Complete replacement of soybean meal with defatted black soldier fly larva meal (BSFLM) in laying hen feeding programs: impact on egg production and quality

By

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ABSTRACT

COMPLETE REPLACEMENT OF SOYBEAN MEAL WITH DEFATTED BLACK SOLDIER FLY LARVA MEAL (BSFLM) IN LAYING HENS FEEDING PROGRAMS: IMPACT ON EGG PRODUCTION AND QUALITY

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A study was carried out in two-phases to investigate the impact of replacing soybean meal with Black Soldier Fly Larvae Meal (BSFLM) in nutritionally balanced corn-soybean meal-based diets for laying hens. Egg production, egg quality and other physiological responses were evaluated. Soybean meal was partially (41%) and totally (100%) replaced by BSFLM in phase 1 and 2, respectively. In phase 1, BSFLM improved yolk color (YC), shell breaking strength (SBS) and shell thickness (ST). However, Hen Day Egg Production (HDEP) and egg mass (EM) were comparable to soybean meal fed birds. BSFLM increased feed intake leading to reduced Feed Conversion ratio (FCR) relative to corn soybean meal-based diet. In phase 2, diets had no effect on HDEP, however, BSFLM decreased EM but improved YC, SBS and ST. Feeding BSFLM decreased ceca and increased liver weight but had no effect on pancreas, gizzard and small intestinal weight. Although complete replacement of soybean meal with BSFLM did not affect HDEP, reduction in EM resulted in poor FCR suggesting further research on nutrient digestibility in BSFLM fed to laying hens. Improved yolk color may be due pigments present in BSFLM while improved shell characteristics may have been attributed to improved calcium metabolism.

DEDICATION

To my children,

Chelsea Wambui and Joseph Karuma

My parents

Elizabeth Wambui and Alfred Mwaniki

My brothers and sisters

Thank you for your love, patience, motivation and prayers. To God be the glory, blessed be the name of the Lord.

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List of Abbreviations

AA	**Amino Acid**
AID	**Apparent Ileal Digestibility**
AME	**Apparent Metabolizable Energy**
AMEn	**Apparent Metabolizable Energy corrected for nitrogen**
AR	**Apparent Retention**
AEW	**Average Egg Weight**
BSF	**Black Soldier Fly**
BSFL	**Black Soldier Fly Larvae**
BSFLM	**Black Soldier Fly Larvae Meal**
BW	**Body Weight**
CP	**Crude Protein**
CF	**Crude Fat**
DM	**Dry Matter**
EW	**Egg Weight**
EQ	**Egg quality**
EM	**Egg Mass**
FI	**Feed Intake**
FCR	**Feed Conversion Ratio**
GE	**Gross Energy**
HU	**Haugh Units**
HDEP	**Hen Day Egg Production**
IEW	**Individual egg weight**

Lys	**Lysine**
MCFA	**Medium Chain Fatty Acids**
Met	**Methionine**
Mg	**Magnesium**
Na	**Sodium**
NDF	**Neutral Detergent Fiber**
SBF	**Shell Breaking Force**
SID	**Standardized Ileal Digestibility**
SBM	**Soybean Meal**
ST	**Shell Thickness**
Thr	**Threonine**
Wk	**Week**
YC	**Yolk Color**

CHAPTER 1

General introduction

In the context of anticipated human population growth, the current animal protein production will need to increase 60% or more by 2050 (FAO, 2011). This increase in animal protein demand will need enormous resources, with feed being the most challenging because of the limited availability of natural resources, climate change and pressure from food–feed–fuel competition (FAO, 2011). Moreover, the sector is under pressure to produce animal food products that are ethical, environmentally sustainable and wholesome. Poultry production represents one of the most economic and easiest means of bridging the supply-demand gap of animal protein, due to their rapid growth rate and superior feed conversion (FAO, 2011). Feed cost accounts for more than 65% of variable cost of producing poultry products, and energy and amino acids account for more than 90% of this cost (Kiarie et al., 2013). Cereal grains (corn, wheat) and soybean meal are by far the most commonly used feed ingredients in the diets for intensively reared poultry in Canada and much of the world. However, in the context of anticipated competition of food, feed and fuel, there is need to characterize the nutritive and economic value of other feedstuffs with potential to serve as alternatives to traditional feedstuffs in poultry diets.

It has been estimated that more than 1.3 billion tons of organic waste is produced on a global scale resulting in enormous environmental, social and economic costs (Makkar, 2017). In Canada, $27 billion worth of food (post-consumer), ends up in landfills or composters each year (Parizeau et al., 2015). The nutrients in the organic waste could be recycled back for animal feeding through insect rearing (Rumpold and Schluter, 2013b; Makkar, 2017). Using insects as a feedstuff can contribute to global food security via feed or as a direct food source for humans

(Schader et al., 2015). Insects contain high amounts of energy, amino acids (**AA**), fatty acids and micronutrients (Rumpold and Schluter, 2013a; Makkar et al., 2014). The insect species with the highest potential for large-scale production are the black soldier fly (**BSF**) (*Hermetia illucens*), common housefly (*Musca domestica*), and yellow mealworm (*Tenebrio molitor*). Specifically, BSF larvae achieve high growth rates and excellent conversion of organic waste to produce a meal (**BSFLM**) with consistent AA concentrations when raised on diverse substrates (Diener et al., 2009; Nguyen et al., 2015; Spranghers et al., 2017).

Black soldier fly products have been approved for use in poultry feed by Canadian Food Inspection Agency. The use of BSFLM as a component of diet has been reported for poultry (De Marco et al., 2015; Marono et al., 2017; Secci et al., 2018), swine (Newton et al., 1977) and for several commercial fish species (St-Hilaire et al., 2007b). However, characterizing insect meal nutritive and functional value, risks associated with using them (e.g. anti-nutritional factors) and potential economic benefits when formulated correctly in practical diets will be pivotal for the feed industry uptake. There is limited research on the use of BSFLM in laying hen diets, specifically when applied in practical poultry diets, to replace conventional feedstuffs and subsequent impact on egg production and quality. Moreover, BSFLM is rich in chitin, a polysaccharide present in arthropod’s exoskeleton, and residual medium chain fatty acids (**MCFA**). Chitin is not digestible by monogastric animals but can be chemically treated (Coward-Kelly, 2006) and microbially fermented (Suresh, 2011) and used as a feed supplement. Chitin has been shown to positively impact gastrointestinal physiology and metabolism of laying hens (Marono et al., 2015). Alternatively, MCFA may impact gastrointestinal ecology. However, there is paucity of information on impact of feeding BSFLM on aspects of poultry visceral organ ecology and physiology. The overall objective of research presented in this thesis was to investigate impact of complete replacement of soybean meal with BSFLM on egg production and egg quality. Additional investigations included, apparent nutrient retention, plasma metabolites and visceral organ weights.

CHAPTER 2

Literature review

2.0 Introduction

The poultry industry is a major contributor to Canada's economy. For example, in 2012, poultry products worth $3.8 billion were produced across Canada (http://www.agr.gc.ca). Poultry products are the most widely consumed animal proteins in Canada, and it is projected that by 2020 it will be the most widely consumed meat worldwide (FAO, 2011). To meet Canadian demand, the supply managed egg system has approximately 25 million layers https://www.eggfarmers.ca/ . Similarly, to meet 32 kg per capita consumption of chicken meat, chicken farmers of Canada raise 700 million broiler chickens https://www.chicken.ca/ . Feed represents more than 65% of the variable cost of producing poultry products with provision of dietary energy and protein (amino acids) representing more than 90% of this cost (Kiarie et al., 2013).

Unpredictable supply and volatile prices of energy and protein feedstuffs is a risk to profitability and sustainability of poultry production. Corn, wheat and soybean meal are the conventional sources of energy and protein for monogastric feeding programs and a possible way to reduce feed cost is by finding alternatives to these conventional sources (Kiarie and Nyachoti, 2009). Currently, soybean meal, other oil seeds such as sunflower and canola and marine by products are the major sources of amino acids in livestock and poultry feed. Due to anticipated increase in human population, animal protein (especially from pig and poultry enterprises) production will need to increase by over 60% in a span of less than three decades (FAO, 2011). This will lead to more pressure on limited available land for cultivation and over exploitation of marine sources for fish meal due to increased demand for animal feed and human food (Marono et al., 2017; Schiavone et al., 2017). Hence the global feed industry needs to come up with alternative sources of protein with high nutrient value, economically viable, and with less risks like antinutritional factors and toxins, environmentally friendly and socially acceptable to effectively replace conventional feed ingredients (Surendra, 2016).

2.1 Organic waste and insect protein production

It has been estimated that more than 1.3 billion tons of organic waste is produced on a global scale resulting in enormous environmental, social and economic effects (Makkar, 2017). In Canada, $27 billion worth of food ends up in landfills or composters each year and most of it occurs at household level in form of rotten fruits and vegetables and left over foods (Parizeau et al., 2015). Moreover, environmental concerns due to high production of poultry and livestock manure calls for use of sustainable and more environmentally friendly production systems (Allegretti et al., 2017). With countries producing more waste and having less disposal options, and landfills filling to capacity, sustainable waste recycling methods should be invented to reduce pressure on landfills, reduce pollution of soil with leachates and air with bad odours, and produce important materials that could be used as feedstuff for animals (Parizeau et al., 2015; Makkar, 2017).

The nutrients in organic waste could be recycled back for human and animal food through insect rearing (Rumpold and Schluter, 2013b; Makkar, 2017). Insects can convert low quality organic waste and manure into nutritious products (Craig Sheppard et al., 1994; Diener et al., 2009; Čičková et al., 2015; Surendra et al., 2016). Insects leave a small ecological footprint in terms of less arable land required to rear them, and less energy and water requirements in comparison to protein rich plants like soybean (Surendra et al., 2016; Makkar, 2017). The insect species with the highest potential for large-scale production are the black soldier fly (*Hermetia* *illucens*), common housefly (*Musca domestica*), and yellow mealworm (*Tenebrio molitor*) (Čičková et al., 2015; Surendra et al., 2016; Makkar, 2017). Black Soldier Fly (**BSF**) has been identified as one insect that can be used for industrial feed production (Sheppard et al., 2002; Newton et al., 2005; Diener et al., 2009a; Čičková et al., 2015; Nguyen et al., 2015; Meneguz et al., 2018b). It has also been used for chitin and biodiesel production (Makkar, 2014). The black soldier fly has been shown to have a high consumption(feeds on organic waste) and reduction rate (breakdown) of animal waste when reared in different animal manure and has been considered as a potential agent of waste management on a large scale (Diener et al., 2009; Nguyen et al., 2015; Spranghers et al., 2017; Meneguz et al., 2018b).

2.3 About the Black Soldier Fly (*Hermetia illucens*)

Black Soldier Fly (*Hermetia illucens*), a *Dipteran,* of the *Stratiomyidae* family is originally native to subtropical and warmer temperate climate of Americas and is currently found in tropics and subtropics all over the world from latitude 46^0N to 42^0S (Makkar et al., 2014; Čičková et al., 2015). Adults are large wasp-like black flies growing up to 20 mm long, who spend most of their time resting on vegetation. The adult fly has a lifespan of 5 to 8 days and has no functioning mouthparts meaning that they do not bite or sting as they are not attracted to human habitats. As adults they do not feed and depend on the fat reserves stored up during the larval stage. In natural breeding sites, females lay their eggs in moist organic material and prefers a wide range of habitats from rotting fruits and vegetables, manures and dead animals (Craig Sheppard et al., 1994). They frequent agricultural settings because the large amounts of organic waste left by livestock offer abundant sites that meet their reproductive needs. In areas where natural habitats are removed (e.g. urbanized areas), the BSF will lay eggs in dumping sites or compost, which provide similar odors and nutrients to naturally occurring organic matter. This is especially true for areas with poor sanitation. The female BSF deposits a mass of about 500 eggs in cracks and crevices adjacent to a feed source with eggs hatching to dull, whitish colour larvae after 22-24 days (Čičková et al., 2015). The larvae can consume 25 to 500 mg of fresh matter/day and in ideal conditions becomes mature in 2 months. The larval stage can last up to 4 months when adequate feed is not available (Makkar et al., 2014). The larvae attains a length of 27 mm, width of 6 mm and weight of 220 mg at maturity (Makkar et al., 2014). At the end of the larval stage (prepupa), the larva empties its digestive tract and stops feeding. The prepupae then migrate in search of a dry and protected pupation site (Čičková et al., 2015). The duration of the pupal stage is about 14 days but can be extremely variable and last up to 5 months (Makkar et al., 2014). The females mate two days after emerging form pupa stage and the cycle begins again.

2.3.1 Ecological value of Black Soldier Fly

Rearing *H. illucens* has been proposed since the 1990's as an efficient way to dispose organic wastes by converting them into a protein-rich and fat-rich biomass suitable for various purposes, including animal feeding, biodiesel and chitin production (Craig Sheppard et al., 1994; Sheppard et al., 2002; Makkar et al., 2014; Čičková et al., 2015; Surendra et al., 2016). The BSF is capable of dealing with demanding environmental conditions, such as drought, food shortage or oxygen deficiency (Craig Sheppard et al., 1994; Tomberlin et al., 2002; Newton et al., 2005). One major advantage of *H. illucens* over other insect species used for biomass production is that the adult does not feed and therefore does not require particular care (Makkar et al., 2014). The flies are closely associated with outdoors, livestock and organic waste and hence pose no health danger to humans in disease transmission (Craig Sheppard et al., 1994). They discourage breeding of other insects like house flies which are carriers of diseases (Newton et al., 2005).

