EFFECT OF VITAMIN E AND C ON SEMEN CHARACTERISTICS, EGGS FERTILITY AND HATCHABILITY IN VILLAGE ROOSTERS

MOHAMAD ASROL BIN KALAM

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER OF AGRICULTURE SCIENCE

FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2018

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: **EFFECT OF VITAMIN E AND C ON SEMEN CHARACTERISTICS, EGGS FERTILITY AND HATCHABILITY IN VILLAGE ROOSTERS**

IJAZAH: **SARJANA (PENGAJIAN BAHASA INGGERIS)**

Saya **Mohamad Asrol Bin Kalam**, Sesi **2015/2017**, mengaku membenarkan tesis Sarjana ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis ini adalah hak Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/):

☐	Sulit	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)
☐	TERHAD	(Mengadungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☑	TIDAK TERHAD	

NURULAIN BINTI ISMAIL
PUSTAKAWAN KANAN
UNIVERSITI MALAYSIA SABAH

MOHAMAD ASROL BIN KALAM
MR1511002T

(Tandatangan Pustakawan)

Tarikh: 22 Mac 2018

(Prof. Dr. Abdul Rashid Bin Baba)
Penyelia

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

22nd March 2018

.....

Mohamad Asrol Bin Kalam
MR1511002T

CERTIFICATION

NAME	:	**MOHAMAD ASROL BIN KALAM**
MATRIC NO.	:	**MR1511002T**
TITLE	:	**EFFECT OF VITAMIN E AND C ON SEMEN CHARACTERISTICS, EGGS FERTILITY AND HATCHABILITY IN VILLAGE ROOSTERS**
DEGREE	:	**MASTER OF AGRICULTURE SCIENCE (LIVESTOCK PRODUCTION)**
VIVA DATE	:	**23rd January 2018**

CERTIFIED BY;

SUPERVISOR
Prof. Dr. Abdul Rashid Bin Baba

SIGNATURE

ACKNOWLEDGEMENT

Alhamdulillah, all praises to Allah for His willing and blessing in completing this thesis. Special appreciation goes to my supervisor, Prof. Dr. Abdul Rashid bin Baba for his supervision and constant support of my thesis project, for his patience, motivation and immense knowledge. His valuable guidance helped me in all the time of process in completing this thesis.

Besides that, my sincere thanks also go to all the laboratory assistants and agriculture officer for helping and giving me the co-operation in preparing the chemicals, instruments and facilities that needed for the experiment.

My best regards to Universiti Malaysia Sabah (UMS) for their assistance in completing this study and allowing me to access the laboratory and facilities in UMS. Last but not least, I would like to thank my family members (Kalam bin Alih, Liyana Abdullah, Mohammad Asrin bin Kalam and Nor Asmida bin Kalam) and colleague for their moral supports and providing me with valuable information sources. Thank you for the unconditional love, supports and sacrifices.

