hossein & Dahlan, 2015; Pauwels *et al*., 2015). Various works had been done around the globe to increase the performance of the village chicken and some gave positive output especially involving feeding and health. Besides, the improvement in village chicken performance needs surveillance on nutritional, breeding, management and health factors to ensure the development of the village chicken and increase the production of the village chicken meat and eggs (Azahan *et al*., 1980; Azahan, 1994; Tadelle *et al*., 2002; Olokosi and Sonaiya, 2003). Thus, constraints were faced by researchers in many aspects such as diseases, nutrition in feed, cost, management system, market output and much more.

2.4.1 Constraints in village chicken farming

The village chicken farming is increasing rapidly due tremendous demand from the consumers, however there are still many constraints that affecting the industry such as in term of the genetics of the village chicken that result in slow-growing chicken. Slow growth chicken not just affect the price of the chicken but also affecting the cost which could affecting many aspects including maintenance cost, feed, and labours cost. The longer the period for the chicken to reach the marketable weight, the higher the cost need to be endured by the farmers. It was known that village chicken slow growth is contributed by several factors such as genetics factor, environmental factor, nutrition, parasites, young mortality and diseases (Cumming, 1991). Although improvement has been made to increase the performance of the purebred village chicken, crossbred village chicken still higher than the purebred village chicken in terms of weight and size (Haitook, 2006). Besides, the slow growth of the village chicken also due to the low nutrient abundance, especially in an extensive system and poor genetic efficiency for feed conversion ratio that result in poor feed utilising, thus resulting in high feed conversion ratio value (Roberts, 1999; Tadelle *et al*., 2003). Huchzermeyer (1973), stated that the nutritional supply for the village chicken was varied due the seasonal variation, thus resulting in limiting supply for energy and protein. One of the major constraints in the village chicken farming was high young chick mortality, where research recorded almost 70% chicks hatched dies during the period of after hatching until brooding. The reason might be due to the several factors such as lack of nutrition, diseases and predators that largely contributed by the extensive system (Cumming, 1991).

Previous study by Kajarern *et al*. (1988) stated purebred village chicken growth was approximately around 1.3 kg/bird at 16 weeks old. Meanwhile, a study made by Azahan (1994) on 2 strains of purebred village chicken, red and black varieties showed

that the weight at 16 weeks old were only 1.22 kg/bird and 1.29 kg/bird respectively. It is largely different from weight of crossbred village chicken which weight more than 2 kg/bird at 16 weeks old (Azahan, 2011; Azahan *et al*., 2011). Furthermore, lack of knowledge among rural community about proper village chicken management contributed to the progress of the village chicken industry. Study showed that majority or 79% of rural farmers did not doing the preventive measures during disease occurrence, 14.4% used traditional medicine, approximately 5% using modern medication and the rest 0.9% using human related medication. However, it was not only due to lack of knowledge but also due to the cost, availability and application procedure (Mapiye & Sibanda, 2005). Among the main constraint in village chicken farming in South-east Asian is diseases. Disease such as Newcastle Diseases was spreading and known as the main diseases in poultry in south-east Asian. The spreading of the disease was largely due to the lack of management system, unconfined housing, lack of systematic rearing of the chicken and difficulty of disease control application due to free ranging system (Aini, 1990; Moges *et al.,* 2010; Dinka *et al.,* 2010). Dirty environment also could invite the spreading of endoparasites, ectoparasites, haemoprotozoa and types of microfilaria (Sani *et al*., 1987; Supraminiam, 1988; Mwale & Masika, 2009). Furthermore, village chicken also low in reproductive production, where most of village chicken only lay up to 80 eggs/chicken/year comparatively with commercial broiler, which could produce more than 300 eggs/chicken/year (Mapiye *et al*., 2008). It could be said that the main constraint of village chicken production is due to lack of genetics potential for the village chicken, especially in indigenous chicken. Thus, crossbreeding with commercial broilers is a good way to improve the genetics potential of the village chicken without compromising the indigenous characteristics within the village chicken. However, unplanned crossing could be diminished the unique characteristics of village chicken such as low in fat content and tasty flavour of the village chicken.

2.4.2 Dietary intake on energy

Generally, the total intake of feed in poultry is based their energy requirement ad libitum. The adjustment of their feed intake to gain sufficient nutrient requirement has resulting in difficulty to determine their exact energy requirement in terms of kilocalories. The concentration of energy in the diet determines the feed consumption (National Research Council, 1984). The need for metabolizable energy (ME) was decreased with the increment of temperature above 21 °C. The effect of temperature to the reduction of ME requirement was mainly due to the declining of the maintenance energy requirement. Although, the usage of high energy during warm weather could result in significant effect on poultry body weight, thus the sufficient amount of amino acid should be given to the poultry to ensure the survival of the poultry (Daghir, 2008).

Table 2.3: Deposition of dietary energy

Deposition of dietary energy	**Kilocalories (kcal)**
Maintenance	1500 ME
Faeces	800
Eggs and tissues	800 ME
Heat increment	600 ME
Urine	300
Total	**4000**

Table 2.2 showed the deposition of dietary energy where from 4000 Kcal obtained from 1 kg of feed can give about 2900 Kcal of metabolizable energy. There are also many factors that affect in the deposition of the dietary energy such as poultry age, genetic, and strains (National Research Council, 1984). Previous research showed that the average of ME around 2600 kcal was the lowest energy that could be given to the poultry and approximately 3200 Kcal of ME was the maximum value to ensure low

deposition of abdominal fat in the chicken (Jackson *et al*., 1982; Holsheimer *et al*., 1992; Wu *et al*., 2005). It was proven by Jackson *et al*. (1982), where in his study significant percenatge of abdominal fat was presence when given high energy feed compared to lower energy feed. However, some study also showed that the energy ranging from 3,023 to 3,383 Kcal ME/kg could result in lower feed intake due to high concentration energy in the feed. Moreover, the decrease of in feed consumption was not correlate with nutrient consumption increment that contains in high energy feed (Bell and Weaver, 2002).

There are few terminologies of dietary energy that are important in determining the energy requirement in poultry. Kilocalories (Kcal) is the standard used of energy unit for poultry, which equal to 1,000 of calorie (cal) and could be defined as required heat to increase the temperature of 1 g of water. The released of energy as heat result from oxidation of substance to carbon dioxide is known as Gross Energy or heat of combustion. Apparent Digestible Energy (DE) is defined as subtraction of gross energy from diet with faecal gross energy. However, in birds DE value is not been practiced due to excretion of faeces and urine from cloaca by birds. Apparent Metabolizable Energy (ME) is the result from subtraction by gross energy from the diet with energy in the faeces, urine and gaseous digestion products. However, gaseous products are not considering much in poultry. The True Metabolizable Energy (TME) is subtraction of gross energy of the diet with excreted product of feed gross energy (National Research Council, 1984). In village chicken, consumption of low energy diet is more economical compared to high energy diet due to insignificant difference in body weight gained and feed conversion ratio (Azahan *et al*., 2011). Village chicken metabolizable energy intake is much affected by the seasonal variation, especially for scavenging village chicken (Tadelle, 1996). However, the amount of energy in scavenge feed resources is

low in term of concentration due to insufficient amount of starch presence in the feed resources (Sonaiya *et al*., 1999).

2.4.3 Dietary intake on protein

Protein is one of the most crucial dietary aspect in poultry that needs to be considered to ensure optimum growth of the village chicken. The requirement of protein for the village chicken is actually for the amino acid contained inside the protein (National Research Council, 1984). The amino acids inside the protein were part of constituents of many parts inside the chicken to accommodate various functions of the chicken body such as feathers, skin, and bone. Furthermore, the level of protein content in feedstuff could affect the amount of feed intake of the animals. There are 2 categories of protein requirement, which are essential amino acid and amino nitrogen, which is used for synthesis and non-essential amino acid (National Research Council, 1984). There are actually less report on the requirement of crude protein (CP) in village chicken feedstuff, however, Chemjor. (1998) reported that 13% of CP was adequate for dietary protein level between 14-21 weeks of age of chicken. In other studies, showed the steady growth of chicken that fed by 10% of CP until 16% of CP could increase body weight gained significantly. Furthermore, the feed conversion ratio (FCR) was significantly lower from 10% to 16% of CP (Kingori *et al*., 2003).

The ability of amino acid absorption is contributed by the factor of gut microflora in the chicken, which largely could be affected by the metabolism of gut tissue. Furthermore, it was already studied that the low digestibility of feed supplementation in chicken diet may increase the fermentation of microbial and also increases the feed digestibility (Hughes, 2003). As the usage of crude protein is very important in chicken growth, there were many researchers in the past of using varies sources of protein such as soybean meal, canola meal, and blood meal (Leeson *et al.,*

1987; Meng & Slominski, 2005; Khawaja *et al.,* 2007; Ahmed *et al.,* 2015). The usage of high percentage of crude protein in poultry could be an advantage in term of growth rate; however, study showed that there were limitations of crude protein level that could be utilised by the chicken. It was studied that 40% is the maximum percentage of dietary protein that could be utilised by chicken (NRC, 1994). Furthermore, previous study also showed that by decreasing the usage of dietary protein level with amino acid supplementations in the chicken diet could increase the chicken overall performances and increase the economic profit, especially in laying chicken (Zeweil *et al*., 2011; Kashani *et al*., 2014).

In other research done by Summers *et al.* (1992) reported that the consumption of balance and level of essential amino acid could affect the amount feed consumed by the chicken, thus affecting the growth and carcass performances of the chicken. Previous research also showed that purebred village chicken could retain same growth rate as commercial broiler with condition of given protein supplementation, thus the usage of protein supplementation in village chicken is important to attain maximum growth. It was also reported that by given either crude protein supplementation by 3.2 g/bird/day or consumption of crude protein by 11.7 g/bird/day during growing period is a good practise for purebred village chicken (Kingori *et al.,* 2007). Furthermore, the weight of organs, carcass performances, and deposition of fat were not affected by restriction of the diets (Fontana *et al.,* 1993).

2.4.4 Dietary intake on fat

One of the beneficial and preferer aspect for consumers to consider village chicken as their daily consumption is due to the low-fat content within the village chicken. Although the deposition of fat is an undesirable aspect in chicken, but there are still desirable percentage of fat needed by the chicken to ensure good palatability by

consumers, as well as for their appearance. The undesirable fats are mainly crop fat and abdominal fat. The abdominal fat account for at least 2% of the body weight in chicken (Leenstra, 1986). There are factors that affecting fat deposition in chicken such as age, gender, and strains (March & Hansen, 1977; Leenstra, 1982). In previous studies, it has been reported that female chicken abdominal fat and total body fat content percentage tends to be much higher compared to male chicken (Edwards *et al*., 1973). Furthermore, Mabray and Waldroup (1983) reported that given low dietary protein content in chicken feed could lower the deposition of fat as well as increasing the percentage of protein in the chicken. Besides, nutritional factor also influenced on the fat deposition has been also reported to be different in different type of chicken strains (Scheele *et al.,* 1981). The dietary fat consumed by chicken usually takes 10 days to be utilised and developed in the chicken body due to the slow lipase secretion from their pancreas (Jin *et al*., 1998). It was reported that village chicken management system also influenced the deposition of fat, where intensive rearing builds more fat compared to scavenge or extensive management system. This was due to the factor of age at sexual maturity (ASM) reached by both chicken reared in the 2 management systems (Kurmaresan *et al*., 2008). Moreover, it has been reported that stress that been caused by heat could significantly decreases the deposition of intermuscular, subcutaneous and abdominal fat. However, feed restricted in the experiment showed enhancement in abdominal fat development in high ambient temperature (Lu *et al.,* 2007).

Research done on jungle fowl showed that the fat content in the chicken was correlated with the level of crude protein in the diet given to the chicken. Furthermore, the fat content in the jungle fowl also recorded the least fat content as compared with commercial broilers and this was correlated with low development of muscle and heavier bone weight; indicating that poor muscle bone ratio in the broilers compared with jungle fowl (Ganabadi *et al.,* 2009). It was also reported by several researchers that

the presence of fat in the feed could enhances the palatability, texture of the meat and lowering dustiness level in broilers (Baião & Lara, 2005; Ayed *et al*., 2015). Furthermore, study also showed that consumption of saturated fat in broilers diet could affect in the decrease in feed gain ratio comparatively with polyunsaturated fatty acid (Abdulla *et al.,* 2016).

2.5 Village Chicken Industry in Malaysia

Poultry is a crucial commodity today as it accounts for 33% of total world meat production and recorded high world per capita consumption at 13.5 kg per capita (International Poultry Council, 2011). In Malaysia, there were about 308.1 million chickens reared in 2016, where per capita consumption (PCC) was 50.1 kg per capita, and village chicken account for 13% of the total number of poultry in the country (Department of Statistic Malaysia, 2016; Supramaniam, 1987). As in 2007, there were 8 million of village chickens reared (Arif *et al.,* 2010), thus it is being expected that the total number of village chicken in Malaysia in 2016 would be approximately 30 million, where it had been increased by 20% in number of population in 9 years.

More than 50% from the total of 334 tonnes of exported village chicken were exported to Singapore (Arif *et al.,* 2010). In Indonesia, the population of village chicken were much higher compared with Malaysia, where the village chicken in Indonesia account for 26% from the total poultry population (877 million) in year 1994 (Ramlah, 1999). The progressiveness of the village chicken industry was slower in Malaysia due to many factors and constraints that had been discussed before in previous sub-chapter. Table 2.4 showed the number of village chicken population in Malaysia by years.

Table 2.4: Number of village chicken in Malaysia based on year (Adapted from: Arif *et al.* 2010)

Years	Number of population (million)
2005	4.9
2007	8.0
2010	11.0
2011	13.9
2012	16.1
2016	~30.0

2.5.1 Local feed sources

Growth of chicken was largely influenced by nutritional factor and genetics. Most of broiler farmers feeding their chicken using commercial starter and commercial finisher diet packages by many brands. These types of diets are expensive due to the amount of crude protein used in the formulation and most of the feedstuff were imported from outside of the country, thus increase the feed cost. However, in village chicken farming, the cost is much higher due to village chicken take longer time to grow (Azahan, 1994). Thus, the usages of local feed sources are much preferable due to their cost and availability.

Palm kernel cake (PKC) is known to be a good supplementation for ruminant animals due to its high metabolizable energy. Most of the PKC produced in Malaysia were produced using expeller method. The only difference between expeller and solvent-extracted PKC is the oil content, where expeller PKC contain at least 4% of oil compare to the solvent-extracted PKC that contain only 1% of oil. Proximate analysis done previously showed that PKC contains 16-18% of crude protein and recorded low metabolizable energy in poultry, which is only 6.5-7.5 MJ/kg. Amino acid in PKC fed to chicken showed at least 62% of availability (Yeong *et al*., 1981). Despite that, the

utilisation of PKC in poultry feed is still less efficient due to the problem such as palatability and digestibility (Alimon, 2004; Sharmila *et al.,* 2014). The level of PKC that could be given to chicken to avoid toxicity is approximately 20% for broilers (Wan Zahari & Alimon, 2004). Research has shown that the low digestibility of PKC in poultry was due to the presence of high level of Non-Starch Polysaccharides (NSP) in the PKC cell wall (Dusterhoft & Voragen, 1991; Choct & Anisson, 1992). However, study done by several researchers showed that the addition of enzyme in PKC could improve its nutrition value and enhances the palatability as well as digestibility for poultry consumption (Sekoni *et al*., 2008; Chong *et al*., 2008).

Other local ingredients that have the potential to be ingredient in poultry feed is coconut meal cake (CMC). There were varieties of data recorded on CMC proximate analysis. Moorthy and Viswanathan (2010) recorded crude protein at 22.75% and 1552 kcal/kg of apparent metabolizable energy in extracted coconut meal. The process of producing coconut milk, oil and many more, result in production of residue as coconut fibre. The coconut fibre has been developed to be a product with high cellulose and low-fat content. Previous research also shown that drying of the coconut fibre could result in proximate data as CP (4.12%), cellulose (37.1%), fat (12.0%) and water (33.0%) However, studies also shown that consumption of coconut meal cake in poultry could result in poor feed conversion ratio (FCR) (Padhi *et al*., 2003; Yulvianti *et al.,* 2015). Despite that, there were also studies that shown an increase in feed conversion ratio (FCR). The researchers conducted an experiment using Leghorn layer chicken recorded increases in FCR level (kg of feed/eggs production) when given coconut meal (Thomas & Scott, 1962; Wignjosoesastro e*t al*., 1972). The production of coconut meal cake without undergoes fermentation process same as production of copra meal. However, copra meal for livestock consumption in Malaysia is limited and expensive, thus result in non-economical option for chicken diet in Malaysia. However, copra meal contains

more nutritive value such as higher crude protein compared to coconut meal cake (Jordan *et al.,* 2006; Ofomaja & Ho, 2007).

Rice is also one of the feedstuff that is easily available in Malaysia due to the availability of rice production in the country. Rice milling activity in the country producing residue that is known as rice bran. Rice is source of energy in poultry diet. However, the energy contained in the rice is only account for 75% of energy that could be gained from corn. Study has shown that high quality of rice bran recorded nutritive value as CP (13%), crude fibre (13%), and fat (13%) (Ravindran and Blair, 1991).

2.6 Poultry Meat Quality

The increase of poultry demand worldwide due to large consumption of world human population has been causing mass production of the meat-based poultry product. However, mass production in poultry has led to many reckless works done by human and this include in slaughtering of the chicken. This could affect the quality of chicken due the presence oxidative reactions. Previous researchers showed many negative effects on chicken quality treated with high temperature due to the oxidative stress and this were worsening as chicken was fed with oxidized feeds (Estevez, 2015). Min *et al.* (2008), has stated that in previous research showed that poultry meat is the most susceptible to oxidative stress compared with other animal meats. There are few factors that cause oxidative damage such as exposure of heat oxidized dietary oil to chicken, handling of chicken, poultry processing and preparation of culinary. The negative effect of oxidation to chicken including low feed intake, poor performance, disease susceptibility, formation of toxic compound and rancidity (Estevez, 2015).

2.6.1 Effect of lipid and amino acid oxidation on meat quality

Oxidative damage could largely affect in lipid content in poultry meat. Lipid is important ingredients in poultry meat that regulate many activities and function. It was known that lipid is functioning in poultry meat by increase the flavour, tenderness, juiciness and aroma profile sensation of the meat. Furthermore, it was known also that lipid oxidation is the factor that decreases the meat quality in poultry during storage. Poultry meat is more susceptible to rancidity due to oxidation of lipid compared with other types of meat, where it could develop during slaughtering period and storage. Amino acid is largely affected by the oxidative damage occur in the chicken body. Trytophan and methionine are known as amino acid that are most susceptible to the presence of oxidative damage (Cadenas & Davies, 2000; Estevez, 2015). Moreover, oxidative damage in chicken may result in drip loss, which was resulted from disruption of the membrane ability to hold water (Weber, 2001). Previous research showed that nutrition plays major role in countering oxidative damage (Estevez, 2015), and selenium is the best trace element that can act as antioxidant in poultry feed as well as playing major role in prevention of cancer, heart and viral complication. The deficiency of selenium in poultry feed might cause cytosolic oxidative stress, and liver oxidative damage in poultry (Zhang *et al*., 2012, Liu *et al.,* 2015).

It was suggested that reactive amino acid side chains are more susceptible to lipid peroxidation products. Furthermore, previous research done also showed that low oxygen could result in cross linkages of derivatives, where 2 carbon-centred radicals reaction produce carbon-carbon cross linkage derivatives (Soladoye *et al.,* 2015).

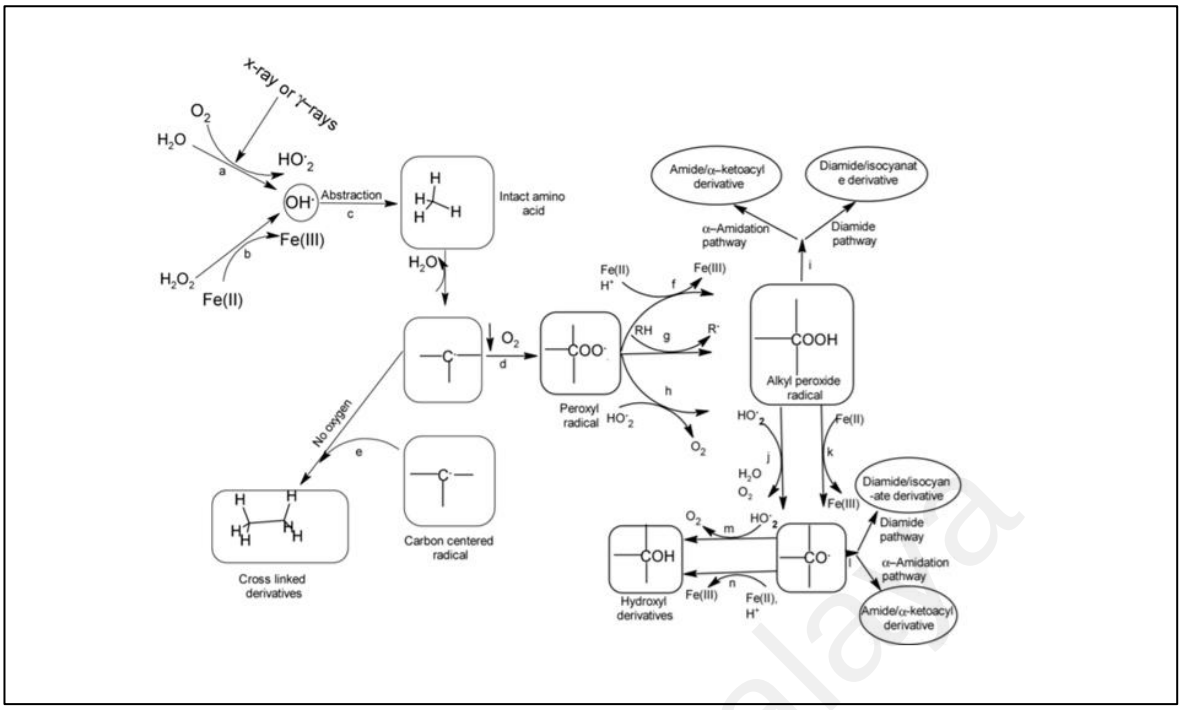

Figure 2.1: Mechanism of protein oxidation in processed poultry meat (Adapted from: Soladoye *et al.,* 2015)

It was known that protein oxidation (PROTOX) could occur during processing, handling and storage of the meat type food. Furthermore, studies also indicate that PROTOX could cause losing of essential amino acids, reducing bioavailability and digestibility as well as contributing in the presence of many health problems. Losing of essential amino acids is also contributed from process of irreversible of essential amino acids modification (Estevez, 2011; Soladoye *et al.,* 2015).

