BLACK SOLDIER FLY LARVAE AS AN ALTERNATIVE PROTEIN SOURCE FOR CANINE AND FELINE DIETS

BY

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DISSERTATION

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ABSTRACT

The pet food industry continues to grow because of increased disposable income and the importance of the human-animal bond. Pet owners are motivated to find foods perceived as being safer, of high quality, and being sustainable. This has resulted in an increased preference for novel ingredients. Black soldier fly larvae (BSFL, *Hermetia illucens*)-derived ingredients have been identified as alternatives to traditional protein sources for dogs and cats. However, the current scientific literature on the use of BSFL in pet food is limited.

The overall objective of this dissertation was to evaluate the nutrient composition of BSFL, its amino acid (AA) digestibilities using a precision-fed cecectomized rooster model, and its effects on palatability, apparent total tract digestibility (ATTD), fecal characteristics, and skin and coat health markers of healthy adult cats. Our first aim was to determine the effects of harvest age on nutrient and AA digestibility and digestible indispensable amino acid score (DIAAS)-like values of BSFL using the precision-fed cecectomized rooster assay. The BSFL were harvested at six different ages (days after hatch; day 0, 11, 14, 18, 23, and 29). Our second aim was to evaluate the effects of BSFL dietary calcium form and concentration on nutrient composition, nutrient and AA digestibilities, and DIAAS-like values for BSFL using the precision-fed cecectomized rooster assay. Calcium chloride ($CaCl_2$) and calcium carbonate ($CaCO_3$) were used to raise BSFL at different concentrations (1.2% of $CaCl_2$, 1.2% of $CaCO_3$, 0.75% of $CaCO_3$, and 0.6% of $CaCl_2$ and $CaCO_3$). Our third aim was to determine palatability and ATTD of BSFL-containing canned diets, fecal characteristics, and skin and coat health markers of healthy adult cats consuming them. Cats were fed the canned diets formulated with poultry by-product meal (PBPM), BSFL meal, whole BSFL, and BSFL oil.

In our first aim, we determined that all harvest ages of BSFL were contained high-quality protein that were well digested, but AA digestibilities were highest at days 14, 18, and 23. Threonine, Met, Cys, and Arg often were the first-limiting AA of BSFL based on DIAAS-like reference values for dogs and cats. In our second aim, we determined that nutrient and AA digestibilities were high (81% to 96% digestibilities), but not different among BSFL fed different calcium sources and concentrations. Aromatic AA (Phe + Tyr) and sulfur-containing AA (Met + Cys) often were the first-limiting AA based on DIAAS-like reference values for dogs and cats.

In our third aim, we reported that the intake ratios were higher in cats fed canned diets containing BSFL meal (1.93:1), whole BSFL (2.03:1), and BSFL oil (1.57:1) compared with a poultry-based control diet. Fecal pH and scores and caloric intake were not different ($P > 0.05$) among diets, but fecal output (as-is, DM, and kcal/d) was highest ($P < 0.05$) for cats fed BSFL meal compared with those fed BSFL oil. The ATTD of acid-hydrolyzed fat (AHF) was not different among treatments, while DM ATTD was greater ($P < 0.05$) for cats fed the BSFL oil diet than for those fed the BSFL meal diet. The ATTD of OM by cats fed control or BSFL oil diets was greater ($P < 0.05$) than for those fed the BSFL meal or BSFL whole diets. For crude protein (CP) and energy, ATTD was greatest ($P < 0.05$) for cats fed the BSFL oil diet and lowest for those fed the BSFL meal diet. Skin and coat health markers, including skin transepidermal water loss (TEWL), skin hydration status, hair imaging score, and skin and coat hair scores were not affected ($P > 0.05$) by treatments. Similarly, hematology and a delayed-type hypersensitivity (DTH) response to saline, phytohaemagglutinin (PHA), and concanavalin A (CONA) showed no differences ($P > 0.05$) among diets. A select serum metabolites were affected by diet ($P < 0.05$), but remained within reference ranges.

This research provided information on the potential for using BSFL in pet foods. Based on our results, the suggested harvest age of BSFL ranges between 14 and 23 days because these ages provide the highest protein quality. Black soldier fly larvae raised with calcium chloride and calcium carbonate accumulate more calcium, but protein quality was similar. Finally, BSFL-derived ingredients hold strong potential for use in pet foods, whether it is included in extruded or canned foods. Further research is needed to determine the optimal concentration of BSFL-derived ingredients in canine and feline diets.

To My Parents and My Wife

Thank you for your endless love and encouragement.

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TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

Pet animals are increasingly regarded as family members and many pet food trends follow human food trends that focus on health and wellness (Sharon et al., 2018). Also, the growing world population is putting greater pressure on the global food production system. Environmental, ethical, and economic concerns about the way we currently produce livestock (beef, chicken, pork, lamb, etc.) are adding to this pressure. Because of this, pet owners are beginning to recognize that sustainability is an important factor when deciding what food to purchase for their animals (Acuff et al., 2021). These views by pet owners have led to the exclusion of certain ingredients, such as animal by-products, corn, soy, and artificial additives. Those ingredients are considered to be lower quality or of poor nutritional value for animals by many pet owners (Carter et al., 2014). Insect-based ingredients can serve as alternative protein and fat sources for dogs and cats because they may appeal to consumer demands for efficient resource use such as low water footprint, land use, and greenhouse gas production (de Vries and de Boer, 2010; Oonincx and de Boer, 2012; Miglietta et al., 2015).

Whole dried black soldier fly larvae (BSFL; *Hermetia illucens*) and BSFL meal were tentatively approved by the Association of American Feed Control Officials (AAFCO, 2021; ingredient 60.117) for use in adult maintenance dog foods and treats. This means that in 2022, pet food manufacturers in the U.S. can use BSFL as a novel ingredient in dog foods and treats. More research is needed to obtain approval for use in cat foods.

Nutritional composition of BSFL depends on the quality of ingested food [crude protein (CP): 38.5 to 47.9%; crude fat: 14.6 to 39.2%] (Zheng et al., 2012; Bosch et al., 2014; Nguyen et al., 2015). Also, harvest age of BSFL may be an important factor because it may lead to fluctuations in nutritional components (Liu et al., 2017). This variation suggests that it is important to evaluate how nutrient content is affected by dietary factors and harvest age of BSFL. Thus, Chapter 3 evaluated different harvest ages of BSFL and Chapter 4 evaluated different dietary calcium forms and concentrations of BSFL on nutrient composition, nutrient and amino acid (AA) digestibilities, and digestible indispensable amino acid score (DIAAS)-like values of BSFL intended for use in pet foods. It was hypothesized that the nutrient and AA digestibilities would be highest for larval stages (day 0; day 11; day 14 and day18) and lowest for pupa stages (day 23 and day 29). However, nutrient and AA digestibility would not be affected by the form or concentration of calcium in the BSFL diet.

Previous studies in pets (Beynen, 2018; Kröger et al., 2020; El-Wahab et al., 2021; Freel et al., 2021) focused on nutrient digestibility, palatability, immune response, and serum metabolites and hematology of dogs fed BSFL-containing extruded diets. For cats, only two studies have evaluated the nutrient digestibility, serum metabolites, and hematology of cats fed BSFL meal-containing extruded diets (Paßlack and Zentek, 2018; Pezzali and Shoveller, 2021). Therefore, Chapter 5 determined the effects of including BSFL meal, whole BSFL, and BSFL oil in canned cat diets on palatability, apparent total tract macronutrient digestibility (ATTD), fecal characteristics, and skin and coat health markers of healthy adult cats. It was hypothesized that when compared with a poultry-based control canned diet, the BSFL-containing canned diets would not negatively affect palatability, ATTD, or fecal characteristics, and that skin and coat health markers would be improved in healthy adult cats.

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CHAPTER 2: LITERATURE REVIEW

IMPORTANCE OF PROTEIN AND AMINO ACIDS IN PET HEALTH

Proteins are composed of one or more long chains of amino acid (AA) residues linked together by peptide bonds. Amino acids are used to synthesize important molecules such as purines, pyrimidines, heme, and various hormones, neurotransmitters, and neuromodulators (NRC, 2006; Monirujjaman and Ferdouse, 2014). There are ten indispensable AA (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) that dogs and cats need to obtain from their diet to maintain normal tissue metabolism and/or growth rate. In addition to these indispensable AA, the cat requires taurine (beta-sulfonic acid) because two essential enzymes (i.e., cysteine dioxygenase and cysteine sulfinic acid decarboxylase) are too low in concentration to synthesize sufficient taurine for health maintenance (Morris et al., 1990). A shortage of taurine induces cardiomyopathy (a severe form of heart disease) and central retinal degeneration and blindness (Sturman, 1993). The remaining twelve AA (alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, hydroxylysine, hydroxyproline, proline, serine, and tyrosine) provide nitrogen and carbon for the synthesis of dispensable AA, glucose synthesis via gluconeogenesis, and serve as structural components for synthesis of other compounds (NRC, 2006).

Many studies have demonstrated that when dogs and cats are fed a diet deficient in any of the indispensable AA, reduced food intake and body weight, decreased plasma AA concentrations, negative nitrogen balance in the body, increased severity of infections (impaired immune function), and loss of muscle mass have occurred (Milner, 1979; Rogers and Morris, 1979; Czarnecki and Baker, 1987; Morris, 1985; Li et al., 2007). Therefore, dietary protein (and the AA they are composed of) is an important nutrient for dogs and cats and required to maintain health.

Important functions of protein and amino acids in the body

Proteins and AA perform a vast array of functions within organisms, including catalyzing metabolic reactions, maintaining proper pH, providing structure to cells, producing digestive enzymes, hormones, and immune substances, and transporting molecules from one location to another. Some of the primary functions of the indispensable AA are listed below.

1. Arginine: arginine is needed for normal protein synthesis and is a precursor for urea and ornithine. Arginine functions in the urea cycle as an allosteric activator of acetylglutamate and carbamoyl-phosphate synthase. The urea cycle allows for a large amount of nitrogen derived from the diet to be converted to urea for excretion from the body (NRC, 2006). However, cats lack the enzyme needed to synthesize sufficient arginine in their own bodies. For example, glutamate and proline are precursors for ornithine synthesis (the immediate precursor for endogenous arginine synthesis), but cats have a low activity of the essential enzymes (i.e., pyrroline-5-carboxylate synthase and ornithine aminotransferase) for ornithine synthesis. In addition, citrulline, which is synthesized from ornithine, cannot be produced by the cat either. Therefore, arginine must be provided in their diet (NRC, 2006).
2. Histidine: histidine is a precursor for several hormones (e.g., thyrotropin-releasing hormone) and regulatory substances such as histamine, anserine, and carnosine (NRC, 2006).
3. Branched-chain amino acids (BCAA): valine (glucogenic), leucine (ketogenic), and isoleucine (ketogenic and glucogenic) regulate blood glucose concentrations,

the mTOR signaling pathway, protein synthesis, and is an important energy source for muscles (NRC, 2006; Monirujjaman and Ferdouse, 2014).

4. Lysine: lysine often is the first limiting AA in cereal-based dog foods. Lysine has an important role in the body, most importantly for protein synthesis, but also in the crosslinking of collagen polypeptides, and in the production of carnitine, which is involved in fatty acid metabolism (NRC, 2006).
5. Methionine: methionine is a sulfur-containing AA and a precursor of S-adenosylmethionine, S-adenosylhomocysteine, homocysteine, cysteine, creatine, and carnitine. Methionine can regulate metabolic processes and activate endogenous antioxidant enzymes (e.g., methionine sulfoxide reductase A and glutathione) (NRC, 2006; Martínez et al., 2017).
6. Phenylalanine: phenylalanine is converted to tyrosine via hydroxylation in the liver. Phenylalanine and tyrosine are aromatic AA containing a benzene ring side chain and phenylalanine is considered an indispensable AA. These AA are needed to synthesize thyroid hormones and catecholamines (NRC, 2006).
7. Threonine: threonine influences the maintenance of intestinal mucosal integrity and barrier function. Threonine does not have specific precursor functions with the exception of producing glycine via threonine aldolase (NRC, 2006; Law et al., 2007).
8. Tryptophan: tryptophan is a precursor of the B vitamin, niacin, in most species and precursor of the neurotransmitters, 5-hydroxytryptophan, serotonin, and melatonin. However, cats cannot synthesize niacin from tryptophan because the activity of

picolinate carboxylase is extremely high, which results in a metabolic diversion toward the synthesis of an alternative end-product, glutamate (NRC, 2006).

9. Taurine: taurine can be synthesized by most animals from cysteine. Cats are not able to synthesize a sufficient amount of taurine to meet daily needs due to the low activity of two enzymes in the taurine synthesis pathway, cysteine dioxygenase and cysteine sulfinic acid decarboxylase (Sturman, 1993; NRC, 2006).

Protein quality and digestibility

Protein quality represents the quantity of indispensable AA available for protein synthesis from what the animal consumes. High-quality proteins are those that contain all of the indispensable AA in a proportion that closely meets the needs of the animal consuming them and are highly digestible. In contrast, low-quality proteins may be defined as those that are lowly digestible and do not contain a sufficient amount of indispensable AA (NRC, 2006).

The digestibility of an ingredient or food provides a measure of its quality because digestibility can be an indicator of how the nutrients in an ingredient correspond to the physiological needs of the animal. Digestibility estimates may be obtained from *in vitro* studies that attempt to simulate a living organism by performing procedures in a controlled environment (Smeets-Peeters et al., 1999; Lankhorst et al., 2007). While some of these systems are expensive, some are less expensive, require less time, and are less likely to invoke ethical animal welfare concerns than research conducted on experimental animals (Grajek and Olejnik, 2004). However, *in vivo* feeding trials are the most appropriate methods to measure the absorption of nutrients in the gastrointestinal tract of animals. Several digestibility methods may be used, with the most common being described below.

Measuring apparent total tract digestibility provides an overall evaluation of nitrogen and AA absorption by analyzing nitrogen and AA concentrations in fecal material and diet consumed (Sauer and Lange, 1992). However, AA and protein may be utilized by microbes in the large intestine leading to conversion to microbial proteins, or degraded leading to nitrogen being lost as ammonia. Therefore, apparent total tract digestibility measures can overestimate protein and AA absorption (Just et al., 1981).

Measuring ileal digestibility is a method allowing for the collection of digesta at the distal ileum. This often is done using a cannula that has been surgically inserted into the intestine. Ileal digestibility for AA may be expressed as apparent (AID), standardized (SID), or true (TID) ileal digestibility. The AID value is calculated from the AA concentrations in ileal effluent, which then is subtracted from the diet AA intake {AID, % = [(AA intake − Ileal AA outflow)/AA intake] × 100}. However, the AID does not include only the AA that are not absorbed in the gastrointestinal tract, but also endogenous AA losses that may be influenced by the dry matter, fiber, and/or anti-nutritional factors present in the gastrointestinal tract. In addition, TID values consider both the basal and specific endogenous losses, even though specific endogenous losses cannot be measured precisely {TID, % = {[AA intake − (ileal AA outflow − total endogenous losses)]/AA intake} × 100} (Moughan et al., 1992; Stein et al., 2007). In the past, valuable ileal cannulation studies were conducted in dogs at a few research institutions (Muir et al., 1996; Hill et al., 1996; Yamka et al., 2003a; Yamka et al., 2003b; Yamka et al., 2004; Yamka et al., 2005; Faber et al., 2010). Because of animal welfare concerns, this procedure is no longer used in dogs. Due to low flow rates and difficulties in cannulating a small animal, ileal cannulation is not feasible in cats either.

The cecectomized rooster assay measures the absorption of feedstuff AA, but does not indicate the metabolism of AA (for growth, maintenance, and production) from the digestive tract of the animal model (Elwell and Soares, 1975). However, according to Nordheim and Coon (1984), precision feeding techniques in the cecectomized rooster assay were simpler, less expensive, and quicker than the chick growth assay. The cecectomized rooster assay has been used for decades as a model of not only dogs and cat, but also that of humans and livestock. Although there are differences in the anatomy and metabolism of mammals and birds, the cecectomized rooster assay often is used to test the protein quality of pet food ingredients because the AA digestibilities and response patterns have been shown to be similar to that of ileal-cannulated dogs (r=0.87 to 0.92) (Johnson et al., 1998). Many studies have been done using the cecectomized rooster assay to evaluate plant-based (de Godoy et al., 2009), animal-based (Johnson et al., 1998; Kerr et al., 2013; Kerr et al., 2014; Deng et al., 2016), and human-grade ingredients (Oba et al., 2019a; Oba et al., 2019b).

Methods for testing protein quality

Various methods have been used for evaluating the protein quality of ingredients for use in dog and cat foods. Several analytical tests, such as chemical score, essential AA index (EAAI), total essential AA content, protein efficiency ratio (PER), biological value (BV), net protein utilization (NPU), protein digestibility corrected amino acid score (PDCAAS), and digestible indispensable amino acid score (DIAAS) have been used to determine protein quality for humans and animals (Kronfeld, 1982; Dust et al., 2005; FAO, 2011; Mathai et al., 2017; Oba et al., 2019a).

The main disadvantage of some methods (chemical score, EAAI, E/T, PER, BV, and NPU) is that the assays are conducted in rodents and the AA requirements of growing rats are not highly correlated with that of growing dogs and cats. In addition, some of the methods (chemical score, EAAI, E/T, BV, and NPU) do not consider the digestibility of protein or the availability of its AA.

For example, heat damaged ingredients may have a decreased amount of available AA, but this change would not be considered when using these methods (NRC, 2006). One limitation with using PDCAAS is that the evaluation of protein quality is based on total tract digestibility of crude protein (CP) and the values generally overestimate the amount of AA absorbed due to hindgut fermentation that is not accounted for (Mathai et al., 2017). To avoid this issue, DIAAS values that are calculated based on the AA digestibility from the end of the small intestine may be used. Therefore, the DIAAS method provides a more accurate estimation of AA absorption than PDCAAS (FAO, 2011; Mathai et al., 2017; Oba et al., 2019a). Calculations for the most common protein quality assessment methods are provided below.

Chemical score: the concentration of each indispensable AA in a feed ingredient is expressed as a percentage corresponding to the same AA pattern of a reference protein.

$$\frac{\text{Limiting AA in the test protein (\%)}}{\text{AA in the reference protein (\%)}}$$

EAAI: the geometrical mean of the ratio of the indispensable AA of the test protein compared with that of a reference protein.

$$\frac{\text{AA in the test protein (\%)}}{\text{Same AA in the reference protein (\%)}}$$

Total essential AA content: the total indispensable AA concentration determines the total quantity of indispensable AA of a particular protein source.

$$\frac{\text{Amount of nitrogen from indispensable AA in a protein source}}{\text{Amount of total nitrogen in the same protein source}}$$

PER: calculation based on the body weight gain of weanling male rats or growing chicks divided by the intake of an adequate diet containing the test protein.

$$\frac{\text{Weight gain of animals (g)}}{\text{Protein consumed by animals (g)}}$$

BV: calculation of the nitrogen used for tissue formation divided by the nitrogen absorbed from an ingredient.

$$\frac{\text{Food nitrogen} - (\text{fecal nitrogen} + \text{urinary nitrogen})}{\text{Food nitrogen} - \text{fecal nitrogen}}$$

NPU: calculation of a protein's BV multiplied by its digestibility.

$$\frac{\text{BV of protein} \times \text{Digestibility of protein}}{}$$

PDCAAS: method adopted by the FDA and FAO in 1993 to evaluate protein quality in human foods. it is a calculation based on limiting AA of a test protein compared with a reference protein, multiplied by the total tract apparent CP digestibility of the test protein.

$$\frac{\text{mg of limiting AA in 1 g of test protein}}{\text{mg of same AA in 1 g of reference protein}} \text{ X total fecal digestibility}$$

DIAAS: proposed for use by the FAO in 2013, this method minimizes the effect of hindgut fermentation on AA digestibility. This calculation measures the AA digestibility of a test protein at the ileum and in comparison to a reference protein.

$$\frac{\text{[(mg of digestible dietary indispensable AA in 1 g of the dietary protein}}{\text{mg of the same dietary indispensable AA in 1g of the reference protein)]}} \text{ X 100}$$

DIAAS-like value: method recently used to estimate protein quality for non-human species (e.g., dogs and cats) and calculated based on data collected using the cecectomized rooster assay (Oba et al., 2019a) and AAFCO and NRC nutrient profiles as the reference proteins for dogs and cats.

$$\frac{\text{[(mg of digestible dietary indispensable AA in 1 g of the dietary protein}}{\text{mg of the same dietary AA in 1g of the reference protein)]}} \times 100$$

IMPORTANT FUNCTIONS OF LIPIDS

Lipids are an important component in pet food because of their high energy density, source of essential fatty acids (EFA) that cannot be synthesized by the body, and many key biological functions (structural component of cell membranes and signaling pathways) (Case et al., 1995). Because dietary lipids present in pet food are highly digestible, often having greater than 90% apparent digestibility, consumption of a more energy-dense diet will decrease the total volume of food consumed. Therefore, if nutrients are not adjusted with regard to fat content (energy density), nutrient deficiencies may result (Case et al., 1995). A minimum lipid concentration is needed in the diet of dogs and cats. According to the current AAFCO nutrient profiles, the minimum fat concentrations in the diet (4,000 kcal ME/kg, DM basis) are 9% for cats (adult maintenance and growth and reproduction) and 5.5% and 8.5% for dogs (adult maintenance and growth and reproduction, respectively).

Classification of dietary lipids

The term “lipid” has been defined as organic compounds that are soluble in an organic solvent, but hardly soluble in water (Bondi, 1987). Lipids include fats, oils, waxes, phospholipids, and cholesterols, and can be classified into simple, complex, and derived lipids (Smith, 2000; Christie, 2003; Fahy et al., 2005). The simple lipids are esters of fatty acids with alcohols (fats, oils, and waxes). The complex lipids are esters of fatty acids, with alcohols containing additional groups such as phosphate, a nitrogenous base, carbohydrate, or protein (phospholipids, glycolipids, and other complex lipids). The derived lipids are the derivatives obtained from the hydrolysis of simple and complex lipids, which possess the characteristics of lipids (fatty acids, monoglycerides, diglycerides, alcohols, and ketone bodies) (Smith, 2000; Christie, 2003; Fahy et al., 2005). Triglycerides (simple lipids) are the most abundant group of lipids and major components of edible oils and fats. Hydrolysis of triglycerides yields glycerol and fatty acids, with fatty acid components affecting the properties of triglycerides (NRC, 2006). Fatty acids can be classified by their number of carbon chain lengths (long, medium, and short-chain), degree of unsaturation (saturated and unsaturated), and location of double bonds (omega-3 and omega-6 fatty acids) (NRC, 2006).

Dogs and cats require omega-6 (linoleic acid; 18:2n-6) and omega-3 (alpha-linolenic acid; 18:3n-3) fatty acids in their diet because they cannot synthesize them on their own. These EFA are long-chain, polyunsaturated fatty acids (LCPUFA) and two enzymes (delta-6-desaturase and delta-5-desaturase) secreted from the liver are involved in the production of other LCPUFA through elongation and desaturation reactions (NRC, 2006). However, dogs and cats cannot interconvert omega-6 and omega-3 fatty acids. The amount of alpha-linolenic acid present in food must be determined relative to the level of linoleic acid because their metabolism requires the same desaturation enzymes, resulting in a competition between the two families (NRC, 2006). The EFA for dogs and cats are listed below.

Omega-6 fatty acids: omega-6 fatty acids include linoleic acid and arachidonic acid. Linoleic acid is essential for both dogs and cats, but arachidonic acid is essential only for cats because of the lack of delta-6 desaturase activity. This enzyme functions at a high enough rate to convert linoleic acid to γ-linolenic acid or tetracosatetraenoic acid to beta-tetracosapaentaenoic acid (NRC, 2006).

Omega-3 fatty acids: omega-3 fatty acids include alpha-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Adult dogs can convert alpha-linolenic acid to both EPA and docosapentaenoic acid (DPA), which is a precursor of DHA. However, cats have lower delta-6 desaturase activity in their liver than dogs, leading to reduced production of EPA and DHA.

Lipid quality

The lipid quality of pet food is important and highly related to the quality of food because lipids contribute to a food's texture, structure, mouthfeel, flavor, color, and smell (Montesano et al., 2018). Lipid quality can be measured by color, fatty acid profile, degree of free fatty acid content, degree of unsaturation, saponification value, and impurities, including moisture, insolubles, and unsaponifiables (MIU) (Shurson et al., 2015). These measurements, however, are not enough to provide information about the feeding value. The feeding value is evaluated by measures of fat oxidation such as peroxide value, thiobarbituric acid reactive substances, and anisidine value (Shurson et al., 2015).

Skin and hair coat health

The skin and hair coat of dogs and cats functions to protect them from physical and infectious injury, temperature control, and immunoregulation. The hair coat is composed almost entirely of protein (more than 90%), with a high concentration of sulfur amino acids (methionine and cystine). The skin consists of two layers, namely the epidermis and dermis.

The epidermis contains specialized skin cells called keratinocytes, which produce keratin that covers the epidermis to prevent the loss of water through the skin. The dermis contains connective tissue, blood vessels, oil and sweat glands, nerves, hair follicles, and other structures. The role of the dermis is to support and protect the skin, assist in thermoregulation, keep the skin and hair lubricated, and prevent friction during movement (Watson, 1998; Lloyd and Marsh, 1999; NRC, 2006).

The epidermal barrier function of the skin is related to the linoleic acid content in ceramide, with many skin and coat disorders (dry, dull, nonpruritic skin disorders, and inflammatory skin diseases) generally dependent on the linoleic acid content of dietary fat sources. This is because linoleic acid improves cohesion between the lipid sheets that make up the intercellular lamellae (Watson, 1998; Lloyd and Marsh, 1999; NRC, 2006). Dietary factors have a major role in maintaining a healthy skin and coat, but nutritional deficiencies are rarely encountered in pet foods. Although many nutrient deficiencies may be associated with skin and coat health, researchers have concentrated largely on EFA (Watson, 1998; Lloyd and Marsh, 1999). The potential benefits of fatty acid supplementation are described below:

Linoleic acid: linoleic acid has a physiological role in maintaining the water permeability barrier of the skin as a constituent of ceramides, which are exuded from keratinocytes into the intercellular spaces. In addition, linoleic acid is a precursor of the LCPUFA in the cell membrane that are important for the formation of phospholipids and other lipid components (NRC, 2006; Kirby et al., 2007). Linoleic acid gives rise to arachidonic acid, which is the major cell membrane fatty acid and a precursor of a series of bioactive metabolites called eicosanoids. The eicosanoids are produced in response to diverse physiological systems and pathological processes (inhibiting inflammation, allergy, fever and other immune responses). The eicosanoids include prostaglandins, prostacyclins, thromboxanes, and leukotrienes. The prostaglandins of the 2-series (prostaglandin E_2) and the leukotrienes of the 4-series (leukotrienes B_4) are proinflammatory and are important mediators of inflammatory and allergic responses (NRC, 2006; Kirby et al., 2007).