Mohamad Asrol bin Kalam
22nd March 2018

ABSTRACT

The aims of this project were to study effect of vitamin E and C on semen production characteristics and fertility in village roosters. This study was conducted from February 2016 to February 2017 at the Faculty of Sustainable Agriculture (FSA), University Malaysia Sabah. First objective of the experiment was to determine the effect of vitamin E or vitamin C supplementation (in vivo) on semen quantity and quality of village roosters. Semen characteristics (sperm gross motility, percentage of live sperm and colour score) after week 4 of supplementation with vitamin E were significantly higher (6.20 ± 0.49, 81.68 ± 2.41 and 2.80 ± 0.13, respectively) in T2 group (P <0.05). Percentages of abnormal tail spermatozoa were significantly (P<0.05) lower in (3.84 ± 1.14) T2 group after supplementation of vitamin E. Gross motility (4.00 ± 0.00) was significantly (P<0.05) higher in group supplement with 4 mg/L vitamin C compared to control groups. Percentage of abnormal head (3.00 ± 1.04) in group that supplemented with 4 mg/L vitamin C was significantly (P <0.05) lower than T0 (0 mg/L) and T1 (2 mg/L) groups. From this study, it can be concluded that higher supplementation of dietary vitamin E (2 IU) or vitamin C (4 mg/L) is beneficial in improving the semen characteristics of village roosters after 4 weeks of supplementation. Second objective was to determine the effect of addition of vitamin E or vitamin C in semen diluent (in vitro) on sperm characteristics undergoing the chilling process in the village roosters. According to the results of this study we conclude that, the most excellent level of vitamin E for addition to the semen diluent of rooster in order to improve the sperm motility and viability and also to reduce the sperm abnormalities of the sperm up to 36 hours cooling time at 5 °C is 2 IU. The most excellent level of vitamin C for addition to the semen diluent of rooster to improve the sperm motility and viability and also to reduce the sperm abnormalities up to 24 hours cooling time at 5 °C is 1% (w/v). Third objective was to determine the effect of vitamin E or vitamin C supplementation (in vivo) on fertility and hatchability after artificial insemination. Fertility and hatchability were increase when the village roosters were supplemented with vitamin E (200 and 400 IU) in their diet in 4 weeks and only fertility was improved the village roosters supplemented with 2 mg/L of vitamin C in the drinking water. The last objective was to determine the effect of addition of vitamin E or vitamin C in semen diluent (in vitro) on fertility and hatchability after artificial insemination. Fertility was improved when the semen of village roosters added with 1 IU of vitamin E. However, fertility and hatchability were not improved when the semen of village roosters was added with vitamin C.

Keywords: Vitamin C and E, roosters, semen characteristics, fertility

ABSTRAK

KESAN VITAMIN E DAN C TERHADAP CIRI-CIRI PENGELUARAN SEMEN DAN KESUBURAN AYAM JANTAN KAMPONG

Kajian ini dijalankan untuk mengkaji kesan vitamin E dan C terhadap ciri-ciri pengeluaran semen dan kesuburan di ayam jantan kampong tempatan. Kajian ini dijalankan daripada Februari 2016 hingga Februari 2017 di Fakulti Pertanian Lestari (FPL), Universiti Malaysia Sabah. Objektif pertama kajian ini adalah untuk mengkaji kesan vitamin E atau vitamin C (in vivo) pada kuantiti dan kualiti semen ayam jantan kampong. Ciri-ciri semen (motiliti kasar sperma, peratusan sperma hidup dan skor warna) selepas 4 minggu pemberian vitamin E adalah lebih ketara (6.20 ± 0.49, 81.68 ± 2.41 dan 2.80 ± 0.13) dalam kumpulan T2 (P <0.05). Peratusan sperma tidak normal (P<0.05) lebih rendah (3.84 ± 1.14) pada kumpulan T2 selepas pemberian vitamin E. Motiliti kasar sperma (4.00 ± 0.00) adalah lebih tinggi (P <0.05) kumpulan yang diberi 4 mg / L vitamin C berbanding kumpulan kawalan. Peratusan kepala sperma yang tidak normal (3.00 ± 1.04) dalam kumpulan yang diberi dengan 4 mg / L vitamin C adalah ketara (P <0.05) lebih rendah daripada kumpulan T0 (0 mg / L) dan T1 (2 mg / L). Kesimpulan daripada kajian ini, pemberian vitamin E (2 IU) atau vitamin C (4 mg / L) yang lebih tinggi memberi manfaat kepada peningkatan ciri-ciri semen ayam jantan kampong tempatan selepas 4 minggu. Objektif kedua kajian ini adalah mengkaji kesan penambahan vitamin E atau vitamin C dalam pencair semen (in vitro) terhadap kualiti sperma ayam jantan kampong tempatan selepas proses penyejukan pada 5 °C. Mengikut keputusan kajian ini dapat disimpulkan bahawa, tahap 2 IU vitamin E adalah optimum untuk meningkatkan motiliti sperma dan peratus sperma hidup serta mengurangkan sperma tidak normal selepas 36 jam penyejukan pada 5 ° C. Tahap 1% (w / v) vitamin C adalah tahap optimum untuk meningkatkan motiliti sperma dan juga untuk mengurangkan sperma tidak normal sehingga 24 jam penyejukan pada 5 ° C. Objektif ketiga kajian ini adalah untuk mengkaji kesan vitamin E atau vitamin C (in vivo) pada kesuburan ayam jantan kampong tempatan dan peratus tetas menggunakan semen ayam jantan kampong tempatan selepas permanian berhadas. Kesuburan dan peratus tetas menunjukan peningkatan apabila ayam jantan kampong tempatan diberi vitamin E (200 dan 400 IU) dalam tempoh 4 minggu dan hanya peratus kesuburan menunjukan peningkatan yang ketara apabila ayam jantan kampong tempatan diberi 2 mg / L vitamin C dalam air minuman. Objektif terakhir kajian ini adalah untuk mengkaji kesan penambahan vitamin E atau vitamin C dalam larutan pencair semen (in vitro) kepada peratus kesuburan dan penetasan selepas permanian berhadas. Kesuburan meningkat dengan ketara apabila semen ayam kampong tempatan ditambah dengan 1 IU vitamin E. Namun, peratus kesuburan dan penetasan tidak menunjukkan perubahan ketara apabila semen ayam jantan kampong tempatan ditambah vitamin C.

