creative commons

COMMONS DEED

저작자표시-비영리-변경금지 2.0 대한민국

이용자는 아래의 조건을 따르는 경우에 한하여 자유롭게

- 이 저작물을 복제, 배포, 전송, 전시, 공연 및 방송할 수 있습니다.

다음과 같은 조건을 따라야 합니다:

저작자표시. 귀하는 원저작자를 표시하여야 합니다.

비영리. 귀하는 이 저작물을 영리 목적으로 이용할 수 없습니다.

변경금지. 귀하는 이 저작물을 개작, 변형 또는 가공할 수 없습니다.

- 귀하는, 이 저작물의 재이용이나 배포의 경우, 이 저작물에 적용된 이용허락조건을 명확하게 나타내어야 합니다.
- 저작권자로부터 별도의 허가를 받으면 이러한 조건들은 적용되지 않습니다.

저작권법에 따른 이용자의 권리는 위의 내용에 의하여 영향을 받지 않습니다.

이것은 이용허락규약(Legal Code)을 이해하기 쉽게 요약한 것입니다.

Disclaimer

dCollection

A Thesis
For the Degree of Master of Science

Supplementation of *Tenebrio Molitor* Larva as a Protein Source on Growth Performance, Nutrient Digestibility and Palatability in Weaning Pigs

이유자돈 사료 내 단백질원으로 거저리 유충의
첨가가 이유자돈의 성장, 영양소 소화율 및
기호성에 미치는 영향

February, 2014

By

Jin, Xing Hao

School of Agricultural Biotechnology
Graduate School of Seoul National University

Supplementation of *Tenebrio Molitor* Larva as a Protein Source on Growth Performance, Nutrient Digestibility and Palatability in Weaning Pigs

이유자돈 사료 내 단백질원으로 거저리 유충의 첨가가 이유자돈의 성장, 영양소 소화율 및 기호성에 미치는 영향

지도교수 **김 유 용**

이 논문을 농학석사 학위논문으로 제출함

2014 년 2 월

서울대학교 대학원 농생명공학부

김 성 호

김성호의 농학석사 학위논문을 인준함

2014 년 2 월

위 원 장 ________________________ (인)

부위원장 ________________________ (인)

위 원 ________________________ (인)

Summary

Increasing demand of protein source for animals and humans is likely to challenge the global capacity for providing enough protein, which leads to increase feed costs for swine production. To resolve this problem, it is very important to find a new alternative protein source for animal feed. Insects can be one of new alternative protein sources, which is able to utilize widely in animal feed without a problem of food safety if its price is acceptable. Consequently, this research was conducted to evaluate the effect of *Tenebrio molitor* larva supplementation on growth performance, nutrient digestibility and palatability in weaning pigs in order to provide an alternative protein source to replace conventional protein sources such as fish meal and soybean meal in animal feed.

A total of 120 weaning pigs (28 ± 3d old and 8.04 ± 0.08 kg of BW) were allotted to one of five treatments in 6 replicates with 4 pigs per in a randomized complete block (RCB) design. Five different levels of *Tenebrio molitor* larva (0, 1.5, 3.0, 4.5 and 6.0 %) were used as dietary treatments. Two phase feeding programs (phase I for 0-14 day, phase II for 15-35 day) were used in this experiment. During phase I, body weight ($P<0.01$), ADG ($P<0.01$) and ADFI ($P<0.01$) were linearly increased as larva level increased in diet. During phase II, body weight was also increased in proportional to dietary level of larva (linear response, $P<0.01$), but ADG was tended to increase ($P=0.08$) when pigs were fed high level of *Tenebrio molitor* larva. In the whole experimental period, there were linear responses in ADG ($P<0.01$) and ADFI ($P<0.05$) as dietary *Tenebrio molitor* larva increased and an increasing trend was also observed in G:F ratio ($P=0.07$). In nutrient digestibility, there were linear improvements in dry matter, crude protein and nitrogen retention was improved by increasing level of *Tenebrio molitor* larva in diet ($P=0.05$). In the results of blood profiles, decrease of blood urea nitrogen ($P=0.05$) and increase of IGF-1 ($P=0.03$) were observed as increasing level of *Tenebrio molitor* larva in diet during phase II. In immune responses, there were no significant differences in concentrations of IgA and IgG by addition of *Tenebrio molitor* larva. These results demonstrated that the use of *Tenebrio molitor* larva as a feed ingredient in weaning diet was feasible and supplementation of *Tenebrio molitor* larva up to 6% could improve growth performance without any detrimental effect, especially in phase I.

Key words: Insect, protein source, *Tenebrio molitor* larva, weaning pigs.

Contents

page

Summary ········· i

Contents ········· iii

List of Tables ········· v

List of Figures ········· vi

List of Abbreviations ········· vii

Ⅰ. **Introduction** ········· 1

Ⅱ. **Literature Review** ········· 3

1. Post-weaning problems in piglets ········· 3

1.1 Growth check after weaning ········· 3

1.2 Anorexia loss of appetite ········· 4

2. Conventional animal protein sources for weaning pigs ········· 5

2.1 Plasma protein ········· 5

2.2 Fish meal ········· 6

2.3 Milk protein ········· 7

2.4 Blood meal ········· 7

3. Use and process of insect as protein source ········· 8

3.1 Using insects as a protein source in feed ································ 8

3.2 Processing insects ·· 10

4. Characteristics of *Tenebrio Molitor* ·· 13

4.1 Life history ·· 13

4.2 Nutritional values ·· 16

4.2.1 Protein quality ·· 17

4.2.2 Fat quality ·· 20

4.2.3 Other nutrients ·· 22

4.2.4 Functional properties ·· 22

4.3 Cost price ·· 25

5. Safety and regulation ·· 26

5.1 Pathogens, heavy metal and toxins ································ 26

5.2 Regulation ·· 28

6. Application of insects in monogastric animals ································ 28

6.1 Growth performance ·· 28

6.2 Nutrient digestibility ·· 29

6.3 Palatability ·· 31

Ⅲ. Supplementation of *Tenebrio Molitor* Larva as a Protein Source on Growth Performance, Nutrient Digestibility and Palatability in

Weaning Pigs

Abstract ···· 33

Introduction ···· 35

Materials and methods ···· 36

Results and discussion ···· 40

Conclusion ···· 45

Ⅳ. **Literature cited** ···· 52

Ⅴ. **Summary in Korean** ···· 69

Ⅵ. **Acknowledgement** ···· 71

List of Tables

Ⅰ. Literature review

Table 1. The growth of *Tenebrio molitor* in optimal temperature and moisture ··· 16

Table 2. Percentage nutritional composition of *Tenebrio molitor* larva, adult, exuvium and excreta ··· 17

Table 3. Amino acid content of *Tenebrio molitor* larva, adult, exuvium and excreta ··· 19

Table 4. Amino Acid Score (AAS) and Chemical Score (CS) of *Tenebrio molitor* larva and pupa ··· 20

Table 5. Fatty acid composition of *Tenebrio molitor* larva, pupa and adult ··· 22

Table 6. Mineral composition of *Tenebrio molitor* larva, adult and waste ··· 23

Table 7. The chitin percentage of *Tenebrio molitor* with different stage ··· 25

Table 8. Prices of different protein sources ··· 26

Table 9. The benzoquinone concentration of different state in different stage of *Tenebrio molitor* ··· 27

Table 10. Apparent faecal digestibility of diets containing insects or soybean meal in broiler chickens ··· 31

Ⅱ. Experiment

Table 1. Formula and chemical compositions of the experimental diets in phase I (0 to 14th day) ··· 46

Table 2. Formula and chemical compositions of the experimental diets in phase II (15^{th} to 35^{th} day) ···································· 47
Table 3. Effect of *Tenebrio molitor* larva supplementation on growth performance in weaning pigs ·· 48
Table 4. Effect of *Tenebrio molitor* larva supplementation on nutrient digestibility in weaning pigs ·· 49
Table 5. Effect of *Tenebrio molitor* larva supplementation on BUN and IGF-1 concentration in weaning pigs ························· 50
Table 6. Effect of *Tenebrio molitor* larva supplementation on IgG, IgA concentration in weaning pigs ····································· 51

List of Figures

Ⅰ. **Literature review**

Figure 1. Use of insects in animal feed chain ································ 10

Figure 2. Possible processing strategies for insect I and II ·········· 13

Figure 3. Morphology of *Tenebrio molitor* ·· 15

Figure 4. Chemical structure of the chitin molecule ························ 24

List of Abbreviation

AAS	:	Amino acid score
ADFI	:	Average daily feed intake
ADG	:	Average daily gain
AOAC	:	Association of official analytical chemists
BUN	:	Blood urea nitrogen
BW	:	Body weight
CP	:	Crude protein
CS	:	Chemical score
DM	:	Dry matter
FAO	:	Food and agriculture organization
FCR	:	Feed conversion ratio
Ig	:	Immonoglobulin
IGF-1	:	Insulin like growth factor-1
ME	:	Metabolizable energy
NRC	:	National research council
RCB	:	Randomized complete block
SAS	:	Statistical analysis system
SBM	:	Soybean meal
WHO	:	World health organization

I. Introduction

The Dutch Ministry of EL&I initiated in early of 2012 to explore using of insects as a sustainable source of feed and identified the additions and limitations amounts for use of insects in the animal feed industry. The use of insects as an feed ingredient of animal protein source in pig and poultry diets was technically feasible because insects could be easily reared in media of grain byproducts with low cost.

Edible insects as animal protein source are generally contained between 30%~70% protein in DM basis and can be good sources of lysine and other amino acids compared with plant proteins. Some insects contained the functional properties in their shells called "chitin" acting as antibiotics or prebiotics to enhance immune responses in rats and chickens (Chen et al., 1999 and 2002). Functional properties of insect-chitin can be supplemented in broiler chickens to influence on hypolipidaemic properties. Insects had a special flavor to increase feed intake in pigs and chickens, in other words, this meant the special flavor to increase palatability (Yang et al., 2010), subsequently its flavor stimulated the secretion of digestive juice and promoted intestine movement. Insects have not only been proposed a high nutritional value, but also were digested and absorbed easily in animal body as a sustainable protein source for animal feed.

Tenebrio molitor was one of species of darking beetle. Like other insects, it had four life stages; egg, larva, pupa and adult. *Tenebrio molitor* contained high protein and optimal animal acid compositions, so it was also generally used as a new animal protein source in animal feed and health food to human. *Tenebrio molitor* could be reared on the dry and bio-waste from fruits, vegetables and cereals. Currently *Tenebrio molitor* is mainly produced by China and approximately 200 tons of dried *Tenebrio molitor* are exported to European, Australian, and Asian markets annually. Four types of *Tenebrio molior* could produce some chemical defence substances. Ramos-Elorduy (2002) reported *Tenebrio molitor* could be used as an protein source to replace soybean meal. Using *Tenebrio molior* to replace fish meal did not show any negative effect on growth performance of piglets (Yang et al., 2010).

Consequently, an experiment was conducted to evaluate the effects of *Tenebrio molitor* larva supplementation on growth performance, blood profiles, nutrient digestibility and palatability in weaning pigs. Moreover, this trial figure out if *Tenebrio molitor* larva can be utilized as an protein source in young animals.

II. Literature Review

1. Post-weaning problems in piglets

1.1. Growth check after weaning

In weaning period, pig growth is affected by many factors. Among them, feed nutrition and the development of intestine are mainly factors. More in detail, the development of intestine in piglet is un-mature after weaning causing a problem that is the weaning pig has a limited digestive capacity to cope with solid feed. Moreover, the weaned pig can secrete the sufficient digestive enzymes for the digestion of milk products but not other feed ingredients, especially plant sources. In animal feed nutrition, using different protein sources such as vegetable and animal proteins are very important to the growth of piglets because pigs are sensitive to the dietary protein source after weaning. Many plant protein sources can lead to produce allergic reactions led to diarrhea, hypersensitivity, low growth rate and mortality. For instance, soybean meal is rich of anti-nutritional factors including glycinine, β -conglycinine and trypsin to strengthen allergic reaction and reduce protein utilization and digestibility, especially in weaning pigs (Miller et al., 1984). The utilization of animal proteins in feed was evaluated by many studies (Hansen et al., 1993c; Kats et al., 1994a; Kennedy et al., 1974a). The results showed using animal protein mostly had positive effects on growth performance and increase feed intake. Stimulating feed intake after weaning is very important to help gut development for increasing the secretion of enzyme. Animal proteins such as fish meal have a special flavor and this flavor can increase palatability (Yang et al., 2010). These beneficial effects on using animal proteins, which are more digestible than plant proteins, also prohibit the fermentation in large intestine by harmful microbes. Piglet growth can be enhanced by optimal ingredients provided and affected by the amount of enzyme output, so degree of digestion must be considered when choosing ingredients used in weaning diet. According to the changes of digestive capacity and feed nutrition in weaning pigs, weaning diet should be made of highly digestible ingredients to avoid growth check.

