Evaluation of cricket meal (*Gryllus sigillatus*) and black soldier fly larvae meal (*Hermetia illucens*) when incorporated in broiler chicken (*Gallus gallus domesticus*) diets

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
April 2021

DEDICATION PAGE

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ABSRACT

Protein is vital for animal growth and health, making it one of the key nutrients for formulating broiler chicken diets. Insect feeds, such as cricket meal (CM, *Gryllus sigillatus*) and black soldier fly larvae meal (BSFLM, *Hermetia illucens*) have potential as protein alternatives to current feed sources, like soybean meal. The nutritional composition of insects could be influenced by rearing conditions, processing, and type of insect. This study investigated the nutrient profile, digestibility, and metabolizable energy of oven-dried and freeze-dried CM, and BSFLM when fed to broiler chickens. The effects of varying dietary inclusion levels of CM, on the growth parameters, health, and meat quality of broiler chickens were examined. The results indicate that oven-dried CM is favourable in comparison to BSFLM, due to its nutritional composition and digestibility, and that CM can successfully be incorporated into broiler diets at an inclusion rate of up to 20% with no detrimental impacts.

LIST OF ABBREVIATIONS USED

1. **BSFLM (Black soldier fly larvae meal)**
2. **BSF (Black soldier fly)**
3. **CM (Cricket meal)**
4. **OD-CM (Oven-dried cricket Meal)**
5. **FD-CM (Freeze-dried Cricket meal)**
6. **NM (Non-medicated)**
7. **M (Medicated)**
8. **SBM (Soybean meal)**
9. **FM (Fish meal)**
10. **DM (Dry matter)**
11. **CP (Crude protein)**
12. **CF (Crude fat)**
13. **GE (Gross energy)**
14. **AME_N (N-corrected apparent metabolizable energy)**
15. **TME_N (True metabolizable energy corrected for nitrogen)**
16. **AIA (Acid-insoluble ash)**
17. **ADFI (Average daily feed intake)**
18. **ADG (Average daily gain)**
19. **FCR (Feed conversion ratio)**
20. **PER (Protein efficiency ratio)**
21. **ADCD (Apparent digestibility coefficient of the diet)**
22. **ADCI (Apparent digestibility coefficient of ingredients)**

ACKNOWLEDGEMENTS

The author would like to acknowledge Dalhousie University and the Verschuren Centre for Sustainability in Energy and the Environment for their support of the project. We are thankful for the funding provided by the Chicken Farmers of Nova Scotia, the Nova Scotia Business Inc Productivity and Innovation Voucher Program and the Mitacs Accelerate grant. We are also grateful to Midgard Insect Farm Inc. for the partnership, donation of the cricket product, and making this research possible. A personal thank you to all the staff and students at the Dalhousie University for their vital assistance with the project, which includes Robin Hilchie, Janessa Henry, Margie Hartling, Vicki Mackintosh, Cassie Stupart, Jamie Fraser, Sarah MacPherson, Krista Budgell, Shima Borzouie, Xujie Li, Mengdi Zhou, Michael McConkey, Janice MacIsaac, and Jing Lu. Thank you to my friends and family members for all the love and support.

CHAPTER 1: INTRODUCTION

The Canadian poultry industry is an essential contributor to Canada's Gross Domestic Product (GDP). Canada produced $2.5 billion worth of chicken products in 2017, with 2836 chicken producers located across Canada (Agriculture and Agri-Food Canada, 2018). The average Canadian consumer eats 33.1 kg of chicken meat each year (Agriculture and Agri-Food Canada, 2018). Live chicken is also exported to other countries, with the United States being the largest market for Canada, purchasing 65% of the chickens sold (Agriculture and Agri-Food Canada, 2018). Although chicken production is one of Canada's top agricultural sectors, the industry faces challenges, such as the changes in antibiotic usage and increasing demand for sustainable feed sources.

Producers desire a supply of sustainable feed sources in response to consumer and retailer demands for more discerning market segments, such as “antibiotic-free” and “grain-fed” labels, and yet broiler chickens (*Gallus gallus domesticus*) require a reliable source of quality protein and energy to sustain efficient growth and meat production. As the poultry industry grows, there is more reliance on plant-based protein sources, like soybean. With the projected increasing human population, there is competing demand for protein, which has increased the need for alternative protein sources for livestock (Leiber et al., 2017). Pressure on producers from retailer branding creates pressure on producers to not only find alternative proteins, but high-quality natural ingredients to maintain health. Of concern also is the increased risk of infectious and noninfectious disease if current production systems cannot adapt, within the limits of stringent biosecurity protocols (Chicken Farmers of Canada, 2014). This has fueled the need to find both protein and anti-microbial alternatives to mitigate the chance of disease and maintain production.

One promising solution for the increasing need for alternative feed sources is entomophagy, which is the use of insects as feed ingredients. Entomophagy is an emerging area of nutritional research with limited available information regarding the inclusions of insects in poultry diets (Biasato et al., 2017; Bovera et al., 2016; Cullere et al., 2016; De Marco et al., 2015; Khusro et al., 2012; Leiber et al., 2017). The nutritional profile of insects such as crickets, mealworms, black soldier fly (BSF), silkworm, and grasshopper vary widely in the literature, but appear to display adequate sources of amino acids for poultry nutrition and could offer other essential dietary components (Bovera et al., 2016; Cullere et al., 2016; Dale, 1994; Józefiak et al., 2016; Khusro et al., 2012). Insects have been a natural feed source for monogastric animals for centuries, and they have been a vital part of the diets of ancestral poultry (Sun et al., 2013). The nutritional profile of insects will vary, depending on the species and methods utilized to rear and process these insects into feed ingredients. However, the protein content of BSF, locusts, and grasshopper are nutritionally comparable to soybeans (Khusro et al., 2012; Liu et al., 2017).

Insects may offer other potential benefits to the poultry industry, as the chitin in their exoskeleton and the internal hemolymph of many species have both shown antimicrobial properties, which could aid in reducing reliance on in-feed antibiotics (Biasato et al., 2017; Cullere et al., 2016; Yi et al., 2014). Although these antimicrobial factors are beneficial attributes, the impact of including insects in broiler chicken diets on growth performance and their meat quality still needs to be determined (Hossain and Blair. 2007; Mareko et al., 2010; Sun et al., 2013; Ullah et al., 2017). Consumer acceptance is also an essential component when considering potential new feed sources and their impact on meat quality. How a feed ingredient will affect the colour, texture,

and cooking of the meat must be determined before chicken producers implement a novel ingredient (Northcutt, 1997). The colour of meat affects a consumer's purchasing habits, and the dietary components of the insect, such as protein and antioxidants, may influence how the chicken products are perceived, because they can influence the colour of the meat (Mercier et al., 1998; Northcutt, 1997).

The research presented in this paper expands on current knowledge in the entomophagy and poultry fields and will explore insects as a potential feed source for broiler chickens. Additionally, this paper will compare cricket meals prepared by two different processing methods to BSFLM, determine their effects on the chicken's growth and digestibility, and provide guidance on the optimal method used in the industry.

CHAPTER 2: LITERATURE REVIEW

Insect production is perceived to be environmentally friendly and could contribute to the reduction of poultry production's environmental impact (Oonincx and De Boer, 2012). Insects, such as crickets and BSF, have protein and amino acid profiles that could meet most of the dietary requirements of broiler chickens. However, insects can be grown using various methods and feed sources, directly influencing their nutrient composition. Processing insects into feed products can further influence the nutrient availability and how broiler chickens digest these nutrients. Certain components found in insects could also offer potential health benefits, such as chitin, a structural component of insects' exoskeleton embedded in a strong protein matrix that provides chemical and physical protection to the insect (Khempaka et al., 2016). Chitin also has an antimicrobial capacity that could alter the gut microbial population of broiler chickens (Khempaka et al., 2016). Other dietary components of insects, like the protein and fat content, could influence the meat quality parameters of broiler chickens, such as the colour and texture, which would thereby alter consumer perception and likely purchasing habits (Northcutt, 1997).

With potential advantages to the poultry industry, there is a keen interest in exploring insects as a potential natural protein source (Bovera et al., 2016; Cullere et al., 2016; De Marco et al., 2015). One of the main hurdles is the lack of knowledge regarding the use of insects in poultry diets, how processing changes their dietary efficiency, and how the nutritional composition and digestibility of insects will affect the internal morphology and meat quality of poultry.

2.1: Environmental impacts of insect production

Environmental concerns, such as land use, freshwater use, and energy consumption associated with feed production, coupled with challenges of weather pattern changes due to climate change, make cereal production unreliable and less sustainable as a feed source for livestock in the coming years. Due to climate change, specific geographical locations may experience an increase in drought, which may affect grain production and is a motivation to research alternatives to current plant-based protein sources (Leng and Hall, 2019). Insects like mealworms, crickets, and BSF could be an environmentally more sustainable alternative to grain production and have a lower resource requirement (Oonincx and De Boer, 2012).

Insect production requires minimal water and feed inputs, which are rarely wasted in the production system (Oonincx and De Boer, 2012). Many insects gain their water requirements from their feed source, and water is only used for cleaning (Oonincx and De Boer, 2012). Insects restrict water evaporation loss by their cuticular lipid layer, restricting water movement through the cuticle (Noble-Nesbiti, 1990). The direct water usage of an insect production facility can be minimal when compared to other systems, such as broiler chicken, which requires 50% more water for one kg of edible protein and beef, which requires five times the amount of water needed to produce one kg of meat (Oonincx and De Boer, 2012).

Inefficiencies in insect production include an elevated level of electricity usage in cold climates to accelerate indoor contained insect production. The electricity used to produce one kg of mealworms is 34 MJ, which is higher than what is required for egg and crop production (Oonincx and De Boer, 2012). This power usage includes transport of feed grains, production emissions, and cleaning, but the majority comes from heating the

facility (Oonincx and De Boer, 2012). Certain insects can be grown in vertical growing systems, which reduces land usage. Even without a vertical system, the space requirement for insect production is minimal, and requires approximately 3.6 m^2 per year for one kg of fresh mealworms (Oonincx and De Boer, 2012). Oonincx and De Boer (2012) found in terms of land used to produce one kg of edible protein, mealworm production requires 43% of the land required to produce milk and 10% of the land required for beef production. Halloran et al. (2017) found that crickets showed similar water usage compared to mealworms. However, Miglietta et al. (2015) suggested that all aspects of insect production need to be considered to understand their water consumption, including the origin of the cricket's feed.

Currently, cricket producers use commercial chicken feed as a growth medium, which negatively impacts their environmental footprint (Halloran et al., 2017). Halloran et al. (2017) suggest that if waste materials like culled vegetables were used as a growth medium, they would reduce the environmental impact of cricket production. With the potential use of organic waste streams as growth medium to rear insects, the reduced environmental impact of inputs makes them favourable compared to current plant-based protein sources for poultry production (Cullere et al., 2016; Myers et al., 2014; Nguyen et al., 2015). Although insect production is considered to have a low environmental impact, the growth medium used, production system, and processing of the insects into a poultry feed can all influence the nutritional composition of these insects, which is of vital consideration for poultry dietary requirements.

2.2: Production of insects

Insects have poikilothermic temperature regulation, and their internal temperature will vary with their environment. Therefore, it is suggested to grow insects in environmentally controlled incubators or rooms (Halloran et al., 2017). While growing insects under environmentally controlled conditions, temperature and humidity can be manipulated to attain optimal production. Crickets like *A. domesticus* require a temperature above 25 °C during growth, and anything under this temperature can be detrimental to growth (Halloran et al., 2017; McCluney and Date, 2008). Crickets have a relative humidity requirement of 60 - 75%, and fluctuations in humidity have been demonstrated to have a negative effect on the growth of bush cricket (*Isophya rizeensis*) (Çağlar et al., 2014). In comparison, BSF larvae have a temperature requirement of 30 °C and relative humidity requirement of 70% for optimal growth (Chia et al., 2018). Both temperature and humidity can influence the growth and nutritional components of crickets and BSF, and should be considered when producing insects for animal feed (Halloran et al., 2017; Hawkey et al., 2021; McCluney and Date, 2008).

Another factor that will influence the nutritional composition of insects is their life stage during metamorphosis. Crickets go through incomplete metamorphosis and have a lifecycle of three months, depending on conditions and feed inputs used (McCluney and Date, 2008). BSF goes through a complete metamorphosis, from egg to fly, in 14 days (Gaffigan, 2017). BSF has one of the most optimal growing systems, the fastest lifecycle, and is favoured by insect producers (Myers et al., 2014; Oonincx et al., 2010). These insects are harvested at the larvae stage and migrate themselves away from their feed supply during metamorphosis (Gaffigan, 2017). Liu et al. (2017) examined BSF nutritional composition over its life cycle and found that crude protein (CP) and crude fats

(CF) fluctuated as the insects aged, with protein varying from 57.6% during the adult stage to 38.0% in 12-day old larvae.

A factor contributing to the nutritional composition of insects is the growth medium that they are provided (Cullere et al., 2019; Halloran et al., 2017; Hawkey et al., 2021; Myers et al., 2014; Nguyen et al., 2015). Tschirner and Simon, (2015) demonstrated that BSF nutritional composition was related to the growth medium reared on and that dried distillers' grains with solubles produced BSF with high CP content and low ash content, as compared with BSF fed a mixture of grain middlings, indicating that body composition of the insects can be manipulated through their diet. Additionally, a standard method used to manipulate the nutritional composition of insects is gut loading, where insects are fed nutrient-dense feeds to increase their nutritional composition (Finke, 2002).

2.3: Processing of insect

Processing method can change the nutritional composition and availability of nutrients in feeds due to modifications in the solubilization of vitamins and minerals, protein denaturation, and Maillard reactions (Melgar-Lalanne et al., 2019; Wiseman et al., 1991; Van Rooijen et al., 2014). The effect that processing has on the nutritional composition of insects has not been thoroughly investigated, and the optimal processing method still needs to be determined (Melgar-Lalanne et al., 2019; Tschirner and Simon 2015). No standard method has been determined for processing insects for animal feed, but when producing a ground meal, there are critical steps like drying, grinding, oil extraction and storage (Van Rooijen et al., 2014).

Various drying methods, such as sun drying, low and high convection oven-drying, freeze-drying, and dehydration, have been examined. Each produced a different

nutritional composition of the insect products being made (Aniebo and Owen, 2010; Bovera et al., 2016; Tzompa-Sosa et al., 2014). Oven drying at 100 °C is a common method used to dry insects. When the insects are exposed to heat, reactions like the Maillard reaction and browning can cause carcinogenic acrylamide formation (Van Rooijen et al., 2014). In terms of animal nutrition, it is key to note that acrylamide is formed by a reducing sugar binding to an amino acid, like lysine (Van Rooijen et al., 2014) blocking the reactive site of the amino acid, making it unavailable for digestion, and lowering the nutritional value of the product (Wiseman et al., 1991; Van Rooijen et al., 2014). Aniebo and Owen (2010) demonstrated how the drying method could affect the nutrient composition of insect products by testing two different drying methods on housefly larvae (*Musca domestica*), which showed that oven-drying produced an insect meal with a lower fat content and a higher protein content when compared to sun drying. Fats may lose moisture through evaporation while drying, due to heat exposure over prolonged time frames. The unique structure of the insect's fat body could also influence the loss of fats during drying (Finke and Oonincx, 2014; Fu et al., 2015).

The high-fat content of insects can also influence the ease of achieving a predetermined particle size during grinding to meals. Insect products contain prominent oils and moisture levels, affecting the grinding of these products, and if these products become compressed it could increase the possibility of heat exchange (Ghosh et al., 2017; Schiavone et al., 2017[a]). To reduce the occurrence of heat exchange, fats can be removed from insects by non-chemical cold pressing or by solvent-extraction, which produces a defatted product that can be more easily ground (Melgar-Lalanne et al., 2019; Tschirner and Simon 2015). Leaving the fat in the product may increase the risk of spoilage during storage (Klunder et al., 2012).

Different processing, growth, and storage methods can also affect the microbial load associated with insects, such as crickets and mealworms (Klunder et al., 2012). Mould formation could lead to the development of mycotoxins, the secondary metabolites of fungus that reduce feed efficiency and have potential adverse health effects (Mngadi et al., 2008). Klunder et al. (2012) investigated the microbial load of crickets (*Acheta domesticus*) after different processing and storage methods. For safe storage, a 5-minute boiling stage was recommended prior to refrigeration with boiled crickets having stable bacteria levels when refrigerated at 4°C for two weeks. Water content negatively influenced spoilage in insect-based products, emphasizing the need for adequate drying and processing and avoid antinutrient formation (Klunder et al., 2012).

The drying, grinding, and storage of insects can have a potential impact on their nutrient composition, and should be evaluated when considering the use of insects in poultry diets. More research is required to understand the effect different processing methods will have on the value of insect products.

2.4: Protein and amino acid content

Protein and amino acid content are dependent on the type of insect and feed source provided to them, and there is a large amount of variation between studies examining the protein and amino acid content of crickets (Rumpold and Schlüter, 2015). With cricket meal (CM) containing ~60% protein, there is potential for this ingredient to be incorporated into the diet of broilers, which have a dietary CP requirement of 18.00 – 23.00% (Dale, 1994; Finke, 2015; Leiber et al., 2017; Nakagaki et al., 1987). Razak et al. (2012) found that the CP of house crickets (*Brachytrupes portentosus*) (60.4%) was similar to soybean meal (SBM) (44.0%) and fish meal (FM) (59.95%).

Dale (1994) advises that broilers have dietary amino acid requirements of 0.60 – 0.90% total sulphur amino acids, and 0.85 - 1.10% lysine. Field crickets (*Gryllus testaceus)* contain 1.928% methionine, 1.011% cysteine, and 4.787% lysine, which exceeds the dietary requirements of broilers (Wang et al., 2004). Józefiak et al. (2016) reported that *Gryllodes sigillatus* contains 1.2% methionine, and *Anabrus simplex*, another genus of crickets, contains 0.93% methionine and 3.48% lysine (Nakagaki et al., 1987). Field crickets have been shown to be markedly deficient in tryptophan and would not meet broiler chicken tryptophan requirements (0.17%), which would make them unsatisfactory for use in broiler diets without supplementation of this limiting amino acid (Wang et al. 2004).

Ghosh et al. (2017) reported that crickets (*Teleogryllus emma*) contain 55.65% protein and have promising levels of arginine (3.71%). Arginine is an essential amino acid for poultry, which is used for protein synthesis and can help with immunity and disease resistance. Chickens require 0.78 - 1.25% arginine in their diets, depending on age and breed, and a deficiency may cause impaired immune functions (Dale, 1994). The total essential amino acid composition of field crickets is comparable to FM, except for histidine, but was higher in lysine, methionine, and cysteine (Wang et al., 2005). Wang et al. (2005) also found that the CP content of field crickets (58.3%) was comparable to FM (60.2%) and SBM (46.8%).

