INSECT MEALS AS NOVEL PROTEIN SOURCES IN RETORTED PET FOOD FOR ADULT CATS

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THESIS

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ABSTRACT: Insect meals are novel and potentially sustainable protein sources. The objective of this study was to determine the chemical composition of 3 selected insect meals [i.e., speckled cockroach (**SC**), madagascar hissing cockroach (**MC**) and superworm (**SW**)] and to determine the effects of these novel ingredients on food intake, apparent total tract digestibility (**ATTD**) of macronutrients, fecal scores and metabolites of adult cats fed insect- or chicken-based retorted diets. This study consisted of a complete randomized design, with 28 adult cats (mean age = 2.1 ± 0.03 yr; mean BW= 4.9 ± 0.8 kg) randomly assigned to 4 experimental retorted diets: Control (chicken-based diet), SC diet, MC diet, or SW diet. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. All diets were formulated according to AAFCO (2018) nutrient profile for adult cats. The experimental period was 28 d, with 7 d of diet adaptation followed by 21 d of dietary treatment. Total fecal collection was completed during the last 4 d of the experimental period. On d21, fresh fecal sample from each cat was collected for determination of fecal metabolites. Food was offered twice daily to maintain the cats' body weight and body condition score. Among the 3 selected insect meals evaluated, oleic acid (113,352-46,353 ug/g), palmitic acid (62,580-19,104 ug/g), linoleic acid (46,265-13,926 ug/g), and stearic acid (21,654-6,473 ug/g) were the most prevalent fatty acids. Branched-chain amino acids and arginine were the most preponderant indispensable amino acids in the insect meals. Apparent total tract digestibility of dry matter (DM), organic matter (OM), acid hydrolyzed fat (AHF), and crude protein (CP) did not differ among treatments ($P > 0.05$) and all diets were well digested by the cats. Similarly, fecal scores did not differ among the treatments and were within the ideal range. Furthermore, no differences ($P > 0.05$) in fecal metabolite concentrations were observed among cats fed different experimental diets. In conclusion, the selected insect meals evaluated herein are rich in linoleic acid, an essential fatty acid for cats. Furthermore, cats fed retorted diets containing these insect meals had comparable ATTD of macronutrients, fecal scores and metabolite concentrations of cats fed the chicken-based diet; suggesting that these novel protein sources might be adequate alternative protein sources in feline diets.

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CHAPTER 1
INTRODUCTION

The global population has increased dramatically, and is estimated to exceed 10 billion before the 22nd century (World Population Prospect, 2019). In the meantime, due to the rapid economic development in some developing countries, especially in China, the total meat consumption is expected to double based on the current food production (Ritchie and Roser, 2017). Similar to the human population, the pet population is also increasing. According to the American Veterinary Medical Association (AVMA), approximately 38.4% and 25.4% of total U.S. households owned a dog or cat, respectively (AVMA, 2019). Many pet owners treat their pets as family members, and are concerned with their health and longevity. They want high quality and humanized ingredients for their pets rather than more traditional pet food protein sources (i.e., animal by-products and rendered ingredients), which leads to a direct competition with human food production (Conway and Saker, 2018). Thus, it is imperative to find sustainable alternative protein sources of good nutritional quality.

Currently, insects or insect meals have gained increasing attention as novel sustainable protein sources in human and animal nutrition. Insects are natural food sources for many animals (i.e., poultry, cats, and fish), have low land and water requirements and a high feed conversion ratio (Sánchez-Muros et al., 2014; Oonincx et al., 2015;Bosch et al., 2014). In addition to the sustainability aspect, good nutrient digestibility and amino acid scores have been reported for some insect species (i.e., housefly pupae and black soldier fly pupae) using in vitro digestibility assays (Bosch et al., 2014; Bosch et al., 2016). Moreover, the chitin, a natural polysaccharide in the exoskeleton of insects, might serve as a substrate for microbial fermentation in the hindgut and support gut health. Chitin seems to be detected by the immune system as an allergy-promoting pathogen-associated molecular pattern (PAMP), thus chitin might have immunomodulatory properties (Komi et al., 2017).

Previous research in poultry (Secci et al., 2018; Dabbou et al., 2018), fish (Henry et al., 2018), swines (Altmann et al., 2018), canines (Jarett et al., 2019) and felines (Lisenko et al, 2018) haveevaluated the effects of insects or insect meals on animal growth performance, nutrient digestibility, or immunity. At large, these studies suggest that diets with a suitable inclusion (5-17% dietary inclusion level) of insects or insect meals could support the normal growth performance and high digestibility of macronutrients compared to conventional protein sources (i.e., soybean meal, chicken meal, etc.). In addition, some researchers also indicated that insect-containing diets might improve the immune function in some species even though the mechanism is still unknown (i.e., fish and poultry) (Borrelli et al., 2017; Henry et al., 2018).

Currently, there is a void in the literature with information pertaining to the chemical composition of different insects or insect meals, and their potential as novel ingredients in pet foods. To our knowledge, no scientific evidence exist on the use of insect meals in retorted pet foods. Therefore, this study aimed to evaluate the the chemical composition of 3 selected insect meals [speckled cockroach (*Nauphoeta cinere*), madagascar hissing cockroach (*Gromphadorhina portentosa*) and superworm (*Zophobas morio*)] and the effects of partial substitution of dietary protein by these selected insect meals in retorted feline diets on ATTD of macronutrients, fecal metabolites, and serum metabolites of adult cats. Our hypothesis is that inclusion of these insect meals would not negatively affect the nutrient digestibility or animal health, and would promote partial fermentation of the exoskeleton components (i.e., chitin), which might lead to a beneficial shift in fecal metabolites (e.g., increased short-chain fatty acids). `

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CHAPTER 2

LITERATURE REVIEW

OVERVIEW OF PROTEIN USED IN PET FOOD

From the data published by the American Veterinary Medical Association (AVMA), in the United States, approximately 48 and 32 million households owned a dog or cat, which corresponds to 38.4% and 25.4% of total U.S. households, respectively (AVMA, 2019). The pet food industry also continues to grow and diversify products to support the pet population and expectations of pet owners. The annual total U.S. pet industry expenditure has grown steadily since the 1990s and reached approximately 75 billion dollars in 2019, with a market share of 32 billion dollars directly related to the food category (APPA, 2019).

Protein is considered the most expensive nutrient on both economic and environmental aspects (Sogari et al., 2019). There are two major functions of protein in food and feed: 1) to provide the indispensable amino acids for the animal, which refers to the amino acids that must be obtained from the food because they are not synthesized or synthesized in insufficient amounts in the body, and 2) to provide dispensable amino acids (source of N) for synthesis of other required molecules and energy (McCusker et al., 2014). Both dogs and cats have ten indispensable amino acids, which are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, and cats also have a dietary requirement for taurine (NRC, 2006). The recommended protein level by the Association of American Feed Control Officials (AAFCO) for adult dogs is 18% dietary crude protein (CP) and for adult cats is 26% CP. Currently, the most common protein sources used in diets for dogs and cats are animal meats, animal by-products and rendered ingredients, and in recent years, human-grade ingredients, and other novel ingredients (i.e., insect meal, micro-algae) have also entered the pet food market (Swanson et al., 2013).

Traditionally, animal by-products comprise the majority of protein sources used in commercial pet foods. By the definition, the meat by-product refers to “a protein source consisting of organ meats, scrap meat, bone, blood, and fatty tissue from mammals, such as cattle or hogs, but not include hair/hide, horns, hoofs or teeth, or intestinal contents” (AAFCO, 2018). Compared to animal meat, animal by-product is an economical ingredient choice. In contrast, animal by-products have limited use in human nutrition, and as such the use of these ingredients in pet foods do not cause a direct competition with human food system, releasing some of the stress on the environment from animal production, and aiding in the sustainability of the pet food industry (Swanson et al., 2013).

Rendered ingredients also are broadly utilized in pet food. Rendering process is that different raw materials from the animal production, which the majority are animal by-products including viscera, bone, feathers and blood, are processed (i.e., cooked) into concentrated protein ingredients, such as meat and bone meal, feather meal, and blood meal, chicken by-product meal. (Meeker and Meisinger, 2015). The rendering process reduces the waste from animal production, prevents water and land pollution, and also protects animal and human health, increasing the sustainability of animal production (Meeker and Meisinger, 2015).

The commercial pet foods are often formulated to exceed the minimum requirements of protein for dogs and cats. This approach is employed to account for possible nutrient loses during food processing, variation in nutrient bioavailability of different ingredients, reduced amount of fecal output and disparities in nutrient requirements of pet animals related to genetics, temperament and physical activity, and physiological status. However, customer’s attitude also is an important factor to consider when formulating diets for companion animals. Besides the animal nutritional requirements, pet food companies aspire to meet the expectation of customers by choosing ingredients and food formats that are appealing to pet owners, and some of those may follow the trends in human foods, such as grain-free, natural and organic, and human-grade food (Conway and Saker, 2018). From an online survey conducted by Bedford during 2016-2018, people were asked whether they agreed with the statement that the natural or organic pet foods were better than standard national brand products. Approximately 50% of respondents chose to strongly agree or somewhat agree with that statement, whereas only 12% of respondents were in disagreement, and the other 37% of respondents had no opinion (Bedford, 2019).

THE IMPORTANCE OF SUSTAINABLE PROTEIN SOURCES

Since the industrial revolution, the global population has increased dramatically, from 1 billion in, the 1800s to approximately 7.8 billion in the 21st century (Current World Population, 2020). However, the total global population continues to grow, and it is estimated to reach 8.6 billion in 2030, and exceed 10 billion before the 22nd century (World Population Prospect, 2019). Meat consumption per capita has increased two-fold during the last forty years, not only due to populational growth, but also because of changes in economic status and food preferences in developing countries, especially China (Ritchie and Roser, 2017). In 2013, the annual total meat production was approximately 320 million tons, which was more than four times the quantity of animal protein produced in 1960s. It is expected that the global demand for meat will continue to increase. As a consequence, sustainability has become a focal point to balance the conflict between increasing human demand for animal protein and limited natural resources.