1.2. Anorexia loss of appetite

The morphological changes including shortening of the villi, a change in the shape of villi and hyperplasia should be the fundamental reason to cause anorexia loss of appetite. The intestinal morphological changes can lead to a decline of intestinal functions, reflecting reduced brush-border enzyme activity and absorption ability (Kenworthy and Allen, 1966; Smith, 1984; Hampson and Kidder, 1986; Miller et al., 1986). Pluske and Williams (1996) presented that changes in gut structure caused a problem with the low levels of voluntary feed intake in weaning period. Early-weaned pigs have low initial feed intake, which was proposed to be a contributing factor to the abruptly-reduced villus height (Cera et al., 1988). It has been reported that a positive relationship exists between feed intake and villous height or villus/crypt ratio (Kelly et al., 1991b; Markkink et al., 1994; Vente Spreeuwenberg et al., 2003). Therefore, decreased villous heights and villus/crypt ratios may be a direct reflection of decreased feed intake in the immediate post-weaning period. Adverse morphological alterations in the intestine have also been ascribed to local transit hypersensitivity reactions caused by dietary antigens (Miller et al., 1984a, b; Newby et al., 1984; Stokes et al., 1987; Li et al., 1990). The digestibility of diets is a determinant of feed intake in weanling pigs since digestibility is highly correlated with the gut filling capacity as well as appetite and has a positive relationship with the feed intake. The Addition of highly digestible ingredient to weaning pig diets can provide adequate nutrients for growth and help to relieve weaning anorexia loss.

2. Conventional animal protein sources for weaning pigs

2.1. Plasma protein

Spray-dried plasma protein is white powder and is produced by the plasma fraction of blood. In a nutritional point of view, spray-dried plasma protein containing 78% protein is high in lysine and tryptophan except methionine (Campbell, 1998). In addition to its high protein content, some special proteins in plasma can improve palatability and growth performance during post-weaning stage. Cromwell (1998) reported dried plasma has been proposed as a highly digestible protein source for weaning pigs. Also, it contains optimal amino acid model that meets the dietary needs of young pig. Furthermore, Pig fed diet containing plasma protein could improve immunity in weaning stage. Gatnau and Zimmerman (1992) reported that the maximum improvement of feed intake and weight gain could be observed by 6% of plasma protein. Kats et al. (1994a) concluded that exceeded 10% plasma protein could be used during phase I (0-14 day) but methionine as first limiting amino acid in plasma protein should be considered to formulate weaning diet. Adding level of plasma protein depends on age, weight of piglets and health status because its price is very expensive.

2.2. Fish meal

Fish meal is used as protein source in early stage of piglets. The quality of fish meal is affected by the type and species of fish. In general, fish meal contains high level of salt and oil. When salt content exceeded 7%, salt must be removed. Fish meals are generally high in protein (50~70%) on dry basis matter and amino acids are sufficient in fish meal compared to other plant protein (Seerley, 1991; Church and Kellems, 1998). Meanwhile, fish meal also contains high content of oil. Newport and keal (1983) presented extracted oil from fish could not increase feed intake and improve growth performance when added in weaning pig diet. Fish meal also is a good source of vitamins and minerals, especially Ca and P, and B vitamins are higher than other protein sources (Seerley, 1991; Church and Kellems, 1998). Many studies were found about addition of fish meal in weaning pig diet had a positive effect on growth performance (Gore et al., 1990; Laksesvela, 1961; Seerley, 1991; Newport and Keal, 1983). Animal protein sources, generally had positive effects on apparent digestibility of nitrogen and amino acids or on nitrogen retention. Addition of 5% fish meal could improve pig performance during the first 3 weeks after weaning (Gore et al., 1990). Stoner et al. (1990) reported pigs fed 8% fish meal showed the maximum weight gain, but addition of 12% fish meal did not seem to increase feed intake. Feed intake was increased because of it also had a special flavor to promote the secretion of digestive juice

(Yang et al., 2010). For pork quality, the amount of fish meal in diet could affect pork quality, exceed 6 or 7% resulting a fishy flavor in pork. Use of fish meal should be considered because the variation in economics is not steady.

2.3. Milk protein

The beneficial effects of increasing milk products in pig diet have been researched for a long time. Dried milk products can be divided into dried whole milk and dried skim milk. Dried skim milk contains no fat and fat-soluble vitamin by processing methods. Both milk products have high digestibility to animals and human. They also are good sources of vitamins and minerals (Seerley, 1991; Cromwell, 1998). Dried milk products are almost good protein source for young pigs. Milk products are very expensive for use in pig diet, but optimal addition of milk products in pig diet can improve growth performance and palatability (Wilson and Leibholz, 1981a,b,c; Walker et al., 1986). Speer et al. (1954) and Diaz et al. (1959) suggested pig diet should contain 40% of dried skim milk during the first week and second week after weaning. The addition of milk protein to starter diet provides sufficient nutrients for growth and helps to diminish the impact of anorexia after weaning. Some studies also reported piglets fed milk products that could maintain villus height and crypt depth to enhance absorption after weaning.

2.4. Blood meal

Blood meal is a good source of protein in animal feed. Generally, blood contains mostly protein (about 83-86 % DM) and small amounts of fat (less than 1% DM) and ash (less than 5% DM), though non-industrial blood meals may include other materials and be richer in ash. Unlike other animal protein sources, blood meal has a bad amino acid balance. Blood meal contains a low in lysine and isoleucine. So when diet for pig is formulated, the level of isoleucine must be enough to meet dietary requirement for performance desired. For mineral composition, blood meal is rich in iron more than 1500 mg/kg on dry matter. Blood meal is generally unpalatable (Miller, 1990) but different processing methods can affect palatability such as spray-drying and flash-drying processing (Miller, 1990; Cromwell, 1998). For piglet growth, Cunha (1977) reported blood meal should not used in weaning pig diet. Instead, some study showed blood meal was an effective protein source for weaning pig, when supplemented in diet during phase I (0-14 days) (Hansen et al., 1993c). Kats et al. (1994a) also reported addition of blood meal in weaning pig diet could decrease feed intake in initial phase after weaning. During phase II (14-28 days), spray dried blood meal could replace fish meal or SDPP as it increase both performance (Tokach et al., 1991). Inclusion rate should be no more than 2.5% of dry matter. In growing pigs, blood meal can replace soybean meal in corn-soybean based diets and then inclusion rate should be at 3 to 4% of dry matter (flash dried blood meal) or at 6% (Ilori et al., 1984).

3. Use and Process of insect as protein source

3.1. Using insects as a protein source in feed

Insects have been estimated as a high nutritional value, utilization and sustainable protein source. Using insects as a protein source could solve global shortage of conventional ingredients. Meanwhile, there was an efficient way to reduce the conventional cost of protein sources such as fish meal and soybean meal (Ng Liew et al., 2001).

Currently, important animal protein ingredients such as fish meal, plasma protein, meat meal and blood meal is used in animal feed. However, in many developed counties the use of the animal proteins is affected by legislation or globally pollutions for fish cultivation. The growing demand of resources to produce these animal proteins has doubled prices in the recent year, so alternative animal protein sources for livestock and swine are pressingly needed. Many researches had been giving the findings on insect supplementation in animal feed.

All over the world 1,500 species of insects were edible and used in animal feed or traditional food (Defoliart, 1992). Processed insects supplied 5~10% of animal protein and other nutrients including fats, vitamins, and minerals (MacEvilly, 2000). European Union have explored application of insects as a protein source in animal feed including black soldier fly, *Tenebrio molitor* and maggots. On the other hand, insects can utilize organic waste to reduce the negative environment impact in animal farms. Furthermore, insects have achieved artificial feeding on bio-wastes. More details on nutritional value, black soldier fly contains high level of protein and fat, which also is a suitable protein source for livestock. Some studies found that a protein source used in animal feed could improve growth of chickens and pigs (Newton, 1977) and fish (Newton et al., 2005; Sheppard et al., 2008). Maggots also can provide a great source of animal protein for poultry. From an economic point of view, Hwangbo (2009) reported maggot could replace fish meal in poultry diet and replacement of fish meal with maggot meal could improve weight gain and protein efficiency in broilers (Awoniyi et al., 2003). In short, not only do using insects as protein source enhance growth performance or improve the carcass quality and feed conversion, but insect contained high content of fat as energy. If insects production could be increased by large-scale rearing, the price of insect will be lower than other animal proteins and can replace expensive protein sources to reduce feed cost. To explore application of insects as a high protein feed ingredient for pig diets was feasible. Use of insects in the animal feed chain is more complex and starts with bio-waste. The interactions in the chains will influence if insect can be used successfully as an alternative animal protein. General, bio-waste is from dried and cooked waste materials from fruits, vegetables and grains and non-edible organic matters. However, Newton et al. (2005) reported insects also can be cultured directly on dry swine manure if urine can be removed by heating or drying treatments. Insects have a potential ability to alleviate a problem of manure accumulation in animal farms.

Bio-waste	→	Rearing insects	→	Processing insects	→	Animal Feed

Figure 1. Use of insects in the animal feed chain

3.2. Processing insects

In analogy to plant protein or other animal proteins, processing insects had a number of steps for application in the animal feed. The possible processing insect could be divided into two strategies.

The first strategy is to obtain the whole insect meal. The first processing step is cleaning of insects. This step separates their excreta and removes a minimum of defensive chemical substances produced by insects. The cleaning step is followed by a drying step. The drying step could remove moisture to be easy stored for a long time. Some researchers presented many different methods for cleaning and drying. Gawaad and Brune (1979) suggested using water at 40~50℃ washed fly larva three times and dried during 24 hours at 60℃. Ramos-Elorduy et al. (2002) suggested dried *Tenebrio molitor* larva at 50℃ for three days. After the drying step, insects were processed by the grinding step to obtain a whole insect meal with full fat (Calvert, 1969; Gawaad and Brune, 1979; Ramos Elorduy et al. 2002). The final step was heating. Unheated insect meal leaded to browning and the digestive enzymes in insects could decrease protein solubility and digestibility (Lwalaba, 2010). High heat treatment also cause a problem about denaturation of protein, it has negative effects on solubility and availability of amino acids. Some insect companies avoid this problem by choosing optimal heating temperature or addition of vitamin C and activated carbon but the cost of insect processing will be increased. So selecting optimal heat treatment was beneficial to increase the safety of insects. Whole insect meal was used directly in animal feed from technological processes.

The second strategy is to separate insect components respectively. After complex-processing, whole insect meal can be smaller particles separated into fat fraction; protein fraction and chitin fraction. Fat removal is thought to be a first step to obtain protein-rich fraction. Insects contain a high level of fat and extracted fat from insect is more expensive and also is used more valuable for human food than usage in animal feed, because insect has special fatty acid composition to ease digestion, which has more positive effects on human health than domestic animal. Removal of fat affected stability of insect meal because insects contain high amounts of unsaturated fatty acid which leads to fat oxidation reactions. Protein content of insect meal was increased by fat removal. Little informations was found about protein composition of insects for whole meal, not for separated protein. Protein-rich fraction also was processed by several extraction methods and then these products could be divided into soluble protein fraction and insoluble fraction. Soluble protein fraction is used in the food industry and insoluble protein fraction also is used in feed industry. Apart from the protein fraction, insect meal contains functional substance that is chitin, which is biodegradable linear polymer. Chitin consists β-(1-4)- linked 2-acetamido-2-deoxy-β-D- glucopyranose units. Chitin is the most abundant polymer in the world, except cellulose and has some physical and chemical properties such as a white, tasteless, odorless and insoluble in water and it dilutes organic solvents, which has a low reactivity. It also mainly exists in the shell of insects and can be processed into chitosan for many applications. For example, chitosan is used in the textile, paper or waste water industries. Chitin fraction can be obtained by two methods of extraction. first method is using NaOH and HCl to extraction, second is using biological methods by fermentation or enzyme treatment, but very expensive. Chitin can improve immune-system of animals and also is used in human medicine and animal feed as antibiotic or prebiotic. Lee (2008) reported that chitin a complex effects on innate and adaptive immune response. If chitin can be supplemented in animal feed, health status of animal will be improved to reduce the use of antibiotics in the feed industry.

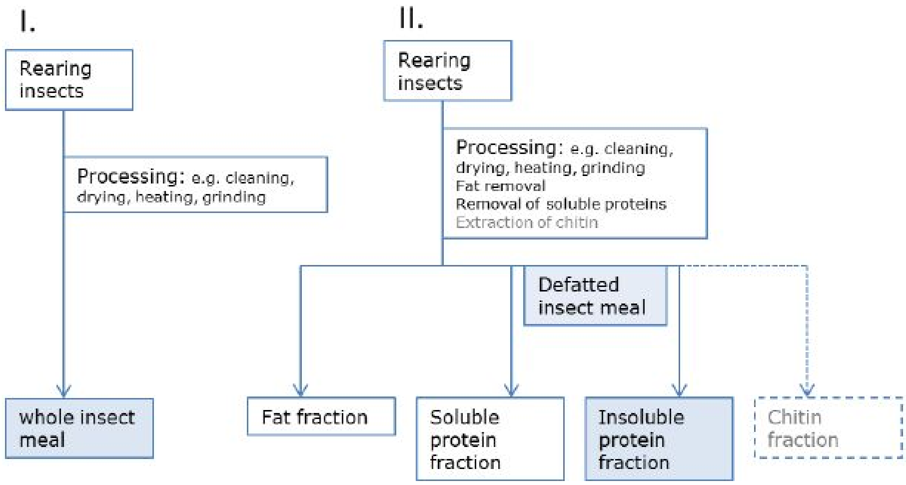

Figure 2. Possible processing strategies for insects I and II.

