EVALUATING MULTIPLE EDIBLE INSECTS AS FEED FOR CATTLE: AN *IN-VITRO* ANALYSIS

by

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DEDICATION

This work is dedicated to my mentor, Dr. Drewery, for walking me through the uncharted territory of the research world and putting up with my naivety and uncertainty. I also dedicate this work to my parents for their relentless, unwavering support throughout this endeavor.

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ABSTRACT

The increasing human population will result in increased consumer demand for animal-derived protein food products. Continued cultivation of traditional protein feeds (e.g., soybean, cottonseed) to feed domestic cattle is unsustainable. Edible insect protein is a promising alternative, requiring fewer resources to produce while also providing a high-quality protein feed source. The objective of this study was to evaluate multiple edible insect species using chemical compositions and *in vitro* digestibility methods to determine the validity of pursuing further research into the use of various edible insect proteins as cattle feed. Insect samples (*n*=14) of different species (*n*=5) were sourced from several private-sector companies along with their product processing information. Ruminal *in vitro* digestibility was analyzed using a DAISYII incubator. Dry matter, organic matter, neutral detergent fiber, acid detergent fiber, crude protein, ether extract (fat), sodium, calcium, and phosphorus were analyzed using industry-validated procedures. Our data indicate a wide margin of protein and fat percentages among and between species. This variation is most likely due to the unstandardized rearing and processing of edible insect products among companies. Neutral and acid detergent fiber also ranged widely among samples, most likely due to chitin content within the exoskeleton of the insects. All samples had more than 65% *in vitro* true digestibility, suggesting high ruminal digestibility. This exploratory study indicates that black soldier fly larvae, mealworms, crickets, and grasshoppers have the potential to be used as a sustainable alternative in cattle feed in place of traditional protein feeds and further research should focus on those species. Our data also suggest that standardizing production practices for edible insects will be vital to ensure quality control and consistency of insect protein products if used wide-scale in the livestock feed industry.

I. LITERATURE REVIEW

A. Growing Human Population & Animal Protein Demands

The U.N. estimates the global human population to increase to approximately 9.5 billion by 2050 (Henchion et al., 2017; Kim et al., 2019; Hopkins et al., 2021). In response, food production will need to increase by 70% to meet this projected population growth (Hopkins et al., 2021). The demand for livestock products and animal-derived protein food products is anticipated to double during this time (Henchion et al., 2017; Jayanegara et al., 2017; Baiano, 2020). Yet, the agriculture sector has limited room for growth with current traditional practices considered unsustainable, limiting total output potential (Hopkins et al., 2021). Expansion of arable land is the most considerable contributor to global warming (Oonincx and de Boer, 2012). The livestock industry, which uses 70% of this arable land, accounts for 14% of global greenhouse gas (GHG) emissions (Oonincx and de Boer, 2012; Gerber et al., 2013).

Currently, 43% of human protein demands globally are met by animal-source protein (Henchion et al., 2017). Between 1990 and 2009, there was a 60% increase in global meat consumption, particularly for beef (Henchion et al., 2017).

The increase in meat consumption is reflected in the number of cattle in the U.S.; as of January 2022, there are 91.9 million head of cattle (USDA-NASS, 2022). While this is 2% lower than the 93.8 million head counted in January 2021, domestic cattle production is an integral and extensive industry in the U.S. (USDA-NASS, 2022). Beef production is environmentally costly, using arable land, water, and energy and inputting chemicals into already depleted soil (Henchion et al., 2017). Husbandry of beef cattle contributes to GHG emissions at a rate of 2850 g/kg mass gained (Oonincx and de Boer, 2012). Production of traditional protein supplements used in feed (e.g., soybean or cottonseed meal) also puts increasing pressure on non-renewable resources (Sogari et al., 2019).

An alternative feed protein source is needed in response to the growing human population and resulting pressures placed on agricultural producers to increase output, especially beef. This alternative protein source should also lessen the environmental footprint associated with beef production. Edible insects have potential to be this alternative feed protein source.

B. Edible Insects

Edible insects are insects that are nutritionally beneficial to humans and domestic animals. Historically, humans have consumed insects in many countries for centuries (Henchion et al., 2017; Kim et al., 2019; Magara et al., 2021; Pasini et al., 2022). Supplementing diets with edible insects is critical in regions with limited access to nutrient-dense foods (Baiano, 2020). The nutrient profile of edible insects depends largely on the species, life stage, and substrate or food on which the insects are grown (Henchion et al., 2017; Pasini et al., 2022).

The most commonly cultivated Orders of insects are Coleoptera (beetles; 31%), Lepidoptera (butterfly and moth caterpillars; 18%), Hymenoptera (sawflies, wasps, bees, and ants; 14%), Orthoptera (grasshoppers, locusts, and crickets); 13%), Hemiptera (true bugs such as cicadas, aphids, planthoppers, leafhoppers); 10%), Blattodea (termites; 3%), Odonata (dragonflies; 3%), Diptera (flies; 3%), and 5% other (Henchion et al., 2017; Kim et al., 2019; Magara et al., 2021).

i. Edible Insects for Human Consumption

Using insects as an alternative protein source has been suggested, researched, and grown in popularity since 2010 (Baiano, 2020; Hopkins et al., 2021). In many countries, such as Africa, China, India, and Thailand, it has been common for centuries to cultivate and consume insects (Magara et al., 2021). Over 2,000 known species of insects are staples in traditional diets for over 2 billion humans worldwide (Henchion et al., 2017). While many customary diets include edible insects, most Western countries maintain an aversion to consuming edible insects (Kim et al., 2019). Globally, the insect rearing market is growing with an estimated worth of 406 million USD (Shahbandeh, 2019). The U.S. comprises 2% of this market, or 8 million USD (Shahbandeh, 2019). By 2023, it is estimated that North America's contribution to the edible insect market will increase by 28% (Shahbandeh, 2019). However, many still view insects as a novelty versus as a legitimate alternative protein food (Kim et al., 2019). Part of the hesitation to consume edible insects comes from the lack of regulation within the market itself (Kim et al., 2019).