2.6.2 The effect preslaughter condition on oxidative stress

It was known that preslaughter process of poultry whether before slaughtering or after slaughtering could increase the oxidative stress. Stressed animals from preslaughter condition usually have condition such as pH drop, fast rigor mortis in muscle and high temperature. Usually product such as meat from stressed animals will become pale, moist, and soft after 18 hours froze, thus affecting in terms of juiciness, cooking losses and yields (Aberle *et al.,* 2001). Heat stress before slaughtering is the prominent factor that could cause oxidative stress in poultry. The increased in body temperature could

result in many metabolic changes toward poultry meat and this usually contributed by acute heat exposure; especially occur to farmers located in hot climate geographical area. High temperature exposure also could result in the increment in concentration of plasma corticosterone. Furthermore, heat stress could result in high oxygen radicals that produce from the break of electron transport chain in the membrane (Ando *et al.,* 1997; Ali *et al.,* 2008).

Besides, shackling and hanging also contributing to the building of oxidative stress in poultry. It was reported that, the procedure could result in the increase of meat redness, production of corticosterone, increased of lactate concentration in breast meat and drop of pH (Kannan *et al.,* 1997; Debut *et al*., 2005; Ali *et al.,* 2008). Furthermore, Debut *et al.* (2005), also stated that struggling of poultry is more affecting to breast muscle compared with thigh muscle by observing lactate concentration in the muscle, where the glycolytic function more. Furthermore, catching and transportation also are factors of stress in poultry. It was reported that the tenderness, water holding capacity, concentration corticosterone, and pH would be affected due to catching and transportation activities in poultry (Ehinger, 1977; Cashman, 1987; Duncan, 1989).

2.7 Effect of Glutathione-S-Transferases Expression on Oxidative Stress in Poultry

Glutathione-S-Transferases (GSTs) are enzymes from dimeric multifunctional enzymes family, which are presence in many living organisms such as animals, plants, bacteria and also human. GSTs are functioning in detoxification process and chemical activation. Furthermore, it is functioning in catalysing the activity of Glutathione (GSH) by catalysing the nucleophilic attack of GSH (Eaton & Bammler, 1999). The ability of GSTs enzymes to interact with xenobiotic substrates is used to identify them into their respective classes. There are few intracellular peptides in GSH such as tripeptide GSH

consisting glycine, cysteine and glutamic acid. Furthermore, GSH also known by its ability to conjugate several exogenous and endogenous compounds (Meister, 1988). The electrophilic site of hydrophobic toxic compounds interacts with group of thiols (-SH) from reduced glutathione by the activation of thiol ester together with GSH (Awasthi *et al*., 2009). Currently, there are 12 subunits of GSTs enzyme that already been identified using classification such as α, μ, π, θ, protein similarities, and cross reactivity of antibodies (Danielson & Mannervick, 1988; Meyer *et al*., 1991).

During pre-slaughter condition of chicken, chicken undergo oxidative stress which could affect the quality of meat. It was known that GSTs could counter the oxidative stress by acting as peroxidase. The GSH-dependent peroxidase reaction used toxic organic hyperoxides and also preventing oxidative damage (Moons, 2005). Moreover, the GSTs also functioning in deactivating certain secondary metabolites such as quinones, unsaturated aldehyde, epoxides, and hydroperoxides that formed result from oxidative stress (Hayes *et al*., 2005). Due to many functions and benefits gained from the GSTs, many studies were conducted to investigate this enzyme capabilities especially for its role in physiologically preventing or protecting against chronic diseases that were caused from damaging of oxidative tissue. Previous study showed that the cytosolic GSTs was involved in oxidative stress protection by activating the antioxidant response element in cGST gene promoter (Hayes *et al.,* 2005). The cGST is functioning in cellular macromolecules protection against oxidative stress. It was known that in mammalian cytosolic GSTs there are few classes that was derived into *alpha* (GSTα), mu (GSTμ), *pi* (GSTπ), *theta* (GSTθ), *sigma* (GSTσ), *zeta* (GSTζ) and *omega* (GSTω) (Saisawang *et al*., 2012). The usual GSTs molecular weight is around 26 kDA that are dimeric, non allosteric enzyme, which contain one active site per monomer (Salinas & Wong, 1999).

2.7.1 Glutathione-S-transferases in poultry

Glutathione-S-transferases are present in various organisms including animals, which play many functions. Among non-protein thiol in mammalian cell GSH is where it is assisting in cellular components like nucleophilic of oxygen and nitrogen in DNA by protecting them from electrophiles where the reaction is low in normal GSH concentration (Salinas *et al*., 1999). Meanwhile, in Avian, GSTs exist in complex isozyme system, where in previous study stated that classes μ and α are the abundant in leghorn chicks (Liu *et al*., 1991). It was said that there were at least 4 cytosolic isoenzymes could be observed in chicken liver (Yeung & Gidari, 1980). GST enzymes from kappa class play major role in defence against chemical and oxidative stress (Morel *et al*., 2004). The GSTs activity could be assayed using many substrates, but the more prominent substrate used is 1-chloro-2,4-dinitrobenzene (CDNB), where it is done by observing the thioether formation with GSH and CDNB (Habiq *et al.,* 1974). Previous research done has reported that the enzyme activity in poultry was much higher compared to the rat, especially in internal organs such as kidney and duodenum. Furthermore, it was also stated that male chicken contain higher GST activity compared with female. The activity of GST could be observed in most organs in chicken such as kidney, liver, duodenum, and testis. (Hayakawa *et al.,* 1972; Maurice *et al.,* 1991). Previous study has shown that purified GSTs enzyme from chicken liver have *p*I value 8.9 (Yeung & Gidari, 1980).

It was known that GSTs could counter the oxidative stress by acting as peroxidase. The GSH-dependent peroxidase reaction uses toxic organic hyperoxides and also preventing oxidative damage (Moons, 2005). Moreover, the GSTs also deactivating certain secondary metabolites such as quinones, unsaturated aldehyde, epoxides, and hydroperoxides that formed result from oxidative stress (Hayes *et al*., 2005). Due to many functions and benefit gain from the GSTs, there were many studies has been made

to investigate this enzyme capabilities, especially for it role, in physiologically preventing or protecting against chronic diseases that were caused from damaging of oxidative tissue. This is possible due to the presence of quinone metabolites inside the GSTs, which is suggesting the GSTs are beneficial for curing diseases. In previous study, GSTs was said to interact with others protein where protein-protein interaction occur. It was being proposed that c-Jun N-terminal Kinase (JNK) formed a complex with Pi-GST as subunit. Theoretically, during oxidative stress the JNK been activated due to the dissociation of class Pi GST and under non-stressed conditions, the JNK will bind with class Pi GST which will help in blocking the signal along the stress kinase pathway (Sherrat & Hayes, 2001).

2.7.2 Glutathione-S-transferases application

Glutathione-S-transferases present in most of organisms on earth and play many roles in metabolically function in the organisms. Scientists have used GSTs enzyme ability in various sectors such as biotechnological, agricultural, medicine and many other economic industries. In agricultural sector, GSTs has been modified to counter problems in plantation, where it was being used in pesticides. Furthermore, GSTs enzymes also capable to detoxify various toxicities produced by human that contribute in environmental pollution. Meanwhile, in medicinal approach, GSTs also capable in prevention of neurodegeneration, where GSTs function in preventing formation of aminochrome and dopachrome as well as Mu GST was responsible for the neuroprotective function (Sherrat & Hayes, 2001). Moreover, GSTs enzyme also functions in detoxifying products from oxidative damage such as acrolein and 4-hydroxylnonenal (Hayes & McLellan, 1999).

Besides, GSTs enzyme also functions in cancer diagnostic in medicinal field. Prada *et al.* (1997) stated that cancer related people would probably expressed high

level of *Pi* class GSTs activities that function in detoxifying the cancer cell. Previous studies also showed that there were anticancer drugs such as 1,3-*bis(*2-chloroethyl)-1-nitrosourea(BCNU), cyclophosphamide, chlorambucil, thiotepa, and melphalan could be detoxified by GSTs enzyme (Tew, 1994; Hayes & Pulford, 1995).

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction

The main objective of this research is to study the effect of local feed sources on village chicken growth performances as well as to establish a biomarker in proper slaughtering method that could be used to identify stress that was induced during slaughtering in village chickens. The research was conducted at 3 locations which were Livestock Science Centre, University of Malaya, Animal Biotechnology-Embryo Laboratory (ABEL), and Veterinary Public Health Laboratory, Bandar Baru Salak Tinggi, Sepang. The research was carried out within 2 years (2016-2017) with the objectives to investigate growth performance of village chickens, proximate analysis on village chicken's meat and proteomic studies on slaughtered village chicken's liver. The aims of the research were to investigate the potential of local ingredients such as palm kernel cake, coconut meal cake, and rice bran in formulation of poultry diet in replacing amount of feedstuffs inside commercial diets from market in term of growth performances, carcass evaluation and the quality of the meat of village chicken using 2 strains. Thus, formulation of the diet was created in the research by mixing commercial ingredients (corn and soybean meal) and local ingredients (palm kernel cake, coconut meal cake, and rice bran). Furthermore, the economic importance of the feedstuffs inside the formulation also studied. At same time, we studied the effect of oxidative stress caused from 2 type of slaughtering methods toward the expression of Glutathione-s-Transferases (GSTs) expression by observing the enzyme activities in village chicken's liver, as well as establishing preliminary biomarker for stressed chicken that could lead to economic importance.

3.2 Experimental Animals

There were 2 strains of village chickens used in this study which were purebred village chicken and crossbred village chicken. The animals were reared and fed in Livestock Science Centre, University of Malaya.

3.2.1 Village chicken

The animals used in this research were from 2 strains purebred village chicken and crossbred village chicken. The chickens were purchased in Kuala Klawang and Hulu Langat from a village chicken farmers. The chicks were ensured to be between around age of 1-day old. Each pen consisted sawdust, aluminium foil and 100-watt bulb to provide heat for the chicks with 50 chicks per pen. Every chick was vaccinated using ND-IB vaccine at 1-day old, 14-days old and subsequent month. The total of animals used in the study were 400 of village chickens.

The research was approved by University of Malaya Institutional of Animal Care and Use Committee (UMIACUC) for experimentation on animals with following animal clearance number I/02022017/03112016-01/R.

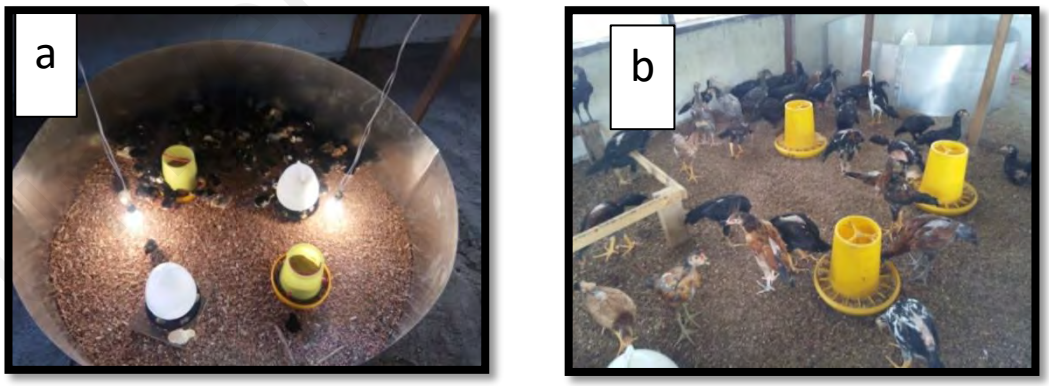

Figure 3.1: Siamese Purebred village chicken were placed in a aluminium foil with sawdust and 100-watt bulb to provide heat insulation; a) 1-day old chicks, b) 3-months old chickens

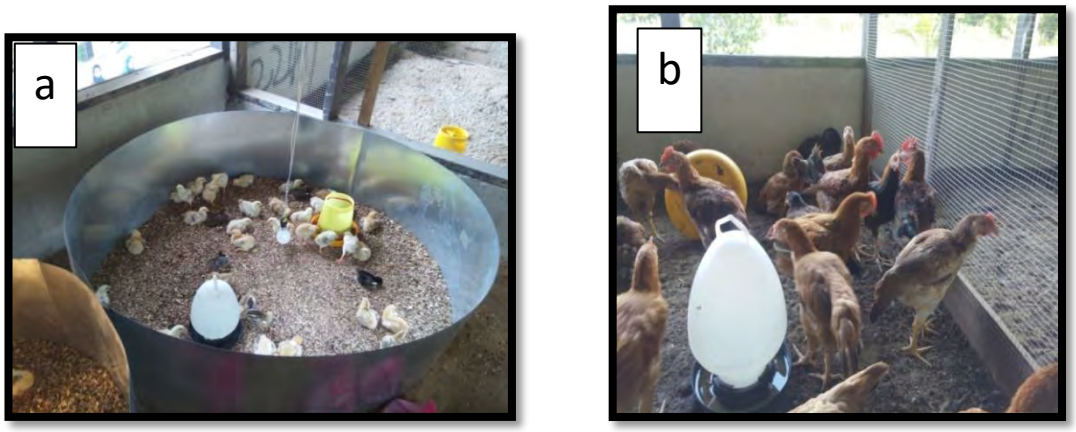

Figure 3.2: Crossbred village chicken were placed in a aluminium foil with sawdust and 100-watt bulb to provide heat insulation; a) 1-day old chicks, b) 3-months old chickens

3.2.2 Animal feeding

The chicks reared in a aluminium foil fed with same commercial starter diet for both strains and treatments with approximately 21% crude protein (CP) and 3000 MJ ME/kg from 1-day old until 21-days old of age. After 21-days they were divided into 2 groups of diets which were Commercial Finisher Diet (CFD) and Formulated Finisher Diet (FFD). The feed were given every morning at 8.00 a.m. where the feed were given in same quantity between both treatments. The number of feed tray used were determined by the number of chickens inside the pen to avoid competition. Water container were cleaned in daily basis and was make sure that there were water 24-hours to avoid dehydration. At 22-days old, the chicks were given 2 types of diet and were weighed every week. The feed intake was also measured weekly by collecting the feed left-over in the feed tray.

3.3 Materials

In this study, the materials used were varies based on experimentation such as nutritional and proteomic studies consisting equipments, chemicals and reagents as well as labwares and disposables.

3.3.1 Proximate study materials

Nutritional study involved the usage of equipments, chemicals and disposables for weight determination and proximate analysis on the village chicken's meat. The equipments were weight balancer, rotatory drum picker, and oven. The materials used for feed formulation were rice bran, soybean meal, corn, palm kernel cake (PKC), coconut meal cake, monodicalcium phosphate (MDCP), vitamin premix and salt. The equipments and materials used for proximate analysis to determine crude protein, gross energy, crude fat, crude fibre, amino acid content and ash content were shown in **Appendix.**

3.3.2 Protein differential analysis

The study on protein differential analysis consisting the usage of specific equipments and chemicals for protein purification, 1-Dimensional electrophoresis, 2-Dimensional electrophoresis and enzyme assay.

3.3.2.1 Protein purification

Protein purification of Glutathione-s-Transferases (GSTs) was done by using Fast Protein Liquid Chromatography (FPLC). The chemicals used in the purification process were used for production of homogenizing buffer, which was used to extract proteins inside the samples before purification process. The ingredients of homogenizing buffer were elution buffer, protease inhibitor, ethylenediaminetetracetic (EDTA), dithiothreitol and propylene thiourea. The usage of centrifuge machine at 13,000 rpm also required to extract the protein out from the sample. The GSTs enzyme were trapped using 1 ml GST column for FPLC and washed using Glutathione.

Figure 3.3: Fast Protein Liquid Chromatography (FPLC) was used to purified GSTs enzyme through 1 ml GST column

3.3.2.2 1-Dimensional electrophoresis

1-Dimensional electrophoresis involved the usage of Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) kit which using the Bio-Rad mini-PROTEAN Tetra system and mixing block. The preparation of sample buffer was done by using the ingredients such as distilled water, Tris-HCl, glycerol, sodium dodecyl sulfate and bromophenol blue.

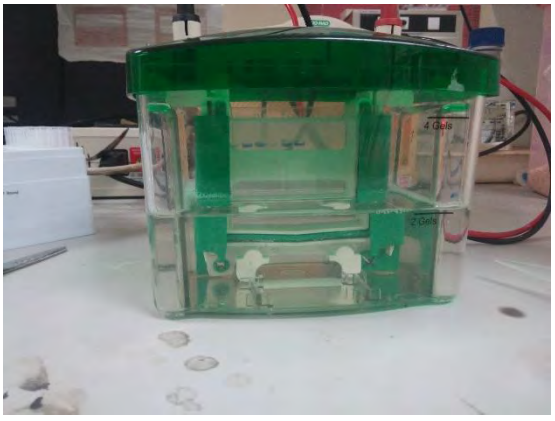

Figure 3.4: SDS-PAGE electrophoresis (1-Dimensional electrophoresis)

3.3.2.3 2-Dimensional electrophoresis

2-Dimensional electrophoresis consisting 2 parts of process which were isoelectric focusing and 2-Dimensional SDS-PAGE electrophoresis. The equipments used during isoelectric focusing were multiphor 2 and Bio-Rad mini-PROTEAN Tetra system for SDS-PAGE. During isoelectric focusing process the preparation of rehydration solution was done by using chemicals such as urea, CHAPS, dithiothreitol (DTT), ampholytes and thiourea. Furthermore, 2-Dimensional electrophoresis also required for the preparation for equilibration solution that used chemicals and reagents such as Tris-HCl, urea, glycerol, sodium dodecyl sulfate (SDS), dithiothreitol (DTT), and iodoacetamide (IA). Equilibration solution used milliQ water to minimize the contamination of unwanted material or microorganisms presence in the distilled water and tools that been used.