Source: Bukkens, 1997; Finke, 2002

4. Characteristics of *Tenebrio molitor*

Tenebrio molitor was the larval form of the mealworm beetle, a species of darking beetle. *Tenebrio molitor* was still the potential as a protein source to be exploited for animal feed and human food.

4.1. Life history

Tenebrio molitor go through four life stages such as egg, larva, pupa and adult. A female adult lays eggs, as many as 500 eggs in one cycle. The egg is white colour and the diameters of the eggs are 1~1.5

mm. The outer shell of eggs are very soft and can be easily broken. The development and hatching of eggs are affected by two main factors, which are temperature and moisture. Some study also reported when the temperature was at 25~30℃ the hatching stage was 5~8 days. However, when the temperature was at 19~22℃, the hatching stage was increased, ranging between 12 and 20 days. Under low temperature condition at 15℃, the eggs could hardly be hatched. So the hatching period should keep in an optimal temperature reducing the hatching stage. In an optimal rearing condition, larva typically emerges from the eggs. The early larva is white colour and is 2~3 mm long and the length of larva was increased by the increase of age. The mature larva is of a light yellow-brown colour and has 12 body segments and the length of adult is generally 28~35 mm. The growth period of larva is generally 80 to 130 days (average 120 days) following the next stage. The growth of larva is affected by many factors such as temperature, water content, rearing density and different feed level. Larva grows faster at a constant temperature of 25℃ and can produce large amounts of adults. The average weight of larva was increased and the growth period was shorten at a constant temperature of 25℃, so the suitable temperature is at 25℃ for larva growth (Manojlovic, 1988). About water content in feed, some results indicated that high water percentage in feed was better for growth, between 5% and 18%. When water percentage beyond 18%, larva was growth slow and the death rate will raise. In rearing density, 1.18 larva/cm^2 is suitable rearing density for larva growth. Meanwhile, larva fed diet with optimal nutrient value rearing in a certain condition because of reasonable nutritional level could stimulate feed intake and improve the growth of larva to reduce the cost for production. The final larva reveals the next stage, the pupa. The pupa did not eat and move and the pupa stage required about 5 days to 11 days to complete its development. (Weaver, 1990). During a period of pupa development, the optimal temperature and humidity is 25~30℃, 50~70% respectively. High temperature can cause a problem, which is the death of un-mature adult inside the pupa. Then the pupa will transform into an adult. In 1 or 2 weeks the pupa splits open and out walks a adult, white at first, but soon turning to brown and finally black after 4~5 days. The length of adult is 15~20 mm. Adult period is 50 days and living period ranges from 50 days to 160 days. Weaver (1990) reported the average lifetime was 57 days and the female adult could lay 15~16 eggs everyday and the cycle repeated.

According to the life story, *Tenebrio molior* has many advantages and it is possible to increase the scale of production at a low price. For instance, there are fast growth, high survival rate, easy to raise, disease resistance and high reproductive capacity, so these advantages can make *Tenebrio molitor* to be a sustainable and low-cost protein source.

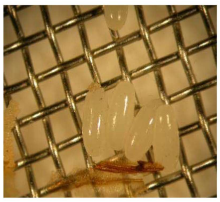			
Eggs	Larvae	Pupae	Adults

Figure 3. Morphology of *Tenebrio molitor*

Table 1. The growth of *Tenebrio molitor* in optimal temperature and moisture

Morphology	Temperature (℃)	Moisture (%)	Hatch, emergence (day)	Growth (day)
Adult	24~34	55~75		60~90
Egg	24~34	55~75	6~9	
Larva	25~30	65~75		85~130
Pupar	25~30	65~75	7~12	

Source: Liu, 2010

4.2. Nutritional values

Tenebrio molitor as an animal protein source used in animal feed is high in protein containing between 18% and 63% protein on a dry matter basis. *Tenebrio molitor* larva has a lower protein content than adult and larva had lowest crude fiber content than other types of *Tenebrio molitor*. However, larva has a highest fat content compared with adult and excreta. Not only larva can be used as protein source, but larva can be used as energy source. When larva is supplemented in animal feed, this characteristic with containing large amount of fat can compensate energy to reduce addition of oil and then reduces feed cost or increases fat digestibility. The adult contained the highest protein content and met requirements of NRC for rat growth (Mark, 2002). Moreover, excreta as a waste product contained incredible protein content by 18.51% , so excreta also was used as a additive for animal feed. Exuvium was high in crude fiber. Crude fiber of insect is made of chitin. Chitin can be processed into chitosan by deacetylation, used in various field as a component such as health food, animal feed and human medicine. The chitin content has not been estimated in different species and stages of insect. Finke (2007) estimated chitin contents in un-processing whole insects. For instance, a *Tenebrio molitor* adults contain chitin by 137.2mg/kg (dry matter basis) and house crickets contain chitin by 67.6mg/kg (dry matter basis). In a word, the nutritive value of *Tenebrio molitor* as potential protein source was available for animal feed.

Table 2. Percentage nutritional composition of *Tenebrio molitor* larva, Adult, Exuvium and Excreta

Items	Larva	Adult	Exuvium	Excreta
Proximal analysis				
Crude protein	46.44	63.34	32.87	18.51
Crude fat	32.70	7.59	3.59	1.30
Crude fiber	4.58	19.96	25.96	13.66
Crude ash	2.86	3.56	3.22	7.29
Moisture	5.33	3.54	13.02	12.20

Sources: Nergui Ravzanaadii, 2012

4.2.1. Protein quality

Improving protein efficiency is important for the growth of weaning pigs. In feed science, Protein efficiency is affected by protein quality. Protein quality is dependent on the amino acid balance for protein source, especially the content of essential animal acid and amino acid composition.

Tenebrio molitor as animal protein source is high in protein content and is a good source of lysine and other amino acid. In general, The amino acid pattern of animal protein is similar to meet the dietary needs of pig. *Tenebrio molitor* larva and adult have higher lysine contents than other types and this characteristic can reduce the addition of artificial-lysine in pig diet for reducing feed cost. Moreover, lysine was the first important essential amino acid (Kerr et al., 1993) and must be supplied in the diet to determine pig growth weight.

Tenebrio molitor larva have many kinds of amino acids and suitable proportion. FAO and WHO recommended the standard of high-quality protein that met two factors. First factor was essential amino acid to total amino acid ratio was 40%, second was essential amino acid to non-essential amino acid was 60% (Pellett, 1980). However, insects as a protein source had low level of methionine and cysteine (Defoliart, 1992). When insect was used in pig diet, deficient amino acids level should be paid to diet formulation, especially methionine level. Amino acid composition of *Tenebrio molitor* larva is similar to the result of Dai et al. (2009), who presented according to the standards of the amino acid score (AAS) and chemical score (CS), the limiting amino acids were evaluated. The first limiting amino acid of larva was the total of methionine and cysteine, in details the value of AAS and CS showed 0.3 and 0.21 respectively. The second limiting amino acid was threonine and the value of AAS and CS was respectively 0.92 and 0.71. So *Tenebrio molitor* larva used in animal feed should consider limiting amino acids within them. In summary, *Tenebrio molitor* larva has various kinds of amino acids and ideal amino acid composition. The use of *Tenebrio molitor* larva containing high protein and suitable amino acid composition as animal protein in pig and poultry diet is feasible.

Some results of previous study for analysis of nutritional value of *Tenebrio molitor* showed *Tenebrio molitor* had high contents of aspartic acid and glutamic acid. Aspartic acid and glutamic acid as flavor amino acids to improve the intensity of feed flavors, when supplemented in animal feed. The functions of these amino acids also showed a protective effects on the liver and heart and had anti-fatigue properties. Li (2007) showed the study that rat fed diet containing whole larva meal could improve growth and feed intake was increased. *Tenebrio molitor* has been described as a high quality protein source and addition of *Tenebrio molitor* larva in swine diet is feasible.

Table 3. Amino acid content of *Tenebrio molitor* larva, Adult, Exuvium and Excreta

Amino acid	Larva[1]	Adult[1]	Exuvium[1]	Excreta[1]	Larva[2]
Isoleucine	3.556	3.918	1.9	0.33	2.6
Leucine	3.405	5.165	1.981	0.368	4.6
Lysine	2.906	2.227	1.009	0.193	1.6
Cysteine+Methionine	1.189	1.134	0.426	0.251	1.6
Phenylalanine +Tyrosine	5.219	3.173	3.016	0.366	7.5
Threonine	1.807	2.153	1.124	0.276	2.7
Valine	2.439	3.368	2.423	0.253	3.8
Histidine	1.527	1.71	1.236	0.438	2.1

Sources: [1]Nergui Ravzanaadii, 2012; [2] E. D. Aguilar-Miranda, 2002

Table 4. Amino Acid Score (AAS) and Chemical Score (CS) of *Tenebrio molitor* larva and pupa

Amino acid	Isoleucine	Leucine	Lysine	Cysteine +Methionine	Phenylalanine +Tyrosine	Threonine
FAO/WHO (mg/g)	40	70	55	35	60	40
Egg protein (mg/g)	50	92	56	50	91	52
Larva (mg/g)	37.5	81.7	63.8	10.5	115.1	36.7
Larva (ASS)	0.94	1.17	1.16	0.3	1.92	0.92
Larva (CS)	0.75	0.89	1.14	0.21	1.26	0.71
Pupa (mg/g)	43.3	81.5	66.0	21.4	130.5	35.7
Pupa (ASS)	1.08	1.16	1.20	1.61	2.18	0.89
Pupa (CS)	0.87	0.89	1.18	0.43	1.43	0.69

Sources: Dai, 2009

4.2.2 Fat quality

Fats are an important part of living and are characterized by the source and quality. Fat sources can be divided into animal fat and plant fat. Animal fat can provide energy to pig diet with low heat increment. In detail, 1g of animal fat can supply 38.60 kJ and is twice as more as protein and sugar supply. Fat quality is determined by fatty acid composition, hardness, color and stability.

Tenebrio molitor used as energy source is high in fat. The energy value of *Tenebrio molitor* (806 kcal) was higher 252 kcal than the house fly (554 kcal), when used in rat feed (Ramos-Eloruy, 1989). Also, *Tenebrio molitor* has a good composition of fatty acid. Fatty acid composition was related to fat digestibility. In general, fat digestion was affected by many factors. For example, the length of the carbon chain, the level of saturated fatty acid. In general, high level of un-saturated fatty acid composition, optimal U:S ratio, short chain of fatty acid also can improve fat digestibility. Braude (1973) reported the absorption rate of fatty acid was relatively correction with the length of the chain of fatty acid, more shorter more digestible and absorbed than long chain fatty acid. Powles (1994) presented the optimal U:S ratio was 5.71 and this value was necessary to maximize fat digestibility in weaning pigs. According to these factors affecting fat digestion, *Tenebrio molitor* has higher level of un-saturated fatty acid and optimal ratio of un-saturated to saturated fatty acids, especially larva compared to other forms in life-stage. *Tenebrio molitor* contains high level of palmitic acid in total saturated fatty acid contents. In addition, *Tenebrio molitor* also has high content of oleic acid and linoleic acid as un-saturated fatty acid. Linoleic acid was an essential fatty acid and was not synthesised in animal body, what got from the diet. The biological function of linoleic acid reduced cholesterol concentration in the blood and increased high density lipoprotein concentration. Li et al. (2007) reported the mice fed diet with oil of *Tenebrio molitor* could improve ability to learn and memorize. Overall, *Tenebrio molior* larva has high level of un-saturated fatty acid and also high ratio of un-saturated to saturated fatty acid ratio. In some insects, for example essential fatty acids such as linolenic acids and linoleic were even higher compared with fish and poultry (Defoliart, 1991). It meant *Tenebrio molitor* larva was high in fat digestibility. In the study of Newton et al. (1977), larva of black soldier fly had better fat digestibility than SBM. The extracted oil from *Tenebrio molitor* larva was feasible to be used more valuable in human diets than used in animal feed because the costs of removal processing is expensive.

Table 5. Fatty acid composition of *Tenebrio molitor* larva, pupa and adult

Component	*Tenebrio molitor*[1]			Larva[2]
	Larva[1]	Pupa[1]	Adult[1]	
Myristic acid	4.87	5.15	2.22	4.60
Palmitic acid	17.13	18.28	18.83	16.40
Palmitoleic acid	2.54	2.21	1.55	2.98
Stearic acid	1.92	2.43	4.67	2.46
Oleic acid	43.85	43.74	40.03	42.19
Linoleic acid	25.31	24.10	27.63	28.92
Linolenic acid	0.68	0.56	0.56	1.13
Saturated fatty acids	23.92	25.86	25.72	23.46
Un-saturated fatty acids	72.38	70.61	69.77	75.22
U/S	3.03	2.73	2.71	3.21

Sources: [1]Huang, 2010, [2]Dai, 2009

4.2.3 Other nutrients

Insect supplements are a good source of protein and fat compared with plant ingredients, they are also good sources of vitamins and minerals. The data of vitamins is not shown and the mineral composition of *Tenebrio molitor* was reported many times (Dai, 2009, Huang, 2010). The exuvium and excreta had high levels of Ca and K than larva and adult. Ca is the most abundant mineral in the body and very important related to the bone growth. Exuvium and excreta as the wastes also are used in human health food and it is a way to recycle the waste products in all life-stages. *Tenebrio molitor* Larva had low content of Ca, but contains high level of P. The contents of many minerals in mealworm were increased as the age increased.