Kata kunci: Vitamin C dan E, ayam penjantan, ciri-ciri semen, kesuburan

TABLE OF CONTENT

CHAPTER 3: MATERIALS AND METHODS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

°C	degree Celsius
%	percentage
×	multiply
±	plus, minus
≥	more and equal than
<	less than
α	alpha
β	beta
γ	gamma
δ	delta
MMOT	mass motility

LIST OF ABBREVIATIONS

CP	crude protein
kcal	kilo calorie
kg	kilogram
ml	milliliter
µL	microliter
g	gram
mg	milligram
L	liter
v	volume
w	weight
IU	international unit
AI	artificial insemination

CHAPTER 1

INTRODUCTION

1.1 Introduction

The poultry sector is one of the livestock industry in Malaysia. The private sector enterprise that push the incredible growth of poultry industry has grown into progressive, organized and developed industry with production of meat and eggs annually (Arshad and Kaur, 2007).

Poultry term commonly involve chicken, duck, geese, turkeys and quails. However, chickens and ducks are main contributor in Malaysia poultry industry. In 2015, total poultry population were constantly contributed by chicken about 95%. Ducks donate comparatively only 3.7% of the poultry population, while geese, quails and turkeys contribute about 1.1%. Commercial broiler breed comprises almost 65% of the chicken population, while layers contribute 23% of the total poultry population. There also some village chicken contributes 4.8% that are largely found in the rural areas (DVS, 2017).

Village chicken (ayam kampong) or indigenous chicken due to their characteristics such as resistance to various of disease, can optimize low quality feed and having their own unique taste are gaining popularity among consumers (Mengesha, 2012). Village chicken usually reared in free range, small scale system and often as hobby in the back garden production. Due to that, nutritional and reproduction knowledge of village chicken especially on fertility were still lacking compared to commercial chicken. Compared to commercial chicken, village chicken

has many advantages and benefit. The indigenous chicken well adapts on climatic zone, less cost on maintenance and feed, resistance to disease and hardy (Ramlah, 1999).

In South-east Asian countries, the production of backyard chicken still contributes the domestic chicken meat consumption although commercial broiler and layer utilize the commercial production. The contribution of village based production is very small in Malaysia based compared to other Asian country such as Indonesia, Thailand and Philippines (Aini, 1990).

The Avian semen are characterized by high concentrations of polyunsaturated fatty acids (PUFAs) (Golzar Adabi *et al.*, 2011; Park and Lynch, 1992), which are linked with increased susceptibility to relative oxygen species (ROS) and lipid peroxidation. Generally, seminal plasma that contain various antioxidants and defend sperm from ROS and lipid peroxidation. However, when ROS is higher than the natural antioxidant defense mechanisms, the sperm will be damage by lipid peroxidation and later can result in fertility (Min *et al.*, 2016).