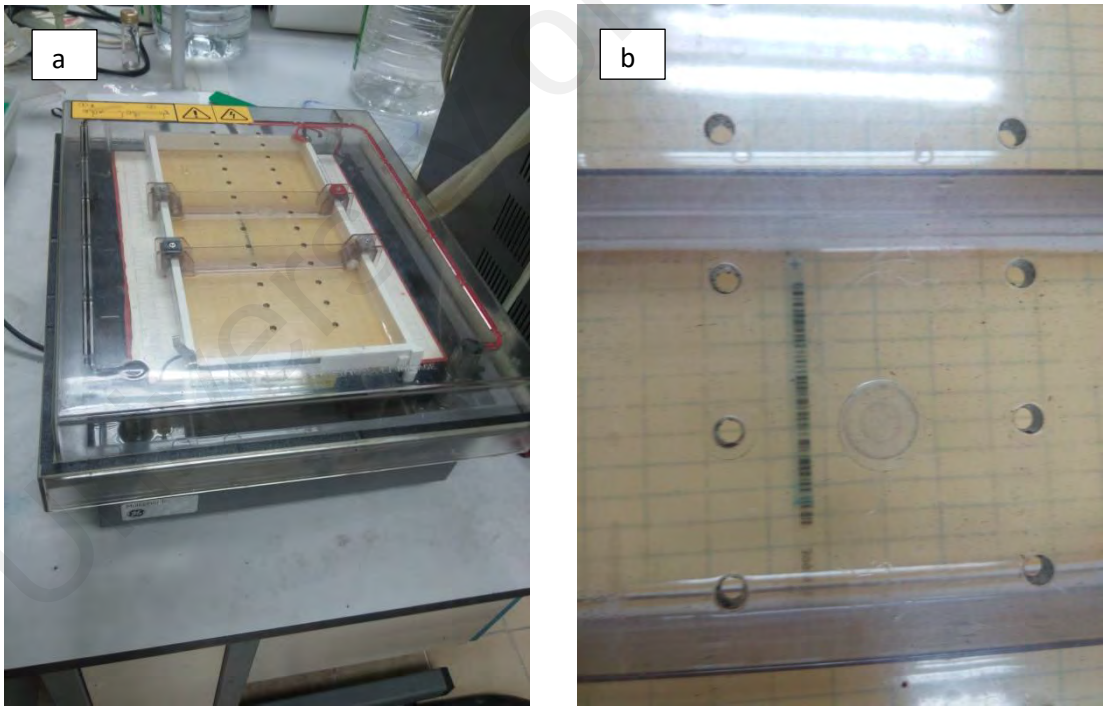

Figure 3.5: Isoelectric focusing (IEF) machine, Multiphor 2 was used in the study to separate protein based on alkalinity and acidity

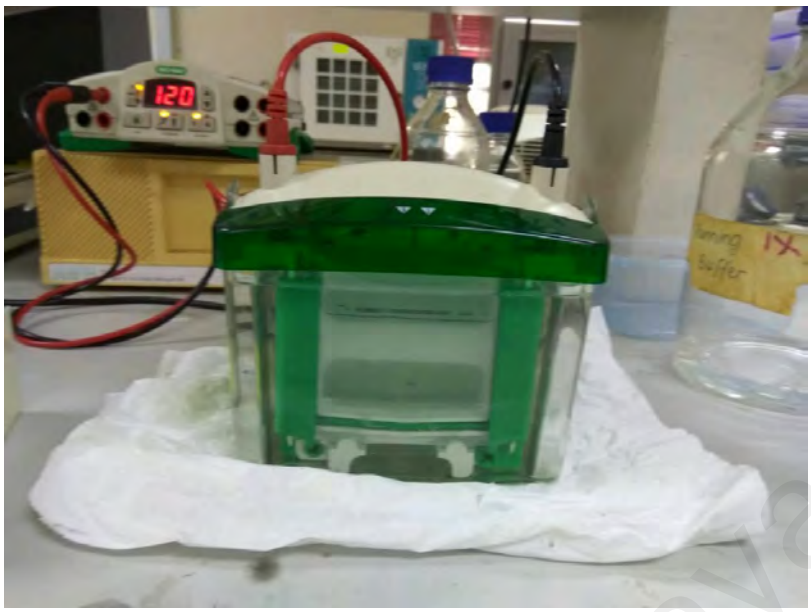

Figure 3.6: SDS-PAGE electrophoresis for 2-Dimensional electrophoresis

3.3.2.4 Enzyme assay

Enzyme assay was conducted by using spectrophotometer to detect the activity of GSTs enzyme presence in the samples as well as pH meter and hot plate for pH adjustment of buffers used in the study. Furthermore, there were also certain chemicals and reagents used for the enzyme assay such as 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, 20 ml of ethanol, 2.85 ml buffer A (500 ml NaOH + 6 g Sodium dihydrogen phosphate) and 50 µl GSH-reduced.

3.4 Methodology

This part will be discussing on the several parts that contributing to the studies; a) standard biosafety and maintenance of research, b) preparation of formulated finisher diet, c) preparation of buffers and solutions for proteomic, d) experimental protocols.

3.4.1 Standard biosafety and maintenance of research

The experiment on animals and laboratory work that involved the use of any hazardous chemicals were monitored specifically to prevent any unrelated stress to the animals, safety to the researchers as well as to prevent equipments broken down.

3.4.1.1 Stress prevention to the experimenting animals

The study was involved the used of village chicken which were purebred and crossbred village chicken. Any unrelated stress that might came from sources such as feed, surrounding situation, and disease infection were prevented thoroughly. The feed given to the chickens were confirmed to be mixed well and free from any spoilage. Furthermore, the feed also given enough to prevent any competition that might caused injury to the animals. The feed tray used also provided enough for all chicken to fed at the same time.

The chicken house provided were sealed with black mesh to minimize human contact from outside and as heat protective materials. The sawdusts were changed every 2 weeks to prevent uncomfortable condition to the animals as well as to prevent any infection of diseases. All of the chickens were vaccinated with ND-IB vaccine as young as 1-day old, 14 days and subsequent month.

3.4.1.2 Researcher safety

Researcher involved in this study were make sure to be free from any contaminants or diseases before entering the animal house to prevent any disease infection to the chicken. Special boot and attire were applied in the premise. During laboratory experimentation, the usage of laboratory coat, glove and face mask must be practised to prevent sample deterioration and contamination especially when handling proteins. Furthermore, when handling any hazardous chemicals and reagents wearing glove and

laboratory coat were compulsory as well as handling specifically chemicals and reagents inside fume cupboard. All researchers also aware of all chemicals specific storage whether inside cold room, hazardous cabinet and chemicals storage room.

3.4.1.3 Equipment, glassware and laboratory maintenance

All equipment used in this study were recorded and maintain according to the guidelines. Equipment such as Fluorescent Performance Liquid Chromatography (FPLC) were cleaned every time an experiment ending with distilled water. The SDS-PAE kit and multiphor 2 also cleaned and washed every time used without leaving anu trace of samples and mineral oils that been used.

All of the glasswares were rinsed before and after used to prevent any contamination from occur. Then, the glasswares were washed using detergent and dried inside oven with 40°C temperature. The workstation at the animal house was cleaned and washed after slaughtering and monthly basis to prevent any infection from diseases that came from the slaughtering blood. Meanwhile, the workstation inside the laboratory was cleaned every time used before and after with 70% of ethanol.

3.4.2 Preparation of experimental materials

Experiment materials for the study were divided into 2 groups which were for the nutritional and proteomic study. The preparation of materials for the nutrtional study involved the preparation of the feed, meat samples and solution used for proximate analysis. Meanwhile, for the proteomic study involved the preparation of specific solution and buffer.

3.4.2.1 Preparation of formulated finisher diet

The formulated finisher diet prepared in the study was based on local ingredient (mixing with imported ingredients (corn and soybean meal) as well as supplementation ingredient such as MDCP, vitamin premix and salt. Tables 3.1 and 3.2 showed the ingredients of formulation finisher diet (FFD) and comparison of nutrient composition between formulation finisher diet and commercial finisher diet as well as the differences in expected market value for both types of feed.

Table 3.1: Formulated Finisher Diet (FFD) ingredients

Ingredients	Amount (Kg)	Dry matter (DM, %)
Corn	29.8	87.0
Rice Bran	11.9	91.0
Palm Kernel Cake	7.4	90.6
Coconut Meat	14.1	43.0
Soybean Meal	26.0	91.0
Vitamin Premix	4.5	-
Mono-Dicalcium Phosphate (MDCP)	4.5	-
Salt	0.9	-
Limestone	0.9	-
TOTAL	100	-

Table 3.2: Comparison of nutrients composition and approximate local price between commercial diet and formulated diet

Nutrients composition	Commercial Finisher Diet (CFD)	Formulated Finisher Diet (FFD)
Dry matter (%, DM)	89.05	81.16
Crude protein (%, DM)	13.40	14.95
Crude fat (%, DM)	3.02	3.78
Crude fibre (%, DM)	3.12	12.22
Ash (%, DM)	4.43	5.18
Gross energy (MJ/kg DM)	16.30	17.13
Metabolizable energy (MJ/kg DM)	9.36	9.84
Approximate local price (USD)/kg	**2.40**	**1.60**

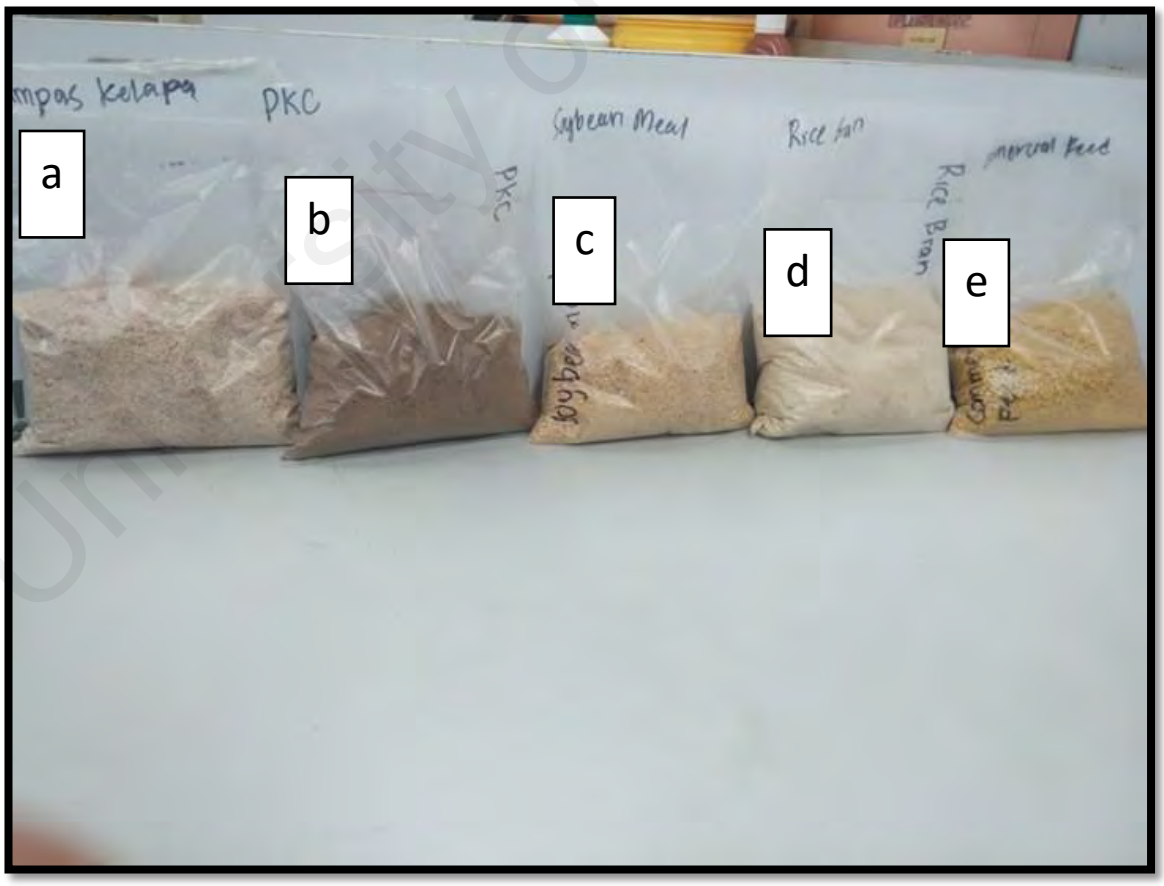

Figure 3.8: Formulated Finisher Diet (FFD) ingredients: a) Coconut Meal Cake b) Palm Kernel Cake c) Soybean Meal d) Rice Bran e) Commercial Feed

3.4.2.2 Preparation of meat samples

Meat samples were used for carcass evaluation that consisting carcass weight, meat to bone percentage, analysis of white and dark meat and proximate analysis. At the age of 8 weeks, 10 randomly chosen chickens with same number of gender were slaughtered. Carcass evaluation was done by evaluating the weight of gizzard, liver, heart, abdominal fat, white and dark meat, and meat to bone ratio. The meat to bone ratio and moisture measurement were done by separating meat from bone for both white and dark meat (Qiao *et al.,* 2001). The meat of the slaughtered chickens was dried inside oven at 98°C for 48 hours. The dried meats were ground into powder form for chemical analyses.

The chemical analysis for the village chicken meat was conducted at Veterinary Public Health Laboratory, Bandar Baru Salak Tinggi, Sepang for 6 months. The analyses included the data for crude protein (CP), crude fat (CF), crude fibre (CFB), amino acid, ash and dry matter. The protocol for this experiment followed the standard operating procedure (SOP) for Department of Veterinary Services Malaysia (FAO, 2011).

3.4.2.3 Purification of Glutathione-S-Transferases in chicken liver

Purification GSTs enzyme was done using Fast Protein Liquid Chromatography (FPLC) with 1 ml GSTs trap column. The protocol of GSTs purification in chicken liver is as following:

a) 0.5 g of village chicken liver sample was grinded or mashed in 5 ml of homogenizing buffer.
b) Homogenized sample was centrifuged for 20 minutes and the supernatant was collected and filtered. (Homogenizing buffer preparation **Appendix)**
c) 5 ml of sample was injected into FPLC with GSTs trap column.

d) Then the column was washed with GSH buffer until showing peak through the FPLC.

e) Collected purified GSTs enzyme was test using SDS-PAGE to confirm their expression.

3.4.2.4 SDS- PAGE protocols

a) Glass plate of SDS kit was clean using acetone.

b) Any leaking was checked after gel casting was set up.

c) APS was prepared (Ammonium Persulfate (APS), 100 mg in 1 mL dH_2O).

d) Gel was let to solidify in 30 minutes and sample was prepared. (Preparation of SDS-PAGE gel **Appendix**)

e) 20 µl of sample buffer was added into cryovial containing 5 µl of sample. (Preparation of sample buffer **Appendix**).

f) The sample was heat up at 95 °C for 4 minutes.

g) Running buffer was poured into the gel casting and tank until full.

h) Protein ladder marker (10 – 200 kD) was loaded. Then, 20 µl of sample was loaded into the well.

i) Electrophoresis was ran at 120 V/150 V/180 V.

j) After that the gel stained using coomasive brilliant blue stain fortnight.

3.4.2.5 2-Dimensional gel electrophoresis protocols

a) 20 µl of sample was mixed with 105 µl of rehydration buffer in cryovial tube. (Preparation of rehydration buffer **Appendix**).

b) The sample was let to rehydrate for 16 to 18 hours on immobilise strip aligner. Isoelectric focusing (IEF).

c) Mineral oil was poured on top of cooling plate and was ensure to cover all part.

d) IEF tray was placed on the cooling plate.

e) The rehydrated immobiline strip was placed side up, where the gel facing up.

f) The electrode was positioned on every strips.

g) Mineral oil was poured into the tray to cover the strips.

h) Milipore was ran as below programme:

   a. Set IS 1 (V = 200V, 5 mA) – (0.01h, 2 W)

   b. Set IS 2 (V = 3500 V, 5 mA) – (1.30 h, 2 W)

i) Then, the strips were removed and the clean up the excess oil.

j) The strips were washed with equilibration solution. Equilibration solution was prepared as shown in the materials part above. (Preparation of equilibration solution **Appendix**)

k) Equilibrated strips were loaded into SDS-PAGE gel and ran until finish.

3.4.3 Proximate analysis protocols

Using the material explained in section 3.3.1 the proximate analyses were done to determine the composition of nutrients inside the feed given to the village chicken. The compositions that been studied were crude protein, crude fat, crude fibre, ash and amino acid content.

3.4.3.1 Crude protein determination protocols

a) 1 gram of powdered dried village chicken meat was weighed into the digestion tube.

b) 2 Kjeldahl tablets were added with 20 ml of sulphuric acid.

c) The tubes were placed inside the digestion unit and the fume removal manifold was connected.

d) The samples were digested at least 1 hour at 420°C.

e) The digestion unit was switched off and the sample was allowed to be cooled for 10-20 minutes.

f) Distilled water was added to each tube to a total volume approximately 80 ml.

g) Conical flask containing 25-30 ml of concentrated boric acid was placed under outlet of the condenser of the distillation unit.

h) Then, 50 ml of NaOH was added and the ammonium was distilled.

i) The content was titrated with hydrochloric acid after adding a few droplets of indicator solution using a titration unit. The amount of titrant used was recorded as the endpoint at the first trace of pink colour appearance.

j) The amount of acid used was recorded.

- ***Calculation***

Nitrogen (%) = Amount acid used for samples – Amount acid used for blank × M(HCl) × 1 × 14.007 ÷ (W × 10)

CP (%) = Nitrogen (%) × 6.25

3.4.3.2 Gross energy determination protocols

1) ***Determination of hyrothermal equivalent (water value) of the bomb calorimeter***

a) Benzoic acid combustion tablet was used to determine the hyrothermal equivalent. The temperature rise was calculated. 4 determinations were made and the mean value was calculated.

b) The benzoic acid was dried at 105 ± 2°C overnight and was cooled in a dessicator. 1 g of dried benzoic acid crystal was reweighed. The temperature rise was determined from the combustion of benzoic acid in the bomb calorimeter.

2) Samples measurement

a) Approximately 1 g of sample was weighed and placed in the combustion cup.

b) 10 cm of platinum wire was attached between the electrode of the bomb and the combustion crucible was set with the sample placed in the loop electrode.

c) 6.5 cm of cotton thread was tied at the middle of the wire. The thread was adjusted to ensure it touched the sample.

d) The bomb was assembled, the screw cap tightened, pressure release valve closed and oxygen was filled to 25-30 atmosphere.

e) 2000 g of distilled water was weighed in the calorimeter bucket and placed in the calorimeter. The bomb was set in the bucket and the clip terminal was attached.

f) Then, the cover was closed the thermometer was lowered and the water circulating motor was start. After that, the cap from the jacket cover was removed and was filled with water, and allowed 1 minute attaining equilibrium.

g) Initial temperature was read and recorded and the sample was ignited. Maximum temperature was used for record.

h) The calorimeter was open and the bomb from the bucket was took and residual pressure of the bomb was released and open.

3) Determination of acidity

a) As burning was completed, the bomb was removed, pressure was released, and open. All inner bomb surfaces were rinsed with stream of distilled water, all washings were collected in a clean beaker and washed until 100 ml.

b) The carbon dioxide was removed by filtering and boiling.

c) The hot filtrate was titrated to phenolphthalein end point with 0.1 N barium hydroxide.

d) 20 ml of 0.1 N sodium carbonate was added, the precipitate was filtered and washed with distilled water.

e) 0.1 N HCl was cooled and titrated using methyl orange as indicator.

- ***Calculation***

Calculation of hydrothermal equivalent of the bomb is

$$He = \frac{W \times A - (L \times C) - 14}{Tf - Ti}$$

Where,

He = hydrothermal equivalent (J/°C)

W = weight of benzoic acid sample (g)

A = joules per gram benzoic acid, i.e. 26442 J/g

L = weigh of cotton thread (g),

C = joules per g cotton, i.e. 17500 J/g.

14 = correction for acid formation (J),

Tf = final temperature, and

Ti = initial temperature.

Gross energy (GE) calculation as below:

$$GE\ \left(\frac{kJ}{g}\right) = \frac{(Tf - Ti) \times He}{W}$$

Where,

Tf = final temperature (°C)

Ti = Initial temperature (°C)

W = weight of sample (g), and

He = hydrothermal equivalent (J/°C)

Result was expressed in kJ/g.

3.4.3.3 Crude fat determination protocols

1) Hydrolysis type fat determination procedure:

a) 5 g of sample was weighed into a beaker to nearest 0.1 mg.

b) 100 ml of hydrochloric acid and silicon carbide was added into the sample and then covered by watch glass.

c) The mixture was let to boil slightly using a heating apparatus and then maintain for 1 hour. The mixture was swirled every 10 minutes to avoid any output from sticking on the wall of the container.

d) The mixture then was filtered using fat free double filter paper in a Buchner funnel with suction.

e) Then, the residue was washed with cold distilled water until a neutral filtrate was obtained.

f) The filter paper with the residue was transferred carefully into an extraction thimble and then dried in a vacuum oven for 60 minutes at 80°C.

g) The thimble was removed from the oven and then been covered with a fat-free cotton wool.

2) Extraction procedure

a) 5 g of sample was weighed into the extraction thimble and was covered with a fat-free wad of cotton wool.

b) Silicon carbide chips was transferred to a dry flask and was weighed to nearest 0.1 mg and was added with 95 ml of petroleum ether.

c) The thimber was placed in the extractor and was connected to a dry flask and reflux unit.

d) Then, the petroleum ether was extracted for 6 hours and the heating apparatus was regulated to obtain at least 10 siphonings per hour.

e) The solvent was distilled until the flask nearly free from the solvent and then was leave for overnight in fume hood for evaporation.

f) The flask residue was dried for 1.5 hour in a vacuum oven at 80°C.

g) The residue was cooled in a desiccator and weighed to nearest 0.1 mg.

- ***Calculation***

Percent of crude fat:

$$\%\ \text{crude fat} = \frac{(\text{w3} - \text{w2}) \times 100}{\text{w1}}$$

Where,

W1 = initial sample weight in grams

W2 = tare weight of flask in grams, and

W3 = weight of flask and fat residue in grams.

3.4.3.4 Crude fibre determination protocols

1) Pretreatment

a) 1 g of village chicken meat sample was weighed into each P100-crucible.

b) The crucible was placed in the filtration equipment and 30 ml of petroleum ether was approximately added to each crucible and was filtered using vacuum.

c) The washing steps was repeated for two times.

d) The residue was dried in air and was transferred quantitatively to a beaker.

2) Digestion

a) 150 ml of sulphuric acid was added to a beaker and was boiled for 30 minutes.

b) Then, the mixture was filtered through crucible using vacuum.

c) The residue was washed 5 times, where each washing using 10 ml of hot distilled water.

d) A volume of acetone was added to cover the residue. Then, the acetone was removed after a few minutes by applying slight suction.

e) The residue was transferred quantitatively to a beaker.

f) 150 ml of sodium hydroxide was added to each beaker and was boiled for 30 minutes.

g) The mixture was filtered through crucible using vacuum.

h) The residue was washed 3 times under vacuum, where each time using 30 ml of acetone. Then, the residue was dried using suction after each washing.