High populations of BSF larvae inhibit growth of house fly populations by limiting oviposition (Craig Sheppard et al., 1994; Tomberlin et al., 2002).

Organic waste disposal and management is quite challenging due to its bulk and high degradability (Makkar, 2017). Management practices using landfills and through anaerobic breakdown and composting negatively impacts the environment due to greenhouse gases emissions and surface and ground water contamination (Surendra et al., 2016). Harmful substances leaching into the soil and infiltrating the water system, bad odors from garbage and manure from large poultry and livestock production operations can be eradicated by use of black soldier fly (Nguyen et al., 2015). The larvae breakdown and use nutrients such as nitrogen and phosphorous from organic wastes (Surendra et al., 2016). The BSF larvae have been shown to be effective manure recyclers and a "Black Soldier Fly Manure Management System" has been proposed to not only reduce livestock waste (Craig Sheppard et al., 1994; Newton et al., 2005), but also reduce house fly populations. The larvae feed on organic waste and break it down to useful biomass that is beneficial to the soil (Diener et al., 2009). Black soldier flies do not concentrate pesticides and mycotoxins from the material they feed on, hence are a healthy and safe source of feed for animals and food for human (Meneguz et al., 2018a; Meneguz et al., 2018b).

Intensive agriculture imposes pressure on available land; this coupled with increasing human population will aggravate the problem (FAO, 2011). Rearing BSF on waste that cannot be consumed by humans and later fed to poultry, livestock, pigs and fish can increase the protein intake of the animals and this is more efficient and environmentally friendly than growing fields of soybean and other grains to meet the protein requirements of the animals (Craig Sheppard et al., 1994; Makkar et al., 2014; Khan, 2018; Meneguz et al., 2018b). Black soldier flies need limited space for rearing and this reduces pressure on available arable land while providing a source of feed and food for animals and humans (Marono et al., 2017). Use of insects as alternative feed source reduces pressure on foodstuffs that are used as food for both animals and humans. This in turn reduces pressure on arable land, deforestation and soil erosion as available land is not overgrazed and over cultivated to meet the high food and feed demand (Schader et al., 2015; Allegretti et al., 2017).

2.3.2 Commercial production of Black Soldier Fly Larvae Meal (BSFLM)

As alluded to, black soldier fly larvae have a diverse range of substrate varying from decaying plant and animal matter to manure, rotting fruits and vegetables. A disadvantage of the BSF for biodegradation is that it requires a warm environment, which may be difficult or energy-consuming to sustain in temperate climates such as Canada. Also and as alluded to, the duration of the life cycle ranges between several weeks to several months, depending on temperatures and the quality and quantity of the diet (Makkar et al., 2014; Makkar, 2017; Meneguz et al., 2018a). The conditions for mating and oviposition are variable ranging from 24-40˚C for temperature and 30-90% for humidity (Sheppard et al., 2002; Makkar, 2017). In this context, for industrial production, it is necessary to maintain a year-round breeding adult colony in a rearing facility (greenhouse) with access to full natural light. Emerging research is indicating optimum conditions include a narrow range of temperature and humidity, as well as a range of suitable levels of texture, viscosity, and moisture content of the diet (Makkar et al., 2014; Meneguz et al., 2018b). Several methods for rearing BSF on substrates such as pig manure (Newton et al., 2005), poultry manure (Craig Sheppard et al., 1994) and artificial substrates (Tomberlin et al., 2002) have been designed.

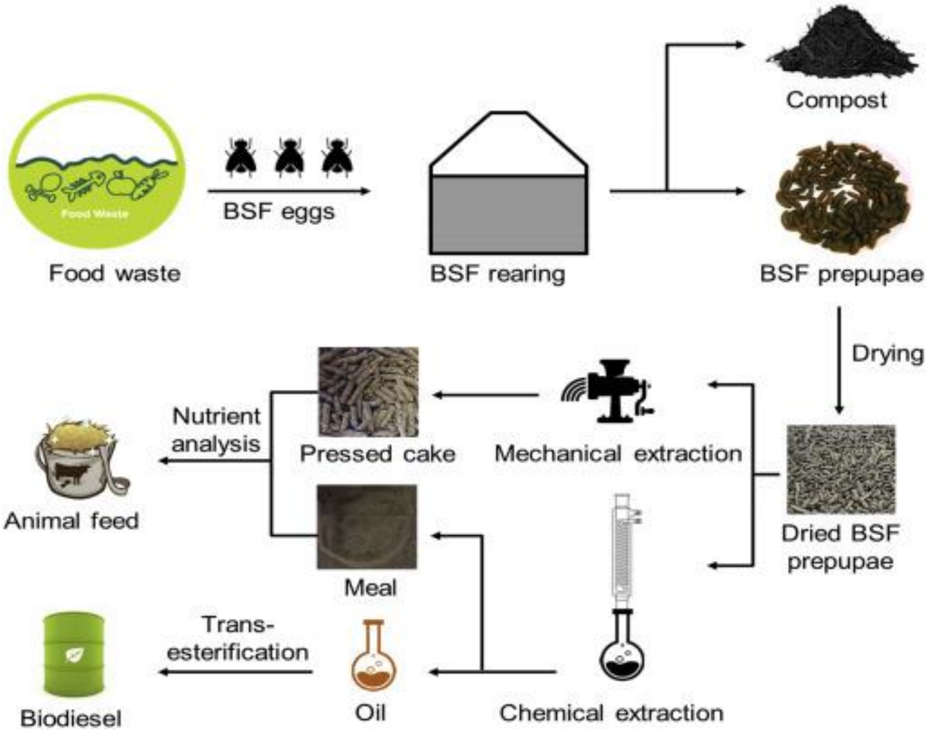

Figure 1: Schematic representation of production of BSF products (Surendra, 2016)

The design of the rearing facilities have containers with an attractive and moist medium to attract egg-laying female adults (Čičková et al., 2015; Meneguz et al., 2018b). Rearing facilities use the migrating behavior of the prepupae for self-collection: larvae climb up a ramp out of a rimmed container to eventually end in a collecting vessel attached to the end of the ramp (Čičková et al., 2015; Meneguz et al., 2018b). Larvae develop through several developmental stages, with the final stage known as the prepupa (Figure 1). The relative optimal humidity in the rearing facilities should fall between 50 and 70%; higher relative humidity makes the diet too wet. The diet should have enough structure (not very deep and well aerated), otherwise the larvae may have a difficult time crawling on it, consuming it and getting adequate oxygen supply (Čičková et al., 2015; Meneguz et al., 2018b). In the latter stage, the larvae stop feeding and the digestive tract is emptied. Then, the prepupa migrates in search of a dry and protected site in preparation for metamorphosis.

Therefore, prepupae are the preferred stage for harvesting, and are used live, chopped, or dried and ground. The live larvae are sold for pets and fish bait, and they can be easily dried for longer storage (Veldkamp and Bosch, 2015). The DM content of fresh larvae is in the range of 35-45%, thus easier and less costly to dehydrate than other fresh by products (Burtle, 2019). In commercial facilities, prepupae is dried for 20 hours in an oven at 60°C and ground into a meal (Figure 1). BSFLM contains about 40-44% crude protein (**CP**); particularly rich in lysine (6 to 8% of the CP) but low in Sulphur amino acids (1.7 to 2.4% of CP), threonine (1.3 to 4.8% of CP) and tryptophan (0.5% of CP) (Makkar et al., 2014). The ash content is relatively high but variable, from 11 to 28% DM; with high concentrations of calcium (5–8% DM) and modest concentrations of phosphorus (0.6–1.5% DM) (Makkar, 2017). Some studies have shown that calcium and fatty acid levels can be enhanced by manipulating the growth substrate (Makkar et al., 2014). For example, the concentration of fat in BSFLM is dependent on rearing substrates: reported values are 15 to 25% for larvae fed on poultry manure, 28% on swine manure, 35% on cattle manure and 42 to 49% on oil-rich food waste (Newton et al., 2005; Nguyen et al., 2015; Spranghers et al., 2017). Notably, the fatty acid profile of BSF prepupae is, in general, high in **MCFA** lauric acid (C12:0, ~>50% of total fat). However, the fatty acid composition of the larvae depends on the fatty acid composition of the diet. Larvae fed on cow manure contained 21% of lauric acid, 16% of palmitic acid, 32% of oleic acid and 0.2% of omega-3 fatty acids, whereas these proportions were respectively 43%, 11%, 12% and 3% for larvae fed 50% fish offal and 50% cow manure (St-Hilaire et al., 2007a).

Commercial BSF rearing facilities often defat the meal mechanically by chopping the frozen larvae and pressing them to extract extracellular fat or use petroleum ether to chemically extract fat to increase protein fraction, improve meal shelf life and to create a fat stream for biodiesel and other applications (Figure 1) (Fasakin et al., 2003; Schiavone et al., 2017). The fat derived from BSFL is converted to biodiesel in a two-step process: by acid catalysed esterification to decrease acidity of crude fat and alkaline catalysed transesterification (Figure 1) (Li et al., 2011; Surendra et al., 2016). Fuel properties of the biodiesel from BSFL were comparable to those of rapeseed (Li et al., 2011), and has the advantage of not competing with food resources or land use and it maximises benefits of waste management by using waste nutrients to grow the insects. Defatting processes produce BSFLM with crude fat content as low as 5% DM depending on fat extraction procedures (Fasakin et al., 2003; Schiavone et al., 2017). Defatting has been shown to increase crude protein from 40-44% DM in whole BSFLM (Makkar et al., 2014; Spranghers et al., 2017) to as high as 65.5 % DM (Schiavone et al., 2017). Moreover, defatted BSFLM was shown to have higher or comparable digestible amino acids concentration to typical animal and plant protein sources used in poultry feed (Schiavone et al., 2017; Mwaniki and Kiarie, 2018). Defatting is also seen as critical control point for BSFLM as fat component has been shown to be the most variable component in larva grown in diverse substrates (Spranghers et al., 2017).

2.3.3 Application of BSFLM in human food and animal feeds

2.3.3.1 Human food

Insects can contribute to global food security via feed or as a direct food source for humans (Schader et al., 2015). Traditionally, edible insects worldwide are believed to have high nutrient content with fat, protein and minerals depending on species and hence used as alternatives food and as substitutes to other protein sources (Rumpold and Schlüter, 2013). Insects have been part of human diet in continents like Africa and Asia for ages; globally over 2 billion people are estimated to consume insects in one form or another (Parizeau et al., 2015).

Insects form part of traditional food and are perceived as high protein sources. Traditional preparation methods of edible insects include steaming, boiling, baking, deep-frying, sun-drying, smoking, and processing into chutney or a paste (Rumpold and Schluter, 2013).

Continued pressure on arable land to grow crops and raise livestock is anticipated to aggravate food insecurity making insect protein a viable mainstream human food particularly in the highly densely populated parts of the world (Parizeau et al., 2015). Most insects consumed today are gathered in the wild and either eaten raw, prepared at home, or sold in local markets (Rumpold and Schluter, 2013a; Kinyuru et al., 2015). Consequently, a tremendous amount of research is still needed on the production and processing methods on an industrial scale as well as on the microbial safety and decontamination of edible insects (Belluco et al., 2015; Kinyuru et al., 2015). Contamination of edible insects can be reduced by managing environmental and farming conditions to avoid accumulation of contaminants. Moreover, there is evidence that the consumption of insects can cause allergic reactions comparable to allergies to crustaceans and house dust mite (Rumpold and Schluter, 2013a; Rumpold and Schlüter, 2013). This is mainly due to the presence of tropomyosin and arginine-kinase, well-known allergens in arthropods that are also present in insects. Cross-reactivity studies suggest that patients allergic to house dust mites and crustaceans may react to food containing yellow mealworm protein (Verhoeckx et al., 2014). More research needs to be done on the allergic cross-reactions resulting from feeding on insects qualifying as possible sources of food to assess their safety (Ricci, 2015).

2.3.3.2 Aquaculture feeding programs

Since the 1990s, the rising demand for fish products for human consumption has been met by aquaculture rather than by wild capture fishery. Fish feeds, notably those of salmonids and marine fish, are usually based on fish meal and fish oil obtained from pelagic species captured for this purpose (Tran et al., 2015). Fish meal is a highly regarded source of protein with an excellent composition of essential AA, while fish oil provides long-chain omega-3 fatty acids favored for their health benefits (St-Hilaire et al., 2007b; Sánchez-Muros et al., 2014; Widjastuti et al., 2014; Tran et al., 2015). However, this reliance on wild fish capture for fish farming is under intense scrutiny. Fishmeal and fish oil may contain contaminants such as polychlorinated biphenyls and dioxins, but consumers are also now interested in sustainability metrics such as the ratio of wild fishery inputs to farmed fish outputs (Tran et al., 2015). Also, the volatility and rise of fish meal prices is a matter of concern for the aquaculture industry. High cost of fishmeal and the ecological impact of over exploitation of fishing ground has led to the need for alternative protein sources for farmed fish diets. Plant protein are cheap alternatives or substitutes for fish meal but have antinutritional factors such as fiber, palatability issues and less balanced AA profile relative to fish requirements.

