
**UNIVERSITI PUTRA MALAYSIA**

***COMPARATIVE STUDY OF THE PESTICIDES AND ANTIBIOTICS RESIDUE IN DUCK MEAT REARED IN CLOSED HOUSE AND OPEN HOUSE FREE RANGE SYSTEM***

**NUR SYAHIRAH BINTI MOHD TAHAR**

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A project paper submitted to the Faculty of Veterinary Medicine, Universiti Putra Malaysia in partial fulfilment of the requirement for the DEGREE OF DOCTOR OF VETERINARY MEDICINE Universiti Putra Malaysia Serdang, Selangor Darul Ehsan

MARCH 2015

## CERTIFICATION

It is hereby certified that I have read this project paper entitled "Comparative Study Of The Pesticides And Antibiotics Residue In Duck Meat Reared In Closed House And Open House Free Range System" by Nur Syahirah binti Mohd Tahar and in my opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999

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## DEDICATIONS

This final year project is dedicated to my beloved family, my supervisor, housemates, classmates, all lecturers and friends that were involve either directly or indirectly in this project

## ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Lokman for his guidance, time and support in this study. I will not have been able to get through this project without his help and guidance. Towards the staff at Makmal Kesihatan Awam Veterinar Salak Tinggi, I send the greatest thanks for their excellent assistance throughout this study. I also thank Perak Duck Food Industry Sdn. Bhd. for their generous and kind heart in providing me the samples as well as a trip to visit their personnel's. I extend my thanks and appreciation to my co-final year project Miss Nur 'Izzaty Halil for her help.

For always believing in me and encouraging me to do the best I can do, I am ever thankful to my family and friends whom although so far away were always behind me. Last but not least, thank you to all my friends class DVM 2015 who has helped me in a way or another I will be forever grateful.

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## ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project

**COMPARATIVE STUDY OF THE PESTICIDES AND ANTIBIOTICS RESIDUE IN DUCK MEAT REARED IN CLOSED HOUSE AND OPEN HOUSE FREE RANGE SYSTEM**

**by**

**NUR SYAHIRAH BINTI MOHD TAHAR**

**2015**

**Supervisor: Dr. Lokman Hakim Idris**

This study compares on antibiotics and pesticides residue in commercial Pekin duck meat from closed house and open house free range system. A total of 16 matured ducks using same type of commercial diet with 8 from each different rearing system were subjected to this study. The antibiotic and pesticides residue experiment was

conducted using six plate test method, ELISA method and QuEChERS method respectively. Six plate test method for screening of qualitative detection of a group of antibiotics in animal tissue; macrolides, aminoglycosides, tetracyclines, sulfonamides, B-lactam and quinolones shows negative result (<2mm or no annular zone around tissue disc) Meanwhile, ELISA method for banned drugs; Chloramphenicol, Nitrofuran, Beta-agonist show negative for all samples. QuEChERS method using GC-ECD/GC-MS for pesticide residue; also shows the negetif result. Thus, the different rearing method does not contribute to the unnecessary usage of antibiotic; either the duck were affected by the pesticides residue subjected to the ponds in open house free range system.

Keyword: *Pekin duck, Closed house, Open house free range, Antibiotics, Pesticides.*

# ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir.

**KAJIAN PERBANDINGAN DARIPADA SISA RACUN PEROSAK DAN SISA ANTIBIOTIK DALAM DAGING ITIKYANG DITERNAK DI SISTEM REBAN TERTUTUP DAN SISTEM REBAN TERBUKA**

**oleh**

**NUR SYAHIRAH BINTI MOHD TAHAR**

**2015**

**Penyelia: Dr. Lokman Hakim Idris**

Kajian ini membandingkan sisa antibiotik dan racun perosak dalam sisa komersial itik Pekin dari sistem reban tertutup dan sistem reban terbuka. Sejumlah 16 itik matang diberikan diet komersial jenis yang sama dengan 8 dari setiap sistem penternakan yang berbeza dipilih untuk kajian ini. Eksperimen sisa antibiotik dan sisa racun perosak telah dijalankan menggunakan kaedah ujian enam plat, kaedah ELISA dan kaedah QuEChERs. Kaedah ujian enam plat digunakan untuk pemeriksaan