The European Union (EU) has regulations in place to monitor insects intended for human consumption (Lorrette and Sanchez, 2022). Insects entering the human food chain must be approved as a Novel Food under Regulation No 2017/893 (Lorrette and Sanchez, 2022). This regulation includes a list of the only species authorized to be sold for human consumption (Lorrette and Sanchez, 2022), such as the tropical house cricket [*Grillodes sigillatus*], Jamaican field cricket [*Gryllus assimilis*], house cricket [*Acheta domesticus*], common, yellow, or lesser mealworm [*Tenebrio molitor*], black soldier fly larvae (BSFL) [*Hermetia Illucens*], house fly [*Musca domestica*], and silkworm [*Bombyx mori*] (Lorrette and Sanchez, 2022).

Regardless of perceptions, incorporating insects into consumer diets may be an answer to the global food and protein shortages (Baiano, 2020). Integrating edible insects as a meal or flour into staple foods can be used to meet protein demands. For example, a recent experiment enriched wheat pasta dough with an edible insect meal made from grasshoppers (Pasini et al., 2022). This grasshopper-enriched dough had increased nutritional value from the insect meal, which adds protein and fatty acids to the pasta (Pasini et al., 2022). In taste tests, the consumer experience was unaltered by the presence of the insect meals (Pasini et al., 2022). In addition to a protein alternative, studies have demonstrated that fat extracted from insects can also be consumed by humans. Some insect fats are liquid at room temperature and have the potential to be used in place of vegetable, olive, peanut, and canola oils (Berezina, 2017). In another study, BSFL fat was implemented in baking recipes, replacing 25-50% of traditional butter without consumer aversion to the final food products (Delicato et al., 2020).

The viability of cultivating edible insects can be seen in the growing success of mealworm production in the EU (Oonincx and de Boer, 2012). Mealworms, the most commonly raised insect in the EU, have an 80% edible and digestible body weight (Oonincx and de Boer, 2012). For comparison, poultry has a 55% edible carcass weight, and beef cattle have an edible carcass weight of 40% (Henchion et al., 2017). The land required to cultivate 1 kg of edible mealworms is one-tenth of that needed to produce the same amount of beef (Oonincx and de Boer, 2012). From an environmental standpoint, edible insects are a sustainable food and feed source as they do not require massive inputs for their cultivation (Oonincx and de Boer, 2012).

Edible insects can also be reared on food waste, an abundant human by-product that contributes heavily to GHG emissions (Pang et al. 2020). Globally, humans create over 1.3 billion tons of food waste yearly, accounting for an economic loss of approximately 1 trillion USD (Pang et al., 2020). Upcycling this food waste by feeding it to insects, which convert it into high-quality protein, can potentially reduce our carbon footprint while creating more food (Pang et al., 2020). Per the Association of American Feed Control Officials (AAFCO), only feed grade or better materials can be fed to insects intended to enter the human food chain in the U.S. (AAFCO, 2021). While this limits the ability of U.S. producers to rear edible insects on most organic wastes, they can still use pre-consumer food waste to be sustainable.

BSFL fed different types of food waste have varying amino acid profiles; see Table 1 (Hopkins et al., 2021). This implies that insects reared on diverse food substrates have different nutrient profiles, so care should be taken if insects are reared for feed or food.

Table 1: Essential amino acid profiles of black soldier fly larvae reared on food waste. ^ indicates the original article presented data as g/kg of BSFL total protein content, + indicates the original article presented data as mg/g of BSFL total protein content. Dashes are used to indicate where data was unreported. ^{WW} - wet weight.

Histidine, His; Isoleucine, Ile; Leucine, Leu; Lysine, Lys; Methionine, Met; Phenylalanine, Phe; Threonine, Thr; Tryptophan, Trp; Valine, Val.

Author	Rearing Substrate (RS) (Mixture Ratio)	Essential Amino Acids								
		His (%)	Ile (%)	Leu (%)	Lys (%)	Met (%)	Phe (%)	Thr (%)	Trp (%)	Val (%)
Liland	RS 1: Wheat	2.8	3.9	6.4	6.2	1.7	4.0	3.9	-	5.8
	RS 2: Wheat, brown algae *A. nodosum*	2.6	4.0	6.6	6.2	1.6	3.9	4.0	-	6.0
	RS 3: Wheat, brown algae *A. nodosum*	2.7	4.0	6.7	5.9	1.7	4.3	4.1	-	6.0
	RS 4: Wheat, brown algae *A. nodosum*	2.4	3.9	6.6	6.0	1.6	3.8	4.0	-	6.0

	RS 5: Wheat, brown algae *A. nodosum*	2.5	4.0	6.6	5.5	1.5	3.7	3.9	-	5.7
	RS 6: Wheat, brown algae *A. nodosum*	2.4	4.0	6.7	5.6	1.4	3.4	4.0	-	5.7
	RS 7: Wheat, brown algae *A. nodosum*	2.5	4.0	6.9	5.5	1.5	3.6	4.1	-	5.6
	RS 8: Wheat, brown algae *A. nodosum*	2.3	4.1	6.3	5.6	1.3	3.0	3.7	-	5.4
	RS 9: Wheat, brown algae *A. nodosum*	2.5	3.8	6.3	5.4	1.4	3.2	3.9	-	5.4
	RS 10: Wheat, brown algae *A. nodosum*	2.5	3.7	6.2	5.5	1.3	3.0	3.9	-	5.5
	RS 11: Brown algae *A. nodosum*	2.7	3.8	6.2	5.6	1.4	3.2	3.9	-	5.5
Shumo +	RS 1: Kitchen waste - potato peelings, carrot, rice, bread debris (ratio unspecified)	0.3	0.3	0.3	0.5	0.8	0.5	-	-	0.1
	RS 2: Brewery by-product spent grain	0.5	0.2	0.4	0.5	0.7	0.2	-	-	0.9
Spanghers ^	RS 1: Restaurant waste - potato peelings, carrot, rice, bread debris (ratio unspecified)	1.4	1.9	3.1	2.3	0.7	1.6	1.6	0.5	2.8
Tschirner	RS 1: Carbohydrate - wheat middlings	3.3	4.2	6.6	5.9	1.6	3.6	3.9	-	5.7
	RS 2: Protein - dried distillers' grains with soluble	-	-	-	-	-	-	-	-	-
	FS 3: Fibre - sugar beet	-	-	-	-	-	-	-	-	-
Cappellozza +	RS 1: Fruit and vegetable mix - zucchini, apple, potato, green beans, carrot, pepper, orange, celery, kiwi, plum, eggplant (unspecified ratio)	1.2	1.6	2.7	2.0	1.8	1.9	2.2	0.4	2.5
Lalander ^	RS 1: Food waste (uncharacterized)	2.9	4.1	6.8	8.3	1.8	4.0	3.9	1.4	5.8
	RS 2: Fruit and vegetable mix - lettuce, apple, potato (5:3:2)	2.6	4.3	6.7	5.1	1.5	3.5	3.5	1.4	6.0
Surendra ww	RS 1: Food waste (uncharacterized)	1.7	1.5	2.3	2.2	0.9	1.5	1.5	-	2.4