Use of insects including BSFLM in aquaculture feed has been approved in many jurisdictions including EU, US and Canada (Bruni et al., 2018). Black soldier fly larvae has been shown to have an essential AA profile that is close to fishmeal and has been proposed as a good alternative to fishmeal (Tran et al., 2015). The BSFLM meal was found to partially replace fishmeal in diet of rainbow trout without any negative effects on survival of the fish, organ development and fillet yield (Bruni et al., 2018). Increased diversity of digestive and mucosa associated microbial communities in rainbow trout was also observed in fish fed diet containing BSFLM (Bruni et al., 2018). Partial inclusion of BSFLM in diet fed to juvenile turbot showed a significantly higher feed efficiency and protein retention at 33% inclusion level of BSFLM but decreased with higher inclusion. Chitin present in BSF affected digestibility and growth implying that BSFLM could partially provide proteins in a turbot diet (Kroeckel, 2012), as diets with >33%

BSFLM had more chitin (g/kg DM) . These studies indicated that while not as ideal as fishmeal, BSFLM may replace part of it in fish diets, usually less than 25 to 30% though greater inclusion rates are possible with synthetic AA fortification (Tran et al., 2015).

2.3.3.3 Swine feeding programs

2.3.3.3.1 Digestibility

There is very limited information on digestibility of BSFLM and insect protein in general in pigs. The apparent fecal digestibility of macronutrients (DM, CP and crude fat) in piglets fed 33% BSFLM was comparable with soybean meal (Newton et al., 1977). In contrast, the highest ileal crude protein digestibility values were observed in piglets fed 4% BSFLM vs. 8% BSFLM (Spranghers et al., 2018). This indicated that providing a limited amount of BSF protein might have a positive effect on the protein digestibility of the diet. However, this effect became negative when a higher amount of BSF protein was provided.

2.3.3.3.2 Production performance

Black soldier fly larvae meal is especially valuable for its AA, lipid and Ca contents and has been evaluated in pig trials. The diets containing BSFLM were as palatable as a soymeal based diet (Newton et al., 1977). Dried BSFLM was fed to early weaned pigs as a replacement of 50 or 100% dried plasma protein with or without AA supplementation in a three phase nursery feeding program. Without AA supplementation, the 50% replacement diet gave slightly better performance during phase 1 (+4% gain, +9% feed efficiency) (Newton et al., 2005). However, the 100% replacement diets did not perform as well as the control (overall performance reduced by 3 to 13%). In a more recent trial, piglets that fed 8% BSFLM consumed the least amount of feed compared with the control (Spranghers et al., 2018). The authors were of the opinion that reduced palatability may be because of the presence of a substantial amount of free MCFAs in the feed. Alternatively, the chitin content may limit feed intake. The immediate post-weaning period is most critical, and generally significantly determines performances in subsequent stages (Pluske, 2016). It has been suggested that perhaps additional refinement (cuticle removal and rendering) to remove chitin may be necessary to make BSFLM meal suitable for early weaned pigs (Newton et al., 2005). Moreover, its relative deficiency in methionine + cystine and threonine requires the inclusion of those AA for the preparation of balanced diets.

2.3.3.4 Poultry feeding programs

2.3.3.4.1 Digestible amino acids and energy

Similar to its application to swine feed, the AA content in BSFLM is comparable or more favourable to conventional feedstuffs used in poultry and implies that BSFLM is an excellent AA source particularly with supplemental sulphur amino acids, the first limiting amino acids in poultry fed corn-soybean meal diets (NRC, 1994). However, in general, there is a dearth of information on digestible AA and AMEn data for BSFLM. Where data do exist, most have been reported based on apparent ileal digestibility (AID) as opposed to standardized ileal digestibility (SID) estimates (De Marco et al., 2015; Schiavone et al., 2017). It has been suggested that SID estimates should be used in formulating poultry diets because these are additive in a mixture of feedstuffs compared with AID estimates (Angkanaporn et al., 1996). Moreover, formulating using SID of AA estimates results in diets that more closely match the birds' requirements and reduce excess nutrients (Adedokun et al., 2007; Moughan et al., 2014; Adeola et al., 2016). Defatted BSFLM was found to have comparable content of SID of essential AA to conventional AA sources for broilers (Schiavone et al., 2017; Mwaniki and Kiarie, 2018). Mwaniki and Kiarie (2018) observed that the SID content of Lys, Met, and Thr in BSFLM was 2.85, 0.82 and 1.98%

DM. These values were higher or comparable to values for soybean meal (2.80, 0.60 and 1.70% DM) (Adedokun et al., 2008; Bandegan et al., 2010; Ullah et al., 2016), fermented soybean meal (2.67, 0.77 and 2.04% DM) and pea protein isolate (3.49, 0.43 and 1.73% DM) (Frikha et al., 2013). Moreover, with few notable exceptions, values for Lys, Met and Thr were respectively higher or comparable with fishmeal (1.62, 0.67 and 1.06% DM) (Ullah et al., 2016), feather meal (1.25, 0.46 and 2.74% DM) and poultry by-products (2.89, 0.90 and 1.71% DM) (Bandegan et al., 2010), meat and bone meal (2.05, 0.57, 1.24% DM) and animal protein blends (2.09, 0.55 and 1.45% DM) (Rochell et al., 2013). Other broiler chicken studies indicated that defatted BSFLM had higher or comparable digestible AA concentration to typical animal and plant protein sources used in poultry feed (De Marco et al., 2015; Schiavone et al., 2017).

The typical crude fat content in whole BSFLM is 15-35 % DM (Makkar et al., 2014) and defatting processes produce meal with crude fat content of as low as 5% DM depending on fat extraction procedures (Schiavone et al., 2017). De Marco et al. (2015) reported a higher level of AMEn (3,967 kcal/kg DM) in full-fat BSFLM (34.3% DM crude fat). Defatted BSFLM samples were determined to have AMEn of 3,554 kcal/kg DM (18.0% DM crude fat; 55.3% DM CP), 2,902 kcal/kg DM (7.1% DM crude fat; 57.5% DM crude protein) and 2,354 kcal/kg DM (4.6% DM crude fat; 65.5% DM CP) (Schiavone et al., 2017; Mwaniki and Kiarie, 2018). This suggested importance of crude fat concentration on assigning accurate AMEn values of BSFLM in practical poultry feed formulation. Moreover, moderate and variable retention of protein and energy in broilers fed BSFLM has been attributed to the negative effects of chitin (De Marco et al., 2015; Schiavone et al., 2017). Broiler chickens fed chitin derived from crustacean shell waste (37.3% CP) digested approximately 50% of chitin protein (Hossain and Blair, 2007). Marono et. al. (2015) demonstrated that *in vitro* CP digestibility was negatively correlated to the chitin content. Surprisingly, chickens have been shown to produce chitinase in the proventriculus and hepatocytes (Suzuki et al., 2002).

2.3.3.4.2 Production performance

Earlier study (as per available literature) showed successful use of BSF larvae and prepupae grown on swine manure or kitchen waste as feedstuffs for young chicks (Hale, 1973). Later studies focused on replacing conventional feedstuffs such as soybean meal with BSFLM. In more recent broiler trials, BSFLM was included at increasing levels (0, 5%, 10% and 15%) to replace soybean meal) in isonitrogenous and isoenergetic diets formulated for 3 feeding phases: starter (1–10 d), grower (10–24 d) and finisher (24–35 d) (Dabbou et al., 2018). The authors reported that increasing levels of dietary BSFLM inclusion improved the body weight gain and feed intake during the starter period. As a component of a complete diet, BSFLM was reported to increase the body weight gain of growing quail driven by increased feed intake (Widjastuti et al., 2014). Increased appetite has been attributed to the improvement of the diet palatability related to BSFLM inclusion. Indeed, a feed-choice test in broiler quails showed that the birds tended to prefer diets containing BSFLM (Cullere et al., 2016) potentially confirming poultry innate behavior of consuming insects.

Numerous studies have also reported negative or no effects on body weight, feed intake and feed conversion. For example, FCR was impaired in broiler chickens fed 15% BSFLM (50% soybean meal replacement)in contrast to control, in nutritionally balanced diet over 10-35 day period (Dabbou et al., 2018). The poor FCR in birds fed 15% BSFLM diet was to a large extent due to body weight gain depression (~10% less than control=0% BSFLM) and to a lesser extent by feed intake (2% less than control). Replacing 45% of soybean meal with BSFLM in a isocaloric and isonitrogenous broiler feed had no impact on feed intake, body weight gain and feed conversion over a 35 day trial (Uushona, 2015). Replacing 100% of soy oil and 25% of soybean meal in a diet for growing broiler quail did not affect body weight gain and meat quality but tended to reduce feed intake (Cullere et al., 2016). Replacing up to 50% of soybean meal with BSFLM showed no effect on live weight and general performance in Barbary partridges (Loponte et al., 2017). Complete replacement of soybean meal with BSFLM in laying hens over 25-45 weeks reduced feed intake by 16% and as a result hens fed BSFLM had reduced egg production, egg mass and body weight (Marono et al., 2017). Other investigations in laying hens indicated BSFLM can partially or fully replace soybean meal with no effect on growth performance (Al-Qazzaz et al., 2016; Maurer et al., 2016). The unpredictable performance effects of BSFLM in practical poultry diets has been to a larger extent attributed to variable effects of chitin on protein digestibility (Makkar et al., 2014). However, other reports have speculated feed refusal based on color as BSFLM tend to be brown (darker) relative to yellow color of typical corn and soybean diets (Marono et al., 2017). Although the feeding value of BSFLM in commercial poultry diets has been reported, this field is its infancy; more research is required to characterize and optimize factors other than nutrients that may influence varied bird responses.

Very few studies are available in the literature on the application of insect meal as a possible alternative protein source for laying hens (Al-Qazzaz et al., 2016; Maurer et al., 2016; Marono et al., 2017). For example, a total replacement of soybean meal with BSFLM in diets for Lohmann Brown classic (week 24-45) reduced feed intake and egg production as earlier explained (Marono et al., 2017). In contrast, egg production and feed intake in Lohmann White Leghorn laying hens fed (week 64-74) diets in which BSFLM replaced 100% of soybean cake were unaffected by dietary treatments (Maurer et al., 2016). The differences in responses could be ascribed to the age and strains of birds (browns consume more feed) (Anderson et al., 2013). The beginning of lay is physically a very challenging time for the young hens. As a result, negative nutrient balances can occur (Leeson, 2005). At the onset of lay, the bird is not only adjusting to her new environment, but she must consume enough energy and nutrients for her body weight development and to reach the high peak in egg production. It is imperative to increase their feed intake from the end of the growing period towards the peak of production in a short time. In this context and given the unpredictable production performance responses of feeding BSFLM, a potential approach is incremental replacement of soybean meal with BSFLM in feeding programs for laying hens, however, such data is lacking in the literature.

2.3.4 Functional value of BSFLM

Chitin is the most common form of fibre in the body of insects contained mainly in their exoskeleton (Finke, 2007). Chitosan, a versatile hydrophilic polysaccharide derived from chitin, has a broad antimicrobial spectrum to which gram-negative, gram-positive bacteria and fungi are highly susceptible (Raafat and Sahl, 2009). The MCFAs are well known for their antimicrobial effects on gut microbiota, while lauric acid is particularly active against Gram positive bacteria (Dierick et al., 2002; Skřivanová et al., 2005). The fat of prepupae reared on organic waste streams with high amounts of starch contains up to 60% lauric acid (Spranghers et al., 2017). Lauric acid, which is known for being a natural antimicrobial agent, acts by disrupting the cell membrane, being thus effective for the control of various foodborne pathogens (Kim and Rhee, 2016). Moreover, the larval secretions of BSF were reported to be very rich in substances with novel antimicrobial properties (Park et al., 2014), which are mainly attributable to the humoral response of insects' immune system, involving the production of peptides with antimicrobial activity that are secreted in the haemolymph. However, there is very little investigations on aspects of functional properties of insects as feed or food constituents.

Chitin has been shown to treat renal failure in humans (Jing et al., 1997). Diabetic rats fed diets with added chitosan had less severe symptoms of diabetes compared to their counterparts with no added chitosan (Algalaly et al., 2015). Dietary BSFLM inclusion has also been reported to positively affect the blood profile of laying hens and Barbary partridges in terms of higher globulin levels, lower albumin-to-globulin ratios and lower creatinine levels (Loponte et al., 2017; Marono et al., 2017). However, there was no effects of BSFLM in haemato-chemical or histological parameters in broiler chickens (Dabbou et al., 2018). Chitin was also found to increase populations of helpful *Lactobacillus* in guts of broilers and reduced the population of *E. coli* and *Salmonella spp*. There was also a notable reduction in ammonia production and increased levels of ceca butyric acid (Khempaka et al., 2011). Cutrignelli et al., (2017) found higher villi height in the duodenum of laying hens fed BSFLM compared with the control. The BSF larvae has also been shown to reduce populations of *E. coli* and *Salmonella spp*. in chicken manure hence avoiding contamination of crops when chicken manure was used as fertilizer and where these pathogens found their way into livestock and human guts. Moreover, it has been demonstrated that fiber produces short-chain fatty acids that lower pH, thus shifting NH_3 to the less volatile NH_4 (Roberts et al., 2007). Chitin may have the same effects in laying hens and thus contribute to NH_3 emission reduction. However, these potential benefits of insect meal have not been evaluated.

