UNIVERSITI PUTRA MALAYSIA

EFFECTS OF HARVESTING TIME ON COMPOSITION OF BLACK SOLDIER FLY LARVAE AND ITS OPTIMUM INCLUSION LEVEL ON GROWTH OF SPRING CHICKENS

DAHIRU SOLI JIBIA

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By

DAHIRU SOLI JIBIA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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DEDICATION

All praises be to Allah who granted my late parent (May Allah grant them Jannatul Firdausi) the opportunities to guide me till this age and afterwards to my brothers and sisters for their endurance to support me in various ways, that I do not have enough words to qualify or describe, specifically Sada Soli, our late brother in person by Muhammad Soli Jibia (May Allah rest his soul in perfect peace).

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

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DAHIRU SOLI JIBIA

February 2017

Chairman : Associate Professor Azhar bin Kassim, PhD
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There is high demand for alternative protein source of animal origin for food and feed, which can sustainably be utilized to meet the growing human population need for food and animals' feed in the near future. Black soldier fly larvae meal was used in this study to substitute the conventional protein ingredient for broiler chicken. The continuous need for a qualitative protein source of animal origin has presented serious struggle for animals and humans. The current study uses black soldier fly larvae for its significant contribution to poultry feed. The bsf larva was established to contain 40% - 60% DM crude protein and metabolizable energy (ME/kcal) that was superior to fishmeal and having a higher amino acid profile than the soybean meal, but equal to the fish meal. Therefore, the study is determined to evaluate the appropriate harvesting age in black soldier fly larva for high nutrient composition and its optimum inclusion level for the performance of young commercial broiler chicks. The set media for larvae harvest were arranged on the same day and harvested according to target age 6 d, 12 d, 18 d and 24 d. Larvae analysis was carried out for percent crude protein, crude fat, ash, dry matter, organic matter and moisture content. Result showed significant difference ($P < 0.05$) among the treatments with T_3 18 d was highly significant 50.47% CP against 6 d, 12 d and 24 d approximately T1 32%, T3 34%, and T4 37% respectively. Amino acid profile was significant ($P < 0.05$) apart from Aspartic acid, glycine, proline and lysine ($P>0.05$). Ninety six day old commercial broiler chick were arranged into four treatment groups, subdivided into four replicates each replicate allotted to six chicks 4 X 4 X 6 (T1 – T4) in a completely randomized design using SAS 9.4 software model, means comparison was carried out by Duncan multiple range test. Four inclusion levels of black soldier fly larvae (bsfl) experimental diets were allocated to each treatment group (0%, 5%, 7.5% & 10%), record of performance were analyzed: where feed intake, body weight gain, daily weight gain, feed conversion ratio and mortality were significantly higher ($P< 0.001$) except the final body weight ($P>0.05$). Sensory and meat characteristics parameters; odor, taste, color (L* a* b*),

shear force and cooking loss were not significant (P>0.05), however, meat color, tenderness and pH (P<0.05). Carcass characteristics and blood parameter analyzed showed (P<0.001) in thigh muscle, liver and (P<0.05) in urea only. While the remaining parameters: carcass weight, dressing percentage, breast muscle, drumstick, wings, cholesterol and total blood protein (TBP) were found to be no significant (P > 0.05) in all the treatments. Conclusively, the study revealed that larvae should best be harvested on 18th d and 5% dietary *Hermetia* inclusion level was the optimum for young commercial broiler chicks.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN PENUAIAN MASA KE ATAS KOMPOSISI ASKAR HITAM LARVA LALAT DAN MEMASUKKAN TAHAP OPTIMUM KE ATAS PRESTASI AYAM SPRING

Oleh

DAHIRU SOLI JIBIA

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Permintaan kepada sumber protin alternatif untuk dijadikan sumber makanan haiwan semakin tinggi untuk memenuhi keperluan populasi manusia yang semakin meningkat. Larva lalat kesatria hitam (Black Soldier fly Larvae – BSFL) telah digunakan dalam kajian ini sebagai bahan mentah untuk dedak ayam pedaging. Permintaan yang berterusan terhadap sumber daging berasaskan haiwan merupakan perkara serius untuk manusia dan haiwan. Kajian ini menggunakan BSFL kerana kontribusinya yang penting dalam ayam industri. Larva BSF emgandungi sumber protein sebanyak 40% - 60% dan tenaga metabolism yang lebih tinggi daripada makanan ikan. Ia juga mengandungi sumber asid amino yang sama dengan makanan ikan dan lebih tinggi berbanding kacang soya.