Adapted from Hopkins et al. (2021)

ii. Edible Insects in Livestock Feeds

While some U.S. consumers would be willing to try edible insects as a novelty food, it does not seem that they can easily be incorporated into most consumers' current diets. A recent survey indicated that 66% of U.S. consumers would be willing to consume animal products from poultry fed an insect-based diet (Higa et al., 2021). This suggests that consumers would be more accepting of edible insects, in indirect forms, such as animal feed, rather than as food for themselves (Higa et al., 2021). These data suggest that utilizing the high-quality protein of edible insects as feed for livestock, versus as food for humans, is promising (Kim et al., 2019).

Cultivating conventional protein supplements used in livestock feeds, such as cottonseed or soybeans, requires large amounts of land, water, and energy in the form of labor and fossil fuels (Chia et al., 2019). Moving away from these traditional protein supplements and toward edible insect proteins can potentially reduce the impact on the environment without sacrificing quality protein meals (Chia et al., 2019)

On top of the environmental impacts of cultivating traditional livestock feeds, feed prices represent 60-70% of the overall cost to livestock producers (Kim et al., 2019). For small-scale farmers, in the developing world particularly, this can be devastating, especially with the price of protein-rich feeds estimated to continue to increase (Chia et al., 2019). Feeding BSFL, mealworms, or houseflies that producers grow to their livestock as a protein supplement has the potential to decrease feed costs without sacrificing high-quality protein needed for animal growth and performance (Chia et al., 2019). Figure 1 outlines a flowchart example of a small-scale livestock operation implementing edible insects into its production and the resulting improvements to the local economy while sustaining minimal environmental impact (Chia et al., 2019).

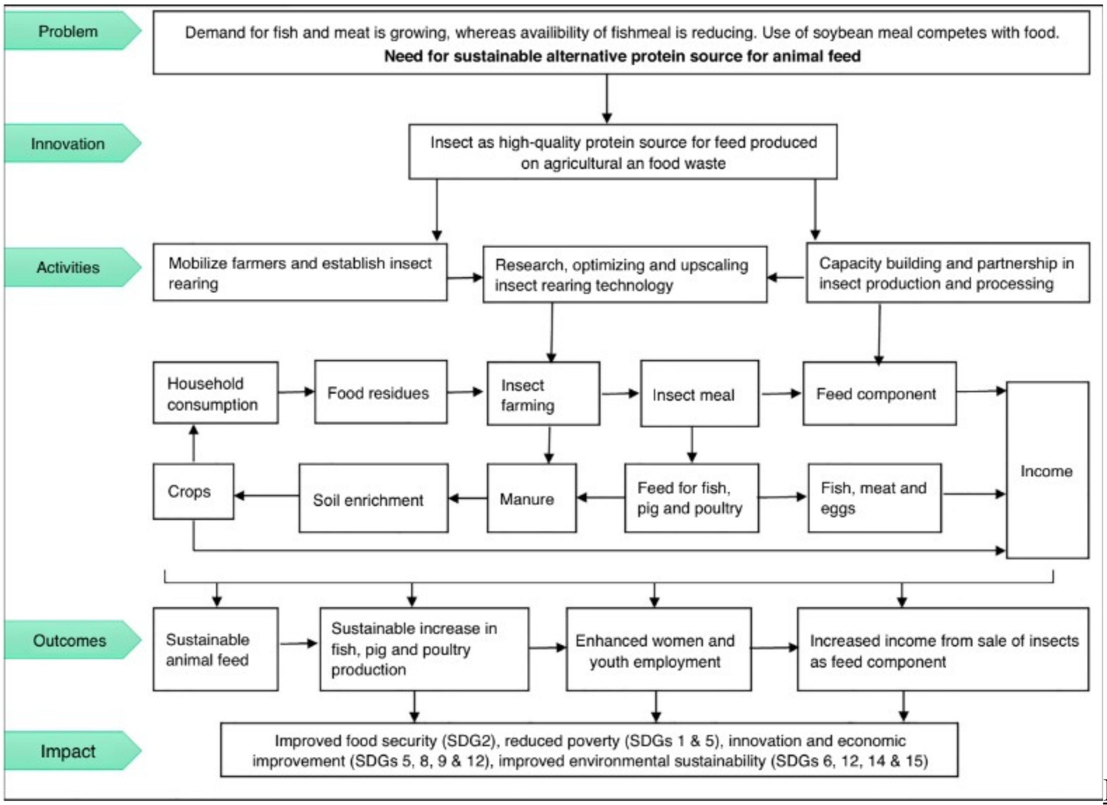

Figure 1: Insect farming for feed in the context of a circular economy (Chia et al. 2019)

iii. Current Research in Edible Insects

Current studies have focused on using edible insects to replace costly soybean and fish meals in livestock feeds (Sogari et al., 2019). Mealworms (*Tenebrio molitor*), BSFL (*Hermetia illucens*), and house flies (*Musca domestica*) are currently considered the species with the most potential (Sogari et al., 2019). All three species have data to support their use as livestock feed, demonstrating positive relationships between feeding and animal health, performance, gut health, and end-product quality (Sogari et al., 2019). Livestock species-specific research has focused on poultry, fish, swine, and ruminants.