Alpha-linolenic acid: alpha-linolenic acid does not have direct functions itself, but provides a sparing effect for linoleic acid. The primary role of alpha-linolenic acid is to synthesize EPA and DHA. Like arachidonic acid, EPA is a precursor for eicosanoids. However, the eicosanoids coming from EPA, such as prostaglandin E_3 and leukotrienes B_5, are less biologically active than those produced from arachidonic acid. EPA and DHA also have anti-inflammatory and immunostimulatory properties (Bauer et al., 1998; NRC, 2006).

Linoleic acid and alpha-linolenic acid produce different families of eicosanoids and compete for the same enzyme systems. The amount and type of eicosanoids synthesized from linoleic acid and alpha-linolenic acid are determined by the type of fatty acids coming from the cell membranes. In addition, the level of linoleic acid and alpha-linolenic acid in cell membranes can be controlled by the amount and ratio of linoleic acid and alpha-linolenic acid present in the diet. The optimal ratio of linoleic acid and alpha-linolenic acid should be between 2.6:1 and 26:1 based on NRC, and below a 30:1 based on AAFCO for adult dogs, but there has been no maximum ratio provided for cats. Therefore, the amount and type of omega-3 and omega-6 fatty acids and their ratio must be considered to maintain skin and coat health for dogs and cats (Bauer et al., 1998; NRC, 2006; Kirby et al., 2007; AAFCO, 2019).

NOVEL INGREDIENTS AND PET FOOD REGULATIONS

Pet health and longevity are top priority to pet owners so they are motivated to find foods perceived as being safer or of higher quality. They also desire alternative ingredients or food types that may provide better nutrition for their dogs and cats compared with diets based on by-products generated from the human food system (Laflamme et al., 2008; Swanson et al., 2013).

Pet foods are formulated with a variety of ingredients, which are selected based on consumer preferences and according to their nutritional needs. In the United States, ingredients, complete pet foods, and drugs are regulated by the Center for Veterinary Medicine (CVM) of the Food and Drug Administration (FDA). The federal Food, Drug, and Cosmetic Act (FD&C Act) defines animal food ingredients or substances added to an animal's drinking water as a "food". There is no separate category for "supplements" when it comes to animals, however, because the Dietary Supplement Health and Education Act (DSHEA) of 1994 did not specify whether the "dietary supplements" definition applied to animals. Therefore, dietary supplements are considered either "foods" or "new animal drugs", depending on the intended use that is determined by CVM using criteria provided in Guide 1240.3605 (FDA, 2017).

A food additive is any substance added to pet food that affects a food's characteristics. The food additives are categorized according to their composition and intended use. For example, substances that supply nutrients, aroma/flavor, aid stability, and substances that modify the food's characteristics, including emulsifiers, sequestrants, anti-caking agents, or enzymes are animal food additives. However, ingredients classified as being generally recognized as safe (GRAS) are not considered to be food additives and do not need approval by FDA. For example, if the intended use of glucosamine sulfate is to improve joint health of animals, this substance cannot be GRAS because it is treated as an animal drug. Any substance that is expected to become a component of animal feed must abide by the food additive regulations. There are three ways for an ingredient to be acceptable for use in pet food, which are briefly described below (Burdock and Carabin, 2004).

Food additive petition (FAP): The FAP is a process reviewed by FDA scientific personnel, who have a variety of expertises such as chemistry, toxicology, nutrition, or microbiology, to verify the general information of substances (its intended effect, safety, and identity) to reach consensus about the safe use of the petitioned material. This protocol is managed by a consumer safety officer (CSO) in the Office of Premarket Approval (OPA). The approval process is composed of several steps:

1. Identification: formal chemical name, common names, synonyms or trade names, chemical abstracts service (CAS) registry number, empirical and structural formulae, molecular or formula weights, composition of the food additive, food additives of natural origin, information about the source, and further characterizing information.
2. Manufacturing process: information about the method of manufacture of a food additive.
3. Specifications of food grade material: description of the substance, identification tests for the substance, an assay of purity for the additive, physicochemical characteristics of the food additive (e.g., ash content, moisture content, melting point, density, refractive index, pH), and limits for impurities and contaminants.
4. Stability of the food additive.
5. Intended technical effect and use: the type of food to which the substance will be added, the amount of the substance that will be added to food, the technical effect, the fate of the substance in food, and any recommendations, suggestions, and directions for use.
6. Analytical methods.

7. Safety evaluation.
8. Proposed tolerance for the food additive.
9. Proposed regulation and environmental assessment.

Once the proposed use of the additive has been approved through scientific review, a substance may obtain final approval after the final rule is published or it may have to wait until completion of the judicial review (Rulis and Levitt, 2008).

Generally recognized as safe (GRAS): The GRAS process to approve ingredients for use in pet foods is similar to that of FAP, but there are a few differences (**Figure 2.1**). First, GRAS uses a self-approval system for companies. For example, the manufacturer prepares all necessary scientific data and information about the use of a substance to obtain agreement that the substance satisfies the GRAS standard from qualified experts. However, the substances that have been used commonly before 1958 without known detrimental effects are considered safe and do not require additional safety evaluation. Second, total time required for the approval of a GRAS ingredient (79 weeks for studies + 37 weeks to complete the GRAS) is shorter to market than that for FAP (1 to 3 years, depending on the petition review) (Burdock and Carabin, 2004).

Ingredient in Association of American Feed Control Officials (AAFCO) Official Publication: AAFCO is a voluntary organization, composed of state, federal, and international regulatory officials who have responsibility for enforcing state laws and regulations concerning the safety of animal feeds, including livestock and poultry feed and pet food. The process of gaining recognition through AAFCO's official publication is similar to the FAP process. In either instance, the FDA requires much information, such as a summary of the request (name of ingredient, intended use, and rationale for the request), proposed definition, description and purpose of the ingredient, manufacturing processes, limitations, data and observations to support intended use, and safety assessment (AAFCO, 2019).

Novel and alternative protein sources

The decision to select a protein source for dog and cat foods should consider many factors, such as AA composition and digestibility, cost, palatability, presence of anti-nutritional factors, and sustainability (Hill, 2004; Swanson et al., 2013). Many pet owners often consider dogs and cats as primarily meat-eaters, and prefer to feed a diet containing a high concentration of animal-derived proteins (Callon et al., 2017). The demand for animal-derived protein sources will likely continue to increase in the future because of the growing population, urbanization, and living standards of developing countries (FAO, 2009; Alexandratos and Bruinsma, 2012). These trends are expected to continue for both humans and pets. Therefore, there has been an increased interest in searching for alternative protein sources to satisfy marketing/consumer demand or to use byproducts from other industries to improve sustainability.

Some novel proteins from animal-based sources (**Table 2.1**; calamari meal, pork peptone, alligator meal, lamb meal, venison meal, and duck meal), plant-based protein sources (**Table 2.2**; maize gluten, soybean meal, adzuki bean, chickpea, cowpea, mung bean, pigeon pea, flaxseed, safflower seed meal, and sunflower seed flour), and insect-based protein sources (**Table 2.3**; cricket, mealworm, superworm, waxworm, housefly, black soldier fly larvae, yellow mealworm, morioworm, and cockroach) are expected to replace many of the traditional protein sources used in the pet food industry (Kendall and Holme, 1982; Yamka et al., 2004; NRC, 2006; USDA, 2007; Brown, 2009; Finke, 2013; Bosch et al., 2014; Deng et al., 2016; Ritala et al., 2017). Although consumers and pet food manufacturers have a great interest in these proteins, few have been well studied in regard to their quality and digestibility in dogs and cats.

BLACK SOLDIER FLY LARVAE

Black soldier fly larvae (BSFL; *Hermetia illucens*) have been studied for their use in waste management, including items such as animal manure, food waste, rice straw, distiller's grains, and kitchen waste (Sheppard, 1983; Zheng et al., 2012; Webster et al., 2016). BSFL may convert a variety of organic wastes to energy because they are polyphagous and secrete many digestive enzymes (α-amylase, lipase, and proteases) from their salivary gland and gastrointestinal tract (Kim et al., 2011). Thus, BSFL have the potential to improve the value of organic waste and animal manure and feces by converting them into a rich fertilizer (Diener et al., 2009; Zheng et al., 2012; Kalová and Borkovcová, 2013; Nguyen et al., 2013). Several studies also have tested whether BSFL could be an alternative protein and/or fat source for swine (Newton et al., 1977; Newton et al., 2005), poultry (Cullere et al., 2016; Marono et al., 2017; Mwaniki et al., 2018), or fish species (Kroeckel et al., 2012; Lock et al., 2015). Recently, insect proteins have received more attention in the pet food industry because of their economic, nutritional, and environmental advantages.

Physical requirements and lifecycle

For optimal growth, BSFL need to ingest a sufficient amount of diet that is nutritionally balanced (Cohen, 2003). Carbohydrates serve as an important energy source for BSFL and are used as a building block for their tissues. Black soldier flies also contain chitin, a long-chain polymer of N-acetylglucosamine, which is part of their exoskeleton (Cohen, 2003). Essential AA (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) have important biological functions, serving as important structural components of larval tissues, for hormone production, and as an energy source (Cohen, 2003). Lipids serve as a building block of cellular membranes, energy storage, cellular communication and protection, and production of hormones (Cohen, 2003). Micronutrients are also indispensable for BSFL, but limited information is available in regard to how dietary micronutrient concentrations affect their development (Cohen, 2003). Dietary calcium has been well studied in BSFL because it is known to be an important micronutrient for the development of their mineralized exoskeleton layer that is rich in $CaCO_3$ (Johannsen, 1922). There are many potential calcium sources in animal feeds (**Table 2.4**), but they have different solubilities, calcium concentrations, and functionalities (Weaver, 1998; Hamdi et al., 2015).

Environmental factors and housing conditions should be considered because BSFL are not active in the winter season. This requires that BSFL need to be reared under laboratory conditions (Zhang et al., 2010), including the factors discussed below.

Light: the lack of sunlight has been shown to negatively influence mating and oviposition in BSFL (Tomberlin and Sheppard, 2002). Tomberlin and Sheppard (2002) used two different artificial lamps (430-watt Pro Ultralight light system or a 40-watt Sylvania Gro Lux system), but these artificial lamps did not affect mating and oviposition of BSFL. According to Zhang et al. (2010), who used two other artificial lamps (500-watt quartz-iodine lamp with a spectrum between 350-2500 nm or 450-watt rare-earth light with a spectrum between 350-450 nm), mating activity was not observed when using the rare-earth light. Approximately 40 mating pairs were observed per day under the quartz-iodine lamp (39% less than sunlight), and when compared with sunlight, larval and pupal development times were not different.

Temperature and humidity: BSFL are native to tropical, subtropical, and warm temperature zones of South America. Thus, maintaining appropriate temperature and humidity is a key factor in determining BSFL survival rate, larval development time and longevity, and egg eclosion rate. When BSFL were reared at 27, 30, or 36˚C, the temperature affected larval and pupal development and survival rate, but prepupal weight gain did not differ across temperatures. The survival rate from larvae to adults was 74 to 97% when BSFL were reared at 27 and 30˚C, but only 0.1% at 36˚C (Tomberlin et al., 2009). Development time from larvae to pupal reared at 27˚C required four more days than for those reared at 30˚C, and larval development time from the day of hatch when reared at 36˚C required seven more days when compared with those reared at 27 or 30˚C (Tomberlin et al., 2009). Chia et al. (2018), who tested nine temperatures (10 to 42˚C), demonstrated that housing temperature affected the BSFL survival rate. Egg eclosion rate was highest for BSFL reared at 30˚C (80%) or 35˚C (75%), and lowest for those reared at 15, 37˚C, or 40˚C (below 11%). The survival rate of the larval and pupal stages also was highest for those reared at 30˚C (90 and 77%) or 35˚C (92 and 75%). Like temperature, relative humidity is also an essential factor affecting BSFL development and survival. Egg eclosion time (124.43 to 87.63 h) and mortality rate (62 to 3%) of the BSFL pupal stage decreased when the relative humidity increased from 25 to 70%. Also, adult longevity (5.17 to 7.94 d) and the emergence rate of BSFL (16 to 93%) increased when they were reared at 40 and 70% relative humidity compared with 25% (Holmes et al., 2012).

Stocking density: larval stocking density has a significant impact on the bioconversion of BSFL (Parra Paz et al., 2015). High stocking density may result in decreased larval development due to the increased competition for feed, but lower density is not always good for their development because high density provides a beneficial effect on food assimilation (Green et al., 2002). Barragan-Fonseca et al. (2017) placed 50, 100, 200, or 400 BSFL in plastic containers (15.5 cm x 10.5 cm x 6 cm), demonstrating that development time was shorter (13 vs. 15 d) at lower larval densities (50, 100, and 200) when raised on a diet containing high nutrient concentrations (14.0% protein, 1.8% fat, and 46% non-cellulose carbohydrate vs. 3.5% protein, 0.7% fat, and 12% non-cellulose carbohydrate).

Life cycle of BSFL can be divided into four phases, including egg, larval, pupa, and adult stages (Tomberlin and Sheppard, 2002), but the length of the BSFL life cycle can be changed depending on the environmental temperature, humidity, and density and quality of diet (Veldkamp et al., 2012; Wang and Shelomi, 2017; Barragan-Fonseca et al., 2017). An adult female BSF lays between 206 and 639 eggs at a time. Each egg is approximately 1 mm in length and has a creamy white color. Once the eggs hatch, the larvae can reach a length of 25 mm and weight of 0.10 to 0.22 g by the end of the larval stage (Diclaro and Kaufman, 2009). After the larval stage, larvae stop feeding and move to a high and clean place for pupa development. As a result, weight loss occurs throughout the pupal stage and is correlated with increased calcium and phosphorus deposition in the exoskeleton (Grodowitz and Broce, 1983).

Nutritional value

Amino acid concentration: AA concentrations of BSFL do not appear to be different among groups raised on different substrates (**Table 2.5**), but BSFL reared on cattle manure seemed to have more AA than for those fed other substrates (chicken feed, biogas digestate, vegetable waste, restaurant waste, swine manure, and chicken manure). Some of the indispensable AA of BSFL reared on cattle manure (histidine, isoleucine, leucine, lysine, and phenylalanine) were higher than for those reared on raw chicken. However, BSFL fed cattle manure had lower concentrations of some indispensable AA, such as methionine, threonine, and tryptophan when compared with those reared on raw chicken (Newton et al., 2005; Oba et al., 2019a). When comparing BSFL reared on swine manure or soybean meal, larvae contain higher concentrations of alanine, methionine, histidine, and tryptophan, and a lower concentration of arginine than for those reared on soybean meal (Arango Gutiérrez, 2005; Newton et al., 2005).

Fatty acid concentration: in comparison with other insects, BSFL contain a high amount of fat in their body, especially in the form of saturated fatty acids (58 to 72% of total fat) and 19 to 40% of total fat in the form of mono- and poly-unsaturated fatty acids (Makkar et al., 2014; Ramos-Bueno et al., 2016). Fatty acids in the larvae are most likely used for energy storage because their energy needs are high for metamorphosis during the pupal stages (Mirth and Riddiford, 2007; Arrese and Soulages, 2010). The fatty acid profile of BSFL also is affected by the diet fed (**Table 2.6**). The most common fatty acids present in BSFL are lauric acid (C12:0), palmitic acid (C16:0), and oleic acid (C18:1 n-9). Lauric acid (C12:0) has been shown to be present at high concentrations (20.9 to 51.8%), with BSFL synthesizing lauric acid from the carbohydrates present in the substrate fed (bread, 51.8%). The ratio of linoleic acid and alpha-linolenic acid in BSFL fed different substrates is between 3.6:1 to 11:1. Feeding a diet containing a linoleic acid: alpha-linolenic acid ratio between 5:1 to 10:1 for dogs showed a higher production of prostaglandin E_2 and leukotrienes B_4 (less inflammatory metabolites) and decreased the inflammatory response (Vaughn et al., 1994).

Mineral concentration: the mineral profiles of BSFL also depend on the substrates they are fed (**Table 2.7**). Many researchers have reported differences in mineral content of BSFL reared on chicken feed, solid aquaculture wastes (SAW), kitchen waste, spent grain, chicken manure, and swine manure (Arango Gutiérrez, 2005; Newton et al., 2005; Finke, 2013; Makkar et al., 2014; Shumo et al., 2019; Schmitt et al., 2019). For instance, calcium concentration was significantly higher in BSFL reared on SAW or swine manure in comparison with BSFL reared on kitchen waste, spent grain, or chicken manure. However, the concentration of other minerals, such as phosphorus, magnesium, sodium, potassium, iron, and zinc were lower. BSFL contain more calcium (1.7 to 8.3%, DM basis) compared with other insects (less than 1%, DM basis) such as teboworms, house flies, Turkestan cockroaches, mealworms, and house crickets (Finke, 2013; Makkar et al., 2014; Shumo et al., 2019; Schmitt et al., 2019). The reason that BSFL contain high calcium concentrations is because of deposition of calcium carbonate ($CaCO_3$), which leads to their high calcium and ash concentrations (Johannsen, 1922; Newton et al., 1977; Barragan-Fonseca et al., 2017).

Sustainability of black soldier fly larvae

Global population growth and increased lifespan, income, and urbanization are predicted to dramatically increase the demand for protein sources around the world (FAO, 2009; Alexandratos and Bruinsma, 2012). Many ingredients for use in dog and cat foods are derived from the human food system (Swanson et al., 2013). Many pet owners treat their animals as family members. They believe “human-grade ingredients” constitute high-quality foods and that quality food is important to the well-being of those that consume it (Swanson et al., 2013; Carter et al., 2014; Sharon et al., 2018). Therefore, increases in pet ownership continues to become more popular, resulting in concerns for sustainability and food security in the future.

Environmental sustainability has become an important issue in recent years. Many animal feed companies expend time and effort on protecting the long-term productivity and health of resources to meet future economic and social needs. Animal-derived proteins have been shown to have a negative impact on the environment. Westhoek et al. (2011) and D’Annunzio et al. (2014)

reported that 12% of greenhouse gas (GHG) emissions are derived from livestock production, and between 1990 and 2010, 5.3 million hectares/year of forest area were lost because of both food and livestock feed crop production for humans and animals. Water use is also a concern. For example, the average water footprint of protein sources is highly variable, and increases from cow's milk (1,020 L/kg protein) to other animal-based sources such as chicken egg (3,265 L/kg protein), chicken (4,325 L/kg protein), goat and sheep (8,763 L/kg protein), pig (5,988 L/kg protein), and beef cattle (15,415 L/kg protein) that are considerably higher (Mekonnen and Hoekstra, 2010).

Insects may be an environmentally friendly alternative to conventional livestock. For instance, the average water footprint of mealworms (23 L/g protein) is lower than pork (57 L/g protein), chicken (34 L/g protein), and beef (112 L/g protein). Also, the global warming potential (represented per kg of edible protein) of mealworms (14 kg of CO_2-eq) is lower than chicken (19 to 37 kg of CO_2-eq), pork (21 to 54 kg of CO_2-eq), and beef (77 to 175 kg of CO_2-eq). Similarly, land use (represented per kg of edible protein) of mealworms (18 m^2) is lower than that of chicken (41 to 51 m^2), pork (46 to 63 m^2), and beef (142 to 254 m^2) (de Vries and de Boer, 2010; Oonincx and de Boer, 2012; Miglietta et al., 2015). BSFL are becoming a popular protein source for animal feed. BSFL have a higher amount of edible fraction content (17.5%, as-is basis) compared with animal proteins such as beef (10.4%), pork (10.6%), and chicken (7.3%) (Koutsos et al., 2019). In addition, BSFL (18.0 per year) have a faster growth cycle (based on the days to reach harvest weight) than beef (0.8 per year), pork (2.0 per year), and chicken (7.8 per year). Also, protein yield (kg/m^2 per year) of BSFL was greater in contrast with beef (0.006 kg/m^2 per year), pork (25.129 kg/m^2 per year), and chicken (21.598 kg/m^2 per year) (Koutsos et al., 2019). Although mealworms are a more sustainable protein source than conventional livestock, BSFL have a higher protein production yield. To our knowledge, however, limited scientific research has been done on how BSFL production affects sustainability.

Legal regulations

In the U.S. and Europe, BSFL must be fed feed-grade ingredients and adhere to standard safe feeding conditions in order to be fed to livestock or companion animals. The restriction to approved, feed-grade materials is intended to ensure the production of safe larvae, which could accumulate pathogens, heavy metals, or other toxins from less regulated inputs (AAFCO, 2016). Ingredient definitions for BSFL according to AAFCO (2021) are provided below.

Dried black soldier fly larvae (whole) is defined for use in adult dogs, poultry, swine, salmonid fish, and wild bird feed as a source of protein and fat – 60.117 and T60.117(C) Dried Black Soldier Fly Larvae.

Dried black soldier fly larvae (ground) has been defatted and is defined for use in adult dogs, poultry, swine, and salmonid fish as a source of protein and fat – 60.117 and T60.117(C) Dried Black Soldier Fly Larvae.

Dried black soldier fly larvae oil is defined for use in swine, fin fish, salmonids, and wild birds as a source of energy –T33.29 Black Soldier Fly Larvae Oil.

CONCLUSIONS

The search for novel protein sources has been a focus for many in the pet food industry over the last decade. Whether it is supported by data or not, pet parents want to feed diets formulated with high-quality, animal-derived protein sources to their animals. Such changes may not be beneficial to the pet food companies, however, who have made changes to reduce concerns for the environment, long-term business profitability, consumer demands, and many aspects of sustainability. Insect protein sources such as BSFL may serve as one of the potential solutions to those problems. There has been a limited amount of research evaluating the growth rates, nutrient composition, and nutrient digestibility of BSFL, especially those reared on different dietary calcium forms and concentrations and those harvested at different ages.

Therefore, the main objectives of this Ph.D. dissertation are: 1) to determine the effects of age on nutrient and AA digestibility of BSFL intended for use in pet foods using the precision-fed cecectomized rooster assay; 2) to investigate the growth performance, nutrient composition, and nutrient and AA digestibility of BSFL fed different forms and concentrations of calcium using the precision-fed cecectomized rooster assay; and 3) to evaluate the palatability, apparent total tract macronutrient digestibility, fecal characteristics, and skin and coat health markers of healthy adult cats fed BSFL-containing diets.

TABLES AND FIGURE

Table 2.1. AA composition of animal-based protein sources for dogs and cats (percentage as-fed)[1]

Ingredient	DM[2]	CP[3]	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Alligator meal	97.7	61.7	4.1	1.3	1.8	3.6	3.8	1.2	2.0	2.2	0.5	2.1
Duck meal no. 1	94.9	60.3	3.7	1.0	1.8	3.5	3.4	1.0	1.9	2.0	0.5	2.2
Lamb meal	95.5	59.1	4.2	0.9	1.6	3.8	3.1	0.8	2.0	2.2	0.4	2.4
Venison meal	93.6	58.7	3.4	1.6	1.1	4.3	3.4	0.7	2.4	2.2	0.5	2.7
Calamari meal	93.8	88.1	5.9	2.1	3.5	5.8	5.1	2.4	3.1	3.3	0.9	3.7
Duck meal no. 2	96.7	60.8	4.4	1.3	2.4	4.4	4.1	1.2	2.3	2.5	0.5	2.9
Pork peptone	96.3	80.5	5.0	1.9	2.9	5.4	5.5	1.4	2.9	2.8	0.8	3.8

[1]Adapted from Deng et al. (2016).
[2]Dry matter.
[3]Crude protein.

Table 2.2. AA composition of plant-based protein sources for dogs and cats (percentage as-fed)[1]

Ingredient	DM[2]	CP[3]	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Maize gluten	87.0	56.3	1.8	1.2	2.3	9.4	1.0	1.3	3.6	1.9	0.3	2.6
Soybean meal	90.0	48.2	3.5	1.3	2.2	3.8	3.0	0.7	2.5	1.9	0.6	2.2
Adzuki bean, raw	87.0	19.9	1.3	0.5	0.8	1.7	1.5	0.2	1.1	0.7	0.2	1.0
Chickpeas, raw	89.0	19.3	1.8	0.5	0.8	1.4	1.3	0.3	1.0	0.7	0.2	0.8
Cowpeas, raw	89.0	23.9	1.7	0.7	1.0	1.8	1.6	0.3	1.4	0.9	0.3	1.1
Mung beans, raw	91.0	23.9	1.7	0.7	1.0	1.9	1.7	0.3	1.4	0.8	0.3	1.2
Pigeon pea, raw	90.0	21.7	1.3	0.8	0.8	1.6	1.5	0.2	1.9	0.8	0.2	0.9
Flaxseed, whole	93.0	18.3	1.9	0.5	0.9	1.2	0.9	0.4	1.0	0.8	0.3	1.1
Safflower seed meal	94.0	35.6	3.9	1.0	1.6	2.5	1.2	0.6	1.8	1.3	0.4	2.3
Sunflower seed flour	93.0	48.1	5.1	1.3	2.4	3.5	2.0	1.0	2.5	2.0	0.7	2.8

[1]Adapted from Kendall and Holme (1982), Yamka et al. (2004), NRC (2006), USDA (2007), and Brown (2009).
[2]Dry matter.
[3]Crude protein.

Table 2.3. AA composition of insect protein sources for dogs and cats (percentage of DM)[1]

Ingredient	CP[5]	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Black soldier fly larvae[2]	56.1	3.7	4.4	4.0	6.1	5.4	1.4	3.1	3.6	5.0
Black soldier fly pupae[2]	52.1	4.2	4.7	4.2	6.5	5.4	1.7	3.3	3.6	5.7
House fly pupae	62.5	4.2	4.8	4.0	6.1	6.2	2.6	5.2	3.8	5.0
House cricket	70.6	5.7	3.4	4.0	6.6	5.8	1.6	3.2	3.6	5.7
Yellow mealworm	52.0	4.6	5.1	4.6	7.3	5.5	1.4	3.4	4.0	6.3
Morioworm	47.0	4.6	4.8	5.0	7.2	5.3	1.6	3.7	4.1	6.5
Argentinian cockroach	64.4	3.5	4.5	3.2	5.3	4.0	1.3	2.7	3.1	5.4
Superworms[3]	50.3	3.5	1.6	2.4	3.7	2.9	0.7	2.0	2.0	3.3
Waxworms[4]	40.1	3.3	0.9	1.8	3.0	2.4	0.7	1.6	1.7	2.7

[1]Adapted from Finke (2013) and Bosch et al. (2014).
[2]Black soldier fly larvae were fed a broiler starter diet.
[3]Superworms were fed a diet formulated with flaxseed, canola oil, fish oil, corn gluten meal, yellow carotenoids, vitamin E, thiamin, and beta-carotene.
[4]Waxworms were fed a diet formulated with flaxseed, canola oil, fish oil, vitamin E, and beta-carotene.
[5]Crude protein.