Table 6. Mineral composition of *Tenebrio molitor* larva, adult and exuvium, and excreta (mgof mineral/kg of sample)

Criteria	Larva	Adult	Exuvium	Excreta
Mineral				
Calcium (Ca)	432.59	484.39	801.14	1,537.97
Phosphorus (P)	7,060.7	8,087.07	5,252.29	14,552.01
Potassium (K)	9,479.73	10,459.8	14,725.66	21,171.75
Iron (Fe)	66.87	78.71	55.86	127.75
Natrium (Na)	3,644.84	4,302.73	6,343.16	3,954.33
Magnesium (Mg)	2,026.88	1,932	1,388.09	7,135.14
Zinc (Zn)	104.28	108.98	265.18	101.31
Copper (Cu)	13.27	18.01	10.04	10.73

Sources: Nergui Ravzanaadii, 2012

4.2.4. Functional properties

There were some studies found that the insect contained a valuable product in its shell, that is called chitin. It is a non-toxic, biodegradable linear polymer. Chitin $(C_8H_{13}O_5N)_n$ consists mainly of β-(1-4)-linked 2-acetamido-2-deoxy-β-D-glucopyranose units and partially of β-(1-4)-linked 2-amino-2-deoxy-β-D-glucopyranose. It was the most abundant polymer in the world instead cellulose. Chitin was insoluble in water and diluted in mineral acids and more organic solvents. Chitin has a low level of reaction and low process ability. Currently, chitin was used in feed additive, human medicine and industrial source. For industry, chitin could be processed into chitosan, which was possible to use in cosmetic, paper or waste water industries and chitin production from insect waste is a new area.

The chitin content of *Tenebrio molitor* is changing with different stages. Most of chitins are remained in exuviate; Exuviate has high percentage of chitin about 18.35%. An adult is produced by metamorphosis at 15~20 time from egg for exuviate accumulation. *Tenebrio molitor* larva contains lowest content of chitin than other types. Recent studies reported that chitin has a complex effect on immune responses (Lee, 2008). Chitin could improve the immune status of the animal acting as antibiotic in rats and chickens (khempaka, 2011). Finke (2007) estimated chitin content in *Tenebrio molior* adult was 137.2mg/kg on dry matter basis.

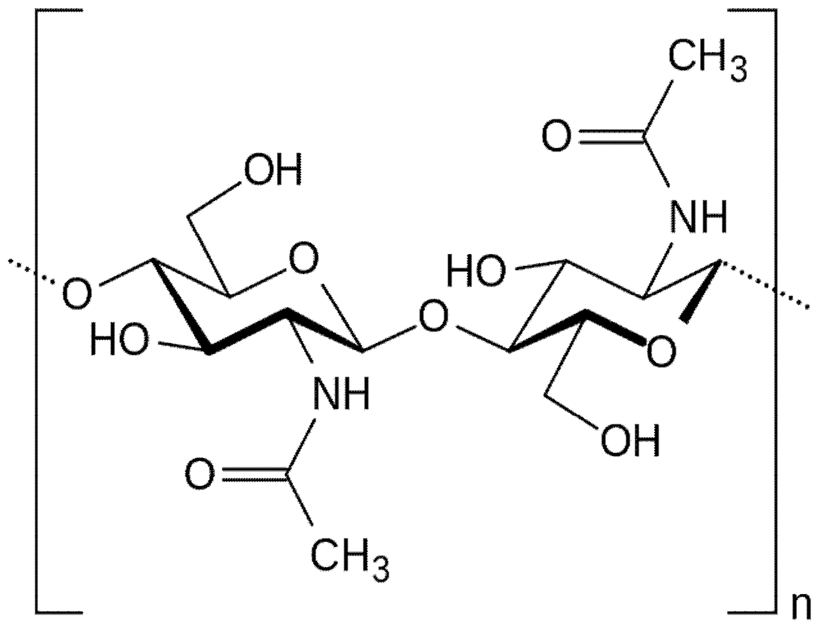

Figure 4. Chemical structure of the chitin molecule
Source: Shahidi, 1999; Campbell, 1996, Morganti, 2012

Table 7. The chitin percentage of *Tenebrio molitor* with different stage

Part	Repetition	Chitosan weight(g)	Chitin percentage	Average Chitin percentage
	1	0.5105	12.865	
Puparium	2	1.5320	13.406	13.772
	3	0.5970	15.044	
	1	0.6930	17.463	
Exuviate	2	0.7391	18.623	18.350
	3	0.7526	18.963	
	1	0.4346	10.950	
Adult	2	0.4593	11.579	11.260
	3	0.4465	11.252	
	1	0.1649	4.158	
Body wall of larva	2	0.1787	4.712	4.364
	3	0.1675	4.221	

Source: Wu, 2007

4.3. Cost price

Prices of feed ingredients for animal feed will rise fast because conventional feed ingredients such as soybean meal or fish meal could not meet the needs for animal feed industry. The FAO is predicting that the prices of many conventional ingredients will rise in the period from 2010 to 2019 (Meuwissen, 2011). Currently, insects are more expensive than other protein sources because insects companies always have low automation and low labour productivity without large scale rearing. The cost price of insect should be reduced by many categories such as reducing feed costs for rearing, labour costs, housing costs and processing costs and increasing insect productivity.

Table 8. Prices of different protein sources (88% of dry matter)

Protein source	Protein content (%)	Price/kg product (€)	Price/kg protein (€)
Mealworm	50	15.80	31.70
Fish meal	65	1.24	1.91
Grain	12	0.14	1.17
Soybean meal	45	0.28	0.62

Source: Meuwissen, 2011

5. Safety and regulation

5.1. Pathogens, heavy metals and toxins

Liu et al. (2008) reported *E. coli* was reduced by the black soldier fly in cow manure. In general studies researched that insects themselves did not have harmful pathogens for humans. However the conditions by the rearing of insects need to pay great attention. Klunder et al. (2012) also reported hygienic handling and correct storage were important to reduce pathogens. *Tenebrio molitor* could produce benzoquinone compounds as chemical defence substances. Some studies pointed out benzoquinone could combine with protein in the cell to produce benzoquinone-adduct and O_3 in rat (unpublish data). Hu (2004) reported the accumulation of large amounts of O_3 led to a decrease in performance of livestock. Wu (2009) reported the benzoquinone concentration of three forms of *Tenebrio molitor* in different growth phase had been evaluated. The results showed the benzoquinone concentration was increased as increasing the age in every form of *Tenebrio molitor* with different growth phase and 70 days larva of

Tenebrio molitor contained highest concentration of benzoquinone (25.257ug/larva) compared to other forms. The benzoquinone concentration of *Tenebrio molitor* is removed by processing methods including cleaning, drying, heating and grinding. Using these methods can separated the benzoquinone from the body of insect and the benzoquinone is volatile substance. Through these methods benzoquinone was easy to remove by evaporation and decomposition. The way of synthesizing methods such as cleaning-drying-heating-grinding removed maximum amount of benzoquinone of 50 days larva of *Tenebrio molitor*, about 95.6% (Wu, 2009), thereby improved edible safety of *Tenebrio molito.* Insect also could accumulate heavy metals from the rearing environment and that could not kill themselves. In general, the accumulation of heavy metals is relative to the feed. The storage of heavy metals in insects may be toxic for animals and human. The use of insect as feed ingredient in pig diets should be determined mostly on safety consideration. For rearing insects it is important to reduce the accumulation of heavy metals.

Table 9. The benzoquinone concentration of different state in different stage of larva of *Tenebrio molitor* L.

Forms	Age	Benzoquinone concentration (ug/larva)
Larva	10	0.701
	50	12.589
	70	25.257
Pupa	1	4.244
	7	5.657
Adult	1	6.325
	10	13.033
	15	20.051

Source: Wu, 2009

5.2. Regulation

Little information was found about the regulation for using insects as feed ingredient in pig and poultry diets in Korea. In EU insect protein may be used as an ingredient in pig and poultry diet. Under new part of EU regulation, insect protein was discussed and might be possible to use in the future. The European commission also completed standards for the use of insects as animal feed ingredient in details and indicated insect producer must improve edible safety. For other countries, it is necessary to develop the regulations or establish new regulations about the usage of edible insects.

6. Application of insects in monogastric animals

Insect as protein source which was supplemented in poultry diet had positive effects on growth performance, nutrient digestibility and palatability, but little information has been available about using *Tenebrio molitor* as protein source in pig diets.

6.1. Growth performance

Using insects as protein source was focus on poultry diet and little information was found about growth performance for pigs. For poultry production, maggots were used as a protein source for poultry. Teguia (2002) reported maggot meal could replace fish meal to reduce feed cost under the large scale of insect production. This indicated the nutrition value of insect was comparable to fish meal. When replaced 25% of fish meal to maggot meal, weight gain was increased and improved protein efficiency rate (Awonyi, 2004). Hwangbo (2009) showed the study that broiler chickens fed diet containing 10 to 15% maggots could improve the carcass quality and growth performance. Another study reported maggot meal supplementation in broiler diet could increase the average weight gain and feed intake compared with corn-soybean basic diet (Pretorius, 2011). Deng (2008) reported using *Tenebrio molitor* meal as protein source in laying hen diet could increase the egg production rate and feed efficiency by 5.06% and 6.9%, respectively. When broiler fed diet with *Tenebrio molitor* meal, the result also improved body weight, feed intake and feed efficiency (Shen, 2006). For pig performance, Ramos-Elorduy et al. (2002) found that *Tenebrio molitor* could potentially be used as an alternative protein source to replace fish meal in pig diet. Zhang et al. (2002) reported using silkworm meal supplementation in finishing pig diet could increase ADG by 23.6% and reduce the finishing period compared with pig fed a conventional diet. In a word, insect meal supplementation as a protein source in pig and poultry diet was feasible from the nutritional and economic point of view.

6.2. Nutrient digestibility

Few studies were found about nutrient digestibility of insect as protein source used in pig or poultry diet. Two studies reported also using housefly to evaluate nutrient digestibility in broiler chickens. Hwangbo (2009) Reported using 4-week old broilers fed a diet with 30% dried housefly larva meal or soybean meal for 7 days to evaluate protein and amino acid digestibility. The results showed housefly larva had a high apparent faecal digestibility value of crude protein compared to soybean meal diet. In details of amino acid digestibility, house fly larva had higher digestibility of most amino acid than soybean meal. It meant housefly larva protein was easy to digestion and had a good amino acid balance to improve protein efficiency. Pretorius (2011) reported using 3-week old broilers fed diet containing 50% dried housefly larva meal or dried housefly pupa meal. The result showed that the digestibility of crude protein of the housefly larva was only 69% and housefly pupa had a higher protein digestibility than larva. This indicated different forms of insect had different nutrient digestibility and how to choose a suitable stage of insect used in pig or poultry diet. Only one study was found Newton (1977), who reported using black solder fly larva as protein source was supplemented in growing pig diet compared to soybean meal diet. The result showed the larva meal treatment had similar apparent digestibility of crude protein compared to soybean meal diet. However, fat digestibility was higher for the larva meal treatment. It may be black soldier fly had a good composition of fatty acid to ease digestion and utilization.

Table 10. Apparent faecal digestibility of diets containing housefly larva, housefly pupa meal or soybean meal in broiler chickens

Criteria	Hwangbo, 2009		Pretorius, 2011	
	Hf larva	SBM	Hf larva	Hf pupa
Nutrient digestibility				
Dry matter	ND	ND	81	83
Crude protein	98.5	98.0	69	79
Crude fat	ND	ND	94	98
Crude ash	ND	ND	83	85
Amino acid digestibility				
Arginine	95.6	93.9	BD	93
Lysine	97.6	92.7	ND	ND
Methionine	95.6	93.0	ND	ND
Threonine	93.3	89.3	93	97
Tryptophan	93.9	93.2	95	99
Leucine	94.7	92.7	ND	ND
Isoleucine	92.2	93.3	ND	ND
Valine	94.5	91.1	91	91
Histidine	93.7	90.1	87	87

Abbreviations: ND = not determined, BD = below detection limit.
Sources: Hwangbo, 2009; Pretorius, 2011

6.3. Palatability

Palatability of feed including taste and texture refers to the reflection of feed intake and feed frequency by animals and it is a comprehensive response to sensory organs in the process of animal foraging and feeding. Palatability of feed could affect animal appetite to adjust feed consumption themselves. Therefore, selecting highly palatable feed ingredient may increase feed intake and growth performance is related to feed consumption. Palatability of feed is affected by many factors. For instance, anti-nutritional factors, moldiness of feed, particle size of feed and special flavor. Some commercial flavor additions has been known to increase feed consumption, although weight gain and feed efficiency were not improved, but a feed ingredient, fish meal was very palatable and many studies showed pig diet containing fish meal could improve growth performance including feed intake (Gore et al., 1990, Stoner et al., 1990). Like fish meal, *Tenebrio molitor* as animal protein also contains a special flavor. Yang (2010) reported replacing fish meal with *Tenebrio molitor* in weaning pig diet could promote the secretion of digestive juice and increase intestinal movement to more strength, so feed consumption was increased.