Oonincx et al. (2015) found that crickets (*Acheta domesticus*) contain 52 - 74% CP and that manipulating the cricket's diet affects their CP content. The same study reported the protein content of four other species, reporting that BSF had the lowest CP content (38 - 46%) when compared to house crickets, yellow mealworms (45 - 69%), and Argentinean cockroaches (59%) (Oonincx et al., 2015). Cullere et al. (2016) found that

BSF contained 40 - 44% protein and stated it had a better amino acid profile for quail diets than SBM. The amino acid profile of BSF was favourable for poultry production with 0.62% methionine, 1.96% lysine, and 1.64% arginine (Cullere et al., 2016). In comparison, Finke, (2015) reported a CP of 60.0% for crickets (*Acheta domestica*) and found that they contained 0.27% methionine, 0.96% lysine, and 1.36% arginine.

The laboratory analysis method used for protein determination can affect the obtained results, as can the nitrogen: protein conversion factor used for insect protein determination, which is still under scrutiny (Janssen et al., 2017). The majority of studies evaluating the CP content of insects use a nitrogen-to-protein factor of 6.25, which was suggested by Finke (2007) and is based on the amino acid composition of mixed proteins (Janssen et al., 2017). Finke (2015) analyzed the amino acid content of crickets to confirm the use of the high nitrogen-to-protein factor. Janssen et al. (2017), however, suggested that the true conversion factor may be around 4.74, but only a limited variety of insects were evaluated during this study.

Poultry growth trials, studying different crickets species, showed that crickets contain high-quality proteins available for efficient conversion by poultry (Finke et al., 1984; Nakagaki et al., 1987; Oonincx et al., 2015; Wang et al., 2005). Interestingly, the study by Oonincx et al. (2015) investigating the variation in insect CP content with diet growth medium, indicates that the insect feed ingredient may to be manipulated to meet the CP and amino acid requirements of poultry. However, with such variation in literature values, it is difficult to estimate true insect protein with any degree of certainty, and more research is required to determine what protein conversion factor should be used.

2.5: Digestibility and effects on growth factors

Although some feeds may offer prominent levels of protein and amino acids, if broiler chickens are unable to digest the protein in the feed, it is not utilized by the birds. The process of digestion of feed converts ingested feed materials into its component nutrients to be absorbed along passage through the digestive tract. This process includes mechanical breakdown of feeds through mastication to reduce the particle size in the gizzard, enzymatic digestion and solubilization of organic materials, pH solubilization of inorganics, and emulsification of lipids (Lloyd et al., 1978). The ability of an animal to digest a feed depends on the quantity of digestive enzymes and fluids to first break down the cell wall of the plant, then cell components to convert them to their molecular form (Lloyd et al., 1978). The anatomical structure of a chicken's digestive tract also plays a crucial role in how this species absorbs its feed.

There are various methods to investigate the digestibility of a feed, one of which is the indicator method. This method requires using an inert substance as an indicator, which is usually diatomaceous earth or chromic acid. Indicators like diatomaceous earth are indigestible and unabsorbed by the animal, pass through the digestive tract uniformly, and the quantity is easily determined chemically (Lloyd et al., 1978). By placing this indicator in the feed at a measured level and measuring the amount excreted in the feces, the digestibility of a feed can be quantified using a ratio formula (Lloyd et al., 1978). There is limited research on the digestibility of insects, but the information currently available, although varied, is promising for the use of insects as a poultry feed (De Marco et al., 2015; Miech et al., 2017; Schiavone et al., 2017[a]; Wang et al., 2005; Wang et al., 2007).

The true amino acid digestibility coefficients of field crickets (*Gryllus testaceus*) range from 82% (cysteine) to 99% (asparagine), with an average true amino acid digestibility of 92.9%, and a true metabolizable energy corrected for nitrogen (TMEn) of 2960 kcal/kg. The researchers who conducted this study concluded that field crickets contained sufficient protein levels for poultry and considerable amounts of digestible amino acids (Wang et al., 2005). Miech et al. (2017) found that the nitrogen retention of CM (*Teleogryllus testaceus;* whole body CM and body CM (legs removed)) was comparable to FM in the diets of castrated male pigs (at a 18.4% CP). Pigs fed the CM gained more weight over the trial than the pigs fed the control diet, and there was no difference among the cricket treatments. The feed conversion was lower for both the cricket treatments when compared to the FM diet, and the study concluded that there was no need to remove the cricket's legs during processing (Miech et al., 2017).

Wang et al. (2007) found that the total tract true amino acid digestibility of methionine, cysteine, and lysine were 97, 84, and 95%, respectively, for cecectomized roosters fed Chinese grasshopper (*Acrida cinerea*). The roosters fed grasshopper meal had a TME_N of 11.34 MJ/kg compared to FM at 11.8 MJ/kg. The average coefficient of total tract true digestibility for the control diet (21.1% CP pretest diet) was 93%, and grasshopper was 94%, with no significant difference between the diets (Wang et al. 2007). The results indicated that when grasshopper meal diets were formulated on equal CP and true metabolizable energy basis, they could replace control diets of broilers, at inclusion levels up to and including 150 g/kg, without any adverse effects on the weight gain, feed intake, and gain to feed ratio (Wang et al., 2007). Wang et al. (2007) stated that grasshoppers were deficient in histidine and that lysine would be the limiting essential amino acid in poultry diets, such that grasshopper should not be the sole source of dietary

protein. Although grasshoppers and crickets are part of the same order of insects, *Orthoptera*, these insects could have varying nutritional compositions, and the amino acid composition of *G. sigillatus* needs to be determined. Amino acid supplementation is used in producing feeds, and it would therefore be possible to add a synthetic amino acid like lysine to chicken feed even if *G. sigillatus* was low in lysine.

When male broilers were fed mealworms (*Tenebrio molitor*) and BSF, both had N-corrected apparent metabolizable energy (AME_N) values of 16.02 and 16.60 MJ/kg with no significant difference ($P < 0.001$) (De Marco et al., 2015). The study analyzed 17 amino acids and found that the average apparent ileal digestibility coefficient was higher in mealworms (86%) when compared to BSF (68%) (due to higher apparent ileal digestibility coefficients of isoleucine, lysine, methionine, phenylalanine, valine, alanine, aspartic acid, glycine, glutamic acid, and tyrosine) (De Marco et al., 2015). The authors noted that the BSF had a low apparent ileal digestibility coefficient for methionine and isoleucine (42 and 45%), which they theorized could have been due to the BSF processing used to convert the insects into a meal, but they were unaware of the processing methods used (De Marco et al., 2015).

Schiavone et al. (2017[a]) included highly and partially defatted BSFLM in broiler chicken diets and found that both tested products can be suitable ingredients for broiler chicken diets. Cullere et al. (2016) found that replacing SBM in broiler quail diets with BSFLM up to 15% provided satisfactory productive performance results. Field cricket was also non-detrimental to broiler chicken growth when included up to 15% (Wang et al., 2005). Research suggests when insects are included in the diet at high concentrations, there can be adverse effects on the growth rates and feed efficiency of poultry, but the optimal dietary inclusion level of each insect is unknown (Wang et al., 2007). The lack of

research regarding crickets in broiler diets, and the unknown digestibility of this insect meal, is a gap of knowledge in this industry.

2.6: Internal morphology

Internal morphology of chicken organs and intestine can be used as indicators of overall health (Biasato et al., 2017; Bovera et al., 2016; Islam and Yang, 2017). In the past, in-feed antibiotics have been used to help prevent disease and promote growth, but Chicken Farmers of Canada has eliminated the preventative use of Category I and II antibiotics in poultry since 2018. This limits mechanisms to mitigate resistance to disease (Chicken Farmers of Canada, 2014). Of significant concern has been the increased intestinal health disorders and production losses associated with chickens raised without in-feed antibiotics, such as the presence of intestinal lesions, which can be an indicator of necrotic enteritis (Keyburn et al., 2006; Parent et al., 2020; Shojadoost et al., 2012). The nutritional components of insect meals may offer natural potential benefits to poultry health and could provide for an in-feed health promoter (Chernysh et al., 2015; Józefiak et al., 2016; Rahnamaeian et al., 2015; Yi et al., 2014).

In addition to being a protein and energy source, insects have antimicrobial peptides found in their hemolymph, stimulating the immune system, and having antimicrobial effects (Chernysh et al., 2015; Yi et al., 2014). Insects produce antimicrobial peptides with a broad spectrum of activity, and over 150 peptides have been identified (Yi et al., 2014). Cationic peptides found in insects are hypothesized to disrupt the bacterial cell envelope by binding with the negatively charged cell membrane of gram-negative bacteria and the lipoteichoic acids in the peptidoglycan layer of gram-positive bacteria (Chernysh et al., 2015; Józefiak et al., 2016; Yi et al., 2014). When these peptides bind onto the bacteria, the bacterial cell wall becomes disrupted/permeable,

which allows for pores to be formed and the free exchange of cellular ions, killing the bacteria (Chernysh et al., 2015; Rahnamaeian et al., 2015). There is great promise for antimicrobial peptides in insects, as it is thought that bacterial resistance will not form quickly to these compounds (Chernysh et al., 2015; Józefiak et al., 2016; Rahnamaeian et al., 2015).

Additional to the antimicrobial peptides found in insects, their exoskeleton is composed of chitin, a polymer of N-acetylglucosamine, which has further antimicrobial properties (Dutta et al., 2012). Chitin is a linear polysaccharide comprised of (1→ 4)- β-linked N-acetylglucosamine units and is considered a form of glucose. Chitin forms strong hydrogen bonds in its chain between the N – H and C = O of each attached chain, making it insoluble in most solvents (Hossain and Blair, 2007). When fed to chickens, Hossain and Blair (2007) found that chitin, extracted from crustacean shells, had an AME_N value of 8.86 MJ/kg, which is 30% lower than common feed grains. They suggested that another use for chitin is its hypolipidaemic and hypocholesterolaemic properties, which reduced the body fat and serum cholesterol of broilers (Hossain and Blair, 2007). There is also evidence to suggest that chitin may work as a prebiotic in broiler diets, allowing for cecal production of butyric volatile fatty acid (Cullere et al., 2016; Khempaka et al., 2011). Butyric volatile fatty acid in poultry diets can provide enterocytes with energy and increase intestinal blood flow, helping with nutrient transport and absorption (Cullere et al., 2016).

Since chitin in insects is not the only beneficial component, it could be suggested that the whole insect should be included, not just the extracted chitin, when incorporating insects into broiler diets. However, Bovera et al. (2016) found that when fed mealworms, the intestinal length and weight increased in broilers compared to a SBM control diet. The

results showed that including mealworms in the diet reduced the protein digestibility by 2% when compared to broilers fed the control diet, and the paper stated that when diets had a lower digestibility, the length and weight of the small intestine increased (Bovera et al., 2016; Smits and Annison, 1996). However, Biasato et al. (2017) demonstrated that mealworms in the diets of poultry did not affect the histological gut morphology and mucin composition. There was also no change in lymphoid system activation nor any increase in duodenal and jejunal morphometric indexes when compared to the control (Biasato et al., 2017). The differences between these studies could be due to the different processing methods used to produce the mealworm feed ingredient.

Insects in broiler chicken diets may have beneficial or negligible effects on the organ index and intestinal morphology. Visual inspection of the internal morphology can indicate overall health and growth and show how a feed affects flock internal health (Kokoszyński et al., 2017; Oviedo-Rondón, 2019; Raji et al., 2017). For example, an increase in bursa of Fabricius and spleen weight can be a sign of an increased immune activity (Oviedo-Rondón, 2019). Islam and Yang (2017) found that the chicken's internal organs remained unaffected, but the bursa of Fabricius reduced in weight in the birds fed super mealworms. Additionally, in both insect treatments, the cecal *E. Coli* and *Salmonella* contents were reduced, which shows excellent promise for insects to be used to reduce pathogenic loads in broiler chickens (Islam and Yang, 2017). Bovera et al. (2016) found that mealworms had an increase in percent spleen weight, which would indicate an immune reaction. However, Shadreck and Mukwanise (2014) found there were no unusual effects on the internal organs of broiler chickens fed *Macrotermes falciger* (termites) and *Encosternum delegorguei* (edible stink bug) at 3% and the organ weights were comparable to those in birds on the control diets. A weight increase in

certain organs can also be an indicator of good protein metabolism, and Ballitoc and Sun (2013) found that when yellow mealworm was included in broiler diets, it increased the heart, abdominal fat, and small intestine weights. The authors suggested that the increase in these organ weights indicated positive health and development effects from including mealworm in the diet (Ballitoc and Sun, 2013).

Variations in the type of insect, production and processing of these insects, level of chitin, and beneficial peptides could all have an impact on the results of these studies, and it is unknown what effects CM will have on the internal morphology of broiler chickens. Although there have been studies showing the effects of insect inclusion on poultry internal morphology, there is limited research on different types of insects as a feed source (Biasato et al., 2017; Cullere et al., 2016).

2.7: Effects on meat quality

The Canadian poultry meat industry is a profitable sector of Canadian economics, and in 2017, Canada produced 1.2 billion kg of eviscerated chicken meat (Agriculture and Agri-Food Canada, 2018). Northcutt (1997) defines the quality of chicken meat by how consumers experience a poultry product through the way it looks, cooks, tastes, and feels in their mouth, and if a product does not meet these expectations, it is considered lower quality. There may be effects of insects on these aspects of meat quality, as this is not clearly explored in the literature.

The colour of meat, both raw and cooked, influences consumer perception when purchasing chicken meat. Chicken meat is unique since it comes both skinless and with skin-on and varies in raw colouration, depending on muscle location (Northcutt, 1997). Multiple factors can influence the colour of chicken meat, such as diet, age, sex, muscle fat levels, pre-slaughter conditions, and processing. The main factor is the myoglobin and

hemoglobin in the muscle, and the pigments found in the blood reflect light off the meat (Northcutt, 1997). Bruising and breaking blood cells can change the colour of the meat and affect saleability if the meat is discoloured and it must be downgraded. Dietary components with antioxidative properties have a beneficial influence on the colour of chicken meat, due to the reduction of rancidity in meat lipids (Mercier et al., 1998).

Another critical factor is the texture of meat products. Texture and tenderness of the meat are defined by shear force, which is considered a measure of the amount of energy required to cut/tear meat. This simulates the action of chewing meat (Bailey, 1972). Texture is influenced by the connective tissue and myofibrillar components and the amount and rate of chemical and physical changes in the muscle as it goes through rigour mortis and begins to soften again (Bailey, 1972). When meat is cooked, the heat will cause chemical changes in the connective tissue and denature the myofibrillar proteins, while coagulation will cause tightening of the myofilaments. This rigour and the softening process can be influenced by slaughtering procedures, stress during production, diet, and muscle formation (Bailey, 1972).

Like texture, the cooking loss of meat is defined by the shrinkage that occurs during cooking. The loss that occurs is from drippings and volatile losses from water evaporation but can also be from the decomposition of fat and volatile aromatic substances (Aaslyng et al., 2003). Drippings are composed of fat, water, nitrogenous and non-nitrogenous extractives. The cooking process of meat causes a loss in humidity, due to steric effects in the muscle, protein denaturing and changes in folding, causing loss of water retention (Aaslyng et al., 2003). Muscle pH is related to cooking loss, and the formation of lactic acid during rigor mortis drops the pH over time (Aaslyng et al., 2003). When muscle proteins are close to their isoelectric point (5.1 pH_I), the negative and

positive charges become equal, and the proteins do not attract water, which increases cooking loss (Aaslyng et al., 2003).

Literature reports on carcass trait responses to insect diets have shown variable results. Insect type and processing method can influence the results found. Schiavone et al. (2019) found that replacing soybean oil with fat from BSF larvae in broiler chicken diets did not lead to significant differences in carcass traits. However, chicken breast fatty acid profile was affected by the inclusion of insect oil, which increased the short-chain fatty acid content of the meat. This was expected, due to the high saturated fatty acid content of the BSF larvae oil, in which 75% of the fatty acid methyl esters are comprised of lauric 52.6%, myristic 8.54%, and palmitic 10.9% fatty acids. The study concluded that the inclusion of insect oil worsened the fatty acid profile of the meat by increasing the monounsaturated fatty acid contents and reducing the of polyunsaturated fatty acids, but this change did not affect the colour of the breast meat. This is a less desirable meat product from the perspective of healthy profiles of meat fatty acid content and could influence consumer purchasing habits (Olmedilla-Alonso et al., 2013; Schiavone et al., 2019).

Bovera et al. (2016) found that some breast meat characteristics were affected by the inclusion of insect meal, leading to muscle pH changes, resulting in significantly greater cooking loss in broilers feed mealworms. Chickens fed a SBM control diet had a muscle pH of 5.95 and a cooking loss of 21.4% compared with chickens fed a mealworm diet (6.12 pH and 23.6%, respectively) (Bovera et al., 2016). However, these values are within the normal range for broiler chicken breast meat and did not classify the meat as being in the pale, soft, and exudative (PSE) or dark, firm, and dry (DFD) ranges (Bovera et al., 2016; Fletcher et al., 2000).

When broiler chickens were placed on pasture during peak grasshopper season, Sun et al. (2013) found that the live weight and amount of abdominal fat was lower than those fed a control indoor diet, but the dressing percent was increased. The breast weights and muscle pH of the pasture birds were also lower, and the cooking loss was reduced (Sun et al., 2013). The breast meat also exhibited increased shear force and, therefore, a reduction in tenderness, which was suggested to be due to the high dietary protein content of grasshopper, and increased bird activity (Sun et al., 2013). There was a significant difference in a* (green - red) colour of the breast meat, but no difference in the L* (lightness) and b* (blue - yellow) values. These observations may result from the interaction of effects from access to an outdoor pasture system, and consumption of grasshopper (Sun et al., 2013). There is limited information on the effect crickets will have on the meat quality of broiler chickens.

2.8: Conclusion

Insects may provide beneficial impacts on the health and growth of broiler chickens at optimal dietary inclusion levels. By understanding how insects influence meat quality, growth parameters, and the physiology of broiler chickens, we will provide evidence on the net benefits of insects as alternative feed ingredients. By assessing the processing methods used to produce insect meal, producers of insect meals will be better informed as to the optimal product specification resulting from meal preparation, providing potential nutritional and environmental advantages over current feed sources. More research is needed to fill the knowledge gaps regarding CM in broiler diets, and this study aims to fill these knowledge gaps. With additional information, the poultry industry can prepare for this emerging feed source and better understand its potential for poultry production.