Table 2.4. Overview of calcium content and solubility of different calcium salts

Product[1]	Calcium content, %[2]	Solubility (in water)	Functionality[3]
Calcium carbonate	40	Insoluble	Fortification, acidity regulator, anti-caking agent, and colorant
Calcium sulphate	23	Insoluble	Acidity regulator, firming agent, fortification
Mono-calcium phosphate	18	Insoluble	Acidity regulator, anti-caking agent, firming agent
Di-calcium phosphate	29	Insoluble	Acidity regulator, raising agent, firming agent
Tri-calcium phosphate	39	Insoluble	Fortification, anti-caking agent, Stabilizer, firming agent
Calcium hydroxide	54	Insoluble	Acidity regulator, firming agent, fortification
Tri-calcium citrate	21	Insoluble	Fortification, stabilizer, acidity regulator
Calcium chloride	27	Soluble	Firming agent, stabilizer, thickener, fortification
Calcium gluconate	9	Soluble	Fortification, acidity regulator, firming agent
Calcium lactate	13	Soluble	Acidity regulator, firming agent, fortification
Calcium lactate gluconate	12	Soluble	Fortification

[1]Adapted from Omya Inc. (http://www.omya.com), Weaver (1998), and Hamdi et al. (2015).
[2] According to chemical composition.
[3] Codex alimentarius (http://www.codexalimentarius.net/gsfaonline).

Table 2.5. AA concentrations of BSFL fed chicken feed, biogas digestate, vegetable waste, restaurant waste, swine manure, cattle manure, and chicken manure (%, DM)[1]

AA	Chicken feed	Biogas digestate	Vegetable waste	Restaurant waste	Swine manure	Cattle manure	Chicken manure
Ala	2.5	2.4	2.4	2.8	2.6	3.7	2.3
Arg	2.0	2.0	2.0	2.0	1.8	2.2	2.1
Asp	3.8	3.4	3.6	3.7	3.0	4.6	1.4
Cys	0.3	0.2	0.2	0.2	0.3	0.1	0.3
Glu	4.2	4.0	4.1	4.6	4.0	3.8	2.8
Gly	2.3	2.3	2.2	2.5	2.1	2.9	2.3
His	1.4	1.4	1.2	1.4	1.0	1.9	1.2
Ile	1.7	1.8	1.7	1.9	1.5	2.0	1.6
Leu	2.9	3.0	2.8	3.1	2.6	3.5	2.6
Lys	2.3	2.6	2.3	2.3	2.2	3.4	2.1
Met	0.8	0.9	0.8	0.7	0.8	0.9	0.8
Phe	1.7	1.9	1.6	1.6	1.5	2.2	1.5
Pro	2.3	2.2	2.1	2.5	2.1	3.3	2.3
Ser	1.7	1.6	1.5	1.6	1.5	0.1	2.8
Thr	1.6	1.7	1.5	1.6	1.4	0.6	1.9
Trp	0.7	0.6	0.6	0.5	0.6	0.2	0.6
Val	2.4	2.5	2.5	2.8	-	-	-

[1]Data taken from Newton et al. (2005), Arango Gutiérrez (2005), and Spranghers et al. (2017).

Table 2.6. Fatty acid concentrations (% of total fatty acids) of BSFL fed different substrates[1]

Fatty acid	Restaurant waste	By-product[2] (high fat)	By-product[2] (low fat)	Chicken feed	Bread	Fish	Cattle manure	Swine manure
Capric acid, C10:00	1.8	0.75±0.07	0.75±0.64	0.9				
Lauric acid, C12:0	23.4	33.65±6.72	49.55±1.63	46.6	51.8±3.3	28.6±2.1	20.92±1.27	49.3
Myristic acid, C14:0	-	7.6±0.28	9.7±0.28	9.2	9.5±0.9	6.1±0.4	2.85±0.07	6.8
Palmitic acid, C16:0	18.2	15.7±1.84	11.7±0.14	12.7	12.7±0.8	12.6±0.4	16.05±0.06	10.5
Palmitoleic acid, C16:1	9.4	3.15±0.35	5.65±1.34	3.4	2.8±0.1	4.8±0.5	-	3.5
Stearic acid, C18:0	5.1	2.6±0.28	1.9±0.14	2.1	1.5±0.3	2.2±0.2	5.68±0.06	2.8
Oleic acid, C18:1	27.1	17±1.56	10.55±0.35	14	12±1.2	25.1±0.7	-	11.8
Linoleic acid, C18:2n-6	7.5	12.7±6.22	4.8±1.7	9.4	7.7±0.3	12.5±0.3	4.51±0.56	3.7
α-Linolenic acid, C18:3	-	1.15±0.49	0.8±0.28	0.8	1.6±0.1	3.4±0.1	0.19±0.01	0.1
Arachidonic acid, C20:4	-	0.15±0.07	0.35±0.35	0.1	-	-	-	-
Docosapentaenoic acid, C22:5 n-3	-	-	-	0.1	-	-	0.03±0.01	-
Docosahexaenoic acid, C22:6 n-3	-	-	-	0.1	-	-	0.006±0.003	-

[1] Data taken from St-Hilaire et al. (2007), Li et al. (2011), Sealey et al. (2011), Kroeckel et al. (2012), Zheng et al. (2012), Oonincx et al. (2015), and Ewald et al. (2020). Values are means ± standard deviations.
[2]By-product derived from food manufacturing (hight fat: 60% of spent grainds, 20% of beer yeast, and 20% of cookie remains; low fat: 50% of beer yeast, 30% of potato steam peelings, and 20% of beet molasses)

Table 2.7. Mineral concentrations (%, DM) of BSFL fed different substrates[1]

Mineral	Chicken feed	SAW90[2]	SAW95[2]	Kitchen waste	Spent grain	Chicken manure	Swine manure
Calcium	3.14	8.29±2.3	6.8±0.3	2±1.4	1.7±0.5	3.2±2.3	5.36
Phosphorus	1.28	1.92±0.4	1.67±0.3	4.1±0.3	4.6±0.6	3.9±0.3	0.88
Magnesium	0.79	0.41±0.1	0.39±0.0	3.3±0.1	3.5±0.1	4.0±0.3	0.44
Sodium	0.27	0.22±0.0	0.19±0.0	2±0.1	2.6±0.1	2.4±0.1	0.13
Potassium	1.96	0.99±0.3	0.98±0.1	5.7±0.0	4.4±0.0	4.9±0.1	1.16
Iron	0.04	0.05±0.0	0.06±0.0	2.2±0.0	0.3±0.0	0.6±0.4	0.08
Zinc	0.02	0.03±0.0	0.02±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.03
Copper	0.002	1.27±0.1	1.16±0.2	0.2±0.0	0.5±0.0	0.4±0.0	0.003
Manganese	0.04	0.02±0.0	0.02±0.0	0.9±0.0	1.1±0.0	1.4±0.0	0.03

[1] Data taken from Arango Gutiérrez (2005), Newton et al. (2005), Finke (2013), Shumo et al. (2019), and Schmitt et al. (2019). Values are means ± standard deviations.
[2]SAW, solid aquaculture wastes (SAW90, CP=21.4%, fat=14.3%, and ash=18.4%; SAW95, CP=12.9%, fat=13.5%, and ash=19.3%).

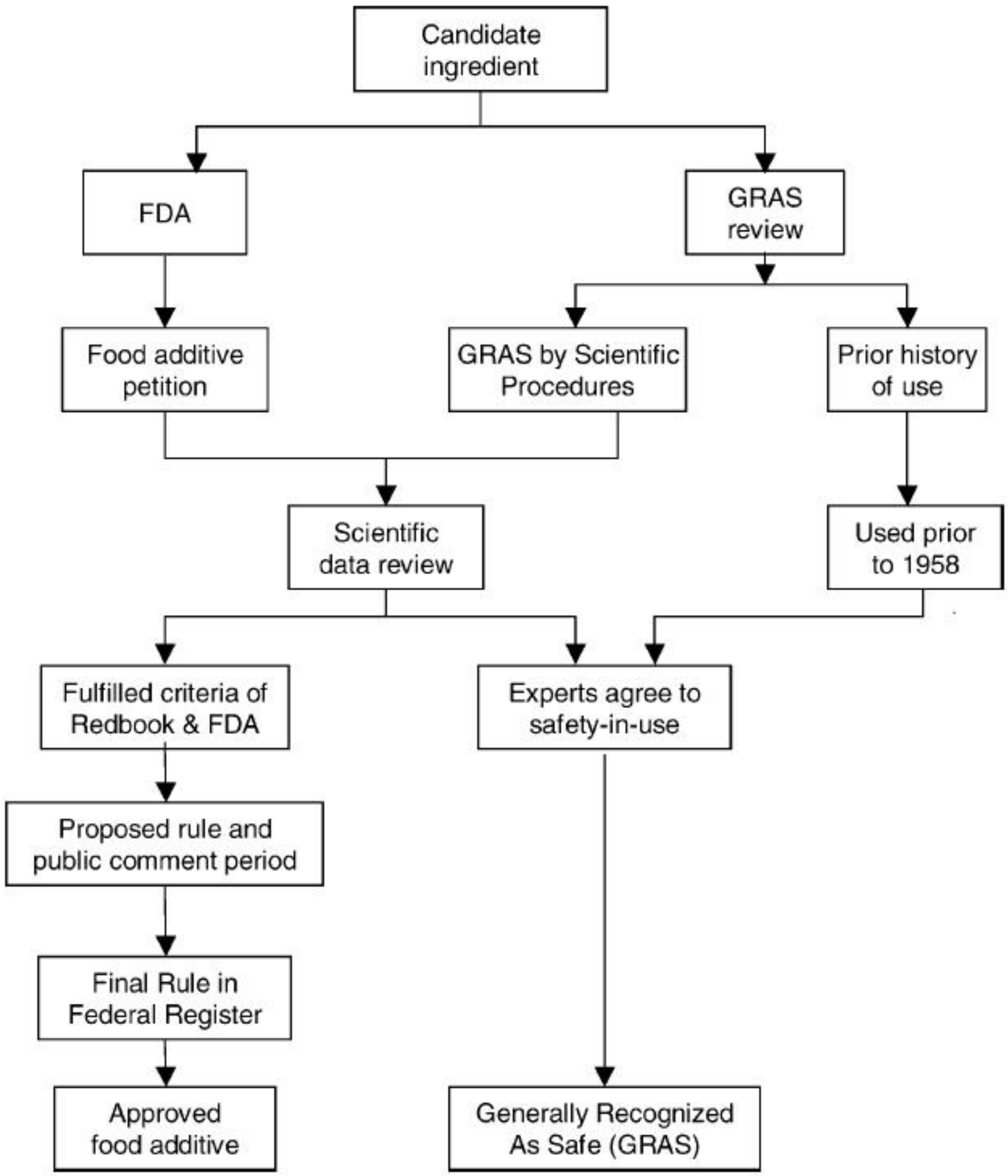

Figure 2.1. Ingredient approval for use in pet food (Burdock and Carabin, 2004).

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***CHAPTER 3:**

NUTRIENT AND AA DIGESTIBILITY OF BLACK SOLDIER FLY LARVAE DIFFERING IN AGE USING THE PRECISION-FED CECECTOMIZED ROOSTER ASSAY

ABSTRACT

Edible insects such as black soldier fly larvae (BSFL) are alternative protein sources for animal feeds due to their high-protein content and potential low environmental footprint. However, protein quality and AA content may vary across insect species and age. Our objective was to determine the effects of age on nutrient and AA digestibility of BSFL intended for use in pet foods using the precision-fed cecectomized rooster assay. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to experimentation. Twenty-four cecectomized roosters (four roosters per substrate) were randomly assigned to test substrates [BSFL0 = day 0 (day of hatch); BSFL11 = day 11; BSFL14 = day 14; BSFL18 = day 18; BSFL23 = day 23; BSFL29 = day 29]. After 24 h of feed withdrawal, roosters were tube-fed 20 g of test substrates. Following crop intubation, excreta were collected for 48 h. Endogenous corrections for AA were made using five additional cecectomized roosters. All data were analyzed using a completely randomized design and the GLM procedure of SAS 9.4. DM and OM digestibilities were not different among substrates, but acid-hydrolyzed fat digestibility tended to be greater ($P < 0.10$) for BSFL23 and BSFL29 than BSFL14 and BSFL18.

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Although all substrates had a high digestibility, BSFL0 and BSFL11 had the lowest ($P < 0.05$) digestibilities for most indispensable and dispensable AA. Digestible indispensable AA score (DIAAS)-like reference values were calculated to determine protein quality according to AAFCO nutrient profiles and NRC recommended allowances for dogs and cats. In general, BSFL18 had the highest, and BSFL11 had the lowest DIAAS-like reference values for most indispensable AA. Threonine, methionine, cysteine, and arginine often were the first-limiting AA. Our results suggest that BSFL are a high-quality protein and AA source, but that age can affect the AA digestibility and protein quality of this alternative protein source.

INTRODUCTION

As the world population increases, the production of animal-derived protein sources must as well and is expected to increase more than 75% by 2050 (Alexandratos and Bruinsma, 2012). This increase in demand will cause ecological strain by increasing greenhouse gas emissions, global freshwater stress, and soil acidification (Miglietta et al., 2015). Insects may serve as a potential solution to those problems. For example, the average water footprint of mealworms (23 L/g protein) is lower than pork (57 L/g protein), chicken (34 L/g protein), and beef (112 L/g protein). Also, the global warming potential of mealworms per kilogram of edible protein (14 kg of CO_2-eq) is lower than chicken (19 to 37 kg of CO_2-eq), pork (21 to 54 kg of CO_2-eq), and beef (77 to 175 kg of CO_2-eq) and the land use (18 m^2) is lower than that of chicken (41 to 51 m^2), pork (46 to 63 m^2), and beef (142 to 254 m^2) (de Vries and de Boer, 2010; Oonincx and de Boer, 2012; Miglietta et al., 2015). Therefore, insects may be considered as a more sustainable protein source compared with traditional animal-derived protein sources for human consumption.

Black soldier fly larvae (BSFL; *Hermetia illucens*) have attracted substantial attention worldwide in recent years because they may serve as an alternative protein source for pet foods given their many economic, nutritional, and environmental advantages. BSFL have several advantages over other insect species, including the number of growth cycles possible per year, the potential for vertical farming, and protein yield (Koutsos et al., 2019). One of their benefits may be due to the variety of digestive enzymes (α-amylase, lipase, and proteases) they secrete by the salivary gland and gastrointestinal tract, leading to more effective digestion and nutrient accumulation than house fly larvae (Kim et al., 2011). For this reason, during the larval stage, BSFL perform well on a variety of organic materials (animal waste and plant material). Despite their ability to grow on waste materials, in the United States and Europe, they must be fed feed-grade ingredients and adhere to standard safe feeding conditions in order to be fed to livestock or companion animals. The restriction to approved, feed-grade materials are intended to ensure production of safe larvae, which could accumulate pathogens, heavy metals, or other toxins from lessregulated inputs (AAFCO, 2016).

The life cycle of BSFL ranges between several weeks to several months depending on the environmental temperature and humidity and quality of diet (Veldkamp et al., 2012; Wang and Shelomi, 2017). Female BSFL lay over 500 eggs in a dry environment. After 4 to 5 d, the eggs hatch and start to consume their diet. Two to 4 wk later, the larvae reach the prepupal stage, stop feeding, and move to sheltered spaces for pupa development (Sheppard et al., 2002; Tomberlin and Sheppard, 2002; Liu et al., 2017). In most previous studies, the prepupal stage of BSFL has been evaluated for nutritional composition analysis for poultry or swine diets (Kroeckel et al., 2012; Cullere et al., 2016; Barragan- Fonseca et al., 2017). To our knowledge, limited scientific data have shown how different harvest age affects BSFL nutrient composition and its bioavailability for animal feeds (Liu et al., 2017). Therefore, it is necessary to evaluate the protein quality of various ages of BSFL for animal feed.

The cecectomized rooster assay has been used as a model for measuring nutrient and AA digestibility of feed ingredients of pet foods because results were shown to be similar to that of ileal-cannulated dogs (Johnson et al., 1998). Like ileal-cannulated animals, cecectomy allows for digestibility estimates to be made with minimal interference from the bacterial fermentation of proteins in the hindgut. Also, the cecectomized rooster assay is less expensive, time-consuming, and labor-intensive than other types of assays (Johnson et al., 1998; Faber et al., 2010; Kerr et al., 2014; Oba et al., 2019). Given these advantages, the cecectomized rooster assay is often a preferred model to evaluate the protein quality of novel ingredients for dogs and cats.

The objective of this study was to determine the effects of the age on nutrient and AA digestibility of BSFL intended for use in pet foods using the precision-fed cecectomized rooster assay.

MATERIALS AND METHODS

Substrates

The BSFL were harvested at six different ages and used for testing in this study. Because the nutritional value of the diet affects BSFL growth rate, the ages (days after hatch) and weights of BSFL studied herein are provided: day 0 (BSFL0): 0.02 mg; day 11 (BSFL11): 0.08 mg; day 14 (BSFL14): 0.14 mg; day 18 (BSFL18): 0.14 mg; day 23 (BSFL23): 0.19 mg; day 29 (BSFL29): 0.21 mg. Industry standard rearing conditions were maintained (Sheppard et al., 2002). All insects were fed a commercial layer ration until 11 d of age. From day 11, they were fed a combination of distiller's dried grains with solubles from a distillery, bakery by-product meal, and calcium chloride. At the time of collection for this trial, larvae were washed, frozen, and shipped to the University of Illinois for further processing and preparation. All ages of BSFL were lyophilized and ground through a 2-mm screen using a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) with dry ice to allow for proper grinding before analysis and feeding to cecectomized roosters.

Cecectomized Rooster Assay

The protocol for the cecectomized rooster assay, including all animal housing, handling, and surgical procedures, was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana- Champaign prior to experimentation. A precision-fed rooster assay using cecectomized Single Comb White Leghorn roosters was conducted as described by Parsons (1985) to determine the nutrient and AA digestibility of the substrates listed above. Prior to the study, the cecectomy surgery was performed on roosters under general anesthesia according to the procedures of Parsons (1985).

Briefly, 24 cecectomized roosters were randomly assigned to the test substrates (four roosters per test substrate evaluated). After 24 h of feed withdrawal, roosters were tube-fed 20 g of test substrates. Following crop intubation, excreta (urine and feces) were collected for 48 h on plastic trays placed under each individual cage. Excreta samples then were lyophilized, weighed, and ground through a 0.25-mm screen prior to analysis. Endogenous corrections for AA were made using five additional cecectomized roosters that had been fasted for 48 h. Macronutrient and AA digestibilities were calculated using the method described by Sibbald (1979).

Chemical Analyses

The substrates and rooster excreta were analyzed for DM (105°C) and ash [organic matter (OM) was calculated based on ash] according to AOAC (2006; DM: method 934.01; OM: method 942.05). Nitrogen and CP were determined using a Leco Nitrogen/Protein Determinator (Model FP-2000, Leco Corporation, St. Joseph, MI) according to the AOAC (2006; method 982.30E). Fat concentrations were measured by acid hydrolysis according to the AACC (1983) followed by diethyl ether extraction (Budde, 1952). Gross energy (GE) was measured using a bomb calorimeter (Model 6200; Parr Instrument Co., Moline, IL). AA were measured at the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) according to the AOAC (2006; method 982.30 E [a, b, c]).

Digestible Indispensable AA Score (DIAAS)-Like Calculations

The calculation of DIAAS-like reference values was followed according to Mathai et al. (2017) and Oba et al. (2019). The digestible indispensable AA reference ratios were calculated for each ingredient using the following equation (FAO, 2011): Digestible indispensable AA reference ratio = digestible indispensable AA content in 1 g protein of ingredient (mg)/mg of the same dietary indispensable AA in 1 g of the reference protein.

The references used were the AAFCO nutrient profiles (AAFCO, 2019) for adults at maintenance (dogs and cats) and growth and reproduction (dogs and cats), and National Research Council (NRC, 2006) recommended allowances for adults (dogs and cats), growing puppies (4 to 14 wk of age), and growing kittens. The DIAAS-like values then were calculated using the following equation adapted from FAO (2011): DIAAS-like % = 100 × [(mg of digestible dietary indispensable AA in 1g of the dietary protein)/ (mg of the minimum recommendation of the same dietary indispensable AA in 1 g of the minimum protein recommendation)].

Statistical Analysis

All data were analyzed as a completely randomized design using the GLM procedure of the Statistical Analysis System 9.4 (SAS Inst., Cary, NC). Substrates were considered to be a fixed effect. Tukey's multiple comparison analysis was used to compare LS means and control for experiment-wise error. Differences were considered significant with $P < 0.05$.

RESULTS

Chemical Composition

The chemical composition of the tested BSFL is presented in **Table 3.1**. In regard to chemical composition, OM was highest in BSFL0 (94.8%, dry matter basis [DMB]) and was steadily reduced with age. CP, acid-hydrolyzed fat (AHF), and GE were variable. CP was highest in BSFL0 (57.2%, DMB), was reduced to half that level by day 11 (26.8%, DMB), and then steadily increased until day 29 (40.3%, DMB). AHF was highest at BSFL14 (39.7%, DMB) and BSFL11 (38.0%), and lower for the other ages (30.2% to 34.5% DMB). The GE values ranged from 5.40 (BSFL23) to 6.29 kcal/g DM (BSFL0). Concentrations of indispensable and dispensable AA are presented in **Table 3.2**. Amino acid patterns were similar to that of CP. The BSFL0 treatment had higher AA concentrations than other stages of BSFL except for tryptophan, valine, tyrosine, and taurine. On the other hand, BSFL11 had lower indispensable AA concentrations than other BSFL ingredients.

Cecectomized rooster assay

Macronutrient digestibilities of tested BSFL ingredients are presented in **Table 3.1.** All animals remained healthy during the study. There were no significant differences in DM and OM digestibility among substrates, but AHF tended to be greater ($P < 0.10$) for BSFL23 and BSFL29 than BSFL14 and BSFL18.

AA digestibilities data are presented in **Table 3.3**, with many differences being observed. Of the indispensable AA, day 0 BSFL had lower ($P < 0.05$) methionine and phenylalanine digestibilities than for those harvested at day 23 of age. Day 11 BSFL had a lower ($P < 0.05$) tryptophan digestibility than day 18 and 23 BSFL. Day 11 BSFL also had a lower ($P < 0.05$) methionine digestibility than day 23 BSFL. Day 29 BSFL had a lower ($P < 0.05$) leucine digestibility than day 18 BSFL. Also, days 0 and 29 BSFL tended to have a lower ($P < 0.10$) histidine digestibility than days 11, 14, 18, and 23 BSFL. Of the dispensable AA, day 29 BSFL had a lower ($P < 0.05$) alanine digestibility than all other BSFL ages except for day 0. Finally, day 0 BSFL had lower ($P < 0.05$) tyrosine digestibility than day 18 BSFL.

DIAAS-Like Calculations

DIAAS-like reference values for growing puppies and kittens are presented in **Table 3.4** and **Table 3.5**, respectively. DIAAS-like reference values for adult dogs and cats at maintenance are presented in **Table 3.6** and **Table 3.7**, respectively. The first limiting AA based on DIAAS-like calculations from AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances are listed in **Table 3.8** and **Table 3.9**, respectively.

Based on the AAFCO recommended allowances for growing puppies, all BSFL ingredients had some DIAAS-like values below 100%. BSFL18, 23 and 29 had the most DIAAS-like reference values over 100% (all except threonine), followed by BSFL14, BSFL0, and BSFL11. Using the NRC recommended allowances for growing puppies, BSFL18 had no DIAAS-like reference values below 100%, and BSFL23 and 29 had only DIAAS-like reference values below 100% for threonine followed by BSFL14, BSFL11, and BSFL0. According to the AAFCO and NRC recommended allowances for growing kittens, all BSFL ingredients had only DIAAS-like reference values below 100% for methionine, sulfur AA (met + cys), and aromatic AA (phe + tyr), with all other indispensable AA values being over 100%.

Based on the AAFCO recommended allowances for adult dogs, all BSFL ingredients had DIAAS-like reference values over 100% except methionine, sulfur AA (met + cys), and tryptophan (for BSFL11). Using the NRC recommended allowances for adult dogs, all BSFL had DIAAS-like values below 100% for leucine, methionine, sulfur AA (met + cys), phenylalanine, and threonine followed. BSFL0 and BSFL11 had DIAAS-like reference values below 100% for isoleucine, aromatic AA (phe + tyr), tryptophan, and valine. The DIAAS-like reference value also was below 100% for BSFL14, BSFL23, and BSFL29.

The first-limiting AA based on DIAAS-like reference values from AAFCO (2019) nutrient profiles for dogs and cats (growth and reproduction; adults at maintenance) are provided in **Table 3.8**. For growing and reproducing dogs, threonine was the first limiting AA for all ages of BSFL ingredients except for BSFL14 that was limited by tryptophan. All of those values were <100, suggesting insufficiency if a diet was formulated with only that protein source and at an inclusion level to meet the nutrient profile. Based on DIAAS-like reference values from AAFCO (2019) nutrient profiles for adult dogs at maintenance, methionine and cysteine were the first limiting AA for all ages of BSFL except for BSFL18 and BSFL23 that were limited by methionine. Based on DIAAS-like reference values from AAFCO (2019) nutrient profiles for growing and reproducing cats, methionine was the first limiting for all ages of BSFL except for BSFL0 that was limited by methionine and cysteine. Based on DIAAS-like reference values from AAFCO (2019) nutrient profiles for adult cats at maintenance, threonine (BSFL0) and arginine (BSFL11; BSFL14, BSFL18; BSFL23 and BSFL29) were the first limiting AA, but all values were >100.

The first-limiting AA based on DIAAS-like reference values from NRC (2006) recommended allowances for growing puppies (4 to 14 wk of age), growing kittens, and adult dogs and cats at maintenance are provided in **Table 3.9**. For growing puppies, methionine, cysteine (BSFL0; BSFL11; BSFL14), and threonine (BSFL18; BSFL23 and BSFL29) were the first limiting AA, but BSFL18 and BSFL23 were >100. Based on DIAAS-like reference values from NRC (2006) recommended allowances for adult dogs at maintenance, methionine and cysteine were the first limiting AA for all ages of BSFL ingredients except for BSFL18 and BSFL23 that were limited by methionine. All of those values were <100. Based on DIAAS-like reference values from NRC (2006) recommended allowances for growing kittens, methionine and cysteine were the first limiting AA for all ages of BSFL ingredients except for BSFL18 and BSFL23 that were limited by methionine. Based on DIAAS-like values from NRC (2006) recommended allowances for adult cats, arginine (BSFL14; BSFL18 and BSFL23), leucine (BSFL29), and phenylalanine and tyrosine (BSFL0; BSFL11) were the first limiting AA, but BSFL14, BSFL18, BSFL23, and BSFL29 were >100.

DISCUSSION

The demand for animal-derived protein sources will continue to increase in coming years because of the growing human population and rising living standards in developing countries (FAO, 2009). This demand will increase the competition for protein sources in human foods, pet foods, and livestock feeds (Bosch et al., 2014). BSFL may be used to address this problem. Compared with other insects, BSFL may provide a higher number of growth cycles per year, a greater potential for vertical farming, and total protein yield (Koutsos et al., 2019). In order to produce high-quality BSFL protein, the quality of food fed to BSFL is important as it correlates with their larval development time and nutrient composition. According to Oonincx et al. (2015), larval development time was increased when BSFL were fed a low-protein diet (over 5 wk) compared with a high-protein, high-fat diet (3 wk). Moreover, the EE and ash content of the substrates fed (chicken feed, digestate, vegetable, and restaurant waste) was highly correlated with EE ($r = 0.942$) and ash ($r = 0.954$) content of BSFL in the prepupa stage (Spranghers et al., 2017). Many other factors also influence the nutrient composition of BSFL, including sex (Sönmez and Gülel, 2008), stage of development (McClement et al., 2003), and environmental factors (temperature and humidity) (Sönmez and Gülel, 2008; Nedvěd and Kalushkov, 2012). Because these factors may have such large effects, research is needed to test the protein quality of different ages of BSFL.