pengesanan kualitatif sekumpulan antibiotik dalam tisu haiwan; macrolides, aminoglycosides, tetracyclines, sulfonamides, B-Laktam dan quinolones menunjukkan keputusan negatif (<2mm atau tiada zon anulus di sekeliling cakera tisu) Sementara itu, kaedah ELISA digunakan untuk dadah terlarang; Chloramphenicol, Nitrofuran, Beta-agonist menunjukkan negatif untuk semua sampel. Kaedah QuEChERS menggunakan GC-ECD / GC-MS untuk residu racun perosak; juga menunjukkan hasil yang negetif. Oleh itu, kaedah penternakan yang berbeza tidak menyumbang kepada penggunaan antibiotik yang tidak diperlukan; hal ini juga sama bagi itik yang diternak di reban terbuka yang terdedah kepada sisa racun perosak.

Kata kunci: *Itik Pekin, Sistem reban tertutup, Sistem reban terbuka, Antibiotik, Racun Perosak.*

# 1.0 INTRODUCTION

## 1.1 Study background

Poultry sector is an integral part of the livestock industry in Malaysia. There were 9 meat duck breeding companies with a total of 20 farms in operation in Penisular Malaysia in 2012. The breeds used were Cherry Valley, Pekin and Muscovy. The subject of this study is Pekin duck (*Anasplatyrhynchos domestica)* and used primarily for egg and meat production. The interest for this study is due to the presumption that in an open house free range system, the duck was more prone to disease due to contact with other wild birds and free access to the contaminated ponds. The farm chosen for this study has two rearing method which is open house free range system and closed house system. The open house free range system and closed house system has up to 10000 ducks populations. Majority of the duck production were exported to the Singapore and the company will be exporting to the Japan in the near future after they stopped exporting since 2007 due to Avian Influenza outbreak in Malaysia. Small number of cases of drugs and medications in excess of set MRLs detected as the poultry industry in Malaysia in a transitional state. Thus, this study will address this issues as well as the presence of pesticides residue due to free access to the ex-mining pond. The use of antibiotics to bring about improved performance in growth and feed efficiency, to synchronize or control of reproductive cycle and breeding performance also often lead to harmful residual effects (Nisha, 2008). On another hand, organochlorine pesticides accumulate in the environment. Organochlorine are very persistent and move long

distances in surface runoff or groundwater. This pesticide may end up in the ponds and affect the animal population within the area.

**1.2 Justifications**

i. The production of duck is for exportation to Singapore and Japan. Thus, it is important for us to closely monitor the quality of our product.

ii. Limited studies done on ducks in Malaysia especially on the antibiotics and pesticides residue.

iii. Antibiotic and pesticide residue was a threat to human health as well as to animal, and can disturb the duck productivity.

**1.3 Study Objectives**

i. To measure the contents of pesticides and antibiotics residue in duck meat

ii. To compare the food safety in duck meat reared in closed house system and open house free range system

**1.4 Hypothesis**

i. There will be higher antibiotic and pesticide residue presence in duck meat reared in open house free range system compared to duck meat reared in closed house system

## 2.0 LITERATURE REVIEW

### 2.1 The duck rearing

Small-scale duck production makes a significant contribution to household economies and food security, especially in developing countries. In addition, it also contributes to improving the nutritional status of the rural poor. Although chickens dominate the poultry egg and meat sector, in some parts of the world, especially Asia, significant amounts of meat and eggs are produced from ducks. (Dinesh, 2008).Rearing of ducks gives maximum return with minimum cost. Ducks are efficient converter of agricultural by- products; kitchen wastes, seeds, grains, garden left over, insects, green grasses and all other human refusal that would otherwise wasted. Ducks occupy second place in comparison with chicken in producing meat and egg in the country. Ducks are traditionally raised under scavenging (Salahuddin et al., 1991) by the smallholders in coastal and low-lying areas, with little or no feed supplementation.