Dalam kajian ini, tempoh penuaian BSFL dikenalpasti bagi memastikan komposisi nutrient berada pada paras tertinggi dan peratusan formulasinya dalam dedak ayam pedaging permula adalah pada paras optimum. Tempoh pematangan larva telah disusun mengikut umur 6 hari, 12 hari, 18 hari, 18 hari dan 24 hari. Komposisi larva yang dianalisis adalah protein, lemak, abu, bahan kering, bahan organik dan kelembapan. Keputusan kajian menunjukkan perbezaan antara rawatan dengan T3 18 hari yang paling tinggi protin 50.47% berbanding 6 hari, 12 hari dan 24 hari iaitu T1 32%, T3 34%, dan T4 37%. Kandungan asid amnio adalah angat berbeza bagi Aspartic acid, glycine, proline and lysine ($P>0.05$). Sebanyk 96 anak ayam berumur sehari telah disusun kepada empat kumpulan rawatan yang mengandungi empat replikasi setiap satu yang mana setiap satu mengandungi enam anak ayam yang telah disusun secara rawak 4x4x6 (T1 – T4) menggunakan SAS model perisian 9.4, bermakna perbandingan telah dijalankan oleh Duncan ujian julat berganda. Empat paras inklusi BSFL (0%, 5%, 7.5% & 10%) telah diuji dan kadar tumbesaran telah direkodkan. Berdasarkan kajian, jumlah pengambilan dedak, berat badan, feed conversion ratio dan kematian adalah lebih tinggi ($P< 0.05$) kecuali berat badan akhir ($P>0.05$). Tiada

perubahan pada rasa dan ciri-ciri daging seperti bau, rasa, warna, shear force dan cooking loss. ($P>0.05$). Tetapi warna daging, kelembutan dan pH ada perbezaan ($P<0.05$). Ciri-ciri karkas dan darah menunjukkan perbezaan pada bahagian thigh muscle, hati ($P<0.001$) dan urea ($P<0.05$) sahaja. Manakala parameter lain seperti berat karkas, peratusan persalinan, otot dada, peha, sayap, kolesterol dan jumlah keseluruhan protin serum adalah tidak berbeza antara semua rawatan ($P > 0.05$). Kesimpulannya kajian ini menunjukkan larva paling optimum dituai pada hari ke 18 dan dimasukkan pada paras 5% dalam formulasi dedak ayam pedaging permula.

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I certify that a Thesis Examination Committee has met on 27 February 2017 to conduct the final examination of Dahiru Soli Jibia on his thesis entitled "Effects of Harvesting Time on Composition of Black Soldier Fly Larvae and its Optimum Inclusion Level on Growth of Spring Chickens" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science.The members of the Supervisory Committee were as follows:

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LIST OF ABREVIATIONS

°C	Degree Celsius
°F	Degree Fahrenheit
a*	Meat Color Redness
Ad lib	ad libitum
ANOVA	Analysis of Variance
ATBU	Abubakar Tafawa Balewa University
ATP	Adenosine Triphosphate
b*	Yellowness
Bokashi	Waste Rice Bran
BPC	British Poultry Council
BSFL	Black Soldier Fly Larvae
BSFLM	Black soldier fly Larvae Meal
BW	Body Weight
BWG	Body weight gain
Ca	Calcium
CF	Crude Fat
CNW	Coconut waste
CP	Crude Protein
CRD	Completely Randomized Design
DFD	Dark Firm Dry
DM	Dry Matter
E. coli	*Eschericia coli*
EAA	Essential Amino Acid
EFSA	European Food Safety Authority

Em	Effective microbes
EPPO	European and Mediterranean Plant Protection Organization
Etc	et cetera
EU	European Union
FAO	Food and Agriculture
FBW	Final Body Weight
FCR	feed Conversion Ratio
FERA	Food and Environmental Research Agency
FI	Feed Intake
g/l	Gram Per liter
GDP	Gross Domestic Product
GDSSJ	Government Day Secondary School Jibia
GGSS	Government Girls Secondary School
GLM	General Linear Model
GSSSS	Government Senior Science Secondary School
H	Hour
HND	Higher National Diploma
HPLC	High performance liquid chromatography
IFFI	International Feed Industry Federation
Kg	Kilogram
KTSB	Katsina Teachers Service Board
L*	Meat Color Lightness
LSD	Least Significant Difference
MT	Metric Ton
ND	National Diploma