CHAPTER 3: OBJECTIVES AND HYPOTHESIS

3.1: Objectives

This study will examine how CM and BSF are digested by broiler chickens, affect broiler chicken growth, and if they impact chicken health and meat quality. The effects of processing method on the nutritional profile and nutrient digestibility of insect meal and the information gathered will help shape future processing methods implemented by insect producers and utilization of this product by poultry producers. The objectives of this project are to:

1) Analyze the nutritional composition of freeze-dried cricket meal and oven-dried cricket meal (FD-CM and OD-CM, *Gryllus sigillatus*) and black soldier fly larvae meal (BSFLM, *Hermetia illucens*) (Chapter 4)
2) Determine the nutrient digestibility of FD-CM, OD-CM, and BSFLM in broiler chickens (Chapter 4)
3) Determine the impact of CM on broiler chicken growth (Chapter 5)
4) Assess the impact of dietary inclusion of CM on visceral organ size and digestive tract morphology (Chapter 5)
5) Investigate the effects of inclusion of CM in the diet on the meat quality of broiler chickens (Chapter 5)

3.2: Hypotheses

1) FD-CM, OD-CM, and BSFLM will provide an efficient nutritional profile for use in poultry feed when compared to current feed sources, such as corn-soy diets (Chapter 4)

2) The processing method will affect the nutritional composition of the CM, and OD-CM will have a lower nutritional value when compared to FD-CM, due to the impact of heat on the nutritional composition (Chapter 4)
3) CM will have no negative effects on the growth parameters measured in chickens when included in a balanced diet (Chapter 5)
4) CM will have no negative effects on the organ weights and intestinal tract of broiler chickens (Chapter 5)
5) CM will have no negative effects on the meat quality of broiler chickens, as measured by shear force and percent cook loss (Chapter 5)

CHAPTER 4: DIGESTIBILITY OF FREEZE-DRIED AND OVEN-DRIED CRICKET MEAL (*GRYLLUS SIGILLATUS*) AND BLACK SOLDIER FLY LARVAE MEAL (*HERMETIA ILLUCENS*) IN BROILER CHICKENS

4.1: Abstract

Protein is an expensive feed component and is vital for animal growth when formulating broiler chicken diets. Soybean meal (SBM) is one of the most used protein sources, but cost and environmental pressures have led to numerous studies evaluating alternatives to this feed ingredient. Knowledge of the nutrient quality and digestibility of potential protein sources like crickets (*Gryllus sigillatus*) and black soldier fly (BSF, *Hermetia illucens*) is required in order to incorporate them into poultry diets. This study investigated the digestibility and metabolizable energy content of oven-dried cricket meal (OD-CM), freeze-dried cricket meal (FD-CM), and black soldier fly larvae meal (BSFLM) when fed to broiler chickens. One of five dietary treatments were randomly assigned to 320, day old, Ross 308 broilers: Basal, BSFLM, OD-CM, and FD-CM. From 15 to 21 days of age, the average daily feed intake, average daily gain, and feed conversion ratio were measured. Excreta was collected on days 19, 20, and 21, analyzed for acid-insoluble ash (AIA) and gross energy, and nitrogen-corrected apparent metabolizable energy (AME_N) was calculated. Both cricket treatments were high for crude protein (CP) (OD-CM 66.9% and FD-CM 61.1%). FD-CM had the highest level of fat (21.2%) compared to OD-CM (16.4%). The apparent digestibility coefficient of ingredients (ADCI) for CP of OD-CM was 60.3%, significantly higher than BSFLM (48.0%). FD-CM had the highest ADCI for GE (86.2%). The AME_N of OD-CM and FD-CM was 5207.8 and 5004.4 kcal/kg, higher than BSFLM (3727.1 kcal/kg). The OD-CM available CP (40.4%) was significantly higher than the BSFLM and FD-CM (30.9 and

33.3%). The OD-CM and FD-CM were comparable in digestibility and nutrient profile, and both had higher digestibility compared to BSFLM in broiler chickens.

4.2: Introduction

Chicken production is one of Canada's top agricultural sectors, but the industry faces challenges, like the sustainable production of feed and production of birds without the use of antibiotics (Agriculture and Agri-Food Canada, 2018; Finke and Oonincx, 2014; Oonincx and De Boer, 2012; Oonincx et al., 2010). Producers are interested in sustainable feed sources, and rapidly growing broiler chickens (*Gallus gallus domesticus*) need a reliable source of protein and energy to sustain growth (Oonincx et al., 2010; Oonincx et al., 2015; Wang et al., 2005; Van Huis, 2013).

A solution for the increasing need for alternative feed sources is entomophagy, which uses insects as feed ingredients. Entomophagy is an emerging area of nutritional research with limited available information regarding the inclusion of insects in poultry diets (Biasato et al., 2017; Bovera et al., 2016; Cullere et al., 2016; De Marco et al., 2015; Khusro et al., 2012; Leiber et al., 2017). The protein content of insects BSF, silkworm, and grasshopper) suggests that they may be adequate sources of protein for poultry nutrition and could offer other essential dietary components, such as fats and micronutrients (Bovera et al., 2016; Cullere et al., 2016; Dale, 1994; Józefiak et al., 2016; Khusro et al., 2012).

The nutritional profile of insects varies, depending on the species, the life cycle they are in, and methods used to produce and process these insects into feed ingredients. There are reports indicating that insects can be nutritionally comparable to current protein sources like soybean meal (SBM) (Leiber et al., 2017; Liu et al., 2017; Nakagaki et al., 1987; Rumpold and Schlüter, 2015; Wang et al., 2004; Van Huis, 2013). Insects like

crickets and BSF could offer an eco-conscious alternative to current feed sources like SBM, with high CP providing a favourable nutritional content (Finke, 2015; Leiber et al., 2017; Oonincx et al., 2010; Van Huis, 2013). Crickets (*Gryllus testaceus*) contain 6.3% - 66.6% CP (Table 1), which on average, is higher than that of SBM at 46.8% (Wang et al., 2004). Ghosh et al. (2017) reported that crickets (*Teleogryllus emma*) contain 55.65% protein, and other studies showed that crickets contain high-quality proteins that are efficient for poultry growth (Nakagaki et al., 1987; Wang et al., 2005). Oonincx et al. (2015) found that crickets (*Acheta domesticus*) contain 52 - 74% CP, and manipulating the cricket's diet, using high and low protein food by-products, affects their CP content. Oonincx et al. (2015) also reported that BSF had a lower CP content (43.8%) than house crickets (57.8%). The protein content of BSF (defatted meal) was reported as 54.8% by Cullere et al. (2016). Cutrignelli et al. (2018) reported that BSF larvae contained 62.7% CP, which was higher than previously reported by Cullere et al. (2016). The varying nutritional contents of BSF and crickets are listed in Table 1. How the poultry digest these protein sources needs to be examined.

Table 1: Literature values for the nutritional composition of black soldier fly and cricket species (on a DM basis)

Reference	Species	Process	DM	CP	CF	Ash	Ca	K	Mg	P	Na	Cu	Mn	Zn	Fe	kcal/kg
			%				mg/100g									
Diptera (Black Soldier Fly)																
Nyakeri et al., (2017)	*Hermetia illucens*	d	-----	39.0	32.6	14.6	100.0	2270.0	-----	-----	3070.0	0.6	560.0	-----	570.0	-----
Oonincx et al., (2015)	*Hermetia illucens*	d	90.0	19.1	-----	-----	-----	-----	-----	670.0	-----	-----	-----	-----	-----	-----
Kamau et al., (2018)	*Hermetia illucens*	d	-----	44.0	25.0	9.5	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Cullere et al., (2016)	*Hermetia illucens*	p	94.6	51.8	15.6	7.7	-----	-----	-----	-----	-----	-----	-----	-----	-----	5510.3
De Marco et al., (2015)	*Hermetia illucens*	p	95.7	38.6	35.8	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	5943.9
Cutrignelli et al., (2018)	*Hermetia illucens*	p	97.8	62.7	4.7	8.0	7055.2	-----	-----	-----	122.7	-----	-----	-----	-----	-----
Marono et al., (2015)	*Hermetia illucens*	p	95.9	50.5	28.4	4.6	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Marono et al., (2015)	*Hermetia illucens*	p	95.9	49.9	29.0	4.7	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Marono et al., (2015)	*Hermetia illucens*	p	98.8	58.8	12.9	6.4	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Marono et al., (2015)	*Hermetia illucens*	p	98.9	58.4	11.6	6.5	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Marono et al., (2015)	*Hermetia illucens*	p	95.1	52.0	11.3	9.9	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Marono et al., (2015)	*Hermetia illucens*	p	94.8	51.8	11.3	10.0	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Nguyen et al., (2015)	*Hermetia illucens*	r	28.2	12.9	2.2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	1050.0
Nguyen et al., (2015)	*Hermetia illucens*	r	33.5	14.7	4.0	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	1300.0
Nguyen et al., (2015)	*Hermetia illucens*	r	44.7	21.0	8.4	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	2140.0
Nguyen et al., (2015)	*Hermetia illucens*	r	46.6	19.4	11.6	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	2330.0
Nguyen et al., (2015)	*Hermetia illucens*	r	-----	21.2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Orthoptera (Cricket)																
Banjo et al., (2006)	*Brachytypes sp*	d	96.6	6.3	-----	1.8	9.2	-----	0.1	126.9	-----	-----	-----	-----	0.7	-----
Adeyeye and Awokunmi, (2010)	*Brachytypes membranaceus*	d	95.0	32.4	3.2	6.6	12.4	112.2	21.3	1093.6	222.6	-----	2.1	103.2	3.1	3586.3
Adeyeye and Awokunmi, (2010)	*Brachytypes membranaceus*	d	98.8	25.8	5.3	4.9	8.6	74.6	21.5	1088.0	103.7	-----	1.5	51.5	10.0	3743.5

Razak et al., (2012)	*Acheta domestica*	d	89.6	60.0	22.7	5.4	1400.0	-----	-----	1000.0	-----	-----	-----	-----	-----	3114.2
Nakagaki et al., (1987)	*Acheta domestica*	d	94.8	62.0	7.5	4.6	190.0	1280.0	110.0	990.0	921.0	2.4	6.4	25.4	15.5	-----
Oonincx et al., (2015)	*Acheta domestica*	d	88.9	17.2	-----	-----	-----	-----	-----	660.0	-----	-----	-----	-----	-----	-----
Kamau et al., (2018)	*Acheta domestica*	d	-----	65.9	12.3	4.8	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Finke, (2002)	*Acheta domestica*	r	30.8	66.6	22.1	3.6	132.1	1126.6	109.4	957.8	435.1	1.9	3.9	21.8	6.2	-----
Finke, (2007)	*Acheta domestica*	r	31.8	22.5	5.9	1.6	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Finke, (2015)	*Acheta domestica*	p	27.5	60.0	28.7	44.4	133.1	1036.4	70.2	796.4	403.6	2.2	3.3	19.6	6.5	-----
Barker, (1997)	*Acheta domestica*	r	26.8	17.3	6.1	1.4	56.3	-----	21.4	209.0	-----	0.2	0.8	5.0	3.0	-----
Hunt et al., (2001)	*Acheta domestica*	r	42.4	-----	-----	-----	46.6	-----	-----	407.0	-----	-----	-----	-----	-----	-----
Punzo, (2003)	*Acheta domestica*	r	28.4	15.8	5.9	1.2	363.5	-----	45.4	232.9	-----	0.2	1.3	4.2	5.5	-----
Yang et al., (2006)	*Acheta confirmata*	r	-----	-----	10.2	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Pennino et al., (1991)	*Gryllidae*	r	27.0	17.4	5.4	1.1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Yhoung-Aree et al., (1997)	*Gryllus bimarculatus*	p	28.6	12.9	5.5	2.1	75.8	305.5	-----	185.3	86.7	-----	-----	-----	9.5	1199.0
Ghosh et al., (2017)	*Gryllus bimarculatus*	d	-----	58.3	11.9	9.7	240.2	1079.9	143.7	1169.6	453.0	4.6	10.4	22.4	9.7	-----
Yhoung-Aree et al., (1997)	*Gryllotalpa africana*	p	28.8	15.4	6.3	2.7	75.7	267.8	-----	254.1	97.0	-----	-----	-----	41.7	1247.0
Gope and Prasad, (1983)	*Gryllotalpa africana*	r	42.3	18.7	49.9	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Yang et al., (2006)	*Gryllotalpa africana*	r	-----	-----	13.4	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Punzo, (2003)	*Gryllus assimilis*	r	31.1	15.9	5.9	1.6	404.3	-----	52.9	230.1	-----	0.3	1.4	4.8	5.6	-----
Bednářová et al., (2009)	*Gryllus assimilis*	r	34.2	-----	11.8	1.5	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Studier and Sevick, (1992)	*Gryllus pennsylvanicus*	r	26.4	25.3	-----	-----	87.4	395.7	38.4	-----	58.3	-----	-----	-----	4.5	-----
Wang et al., (2004)	*Gryllus testaceus*	d	95.0	58.3	10.3	3.0	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Ajai et al., (2013)	*Gymnogryllus lucens*	d	-----	-----	-----	-----	-----	28.8	153.9	-----	15.6	69.1	-----	25.7	51.9	-----
Ghosh et al., (2017)	*Teleogryllus emma*	d	-----	55.7	25.2	8.2	193.5	895.5	152.5	1085.4	278.2	2.2	5.9	18.5	10.8	-----
Nurhasan et al., (2010)	*Teleogryllus testaceus*	r	35.2	25.1	4.6	1.6	34.0	-----	-----	-----	-----	-----	-----	8.8	21.	

Christensen et al., (2006)	*Onjiri mammon*	d	5.5	-----	-----	7.8	341.0	-----	-----	-----	-----	-----	-----	25.1	1562.0	-----

R= raw, d= dried, p= processed.

The exoskeleton of insects contains chitin, and has shown antimicrobial properties, reducing reliance on in-feed antibiotics (Biasato et al., 2017; Cullere et al., 2016; Islam and Yang, 2017; Karavolias et al., 2018). In addition to chitin, insects have antimicrobial peptides in their hemolymph, which stimulate the immune system and have antimicrobial effects (Chernysh et al., 2015; Yi et al., 2014). There is a keen interest in exploring this alternative protein source with multiple potential benefits to the poultry industry.

It is essential to know the available nutrient composition of an ingredient when formulating a diet instead of relying on the nutrient profile. While the nutrient content may be high, utilization and absorption of these nutrients by the animal may be considerably lower if digestibility is low (Leeson and Summers, 2002; Lloyd et al., 1978). For example, the intestinal tract cannot efficiently break down components of certain feed types, such as the cell wall (Leeson and Summers, 2002; Lloyd et al., 1978). The membrane composition of insect cells is closer to mammalian cells than other eukaryotic cells (Koth and Payandeh, 2009). Therefore, the breakdown and digestibility of insect cells are expected to be more similar to that of animal-based feed sources rather than plant-based sources when considering their digestibility.

Studies have investigated *in vitro* digestion to simulate the digestion of these potential feeds in monogastric animals. For example, Marono et al. (2015) completed an *in vitro* assay of BSF and found that BSF had a CP digestibility (49.9%). There was also a negative correlation between CP digestibility and the chitin content (Marono et al., 2015). The study stressed the importance of reducing the chitin content of insect meals when considering them as an animal feed ingredient, as it was most significantly associated

with lower CP *in vitro* digestibility (Marono et al., 2015). De Marco et al. (2015) investigated the average apparent ileal digestibility coefficient of BSF and mealworms in broiler chickens and found that the coefficient was higher in mealworms (86%) than in BSF (68%). It was also suggested that the chitin content in harder exoskeleton insects, like BSF larvae, affected the apparent digestibility coefficients of nutrients (De Marco et al., 2015). This research suggests that components of chitin from insects can influence how broiler chickens digest other nutrients and that certain insects with lower chitin content could be more digestible feed sources (De Marco et al., 2015; Marono et al., 2015).

Each insect species will have different nutrient profiles and digestibility, depending on its growing conditions, age, sex, physiology, and how they are processed (Aniebo and Owen, 2010; Adeyeye and Awokunmi, 2010; Barker, 1997; Bednářová et al., 2009; Finke, 2002; Liu et al., 2017; Rumpold and Schlüter, 2015; Schiavone et al., 2017[a]; Wang et al., 2004; Van Huis, 2013). Insect products have been produced through various drying methods such as sun-drying, low and high convection oven-drying, freeze-drying, and dehydration. Each of these methods produce variations in nutritional content and availability (Aniebo and Owen, 2010; Bovera et al., 2016; Tzompa-Sosa et al., 2014; Rumpold and Schlüter, 2015; Van Huis, 2013). Cricket meal (CM) is a promising feed source, but the most effective processing method for insect meal requires further study. The information previously found is vital to move this potential feed ingredient forward, but there are gaps in the present knowledge.

The objectives of this paper are to determine the nutrient composition and digestibility of crickets (*Gryllus sigillatus*) processed by two separate methods: freeze-

dried cricket meal (FD-CM), oven-dried cricket meal (OD-CM), as well as BSFLM (*Hermetia illucens*) in broiler chickens.

4.3: Materials and methods

4.3.1: Ingredients

BSFLM (a defatted meal) was sourced from Enterra Inc. (Langley City, BC, Canada). FD-CM and OD-CM were sourced from Midgard Insect Farm Inc. (Windsor, NS, Canada).

4.3.2: Proximate analyses of ingredients and diets

The OD-CM, FD-CM, BSFLM and the sample diets were frozen at −20 °C, freeze-dried, and ground to a 1 mm particle size. Procedures were followed to measure the dry matter (DM; 100-moisture; AOAC, 2005; method no. 934.01), CP (AOAC, 2005; method no. 990.03; Leco protein/N analyzer (Model FP-528, Leco Corp., St. Joseph, MI, USA)), and gross energy (GE). To determine the GE content of the samples, a Parr adiabatic bomb calorimeter was used (Parr Adiabatic Calorimeter, Model 6300, Parr Instrument Co., Moline, IL, USA) (Model 6520A, Parr Instrument Co., Miline, IL, USA)). The Nova Scotia Department of Agriculture (Truro, NS, Canada) determined the crude fat (CF) level of the OD-CM, FD-CM, and BSFLM (AOCS, 2005; method AM 5–04; ANKOM XT15 extraction system (ANKOM Technology, Macedon, NY, USA)) and the mineral analysis using an Inductively Coupled Argon Plasma analyzer (Varian 725-ES, Agilent Technologies, Inc., Santa Clara, Cal, USA) (AOAC, 2003; method no. 968.08) (Horwitz and Latimer, 2005).

4.3.6: Performance data

Performance parameters measured were: average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR). On days 14 and 21, average body weight was recorded by batch weighing each cage. Bodyweight gain and feed consumption data were used to calculate FCR and were corrected for mortalities. All weight measurements were recorded using a high-precision electronic scale. Growth parameters were calculated as follows: average daily feed intake (ADFI) = (Total feed consumed (g)/Number of birds per cage)/(Number of days), average daily gain (ADG) = (ΔWeight (g)/Number of birds per cage)/(Number of days), and feed conversion ratio (FCR) = (Feed intake(g)/Number of birds per cage)/(Weight gain (g)).

4.3.3: Diet preparation

Diets (Table 2) were prepared at the Chute Animal Nutrition Center (Truro, NS, Canada). Four diets in mash form were made: a basal grower diet (formulated according to the National Research Council (Dale, 1994) recommendations for Broilers), and three experimental grower diets: FD-CM, OD-CM, or BSFLM at a 70:30 (basal diet: test ingredient) ratio (Bryan et al., 2017). Each diet contained 0.8% celite (diatomaceous earth) as an inert marker. The basal diet was formulated to have 2917 $kcal \cdot kg^{-1}$ metabolizable energy with 20% protein. Diets were mixed using a Marion mixer (Rapids Machinery Company, Marion, Iowa, USA), and the vitamin-mineral premix was formulated and produced on-site.