3) Drying and incineration

a) The crucibles were placed into an oven and was adjusted to 103°C and dried for 4 hours. The drying time starts when the oven has reached 103°C.

b) Then, crucibles were placed in a desiccator and allow to cool.

c) The crucible was weighed directly after removing from desiccator.

d) Then crucibles were placed in a muffle furnace and the samples were incinerated for 2 hours at 550°C. The incineration time starts when the furnace has reached 550°C.

e) The crucibles were placed in desiccator and allow cooling.

f) Directly the crucibles were weighed after removing from the desiccator.

- ***Calculation***

Percent of Crude Fibre (%CF):

$$\%\text{CF} = \frac{(W2 - W3) \times 100}{W1}$$

Where,

W1 = weight of the sample (g),

W2 = weight crucible and residue after drying (g), and

W3 = weight crucible and residue after incineration (g).

3.4.3.5 Amino acid determination protocols

1) ***Sample preparation***

a) 0.1 g of sample was weighted into stopperred tube

b) 5 ml of 6N HCl hydrolysate was added and then sonicated for 5 minutes

c) The samples then were heated at 110 °C for 24 hours and then cold at room temperature

d) 400 µl of 50 µmole/ml of AABA was added and mark up until 100 ml with distilled water. Then was mixed properly.

e) The mixed solution then was filtered through whatman 541 filter paper

f) The aliquot was filtered again using syringe filter

g) 10 µl of derivatisation (=2 nmole AABA =20.62 ng AABA) was pippeted into 1.5 ml Eppendorf tube.

h) AccQ Fluor Reagent was prepared (1 ml of acetonitrile was added into bottle 2A and mixed properly and then heated at 55°C for less than 10 minutes).

i) 20 µl of prepared AccQ Fluor Reagent was added.

j) 70 µl of borate buffer was added and the vortex for 2 minutes.

k) Wait for the solution to derivatise for 1 minute.

l) 150 µl low insert was transferred and inserted into 1 ml HPLC vial cap.

m) The sample was ready to inject into HPLC (10 µl).

3.4.3.6 Ash determination protocols

Ash determination was done by exposing sample with high temperature and also known as ashing between 500 and 600ºC. Generally, any water and organic substances within the samples were burned and vaporized in the presence of the oxygen in air to CO^2, H_2O and N^2.

The ash was determined using below formulation where it could be expressed either wet or dry basis:

$$\%\ Ash\ (Dry\ basis) = \frac{M_{\text{ash}}}{M_{dry}} \times 100$$

$$\%\ Ash\ (Wet\ basis) = \frac{M_{ash}}{M_{wet}} \times 100$$

3.5 Experimental Design

There were 2 experiments conducted in this study which generally involved the nutritional study for Experiment 1 and proteomic study for Experiment 2. Both experiments were designed with different objectives such as Experiment 1 was designed to evaluate the effect of local feed sources (PKC and coconut meal cake (milk extracted meat)) on the growth performance of village chicken, while Experiment 2 was to study the effect of slaughtering method on the expression of glutathione-s-transferases.

3.5.1 The effect of local feed sources on the growth and carcass performance of village chicken (Experiment 1)

The objective of study was to investigate the potential of using local feed sources as poultry feed to village chicken and its possibility to replace the commercial ingredients

such as corn and soybean meal by decreasing the usage of it. In addition, this experiment was also conducted to study the growth and carcass performances using the two types of diet in two strains of village chicken, which were purebred village chicken and crossbred village chicken. This experiment involved the collection of data of body weight, feed intake, carcass evaluation, feed conversion ratio (FCR), and meat to bone ratio. The formulation of formulated finisher diet (FFD) was explained in previous section and shown in the Tables 3.1. and 3.2.

The body weight of the village chickens was measured using electronic balance weekly. Feed and water were removed overnight before body weight measurement. The total feed intake was calculated based on daily feed leftover (grams) subtracting with total feed given. Feed intake data was obtained on a pen basis to estimate feed conversion ratio of the two dietary treatments for both strains. Feed intake was determined weekly by measuring the difference of the total amount of feed given from feed residue. Total feed intake via weekly basis was measured based calculation below:

$$Daily\ Feed\ Intake = Daily\ Feed\ Given\ (grams) - Daily\ Feed\ Leftover$$

All the crossbred birds in each dietary treatment were slaughtered after 8 and 12 Weeks of age and all the purebred birds in each dietary treatment be slaughtered after 12 and 16 Week of ages. The birds were starved overnight prior to slaughtering. For carcass yield evaluation, 10 birds per treatment (5 males and 5 females) per replicate were randomly selected. The slaughtered birds were dressed by semi-scald method and the feathers were removed manually in rotary drum picker. The head, crop, lower legs, viscera were removed. The weights of warm eviscerated carcasses were taken. The weight of liver, gizzard, heart and abdominal fat as well as dressed edible yield of breast, thigh and drumstick as percentages of live weights were taken individually. The percent of meat quantity to bone and meat quantity were determined.

Feed conversion ratio is calculation to measure the efficacy of feed given in converting into beneficiary output such as meat and bone. The calculation of FCR was as below:

$$Feed\ conversion\ ratio\ (FCR) = \frac{Weekly\ Feed\ Intake\ (grams)}{Weekly\ Weight\ Gained\ (Grams)}$$

Meat to bone ratio is calculation of edible meat possessed to bone via white and dark meat. Meat to bone ratio was calculated in percentage.

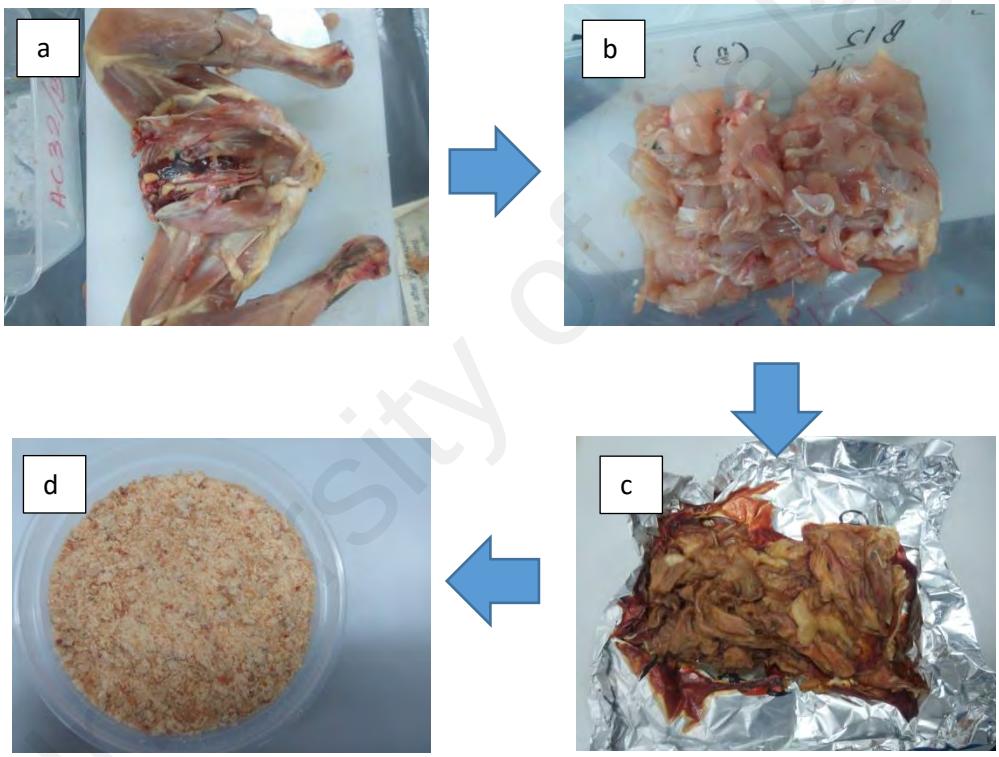

Figure 3.8: Grind process of chicken meat; a) Lower leg carcass, b) chicken meat without bone, c) dried chicken meat, d) dried powderchicken meat (proximate purpose)

Figure 3.9: Dark meat and white meat of chicken

3.5.2 The effect of different slaughtering method on the expression of Glutathione-S-Transferases (Experiment 2)

At 2 months of age the 10 chickens were slaughtered using 2 methods of slaughtering which were Non-Stress Slaughtering Method (NSSM) and Stress Slaughtering Method (SSM). Meanwhile, NSSM was done by slaughtering the chicken by making sure respiratory tract, oesophagus, carotid arteries and jugular vein were cut. SSM was done by cutting only respiratory tract. The chickens were let die due respiratory complication. After the chicken dead, liver and meat samples were collected for proteomics analysis. Proteomic analysis comparison was carried out by comparing between NSSM and SSM village chickens.

An amount of 0.5 g of liver samples was manually homogenized using homogenizing buffer up to 5 ml. Then, the samples were filtered by using syringe filter. A volume of 2-3 ml of samples were injected into FPLC for purification of Glutathione-S-Transferases (GSTs). The purified samples were directly analysed by using SDS-PAGE to determine and confirmed the specific molecular weight of GSTs present. The concentration of protein was determined using spectrophotometer at wavelength 595 nm

using Bradford assay. Low concentration of GSTs enzyme was concentrated using protein concentrator until reach 100 µl of the total samples. A volume of 20 µl of each sample were rehydrated with 105 µl of rehydration buffer on IEF gel strip for 16-18 hours. Rehydrated gel strip undergoes isoelectric focusing for 3 hours using multiphor 2 and then was equilibrated by using equilibration 1 (EB 1) and equilibration 2 (EB 2) solutions for 20 minutes each. The equilibrated gel strip was loaded into SDS-PAGE and ran for 2 hours. The gel was stained using coomasive brilliant blue staining for 4 days. Expression of protein GST was observed by looking at the present of spot of the gel.

Purified samples of GSTs enzyme were immediately tested for their enzyme activity by using spectrophotometer at wavelength 280 nm (Mannervik, 1985). Fresh 1-chloro-2,4-dinitrobenzene (CDNB) was used as substrate. The preparation of CDNB was done by diluting 0.2430 g of CDNB into 20 ml of ethanol. The enzyme activity was evaluated by mixing 4 substrates, which were buffer A (2.85 ml), sample of GST (50 µl), GSH-reduced (50 µl) and CDNB (50 µl) by triplicate. (GSH-reduced = 0.0553 g in 3 ml of buffer A; buffer A = 6 g sodium dihydrogen phosphate in 500 ml with pH 4.25-6.50 by NaOH). Following calculation was used to determine the enzyme activity:

$$A = \varepsilon c L$$

A = absorbance, ε = coefficient, c = protein concentration, L = cuvette length

$$Specific\ Total\ Activity = \frac{Total\ activity}{Total\ amount\ protein}$$

3.6 Flow Chart of Methodology

The flow chart of methodology for Experiment is given in Figure 3.10.

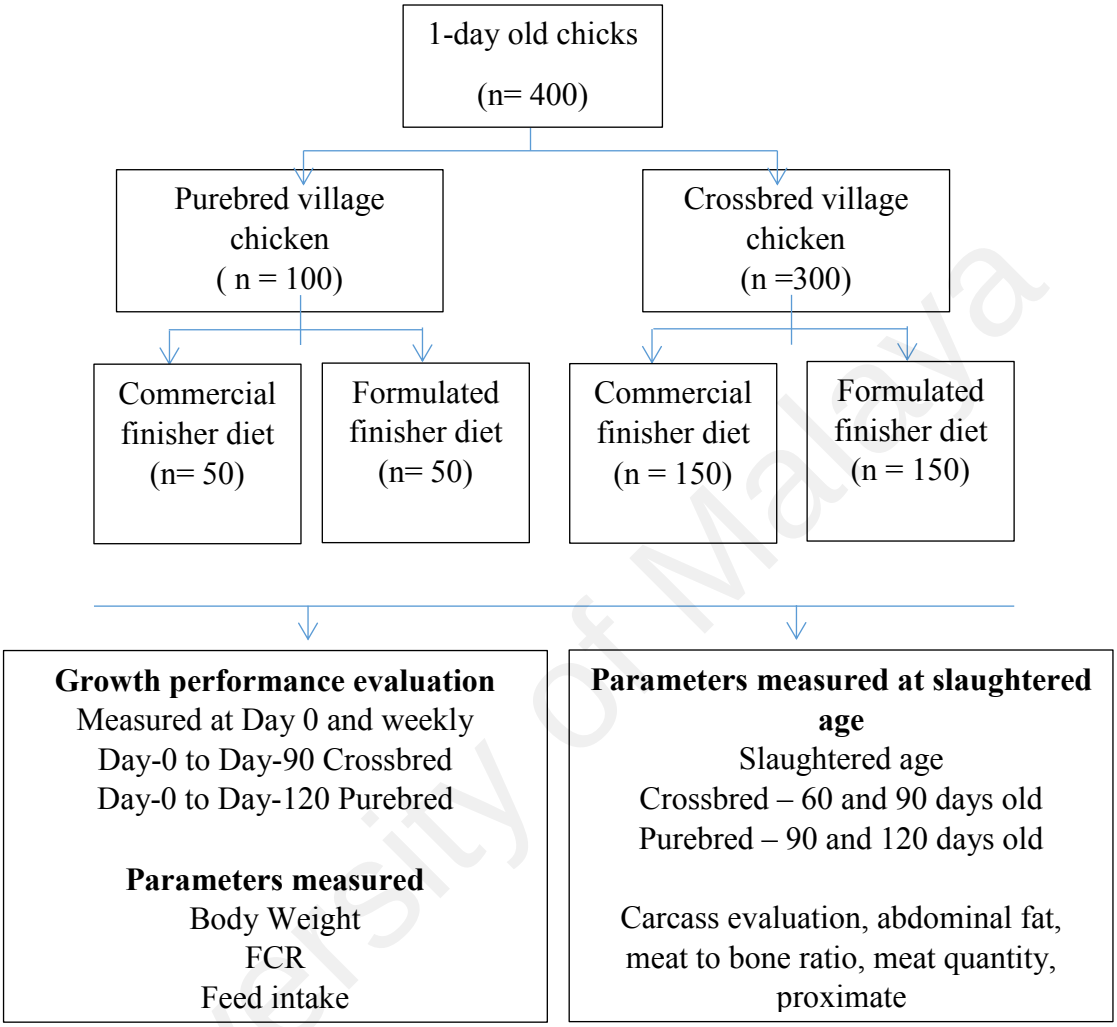

Figure 3.10: The effect of local feed sources on the growth and carcass performance of village chicken (Experiment 1)

The flow chart of methodology for Experiment 2 is shown in Figure (3.11)

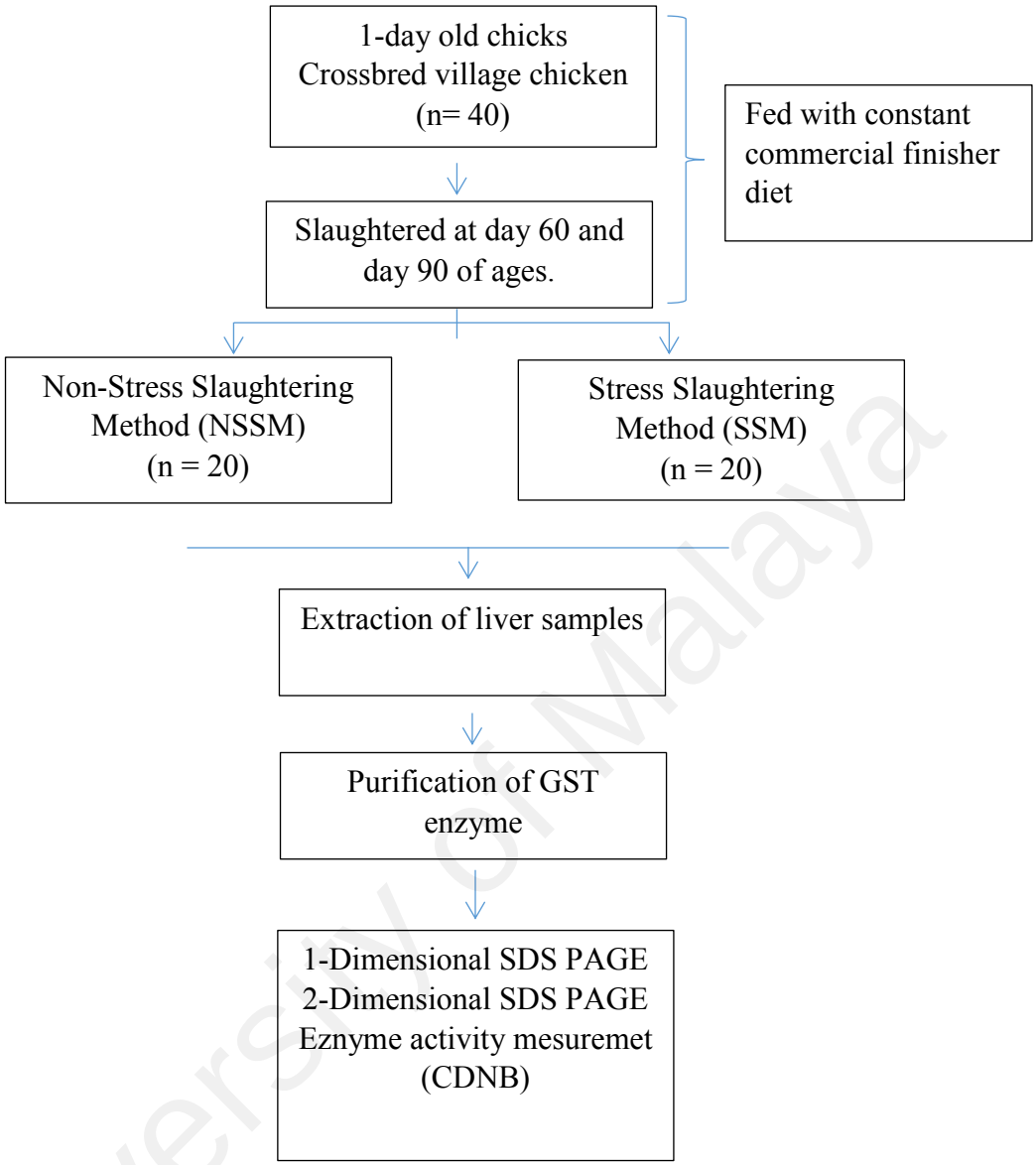

Figure 3.11: The effect of different slaughtering method on the expression of Glutathione-s-transfererases (Experiment 2)

3.7 Statistical Analysis

The data were evaluated using SPSS software either ANOVA with Duncan Multiple Range Test (DMRT) and T-test.

Nutritional experimental

A 2x2x2 factorials experiments were conducted for the treatments as follows: 2 breeds vs. 2 diets and 2 ages. Parameters measured were body weight, carcass weight, offals weight, abdominal fat, meat quantity, feed intake, and FCR.

Molecular Experimental

Factor: slaughtering method, Parameters measured: specific enzyme activity.

CHAPTER 4: RESULTS

4.1 The Effect of Local Feed Sources on The Growth and Carcass Performance of Village Chicken (Experiment 1).

Generally, the growth performance of purebred village chicken was slower compared with crossbred village chicken. There were no significant differences in body weight showed for purebred given commercial finisher diet (CFD) and formulated finisher diet (FFD). However, at 3 and 12 Week of age of purebred village chicken, there were significant differences (Figure 5) as shown in Table 4.1. FFD fed chicken for purebred strain showed low body weight compared with CFD fed purebred chicken. Moreover, the gap differences between CFD and FFD fed chicken in purebred village chicken was large compared with crossbred village chicken.

Meanwhile, the bodyweight for crossbred village chicken for chicken fed with CFD and FFD showed no significant difference, generally, except for Week 7 of age as shown in Table 4.2. The gap difference of body weight for crossbred village chicken for chicken fed with CFD and FFD were much lower compared in purebred village chicken. Furthermore, both of the diets reached village chicken marketable weight (1 kg) at Week 9 of age. FFD diet showed more promising outcome in crossbred village chicken compared with purebred village chicken in terms of body weight.

4.1.1 Body weight of purebred and crossbred village chicken by weeks

Table 4.1 shows the results of body weight for purebred village chicken given 2 types of diets, commercial finisher diet (CFD) and formulated finisher diet (FFD).

Table 4.1: Purebred village chicken body weight given 2 types of diet

Diet / Week	Commercial finisher diet (CFD)(SEM) (g)	Formulated finisher diet (FFD)(SEM) (g)	Significant
3	143.6±3.4	91.9±2.5	*
4	239.0±6.0	165.9±4.7	
5	350.6±8.0	267.9±7.6	
6	471.4±15.7	437.9±11.4	
7	577.8±14.5	499.4±14.6	
8	770.0±17.4	611.9±21.2	
9	908.4±22.2	735.6±22.9	
10	1074.2±27.7	834.2±24.3	
11	1235.9±30.5	868.9±25.7	
12	1307.6±33.8	923.9±30.0	*
14	1482.2±42.4	1080.4±37.0	
16	1768.5±50.1	1343.8±41.9	

*Note = * indicate significant difference ($p<0.05$).*

Table 4.2 shows the results of body weight of crossbred village chicken given 2 types of diet, commercial finisher diet (CFD) and formulated finisher diet (FFD).