NVRI	National Veterinary Research Institute
OM	Organic Matter
P	Phosphorus
PGD	Post Graduate Diploma
pH	Hydroxyl ion Concentration
PSE	Pale Soft and Exudative
SAS	Statistical Analysis System
SEM	Standard Error Mean
T	Treatment
TBP	Total Blood Protein
TETFUND	Tertiary Education Trust Fund
UK	United Kingdom
UN	United Nations
US	United states
WHC	Water Holding Capacity

CHAPTER ONE

GENERAL INTRODUCTION

Poultry has essentially supported human sustainability through provision of good protein source by means of their meat, egg among others and its by-products for insect larvae substrate and poultry feedstuff such as feces and feathers (Makkar, *et al.*, 2014). But to achieve this the chickens' nutrient requirements have to be fulfilled, purposely to certify their ideal productivity which leads to inclusion of large quantity of high value protein source ingredients, principally in their first few days of live (Prestorius, 2011).

Poultry meat, predominantly chicken is basically the cheap source of animal protein in the foods of the Malaysians,' which consisted nearly 72% out of the overall Malaysia's meat requirements in 2008, when related to 58% in the year 1990 (Bisant, 201I) given the 35.3kg per capita consumption of broiler meat, and that malaysia has reached or attained a self-sufficiency level of 128.1% (Jayaraman *et al.*, 2013). Despite the broiler industry development, Malaysia as a developing country has been importing most of its poultry feed inputs, where soybean and corn account for up to 65% for the production cost (USDA, 2014). Since, feed is the primary items in any system of poultry production in terms of cost, and the beneficiaries of poultry development have been few in underdeveloped nations, similarly the availability of low-priced, high-quality feeds is vital if poultry production is to remain competitive and continue to grow to meet the demand for animal protein (Mengesha, 2012). When high competition for feed resources, becomes obvious the prices for poultry feeds intensify. Thus, it compels the producers to search for an alternative and locally available feed source. The availability and digestibility of insects which have made them to be referred as the promising future insects for feed industries (Cullere *et al.*, 2016; Rumpold & Schlüter, 2015), making use of insects as unconventional feed material was due to the fact that insect's larvae have a huge prospect to offer a useful protein source in livestock feed (Kenis, *et al.*, 2014; Maurer *et al*., 2015; Cullere *et al*., 2016). It was established that bsf larvae production can associate friendly with environment by reducing the risks caused as a result of waste accumulation and at the same time would provide a nutrient-rich resource (Barry, 2004; Diener *et al.*, 2011; Zhou *et al*., 2013; Makinde, 2015).

Nonetheless, insects have in recent times started being studied as innovative feed ingredients to better achieve the desired goals in poultry (Schiavone *et al.*, 2016), since, it has balance of nutrients, which directly involved in optimizing the production function (i.e. feed conversion, body weight or breast muscle buildup) and variation in the nutrient levels that can result to significant impact on the production output (Kidd, 2004). And like other insect sources of protein, such as house fly larvae meal, black soldier fly larvae also were used as alternative replacement of the conventional sources (Ojewola, *et al.,*2005), because, it is practicable to produce insects on a large scale for sustainable protein rich ingredient in poultry diets, as they can sustain on substrates of bio-waste and organic substances (Veldkamp & Bosch, 2015). Also proximately, it

composed of a considerable amount of methionine and lysine of 2.1%, 6.6% respectively, when compared to soymeal 1.32% methionine and 6.18 % lysine. Soldier fly larvae was also one of the five most currently studied and produced insect in the world (Makkar, *et al.,* 2014; Veldkamp & Bosch, 2015).