Table 2: Ingredient and nutrient composition of starter diet, basal, black soldier fly larvae meal (BSFLM), oven-dried cricket meal (OD-CM), and freeze-dried cricket meal (FD-CM) grower diets

	Starter Diet	Basal	BSFLM	OD-CM	FD-CM
Ingredients as fed basis (%)					
Corn	44.50	62.10	43.13	43.13	43.13
Corn starch	-----	-----	-----	-----	-----
SBM	38.70	33.25	22.26	22.26	22.26
Test ingredient	-----	-----	30.00	30.00	30.00
Wheat	10.00	-----	-----	-----	-----
Tallow-grease blend	3.20	-----	-----	-----	-----
Limestone ground	1.70	1.83	1.83	1.83	1.83
Dicalcium phosphate	0.60	1.00	1.00	1.00	1.00
Celite[Z]	-----	0.80	0.80	0.80	0.80
Vitamin/mineral premix[Y, X]	0.50	0.50	0.50	0.50	0.50
Iodized salt	0.40	0.39	0.39	0.39	0.39
Methionine premix[W]	0.40	0.13	0.09	0.09	0.09
Total	100.00	100.00	100.00	100.00	100.00
Analyzed Results					
GE (kcal/kg)	4298.6	4151.8	4452.2	4764.2	4793.2
DM (%)	88.3	90.5	92.4	88.2	94.3
CP (%)	23.3	19.9	30.5	31.7	32.7

[Z]Hyflo Super Cel, Food chemical codex grade (Van Waters and Rogers Ltd. Richmond, BC, Canada).

[Y]Starter premix (amount per tonne), vitamin A (650×106IU kg), 15g, vitamin D3 premix (50×106 IU kg-1), 40g; vitamin E (500,000 IU kg), 50g; vitamin K (33%), 9g; riboflavin (95%), 8g; DL Ca- pantothenate (45%), 30g; vitamin B12 (l000 mg kg), 23g; Niacin (99%), 30; folic acid (3%), 133g; choline chloride (60%), 1335g; biotin (0.04%), 750g; pyridoxine (990,000 mg kg), 5g; thiamin (970,000 mg kg), 3g; manganous oxide (60%), 117g; zinc oxide (80%), l00g; copper sulphate (25%), l00g; selenium premix (675 mg kg), 220g; ethoxyquin (50%), l00g; wheat middlings 1432g; ground limestone (38%), 500g.

[X]Grower premix, vitamin A (650×106 IU kg), 15g,vitamin D3 premix (50×106 IU kg), 40g; vitamin E (500,000 IU kg), 50g; vitamin K (33%), 9g; riboflavin (95%), 8g; DL Ca- pantothenate (45%), 30g; vitamin B12 (l000mg kg), 23g; Niacin (99%), 30; Folic acid (3%), 133g; choline chloride (60%), 1335g; biotin (0.04%), 750g pyridoxine (990,000 mg kg), 5g; thiamin (970,000 mg kg), 3g; manganous oxide (60%), 117g; zinc oxide (80%), l00g; copper sulphate (25%), l00g; selenium premix (675 mg kg), 220g; ethoxyquin (50%), l00g; wheat middling's 1532g; ground limestone (38%), 500g.

[W]Methionine premix contained 500g kg DL- Methionine and 500g kg wheat middlings

4.3.4: Animal husbandry

A total of 320 one-day-old male Ross 308 broiler chicks were obtained from Cox Bros. Poultry Farm Ltd. (Maitland, NS, Canada). When the birds arrived, they were randomly selected and placed in 40 cages (60cm x 48cm). The cages were randomly assigned one of four dietary treatments (Basal, FD-CM, OD-CM, or BSFLM) (8 replicate cages per treatment, 8 chicks per cage). The trial was conducted in an environmentally controlled room at the Atlantic Poultry Research Centre (Truro, NS, Canada). The broilers were placed under continuous light for 48 h initially. After 48 h, the photoperiod was reduced to 18 light hours and was controlled with a rheostat. From day 5 to 21, the light intensity (lux) gradually reduced from 20 to 5 lux. The temperature was set to 32°C from days 0 to 7 and was reduced 3°C a week until the temperature reached 26°C on day 21. Feed and water were provided to the birds on arrival, and throughout the trial, it was provided *ad libitum*. From day 1 through 14, the birds were fed a standard broiler starter diet. From day 15 to 21, each cage was fed either the basal diet or the test diet. Feed was provided twice daily, and feed consumption was recorded. Health checks were performed during the same period. Feed was weighed back on days 14, 21 and when a mortality occurred. Mortalities were weighed, recorded, and sent for a necropsy to a veterinary pathologist. All animals were managed according to the Canadian Council of Animal Care Codes of Practice (2009) under research approved by the Dalhousie University Faculty of Agriculture's Animal Care and Use Committee guideline (ACUC File: 2017-092).

4.3.5: Sample collection and analysis

On days 19, 20, and 21, representative samples of excreta were collected from trays under each cage at 8 AM and 2 PM. On day 21, the birds were euthanized by cervical dislocation. Ileal contents were collected from the point of the Meckel's diverticulum to approximately 1 cm anterior to the ileal-cecal junction. Digesta collected was pooled for each cage. Feed samples were collected for each period and were stored at -20 ºC until analysis could be performed. The ileal contents and excreta were freeze-dried and analyzed in duplicate for GE content (Parr Adiabatic Calorimeter, Model 6300, Parr Instrument Co., Moline, IL, USA) and CP (AOAC, 2005; method no. 990.03; Leco protein/N analyzer (Model FP-528, Leco Corp., St. Joseph, MI, USA)). Dried excreta and the test diets were analyzed for acid-insoluble ash (AIA) using the 2N HCl method to estimate the content of celite in the samples (McCarthy et al., 1974; Vogtmann et al., 1975). By estimating the content of the marker in both the diets and the excreta, and by measuring their respective GE and CP, corrected for uric acid, the apparent metabolizable energy nitrogen corrected (AME_N) value for the test ingredients was calculated using the method of Leeson and Summers (2002). The calculations are as follows: Excreta GE / g of diet = Excreta GE x (Diet AIA / Excreta AIA), Nitrogen retained per g of diet = Diet Nitrogen - (Excreta nitrogen x (Diet AIA / Excreta AIA)), Nitrogen corrected = Nitrogen retained per g of diet x 8.22, Metabolizable energy of basal diet = Diet GE - (Excreta GE / g of diet + nitrogen correction), AME_N = Metabolizable energy of basal diet - (Metabolizable energy of basal diet - Metabolizable energy of test diet) / Level of test ingredient in test diet. The digestibility calculations followed the methods outlined by Lloyd et al. (1978) and were as followed: Apparent digestibility coefficient of the diet (ADCD) (%) = [1-((% Diet AIA) x (% Nutrient in excreta)) / ((% Excreta AIA) x (%

Nutrient in diet))] x 100, Apparent digestibility coefficient of the ingredient (ADCI) (%) = [{((Level of nutrient in reference diet) x (100 - Level of test ingredient in test diet)) + ((Level of nutrient in test ingredient) x (Level of test ingredient in test diet))} x (ADC of nutrient in test diet) - ((Level of nutrient in reference diet) x (100 - Level of test ingredient in test diet)) x (Apparent digestibility coefficient of nutrient in reference diet)] x ((Level of nutrient in test ingredient) x (Level of test ingredient in test diet) $^{-1}$).

4.3.7: Statistical analysis

The experiment was run as a completely randomized design. All statistical analyses were performed on IBM SPSS Statistics 25 (IBM, Armonk, NY, USA). Data were tested for normality using the Shapiro-Wilks test (Wagner, 2011). Extreme observations were found using the interquartile range rule, with a multiplier of 3, and were used as indicators of outliers, which were removed (Wagner, 2011; Hoaglin and Igkewicz, 1987). Average weight, ADFI, ADG, FCR, apparent digestibility coefficient of diets (ADCD), apparent digestibility coefficient of ingredients (ADCI), and available nutrients were subjected to a one-way ANOVA, and statistical significance was determined using the Ryan-Einot-Gabriel-Welsch F Significant Differences test (α=0.05) (Wagner, 2011).

4.4: Results and discussion

4.4.1: Nutrient composition

The nutrient composition of the BSFLM, OD-CM, and FD-CM varied. The OD-CM had the lowest DM content (95.1%), compared to crickets (98.9%) and BSF (96.3), which suggests that higher water content may result in an increased risk of spoilage if stored poorly, and thus has reduced storage potential (Kamau et al., 2018; Klunder et al., 2012). Kamau et al. (2018) stated that cricket and BSFLM dried to 5% moisture content could be stored at 25 °C for 220 days without spoilage, but that if the temperature were 35 °C, CM would only last 63 days. If the moisture content were reduced, the shelf life of the product would increase (Kamau et al., 2018). The BSFLM, OD-CM, and FD-CM all had DM above 95% (Table 3), which would reduce their chance of spoiling (Kamau et al., 2018; Klunder et al., 2012).

OD-CM had a higher CP content (66.9%) than the FD-CM (61.1%), and BSFLM was in between (64.5% CP; Table 3). These results were in line with previously published values for crickets (57.8% CP; Oonincx et al., 2015). For defatted BSFLM, a previous study reported a CP value of 51.8% (Cullere et al., 2016). The insect products all had a protein content higher than SBM, which is on average 44% CP (de Coca-Sinova et al., 2008; Pacheco et al., 2013; Wang et al., 2004). The OD-CM protein content was the highest of all three test ingredients, but the increased temperature from oven-drying could have impacted the protein structures found in the OD-CM (Aniebo and Owen, 2010; Wiseman et al., 1991; Van Rooijen et al., 2014).

Each ingredient's fat content differed, with BSFLM having the lowest fat content at 11.3% (a defatted product). OD-CM had a fat content of 16.4%, which was lower than FD-CM at 21.2%. Oven-drying requires increased temperatures, which may cause fats to

render from the crickets during this process (Aniebo and Owen, 2010; Józefiak et al., 2016). FD-CM would not have been exposed to high heat during production, which allowed the fats to stay in the product, increasing the fat content. Reduced fat content in the ingredients could lower the risk of spoilage factors (Kamau et al., 2018; Klunder et al., 2012). The peroxide values reflected this, and the defatted BSFLM had a lower level of spoilage at 9.4 versus 22.6 (OD-CM) and 23.7 (FD-CM) mEQ/kg. Increased fat in-feed ingredients leads to an increased chance of rancidity, and the levels in CM could lead to rancidity and the formation of free radicals (Bishawi, 1993; Klunder et al., 2012). Oxidized/rancid feeds have a decreased nutritive value and can reduce the performance of broiler chickens (Engberg et al., 1996). Although antioxidants are added to feeds to reduce rancidity, this factor should be considered when producing these products and incorporating them into poultry diets (Bishawi, 1993).

GE levels were lower in the BSFLM at 4919.5 kcal/kg in comparison to the OD-CM and FD-CM (5891.1 and 5630.8 kcal/kg). The energy levels could be due to the higher fat content found in the CM. The BSFLM was a defatted product, which would lower the energy content of the product.

The mineral content of the insect products was similar, with only a few exceptions seen in Table 3. The insect's exoskeleton could explain the increased calcium level (2707.7 mg/100g) of BSFLM. BSF has a mineralized exoskeleton that binds with calcium, which increases the calcium content of the ingredient (Roncarati et al., 2015). Although BSFLM had a high calcium content, it is unknown if this mineral is available for digestion because it is bound to the chitin and proteins found in the exoskeleton (Finke and Oonincx. 2014). The calcium content of the crickets was 166.0 (OD-CM) and 198.5 (FD-CM) mg/100g. Adámková et al. (2014) found that *Gryllus assimillis* contained

78.2 mg/100g calcium, which was lower than the tested samples but higher than some literature reports. OD-CM had a higher sodium content of 322.0 mg/100g than FD-CM and BSFLM, and BSF is reported to have lower sodium levels than other insects (Barragan-Fonseca et al., 2017).

Potassium levels were also higher in the BSFLM (1450.7 mg/100g), whereas the crickets contained 963.0 (OD-CM) and 783.0 (FD-CM) mg/100g potassium. Crickets contain ~28.2 mg/100g potassium (Ajai et al., 2013), which is much lower than the values reported in the current study. However, BSF larvae had a potassium level of 2270.0 mg/100g, which was higher than the level determined in this study (Nyakeri et al., 2017). Magnesium was higher in the BSFLM at 362.7 mg/100g, compared to 93.5 (OD-CM) and 104.5 (FD-CM) mg/100g. The values were similar to previous studies (Ajai et al., 2013: Barragan-Fonseca et al., 2017). There were higher copper levels in the FD-CM and OD-CM (6.7 and 6.1 mg/100g respectively), compared to the BSFLM (1.6 mg/100g), which could be associated with the feed given to the crickets during production.

Potassium, magnesium, copper, and sodium are water-soluble minerals and could have been affected by the processing method (Aniebo and Owen, 2010; Rumpold and Schlüter, 2015; Van Huis, 2013). The reduced manganese content of the OD-CM crickets (4.6 mg/100g) could be explained by the fact that manganese is fat-soluble and the reduced fat content in the OD-CM crickets would have led to a reduction in this micronutrient. The difference between BSFLM manganese content (36.3 mg/100g) was large, and could be due to species differences or feed used, but was lower than what was found in wild BSF (560 mg/100g; Nyakeri et al. 2017). The zinc content in the OD-CM was higher (25.8 mg/100g, Table 3) than the other samples and was similar to other studies shown in Table 1, so the processing method would explain the difference between

OD-CM and FD-CM crickets (Adeyeye and Awokunmi, 2010; Christensen et al., 2006; Rumpold and Schlüter, 2015; Van Huis, 2013).

Species and processing methods influence the nutritional content of insect feed products (Rumpold and Schlüter, 2015; Wang et al., 2004; Van Huis, 2013). Published nutritional information on insects found in the literature vary and are provided in Table 1. Differences are found even within the same species, these are due to the rearing conditions, including feed source (Aniebo and Owen, 2010; Rumpold and Schlüter, 2015; Van Huis, 2013). The current research emphasizes the effect that processing methods can have on the nutritional composition of insects.

Table 3: Proximate analysis of black soldier fly larvae meal (BSFLM), oven-dried cricket meal (OD-CM), and freeze-dried cricket meal (FD-CM) on an as fed basis

	BSFLM	OD-CM	FD-CM
Nutrient			
Gross energy (kcal/kg)	4919.5	5891.1	5630.8
DM (%)	96.3	95.1	98.9
CP (%)	64.5	66.9	61.1
CF (%)	11.3	16.4	21.2
Calcium (mg/100g)	2707.7	166.0	198.5
Copper (mg/100g)	1.6	6.7	6.1
Magnesium (mg/100g)	417.1	93.5	104.5
Manganese (mg/100g)	36.3	4.6	6.1
Phosphorus (mg/100g)	881.2	854.5	787.5
Potassium (mg/100g)	1450.7	963.0	783.0
Sodium (mg/100g)	124.5	322.0	233.5
Zinc (mg/100g)	14.9	25.8	18.2
Peroxide (mEQ/kg)	9.4	22.6	23.7

BSFLM= Enterra Feed Corporation.
OD= Midgard Insect Farm Inc.
FD= Midgard Insect Farm Inc.

4.4.2: Nutrient digestibility

There was a difference in the CP ADCD, with OD-CM having the highest value (55.8%), which was significantly different from all the other diets, except the FD-CM diet (52.2%) (Table 4). The BSFLM diet had an ADCD of 48.5% for CP. In a previous study, BSFLM had a CP apparent digestibility of 34.0% when included in broiler quail diets at 15% but had a higher digestibility at 10% inclusion, 42.9% (Cullere et al., 2016). The GE ADCD was also affected by the diet, with the highest value being the FD-CM diet (76.7%), whereas BSFLM (73.1%) and OD-CM (72.7%) were not significantly different. The metabolizable energy ADCD was also significantly different between the diets, and the OD-CM and FD-CM cricket diets had increased metabolizable energy (3870.5 and 3811.3 (AME_N)kcal/kg, respectively) compared to the BSFLM diet (3188.5 (AME_N)kcal/kg).

The ADCI in (Table 4) showed no difference among treatments for DM content. The CP ADCI was significantly higher for the OD-CM (60.3%) than the BSFLM (48.0%). The use of heat to make the OD-CM may have caused a Maillard reaction to occur, negatively affecting the digestibility of protein in the OD-CM by binding the amino acids to the reducing sugars found in the exoskeleton and hemolymph (Van Rooijen et al., 2014; Yu et al., 2008). However, the OD-CM CP ADCI was not significantly different from the FD-CM (54.4%). The amino acid digestibility could illuminate if the heat during processing affected the protein digestibility, and future research could further evaluate this factor. The CP ADCI of each ingredient was low compared to SBM, limiting this feed as a protein alternative (de Coca-Sinova et al., 2008; Pacheco et al., 2013).

The current study showed that the FD-CM had a higher fat content and increased GE digestibility than OD-CM (Table 4). The metabolizable energy of the OD-CM and FD-CM ingredients (5207.8 and 5004.4 (AME_N)kcal/kg, respectively) was also higher than the BSFLM (3727.1 AME_N)kcal/kg). The CM had an improved metabolizable energy digestibility compared to BSFLM, which was due to the increased crude fat content. Schiavone et al. (2017[a]) found comparable results when they tested the AME_N of highly and partially defatted BSFLM, and the partially defatted BSFLM had a higher AME_N. This suggests that the CM's high-fat content would have slowed down the rate of passage of feed, giving more time for digestion and better nutrient absorption, increasing the GE and metabolizable energy digestibility (Poorghasemi et al., 2013; Ravindran et al., 2016).

The available nutrient composition, seen in Table 4, is based on the digestibility and indicates what percentage of the nutrient is absorbed by the broiler chicken. The OD-CM had a higher digestibility and CP content, resulting in an available 40.4% CP level, and was significantly higher than the BSFLM and FD-CM (30.9 and 33.3%). The available GE was higher in the FD-CM crickets (4851.9 kcal/kg), whereas OD-CM crickets (4454.2 kcal/kg) were higher than the BSFLM (3794.1 kcal/kg). The higher FD-CM available GE value was due to the high digestibility (86.2%) of GE and its high-fat content of 21.2%. The processing and lyophilization during freeze-drying allowed for the fats to stay in the product and increased its digestibility. The increased fats and available GE of the FD-CM could reduce the need to add additional fats to poultry diets while incorporating this ingredient. However, FD-CM's fatty acid profile would need to be determined to see if it meets the dietary requirements of broiler chicken (Schiavone et al., 2017).

Fats are required for hormone production and cellular membrane integrity, and the linoleic acid requirement of broilers is estimated to be 1% of the diet because poultry cannot synthesize this fatty acid (Dale, 1994). Mole crickets (*Gryllotalpa africana*) and ground crickets (*Acheta confirmata*) contain 1541.6 and 2739.6 mg/100 g of linoleic acid (Yang et al., 2006). Ghosh et al. (2017) reinforced the high levels of linoleic acid and found that cricket species *Teleogryllus emma* and *Gryllus bimaculatus* had a linoleic acid content of 9610 and 4150 mg/100 g. Yang et al. (2006) suggested that the prominent levels of linoleic acid found in insects were due to the green leaves the crickets were eating, which contained precursors to n-3 PUFA and increased the linoleic acid content of the crickets. While examining how the growth medium of insects affects their nutrition profile, Oonincx et al. (2015) found that the linoleic acid profile changed depending on the diet of the insect, reinforcing the effect the production of insects has on their nutrient composition. Crickets had prominent levels of linoleic acid, and FC-CM could offer a fat alternative in poultry diets.