Sustainability can be broadly defined as practices that meet current needs without compromising future generations to meet theirs. A sustainable system is comprised of environmental, social, and economic components (Swanson et al., 2013). Specifically to food systems, nutritional sustainability is complex and involves many aspects of the food system such as management of waste and inputs (e.g., water, energy, land), cost of consumables and final products and profit to manufacturers and producers, and food quality, quantity, safety, as well as health, welfare, and nutrition. According to Swanson et al. (2013), nutritional sustainability can be defined as "the ability of a food system to provide sufficient energy and the amounts of essential nutrients required to maintain good health in the population without compromising the ability of future generations to meet their nutritional needs". Nutritional sustainability of different protein sources include measurements of carbon and water footprint, and generally, plant-based proteins cause less pollution than animal proteins, and among different animal species, ruminant animals have the highest carbon and water footprint in contrast with other animal-based protein sources (e.g., poultry; Swanson et al., 2013).

Compared to conventional animal-protein sources, plant-based proteins require lower land resources, produce less greenhouse gases, and also are considered healthier due to lower fat content (Wang and Peng, 2008). Traditionally, the most common dietary vegetal protein used in both human and animal food system have focused on soybean and cereal proteins (i.e., corn, rice and wheat), but pulses are also very important sources of dietary protein, especially in developing countries to overcome malnutrition (Henchion et al., 2017). From the nutritional perspective, pulses contain, on average, 20-25% crude protein, 60-65% carbohydrates with low concentrations of lipids (1-1.5%) and ash (2.5-4%) (Henchion et al., 2017). However, there are still some concerns with plant-based proteins. Vegetal proteins, unlike animal protein, may not contain all 9 essential amino acids (i.e., histidine, leucine, isoleucine, valine, threonine, methionine, phenylalanine, tryptophan and lysine) required by humans, and pulses also have low concentration of methionine, and some anti-nutritional factors (i.e., hydrolase inhibitors and lectins) (Elorinne et al., 2016). One strategy to overcome nutrient imbalances is to use complementary ingredients in diet formulations. Cereal grains when combined with pulses provide a more balanced amino acid profile because the pulse is enriched in lysine and folate while limited in methionine, tryptophan and cystine which perfectly make the composite with cereals whose the first-limiting amino acid is lysine (Singh, 2017).

In addition to traditional plant-based protein, seaweed and micro-algae are novel protein sources. By the definition, seaweeds are multicellular while microalgae are single-cell organisms. In regards to sustainability, micro-algae have a lower land requirement (<2.5 m^2 per kg of protein) than some plant proteins(i.e., soybean meal and pea protein meal) and much lower than beef that requires 144-258 m^2 of land to produce 1 kg of meat (Caporgno and Mathys, 2018). Some micro-algae species (i.e., *Chlorella* and *Arthrospira*) contain high quality protein, providing all essential amino acids required by humans (Caporgno and Mathys, 2018). In addition to the protein, microalgae are also rich sources of polyunsaturated fatty acids (PUFA) (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), which has health benefits for both human and animals (Fleurence, 1999). Currently, around 30% of microalgae production is sold for animal feed and is a competitive ingredient to soybean meal in poultry and swine diets due to their high sustainability and potential health benefits (Becker, 2017; Henchion et al., 2017).

In addition to plant-based protein and micro-algae, insects are also considered novel protein sources. Insects have a very long history as traditional foods in human nutrition throughout Asia, Australia and Africa, and also are part of natural diets in many animal species including fish, poultry, and some wild mammals (Jongema, 2017; van Huis, 2013; DiGiacomo and Leury, 2019). The nutritient profile in different insect species vary, but generally they contain high protein content, with the first-limiting amino acids being methionine and cystine (Bosch et al., 2014). Black soldier fly larvae has been one of the most researched insect sources in animal nutriton. Besides its beneficial nutritional properties, black soldier fly larvae also has a few sustainable advantages including numerous growth cycles per year, lower land requirement and high feed conversion ratio (Do et al., 2020). The following sections of this chapter will focus on the regulatory constraints and application of insects and insect meals in farm animal and companion animal nutrition.

REGULATORY CONSTRAINTS AND DEVELOPMENTS AROUND USE OF INSECTS IN ANIMAL FEED AND PET FOOD

In recent years, insects and insect meals have been in the spotlight as a potential sustainable protein sources in farm animal feed and pet food, and substantial economical investments also have been put into insect production (DiGiacomo and Leury, 2019). From the prediction of International Platform of Insects for Food and Feed (IPIFF), insect production will reach 200,000 tones in 2020, and 1.2 million tones in 2025 (IPIFF, 2019). However, since insect production is a new emerging sector, specific laws are lacking and existing regulations also largely differ worldwide. Lähteenmäki-Uutela et al. (2017) summarized the legalities in some key region over the world. According to those authors, in the U.S., there is lack of clear guidelines from the government, but edible insects are considered feed additives in food, pet food and feed, which requires the approval status of Generally Regarded As Safe (**GRAS**) from the Food and Drug Adminstration (**FDA**). In the European Union (**EU**), the insect food is considered as novel foods.

The specific rules are massively missing on rearing of insects and use insects as food and feed, but some European countries had their own regulation framework. In 2017, the EU regulation also allowed insect meals as ingredients in diets of farmed fish. However, under EC Regulation 1069/2009 and EC Regulation 767/2009, feeding farmed animals with processed animal protein are not permitted which indicates some organic by-products (i.e., household waste and manure) cannot be used as substrates for insect production, even if the insect can convert manure in a high quality nutrient (Schrogel and Watjen, 2019; van Huis and Oonincx, 2017). In Mexico and Australia, some insect species have a long history as medicines and traditional food, and generally do not require the registration of insects as feed materials (Lähteenmäki-Uutela et al., 2017).

In addition to legalities in food and feed use, the animal welfare of insects is also a concern, but there are no specific welfare regulations a living conditions and the culling process of insects in most countries (DiGiacomo and Leury, 2019). In the EU, insects are classified as non-vertebrates, so animal welfare regulations are not applied to them. However, if the insects are produced in large-scale, the animal welfare of insects should be taken into accountto minimize pain during handling and slaughtering practices (Lähteenmäki-Uutela et al., 2017).

DEFINITION AND CLASSIFICATION OF INSECTS AND INSECT MEALS

Generally, insects are considered high-quality dietary protein sources. The nutrient content and amino acid profile of ten different insect sources, including housefly pupae (*Musca domestica*), adult house cricket (*Acbeta domesticus*), yellow mealworm larvae (*Tenebrio molitor*), lesser mealworm larvae (*Alphitobius diaperinus*), Morio worm larvae (*Zophobas morio*), black soldier fly (*Hermetia illucens*) larvae and pupae, adult six spot roach (*Eublaberus distanti*), adult death's head cockroach (*Blaberus craniifer*) and adult female Argentinean cockroach (*Blaptica dubia*) were determined and compared with poultry meal, fish meal and soybean meal as reference substrates (Bosch et al., 2014). Different insect sources contain varied amounts of protein and fat, but in general, insect meals have higher CP than soybean meal, and house crickets contain as high as 71% CP while Morio worm had the lowest CP of 47% (Bosch et al., 2014). In addition, those authors also evaluated the amino acid composition of these insect substrates and used calculated amino acid scores based on amino acid minimum requirements for kittens and puppies (NRC, 2006) to determine if the insect sources contained suitable amino acid profile for dogs and cats. Those results showed that methionine and cystine were the first limiting amino acids for most insect substrates and housefly pupae, black soldier fly pupae and Morio worm had higher amino acid scores compared to other insect substrates (Bosch et al., 2014). Although the fat content in the insects is variable, the saturated/unsaturated fatty acid ratio, which is usually less than 0.4, is also favorable in most insects (van Huis, 2013). The nutrition composition of insects, however, depends on the type of the diet that the insects are reared from (Oonincx, 2015). When fed with a high protein, low fat diet (22.9% CP and 1% fat), Argentinean cockroaches contained 72.5% CP and 16.1% of fat, in contrast of when they were fed with a low protein, high fat diet (12.9% CP and 14.6% fat), these insects contained 37.5% of CP with 40% of fat (Oonincx, 2015).

There are two major methods for harvesting insects as feed ingredients; 1) harvesting insects from the wild or 2) farming insects using small-scale or large-scale industrialized facilities (van Huis and Oonincx, 2017). Insects directly harvested from the wild can lead to overexploitation and changes to natural eco-system threatening this food resource, but it may also be beneficial in the control of edible insects considered pests in agro-systems (van Huis and Oonincx, 2017). Insect farming is also a suitable choice, because the feed conversion efficiency is high in several insect species, for example, in black soldier fly larvae the feed conversion ratio ranges from 0.43 to 0.55, while the maximum ratio in poultry is approximately 0.3 (Oonincx et al. 2015). Besides the high feed efficiency, some insects also can rely on organic by-product , and convert those low-value feed to high quality protein, this seems to be the case for mealworms and oriental ground crickets (*Teleogryllus testaceus*) (van Huis and Oonincx, 2017). However, the attention should also be paid on the legislation and safety issues when choosing organic by-product as substrates, for example, the EU prohibited the use of manure as animal feed (van Huis and Oonincx, 2017).