Weaning exposes piglets to multiple stressors (both nutritional and environmental) which results in a reduced feed intake, gut alterations and reduced digestive capacity, frequently associated with the proliferation of pathogens, such as enteropathogenic *E. coli* (Pluske, 2016).

To manage weaning processes, baby pig diets are fortified with antimicrobial feed additives, a practice that is under scrutiny (Kiarie et al., 2013; Kiarie et al., 2016). Since early weaned piglets are prone to bacterial infections, the effects of BSF fat on the porcine gut microbiota was assessed in simulated piglet small intestine (Spranghers et al., 2018). Different amounts of BSF fat were added to an incubation medium, which contained a synthetic diet, a phosphate buffer (pH 5) and a microbial inoculum from one donor piglet. The medium was incubated at 37°C for 4 h. Using selective media, coliforms, D-*streptococci*, *lactobacilli* and total anaerobic bacteria were counted on aliquots taken at the end of the incubations. The BSFLM fat at 0.58 g C12:0/100 mL, suppressed growth of *lactobacilli*, but the most substantial antibacterial effects were recorded against D-*streptococci*. At the highest inclusion level (equivalent to 0.87 g C12:0/100 mL), around 2 log fold reductions of D-*streptococci* were observed. Further investigations are needed to evaluate the gut health benefits of BSFLM components in laying hens.

2.3.5 Summary

Soybean and corn are the main energy and protein sources in poultry feed formulations. Pressure from competition as food for humans and feed for other animals, raw materials for biofuel industries and volatile prices have prompted the global feed industry to seek alternative feed ingredients.

At the same time, the anticipated increase in human population implies a need to increase animal derived products to curb food insecurity. Eggs are inexpensive sources of high-quality protein, vitamins and minerals. Appropriate nutrition for laying hens is crucial for optimal egg production. Diets must be formulated to meet the hens' protein, energy, mineral and vitamin requirements. Protein and energy rich ingredients are mainly plant based like soybean meal, wheat and corn. High prices of these feedstuffs ultimately affect egg production costs. Insects have been shown to be competitive candidates to replace soybean due to their superior amino acid profiles, high calcium and phosphorus and fatty acids. Several studies have shown that BSFLM improved egg production, egg quality and antimicrobial effect in guts of poultry. However, incorporation in poultry diets has been shown to lead to poor feed conversion as well as low protein digestibility due to chitin. Most studies in laying hens have been done in brown layers and broilers but there is limited study on the use of BSFLM in White leghorns, the dominant egg producing strain in Canada and the US. There is also no clear recommended inclusion level of the insect meal in white layers diets. This thesis will investigate the effect of substituting soybean meal partially and wholly with BSFLM on egg production and egg quality and other physiological effects in Shavers white layers from onset of lay (19 weeks) to 43weeks of age.

CHAPTER 3

Hypothesis and objectives

Hypothesis

Insect meal (BSFLM) can completely replace soybean meal in practical diets for laying hens without negative effects on egg production, egg quality and select physiologic responses.

Overall objective:

Evaluate the impact of complete replacement of soybean meal with BSFLM in diets fed to white leghorn layers from 19 to 43 weeks of age.

Specific objectives:

At the onset of lay, the bird is not only adjusting to her new environment, but she must consume enough energy and nutrients for her body weight development and to reach the high peak in egg production. It is imperative to increase feed intake from the end of the growing period towards the peak of production in a short time (Leeson and Summers, 2005). In this context, the study was divided into phases to capture incremental replacement of BSFLM in practical laying hen diets:

1) Impact of replacing 41% soybean meal with BSFLM in a corn-based diet on egg production and egg quality in laying hens from 19 to 27 weeks of age.
2) Impact of complete (100%) replacement of soybean meal with BSFLM in a corn-based diet on egg production, egg quality, visceral organ weight, plasma metabolites and apparent nutrient retention in laying hens from 28 to 43 weeks of age.

CHAPTER 4:

Impact of replacing 41% soybean meal with BSFLM in a corn-based diet on feed efficiency, egg production and egg quality in laying hens from 19 to 27 weeks of age [1]

4.1 Abstract

A corn-soybean meal diet was formulated with 0 or 5.0 or 7.5% BSFLM and fed (n=6) to a total of 108, 19-wk old Shaver white pullets placed in conventional cages (6 birds/cage). The birds had free access to feed and water. Hen day egg production (**HDEP**) and average egg weight (**AEW**) was monitored daily and feed intake (**FI**) weekly. Egg quality (**EQ**) parameters were assessed on individual eggs collected on the 5th day of weeks 22, 24 and 26 and included individual EW (**IEW**), albumen height (**HU**), yolk color, egg shell breaking strength (**SBS**) and thickness (**ST**). A quadratic response ($P < 0.02$) was observed for HDEP, EW and egg mass. Specifically, birds fed 0 and 7.5% BSFLM diets had similar ($P > 0.05$) values for these parameters with birds fed 5.0% BSFLM showing lower ($P < 0.05$) HDEP than 0 or 7.5% BSFLM fed birds. The HDEP was 89.4, 84.8 and 87.8 for 0, 5.0 and 7.5% BSFLM, respectively. Feeding BSFLM linearly ($P < 0.01$) increased FI and FCR (FI/egg mass). There was no diet effect ($P > 0.05$) on IEW and HU, however, BSFLM linearly ($P = 0.02$) reduced IEW: 53.7, 52.3 and 53.0 g for 0, 5.0 and 7.5% for BSFLM fed birds, respectively. Feeding BSFLM linearly ($P < 0.01$) increased YC, SBS and ST. In conclusion, birds fed 7.5% BSFLM had similar HDEP and egg mass but poorer FCR relative to corn-soybean meal diet without BSFLM.

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[1] Z. Mwaniki, M. Neijat, and E. Kiarie. Egg production and quality responses of adding up to 7.5% defatted black soldier fly larvae meal in a corn–soybean meal diet fed to Shaver White Leghorns from week 19 to 27 of age. 2018 Poultry Science 97:2829–2835.

4.2 Introduction

Very few studies are available in the literature on application of insect meal as a possible alternative protein source for laying hens (Al-Qazzaz et al., 2016; Maurer et al., 2016; Marono et al., 2017). For example, a total replacement of soybean meal with BSFLM in diets for Lohmann Brown classic (week 24-45) reduced feed intake and egg production (Marono et al., 2017). In contrast, egg production and feed intake in Lohmann White Leghorn laying hens fed (week 64-74) diets in which BSFLM replaced 100% of soybean cake were unaffected by dietary treatments (Maurer et al., 2016). The differences in responses could be ascribed to the age and strains of birds (brown consumed more feed) (Anderson et al., 2013). The beginning of lay is physically a very challenging time for the young hens. As a result, negative nutrient balances can occur (Leeson, 2005). At the onset of lay, the bird is not only adjusting to her new environment, but she must consume enough energy and nutrients for her body weight development and to reach the high peak in egg production. It is imperative to increase their feed intake from the end of the growing period towards the peak of production in a short time. In this context and given the unpredictable production performance responses of application of BSFLM, a potential approach is incremental replacement of soybean meal with BSFLM in feeding programs for laying hens, however, such data is lacking in the literature. Moreover, most studies on BSFLM have been done on broiler chicken and brown layers and there is a knowledge gap in the impact of BSFLM in white leghorns on egg production, egg quality characteristics and calcium uptake and use. Therefore, the objective of the present study was to evaluate effects of 0, 5.0 and 7.5% (41% soybean meal replacement) inclusion of defatted BSFLM in practical corn–soybean diet fed to laying hens from onset of laying to peak lay.

4.3 Materials and methods

The experimental protocol was approved by the University of Guelph Animal Care Committee which complies with the Canadian Code of Practice for the Care and Use of Animals for Scientific Purposes (CCAC, 2009).

4.3.1 Insect meal (BSFLM) and diets

Defatted BSFLM (approximately 6% crude fat as fed) was procured from a commercial manufacturer and vendor (Enterra feed Corp., Vancouver, BC, Canada). The meal is a dry, powder product derived from larvae of the BSF (*Hermetia illucens*) reared on pre-consumer recycled food collected from local farms, food processors and grocery stores. The meal is approved by the Canadian Food Inspection Agency for feeding poultry. A standard corn-soy bean meal (0% BSFLM) diet was formulated to meet the nutrient requirements for 19 weeks of age pullets in accord to Shaver White commercial management guidelines (ISA, 2016). The BSFLM was included at 5.0 and 7.5% to maintain iso-caloric and iso-nitrogenous specification (Table 4.2). The nutrients specification of BSFLM was derived from published data (De Marco et al., 2015; Schiavone et al., 2017). The nutrients specification of other feedstuffs were from Evonik Aminodat https://animal-nutrition.evonik.com/product/animal-nutrition/aminodat. All diets were prepared in pellet form at the Arkell research station feed mill, University of Guelph.

4.3.2 Birds, housing and experimental procedures

One hundred and eight, 19-week-old pullets (Shaver White Leghorns) were placed in cages (6 birds per cage) based on BW. Cage dimensions were 66.0 cm × 62.2 cm × 49.5/46.4 cm (front/rear) for depth × width × height, respectively and were housed in an environmentally controlled room (20°C) at the Arkell Poultry Research Station, University of Guelph. The birds received 14 h of incandescent light (15 lux, 06:00 to 19:00 hr) and 10 h of dark per day. The diets were allocated to cages based on BW in a completely randomized design to give 6 replicates per diet. The diets were fed from week 19 to 27. The birds had free access to feed and water throughout the experimental period. Total number of eggs laid and total egg weight per cage were recorded daily. Feed intake was determined on weekly basis and BW was collected at the end of weeks 21, 23, 25 and 27. All eggs collected on the 5th day of week 22, 24 and 26 were submitted for egg quality analyses on the same day.

4.3.3 Sample analysis

4.3.3.1 Egg quality measurements

The individual egg weight (**IEW**), height of albumin (Haugh units, **HU**) and yolk color were determined by egg Analyzer™ (ORKA Food Technology Ltd, Ramat HaSharon, Israel). The systems detect, calculates and reports values for yolk color (1-15 colors scale based on DSM/Roche yolk color fan), HU and egg weight (g). Prior to measurements, the unit was calibrated as per manufacturer recommendations. The egg shell thickness (**ST**) was measured using a high-resolution non-destructive device that measures ST without breaking using precision ultrasound (ESTG-1, ORKA Food Technology Ltd.). Briefly, a gel is applied on the egg followed by placement of the egg on cradle to read shell thickness in mm. Shell breaking strength (**SBS**, kgf) was measured by Force Reader (ORKA Food Technology Ltd.), the unit measures accurately the breaking point of the egg shell by applying mechanical force on vertically placed egg on the cradle.

4.3.3.2 Chemical analyses

Samples of BSFLM and diets were finely ground in a coffee grinder and thoroughly mixed for analyses. Samples were analyzed for DM, CP, gross energy (**GE**), crude fat, starch, ethanol soluble carbohydrates, neutral detergent fiber (**NDF**) and minerals. Dry matter determination was carried out according to standard procedures method 930.15 (AOAC, 2005). Nitrogen was determined by the combustion method 968.06 (AOAC, 2005) using a CNS-2000 carbon, N, and sulfur analyzer (Leco Corporation, St. Joseph, MI). The CP values were derived by multiplying the assayed N values by a factor of 6.25. Gross energy was determined using a bomb calorimeter (IKA Calorimeter System C 5000; IKA Works, Wilmington, NC). The NDF contents were determined according to (Van Soest et al., 1991) using Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY). Crude fat content was determined using ANKOM XT 20 Extractor (Ankom Technology, Fairport, NY). Samples for AA analysis were prepared by acid hydrolysis according to the method of AOAC (2005, method 982.30), and as modified by (Mills et al., 1989). Briefly, about 100 mg of each sample was digested in 4 mL of 6 N HCl for 24 h at 110°C, followed by neutralization with 4 mL of 25% (weight/volume) NaOH and cooled to room temperature. The mixture was then equalized to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using an AA analyzer (Sykam, Germany). Samples for analysis of sulfur containing AA (Methionine and Cystine) were subjected to performic acid oxidation prior to acid hydrolysis. Tryptophan was not determined. Minerals (Ca, P, K, Mg and Na), ethanol soluble carbohydrates and starch were analyzed in a commercial laboratory (SGS Canada Inc, Guelph, ON, Canada).