Table 4.2: Crossbred village chicken body weight given 2 types of diet

Diet / Week	Commercial finisher diet (CFD)(SEM)	Formulated finisher diet (FFD)(SEM)	Significant
3	197.1±4.6	196.7±3.5	
4	334.6±7.1	313.8±6.7	
5	461.3±9.7	434.7±9.6	
6	659.7±15.0	647.4±15.3	
7	908.3±23.4	845.5±20.0	*
9	1210.3±33.0	1155.0±27.6	
10	1495.9±43.8	1324.2±32.5	
11	1672.3±55.4	1500.0±45.0	
12	1876.2±64.2	1668.6±49.3	

4.1.2 Body weight of village chicken (purebred and crossbred village chicken) based on genders.

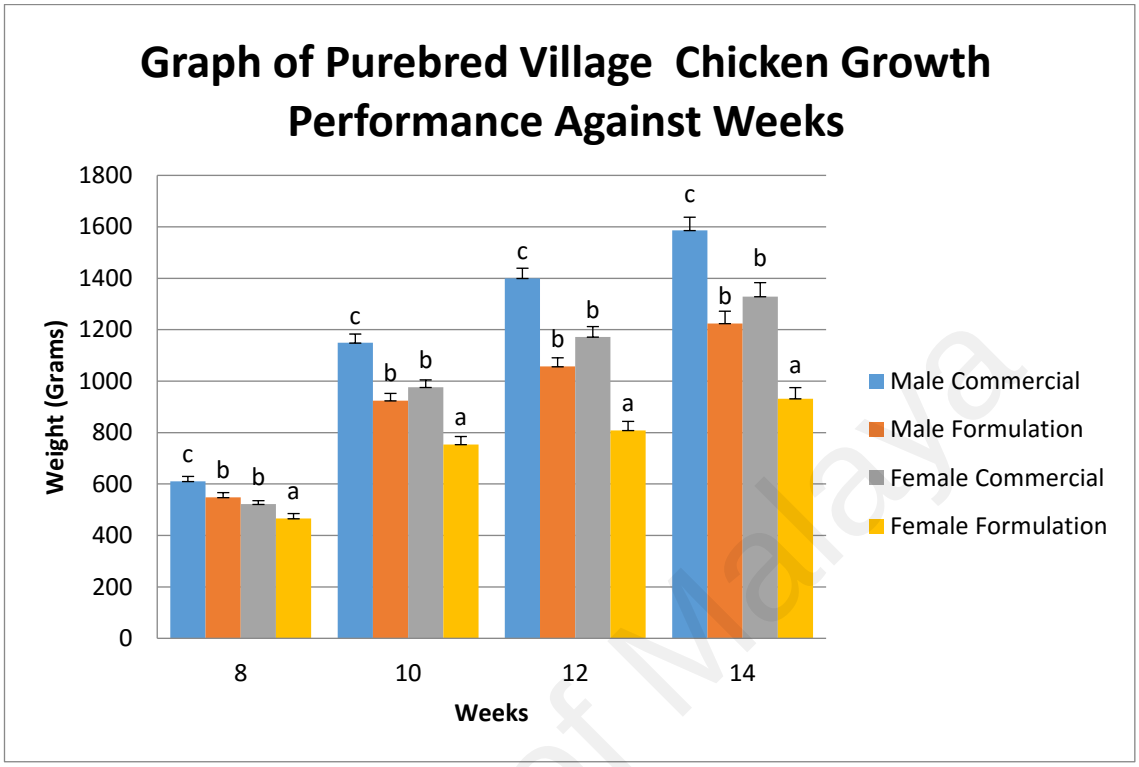

Figure 4.1: Graph of body weight against week (purebred village chicken) using 2 types of diet by genders

Figure 4.1 shows that body weight of purebred village chicken according to genders. Male chickens from each treatment were dominantly heavier compared with female chickens. CFD male chickens were significantly heavier compared with other treatment of male and female chickens. There were no significant differences between female CFD and male FFD village chickens, despite of male FFD gained more weight than female CFD village chickens. Meanwhile, female chickens fed with FFD recorded the least body weight for 14 weeks of research duration. This indicated that FFD diet did not contribute significantly in the growth of purebred village chickens, especially in female purebred chickens.

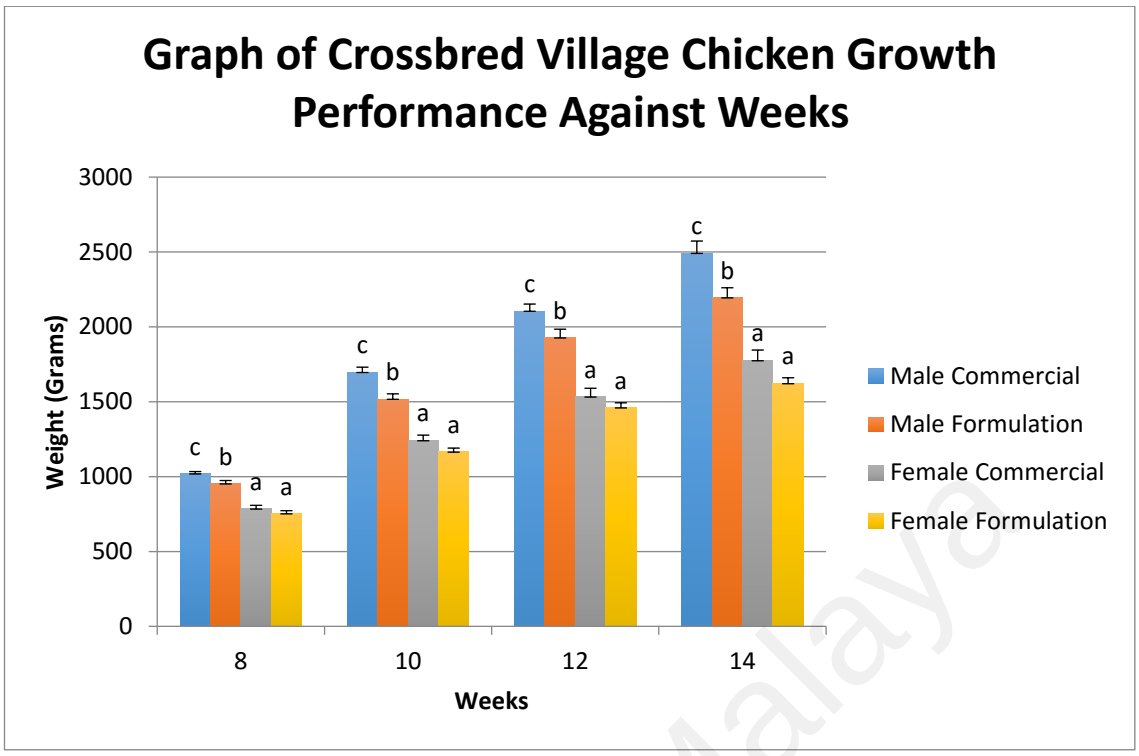

Figure 4.2: Graph of body weight against week (crossbred village chicken) using 2 types of diet by genders

Figure 4.2 shows the graph of body weight against week for crossbred village chicken using 2 types of diet, namely CFD and FFD. Male chickens given CFD showed significant differences in all weeks comparatively with FFD male and female chickens as well as CFD female chickens. However, male chickens given FFD were significantly heavier compared with female chickens given CFD and FFD. Meanwhile, female chickens given CFD and FFD for crossbred village chicken showed no significant differences. Both of male chickens given CFD and FFD reached marketable weight at 1.5 kg at 10 weeks of age.

4.2 Carcass Performances of Village Chicken

From the research, there were significant findings in carcass performance compared with live body weight of village chicken, especially for crossbred village chicken, which was contributed by the inclusion of the local feedstuff such as PKC and coconut meal cake. Generally, the internal organs such as gizzard, liver, and heart weight showed no significant differences and only been differentiated according to age, gender and strain of chickens. However, within the strains and ages, there were no significant differences and did not influence much in the growth of village chicken. Male chickens were dominated in all aspects in both type of diets compared with female chickens.

The meat quantity from first slaughtering at Week 8 of age for the crossbred village chickens was significantly higher at Week 12 of age. The same trend showed for the purebred village chicken. The interesting findings from this research were the carcass weight, abdominal fat, white and dark meat and meat quantity at both slaughtering ages (Weeks 8 and 12 of age for crossbred village chicken) and (Weeks 12 and 16 of age for purebred village chicken). Although, CFD gave a promising result in term of live body weight, however the carcass and meat quantity showed for FFD diet showed the potential for poultry feed usage. In addition, CFD fed chickens could affect toward the economic aspects in village chicken industry such as the production of meat compared with unusable materials such as feathers, fats and bones.

4.2.1 Carcass performances of crossbred village chicken

Table 4.3: Effect of diets on carcass performances between genders of village chickens (crossbred)(Grams)

Diet / Parameter	Commercial diet, (g)		Formulated diet (g)	
	Male (CM)	Female (CF)	Male (FM)	Female (FF)
8th weeks age				
Live weight	1718.0±47.4^{d}	1260.4±60.0^{b}	1408.0±28.0^{c}	1127.0±23.4^{a}
Carcass weight	1321.0±85.6^{c}	946.0±39.9^{ab}	1020.0±25.9^{b}	815.8±21.4^{a}
Gizzard	43.0±0.9^{a}	35.8±1.1^{a}	43.2±3.7^{a}	37.0±4.1^{a}
Liver	42.4±3.6^{b}	30.0±2.7^{a}	43.2±1.1^{b}	32.0±3.4^{a}
Heart	10.6±1.5^{b}	6.0±1.0^{a}	6.0±1.0^{a}	5.0±0.0^{a}
Abdominal fat	15.6±4.5^{b}	7.0±3.1^{ab}	3.9±1.8^{a}	2.1±0.6^{a}
White Meat	609.5±22.6^{c}	429.7±15.4^{ab}	462.4±14.1^{b}	378.2±13.9^{a}
Dark Meat	513.2±24.7^{c}	358.5±16.9^{ab}	407.2±14.1^{b}	309.5±5.4^{a}
Meat Quantity -White meat -Dark Meat	 284.9±11.5^{b} 261.2±9.2^{c}	 214.0±5.9^{a} 187.1±8.9^{ab}	 224.2±11.7^{a} 211.7±12.5^{b}	 192.3±11.9^{a} 168.6±1.9^{a}
12th weeks age				
Live weight	2791.0±149.9^{b}	2495.0±88.5^{b}	)±116.8^{a}	)±43.8^{a}
Carcass weight	2146.0±109.6^{c}	1500.0±78.9^{a}	1818.0±83.0^{b}	1332.0±32.5^{a}
Gizzard	64.4±1.5^{b}	56.0±1.7^{a}	67.0±3.1^{b}	64.6±2.0^{b}
Liver	51.3±5.7^{ab}	36.5±3.1^{a}	61.8±7.7^{b}	46.3±6.3^{ab}
Heart	12.7±1.0^{c}	7.9±0.4^{a}	10.7±0.7^{b}	7.4±0.3^{a}
Abdominal fat	31.0±7.0^{c}	24.1±9.7^{bc}	9.9±9.5^{b}	6.5±6.6^{a}
White Meat	1073.7±56.7^{c}	806.1±42.9^{ab}	940.0±53.1^{bc}	715.6±15.2^{a}
Dark Meat	1008.9±70.6^{c}	648.4±37.0^{a}	826.2±39.1^{b}	567.9±19.5^{a}
Meat Quantity -White meat -Dark Meat	 453.5±36.7^{a} 484.1±40.5^{b}	 378.9±29.4^{a} 317.5±19.9^{a}	 436.0±48.0^{a} 415.2±41.3^{b}	 349.0±19.7^{a} 302.2±17.7^{a}

^{a,b,c,d} means with the different superscripts letters in same row showed significant differences at ($P<0.05$).

Table 4.3 shows the data on the effect of diets on carcass performances between genders in crossbred village chickens. At Week 8 of age, for the crossbred village chicken at slaughtered age showing that male CFD contains significantly higher live weight (1718.0 ± 47.4 g) compared with others, followed by male FFD, female CFD and female FFD village chickens. For carcass weight at 8th week of age, the female CFD and FFD village chickens showed no significant differences (9946.0 ± 39.9 g and 815.8 ± 21.4 g) respectively. The abdominal fat for CFD male village chickens was in tandem

with their live weights, which were significantly higher compared with others (15.6 ± 4.5 g). Female village chickens fed with CFD having cumulative white and dark meats weight quantity of 429.7 ± 15.4 g and 358.5 ± 16.9 g respectively, showing no significant differences to female village chickens fed with FFD cumulative white and dark meats weight quantity at 378.2 ± 13.9 g and 309.5 ± 5.4 g respectively, as well as showed no significant differences in male FFD village chickens, although male FFD village chickens were much higher in average for white and dark meats quantity (462.4 ± 14.1 g and 407.2 ± 14.1 g). The meat quantity showed variable data, where for white meat CFD male chickens (284.9 ± 11.5 g) was significantly higher compared with others. Meanwhile, FFD male chickens, CFD female chickens and FFD female chickens showed no significant differences (224.2 ± 11.7 g, 214.0 ± 5.9 g and 192.3 ± 11.9 g, respectively). For dark meat, the meat quantity for FFD male chickens was significantly higher (211.7 ± 12.5 g) compared to female FFD chickens (168.6 ± 1.9 g) and no significant differences with female CFD village chickens (187.1 ± 8.9 g), although male FFD village chickens were much higher compared to CFD female village chickens.

At 12th week of age, the carcass weights for male CFD chickens (2146.0 ± 109.6 g) were significantly higher compared to male FFD chickens (1818.0 ± 83.0 g). However, there were huge different between the live weight (2791.0±149.9 g) and carcass weight (2146.0 ± 109.6 g) in male CFD fed village chickens compared with male FFD fed chickens. Male FFD fed chickens contained significantly higher abdominal fat compared with male and female FFD fed chickens, however at this age there were no significant difference in abdominal fat between CFD male chickens (31.0 ± 7.0 g) and CFD female chickens (24.1± 9.7 g). There were no significant differences in female CFD and FFD chickens for white and dark meats at 12th week of age. However, for male CFD village fed chickens only white meat showed no significant difference (1073.7 ± 56.7 g) compared with male FFD fed village chickens (940.0 ±

53.1 g). There were no significant differences between male and female village chickens for both fed with CFD and FFD for white meat quantity. For dark meat there were no significant differences between male CFD and FFD fed chickens.

4.2.2 Carcass performances of purebred village chicken

Table 4.4: Effect of diets on carcass performances between genders of village chickens (Purebred)(Grams)

Diet / Parameter	Commercial diet, (g)		Formulated diet, (g)	
	Male (CM)	**Female (CF)**	**Male (FM)**	**Female (FF)**
12th weeks age				
Live weight	2359.0±92.3^{c}	1826.0±115.3^{b}	1846.0±100.9^{b}	1426.0±31.7^{a}
Carcass weight	1761.0±67.2^{c}	1382.0±88.7^{b}	1332.0±73.6^{b}	1055.0±13.9^{a}
Gizzard	59.6±4.2^{a}	49.6±2.3^{a}	56.9±3.9^{a}	48.1±3.6^{a}
Liver	36.5±1.6^{ab}	32.5±2.7^{a}	41.5±1.9^{b}	34.5±2.2^{a}
Heart	10.7±0.6^{c}	7.7±0.8^{b}	7.6±1.1^{b}	5.0±0.4^{a}
Abdominal fat	4.5±1.7^{ab}	12.0±3.7^{b}	5.4±1.6^{ab}	5.6±1.0^{ab}
White Meat	932.3±45.6^{c}	802.3±26.3^{bc}	707.6±38.3^{ab}	587.0±9.1^{a}
Dark Meat	742.3±27.2^{c}	626.7±44.3^{b}	540.3±33.0^{b}	417.1±28.8^{a}
Meat Quantity -White meat -Dark Meat	 334.9±23.8^{ab} 320.8±33.3^{b}	 360.8±25.6^{b} 329.5±13.2^{b}	 302.1±43.4^{ab} 289.6±24.6^{ab}	 270.6±8.7^{a} 216.8±20.3^{a}
16th weeks age				
Live weight	2295.0±196.2^{b}	1635.0±116.0^{a}	2303.0±121.0^{b}	1708.0±65.7^{a}
Carcass weight	1837.0±165.9^{b}	1302.0±103.0^{a}	1754.0±105.9^{b}	1321.0±64.4^{a}
Gizzard	46.6±7.9^{a}	42.7±4.2^{a}	57.5±4.4^{a}	54.5±4.8^{a}
Liver	24.0±2.1^{a}	38.1±2.6^{a}	18.8±3.1^{b}	25.5±1.2^{a}
Heart	11.5±1.9^{ab}	7.8±1.3^{a}	13.7±1.1^{b}	8.5±0.9^{a}
Abdominal fat	24.1±6.6^{c}	16.4±3.5^{ab}	7.5±1.7^{ab}	15.3±3.4^{a}
White Meat	876.1±96.2^{b}	697.6±65.4^{ab}	883.0±48.1^{b}	654.2±46.1^{a}
Dark Meat	785.3±72.7^{b}	570.2±70.9^{a}	782.7±33.1^{b}	544.0±37.1^{a}
Meat Quantity -White meat -Dark Meat	 459.0±43.9^{b} 411.1±52.9^{ab}	 364.4±38.9^{ab} 313.5±41.7^{ab}	 473.8±25.8^{b} 425.4±28.8^{b}	 339.7±31.5^{a} 287.6±28.2^{a}

^{a,b,c,d} means with the different superscripts letters in same row showed significant differences at ($P<0.05$).

Table 4.4 shows the data of effect of diets on carcass performances between genders in purebred village chickens. At 12th Week of age, the live weight for the purebred village chicken at slaughtered age showing that male village chickens fed with CFD significantly higher (2359.0 ± 92.3g) compared with others followed by male FFD, female CFD and female FFD of purebred village chickens. For carcass weight at 8th

Weeks of age, the female village chickens fed with CFD and FFD showed significant differences (1382.0 ± 88.7 g and 1055.0 ± 13.9) respectively. There were no significant differences in abdominal fat for male CFD and FFD fed village chickens (4.5 ± 1.7 g and 5.4 ± 1.6 g respectively). However, female CFD fed village chickens contained highest abdominal fat at 8th Week of age. Male purebred village chickens fed with CFD and FFD contain no significant difference for white and dark meat quantity (334.9 ± 23.8 g and 302.1 ± 43.4g respectively). Meanwhile female CFD fed village chickens contained significantly higher meat quantity for white and dark meat (360.8 ± 25.6 g and 329.5 ± 13.2 g, respectively) compared with female FFD fed village chickens (270.6 ± 8.7 g and 216.8 ± 20.3 g, respectively). Moreover, the dark meat showed no significant differences for CFD male and female and FFD male fed village chickens (320.8 ± 33.3 g, 329.5 ± 13.2 g and 289.6 ± 24.6 g, respectively).

At 16th Weeks of age, the carcass weights for male CFD and FFD fed village chickens showed no significant differences (1837.0 ± 165.9 g and 1754.0 ± 105.9 g, respectively) as well as female village chickens fed with CFD and FFD also showed no significant differences (1302.0 ± 103.0 g and 1321.0 ± 64.4 g , respectively). Male CFD village chickens contained significantly high abdominal fat (24.1 ± 6.6 g) compared with male and female FFD fed village chickens, however at this age there were no significant difference in abdominal fat between CFD female, FFD male and female of purebred village chickems. There were no significant differences male CFD and FFD for white and dark meat quantity at 16th Weeks of age as well as for female village chickens fed with CFD and FFD. There were no significant differences between male CFD and FFD fed chicken for white and dark meat quantity (459.0 ± 43.9 g, 411.1 ± 52.9 g and 473.8 ± 25.8 g, 425.4 ± 28.8 g, respectively). The data also showed same trend for female for both CFD and FFD fed village chickens for white and dark meat

quantity (364.4 ± 38.9 g, 313.5 ± 41.7 g and 339.7 ± 31.5 g, 287.6 ± 28.2 g, respectively).

4.2.3 Percentage of meat production

Table 4.5: Percentage of meat quantity for purebred village chicken and crossbred village chicken given 2 types of diet

Strains	**CFD (Mean ± SEM)**		**FFD (Mean ± SEM)**	
	Male	Female	Male	Female
PVC (12 Weeks), (%)	27.8±0.6^{a}	37.8±0.3^{b}	32.1±0.7^{ab}	34.2±0.9^{b}
CVC (8 Weeks), (%)	31.8±0.4^{a}	31.8±0.3^{a}	31.0±0.9^{a}	32.0±0.6^{a}
PVC (16 Weeks), (%)	37.9±0.5^{a}	41.5±0.7^{b}	39.0±0.5^{a}	36.7±0.9^{a}
CVC (12 Weeks), (%)	33.6±0.5^{b}	27.9±0.6^{a}	43.6±0.8^{c}	36.1±0.03^{b}

^{a,b,c} means with the different superscripts letters in same row showed significant differences at ($P<0.05$).

Table 4.6: Percentage of meat to bone quantity for purebred village chicken and crossbred village chicken given 2 types of diet

Strains	**CFD (Mean)**		**FFD (Mean)**	
	Male	Female	Male	Female
PVC (90 days),(%)	39.2	48.3	47.4	48.5
CVC (60 days), (%)	48.6	50.9	50.1	52.5
PVC (120 days), (%)	52.4	53.5	60.0	52.4
CVC (90 days), (%)	45.0	47.9	48.2	50.7

Table 4.5 showed the data of meat quantity percentage produced by purebred village chicken (PVC) and crossbred village chicken (CVC) given 2 types of diets. Apparently, female PVC (37.8 ± 0.3 g) produced significantly higher meat quantity compared to

male PVC given CFD (27.8 ± 0.6 g). In addition, Table 4.6 showed the percentage of meat to bone quantity for both purebred and crossbred village chickens fed using both diet.