Digestibility and nutrient profile are essential when considering insect type and processing method. Both CM products provided essential nutrients to the birds, and the digestibility of the ingredients was comparable. However, the CP digestibility of the insect products was lower than SBM and limits them as protein alternatives. The BSFLM had the lowest ADCI of CP, GE, and metabolizable energy. There was a difference between FD-CM and OD-CM regarding the available GE and protein. Although the FD-CM crickets had an improved available energy content, OD-CM had a higher available CP.

Table 4: Apparent digestibility coefficients of diets (ADCD), apparent digestibility coefficients of ingredients (ADCI), and available nutrients of black soldier fly larvae meal (BSFLM), oven-dried cricket meal (OD-CM), freeze-dried cricket meal (FD-CM), and basal diet

Ingredient	Basal	BSFLM	OD-CM	FD-CM	ᵉSEM	P-value
ADCD (%)						
Dry matter	92.5	92.3	92.4	92.2	0.16	0.94
Crude protein	49.3^{b}	48.5^{b}	55.8^{a}	52.2^{ab}	0.95	0.02
Gross energy	70.8^{b}	73.1^{b}	72.7^{b}	76.7^{a}	0.55	0.00
Metabolizable energy (AME_N)kcal/kg	3188.5^{c}	3401.4^{b}	3870.5^{a}	3811.3^{a}	53.30	0.00
ADCI (%)						
Dry matter	-----	91.9	92.2	91.5	0.59	0.91
Crude protein	-----	48.0^{b}	60.3^{a}	54.4^{ab}	1.89	0.02
Gross energy	-----	77.1^{b}	75.6^{b}	86.2^{a}	1.46	0.00
Metabolizable energy (AME_N)kcal/kg	-----	3727.1^{b}	5207.8^{a}	5004.4^{a}	145.11	0.00
Available nutrient						
Dry matter (%)	-----	89.3	87.7	87.3	0.57	0.81
Crude protein (%)	-----	30.9^{b}	40.4^{a}	33.3^{b}	1.32	0.01
Gross energy (kcal/kg)	-----	3794.1^{c}	4454.2^{b}	4851.9^{a}	109.32	0.00

a, b, c Means within rows with different superscripts are significantly different ($P < 0.05$). ᵉSEM = Standard error of the mean.

4.4.3: Animal performance

The growth performance of broilers fed BSFLM, NF, OD-CM, and FD-CM diets are reported in Table 5. The processing method and insect species did not influence the ADFI. The average weight of the birds was not significantly different on days 0, 14, and 21. Cullere et al. (2016) found no difference in body weight between chickens fed a control diet than 10% and 15% BSFLM diets. Other research showed no difference in broiler quail feed intake when fed BSFLM up to a 15% dietary inclusion level (Cullere et al., 2016).

During the grower phase, the ADG of the birds fed the OD-CM, and FD-CM diets had the highest values at 73.9 g/day and 78.2 g/day, respectively. The type of insect did play a role in the ADG of the broiler chickens and the BSFLM had a lower ADG than the OD-CM and FD-CM. Other studies have shown that CM increased the weight gain of broilers at a 16.56% dietary inclusion compared to broilers fed a SBM diet (Razak et al., 2012). Cullere et al. (2016) found no difference when feeding BSFLM diets to broiler quails compared to a control diet, which was similar to the results in Table 5, with BSFLM having a similar weight compared to the basal diet. The OD-CM and FD-CM had significantly lower FCR (1.4 and 1.4) than the basal diet (1.8). Razak et al. (2012) found that CM increased the FCR of broilers, which contradicted this study's results. This could be due to the difference in crickets used for the study, in terms of processing method, age, or species of cricket.

The processing method did not affect the growth parameters of broiler chickens, and the FD-CM and OD-CM had similar results for all parameters. However, the type of insect did influence the results, with the BSFLM having a lower ADG in the grower phase. This could be related to the low CP ADCI shown in Table 4, and the BSFLM did

not provide the same level of protein as the CM. The diets were not formulated to be isonitrogenous and isocaloric though, so any inference on the growth parameters cannot be fully determined with this study.

Table 5: Average weight, average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) of broiler chickens fed basal, black soldier fly larvae meal (BSFLM), oven-dried cricket meal (OD-CM), and freeze-dried cricket meal (FD-CM)

	Basal	BSFLM	OD-CM	FD-CM	ᶜSEM	P-value
Average weight (g)						
0	40.2	39.5	40.3	40.1	0.36	0.88
14	396.9	386.6	361.5	392.3	9.74	0.60
21	833.3	832.2	878.8	939.6	19.15	0.15
ADFI (g/day)						
Starter (0 – 14)	36.6	37.5	38.9	37.3	0.61	0.61
Grower (15 – 21)	109.6	99.3	103.2	106.1	1.64	0.13
ADG (g/day)						
Starter (0 – 14)	25.5	24.7	22.9	25.2	0.70	0.60
Grower (15 – 21)	62.4[b]	63.8[b]	73.9[a]	78.2[a]	1.77	0.00
FCR						
Starter (0 – 14)	1.450	1.602	1.705	1.577	0.07	0.69
Grower (15 – 21)	1.768[a]	1.572[b]	1.396[b]	1.374[b]	0.03	0.00

a, b, c Means within rows with different superscripts are significantly different ($P < 0.05$). ᶜSEM = Standard error of the mean.

4.5: Conclusion

Digestibility and nutrient profile are important when considering the type of insect and processing method. Both cricket meals provided essential nutrients to the birds and the digestibility of the ingredients were comparable. The BSFLM had a lower CP digestibility than OD-CM, lower GE digestibility than FD-CM, and a metabolizable energy digestibility lower than OD-CM. There was a difference seen between FD-CM and OD-CM regarding the available gross energy and protein. Although the FD-CM crickets had an improved available energy content, OD-CM had a higher available CP. Expanding on the cost of production regarding both OD-CM and FD-CM would give cricket producers further knowledge on the optimal processing methods for CM.

CHAPTER 5: THE IMPACT OF CRICKET MEAL (GRYLLUS SIGILLATUS) ON THE MEAT QUALITY, GROWTH, AND INTERNAL MORPHOLOGY OF BROILER CHICKENS

5.1: Abstract

Access to protein sources for animal feeds is vital to ensuring efficient food production. This study investigates the use of cricket (*Gryllus sigillatus*) meal (CM) at dietary inclusion rates of 0% (non-medicated control; NM), 0% (medicated control), 5, 10, 15, and 20% CM (all non-medicated), and its impact on the growth performance, internal morphology, and meat quality of Ross 308 broiler chickens (n=624 total; 26 birds/pen). Bird weight and feed intake were recorded weekly, and growth parameters were calculated. On days 13, 20, and 35, organ indices were calculated for three birds/pen. On day 35, meat quality was analyzed. The final average live weight of broilers fed 5% CM (1933.4 g) was lower than broilers fed the 10% CM (2063.5 g; $P<0.05$) and the 0% NM diets (2095.6 g; $P<0.05$). The total weight gain of chickens fed 5% CM (54.1 g/day) was lower than that of chickens fed all other treatments ($P<0.05$). A significant difference was observed in the small intestine of the chickens fed 5% CM (7.9%) on day 20 compared to all other treatments. Feed treatments did not influence meat texture or colour. Cooking loss in birds fed the 10% CM diet (35.5%) was significantly higher than that of birds fed the 0% NM control (31.9%). Results indicate that CM included in up to 20% of the diet had no detrimental impact on the growth, internal morphology, and meat quality of broiler chickens. Further research is required to determine whether a dietary inclusion of >20% CM will produce the same results.

5.2: Introduction

Interest in including insects in poultry diets is increasing. Due to the sustainability issues associated with current feed sources, such as water usage, soil degradation, and greenhouse gas emissions, researchers and producers are investigating insects as potential feed ingredients (Bovera et al., 2016; Islam and Yang, 2017; Oonincx et al., 2012; Shadreck, 2014; Wang et al., 2005). The production of insects could reduce environmental concerns associated with current poultry feed production (Oonincx et al., 2012). Insects are a viable source of nutrients, comparable to protein sources that are currently used in poultry diets, like soybean meal (SBM) (Wang et al., 2005). The nutritional composition of insects can vary, depending on taxonomic order, rearing conditions, age, feed source, and processing (Rumpold and Schlüter, 2015). Cricket producers favour common mass-produced crickets (*Gryllus sigillatus*) due to their hearty production, ease of care, and resistance to the *Acheta domesticus* densovirus, which negatively affects cricket producers by causing widespread mortality (Wang et al., 2005; Weissman et al., 2012). However, there is a lack of nutritional information for *Gryllus sigillatus* and their use in broiler chicken feed and subsequent effects on broiler growth, internal morphology, and meat quality (Wang et al., 2005; Weissman et al., 2012).

Crickets show considerable variation in their nutritional content based on species, rearing conditions, and nutrient sources, but on average, contain ~60% CP (dry matter basis) and have the potential to meet the CP requirements of broiler chickens (18.00 – 23.00%) (Dale, 1994; Finke, 2015; Leiber et al., 2017; Nakagaki et al., 1987; Rumpold and Schlüter, 2015). Crickets also contain ~16% crude fat, but their fatty acid profile largely depends on their diet (Rumpold and Schlüter, 2015; Finke, 2015). The mineral and vitamin content of crickets is also favourable for poultry diets; however, they do not meet the manganese requirements of poultry (Ajai et al., 2013; Finke, 2015; Rumpold and

Schlüter, 2015). Previous research investigating the nutrient composition of insects and their effect on chicken growth and production has proven variable (Ballitoc and Sun, 2013; Shadreck, 2014; Wang et al., 2005; Weissman et al., 2012). For example, a study included cricket meal (CM, *Gryllus testaceus*) in broiler diets at dietary inclusion levels of 5, 10, and 15%, substituting this ingredient for corn and SBM and monitored the growth performance of the chickens from day 8 to day 20 (Wang et al., 2005). The nutritional composition of the CM used in their study was 58.3% CP and 10.3% fat, which was higher than the SBM used in the study (46.8% CP and 1.84% fat) (Wang et al., 2005). Weight gain and feed to gain ratio were not different among broilers fed any treatment, indicating that CM could effectively replace SBM (Wang et al., 2005). Improved feed efficiency in broiler chickens when fed diets containing insects such as *G. sigillatus* would benefit sustainable food production.

Another contributing factor to the use of CM as a poultry feed ingredient is the movement away from medicating broiler diets and the ongoing investigation into the use of functional feed ingredients (Islam and Yang, 2017; Lokman et al., 2019). Bioactive compounds found in insects like melanin and chitin have exhibited antimicrobial and antibiotic capabilities (Islam and Yang, 2017). When cricket chitin was extracted from the exoskeleton and fed to broiler chickens at a 0.05% dietary inclusion level, it improved the growth and carcass quality (Lokman et al., 2019).

Bird health can affect feed efficiency, growth, and meat quality (Ballitoc and Sun, 2013; Bovera et al., 2016; Shadreck, 2014; Wang et al., 2005; Weissman et al., 2012). Visual inspection of the internal organs, like the bursa of Fabricius (bursa), can indicate health and efficient growth (Kokoszyński et al., 2017; Raji et al., 2017). Internal morphology has implications for production efficiency because it can indicate overall

flock health, and birds suffering from acute disease could reduce profit margins for producers (Oviedo-Rondón, 2019). Feed quality and digestibility directly link with intestinal health and including insect meal in broiler chicken diets can affect the internal organs and intestinal tract (Ballitoc and Sun, 2013; Bovera et al., 2016; Oviedo-Rondón, 2019; Shadreck, 2014;). For example, increased spleen weight can indicate an increase in immune system activity (Oviedo-Rondón, 2019). Researchers have found that the chitin component of an insect's exoskeleton showed bacteriostatic, antifungal, and antimicrobial proprieties, which lowers internal stress and reduces the increase of plasma corticosterone that slows the growth of lymphoid organs such as the spleen (Ballitoc and Sun, 2013; Bovera et al., 2016; Shadreck, 2014). Although the effect of crickets on internal organ weights and the digestive tract of broiler chickens have not been researched in-depth, chitin could modulate the structure, histology, and microbiota of the intestinal tract by reducing nutrient digestibility while improving prebiotic activity (Bovera et al., 2016; Lokman et al., 2019). While previous studies indicate that insect meal inclusion can have a variable influence on broiler organ weight and the intestinal tract, studies have not specifically investigated the impact of *Gryllus sigillatus* on the internal organs and intestinal tract of broiler chickens.

The poultry meat industry is a leading source of income for many countries, but the meat quality of chickens fed insect meals varies, depending on the insects used (Agriculture and Agri-Food Canada, 2018; Bovera et al., 2016; Hwangbo et al., 2008; Schiavone et al., 2017[a]; Schiavone et al., 2019; Sun et al., 2013). As defined by Northcutt (1997), meat quality is measured by the way meat looks, cooks, tastes, and feels, and if a product does not meet these expectations, it is considered lower quality. Consumers are concerned with the appearance of chicken products, like skinless chicken breast, and a

breast that is pale pink in colour is associated with freshness (Northcutt, 1997). The texture and cook yield of chicken breast meat are also related to its quality by consumers, and if a breast shrinks and has a tough texture, it is viewed as low quality (Northcutt, 1997). Bovera et al. (2016) reported that chickens fed a SBM control diet had a cook loss of 21.4%, versus chickens fed a 30% mealworm (*Tenebrio molitor*) diet that had a 23.6% cook loss. Cullere et al. (2019) fed *Hermetia illucens* to quail, and the cook loss and toughness of the meat was highest in the birds fed black soldier fly (BSF, *Hermetia illucens*) at a 15% dietary inclusion (Cullere et al., 2016). There is, however, no information available about the effect of crickets on the meat quality of broiler chickens.

This study aims to examine the influence of cricket meal on the growth, internal morphology, and meat quality of broiler chickens compared to a medicated and non-medicated control diet.

5.3: Materials and methods

5.3.1: Diet preparation

All diets were made at the Chute Animal Nutrition Centre located at Dalhousie University Faculty of Agriculture (Truro, NS, Canada). Six experimental diets (Table 6) were formulated to be isonitrogenous and isocaloric for the starter (days 0 – 21), grower (days 21 – 28), and finisher (days 28 – 35) phases and to meet the nutrient requirements of broiler chickens at each phase (Dale, 1994). The CM was provided by Midgard Insect Farm Inc. (Windsor, NS, Canada) and was included in non-medicated (NM) diets at dietary inclusion levels of 0, 5, 10, 15, and 20%. As the percent inclusion of CM increased, SBM, tallow-grease blend, dicalcium phosphate, methionine, and iodized salt decreased to balance the nutritional value of the crickets (Table 6). An NM and a medicated (M) (*Virginiamycin* and *Coban*) control diet containing CM at 0% were also

included. Diets were mixed using a Marion mixer (Rapids Machinery Company, Marion, Iowa, USA) and fed in a mash form (Chiba, 2013; MacIsaac et al., 2005; Parsons et al., 2006).

Table 6: Ingredient composition, calculated, and analyzed nutrient composition of starter, grower, and finisher diets fed in this experiment

	Starter diets						Grower diets						Finisher diets					
	0% NM	0% M	5%	10%	15%	20%	0% NM	0% M	5%	10%	15%	20%	0% NM	0% M	5%	10%	15%	20%
Ingredients as fed basis (%)																		
Corn ground	41.58	41.43	43.05	44.53	46.01	47.50	43.89	43.74	45.38	46.84	48.33	49.82	49.79	49.66	51.27	52.71	54.15	55.59
SBM	40.18	40.20	34.21	28.24	22.27	16.300	36.59	36.61	30.62	24.65	18.68	12.71	31.36	31.38	25.39	19.43	13.46	7.50
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cricket meal	-----	-----	5.00	10.00	15.00	20.00	-----	-----	5.00	10.00	15.00	20.00	-----	-----	5.00	10.00	15.00	20.00
Tallow-grease blend	3.12	3.17	2.75	2.38	2.02	1.65	4.82	4.87	4.45	4.09	3.72	3.35	4.44	4.49	4.10	3.76	3.43	3.09
Limestone ground	1.76	1.76	1.80	1.84	1.88	1.91	1.61	1.61	1.65	1.69	1.72	1.76	1.49	1.48	1.52	1.56	1.60	1.63
Dicalcium Phosphate 21 p	1.33	1.33	1.26	1.18	1.10	1.02	1.16	1.16	1.08	1.01	0.93	0.85	1.02	1.02	0.94	0.86	0.78	0.71
Dl Methionine premix[w]	0.50	0.62	0.54	0.46	0.38	0.29	0.54	0.54	0.45	0.37	0.29	0.21	0.50	0.50	0.41	0.33	0.25	0.17
Vitamin/mineral premix[y,x]	0.62	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Pellet binding agent	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Iodized salt	0.41	0.41	0.39	0.37	0.35	0.32	0.39	0.39	0.37	0.35	0.33	0.30	0.40	0.40	0.37	0.35	0.33	0.31
Stafac[v]	-----	0.05	-----	-----	-----	-----	-----	0.05	-----	-----	-----	-----	-----	0.05	-----	-----	-----	-----
Coban[u]	-----	0.03	-----	-----	-----	-----	-----	0.03	-----	-----	-----	-----	-----	0.03	-----	-----	-----	-----
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Analysis																		
Metabolizable energy (Kcal/kg)	3025	3025	3025	3025	3025	3025	3150	3150	3150	3150	3150	3150	3200	3200	3200	3200	3200	3200
Crude protein (%)	23.00	23.00	23.00	23.00	23.00	23.00	21.50	21.50	21.50	21.50	21.50	21.50	19.50	19.50	19.50	19.50	19.50	19.50
Calcium (%)	0.96	0.96	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.87	0.87	0.79	0.79	0.79	0.79	0.79	0.79
Available phosphorus (%)	0.48	0.48	0.48	0.48	0.48	0.48	0.44	0.44	0.44	0.44	0.44	0.44	0.40	0.40	0.40	0.40	0.40	0.40
Sodium	0.19	0.19	0.19	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Lysine (%)	1.45	1.45	1.46	1.47	1.49	1.52	1.34	1.34	1.35	1.36	1.37	1.39	1.18	1.18	1.19	1.20	1.22	1.23
Methionine + Cystine %	1.08	1.08	1.08	1.08	1.08	1.08	0.99	0.99	0.99	0.99	0.99	0.99	0.91	0.91	0.91	0.91	0.91	0.91
Analyzed Results																		
Gross energy (cal/g)	4330.7	4470.3	4656.2	4638.8	4632.6	4776.6	4551.9	4571.8	4554.5	4607.3	4694.7	4740.6	4542.6	4541.5	4635.3	4606.7	4708.6	4735.5

Dry matter (%)	88.0	88.2	88.3	88.8	88.9	89.3	87.3	89.1	88.8	88.0	88.1	88.5	88.7	88.5	89.4	89.5	89.2	90.0
Crude protein (%)	23.3	23.4	18.3	25.3	25.2	25.0	22.0	22.0	22.8	23.1	23.7	23.7	21.1	20.7	21.8	21.7	22.4	22.0
Crude fat (%)	5.8	5.8	6.5	7.5	7.4	9.1	7.2	7.4	7.9	8.4	9.0	9.8	7.3	7.2	7.7	8.3	8.8	9.6
Calcium (%)	1.1	1.0	0.9	0.4	0.9	0.8	0.8	0.9	0.9	1.0	0.9	0.9	0.8	0.8	0.8	0.8	0.5	0.5
Potassium (%)	1.0	1.0	0.7	0.9	0.8	0.6	0.9	0.9	0.9	0.9	0.8	0.7	0.9	0.9	0.8	0.8	0.7	0.6
Magnesium (%)	0.2	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1
Phosphorus (%)	0.7	0.7	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.5	0.5
Sodium (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Copper (ppm)	23.4	28.0	35.9	31.1	30.2	32.6	19.5	21.9	28.6	28.7	27.7	29.3	24.7	22.8	22.7	23.8	27.5	29.7
Manganese (ppm)	143.5	139.8	131.7	134.3	133.1	128.6	110.8	133.5	134.0	134.1	130.6	128.0	128.9	117.2	125.9	131.6	136.9	129.0
Zinc (ppm)	132.2	124.3	131.0	142.0	139.4	160.4	119.2	136.4	144.9	150.5	157.8	162.1	133.6	119.8	135.8	156.7	148.3	152.0

NM= Non-medicated. M=Medicated.