The following are examples of studies using edible insect meals in poultry feed, with the research predominantly centered around broiler hens. House fly larvae meal replaced 4% of the fishmeal in a broiler diet with no change in growth or performance

(Awoniyi et al., 2003). A set of broiler diets containing soybean meal at 31%, 26%, and 20% were altered to have dried mealworm meal at 0%, 5%, and 10%, with no negative effects on animal growth or performance (Ramos-Elorduy et al., 2002). Edible insect fats have also been used to replace traditional fats or oils in poultry diets. BSFL fat was added to a finishing broiler diet and did not affect growth or performance (Schiavone et al., 2017). BSFL oil replaced 50-100% of the soybean oil in broiler diets with no adverse health effects, changes to carcass quality, or histological changes (Schiavone et al., 2017). Another study reported that replacing soybean oil with mealworm and superworm (*Zophobas morio*) oil positively affected digestibility, gut health, improved carcass quality, and improved fatty acid profiles of broiler hens (Benzertiha et al., 2019). There is less data for laying hens but, in the available literature, completely replacing protein sources with edible insect protein did not negatively affect production (Marono et al., 2017). Further, a laying diet using fishmeal substituted it with dried mealworm meal, and egg production increased by 2.4% (Wang et al., 1996).

Examples of research in fish are also widely available as edible insects are frequently used in fish diets. Nile tilapia (*Oreochromis niloticus*) were fed a diet with house fly larvae meal replacing 75% of the fishmeal with no negative effects on growth or performance (Wang et al., 2017). The use of BSFL meal in feed for farmed Atlantic salmon (*Salmo sala*) did not change the odor, flavor, or texture of the finished product (Lock et al., 2016). Mealworms can replace 40-80% of a standard meal made of soybean meal and corn gluten for farmed catfish (*Ameiurus melas Raf.*) without adversely affecting growth or performance (Roncarati et al., 2015).

The evaluation of edible insect meals for feeding other livestock species is less well documented. Newton et al. (1997) created two swine diets containing 20% crude protein and 13% either extract (fat) from a mixture of dried BSFL meal, soybean meal, and brown grease to bind the feed together. They determined that BSFL meal is a viable and valuable ingredient for swine feed as it contains essential amino acids, a good fatty acid profile, and abundant calcium (Newton et al., 1977).

The least researched livestock animals with regards to edible insect intake are ruminants. Two *in vitro* studies by Jayanegara et al. (2017; 2020) determined the ruminal digestibility of BSFL meal. Both studies concluded that chitin had a negative effect on digestibility (Jayanegara et al. 2017; 2020). Jayanegara et al. (2017) suggested further research into removing chitin completely from feeds to improve nutrient utilization; however, their follow-up study, where chitin was enzymatically converted to chitosan, did not positively affect digestibility (Jayanegara et al. 2020). To our knowledge, the only *in vivo* study conducted on cattle using edible insects tested the viability of BSFL meal in cannulated steers (Fukuda et al., 2022). The authors concluded that BSFL meal is a viable protein supplement that can replace cottonseed meal, particularly for cattle consuming low-quality forage (Fukuda et al., 2022). In a comparable experiment using *in situ* methods, Merino ewes were fed mealworm, superworm, lesser mealworm (*Alphitobius diaperinus*), and house cricket meals (Toral et al., 2022). The authors concluded these meals were viable feed alternatives for small ruminants (Toral et al., 2022).

C. Continuing Research for Edible Insects

Additional research is ongoing to expand the market, cultivation, and scale of the edible insect sector of agriculture. One avenue of research being explored is using edible insects to assist in sanitation (Banks et al., 2014). BSFL are non-vector, non-pest insects that can be reared on a wide variety of waste ranging from food to fecal (Bank et al., 2014). It has been proposed that in areas with minimal sanitation infrastructure, BSFL colonies should be established to remove excess waste (Bank et al., 2014). The mature larvae can then be harvested and fed to livestock as a protein source (Bank et al., 2014). However, in the U.S., AAFCO restricts this practice for edible insect products intended to enter the human food or animal feed chain(AAFCO, 2021).

Using BSFL to reduce GHG emissions and volatile organic compounds (VOC) in poultry, swine, and dairy cattle manure has also been investigated (Beskin et al., 2018). VOCs across all types of manure had an 87% reduction when fed to BSFL (Beskin et al., 2018). Using this system on farms can be beneficial to offset the GHG emissions produced by the livestock industry (Beskin et al., 2018). The BSFL are reared on livestock manure, harvested when mature, and fed back to the livestock as a high-quality protein source (Beskin et al., 2018).

Current research is primarily focused on mealworms, BSFL, and house flies as protein alternatives for livestock. Our study proposes that other edible insects could be considered viable options for supplementing livestock feed. Additionally, there is minimal research on edible insects in large ruminants. However, beef cattle are the most costly livestock animals in environmental use and feed costs. Our study aims to fill a deficit in the current literature and suggests further evaluation into the potential for edible insects to be used as cattle feed.

II. OBJECTIVE

The objective of this study was to determine the nutritional value and *in vitro* digestibility of multiple edible insect species as protein supplements for large ruminants. This study also aimed to assess the effects of upstream processing of the insects on the final product's nutritive characteristics. These objectives support our ultimate goal of determining the validity of various edible insects as feed for cattle.

III. MATERIALS & METHODS

A. Sample Collection

Two-pound samples of various species of edible insect products were obtained from private sector companies sourced from the member directory of the North American Coalition for Insect Agriculture. Prospective companies were contacted via email with a pre-written statement explaining the project's purpose, requesting a sample shipment of edible insect products, and requesting specific rearing and processing information (e.g., insect diet, housing/growing conditions, drying temperature) for each sample. The initial email also briefly described the benefits of collaborating with the laboratory for this study (i.e., providing a free nutritional characterization of their samples).

Fourteen (*n*=14) insect samples were received from several companies. The collected samples were from five (*n*=5) insect species: house cricket (*Acheta Domesticus*), banded cricket (*Gryllodes sigillatus*), grasshopper (*Locusta migratoria*), mealworm (*Tenebrio molitor*), and black soldier fly larvae (*Hermetia illucens*).