Ⅲ. Supplementation of *Tenebrio Molitor* Larva as a Protein Source on Growth Performance, Nutrient Digestibility and Palatability in Weaning Pigs

Abstract: This experiment was conducted to investigate the effects of dietary *Tenebrio molitor* larva on growth performance, nutrient digestibility and blood characteristics in weaning pigs. A total of 120 weaning pigs (28 ± 3 days and 8.04 ± 0.08 kg of BW) were allotted to one of five treatments, based on sex and body weight, in 6 replicates with 4 pigs per pen by a randomized complete block (RCB) design. Five different levels of *Tenebrio molitor* larva (0, 1.5, 3.0, 4.5 and 6.0 %) were used as dietary treatments. Two phase feeding programs (phase I for 0-14 day, phase II for 15-35 day) were used in this experiment. During phase I, increasing level of *Tenebrio molitor* larva in diet linearly improved the body weight ($P<0.01$), ADG ($P<0.01$) and ADFI ($P<0.01$). During phase II, ADG also tended to increase when pigs were fed higher level of *Tenebrio molitor* larva ($P=0.08$). Therefore, increasing level of *Tenebrio molitor* larva improved the ADG ($P<0.01$) and ADFI ($P<0.05$) and tended to increase G:F ratio ($P=0.07$) during overall experimental period. As dietary *Tenebrio molitor* larva was higher, nitrogen retention and digestibilities of dry matter as well as protein were increased, respectively ($P=0.05$). In the results of blood profiles, decrease of blood urea nitrogen ($P=0.05$) and increase of

IGF-1 (P=0.03) were observed as dietary *Tenebrio molitor* larva was increased in diet during phase II. In immune responses, there were no significant differences in IgA and IgG concentration by addition of *Tenebrio molitor* larva. Consequently, supplementation of *Tenebrio molitor* larva up to 6% in weaning pig diet could improve growth performance and nutrient digestibility without any detrimental effect on immune responses.

Key Words: Insect, Protein source, *Tenebrio molitor* larva, Weaning pigs.

Introduction

More than 10 millions of insect species have been identified, which account for a half of all creatures in earth. Among of them, approximately 1,500 species of insects are known to edible protein source for human and animal food (Ng Liew, 2001). Generally, insects contain high level of protein, 30.8~81% DM basis, and 18 kinds of amino acids including 4 essential amino acids, some vitamins and minerals properties have been evaluated in details (MacEvilly et al., 2000). Furthermore, insects have several physiological traits such as high efficiency of reproductive ability, high organic conversion and easy rearing with cheap cost of their feed. Therefore, insects have been proposed as a sustainable alternative protein source for animal feed.

Insects market for animal feed is continually increasing in China, especially focused on *Tenebrio molotor*. *Tenebrio molitor* has been known to be an acceptable protein source in poultry diet (Ramos-Elorduy et al., 2002), and the nutrition value of *Tenebrio molitor* was also reported (Nergui, 2012).

Previously, various of kinds of studies had been conducted to evaluate the nutritional value of *Tenebrio molitor* and developed the methods for large-scale production (Hemandez 1987, Legunes and Garcia 1994). In poultry nutrition, the effects of *Tenebrio molitor* are relatively well known by several researchers (Ramos-Elordoy et al., 2002; Liu et al., 1994; Deng et al., 2008). However, little information is available about using *Tenebrio molitor* in pig diet.

Consequently, the present study was conducted to investigate the effects of *Tenebrio molitor* larva supplementation as protein source on growth performance, nutrient digestibility, blood profiles and palatability in weaning pig diet.

Materials and methods

Animal and Experimental design

A total of 120 crossbred ([Yorkshire × Landrace] × Duroc) weaner pigs (28 ± 3 d of age, 8.04 ± 0.08 kg BW) were assigned to one of five treatments considering sex and initial body weight in 6 replicates with 4 pigs per pen in a randomized complete block design. Pigs were reared on concrete-slot floor weaner facility (concrete-slot floor, 0.90×2.15 m^2) during the whole experimental peirod. Control treatment group was corn-SBM-barley based diet and the other groups were provided diet containing 1.5, 3.0, 4.5 or 6.0% of *Tenebrio molitor* larva during 35 days after weaning. During experimental periods, feed and water were provided *ad-libitum* through a feeder and a nipple installed in each pen. The environmental temperature was kept at 30℃ during the first 7 days and lowered 1℃ every week. Experimental period consist of two phases (Phase I: 14 days, Phase II: 21 days) and body weight and feed intake were recorded at the end of each phase to calculate average daily gains (ADG), average daily feed intakes (ADFI) and gain to feed ratio (G/F ratio).

Experimental diets

A corn-SBM-barley meal based control diet was formulated, then the other 4 treatment diets were formulated with addition of 1.5%, 3.0%, 4.5% or 6.0% of *Tenebrio molitor* larva in basal diet and other feed ingredients were adjusted for identical nutrient composition of each experimental diet. All nutrient composition of experimental diets were met or exceeded the nutrient requirements of NRC (2012).

Experimental *Tenebrio molitor* larva was harvested approximate 70 d of age and air-dried prior to utilization in each treatment diet. *Tenebrio molitor* larva contained approximately 5.84% of moisture; 43.27% of crude protein; 32.93% of fat and 4.86% of crude ash, respectively. Formulas and chemical composition of experimental diets were presented in Tables 1 and 2.

Digestibility trial

To evaluate the nutrient digestibility and nitrogen retention of *Tenebrio molitor* larva containing weaner feed, a total of 20 crossbred Phase II barrows (10.05 ± 0.98 kg BW) were assigned to in an individual crate and allotted to one of treatments with 4 replicates in completely randomized design. Each pig was fed 100g of phase II diet twice a day at 7:00 and 19:00. After 5 days adaptation period, 5 days of collection period was started with addition of 1% chromium oxide in experimental diets as an initial marker. As a finishing marker, 1% ferric oxide was added in each experimental diet at 6th day of experimental period. Collection of feces was started when the chromium oxide appeared in the feces and kept until the next appearance of ferric oxide in the feces. Urine samples were collected during collection period in plastic container containing 50 ml of 4N H_2SO_4 to avoid evaporation of nitrogen for nitrogen retention analysis. Fecal and urinary samples were stored at -20℃ until the end of collection period and the feces was dried in a drying oven at 60℃ for 72 h and then ground to 1mm in a Wiley mill for chemical analysis including moisture, protein, fat and ash contents by AOAC methods (1995).

Blood sampling

In each treatment, 6 pigs near average body weight were bled through the anterior vena cava to analyze blood urea nitrogen (BUN), insulin-like growth factor (IGF-1) and serum immunoglobulins (IgA, IgG). Blood sampling was done at initial day and the ends of each phase. Collected blood samples were in disposable culture tube and centrifuged for 15 min by 3,000rpm at 4℃ (Eppendorf centrifuge 5810R, Germany). The sera were carefully transferred to 1.5 ml plastic tubes and stored at -20℃ until later analysis. Total BUN concentration was analyzed using a blood analyzer (Ciba-Corning model, Express Plus, Ciba Corning diagnostics Co.), and IGF-1 concentration was analyzed with hormone analyzer (Immulite 2000, DPC, SUA). Serum IgG and IgA concentration were analysed by ELISA assay by the manufacture’s protocols (ELISA Starter Accessory Package, Pig IgG ELISA Quantitation Kit, Pig IgA ELISA Quantitation Kit; Bethyl).

Chemical analysis

Diets were ground by a Cyclotec 1093 Sample Mill (Foss Tecator, Hillerod, Denmark) and ground diets and feces were analyzed.

The contents of dry matter (DM) (procedure 967.03; AOAC, 1990), ash (procedure 923.03; AOAC, 1990) nitrogen was analyzed by using the Kjeldahl procedure with Kjeltec (KjeltecTM 2200, Foss Tecator, Sweden) and calculating the CP content (Nitrogen × 6.25; procedure 981.10; AOAC, 1990). Nitrogen in urine was determined by Kjeltec (KjeltecTM 2200, Foss Tecator, Sweden).

Statistical analysis

Statistical analysis was carried out by least squares mean comparisons using General Linear Model (GLM) procedure in SAS (SAS Inst. Inc., Cary, NC). Orthogonal polynomial contrasts were performed to analyze the linear or quadratic effects of increasing levels of *Tenebrio molitor* larva in weaning pig diet. Each pen was considered as an experimental unit in measuring growth performance, while individual pig was also served as an experimental unit for analyzing nutrient digestibility, nitrogen retention and blood characteristics. Statistical responses and suggestive responses of dietary *Tenebrio molitor* larva were considered at $P < 0.05$ and $P < 0.10$, respectively.

Results and discussion

Growth performance

Table 3 presented the effects of supplementation of *Tenebrio molitor* larva on growth performance in weaning pigs. Increasing *Tenebrio molitor* larva in weaning pig diet significantly improved the body weight during 35 days of experimental period (linear, $P<0.01$). During phase I (0 to 14th day), ADG and ADFI were linearly increased as larva level increased (Linear, $P<0.01$). During Phase II (15 to 35th day), increasing *Tenebrio molitor* in weaning pig diet tended to improve the ADG of pigs (linear, $P=0.08$) but ADFI was not altered by addition of *Tenebrio moiltor* larva. Although, significant improvement was not observed in G:F ratio during phases I and II, numerical improvement was observed by increasing *Tenebrio moiltor* level in diet which resulted in tendency of improvement during overall experimental period (linear, $P<0.07$).

In poultry nutrition, the effects of *Tenebrio molitor* are relatively well known by several researchers (Ramos-Elordoy et al., 2002; Liu et al., 1994; Deng et al., 2008). *Tenebrio molitor* has been known to be an acceptable protein source in pig and poultry diet (Ng Liew et al., 2001) but little information has been available about using *Tenebrio molitor* as protein source in pig diets. Recently, Chen et al. (2012) reported that increasing *Tenebrio molitor* protein concentrate up to 6% in weaning pig diets linearly improved body weight and body weight gain. Although current study used full-fat *Tenebrio molitor* larva, the results of current study also showed dietary *Tenebrio molitor* have benefits as a protein source for weaning pigs.

Moreover, improvement of ADFI by increasing *Tenebrio molitor* larva in current study can be explainable with the study of Yang et al. (2010) which demonstrated that *Tenebrio molitor* had a special flavor which increased the secretion of digestive juice, resulting in increase of feed intake in weaning pigs.

In addition, piglets had to faced on several stresses which caused lower feed consumption and growth retardation (Bark et al., 1986) after weaning. Subsequently, animal origin protein sources are widely utilized in weaning pig diet rather than soybean meal or other plant-derived ingredients although its price is much more expensive. In the current study, increasing level of *Tenebrio molitor* larva in diet showed higher growth performance during phase I than that of phase II. Consequently, the use of *Tenebrio molitor* larva as a feed ingredient was expected to be feasible if its price will be competitive with fish meal.

Nutrient digestibility

Addition of *Tenebrio molitor* larva in weaning pig diet showed notable improvements in nutrients digestibility and nitrogen retention (Table 4). There were linear increase in dry matter (linear, P=0.05) and crude protein (P=0.05) digestibility by increasing level of *Tenebrio molitor* larva in diet and crude ash digestibility also tended to improve by dietary *Tenebrio molitor* larva (linear, P=0.06).

In nitrogen retention, pigs fed higher amount of *Tenebrio molitor* showed reduction of nitrogen excretion through feces (linear, P=0.05), which resulted in linear increase of nitrogen retention (linear, P=0.05).

In addition, decrease of fecal nitrogen excretion (linear, P=0.05) was observed by addition of *Tenebrio molitor* larva in diet subsequently there was a linear increase of nitrogen retention (linear, P=0.05).

Nutrient digestibility of *Tenebrio molitor* larva in swine had not been well documented. In the other insect studies, Newton et al. (1977) reported that addition of black soldier fly larva in pig diet showed similar apparent crude protein digestibility with soybean meal. Similarly, Hwangbo et al. (2009) showed broilers fed diet containing 30% housefly larva meal showed a higher apparent crude protein digestibility with improvement in the most of amino acids digestibility than broilers fed soybean meal based diet. Generally, animal protein sources had better protein efficiency than plant-derived protein sources, because animal protein contained high level of essential amino acids with balanced amino acid composition (Cromwell, 1998).

In the current study, supplementary *Tenebrio molitor* larva did not alter crude fat digestibility. Insect larva contained high level of energy, because larva accumulated lipid in their body for pupa stage. Therefore, addition of *Tenebrio molitor* larva in diet caused decrease in addition of soy oil in the present study. On the contrary to protein digestibility, plant derived fat has higher amount of unsaturated fatty acids than animal derived fat sources such as tallow and lard, which resulted in higher digestibility in mono-gastric animals. However, Newton et al. (1977) demonstrated that black soldier fly larva had better fat digestibility than that of SBM and Nergui et al. (2012) represented that *Tenebrio molitor* larva contained high level of unsaturated fatty acid. Therefore, equivocal result in fat digestibility indicated that *Tenebrio molitor* larva is not only a superior protein source but also a suitable fat source for weaning pigs.