[u] Coccidiostat - Pfizer Animal Health, London, ON, Canada.

[v] Antibiotic - Elanco Animal Health, Guelph, ON, Canada.

[w] Pellet Binder - Uniscope, Inc., Johnstown, CO, USA.

[y] Starter premix (amount per tonne), vitamin A (650×106IU kg),15g, vitamin D3 premix (50×106 IU kg), 40g; vitamin E (500,000 IU kg), 50g; vitamin K (33%), 9g; riboflavin (95%), 8g; DL Ca-pentothenate (45%), 30g; vitamin B12 (l000 mg kg), 23g; niacin (99%), 30; folic acid (3%), 133g; choline chloride (60%), 1335g; biotin (0.04%), 750g; pyridoxine (990,000 mg kg), 5g; thiamin (970,000 mg kg), 3g; manganous oxide (60%), 117g; zinc oxide (80%), l00g; copper sulphate (25%), l00g; selenium premix (675 mg kg), 220g; ethoxyquin (50%), l00g; wheat middlings 1432g; ground limestone (38%), 500g.

[x] Grower premix, vitamin A (650×106 IU kg), 15g,vitamin D3 premix (50×106 IU kg), 40g; vitamin E (500,000 IU kg), 50g; vitamin K (33%), 9g; riboflavin (95%), 8g; DL Ca- pentothenate (45%), 30g; vitamin B12 (l000mg kg), 23g; niacin (99%), 30; folic acid (3%), 133g; choline chloride (60%), 1335g; biotin (0.04%), 750g pyridoxine (990,000 mg kg), 5g; thiamin (970,000 mg kg), 3g; manganous oxide (60%), 117g; zinc oxide (80%), l00g; copper sulphate (25%), l00g; selenium premix (675 mg kg), 220g; ethoxyquin (50%), l00g; wheat middlings 1532g; ground limestone (38%), 500g.

[z] Supplied/kg premix: DL-Methionine, 0.5 kg; wheat middlings, 0.5 kg.

5.3.2: Chemical Analysis

Dry matter, gross energy, and crude fat of the CM and experimental diets were analyzed according to the procedures reported in Fisher et al. (2020). The Nova Scotia Department of Agriculture (Truro, NS, Canada) determined the CP level of the samples by the Dumas method and performed the mineral analysis using an Inductively Coupled Argon Plasma analyzer (Horwitz and Latimer, 2005). The proximate analysis of the CM is provided in Table 3.

5.3.3: Animal Husbandry

Six hundred and twenty-four mixed-sex, fast-feathering Ross 308 broilers, with an average live weight of 39 ± 0.8 g, were used. Chicks were obtained from Cox Bros. Poultry Farm Ltd. (Maitland, NS, Canada). The chicks were transported to the Atlantic Poultry Research Centre (APRC), located at the Faculty of Agriculture, Dalhousie University (Truro, NS, Canada). On the day the chicks arrived at the APRC, they were randomly placed in 24 pens (26 birds/pen; 4 replicates/diet) that were 1.4 m x 2.14 m in dimension, with pine shavings for bedding (4 cm), and located in a climate-controlled room. The broilers were placed under continuous light for 48 h initially, and the photoperiod was reduced to 18 light hours for days 5 to 35. Lighting was controlled with a rheostat that gradually reduced light intensity (lux) from 20 lux on day 0, to 5 lux on day 35. The temperature was set to 32°C from days 0 to 7 and was reduced 3°C each week until the temperature reached 21°C on day 28 and remained consistent until day 35. Feed and water were provided *ad libitum*.

A necropsy was completed by a veterinary pathologist (Animal Health Laboratory, Truro, NS, Canada) when mortalities occurred. All animals were managed following the Canadian Council on Animal Care Codes of Practice, (2009) under a

research protocol that was approved by Dalhousie University Faculty of Agriculture's Animal Care and Use Committee (ACUC File #: 2018-043).

5.3.4: Performance data

On days 0, 7, 14, 21, 28, and 35, average body weight was recorded by batch weighing each cage and recording feeder residual weights. Mortalities were recorded as a percentage of the total number of birds in each pen. Growth and feed intake parameters were calculated as follows: average daily feed intake (ADFI) = (Total feed consumed (g)/Number of birds per pen)/(Number of days), average daily gain (ADG) = (ΔWeight (g)/Number of birds per pen)/(Number of days), feed conversion ratio (FCR) = (Feed intake(g)/Number of birds per pen)/(Weight gain (g)), and protein efficiency ratio (PER) = (Weight gain (g))/(Feed intake (g)/Number of birds)*Protein content (%)).

5.3.5: Sample collection

Three birds/pen were randomly selected for sampling (days 13, 20, and 35). The broilers to be sampled were euthanized by cervical dislocation, after which their body weights were recorded. The internal organs (crop, liver, pancreas, spleen, and bursa) were removed, and the organ index was calculated as a ratio of organ weight to carcass body weight. The small intestine from the pyloric junction to the ileal-cecal junction was removed, weighed, and calculated as a ratio to carcass body weight. Relative organ weights were calculated as: Organ (%) = Organ (g)/ Live weight (g) x 100.

Ileal contents were collected from the Meckel's diverticulum to approximately 1 cm anterior to the ileal-cecal junction, and a portion of the sample was frozen for future use. The pH of the digesta was taken (Oakton™ Waterproof Big Display pHTestr™ 30, Fisher Scientific). On sampling days 20 and 35 only, the section of the intestine between the pylorus and the ileal-cecal junction was removed from each bird and weighed. The

section of the small intestine between the Meckel's diverticulum and the ileal-cecal junction underwent gross pathological diagnosis for lesions. The scoring was based on the presence of intestinal lesions, and scored on a 0 to 6 scale: 0= no gross lesions; 1= thin or brittle walls, but removable fibrin; 2= localized necrosis or ulceration (1 to 5 foci); 3= localized necrosis or ulceration (6 to 15 foci); 4= localized necrosis or ulceration (16 or more foci); 5= patches of necrosis 2 to 3 cm long; 6= diffuse necrosis (Keyburn et al., 2006; Shojadoost et al., 2012). On sampling day 35 only, breast (pectoralis major and minor muscles) and liver samples were weighed, collected in whirl pack bags, and stored at 4°C until analysis 24 h postmortem.

5.3.6: Meat quality

On day 35, the breasts and livers were removed for quality assessment and analyzed for colour using a Hunterlab MiniScan EZ 4500L spectrophotometer (HunterLab, Reston, Va., USA). The spectrophotometer analyzed L* a* b*; lightness, red/green, yellow/blue, respectively. The livers were placed in a crystal cuvette to allow for a representative analysis of the liver tissue colour. Raw breasts were measured by placing the spectrophotometer directly to the exterior surface of the caudal part of the Pectoralis major muscle. All the samples were scanned, rotated 90°, and scanned again.

The texture of both raw and cooked meat was measured using a TA.XTPlus Texture Analyzer (Stable Micro Systems, Surrey, UK). The left breasts of the sampled chickens were used to measure the raw texture, and the right breasts were cooked as described in the following paragraph to determine the cooked texture of the chicken breast. Shear force was determined according to the chicken breast application settings for the TA. XTPlus Texture Analyzer set for muscle shear force. All measurements were taken at room temperature.

Cook yield was determined by pre-weighing and batch-cooking the right chicken breasts previously analyzed for colour. The chicken breasts were placed in a preheated convection oven set to 77°C. A core temperature of 75°C was reached, and the samples were cooled to room temperature before cooked weight was measured. Cooking loss was calculated by 100 × [cooked sample weight (g)/raw sample weight (g)].

5.3.7: Statistical analysis

The experiment was run as a completely randomized design. All statistical analyses were performed on IBM SPSS Statistics 25 (IBM, Armonk, NY, USA). Data were tested for normality using the Shapiro-Wilks test (Wagner, 2011). Extreme observations were found using the interquartile range rule, with a multiplier of 3, and were used as indicators of outliers, which were removed (Wagner, 2011; Hoaglin and Igkewicz, 1987). Performance, internal morphology, and meat quality parameters were subjected to a one-way ANOVA. Internal morphology parameters were further analyzed as two-way ANOVA for sex, diet, and sex * diet. Statistical significance was determined using the Ryan-Einot-Gabriel-Welsch F Significant Differences test (α=0.05) (Wagner, 2011). Linear and quadratic regression analysis was also performed on all data (excluding the 0% M diet) to evaluate the relationship between CM inclusion rate and the measured parameters, and if both were significant, the higher order equation was used. Differences between means were considered significant when $P < 0.05$. Lesion scores were subjected to a chi-square test to determine any significant effects the diet had on the presence of lesions ($\alpha = 0.05$) (Wagner, 2011).

5.4 Results and discussion

5.4.1: Performance parameters

Performance parameters recorded for ADFI, ADG, FCR, PER, and mortality are reported in Table 7. On day 21, birds fed 15 (808.7 g) and 20% (834.3 g) CM weighed significantly more than the 5% CM-fed birds (689.4 g). This trend continued to day 28, where the 5% CM-fed birds had the lowest average live weight (1253.9 g), which was significantly different from all other treatments, except the 0% M diet (1345 g). By day 35, the 5% CM-fed broilers had the lowest average live weight (1933.4 g), which was different from those fed all other diets. The response of the average live weight of the birds to increasing dietary inclusion levels of CM on days 7, 14, and 21 were significantly correlated as indicated by positive quadratic equations, as reported in Table 8. Hwangbo et al. (2008) indicated that Ross broilers fed house fly maggots (*Musca domestica*) did not differ in live weight until day 28, where the birds had a higher weight at 10 and 15% inclusion, and that birds fed the 5% inclusion level had the lowest live weight. These results were similar to the data reported in this study, indicating that a lower dietary inclusion level of CM led to lower body weight in relation to the control and higher dietary levels of CM (>5%).

There was a significant difference in ADG during the starter phase (Table 7). Birds fed 20% CM had the highest ADG at 37.9 g/day, which was significantly higher than the 5% CM at 31.0 g/day. When the chickens were switched to the grower diets, the 20% CM diet had the lowest ADG at 77.4 g/day compared to the 0% NM, 0% M, and 10% CM diets (89.9, 85.5, and 84.5 g/day, respectively). Following the finisher phase, the ADG of birds fed the 20% diet (96.2 g/day) was no different from those fed the other diets, except for the 0% NM (102.6 g/day), indicating possible compensatory growth. The

total ADG showed that the 5% CM diet (54.1 g/day) underperformed in comparison to all other treatments ($P<0.05$). There was a significant positive relationship associated with increasing dietary inclusion levels of CM during the starter phase (Table 8). However, during the grower and finisher phases, a negative relationship was associated with increasing dietary CM levels.

There was a significant difference among treatments in the ADFI during the finisher phase. The highest ADFI was observed in broilers fed the 10% CM diet with a 155.5 g/day value, which was significantly different from the 15 and 20% CM diets (119.2 g/day and 115.0 g/day). As the chickens aged, FCR decreased, except the 10% CM, which went from 1.7 in the starter phase, down to 1.4 during the grower phase, and back up to 1.6 in the finisher phase. This result was significantly different from broilers fed the 15 and 20% CM diets in the finisher phase (1.3 and 1.2). The FCR of broilers increased with age, suggesting that 10% dietary inclusion of CM may offer benefit in terms of feed conversion during the grower phase (Chiba, 2013). Throughout the trial, PER increased with the dietary inclusion level of CM, but the PER of broilers fed the 5% diet (2.9) was significantly higher than all the other diets during the starter phase. These results contradicted Wang et al. (2005), who found no difference in the weight gain, ADFI, and gain to feed ratio of the Arbor Acres broilers fed field crickets up to 15% inclusion. In another study, crossbred broilers fed a diet containing house crickets (*Brachytrupes portentosus*) had a higher weight gain and higher gain to feed ratio than the SBM control diet (Razak et al., 2012). The study also found that house crickets had a higher PER than SBM and suggested that the difference was due to the higher protein content and lower amino acid digestibility of the crickets (Razak et al., 2012). The

variation observed in the results could be due to different insect species, rearing conditions, or the broiler stock variation (Cullere et al., 2016; Wang et al., 2005).

Table 7: Average live weight, average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR), protein efficiency ratio (PER), and mortality of broiler chickens fed cricket meal intake, related parameters of broiler chickens fed cricket meal

	0% NM	0% M	5% CM	10% CM	15% CM	20% CM	SEM	P-value
Average live weight (g)								
0	39.4	39.2	38.9	39.1	39.4	39.0	0.16	0.63
7	136.7^{b}	125.8^{c}	127.5^{c}	143.2^{ab}	147.3^{a}	149.9^{a}	2.08	0.00
14	362.6^{bc}	349.0^{c}	306.8^{d}	383.0^{ab}	390.8^{ab}	405.8^{a}	7.32	0.00
21	767.9^{abc}	756.2^{abc}	689.4^{c}	745.6^{bc}	808.7^{ab}	834.3^{a}	12.02	0.00
28	1377.6^{a}	1344.9^{a}	1253.9^{b}	1390.1^{a}	1363.9^{a}	1376.2^{a}	12.03	0.00
35	2095.6^{a}	2043.5^{ab}	1933.4^{b}	2063.5^{a}	2024.9^{ab}	2049.3^{ab}	14.90	0.02
ADFI (g)								
Starter	62.1	61.4	59.1	62.4	64.9	65.2	0.95	0.46
Grower	130.7	136.6	115.7	112.4	112.6	123.8	3.48	0.21
Finisher	149.6^{ab}	131.4^{abc}	131.2^{abc}	155.5^{a}	119.2^{bc}	115.0^{c}	4.16	0.01
Total	93.4	90.4	84.8	91.0	85.3	86.9	1.10	0.12
ADG (g)								
Starter	33.8^{bcd}	33.7^{cd}	31.0^{d}	36.2^{abc}	36.7^{ab}	37.9^{a}	0.56	0.00
Grower	89.9^{a}	85.5^{ab}	80.6^{cd}	84.5^{bc}	79.3^{d}	77.4^{d}	0.98	0.00
Finisher	102.6^{a}	99.8^{ab}	97.1^{ab}	96.2^{ab}	94.5^{b}	96.2^{b}	0.81	0.02
Total	58.7^{a}	57.3^{a}	54.1^{b}	57.8^{a}	56.8^{a}	57.4^{a}	0.39	0.00
FCR								
Starter	1.743	1.822	1.906	1.727	1.773	1.722	0.03	0.28
Grower	1.456	1.596	1.528	1.426	1.417	1.599	0.04	0.57
Finisher	1.459^{ab}	1.317^{ab}	1.349^{ab}	1.617^{a}	1.263^{b}	1.196^{b}	0.04	0.01
Total	1.589	1.578	1.567	1.574	1.502	1.513	0.02	0.45
PER								
Starter	2.4^{b}	2.4^{b}	2.9^{a}	2.3^{b}	2.3^{b}	2.3^{a}	0.06	0.00
Grower	3.2	2.9	3.1	3.3	3.1	2.6	0.10	0.43
Finisher	3.4^{ab}	3.6^{a}	3.4^{ab}	2.9^{b}	3.5^{a}	3.5^{a}	0.07	0.02
Total	2.7^{b}	2.7^{b}	3.5^{a}	2.5^{b}	2.6^{b}	2.7^{b}	0.07	0.00
Mortality (%)								
Starter	4.6	1.0	2.8	2.8	0.9	1.9	0.66	0.63
Grower	1.3	0.0	0.0	0.0	0.0	1.3	0.30	0.56
Finisher	0.0	0.0	0.0	0.0	0.0	0.0	0.00	1.00

^{a,b,c,d,e,f,g,h} Rows and columns with different letters differed significantly ($P<0.05$).
NM=Non-medicated, M= medicated, CM=cricket meal, SEM= standard error of the mean, ADFI=average daily feed intake, ADG=average daily gain, FCR=feed conversion ratio, PER=protein efficiency ratio.