Ugwumba (2001) and Sogbesan *et al.* (2005) Conveyed that a routine of studies were conducted on the prospects of insects' larvae or maggot meal as a future alternative animal protein, to conventional fish meal or soy meal. Among these insects black soldier fly larvae was found to be universal in various aspects: geographical distribution which is worldwide (Brammer, C. A., and von Dohlen, 2007), feed for livestock production such as pig, poultry and fish (Józefiak & Engberg, 2015) as well as waste management. Also it was cheaply used for control of certain vector insects, specifically the house fly (Sheppard *et al*., 2002; Neill *et al*., 2013) and reduce feed competition which could eventually cut down the cost of production and enhance food security (Huis *et al*., 2013).

Moreover, it can overcome the worrisome of using fish meal in poultry feeding. The reason was due to adulterations in the fish meal conceivably as a result of processing procedures involved, as recently experienced in some tropical developing countries as cited by Heuzé *et al.(*2015), similarly it was reported that this has resulted to a significantly high levels of ash and to a certain degree low protein content (Heuzé *et al.,* 2015). Considering these, it could pave ways to enable black soldier larvae a wider perspective, despite the recent classification by researchers as the most potentially capable animal protein source with a very rich amino acid (AA) profile where all its essential amino acids (EAA) for instance methionine 2.1%, lysine 6.6% were higher than soymeal 1.3% methionine, 6.18% lysine and that of food and agricultural organization (FAO) reference protein methionine and lysine 2.50%, 5.80% accordingly. Also the mineral contents were positively satisfactory, most especially calcium (Ca) 7.56% and phosphorus (P) 0.9% compositions were excellently rich (Makkar *et al*., 2014).

Furthermore, larvae were harvested at different ages and for various purposes, which include protein supplementation in feed for broiler chicken, layers, quails, swine and fish, pets/ reptiles. Yet until now, time (age) at harvesting larvae continued to be one of the challenging tasks due to these differences in the proximate composition in research and industrial atmospheres (Atteh and Ologbenla, 1993) and (Aniebo and Owen, 2010). Probably because most of the time little or no attention was paid to this aspect, as such, it becomes a common practice amongst; researchers, university student experiments and projects, who mostly sourced the larvae from stores (supplier). On the other hand, some of the industries do not take into considerations on the time of harvest and drying temperature, which also further affect the quality of the product and then lead to variations in its chemical composition (Prestorius, 2011).

The Black soldier flies (Bsf) *Hermetia illucens Linnaeus* are small, harmless insects that have the potential to provide promising solutions to two of modern agriculture's growing problems: the high cost of animal feed and the disposal of large amounts of

animal waste. which indicated that black soldier fly might be helpful in bridging the gap linking animal waste and animal feed (Watson *et al*., 2005). It belongs to the Order *Diptera*, family *Stratiomyidae,* Genus *Hermetia* and Species *Hermetia illucens* (*L)*, where many adult members *Stratiomyids* are attractively colored ranging between black, bluish, green and yellow, sometimes having a glistening external features, particularly the wings. There are also several members which resembled other different types of flying insects like wasp and bees (Sheppard *et al*., 2002) fairly large about 3-30 mm long (Bayless, 2008). It was reported that scarcity of the insect in the field may have been the reason for not drawing much attention the same degree on research like other large families of flies, such as *Asilidae* and *Syrphidae* have received (Woodley, 1989), furthermore, their distribution extends nearly to all parts of the globe, with the wider range in Africa, Australia, Equatorial Tropics, Europe and Western Hemisphere (Brimley, 1938; Brammer and von Dohlen, 2007).

The sustainability of soldier fly larvae on decayed organic substances such as kitchen waste, feed/ food waste and wheat bran (Bondari and Sheppard, 1981, 1984 and 1987; Brammer and Dohlen, 2010; Neill *et al*., 2013) were largely documented in a diverse publications; books, journals, newsletters, unpublished papers and even the unpublished work. Even though, in the recent times bsf larvae are reared, harvested and used for various purposes, which involve use for protein supplementation and replacement in feed for broiler chicken, layers, Quails, fish, pets and reptiles. But, until nowadays, data with reference to the harvesting period, age or time on larvae are not sufficiently available, and the only few literature that we came across in the course of this study, which discussed about the larvae age at harvest, were either not clearly specified or questionable.