4.3.4. Calculations and statistical analyses

Hen–day egg production (**HDEP, %**) was calculated as total number of eggs laid per cage divided by the number of hens per cage. Average egg weight (**AEW, g/bird**) was calculated as total egg weight per cage divided by the number of hens housed per cage. The egg mass (**EM**) was calculated as HDEP multiplied by AEW. Weekly feed intake per cage was divided by seven and number of hens per cage to derive daily feed intake per day per bird (**FI, g/bird**). Feed conversion ratio (**FCR**) was calculated by dividing FI by EM. The egg data were analyzed using GLM procedures (SAS Inst. Inc., Cary, NC) and the cage was the experimental unit. Data on egg production, egg quality and body weight were subjected to a 2-way ANOVA according to the following model; $Y_{ijk}=\mu + D_i + W_j + DWij + e_{ijk}$, where Y is one observation, μ is general mean, D is the diet effect (i =0, 5 or 7.5% BSFLM), W is the week effect (j=from19 to 27 for egg production or 22, 24 and 26 for egg quality or 21, 23, 25 and 27 for bodyweight), DW is diet x Week interaction and e is the error term. Contrast coefficients from unequally spaced BSFLM were generated using the interactive matrix language procedure of SAS. An α level of $P \leq 0.05$ was used as the criterion for statistical significance.

4.4 Results and discussion

The analyzed chemical composition of BSFLM sample and experimental diets are shown in Tables 4.1 and 4.3, respectively. The crude fat concentration was lower than values of 15-35% DM reported for non-defatted BSFLM (Makkar et al., 2014) but comparable to defatted BSFLM sample (Marono et al., 2017; Schiavone et al., 2017). The concentration of CP was higher than values of 40-44% DM for whole BSFLM (Makkar et al., 2014; Spranghers et al., 2017) but within the range of 47.6 to 65.5% DM for defatted BSFLM (Marono et al., 2017; Schiavone et al., 2017). Crude protein variation in BSFLM are indications of variable fat and chitin concentrations as well as growth substrates (Liu et al., 2012). The concentrations of Lys, His and Val were higher than values reported for defatted BSFLM (~crude fat 4.7% DM) (Schiavone et al., 2017). However, the concentrations of other AA were comparable to defatted samples (Schiavone et al., 2017). The concentration of Ca was somewhat lower (5–8% DM) whereas concentration of P was comparable (0.6–1.5% DM) to literature values (Makkar et al., 2014). Black soldier fly larvae are converters of organic waste into edible biomass, of which the composition of the meal depend on the substrate (Diener et al., 2009; Nguyen et al., 2015). However, in a recent study, it was demonstrated that the concentration of CP and AA is very consistent, and fat was variable in a meal from larvae grown on diverse substrates (chicken feed, vegetable waste, biogas digestate and restaurant waste) (Spranghers et al., 2017). This suggested a defatted BSFLM could be a very attractive protein feed ingredient for poultry diets. The diet with 5% BSFLM assayed slightly lower CP relative to other diets but was above formulation target of 17% (Table 4.2). However, the calculated AME and AA concentrations were comparable across all diets.

The effects of BSFLM inclusion on HDEP is shown in Table 4.4. There was a quadratic effect ($P< 0.01$) on HDEP with birds fed 5% BSFLM showing lower HDEP than birds fed 0 or 7.5% BSFLM. Similarly, BSFLM inclusion had quadratic ($P< 0.021$) response on AEW and egg mass with 5% BSFLM fed birds showing lower EW relative to 0% BSFLM. Feed intake was linearly and quadratically increased ($P<0.01$) by inclusion of BSFLM with birds fed 7.5% BSFLM showing the highest feed intake relative to the 0 or 5% BSFLM. As a result, a linear ($P =0.003$) increase in FCR was observed with increasing level of BSFLM. Generally, HDEP, AEW, egg mass and FI increased as expected from week 19 to 27. However, significant interaction ($P< 0.05$) between diet and week was observed for egg mass and FI.

Energy and AA intakes are the greatest driver of egg production and egg size (Leeson, 2005). The 5% BSFLM diet had comparable calculated AME and AA with other diets, it is thus rather difficult to explain why we observed reduced HDEP and EW in birds fed this diet. A total replacement of soybean meal with defatted BSFLM (17% BSFLM) in diets for Lohmann Brown classic (week 24-45) indicated birds fed BSFLM had lower HDEP, EW, EM, and FCR than birds fed soybean meal (Marono et al., 2017). In contrast, egg production, feed intake, and FCR of

Lohmann White Leghorn laying hens fed (week 64-74) diets in which defatted BSFLM replaced 100% of soybean cake (24% BSFLM) were unaffected by dietary treatments (Maurer et al., 2016). The differences in responses in the current study and others (Maurer et al., 2016; Marono et al., 2017) could be ascribed to the age and strains of birds (brown consume more feed). Thus, feed intake increased with inclusion of BSFLM. The beginning of lay is physically a very challenging time for the young hens. As a result, negative nutrient balances can occur (Leeson, 2005). At the onset of lay, the bird is not only adjusting to her new environment, but she must consume enough energy and nutrients for her body weight gain and to reach the high peak in egg production. It is imperative to increase their FI from the end of the growing period towards the peak of production in a short time.

Increased feed consumption was also observed in laying quails fed up to 10% BSFLM in practical diets (Widjastuti et al., 2014). The high FI of birds fed BSFLM might be due to higher fiber content in form of chitin (Liu et al., 2012). Feed composition in terms of nutrient content and nutrient balance is an important determinant of voluntary feed intake in poultry. In general, when factors such as ingredient composition (e.g. fiber), health and genotype are standardized, evidence suggests that, chickens offered feed *ad libitum* will consume feed to meet their requirement of the first limiting nutrient, which in most cases are energy yielding nutrients (Newcombe and Summers, 1984). In poultry, dietary fibre affects availability of energy and nutrients, and thus birds fed fibrous diet will consume more of that diet. The implications for the current study were such that the birds fed 7.5% BSFLM consumed more feed to meet energy requirements. In this context, it is noteworthy that 7.5% BSFLM diet had slightly lower crude fat and gross energy concentration compared to other diets. Feeding BSFLM linearly increased BW (Table 4.5). In contrast, Lohmann Brown classic (week 24-45) hens fed 17% BSFLM had lower body weight because of depressed feed intake (Marono et al., 2017). Specific studies in applications of BSFLM in poultry feeding have focused on growing poultry and limited studies exists in layers. As a component of a complete diet, BSFLM was reported to increase quails body weight gain driven by increased FI (Widjastuti, 2014).

The data for individual eggs collected on weeks 22, 24 and 26 are shown in Table 4.6. There was no diet effect ($P > 0.05$) on IEW and HU. The IEW values were 53.7, 52.3 and 53.0 g for 0, 5.0 and 7.5% BSFLM, respectively. This observation suggested that feeding BSFLM improved uniformity of egg size an important metric for egg producers. Feeding BSFLM linearly ($P < 0.01$) increased yolk color, SBS and ST. The yolk color improvement suggested the meal had pigments that increased intensity of yolk color. Indeed recent report demonstrated that feeding laying hens BSFLM increased concentration of γ-tocopherol, lutein, β-carotene and total carotenoids compared with egg yolks from birds fed soybean meal (Secci et al., 2018). The improved egg shell characteristics were indicative of either improved Ca absorption in the gut and improved Ca metabolism or both. We however observed that 5% inclusion level of BSFLM resulted to stronger egg shells as compared to the control and 7.5% inclusion levels. This could be explained by the relationship between small egg size and stronger egg shells. Indeed, although egg shell quality was not reported, feeding laying hens (week 24-45) 17% BSFLM increased circulating serum Ca levels relative to the control (0% BSFLM) despite the two diets having similar Ca concentration (Marono et al., 2017). Egg shell is 99% Ca and daily egg shell formation equate to a removal of 2-3 grams of Ca equivalent to 10% of the hen body Ca reserve (Gilbert, 1983; Etches, 1987). About 60-75% of Ca in egg shell is derived from diet and 25-40% was from the skeletal stores (Comar and Driggers, 1949). Calcium homeostasis is created through a balance between intestinal absorption, renal excretion, and bone mineral metabolism to meet the bird's requirements (Elaroussi et al., 1994). Although we did not quantify chitin in the present study, dietary NDF content increased with addition of BSFLM (Table 4.3). It is plausible that the high fiber may have increased ceca fermentation (Kiarie et al., 2014). Increased hindgut fermentation has been shown to increase mineral absorption (Metzler-Zebeli et al., 2010) and thus better egg shell quality in hens fed BSFLM. Indeed, total replacement of soybean meal with BSFLM in laying hens' diet from 24 to 45 weeks of age resulted in a higher cecal production of butyric acid (Cutrignelli et al., 2017). It is also possible other mechanisms related to feeding BSFLM may have influenced strong egg shell characteristics.

Table 4.1. **Chemical composition of defatted black soldier fly larva meal, as fed**

Item	Amount
DM, %	97.5
CP, %	56.1
Gross energy, kcal/kg	4,973
Fat, %	6.84
Starch, %	5.97
Ethanol soluble carbohydrates, %	4.55
Ca, %	1.21
P, %	0.95
K, %	1.58
Mg, %	0.39
Na, %	0.20
Amino acids, % (% of CP)	
Indispensable	
Arg	2.72 (4.8)
His	5.51 (10.1)
Ile	2.38 (4.2)
Leu	3.81 (6.8)
Lys	3.22 (5.7)
Met	0.9 (1.6)
Met + Cys	1.3 (2.3)
Phe	2.11 (3.8)
Thr	2.26 (4.0)
Val	3.38 (6.0)
Dispensable	
Ala	3.8 (6.8)
Asp	5.13 (9.1)
Cys	0.40 (0.7)
Glu	6.67 (11.9)
Gly	2.99 (5.3)
Pro	3.34 (6.0)
Ser	2.5 (4.5)
Tyr	2.76 (4.9)

Table 4.2. Composition of experimental diets, *as fed*

Item	Black fly soldier larvae meal, %		
	0.0	5.0	7.5
Corn	43.3	46.7	48.2
Soy bean meal	25.4	18.5	15.1
Wheat	15.0	15.0	15.0
Black fly soldier larvae meal	0.00	5.00	7.50
Soy oil	3.27	1.92	1.30
Limestone fine, <1 mm	7.29	7.21	7.17
Limestone course, 2-4 mm	2.19	2.16	2.15
Mono calcium phosphate	2.00	1.98	1.98
Vitamin-trace premix[1]	1.00	1.00	1.00
Salt	0.37	0.29	0.33
DL-Methionine	0.2	0.21	0.22
Sodium bicarbonate	0.04	0.05	0.05
L-Lysine	0.01	-	-
L-Threonine	-	0.01	0.02
Calculated provisions			
AME, kcal/kg	2,800	2,800	2,800
Crude protein, %	17.0	17.0	17.0
Crude fat, %	5.14	4.32	3.96
SID Lys, %	0.75	0.75	0.75
SID Met, %	0.43	0.46	0.48
SID Met + Cys	0.67	0.67	0.67
SID Try, %	0.20	0.17	0.16
SID Thr, %	0.52	0.52	0.53
Ca, %	4.10	4.10	4.10
Available P, %	0.44	0.44	0.44
Na, %	0.18	0.18	0.18
Cl, %	0.25	0.20	0.23

[1] Vitamin mineral premix provided per kilogram of premix: vitamin A, 880,000 IU; vitamin D3, 330,000 IU; vitamin E, 4,000 IU; vitamin B12, 1,200 mcg; biotin, 22,000 mcg; menadione, 330 mg; thiamine, 400 mg; riboflavin, 800 mg; pantothenic acid, 1500 mg; pyridoxine, 300 mg; niacin, 5,000 mg; folic acid, 100 mg; choline, 60,000 mg; iron, 6,000 mg; and copper, 1,000 mg

Table 4.3. Analyzed chemical composition of experimental diets, *as fed basis*

	Black fly soldier larvae meal, %		
Item	0	5	7.5
Dry matter, %	89.3	88.8	89.2
Gross energy, kcal/kg	3,559	3,533	3,482
Crude protein, %	18.5	17.3	18.1
Crude fat, %	4.32	4.13	3.44
Starch, %	37.9	37.8	37.7
Ethanol soluble carbohydrates, %	3.79	3.53	3.15
NDF, %	7.97	8.30	10.68
Ca, %	3.73	3.66	4.08
P, %	0.74	0.73	0.80
K, %	0.77	0.73	0.77
Mg, %	0.18	0.18	0.20
Na, %	0.16	0.16	0.17
Indispensable amino acids, %			
Arg	1.08	1.00	1.03
His	0.54	0.67	0.77
Ile	0.66	0.70	0.72
Leu	1.46	1.35	1.41
Lys	0.93	0.91	0.96
Met	0.47	0.49	0.44
Met + Cys	0.74	0.74	0.65
Phe	0.89	0.71	0.75
Thr	0.67	0.64	0.67
Val	0.76	0.85	0.88
Dispensable amino acids, %			
Ala	0.88	0.74	0.94
Asp	1.77	1.61	1.69
Cys	0.28	0.25	0.22
Glu	3.46	3.07	3.21
Gly	0.74	0.73	0.77
Pro	1.12	1.09	1.10
Ser	0.95	0.85	0.92
Tyr	0.67	0.56	0.67