Commercial duck meat farms are intensive operations similar to chicken meat farms. Ducks are raised in sheds which vary from open-sided naturally ventilated sheds to fully enclosed climate controlled tunnel ventilated houses. Commercial duck meat production is therefore a full-time specialised business requiring significant investment in both time and money. Whilst the duck meat industry is quite small in comparison to chicken meat production, it is expanding rapidly at a growth rate of 10-15% annually. The size of commercial farms is typically measured by the number of birds reared at any one time. Commercial operations vary in size from relatively small farms of 6,000 ducks

per batch to large operations with 50,000 to 100,000 ducks per batch. Most current commercial duck farms house between 10,000 to 50,000 birds at a time (Stein, 2012).In Malaysia, duck commonly were reared semi-intensively, but as for now we have 4 closed house system for duck rearing in Malaysia (DVS,2013)

**2.2 Chemical residue in duck meat**

The antibiotics nowadays used for improved performance in growth especially in broilers andfatteners. They may produce improved growth rate because of thinning of mucous membrane of the gut, facilitating better absorption, altering gut motility to enhance better assimilation, producing favorable conditions to beneficial microbes in the gut of animal by destroying harmful bacteria and partitioning proteins to muscle accretion by suppressing monokines. Antibiotics also favour growth by decreasing degree of activity of the immune system, reduced waste of nutrients and reduce toxin for mation. In most of the cases only young growing animals and poultry are responsive to antibiotic mediated growth promotion. Indiscriminating use of antibiotics in all cases of pyrexia, inflammation, wounds and viral diseases have widespread residual effects on edible tissues. The use of antibiotics only in specific conditions is justified because the roll of microbial agents is mainly to kill the rapidly dividing invading cells. Animals and poultry are receiving sub therapeutic levels of antibiotics to prevent possible infection. But the antibiotics are specific to their. spectrum of activity only in the active multiplying stage of bacteria. But it will not provide overall protection. Only in certain cases like dry cow therapy and surgical

procedures are wanting of antibiotic prophylaxis. Antimicrobials are used either directly or indirectly during the production processing and storage of milk and milk products. Direct contamination of milk may occur from air and water during processing, storage and transportation. Besides feed given to animals is also source of indirect contamination. Man will be the ultimate consumer of these antibiotic residues. There are some causes of miscellaneous use like lack of awareness, lack of extension activities, inadequate literature supplied by manufacturers, lack of safer drugs and exploitation of more production and profit from animals. FDA prohibits the extra label use of chloramphenicol, furazolidone, nitrofurazone, sulphonamide drugs, and flouroquinolones in lactating animals (Nisha, 2008)

Organochlorine pesticides (OCPs) are synthetic, non-polar, toxic and environmentally persistent dichlorodiphenylethanes, cycodienes or chlorinated benzenes that are used for pests control. Synthetic pesticides were introduced into the Nigerian markets in the 1950s (Adeyeye and Osibanjo, 1990) and farmers have been using them for control of weeds; weevils of cotton, beans and cereals; borers of plant stalks and yams; and disease vectors like tsetse flies and mosquitoes. The persistent nature of the OCPs is advantageous for the control of pests such as termites around buildings. Pesticides applications as enumerated above have brought about increased output of agricultural products and better life quality generally. The use of pesticides and copper fungicides for the treatment of diseases by farmers over the years would no doubt have led to the accumulation of their residues in soils, water and fish of the agricultural areas, as have been reported elsewhere in literature

(Merry et al., 1983; Osibanjo and Bamgbose, 1990; Akinnifesi et al., 2006). However, the challenges of environmental degradation with its associated health problems provoked by the ubiquitous OCPs are daunting owing to their persistent nature and high lipid solubility. In turn, these factors have led to the problems of bioconcentration and biomagnification of the OCPs in animal tissues that live within the environment where the OCPs are found. For example, in the aquatic environment, OCPs are not readily metabolized and excreted by fish hence, they "biomagnify" up the food chain. When pesticides reach water bodies, they are rapidly absorbed and accumulated by the bottom sediment, plankton, algae, aquatic invertebrates, aquatic vegetation and fish. Ecological effects of OCPs include interference with reproductive success of organisms high on the food chain, especially fish eating birds such as osprey, pelicans, falcons and eagles. OCPs especially DDT have estrogenic and enzyme inducing properties. The adverse effects of DDT demonstrated in experimental animals include infertility (Jonsson et al., 1975), a decrease in the number of implanted ova (Lundberg, 1974), intrauterine growth retardation (Fabro et al., 1984), cancer (Cabral et al., 1982; Muino et al., 1995), neurologic developmental disorders (Eriksson et al., 1990; Muino et al., 1995) and mortality (Clement and Okey, 1974). Ortho and para isomers of DDT compete with estradiol for binding to estrogen receptors in uterine cytosol thus causing changes in steroid metabolism and alter the ability of birds to mobilize calcium to produce strong egg shells (Close, 1996; Allen and Otis, 1998; Cohn, 1999; Creekmore et al., 1999; O'Shea et al., 2001). This has led to the prescription of tolerances such as