In the current study, day 0 BSFL had the highest CP content, and from days 11 to 29, CP content linearly increased because BSFL tends to store nutrients in their body for the adult stage. Also, ash content linearly increased from days 0 to 29 due to the development of their exoskeleton. BSFL AHF composition varied considerably from days 0 to 29, but was typically changing over time in a manner that was opposite to that of CP. This pattern of CP and AHF deposition has been reported previously. According to Liu et al. (2017), the CP content of BSFL increased after hatching, and then it slowly decreased until day 14, while it increased in pupal and adult stages. The diet fed after day 11 for BSFL in the present study may have affected CP content during the larval stage. The previous study reported that BSFL CP content was affected by CP content of test substrates. The CP content of BSFL increased when fed a low-protein diet (10% CP) compared with a high-protein diet (17% CP) (Barragan-Fonseca et al., 2019), and other studies also showed that CP content of BSFL decreased with increased CP content in the BSFL diet (Tschirner and Simon, 2015; Barragan-Fonseca et al., 2017). The reason for the rapid decrease in CP from days 0 to 11 BSFL may be due to the normal growth and development of the body that occurs at that time. The body size of BSFL increases from only 1 mm at hatching to ~27 mm in length and 6 mm in width before the prepupal stage (Park, 2016).

Crude fat (CF) content of BSFL is also affected by the protein and carbohydrate content of the diet fed. According to Barragan- Fonseca et al. (2019), BSFL CF content increased when BSFL were fed a diet containing high CP (24%) and carbohydrate (55%). BSFL CF content of different life stages was evaluated by Liu et al. (2017), and CF content tended to fluctuate. They reported that CF firstly decreased after hatching (15.8%) to the neonate larvae (11.8%) and gradually increased in the larval phase (28%), and then reduced in pupal stages. In the current study, the AHF content of BSFL increased from days 0 to 14 and then decreased from days 18 to 29. The reason for the increase of fat content from days 0 to 14 may be due to their energy needs for metamorphosis during the pupal stages (Mirth and Riddiford, 2007; Arrese and Soulages, 2010). Moreover, because adult BSFL do not feed during the time of mating and oviposition, the amount of nutrients accumulated in the larval stage is essential for adult life (Arrese and Soulages, 2010; Liu et al., 2017). In this study, ash content linearly increased from days 0 to 29, and this result was similar to the value previously reported by Liu et al. (2017). The possible reason for a higher level of ash in pupal than in larval stage is due to the formation of the cuticle layer in their body (Veldkamp et al., 2012; Liu et al., 2017).

BSFL contain about 41% to 44% CP, but less than house flies (45% to 55% CP) and mealworms (49% to 57% CP) (Klasing et al., 2000; St-Hilaire et al., 2007; Makkar et al., 2014). St-Hilaire et al. (2007) compared AA concentrations of the BSFL prepupal and pupal stages of houseflies. Most of the indispensable and dispensable AA concentrations of BSFL were lower than that of house flies, but not significantly. Of course, the methodology used to rear the insects and analyze the composition in the lab will impact nutrient concentrations measured. Compared with the AA concentrations of larval and prepupal stages of BSFL fed household organic waste evaluated by Kawasaki et al. (2019), some of indispensable AA of prepupal stage (arginine, histidine, leucine, methionine, threonine, and tryptophan) were higher than day 23 BSFL in this study. Also, some of the indispensable AA in the larval stage (arginine, histidine, isoleucine, and leucine) were higher than day 18 BSFL in this study. However, the prepupal stages of BSFL fed swine manure had lower indispensable AA such as leucine, methionine, and tryptophan than day 23 BSFL (Newton et al., 2005). Additionally, prepupa reared on dairy cow manure showed lower indispensable AA (isoleucine, methionine, phenylalanine, and threonine) than day 23 BSFL (Sealey et al., 2011). These results demonstrate the importance of diet and environment on BSFL AA and protein composition.

When evaluating protein quality of feed ingredients, AA and macronutrient composition and digestibility are necessary. Therefore, ileal-cannulated animals and the cecectomized rooster assay have been used to determine the quality of protein ingredients (Johnson et al., 1998; Faber et al., 2010; Kerr et al., 2014; Oba et al., 2019). Although the precision-fed cecectomized rooster assay includes a surgical procedure and does not allow the measure of AA metabolism, this assay is a good model to measure the nutrient and AA digestibility of pet food ingredients because ileal-cannulated dog and cat models have issues related to expense, animal welfare, and length of time

(Engster et al., 1985; Kerr et al., 2014; Deng et al., 2016). Additionally, the results from the cecectomized rooster assay and ileal-cannulated dogs have been shown to be highly correlated. According to Johnson et al. (1998), they used six ileal-cannulated dogs (6 × 6 Latin square design) and 24 cecectomized roosters (completely randomized design) to test six animal by-product foods, with results showing a high correlation between roosters and dogs (r = 0.87 to 0.92). Therefore, the cecectomized rooster assay is an appropriate model to evaluate the protein quality of different ages of BSFL ingredients (Johnson et al., 1998; Kerr et al., 2014; Deng et al., 2016; Oba et al., 2019).

For all AA evaluated in this study, days 14, 18, and 29 BSFL had the higher AA digestibilities than other stages of BSFL. Of the indispensable AA, days 14, 18, and 23 BSFL had a digestibility of over 90% except for histidine, threonine, and valine. Besides days 0, 11, and 29, BSFL had an indispensable AA digestibility of over 85% except for histidine and valine. Although days 14, 18, and 23 BSFL had higher indispensable AA digestibilities than other stages of BSFL for most AA, all BSFL ingredients could be easily absorbed and utilized by roosters.

Little information is available about the AA digestibility of different ages of BSFL. When compared with the data conducted by De Marco et al. (2015), all AA digestibilities of the BSFL meal had lower values (<85%) because the larvae were dried for 20 h at low temperature (60 °C) and ground to meal and also, they were reared on a different diet (cereal by-product) compared with the current study. The defatted BSFL reported by Mwaniki and Kiarie (2018) had similar AA digestibility to the day 11 BSFL in the present study but lower than days 14, 18, and 29 BSFL except for valine and glycine. BSFL diet, processing methods, chitin contents in the prepupal and pupal stage, experimental design and environmental factors (temperature and humidity) may affect AA digestibility of BSFL.

DIAAS-like reference values have recently been used to evaluate the protein quality of feed ingredients for dogs and cats (Oba et al., 2019). Based on the DIAAS-like reference values in this study, if a diet was formulated with only BSFL protein for growing puppies by using AAFCO nutrient requirements, day 14 BSFL may not provide sufficient arginine (DIAAS-like reference value = 98.68), met + cys (DIAAS-like reference value = 84.29), phenylalanine (DIAAS-like reference value = 95.12), and threonine (DIAAS-like reference value = 75.26), while days 18 and 23 BSFL need more threonine (DIAAS-like reference value = 80.34; 77.7) to meet nutrient requirement. If NRC recommendations are used as a reference, only met + cys (DIAAS-like reference value = 84.29) and threonine (DIAAS-like reference value=96.63) were the limiting AA in day 14 BSFL. For the growth and reproduction of cats and kittens, met + cys (DIAAS-like reference value = 61.51 to 98.29) and phe + tyr (DIAAS-like reference value = 79.96 to 95.45) were not sufficient if a diet was formulated based on AAFCO and NRC recommendations. As a similar pattern was observed in adult dogs using AAFCO recommendations, all BSFL ingredients fulfill the AA requirements except for met + cys (DIAAS-like reference value = 62.46 to 95.81). However, based on the NRC recommendations, day 14 BSFL had sufficient arginine (DIAAS-like reference value = 125.31), histidine (DIAAS-like reference value = 140.78), isoleucine (DIAAS-like reference value = 102.88), lysine (DIAAS-like reference value = 176.10), phe + tyr (DIAAS-like reference value = 137.55), and valine (DIAAS-like reference value = 114.70). Days 18 and 23 BSFL also showed a similar pattern with day 14 BSFL. For adult cats, all BSFL ingredients had DIAAS-like reference values over 100%.

In conclusion, this study provided the macronutrient and AA digestibilities data of different ages of BSFL intended for use in dog and cat foods. DM and OM digestibilities had no difference, but AHF digestibility tended to vary and AA digestibilities were different among substrates. For evaluating protein quality of different ages of BSFL, our data showed that AA digestibilities were highest in days 14, 18, and 23 BSFL and threonine, methionine, and tryptophan often were the first-limiting AA of BSFL based on DIAAS-like reference values for dogs and cats. However, all ages of BSFL ingredients had high-quality protein, AA concentrations, and digestibilities. Therefore, BSFL appears to be a high-quality protein source for pet food. These data provide more information into the potential for BSFL to be applied to pet food and other animal feeds. Differences in rearing conditions, diet, and processing characteristics need to be taken into account when utilizing a commercial source of BSFL, but it is clear that producers and their customers may be able to modify the nutrient content and subsequent digestibility of insect-derived ingredients for a particular application. Further research such as product safety, pet owner perception, processing effects (extrusion and retort), and nutrient digestibility of a complete and balanced diet including BSFL as a significant protein source should be done to determine how much BSFL may be used in pet food formulations.

TABLES

Table 3.1. Chemical composition and macronutrient digestibilities of BSFL differing in life stage using the precision-fed cecectomized rooster assay

Item	BSFL0[1]	BSFL11	BSFL14	BSFL18	BSFL23	BSFL29	SEM	P-value
Chemical composition								
DM, %	96.02	93.97	93.93	93.74	89.26	91.96	-	-
OM, % DM	94.82	91.38	91.49	89.65	86.54	85.87	-	-
CP, % DM	57.20	26.77	31.31	37.72	39.74	40.34	-	-
AHF, % DM	31.44	37.98	39.74	34.45	30.20	32.45	-	-
GE, kcal/g DM	6.29	5.95	6.10	5.90	5.40	5.57	-	-
Nutrient digestibility								
DM, %	54.61	63.24	58.43	55.73	61.11	58.26	1.312	0.4386
OM, %	64.26	75.57	69.85	68.49	71.64	67.64	1.299	0.1952
AHF, %	83.58^{xy}	82.00^{xy}	80.27^{y}	80.77^{y}	88.26^{x}	86.36^{x}	0.904	0.0401

[1]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae; AHF, acid-hydrolyzed fat.

$^{x\text{-}y}$Within a row, means lacking a common superscript letter differ (P<0.10); n, 4 roosters per treatment.

Table 3.2. Indispensable and dispensable AA concentrations (% DM) of BSFL differing in life stage

Item	BSFL0[1]	BSFL11	BSFL14	BSFL18	BSFL23	BSFL29
CP, % DM	57.20	26.77	31.31	37.72	39.74	40.34
Indispensable AA						
Arginine	3.18	1.22	1.46	1.82	1.86	2.05
Histidine	1.45	0.78	0.94	1.19	1.18	1.29
Isoleucine	2.49	1.10	1.35	1.67	1.79	1.82
Leucine	3.71	1.68	2.02	2.58	2.76	2.80
Lysine	3.81	1.65	2.12	2.67	2.67	2.71
Methionine	0.93	0.40	0.48	0.67	0.70	0.74
Phenylalanine	1.95	0.97	1.22	1.67	1.76	1.73
Threonine	2.10	1.00	1.23	1.52	1.54	1.57
Tryptophan	0.57	0.26	0.40	0.57	0.58	0.50
Valine	3.13	1.58	2.10	2.91	3.03	3.23
Selected dispensable AA						
Alanine	3.71	1.96	2.24	2.71	2.56	2.35
Aspartic acid	4.32	1.88	2.49	3.39	3.52	3.63
Cysteine	0.60	0.36	0.35	0.36	0.31	0.28
Glutamic acid	6.38	2.95	3.30	3.80	3.70	3.63
Glycine	2.87	1.28	1.59	1.97	2.30	2.43
Proline	2.87	1.73	1.97	2.39	2.44	2.30
Serine	2.21	1.04	1.28	1.55	1.58	1.62
Tyrosine	2.14	1.20	1.79	2.48	2.63	2.64
Taurine	0.05	0.10	0.09	0.08	0.08	0.07

[1]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae.

Table 3.3. AA digestibilities (%) of BSFL differing in life stage using the precision-fed cecectomized rooster assay

Item	BSFL0[1]	BSFL11	BSFL14	BSFL18	BSFL23	BSFL29	SEM	*P*-value
CP, % DM	57.20	26.77	31.31	37.72	39.74	40.34	-	-
Indispensable AA								
Arginine	90.77	93.42	94.05	94.09	95.52	90.64	0.647	0.1623
Histidine	82.99^{y}	89.58^{x}	89.09^{x}	90.91^{x}	87.82^{x}	82.87^{y}	0.959	0.0301
Isoleucine	86.97	89.29	90.67	92.06	92.52	86.72	0.718	0.0504
Leucine	87.02^{ab}	91.22^{ab}	92.04^{ab}	93.58^{a}	92.90^{ab}	85.99^{b}	0.852	0.0186
Lysine	87.75	89.12	91.03	90.49	91.36	90.16	0.721	0.7619
Methionine	89.43^{b}	89.15^{b}	90.79^{ab}	92.63^{ab}	96.04^{a}	92.78^{ab}	0.670	0.0108
Phenylalanine	85.81^{b}	88.46^{ab}	90.05^{ab}	92.34^{ab}	93.98^{a}	90.18^{ab}	0.764	0.0165
Threonine	87.37	87.01	88.55	92.16	92.67	88.08	0.921	0.3176
Tryptophan	89.98^{ab}	89.50^{b}	92.09^{ab}	94.43^{a}	94.46^{a}	92.56^{ab}	0.551	0.0109
Valine	80.88	79.75	83.79	85.29	81.64	75.34	1.117	0.1383
Selected dispensable AA								
Alanine	89.99^{ab}	92.09^{a}	92.53^{a}	93.95^{a}	91.46^{a}	82.15^{b}	1.024	0.0023
Aspartic acid	89.88	88.02	90.22	92.55	94.28	91.00	0.686	0.1134
Cysteine	79.22	81.44	78.38	79.00	87.40	81.89	1.809	0.7679
Glutamic acid	90.42	89.28	88.41	89.85	92.08	88.01	0.784	0.7484
Glycine	76.29	82.56	70.27	78.37	84.96	73.72	2.259	0.4602
Proline	85.82	88.57	89.25	91.39	90.18	83.04	0.957	0.1039
Serine	86.71	86.87	89.51	90.42	91.12	81.47	1.111	0.1112
Tyrosine	85.06^{b}	89.31^{ab}	91.03^{ab}	93.39^{a}	91.77^{ab}	86.94^{ab}	0.807	0.0097

[1]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae.

[a-b]Within a row, means lacking a common superscript letter differ ($P<0.05$); n, 4 roosters per treatment.

[x-y]Within a row, means lacking a common superscript letter differ ($P<0.10$); n, 4 roosters per treatment.

Table 3.4. Digestible indispensable AA score (DIAAS)–like reference values[1] of BSFL differing in life stage for growing puppies[2]

	AAFCO (2019)						NRC (2006)					
Item	BSFL0[3]	BSFL11	BSFL14	BSFL18	BSFL23	BSFL29	BSFL0	BSFL11	BSFL14	BSFL18	BSFL23	BSFL29
Indispensable AA												
Arginine	113.54^{a}	95.78^{c}	98.68^{bc}	102.14^{bc}	100.59^{bc}	103.65^{b}	143.72^{a}	121.24^{c}	124.91^{bc}	129.30^{bc}	127.33^{bc}	131.20^{b}
Histidine	107.58	133.46	136.78	146.66	133.34	135.51	121.37^{b}	150.57^{a}	154.32^{a}	165.46^{a}	150.44^{a}	152.89^{a}
Isoleucine	119.98^{bc}	116.25^{c}	123.89^{abc}	129.16^{ab}	132.06^{a}	123.99^{abc}	131.05^{bc}	126.98^{c}	135.33^{abc}	141.08^{ab}	144.25^{a}	135.43^{abc}
Leucine	98.45^{c}	99.83^{c}	103.57^{bc}	111.63^{ab}	112.54^{a}	104.11^{abc}	98.45^{c}	99.83^{c}	103.57^{bc}	111.63^{ab}	112.54^{a}	104.11^{abc}
Lysine	146.13^{bc}	137.31^{c}	154.09^{ab}	160.12^{a}	153.45^{ab}	151.42^{ab}	149.45^{bc}	140.43^{c}	157.59^{ab}	163.76^{a}	156.94^{ab}	154.86^{ab}
Methionine	93.47^{b}	85.63^{c}	89.48^{bc}	105.76^{a}	108.75^{a}	109.41^{a}	99.14^{b}	90.81^{c}	94.90^{bc}	112.17^{a}	115.34^{a}	116.04^{a}
Met + Cys	72.50^{b}	77.82^{b}	84.29^{b}	106.11^{a}	111.21^{a}	106.95^{a}	72.50^{b}	77.82^{b}	84.29^{b}	106.11^{a}	111.21^{a}	106.95^{a}
Phenylalanine	79.30^{e}	86.88^{d}	95.12^{c}	110.82^{ab}	112.83^{a}	104.84^{b}	101.26^{e}	110.93^{d}	121.46^{c}	141.51^{ab}	144.08^{a}	133.87^{b}
Phe + Tyr	105.73^{e}	124.69^{d}	176.17^{c}	249.13^{b}	263.57^{a}	250.18^{ab}	105.73^{e}	124.69^{d}	176.17^{c}	249.13^{b}	263.57^{a}	250.18^{ab}
Threonine	69.39^{c}	70.30^{bc}	75.26^{abc}	80.34^{a}	77.70^{ab}	74.17^{abc}	89.10^{c}	90.27^{bc}	96.63^{abc}	103.16^{a}	99.76^{ab}	95.23^{abc}
Tryptophan	100.88^{c}	97.77^{c}	132.35^{b}	160.53^{a}	155.10^{a}	129.06^{b}	87.72^{c}	85.02^{c}	115.89^{b}	139.59^{a}	134.87^{a}	112.23^{b}
Valine	146.44^{c}	155.73^{c}	185.96^{b}	217.72^{a}	205.97^{ab}	199.61^{ab}	146.44^{c}	155.73^{c}	185.96^{b}	217.72^{a}	205.97^{ab}	199.61^{ab}

[1]DIAAS-like reference values were calculated from the digestibility of amino acids in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles of amino acids for growth and reproduction of dogs, and NRC (2006) recommended allowances of amino acids for growing puppies (4 to 14 wk of age).
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae. SEM values for AAFCO (2019) nutrient profile data: arginine, 1.307; histidine, 2.704; isoleucine, 1.336; leucine, 1.327; lysine, 1.858; methionine, 2.052; met+cys, 3.415; phenylalanine, 2.633; phe+tyr, 13.222; threonine, 1.034; tryptophan, 5.023; valine, 5.877. SEM values for NRC (2006) recommended allowance data: arginine, 1.654; histidine, 3.051; isoleucine, 1.459; leucine, 1.327; lysine, 1.900; methionine, 2.177; met+cys, 3.145; phenylalanine, 3.362; phe+tyr, 13.222; threonine, 1.327; tryptophan, 4.368; valine, 5.877.
$^{a-e}$Within a row, means lacking a common superscript letter differ ($P < 0.05$); n, 4 roosters per treatment.

Table 3.5. Digestible indispensable AA score (DIAAS)–like reference values[1] of BSFL differing in life stage for growing kittens[2]

	AAFCO (2019)						NRC (2006)					
Item	**BSFL0**[3]	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**	**BSFL0**	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**
Indispensable AA												
Arginine	122.08^{a}	102.99^{c}	106.11^{bc}	109.83^{bc}	108.16^{bc}	111.45^{b}	131.41^{a}	110.86^{c}	114.21^{bc}	118.22^{bc}	116.43^{bc}	119.96^{b}
Histidine	191.25^{b}	237.26^{a}	243.16^{a}	260.73^{a}	237.05^{a}	240.91^{a}	159.38^{b}	197.71^{a}	202.64^{a}	217.27^{a}	197.55^{a}	200.76^{a}
Isoleucine	202.82^{bc}	196.52^{c}	209.43^{abc}	218.33^{ab}	223.25^{a}	209.60^{abc}	175.27^{bc}	169.83^{c}	180.99^{abc}	188.68^{ab}	192.93^{a}	181.13^{abc}
Leucine	132.29^{c}	134.15^{c}	139.17^{bc}	150.01^{ab}	151.22^{a}	139.90^{abc}	110.24^{c}	111.79^{c}	115.97^{bc}	125.01^{ab}	126.02^{a}	116.58^{abc}
Lysine	146.13^{bc}	137.31^{c}	154.09^{ab}	160.12^{a}	153.45^{ab}	151.42^{ab}	171.91^{bc}	161.54^{c}	181.28^{ab}	188.38^{a}	180.53^{ab}	178.14^{ab}
Methionine	70.35^{b}	64.45^{c}	67.35^{bc}	79.61^{a}	81.86^{a}	82.35^{a}	82.61^{b}	75.68^{c}	79.09^{bc}	93.48^{a}	96.12^{a}	96.70^{a}
Met + Cys	61.51^{b}	66.03^{b}	71.51^{b}	90.03^{a}	94.36^{a}	90.74^{a}	64.08^{b}	68.78^{b}	74.50^{b}	93.78^{a}	98.29^{a}	94.52^{a}
Phenylalanine	168.77^{e}	184.89^{d}	202.43^{c}	235.85^{ab}	240.13^{a}	223.12^{b}	146.27^{e}	160.24^{d}	175.44^{c}	204.40^{ab}	208.11^{a}	193.37^{b}
Phe + Tyr	95.45^{e}	112.56^{d}	159.05^{c}	224.91^{b}	237.94^{a}	225.86^{ab}	79.96^{e}	94.29^{d}	133.23^{c}	188.41^{b}	199.32^{a}	189.20^{ab}
Threonine	131.82^{c}	133.55^{bc}	142.96^{abc}	152.62^{a}	147.59^{ab}	140.88^{abc}	123.37^{c}	124.99^{bc}	133.79^{abc}	142.83^{a}	138.13^{ab}	131.85^{abc}
Tryptophan	107.60^{c}	104.29^{c}	141.18^{b}	171.23^{a}	165.44^{a}	137.67^{b}	140.10^{c}	135.80^{c}	183.82^{b}	222.95^{a}	215.41^{a}	179.25^{b}
Valine	207.46^{c}	220.61^{c}	263.45^{b}	308.43^{a}	291.79^{ab}	282.78^{ab}	172.88^{c}	183.74^{c}	219.54^{b}	257.03^{a}	243.16^{ab}	235.65^{ab}

[1]DIAAS-like reference values were calculated from the digestibility of amino acids in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles of amino acids for growth and reproduction of cats, and NRC (2006) recommended allowances of amino acids for growing kittens.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23; day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae. SEM values for AAFCO (2019) nutrient profile data: arginine, 1.405; histidine, 4.808; isoleucine, 2.258; leucine, 1.783; lysine, 1.858; methionine, 1.545; met+cys, 2.898; phenylalanine, 5.603; phe+tyr, 11.937; threonine, 1.964; tryptophan, 5.358; valine, 8.326. SEM values for NRC (2006) recommended allowance data: arginine, 1.512; histidine, 4.006; isoleucine, 1.952; leucine, 1.486; lysine, 2.186; methionine, 1.814; met+cys, 3.019;phenylalanine, 4.856; phe+tyr, 9.999; threonine, 1.838; tryptophan, 6.977; valine, 6.934.
$^{a-e}$Within a row, means lacking a common superscript letter differ ($P < 0.05$); n, 4 roosters per treatment.

Table 3.6. Digestible indispensable AA score (DIAAS)–like reference values[1] of BSFL differing in life stage for adult dogs[2]

	AAFCO (2019)						NRC (2006)					
Item	**BSFL0**[3]	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**	**BSFL0**	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**
Indispensable AA												
Arginine	178.10[a]	150.24[c]	154.79[bc]	160.23[bc]	157.79[bc]	162.85[b]	144.17[a]	121.62[c]	125.31[bc]	129.71[bc]	127.74[bc]	131.61[b]
Histidine	199.31[b]	247.25[a]	253.40[a]	271.70[a]	247.04[a]	251.06[a]	110.73[b]	137.36[a]	140.78[a]	150.95[a]	137.24[a]	139.48[a]
Isoleucine	179.33[bc]	173.76[c]	185.18[abc]	193.05[ab]	197.40[a]	185.33[abc]	99.63[bc]	96.53[c]	102.88[abc]	107.25[ab]	109.67[a]	102.96[abc]
Leucine	149.41[c]	151.51[c]	157.18[bc]	169.42[ab]	170.79[a]	158.00[abc]	83.01[c]	84.17[c]	87.32[bc]	94.12[ab]	94.88[a]	87.78[abc]
Lysine	167.00[bc]	156.92[c]	176.10[ab]	182.99[a]	175.38[ab]	173.05[ab]	167.00[bc]	156.92[c]	176.10[ab]	182.99[a]	175.38[ab]	173.05[ab]
Methionine	79.31[b]	72.65[c]	75.92[bc]	89.74[a]	92.28[a]	92.84[a]	44.06[b]	40.36[c]	42.18[bc]	49.85[a]	51.26[a]	51.58[a]
Met + Cys	62.46[b]	67.05[b]	72.62[b]	91.42[a]	95.81[a]	92.14[a]	34.70[b]	37.25[b]	40.34[b]	50.78[a]	53.23[a]	51.19[a]
Phenylalanine	117.01[e]	128.19[d]	140.35[c]	163.52[ab]	166.49[a]	154.70[b]	65.01[e]	71.22[d]	77.97[c]	90.85[ab]	92.49[a]	85.94[b]
Phe + Tyr	148.60[e]	175.24[d]	247.59[c]	350.13[b]	370.42[a]	351.61[ab]	82.55[e]	97.35[d]	137.55[c]	194.52[b]	205.79[a]	195.34[ab]
Threonine	120.28[c]	121.86[bc]	130.45[abc]	139.26[a]	134.68[ab]	128.56[abc]	74.59[c]	75.57[bc]	80.90[abc]	86.36[a]	83.52[ab]	79.72[abc]
Tryptophan	100.88[c]	97.77[c]	132.35[b]	160.52[a]	155.10[a]	129.06[b]	64.05[c]	62.08[c]	84.03[b]	101.92[a]	98.47[a]	81.94[b]
Valine	162.58[c]	172.89[c]	206.46[b]	241.71[a]	228.67[ab]	221.61[ab]	90.32[c]	96.05[c]	114.70[b]	134.28[a]	127.04[ab]	123.12[ab]

[1]DIAAS-like reference values were calculated from the digestibility of amino acids in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances of amino acids for adult dogs at maintenance.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae. SEM values for AAFCO (2019) nutrient profile data: arginine, 2.050; histidine, 5.010; isoleucine, 1.997; leucine, 2.014; lysine, 2.123; methionine, 1.741; met+cys, 2.942;phenylalanine, 3.884; phe+tyr, 18.583; threonine, 1.792; tryptophan, 5.023; valine, 6.525. SEM values for NRC (2006) recommended allowance data: arginine, 1.659; histidine, 2.783; isoleucine, 1.109; leucine, 1.119; lysine, 2.123; methionine 0.967; met+cys, 1.635;phenylalanine, 2.158; phe+tyr, 10.324; threonine, 1.111; tryptophan, 3.189; valine, 3.625.
[a-e]Within a row, means lacking a common superscript letter differ ($P<0.05$); n, 4 roosters per treatment.