The antioxidants have important roles in avian reproduction. Vitamin E reduce oxidative stress in sperm that can optimize fertilizing ability. Vitamin E supplements in poultry diets popularly used to increase production and reproductive performances. Vitamin E is a natural antioxidant that enhances semen quantity and quality as well fertilizing capacity of chickens, when it is provided at a level some 500 times greater than the NRC requirements (15 IU/kg diet) (Tabatabaei *et al.*, 2011). There is little information available about their effect through diet, nor the mechanisms and vitamin E in improving the fertility of roosters especially indigenous chicken even though physiological responses of poultry to vitamin E have been well established.

Furthermore, vitamin E requirements must be met from dietary source because poultry cannot synthesize vitamin E (Bölükbaşi *et al.*, 2007). Therefore, to

study the dietary level of vitamin E in the diet of indigenous chicken is quite interesting.

Vitamin C (Ascorbic Acid) is a water soluble vitamin which is important for normal body function. It has been linked with fertility for many years and may have evolutionary significance. However the exact physiological function in reproduction has been uncertain (Tabatabaei, 2012). Vitamin C is antioxidant that exist naturally in chicken spermatozoa and seminal plasma. It is well established that dietary supplementation of vitamin C improves quality of poultry semen (Jabbar *et al.*, 2015).

1.2 Problem Statement

Semen quality had a prominent role in reproduction of the chicken. The quality of semen will affect the reproductive performance especially in the application of artificial insemination. Oxidative stress is great concern to poultry industry with breeder rooster. The sperm of the rooster will be affected by oxidative stress and resulting infertility. Antioxidants such as vitamin E and vitamin C has a strong impact on oxidative stress.

1.3 Objective

i. To determine the effect of vitamin E or vitamin C supplementation (in vivo) on semen quantity and quality of village roosters.

ii. To determine the effect of addition of vitamin E or vitamin C in semen diluent (in vitro) on sperm characteristics undergoing the chilling process in the village roosters.

iii. To determine the effect of vitamin E or vitamin C supplementation (in vivo) on fertility and hatchability after artificial insemination of hen.

iv. To determine the effect of addition of vitamin E or vitamin C in semen diluent (in vitro) on fertility and hatchability after artificial insemination of hen.

1.4 Hypothesis

i. Ho: There are no effect of vitamin E or vitamin C supplementation (in vivo) on semen quantity and quality of village roosters.

Ha: There are significant effect of vitamin E or vitamin C supplementation (in vivo) on semen quantity and quality of village roosters.

ii. Ho: There are no effect of addition of vitamin E or vitamin C in semen diluent (in vitro) on sperm characteristics undergoing the chilling process in the village roosters.

Ha: There are significant effect of addition of vitamin E or vitamin C in semen diluent (in vitro) on sperm characteristics undergoing the chilling process in the village roosters.

iii. Ho: There are no effect of vitamin E or vitamin C supplementation (in vivo) on fertility and hatchability after artificial insemination of hen.

Ha: There are significant effect of vitamin E or vitamin C supplementation (in vivo) on fertility and hatchability after artificial insemination of hen.

iv. Ho: There are no effect of addition of vitamin E or vitamin C in semen diluent (in vitro) on fertility and hatchability after artificial insemination of hen.

Ha: There are significant effect of addition of vitamin E or vitamin C in semen diluent (in vitro) on fertility and hatchability after artificial insemination of hen.

CHAPTER 2

LITERATURE REVIEW

2.1 The Poultry

Poultry are referring to domesticated birds kept by human to satisfy human needs particularly food. Chicken, duck, turkey, goose, guinea fowls, quail, pea fowl, pigeons, pheasants, ostriches and other game birds are species that widely accepted for poultry species. Poultry keeping is mainly for their meat, their egg, feather and sometimes pet (Lambio and Arbodela, 2010).