maximum residue level (MRL) and no observable adverse effect level (NOAEL) for various pesticides in food and water, especially by the Codex Alimentarius Commission (CODEX, 2004).

## 3.0 MATERIALS AND METHODS

### 3.1 Ducks

The sampling was done at Perak Duck Food Industry farm located at Kampung Nibung, Temerloh Trong, Taiping, Perak. Eight sample of duck carcass were taken from each housing system, the open house free range and the closed house. The total of 16 Pekin duck is from the strain Cherry Valley (CV2000). Random sampling was done at the slaughter house whereby the sex of the duck was not included in selection criteria. All of the ducks from both system were fed commercial poultry feed. Prior to the slaughtering, all ducks received similar pre slaughter treatment, as they were relaxed and fasted for 24 hours but the water was provided. All of the ducks were slaughtered at $54^{th}$ day through halal slaughtering procedure.

### 3.2 Experimental Design

The sample carcasses from the slaughtering house were used for the experiment to be conducted in MakmalKesihatanAwamVeterinarSalakTinggi. Prior to the study, the carcass were stored at -25°C for 24 hours before the experiment was conducted. The frozen sample of the breast muscle, pectoralis major was used for the screening test and

the rest of the muscle was minced using food processor for ELISA test and pesticides residue determination test.

### 3.3 Screening for antibiotic using six plate method

This method uses 6 media that had been cultured separately with different species of bacteria at certain pH level. The test was carried out as agar diffusion test in which the utilization of bacteria that are relatively sensitive or resistant to a particular class of antibiotic. The media prepared for this test contains nutrient that help to enhance the growth and multiplication of bacteria rapidly in 18 to 24 hours of incubation period. The six group of antibiotics which can quantitatively be identified from various tissue sample with this method are: macrolides, aminoglycosides, tetracycline, sulphonamides, β-lactams, and quinolones. In this method, the sliced meat sample was placed on the media inoculated with *Bacillus cereus* spore ( at pH 6.0 ), *Bacillus subtilis* spore ( at pH 6.0, pH 7.2, pH 8.0 ), *K. rhizophilla* ( pH 8.0 ), *Escherichia coli* ( pH 8.0 ). Trimethoprime were added in pH 7.2 media to increase sensititvity detection of sulphanomide residue. This method apply the principle of observation microbial inhibition zone created by diffusion of antibiotics from the test sample onto the bacterial cultured agar plate.