Sample identification codes were assigned to each product to maintain company anonymity and referenced throughout this paper. The IDs associated with each product, product type, and processing information received from the manufacturer are in Table 2. Table 3 contains physical descriptions of each sample on a received basis and after in-house processing.

Table 2: Insect sample IDs, associated species, product type, and processing information.

Sample ID	**Insect Species**	**Product Type**	**Processing Information**	
			Insect Diet	**Product Processing**
1	Black Soldier Fly Larvae (Hermetia illucens)	Ground, dry meal	No information available.	No information available.

2	Black Soldier Fly Larvae (Hermetia illucens)	Dry Frass	No information available.	No information available.
3	Black Soldier Fly Larvae (Hermetia illucens)	Defatted, pelleted meal	Dried beer mash	Larvae harvested using a large shaker table. Meal made by vacuum drying larvae at a low temperature. Screw press used to separate protein from the oils with the addition of CO_2 liquid to reach a lower fat content.
4	Black Soldier Fly Larvae (Hermetia illucens)	Defatted, pelleted meal	Dried beer mash	Larvae harvested using a large shaker table. Meal made by vacuum drying larvae at a low temperature. Screw press used to separate protein from the oils with the addition of CO_2 liquid to reach a lower fat content.
5	Grasshopper (Locusta migratoria)	Whole, dried insects	Preformulated diet	Assumption - no data given: Eggs acquired from adult oviposition sites, incubated for 9 days, and allowed to hatch. After hatching, juveniles (i.e. pinheads, young nymphaea) were placed into grow-out containers for 35 - 45 days (adulthood) and fed a preformulated diet. The adults were mechanically sifted from the remaining diet and frass. The meal was dried and mechanically ground into a fine powder.
6	House Cricket (Acheta domesticus)	Ground, dry meal	Preformulated diet	Eggs acquired from adult oviposition sites, incubated for 9 days, and allowed to hatch. After hatching, juvenile crickets (i.e. pinheads, young nymphaea) were placed into grow-out containers for 35 - 45 days (adulthood) and fed a preformulated diet. The adults were mechanically sifted from the remaining diet and frass. The meal was dried and mechanically ground into a fine powder.
7	Mealworm (Tenebrio molitor)	Defatted, dry meal	Preformulated growing substrate	Eggs oviposited into a preformulated growing substrate; hatch within 7-10 days. Reared in “Grow-Out” containers for 8-10 weeks on a preformulated substrate until right before pupation. Before pupation, mechanically shifted from substrate and frass. The meal was dried and mechanically ground into a fine powder.

8	Black Soldier Fly Larvae (Hermetia illucens)	Ground, dry meal	Preformulated nursery diet	Eggs acquired from adult oviposition sites, placed on a preformulated nursery diet, allowed to hatch, then grow for 5-7 days. Larvae mechanically sifted from the remaining nursery diet and frass and placed in grow-out containers for 8-24 days. Larvae fed a mixture of pre-consumer food waste and a preformulated diet. Larvae mechanically sifted from the remaining grow-out diet and frass. The meal was dried and mechanically ground into a fine powder.
9	Black Soldier Fly Larvae (Hermetia illucens)	Ground, dry meal	Preformulated nursery diet	Eggs acquired from adult oviposition sites, placed on a preformulated nursery diet, allowed to hatch, then grow for 5-7 days. Larvae mechanically sifted from the remaining nursery diet and frass and placed in grow-out containers for 8-24 days. Larvae fed a mixture of pre-consumer food waste and a preformulated diet. Larvae mechanically sifted from the remaining grow-out diet and frass. The meal was dried and mechanically ground into a fine powder.
10	Banded Crickets (Gryllodes sigillatus)	Refrigerated, (wet) whole insects	Mixture of dry chicken and dog food Supplemented with potatoes and cabbage Ad libitum access to water crystals	Raised in tubs approx 18" x 30" on vermiculite substrate. In-house: dried at 55 °C for approx 144 hours.
11	House Cricket (Acheta domesticus)	Ground, dry meal	Meat-based, insect-specific diet made by Bio-Forge Labs	Crickets killed by freezing, then flash boiled, rinsed, and dehydrated via an Excalibur Food Dehydrator.
12	House Cricket (Acheta domesticus)	Whole, roasted insects	Basic grain diet Ad libitum access to water and vitamin mix	Crickets killed using CO_2 or drying ovens. Dehydrated in ovens.
13	Mealworm (Tenebrio molitor)	Defatted, dry meal	Organic oats and carrots	Mealworms were oven toasted. Defatted using a screw press. Ground into a fine powder using a Burr Mill.

14	Black Soldier Fly Larvae (Hermetia illucens)	Whole, dried larvae	Organic and industrial waste Organic waste is primarily grain-based	Starting moisture of 65%; Dried to less than 10% moisture. (As low as 6%) Drying was the killing method. Oven Room Drying: Longer drying time with lower temperature. Product dried from the outside in - end product is shriveled / hardened.

Table 3: Physical descriptions for samples based on product type, on an as-received basis, and after in-house processing.

In-house processing consisted of grinding samples with a NutriBullet blender with a mill blade attachment until homogeneity was achieved and the particles were small enough to pass through a 2mm screen.

Sample ID	**Insect Species**	**Product Type**	**Physical Description**	
			As Received	**After In-House Processing**
1	Black Soldier Fly Larvae (Hermetia illucens)	Ground, dry meal	Coarse, clumped together.	Fine meal, slight sand texture.
2	Frass from Black Soldier Fly Larvae (Hermetia illucens)	Dry meal	Coarse, flake-like.	Fine powdered meal, with some fibrous material.
3	Black Soldier Fly Larvae (Hermetia illucens)	Defatted, pelleted meal	Pelleted feedstuffs - compressed and cylindrical.	Fine, flour-like meal.
4	Black Soldier Fly Larvae (Hermetia illucens)	Defatted, pelleted meal	Pelleted feedstuffs - compressed and cylindrical.	Fine, flour-like meal.
5	Grasshopper (Locusta migratoria)	Whole, dried insects	Whole, dried grasshoppers.	Fine powdered meal, with some fibrous material.
6	House Cricket (Acheta domesticus)	Ground, dry meal	Coarse, clumped together.	Fine powdered meal, with some fibrous material.
7	Mealworm (Tenebrio molitor)	Defatted, dry meal	Coarse, clumped together.	Fine, flour-like meal.
8	Black Soldier Fly Larvae (Hermetia illucens)	Ground, dry meal	Coarse, clumped together.	Wet, sand-like texture; prone to sticking together.
9	Black Soldier Fly Larvae (Hermetia illucens)	Ground, dry meal	Coarse, clumped together.	Fine powdered meal, with some fibrous material; prone to sticking together.
10	Banded Crickets (Gryllodes sigillatus)	Refrigerated, (wet) whole insects	Coarse.	Fine powdered meal, with some fibrous material.