4.3 Feed Intake, Feed Conversion Ratio, and Daily Weight Gained

Table 4.7 showed the mean data for feed intake and feed conversion ratio (FCR) in mixed gender for purebred village chicken. Generally, there were no significant differences in feed intake for purebred village chickens given CFD and FFD (363.8±46.3 g and 378.3±52.5 g respectively). Although average weekly gained recorded for CFD is much higher (129.3±12.8 g) compared to FFD (92.4±13.7 g), but there were no significant differences recorded. Apparently, purebred village chicken fed with CFD (2.9) could utilise more feed compared with FFD (5.2) based the feed conversion ratio recorded.

Table 4.7: Effect of diets on feed intake and feed conversion ratio in mixed gender purebred village chicken (Mean ± SEM)

Parameter	Commercial Finisher Diet (CFD) (n = 150)	Formulated Finisher Diet (FFD) (n = 150)	T-test significance
Feed Intake (Weeks 3–12) , (g/chick)	363.8±46.3	378.3±52.5	*
Average Weekly Weight Gained (Weeks 3–12) (g/chick) Average Daily Gained (Weeks 3-12)(g/chicks)	129.3±12.8 18.5±1.8	92.4±13.7 13.2±2.0	* *
Feed Conversion Ratio /week (FCR) (Weeks 3–12)	2.9±0.6	5.2±1.6	*

*Note = * indicate no significant differences at (p >0.05).*

Table 4.8: Effect of diets on feed intake and feed conversion ratio in mixed gender crossbred village chicken (Mean ± SEM)

Parameter	Commercial Finisher Diet (CFD) (n = 50)	Formulated Finisher Diet (FFD) (n = 50)	T-test significance
Feed Intake (Weeks 3–12) , (g/chick)	446.6±73.3	432.2±70.9	*
Average Weekly Weight Gained (Weeks 3–12) (g/chick)	187.7±22.2	175.4±20.8	*
Average Daily Gained (Weeks 3-12)(g/chicks)	26.8±3.2	25.1±3.0	*
Feed Conversion Ratio /week (FCR) (Weeks 3–12)	2.4±0.4	2.6±0.4	*

*Note = * indicate no significant differences at (p >0.05).*

Table 4.8 showed the mean data for feed intake and feed conversion ratio in crossbred village chickens fed with both types of diet. Crossbred village chicken recorded almost same trend with purebred village chicken, where CFD fed chicken (446.6±73.3 g) consumed more feed compared with FFD fed chickens (432.2±70.9 g). However, there were still no significant differences in feed intake for chicken that been fed with both diets. The average weekly weight gained for CFD fed chicken (187.7±22.2 g) and FFD fed chicken (175.4±20.8 g) showed no significant differences. Meanwhile, the FCR recorded for CFD and FFD fed chickens were 2.4 and 2.6, respectively. The FCR recorded were much lower compared in the purebred village chicken.

4.4 Proximate Analysis

Table 4.9: Proximate analysis of crossbred village chicken meat using 2 types of diet

Composition	**CFD**	**FFD**	**T-test significance**
Dry matter, DM (%)	97.5±0.04	97.9±0.3	
Crude Protein, CP (%)	78.4±2.5	84.5±1.1	*
Crude Fat, CF (%)	4.9±0.9	5.3±0.6	
Crude Fibre, CFb (%)	1.8±1.3	3.3±3.3	
Ash, (%)	9.4±1.6	5.3±0.4	*

*Note = * indicate significant difference (p<0.05).*

Table 4.10: Proximate analysis of purebred village chicken meat using 2 types of diet

Composition	**CFD**	**FFD**	**T-test significance**
Dry matter, DM (%)	97.2±0.4	97.6±0.2	
Crude Protein, CP (%)	87.7±0.3	89.3±2.2	
Crude Fat, CF (%)	5.5±0.1[b]	3.3±0.8	*
Crude Fibre, CFb (%)	2.4±1.8	5.2±3.1	*
Ash, (%)	6.8±0.3	4.9±0.3	

*Note = * indicate significant difference (p<0.05).*

Tables 4.9 and 4.10 showed the proximate analysis result of crossbred and purebred village chicken's meats fed with CFD and FFD respectively.

4.5 Amino Acid Content in Village Chicken Meat Between Diets

Table 4.11 showed the analysed data of amino acids content in crossbred village chicken's meat fed with CFD and FFD.

Table 4.11: Amino acid content in crossbred village chicken meat fed on 2 types of diets

Amino Acid	Amount (Mean±SEM)		T-test significance
	CFD (n=10)	FFD (n=10)	
Ammonia, NH_3	0.48±0.05	0.79±0.07	*
Histidine	5.15±2.10	6.89±2.13	
Serine	4.91±3.00	9.35±4.10	*
Glycine	5.17±0.66	6.89±2.13	
Arginine	5.62±0.31	8.61±0.80	*
Proline	3.66±0.47	5.41±0.41	*
Cysteine	11.19±1.34	14.31±1.39	
Lysine	0.16±0.03	2.41±0.62	*
Valine	1.87±0.25	2.52±0.33	
Aspartate	8.33±0.83	13.52±0.92	*
Glutamate	13.52±1.55	15.92±1.92	
Threonine	4.14±0.50	6.04±0.57	*
Alanine	4.77±0.36	8.24±0.62	*
Methionine	3.52±0.51	4.79±0.32	*
Tyrosine	5.09±0.58	10.59±0.79	*
Isoleucine	7.88±0.52	8.99±0.60	
Leucine	3.89±0.27	10.50±1.39	*
Phenyalanine	3.09±0.18	6.19±0.60	*

*Note = * indicate significant difference ($p<0.05$).*

4.6 Economical Analysis

Table 4.12: Economic analysis for production of village chicken

Week	Daily Feed Intake (g/day/bird)		Average Live Weight (g)		Cost (USD/Week)		Profit Gained Each Week (USD)	
	CFD	**FFD**	**CFD**	**FFD**	**CFD**	**FFD**	**CFD**	**FFD**
4	30.8	31.3	334.6[a]	313.8[a]	0.12	0.08	0.82	0.79
5	34.4	31.3	461.3[a]	434.7[a]	0.13	0.08	1.16	1.14
6	52.8	51.0	659.7[a]	647.4[a]	0.20	0.13	1.65	1.68
7	63.5	61.6	908.3[a]	845.5[a]	0.24	0.16	2.30	2.21
9	82.3	80.8	1210.3[a]	1155.0[a]	0.31	0.20	3.08	3.03
10	84.8	80.0	1495.9[a]	1324.2[b]	0.32	0.20	3.87	3.51
11	**89.2**	**85.1**	1876.2[a]	1668.6[a]	0.34	0.21	4.91	4.46
12	94.2	96.2	2193.3[a]	1877.6[a]	0.36	0.24	5.78	5.02
Total	**532.0**	**517.3**			**2.02**	**1.30**	**23.57**	**21.84**

[a,b,] means with the different superscripts letters in same row showed significant differences at ($P<0.05$).

Note: Considering the price of local CFD 0.54 USD/kg and FFD 0.36 USD/kg and the price for 1 kg of village chicken is 2.8 USD/kg based local market price in Malaysia. The average live weight was used to determine the price of the village chicken.

Table 4.12 showed the economic analysis based on feed for the production of village chicken using CFD and FFD. Based on the crossbred village chicken performances, the FFD total feed intake for CFD and FFD fed chickens were 532.0 g/day/bird and 517.3 g/day/bird, respectively. The final average live weight at Week 12 for FFD fed chicken were much lower compared with CFD fed chickens causing low production cost for FFD fed chicken. Despite that, FFD fed chicken still having low profit compared with profit gained by CFD fed chicken. Table 4.9 also showed assumption of profit gained for each week where, CFD diet was assumed to gain more profit, which was in lined with their average live weight.

4.7 The Expression of Glutathione-S-Transferases in Slaughtered Village Chicken Liver (Exp. 2)

The expression of Glutathione-S-Transferases (GSTs) was detected within the liver in Non-stress slaughtered village chicken (NSSM) and Stress slaughtered village chicken (SSM). The expression of GSTs in the village chicken liver was detected at molecular weight of 25-26 kD. The expression of GSTs was clearly visible on the SDS gel after purification using FPLC and also expressed on 2-Dimensional electrophoresis gel.

4.7.1 1st Dimensional SDS-PAGE of GSTs expression in village chicken liver

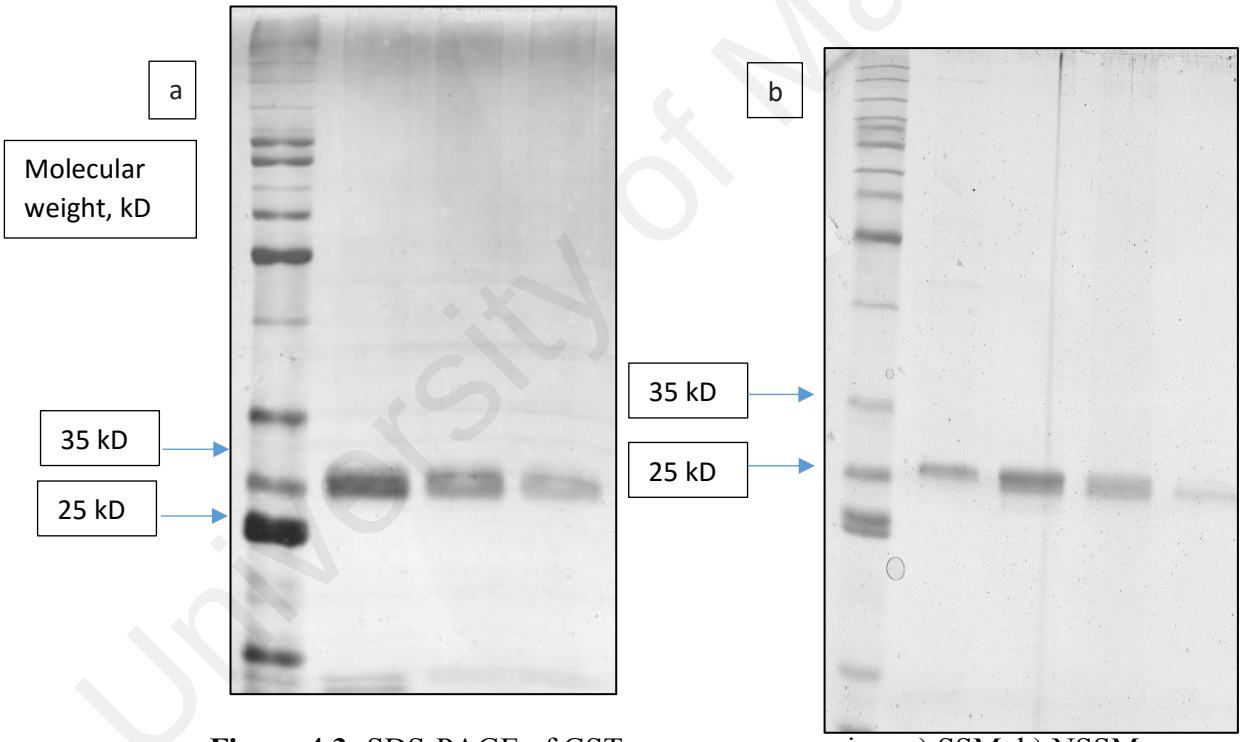

Figure 4.3: SDS-PAGE of GSTs enzyme expression; a) SSM, b) NSSM

The expression of GSTs could be spotted in both NSSM and SSM chicken at 25-26 Kd as shown in Figure 4.5. The intensity of the expression could not be used to assume the quantity of protein enzymes present within the chicken liver.

4.7.2 2nd Dimensional gel electrophoresis of GSTs enzyme in village chicken with treatments SSM against NSSM

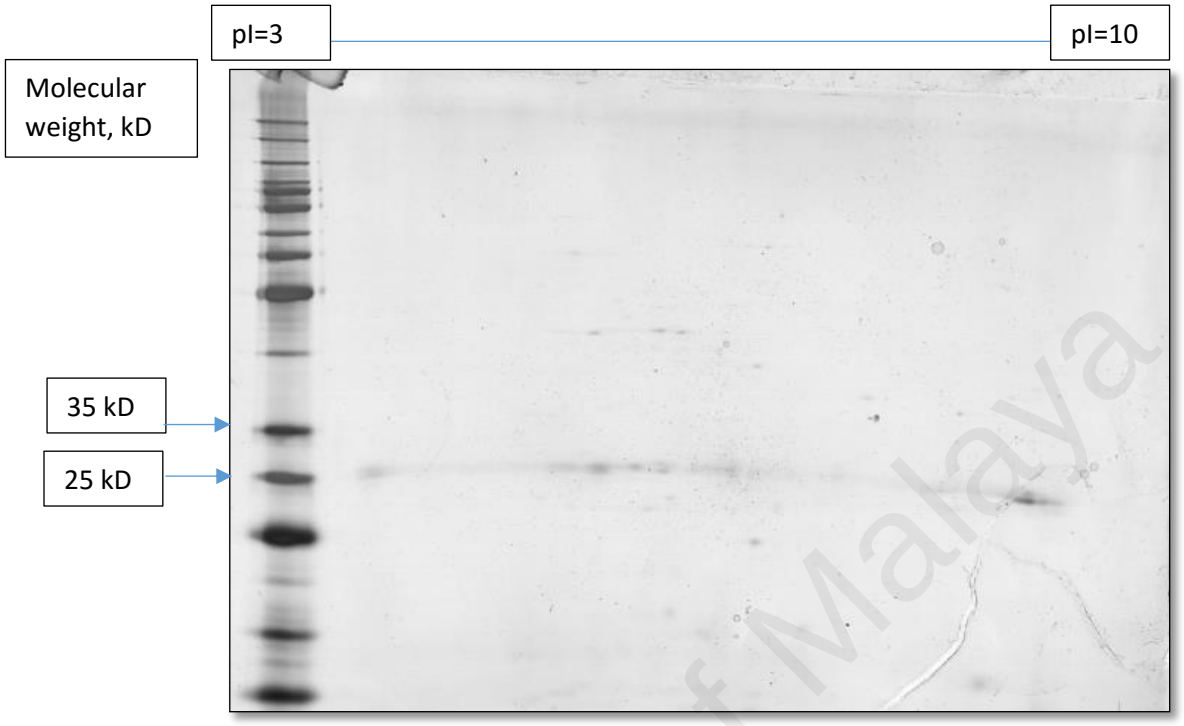

Figure 4.4: Spot of NSSM showed on 2-D gel

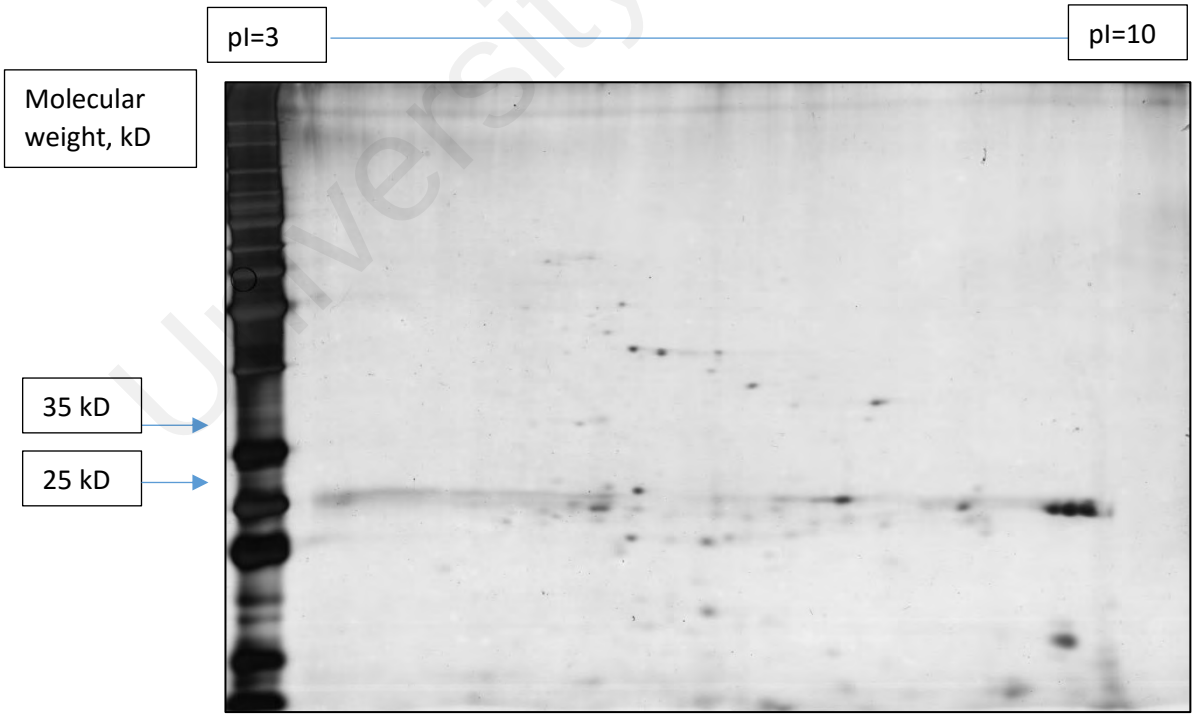

Figure 4.5: Spot of SSM showed on 2-D gel

4.7.3 Enzyme activity of SSM against NSSM treated chicken

Table 4.13: Enzyme activity, total amount of protein and specific total activity of GSTs enzyme between NSSM and SSM

Replication	**NSSM (n = 10)**	**SSM (n = 10)**
Enzyme activity (µmol/min)	0.54±0.16[a]	0.84±0.21[a]
Total activity (µmol/min)	0.72±0.17[a]	1.34±0.24[a]
Protein (mg/mL)	0.10±0.03[a]	0.11±0.03[a]
Total amount of protein (mg)	0.11±0.03[a]	0.19±0.03[a]
Specific total activity (µmol/min/mg)	5.57±0.34[a]	7.19±0.08[b]

[a,b]Means with the same superscript letter in a row showed significant difference at ($P<0.05$).

CHAPTER 5: DISCUSSION

5.1 Introduction

The village chicken industries have been tremendously developed due to the increment of consumer demand for nutritious poultry's meat. Increase of awareness among people about health measures as well as the benefits of consumption village chicken also contributing to the large demand. The consumer's preference for the village chicken is due to their organic sources, flavour, and leanness of their meat (Kingori *et al.,* 2010). Ganabadi *et al.* (2009), reported that jungle fowl contained the lowest fat content compared with other types of chicken. Furthermore, village chicken farming is useful for the farmer from rural area, especially for a poor household to increase their income. This is due to the reason that village chicken does not require such intensive facilities to accommodate them, as well as could regenerate income by minimizing the management and production cost. One of the advantages of the village chicken that allowing low production cost is the needing of high facilitated housing for the village chicken, where most of the village chicken are reared either free-range or semi-intensive systems. Village chicken was known for their good adaptation to the surrounding environment and also for their survival rate in harsh environmental and nutritional condition such as low and unbalanced diets (Gichohi & Maina, 1992; Dessie *et al.,* 2013).

Despite that, slow growth of village chicken especially for the purebred strains has become a major constraint for farmers to gain more profit due to the long growing period and low body weight gained, thus increasing the production and management cost. The slow growth of village chicken has been recorded by various researchers, where the main reason was due to the genetics factor (Ajuyah, 2013; Francis *et al.,* 2016; Khawaja *et al.,* 2016). Besides, diseases have become a crucial constraint in village chicken industry. Newcastle disease is the most prominent disease in the poultry

industry, especially to chicken reared in South-East Asian (ASEAN) countries that have high humidity, fluctuation of temperature, differences in geographical areas and strong wind. This was worsening during the raining or monsoon season due to the high humidity and strong wind, which decreasing the survival rate of the chicks and causing the chickens to be more susceptible to diseases (Aini, 2013; Ibrahim *et al.,* 2016). Thus, intensive and semi-intensive rearing of village chicken could solve the problem of diseases susceptibility. The high susceptible rate of chicken to the diseases was due to the fre-range system that exposing chickens to the various diseases, which originated from many sources such as feed, environment and other animals (Aini, 1990; Moges *et al.,* 2010; Dinka *et al.,* 2010).

Mass production of chicken today has increase consumers anxiety about the quality of slaughtered chickens. Previous research already reported about the effect of pre-slaughtered stress condition on meat quality on livestock such as in poultry, where the stress could be induced from heat stress, shackling during processing, transportation and withdrawal of feed (Ali *et al.,* 2008). However, there were not many researchers study on the effect of slaughtering method on village chicken's meat quality using chemical and proteomic approaches. Slaughtering was conducted to remove blood as fast as possible to ensure no flow of oxygen to the brain (Gregory, 2007). Non-proper slaughtering could increase the duration of blood removal, thus increasing the time of death of the animals. This could be happening in the industry and has been numerous reported by consumers and researchers such as done by Farouk *et al.* (2014), who reported that the mechanical slaughtering used in mass production tends for missed slaughtered after period of time of running. Moreover, this situation could lead to the build-up of oxidative stress, which would affect the quality of the meat and their shelf-life, which could lead to food spoilage. The oxidative damage was occurring due to the effect of lipid and protein oxidation occurrence, thus resulting in diseases susceptibility,

rancidity and formation of various toxic compounds in poultry. Furthermore, it was also reported that oxidative damaged could cause severe loss of fatty acid and essential amino acid. Thus, protection against the oxidative stress was needed to overcome the stress and loss of fatty acid as well as the essential amino acid, which is irreversible. The endogenous enzyme such as glutathione peroxidase could counter the stress caused by the oxidation of lipid and protein (Estevez, 2015).