Table 3.7. Digestible indispensable AA score (DIAAS)–like reference values[1] of BSFL differing in life stage for adult cats[2]

	AAFCO (2019)						NRC (2006)					
Item	**BSFL0**[3]	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**	**BSFL0**	**BSFL11**	**BSFL14**	**BSFL18**	**BSFL23**	**BSFL29**
Indispensable AA												
Arginine	126.15^{a}	106.42^{c}	109.65^{bc}	113.49^{bc}	111.77^{bc}	115.16^{b}	131.07^{a}	110.57^{c}	113.92^{bc}	117.92^{bc}	116.12^{bc}	119.65^{b}
Histidine	176.45^{b}	218.89^{a}	224.34^{a}	240.54^{a}	218.70^{a}	222.26^{a}	161.83^{b}	200.76^{a}	205.75^{a}	220.61^{a}	200.58^{a}	203.85^{a}
Isoleucine	189.30^{bc}	183.42^{c}	195.47^{abc}	203.78^{ab}	208.37^{a}	195.62^{abc}	176.09^{bc}	170.62^{c}	181.83^{abc}	189.56^{ab}	193.83^{a}	181.98^{abc}
Leucine	118.35^{c}	120.01^{c}	124.50^{bc}	134.20^{ab}	135.29^{a}	125.15^{abc}	110.67^{c}	112.23^{c}	116.43^{bc}	125.50^{ab}	126.51^{a}	117.04^{abc}
Lysine	183.10^{bc}	172.05^{c}	193.07^{ab}	200.63^{a}	192.28^{ab}	189.73^{ab}	343.82^{bc}	323.07^{c}	362.56^{ab}	376.75^{a}	361.07^{ab}	356.27^{ab}
Methionine	189.02^{b}	173.15^{c}	180.95^{bc}	213.88^{a}	219.92^{a}	221.26^{a}	171.06^{b}	156.70^{c}	163.76^{bc}	193.55^{a}	199.03^{a}	200.23^{a}
Met + Cys	146.61^{b}	157.38^{b}	170.45^{b}	214.58^{a}	224.90^{a}	216.27^{a}	132.68^{b}	142.42^{b}	154.25^{b}	194.49^{a}	203.52^{a}	195.72^{a}
Phenylalanine	190.15^{e}	208.31^{d}	228.07^{c}	265.72^{ab}	270.54^{a}	251.38^{b}	146.27^{e}	160.24^{d}	175.44^{c}	204.40^{ab}	208.11^{a}	193.37^{b}
Phe + Tyr	103.81^{e}	122.42^{d}	172.97^{c}	244.61^{b}	258.78^{a}	245.64^{ab}	79.86^{e}	94.17^{d}	133.06^{c}	188.16^{b}	199.06^{a}	188.95^{ab}
Threonine	114.24^{c}	115.74^{bc}	123.90^{abc}	132.27^{a}	127.91^{ab}	122.10^{abc}	123.37^{c}	124.99^{bc}	133.79^{abc}	142.83^{a}	138.13^{ab}	131.85^{abc}
Tryptophan	145.71^{c}	141.23^{c}	191.78^{b}	231.87^{a}	224.03^{a}	186.42^{b}	137.95^{c}	133.71^{c}	181.00^{b}	219.52^{a}	212.10^{a}	176.49^{b}
Valine	185.60^{c}	197.36^{c}	235.68^{b}	275.93^{a}	261.04^{ab}	252.98^{ab}	173.56^{c}	184.56^{c}	220.40^{b}	258.04^{a}	244.11^{ab}	236.58^{ab}

[1]DIAAS-like reference values were calculated from the digestibility of amino acids in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances of amino acids for adult cats at maintenance.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae. SEM values for AAFCO (2019) nutrient profile data: arginine, 1.452; histidine, 4.436; isoleucine, 2.108; leucine, 1.595; lysine, 2.328; methionine, 4.150; met+cys, 6.906;phenylalanine, 6.312; phe+tyr, 12.982; threonine, 1.702; tryptophan, 7.256; valine, 7.449. SEM values for NRC (2006) recommended allowance data: arginine, 1.508; histidine, 4.068; isoleucine, 1.961; leucine, 1.492; lysine, 4.371; methionine, 3.756; met+cys, 6.250;phenylalanine, 4.856; phe+tyr, 9.986; threonine, 1.838; tryptophan, 6.870; valine, 6.966.
[a-e]Within a row, means lacking a common superscript letter differ (*P*<0.05); n, 4 roosters per treatment.

Table 3.8. First-limiting AA based on digestible indispensable amino acid score (DIAAS)–like reference values[1] of BSFL differing in life stage from AAFCO (2019) nutrient profiles[2]

Item[3]	**Growth and Reproduction**		**Adults**	
	Dogs	**Cats**	**Dogs**	**Cats**
BSFL0	69 (Thr)	62 (Met + Cys)	62 (Met+ Cys)	114 (Thr)
BSFL11	70 (Thr)	64 (Met)	67 (Met+ Cys)	106 (Arg)
BSFL14	75 (Trp)	67 (Met)	73 (Met+ Cys)	110 (Arg)
BSFL18	80 (Thr)	80 (Met)	90 (Met)	113 (Arg)
BSFL23	78 (Thr)	82 (Met)	92 (Met)	112 (Arg)
BSFL29	74 (Thr)	82 (Met)	92 (Met+ Cys)	115 (Arg)

[1]DIAAS-like reference values were calculated from the digestibility of amino acids in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles of amino acids for dogs and cats.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae.

Table 3.9. First-limiting AA based on digestible indispensable amino acid score (DIAAS)–like reference values[1] of BSFL differing in life stage from NRC (2006) recommended allowances[2]

			Adult	
Item[3]	**Puppies (4 to 14 wk of age)**	**Kittens**	**Dogs**	**Cats**
BSFL0	73 (Met + Cys)	64 (Met + Cys)	34 (Met + Cys)	80 (Phe + Tyr)
BSFL11	78 (Met + Cys)	69 (Met + Cys)	37 (Met + Cys)	94 (Phe + Tyr)
BSFL14	84 (Met + Cys)	75 (Met + Cys)	40 (Met + Cys)	114 (Arg)
BSFL18	103 (Thr)	93 (Met)	50 (Met)	118 (Arg)
BSFL23	100 (Thr)	96 (Met)	51 (Met)	117 (Arg)
BSFL29	95 (Thr)	94 (Met + Cys)	52 (Met + Cys)	117 (Leu)

[1]DIAAS-like reference values were calculated from the digestibility of amino acids in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the NRC (2006) recommended allowances of amino acids for dogs and cats.
[3]BSFL0, day 0, day of hatch of black soldier fly larvae; BSFL11, day 11 of age of black soldier fly larvae; BSFL14, day 14 of age of black soldier fly larvae; BSFL18, day 18 of age of black soldier fly larvae; BSFL23, day 23 of age of black soldier fly larvae; BSFL29, day 29 of age of black soldier fly larvae.

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***CHAPTER 4:**

AMINO ACID DIGESTIBILITY AND DIGESTIBLE INDISPENSABLE AMINO ACID SCORE-LIKE VALUES OF BLACK SOLDIER FLY LARVAE FED DIFFERENT FORMS AND CONCENTRATIONS OF CALCIUM USING THE PRECISION-FED CECECTOMIZED ROOSTER ASSAY

ABSTRACT

Black soldier fly larvae (BSFL) are an alternative protein source for animals, including dogs and cats. Dietary calcium source is an essential nutrient for BSFL development in the pupal stage. Calcium carbonate ($CaCO_3$) and calcium chloride ($CaCl_2$) are common calcium sources but differ in solubility, acid-binding capacity, and calcium concentration. A high calcium concentration in BSFL may affect how well nitrogen and amino acids (AA) are digested by animals consuming them, thereby affecting feed conversion efficiency. Our objective was to determine the effects of dietary calcium form and concentration on nutrient composition, AA digestibility, and digestible indispensable amino acid score (DIAAS)-like values of BSFL intended for use in animal feeds using the precision-fed cecectomized rooster assay. All BSFL tested in this study were harvested at 18 d after hatch. Industry standard rearing conditions were maintained and a commercial layer ration was fed to all BSFL until 11 d post-hatch. From day 11 to 18, BSFL were fed a combination of distiller's dried grains with solubles from a distillery, bakery byproduct meal, and varied calcium sources.

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All BSFL diets contained 0.2% calcium in the basal diet plus additional calcium in the following amounts and forms: BSFLA: 1.2% $CaCl_2$, BSFLB: 1.2% $CaCO_3$, BSFLC: 0.75% $CaCO_3$, and BSFLD: 0.6% $CaCO_3$ + 0.6% $CaCl_2$. On day 18, BSFL were washed and frozen. Prior to the rooster assay, BSFL were lyophilized and ground. In total, 16 cecectomized roosters (4 roosters per substrate) were randomly assigned to test substrates. After 24 h of feed withdrawal, roosters were tube-fed 20 g of test substrates. Following crop intubation, excreta were collected for 48 h. Endogenous corrections for AA were made using five additional cecectomized roosters. All data were analyzed using a completely randomized design and the GLM procedure of SAS 9.4. Nutrient and AA digestibilities were not different among substrates. DIAAS-like values were calculated to determine protein quality according to the Association of American Feed Control Officials nutrient profiles and National Research Council recommended allowances for dogs and cats. Although AA digestibilities did not differ, those containing $CaCO_3$ generally had higher DIAAS-like reference values than the diet containing $CaCl_2$ alone (BSFLA). Aromatic AA (Phe + Tyr) and sulfur AA (Met + Cys) often were first-limiting AA. Our results suggest that calcium sources fed to BSFL did not affect AA digestibility and protein quality.

INTRODUCTION

Black soldier fly larvae (BSFL; *Hermetia illucens*) have been shown to efficiently convert various organic materials such as livestock manure, fruits, and vegetable waste into alternative protein and fat sources for swine (Newton et al., 1977, 2005), poultry (Cullere et al., 2016; Marono et al., 2017; Mwaniki et al., 2018), and fish species (Kroeckel et al., 2012; Lock et al., 2015). The environmental factors and housing conditions (e.g., temperature, humidity, and stocking density) used and dietary ingredient and nutrient composition fed to BSFL require consideration because they affect their growth rate, survival rate, and nutrient composition (Sheppard et al., 2002; Newton et al., 2005; Diener et al., 2009; Tomberlin et al., 2009; Holmes et al., 2012).

Chia et al. (2018), who tested nine temperatures (10 to 42 °C), demonstrated that housing temperature affected BSFL survival rate. Egg eclosion rate was highest at 30 °C (80%) and 35 °C (75%) and lowest at 15, 37, and 40 °C (below 11%). The survival rate of the larval and pupal stages also was highest at 30 °C (90% and 77%) and 35 °C (92% and 75%). Like temperature, relative humidity is also an essential factor affecting BSFL development and survival. Egg eclosion time (124.43 to 87.63 h) and mortality rate (62% to 3%) of the BSFL pupal stage decreased when the relative humidity increased from 25% to 70%. Also, adult longevity (5.17 to 7.94 d) and the emergence rate of BSF (16% to 93%) increased when they were reared on 40% and 70% of relative humidity compared with 25% (Holmes et al., 2012). Another aspect under consideration is stocking density. Barragan-Fonseca et al. (2018) placed 50, 100, 200, or 400 BSFL in plastic containers (15.5 × 10.5 × 6 cm), demonstrating that development time was shorter (13 vs. 15 d) at lower larval densities (50, 100, and 200) when raised on a diet with high nutrient concentrations (14.0% protein and 1.8% fat vs. 3.5% protein and 0.7% fat).

Dietary factors not only influence BSFL performance but also their nutrient composition. Oonincx et al. (2015) observed that when BSFL were fed a chicken feed diet (control diet), they had a shorter development time (20 d) than larvae fed animal manures (chicken: 144 d, pig: 144 d, and cow: 214 d). According to Nguyen et al. (2015), protein and fat concentrations of BSFL fed rendered fish (protein: 50% and fat: 36.2%) or pig liver (protein: 76.7% and fat: 12.8%) were higher than BSFL fed chicken feed (protein: 18% and fat: 2.52%). However, Bosch et al. (2014) reported that BSFL fed a broiler starter diet had protein (56.1%) and fat (12.8%) concentrations that were much higher than for those fed a similar diet in the Nguyen et al. (2015) study. The mineral content of BSFL also depends on the diet fed. For instance, phosphorus and calcium concentrations of BSFL reared on poultry manure (1.5% and 7.8%) were higher than BSFL reared on swine manure (0.88% and 5.36%) or chicken feed (1.28% and 3.14%) (Newton et al., 2005; Dierenfeld and King, 2008; Finke, 2013).

BSFL contain high calcium concentrations and greater calcium, magnesium, and potassium concentrations than other insects, such as tebo worms, Turkestan cockroaches, and house flies (Finke, 2013). The reason that BSFL contain high calcium content is because their exoskeleton layer is rich in $CaCO_3$ (Johannsen, 1922). Dietary calcium sources for BSFL may impact the development of their epidermis in the prepupal to pupal stages. There are many dietary calcium options when it comes to feeding BSFL, with variability in acid-binding capacity, water solubility, and calcium concentration. To our knowledge, no scientific research has been conducted to test how dietary calcium form and concentration affect the growth rate of BSFL and their protein quality for use in animal feed. For this reason, our objective was to determine the effects of dietary calcium form and concentration on nutrient composition, amino acid (AA) digestibility, and digestible indispensable amino acid score (DIAAS)-like values of BSFL intended for use in animal feeds using the precision-fed cecectomized rooster assay. We hypothesized that the different forms and concentrations of dietary calcium sources used in this study would affect the nutrient composition of BSFL. However, we hypothesized that the protein quality of BSFL would not be changed.

MATERIALS AND METHODS

Substrates

All BSFL tested in this study were harvested at 18 d after hatch. Industry standard rearing conditions were maintained (Sheppard et al., 2002) and a commercial layer ration was fed to all BSFL until 11 d post-hatch. From day 11 to 18, they were fed a combination of distiller's dried grains with solubles from a distillery, bakery byproduct meal, and calcium sources ($CaCl_2$ and $CaCO_3$). Treatment groups included the following:

1 BSFLA (0.2% calcium in basal diet + 1.2% Ca from $CaCl_2$)

2 BSFLB (0.2% calcium in basal diet + 1.2% Ca from $CaCO_3$)

3 BSFLC (0.2% calcium in basal diet + 0.75% Ca from $CaCO_3$)

4 BSFLD (0.2% calcium in basal diet + 0.6% Ca from $CaCO_3$ +
0.6 % Ca from $CaCl_2$)

Although $CaCl_2$ is the typical calcium source used to raise BSFL in production, it is expensive, so a more economical option was tested at various inclusion levels. Because calcium sources differ in solubility, calcium content, and acid-binding capacities, numerous options exist. In this initial study, it was of interest to test whether 50% or total replacement of the 1.2% $CaCl_2$ was possible without detrimental effects. On day 18, larvae were washed and frozen. All BSFL then were lyophilized and ground through a 2-mm screen with dry ice prior to chemical analysis and feeding to cecectomized roosters.

Cecectomized Rooster Assay

The protocol for the cecectomized rooster assay, including all animal housing, handling, and surgical procedures, was reviewed and approved by the Institutional Animal Care and Use

Committee at the University of Illinois at Urbana- Champaign prior to experimentation. A precision-fed rooster assay using cecectomized Single Comb White Leghorn roosters was conducted as described by Parsons (1985) to determine the dry matter (DM), organic matter (OM), acid-hydrolyzed fat (AHF), and AA digestibility of the substrates listed above. Prior to the study, the cecectomy surgery was performed on roosters under general anesthesia according to the procedures of Parsons (1985).

Briefly, 16 cecectomized roosters were randomly assigned to the test substrates (4 roosters per test substrate evaluated). After 24 h of feed withdrawal, roosters were tube-fed 20 g of test substrates. Following crop intubation, excreta (urine and feces) were collected for 48 h on plastic trays placed under each individual cage. Excreta samples then were lyophilized, weighed, and ground through a 0.25-mm screen prior to analysis. Endogenous corrections for AA were made using five additional cecectomized roosters that had been fasted for 48 h. Nutrient and AA digestibilities were calculated using the method described by Sibbald (1979).

Chemical Analyses

The substrates and rooster excreta were analyzed for DM (105 °C) and ash (OM was calculated based on ash) according to AOAC (2006; DM: method 934.01; OM: method 942.05). Nitrogen and crude protein (CP) were determined using a Leco Nitrogen/ Protein Determinator (Model FP-2000, Leco Corporation, St. Joseph, MI) according to AOAC (2006; method 982.30E). Fat concentrations were measured by acid hydrolysis according to AACC (1983) followed by diethyl ether extraction (Budde, 1952). Gross energy (GE) was measured using a bomb calorimeter (Model 6200; Parr Instrument Co., Moline, IL). AA and calcium were measured at the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO) according to the AOAC (2006) method 982.30 E [a, b, c] and method 968.08.

Digestible Indispensable AA Score (DIAAS)-Like Calculations

The calculation of DIAAS-like values was followed according to Mathai et al. (2017) and Oba et al. (2019). The digestible indispensable AA reference ratios were calculated for each ingredient using the following equation (FAO, 2011): Digestible indispensable AA reference ratio = digestible indispensable AA content in 1 g protein of food (mg)/mg of the same dietary indispensable AA in 1 g of the reference protein.

The references used were the Association of American Feed Control Officials (AAFCO, 2019) nutrient profiles for adults at maintenance (dogs and cats) and growth and reproduction (dogs and cats) and National Research Council (NRC, 2006) recommended allowances for adults (dogs and cats), growing puppies (4 to 14 wk of age), and growing kittens.

The DIAAS-like values then were calculated using the following equation adapted from the Food and Agriculture Organization (FAO, 2011): DIAAS-like % = 100 × [(mg of digestible dietary indispensable AA in 1 g of the dietary protein)/(mg of the minimum recommendation of the same dietary indispensable AA in 1 g of the minimum protein recommendation)].

Statistical Analysis

All data were analyzed as a completely randomized design using the GLM procedure of the Statistical Analysis System 9.4 (SAS Inst., Cary, NC). Substrates were considered to be a fixed effect. Tukey's multiple comparison analysis was used to compare LS means and control for experiment-wise error. Differences were considered significant with $P < 0.05$.

RESULTS

Chemical Composition

The chemical composition of the tested BSFL is presented in **Table 4.1**. The chemical composition of all four BSFL treatments was similar, containing 87.8% to 89.5% OM, 40.0% to 41.7% CP, 31.1% to 33.5% AHF, and 2.87% to 3.47% calcium, and GE content between 5.61 and 5.85 kcal/g. Concentrations of indispensable and dispensable AA are presented in **Table 4.2**. AA concentrations and patterns of all BSFL were similar.

Cecectomized rooster assay

The digestibilities of DM, OM, and AHF were not different among BSFL tested (**Table 4.1**). There were no differences in indispensable and dispensable AA digestibilities for all BSFL tested (**Table 4.3**). All indispensable AA digestibilities were higher than 90%, with the exception of valine (81.29% to 84.03%). For most of the dispensable AA, digestibilities were greater than 90%. The exceptions were for cysteine (76.88% to 81.95%), glycine (79.1% to 86.38%), and serine (87.92% to 91.18%).

DIAAS-Like Calculations

DIAAS-like reference values for growing puppies and kittens are presented in **Table 4.4** and **Table 4.5**, respectively. DIAAS-like reference values for adult dogs and cats at maintenance are presented in **Table 4.6** and **Table 4.7**, respectively. With the exception of threonine and sulfur AA (methionine + cysteine), most of the BSFL tested had DIAAS-like values over 100%. For BSFLA, DIAAS-like reference values for arginine, sulfur AA (methionine + cysteine), and threonine were less than 100% when using the AAFCO recommended allowances for growing puppies. Based on the NRC recommended allowances for growing puppies, all BSFL had DIAAS-like reference values above 100%, with the exception of threonine and sulfur AA (methionine + cysteine). According to the AAFCO and NRC recommended allowances for growing kittens, all BSFL had DIAAS-like reference values above 100% with the exception of sulfur AA (methionine + cysteine) and aromatic AA (phenylalanine + tyrosine).

All BSFL ingredients had DIAAS-like reference values over 100%, with the exception of methionine and sulfur AA (methionine + cysteine), when using the AAFCO recommended allowances for adult dogs. Based on the NRC recommended allowances for an adult dog at maintenance, however, DIAAS-like reference values were lower than 100% for leucine, sulfur AA (methionine + cysteine), aromatic AA (phenylalanine + tyrosine), threonine, and tryptophan for most of the BSFL tested. According to the AAFCO and NRC recommended allowances for adult cats, all BSFL tested had DIAAS-like reference values over 100% with the exception of sulfur AA (methionine + cysteine) and aromatic AA (phenylalanine + tyrosine).

The first-limiting AA based on DIAAS-like reference values from AAFCO (2019) nutrient profiles for dogs and cats (growth and reproduction; adults at maintenance) are provided in **Table 4.8**. Sulfur AA (methionine + cysteine) was the first-limiting AA for all BSFL when calculating DIAAS-like reference values using AAFCO (2019) nutrient profiles for growing and reproducing dogs. Sulfur AA (methionine + cysteine) also was the first-limiting AA for all BSFL when calculating DIAAS-like reference values using AAFCO (2019) nutrient profiles for adult dogs and cats at maintenance. For these categories, all of the DIAAS-like reference values for limiting AA were less than 100, suggesting insufficiency if a diet was formulated with only that protein source and at an inclusion level to meet the nutrient profile. Sulfur AA (methionine + cysteine) was the first-limiting AA when calculating DIAAS-like reference values using the AAFCO (2019) nutrient profiles for adult cats at maintenance, with all values being greater than 100, suggesting sufficiency.

The first-limiting AA based on DIAAS-like reference values from NRC (2006) recommended allowances for growing puppies (4 to 14 wk of age), growing kittens, and adult dogs and cats at maintenance are provided in **Table 4.9**. Sulfur AA (methionine + cysteine) was the first-limiting AA for all BSFL for growing puppies and kittens and adult dogs and cats at maintenance, whereas aromatic AA (phenylalanine + tyrosine) was the first-limiting AA for BSFLC and BSFLD for adult cats at maintenance. For these categories, most of the DIAAS-like values for limiting AA were less than 100, suggesting insufficiency if a diet was formulated with only that protein source and at an inclusion level to meet the nutrient profile.

DISCUSSION

BSFL have been of substantial interest over the past couple of decades as a means for organic waste management as well as their ability to convert these food sources into a high-quality nutrient source for livestock feeds and pet foods (Newton et al., 1977, 2005; Sheppard et al., 2002; Tomberlin et al., 2009; Holmes et al., 2012; Kroeckel et al., 2012; Cullere et al., 2016; Marono et al., 2017; Barragan-Fonseca et al., 2018; Chia et al., 2018; Mwaniki et al., 2018; Do et al., 2020; Freel et al., 2021). Similar to mammals, BSFL must be fed a sufficient amount of essential nutrients to meet their nutrient and metabolic requirements (Cohen, 2003). Carbohydrates can be used as an energy source and building block for BSFL tissues. Essential AA such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are important for the production of BSFL tissues, hormones, transport proteins, and energy (Cohen, 2003). Lipids also play several roles in these organisms, such as serving as a rich energy source, as a structural component of cell membranes, and as chemical messengers (Cohen, 2003). Micronutrients are indispensable for BSFL development, but limited information is available in regard to how dietary micronutrients affect BSFL development (Cohen, 2003).

BSFL can be a good source of calcium, but their calcium concentration is very responsive to diet and contain as little as 0.12% Ca (DM basis) up to 6.61% (Dierenfeld and King, 2008; Tschirner and Simon, 2015; Spranghers et al., 2016). If the diet was formulated with 20% of BSFL, the minimal requirement of calcium for kittens and puppies (NRC, 2006) would be met easily (providing 250% and 230% of the requirement). BSFL also have a calcium to phosphorus ratio (2.6:1) that is relatively easy to use in dietary formulations (Finke, 2013). Conversely, tebo worms (11%), Turkestan cockroaches (33%), and house flies (66%) contain low calcium concentrations and calcium and phosphorus ratios (1:18, 1:4.6, and 1:4.9) that are not easy to use in dietary formulas (Finke, 2013). Compared with other insects, BSFL can accumulate high calcium concentrations because they have a mineralized exoskeleton. Therefore, like face flies (*Musca autumnalis*), dietary calcium is positively linked with calcium accumulation in BSFL due to the formation of the cuticle layer during pupation (Roseland et al., 1985; Tomberlin et al., 2002; Finke, 2013). For those reasons, dietary calcium source may be an important consideration for BSFL development.

The gastrointestinal tract of BSFL is composed of three parts, including the foregut, midgut, and hindgut, with the midgut serving as the most important section for nutrient absorption (Kim et al., 2011). The Malpighian tubules located in the midgut and hindgut junction are essential for the excretion of nitrogenous products and other metabolites and maintaining nutrient balance (Murakami and Shiotsuki, 2001; Chapman, 2013; Gold et al., 2018). According to Grodowitz et al. (1987) and Krueger et al. (1988), dietary calcium in face flies is transported from the

Malpighian tubules to the cuticle via hemolymph. They have mineralization of the epidermis during the pupal stage because mineralized granules, which are composed of calcium, magnesium, phosphate, and carbonate, accumulate in the lumen of the larval Malpighian tubules. Therefore, as with face flies, BSFL may have a similar mechanism of dietary calcium absorption.