**Figure 1: Bioassay dish illustration**

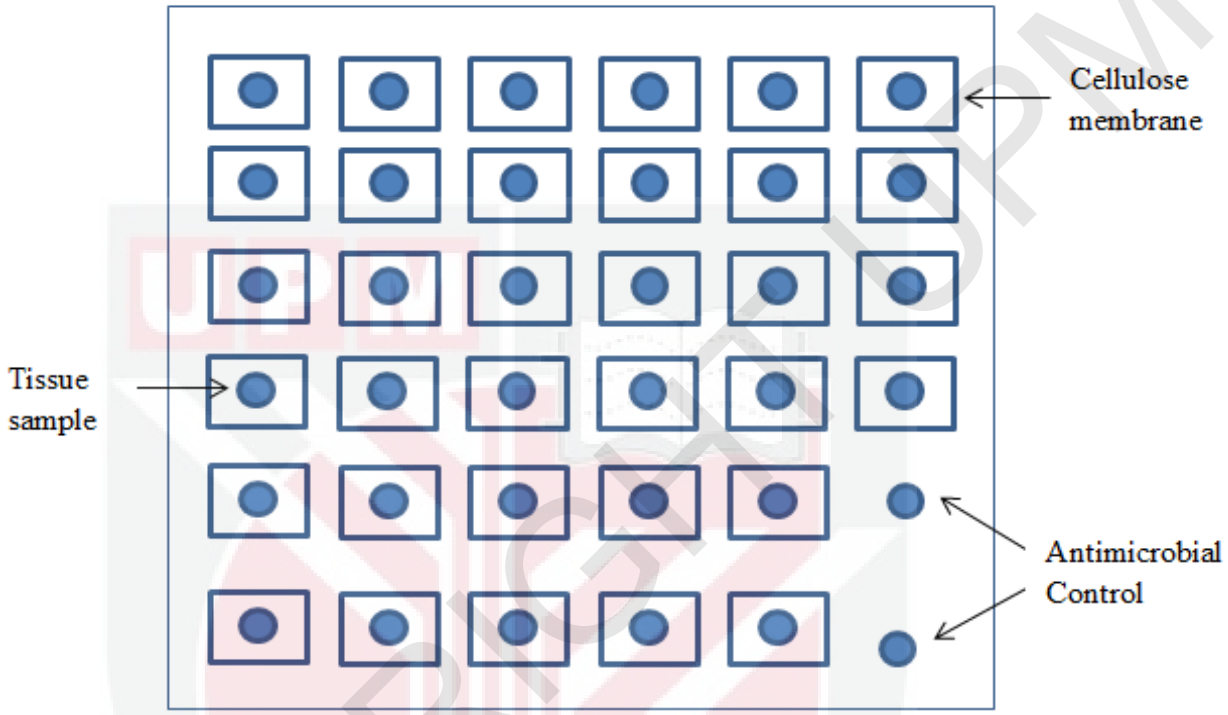

**Table 1: Determination of antibiotic group based on the positive(+ve) / negative (-ve) result of sample on agar plates**

| | *B. subtilis* pH 6.0 | *B. subtilis* pH 7.2 | *B. subtilis* pH 8.0 | *K.rhizophilla* pH 8.0 | *B. cereus* pH 6.0 | *E. coli* pH 8.0 |
|---|---|---|---|---|---|---|
| Sulphonamide | | +ve | | | | |
| Aminoglycosides | | -ve/+ve | +ve | | | |
| Macrolide | | | | +ve | | |
| Tilmicosin | -ve | | | +ve | | |
| Tetracycline | +ve | | | | +ve | |
| B-lactam | +ve | | | +ve | | |
| Penicilin | +ve | +ve | +ve | +ve | | |
| Quinolone | | | | | | +ve |

### 3.4 Screening for Banned Drugs using ELISA method

ELISA method was performed using ELISA Kit. The principle for this method is the direct competitive enzyme-linked immunosorbent assay (ELISA) which operates on the basis of competition between the drug/metabolite in the sample and the drug enzyme conjugate for the limited number or specific antibody binding sites. The procedure for this method:

1. Drug in the tissue samples is first derivatised with 2-nitrobenzoic aldehyde.
2. Extraction method was done by ethyl acetate to increases the sensitivity and specificity of the test.
3. Followed by clean-up step involving the use of iso-octane.
4. The plate was then read using micro plate reader.

The test kit is easy to use, highly specific, cost effective screening method. The measurement of drug residues in various matrices in urine, serum, meat, feed, milk, honey, feather, hair, egg and retina. It has intra and inter assay precision typically <10% CV. The test kit also has a lower set-up costs as no expensive equipment required. The kit works faster , which is up to 80 samples can be analysed in 2 to 3 hours with ELISA.

### 3.5 Organochlorine determination using GC/MS Method

Gas chromatography–mass spectrometry (**GC-MS**) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. In this study, the sample was tested for organochlorine pesticides. The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. AÒlibraryÓ of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector so far. The QuEChERS Procedure for this method were as follow:

1. Five gram of sample weighed in 50 ml centrifuge tube, five ml acetonittrile was added and shake vigorously
2. Buffer solution was added and the tube was shake vigorously
3. The tube was centrifuged at 3000rpm for 5 minutes

4. One ml of supernatant solution transferred to two ml dSPE tube that contain salts and mixed well. Centrifuge at 3000rpm for five minutes
5. 500 microlitre supernatant was transferred to GC vial and 5% formic acid was added.
6. It was then analyzed using GC-MS.