Glutathione-S-transferases (GSTs) acts as the catalyse for glutathione to increase its activity, thus could possibly minimizing the oxidative stress occurred. Previous researchers have studied that GSTs has the potential in many aspects such as medical, agricultural and research. Oxidative damage which occurs in certain organism could be prevented by GSH-dependent peroxides reaction (Moons, 2005). Furthermore, GSTs also functioning in deactivating secondary metabolites such as quinones, unsaturated aldehyde, epoxides, and hydroperoxides that formed resulted from the oxidative stress (Hayes *et al*., 2005). There were also studied involved GSTs in liver of broiler to monitor the oxidative stress when given certain antioxidants (Maurice *et al*., 1991; Elamaran *et al.,* 2015). This dissertation is aiming to study the potential of using local feed sources for local village chicken for growth and ability to market them as good as using commercial feed. In addition, this study is aiming also to evaluate the benefits of rearing village chicken in terms of economic and nutritionally. Lastly, the study of quality of chicken meat, which is specifically focused on slaughtering method hopefully, could give a good understanding of certain issues.

5.2 The Effect of Local Feed Sources on the Growth and Carcass Performances of Village Chicken (Exp. 1)

The body weights for village chickens fed with feed that mixed with PKC and coconut meal cakes (FFD) were better in crossbred village chickens compared in purebred

village chickens. The usage of FFD diet was important due to the ingredients, which included coconut meal cake (CMC) (extracted milk coconut meat) and palm kernel cake (PKC) that were highly available and cheap in this country. Furthermore, based on calculation, FFD could be beneficial economically as the diet could save at least 30% from the total commercial feed cost. This is due to the body weight of purebred village chickens fed with FFD was lighter compared given CFD diet. Most of the data showing the differences of body weight were no significant between purebred village chickens given CFD and FFD, however the gap of body weight between both diets were much severe compared in crossbred strain. Moreover, the gap of body weight between CFD and FFD chickens were increased proportionally with age. This may be indicating that the digestibility and palatability level of FFD compared with CFD. Although FFD diet (14.95 %, DM) contained higher CP compared in CFD diet (13.4%, DM), the body weight of FFD fed chicken were lower compared with CFD fed chicken. Meanwhile, the carcass performances for both strains showed various result. As stated in results, generally the weight for internal organs such as gizzard, liver and heart showed no significant differences among strains, ages and genders. The data was supported by the previous study using broilers fed with 5 types of diet with different CP recorded (Ojewola *et al.,* 2005). Furthermore, the data were also in lined with research done by Goromela *et al.* (2008), where they showed that village chicken's gizzard, liver and liver weight have no significant differences despite the feeding different regime of diets.

5.2.1 Body weight

The body weight recorded for purebred village chickens either given CFD or FFD diets were lower compared in crossbred village chickens. Generally, there were no significant differences between CFD and FFD fed chicken for both strains, except at Week 3 and Week 12 of ages for purebred village chicken and Week 7 of age for crossbred village

chickens. Although, from figure 4.1 and 4.2 showed CFD fed chicken's body weight dominating FFD, but it could be seen that the differences between body weight in purebred village chicken were more severe compared in crossbred village chickens. From Table 4.1, the body weight of purebred village chicken at 12 weeks of age given CFD and FFD were 1307.6 ± 33.8 g and 923.9 ± 30.0 g, respectively. Meanwhile, for crossbred village chicken, the body weight recorded at Week 12 given CFD and FFD were 1876.2 ± 64.2 g and 1668.6 ± 49.3 g, respectively.

The body weight recorded from both strains showed that the FFD fed village chickens having lower body weight compared with CFD fed chicken. However, the live body weight could be influenced by the weight of bone formation, feathers and fat, which are unfavourable parts in chickens for commercial market. Nutrient composition from Table 3.2 has shown that the CP for CFD diet was much lower (13.4%, DM) compared with FFD diet (14.95%, DM). However, high fibre content recorded in FFD (12.22%, DM) might be the reason of low digestibility, which resulting low body weight of FFD chicken. Furthermore, high fibre content also could result in low palatability in chicken. Low digestibility that results from high fibre content also affects in feed intake of the chicken. This was shown in Table 4.6, where the feed intake from Weeks 3 to 12 of age from purebred village chickens, showed that FFD diet was consumed more (378 ± 52.5 g/chick) compared with CFD fed chicken (363.8 ± 46.3 g/chick). However, crossbred village chicken showed feed intake from Week 3 to 12 of age of FFD diet lower (432.2 ± 70.9 g/chick) compared with CFD diet (446.6 ± 73.3 g/chick). The differences between purebred and crossbred village chickens given CFD and FFD diets in term of feed intake were actually due to genetics factor. From previous studied done by Walugembe *et al.* (2014) recorded difference high body weight gained of layer strain chicken fed with high fibre content compared with low fibre content diet. Furthermore, studied on digestibility on the different line of broilers also showed varies

of digestibility between the strains (Carre´ *et al*., 2002; Mignon-Grasteau, 2004). The result was supported by the previous study, that stated that high fibre content in chicken diets could decrease body weight gain, as well as contained low body weight compared with chicken fed with low fibre diet (Lumpkins *et al,* 2004; Loar *et al.,* 2010; Walugembe *et al.,* 2014).

5.2.2 Effect of gender on body weight given 2 types of diet

Moreover, the body weight between genders showed that male CFD fed village chickens were significantly heavier compared with male FFD, female CFD and FFD fed village chickens in both strains. Female purebred village chickens fed with CFD showed significantly heavier weight compared with female FFD fed village chickens. However, in crossbred village chickens, female CFD and FFD fed village chickens showed no significant differences on body weight. Male chickens obviously contained highly significant weight compared with female chickens. This might be due to the factor of gender, where male village chicken tends to grow faster compared to female chicken (Azahan, 2011). Thus, increase the difference of gap body weight between male and female chickens fed with CFD and FFD fed chicken. Despite that, male from both strains reached the marketable weight at 10 weeks of age (1.5 kg). Compared between purebred and crossbred village chicken given both types of diet, the differences in body weight between CFD and FFD fed village chickens were much lower in crossbred chickens compared with purebred village chickens. This indicated that the FFD diet was more suitable for fast growing village chicken such as the crossbred strain.

5.2.3 Carcass weight

Economically, based on the data of carcass performances, crossbred village chicken could utilise on FFD diets better compared with purebred village chicken. This was due

to the carcass weight for crossbred village chickens that were heavier compared to purebred village chickens. The carcass weight for male purebred village chickens at 12th Weeks of age was 1761 ± 67.2 g, while for male crossbred village chickens were 214 6 ± 109.6 g for chickens fed with CFD. It was also same with the data for male purebred and crossbred village chickens given FFD diet, which recorded 1020 ± 25.9 g and 1754.0 ± 105.9 g (Table 4.4), respectively. The slow growth of purebred village chickens was due to the genetics factor, which had been discussed before from previous study (Ajuyah, 2013; Francis *et al.,* 2016; Khawaja *et al.,* 2016). Furthermore, low weight recorded for carcass weight in FFD fed chickens might be due to the reason of chicken is a type of monogastric animal species that limit their digestibility on the diet that contained high fibre (Rodriquez & Preston, 1997). FFD was formulated using PKC, although the amount was adequate, but it could still affluence the digestibility of the chickens, especially for purebred village chickens. In previously studied using broilers, it was reported that palm kernel meal contained high fibre level that could decrease the feed digestibility as well as increased feed intake, thus recommended level usage of palm-based diet was up to 40% from the studied (Sundu *et al.,* 2006). Meanwhile, from previous studied also, it was stated that PKC contained lower protein digestibility which was below 80% compared with other types of feed sources (Heuze *et al.,* 2016). It was also stated previously that PKC contain high amount of insoluble fibre and non-starch polysaccharides (NSPs) (Sundu & Dingle, 2002; Alimon 2004; Fransech & Brufau, 2004). The accumulation of fibre might also occur in the chicken's gizzard due to the consumption of PKC, which occur due to the structure of the PKC which is coarse and ground resulting poor digestibility and increase of hardness (Hetland *et al.,* 2005). In this study, the proximate analysis of the diets showed that the FFD diet contained higher fibre content compared with CFD diet 3.12%, DM and 12.22%, DM, respectively.

5.2.4 Abdominal fat content

One of the important aspect in village chicken industry in Malaysia is the nutritive value of the chicken. Village chicken was known for their good nutritive value, where it recorded low abdominal fat, thus increasing the demand by the consumers (Ahn *et al.,* 1997; Musa *et al.*, 2006). Higher weight of abdominal fat recorded in CFD fed chickens compared with FFD fed chickens in both strains, where in crossbred village chickens, the abdominal fat recorded at 12th Weeks of age were 31.0 ± 7.0 g and 24.1 ± 9.7 g for both male and female fed on CFD diet, where CFD male was significantly heavier compared with other treatments. Meanwhile, crossbred village chicken that fed on FFD diet recorded 9.9 ± 9.5 g and 6.5 ± 6.6 g, for male and female, respectively. Apparently, there were significant differences between female CFD and female FFD in abdominal fat content, where CFD female chickens abdominal fat content were significantly higher compared in female FFD. The differences in abdominal fat content might be due to the differences in protein content between CFD (13.4%, DM) and FFD (14.95%, DM). This was supported from previous studied, which suggested that the similarity in energy content but different in crude protein in animal diet could affect the deposition of fat in poultry (Summers & Leeson, 1979).

Moreover, for purebred village chicken the abdominal fat at 12th Weeks of age showed no significant differences between the treatments. However, at 16th Weeks of age, the abdominal fat for male CFD (24.1 ± 6.6 g) were significantly higher compared with female CFD, male FFD and female FFD (16.4 ± 3.5 g, 7.5 ± 1.7 g, 15.3 ± 3.4 g) respectively. The data from this research was contradicted to previous research done by Velasco *et al.* (2010), whose recorded high deposition of fat in chicken fed on palm oil source diet. Generally, the deposition of abdominal fat in this study relatively low, which was only around 1-2% of abdominal fat/live weight. This was supported from previous research using cockerel strain chicken, where they recorded low subcutaneous

fat and abdominal fat content compared with broiler. It was suggested that, the effect from physical activity done by the chicken, could increase muscle metabolism, thus lowering fat accumulation (De Silva *et al.,* 2016). In addition, it was suggested that the high accumulation of the abdominal fat in CFD fed chicken compared with FFD fed chicken was due to the digestibility of CFD diet that was better compared with FFD. Higher level of fibre in diet could cause reduction of energy (Zahari & Alimon, 2005). The significance of abdominal fat in CFD compared with FFD was also supported by the data from proximate analysis (crude fat), where CFD fed purebred village chickens recorded high significant differences (5.5 ± 0.1 %) compared with FFD fed chicken (3.3 ± 0.8 %).

From previous studied done on Korean Native Chicken (KNC), it was shown that the crude fat was significantly lower than commercial broiler. From the study, the KNC and commercial broiler recorded crude fat at 4% and 5.8%, respectively. Compared with this research, FFD fed chickens for purebred village chicken (3.3 ± 0.8 %) was lower than KNC and commercial broiler. However, CFD (4.9 ± 0.9 %) and FFD (5.3 ± 0.6 %) fed chicken's fat content were higher than KNC but lower than commercial broilers (Jung *et al.,* 2011).

5.2.5 Meat quantity

Although the live body weight of chicken is one of the crucial aspects in determining the price of the chicken, but the production of meat also important. Differ with the commercial broilers that produce more meat in short period of time, village chicken tends to produce non-profitable aspects such as feathers and bones. Certain market requires only meat that will further process into various food such as nuggets, fillets, meat burger and many more. When comparing with commercial broilers, village chicken builds up more bone up to 36% than the broilers, which bone only account

24%. Scavenging characteristics promote the high percentage of bone in village chicken (Van Marle-Koster & Casey, 2001).

In this study, meat quantity was separated into two parts which were white and dark meat after deboning to investigate the percentage of meat produced as compared with bone. At 8th Weeks of age of crossbred village chicken, the meat quantity for male CFD fed chickens were significantly higher compared with male FFD fed chickens, female CFD and FFD fed chickens for both white and dark meat (Table 4.3). The male CFD fed chicken accounts for 48.6% of meat, while male FFD fed chicken meat accounts 50.1% of meat. In other hand, female CFD and FFD showed no significant differences on meat quantity for white and dark meat, which account for 50.9% and 52.5% of meat, respectively. Both male and female CFD chickens recorded higher percentage of meat compared with FFD fed chicken. Meanwhile, at 12th Weeks of slaughter age, the meat quantity for FFD fed chicken was improved, where they showed no significant differences for all treatments. Male CFD and FFD meat account for 45.0% and 48.2% of meat produced, while female CFD and FFD meat recorded 47.9% and 50.7%, respectively. FFD fed chicken showed higher meat percentage compared with CFD fed chicken. It could be assumed that CFD fed village chickens developed more bone than meat compared with FFD fed chicken.

Meanwhile the purebred village chicken recorded showed no significant differences in meat quantity at 8th Weeks of age for male CFD and FFD fed chickens for both white and dark meats. Only female CFD chickens showed significant differences compared with FFD female chickens (Table 4.4). Moreover, it was also recorded that indigenous chicken such as the purebred village chicken developed lesser bone weight and high muscle development compared with commercial broilers (Ganabadi *et al.,* 2009). Male CFD and FFD chickens recorded 39.2% and 47.4% of meat produced compared to bone, while female CFD and FFD chickens recorded 48.3% and 48.5%,

respectively. The data for 16th Week of age also showed a similar trend for the purebred village chickens, where there were no significant differences between the diets whether white or dark meats. The meat at this slaughter age showed that male and CFD and FFD fed chicken produced 52.4% and 60.0% of meat, respectively, meanwhile female CFD and FFD fed chicken recorded 53.5% and 52.4% of meat, respectively. This indicated that meat quantity was improved as the village chicken getting older, which proved that more than half of the carcass weight was meat. Moreover, the ability of FFD to produce more meat than bone showed that the diet has the potential to be marketed but the quantity of the meat produced was still low compared with CFD, despite producing more bone compared to meat. This might be due to the digestibility of the FFD that was influenced by high fibre (12.22%) and ash content (5.18%) compared with CFD fed chickens. It was stated that previously, higher ash content could result in lower quality of protein and amino acid digestibility, thus affecting the muscle build up in the chicken (Carl, 1999).

5.3 Feed Conversion Ratio, Feed Intake, and Weekly Weight Gained of Village Chicken Given 2 Types of Diets

High feed conversion ratio (FCR) was recorded for FFD fed chickens, especially for purebred village chicken, where it recorded 5.2 and 2.9 for CFD and FFD, respectively. This could be due to the genetics factor, where purebred growth slower compared to crossbred village chicken, that recorded FCR at 2.4 and 2.6 for CFD and FFD fed chicken, respectively. The high FCR recorded was also due to the factors of digestibility and palatability for FFD. From the FCR data, it could be seen that crossbred village chicken could adapt more to FFD. The data was supported from previous study, where stated that high level PKC could reduce the body weight of chicken (Pushpakumara *et al.,* 2017). Furthermore, the utilization of PKC based feed such as FFD was limited due

to the high fibre content and low metabolizable energy (Sharmila *et al.,* 2014). Moreover, the availability of amino acid and palatability could be reduced as the level of PKC and fibre content were increased (Onwudike, 1986). Previous study also reported that there were 30% of β-Mannan in the PKC that could largely affect the FCR (Ariff *et al*., 1998)

The inclusion of high fibre feed sources into the diet also could affect the feed intake and weekly weight gained, where the weekly weight gained recorded for purebred village chicken were 129.3 ± 12.8 g/chick and 92.4 ± 13.7 g/chick given CFD and FFD fed chickens, respectively. Meanwhile, in crossbred village chickens recorded that the weekly weight gained 187.7 ± 22.2 g/chick and 175.4 ± 20.8 g/chick for CFD and FFD fed chicken. The weight gained for FFD fed chickens were lower compared to CFD fed chickens for both strains, this might be due the inclusion of high fibre source of feed such as PKC, where in previous study it was stated that β-Mannan in the PKC could reduce weight gained at 20-25% in poultry (Ariff *et al*., 1998). The feed intake for strains showed no significant differences in the chickens that been fed on CFD and FFD. However, purebred village chicken recorded lower feed intake fed on FFD compared to CFD. This could be due to the palatability and digestibility in FFD, where crossbred village chicken could utilise the feed better compared to purebred village chicken.

5.4 Proximate Analysis on Village Chicken Meat

The proximate analysis was done using meat sample from both strains to investigate the level of crude protein (CP), crude fibre (CFb), crude fat (CF), and ash. High CP was recorded for FFD fed chicken's meat compared with CFD in both strains of village chicken. Furthermore, FFD chicken's meats also recorded high level of CFb compared with chickens fed with CFD in both strains, where purebred village chicken recorded

significantly higher CFb. Meanwhile, CF was significantly higher in CFD chicken meats compared with FFD for purebred village chicken and no significant difference in crossbred village chicken. There was significantly higher ash content in CFD chicken meat compared with FFD, especially in crossbred village chicken. This analysis was correlated with the data from carcass performances and gave a good indication of the potential of FFD usage in village chicken industry.

5.4.1 Crude protein

From the data in Table 4.8, it was being observed that FFD fed chicken meat (84.5±1.1%) contained significantly higher CP in crossbred village chicken compared with CFD fed chicken (78.4 ± 2.5%). However, crude protein in purebred village chicken meats showed no significant differences between CFD (87.7 ± 0.3%) and FFD, but FFD fed chicken meat have higher percentage of CP (89.3±2.2%) apparently. The data was supported by the percentage of meat produced by village chicken fed on FFD, which was higher compared CFD fed chicken. High protein produced in the chicken's meat given FFD was an indicator that FFD could be a good nutritive feed, especially to village chicken.

Furthermore, the CP recorded for both strains showed that purebred village chicken contained higher CP compared to crossbred village chicken based on Table 4.8 and 4.9. This was supported by research done Lonergan *et al.* (2001), who reported that high significant difference between the genetics group and recorded low protein content in broiler breast compared with cross strain. Moreover, from previous study it was also stated that broiler lines such as crossbred village chicken or fast-growing chicken have a reduction in protein catabolism to govern the increase in growth rate and muscle mass compared with slow-growing chicken (Dransfield & Sosnicki, 1999).

When compared with FFD meat composition (Table 3.1), it showed that FFD fed chicken meats contained higher CP compared with CFD. Thus, it could be considered that the amount of CP in the feed influenced the composition of CP in the meat of the village chicken. Furthermore, CP was higher in purebred village chicken may be indicated that CP was influenced by genetics factor and muscle mass that produced by the chicken, as well as the metabolism of the chicken itself, where purebred village chickens were more actively move compared with crossbred village chicken.

5.4.2 Crude fibre

The crude fibre (Cfb) content in the village chicken meat is an important factor in determining the quality of meat. In this study, the CFb recorded significantly higher for FFD fed chicken's meat (5.2 ± 3.1%) compared with CFD (2.4 ± 1.8%) in purebred village chickens. Meanwhile, there were no significant differences recorded in crossbred village chickens for CFD (1.8 ± 1.3%) and FFD (3.3 ± 3.3%) fed chickens.

It has been good attention nowadays to the level of dietary fibre in meat, especially to most consume meat such as poultry meat. It has been stated that fibre within the meat was beneficial due to their function in reducing cooking loss, modification in texture and flavour, retention in water, and lubricant in the meat (Jimenez-Colomenero, 1996; Akoh, 1998). Furthermore, tenderization in village chicken also been influenced by weakening muscle fibre within the chicken. Low proteolytic and less activity in fast growing chicken could reduce the tenderization such as in crossbred village chicken (Dransfield & Sosnicki, 1999). However, the data in this research was contradicted with the research done by Ogunmole *et al.* (2013), who recorded low fibre content in their local chicken (1.96%) and no detection limit in exotic chicken in their research.

5.4.3 Crude fat

CFD fed chickens for purebred (5.5 ± 0.1%) contained the highest crude fat in meat, which showed highly significant difference with FFD fed chickens (3.3 ± 0.8%). However, there was no significant difference of crude fat in crossbred village chicken between CFD (4.9 ± 0.9%) and FFD (5.3 ± 0.6%). The data of crude fat in the meat was inlined with the data for abdominal fat (Table 4.3 and 4.4), where CFD fed chickens for both strains showed significant difference with FFD fed chickens.