11	House Cricket (Acheta domesticus)	Ground, dry meal	Fine, flour-like meal.	Fine, flour-like meal.
12	House Cricket (Acheta domesticus)	Whole, roasted insects	Whole roasted crickets.	Fine, flour-like meal.
13	Mealworm (Tenebrio molitor)	Defatted, dry meal	Fine, flour-like meal.	Fine, flour-like meal.
14	Black Soldier Fly Larvae (Hermetia illucens)	whole, dried larvae	Dried whole larvae, most likely Oven Room Drying Technique. Arrived in vacuum sealed package	Very wet, sand-like in texture.

Samples, excluding sample 10 (banded crickets), were received as dried products. Samples that were acquired whole or pelleted were processed into a meal to pass through a 2-mm screen, ensuring homogenous distribution of particles.

B. Laboratory Analysis

Samples were dried in a forced-air oven at 105°C for 24 h, allowed to air equilibrate for 20 minutes, then weighed to determine dry matter (DM). Organic matter (OM) was evaluated by the loss of dry weight upon combustion of the sample for 8 h at 450°C.

Analysis for neutral detergent fiber (NDF) and acid detergent fiber (ADF) were completed using the Ankom Fiber Analyzer A200, with sodium sulfite and amylase omitted (Ankom Technology Corp. Macedon, NY). Before conducting the NDF or ADF analyses, due to the high-fat content of the samples, an acetone pre-ether extract protocol was executed following Ankom procedures (Ankom Technology Corp. Macedon, NY). The pre-ether extract protocol was performed for all samples, excluding samples 5 and 13, due to the approximate less than 5% ether extract (fat) content.

Ruminal digestibility was determined using the standard DAISY^{II} incubator protocols (Holden, 1999). Samples were inserted into DAISY^{II} digestion vessels containing a 1:5 ratio mixture of two buffer solutions and rumen fluid collected from steers fitted with a rumen cannula using the rumen fluid collection technique outlined in by the Ankom DAISY^{II} protocols, held at 39.5°C (Ankom Technology Corp. Macedon, NY; Holden, 1999). Collected rumen fluid and rumen mat were blended while being purged continually with CO_2, then strained through five layers of cheesecloth before incubation (Holden, 1999).

Additional nutritional characteristics were evaluated by the external company SDK Laboratories. The samples were analyzed on an as-received and dry matter basis for crude protein (CP), ether extract (EE), sodium (Na), calcium (Ca), phosphorous (P), dry matter (DM), and moisture (M) using industry-validated methods.

C. Calculations

Calculations were performed for acid detergent fiber and neutral detergent fiber using the provided protocols for the Ankom Fiber Analyzer A200 (Ankom Technology Corp. Macedon, NY). Calculations for *in vitro* true digestibility (as received basis) [IVTD] and *in vitro* true digestibility (dry matter basis) [$IVTD_{DM}$] followed Daisy^{II} protocols (Ankom Technology Corp. Macedon, NY; Holden, 1999).

% ADF / NDF (as-received basis) = $100 \times (W_3 - (W_1 \times C_1)) / W_2$

<u>Where:</u> W_1 = Bag tare weight. W_2 = Sample weight. W_3 = Dried weight of the bag with fiber after the extraction process. C_1 = Blank bag correction (average of final oven-dried weight divided by original blank bag weight.)

% IVTD (as received basis) = $100 - (W_3 - (W_1 \times C_1)) \times (100 / W_2)$

% $IVTD_{DM}$ (DM basis) = $100 - (W_3 - (W_1 \times C_1)) \times (100 / (W_2 \times DM))$

Where: W_1 = Bag tare weight. W_2 = Sample weight. W_3 = Final bag weight after In Vitro digestion and sequential Neutral Detergent fiber treatments. C_1 = Blank bag correction (average of final oven-dried weight divided by original blank bag weight.)

IV. RESULTS

Nutrient profile results obtained from SDK Laboratories are in Table 4. All samples were evaluated for DM, CP, EE, Ca, P, and Na percentages. As of the submissions of this thesis, the results for sample 14 are pending.

Table 4: SDK Laboratories Nutrient Compositions

Sample ID	**DM (%)**	**CP (%)**		**EE (%)**		**Ca (%)**		**P (%)**		**Na (%)**	
	As Received	Dry Basis	As Received	Dry Basis	As Received	Dry Basis	As Received	Dry Basis	As Received	Dry Basis	As Received
1	91.40	45.01	41.14	36.28	33.16	2.18	1.99	0.38	0.35	0.07	0.06
2	93.47	25.11	23.47	5.53	5.17	2.73	2.55	0.83	0.78	0.53	0.50
3	94.10	50.90	47.90	10.38	9.77	1.94	1.83	0.90	0.85	0.22	0.21
4	90.21	52.09	46.99	9.67	8.72	1.96	1.77	0.89	0.80	0.22	0.20
5	92.47	80.12	74.09	4.67	4.32	0.11	0.1	0.59	0.55	0.13	0.12
6	91.41	66.40	60.70	21.65	19.79	0.16	0.15	0.67	0.61	0.28	0.26
7	92.21	54.39	50.15	22.20	20.47	0.12	0.11	0.80	0.74	0.22	0.20
8	93.90	38.82	36.45	32.94	30.93	3.39	3.18	0.62	0.58	0.27	0.25
9	95.18	46.36	44.13	33.98	32.34	1.38	1.31	0.57	0.54	0.18	0.17
10	95.01	57.00	54.16	29.97	28.47	0.22	0.21	0.82	0.78	0.38	0.36
11	97.12	69.51	67.51	16.71	16.23	0.16	0.16	0.76	0.74	0.37	0.36
12	96.28	70.15	67.54	15.31	14.74	0.18	0.17	0.89	0.86	0.38	0.37
13	95.76	72.85	69.76	6.00	5.75	0.04	0.04	1.12	1.07	0.23	0.22
14		Waiting on Results									