## 4.0 RESULTS

### 4.1 Screening for antibiotic using six plate method

The total of 16 samples, 8 from the closed house system and another 8 from open house free range system, the samples was tested negative.

### 4.2 Screening for Banned Drugs using ELISA method

The total of 16 samples, 8 from the closed house system and another 8 from open house free range system, the samples was tested negative.

### 4.3 Organochlorine determination using GC/MS Method

The total of 16 samples, 8 from the closed house system and another 8 from open house free range system, the samples was tested negative.

## 5.0 DISCUSSION

### 5.1 Antibiotic and Pesticides residue in duck meat rearedin ClosedHouse versus Open House Free System

In the open house free range system, there is an increased risk of disease due to contact with droppings and contact with wild birds. Meanwhile in closed house system, there is relatively low risk of disease and parasitism associated with better hygiene and biosecurity. From this understanding, we might think the duck reared in open house free range system will be introduced to more drugs usage, i.e; antibiotics. The screening of poultry meat showed detectable levels of antibiotic residues which may indicate the widespread misuses of antibiotic, but in this study we can said that there are no usage of antibiotic at all or the farmer follows the recommended withdrawal periods of drugs.

Duck reared at open house free range system has an open access to the pond (ex-mining pond). Our concern are the continuing rapid usage of pesticide lead to pesticide contaminant accumulation in the environment but from our result there is no pesticide residue found in tissues sample. Thus we can conclude that the ponds are not contaminated with pesticide residue

### 5.2 Risk factors of antibiotic and pesticides residual

Non-observence withdrawal period is the most common factor that lead to antibiotic residue. The usage of antibiotics was known in poultry industry. Many antimicrobial agents are used in farm animals for therapeutic and prophylactic purposes,

and as a result of this application, trace quantities of the drugs or their metabolites may be present as residues in food. The residues of these substances or their metabolites in meat and other foods of animal origin may cause adverse effects on consumers' health (Moreno-Bondi et al., 2009). The common use of antimicrobial agents for therapeutic and nontherapeutic purposes has been mentioned as a reason for the occurrence of antibiotic resistance among human beings (Ghosh and LaPara, 2007). The withdrawal period must be observed carefully as this is important before the meat is marketed. When antibiotics are used in livestock and poultry production, strict withdrawal periods must be followed before the animals are processed for foods. The U.S. Department of Agriculture (USDA) monitors meat and poultry to ensure that in the unlikely event that antibiotic residues are present, they do not exceed the tolerance levels deemed unsafe by FDA and USDA. The withdrawal period approved for antibiotic is two weeks before the slaughtering. Thus, it is important to carefully observe the period after administration of antibiotics.

The exposure to the contaminated ponds with pesticides will be another factor that might lead to the chemical residue in meat. Organochlorine(OC) pesticides are among the agrochemicals that have been used extensively for long periods. They have been used widely in agriculture, as well as, in mosquito, termite and tsetse fly control programs (Guo, 2008) OC pesticides are characterized by low polarity, low aqueous solubility (lipophilicity) and as a result they have a potential for bioaccumulationin the food chain posing a great threat to human health and the environment globally (Afful, 2010)

The misuse or overuse of antibiotics also results in chemical residue in duck meat. There are some causes of misuse like lack of awareness, lack of extension activities, inadequate literature supplied by manufacturers, lack of safer drugs and exploitation of more production and profit from animals (Nisha, 2008). The responsibility for residue control and prevention cannot lie solely within a governmental agency; rather the responsibility must be shared by the government, producers, veterinarians, teachers and academicians, marketing associations, and other interested parties, who must strive for both healthy and efficiently grown animals as well as a safe food supply (Hisham, 2013)

There is no antibiotic residue found in the duck meat produced found in this farm as the company strictly follow the export regulation of antibiotic free residue duck meat. Instead of using antibiotics as prophylactic drugs, this farm practice a good hygiene and strict biosecurity to reduce risk of disease.

## 6.0 CONCLUSION

In conclusion, there are no significant difference for antibiotic and pesticide residue presence in duck meat reared in open house free range system and duck meat reared in closed house system

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