Blood profiles

Blood urea nitrogen and Insulin like growth factor-1

Changes of blood urea nitrogen (BUN) and serum insulin like growth factor (IGF-1) concentration during feeding trial were presented in Table 5. Increasing level of *Tenebrio molitor* larva linearly decreased BUN concentration at 35th day after weaning (linear, $P<0.05$) and similar decreasing tendency was observed at 14th day (linear, $P=0.08$). In contrast to BUN concentration, serum IGF-1 concentration was linearly increased by addition of *Tenebrio molitor* larva at 35th day after weaning (linear, $P<0.05$).

Generally, BUN concentration is considered as an indicator for determination of protein quality and protein intake by pigs (Eggum, 1970). High level of BUN represented that excessive amino acids were metabolized and circulated in the blood during the excretion. In addition IGF-1 is secreted by stimulation of growth hormones which related to growth and differentiation of tissue (Bayes-genis, 2000). Therefore, decrease of BUN concentration and increase of serum IGF-1 can be used to predict the trend of protein utilization and animal growth. The current results in BUN and serum IGF-1 support the improvement of growth performance and nutrient digestibility with supplementation of *Tenebrio molitor* larva.

Immune response (IgA* and *IgG)

Table 6 showed the effects of increasing *Tenebrio molitor* larva in diet on serum IgA and IgG concentration. Increase of dietary

Tenebrio molitor larva in diet did not alter serum immunoglobulins. Effects of supplementary insects on immune responses are conflicted by researches. Insects contained high level of chitin as a component of exoskeleton (Finke, 2002). Some studies reported that chitin had complex effects on immune response (Lee et al., 2008), which improved the immune status of the animals (Harikrishnan et al., 2012). However, some researches demonstrated that using insects should increase concerns about edible safety because insects contained chemical defence substances as toxin produced by exocrine gland (Wang et al., 2001). Although, Wu et al. (2009) represented that *Tenebrio molitor* larva contained benzoquinone as a toxin which could cause health problem in animal feed, in the results of the current study, however, showed no detrimental effects on serum IgG and IgA concentration by supplementation of *Tenebrio molitor* larva in weaning pigs.

Conclusion

In the results of growth performance, body weight (linear, $P<0.01$) was increased as larva level increased during the whole experimental period. There were significant differences in nutrient digestibility of crude dry matter ($P=0.05$) and crude protein ($P=0.05$), resulting in linear response. When pigs were fed diets containing high level of larva, a decrease of BUN (linear, $P=0.05$) and increase of IGF-1 (linear, $P=0.03$) were observed in phase II. Addition of *Tenebrio molitor* larva by 6% in weaning pig diet showed positive effects on growth performance, nutrient digestibility and blood profiles. In immune responses, there was no detrimental effects in serum IgG and IgA concentration by addition of *Tenebrio molitor* larva in weaning pig diet. *Tenebrio molitor* larva was considered an applicable protein source in weaning pig diet and addition of *Tenebrio molitor* larva up to 6% in weaning pig diet could improve growth performance and nutrient digestibility without detrimental effect on immune response.

Table 1. Formula and chemical compositions of the experimental diets in phase I (0 to 14th day)

Ingredients, %	*Tenebrio molitor* larva levels				
	0	1.5	3.0	4.5	6.0
Corn	30.95	31.03	31.02	31.05	31.04
SBM(44%)	35.10	33.34	31.51	29.80	28.01
Molitor Larva	0.00	1.50	3.00	4.50	6.00
Whey powder	3.00	3.00	3.00	3.00	3.00
Lactose	12.00	12.00	12.00	12.00	12.00
Barley	12.42	13.32	14.37	15.26	16.29
Soy-oil	3.22	2.51	1.79	1.10	0.39
MCP	1.22	1.22	1.20	1.20	1.20
Limestone	0.94	0.94	0.94	0.96	0.96
L-Lysine·HCl	0.40	0.39	0.39	0.39	0.38
DL-methionine	0.40	0.39	0.39	0.39	0.39
Vit. Mix[1)]	0.12	0.12	0.12	0.12	0.12
Min. Mix[2)]	0.12	0.12	0.12	0.12	0.12
Salt	0.20	0.20	0.20	0.20	0.20
Choline-Cl(25%)	0.10	0.10	0.10	0.10	0.10
ZnO	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Chemical Composition[3)]					
ME (kcal/kg)	3,400.00	3,400.00	3,400.00	3,400.00	3,400.00
CP (%)	20.56	20.56	20.56	20.56	20.56
Lysine (%)	1.35	1.35	1.35	1.35	1.35
Methionine (%)	0.39	0.39	0.39	0.39	0.39
Ca (%)	0.80	0.80	0.80	0.80	0.80
Total P (%)	0.65	0.65	0.65	0.65	0.65

1) Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D_3,1,600IU; vitamin E, 32IU; d-biotin, 64g; riboflavin, 3.2mg; calcium pantothenic acid, 8mg; niacin, 16mg; vitamin B_{12}, 12g; vitaminK, 2.4mg.

2) Provided the following per kilogram of diet : Se, 0.1mg; I, 0.3mg; Mn, 24.8mg; $CuSO_4$, 54.1mg; Fe, 127.3mg; Zn, 84.7mg; Co, 0.3mg.

3) Calculated values

Table 2. Formula and chemical compositions of the experimental diets in phase II (15th to 35th day)

Ingredients, %	*Tenebrio molitor* larva levels				
	0	1.5	3.0	4.5	6.0
Corn	32.55	33.49	34.44	35.38	36.29
SBM(44%)	27.28	25.61	23.95	22.27	20.63
Molitor Larva	0.00	1.50	3.00	4.50	6.00
Lactose	4.00	4.00	4.00	4.00	4.00
Barley	30.00	30.00	30.00	30.00	30.00
Soy-oil	3.42	2.65	1.87	1.10	0.34
MCP	1.03	1.00	1.01	1.00	1.00
Limestone	0.66	0.69	0.68	0.70	0.71
L-Lysine·HCl	0.43	0.43	0.42	0.42	0.41
DL-methionine	0.09	0.09	0.09	0.09	0.08
Vit. Mix[1]	0.12	0.12	0.12	0.12	0.12
Min. Mix[2]	0.12	0.12	0.12	0.12	0.12
Salt	0.20	0.20	0.20	0.20	0.20
Choline-Cl(25%)	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Chemical Composition[3]					
ME (kcal/kg)	3,350.00	3,350.00	3,350.00	3,350.00	3,350.00
CP (%)	18.88	18.88	18.88	18.88	18.88
Lysine (%)	1.23	1.23	1.23	1.23	1.23
Methionine (%)	0.36	0.36	0.36	0.36	0.36
Ca (%)	0.70	0.70	0.70	0.70	0.70
Total P (%)	0.60	0.60	0.60	0.60	0.60

[1] Provided the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D_3,1,600IU; vitamin E, 32IU; d-biotin, 64g; riboflavin, 3.2mg; calcium pantothenic acid, 8mg; niacin, 16mg; vitamin B_{12}, 12g; vitaminK, 2.4mg.

[2] Provided the following per kilogram of diet : Se, 0.1mg; I, 0.3mg; Mn, 24.8mg; $CuSO_4$, 54.1mg; Fe, 127.3mg; Zn, 84.7mg; Co, 0.3mg.

[3] Calculated values

Table 3. Effect of *Tenebrio molitor* larva supplementation on growth performance in weaning pigs[1]

Levels	*Tenebrio molitor* larva 0%	1.5%	3.0%	4.5%	6.0%	SEM[2]	Lin.[3]	Quad.[3]
Body weight, kg								
Initial	8.05	8.04	8.05	8.04	8.04			
14th day	9.63	9.80	10.01	10.51	10.66	0.310	0.01	0.83
35th day	17.78	18.34	19.10	19.77	20.22	0.531	0.01	0.90
Average daily gain, g								
0-14th day	113	126	140	176	188	11.570	0.01	0.81
14-35th day	388	407	433	441	455	15.153	0.08	0.82
Overall	278	294	316	335	348	10.986	0.01	0.91
Average daily feed intake, g								
0-14th day	250	270	283	336	349	13.338	0.01	0.77
14-35th day	721	736	754	753	773	25.355	0.44	0.96
Overall	532	550	566	586	604	17.266	0.05	0.96
Gain/feed rate								
0-14th day	0.452	0.467	0.495	0.524	0.539	0.028	0.25	0.28
14-35th day	0.539	0.552	0.575	0.586	0.589	0.015	0.24	0.89
Overall	0.521	0.538	0.565	0.573	0.576	0.011	0.07	0.62

1 A total of 120 crossbred pigs were fed from average initial body weight 8.04 ± 0.08 kg

2 Standard error of mean.

3 Lin.: linear value, Quad.: quadratic value.

Table 4. Effect of *Tenebrio molitor* larva supplementation on nutrient digestibility in weaning pigs[1]

Levels	*Tenebrio molitor* larva					SEM[2]	Lin.[3]	Quad.[3]
	0%	1.5%	3.0%	4.5%	6.0%			
Nutrient digestibility, %								
Dry matter	90.13	92.33	92.93	93.80	94.22	0.715	0.05	0.53
Crude protein	86.29	90.25	91.27	92.17	93.04	1.141	0.05	0.47
Crude ash	67.62	67.17	71.11	72.20	76.01	1.841	0.06	0.66
Crude fat	81.40	81.67	82.96	82.79	81.55	1.328	0.87	0.65
N-retention, g/day								
N-intake	5.30	5.34	5.34	5.31	5.36			
N-feces	0.73	0.52	0.47	0.42	0.37	0.060	0.05	0.48
N-urine	2.37	2.55	2.55	2.51	2.56	0.155	0.80	0.85
N-retention	2.20	2.27	2.33	2.38	2.42	0.156	0.05	0.98

[1] A total of 20 crossbred pigs were fed from average initial body weight 10.05 ± 0.98 kg / 42±5 days of age.

[2] Standard error of mean.

[3] Lin.: linear value, Quad.: quadratic value.

Table 5 Effect of *Tenebrio molitor* larva supplementation on BUN and IGF-1 in weaning pigs

Levels	*Tenebrio molitor* larva					SEM[1]	Lin.[2]	Quad.[2]
	0%	1.5%	3.0%	45%	6.0%			
BUN, mg/dL								
Initial	9.40	9.40	9.40	9.40	9.40			
2 week	14.87	14.27	12.15	12.03	12.08	0.617	0.08	0.52
5 week	12.02	11.12	10.85	10.32	10.28	0.658	0.05	0.85
IGF-1. ng/mL								
Initial	75.12	75.12	75.12	75.12	75.12			
2 week	83.77	87.77	86.10	87.13	93.08	3.169	0.37	0.78
5 week	107.67	123.00	129.58	131.48	136.34	4.007	0.03	0.44

[1] Standard error of mean.

[2] Lin.: linear value, Quad.: quadratic value.

Table 6. Effect of *Tenebrio molitor* larva supplementation on serum IgG and IgA concentration in weaning pigs

Levels	*Tenebrio molitor* larva					SEM[1]	Lin.[2]	Quad.[2]
	0%	1.5%	3.0%	4.5%	6.0%			
IgG, mg/ml								
Initial	2.09	2.09	2.09	2.09	2.09			
2 week	1.89	2.04	1.93	2.18	2.11	0.102	0.20	0.911
5 week	4.56	4.43	4.54	4.50	4.45	0.309	0.90	0.251
IgA, mg/ml								
Initial	1.29	1.29	1.29	1.29	1.29			
2 week	1.47	1.60	1.55	1.63	1.56	0.069	0.15	0.964
5 week	2.48	2.34	2.41	2.39	2.52	0.154	0.95	0.933

[1] Standard error of mean.

[2] Lin.: linear value, Quad.: quadratic value.

IV. Literature Cited

Aguilar-Miranda ED, Lopez MG, Escamilla-Santana C, Barba De La Rosa AP (2002).

Aniebo, A. O., E. S. Erondu, O. J. Owen. 2009. Replacement of fish meal with maggot meal in African catfish (Clariusgariepinus) diets. Revista UDO Agrícola 9:666-671.

AOAC. 1995. Official methods of analysis (1th ed). Association of official analytical chemists. Washington, D. C., USA.

Awoniyi, T. A. M., V. A. Aletor, et al. 2003. "Performance of broiler-chickens fed on maggot meal in place of fishmeal." International Journal of Poultry Science 2(4): 271-274.

Bark. L. J., T. D. Crenshaw, and V. D. Leibbrandt. 1986. The effect of meal intervals and weaning on feed intake of early weaned pigs. *J. Anim. Sci.* 62, 1233-1239.

Bayes-Genis A, Conover CA, and Schwartz RS. 2000. The insulin-like growth factor axis: a review of atherosclerosis and restenosis. Circ Res 86:125-130.

Braude, R. and M. J. Newport. 1973. Artificial rearing pigs. Br. J.

Nutr., 29:447.

Bukkens, S.G.F. 1997. The nutritional value of edible insects. Ecology of Food and Nutrition 36: 287-319.

Calvert, C., R. Martin, N. Morgan. 1969. House fly pupae as food for poultry. Journal of Economic Entomology 62:938-939.

Campbell, J. M. 1998. The use of plasma in swine feeds, Discoveries. A Quarterly Tech. Update, American Protein Corp., Ames, 4.

Cera, K. R., D. C. Mahan, R. F. Cross, G. A. Reinhart and R. E. Whitmoyer. 1988. Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. J. Anim. Sci. 66:574-584.