The overall average CP was 56.05% (DM basis), with the highest between species samples seen in sample 5 (grasshopper meal) with 80.12% and overall lowest from sample 8 (BSFL meal) with 38.82%. Sample 2, BSFL frass, had the lowest overall CP of 25.11%, but cannot be compared to the other samples as it is the frass from the BSFL, not the BSFL itself. The BSFL sample average CP was 44.53% (DM basis). Sample 3, a BSFL pelleted meal, had the highest CP of 52.09%. Sample 8, a mealed product, had the lowest CP of 38.82%. The cricket samples averaged a CP of 65.77%. Sample 12, a finely processed flour of crickets, had the highest CP of 70.15%. While sample 10, the only banded cricket (*Gryllodes sigillatus*) sample, had the lowest CP of 57.0%. The two mealworm samples averaged a CP of 65.12%. Sample 13, a defatted powder, had a DM CP of 72.85, while sample 7, which was less processed, had a CP of 54.39%. Sample 5, the only grasshopper sample, had a CP of 80.12%.

Among species, the EE average was 18.87% (DM basis), the overall highest percentage was seen in Sample 1 (BSFL meal) with 36.28%, and the overall lowest was sample 5 (grasshopper meal) with 4.67%. Between BSFL samples, the average EE was 21.46%, with the highest percentage coming from sample 1 with 36.28%. The sample with the lowest EE was sample 2 with 5.53%. The cricket samples averaged 20.91% EE. The highest EE percentage came from sample 10 with 29.97%, and the lowest percentage from sample 12 with 15.31%. The average EE for mealworm samples was 14%. Sample 7 had the highest EE with 22.20%, and sample 13 had the lowest with 6.00%. The grasshopper sample (5) had an EE of 4.67%.

On a DM basis, the mineral components of the tested edible insect samples were similar to those found in soybean and cottonseed meals. Samples 5, 6, 7, 10, 11, 12, and 13 had average Ca values less than 1%. Samples 3, 4, and 9 had an average Ca less than 2%, and samples 1 and 2 had an average greater than 2%. Only sample 8 had a Ca percentage above 3%. P values of all samples were less than 1%, excluding sample 13, which has a value of 1.12%. All samples had a Na percentage of less than 1%.

In-house chemical composition profiles of DM, OM, NDF, ADF, IVTD, and $IVTD_{DM}$ percentages are detailed in Table 5.

Table 5: In-House Chemical Compositions

Sample ID	**DM (%)**	**OM (%)**	**NDF (%)**	**ADF (%)**	**IVTD (%)**	**$IVTD_{DM}$ (%)**
1	90.37	91.25	40.27	19.15	73.26	70.41
2	92.15	85.88	54.41	19.40	71.56	69.14
3	95.00	90.20	38.68	11.72	81.81	80.86
4	90.29	89.58	41.68	17.75	81.58	79.60
5	92.99	95.85	35.08	40.36	70.32	68.08
6	91.36	93.88	39.66	23.81	74.21	71.77
7	92.56	93.70	37.37	17.19	77.00	75.15
8	94.64	85.39	16.19	15.29	84.81	83.95
9	95.56	92.10	24.32	12.97	79.13	78.16
10	95.59	94.22	25.11	16.59	74.68	73.51
11	98.01	95.57	38.54	18.62	75.60	75.11
12	97.53	94.59	49.84	23.92	73.57	72.90
13	96.23	93.94	55.43	25.40	81.79	81.07
14	95.37	94.36	14.19	9.05	82.79	81.96

The in-house calculated DM average across all insect samples was 94.12%. OM average of all insect samples was 92.18%. The BSFL average OM was 93.34%, the highest sample percentage being 94.36% from sample 14 and the lowest being 85.39% from sample 4. Cricket samples averaged 94.57% for OM, the highest percentage in sample 11 with 95.57%, and the lowest in sample 6 with 93.88%. The two mealworm samples averaged 93.82% OM with less than a 1% difference. The grasshopper sample had an OM of 95.85%.

The overall average NDF for all samples was 36.48%. There was a wide variation among samples, both within and between species. Sample 13 had the highest NDF percentage of 55.43%, and sample 14 had the lowest at 14.19%. The average ADF percentage for all samples was 19.37%. There was also variation in ADF, but not as wide a margin as NDF, excluding major outliers. The highest ADF percentage overall was sample 5 with 40.36%, and the lowest was sample 14 with 9.05%.

For BSFL the average NDF was 32.82%. The ADF average for the BSFL samples was 15.05%. The sample with the highest percentage for both was sample 2 with 54.41% NDF and 19.40% ADF. Among the cricket samples, the average NDF percentage was 38.29% and the average ADF percentage was 20.74%. The highest NDF and ADF were in sample 12 with 49.84% and 23.91%, respectively. While the lowest NDF and ADF were in sample 10 with 25.11% and 16.59%, respectively. The mealworm samples' NDF percentage average was 46.40%. The ADF percentage average was 21.30%. The highest mealworm percentages were for sample 13, with an NDF of 55.43% and ADF of 25.40%. While the lowest was sample 7, with an NDF of 37.37% and ADF of 17.19%. The grasshopper sample, sample 5, had an NDF of 35.08% and an ADF of 40.36%.