Table 4.4. Effects of inclusion of black soldier fly larva meal (BSFLM) in corn-soybean meal diet fed to laying pullets (19-27 week of age) on egg production, average egg weight, egg mass and FCR

Item	Hen day egg production, %	Average egg weight, g/bird/day	Egg mass, g/day	Feed intake, g/bird/day	FCR
Main effect of BSFLM inclusion, %,					
0.0	89.4^{a}	50.5^{a}	45.8^{a}	92.2^{b}	2.256^{b}
5.0	84.8^{b}	50.1^{b}	43.0^{b}	92.0^{b}	2.385^{ab}
7.5	87.8^{a}	50.4^{ab}	44.8^{ab}	95.7^{a}	2.430^{a}
SEM	0.642	0.123	0.327	0.350	0.042
Main effect of age, week					
19	46.0^{d}	42.3^{g}	19.4^{g}	63.8^{f}	4.250^{a}
20	73.9^{c}	44.2^{f}	32.7^{f}	89.4^{d}	3.050^{b}
21	89.0^{b}	46.5^{e}	41.4^{e}	76.7^{e}	1.904^{c}
22	94.4^{a}	49.8^{d}	47.0^{d}	94.6^{c}	2.044^{c}
23	96.3^{a}	51.8^{c}	49.9^{c}	96.5^{c}	1.954^{c}
24	96.0^{a}	53.4^{b}	51.3^{b}	97.0^{c}	1.905^{c}
25	96.2^{a}	54.4^{ab}	52.3^{ab}	102.2^{b}	1.973^{c}
26	96.6^{a}	54.9^{a}	53.1^{a}	110.2^{a}	2.089^{c}
27	97.2^{a}	55.3^{a}	53.7^{a}	109.1^{a}	2.045^{c}
SEM	1.029	0.197	0.511	0.561	0.067
Probabilities					
BSFLM	<0.010	0.038	<0.010	<0.010	0.010
Week	<0.010	<0.010	<0.010	<0.010	<0.010
BSFLM*week	0.089	0.237	0.031	<0.010	0.797
Response to BSFLM inclusion					
Linear	0.005	0.263	0.002	<0.010	0.003
Quadratic	<0.010	0.021	<0.010	<0.010	0.794

Means assigned different letters within a factor of analysis (BSFLM, week) are significantly different, $P < 0.05$

Table 4.5. Effects of inclusion of black soldier fly larva meal (BSFLM) in corn-soybean meal diet fed to laying pullets (19-27 week of age) on body weight (kg).

Main effect of BSFLM inclusion, %	
0	1.496^{b}
5	1.548^{a}
7.5	1.536^{a}
SEM	0.007
Main effect of age, week	
19[1]	1.384^{c}
21	1.407^{c}
23	1.469^{b}
25	1.675^{a}
27	1.700^{a}
SEM	0.010
Probabilities	0.017
BSFLM	<0.010
Week	<0.010
BSFLM*week	0.011
Response to BSFLM inclusion	
Linear	<0.010
Quadratic	0.008

[1] Intial body weight

Means assigned different letters within a factor of analysis (BSFLM) are significantly different, $P < 0.05$

Table 4.6. Effects of inclusion of black soldier fly larva meal (BSFLM) in corn-soybean meal diet fed to laying pullets (19-27 week of age) on egg quality characteristics[1]

Item	Individual egg weight g	Haugh units mm	Yolk color	Shell breaking strength kgf	Shell thickness mm
Main effects of BSFLM inclusion, %					
0	53.7	64.6	4.32^{b}	4.70^{b}	0.404^{b}
5	52.3	67.0	4.68^{a}	5.23^{a}	0.427^{a}
7.5	53	68.1	4.83^{a}	4.95^{b}	0.431^{a}
SEM	0.4	2.02	0.091	0.083	0.006
Main effects of age, week					
22	50.2^{c}	58.9^{b}	4.22^{b}	5.10^{a}	0.406^{b}
24	53.2^{b}	72.7^{a}	4.87^{a}	5.02^{ab}	0.434^{a}
26	55.6^{a}	68.1^{a}	4.73^{a}	4.75^{b}	0.421^{ab}
SEM	0.4	2.02	0.091	0.082	0.006
Probabilities					
BSFLM	0.070	0.464	<0.010	<0.010	0.003
Week	<0.010	<0.010	<0.010	0.010	0.005
BSFLM*week	0.133	0.893	0.191	0.038	0.047
Response to BSFLM inclusion					
Linear	0.123	0.218	<0.010	0.007	<0.010
Quadratic	0.081	0.978	0.858	<0.010	0.515

Means assigned different letters within a factor of analysis (BSFLM, week) are significantly different, $P < 0.05$

CHAPTER 5

Impact of complete (100%) replacement of soybean meal with BSFLM in a corn-based diet on egg production, egg quality, visceral organ weight, plasma metabolites and apparent retention of nutrient in laying hens from 28 to 43 weeks of age

5.1 Abstract

Effects of-total replacement of SBM with BSFLM in a corn-based diet fed from 28 to 43 weeks of age was investigated. A control diet was formulated to meet specifications, 2 additional diets were made by inclusion of either 10 or 15% BSFLM (total replacement). The BSFLM sample used was the same as in Chapter 4 and the same birds were used as this was not an independent experiment but a continuation. Titanium dioxide (0.3%) was added in the diets for determination of apparent retention (**AR**) of nutrients. The birds had *ad libitum* access to feed and water. Egg production/cage was monitored daily. Feed intake and body weight were monitored in 4-week intervals. All eggs laid on the 6th day of weeks 31, 35, 39 and 43 were analyzed for HU, YC, SBF and ST. Grab excreta samples were collected on week 33 for three consecutive days for AR of nutrients. Two birds/cage were sacrificed at the end of week 43 for organ weight. There was no ($P > 0.05$) diet effect on HDEP, FI and HU. Egg weight decreased quadratically ($P < 0.003$), EM decreased linearly ($P<0.03$) and FCR, YC and SBS linearly increased ($P <0.05$) with increasing BSFLM levels. Inclusion of BSFLM quadratically increased ($P<0.005$) liver weight and had a linear and quadratic increase ($P<0.05$) in AME. Complete replacement of soybean meal with defatted BSFLM resulted in poor FCR linked to lower EW suggesting some amino acids may have been limiting. Improved YC suggested BSFLM had pigments that increased intensity of yolk color and better shell quality indicated potential role in Ca metabolism.

5.2 Introduction

Most research on the use of BSFLM in diets of layers have focused on partial replacement of soybean meal or protein feedstuffs. A study by Maurer et al. (2016) showed no effect when BSFLM fully replaced (100%) soybean cake on FI, laying performance and EW in white Leghorns (64-74wks old). Marono et al. (2017) reported that full replacement by BSFLM did not affect egg production but negatively influenced FI and final body weight in Lohmann Brown hens (24-45wks old). Laying hens have different nutrient requirements depending on strain and age amongst other factors (Leeson and Summers, 2005). Young pullets, particularly white leghorns, have high energy requirements at onset of lay to meet body growth requirements as well as egg production, yet they have much less capacity for consumption. However, brown hens eat more as compared to their white counterparts (Leeson and Summers, 2005). Based on available literature search, complete replacement of SBM with BSFLM in diets for white leghorns have not been reported. Therefore, this study was done to investigate effect of full replacement of soybean meal with BSFLM on Shaver White hens from 28-43 weeks of age.

5.3 Materials and methods

5.3.1 Birds, diets and experimental procedures

The experiment was a continuation of phase 1 (chapter 4); the birds were maintained on the same diets with exception of incremental BSFLM. The birds that received 5 and 7.5% BSFLM in phase 1 received 10 and 15% BSFLM, respectively in phase 2. Inclusion of 15% BSFLM replaced soy bean meal completely (Table 5.1). A standard corn-soy bean meal (0% BSFLM) diet was formulated to meet the nutrient requirements for 28 weeks of age hens in accord to Shaver White commercial management guidelines (ISA, 2016). The BSFLM was included at 10 and 15% to maintain iso-caloric and iso-nitrogenous specification (Table 5.1). The birds were allowed free access to feed and water to week 43 of age. Egg production on a cage basis were monitored daily. Feed intake and body weights were monitored in 4-week intervals. Egg weight and quality characteristics were assessed on individual eggs collected on the 6th day of weeks 31, 35, 39 and 43. Grab fresh excreta samples were collected on week 33 for three consecutive days. Briefly, excreta collection boards were placed under each cage every morning and sample collection done at the end of the day. Feathers and feed particles were removed, and samples put in sealable labelled polythene bags and frozen at -20˚C for later analyses. Blood samples were collected from the wing vein of two birds per replicate in heparin coated tubes and centrifuged at 1,500 x g at 4˚C for 15 minutes to separate plasma and stored at -20˚C for later analysis. At the end of the experiment two birds per cage were randomly selected, weighed and sacrificed by cervical dislocation. The liver, pancreas and empty gizzard, small intestine and ceca were weighed.

5.3.2 Sample analyses

5.3.2.1 Egg quality measurements

Egg quality measurements were done in the same way as described in Chapter ~~4.~~

5.3.2.2 Chemical analyses

Daily excreta samples were pooled for each cage and oven-dried at 60°C. Samples of diets and excreta were finely ground in a coffee grinder (CBG5 Smart Grind, Applica Consumer Products Inc., Shelton, CT) and thoroughly mixed for analysis. Diets and excreta samples were analyzed for DM, CP, gross energy, crude fat and neutral detergent fibre (NDF) in the same way as described in chapter 4. Diets were further analyzed for AA, starch, ethanol soluble carbohydrates, K, Mg and Na as described in chapter 4. Titanium content in the diet and excreta was measured on a UV spectrophotometer following the method of (Myers et al., 2004). The diets and excreta samples were wet acid digested with nitric and perchloric acid mixture (AOAC International, 2005; method 990.08) and concentrations of Ca and P in the supernatant read on an inductively coupled plasma mass spectrometer (Varian Inc, Palo Alto, CA) at the School of Environmental Sciences, University of Guelph. Plasma samples were submitted to Animal Health Laboratories, University of Guelph for plasma creatinine analyses.

5.4. Calculations and statistical analyses

The HDEP, AEW, EM and FCR were calculated as described in chapter 4 in 4-week periods (weeks 28 to 31, 32 to 35, 36 to 49 and 40 to 43). Organ weights (liver, pancreas, gizzard, small intestine and ceca) were standardized by individual bird BW. Apparent retention (**AR**) of dietary components was calculated as follows:

100- ((100 x (marker in diet x component in excreta) / marker in excreta x component in diet))

The marker for this case was Titanium dioxide. A component could be: DM, gross energy, NDF, Ca or P.

The data were analyzed using GLM procedures (SAS Inst. Inc., Cary, NC) and the cage was the experimental unit. Data on egg production, egg quality and body weight were subjected to a 2-way ANOVA according to the following model; $Y_{ijk}=\mu+ D_i + W_j +DWij + e_{ijk}$, where Y is one observation, μ is general mean, D is the diet effect (i =0, 10 or 15% BSFLM), W is the 4 week period effect (j=28 to 31, 32 to 35, 36 to 49 and 40 to 43), DW is the Diet x Week interaction and e is the error term. Data on organ weight, plasma creatinine and AR of components were subjected to a 1-way ANOVA according to the following model; $Y_{ij}=\mu+ D_i +$

e_{ij}, where Y is one observation, μ is general mean, D is the diet effect (i =0, 10 or 15% BSFLM) and e is the error term. Contrast coefficients from unequally spaced BSFLM were generated using the interactive matrix language procedure of SAS. An α level of $P \leq 0.05$ was used as the criterion for statistical significance.

5.5 Results and discussion

The analyzed chemical composition of the diets is shown in table 5.2. The analyzed gross energy concentration in 10% BSFLM diet was 2% (-76 kcal/kg) lower relative to the control, however, the value for 15% BSFLM was 3% (+96 kcal/kg) higher than control. This discrepancy in gross energy concentration could not be explained by trends in the concentration of energy yielding components (CP, starch, crude fat and NDF). Addition of BSFLM increased concentration of NDF indicating increased chitin as BSFLM was added in the diet. Specifically, relative to the control, the concentration of NDF was 30 and 40% more in 10 and 15% BSFLM diets, respectively (Table 5.2).