Chitin is a natural polysaccharide in the exoskeleton of insects, and its chemical structure consist of N-acetylglucosamine residues linked by β-(1,4)-glycosidic bonds (Komi et al., 2017). In insects, the chitin exists as protein-chitin cross-linked fibers. However, mammals do not synthesize chitin and lack the necessary enzyme (chitinase) to degrade it. As a consequence, this protein stays largely intact in the gastrointestinal tract upon insect consumption, and because of that chitin has the potential to be sensed by the immune system as an allergy-promoting pathogen-associated molecular pattern (PAMP) (Komi et al., 2017). Even though the mechanism of action of chitin on the immune system is complex and not fully understood, it has been reported in the literature that chitin in the diet may improve the immune system of fish and dogs (Henry et al., 2018; Lei et al., 2019). Henry et al. (2018) reported that fish fed with yellow mealworm (*Tenebrio molitor)* containing diets, the myeloperoxidase activities and nitric oxide concentration decreased significantly which indicates anti-inflammatory activity, and also increased anti-parasitic defense (Henry et al., 2018). The immune effects of chitin might be related to the polymer size of chitin; large polymers (> 70 μm) may not have effect on the immune system, but intermediate polymer size (40-70μm) may cause inflammation, whereas small polymers (< 40μm) may trigger an anti-inflammatory reaction, with these differencesprobably because different sized-particles activate different receptors and signaling pathways (Lee et al., 2008). In addition, a diet containing black soldier fly (*Hermetia illucens*) in laying hens modulated the gut microbiota and resulted in higher short chain fatty acids [**SCFA**], and the authors suggested that the insect diet might have prebiotic-like effects while the chitin might be the key regulator, since the activity of chitinase in hens fed the insect-based diet was 4-fold higher than hens in the control group (Borrelli et al., 2017).

Despite the increasing interest of insects as alternative protein sources in human and pet foods and animal feeds, there are also some concerns, and these involve issues pertaining to food safety (accumulation of heavy metals), consumer acceptance, and management of insect farming and environmental risks (Biancarosa et al, 2017; van Huis and Oonincx, 2017). When black soldier fly larvae are fed with seaweed-enriched media, which naturally accumulates heavy metals, a portion of the heavy metals including cadmium, lead, mercury, and arsenic can be transferred from the feeding materials to insects. When seaweed comprises more than 20% of the diet of these insects, it can increase the concentration of heavy metals to be above the current EU maximum level in feed diet (Biancarosa et al, 2017; EU, 2017). Therefore, careful consideration of feeding media to insects is crucial to determine their nutritional adequacy and avoid accumulation of heavy metals and other toxic compounds that may cause health problems in animals and humans.

Consumer opinion and acceptance is also a challenge in including insects as food or ingredients in human nutrition and pet foods, as entomophagy, which means the practice of eating insects, is not widely accepted in Western countries (van Huis, 2013). In addition, the animal welfare of insects is another consideration if insect farming becomes a reality. With the current knowledge and practices applied to insect farming, it would be difficult to control the density of insects like in some traditional animal farming systems and to protect the animal welfare of insects during the harvesting and processing condition as well as to guarantee food safety (van Huis, 2013). Moreover, some insects are harmful to different natural habitats, so it is also vital to ensure environmental risks are minimized in insect farming practices (van Husi and Oonincx, 2017).

INSECTS IN MONOGASTRIC NUTRITION

Currently, the major protein sources used in farm animal nutrition are plant-based, such as soybean meal, which contains anti-nutritional factors and the soybean crop requires large land and water resources (Maurer et al., 2016). Insects are part of natural diets of a variety of animal species, and are good sources of protein and amino acids, and depending on the species, are also lipid-rich ingredients with a low carbon footprint (Sogari et al., 2019). Thus, insects are recognized as a potential alternative protein source in animal feeds. To date, limited scientific evidence is available on the use of insects in diets for monogastric animals, with a few studies conducted in fish, poultry, swine, and dogs. Monogastric animals, at large, have similar gastrointestinal physiology, despite some differences in gut anatomy. Thus, scientific findings from monogastric animals fed diets containing insects or insect meals can be used as a foundation to explore the nutritional value of insects in pet foods.

Use of Insects in Poultry Nutrition

Trends in U.S. meat consumption,indicated that there has been a shift from red meat towards higher poultry consumption since 1960s (Daniel et al., 2010). Soybean cake and soybean meal are main sources of protein in poultry nutrition. However, soybean crops require large areas of cultivation and several inputs for optimal production (e.g., fertilizers, pesticides, water) (Maurer et al., 2016). Insects are natural feed of birds with high feed conversion ratio and good nutrition profile (i.e,. amino acids and minerals), so insect meal could be a good candidate as alternative protein ingredients in poultry diets (Secci et al., 2018).

A recent study aimed to determine the optimal inclusion level of black soldier fly (*Hermetia illucens* L; **HI**) larva meal in poultry diets. The effects of different inclusion levels of HI larva meal (i.e., 0, 5, 10, or 15%) on growth performance, blood parameters, and gut morphology of broiler chickens were evaluated. In the study, 256 male birds were fed one of four diets: HI0, HI5, HI10 and HI15. At d10, 24 and 35, the live weight and average daily gain of chicks showed a linear and quadratic correction to HI concentration in the meal (maximum for HI10 group), but decrease in HI15 group. In addition, at the end of the experimental period, birds on HI15 group had lower villus high and greater crypt depth in contrast to other treatmnets groups, which indicates that 15% of HI larva meal inclusion may negatively impact intestine morphology. Overall, lower inclusion levels (< 10%) of HI larva meal added in the poultry diet might promote to performance of broiler chickens while higher inclusion levels (15%) had negative effects on the growth parameter and intestine morphology (Dabbou et al., 2018).

A similar study conducted by Secci et al. (2018) with 108, 24-week-old Lohmann Brown Classic laying hens, evaluated the effects of black soldier fly (*Hermetia illucens*) larva meal as an alternative protein source on egg quality. The researchers wanted to compare a control diet containing soybean meal as the main protein source to the experimental diet with black soldier fly larvae added at the expense of soybean meal as the main protein source. The animal trail lasted 21 weeks, and a total of 88 eggs collected in 8 sampling days throughout the experiment. The eggs from hens fed the insect meal diet had larger yolk size compared to control group. The fatty acid profile of yolks in both groups were similar, but yolks of hens fed the insect meal diet had significantly higher levels of γ-tocopherol, lutein, β-carotene and total carotenoids, but11% lower level of cholesterol than control group. In the conclusion, the eggs from hens fed diets containing insect meal were of good quality, suggesting that black soldier fly larvae meal could be a sustainable alternative protein source in Lohmann Brown Classic laying hens.

Borrelli et al (2017) evaluated the effects of an insect-based (defatted black solider fly *H. illucens* larvae meal) diet on gut microbiota and fermentation products in laying hens. A total of 24 hens were randomly assigned into 2 treatments: 1) corn and soybean meal-based diet (23.5% soybean meal; **SD**) and 2) corn and insect meal (17% of defatted *H. illucens* larvae meal; **ID**). These two diets were formulated based on Lohmann Brown classic Management Guides (2013) and had a similar nutrient profile and metabolizable energy level. The study period lasted 21 weeks, and the starting age of hens was 24 weeks. During the study, feed intake and body weight were recorded weekly. Cecal samples were collected at the end of the experimental period for microbiota and SCFA analyses. The health status of all hens remained good throughout the study and the egg quality did not differ between two groups. For cecal microbiota, ID hens had a more diverse microbiota population, and there was a strong difference between the microorganism communities in two groups. In addition, ID hens also produce significantly higher concentration of acetate (10.8 mmol/L in ID; 6.9 mmol/L in SD), propionate (5.8 mmol/L in ID; 3.0 mmol/L in SD), butyrate (4.4 mmol/L in ID; 1.5 mmol/L in SD) and total SCFA (22.5 mmol/L in ID; 12.3 mmol/L in SD), and it was related to the shift observed in the cecal microbiota. Furthermore, those researchers suggested that the insect meal could have a potential prebiotic effect, and chitin would be the key regulator because they reported that the enzymes (N-acetylglucosamine 6-phosphate deacetylase and β-N-acetylhexosaminidases) related to chitin degradation were enriched in SD hens compared to the ID group. In addition, a few bacterial genera (i.e., *Flavonifractor plautii*, *Christensenella minuta* and *Alkaliphilus transvaalensis*) involved in chitin degradation were correlated with high SCFA concentrations. These results suggest that the insect meal and chitin may promote beneficial shifts in the gut microbiota in laying hens.

Use of Insects in Aquaculture

Since the last century, the demand for fish products continues to rise (Henry et al., 2015) However, in the meantime, decline in wild fish harvest and expansion of fish farming, has resulted in a steady decrease in the availability of fishmeal and fish oil, the two major aquaculture ingredients (FAO, 2014). In aquaculture, the cost of fish feeds can exceed 50% of the overall production cost, especially in carnivorous fish farming (Wilson, 2002; Rana et al., 2009). Insects are part of natural feed of fish, and environmental-friendly with limited resource requirement for production, making insects attractive protein sources in fish diets.

In addition to a good protein source, some insect meals also are beneficial to fish immune system. Henry et al. (2018) evaluated the effect of mealworm meal(*Tenebrio molitor*) as feed on the immune system of European sea bass (*Dicentrarchus larbrax).* In this experiment, a total of four diets with similar CP concentration (53% CP) and caloric density (5139 kcal/kg DM) were manufactured. The control diet contained 70% fishmeal, and the other three diets replaced 36% of fishmeal with *Tenebrio molitor* meal, in combination with no additional synthetic digestive enzymes, with addition of synthetic proteases, or with addition of synthetic carbohydrase, respectively. A total of 180 fish in 12 different tanks were randomly assigned to these 4 different diets for a period of 6 wk. In their previous study, it was found that final body weight of fish were not affected by the dietary treatment, and fish being fed insect mea without additional enzymes had higher digestibility of protein than other 3 dietary groups (Gasco et al., 2016) The current experiments showed that the fish fed any of the diets containing the insect meal had higher anti-inflammatory response, because the concentration of myeloperoxidase, nitric oxide and ceruloplasmin, which are involved in inflammatory processes, were significantly decreased compared to control group. Fish fed with insect meal without additional synthetic digestive enzymes or with addition of synthetic carbohydrase had higher serum concentration of trypsin inhibition, which indicated higher anti-parasite activity, compared to other two groups, and authors hypothesized that it probably was because insect chitin has similar components as exoskeleton of parasites, so the insect meal in diet could be an immune stimulant to improve anti-parasitic ability, and exogenous proteases probably degraded some proteins involved in this immune response. Thus, the mealworm meal could improve the anti-inflammatory and anti-parasitic function without affecting nutrient digestibility, suggesting that this ingredient could be a good alternative protein source in aquaculture.