There are many potential calcium sources in animal feeds, such as organic salts (tricalcium citrate, calcium lactate, calcium lactate gluconate, and calcium gluconate) and inorganic salts (calcium chloride, calcium carbonate, and calcium phosphate; Trailokya et al., 2017). In the current study, we compared $CaCO_3$ and $CaCl_2$ as dietary calcium sources for BSFL and they have different solubilities (insoluble vs. soluble; pH range between 3 and 6), calcium concentrations (40% vs. 27%), and acid-binding capacities (244 vs. 2.4 mEq/kg to reach pH of 3) (Weaver, 1998; Hamdi et al., 2015). Several studies have determined that calcium citrate (soluble) has a higher absorption rate than $CaCO_3$ (insoluble) due to its solubility in water (Nicar and Pak, 1985; Heller et al., 1999). Heaney et al. (1990), however, tested seven different calcium salts (calcium oxalate, hydroxyapatite, tricalcium phosphate, calcium citrate, calcium citrate malate, bisglycinocalcium, and $CaCO_3$) to evaluate their relationship between solubility and absorption and were unable to identify a significant correlation. Another in vitro study conducted by Goss et al. (2007) reported that while $CaCO_3$ (3.6 mg mL^{-1}) had a greater solubility in the gastrointestinal tract than calcium citrate (0.2 mg mL^{-1}) at a pH of 6, $CaCO_3$ (0.12 mg mL^{-1}) had a lower solubility than calcium citrate (0.24 mg mL^{-1}) at a pH of 7.5. The solubility of dietary calcium in water is affected by pH because it impacts its form (anion vs. cation). Secretion of hydrochloric acid and sodium bicarbonate from the stomach and intestines affects the pH in the gastrointestinal tract. Thus, the solubility values determined from the gastrointestinal tract would be a key factor to understand the effect of the solubility on calcium absorption rather than simple aqueous solubility. Dietary calcium sources used in the current study have different pH-dependent solubilities and may have different absorption rates in the midgut of BSFL. The pH of the BSFL midgut changes according to region (anterior midgut: pH = 7, mid-midgut: pH = 2, and posterior midgut: pH = 6.3 to 9.3; Espinoza-Fuentes and Terra, 1987; Overend et al., 2016; Gold et al., 2018). Although $CaCO_3$ has a higher calcium content than $CaCl_2$, $CaCl_2$ may be more bioavailable to BSFL because of its higher solubility in the midgut, and, in this trial, the combination of both forms resulted in the highest numerical concentration of BSFL calcium.

The nutrient concentrations, including indispensable AA, were similar for all BSFL tested in the current study. Shumo et al. (2019) reared BSFL on three different organic wastes, including chicken manure (high ash: 20% and low fat: 2.7%), brewer's spent grain (high neutral detergent fiber [NDF]: 50% and high acid detergent fiber [ADF]: 39%), and kitchen waste (high protein: 20%). BSFL fed kitchen waste had higher DM (87.5%) and fat (34.3%) concentrations and lower CP (33%) concentrations than BSFL fed chicken manure or brewer's spent grain. Also, BSFL reared on brewer's spent grain had higher ADF (15%) and NDF (28.6%) concentrations than larvae fed chicken manure (ADF: 12.6% and NDF: 21.9%) or kitchen waste (ADF: 13.2% and NDF: 20.4%). Barragan-Fonseca et al. (2019) reported that BSFL CP concentration increased when larvae were fed a diet containing 10% CP concentration compared with a high-protein diet (17%). Similarly, Tschirner and Simon (2015) reported that larvae fed a high-fiber diet containing only 8.5% CP had a higher CP content (52.3%) than larvae (44.6%) fed a high-protein diet (31.2% CP).

BSFL crude fat concentration was shown to increase as dietary carbohydrate concentration increased (Barragan-Fonseca et al., 2019). Compared with AA concentrations of BSFL reported by Shumo et al. (2019), BSFL fed kitchen waste had higher concentrations of indispensable AA (arginine and phenylalanine) and dispensable AA (proline, hydro-proline, and tyrosine) than BSFL fed chicken manure or brewer's spent grain. Based on the previous studies, the nutrient composition (DM, CP, fat, ADF, and NDF) of the substrates affected the chemical composition and AA concentration of BSFL. In the current study, different forms and concentrations of dietary calcium sources influenced the calcium concentrations of BSFL, but there was no significant difference in the nutrient composition of BSFL among treatments.

The precision-fed cecectomized rooster assay is often used to test the protein quality of pet food ingredients because the AA digestibilities and response patterns have been shown to be similar to that of ileal-cannulated dogs (r = 0.87 to 0.92; Johnson et al., 1998; Faber et al., 2010; Kerr et al., 2014; Oba et al., 2019). Other methods of testing AA digestibilities have drawbacks when compared with the cecectomized rooster assay. For example, the total collection method that uses fecal analysis is heavily influenced by the fermentative activity of the large intestinal microbiota and is a time-consuming, labor-intensive, and expensive method (Hendriks and Sritharan, 2002; Hendriks et al., 2013). Intestinal cannulas have been used to estimate nutrient digestion at specific points in the gastrointestinal tract for many years. Cannulation, however, also has many disadvantages, such as leakage of chyme from the cannula, skin ulceration and infection, and the discomfort of animals (Hill et al., 1996). Therefore, the cecectomized rooster assay is an appropriate model to evaluate the protein quality of ingredients because it minimizes the influence of microbes in the hindgut.

Because BSFL have a high calcium concentration due to their mineralized exoskeleton (Grodowitz and Broce, 1983; Krueger et al., 1988) and high dietary calcium concentrations may influence the digestibility of nitrogen and AA (Selle et al., 2009; Wilkinson et al., 2014), the current study was conducted to test whether dietary calcium source or concentration affected nutrient digestibility. It was interesting to note that nutrient and AA digestibilities were similar among all BSFL tested in the current study. According to McDonald and Solvyns (1964), increasing dietary calcium ($CaCO_3$) concentrations from 9 to 25 g/kg resulted in a greater small intestinal pH from 5.6 to 6.0 and a reduction in weight gain of chickens from 77 to 63.5 g/d. Wilkinson et al. (2014) demonstrated that increasing dietary calcium concentration ($CaCO_3$) from 2.5 to 10 g/kg led to a reduction in the apparent ileal digestibility of DM, nitrogen, and AA in broiler chickens. The possible reason for these results is that the increased intestinal pH resulting from the high dietary calcium concentrations reduced the action of pepsin. In the current study, the calcium form and concentration fed to BSFL had no adverse effects on BSFL protein quality or calcium concentrations, which may have been due to a low acid-binding capacity, impact on intestinal pH, and consequent nutrient absorption.

DIAAS-like reference values are an indicator of protein quality, providing a more accurate measure than the protein digestibility-corrected AA score because it uses ileal rather than fecal digestibility in its calculation (FAO, 2011; Mathai et al., 2017; Oba et al., 2019). Based on the DIAAS-like reference values in the current study, all BSFL tested seem to provide sufficient AA for growing puppies and kittens except for threonine and sulfur AA (methionine + cysteine). Because the AAFCO and NRC recommended allowances are lower for adult dogs and cats, more AA would be insufficient for those life stages if diets were only formulated using BSFL as the sole protein source and at a rate to meet the recommended CP concentrations.

This study had a couple of limitations that should be discussed briefly. First, although the precision-fed cecectomized rooster assay is a highly repeatable method that has been used to study many novel proteins in recent years (Kerr et al., 2013, 2014; Deng et al., 2016; Oba et al., 2019; Do et al., 2020), the number of replications in this study (4 to 5 roosters per treatment) was low. This replication number may have limited our ability to detect differences among treatments. Also, the DIAAS-like values used to estimate protein quality for dog and cat foods are based on the assumption that diets would be formulated with that single protein source and contain an inclusion level to meet the CP recommendation. Dog or cat foods formulated using a different strategy would need to keep this in mind when assessing the value of BSFL.

In conclusion, our data show that BSFL contain a relatively high concentration of calcium and that BSFLD fed $CaCl_2$ and $CaCO_3$ accumulated more calcium than the other BSFL. Despite their differences in nutrient composition, nutrient and AA digestibilities among all BSFL sources were similar when tested in the precision-fed cecectomized rooster assay. Sulfur AA (methionine + cysteine) and aromatic AA (phenylalanine + tyrosine) were estimated to be the first-limiting AA of BSFL based on DIAAS-like reference values for dogs and cats. All BSFL ingredients, however, showed very high AA digestibilities, with most exceeding 90%. Therefore, although dietary calcium form and concentration may affect the calcium concentrations of BSFL, they all serve as high-quality protein sources for use in livestock feeds and pet foods.

TABLES

Table 4.1. Chemical composition and macronutrient digestibility of BSFL fed different forms and concentrations of calcium using the precision-fed cecectomized rooster assay

Item	BSFLA[1]	BSFLB[2]	BSFLC[3]	BSFLD[4]	SEM	P-value
Chemical composition						
DM, %	93.34	94.09	93.16	93.56	-	-
OM, % DM	88.07	88.06	89.49	87.84	-	-
CP, % DM	41.68	40.15	41.58	41.04	-	-
AHF, % DM	31.15	33.45	33.05	32.73	-	-
GE, kcal/g DM	5.63	5.84	5.85	5.61	-	-
Ca, % DM	3.20	3.42	2.87	3.47	-	-
Nutrient digestibility						
DM, %	62.30	60.39	62.55	59.77	1.403	0.8926
OM, %	74.19	71.02	74.19	68.63	1.173	0.3548
AHF, %	85.30	82.94	85.91	83.21	0.929	0.6373

[1]BSFLA, 0.2 % calcium in basal diet + 1.2 % $CaCl_2$
[2]BSFLB, 0.2 % calcium in basal + 1.2 % $CaCO_3$
[3]BSFLC, 0.2 % calcium in commercial diet + 0.75 % $CaCO_3$
[4]BSFLD, 0.2 % calcium in commercial diet + 0.6 % $CaCO_3$ + 0.6 % $CaCl_2$

Table 4.2. Indispensable and dispensable AA concentrations (% DM) of BSFL fed different forms and concentrations of calcium

Item	BSFLA[1]	BSFLB[2]	BSFLC[3]	BSFLD[4]
CP, % DM	41.68	40.15	41.58	41.04
Indispensable AA				
Arginine	1.92	1.99	2.02	2.00
Histidine	1.24	1.27	1.31	1.29
Isoleucine	1.71	1.85	1.90	1.78
Leucine	2.60	2.76	2.81	2.69
Lysine	2.78	2.91	2.89	2.89
Methionine	0.69	0.72	0.73	0.73
Phenylalanine	1.71	1.81	1.83	1.78
Threonine	1.52	1.61	1.63	1.58
Tryptophan	0.58	0.60	0.60	0.58
Valine	2.60	2.91	3.01	2.81
Selected dispensable AA				
Alanine	2.45	2.76	2.74	2.63
Aspartic acid	3.54	3.69	3.74	3.65
Cysteine	0.33	0.36	0.34	0.35
Glutamic acid	3.79	3.95	3.89	3.88
Glycine	1.97	2.14	2.19	2.06
Proline	2.27	2.41	2.47	2.36
Serine	1.43	1.55	1.56	1.48
Tyrosine	2.58	2.69	2.57	2.73
Taurine	0.08	0.07	0.06	0.07

[1]BSFLA, 0.2 % calcium in basal diet + 1.2 % $CaCl_2$
[2]BSFLB, 0.2 % calcium in basal + 1.2 % $CaCO_3$
[3]BSFLC, 0.2 % calcium in commercial diet + 0.75 % $CaCO_3$
[4]BSFLD, 0.2 % calcium in commercial diet + 0.6 % $CaCO_3$ + 0.6 % $CaCl_2$

Table 4.3. AA digestibilities (%) of BSFL fed different forms and concentrations of calcium using the precision-fed cecectomized rooster assay

Item	**BSFLA**[1]	**BSFLB**[2]	**BSFLC**[3]	**BSFLD**[4]	**SEM**	***P*-value**
CP, % DM	41.68	40.15	41.58	41.04	-	-
Indispensable AA						
Arginine	95.05	93.54	94.58	95.77	0.600	0.6539
Histidine	91.85	90.79	91.43	91.32	0.409	0.8653
Isoleucine	92.53	91.48	92.25	92.75	0.531	0.8740
Leucine	93.06	92.42	92.52	93.47	0.576	0.9271
Lysine	92.28	91.15	91.15	92.25	0.570	0.8438
Methionine	93.71	92.50	93.25	93.52	0.500	0.8515
Phenylalanine	92.80	91.91	92.00	92.47	0.523	0.9397
Threonine	91.62	90.56	90.76	92.11	0.738	0.8910
Tryptophan	95.50	94.40	94.44	95.48	0.411	0.6832
Valine	81.29	83.22	82.00	84.03	1.165	0.8704
Selected dispensable AA						
Alanine	92.69	91.73	92.62	93.29	0.530	0.8137
Aspartic acid	93.68	92.49	93.37	93.57	0.459	0.8278
Cysteine	80.38	76.88	78.08	81.95	1.914	0.8220
Glutamic acid	91.60	89.51	91.44	91.57	0.615	0.6088
Glycine	81.08	79.10	84.72	86.38	1.466	0.2947
Proline	90.31	90.62	91.07	91.67	0.543	0.8576
Serine	89.52	87.92	89.37	91.18	0.939	0.7240
Tyrosine	93.66	93.26	92.64	93.85	0.395	0.7535

[1]BSFLA, 0.2 % calcium in basal diet + 1.2 % $CaCl_2$
[2]BSFLB, 0.2 % calcium in basal + 1.2 % $CaCO_3$
[3]BSFLC, 0.2 % calcium in commercial diet + 0.75 % $CaCO_3$
[4]BSFLD, 0.2 % calcium in commercial diet + 0.6 % $CaCO_3$ + 0.6 % $CaCl_2$

Table 4.4. DIAAS–like reference values[1] of BSFL fed different forms and concentrations of calcium for growing and reproducing dogs and growing puppies[2]

Item	AAFCO (2019)[3]					NRC (2006)[3]				
	BSFLA[3]	BSFLB	BSFLC	BSFLD	SEM	BSFLA	BSFLB	BSFLC	BSFLD	SEM
Indispensable AA										
Arginine	98.51[b]	104.32[ab]	103.39[ab]	105.03[a]	0.906	124.70[b]	132.05[ab]	130.88[ab]	132.95[a]	1.147
Histidine	139.73[b]	146.86[a]	147.31[a]	146.79[a]	1.030	157.65[b]	165.69[a]	166.20[a]	165.61[a]	1.162
Isoleucine	120.30[a]	133.59[a]	133.60[a]	127.50[a]	1.600	131.41[b]	145.92[a]	145.93[a]	139.27[a]	1.748
Leucine	101.25[b]	110.81[a]	109.07[a]	106.87[ab]	1.148	101.25[b]	110.81[a]	109.07[a]	106.87[ab]	1.148
Lysine	153.87[b]	165.17[a]	158.41[ab]	162.42[ab]	1.460	157.37[b]	168.93[a]	162.01[ab]	166.11[ab]	1.493
Methionine	99.72[b]	106.59[a]	105.25[a]	106.94[a]	0.930	105.77[b]	113.05[a]	111.63[a]	113.43[a]	0.986
Met + Cys	49.89	51.38	51.51	53.24	0.751	49.89	51.38	51.51	53.24	0.752
Phenylalanine	103.20[b]	112.33[a]	109.77[a]	108.74[ab]	1.063	131.78[b]	143.44[a]	140.17[a]	138.85[ab]	1.357
Phe + Tyr	121.01[b]	126.07[ab]	122.91[ab]	127.13[a]	0.867	121.01[b]	126.07[ab]	122.91[ab]	127.13[a]	0.867
Threonine	72.28[b]	78.57[a]	76.98[ab]	76.73[ab]	0.862	92.80[b]	100.88[a]	98.84[ab]	98.52[ab]	1.106
Tryptophan	149.50[b]	158.72[a]	153.32[ab]	151.82[b]	1.085	130.00[b]	138.02[a]	133.32[ab]	132.01[b]	0.944
Valine	167.77[b]	199.59[a]	196.42[a]	190.39[ab]	4.174	167.77[b]	199.59[a]	196.42[a]	190.39[ab]	4.174

[1]DIAAS-like reference values were calculated from the true digestibility of AA in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles of AA for growth and reproduction of dogs and NRC (2006) recommended allowances of AA for growing puppies (4 to 14 wk of age).
[3]BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.
[a,b]Within a row, means lacking a common superscript letter differ ($P < 0.05$); n, 4 roosters per treatment.

Table 4.5. DIAAS–like reference values[1] of BSFL fed different forms and concentrations of calcium for growing and reproducing cats and growing kittens[2]

Item	AAFCO (2019)[3]					NRC (2006)[3]				
	BSFLA[3]	BSFLB	BSFLC	BSFLD	SEM	BSFLA	BSFLB	BSFLC	BSFLD	SEM
Indispensable AA										
Arginine	105.93[b]	112.17[ab]	111.18[ab]	112.93[a]	0.974	114.02[b]	120.74[ab]	119.67[ab]	121.56[a]	1.049
Histidine	248.41[b]	261.09[a]	261.89[a]	260.96[a]	1.831	207.01[b]	217.58[a]	218.24[a]	217.47[a]	1.526
Isoleucine	203.37[b]	225.83[a]	225.85[a]	215.53[a]	2.705	175.75[b]	195.17[a]	195.18[a]	186.26[a]	2.337
Leucine	136.05[b]	148.91[a]	146.57[a]	143.61[ab]	1.542	113.38[b]	124.09[a]	122.14[a]	119.67[ab]	1.285
Lysine	153.87[b]	165.17[a]	158.41[ab]	162.42[ab]	1.460	181.03[b]	194.32[a]	186.36[ab]	191.09[ab]	1.718
Methionine	75.06[b]	80.23[a]	79.22[a]	80.50[a]	0.700	88.14[b]	94.21[b]	93.03[a]	94.52[a]	0.822
Met + Cys	42.33	43.60	43.70	45.18	0.638	44.10	45.41	45.52	47.06	0.664
Phenylalanine	219.63[b]	239.06[a]	233.62[a]	231.42[ab]	2.261	190.35[b]	207.19[a]	202.47[a]	200.56[ab]	1.960
Phe + Tyr	109.25[b]	113.81[ab]	110.96[ab]	114.77[a]	0.782	91.52[b]	95.34[ab]	92.95[ab]	96.15[a]	0.655
Threonine	137.30[b]	149.25[a]	146.23[ab]	145.75[ab]	1.637	128.50[b]	139.68[a]	136.86[ab]	136.41[ab]	1.532
Tryptophan	159.46[b]	169.30[a]	163.54[ab]	161.94[b]	1.158	207.63[b]	220.44[a]	212.95[ab]	210.86[b]	1.507
Valine	237.67[b]	282.75[a]	278.27[a]	269.72[ab]	5.913	198.06[b]	235.63[a]	231.89[a]	224.76[ab]	4.928

[1]DIAAS-like reference values were calculated from the true digestibility of AA in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles of AA for growth and reproduction of cats and NRC (2006) recommended allowances of AA for growing kittens.
[3]BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.
[a,b]Within a row, means lacking a common superscript letter differ ($P < 0.05$); n, 4 roosters per treatment.

Table 4.6. DIAAS–like reference values[1] of BSFL fed different forms and concentrations of calcium for adult dogs[2]

Item	AAFCO (2019)[3]					NRC (2006)[3]				
	BSFLA[3]	BSFLB	BSFLC	BSFLD	SEM	BSFLA	BSFLB	BSFLC	BSFLD	SEM
Indispensable AA										
Arginine	154.53[b]	163.64[ab]	162.19[ab]	164.75[a]	1.422	125.09[b]	132.48[ab]	131.29[ab]	133.37[a]	1.151
Histidine	258.87[b]	272.08[a]	272.92[a]	271.95[a]	1.901	143.81[b]	151.16[a]	151.62[a]	151.08[a]	1.060
Isoleucine	179.82[b]	199.69[a]	199.70[a]	190.57[a]	2.392	99.90[b]	110.94[a]	110.94[a]	105.87[a]	1.329
Leucine	153.66[b]	168.18[a]	165.53[a]	162.19[ab]	1.742	85.37[b]	93.43[a]	91.96[a]	90.11[ab]	0.968
Lysine	175.85[b]	188.77[a]	181.04[ab]	185.63[ab]	1.669	175.85[b]	188.77[a]	181.04[ab]	185.63[ab]	1.669
Methionine	84.61[b]	90.44[a]	89.31[a]	90.74[a]	0.789	47.01[b]	50.24[a]	49.61[a]	50.41[a]	0.438
Met + Cys	42.98	44.27	44.37	45.87	0.648	23.88	24.59	24.65	25.48	0.360
Phenylalanine	152.28[b]	165.75[a]	161.98[a]	160.45[ab]	1.568	84.60[b]	92.08[a]	89.99[a]	89.14[ab]	0.871
Phe + Tyr	170.07[b]	177.18[ab]	172.74[ab]	178.68[a]	1.218	94.49[b]	98.43[ab]	95.96[ab]	99.26[a]	0.677
Threonine	125.28[b]	136.19[a]	133.44[ab]	133.00[ab]	1.494	77.70[b]	84.46[a]	82.75[ab]	82.48[ab]	0.926
Tryptophan	149.50[b]	158.72[a]	153.32[ab]	151.82[b]	1.085	94.92[b]	100.77[a]	97.35[ab]	96.39[b]	0.689
Valine	186.26[b]	221.59[a]	218.07[a]	211.37[ab]	4.634	103.48[b]	123.10[a]	121.15[a]	117.43[ab]	2.574

[1]DIAAS-like reference values were calculated from the true digestibility of AA in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances of AA for adult dogs at maintenance.
[3]BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.
[a,b]Within a row, means lacking a common superscript letter differ ($P < 0.05$); n, 4 roosters per treatment.

Table 4.7. DIAAS–like reference values[1] of BSFL fed different forms and concentrations of calcium for adult cats[2]

Item	AAFCO (2019)					NRC (2006)				
	BSFLA[3]	BSFLB	BSFLC	BSFLD	SEM	BSFLA	BSFLB	BSFLC	BSFLD	SEM
Indispensable AA										
Arginine	109.46[b]	115.91[ab]	114.88[ab]	116.70[a]	1.001	113.72[b]	120.43[ab]	119.36[ab]	121.24[a]	1.046
Histidine	229.17[b]	240.88[a]	241.61[a]	240.76[a]	1.689	210.19[b]	220.92[a]	221.60[a]	220.82[a]	1.549
Isoleucine	189.81[b]	210.78[a]	210.79[a]	201.16[a]	2.524	176.57[b]	196.07[a]	196.09[a]	187.13[a]	2.348
Leucine	121.72[b]	133.22[a]	131.12[a]	128.47[ab]	1.380	113.82[b]	124.58[a]	122.62[a]	120.14[ab]	1.290
Lysine	192.80[b]	206.96[a]	198.48[ab]	203.52[ab]	1.829	362.05[b]	388.64[a]	372.72[ab]	382.17[ab]	3.435
Methionine	201.67[b]	215.54[a]	212.84[a]	216.27[a]	1.880	182.50[b]	195.06[a]	192.62[a]	195.71[a]	1.701
Met + Cys	100.89	103.91	104.16	107.67	1.520	91.31	94.03	94.26	97.44	1.375
Phenylalanine	247.45[b]	269.34[a]	263.21[a]	260.73[ab]	2.548	190.35[b]	207.19[a]	202.47[a]	200.56[ab]	1.960
Phe + Tyr	118.82[b]	123.78[ab]	120.68[ab]	124.83[a]	0.851	91.40[b]	95.22[ab]	92.83[ab]	96.02[a]	0.655
Threonine	118.99[b]	129.35[a]	126.73[ab]	126.32[ab]	1.419	128.50[b]	139.68[a]	136.86[ab]	136.41[ab]	1.532
Tryptophan	215.94[b]	229.26[a]	221.46[ab]	219.29[b]	1.568	204.44[b]	217.05[a]	209.67[ab]	207.61[b]	1.484
Valine	212.63[b]	252.96[a]	248.94[a]	241.29[ab]	5.290	198.84[b]	236.55[a]	232.80[a]	225.65[ab]	4.947

[1]DIAAS-like reference values were calculated from the true digestibility of AA in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles and NRC (2006) recommended allowances of AA for adult cats at maintenance.
[3]BSFLA: 0.2% calcium in basal diet + 1.2% $CaCl_2$; BSFLB: 0.2% calcium in basal + 1.2% $CaCO_3$; BSFLC: 0.2% calcium in basal diet + 0.75% $CaCO_3$; BSFLD: 0.2% calcium in basal diet + 0.6% $CaCO_3$ + 0.6% $CaCl_2$.
[a,b]Within a row, means lacking a common superscript letter differ ($P < 0.05$); n, 4 roosters per treatment.

Table 4.8. First-limiting AA based on DIAAS–like reference values[1] of BSFL fed different forms and concentrations of calcium from AAFCO (2019) nutrient profiles[2]

Item[3]	Growth and Reproduction		Adults	
	Dogs	**Cats**	**Dogs**	**Cats**
BSFLA	50 (Met + Cys)	42 (Met + Cys)	43 (Met + Cys)	101 (Met + Cys)
BSFLB	51 (Met + Cys)	44 (Met + Cys)	44 (Met + Cys)	104 (Met + Cys)
BSFLC	51 (Met + Cys)	44 (Met + Cys)	44 (Met + Cys)	104 (Met + Cys)
BSFLD	53 (Met + Cys)	45 (Met + Cys)	46 (Met + Cys)	107 (Met + Cys)

[1]DIAAS-like reference values were calculated from the true digestibility of amino acids in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the AAFCO (2019) nutrient profiles of AAs for dogs and cats.
[3]BSFLA, 0.2 % calcium in basal diet + 1.2 % $CaCl_2$; BSFLB, 0.2 % calcium in basal + 1.2 % $CaCO_3$; BSFLC, 0.2 % calcium in commercial diet + 0.75 % $CaCO_3$; BSFLD, 0.2 % calcium in commercial diet + 0.6 % $CaCO_3$ + 0.6 % $CaCl_2$

Table 4.9. First-limiting AA based on DIAAS–like reference values[1] of BSFL fed different forms and concentrations of calcium from NRC (2006) recommended allowances[2]

Item[3]	Puppies (4 to 14 wk of age)	Kittens	Adult	
			Dogs	Cats
BSFLA	50 (Met + Cys)	44 (Met + Cys)	24 (Met + Cys)	91 (Met + Cys)
BSFLB	51 (Met + Cys)	45 (Met + Cys)	25 (Met + Cys)	94 (Met + Cys)
BSFLC	51 (Met + Cys)	46 (Met + Cys)	25 (Met + Cys)	93 (Phe + Tyr)
BSFLD	53 (Met + Cys)	47 (Met + Cys)	25 (Met + Cys)	96 (Phe + Tyr)

[1]DIAAS-like reference values were calculated from the true digestibility of amino acids in cecectomized roosters.
[2]DIAAS-like reference values were calculated using the NRC (2006) recommended allowances of AA for dogs and cats.
[3]BSFLA, 0.2 % calcium in basal diet + 1.2 % $CaCl_2$; BSFLB, 0.2 % calcium in basal + 1.2 % $CaCO_3$; BSFLC, 0.2 % calcium in commercial diet + 0.75 % $CaCO_3$; BSFLD, 0.2 % calcium in commercial diet + 0.6 % $CaCO_3$ + 0.6 % $CaCl_{2\backslash}$.