There was no (P>0.05) diet effect on HDEP (Table 5.3) suggesting that the noted differences in the analyzed concentration of gross energy in the diets had no impact on egg production (Leeson and Summers, 2005). However, EW decreased quadratically (P=0.003) with BSFLM inclusion and as a result there was a significant linear decrease in EM (P =0.03); the EM for the control, 10% BSFLM and 15% BSFLM was 55.9, 53.9 and 54.0 g/bird/day, respectively. Feeding BSFLM linearly increased (P=0.045) FCR (FI/EM) attributed to reduced EM as FI was not influenced by the diet (P=0.442). The observed FCR was 1.91, 1.98 and 2.02 for the control, 10% BSFLM and 15% BSFLM, respectively. The FCR was also shown to increase in Lohmann Brown Classic laying hens fed with diet containing BSFLM as a total replacement of SBM

(Marono et al., 2017) and with partial replacement of SBM in chapter 4. The supply of adequate protein (amino acids) is critical for egg size in laying hens (Leeson and Summers, 2005). It appears that BSFLM meal somewhat has a negative impact on amino acid utilization. Moderate and variable retention of protein and energy in broilers fed BSFLM has been attributed to the negative effects of chitin (De Marco et al., 2015; Schiavone et al., 2017). Broiler chickens fed chitin derived from crustacean shell waste (37.3% CP) digested approximately 50% of chitin protein (Hossain and Blair, 2007). Marono et al. (2015) demonstrated that *in vitro* CP digestibility was negatively correlated to the chitin content. Although chitin concentration was not measured in the current study, the increased concentration of NDF with addition of BSFLM suggested increased dietary concentration of chitin. There was a significant increase in HDEP (P=0.05), egg weight (P<0.001) and egg mass (P=0.028) with increase in age of the birds. There was a quadratic increase in body weights of birds fed BSFLM (P=0.042) (Table 5.3). This was attributed to increased feed intake in the first phase as the birds ate more to meet the energy requirements for maintenance at start of lay. Body weights increased significantly with age of birds (P<0.001). There was however no interaction effect between diet and age on the body weights (P=0.995) suggesting no impact of BSFLM inclusion.

Feeding BSFLM linearly (P<0.001) increased YC intensity (Table 5.4). A linear (P < 0.001) and quadratic (P=0.028) increase in SBF and ST was observed with increasing BSFLM levels. However, individual egg weight increased significantly with advancement of laying cycle (P<0.0001) but decreased with inclusion of BSFLM (P=0.01). This could be due to low analyzed values of Methionine + Cystine and Lysine in 10 and 15% BSFLM diets, (Table 5.3). Increase in egg weight with age can be explained by increased proportion of yolk at the expense of albumen as the bird ages (Whitehead et al., 1991). BSFLM quadratically (P=0.005) increased liver weight. Inclusion of BSFLM reduced empty ceca weight linearly (P=0.003) and quadratically (*P*=0.002). There was however no effect (*P*>0.05) on pancreas, small intestine and gizzard weights (Table 5.5). Creatinine is indicator of kidney function and high levels are an indication of kidney problems (Greenacre et al., 2008). Total replacement of soybean meal with BSFLM did not affect the levels of blood creatinine (*P*>0.05) (Table 5.5). This agreed with a study by Marono et. al (2017) where dietary inclusion of BSFLM did not affect plasma metabolites in Lohmann brown layers. A study on barbary partridges fed BSFLM showed that levels of creatinine were significantly reduced compared to those fed on soy bean meal (Loponte et al., 2017). Jing et al., (1997) found that chitosan, a derivative of chitin present in insects improved renal function of diabetic rats evidenced by reduced plasma creatinine levels.

There was no significant increase in apparent retention of DM *(P*=0.399), GE (*P*=0.275) and crude fat (*P*=0.946) on replacement of soybean meal with BSFLM (Table 5.6). However, the AR of NDF increased linearly (*P*< 0.0001) and quadratically (*P*=0.036) with inclusion of BSFLM (Table 5.6). BSF contains fiber in the form of chitin and birds possess the ability to break down chitin due to presence of endogenous chitinase in the proventricular mucosa (Jeuniaux 1963). Laying birds require Ca for egg shell formation, bone formation and normal metabolic functions (Leeson and Summers, 2005). Dietary sources are not usually enough to meet the bird's requirements and, as such, inorganic sources of Ca and P are added to diets (Akbari Moghaddam Kakhki et al., 2018b; Akbari Moghaddam Kakhki et al., 2018c). Feeding BSFLM had a quadratic *(P*=0.003) response on AR of Ca, with 10% BSFLM showing lower AR of Ca relative to control or higher 15% BSFLM (Table 5.6). Based on observations on shell quality (SBS and ST), reduced AR of Ca in birds fed 10% BSFLM was surprising and could point at inaccuracies in Ca analyses in the diet and/or excreta samples. Replacing soybean meal with BSFLM did not affect AR of P (*P*>0.05). The AME (Apparent Metabolizable Energy) increased linearly *(P*=0.001) and quadratically (*P*=0.007) with inclusion of BSFLM (Table 5.6). Since AR of GE was not affected by the diets, effects on AME were due to higher GE in 15% BSFLM diet.

Complete replacement of soybean meal with defatted BSFLM had no effect on hen day egg production but it resulted in poor FCR linked to lower egg weight suggesting some amino acids may have been limiting. Improved yolk color suggested BSFLM had pigments that increased intensity of yolk color and better shell quality indicated potential role in Ca metabolism.

Table 5.1. Composition of experimental diets, *as fed basis*

Ingredient Name	Control	BSFLM 10%	BSFLM 15%
Corn	45.3	50.6	51.1
Wheat	20.0	20.0	20.0
Soy bean meal 46%	18.8	5.24	0.00
Black fly soldier larvae meal	0.00	10.0	15.0
Soy oil	1.48	0.00	0.00
Limestone fine	7.14	6.99	6.91
Limestone coarse	2.86	2.79	2.76
Monocalcium phosphate	2.11	2.08	2.04
Vitamin and trace element premix[1]	1.00	1.00	1.00
Salt	0.30	0.31	0.32
DL-Methionine	0.25	0.27	0.28
L-Lysine HCL	0.20	0.18	0.13
L-Threonine	0.09	0.11	0.11
L-Tryptophan	0.00	0.03	0.05
Sodium bicarbonate	0.13	0.07	0.01
Titanium dioxide	0.30	0.30	0.30
Calculated provisions			
AME, kcal/kg	2,800	2,800	2,800
Crude protein, %	15.0	15.0	15.0
Crude fat, %	3.63	3.10	3.46
Linoleic acid, %	1.95	1.29	1.27
SID Lys, %	0.75	0.75	0.75
SID Meth, %	0.45	0.52	0.55
SID Met + Cys, %	0.67	0.67	0.67
SID Try, %	0.17	0.15	0.15
SID Thr, %	0.52	0.52	0.52
Ca, %	4.30	4.30	4.30
Available P, %	0.45	0.45	0.45
Na, %	0.18	0.18	0.18
Cl, %	0.25	0.25	0.25

[1] Vitamin mineral premix provided per kilogram of premix: vitamin A, 880,000 IU; vitamin D3, 330,000 IU; vitamin E, 4,000 IU; vitamin B12, 1,200 mcg; biotin, 22,000 mcg; menadione, 330 mg; thiamine, 400 mg; riboflavin, 800 mg; pantothenic acid, 1500 mg; pyridoxine, 300 mg; niacin, 5,000 mg; folic acid, 100 mg; choline, 60,000 mg; iron, 6,000 mg; and copper, 1,000 mg

Table 5.2. Analyzed chemical composition of experimental diets, *as fed basis*

	Black fly soldier larvae meal, %		
Item	0	10	15
Dry matter, %	89.24	89.82	90.52
Gross energy, kcal/kg	3,411	3,335	3,507
Crude protein, %	15.11	14.70	14.79
Crude fat, %	3.36	2.65	2.81
Starch, %	39.63	43.29	43.41
Ethanol soluble carbohydrates, %	3.64	1.79	1.43
NDF, %	6.90	9.00	9.68
Ca, %	4.36	4.22	4.56
P, %	0.72	0.74	0.74
K, %	0.67	0.57	0.52
Mg, %	0.18	0.18	0.19
Na, %	0.20	0.20	0.20
Indispensable amino acids, %			
Arg	0.94	0.78	0.61
His	0.45	0.70	0.84
Ile	0.59	0.59	0.50
Leu	1.23	1.24	1.14
Lys	0.92	0.84	0.64
Met	0.47	0.45	0.57
Met + Cys	0.72	0.64	0.76
Phe	0.72	0.71	0.57
Thr	0.63	0.62	0.56
Val	0.68	0.78	0.69
Dispensable amino acids, %			
Ala	0.75	0.88	0.87
Asp	1.47	1.29	1.01
Cys	0.25	0.20	0.19
Glu	3.08	2.69	2.34
Gly	0.62	0.66	0.60
Pro	1.07	1.05	1.02
Ser	0.83	0.75	0.66
Tyr	0.51	0.57	0.56

Table 5.3. Effects of replacing soybean meal with black soldier fly larva meal (BSFLM) in diets fed to shaver white layers (28-43 week of age) on egg production, FCR and body weights

BSFLM inclusion, %	Hen day egg production, %	Egg weight, g/bird/day	Egg mass, g/day	Feed intake, g/bird/day	FCR	Body weight, kg
0	96.7	57.8^{a}	55.9	106.9	1.913	1.548^{b}
10	94.6	56.9^{b}	53.9	106.0	1.976	1.586^{a}
15	95.5	56.5^{b}	54.0	109.0	2.022	1.556^{a}
SEM	0.960	0.313	0.693	1.667	0.038	0.011
Period, week						
28 to 31	96.6^{b}	54.9^{c}	53.1^{b}	94.6^{d}	1.791^{b}	1.506
32 to 35	96.8^{b}	56.9^{b}	55.1^{ab}	105.3^{c}	1.913^{b}	1.536
36 to 39	92.9^{b}	57.9^{ab}	53.8^{b}	110.8^{b}	2.064^{a}	1.591
40 to 43	96.2^{a}	58.6^{a}	56.3^{a}	118.6^{a}	2.114^{a}	1.621
SEM	1.109	0.362	0.800	1.925	0.044	0.013
Probabilities						
BSFLM	0.312	0.010	0.065	0.442	0.130	0.042
Week	0.050	<0.001	0.028	<0.001	<0.001	<0.001
BSFLM*week	0.906	0.933	0.963	0.453	0.746	0.995
Response to BSFLM inclusion						
Linear	0.270	0.884	0.030	0.498	0.045	0.686
Quadratic	0.291	0.003	0.377	0.280	0.849	0.049

Means assigned different letters within a factor of analysis (BSFLM, period) are significantly different, $P < 0.05$

Table 5.4. Effects of replacing soybean meal with black soldier fly larva meal (BSFLM) in diets fed to shaver white layers (28-43 week of age) on egg quality characteristics

BSFLM inclusion, %	Yolk color	Haugh units	Shell breaking strength, kgf	Shell thickness, mm	Individual egg weight, g
0	4.27^{c}	65.3	4.690	0.436	57.8^{a}
10	4.85^{b}	66.5	4.775	0.434	56.9^{b}
15	5.03^{a}	66.3	4.872	0.438	56.5^{b}
SEM	0.046	1.325	0.068	0.002	0.313
Period, week					
28 to 31	5.04^{a}	66.5	5.12^{a}	0.433^{c}	54.9^{b}
32 to 35	4.82^{b}	67.4	4.58^{c}	0.438^{ab}	56.9^{a}
36 to 39	4.29^{c}	64.7	4.54^{c}	0.432^{bc}	57.9^{a}
40 to 43	4.72^{b}	65.4	4.87^{b}	0.441^{a}	58.6^{a}
SEM	0.053	1.530	0.079	0.002	0.362
Probabilities					
BSFLM	<0.001	0.596	0.176	0.273	0.010
Week	<0.001	0.785	<0.001	0.273	<0.0001
BSFLM*week	0.561	0.328	0.782	0.684	0.933
Response to BSFLM inclusion					
Linear	<0.001	0.506	<0.001	0.863	0.863
Quadratic	0.993	0.266	0.129	0.028	0.028

Means assigned different letters within a factor of analysis (BSFLM, period) are significantly different, $P < 0.05$

Table 5.5. Effects of replacing soybean meal with black soldier fly larva meal (BSFLM) in diets fed to shaver layers (28-43 week of age) on organ weights and Plasma creatinine.