Use of Insects in Swine Nutrition

The use of insects in swine diets was first evaluated in 1970s by Newton et al. (1977). In that study the amino acid profile, nutrient digestibility, calcium and phosphorus balance and palatability of diets containing black soldier fly larvae meal (33%) were determined. A total of 6 5-wk-old, littermate barrow pigs were used in a triplicated 2x2 Latin square design and fed with two different diets: control diets with 25.5% of soybean meal and larvae meal diet with 33% of black soldier fly larvae. Pigs fed with insect meal diet showed lower digestibility of ash and nitrogen-free extract than control groups. For the amino acid profile, both diets were low in methionine and cystine, but in the larvae meal, the concentration of threonine (0.55% DMB) and tryptophan (0.2% DMB) were lower than the requirement (threonine: 0.86% DMB and tryptophan: 0.24% DMB). The authors concluded that the black soldier fly larvae had the potential to server as a protein source, but lower inclusion levels in combination with other protein sources (e.g., soybean meal) would be needed to balance the diets, and to control for the high ash and ether extract, and lack of some essential amino acids of the dried larvae meal.

The pork product quality also is a focus of the pork industry since pork is one of top 3 animal proteins consumed in the world. Altmann et al. (2018) conducted a study to evaluate the pork quality characteristics when soybean meal in swine diets were replaced by micro-algae or partly-defatted *Hermetia illucens* larval meal. A total of 48 barrows were used and fed with three different diets with 2 experimental replicates. Based on body weight, animals were divided into three phases: 25-50 kg, 50-75 kg, and over 75 kg respectively. In the first two phases, the soybean meal was replaced by 50% with the insect meal in replicate 1, and 75% in replicate 2. In the third phase (over 75 kg), soybean meal was 100% replaced by the insect meal in both replicates. Partial or full substitution of soybean meal by the insect meal did not affect most of pork quality parameters (e.g., instrumental tenderness, lean color, and intramuscular fat), except lower backfat lightness in pigs fed the insect meal diet when samples were collected in the final phase. In that same study, inclusion of insect meal impacted sensorial parameters, resulting in stronger odor and higher juiciness than the control group. It also altered the fatty acid composition of backfat. Pigs fed the insect meal diet had higher linoleic acid and gamma-linolenic acid concentrations than pigs fed the control diet. Inclusion of insect meal also resulted in a 5-fold increase in lauric acid (C12:0) concentration in backfat.

From the current literature, some insect meals (i.e., black soldier fly larvae meal and *Tenebrio molitor* meal) are promising alternative protein sources in farm animals without compromising growth performance and nutrient digestibility. However, conflicting literature exists when using high inclusion level (> 15%) of insect meals showing negative effects on feed conversion ratio and intestine morphaology (i.e. lower villus height) in broiler chickens, so future research could focus on the appropriate inclusion levels of specific insect meals on different animal species and pohysiological stages. Moreover, most of the current literatures has evaluated black soldier fly larvae as a novel insect protein source in feeds and pet foods, but from Bosch et al. (2014), there were some other insects with high amino acid scores (i.e., housefly pupae and Morio mealworm) and good nutrient profile, so new insect species should be evaluated in future studies. In addition, some researchers hypothesized that the insect meal and chitin might have beneficial effects on immune function of poultry and fish (Borrelli et al., 2017; Henry et al., 2018). Even though the mechanisms of how insects supplemented in diets may stimulate the immune system are still unknown, it would still be valuable to determine if similar effects would be observed in mammals.

INSECTS IN COMPANION ANIMAL NUTRITION

Currently, there is limited scientific evidence on the nutrient profile and nutritional adequacy of insects in companion animal nutrition. Most of the research evaluating insects in diets of dogs and cats have been done during the last decade.

Bosch et al. (2014) evaluated *in vitro* digestibility of organic matter (OM) and nitrogen (N) and nutrient composition of 10 selected insects. It could be concluded that the general first-limiting amino acids of insects were methionine and cystine, and the macronutrient profile, amino acid scores and OM and N digestibility were varied among different insect species. The same group of researchers (Bosch et al., 2016) further evaluated the black soldier fly larvae, housefly larvae, and yellow mealworm larvae from the previous 10 selected insects species and measured the *in vitro* digestibility and fermentability of these 3 insects using *in vitro* simulated canine gastric and small intestinal digestion. Although the amino acids profile varied among these 3 insects; housefly larvae contain higher methionine and lysine than black soldier fly larvae and yellow mealworm larvae, overall these 3 larvae contained high quality protein and high amino acid bioavailability. During *in vitro* fermentation stage using canine inoculum, similar gas production was observed between the yellow mealworm larvae and the shrimp chitin (used as control). It was also observed that the inoculum of 1 out of 3 dogs was better capable of fermenting undigested residues from the yellow mealworm larvae. Thus, further research is needed to further evaluate chitin fermentation characteristics in dogs and the processing effects on bioavailability of nutrients for pet animals.

After evaluation of *in vitro* digestibility and amino acid scores of selected insect species, Do et al. (2020) determined the macronutrient and amino acid digestibility of black soldier fly larvae (BSFL) at different ages using the precision-fed cecectomized rooster assay. BSFL were harvested in six different ages (0, 11, 14, 18, 23 and 29 d), and grinded through 2-mm screen and fed to 24 cecetomized roosters. The digestibility of DM and OM did not differ and were high (54.61-58.26%) for all substrates, AHF digestibility varied (80.27-88.26%, $P < 0.05$) in different life stages, and tend to be greater in BSFL23 and BSFL29 (88.26% and 86.36%, respectively). For amino acids digestibility, BSFL0 and BSFL11 hold the lower digestibility for some indispensable and dispensable amino acids (i.e. methionine, tryptophan and tyrosine). For different life stages in dogs or cats, the first-limiting amino acid (AA) were different: threonine (dogs in growth and reproduction), methionine (adult dogs; cats in growth and reproduction) and arginine (adult cats). Generally, all ages of BSFL had high AA scores and remained high digestibility, so there is potential of BSFL as a protein source in pet food, but future research needed for inclusion level and processing effects.

In the previous research, the insect meal, especially the black soldier fly has shown positive effects on fish immune system (Esteban et al., 2001), so Lei et al. (2019) performed a study that aimed to evaluate the effects of supplementation of defatted black soldier fly larvae (BSFL at 0%, 1%, or 2 %) meal for adult dogs on ATTD of macronutrients and immunological parameters (i.e., [i.e., serum tumor necrosis factor-α (**TNF-α**), interleukin-6 (**IL-6**), superoxide dismutase (**SOD**) and glutathione peroxidase (**GPx**)]. All animals were randomly assigned into three groups and fed grain-based, complete and balanced commercial extruded diets with inclusion of either 0%, 1% or 2% BSFL for 42 d during the feeding trial. During the last 3 d of experiment, total fecal samples were collected, and at end of the feeding period, all dogs were intraperitoneally challenged with *Escherichia coli* lipopolysaccharide (LPS) at 100 μg/kg of body weight, and blood samples were collected before and at 3 and 6 h post-challenge to evaluate TNF-α, IL-6, SOD and GPx. Increasing levels of BSFL increase the ATTD of DM and N, but had no effect on lipid ATTD. A linear increase in serum concentrations of SOD and GPx after LPS challenge was observed in response to BSFL supplementation, indicating greater antioxidative capacity in dogs fed BSFL. In addition, serum TNF-α concentration, a pro-inflammatory cytokine, linearly decreased with BSFL supplementation, suggesting that BSFL may have anti-inflammatory properties. Overall, it could be concluded that the insect meal in canine diets could increase DM and N digestibility and exert antioxidant and anti-inflammatory properties in adult dogs.

In addition to the work done to evaluate the nutritional and functional characteristics of BSFL in canine nutrition, a recent study investigated the effect of banded crickets (*Gryllodes sigillatus*) supplementation on the canine gut microbiome (Jarett et al., 2019). Thirty-two adult male and female Beagles were used in this study, and randomly assigned to 1 of 4 dietary treatments. All diets were formulated to be complete and balanced with whole cricket meal partially replacing chicken at either 0%, 8%, 16% or 24%, respectively. The experimental period was comprised of 29 d, and fresh fecal samples were collected on 0, 14, and 29 d. Fecal microbiota was analyzed using high-throughput sequencing of 16S rRNA gene. There were 12 amplicon sequence variants that differed among dietary groups. An increase of *Catenibacterium* while a decrease of *Bacteroides* were observed in fecal microbiota of dogs fed the 24% cricket diet. However, overall fecal microbiota diversity fed experimental diets were similar to control diets. Thus, those authors suggested that the effects of banded cricket (up to 24% inclusion level) on dog's gut microbiota were minimal, and banded cricket supplementation could be a sustainable alternative protein source in canine diet.