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***CHAPTER 5:**

PALATABILITY AND APPARENT TOTAL TRACT MACRONUTRIENT DIGESTIBILITY OF RETORTED BLACK SOLDIER FLY LARVAE-CONTAINING DIETS AND THEIR EFFECTS ON THE FECAL CHARACTERISTICS AND SKIN AND COAT HEALTH MARKERS OF CATS CONSUMING THEM

ABSTRACT

There is growing interest in using black soldier fly larvae (BSFL) as an alternative protein and fat source in animal feeds due to its sustainability and nutritional qualities. Because little research has been conducted to evaluate the use of BSFL in cats, our objective was to determine the palatability and apparent total tract macronutrient digestibility (ATTD) of BSFL-containing canned diets and the fecal characteristics and skin and coat health markers of healthy adult cats consuming them. First, three palatability tests were conducted to compare the following diets: 1) diet with poultry byproduct meal (PBPM) and chicken serving as the primary protein sources (control) vs. diet with BSFL meal replacing PBPM (BSFL meal); 2) control vs. diet with whole BSFL replacing some PBPM and poultry fat (BSFL whole); and 3) control vs. diet with BSFL oil replacing poultry fat (BSFL oil). All diets were formulated to meet Association of American Feed Control Officials nutrient profiles (2019) for adult cats and were produced using a still retort.

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A paired t-test was conducted to analyze data from each palatability test, with a higher ($P < 0.05$) consumption ratio being observed for BSFL meal (1.93:1), BSFL whole (2.03:1), and BSFL oil (1.57:1). Second, 32 adult cats (20 females; 12 males; BW: 4.19 ± 0.55 kg; age: 3.3 ± 0.38 yr) were used in a complete randomized design study composed of a 21-d baseline period and a 70-d experimental period. Cats consumed the control diet during the baseline and then were allotted to 1 of 4 experimental diets (n=8/group): 1) control, 2) BSFL meal, 3) whole BSFL, and 4) BSFL oil. Fecal and hair samples were collected after baseline and experimental periods for ATTD, fecal characteristics, and skin and coat health marker analysis. Fecal output was higher ($P < 0.05$) and fecal dry matter percentage lower ($P < 0.05$) for cats fed BSFL meal than for those fed BSFL oil. Organic matter, crude protein (CP), and energy ATTD were lower ($P < 0.05$) for cats fed BSFL meal than for those fed BSFL oil or control. CP and energy ATTD were lower ($P < 0.05$) for cats fed BSFL whole than for those fed BSFL oil. A select serum metabolites were affected by diet ($P < 0.05$), but remained within reference ranges. Hematology and skin and coat markers were not affected by diet ($P > 0.05$). Overall, our results suggest that BSFL-containing diets are palatable and do not negatively affect fecal characteristics, serum chemistry, or skin and coat markers, but may result in slightly lower nutrient digestibilities.

INTRODUCTION

Interest in black soldier fly larvae (BSFL; *Hermetia illucens*) as an alternative protein and fat source for pet food has increased recently due to their low environmental footprint and high nutritional value [crude protein (CP): 38.5 to 47.9%; crude fat: 14.6 to 39.2%] (Zheng et al., 2012; Bosch et al., 2014; Nguyen et al., 2015). Previous studies using a cecectomized rooster model have demonstrated that BSFL has high amino acid (AA) digestibility and protein quality (Do et al., 2020;

Do et al., 2021). In addition to serving as a high-quality protein source, the biofunctionalities of BSFL-derived proteins have been evaluated recently. These proteins have been shown to have anti-inflammatory, antioxidant, immunomodulatory, and antibacterial properties (Firmansysah and Abduh, 2019; Zhu et al., 2020; Mouithys-Mickalad et al., 2020). For example, several peptide sequences present in BSFL hydrolysates that have alanine, lysine, isoleucine, phenylalanine, leucine, and valine residues at the C- and N-termini have been shown to enhance free radical scavenging ability (Zhu et al., 2020). BSFL oil is also a unique fat source because it is high in medium-chain fatty acids such as lauric acid (C12:0). These fatty acids have antibacterial properties and are an important component of sebaceous lipids (sebum) that provide protection to the skin from pathogens and water loss (Richman and Griffin, 2018; Fischer, 2020). Moreover, chitin which is present in the cuticle of BSFL (structurally identical to cellulose), has been suggested to have prebiotic potential that may benefit gut health (Roseland et al., 1985; Tomberlin et al., 2002; Finke, 2013). These potential impacts on health require further study in general and in the target species.

A few studies have evaluated the use of BSFL in pet foods, with the main focus being on dogs thus far. With most novel ingredients, palatability, gastrointestinal tolerance, and apparent total tract digestibility (ATTD) are the initial outcomes of interest. Compared with dog foods based on traditional protein sources, foods containing various insects such as BSFL, mealworm (*Tenebrio molitor*), crickets (*Gryllodes sigillatus*), and cockroaches (*Shelfordella lateralis*) have been shown to have similar smell and consumption preferences by dogs. For example, when beagle dogs had free access to commercial dry dog foods with aromas from four insect species, results showed no differences ($P > 0.05$) in the frequency of first choice (Kierończyk et al., 2018). Beynen (2018) reported food consumption results, with dogs slightly preferring a BSFL-containing diet over a yellow mealworm-containing diet (intake ratio = 60:40), while the reverse was noted in cats (intake ratio = 40:60).

Four published studies have tested the digestibility of BSFL-containing diets in dogs. In one study, adult female beagles (n=9) fed extruded diets containing 0, 1, and 2% of BSFL meal for 42 d had linear increases in dry matter (DM) and CP ATTD, but fat ATTD was not changed (Lei et al., 2019). In another study, DM ATTD was slightly greater ($P < 0.05$; 83% vs. 82%) while CP digestibility was slightly lower ($P < 0.05$; 77% vs. 79%) in beagle dogs fed a BSFL meal-containing extruded diet [20% in diet, DM basis (DMB)] than for those fed a control diet based on lamb meal (Kröger et al., 2020). When adult dogs were fed an extruded diet containing 28% BSFL meal (DMB), they had higher ($P < 0.05$) CP (82.3%) and fat (94.5%) ATTD compared with those fed a poultry-based diet (33% in diet, DM) (El-Wahab et al., 2021). Finally, Freel et al. (2021) tested BSFL meal-based extruded diets (5, 10, and 20% in diet, as-is) and BSFL oil-based diets (2.5 and 5.0% in diet, as-is) in adult dogs, reporting that there were no differences in the ATTD of DM, CP, fat, and energy among treatments.

Other than two studies testing extruded diets (Paßlack and Zentek, 2018; Pezzali and Shoveller, 2021), limited research has been conducted to evaluate BSFL-containing in cats and with no studies testing canned diets. For this reason, the objective of this study was to determine the palatability and ATTD of BSFL-containing canned diets and the fecal characteristics and skin and coat health markers of healthy adult cats consuming them. We hypothesized that when compared with a poultry-based control diet, BSFL-containing canned diets would not negatively affect palatability, ATTD, or fecal characteristics, and that skin and coat health markers would be improved in healthy adult cats.

MATERIALS AND METHODS

Palatability Studies

Three 2-d palatability tests were conducted at Kennelwood Inc. (Champaign, IL). In each test, 20 adult cats (BW: 4.56 ± 1.18 kg) were used. Two stainless steel bowls, each containing approximately 150 g of canned diet, were offered once daily for 2 d. Bowl placement was reversed daily to prevent left–right bias and both bowls were presented for 30 min. If one diet was completely consumed prior to the end of the 30 min, both bowls were removed. Food consumption and first choice preference were recorded for each cat. The following experiments were performed: 1) canned diet with poultry byproduct meal (PBPM) and chicken serving as the primary protein sources (control) vs. canned diet with BSFL meal replacing PBPM (BSFL meal); 2) control vs. canned diet with whole BSFL replacing some PBPM and poultry fat (BSFL whole); and 3) control vs. canned diet with BSFL oil replacing poultry fat (BSFL oil). A paired t-test was conducted to analyze data from the palatability tests, with statistical significance set as $P < 0.05$.

Digestibility Study

Animals and Housing

Healthy adult cats (20 females; 12 males; BW: 4.19 ± 0.55 kg; age: 3.3 ± 0.38 yr) were used in a completely randomized design. All cats were housed in cages (approximately 1.02 m deep; 0.76 m wide; 0.71 m high) during feeding times at the University of Illinois in a temperature (21°C) and light-controlled (14 h light:10 h dark) room. At other times, cats were group-housed outside of their cages. Cats were allowed access to various toys and scratching poles for environmental enrichment and were socialized with human interaction at least two times per wk. On the basis of the estimated maintenance energy requirement for adult cats and information from previous feeding records, an amount of food to maintain BW was offered and intake was measured twice daily (08:00-09:00 am and 15:00-16:00 pm). Cats had free access to fresh water at all times.

Experimental Periods and Diets

This study was composed of 21d baseline period followed by a 70d experimental period. During the baseline, all cats consumed the control diet (**Table 5.1**). After baseline measurements, cats were randomly assigned to 1 of 4 experimental canned diets (n=8/group): 1) diet based on fresh/frozen poultry, PBPM, corn gluten meal, white rice, chicken liver, and poultry fat (control); 2) diet with BSFL meal replacing PBPM (BSFL meal; providing ~42% of CP and ~36.5% of fat); 3) diet with whole BSFL replacing some PBPM and poultry fat (BSFL whole; providing ~17% of CP and ~38% of fat); 4) diet with BSFL oil replacing poultry fat (BSFL oil; providing ~22% of fat). All experimental diets were formulated to meet all Association of American Feed Control Officials (AAFCO; 2019) nutrient recommendations for adult cats, included fresh/frozen poultry, PBPM, white rice, chicken liver, and poultry fat, and were formulated to contain approximately 40% CP and 20% crude fat on DMB. Diets were manufactured at the University of Illinois Integrated Bioprocessing Research Laboratory. A still retort (Allpax, 30802 series) was used to achieve the desired cook and sterilize all diets. Cats were fed to maintain BW throughout the study. Food offered and refusals were measured daily to calculate intake.

Fecal and Blood Sample Collection and Analyses

During the fecal collection phase, total fecal samples were collected for 5 d. At these times, total feces were weighed and frozen at -20^{o}C until analyses were completed. Fresh feces were collected for measurement of pH and moisture content. Fecal pH was measured immediately using an AP10 pH meter (Denver Instrument, Bohemia, NY) equipped with a Beckman Electrode (Beckman Instruments Inc., Fullerton, CA). An aliquot then was collected for DM determination. All fecal samples during the collection phase were scored according to the following scale: 1 = hard, dry pellets, small hard mass; 2 = hard, formed, dry stool; remains firm and soft; 3 = soft, formed, and moist stool, retains shape; 4 = soft, unformed stool, assumes shape of container; 5 = watery, liquid that can be poured.

On the last day of each collection period, approximately 4 mL of blood was collected via jugular or cephalic puncture. Cats were fasted for 12 h prior to blood collection. Cats were sedated by intramuscular injection of dexmedetomidine (0.5 mg/mL IM) for sedation. After blood was collected, an injection of the reversal agent for dexmedetomidine, atipamezole (0.5 mg/mL intramuscular), was given. Samples were immediately transferred to appropriate vacutainer tubes. The blood tubes for serum isolation were centrifuged at 1,300 × g at 4^{o}C for 10 min (Beckman CS-6R centrifuge; Beckman Coulter Inc., Brea, CA). Serum then was transported to the University of Illinois Veterinary Medicine Diagnostics Laboratory for serum chemistry analysis. K_2EDTA tubes were cooled (but not frozen) and then transported to the University of Illinois Veterinary Medicine Diagnostics Laboratory for hematology. Cats received their regular daily meal after blood collection.

Chemical Analysis and Apparent Total Tract Macronutrient Digestibility Calculations

Diet subsamples were collected, lyophilized, and ground through a 2-mm screen using a Wiley Mill (model 4, Thomas Scientific, Swedesboro, NJ) with dry ice to allow for proper grinding before analysis. Total fecal samples were composited and dried at 57°C for 1 wk. Fecal samples then were ground through a 2-mm screen using a Wiley Mill (model 4). DM and organic matter

(OM) concentrations were analyzed according to the Association of Official Analytical Chemists (AOAC, 2006; DM: method 934.01; OM: method 942.05). Fat concentrations were measured by acid hydrolysis according to the AACC (1983) followed by diethyl ether extraction (Budde, 1952). CP concentration was calculated from Leco total nitrogen values (TruMac N, Leco Corporation, St. Joseph, MI; AOAC, 2006). Gross energy (GE) was measured using an oxygen bomb calorimeter (model 6200, Parr Instruments, Moline, IL). Total dietary fiber (TDF) concentration of the diet samples was determined according to Prosky et al. (1985). ATTD of nutrients and energy was calculated using the following equation: Digestibility (%) = [Nutrient intake (g/d) – Fecal output (g/d)]/ Nutrient intake (g/d) x 100%.

Skin and Hair Collection and Analyses

First, the skin and hair of cats were scored by blinded researchers according to Rees et al. (2001). Hair was scored as follows: 1 = dull, coarse, dry; 2 = poorly reflective, non-soft; 3 = medium reflective, medium soft; 4 = highly reflective, very soft; 5 = greasy. Skin was scored as follows: 1 = dry; 2 = slightly dry; 3 = normal; 4 = slightly greasy; 5 = greasy.

To assess hair using microscopy, hair samples were collected from the back using an electric clipper (Oster, McMinnville, TN). A 2-inch square of hair was clipped, with hair placed into Whirl Pak bags (Nasco, Fort Atkinson, WI) and stored at -20^{o}C until analysis. Eight hairs per cat were cut at a 45° angle and fixated by carbon tape to a 2.5 cm disk or a 1.5-inch disk. These disks received a gold and lead sputter coating and were imaged at the Beckman Institute for Advanced Science and Technology using a scanning electron microscope (FEI Quanta FEG 450 ESEM; FEI Company, Hillsboro, OR) for photographic surveying. Hairs were scored by three blinded researchers using a grading system similar to that established by Kim et al. (2010) to assess damage to the hair surface and cortex. Hair surface damage was scored as follows: 0 = intact hair, regular overlay of the cuticle; 1 = irregular overlay of the cuticle; 2 = lift-up of the cuticle; 3 = severe lift-up of the cuticle; or over half scale size gone; 4 = partial presence of cuticle. Hair cortex damage was scored as follows: 0 = intact and thick and dense cortex, regular overlay of the cortex; 1 = thick and dense cortex, with minor damage to the cortex, presence of crack; 2 = thick cortex, with moderate damage to the cortex, presence of crack; 3 = thick cortex, with severe damage to the cortex, presence of crack and hole; 4 = thin cortex, with severe damage to the cortex, presence of crack, blurry separation between cortex and medulla; 5 = very thin cortex with severe damage to the cortex, presence of crack, loss of distinction between cortex and medulla.

To assess skin transepidermal water loss (TEWL) and hydration status, the left side of the groin area and the back were first shaved 24 h before testing using an electric clipper. Assessment was conducted in 3 body areas: left side of groin, inner surface of left auricle (outer ear), and back. TEWL was measured using a Tewameter TM 300 MDD (Courage + Khazaka Electronic GmbH, Cologne, Germany). Hydration status was measured using a Corneometer CM 825 (Courage + Khazaka Electronic GmbH, Cologne, Germany). Each measurement was conducted 3 times by the same operator and the average result of the 3 measurements was used for analysis. The temperature (20 to 25°C) and relative humidity (40 to 60%) in the testing room was maintained as recommended by the manufacturer.

After skin and hair had been evaluated, a delayed-type hypersensitivity (DTH) test as described by Kim et al. (2000) was conducted. First, a 10 cm^2 area was shaved 24 h before DTH using an electric clipper on the side of the thorax. On testing day, cats were injected intradermally in the flank area with saline (8.5 mg/mL; control), phytohaemagglutinin (PHA; 0.5 mg/mL), and concanavalin A (CONA; 0.5 mg/mL). Both PHA and CONA function as non-specific antigens.

The injection site was clipped and wiped with 70% ethyl alcohol. Skin induration was measured at baseline (0) and 15, 30, 45, 60 min, 24 h, 48 h, and 72 h after injection using a digital caliper. To determine the average diameter, the mean values of the longest and midpoint orthogonal diameter (mm) of the wheal (swollen area) were measured (van der Valk et al., 2015).

Statistical Analysis

All data were analyzed using the Mixed Models procedure of SAS (version 9.4; SAS Institute, Cary, NC) with treatment as a fixed effect and cat as a random effect. Differences among treatments were determined using a Fisher-protected least significant difference with a Tukey adjustment to control for experiment-wise error. Data are reported as means ± pooled SEM with statistical significance set a $P < 0.05$.

RESULTS

Chemical Composition

The chemical composition of the diets tested is presented in **Table 5.2**. The DM content among experimental diets was similar (25.53 to 28.76% DMB). On a DMB, OM content was highest in the BSFL meal diet (95.93%) and lowest in the control diet (92.41%). The acid-hydrolyzed fat (AHF) and gross energy were lowest for the control diet (15.16%, 4.75 kcal/g DMB) and similarities were observed in the BSFL meal (17.17%, 4.93 kcal/g DMB), BSFL whole (18.19%, 4.93 kcal/g DMB), and BSFL oil (17.08%, 4.93 kcal/g DMB) diets. The control and BSFL oil diets had a higher CP content (42.17 and 42.69% DMB) than the BSFL whole diet (39.37% DMB), followed by the BSFL meal diet (33.39% DMB). The TDF content was highest for the

BSFL whole diet (7.60% DMB), followed by the BSFL meal (7.30% DMB) and control (5.0% DMB) diets, and lowest in the BSFL oil diet (4.7% DMB).

Palatability Testing

Data from the palatability tests are presented in **Figure 5.1**. In Experiment 1, the BSFL meal diet was preferred ($P < 0.05$) by a 1.93:1 intake ratio over the control diet. In addition to having higher consumption, the BSFL meal diet was first approached 28 out of 40 occasions and first consumed 30 out of 40 occasions over the 2 d. For Experiment 2, the BSFL whole diet was preferred ($P < 0.05$) by a 2.03:1 intake ratio over the control diet. Over the 2 d, the BSFL whole diet was first approached 25 out of 40 occasions and first consumed 27 out of 40 occasions. For Experiment 3, the BSFL oil diet was preferred ($P < 0.05$) by a 1.57:1 intake ratio over the control diet. Over the 2 d, the BSFL oil diet was first approached on 24 out of 40 occasions and first consumed 24 out of 40 occasions.

Food Intake, Fecal Output, Fecal Characteristics, and Apparent Total Tract Macronutrient Digestibility

Food intake, fecal characteristics, and ATTD data are presented in **Table 5.3**. There were no differences in food or caloric intake ($P > 0.05$) among diets. However, cats fed the BSFL meal diet had a higher ($P < 0.05$) fecal output (as-is and DMB) than cats fed the BSFL oil diet. Similarly, cats fed the BSFL meal diet had a higher ($P < 0.05$) fecal caloric output (kcal/d) than cats fed the control or BSFL oil diets. Fecal pH and scores were not different among diets. However, cats fed the BSFL oil diet had a higher ($P < 0.05$) fecal DM than cats fed the BSFL meal diet. The ATTD of DM was greater ($P < 0.05$) for cats fed the BSFL oil diet than for those fed the BSFL meal diet.

The ATTD of OM was greater ($P<0.05$) for cats fed the control or BSFL oil diets than for those fed the BSFL meal or BSFL whole diets. For CP, ATTD was greater ($P < 0.05$) for cats fed the BSFL oil diet than for those consuming the BSFL meal or BSFL whole diets. ATTD of CP also was greater ($P < 0.05$) in cats fed the control or BSFL whole diets than for those fed the BSFL meal diet. The ATTD of energy was greater ($P < 0.05$) for cats fed the BSFL oil diet than for those consuming the BSFL meal or BSFL whole diets. The ATTD of energy also was greater ($P < 0.05$) for cats fed the control diet than for those consuming the BSFL meal diet. There were no differences in the ATTD of AHF among groups ($P > 0.05$).

Skin Transepidermal Water Loss (TEWL), Skin Hydration Status, Hair Imaging Scores, and Skin and Coat Hair Scores

There were no differences in skin TEWL, skin hydration status, hair imaging scores, and skin and hair coat scores ($P > 0.05$) among diets (**Table 5.4**). Using the open-chamber method, TEWL on the back, groin and auricle skin of cats was 12.32 ± 1.05 $g/h/m^2$ (range: 11.24 to 13.76 $g/h/m^2$), 10.42 ± 0.65 $g/h/m^2$ (range: 9.92 to 11.29 $g/h/m^2$), and 11.35 ± 0.84 $g/h/m^2$ (range:10.11 to 11.96 $g/h/m^2$), respectively. When examining skin hydration status, the auricle showed the highest hydration [range: 27.50 to 34.37 arbitrary units (AU)]. The other body areas such as the back and groin showed low skin hydration values (0.00 to 0.04 AU; 0.43 to 0.94 AU). When grading the hair surface and cortex using microscopy, no differences were observed ($P > 0.05$) among diets. The condition of the skin and hair also did not differ ($P > 0.05$) among treatments during the experiment, with all cats having hair that remained in a state described as having a medium level of reflectivity and softness, and skin that was scored as being normal.

A Delayed-Type Hypersensitivity (DTH) Response

The DTH response to PHA and CONA showed no differences ($P > 0.05$) among diets (**Table 5.5**). As expected, there was no DTH response to saline from 0 to 72 h (data not shown). However, PHA led to a quick response that provided a peak in induration response after 30 min (range: 4.56 to 9.49 mm), which subsided and was barely measureable after 48 h. A similar response was observed in induration response to CONA, with a high response observed within 15 min, which lasted for about 24 h and then progressively declined thereafter.

Serum Chemistry Profiles and Hematology

Serum chemistry profiles and hematology of cats fed all diets were within the reference ranges for adult cats, with the exception of a slightly higher albumin to globulin ratio (reference range: 0.6 to 1.1; baseline: 1.15, BSFL oil: 1.13), slightly lower blood Ca (reference range: 8.8 to 10.2 mg/dL; BSFL whole: 8.64 mg/dL, control: 8.70 mg/dL), slightly higher Na to K ratio (reference range: 28-36; baseline: 38.28, control: 40.25), slightly higher cholesterol (reference range: 66-160 mg/dL; BSFL meal: 189.37 mg/dL, BSFL whole: 173.13 mg/dL), and slightly higher eosinophils (reference range: 0-0.8 10^3/uL; baseline: 0.88 10^3/uL, control: 0.91 10^3/uL, BSFL meal: 0.86 10^3/uL) (**Table 5.6** and **Table 5.7**). Cats fed the control diet had greater ($P < 0.05$) serum creatinine concentrations than for those fed the BSFL meal or BSFL whole diets. Cats fed the control or BSFL meal diets had higher ($P < 0.05$) blood urea nitrogen concentrations than for those fed the BSFL whole diet. Cats fed the BSFL meal diet had higher ($P < 0.05$) serum bicarbonate concentrations than for those fed the control diet. All other blood metabolites and cells were not different among groups ($P > 0.05$).

DISCUSSION

The use of BSFL-based ingredients in animal feed is promising due to their environmental footprint and nutritional quality (contains up to 40% of CP and 28% lipids) (Zheng et al., 2012; Bosch et al., 2014; Makkar et al., 2014; Nguyen et al., 2015; Wang and Shelomi, 2017). In previous studies, our lab used a cecectomized rooster model to evaluate the amino acid (AA) digestibility and protein quality of BSFL-based ingredients (Do et al., 2020; Do et al., 2021). Digestible indispensable AA score (DIAAS)-like values were calculated according to Mathai et al. (2017) using AAFCO nutrient profiles and NRC recommended allowances for dogs and cats and were shown to be of high quality, with limiting AA being methionine and cysteine for most ingredients (Do et al., 2020; Do et al., 2021). In regard to fat, BSFL oil derived from the defatting process is high in saturated fatty acids (52.5 to 68.7% of total fatty acids), followed by monounsaturated fatty acids (12.2 to 22.2% of total fatty acids) and polyunsaturated fatty acids (7.8 to 24.2% of total fatty acids) (Barroso et al., 2017; Liland et al., 2017; Kierończyk et al., 2020).

A few studies have recently evaluated BSFL-containing diets in dogs. El-Wahab et al. (2021) reported that dogs fed a BSFL meal-based diet (30% of BSFL meal, DMB) had higher ($P < 0.05$; 114 g/d as-is) fecal output than dogs fed a PBPM-based diet (30% of PBPM, DMB; 98.4 g/d as-is). Even though ATTD of CP (80.5 vs. 82.3%) and crude fat (CF; 91.6 vs. 94.5%) were higher ($P < 0.05$) for dogs fed a BSFL meal-based diet than for those fed a PBPM-based diet, no differences ($P > 0.05$) were observed in ATTD of OM and nitrogen free extract (NFE) in that study. Kröger et al. (2020) reported that dogs eating a BSFL-meal diet (20% of diet, DMB) had a lower ($P < 0.05$) daily fecal output (109 g/d, as-is) than for those fed the control diet (lamb meal as the main protein source; 137 g/d, as-is). In that study, dogs fed a BSFL-meal diet had a lower ($P > 0.05$) ATTD of CP (79.2 vs. 77.3%) than dogs fed the control diet. ATTD of DM and NFE showed an opposite result, with dogs eating a BSFL-meal diet having higher (P < 0.05) ATTD of DM (81.8 vs. 83.2%) and NFE (90.7 vs. 92.9%) than for those fed the control diet. The ATTD of OM, ether extract, crude ash, and crude fiber was not different (P > 0.05) among treatments. Kilburn et al. (2020) reported that dogs fed extruded diets containing 8, 16, or 24% (as-is) banded cricket meal had a linear increase in fecal output (64.8 to 93.4 g/d as-is; 23.4 to 33.6 g/d DMB). Not surprisingly, the ATTD of DM (88.9 to 83.9%), OM (91.5 to 86.8%), CP (88.2 to 82.1%), fat (96.4 to 94.8%), and GE (92.4 to 88.3%) was linearly decreased (P < 0.05) with increased banded cricket meal inclusion. Lei et al. (2019) determined the ATTD of three dietary BSFL meal inclusion levels (0, 1, or 2% as-is). Even though ether extract digestibility was not affected (P > 0.05), linear increases (P < 0.05) in the ATTD of DM (71.97 to 75.21%) and nitrogen (73.16 to 78.51%) were observed. Finally, Freel et al. (2021) reported that no differences (P > 0.05) were observed in ATTD of DM, CP, fat, or GE for diets containing 0, 5, 10, or 20% (as-is) BSFL meal or 2.5 or 5.0% (as-is) BSFL oil fed to adult dogs. Discrepancies among the studies reported above may be due to BSFL inclusion levels, the chitin content in BSFL ingredients tested, or other unknown variables affecting outcome measures.

Despite the interest that exists in using BSFL-based ingredient in pet foods, they have not been well tested in cats, especially in canned diets. Therefore, we investigated the effects of BSFL-containing canned diets on palatability, ATTD, fecal characteristics, skin and coat health, serum metabolites, and hematology of adult cats in the current study. Experimental diets were formulated to contain a similar ingredient composition, similar nutrient and calorie content, and with practical inclusion levels of BSFL-based ingredients. Because CP content was lower and AHF, ash, and TDF concentrations were higher than expected in BSFL ingredients, the diets were not as consistent as desired. The TDF variability is likely due to the chitin content of BSFL-based ingredients (Bosch et al., 2014). Chitin (poly-N-acetyl-d-glucosamine) is an important component of BSFL formed in the cuticle layer during pupation and serves as an insoluble fiber in the diet (Roseland et al., 1985; Tomberlin et al., 2002; Finke, 2013).