Table 8: Linear and quadratic regression of average live weight, average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR), protein efficiency ratio (PER), and mortality with *P* and r^2 values

Parameter	Regression	Equation	*P*	r^2
Average live weight (g)				
0	Linear	$y = - 0.126x + 39.276$	0.461	0.056
	Quadratic	$y = 0.055x^2 - 0.504x + 39.761$	0.687	0.080
7	Linear	$y = 3.880x + 126.276$	0.016	0.455
	Quadratic	$\mathbf{y = 0.994x^2 - 2.952x + 135.053}$	0.035	0.524
14	Linear	$y = 14.057x + 315.782$	0.029	0.393
	Quadratic	$\mathbf{y = 6.067x^2 - 27.655x + 369.376}$	0.024	0.563
21	Linear	$y = 23.597x + 675.893$	0.032	0.384
	Quadratic	$\mathbf{y = 12.119x^2 - 59.719x + 782.941}$	0.013	0.619
28	Linear	$y = 6.892x + 1325.111$	0.530	0.041
	Quadratic	$y = 11.164x^2 - 69.863x + 1423.729$	0.218	0.287
35	Linear	$y = - 2.978x + 2043.939$	0.823	0.005
	Quadratic	$y = 14.510x^2 - 102.734x + 2172.109$	0.208	0.295
ADFI (g)				
Starter	Linear	$y = 0.025x + 664.359$	0.967	0.000
	Quadratic	$y = 0.624x^2 - 4.266x + 69.872$	0.266	0.255
Grower	Linear	$y = - 4.177x + 136.293$	0.202	0.157
	Quadratic	$y = 3.175x^2 - 26.005x + 164.338$	0.127	0.367
Finisher	Linear	$y = - 4.075x + 149.048$	0.190	0.165
	Quadratic	$y = - 1.097x^2 + 3.466x + 139.360$	0.193	0.381
Total	Linear	$y = - 1.35x + 95.684$	0.058	0.314
	Quadratic	$y = 0.90x^2 - 7.067x + 102.663$	0.051	0.484
ADG (g)				
Starter	Linear	$y = 1.132x + 30.732$	0.023	0.420
	Quadratic	$\mathbf{y = 0.393x^2 - 1.571x + 34.204}$	0.031	0.537
Grower	Linear	$y = - 2.386x + 91.488$	0.000	0.747
	Quadratic	$\mathbf{y = 0.406x^2 - 5.180x + 95.077}$	0.001	0.797
Finisher	Linear	$y = - 1.410x + 102.690$	0.056	0.319
	Quadratic	$y = 0.478x^2 - 4.696x + 106.911$	0.098	0.403
Total	Linear	$y = - 0.080x + 57.274$	0.833	0.005
	Quadratic	$y = 0.413x^2 - 2.918x + 60.920$	0.213	0.291
FCR				
Starter	**Linear**	$\mathbf{y = - 0.061x + 2.081}$	0.029	0.394
	Quadratic	$y = 0.002x^2 - 0.044x + 2.060$	0.104	0.395
Grower	Linear	$y = - 0.007x + 1.486$	0.859	0.003
	Quadratic	$y = 0.031x^2 - 0.222x + 1.762$	0.446	0.164
Finisher	Linear	$y = - 0.022x + 1.456$	0.436	0.062
	Quadratic	$y = - 0.019x^2 + 0.108x + 1.289$	0.448	0.164
Total	**Linear**	$\mathbf{y = - 0.026x + 1.671}$	0.022	0.424
	Quadratic	$y = 0.002x^2 - 0.042x + 1.690$	0.079	0.431
PER				
Starter	Linear	$y = - 0.006x + 2.350$	0.896	0.002
	Quadratic	$y = - 0.041x^2 - 0.274x + 1.990$	0.415	0.177
Grower	Linear	$y = - 0.012x + 3.067$	0.902	0.002
	Quadratic	$y = - 0.067x^2 + 0.449x + 2.473$	0.597	0.108
Finisher	Linear	$y = 0.006x + 3.358$	0.915	0.001
	Quadratic	$y = 0.053x^2 - 0.361x + 3.830$	0.364	0.201
Total	Linear	$y = - 0.048x + 2.917$	0.412	0.068
	Quadratic	$y = - 0.055x^2 + 0.331x + 2.430$	0.226	0.281

5.4.2: Internal morphology

The average body weight of the birds fed the 5% CM diet was lower on day 13 and 20 (290.6 g and 668.4 g) than those fed the other dietary treatments, which could have been caused by the lower protein content in this test diet. By the last sampling date, all treatments had similar weights (Table 9). Sex did influence the weights of the birds, with males weighing more (2233.6 g) than females (1943.6 g) ($P<0.05$). This sexual dimorphism is in agreement with previous research (Mignon-Grasteau et al., 1998). There was a significant difference in liver organ index on day 13, where the 20% CM-fed broilers (3.6%) had a significantly higher liver organ index than broilers fed the 10% (3.3%) diet. A significant difference was observed organ index of the small intestine of the chickens fed 5% CM (7.9%) on day 20 compared to all other treatments. Ballitoc and Sun (2013) suggested that the increase in the heart and small intestine weight of broilers fed mealworms up to a dietary inclusion level of 10% indicates improved muscle and fat mass. Other studies found a significantly higher length and weight of the gastrointestinal tract of broiler chickens fed diets containing 30% mealworms (*Tenebrio molitor*) (Bovera et al., 2016). The length and weight of the small intestine can increase when a diet has a low protein digestibility, explaining the increase in small intestine weight in Table 9 (Bovera et al., 2016). Bovera et al. (2016) suggested that the increase in small intestine length was due to the indigestible chitin content in insects. The broiler chickens fed higher dietary inclusion levels of CM had lower intestinal weights, suggesting that the CM was more digestible than the SBM included in the 0% NM, 0% M, and 5% CM diets, although future research will be required to confirm this.

On day 13, the 10% CM-fed birds had an ileal pH of 6.4 versus all the other diets ($P<0.05$). When 0.1 and 0.2% *Gryllodes sigillatus* and *Gryllus assimilis* were fed to

female Ross 308 birds, they decreased the digesta and caecal pH, which was the opposite of this study (Józefiak et al., 2018). Lesion scores in Table 11 indicate no significant difference among treatments on days 21 and 35. The chi-square results for observations also show no difference between the observation dates. There was no significant difference in treatments, but it is of interest that on day 35, broilers fed the 20% CM diet had the highest frequency of 0 scores in comparison to the other treatments (Figure 2). These results indicate that CM (at any dietary inclusion level) was as effective as the medicated diet at reducing intestinal lesions. Future studies could challenge the birds in order to test the effectiveness of CM at reducing intestinal lesions.

Table 9: Body weight, crop, liver, pancreas, spleen, and bursa (as reported as a % of body weight) of broiler chickens fed the experimental diets

	Sex		Diet						SEM		P-value		
Day	**F**	**M**	**0% NM**	**0% M**	**5% CM**	**10% CM**	**15% CM**	**20% CM**	**Sex**	**Diet**	**Sex x diet**	**Sex**	**Diet**
Average body weight (g)													
13	347.6	354.4	358.6^{bc}	331.1^{d}	290.6^{e}	353.6^{cd}	387.0^{a}	382.3^{ab}	5.39	7.22	0.54	0.11	0.00
20	718.0^{b}	774.0^{a}	753.6^{abc}	715.0^{bc}	668.4^{c}	804.5^{ab}	761.6^{ab}	829.0^{a}	11.01	13.45	0.14	0.03	0.00
35	1943.6^{b}	2233.6^{a}	2160.0	2100.6	2063.8	2126.8	2125.3	2082.6	31.93	20.60	0.53	0.00	0.85
Crop (% of body weight)													
13	1.0^{b}	1.4^{a}	0.9^{ab}	1.4^{ab}	1.5^{a}	0.7^{b}	1.5^{a}	1.1^{ab}	0.29	0.09	0.43	0.02	0.02
20	0.7	0.7	0.7	0.7	0.7	0.9	0.6	0.8	0.04	0.04	0.51	0.90	0.42
35	0.7	0.7	0.8	0.5	0.9	0.6	0.5	0.6	0.05	0.06	0.10	0.54	0.21
Liver (% of body weight)													
13	3.5	3.3	3.5^{ab}	3.2^{b}	3.5^{ab}	3.3^{ab}	3.4^{ab}	3.6^{a}	0.06	0.04	0.03	0.71	0.05
20	2.8	2.7	2.9^{a}	2.7^{ab}	2.8^{a}	2.5^{b}	2.7^{ab}	2.7^{ab}	0.03	0.03	0.08	0.35	0.01
35	2.2	2.0	2.0	2.0	2.2	2.1	2.1	2.1	0.03	0.03	0.09	0.05	0.68
Pancreas (% of body weight)													
13	0.5	0.6	0.5	0.4	0.5	0.5	0.5	0.5	0.06	0.01	0.21	0.37	0.72
20	0.4^{a}	0.4^{b}	0.4	0.4	0.4	0.4	0.4	0.4	0.01	0.01	0.16	0.00	0.78
35	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.01	0.01	0.53	0.56	0.50
Spleen (% of body weight)													
13	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.00	0.00	0.11	0.86	0.09
20	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.00	0.01	0.24	0.68	0.74
35	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.00	0.00	0.97	0.68	0.54
Small intestine (% of body weight)													
13	8.9	8.8	9.0	9.1	8.7	9.3	8.7	8.3	0.10	0.11	0.09	0.67	0.11
20	7.3	7.0	7.1^{b}	7.0^{b}	7.9^{a}	6.7^{b}	6.9^{b}	6.8^{b}	0.08	0.11	0.57	0.07	0.00
35	4.7^{b}	5.0^{a}	4.9	4.7	4.9	4.7	4.9	4.8	0.07	0.05	0.34	0.04	0.43
Bursa (% of body weight)													
13	0.2^{b}	0.2^{a}	0.2	0.2	0.2	0.2	0.2	0.2	0.01	0.01	0.60	0.02	0.37
20	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.01	0.01	0.54	0.10	0.20
35	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.01	0.01	0.24	0.30	0.07
Ileal pH													
13	7.1^{a}	6.8^{b}	6.9^{a}	7.0^{a}	6.9^{a}	6.4^{b}	7.0^{a}	7.0^{a}	0.05	0.06	0.85	0.00	0.02

20	6.5	6.4	6.0	6.4	6.5	6.7	6.5	6.4	0.06	0.06	0.89	0.39	0.05
35	7.0[a]	6.7[b]	6.7	7.1	6.6	6.6	6.8	6.8	0.06	0.07	0.30	0.04	0.27

[a,b,c,d] Rows with different letters differed significantly (P<0.05). F= Female, M= male, NM= non-medicated, M= medicated, CM= cricket meal, SEM= standard error of the mean

Table 10: Linear and quadratic regression of body weight, crop, liver, pancreas, spleen, and bursa (as reported as a % of body weight) *P* and r^2 values

Parameter	Regression	Equation	*P*	r^2
Average body weight (g)				
13	Linear	$y = 11.750x + 310.750$	0.095	0.253
	Quadratic	**$y = 7.483x^2 - 39.697x + 376.851$**	0.048	0.492
20	Linear	$y = 18.271x + 688.806$	0.072	0.289
	Quadratic	$y = 8.000x^2 - 36.727x + 759.469$	0.088	0.417
35	Linear	$y = -22.760x + 2199.597$	0.294	0.109
	Quadratic	$y = 19.108x^2 – 154.128x + 2368.385$	0.217	0.288
Crop (% of body weight)				
13	Linear	$y = - 0.013x + 1.242$	0.878	0.002
	Quadratic	$y = - 0.045x^2 + 0.300x + 0.841$	0.701	0.076
20	Linear	$y = - 0.035x + 0.901$	0.402	0.071
	Quadratic	$y = - 0.007x^2 + 0.012x + 0.543$	0.695	0.078
35	Linear	$y = - 0.062x + 1.032$	0.215	0.149
	Quadratic	$y = 0.018x^2 - 0.186x + 1.192$	0.413	0.178
Liver (% of body weight)				
13	Linear	$y = - 0.030x + 3.623$	0.583	0.031
	Quadratic	$y = 0.004x^2 - 0.056x + 3.656$	0.863	0.032
20	Linear	$y = - 0.050x + 2.903$	0.054	0.323
	Quadratic	$y = 0.019x^2 - 0.182x + 3.072$	0.078	0.432
35	Linear	$y = 0.031x + 1.927$	0.359	0.084
	Quadratic	$y = - 0.026x^2 + 0.209x + 1.699$	0.326	0.221
Pancreas (% of body weight)				
13	Linear	$y = 0.006x + 0.455$	0.500	0.047
	Quadratic	$y = 0.000x^2 + 0.008x + 0.452$	0.804	0.047
20	Linear	$y = 0.003x + 0.373$	0.504	0.046
	Quadratic	$y = 0.005x^2 - 0.029x + 0.414$	0.246	0.268
35	Linear	$y = - 0.002x + 0.295$	0.682	0.017
	Quadratic	$y = 0.002x^2 - 0.018x + 0.315$	0.754	0.061
Spleen (% of body weight)				
13	Linear	$y = 0.001x + 0.087$	0.775	0.009
	Quadratic	$y = 0.001x^2 - 0.005x + 0.094$	0.873	0.030
20	Linear	$y = 0.001x + 0.070$	0.503	0.046
	Quadratic	$y = 0.001x^2 - 0.004x + 0.077$	0.692	0.079
35	Linear	$y = 0.001x + 0.084$	0.507	0.045
	Quadratic	$y = - 0.002x^2 + 0.016x + 0.066$	0.291	0.240
Small intestine (% of body weight)				
13	Linear	$y = - 0.086x + 9.038$	0.367	0.082
	Quadratic	$y = - 0.063x^2 + 0.350x + 8.478$	0.398	0.185
20	Linear	$y = - 0.134x + 7.502$	0.198	0.159
	Quadratic	$y = - 0.047x^2 + 0.189x + 7.086$	0.356	0.205
35	Linear	$y = - 0.053x + 5.130$	0.206	0.155
	Quadratic	$y = - 0.002x^2 - 0.040x + 5.113$	0.468	0.155
Bursa (% of body weight)				
13	Linear	$y = 0.001x + 0.192$	0.913	0.001
	Quadratic	$y = - 0.002x^2 + 0.017x + 0.171$	0.786	0.052
20	Linear	$y = 0.002x + 0.201$	0.710	0.014
	Quadratic	$y = 0.000x^2 + 0.000x + 0.204$	0.933	0.015
35	Linear	$y = 0.008x + 0.165$	0.304	0.105

	Quadratic	$y = -0.001x^2 + 0.017x + 0.154$	0.588	0.111
Ileal pH				
13	Linear	$y = -0.025x + 6.864$	0.700	0.015
	Quadratic	$y = 0.021x^2 - 0.168x + 7.047$	0.830	0.041
20	**Linear**	**$y = 0.094x + 6.150$**	0.025	0.408
	Quadratic	$y = -0.021x^2 + 0.235x + 5.968$	0.066	0.453
35	Linear	$y = 0.084x + 6.398$	0.070	0.291
	Quadratic	$y = 0.023x^2 - 0.073x + 6.600$	0.153	0.341

Table 11: Average lesion scores in broiler chickens fed experimental diets

Day	0% NM	0% M	5% CM	10% CM	15% CM	20% CM	SEM	P-value	Chi-square
20	0	0	0	0	0	0	0.19	0.57	0.38
35	1	1	1	1	1	0	0.29	0.09	0.04

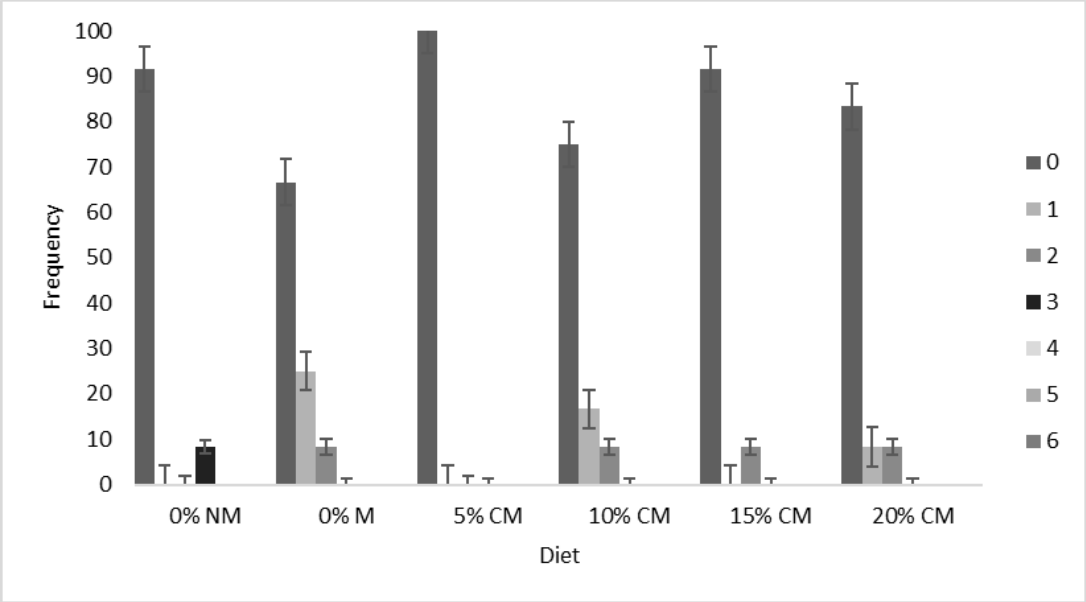

Figure 1: Frequency (%) of lesion scores of broiler chickens fed cricket meal on Day 20

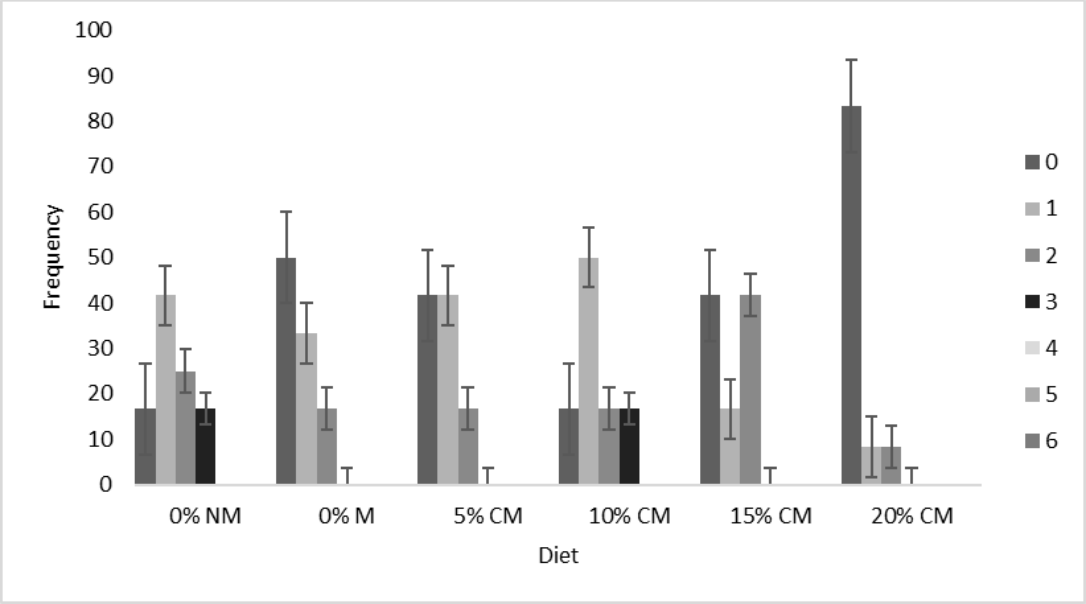

Figure 2: Frequency (%) of lesion scores of broiler chickens fed cricket meal on day 35

5.4.3: Meat quality

There was no significant difference among breast weights. This was similar to research in BSF, which found no difference in breast weight of broilers fed BSF at dietary inclusion levels of 10 and 15% (Schiavone et al., 2019). Bovera et al. (2016) found no change in the breast weight of broilers fed a 30% mealworm diet. The same study reported no change in the chicken breast colour, which was similar to the current study results provided in Table 12 (Bovera et al., 2016). Cullere et al. (2016) did find a difference in the redness (a*) of broiler quail breast meat, observing the highest value (1.13) in quail fed the highest dietary inclusion level of 15% BSF. Leiber et al. (2017) and Schiavone et al. (2019) found no change in the breast colour of broilers fed BSF. The redness (a*) and lightness (L*) of the chicken breast are the leading indicators of the light pinkish hue consumers desire, and all the treatments had an above-average red (a*) colouration (Qamar et al., 2019; Wideman et al., 2016). The lack of colour change in the CM diets is beneficial, since an off-colour (green (low a*) or pale (high L*)) will often deter consumers from purchasing a product (Cullere; et al., 2016; Qamar et al., 2019; Qiao et al., 2001; Wideman et al., 2016)

There was a difference observed in cooking loss between broilers fed the 10% diet (35.5% loss) and the 0% NM diet (31.9% loss). When fed 15% BSF, broilers displayed no change in breast meat cooking loss (Schiavone et al., 2019). However, feeding 30% mealworms showed similar results to the current study, and Bovera et al. (2016) found a significant increase in the cooking loss of breast meat. Cooking loss can negatively affect profitability for producers and processors when making value-added products, and the loss of moisture can negatively affect the sensory feel while consuming the meat (Northcutt, 1997). Breast meat texture, both raw and cooked, was not influenced by the

CM diets. Other studies have found an increase in breast meat shear force in broilers fed 15% BSF, which was associated with an increase in cooking loss (Cullere et al., 2016). In terms of visual and texture parameters, the results indicate that CM is suitable for use on a commercial scale at dietary inclusion levels up to 20%.