The crude fat recorded for purebred village chickens fed on FFD (3.3±0.8%) was approximately similar with crude fat recorded by native chicken from India (3.13 ± 0.35%), as well as lower than broiler from the country (7.57 ± 0.47%) compared with crude fat from crossbred and purebred village chickens given CFD and FFD (Valavan *et al.,* 2016). Nutritional factor was one of the factors that could affect the deposition of fat in poultry (Summers & Leeson, 1979; Fouad & El-Senousey, 2014). The low crude fat of chicken that fed on FFD using palm source was contradicted with Velasco *et al.* (2010), whose recorded high deposition of fat in chicken fed on palm oil source diet. Besides, sex, ages, genotype and environment factors also influenced fat deposition in poultry (Leenstra, 1986). In this study, there were increased in the fat depots compared in village chicken compared with others native chicken that were recorded from previous research. This might be due to the reason of intensive farming system and ad libitum feed given, which could promote the deposition of fat. The Intensive system limits the movement of the village chicken, thus convert the excess feed intake into fat depots (Summers & Leeson, 1979). This was also supported by previous research using Taiwanese Native Chicken, which had been observed that free range chicken contained high crude fat content compared conventional method and purebred village chicken contained similar trend with the Taiwanese Native Chicken, where its crude protein was high as the crude fat lower (Cheng *et al.,* 2008). Furthermore, the fat content in the

jungle fowl also recorded the least fat content as compared with commercial broilers and this was correlated with low development of muscle and heavier bone weight; indicating that poor muscle bone ratio in the broilers compared with jungle fowl (Ganabadi *et al.,* 2009).

5.4.4 Ash

Ash content is important as indicator or measurement of the mineral content within the meat, where mineral such as calcium, phosphorus and potassium present in the meat, thus it is important to know the ash content for meat quality evaluation (Ismail, 2017). In crossbred village chicken meat, ash content in CFD (9.4 ± 1.6%) was significantly higher compared to FFD (5.3 ± 0.4%), meanwhile, there were no significant difference between CFD (6.8 ± 0.3%) and FFD (4.9 ± 0.3%) fed chicken meats in purebred village chicken. CFD recorded higher ash content, which indicated higher mineral content in the meat. However, the data of ash content from the study was high compared to ash content from previous studies using Korean Native Chicken (KNC), Slovenian crossbred and commercial broilers (Al-Najdawi & Abdullah, 2002; Holcman *et al.,* 2003; Probst, 2008; Choe *et al.,* 2010).

The ash content was higher compared to previous study and this could be influenced by the fact that the analysis was done using male village chickens. It was studied that ash content could be affected by genders, where male chicken contained higher ash content compared to female. Furthermore, the ash content from broilers was the result from meat that was reared in short time compared with village chicken. The ash content was also influenced by their age, as the chicken growth, the ash content will also increase (Souza *et al.,* 2011).

5.4.5 Amino Acid Profile in Crossbred Village Chicken

The data of amino acid content in crossbred village chicken indicating the differentiation of amino acid content in village chicken fed on CFD and FFD. Nutrition is one of the factors in the amino acid composition in poultry meat. Amino acid is important for protein utilisation beside of nitrogen content in the feed. Unlike another animal, poultry cannot produce certain amino acids to fulfil the need in the poultry body. There are 10 amino acids that cannot be synthesised in poultry body, which are methionine, lysine, threonine, tryptophan, isoleucine, arginine, leucine, histidine, valine and phenylalanine, which must be supplied in the daily diet for growth and maintenance (Ravindran & Bryden, 1999).

In Table 4.10 showed variable data of amino acid content in crossbred village chicken meat. A significant result was shown for FFD fed village chicken meat for ammonia, serine, arginine, proline, lysine, aspartate, threonine, alanine, methionine, tyrosine, leucine, and phenylalanine compared to CFD chicken. Meanwhile, amino acid such as histidine, glycine, cysteine, valine, glutamate and isoleucine showed no significant difference between CFD and FFD fed crossbred village chicken meat. Arginine is important for blood circulation in arteries at heart, which can prevent from coronary heart disease and in this study FFD fed chicken meat contained significant level of arginine (8.61 ± 0.80%) compared with CFD fed chicken meat (5.62 ± 0.31%). When compared with broilers (white meat) done by Beach *et al.* (1946), amount of arginine (6.91%) was lower than FFD but higher than CFD. Furthermore, arginine content also lower for both CFD and FFD crossbred village chicken's meat compared to meat (pectoralis major) from Thai indigenous chickens (4.58%) (Wattanachant, 2008). Serine is a conditionally essential amino acid that could be changed to essential with certain condition to fulfil the serine demand, which functioning in cell proliferation and important for nerve system (de Koning *et al.,* 2003). Serine in the crossbred village

chicken meat was significantly higher in FFD (9.35 ± 4.10%) compared in CFD fed chicken meat (4.91 ± 3.00%). Obviously, serine in FFD meat was higher compared to Thai indigenous chicken, as well as in broilers (Beach *et al.,* 1946; Wattanachantm 2008).

Meat from FFD fed chickens also has higher arginine content, where FFD fed chicken recorded 8.61 ± 0.80%, significantly higher compared to CFD meat, 5.62 ± 0.31%. This was also similar to proline, lysine, aspartate and threonine, where recorded significant difference between FFD (5.41 ± 0.41%, 2.41 ± 0.62%, 13.52 ± 0.92% and 6.04 ± 0.57%) compared to CFD (3.66 ± 0.47%, 0.16 ± 0.03%, 8.33 ± 0.83% and 4.14 ± 0.50%). Furthermore, alanine, methionine, tyrosine, leucine and phenylalanine also showed significantly higher in FFD (8.24 ± 0.62%, 4.79 ± 0.32%, 10.59 ± 0.79%, 10.50 ± 1.39% and 6.19 ± 0.60%) compared to CFD fed chicken meats (4.77±0.36%, 3.52 ± 0.51%, 5.09 ± 0.58%, 3.89 ± 0.27% and 3.09 ± 0.18%). Most of amino acid recorded in this study contained higher amount of amino acid contents compared to previous research done on Korean native chickens, Thai indigenous chickens, and commercial broilers (Wattanachant, 2008; Choe *et al.,* 2010). Lysine (2.41 ± 0.62%) recorded lower compared to previous study done by Beach *et al.* (1943) and Wattanachant (2008) who recorded 8.44% and 3.41%, respectively. Most of amino acid recorded higher amount of amino acid compared to previous study, this might be due to the condition of the meat that was fresh and dried. Furthermore, the meat used was thigh meat compared to previous study that using breast, drumstick and leg meat. FFD fed chickens from crossbred strain recorded high amino acid content and this was correlated with data of crude protein, which recorded that FFD fed chicken meat contained significant amount of crude protein compared to CFD fed chicken meat. It was stated that at least 90% of amount of amino acid content representing the amount of crude protein presence in poultry (Hunton, 1995). Besides, it was known that village chicken exhibit unique taste

that contributing for their demand and choice by consumers, thus, from this study the amino acid that responsible for taste and flavour in poultry, glutamate was higher in both diets compared to previous research done on broilers, Korean native chicken and Thai indigenous chicken (Farmer, 1999; Wattanachant, 2008; Choe *et al.,* 2010).

5.6 Expression and Activity of Glutathione-S-Transferases From Village Chicken Liver Based on Slaughtering Method

Purification of Glutathione-s-transferases using crossbred village chicken's liver was done to investigate stress endure by the chicken during slaughtering process that could affect the quality of the meat. It was known that manual slaughtering could remove the blood from vein in just few second. However, mass slaughtering could increase human error, especially during slaughtering and the incidence non-proper slaughtering could happen where the veins did not been cut properly, thus slowing the removal of blood and increase time of death. This could induce oxidative stress in chicken and could reduce the quality and shelf-life of the meat as well as exposed the meat to spoilage.

Glutathione-s-transferases (GSTs) was known for their function in countering oxidative stress that was induced by many factors such as heat stress, nutritional factor and slaughtering process. Slaughtering method was studied to contain significant effect in meat quality of poultry (Hafiz *et al.,* 2015). GSTs that conjugated with GSH-dependent peroxidase could prevent oxidative damage. The prevention of oxidative stress was done, where secondary metabolites such as quinones, unsaturated aldehyde, epoxides and hydroperoxides by GSTs (Moon, 2005; Hayes *et al.,* 2005). The expression of GSTs using crossbred liver slaughter using NSSM and SSM could be observed at 25-26 kD on 1st dimensional SDS-PAGE electrophoresis. Hayes *et al.* (2005), stated that GSTs can be seen at molecular weight 20-28 kD. It was known that GSTs derived into six enzyme classes comprised of alpha (GSTα), mu (GSTμ), pi

(GSTπ), theta (GSTθ), sigma (GSTσ), zeta (GSTζ) and omega (GSTω) (Saisawang *et al*., 2012). In 2-Dimensional electrophoresis gel showed that the isoelectric point (p*I)* was high indicating higher pH value of the enzyme's presence. Previous studied also reported that GSTs in chicken liver exhibited optimum pH at 8.8 and could also ranging between 6 to 9 (Yeung & Gidari, 1980; Irzyk & Fuerst, 1993). In current study the pI value of GSTs presence was between 8.6 to 8.9 which was in lined with other studied done by Sun *et al.* (1996), who reported that GSTs in liver of rat was between 8.8 and 8.9.

The GSTs activity showed significant differences in the enzyme activity, total protein content and specific total activity. Apparently, the data of SSM 7.19±0.08 µmol/min/mg) showed significantly higher in specific total activity compared to NSSM (5.57±0.34 µmol/min/mg). thus, it could be assumed that SSM could influenced the production of oxidative stress in poultry, which could be affecting the meat quality and also lowering shelf-life of the meat. The deterioration of meat quality due to oxidative stress was also influenced by the occurrence of lipid oxidation (LOX), which occur due to the slow drainage of blood, thus leaving accumulation of haemoglobin inside the meat (Ali *et al.,* 2011). This deterioration of meat quality could also be affecting the flavour, colour of the meat and texture of the meat (Min & Ahn, 2005). In other research done by Maurice *et al*. (1991), showed much lower total enzyme activity of GSTs (CDNB substrate) at 0.89 µmol/min/mg in Barred Plymouth Rock chicken compared to Malaysia village chicken recorded 6.85 µmol/min/mg done in this research. However, the specific total activity of the enzymes might also have influenced by the environmental condition such as hot climate during the study period. In addition, the usage of crossbred village chicken in the study might also cause the occurrence of oxidative stress within the chicken due to the genetics selection factor (Estevez, 2015; Sihyo *et al.,* 2013). The increase of activity of the enzymes also could be influenced by

the increase of rigorous movement after slaughtering. The removal of the respiratory tract in SSM treated chickens could result in respiratory complication, which could result in the increment of body temperature. Lack of oxygen and increase time of death could result in hypoxia. Hypoxia could influence the increment of reactive oxygen species (ROS) and increase the cytosolic cytochrome *c* as well as result in the oxidation of lipid and protein (Coimbra-Costa *et al.,* 2017).

5.7 General Discussion

The production of village chicken in the country is increasing but it is still slower compared to commercial broilers and this was influenced by few factors such as feed cost, strains, management system, slow growth traits of the village chicken, quality of village chicken meat, and many more. Despite of the constraints, the demand for the village chicken is still high, thus more research should be conducted to increase the performances of the village chicken, as well as to find out a good alternative in term of feed type to reduce the cost and increase the village chicken performance. Most of village chicken farmers used crossbred village chicken instead of purebred village chicken due to their fast-growing trait, as well as feeding their chicken with commercial feed, which is costly and give negative influence in nutritive value of chicken. As for village chicken, commercial feeding resulting the village chicken resemble with commercial broilers in term of weight, fat content and other nutritive value, thus decrease people preference to the commodity, as well as give bad image to the village chicken industry.

The detection of high quality and correct slaughter method of poultry should be emphasized today due to mass production of poultry in the country. Although, the preliminary study only includes method of slaughtering as a study area, but the study has given an insight about study method so that it could be widely used in the area of

study that relating to the evaluation of meat quality and health. This insight also can be used form muslim for evaluating correct slaughtering method according to Islamic way, thus can increase people trust to muslim products and services. Limitation in knowledge, fund and time have become major problem in this kind of preliminary study but the limitation has becoming a good idea for future research.

5.7.1 Growth performances, carcass evaluation and meat quantity of village chicken fed CFD and FFD

Crossbred village chicken growth faster compared to purebred village chicken and CFD showed a better result in term of body weight compared to FFD fed chickens. The slow growth of the purebred line was largely due to the genetics factor (Ajuyah, 2013; Francis *et al.,* 2016; Khawaja *et al.,* 2016). Despite that, FFD showed more potential when fed by crossbred village chicken as lower FCR had been recorded (2.6) compared in purebred village chicken that recorded high FCR value (5.2). This indicates the ability of the village chickens to utilise the feed and in crossbred village chicken the difference of FCR between CFD and FFD chickens was lower. Generally, the body weight for male CFD for both strains dominating the result but female crossbred village chicken recorded no significant differences between CFD and FFD fed chickens. Despite of FFD fed chicken have lower body weight compared to CFD fed chicken, but the abdominal fat showed significantly lower compared to CFD fed chicken, especially in male chickens for both strains. The high demand of the village chicken was largely influenced by their good nutritive value and unique taste (Ahn *et al.,* 1997; Musa *et al.*, 2006). The low abdominal fat recorded for FFD fed chicken has become a beneficial advantage to the feed as on if it speciality, thus could increase consumer preference.

The carcass weight showed significantly heavier in CFD fed chicken especially in crossbred village chicken, while no significant difference in purebred village chicken

recorded at 16th Week of age compared with FFD fed chicken. The low efficiency of FFD to utilise the nutrient to be meat was due to the high fibre content in the diet, where it could result in reduction in body weight gain if given in non-considerate amount (Lumpkins *et al,* 2004; Loar *et al.,* 2010; Walugembe *et al.,* 2014), which in this study the large amount of fibre was due to the usage of palm kernel cake (PKC) in the FFD and the high usage of PKC can cause toxicity, only 20% can be said considerate amount to be used in poultry diet (Alimon, 2004). Although, in the study the percent of PKC used was lower than 20%, but inefficiency in mixing the grains daily might influence the amount of PKC in the diet, thus affecting the digestibility and palatability of the diet. However, it is an interesting finding, where the amount of meat quantity produces showed no significant differences between CFD and FFD fed chickens in both strains, despite of CFD fed chicken showed significant heavier compared with FFD fed chicken, especially in crossbred village chicken. Moreover, the study also showed high percentage in meat to bone percentage in FFD fed chicken compared to CFD fed chicken. This indicating that FFD could utilise the production of meat more compared to CFD, while CFD utilises their nutrient by converting the nutrient into non-essential aspect such as bone. Furthermore, it could be said that the high weight recorded in CFD fed chicken might be due to the amount of fat and bone mass.

5.7.2 Relationship between proximate and amino acid analysis in village chicken

There was a relationship between the data recorded in the previous sub-chapter with proximate and amino acid profile recorded in this research. It could be observed through the data from the meat to bone percentage, where FFD fed chicken recorded higher percent in meat to bone compared to CFD fed chicken and through proximate analysis done it could be observed that the crude protein in the meat was significantly higher in FFD compared to CFD fed chicken meat, especially crossbred village chicken.

Moreover, it was an outstanding result as analysis of amino acid profile indicating FFD fed chicken in crossbred village chicken have higher nutrition value, where the amino acids amount in the meat were higher compared in CFD fed chicken. There were 12 amino acid that showed significantly higher in FFD fed chicken compared with CFD fed chicken, where the amino acids were ammonia, serine, arginine, proline, lysine, aspartate, threonine, alanine, methionine, tyrosine, leucine, and phenylalanine.

From the study, it could be said that FFD could produce high quality of village chicken, where the nutrition value was higher compared to the CFD fed chicken. Although, the FCR is less than CFD, but the differences in crossbred village chicken was lower and showed probability for improvement in term of digestibility and palatability of FFD. Furthermore, glutamate that responsible for the village chicken unique taste was higher in chicken that fed on FFD compared with CFD (Fujimoto *et al.,* 1996; Farmer, 1999; Wattanachant, 2008) and it was also higher compared to broiler and other countries native chicken, thus it could be a good aspect to increase consumers preference to village chicken meat. The crude fat also lowers in the FFD, especially in purebred village chicken, where significant differences recorded. Meanwhile, higher crude fibre recorded in purebred fed on FFD compared to CFD fed chicken. Data from this research showed higher crude protein but lower in crude fat compared with commercial broilers (Ogunmola *et al.,* 2013). The high amino acids content in the meat is important to ensure high quality of meat and for indicative of crude protein content, Hunton, (1995), stated that 90% of the amino acid amount reflecting the amount of crude protein within poultry meat. The percentage of meat to bone of CFD was higher compared in FFD fed chicken and this can be supported by the data from ash content, where the ash content recorded significantly higher in CFD fed chicken compared with FFD in crossbred village chicken.

5.7.3 Stress detection using proteomic approach

The preliminary study on the effect of slaughtering method on the expression of GSTs enzyme showed significant differences in specific total activity, where SSM contained significantly higher specific total activity compared to NSSM chicken in the liver. Furthermore, this research also managed to locate and purified GSTs enzyme at 25-26 kD. 2-Dimesnional SDS-PAGE electrophoresis is able to locate the differences expression of different classes of expression based on spot present on the 2D gel. There were obvious differences in term of quantity and intensity of the spot presence between NSSM and SSM, where SSM spot more intense compared to NSSM, however, more research should be done in this aspect to specifically discussed in this matter.

5.8 Constraints and Future Direction

As the production of village chicken increase in Malaysia, more study has been conducted to investigate the best diet in poultry and many constraints face by the researcher. The constraint that had been encounter during this research such as:

a) Facilities: the analysis for proximate and amino acid profile need to be done at Veterinary laboratory in Bandar Salak Tinggi due to limitation of facilities in the campus.
b) Work load: the study requires physical work such repairing the chicken house, feeding and feed preparation that require time and energy to be done.
c) Uneven feed mixing: the village chicken having problem in utilising the feed due to uneven mixing that had been done manually using hand.
d) Optimisation time: the proteomic method needs to be optimised according to sample and objectives, thus took more time to locate the best optimisation method.

Even though the research face few of constraint but this research has been succeed to locate new findings, as well as has open new ideas and research methodology for future research, which could be suggested in future studies:

a) To use other than PKC or treated PKC with an enzyme to increase their digestibility and palatability in poultry.
b) To compare the growth performance, carcass evaluation, proximate and amino acid content with commercial broilers.
c) Using the optimisation done in this research to analyse the expression of GSTs enzyme various organs in the poultry with variation of treatment such as heat stress, slaughtering method, and pre-slaughter handling.
d) The classes of GSTs enzyme from study in future should be investigated more about their properties and function.

CHAPTER 6: CONCLUSIONS

This research was conducted to identify the potential of using local feedstuffs (PKC and coconut meal cake) in poultry formulation diet focusing more for village chicken industry. Furthermore, this study also showed specifically review on village chickens' performances in term of growth, carcass and feed proximate as well as meat proximate data when fed with the 2 types of diet (CFD and FFD). It can be concluded that FFD could be used in poultry diet and reducing the usage of commercial feedstuffs such as corns and soybean meal, thus could reduce the cost of the feed itself. FFD contained few characteristics based on the data from the study that can be contributed efficiently compared with CFD in the village chicken's industry, where it can be concluded specifically as follows:

a) FFD in village chickens resulted in low subcutaneous and abdominal fat based on the carcass evaluation and proximate analysis in meat compared to CFD.

b) Village chickens that were fed on FFD develop significantly higher crude protein and ash for crossbred village chickens, as well as significantly higher in crude fat and fibre content in the meat.

c) FFD in village chickens also developed more meat compared to bone based on meat to bone percentage, as well as no significant difference was shown in meat production at Week 16 for purebred and Week 12 for crossbred village chickens.

d) FFD in village chickens recorded significantly higher amount in 12 amino acids in the meat, indicating a high quality of meat.

e) There was an apparent increase in FFD village chicken's meat glutamate content that is responsible for taste in meat, which can be a good aspect for good taste in village chickens.

f) FFD in village chickens can cut at least 30% of feed cost compared to CFD village chickens, as well as can be located, available and accessible easily in the country.

The preliminary study on the effect of slaughtering method on the expression of Glutathione-s-Transferases could draw to the following conclusions:

a) There was significant amount of GSTs specific enzyme activities in SSM slaughtered village chicken's livers compared with NSSM slaughtered village chicken's liver. Thus, the technique could be developed more to be used in poultry industries for better production.

b) Purification of GSTs was a success where GSTs expression could be detected at 25-26 kD on SDS-PAGE gel.

c) There were also differences in expression of GSTs enzyme on 2-Dimensional electrophoresis, indicating differences of GSTs classes expression.

In a nutshell, FFD in village chickens has the potential to provide alternative choice to the commercial feed, however it could be refinely researched before it could recommend being used in village chicken routinely in the future. In addition, even though the present findings of FFD in village chickens apparently did not influence the body weight similar to CFD, the village chicken could produce high quality meat that contains a high nutritive value, which could be a value-add for marketability and consumer's preferences, particularly in ASEAN countries.

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LIST OF PUBLICATION AND PAPER PRESENTED

Manuscript published

1. **Mazlishah, M. S. H**., Alias, Z., Khadijah, W. E., and Abdullah, R. B. (2018). Effect of palm kernel cake and coconut-based formulated diet on Malaysia Village Chicken growth performances and meat quality. *Journal of Tropical Agricultural Science, 41*(4), 1703-1716.

Paper presented

1. Production of village chicken using local feed sources. *Halal Research University of Malaya (HARUM), September 26 2016, Kuala Lumpur*.