In the current studies, BSFL-containing diets were shown to have sufficient palatability. Food and calorie intake did not differ among treatments, but cats fed BSFL meal had higher ($P < 0.05$) fecal output and reduced nutrient digestibility. Paßlack and Zentek, (2018) fed diets consisting of 22 or 35% BSFL meal (as-is) to cats and reported that ATTD of CP (77.0 and 73.4%; $P < 0.05$) was reduced, while ATTD of crude fat (96.0 vs. 92.6%) was increased with increasing BSFL meal inclusion. As mentioned previously, the reduction in nutrient digestibility by cats fed BSFL meal in the current study may have been related to the chitin content of BSFL meal, which can negatively affect nutrient digestibility (Lei et al., 2019; Penazzi et al., 2021). Serum chemistry and complete blood count analyse were within reference ranges for adult cats. These results indicate that the treatments did not negatively affect any health outcomes. Similarly, Pezzali and Shoveller (2021) reported that serum chemistry and complete blood count parameters remained within reference ranges for adult cats fed a diet containing 4.6% BSFL meal for 21 d.

The fatty acid profile of BSFL oil is rich in lauric acid (12:0), making up to 23.9-42.1% of total fatty acids depending on rearing conditions and diet (Liland et al., 2017; Kierończyk et al., 2020). Lauric acid may serve as an antimicrobial agent, against Gram-positive bacteria, which naturally occur on the skin, in mucosal membranes, and in sweat. Also, lauric acid is one of the sebaceous triglycerides that is important for the epithelial barrier function of skin and moisture retention of the inner layer of hair (Burtenshaw, 1945; Thormar and Hilmarsson, 2007; Brasser et al., 2011; Czyz et al., 2012; Lin et al., 2018; Richman and Griffin, 2018; Fischer, 2020). In the current study, no differences were observed in skin TEWL, skin hydration status, hair imaging score, and skin and hair scores. The lack of response may be related to the cats used in the current study, which remained healthy and had no skin diseases. However, there were regional differences in TEWL and skin hydration, which may have been due to differences in sweat gland activity, skin thickness, skin surface temperature, or corneocyte size (Alexander et al., 2018).

The DTH response is a cell-mediated immune response that can be induced by the intradermal injection of antigens (e.g., CONA and PHA) and is due to T-cells and activated macrophages (Grabbe et al., 1995; McLellan et al., 1998; Roychowdhury and Svensson, 2005; Mathers and Larregina, 2006; Wittmann and Werfel, 2006). BSFL-derived proteins can be hydrolyzed by many enzymes, including alcalase, trypsin, neutrase, pepsin, and protamex to produce bioactive short-chain peptides and free AA. These molecules have various biological activities such as anti-inflammatory, antioxidant, immunomodulatory, and anti-bacterial properties (Firmansysah and Abduh, 2019; Zhu et al., 2020; Mouithys-Mickalad et al., 2020). Also, BSFL oil contains polyunsaturated fatty acids (7.8 to 24.2% of total fatty acids) that are known to attenuate inflammatory response (Calder and Grimble, 2002; Barroso et al., 2017; Liland et al., 2017; Kierończyk et al., 2020). In the current study, we hypothesized that BSFL protein derivatives would scavenge reactive oxygen species, which are produced from neutrophils at the site of inflammation, or inflammatory response would be reduced by the fatty acid composition of BSFL oil, ultimately decreasing DTH response. No differences in DTH response were observed among the treatments, however, which may have been due to the health of the cats used in the study.

In conclusion, the addition of BSFL-based ingredients (BSFL meal, whole BSFL, and BSFL oil) had no detrimental effects on animal health, fecal quality, or skin and coat health. Inclusion of BSFL-based ingredients in canned diets improved palatability when compared with a poultry-based control diet. However, the inclusion of BSFL meal resulted in decreased ATTD of

DM, OM, CP, and energy and the inclusion of whole BSFL reduced ATTD of OM, CP, and energy. Overall, dietary supplementation of BSFL ingredients were well accepted by healthy adult cats without any detrimental effects on fecal characteristics, skin and coat health, and serum chemistry, or hematology.

TABLES AND FIGURE

Table 5.1. Ingredient composition of the experimental diets fed to adult cats (%, as-is)

Ingredient	Control	BSFL meal	BSFL whole	BSFL oil
Water	63.33	63.65	63.36	62.63
Black soldier fly larvae (BSFL) meal	-	10.00	-	-
BSFL (whole)	-	-	5.00	-
BSFL oil	-	-	-	1.50
Poultry byproduct meal	9.34	-	4.00	8.42
Chicken, deboned without skin	10.50	10.50	10.50	11.50
Corn gluten meal	4.00	5.00	5.20	4.62
Chicken liver	4.00	4.00	4.00	4.00
Poultry fat	1.50	1.50	1.00	-
White rice	5.00	3.03	4.62	5.00
Guar gum	0.60	0.60	0.60	0.60
Rice flour	1.50	1.50	1.50	1.50
Mineral premix[1]	0.07	0.07	0.07	0.07
Vitamin premix[2]	0.06	0.06	0.06	0.06
L-taurine	0.05	0.05	0.05	0.05
Salt	0.05	0.05	0.05	0.05

[1]Provided per kg diet: Cu (as $CuCO_3$), 7.0 mg; K (as KIO_3), 0.7 mg; Fe (as $C_6H_5FeO_7$), 52.5 mg; Mn (as $MnCO_3$), 7.0 mg; Na (as Na_2SeO_3), 154.0 mg; Zn (as $ZnCO_3$), 70.0 mg; Co (as $CoSO_4$), 1.5 mg
[2]Provided per kg diet: vitamin A, 6,000 IU; vitamin D3, 900 IU; vitamin E, 48 IU; vitamin K3, 0.72 mg; biotin, 0.04 mg; vitamin B12, 38.33 ug; folic acid, 0.36 mg; nicotinic acid, 41.40 mg; vitamin B5, 16.80 mg; pyridoxine, 10.20 mg; riboflavin, 10.20 mg; thiamin, 10.20 mg

Table 5.2. Analyzed chemical composition of experimental diets fed to adult cats

Item	Treatment			
	Control	BSFL meal	BSFL whole	BSFL oil
Dry matter (DM), %	28.37	28.18	25.53	28.76
	-------------------- %, DM basis -------------------			
Organic matter	92.41	95.93	94.44	93.24
Ash	7.59	4.07	5.56	6.76
Acid-hydrolyzed fat	15.16	17.17	18.19	17.08
Crude protein	42.17	33.39	39.37	42.69
Total dietary fiber	5.00	7.30	7.60	4.70
Choline, mg/kg	2020	2060	2040	1930
Taurine, %	0.19	0.24	0.27	0.32
Chitin[1], %	0.00	0.85	0.96	0.00
Gross energy, kcal/g	4.75	4.93	4.93	4.93

[1]Chitin content of diets estimated based on chitin content of BSFL meal (2.41%) and BSFL whole (4.91%).

Table 5.3. Food intake, fecal characteristics, and apparent total tract macronutrient digestibility of adult cats fed black soldier fly larvae (BSFL)-containing canned diets

Item	Treatment					
	Control	BSFL meal	BSFL whole	BSFL oil	SEM[1]	P-value
Food intake						
g food/d (as-is)	216.00	208.05	205.01	202.62	4.131	0.7042
g food/d (DM basis)	61.28	58.63	52.34	58.78	1.271	0.0778
kcal/d	290.86	289.32	258.00	287.32	6.038	0.1679
Fecal output						
g feces/d (as-is)	30.55[ab]	39.94[a]	30.40[ab]	23.82[b]	1.593	0.0015
g feces/d (DM basis)	8.88[ab]	10.98[a]	8.86[ab]	7.83[b]	0.400	0.0327
kcal/d	36.06[b]	49.58[a]	41.25[ab]	32.88[b]	1.890	0.0053
Fecal characteristics						
Fecal pH	6.56	6.63	6.64	6.68	0.024	0.3619
Fecal score[2]	2.88	2.50	2.75	2.31	0.083	0.0674
Fecal DM, %	28.92[ab]	27.91[b]	29.65[ab]	33.00[a]	0.701	0.0499
Digestibility, %						
Dry matter	85.47[ab]	81.19[b]	83.16[ab]	86.51[a]	0.655	0.0118
Organic matter, %	88.63[a]	83.34[b]	84.80[b]	89.15[a]	0.651	0.0005
Acid-hydrolyzed fat, %	88.94	87.64	87.75	90.61	0.552	0.1976
Crude protein, %	86.71[ab]	77.73[c]	82.87[b]	87.64[a]	0.888	<.0001
Energy, %	87.57[ab]	82.79[c]	84.10[bc]	88.52[a]	0.645	0.0007

[1]Pooled standard error of the mean.
[2]Fecal scores: 1 = hard, dry pellets; small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured.
[a-c]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

Table 5.4. Skin transepidermal water loss (TEWL), skin hydration status, hair imaging scores, and skin and coat hair scores of adult cats fed black soldier fly larvae (BSFL)-containing canned diets

Item	Treatment				SEM[1]	P-value
	Control	BSFL meal	BSFL whole	BSFL oil		
TEWL, g/h/m^2						
Back	12.13	11.24	12.16	13.76	0.565	0.4802
Groin	10.52	9.92	9.93	11.29	0.352	0.4882
Auricle	11.96	10.11	11.79	11.54	0.466	0.5024
Hydration status, arbitrary units						
Back	0.04	0.00	0.00	0.00	0.009	0.4074
Groin	0.94	0.43	0.43	0.45	0.167	0.6596
Auricle	33.38	34.37	27.50	32.28	1.567	0.4366
Hair imaging scores[2]						
Hair surface damage	1.75	1.56	1.98	1.56	0.173	0.8209
Hair cortex damage	2.63	2.50	3.15	2.52	0.169	0.5090
Skin and hair scores[3]						
Skin	3.13	3.38	3.23	3.23	0.045	0.2761
Hair	2.83	2.83	2.96	2.83	0.039	0.6131

[1]Pooled standard error of the mean.

[2]Hair surface: 0 = intact hair, regular overlay of the cuticle; 1 = irregular overlay of the cuticle; 2 = lift-up of the cuticle; 3 = severe lift-up of the cuticle; or over half scale size gone; and 4 = partial presence of cuticle; Hair cortex: 0 = intact and thick and dense cortex, regular overlay of the cortex; 1 = thick and dense cortex, with minor damage to the cortex, presence of crack; 2 = thick cortex, with moderate damage to the cortex, presence of crack; 3 = thick cortex, with severe damage to the cortex, presence of crack and hole; or over half scale size gone; 4 = thin cortex, with severe damage to the cortex, presence of crack, blurry separation between cortex and medulla; and 5 = very thin cortex with severe damage to the cortex, presence of crack, loss of distinction between cortex and medulla.

[3]Hair: 1 = dull, coarse, dry; 2 = poorly reflective, non-soft; 3 = medium reflective, medium soft; 4 = highly reflective, very soft; and 5 = greasy; Skin: 1 = dry; 2 = slightly dry; 3 = normal; 4 = slightly greasy; and 5 = greasy.

Table 5.5. Delayed-type hypersensitivity (DTH) response of adult cats fed black soldier fly larvae (BSFL)-containing canned diets

Item	Treatment				SEM[1]	P-value
	Control	BSFL meal	BSFL whole	BSFL oil		
PHA, mm[2]						
15 min	6.06	6.98	4.86	5.71	0.603	0.6842
30 min	6.49	4.56	4.63	4.68	0.702	0.7409
45 min	4.35	3.98	3.99	4.15	0.772	0.9983
60 min	2.95	3.42	2.47	2.67	0.702	0.9709
24 h	2.17	2.96	0.24	3.33	0.563	0.2168
48 h	0.68	1.44	0.00	0.48	0.277	0.3307
72 h	0.00	0.00	0.00	0.00	-	-
CONA, mm[3]						
15 min	7.61	5.52	3.72	6.73	0.607	0.1266
30 min	5.72	4.30	4.06	5.85	0.735	0.7722
45 min	5.34	4.12	3.15	6.34	0.795	0.5336
60 min	5.39	3.48	3.61	4.66	0.795	0.8382
24 h	7.86	6.44	3.06	4.22	0.922	0.2594
48 h	2.99	2.45	1.48	3.69	0.641	0.6884
72 h	0.95	0.91	0.4	0.88	0.308	0.9365

[1]Pooled standard error of the mean.

[2]Phytohaemagglutinin (PHA; 0.5 mg/mL); the average diameter = (mean value of the longest + midpoint orthogonal diameter)/2).

[3]Concanavalin A (CONA; 0.5 mg/mL); the average diameter = (mean value of the longest + midpoint orthogonal diameter)/2).

Table 5.6. Serum chemistry profiles for adult cats fed black soldier fly larvae (BSFL)-containing canned diets

Item	Reference range	Treatment				SEM[1]	P-value
		Control	BSFL meal	BSFL whole	BSFL oil		
Creatinine (mg/dL)	0.4-1.6	1.43[a]	1.15[b]	1.13[b]	1.30[ab]	0.035	0.0047
Blood urea nitrogen (mg/dL)	18-38	28.13[a]	28.88[a]	23.00[b]	26.63[ab]	0.668	0.0044
Total protein (g/dL)	5.8-8.0	6.18	6.08	5.86	6.24	0.080	0.3838
Albumin (g/dL)	2.8-4.1	3.20	3.04	3.06	3.29	0.047	0.1966
Globulin (g/dL)	2.6-5.1	2.98	3.04	2.80	2.95	0.060	0.5755
Albumin:globulin ratio	0.6-1.1	1.09	1.01	1.10	1.13	0.027	0.5271
Ca (mg/dL)	8.8-10.2	8.70	8.84	8.64	8.79	0.058	0.6440
P (mg/dL)	3.2-5.3	3.96	3.88	3.68	4.23	0.102	0.3032
Na (mmol/L)	145-157	147.13	148.25	147.37	147.25	0.258	0.4179
K (mmol/L)	3.6-5.3	3.77	4.49	4.23	4.23	0.106	0.1167
Na:K ratio	28-36	40.25	33.88	35.38	34.75	1.186	0.2296
Cl (mmol/L)	109-126	118.00	117.38	117.38	116.25	0.327	0.3017
Alkaline phosphatase (U/L)	10-85	20.50	23.00	22.38	24.75	1.327	0.7977
Alanine transaminase (U/L)	8-65	57.75	46.13	38.25	64.25	4.830	0.2300
Gamma glutamyltransferase (U/L)	0-3	0.00	0.00	0.13	0.13	0.043	0.5796
Total bilirubin (mg/dL)	0.0-0.3	0.14	0.15	0.13	0.15	0.009	0.7296
Creatine phosphokinase (U/L)	10-250	140.25	125.00	168.13	153.50	12.562	0.6773
Cholesterol (mg/dL)	66-160	145.62	189.37	173.13	158.00	6.830	0.1187
Triglycerides (mg/dL)	21-166	33.00	28.75	50.13	34.50	4.549	0.3813
Bicarbonate (mmol/L)	12-21	17.75[b]	20.38[a]	19.00[ab]	19.00[ab]	0.316	0.0255
Anion gap	10-27	15.00	15.00	15.25	16.00	0.275	0.5474

[1]Pooled standard error of the mean.

[a-b]Within a row, means lacking a common superscript letter differ ($P < 0.05$).

Table 5.7. Hematology of adult cats fed black soldier fly larvae (BSFL)-containing canned diets

Item	Reference range	Treatment[1]				SEM[1]	P-value
		Control	BSFL meal	BSFL whole	BSFL oil		
Red blood cells (10^6/uL)	5.00-10.00	8.53	8.62	7.86	8.26	0.130	0.1567
Reticulocyte count (%)	-	0.11	0.12	0.08	0.12	0.012	0.4768
Hemoglobin (g/dL)	8.0 - 15.0	12.34	12.40	11.64	11.8	0.206	0.4803
Hematocrit (%)	30.0 - 55.0	35.28	36.28	33.34	33.74	0.576	0.2442
Mean cell volume (fi)	37.0 - 55.0	41.41	42.43	42.36	40.9	0.372	0.5084
Mean corpuscular hemoglobin (pg)	13.0 - 18.0	14.50	14.41	14.79	14.3	0.154	0.7303
Mean corpuscular hemoglobin (g/dL)	29.0 - 38.0	34.98	34.23	34.90	34.93	0.211	0.5647
White blood cells (10^3/uL)	5.50-19.50	12.88	12.31	10.59	11.95	2.876	0.7412
Lymphocytes (10^3/uL)	1.70-7.00	3.68	3.63	3.22	4.71	0.317	0.4016
Lymphocytes (%)	-	28.98	29.26	32.46	39.63	1.905	0.1651
Monocytes (10^3/uL)	0.00-0.90	0.42	0.30	0.41	0.35	0.026	0.3671
Monocytes (%)	-	3.36	2.89	4.30	3.11	0.300	0.3737
Eosinophils (10^3/uL)	0.00-0.80	0.91	0.86	0.70	0.69	0.058	0.4544
Eosinophils (%)	-	7.80	7.48	7.19	6.03	0.660	0.8103
Basophils (10^3/uL)	0.0-2.0	0.07	0.01	0.03	0.03	0.008	0.0673
Basophils (%)	-	0.73	0.13	0.34	0.26	0.098	0.1594

[1]Pooled standard error of the mean.

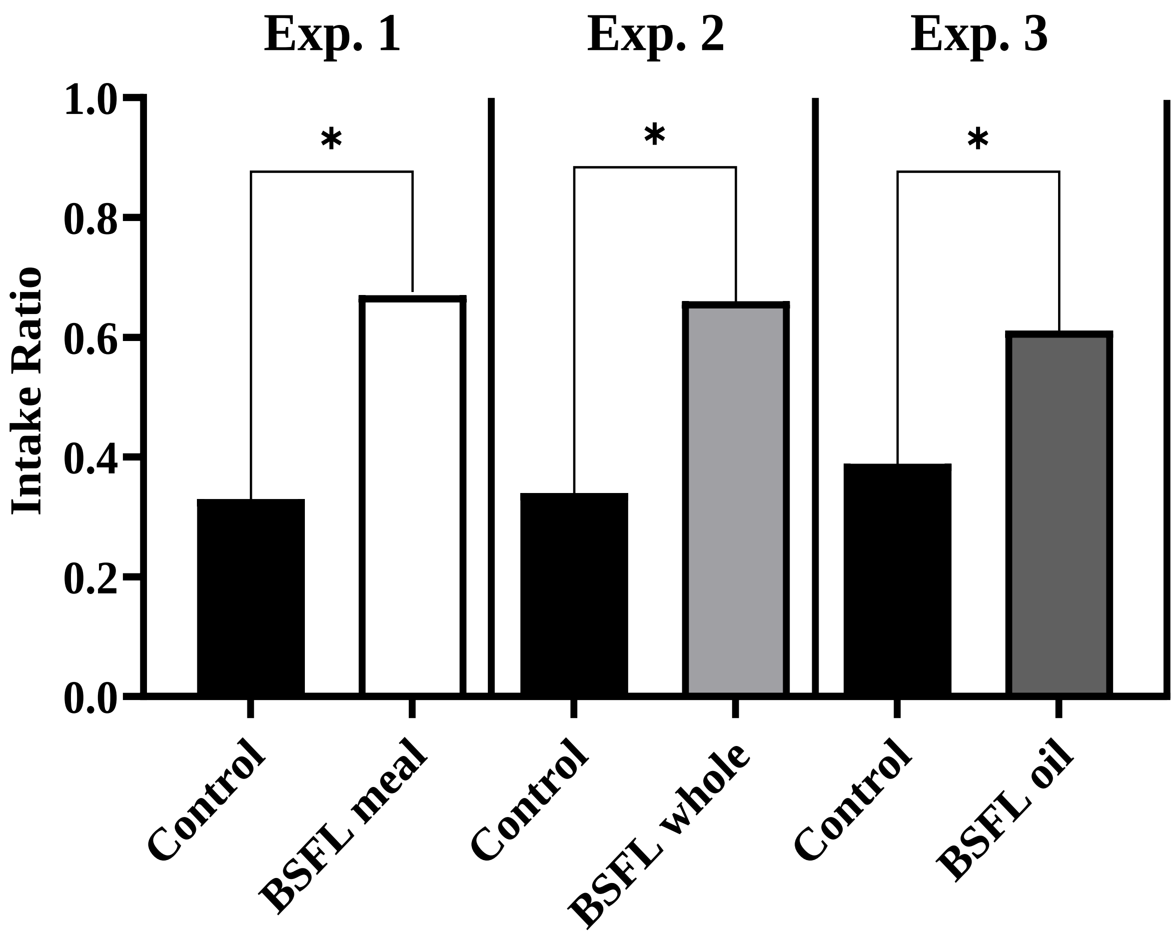

Figure 5.1. Food intake ratios of cats fed black soldier fly larvae (BSFL)-containing canned diets (Exp. 1: control vs. BSFL meal; Exp. 2: control vs. BSFL whole; Exp. 3: control vs. BSFL oil); * indicates the significant difference (P < 0.05).

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CHAPTER 6: SUMMARY

Black soldier fly larvae (BSFL) are attracting the attention of the pet food industry as an alternative protein and energy source for dog and cat. BSFL can efficiently break down a wide variety of organic resources and convert them into high quality protein and fat sources (Diener et al., 2009; Makkar et al., 2014; Liu et al., 2017). However, the nutritional composition of BSFL varies considerably depending on rearing conditions, harvest age, and feed provided. Therefore, it is important to determine the optimal rearing conditions for increased fecundity, larval size, feed conversion, and a quick growth cycle of BSFL. BSFL ingredients such as dried whole BSFL and BSFL meal were tentatively approved by AAFCO (2021) for use in adult dog foods, but these ingredients are not yet approved for cat foods. Also, no studies have evaluated BSFL-containing canned diets by cats.

To our knowledge, limited scientific data have shown how different harvest ages and dietary calcium sources or concentrations affect BSFL nutrient composition and its bioavailability for animal feeds. Also, no studies have determined how BSFL-containing canned diets affect palatability, apparent total tract digestibility (ATTD), fecal characteristics, and skin and coat health makers of cats. This thesis was conducted to address some of these gaps in knowledge.

In our first aim, we determined that harvest age on days 14, 18, and 23 of BSFL showed the highest AA digestibilities compared with days 0, 11, and 29 in precision-fed cecectomized roosters. No differences were observed in dry matter (DM) or organic matter (OM) digestibility, while acid-hydrolyzed fat (AHF) digestibility tended to be higher ($P < 0.05$) for days 23 and 29 than days 14 and 18. Based on digestible indispensable amino acid score (DIAAS)-like reference values, which were calculated from AAFCO nutrient profiles and NRC recommended allowances for dogs and cats, day18 had the highest and day 11 had the lowest DIAAS-like reference values for most indispensable AA (threonine, methionine, cysteine, and arginine).

In our second aim, we determined that different dietary calcium sources ($CaCO_3$ and $CaCl_2$) and concentration fed to BSFL did not affect digestibilities of DM, OM, or AHF by precision-fed cecectomized roosters. Similarly, there were no differences in indispensable and dispensable AA digestibilities for all BSFL treatments. Sulfur AA (methionine + cysteine) and aromatic AA (phenylalanine + tyrosine) were estimated to be the first-limiting AA of BSFL based on DIAAS-like reference values for dogs and cats.

In our third aim, an experiment was conducted to determine the palatability, apparent total tract digestibility (ATTD), fecal characteristics, and skin and coat health markers of healthy adult cats consuming BSFL-containing canned diets. BSFL-containing canned diets were formulated with poultry by-product meal (PBPM), BSFL meal (10.0%, as-is), whole BSFL (5.0%, as-is), and BSFL oil (1.5%, as-is). In the palatability tests, BSFL meal (1.93:1), BSFL whole (2.03:1), and BSFL oil diets (1.57:1) resulted in a higher ($P < 0.05$) consumption ratio than the control diet. Fecal pH and scores and caloric intake were not different ($P > 0.05$) among diets, while fecal output (as-is, DM, and kcal/d) was highest ($P < 0.05$) for BSFL meal compared with BSFL oil. The ATTD of AHF was not different among treatments, but DM ATTD was greater ($P < 0.05$) for cats fed the BSFL oil diet than for those fed the BSFL meal diet. The ATTD of OM for control or BSFL oil was greater ($P < 0.05$) compared with the BSFL meal or BSFL whole diets. For CP and energy, ATTD was greatest ($P < 0.05$) for BSFL oil and lowest for BSFL meal. Skin and coat health markers, including skin transepidermal water loss (TEWL), skin hydration status, hair imaging score, and skin and coat hair scores were not affected ($P > 0.05$) by treatments. Similarly, hematology and a delayed-type hypersensitivity (DTH) response to phytohaemagglutinin (PHA) and concanavalin A (CONA) showed no differences ($P > 0.05$) among diets. A select serum metabolites were affected by diet ($P<0.05$), but remained within reference ranges.

This research provided a broader knowledge of BSFL, namely how harvest age and different calcium sources and concentrations affect nutrient composition of BSFL and its digestibility for use in dog and cat foods. The primary emphasis was on the protein and amino acid content and digestibilities and how this knowledge may be applied to improve the larval body composition of BSFL in production systems, with the goal of maximizing protein content and quality of BSFL. Finally, BSFL-derived ingredients such as BSFL meal, whole BSFL, and BSFL oil were shown to be well consumed, tolerated, and digested by adult cats. Our data support their use in pet foods as an alternative protein and/or fat source once regulatory approvals are in place. Further research is needed, as the potential benefits of long-term feeding on pet health are unknown. Identifying optimal concentrations of BSFL-derived ingredients in canine and feline diets may also be of interest in the future.

Based on these findings and those from previous studies, pet food formulators must consider using complementary protein sources with BSFL to compensate for the limiting AA for dog and cat foods, including the sulfur AA (methionine + cysteine) and aromatic AA (phenylalanine + tyrosine). In dogs, dry diets containing BSFL meal at up to 20% (as-is) and BSFL oil at up to 5% (as-is) inclusion appears to not negatively affect hematology, serum chemistry, or nutrient digestibility (El-Wahab et al., 2021; Freel et al., 2021). Our research suggests that in cats, BSFL may be included up to 10% of BSFL meal (as-is) in a wet food without negatively affecting fecal characteristics or serum chemistry. At this rate, however, a reduction in nutrient digestibility may be expected. Long-term feeding studies testing the potential biofunctionalities of BSFL-based components such as chitin, bioactive peptides, and lauric acid have not been conducted in dogs and cats and are justified.

Pet owner interest in alternative proteins has grown over time along with their interest in sustainability, pet health, and ethical considerations. Many alternatives such as plant-based proteins and single cell (bacteria; yeast) protein sources are already being used in pet foods and are expected to replace more of the conventional protein sources in the future. A variety of insect-based protein sources are expectred to compete in this space (Bosch and Swanson, 2021). Current impediments of their use in pet foods include the technical difficulties of mass production, the cost of product development, quality control, and legal and regulatory approval and guidance. Another challenge related to insect-based proteins is pet owner acceptance because insects are not common food items in Western cultures. Despite the current challenges, insect-based proteins have a promising future in the pet food and livestock industries.

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