Black soldier fly larvae meal differ significantly in their protein compositional property according to the stage of life 42% - 60% CP and were considered as complete diet, if the diet was said to be complete and support performance of the chicks. Therefore when these compositions are affected, then the function of the diet is defeated (Sheppard *et al*., 2007). The inconsistent larvae composition effect, that were due to varying harvesting age is a challenge which could begin to raise concern in the near future, particularly in areas of research and industry. As a result, the effect would demonstrate itself by the continuous fluctuation in larval protein composition and eventual imbalance in the nutritive potential of the dietary larvae. Thus, results in a varied impact on the performance of the dietary larvae meal is fed to poultry (Waterlow, & Stephen, 1968). showing varied compositions in the percent crude protein and crude fat include; 63.1% CP and 15.5% CF (Calvert and Martin, 1969), 37.5% CP and 19.8% CF (Ogunji *et al*., 2006), 50.4% and 20.6% CF (Sogbesan *et al.,* 2006) and (Aniebo *et al*., 2008) 42%, 47.1 % - 55.4% and 25.3% CF. These differences indicate that the larvae may have been harvested at different ages, as such, definite time of the harvested larvae were not mentioned.

Nevertheless, there were a few exceptions whom have reported the period at harvest, such as Min & Logan (2004) harvesting on the 6^{th} day and larvae yield was 48% CP, also Aniebo *et al*. (2008) have obtained 42% - 55% CP and 25% CF compositions of the larvae that were harvested on the 6^{th} day after eggs were hatched. It was reported

by Odesanya *et al.* (2011) after the larval harvest result showed that the larvae meal contained 48% CP composition after the 6th day, no precise number of days were stated, according to the report. Recently, Widjastuti *et al.* (2014) have similarly harvested the larvae and obtained 46.58 % CP without specific date, but it was revealed that from 3rd - 4th day. It was revealed that the effect of varied chemical contents of larvae meal could only be quantified after when its fed to animal (Waterlow & Stephen, 1968). However, the levels of inclusion of feed ingredients in poultry diets, has become a common phenomenon, thus, several studies have reported different inclusion levels using various forms of dietary protein, either combined two ingredients (Widjastuti et al., 2014) or a single ingredient (Kassim & Suwanpradit, 1996) and the ranges could be at least 2% - 100% levels. Larvae meal was used in the various animals feed as an unconventional protein replacement, for soy meal, and occasionally as a substitute for fish meal or combining larvae and Soymeal or larvae and fishmeal (Makkar *et al.*, 2014; Widjastuti *et al*., 2014) and 12g & 24g replaced 50% and 100% levels of soybean (Maurer et al., 2015). In view of these, the current work will attempt to investigate the best period for larval nutrient composition, chiefly crude protein and crude fat which will enable its prompt harvest.

1.1 Problem statement

The continuous demand for qualitative animal protein source for chicken has presented serious struggle for animals and humans in search of food from animal protein origin and persistent need for a renewable feed resources in livestock nutrition (Prestorius, 2011).

1.2 Justification

It was established that nature has continuously offered the natural feed in the insect kingdom, such as larvae, which provide a source of food in the wild environments, and presented an extraordinary dietary structures (Scholtz and Holm, 1985; Acorn *et al*., 2003; Pretorius, 2011). Also the insects represent a possible alternative nutrient source for the livestock sector, which could help to face the increasing demand and price for conventional feedstuffs in a more sustainable approach. up till now, for the industrialized countries, they do not have a defined and understandable legislation or standardized guidelines for using insects as a component of feed that impedes the industrial development of this emerging sector (Cullere *et al.*, 2016). According to FAO 1995 and quoted by Mengesha (2012) has reported that despite the increased monogastric animal production and improved production systems, yet, the underdeveloped nations are still unable to enjoy these poultry resource development as a result of high cost of feed items. But the scavenging chicken production as one of largest and most efficient energy source in poultry sector in the world. To close this gap, alternative feed resource that is locally available and practically obtainable is needed (Mengesha, 2012).

1.3 Hypothesis

The study hypothesized that quality nutrient composition of black soldier fly larvae is influenced by its stage of growth and the amount which could affect broiler performance.

1.4 General Objective

The study was focused to use black soldier fly larvae for quality nutrient to provide with alternative protein feed ingredient for growth performance in young chicken.

1.5 Specific Objectives

1. To determine the most appropriate harvesting time for the nutrient composition in black soldier fly larvae
2. To determine the optimum inclusion level of larvae meal for growth performance and carcass quality.

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