Chicken were originated in Southeast Asia before the trader and sailor brought them to the rest of the world. Indigenous village chicken nowadays was come from cross-breeding with exotic breeds and random breeding within the flock. Due to that, it is impossible to make the characteristic and productive performance to be standardize. There are no exact list of breed and varieties of chicken reared by the rural smallholders. However, there were a quite information regarding the indigenous chicken population from part of the world (Lambio and Arbodela, 2010).

Turkey is the descendant North-American wild turkey (*Meleagris gallopavo*). Then the turkey domesticated in Mexico and around 1530 AD turkey were brought to Europe (Macjowski and Zieba, 1982). Turkeys are believed native to Latin America. Turkey can be said the largest birds in farming system after ostriches and geese. The males weight about 7 to 8 kg and females about 4 to 5 kg. The meat conformation is good, and they can lay eggs about 90 eggs per year. The turkey also has a good

hatchability. However, compared to chicken and duck turkeys are more susceptible to disease (Sonaiya and Swan, 2004).

The domestic duck originated from the Mallard (*Anas platyrhynchos*) which inhabits all Europe, Africa, Asia and North America. It nests in the vegetation surrounding ponds or rivers and is a migrant species, over wintering in warmer climates. Males weight 1.7–2 kg while females weight much less. Females lay 6–14 eggs in nests (Cherry and Morris, 2008).

The guinea fowl (*Numida meleagris*) are native to West Africa but now found in many part of the world. Guinea fowl are seasonal breeder laying eggs only during rainy season, under free range condition. Guinea fowl are very timid bird that at the night they are roosting in tree. They fly very little and also a great walker. Guinea fowl can adapt both cool and hot condition and they have potential to increase meat and egg production (Sonaiya and Swan 2004).

2.2 Indigenous Chicken

Malaysian indigenous chicken (*Gallus gallus domesticus*) popularly known 'ayam kampung' that means as village are from the cross breeding of the Red jungle fowl (*gallus gallus*) with mixed exotic domestic breed that believe come from Europe especially British (Azahan and Zahari, 1983). The characteristics of the village chicken are varying between the individual which hard to fit the description to the entire flock. Generally, the common characteristics share by the village chicken are they tend to have small body size (Khadim *et al.*, 2014; Azahan, 1994) and their growth rate and egg production are poor (Azahan *et al.*, 1980). Village chicken also show different colour of plumage and body conformation and their physical characteristics are varies among them (Khadim *et al.*, 2014).

2.3 Important of Indigenous Chicken

Indigenous chicken is more resistance to disease and can utilizing low quality feed due to consequence of natural selection. Indigenous chicken also remains predominant in the rural area because of its hardy, adaptive and are preferred by consumer. The indigenous chickens always fetcher better price than exotics because it's unique taste and flavor (Mupeta *et al.*, 2003).

Indigenous chickens have important role for smallholders and donate largely to food security of households in rural and semi-urban communities. Almost all rural and semi-urban families in developing countries rear a small flock of indigenous chickens in the backyard (Al-qamashoui, 2014).

Local chicken products are consumed at household level. Backyard poultry contributes for many non-economic benefits such as manure production, weed and pest control, waste and agricultural byproducts recycling and conservation of valuable genetic resources. The local chickens, which are commonly classified worldwide as non-descriptive types due to lack of information, vary widely in body size, body conformation, plumage colour and many other phenotypic characteristics, which is important in income and family food security in rural farm households (Liyanage *et al.*, 2015).

2.4 Taxonomic of Indigenous Chicken

The village chicken can be mix of black, red and brown plumage. Their feathers are frizzle and silkie. The characteristic of the village chicken also that they had broad head with short beak with slightly curved downward. They have smooth face and the eye are deep-set. The spur on the legs are present only on males' chicken. The roosters that have already mature weight are about 2.0 kg and the hens is 1.5 kg (Azahan, 1994).