CHEN Zai-Bin. 2012. Analysis for nutritional value of four kinds of insects and use of *Tenebrio molitor* power in weaning pig production. [D]. Shandong agricultural university, China. China Knowledge Resource Integrated Database (CNKI).

Church, D. C., and R. O. Kellems. 1998. Supplemental protein sources. In Livestock Feeds and Feeding, Kellems, R. O. and D. C. Church, Eds., Prentice-Hall, Upper Saddle River, NJ, 135.

Cromwell, G. L. 1998. Feeding swine. In Livestock Feeds and Feeding, 4th ed., Kellems, R. O., and D. C. Church, Eds., Prentice-Hall, Upper

Saddle River, NJ, 354.

Cunha, T. J. 1977. Swine Feeding and Nutrition, Academic Press, New York.

DAI Chun-hua, MA Hai-le, SHEN Xiao-kun, JIANG Zhe, GU Xiao-hong, TANG Jian. 2009. Analysis on the nutrient conposition in the larva and pupa of Tenebrio molitor. Science and Technology of Food Industry Vol.30, No.04, 2009.

Defoliart GR. 1991. Insect fatty acids: similar to those of poultry and fish in their degree of unsaturation, but higher in thepoly-unsaturate. Food Insects Newsletter 4(1), 1-4.

Defoliart GR. 1992. Insects as Human food. Gene DeFoliart discusses some nutritional and economic aspects. Crop protection 11, 395-399.

DENG Qing-yun, JIANG Yi-quan, LI Guo-yuan. 2008. The laying effect of Insect protein fodder on layer. Livestock and poultry industry. 10.3969/ j. issn. 1008-0414. 2008. 10. 009.

Despins, J. L., R. C. Axtell. 1995. Feeding behavior and growth of broiler chicks fed larvae of the darkling beetle, Alphitobiusdiaperinus. Poultry Science 74:331-336.

Dhaliwal, J., R. Virk, A. Atwal. 1980. The use of house fly (Musca domestica Linnaeus) pupae meal in broiler mash. Indian Journal of

Poultry Science 15:119-122.

Diaz, F., V. C. Speer, P. G. Homeyer, V. W. Hays, and D. V. Catron. 1959. Comparative performance of baby pigs fed infant and baby pig diets, J. Nutr., 68:131.

Diener, S., C. Zurbrügg, K. Tockner. 2009. Conversion of organic material by black soldier fly larvae: Establishing optimal feeding rates. Waste Management and Research 27:603-610.

Đ.orđ.ević., M., B. Radenković.-Damnjanović., M. Vuč.inić., M. Baltić., R. Teodorović., L. Janković., M. Vukaš.inović., M. Rajković.. 2008. Effects of substitution of fish meal with fresh and dehydrated larvae of the house fly (Musca domestica L.) on productive performance and health of broilers. Acta Veterinaria 58:357-368.

Eggum, B. O. 1970. Blood urea measurement as a technique for assessing protein quality. Br. J. Nutr. 24:983.

Fasakin, E. A., A. M. Balogun, O. O. Ajayi. 2003. Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish Clariasgariepinus fingerlings. Aquaculture Research 34:733-738.

Finke, M. D. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology 21:269-285.

Finke, M. D. 2007. Estimate of chitin in raw whole insects. Zoo Biology 26:105-115.

Gatnau, R., and D. R. Zimmerman. 1992. Determination of optimum levels of inclusion of spray-dried porcine plasma (SDPP) in diets for weanling pigs fed in practical conditions, J. Anim. Sci., 70(Suppl. 1):60 (Abstr.).

Gawaad, A. A. A., H. Brune. 1979. Insect protein as a possible source of protein to poultry. Zeitschrift für Tierphysiologie Tierernährung und Futtermittelkunde 42:216-222.

Ghaly, A., F. Alkoaik. 2009. The yellow mealworm as a novel source of protein. American Journal of Agricultural and Biological Sciences 4:319-331.

Gore, A. M., R. W. Seerley, and M. J. Azain. 1990. Menhaden fish meal and dried whey levels in swine starter diets, Special Publ. No. 67, Georgia Agric. Exp. Sta., Athens, 11.

Goulet, G., P. Mullier, P. Sinave, G. J. Brisson. 1978. Nutritional evaluation of dried Tenebrio molitor larvae in the rat. Nutrition Reports International 18:11-15.

GUO Ai-wei, XIONG Chun-mei. 2008. The Application of Insect Protein in the Animal Production. China Animal Husbandry &

Veterinary Medicine, 2008(1).

Hampson, D. J. and D. E. Kidder. 1986. Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. Res. Vet. Sci. 40:24-31.

Hale, O.M., 1973. Dried Hermetia illucens larvae (Stratiomyidae) as a feed additive for poultry. Journal of the Georgia Entomological Society 8:16-20.

Han, B. K., Lee, W. J., Jo, D. H. 1997. Chitinolytic enzymes from the gizzard and the chyme of the broiler (Gallus gallus L.). Biotechnology Letters 19: 981−984.

Han, B. K., Moon, J. K., Ryu, Y. W., Park, Y. H., Jo, D. H. 2000. Purification and characterization of acidic chitinases from gizzards of broiler (Gallus gallus L.). Journal of Biochemistry and Molecular Biology 33: 326-331.

Hansen, J. A., J. L. Nelssen, R. D. Goodband, and T. L. Weeden. 1993c. Evaluation of animal protein supplements in diets of early-weaned pigs, J. Anim. Sci., 71:1853.

Hwangbo, J., E. C. Hong. 2009. "Utilization of house fly-maggots, a feed supplement in the production of broiler chickens." J Environ Biol. 30(4): 609-614.

HUANG Qiong, ZHOU Zu-ji, ZHOU Ding-gang, HU Jie, YANG Wei, YANG Chun-ping, 2006. Analysis of Nutritional Component of Tenebrio molitor (L.) Pupa. Sichuan Journal of Zoology Vol.25 No.4 2006.

HUANG Zi-zan, ZHANG Nai-zhong, LU Yuan-da. 1984. Development of new source of protein feed - rearing maggots. [J.] Feed Research. 1987 (3):17-21. China Knowledge Resource Integrated Database (CNKI).

HUANG Zi-zan, ZHANG Zu-sheng, LI Yi-ning, CHEN Zi-gao. 1993. Maggot farming is a good way to solve the source of animal protein. Guangxi Journal of Animal Husbandry & Veterinary Medicine, Editorial E-mail, 1993-04. Page: 22-23. China Knowledge Resource Integrated Database (CNKI).

Hu Wen-Qin, Meng Qing-Li. 2004. Mechanisms of generating and scavenging reactive oxygen species in animals. Journal of ecology of dimestic animal, 2004, 25(3).

Ilori, J. O., E. R. Miller, D. E. Ullrey, P. K. Ku, and M. G. Hogberg. 1984. Combinations of peanut meal and blood meal as substitutes for soybean meal in corn-based growing-finishing diets, J. Anim. Sci., 59:394.

JIA Sheng-fu, MA Yan-biao. 2007. Effect of Fly Maggot on Weight Gain of Broiler. [J.] CHINA POULTRY, 2007,29(12):39~41.

Jones, L. D., R. W. Cooper, R. S. Harding. 1972. Composition of mealworm Tenebrio molitor larvae. The Journal of Zoo Animal Medicine 3:34-41.

Kats, L. J., J. L. Nelssen, M. D. Tokach, R. D. Goodband, T. L. Weeden, S. S. Dritz, J. A. Hansen and K. G. Frisen. 1994. The effects of spray-dried blood meal on growth performance of the early-weaned pig. J. Anim. Sci. 72:2860-2869.

Kats, L. J., J. L. Nelssen, M. D. Tokach, R. D. Goodband, J. A. Hansen, and J. L. Laurin. 1994a. The effect of spray-dried porcine plasma on growth performance of the early-weaned pig, J. Anim. Sci., 72:2075.

Kelly, D., J. A. Smith and K. J. McCracken. 1991b. Digestive development in the early-weaned pig. II. Effect of level of food intake on digestive enzyme activity during the immediate post-weaning period. Br. J. Nutr. 65:181-188.

Kennedy, J. J., F. X. Aherne, D. L. Kelleher, and P. J. Caffrey. 1974a. An evaluation of the nutritive value of meat-and-bone meal. 1. Effects of level of meat-and-bone meal and collagen on pig and rat performance, Ir. J. Agric. Res., 13:l.

Kenworthy, R. and W. D. Allen. 1966. Influence of diet and bacteria on small intestine morphology, with special reference to early weaning and Escherichia coli. J. Comp. Patho. 76:291-298.

Kerr, B. J. 1993. Optimizing Lean Tissue Deposition in Swine. BioKyowa Tech. Rev. Vol. 6. Nutri-Quest, Inc., Chesterfield, MO.

Khempaka, S., C. Chitsatchapong, W. Molee. 2011. Effect of chitin and protein constituents in shrimp head meal on growth performance, nutrient digestibility, intestinal microbial populations, volatile fatty acids, and ammonia production in broilers. Journal of Applied Poultry Research 20:1-11.

Klasing, K. C., P. Thacker, M. A. Lopez, C. C. Calvert. 2000. Increasing the calcium content of mealworms (Tenebrio molitor) to improve their nutritional value for bone mineralization of growing chicks. Journal of Zoo and Wildlife Medicine 31:512-517.

Laksesvela, B. 1961. Graded levels of herring meal to bacon pigs, effect on growth rate, feed efficiency and bacon quality, J. Agric. Sci. (Cambridge), 56:307

Lee, C.G., Da Silva, C.A., Lee, J.-Y., Hartl, D., J.A. Elias. 2008. Chitin regulation of immune responses: an old molecule with new roles. Current Opinion in Immunology 20: 1-6.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, J. D. Hancock, G. L. Allee, R. D. Goodband and R. D. Klemm. 1990. Transient hypersensitivity to soybean meal in early-weaned pigs. J. Anim. Sci.

68:1790-1799.

LI Han-chen, JI Zhi-xin, GAO Su-hong, LIU Shao-jun. 2007. The Nutrition and Health Functions of *Tenebrio molitor*. Journal of Hebei University of Science & Technology Vol.21. No. 3 Sep.

LIU Yu-Sheng, WANG Fu-Bin, CUI jun-Xia. .2010. Recent status and advances on study and utilization of *Tenebrio molitor*. Journal of Environmental Entomology, 2010, 23(1).

Liu Q, J.K. Tomberlin, J.A. Brady, M.R. Sanford, Z. Yu, 2008. Black Soldier Fly (Diptera: Stratiomyidae) Larvae Reduce Escherichia coli in Dairy Manure. Environ. Entomol., 37: 1525-30.

Lwalaba, D. Hoffmann, K.H., J. Woodring. 2010. Control of the release of digestive enzymes in the larvae of the fall armyworm, Spodopterafrugiperda. Archives of Insect Biochemistry and Physiology 73: 14-29.

MacEvilly C. 2000. Bugs in the system. Nutrition Bulletin 25, 267-268.

Manoilovic. B. 1988. Influence of food and temperature on post-embryonal survival of the *Tenebrio molitor* L. Zastita-bilia (Yugoslavia), 1988, 39(1): 43-53.

Mark DF. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology 21, 269-285.

Makkink, C. A., G. P. Negulescu, G. X. Qin and M. W. A. Verstegen. 1994. Effect of dietary protein source on feed intake, growth, pancreatic enzyme activities and jejunal morphology in newly-weaned piglets. Br. J. Nutr. 72:353-368.

Meuwissen, P. 2011. Insecten als nieuwe eiwitbron. The Netherlands.

Miller, B. G., T. J. Newby, C. R. Stokes and F. J. Bourne. 1984a. Influence of diet on postweaning malabsorption and diarrhea in the pig. Res. Vet. Sci. 36:187-193.

Miller, B. G., T. J. Newby, C. R. Stokes and F. J. Bourne. 1984b. Immune hypersensitivity and post-weaning diarrhoea in the pig. Proc. Nutr. Soc. 43:116A

Miller, B. G., P. S. James, M. W. Smith and F. J. Bourne. 1986. Effect of weaning on the capacity of pig intestine villi to digest and absorb nutrients. J. Agric. Sci. Camb. 107:579-589.

Miller, E. R. 1990. Blood meal: flash-dried. In Nontraditional Feed Sources for Use in Swine Production, Thacker, P. A., and R. N. Kirkwood, Eds., Butterworths, Boston, 53.

MU Li-xia, LIAO Sen-tai, Geng-sheng, SUN Yuan-ming, ZOU Yu-xiao, SHI ying. 2011. Research Status of Development and Utilization for

Isect Protein. Modern Food Science and Technology. 2011, Vol. 27, No. 12.

Newby, T. J., B. Miller, C. R. Stokes, D. Hampson and F. J. Bourne. 1984. Local hypersensitivity response to dietary antigens in early weaned pigs. In Recent Advances in Animal Nutrition (Ed. W. Haresign and D. J. A. Cole). Butterworths, London, pp. 49-59.

Newport, M. J., and H. D. Keal. 1983. Effect of protein source on performance and nitrogen metabolism ofpigs weaned at 21 days of age, Anim. Prod., 37:395.

Newton, G. L., C. V. Booram, R. W. Barker, O. M. Hale. 1977. Dried Hermetia illucens larvae meal as a supplement for swine. J. Anim. Sci. 44: 395-400.