A gap in the literature exists pertaining to the use of insects in feline nutrition. Our laboratory, in collaboration with the Federal University of Lavras in Brazil, have evaluated the impact of 3 different dietary insect meals (Madagascar cockroach, *Gromphadorhina portentosa*; Cinerea cockroach, *Nauphoeta cinérea;*and Superworm, *Zophobas morio*) and 2 inclusion levels (7.5% or 15%) in extruded diets of adult cats on the nutrient digestibility, serum metabolites and hematology, and fecal parameters (i.e., score, pH, metabolites) and microbiota. Overall, ATTD of macronutrients did not differ based on insect meal type and inclusion level, and only ATTD of chitin was greater in cats fed diets containing the Madagascar cockroach meal. Fecal SCFA concentrations and microbiota were also not affected by insect meal supplementation and inclusion levels. Overall, the data from that study suggests that supplementation of these select insect meals up to 15% in extruded diets had no negative effects on ATTD digestibility, hindgut microbiota and overall health of adult cats (Lisenko et al., 2018). Moreover, extruded and retorted are two major processing methods in commercial pet food market, and different processing methods may impact the nutritional properties of various insect sources or insect meals. Currently, there is a void in the literature in this area.

THESIS OBJECTIVES

The aim of this thesis was to evaluate the effects of partial substitution of dietary protein by select insect meals (speckled cockroach (*Nauphoeta cinere*), madagascar hissing cockroach (*Gromphadorhina portentosa*) and superworm (*Zophobas morio*)) in retorted feline diets on ATTD of macronutrients, fecal metabolites, and serum metabolites in adult cats. It was hypothesized that inclusion of insect meals would be a comparable protein source to chicken meal in retorted diets for adult cats, without negatively affecting nutrient utilization and animal health. It was also expected augmented fecal metabolite concentrations, due to partial fermentation of exoskeleton components (e.g., chitin) of these insect sources.

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CHAPTER 3

NUTRITIONAL COMPOSITION AND APPARENT TOTAL TRACT DIGESTIBILITY OF SELECTED INSECT MEALS AS NOVEL PROTEIN SOURCES IN RETORTED PET FOODS FOR ADULT CATS

ABSTRACT

Insect meals are novel and potentially sustainable protein sources. The objective of this study was to determine the chemical composition of 3 selected insect meals [i.e., speckled cockroach (**SC**), madagascar hissing cockroach (**MC**) and superworm (**SW**)] and to determine the effects of these novel ingredients on food intake, apparent total tract digestibility (**ATTD**) of macronutrients, fecal scores and metabolites of adult cats fed insect- or chicken-based retorted diets. This study consisted of a complete randomized design, with 28 adult cats (mean age = 2.1 ± 0.03 yr; mean BW= 4.9 ± 0.8 kg) randomly assigned to 1 of 4 experimental retorted diets: Control (chicken-base diet), SC diet, MC diet, or SW diet. All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. All diets were formulated according to AAFCO (2018) nutrient profile for adult cats. The experimental period was 28 d, with 7 d of diet adaptation followed by 21 d of dietary treatment. Total fecal collection was completed during the last 4 d of the experimental period. On d21, a fresh fecal samples from each cat was collected for determination of fecal metabolites. Food was offered twice daily to maintain body weight and body condition score. Among the 3 selected insect meals evaluated, oleic acid (113,352-46,353 ug/g), palmitic acid (62,580-19,104 ug/g), linoleic acid (46,265-13,926 ug/g), and stearic acid (21,654-6,473 ug/g) were the most prevalent fatty acids. Branched-chain amino acids and arginine were the most preponderant indispensable amino acids in these insect meals. Apparent total tract digestibility of dry matter (DM), organic matter (OM), acid hydrolyzed fat (AHF), and crude protein (CP) did not differ among treatments ($P > 0.05$) and all diets were well digested by the cats. Similarly, fecal scores did not differ among the treatments and were within ideal range. Furthermore, no differences ($P > 0.05$) in fecal metabolite concentrations were observed among cats fed different experimental diets. In conclusion, the selected insect meals evaluated herein are rich in linoleic acid, an essential fatty acid for cats. Furthermore, cats fed retorted diets containing these insect meals had comparable ATTD of macronutrients, fecal scores and metabolite concentrations of cats fed the chicken-based diet; suggesting that these novel protein sources might be adequate alternative ingredients in feline diets.

Keywords: alternative protein, chemical composition, fatty acid, feline, insect, nutrient digestibility

INTRODUCTION

Insect meals are considered high-quality protein sources. From an *in vitro* study, insect meals have been shown to have high protein and fat concentrations, and also good amino acid profile which makes them potential ingredients for feline and canine foods (Bosch et al., 2014; van Huis, 2013). Moreover, the ratio of saturated/unsaturated fatty acids is also favorable in some insect species (van Huis, 2013). Additionally, insect meals can be sustainable protein sources with high feed efficiency and a lower nutrition requirement for production. The feed convertion ratio of poultry which has the highest feed efficiency among traditional animal protein sources is around0.3, but the feed conversion ratio of black soldier fly larvae, for example, is around 0.43-0.55 (Oonincx et al., 2015).

Despite increasing interest in the utilization of insect protein in pet foods, there is limited information on the chemical composition and nutritional adequacy and safety of these ingredients when incorporated in diets for companion animals. However, in recent decades, insect meals were already used as novel protein sources in diets of some farm animals such as swine, fish, and poultry. From previous research studies, the specific inclusion of selected insect meals in diets of farm animals did not result in adverse effects on growth performance, digestibility of macronutrients and product quality in poultry (0-15% black soldier fly larvae meal; Dabbou et al., 2018), swine (33% black soldier fly larvae meal; Newton et al., 1977) and aquaculture nutrition (36% fishmeal replaced by mealworm meal; Henry et al., 2018). Moreover, the chitin, which is a natural polysaccharide in the exoskeleton of insects, has similar properties of dietary fibers to promote fecal metabolites production and may also exert an immune-modulatory effect, even though mechanisms are still undetermined (Komi et al., 2017; Henry et al., 2018; Lei et al., 2019). However, most of the current literature on the evaluation of insects in animal nutrition has focused on determining the chemical composition and nutrition adequacy of black soldier fly larvae. Yet, different insects contain distinct amino acid and fatty acid profiles, with variable nutritional value and functionality. Therefore, the objective of this study was to determine the chemical composition of 3 selected insect meals (i.e., speckled cockroach, madagascar hissing cockroach and superworm) and to determine the effects of these novel ingredients on food intake, macronutrient apparent total tract digestibility (ATTD), fecal scores and metabolites of adult cats fed insect- or chicken-based retorted diets .

MATERIALS AND METHODS

All animal care procedures were approved by the University of Illinois Animal Care and Use Committee. All methods were performed in accordance with the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Animals and Diets

In this study, a total of 28 cats were used, with 18 spayed female and 10 neutered male adult domestic shorthair cats with average age of 2.1 ± 0.03 yr, mean body weight of 4.9 ± 0.8 kg; and mean body condition score of 5.4 ± 0.4 using a 9-point scale (Laflamme, 1997). All cats were housed in Edward R. Madigan Laboratory at the University of Illinois at Urbana-Champaign in a temperature and light controlled room, following a 14 h light: 10 h dark schedule. Cats were individually housed during feeding (twice daily: 0800 – 1000 and 1500 – 1700) and fecal collection periods, and group housed during remainder of the experimental period. Water was available ad libitum during all the times. During the study, body weight and body condition score were recorded weekly, and food intake was recorded daily. Cats were fed to maintain ideal body weight and body condition score throughout the study.

Three selected insect meal sources [i.e., speckled cockroach (SC) *Nauphoeta cinerea*; madagascar hissing cockroach (MC), *Gromphadorhina portentosa;* superworm (SW), *Zophobas morio larvae*] were obtained from the Laboratory of Entomology of the Institute of Agricultural Sciences at the Federal University of Minas Gerais, Montes Carlos, Brazil. All insects were reared on a plant-based diet (i.e., soybean, corn, and wheat) and harvested by immersion in boiling water, followed by a 48 h drying period in a forced-air oven at 50 ^{o}C, and then ground using an electric screw meat grinder (Botini 1/3cv, model 1645, Grupo Botini, São Paulo, Brazil). The two species of coackroaches were harvested during the adult phase (60 d of age), whereas the superworm was harvested during the larval stage at approximately 90 d of age. The chemical composition of the test ingredients is shown in **Table 3.1**. Four retorted diets were used in this experiment. All diets were formulated to have similar ingredient and chemical composition and to meet or exceed the nutrient profile for adult cats according to AAFCO (2018). In these experimental diets, most ingredients remained at a constant inclusion level among dietary treatments with the exception of the test protein sources (i.e., SC, MC and SW) that were added at 4% inclusion level at the expense of 3.5% chicken meal and 0.5% corn gluten meal of the control diet (**Table 3.1**). All experimental diets were manufactured at the Food Science and Human Nutrition Pilot Plant at University of Illinois at Urbana-Champaign. Briefly, ingredients for each dietary treatment were mixed by hand prior to being homogenized with opposite turning agitators in a jacketed steam kettle heated to 65.5^{o}C for 10 min. The pre-heated mixture then was added to cans (500 ± 5 g) and steam-flushed prior to sealing. Sealed cans then were statically retorted to reach an Fo value of 8 in an Allpax

Gentle Motion Retort (Allpax, Covington, LA.). Wireless DataTrace temperature probes (Mesa Labs, Lakewood, CO) were used to calculate Fo values during the retort process.

Experimental Design and Sample Collection

This experiment followed a complete randomized design, and the experimental period consisted of a 7-d adaptation period to the control diet, followed by a 21-d feeding period in which cats were randomly assigned to 1 of 4 experimental diets. At baseline (d-0) and on d-21, all cats were fasted overnight, and a total of 5 ml blood was collected from each cat into BD Vacutainer serum separator and EDTA tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) for serum chemistry and complete blood count analyses, respectively. All samples were analyzed by the University of Illinois Veterinary School Diagnostics Laboratory (Urbana, IL).