BSFLM inclusion, %	Organ weight, g/kg body weight[1]					Creatinine (μmol/L)
	Gizzard (empty)	Small intestines (empty)	Ceca (empty)	Liver	Pancreas	
0	8.81	13.82	3.13^{a}	23.96^{b}	2.27	16.67
10	8.55	13.53	2.55^{b}	27.85^{a}	1.99	19.80
15	8.82	13.17	2.47^{c}	29.77^{a}	1.96	14.67
SEM	0.37	0.58	0.09	1.09	0.11	2.49
Probabilities	0.853	0.739	0.0004	0.006	0.117	0.378
Response to BSFLM inclusion						
Linear	0.605	0.838	0.003	0.074	0.135	0.303
Quadratic	0.839	0.461	0.002	0.005	0.115	0.331

[1] Values are average of two birds sacrificed at the end of week 43 of age

Means assigned different letters within a column are significantly different, $P < 0.05$

Table 5.6. Effects of replacing soybean meal with black soldier fly larva meal (BSFLM) in diets fed to shaver white layers (28-43 week of age) on apparent retention (AR, %) of components and AME (kcal/kg)

BSFLM inclusion, %	ARDM[1]	ARGE[2]	ARFat[3]	ARNDF[4]	ARCa[5]	ARP[6]	AME as fed[7]	AMEDM[8]
0	77.76	84.73	86.64	40.792^{c}	36.11^{a}	29.19	2913.47^{b}	3215.53^{b}
10	78.92	85.35	86.20	64.85^{b}	28.57^{b}	25.43	2921.15^{b}	3208.31^{b}
15	79.11	86.10	86.57	68.89^{a}	41.72^{a}	29.44	3042.69^{a}	3328.99^{a}
SEM	0.74	0.58	0.99	1.86	2.52	5.11	20.27	22.21
Probabilities	0.399	0.275	0.946	<0.0001	0.008	0.826	0.0006	0.0024
Response to BSFLM inclusion								
Linear	0.191	0.124	0.909	<0.0001	0.375	0.935	0.001	0.003
Quadratic	0.784	0.686	0.757	0.036	0.003	0.547	0.007	0.033

[1] ARDM: Apparent Retention of Dry Matter

[2] ARGE: Apparent Retention of Gross Energy

[3] ARFat: Apparent Retention of Crude Fat

[4] ARNDF: Apparent Retention of Neutral Detergent Fibre

[5] ARCa: Apparent Retention of Ca

[6] ARP: Apparent Retention of Phosphorus

[7] AME: Apparent Metabolizable Energy as fed basis

[8] AMEDM: Apparent Metabolizable Energy, dry matter basis

Means assigned different letters within a column are significantly different, $P < 0.05$

CHAPTER 6

General discussion and conclusions

The anticipated rise in human population calls for an increase in animal protein production by over 60% in the next three decades (FAO, 2011). High animal protein demand will require more resources, feed being most challenging due to food-feed-fuel competition, limited land for cultivation and change in climate. Poultry products are good sources of animal protein, for instance, eggs are inexpensive high-quality protein sources (Miranda, 2015), but faces challenge of feed cost which accounts to over 65% cost of producing eggs and meat. Energy and amino acids account for over 90% of feed cost in poultry production (Kiarie et al., 2013). Soybean meal, corn and wheat are the most commonly used feed ingredients for poultry diets in most parts of the world but due to the volatile prices, competition with human food and biofuel industries, there is need for the global feed industry to investigate alternative feed ingredients that do not affect poultry production and product quality, are economically feasible and socially acceptable.

A lot of research has shown that insects can be excellent feed ingredients and sources of human food (Schader et al., 2015). Insects have been shown to have high concentrations of amino acids, fatty acids and micronutrients (Rumpold and Schluter, 2013; Makkar et al.,2014). Black soldier fly (*Hermetia illucens),* common house fly (*Musca domestica*), and yellow meal worm, (*Tenebrio molitor*) are the insects with high potential for large scale production. For the purpose of this research, black soldier fly was assessed. Its larva has high growth rates and efficiently converts organic waste to produce a meal (Black Soldier Fly Larvae Meal, BSFLM) which has a consistent AA concentration when larvae is raised on diverse substrates (Diener et al., 2009; Nguyen et al., 2015; Spranghers et al., 2017).

Black soldier fly product use in poultry feed has been approved by Canadian Food Inspection Agency. The use of BSFLM as feed ingredient has been reported for poultry (De Marco et al., 2015; Marono et al., 2017; Secci et al., 2018; Mwaniki and Kiarie, 2018), swine (Newton et al., 1977; 2005; Spranghers et al., 2018) and some fish species (St-Hilaire et al., 2007; Sánchez-Muros et al., 2014; Widjastuti et al., 2014; Tran et al., 2015). Most of the studies done on BSFLM incorporation in poultry diets has been in broilers and brown layers and few studies have been done on its use in white layer diets and the impact it has on egg production and quality are largely unknown. Proper nutrition for laying hens is crucial for optimal growth and egg production as well as reduction of adverse effects to the environment caused by excretion of excess nutrients (Leeson, 2005; Alagawany et al., 2016). This thesis investigated the effects of partial and total replacement of soybean meal with BSFLM in diets of Shaver white leghorns on egg production, egg quality, visceral organ weights, plasma metabolites and apparent component retention.

The research was done in two phases to assess the effect of incremental replacement of soy bean meal with defatted black soldier fly larvae meal. A total of 108, 19-week-old pullets (Shaver White Leghorns) were allocated to three diets (pellet form) containing 0, 5 and 7.5% BSFLM up-to week 27 (Chapter 4). The birds had *ad libitum* access to feed and water. Hen day egg production was monitored, and feed intake data was collected on a weekly basis. Egg quality parameters were measured on individual eggs collected on day 5 of weeks 22, 24 and 26. The quality parameters assessed were individual egg weight, albumen height (expressed as Haugh Units), yolk color, egg shell breaking strength and shell thickness. There was a quadratic reduction of HDEP, Egg weight and Egg mass on inclusion of BSFLM. It is however important to note that the 5% inclusion level had lesser values for these parameters in comparison to control diet and 7.5% inclusion levels. Feeding BSFLM significantly increased feed intake and FCR probably due to the high chitin content in the meal hence the birds ate more feed in order to meet their energy requirements. Feeding BSFLM increased body weights especially on week 23, 25 and 27. There was a significant increase in yolk color, shell breaking strength and shell thickness upon inclusion of BSFLM. The second study was done with the same birds (Chapter 5) from week 28 to week 43 with control birds in phase I continuing with control and BSFLM fed birds continuing with BSFLM by doubling the amount of BSFLM fed in study 1. A 15% inclusion of BSFLM completely replaced soybean meal and the effects on egg production, egg quality, nutrient retention, plasma metabolites and visceral organ weights were evaluated. The birds were fed three diets with 0, 10 and 15% (total replacement) inclusion levels of BSFLM and had *ad libitum* access to feed and water up-to week 43 of age. Egg production was monitored daily while feed intake and body weights were monitored in 4-week intervals. All eggs laid on day 6 of weeks 31, 35, 39 and 43 were subjected to quality analyses as in chapter 4. At the end of the study 2, blood samples were taken for plasma creatinine analysis and weights of liver, pancreas, gizzard, small intestine and ceca were recorded. No dietary effect on HDEP and feed intake was observed, but feed intake and FCR increased as the birds grew older. Body weight increased with BSFLM but surprisingly the diet with 10% BSFLM had the heaviest birds. Increase in bodyweights with age could be attributed to the fact that the older birds need energy for egg production only. There was however significant increase in HDEP, egg weight and mass with age of the birds. Dietary effect on egg weight and egg mass was observed. Individual egg weight was observed to increase with advancement of lay cycle. Black soldier fly larvae meal significantly increased yolk color, shell breaking strength and shell thickness. Liver weight increased with BSFLM while ceca weights reduced. No dietary effect was observed on pancreas, small intestine and gizzard weight as well as plasma creatinine concentration. The apparent retention of neutral detergent fiber was significantly increased by BSFLM as compared to control diet. Apparent retention of calcium was lower at 10% BSFLM inclusion level. There was no dietary effect on retention of dry matter, gross energy and fat. Complete replacement of soybean meal with BSFLM significantly increased Apparent metabolizable energy, which can be attributed to higher concentration of gross energy in the diet.

Results from the study showed that BSFLM improved egg quality characteristics such as yolk color, shell thickness and shell breaking strength. The study also demonstrated that BSFLM had no effect on hen day egg production in comparison to diet with soybean meal as main amino acids source, but reduced egg weight and egg mass were observed when birds were fed BSFLM. Increased liver weights could have resulted from increased feed intake and paralleled high body weight. Liver is important in intermediary metabolism as it absorbs and alters nutrients after absorption from the gut and releases them when needed. Liver weight changes in relation to amount of feed taken and to changes in metabolism (Battley, 2005). Older birds require energy for egg production only and hence excess energy is diverted to storage reserves in the liver. This was evidenced in this study by significant increment in liver weights with replacement of BSFLM. Diets high in fiber are known to increase length and weight of small intestines and ceca due to low digestibility, (Loponte, 2017). Reduced empty ceca and small intestinal weights for birds fed BSFLM in contrast to soy bean meal diet was unexpected. We speculated that the birds fed BSFLM were able to digest chitin in BSFLM due to increased production of chitinase in the proventriculus. Further studies however need to be done to ascertain this. Plasma creatinine levels were not affected by BSFLM suggesting that BSFLM had no adverse effects on kidney function.

Protein and amino acid requirements are greatest from onset of production up to peak egg mass production because this is the period when body weight, egg weight and egg production are all increasing. This explains why there was partial replacement of soybean meal with BSFLM from week 19-27 in Chapter 4 to ensure the pullets had enough nutrients for growth and egg production. Total replacement with BSFLM increased apparent retention of neutral detergent fiber. Calcium is very crucial in laying hens for egg shell formation and bone strength. Modern layers lay an egg daily meaning that they need 4-5grams of calcium per day in order to produce eggs with good quality shells (Bar, 2009). Eggs with strong shells ensures that the product meets the demands of egg traders, processing plants, hatcheries and consumers and prevent income loss to egg farmers. In the current study, improved shell thickness and shell breaking force was attributed to improved calcium metabolism. Total replacement with BSFLM increased apparent metabolizable energy significantly. Metabolizable energy requirements in laying hens is partitioned into energy for maintenance and egg production. Energy in diets come from carbohydrates, proteins and fat sources. Proper diet composition at onset of lay is important as the pullets need more energy for body weight gain as well as meet energy requirements for peak lay. Laying hens obtain this energy from proteins in feed as well as energy giving feedstuff. Demand for maintenance energy precedes that for egg production, hence small reductions in energy intake or low energy diets may significantly affect energy diverted to egg production and egg size. Low energy diets lead to increased feed intake to compensate for the insufficient amount of energy in the feed ingredient. High dietary energy levels reduce feed intake hence improving FCR without affecting performance parameters and egg quality traits (Nahashon,2007).

The egg industry in Canada operates under a national supply management system that was introduced in 1972 in order to provide fair returns to producers and reasonably priced supply to consumers (Heminthavong, 2018). Under the regulations, any egg that is sold from a location other than the farm gate must be graded in a federally registered egg grading station inspected by the Canadian Food Inspection Agency. The Canadian eggs are graded as: pee wee (<42 g), small (42–49 g), medium (49–56 g), large (56–63 g), extra-large (63–70 g), and jumbo (≥70 g). Under this system producers are paid the highest and similar price for any egg equal to or above 56 g (EFO, 2016). This has two implications, one is that there is no economic advantage of producing an egg of less than 56 g and arguably an egg larger than 56 g will be costly from feed cost and egg shell quality perspectives (Akbari Moghaddam Kakhki et al., 2018a). The genetics of modern white leghorns is such that these birds will achieve an egg of >56 g very early in lay cycle for example 40 weeks of age for LSL lite (Lohmann, 2016) and 27 weeks of age for Shaver White (ISA, 2011). Therefore, phase 2 of the current study coincided with a time period when Shaver white pullets start laying eggs in the large category. The present study indicated that with diets formulated to breed specifications, BSFLM can replace soybean meal in laying hen diets without any impact on egg production rate (HDEP). However, birds on BSFLM had lighter eggs as compared with those fed on soybean meal diet. Indeed, over 28-43 weeks period the egg weight for the control, 10% BSFLM and 15% BSFLM was 57.8, 56.9 and 56.5 g/bird per day. While these weights were within large category as per industry standards the eggs for BSFLM, they were trending towards the lower end of large category. This aspect combined with poor FCR indicates an economic risk of feeding hens BSFLM. A study carried out by Faria, (2002) found that decreasing dietary levels of the most limiting amino acid reduced egg weight. Because the diets in the current study were formulated to be equal in amino acids particularly Met + Cys to the control, it is possible chitin may have interfered with amino acids utilization as discussed in chapter 5. There is need to identify factors that could limit utilization of amino acids in BSFLM in laying hens.

Although commercial production of BSFLM is emerging in some countries (AgriProtein in South Africa, Enviroflight in Ohio, USA and Enterra, BC, Canada) significant insect production is carried out at a small scale in Asian and African countries. Thus, limited quantities of insect feed products are a big challenge (Loponte, 2017). In this context, the current insect products are expensive making it uneconomical to use as feed ingredients (e.g. BSFLM is $10-15/kg vs. soybean meal $0.50/kg, Kiarie personal communication). For insect meal to become a mainstream feedstuff for the animal feed industry, it will be necessary to optimize production conditions to reduce cost, guarantee a constant availability at a reasonable price and define environmental impact of their production (Sánchez-Muros et al., 2014). This would ultimately improve the competitiveness and the economic perspective of insect meals as sustainable feedstuffs. Moreover, investigations of other functional attributes of BSFLM, such as antimicrobial properties, need to be explored to add value of whole BSF prepupae compared to conventional protein sources. Perhaps the greatest challenge of application of insect meal in feed and food systems is rejection of animal products fed with insects because of consumers' prejudices (Rumpold and Schluter, 2013a; Verbeke et al., 2015). Moreover, research is also needed to grow microbially safe insects that will not transmit pathogens, diseases and toxins to humans and animals when used as source of feed and food. Chitin, which forms the main constituent of insect body, affects protein digestibility and estimation of chitin content needs to be done when formulating diets using insect meal to improve nutrient digestibility and uptake by the birds (Marono et al., 2015). Chitin has also been shown to have a positive influence on the gastrointestinal tract of chicken. Research on how to harvest chitin need to be done to reap the full benefit of black soldier fly larvae. In view of foregoing, the knowledge about insect products is still scarce, and much more information is needed regarding the optimal production, composition and digestive use, food safety criteria and product quality, before considering using insects as a viable alternative ingredient.

CHAPTER 7

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