Newton, G. L., D. C. Sheppard, D.W. Watson, G.J. Burtle, C.R. Dove, J.K. Tomberlin, E.E. Thelen. 2005. The black soldier fly, Hermetia illucens, as a manure management / resource recovery tool." State of the Science, Animal Manure and Waste Management. Jan. 5-7, San Antonio, TX.

Newton, L., C. Sheppard, D.W. Watson, G. Burtle, 2005. Using the black soldier fly, Hermetia illucens, as a value-added tool for the management of swine manure. Report for Mike Williams, Director of the Animal and Poultry Waste Management Center, North Carolina

State University, Raleigh, Nc.

Nergui Ravzanaadii, Seong-Hyun Kim, Won Ho Choi, Seong-Jin Hong, and Nam Jung Kim. 2012. Nutritional Value of Mealworm, *Tenebrio molitor* as Food Source. Int. J. Indust. Entomol. Vol. 25, No. 1, 2012, pp. 93~98

Ng WK, Liew FL, Ang LP, Wong KW. 2001. Potential of mealworm (Tenebrio molitor) as an alternative protein source in practical diets for African catfish, Clarias gariepinus. Aquaculture Research 32, 273-280.

NRC. 2012. Nutrient Requirements of Swine, National Academy Press, Washington, D.C.

Ottevanger, E. 2012. Insects as alternative protein sources in animal feed, a literature review. MSc. Thesis Wageningen University. ANU-80424.

Pellett. P. L. Yong. V. R. 1980. Nutritional evaluation of protein food [M]. Tokyo: The United National University, 1980. 26-29.

Pluske, J. R. and I. H. Williams. 1996. The influence of feeder type and the method of group allocation at weaning on voluntary food intake and growth in piglets. Anim. Sci.62:115-120.

Powles J, Wiseman J, Cole Dja, Hardy B: Effect of chemical structure of fats upon their apparent digestible energy value when given to young pigs. Anim Prod 58: 411-417, 1994.

Pretorius, Q. 2011. The evaluation of larvae of Musca domestica (common house fly) as protein source for broiler production, Stellenbosch University, Stellenbosch.

QI Fang, LI Gang-sheng. 2001. Insect Nutrient Composition and Feeding Value. Journal of Zhangjiakou Agricultural College Vol. 17. No. 2

Ramos-Elorduy J, Pino MJM 1989. Los insectos comestibles en el México antiguo (Estudio Etnoentomológico). Mexico: AGT Editor.

Ramos-Elorduy, J., E. A. Gonzalez. 2002. "Use of *Tenebrio molitor* (Coleoptera : Tenebrionidae) to recycle organic wastes and as feed for broiler chickens." Journal of Economic Entomology 95(1):214-220.

SAS Institute, 2002-2003. SAS/STAT User's Guide: Version 9.1. SASInstitute, Cary, NC.

Seerley, R. W. 1991. Major feedstuffs used in swine diets. In Swine Nutrition, Miller, E. R., D. E. Ullrey, and A. J. Lewis, Eds., Butterworth-Heinemann, Boston, 451.

SHEN Hong, PAN Xiao-Liang, WANG Jun-Gang. 2006. Effect of

Tenebrio molitor supplementation on growth performance and protein deposition in broilers. [J]. Sichuan Animal & Veterinary Science. 2006(07):61-62

Sheppard, D. C, G. L. Newton, G. Burtle. 2008. Black soldier fly prepupae.

Smith, M. W. 1984. Effect of postnatal development and weaning upon the capacity of pig intestinal villi to transport alanine. J. Agric. Sci. Camb. 102:625-633.

Speer, V., G. Ashton, F. Diaz, and D. Catron. 1954. New I.S.C. Pre-starter “75,” Ia. Farm Sci., 8:3.

Stokes, C. K., B. G. Miller, M. Bailey, A. D. Wilson and F. J. Bourne. 1987. The immune response to dietary antigens and its influence on disease susceptibility in farm animals. Vet. Immunol. Immunopathol. 17:413-423.

Stoner, G. R., G. L. Allee, J. L. Nelssen, M. E. Johnston, and R. D. Goodband. 1990. Effect of select menhaden fish meal in starter diets for pigs, J. Anim. Sci., 68:2729.

Téguia, A., M. Mpoame. (2002). "The production performance of broiler birds as affected by the replacement of fish meal by maggot meal in the starter and finisher diets." Tropicultura 20(4): 187-192.

Tokach, M. D. ; Goodband, R. D. ; Nelssen, J. L. ; Hansen, J. A., 1991. Comparison of protein sources for phase II starter diets. In: Goodband, B.; Tokach, M. (Eds.), Kansas State University Swine Day 1991. Report of Progress 641: 57-60 1991.

Vente Spreeuwenberg, M. A. M., J. M. A. J. Verdnok, A. C. Beynen and M. W. A. Verstegen. 2003. Interrelationships between gut morphology and feces consistency in newlyweaned piglets. Anim. Sci. 77:85-94.

Walker, W. R., C. V. Maxwell, F. N. Owens, D. S. Buchanan. 1986. Milk versus soybean protein sources for pigs. I. Effects on performance and digestibility, J. Anim. Sci., 63:505.

WANG Xiang-Ping, LEI Chao-Liang, NIU Chang Ying, 2001. The defensive secretion from insects. Institute of insect sources, Huazhong Agricultural University, Wuhan, China.

Weaver-DK, McFarlane-JE. 1990. The effect of larval density on growth and development of Tenebrio molitor. Journal of insect physiology 1990, 36(7): 531~536.

Wilson, R. H., and J. Leibholz. 1981a. Digestion in the pig between 7 and 35 days. 1. The performance of pigs given milk and soyabean proteins, Br. J. Nutr., 45:301.
Wilson, R. H., and J. Leibholz. 1981b. Digestion in the pig between 7

and 35 days of age. 2. The digestion of dry matter and the pH of digesta in pigs given milk and soyabean proteins, Br. J. Nutr., 45:321.

Wilson, R. H., and J. Leibholz. 1981c. Digestion in the pig between 7 and 35 days of age. 3. The digestion of nitrogen in pigs given milk and soyabean proteins, Br. J. Nutr., 45:337.

Wirtz, R.A. 1984. "Allergic and toxic reactions to non-stinging arthropods." Annual Review of Entomology, 29: 47-69.

Wu Shu-Xia, Lin Hua-feng, 2009. Studies on Optimization of Rearing Condition and Nutrient Content of larvae of *Tenebrio molitor* L. Anhui Agricultural University Hefei, Anhui, China.

YANG Zai-Bin, YANG Hai-ying. 2010. Effect of addition of Tenebrio molitor and maggot powder replacing fish meal on growth performance in weaning pigs. Journal of Jiangxi feed. 2010, (2). China Knowledge Resource Integrated Database (CNKI)

Zuidhof, M., C. Molnar, F. Morley, T. Wray, F. Robinson, B. Khan, L. Al-Ani, L. Goonewardene. 2003. Nutritive value of house fly (Musca domestica) larvae as a feed supplement for turkey poults. Animal Feed Science and Technology 105:225-230.

Ⅴ. Summary in Korean

실험: 자돈 사료 내 거저리 유충의 첨가가 이유자돈의 성장성적과 혈액성상, 영양소 소화율 및 기호성에 미치는 영향

본 연구는 자돈 사료 내 거저리 유충의 수준별 첨가가 이유자돈의 성장성적과 혈액성상, 영양소 소화율 및 기호성에 미치는 영향을 규명하기 위해 수행하였다. 평균 체중 8.04 ± 0.08kg인 삼원 교잡종 ([Yorkshire x Landrace] x Duroc) 이유자돈 120두를 공시하였으며, 총 5주 동안 사양실험을 수행하였다. 사양실험은 총 5처리 6반복으로 돈방 당 4두씩 성별과 체중에 따라 난괴법 (RCBD; Randomized Completely Block Design)으로 배치하였다. 처리구는 거저리 유충을 첨가한 수준을 0%, 1.5%, 3.0%, 4.5%, 6.0% 따라 설정하였으며 실험을 수행하였다. 성장성적 결과를 보았을 때, 2주차와 5주차의 체중과, 0-2주와 0-5주의 일당증체량에서 거저리 유충의 첨가수준이 증가함에 따라 linear하게 증가하는 결과가 나타났으며($P < 0.01$), 2-5주의 일당증체량은 거저리 유충 첨가수준이 높일수록 linear하게 증가하는 경향이 나타났다 ($P=0.09$). 0-2주의 사료섭취량이 거저리 유충 첨가수준이 증가함에 따라 linear하게 증가하였으며 ($P < 0.01$), 0-5주의 사료섭취량도 linear하게 증가하였다($P < 0.05$). 사료효율에서는 0-5주에만 linear하게 증가하는 추세가 나타났으며($P=0.07$), 0-2주와 2-5주에서는 통계적으로 유의적인 차이가 나타나지 않았지만 첨가수준이 증가할수록 사료효율이 수치적으로 높아지는 결과가 나타났다.

전체적으로 성장성적을 봤을 때 유충의 첨가량이 증가함에 따라서 phase I에서 더 좋은 성장효과를 나타났다.

영양소 소화율에서는 거저리 유충의 첨가수준이 높아질수록, 건물과 조단백질 소화율이 유의적으로 높아지는 것으로 나타났고 ($P<0.05$) 조회분의 소화율에서는 소화율이 증가하는 경향이 나타났다 ($P=0.06$). 반면에 조지방의 소화율에서는 처리구간 통계적 유의차가 발생하지 않았다. 질소의 이용률 측면에서는 거저리 유충 첨가량이 증가할수록 분내 질소배출량이 linear하게 감소하였으며($P<0.05$), 질소의 축적량은 거저리 유충 첨가량이 높아질수록 linear하게 증가하고 반면에 뇨 중 질소배출량은 거저리 유충 첨가량에 영향을 받지 않는 것으로 사료된다. 혈액성상을 분석한 결과, BUN의 농도는 2주차에 거저리 유충이 6% 첨가된 처리구가 다른 처리구들에 비해 통계적으로 유의미하게 낮아지는 경향을 보였다 ($P=0.08$). 또한, 5주차에서는 거저리 유충의 첨가량이 증가함에 따라 혈중 BUN 농도가 linear하게 감소하였다($P<0.05$). 혈중 IGF-1 농도를 측정한 결과, 5주차에 거저리 유충의 첨가수준이 증가함에 따라 IGF-1 농도가 linear하게 증가하는 결과가 나타났다($P<0.03$). 이는 자돈 사료내 거저리 유충의 첨가는 단백질 이용효율을 높혀 BUN농도를 감소시키고, 자돈의 성장을 촉진시키는 IGF-1분비를 자극하는 영향을 주는 것으로 사료된다. 면역지표에서는 2주차와 5주차의 IgG 와 IgA의 농도를 살펴보면 거저리 유충의 첨가량이 증가하도 유의적인 차이가 나타나지 않았고 거저리 유충의 첨가가 면역지표에 영향을 미치지 않는 것으로 사료된다. 결론적으로 자돈 사료 내 거저리 유충의 첨가는 자돈의 영양소 이용률을 개선하고 혈중 IGF-1의 분비를 촉진시켜 자돈의 성장성적을 향상시킬 수 있을 것으로 사료되며, 본 실험결과 자돈 사료 내 거저리 유충을 6% 이상 첨가하여도 긍정적인 효과가 나타날 수 있다고 사료된다 .

Ⅵ. Acknowledgement

I would like to gratefully and sincerely thank Dr. Yoo Yong Kim for his guidance, understanding, patience, and most importantly, his friendship during my graduate studies at SNU. His mentorship was paramount in providing a well rounded experience consistent my career goals. He encouraged me not only grow as an experimentalist or a chemist but also as an instructor and an independent thinker. I am sure many graduate students are given the opportunity to develop their own individuality and self-sufficiency by being allowed to work with such independence. For everything you've done for me, Dr. Yoo Yong Kim, I thank you.

I would also like to thank all of the members of the my research group and colleagues at the laboratory of Animal Nutrition and Biochemistry, especially to Dr. Young Dal Jang, Sung Kwon Jang, Pil Seung Heo, Dong Hyuk Kim, Kwang Ho Kim, Song San Jin, Jae Cheol Jang, Kyung Young Jin, Dong Wook Sin, Yun Yeong Jo, Sung Woong Jung, Geon Il Lee, Jin Su Hong, Lin Hu Fang, Jae Hark Jeong, Young Jun Ji, Soo Duck Noh, Ki Hyun Kwon, Seong A Yu, Jeong Hyun Moon, Kyung Won Kang, Min Seong Yoon, Yong Il Lee, Hyo Sim Choi, Chang Woo Park, Joo Min Kim, Jong Seon Lim, Yoon Kyung Hyun, Hyun Bong Choi. I will always remember their encouragement and every great moment we had together.

Finally, and most importantly, I would like to thank my family.

Their support, encouragement, quiet patience and unwavering love were undeniably the bedrock upon which the past 2 years of my life have been built. Their tolerance of my occasional vulgar moods is a testament in itself of their unyielding devotion and love. I thank my parents, Ji-lian Jin and Yong-ai Nan for their faith in me and allowing me to be as ambitious as I wanted. It was under their watchful eye that I gained so much drive and an ability to tackle challenges head on.