At the last 4 d of the experimental period, total fecal output was collected from all cats. During the same period, a fresh fecal sample from each cat was collected within 15 min of defecation. Fecal samples were scored using a 5-point scale: 1= day, hard pellets; 2- firm, retains shape but pliable; 3= soft and moist, but retains a shape; 4= soft, unformed; 5= watery liquid; a score of 2-3 is considered ideal. Fecal pH was measured in fresh samples with a Denver Instrument AP10 pH meter (Denver Instrument, Bohemia, NY) with a Beckman electrode (Beckman Instruments, Inc., Fullerton, CA). These were then aliquoted out to be measured for dry matter (**DM**), fermentative end-product concentrations, including short-chain fatty acids (**SCFA**), branched-chain fatty acids (**BCFA**), phenols, indoles, and ammonia. Briefly, 2 g of each fresh fecal sample was weighed in duplicate for DM, and dried in 105°C oven for 48 h. A fecal aliquot of 3 g was weighed and placed in a 30 ml Nalgene bottle with 2 N hydrochloric acid and frozen at -20°C until analysis of SCFA, BCFA, and ammonia concentrations. Aliquots of 2 g were weighed in duplicates and placed in 16 ml plastic tubes for phenols and indoles and stored at -20°C until analysis. During the total fecal collection, all feces were weighed, scored and stored in a -20°C freezer until analysis to **ATTD** of macronutrients.

Sample Preparation and Chemical Analysis

The four retorted experimental diets were freeze-dried in a FTS Systems Dura-Dry MP freeze-dryer (SP Scientific, Warminster, PA) and fecal samples were dried in a 57°C oven for approximately 7 d. Then, diet and fecal samples were ground in a Wiley Mill (model 4; Thomas Scientific, Swedesboro, NJ) through a 2-mm screen size in preparation for chemical analyses.

Insect meals, experimental retorted diets, and fecal samples were analyzed for dry matter (DM), and ash according to AOAC (2006; methods 934.01 and 942.05). Crude protein (**CP**) was calculated based on total N concentration determined through Leco (TruMac N, Leco Corporation, St. Joseph, MI) according to AOAC (2006; method 992.15). Acid hydrolysis followed by ether extraction was used to determine total lipid content according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Gross energy (GE) was analyzed through use of bomb calorimetry (Model 6200, Parr Instruments Co., Moline, IL). Complete amino acid profile was determined for the 3 selected insect meals according to AOAC (2007). Chitin concentration of insect meals was determined according to Hornung and Stevenson (1971), and Ma and Zuazaga (1942) at the Pet Animal Nutrition Study Center in the Department of Animal Sciences at the Federal University of Lavras, Minas Gerais, Brazil.

Short-chain fatty acid and BCFA concentrations in fresh fecal samples were determined through the use of gas chromatography according to the method of Erwin et al. (1961) and Goodall and Byers (1978). Volatile fatty acid concentrations were determined using a Hewlett-Packard (HewlettPackard, Avondale, PA) Model 5890A gas chromatograph equipped with a flame ionization detector (**FID**) on a glass column (1.8 m x 4 mm i.d.) packed with GP 10% SP-1200/1% H3PO$_4$ on 80/100 chromosorb W AW (Supelco, Bellefonte, PA). Nitrogen was the carrier gas and had a flow rate of 45 ml/min. The temperatures for the oven, injection port, and detector port were 125, 175, and 180 ^{0}C, respectively. The analyzed concentration from each set of duplicate tubes for acetate, propionate, butyrate, isobutyrate isovalerate, valerate acid, total SCFA and BCFA were averaged. Fecal phenol and indole concentrations were determined by gas chromatography according to the method of Flickinger et al. (2003) with modifications. Phenol and indole analysis utilized a Thermo Scientific TRACE 1300 gas chromatograph coupled with FID detector. A 1 µl sample was injected at 220^{0}C, at splitless mode. A Nukol Supelcol column (60 m length, 0.32 mm diameter) with a film thickness of 0.25 µm was used to separate phenolic compounds. The oven temperature was initially 150^{0}C which was held for 1 min, and then it was increased at 25^{0}C per min to 200^{0}C and held for 35 min. The internal standard used was 5-methylinodle and all samples were analyzed in duplicates. Ammonia concentrations were measured according to the method of Chaney and Marbach (1962).

Fatty Acid Analysis of Select Insect Meals and Experimental Diets

Fatty acid profile of the 3 selected insect meals and the 4 experimental diets were determined according to Lepage and Roy (1986) and Masood et al (2005) with modification in house. Acetyl chloride, butylated hydroxytoluene (BHT), potassium carbonate, HPLC-grade methanol and hexane were purchased from Sigma-Aldrich (St. Louis, MO), while the internal standard (nonadecaoic acid, 19:0) and external fatty acids methyl ester standards were purchased from Supelco Sigma-Aldrich (St. Louis, MO). A sample size of 0.1 g was used for each duplicate. To prepare methanol-BHT solution with ratio of 50 µg BHT/ml methanol to prevent the oxidation of fatty acids, and add internal standard into methanol-BHT regent at concentration of 0.1 mg/ml.

Then, 100 μl internal standard solution was mixed with the test substrates, and 2 ml methanol-hexane (4:1, v/v) mixture was added. Tubes were vortexed and put on the ice. Then, 200 μl acetyl chloride were slowly added to the tubes. They were placed under N immediately. The samples were heated for 10 min at 100^0C, vortexed briefly, and heated for an additional 50 min. After heating, the tubes were placed on ice and allowed to cool and neutralized by adding 5 ml of a 6% Na_2CO_3 solution. The tubes were vortexed for 1 min and centrifuged at 2,300 *g* at 4^0C for 3 min to separate the mixture into two phases. The upper organic phase was collected into a test tube and the extraction was repeated once more by adding 0.5 ml of hexane, vortexing, and centrifuging for another 3 min at the same speed and temperature. The organic phase was collected again and combined with the first extraction. The combined extraction was evaporated under N to 300 μL, then transferred to a gas chromatography vial with a 300 μL glass insert, and crimped under N for fatty acid methyl ether (**FAME**) analysis by gas chromatography. Thermo Scientific TRACE 1300 Gas Chromatograph coupled with FID was used for analyzing individual FAME. Samples (1 ul) were injected into GC and separated on a fused silica capillary column (SP-2560, 100 m length, 0.25 mm I.D., 0.2 um film thickness). The carrier gas was helium, and the flow rate was 20 cm/sec, at split ratio of 100:1. The temperature was at 140^0C initially for 5 min, then increased at 4 ^{0}C /min to a final temperature of 240 ^{0}C and held for 15 min. The temperatures for the injector and detector were 250 ^{0}C and 260 ^{0}C, respectively. The internal standard was nonadecanoic acid (C19:0, Nuchek Prep, Elysian, MN). Fatty acid methyl ester standards (Supelco 37 Component FAME Mix, Sigma Aldrich) were used as an external standard to identify the fatty acid peaks in the samples by comparing with retention time.

Statistical Analysis

Data were analyzed using SAS (SAS Institute Inc., version® 9.4, Cary, NC), with a fixed effect of diet and a random effect of animal. Data normality was verified using PROC UNIVARIATE. Differences among treatments were determined using a Fisher-protected least significant difference test with a Tukey adjustment to control for type-1 experiment-wise error. A probability of $P < 0.05$ was accepted as statistically significant and reported pooled standard errors of the mean (**SEM**) were determined according to the Mixed Models procedure of SAS.

RESULTS AND DISCUSSION

Insect Meal and Experimental Diet Proximate Analysis, Food Intake, and Fecal Characteristics

The 3 selected insect meals had similar DM (range: 92.1 – 93.5%) and OM (range: 94.9 – 96.9%) (**Table 3.2**). Crude protein and acids hydrolyzed fat (**AHF**) concentrations varied among insect meal sources; MC meal had the highest concentration of CP (85.6%), in contrast with SC meal (61.3%) and SW meal (53.4%). The SC meal and SW meal, however, had the highest AHF concentration at 34.8%. The SC meal had slightly lower AHF concentration at 33.1%, and MC meal had the lowest concentration at 14.3%. Gross energy also varied among insect meals, but was reflective of the lipid content of each substrate. Gross energy of SW meal (6.8 kcal/g) and SC meal (6.6 kcal/g) were comparable, whereas MS meal had lower (5.6 kcal/g) GE content. Chitin concentration was highest for MC meal (10.3%), and SC meal and SW meal had similar concentration of 8.7% and 8.0%, respectively. Nutrient composition of edible insects varies substantially, depending on the species, origin, stage of life, and rearing feed (Finke and Oonincx, 2014).

To date, limited scientific information is available on the chemical composition and nutritional adequacy of insects in pet foods. Bosch et al. (2014) determined the proximate composition of 10 insect substrates; in general, all insects had high crude protein (47-71%) and fat (13-40%) concentrations. In that same study, 3 species of cockroaches [i.e., six spot cockroach (*Enblaberus distanth*), death's head cockroach (*Blaberus craniifer*) and Argentinean cockroach (*Blaptica dubia*)] and Morio worm larvae (*Zophobas morio*) were evaluated. Crude protein for those 3 species of cockroaches ranged from 64-66%, which is similar to the CP concentration of the SC meal (61.3%) but lower than the MC meal (85.6%) in this study. Fat content among the 3 cockroach species evaluated by Bosch et al. (2014) was fairly consistent varying from 22-25%. In the present study, AHF concentration for the MC meal was lower (14.3%), but higher for the SC meal (33.1%). Fat and CP concentration of SW meal (53.4% and 34.8%) differed from values previously reported of 47 and 40%, respectively (Bosh et al., 2014). A wide variation in amino acid composition among insect species and within species across life stages have been reported (Ramos-Elorduy et al., 2002; Rumpold and Schluter, 2013; Bosch et al., 2014; Ghosh et al., 2017; Do et al., 2020). Higher indispensable amino acid concentrations were reported by Bosch et al. (2014) for Morio worm larvae and the 3 species of cockroaches analyzed in that study in contrast with our findings. Ghosh et al. (2017) reported similar (i.e., arginine, isoleucine, leucine, threonine, and valine) or slightly lower (i.e., lysine, methionine, and phenylalanine) concentrations of indispensable amino acids of larvae of different species of edible beetles and crickets. In general, black soldier fly larvae at 0 d and 18-29 d of age had comparable indispensable amino acid concentrations with the selected insects evaluated in this study, however lower concentrations of indispensable amino acids were reported at 11 d and 14 d of age (Do et al., 2020). Discrepancies observed in the chemical composition can be related to intrinsic differences in nutrient composition among insect species, feed that these insects were reared off, as well as age, harvesting and processing methods.