Table 12: Breast weight, % cook loss, breast colour, breast texture, and liver colour of broiler chickens fed experimental diets

	Diet							
	0% NM	**0% M**	**5% CM**	**10% CM**	**15% CM**	**20% CM**	**SEM**	**P-value**
Breast weight (% of live weight) (raw)								
	19.1	19.43	16.8	17.9	18.4	18.8	0.36	0.06
Breast % cook loss								
	31.9[b]	32.8[ab]	35.0[ab]	35.5[a]	34.1[ab]	33.0[ab]	0.37	0.02
Breast colour (raw)								
L*	56.0	58.0	59.0	57.7	57.9	57.7	0.41	0.60
a*	9.1	9.1	8.8	8.9	9.1	8.9	0.16	1.00
b*	20.8	20.3	21.4	19.8	19.8	19.6	0.44	0.85
Raw breast texture (g)								
	1147.0	1158.9	1245.3	1137.5	1088.3	1177.3	25.02	0.66
Cooked breast texture (g)								
	1942.3	1546.6	1827.6	1713.5	1769.2	1716.7	58.36	0.55
Liver colour								
L*	33.6	31.7	35.2	34.5	35.0	33.8	0.67	0.75
a*	16.3	17.8	16.2	17.2	16.6	17.3	0.21	0.20
b*	19.4	20.0	20.7	20.6	20.6	21.0	0.38	0.91

[a,b,c,d] Rows with different letters differed significantly ($P<0.05$).
NM=Non-medicated, M=medicated, CM=cricket meal, SEM= standard error of the mean, L*=lightness, a*=red/green, b*=yellow/blue.

Table 13: Linear and quadratic regression of breast weight, % cook loss, breast colour, breast texture, and liver colour *P* and r^2 values

Parameter	Regression	Equation	*P*	r^2
Breast weight (% of live weight) (raw)				
	Linear	$y = 0.106x + 17.692$	0.673	0.018
	Quadratic	**$y = 0.344x^2 - 2.257x + 20.728$**	0.048	0.467
Breast % cook loss				
	Linear	$y = 0.391x + 32.329$	0.357	0.085
	Quadratic	$y = - 0.541x^2 + 4.110x + 27.552$	0.061	0.463
Breast colour (raw)				
L*	Linear	$y = 0.219x + 56.223$	0.458	0.056
	Quadratic	**$y = - 0.408x^2 + 3.027x + 52.615$**	0.041	0.508
a*	Linear	$y = - 0.024x + 9.340$	0.866	0.003
	Quadratic	$y = 0.050x^2 - 0.370x + 9.785$	0.853	0.035
b*	Linear	$y = - 0.178x + 20.689$	0.714	0.014
	Quadratic	$y = - 0.537x^2 + 3.514x + 15.945$	0.190	0.309
Breast texture (raw)				
	Linear	$y = 11.429x + 1076.791$	0.629	0.024
	Quadratic	$y = - 11.318x^2 + 89.244x + 976.811$	0.689	0.079
Breast texture (cooked)				
	Linear	$y = - 35.619x + 1864.089$	0.484	0.050
	Quadratic	$y = - 9.525x^2 + 29.868x + 1779.948$	0.763	0.058
Liver colour				
L*	Linear	$y = - 0.493x + 37.251$	0.326	0.096
	Quadratic	$y = 0.124x^2 - 1.348x + 38.351$	0.590	0.111
a*	Linear	$y = 0.384x + 15.037$	0.068	0.296
	Quadratic	$y = 0.001x^2 + 0.379x + 15.044$	0.206	0.296
b*	Linear	$y = 0.162x + 20.380$	0.644	0.022
	Quadratic	$y = 0.225x^2 - 1.384x + 22.367$	0.560	0.121

5.5: Conclusion

The effect of dietary CM inclusion on growth parameters (average live weight, ADFI, ADG, FCR, and PER), internal morphology, and meat quality of broiler chickens was determined. The average live weight, ADFI, ADG, and FCR of the birds were significantly influenced by the CM, with 5% having the lowest growth performance level. CM did not influence the texture and colour of the breast meat, but the 10% CM diet did affect the cooking loss of breast meat, which could cause the product to become less desirable for consumers. The results indicated that there was no detrimental dietary impact on growth, meat quality, and internal morphology when CM is included at a dietary inclusion level up to 20% in an NM broiler chicken diet. More research is required to determine whether a dietary inclusion of >20% CM in broiler chicken diets will affect the growth, meat quality, and internal morphology of broilers.

CHAPTER 6: CONCLUSION AND FUTURE DIRECTION

This research provides key information for the potential use of crickets in broiler chicken diets, and demonstrates that CM provides a feasible feed source. Insect meal has an effective nutritional profile for use in poultry diets and BSFLM, OD-CM, and FD-CM are nutritionally comparable to traditional protein sources such as SBM and FM. This study reinforced the fact that insect species have differing nutritional compositions. FD-CM had a higher gross energy content and metabolizable energy than the BSFLM. The BSFLM calcium, magnesium, and potassium content were higher than both OD-CM and FD-CM. This difference could be due to species life cycles, physiology, or production methods, and all these factors need to be considered when selecting an insect to include in broiler chicken diets.

Processing method impacted the nutritional profile of the CM, but the CP digestibility was not lowered in the OD-CM as had been predicted. It was anticipated that the OD-CM was exposed to high heat during oven-drying, which would lower the nutritional value when compared to freeze-drying. The CP digestibility of FD-CM was lower than the OD-CM, but both had a significantly higher CP than the BSFLM, suggesting the heat exposure during the drying phase of OD-CM did not negatively affect CP digestibility. BSFLM, FD-CM, and OD-CM all had lower CP digestibility in comparison to literature values for SBM, which should be considered when insect meals are used as alternatives to current feed sources.

OD-CM had less fat than FD-CM as well as less available gross energy content. Freeze-drying did result in increased fat content, due to the lack of heat exposure, which could help with digestion of other nutrients, and reduce additional fats being added to broiler feeds. Storage of this product should be considered, as the peroxide levels

increased in the higher fat FD-CM. Cost of production should be considered if freeze-drying is to be used on a mass scale.

CM influenced the average live weight, ADG, ADFI, FCR, and PER of broiler chickens. There was a significant positive correlation between average live weight of the broilers fed CM and dietary inclusion levels of CM, with higher inclusion levels resulting in higher average live weights. A significant positive relationship was observed in ADG during the starter phase, and broilers fed the 20% CM diet had the highest ADG. However, during the grower and finisher phases, there was a negative relationship between ADG and dietary CM levels. Broilers fed the control diets and lower CM inclusion levels (5 and 10%) gained more weight for each kg of feed eaten. The lower weight gain could be due to the low digestibility of the CM compared to SBM that was in the control diets at a higher inclusion level than the test diets. Overall, CM had no detrimental effects on broiler chicken growth parameters at dietary inclusion levels up to 20%. More research on ideal incorporation level would effectively optimize this feed.

Examining the full effects of CM on broiler health could illuminate potential negative or positive impacts. There were differences in the crop, small intestine, and liver organ index of broiler chickens fed the CM diets. However, there were no effects at any tested inclusion levels of CM on other internal morphology parameters measured (bursa, spleen, and intestinal lesions). These results suggest no increased immune response of the birds fed CM, and the broilers fed CM diets were comparable to those fed the medicated diets. Further research on the influence of these diets on the gut microbiota and intestinal histology would illuminate whether additional health effects can result from CM dietary inclusion.

An objective was to investigate the dietary inclusion of CM on the meat quality of broiler chickens. Results from Chapter 5 indicate that CM does not affect the breast weight, colour, and texture of broiler breast muscle. The initial hypothesis was that CM would not negatively influence the meat quality of broiler chickens. However, broilers fed the 10% CM diets had a higher cooking loss than the 0% NM diet. Increased cooking loss affects meat quality and could negatively impact the sensory feel of meat. If the digestibility of the CM amino acid content were low, it would have affected muscle synthesis, and therefore affected the cooking loss. Determining the amino acid content and amino acid digestibility of *G. sigillatus* would help determine if this is what caused the increase in cooking loss.

Insects could be an environmentally friendly protein and energy source, but all aspects of their production and processing must be considered. Although the nutritional composition of BSFLM and CM was comparable to SBM, the digestibility of these insects was lower than reference values for SBM. The decreased digestibility and reduced available protein could be a reason for the effects CM had on the growth parameters and meat quality of broiler chickens. If indigestible protein is included in poultry diets, it will be excreted in the faeces, which negates the environmental gains insect production provides. Before CM can be thought of as the eco-conscious and effective protein and energy source, dietary inclusion levels (above and below the levels assessed in this study) should be assessed to find the optimal inclusion level.

The findings reported in this document can be used to move this potential feed ingredient forward, and research expanding this topic will be beneficial for the poultry industry and Canadian consumers. It is recommended that when incorporating CM into broiler chicken diets, the diet is formulated on a digestible nutrient basis. The formulated

diets and regression curves provided can be used by poultry producers to predict broiler performance when incorporating CM into broiler diets and will help determine the optimal dietary inclusion level of *G. sigillatus.* Insect type and processing method affect the nutritional composition of the insect meals, and it is vital for insect producers to understand this when producing insect meals for broiler chicken feeds. The nutritional composition and digestibility of BSFLM, OD-CM, and FD-CM can be used by insect producers to evaluate which processing method will be implemented. The OD-CM was the preferred insect product because of processing method, nutrient composition, and digestibility. When OD-CM was used in broiler chicken diets, it influenced the growth parameters, internal organs, and meat quality. However, the effects seen were not detrimental, and it is suggested that CM can be included in broiler diets at dietary inclusion levels up to 20%.

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APPENDICES

Appendix A: Nitrogen free diet inclusion and effects on broiler growth parameters

Ingredients as fed basis (%)	NF
Corn	-----
Corn starch	20
SBM	-----
Test ingredient	-----
Wheat	-----
Dextrose	63.6
Cellulose	5
Soybean oil	5
Tallow-grease blend	-----
Limestone ground	1.3
Dicalcium phosphate	1.9
Celite	0.8
Vitamin/mineral premix	0.5
Iodized salt	-----
Methionine premix	-----
Sodium hydrogen carbonate	0.8
Potassium chloride	0.3
Potassium carbonate	0.3
Magnesium oxide	0.2
Choline chloride	0.3
Total	100
Analyzed Results	
GE (kcal/kg)	3480.1
DM (%)	91.5
CP (%)	0.2
Growth Parameters	
Average weight (g)	
0	39
14	382.5
21	349.3
ADFI (g/day)	
Starter (0 – 14)	40.2
Grower (15 – 21)	62.6
ADG (g/day)	
Starter (0 – 14)	24.5
Grower (15 – 21)	-4.7
FCR	
Starter (0 – 14)	1.7
Grower (15 – 21)	-14

The nitrogen-free (NF) diet was included to measure the true ileal digestibility, but there was a limited amount of ileal contents, so the assay could not be completed. The data was included to demonstrate the effect of this diet on broiler chickens. The reduced ADFI in birds fed the NF diet indicated that the birds were not attracted to the feed, leading to weight loss. In the grower phase, the NF FCR was significantly lower (-14.0) than the basal diet. The visual spectrum of birds attracts them to colours like red, but the NF diet was white (Prescott and Wathes, 1999). The diet contained white diatomaceous earth instead of chromic oxide, which is used in digestibility trials and produces a green hue, which could attract chickens to this diet because of their visual spectrum (Prescott and Wathes, 1999). The texture of the diet was also soft and powder-like, which could have influenced the feed intake of the broiler chickens. It is suggested that in test NF diets used for experimental diets, pelleting, or addition of attractants be employed to try to mitigate the reduction in ADFI.

Appendix B: Mortality reports of broiler chickens fed basal, black soldier fly larvae meal (BSFLM), oven-dried cricket meal (OD), freeze-dried cricket meal (FD), and nitrogen-free (NF) digestibility diets

Date	Age (day)	Weight (g)	Death	Notes	Necropsy
NF					
2018/18/08	5	103	Found dead during morning feeding.	Male. The carcass is in normal neonatal body condition with a full digestive tract. The yolk sac is distended, hyperemic with prominent vessels, and filled with watery brown contents.	Omphalitis/yolk sac infection.
2018/28/08	15	438	Found with bloody wings that looked damaged, so culled.	Focal hemorrhages are noted on the wing tips, with dried blood on the skin surface and mild subcutaneous hemorrhage and edema No other abnormal findings are identified at gross post-mortem. Histology: The following tissues show no significant abnormal findings: Heart, Lung, Liver, Kidney, Bursa, Brain.	Skin, hemorrhage multifocal (wing tips), acute, mild to moderate. Other than focal trauma to wing tips, no other lesions are identified in this bird. No evidence of infectious or inflammatory disease is present- possible trauma from other birds or equipment in the facility?
BSFLM					
2018/23/08	10	75	Small and weak, so called.	Male. The carcass is in normal neonatal body condition with an empty digestive tract. Pectoral musculature is markedly pale. The yolk sac is distended, hyperemic with prominent vessels, and filled with watery brown contents.	Omphalitis/yolk sac infection.
FD					
2018/26/08	13	391	Found dead during morning feeding.	Bird is in moderate to good body condition, with moderate amount of subcutaneous and visceral adipose tissue. No visible abnormal findings are noted at gross post-mortem. Histology: Lung: Moderate congestion. Small amounts of mucus are noted in secondary bronchi. Bursa: Moderate lympholysis is noted. The following tissues show no significant abnormal	No primary cause of death identified.

				findings: Heart, Spleen, Liver, Kidney.	

Omphalitis/Yolk Sac peritonitis: Multisystemic fibrinous or heterophilic inflammation is most likely due to bacterial infection originating in the yolk sac and E. coli is most often isolated from these infections. This infection may cause sudden death in birds during the early post-hatching period or may cause stunting, weakness or additional deaths in the first weeks of life if birds survive the initial insult.

Appendix C: Mortality reports of broiler chickens fed cricket meal at dietary inclusion levels of 0, 5, 10, 15 and 20%

Date	Age (day)	Weight (g)	Death	Symptoms	Necropsy
0% NM					
2019/06/01	3	56.2	Found dead	Pericardial sac is filled with a moderate amount of loosely adherent fibrin. Vessels on the surface of the yolk sac are prominent.	Pericarditis, fibrinous, moderate to severe, acute.
2019/07/01	4	46	Found dead	Yolk sac is enlarged, vessels are prominent, and the surface is covered by a thin layer of fibrin. Navel is reddened.	Omphalitis, fibrinous, acute.
2019/11/01	8	89	Found dead	Yolk sac is enlarged, vessels are prominent, and the surface is covered by a thin layer of fibrin. Navel is reddened. Abdomen contains a moderate amount of cloudy red/tan fluid and flecks of fibrin. Pericardial sac is filled with a moderate amount of loosely adherent fibrin.	Peritonitis, pericarditis, fibrinous, moderate to severe, acute.
2019/29/01	26	1204	Found dead	Heart has an elongated contour. Bilaterally lungs show moderate to severe congestion and mild edema. Spleen is mildly enlarged, and petechial hemorrhages are visible on the capsular surface. Spleen: Light growth *E. coli*, *Enterococcus sp*.	Flip Over or Sudden Death Syndrome.

				Probable Sudden Death Syndrome; bacteria isolated considered post-mortem contaminants in this case (low numbers and multiple species isolated).	
0% M					
2019/25/01	22	293	Culled- trouble walking, small	Abdomen contains a moderate amount of cloudy red/tan fluid and flecks of fibrin. A thick layer of fibrin is partially adherent over the capsular surface of the liver and similar material is noted within the pericardial sac, with moderately firm adhesions. An approximately 2.5 cm x 1 cm yolk sac remnant is present, material is red and inspissated with fibrin adherent over the surface.	Polyserositis, fibrinous, moderate to severe, acute
5%					
2019/05/01	2	43.5	Found dead	Yolk sac is enlarged, vessels are prominent, and the surface is covered by a thin layer of fibrin. Navel is reddened.	Omphalitis, fibrinous, acute.
2019/08/01	5	50	Found dead	Yolk sac is enlarged, vessels are prominent, and the surface is covered by a thin layer of fibrin. Navel is reddened. Abdomen contains a moderate amount of cloudy red/tan	Pericarditis, peritonitis, fibrinous, moderate to severe, acute Omphalitis, fibrinous, acute.

				fluid and flecks of fibrin. Pericardial sac is filled with a moderate amount of loosely adherent fibrin.	
2019/08/01	5	48	Found dead	Yolk sac is enlarged, vessels are prominent, and the surface is covered by a thin layer of fibrin. Navel is reddened. Abdomen contains a moderate amount of cloudy red/tan fluid and flecks of fibrin. Pericardial sac is filled with a moderate amount of loosely adherent fibrin.	Pericarditis, peritonitis, fibrinous, moderate to severe, acute Omphalitis, fibrinous, acute.
10%					
2019/05/01	2	45.3	Found dead	Yolk sac is enlarged, vessels are prominent, and the surface is covered by a thin layer of fibrin. Navel is reddened. Swab, yolk sac: E. coli heavy growth.	Omphalitis, fibrinous, acute.
2019/21/01	18	200	Culled- small & weak	Subcutaneous tissue over the ventral abdomen is moderately edematous, with small amounts of fibrin, particularly in the region adjacent to the liver. Abdomen contains a moderate amount of cloudy red/tan fluid and flecks of fibrin. A thick layer of fibrin is partially adherent over the capsular surface of the liver.	Peritonitis, perihepatitis, fibrinous, moderate to severe, acute to subacute. Cellulitis, locally extensive, fibrinous, acute
15%					

2019/05/01	2	34.8	Found dead	Undersized bird Gastrointestinal tract is empty. No other significant abnormal findings.	Maladjustment.
2019/07/01	4	57	Found dead	Pericardial sac is filled with a moderate amount of loosely adherent fibrin. Vessels on the surface of the yolk sac are prominent. Swab, pericardial sac: *E. coli* heavy growth.	Pericarditis, fibrinous, moderate to severe, acute.
20%					
2019/05/01	2	47.2	Found dead – twister neck	Yolk sac is enlarged, vessels are prominent, and the surface is covered by a thin layer of fibrin. Navel is reddened. No abnormalities identified in neck or head	Omphalitis, fibrinous, acute.
2019/06/01	3	60.7	Found dead	Yolk sac is enlarged, vessels are prominent, and the surface is covered by a thin layer of fibrin. Navel is reddened. The abdomen contains a moderate volume of cloudy red/tan fluid with small flecks of fibrin.	Omphalitis, peritonitis, fibrinous, acute.

"Flip Over" or Sudden Death Syndrome. This syndrome occurs in broiler type birds, most often between the ages of 2-3 weeks. The pathogenesis is incompletely understood and may involve aspects of bird genetics, nutrition, and environmental factors.

Pericarditis is inflammation of the pericardium (the fibrous sac surrounding the heart).

Perihepatitis is inflammation of the serous or peritoneal coating of the liver.

Omphalitis/Yolk Sac peritonitis: Multisystemic fibrinous or heterophilic inflammation is most likely due to bacterial infection originating in the yolk sac and *E. coli* is most often isolated from these infections. This infection may cause sudden death in birds during the early post-hatching period or may cause stunting, weakness or additional deaths in the first weeks of life if birds survive the initial insult.