Fatty acid profile (ug/g on a DM basis) of insect meals varied (**Table 3.2**). Among the 3 selected insect meals evaluated herein, the most predominant fatty acids were: oleic acid (113,352-46,353 ug/g), palmitic acid (62,580-19,104 ug/g), linoleic acid (46,265-13,926 ug/g), and stearic acid (21,654-6,473 ug/g). The SW meal had higher levels of caprylic (4,052 ug/g) and capric (674 ug/g) acids compared with SC meal (9.3 and 13.7 ug/g, respectively) or MC meal (6.5 and 5.3 ug/g, respectively). Myristic acid concentration was similar between SC (1,627 ug/g) and SW (1,684 ug/g) meals, but lower for MC meal (402.0 ug/g). A greater concentration of palmitoleic acid was present in the SC meal (12,879 ug/g) in contrast with MC meal (3,433 ug/g) and SW meal (1,321 ug/g). Similarly, α- linolenic acid concentration was higher in the SC meal (2,060 ug/g) in comparison with SW meal (1,739 ug/g) and MC meal (848.7 ug/g). Arachidonic acid was present at low concentration in the 3 select insect meals varying from 31.9-264.7 ug/g. Eicosapentaenoic and docosahexaecoic acids were not present at detectable concentrations in these substrates.

While a few studies have examined the proximate and amino acid composition of a wide variety of insect species, including cockroaches (Bosh et al., 2014), different sources of worms and beetles (Bosch et al., 2014; Ghosh et al., 2017), and black soldier fly larvae and pupae (Bosch et al., 2014; Liu et al., 2017; Do et al., 2020), the fatty acid profile of most edible insect sources still largely unknown despite these ingredients containing copious levels of lipids in addition to being protein-rich ingredients. Most insects can biosynthesize palmitic, stearic and oleic acids

(Paul et al., 2017; Benzertiha et al, 2019). Our findings are in agreement with previous literature that reported palmitic, stearic, oleic acids, and linoleic acid being among the most prevalent fatty acids in a variety of insect substrates. Linoleic acid is an essential fatty acid for dogs and cats (NRC, 2006). Dietary supplementation of this fatty acid has been shown to improve skin and coat scores in dogs, as this fatty acid seems not to be extensively metabolized and unsaturated, with a large proportion being deposited in the skin and fur of pet animals (Marsh et al., 2000; Fu et al., 2001).

The chemical composition of the retorted experimental diets is shown in **Table 3.3**. Experimental diets had comparable concentrations of macronutrients; DM and OM concentrations varied from 20.4% to 24.4% and 90.9% to 93.1%, respectively. The SC and SW diets had lower CP concentrations (39.5% and 39.1%, respectively, but higher concentrations of AHF (26.7% and 27.4%, respectively) compared with control or MC diets (CP: 43.6% and 44.4%, respectively; AHF: 23.6% and 24.0%, respectively). Gross energy of experimental diets were on average 6.1 kcal/g (range: 5.9 – 6.3 kcal/g). Fatty acid profile of experimental diets is also depicted in **Table 3.3**. In general, less variation was observed in the fatty acids profile of the experimental diets in contrast with the insect meals. This was expected as the inclusion of insect meal corresponded to only 4% of the ingredient composition of these diets, with chicken and chicken liver comprising the main ingredients in these diets and were kept at constant inclusion level. The 4 most predominant fatty acids among the experimental diets were: oleic acid (81,150-66,778 ug/g), palmitic acid (57,038-45,034 ug/g), linoleic acid (43,461-33,751 ug/g), and stearic acid (15,990-13,799 ug/g). Overall, the variation in chemical composition among the experimental diets were likely due to the fixed rate of inclusion of select insect meals (4%) added at the expense of 3.5% of chicken meal and 0.5% of corn gluten meal (**Table 3.1**).

Daily food intake (g/d, as is or g/d, DMB) did not ($P > 0.05$) differ among dietary treatments (**Table 3.4**). However, a wide variation in food intake was observed among cats with this behavior being independent of dietary treatment. Throughout the experimental period, some cats consistently consumed 100% of their daily food ration, whereas others had variable daily consumption ranging from 30-100% of the daily food ration. Cats can be peculiar about food flavor profile, texture, shape, and temperature (Zaghini et al., 2005, German et al., 2015). Therefore, it is possible that these cats were demonstrating a neophobic behavior towards the retorted diets, as these animals had been strictly fed extruded diets during their lifespan prior to this study. While cats may show neophilia towards novel diets, it seems that food preference for novel diets (also known as monotony effect) is more prevalent in free-ranging cats than cats raised exclusively on nutritionally complete diets (Church et al., 1996). Despite intermittent inappetence, all cats remained healthy based on cats' daily behavior (only observational), and serum metabolites and complete blood count results (data not shown). Serum chemistry did not differ among dietary treatments, and all values, with exception of creatinine and glucose concentrations, were within the reference range provided by the University of Illinois Veterinary Diagnostics Laboratory. The creatinine concentration of all treatments was approximately 1.7 mg/dL, which is slightly higher than its reference range (0.4 – 1.6 mg/dL). However, the concentration of creatinine was the same on d 0 (baseline) and d 21 (end of experimental period). Similarly, serum glucose concentrations among cats fed experimental diets were above the reference range on both collection periods without any significant differences among treatments. Transient hyperglycemia observed in these cats is attributed to sedative used prior to blood collection.

Fecal scores were not affected ($P > 0.05$) by dietary treatment and were within ideal range using a 5-point scale; ranging, on average, from 1.9 to 2.3 for cats fed the MC and SC diets, respectively. Fecal pH ranged from 6.4 for cats fed the control diet to 7.0 for cats fed the MC diet. Fecal output (g/d) on as is or DM basis also did not differ (P > 0.05) among dietary treatments (**Table 3.4**). Ideal fecal scores have also been reported in kittens fed raw diets containing (Hamper et al., 2016). Kerr et al. (2013) reported similar average fecal score of 2.6, using the same 5-point scale as of the present study, when African wildcats (*Felis silvestris lybica*) were fed a retorted feline diet.

Apparent Total Tract Digestibility of Macronutrients, Digestible Energy, and Fecal Fermentative End-Products

Inclusion of selected insect meals at the expense of chicken meal and corn gluten meal resulted in comparable (P > 0.05) ATTD of macronutrients and digestible energy of retorted diets when fed for adult cats for a 21 d period (**Table 3.5**). On average, DM ATTD varied between 86.5% to 88.1%. Similarly, the ATTD of OM, CP, and AHF were high and between 88.9%-90.6%, 86.3%-89.4%, and 90.1%-92.3%, respectively. Digestible energy did not differ among dietary treatments and, on average, had a coefficient of digestibility of 90% across dietary treatments, resulting on a caloric content of 5.5 kcal/g on average. Unfortunately, limited scientific data on in vivo macronutrient ATTD of pet foods containing insects is available. *In vitro* OM and N digestibility of Morio worm of 91% and 92%, respectively, was reported by Bosch et al. (2014). Those same authors, reported lower *in vitro* OM and N digestibility values for six spot cockroach and death's head cockroach (78% and 76% and 79% and 78%, respectively), but similar in vitro OM (84%) and N (84%) digestibility for Argentinean cockroach in comparison with our findings. Previous research in our laboratory, demonstrated that when these insect meals were added at 7.5% and 15% inclusion levels in extruded diets of adult cats and dogs, macronutrient ATTD was not negatively affected (Lisenko et al., 2018 a,b).

The fecal metabolites analyzed included ammonia, phenols, indoles, SCFA and BCFA (**Table 3.6**). Fecal metabolites of cats fed diets containing selected insect meals did not differ ($P > 0.05$) from cats fed the control diet. Generally, SCFA are considered to be beneficial to gut health, and SCFA production is associated microbial hindgut saccharolytic fermentation (Gross et al. 2019). Borrelli et al. (2017) found when replacing soybean meal (23.5%) defatted*hermetia illucens* larvae meal (17%), the ceca SCFA concentration in in these birds increased (acetate:10.8 mmol/L in ID and 6.9 mmol/L in SD, $P<0.001$; propionate: 5.8 mmol/L in ID and 3.0 mmol/L in SD, $P<0.001$; butyrate: 4.4 mmol/L in ID and 1.5 mmol/L in SD, $P<0.001$). Branched-chain fatty acids, phenol, indoles and ammonia are putrefactive compounds from proteolytic microbial fermentation in the hindgut, increased concentration of those metabolites may result in undesirable fecal characteristics and increased the fecal output (Gross et al. 2019; Miner and Hazen, 1969).

CONCLUSIONS

Overall, the 3 selected insect meals evaluated in this study are lipid and protein-rich ingredients; containing high concentrations of linoleic acid, an essential fatty acid for cats with potential benefits for skin and coat health, and high concentrations of branched-chain amino acids and arginine. Inclusion of 4% of these insect meals in replacement of chicken meal and corn gluten meal in retorted diets for adult cats resulted in no negative effects on ATTD of macronutrients, fecal scores and metabolites, and overall animal health. Therefore, the data gathered herein suggest that these selected insect meals are adequate alternative ingredients for feline diets. Further research should evaluate potential beneficial effects of these ingredients in skin and coat of pet animals.

TABLES

Table 3.1. Ingredient composition of retorted experimental diets

	Treatments[1]			
Item, % as-is basis	Control	SC Diet	MC Diet	SW Diet
Water	39.49	39.49	39.49	39.49
Chicken	33.00	33.00	33.00	33.00
Steam	10.00	10.00	10.00	10.00
Rice flour	5.00	5.00	5.00	5.00
Corn gluten meal	**4.00**	**3.50**	**3.50**	**3.50**
Chicken liver	4.00	4.00	4.00	4.00
Chicken meal	**3.50**	**0.00**	**0.00**	**0.00**
Speckled cockroach; SC[1]	0.00	**4.00**	0.00	0.00
Madagascar hissing cockroach; MC[1]	0.00	0.00	**4.00**	0.00
Superworm; SW[1]	0.00	0.00	0.00	**4.00**
Guar gum	0.35	0.35	0.35	0.35
Carrageenan	0.15	0.15	0.15	0.15
Potassium chloride	0.13	0.13	0.13	0.13
Chelated mineral mix	0.10	0.10	0.10	0.10
Choline chloride 70%	0.09	0.09	0.09	0.09
Vitamin premix	0.08	0.08	0.08	0.08
Taurine	0.05	0.05	0.05	0.05
Salt, plain	0.05	0.05	0.05	0.05
Thiamine mononitrate	0.01	0.01	0.01	0.01

[1] SC = Speckled cockroach, *Nauphoeta cinerea* ; MC = Madagascar hissing cockroach, *Gromphadorhina portentosa*; SW = Superworm, *Zophobas morio larvae*

Table 3.2. Proximate analysis and indispensable amino acid and fatty acid composition of selected insect meal sources

	Insect Meals[1]		
Item	SC Meal	MC Meal	SW Meal
Dry matter (%)	92.6	92.1	93.5
		Dry matter basis	
Organic matter (%)	96.1	94.9	96.9
Crude protein (%)	61.3	85.6	53.4
Acid-hydrolyzed fat (%)	33.1	14.3	34.8
GE[2] (kcal/g; measured)	6.6	5.6	6.8
Chitin (%)	8.7	10.3	8.0
Indispensable amino acid (%)			
Arginine	3.0	2.8	2.7
Histidine	1.5	1.6	1.6
Isoleucine	2.1	2.0	2.4
Leucine	3.5	3.4	3.6
Lysine	3.0	2.7	3.2
Methionine	0.9	0.7	0.6
Phenylalanine	2.2	2.1	2.2
Threonine	1.9	1.8	2.0
Tryptophan	0.5	0.4	0.8
Valine	3.6	3.9	3.4
Taurine	0.0	0.0	0.1
Fatty acid (ug/g)			
Caprylic; C8:0	9.3	6.5	4051.7
Capric; C10:0	13.7	5.3	673.7
Lauric; C12:0	177.4	43.8	127.7
Myristic; C14:0	1,626.8	402.0	1,684.2
Myristoleic; C14:1	240.6	26.7	13.2
Pentadecanoic; C15:0	179.3	74.9	302.4
Palmitic; C16:0	62,580.2	19,104.0	58,730.9
Palmitoleic; C16:1	12,878.7	3,432.9	1,321.1
Heptadecanoic; C17:0	347.4	229.3	663.1
Stearic; C18:0	14,638.6	6,473.1	21,653.7
Oleic; C18:1n9	113,351.8	46,352.6	78,562.5
Linoleic; C18:2n6	21,882.5	13,925.9	46,265.2

Table 3.2. (cont.). Chemical composition and amino acid and fatty acid profiles of select insect meal sources

Fatty acid (ug/g)			
α-Linolenic; C18:3n3	2,060.0	848.7	1,739.1
Arachidic; C20:0	557.3	365.3	605.8
Arachidonic; C20:4n4	264.7	71.8	31.9
Eicosapentaenoic; C20:5n3[2]	n.d	n.d	n.d
Docosahexaenoic; C22:6n3[2]	n.d	n.d	n.d

[1] SC = Speckled cockroach, *Nauphoeta cinerea* ; MC = Madagascar hissing cockroach, *Gromphadorhina portentosa*; SW = Superworm, *Zophobas morio larvae*

[2] GE = gross energy; n.d. = not detected

Table 3.3. Chemical composition and fatty acid profile of retorted experimental feline diets containing select protein sources

	Treatments[1]			
Item	Control	SC Diet	MC Diet	SW Diet
Dry matter (%)	24.4	22.3	22.3	20.4
		%, Dry matter basis		
Organic matter (%)	93.1	93.1	90.9	91.3
Crude protein (%)	43.6	39.5	44.4	39.1
Acid-hydrolyzed fat (%)	23.6	26.7	24.0	27.4
GE[2] (kcal/g; measured)	5.9	6.1	6.1	6.3
Fatty Acid (ug/g)				
Caprylic; C8:0	38.2	32.9	33.0	962.5
Capric; C10:0	23.2	18.0	12.1	158.7
Lauric; C12:0	75.3	98.3	70.8	89.1
Myristic; C14:0	1,244.6	1,269.3	1,032.8	1,428.1
Myristoleic; C14:1	343.6	319.7	262.3	291.6
Pentadecanoic; C15:0	154.7	155.3	133.3	195.3
Palmitic; C16:0	52,864.1	52,404.4	45,034.3	57,038.1
Palmitoleic; C16:1	12,248.3	12,100.2	9,852.1	10,607.1
Heptadecanoic; C17:0	295.8	292.1	279.0	382.7
Stearic; C18:0	14,371.3	13,792.1	13,799.4	15,989.8
Oleic; C18:1n9	77,462.6	79,804.6	66,777.5	81,150.4
Linoleic; C18:2n6	43,263.9	36,482.2	33,751.1	43,460.8
α-Linolenic; C18:3n3	1,909.3	1,803.7	1,464.9	1,845.2
Arachidic; C20:0	212.9	280.0	271.1	295.3
Arachidonic; C20:4n4	1,795.2	1,319.4	2,621.8	1,168.8
Eicosapentaenoic; C20:5n3[2]	n.d.	n.d.	n.d.	n.d.
Docosahexaenoic; C22:6n3	123.0	102.8	152.3	105.8

[1] SC = Speckled cockroach, *Nauphoeta cinerea* ; MC = Madagascar hissing cockroach, *Gromphadorhina portentosa*; SW = Superworm, *Zophobas morio larvae*

[2] GE = gross energy; n.d. = not detected

Table 3.4. Food intake and fecal characteristics of cats fed retorted experimental diets containing select protein sources

	Treatments[1]					
Item	Control	SC Diet	MC Diet	SW Diet	SEM	P-value
Food Intake (g/d, as-is)	168.3	212.9	156.6	162.3	18.655	0.1722
Food Intake (g/d, DMB)	41.1	47.4	34.9	33.0	4.265	0.1132
Fecal characteristics/output						
Fecal score[2]	2.2	2.3	1.9	2.2	0.161	0.2647
Fecal pH	6.4	6.8	7.0	6.6	0.202	0.2449
Fecal output, as-is (g/d)	16.9	20.0	11.4	12.7	3.320	0.2792
Fecal output, DMB (g/d)	6.0	6.3	4.1	4.3	0.867	0.1971

[1] SC = Speckled cockroach, *Nauphoeta cinerea* ; MC = Madagascar hissing cockroach, *Gromphadorhina portentosa*; SW = Superworm, *Zophobas morio larvae*

[2] Fecal scores: 1= day, hard pellets; 2- firm, retains shape but pliable; 3= soft and moist, but retains a shape; 4= soft, unformed; 5= watery liquid

Table 3.5. Total tract apparent macronutrient and energy digestibility of cats fed retorted experimental diets containing select protein sources

	Treatments[1]					
Item	Control	SC Diet	MC Diet	SW Diet	SEM	*P*-value
Dry matter (%)	87.88	86.92	88.12	86.49	1.334	0.803
	%, DM basis					
Organic matter (%)	90.64	89.22	89.88	88.86	1.085	0.656
Crude protein (%)	89.43	86.64	88.62	86.31	1.333	0.294
Acid-hydrolyzed fat (%)	90.14	90.33	92.27	90.53	1.260	0.625
Digestible energy (%)	90.14	89.23	90.49	89.35	1.140	0.841

[1] SC = Speckled cockroach, *Nauphoeta cinerea* ; MC = Madagascar hissing cockroach, *Gromphadorhina portentosa*; SW = Superworm, *Zophobas morio larvae*

Table 3.6. Fecal fermentative end-products of cats fed retorted experimental diets containing select protein sources

	Treatments[1]					
Item (µmole/g DM basis)	Control	SC Diet	MC Diet	SW Diet	SEM	P-value
Ammonia	166.2	161.2	142.3	187.5	20.43	0.7343
Phenols & Indoles						
Total Phenols/Indoles	4.4	3.2	2.9	4.6	0.60	
Phenols	0.1	0.2	0.1	0.1	0.06	0.4443
Indoles	4.2	3.0	2.8	4.5	0.59	0.2757
SCFA[2]						
Acetate	170.4	207.9	117.7	218.3	36.65	0.3119
Propionate	68.6	92.5	40.0	94.0	17.35	0.1106
Butyrate	41.6	43.5	31.0	49.0	6.20	0.6377
BCFA[2]						
Isobutyrate	8.9	8.4	9.0	10.5	1.33	0.3460
Isovalerate	15.7	13.5	13.5	18.9	2.24	0.4333
Valerate	10.4	11.1	8.8	10.7	1.27	0.8580

[1] SC = Speckled cockroach,*Nauphoeta cinerea* ; MC = Madagascar hissing cockroach, *Gromphadorhina portentosa*; SW = Superworm, *Zophobas morio larvae*

[2] SCFA = short-chain fatty acid; BCFA = branched-chain fatty acid

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