The overall IVTD average across all samples was 77.29%. The average $IVTD_{DM}$ across all samples was 75.83%. Sample 8 had the highest percentages of IVTD and $IVTD_{DM}$ overall, with 84.81% and 83.95%, respectively. The lowest overall IVTD and $IVTD_{DM}$ percentages were from sample 5 with 70.32% and 68.08%, respectively. The IVTD average for BSFL was 79.28%, and the $IVTD_{DM}$ average was 77.73%. The sample with the highest percentage for both was sample 8, with an IVTD of 84.81% and $IVTD_{DM}$ of 83.95%. The lowest percentages were from sample 2 with an IVTD of 71.56% and $IVTD_{DM}$ of 69.14%. The cricket samples had an IVTD average of 74.52% and an $IVTD_{DM}$ average of 73.32%. The highest percentage for both was sample 11 with an IVTD of 75.60% and an $IVTD_{DM}$ of 75.11%. The lowest percentage for both was sample 12 with an IVTD of 73.57% and $IVTD_{DM}$ of 72.90%. The two mealworm samples had an average of 79.40% for IVTD and 78.11% for IVTD_{DM}. The higher percentages were from sample 13, with an IVTD of 81.79% and an IVTD_{DM} of 81.07%. Sample 7 had lower percentages, with an IVTD of 77.00% and an IVTD_{DM} of 75.15%. The grasshopper sample (5) had an IVTD of 70.32% and an IVTD_{DM} of 68.08%.

The DM, CP, EE, NDF, ADF, Ca, P, and Na values of soybean and cottonseed meal, conventional protein supplements for cattle feed, are listed in Table 6 as a point of comparison (Nutrient Requirements of Beef Cattle, 2000).

Table 6: Means for the Composition Data of Feeds Commonly Used in Beef Cattle Diets

Feed Name	**DM (%)**	**CP (%)**	**EE (%)**	**NDF (%)**	**ADF (%)**	**Ca (%)**	**P (%)**	**Na (%)**	**IVTD_{DM} (%)**
Soybean Meal	90.9	51.8	1.67	10.3	7.0	0.46	0.73	0.07	77.5
Cottonseed Meal	90.2	46.1	3.15	28.9	17.9	0.10	0.10	0.01	56.6

Adapted from National Research Council, 2000 & Mabjeesh et al. 2000

V. DISCUSSION

This study evaluated fourteen edible insect samples for their nutrient composition, ruminal digestibility, and ultimate potential value for use in cattle feed. The results of this *in vitro* trial suggest that the insect samples were raised and/or processed using various methods based on the nutrient profile data. This data is corroborated by the rearing and processing data delineated by the manufacturing companies and previous studies on nutrient characteristics based on rearing conditions; see Table 2. These factors at least partially explain why insects of the same species have varying degrees of CP, EE, and digestibility characteristics (NDF, ADF, IVTD, and IVTD_{DM}).

Nutrient composition disparities were apparent for CP values between all samples, which have a variation of ± 55%. The BSFL samples, not including the frass sample, varied by ± 14%. The cricket samples only vary by ± 13% and the mealworm samples by ± 15%. The outlier in the samples is the grasshopper meal (5), with a CP of 80.12%. By comparison, sample 13, with the next highest CP, is a defatted mealworm meal, containing 72.85%. This demonstrates the point that species, life stage, and rearing substrate greatly influence the nutrient profile of edible insects (Hopkins et al., 2020).

The variance in EE values are also notable, with a variation among all samples of ± 32%. The EE variances within insect species were more consistent with BSFL varying by ± 20%, cricket by ± 15%, and mealworm by ± 16%. The grasshopper sample was not as much of an outlier, with less than 10% overall EE. However, several other samples (2, 4, 13) also had less than 10% EE. For example, samples 1 and 4 are both BSFL, with the former having 36.28% EE and the latter having 9.67%. Sample 1 was a mealed product with a damp, sand-like texture, while sample 4 was a processed, defatted, and pelleted meal. The variation in EE values most likely has to do with this defatting and processing of the insect products. Samples that were not processed generally had high fat content, which is consistent with insect meals (Lorrette and Sanchez, 2022). While the high fat content of insect meals can be unfavorable for ruminant diets, processing and defatting can make them more bioavailable to target species. The extracted fat or oils also have potential uses in other sectors of industry, including human consumption and biodiesel (Berezina, 2017; Delicato et al., 2021; Lee et al., 2021).

The mineral components of the tested edible insect samples were similar to those found in soybean and cottonseed meals; see Table 6. Samples 5, 6, 7, 10, 11, 12, and 13 had average Ca similar to soybean and cottonseed meals, which average 0.46% and 0.10%, respectively (National Research Council, 2000). Samples 1, 2, 3, 4, 8, and 9 had greater than 1% Ca, with sample 8 having the highest average of 3.39%. Sample 13 had a P of 1.12%, which is slightly higher than that of soybean and cottonseed meal (0.73% and 0.10%, respectively) and all other samples had less than 1% P. Comparatively, all samples had less than 0.5% Na which was higher than the 0.07% average for soybean meal and 0.01% for cottonseed meal.

Neutral detergent fiber (NDF) analysis measured the fiber residues remaining when samples were treated with heat, agitation, and a detergent solution. These residues are predominantly hemicellulose, cellulose, and lignin. High NDF percentages equate to more materials that can be fermented in the rumen but have low nutritional value within a feed sample (Ankom Technology Corp. Macedon, NY). Acid detergent fiber (ADF) analysis measured the remaining fiber residues after samples were treated with heat, agitation, and digested by an acid detergent solution. These remnants are predominantly cellulose and lignin (Ankom Technology Corp. Macedon, NY). ADF is inversely related to digestibility, meaning a low percentage implies a higher energy feedstuff.

Overall, the samples had varying degrees of NDF and ADF indicating the amount of insoluble or indigestible material within the edible insect meal. The NDF range for all samples was ± 41%, and the range for ADF was ± 31%. The most indigestible hemicellulose, cellulose, and lignin remnants were found in sample 13, defatted mealworm meal. Sample 13, however, had a moderate ADF percentage of 25.40%. Hemicellulose remnants increased the overall NDF values across all samples. The hemicellulose most likely came from chitin. The sample with the most indigestible cellulose and lignin remnants was sample 5, grasshopper meal.

Chitin is found in the exoskeleton of insects. It is the most likely cause of the elevated NDF and ADF values. Chitin is a crude protein made of a modified polysaccharide, N-acetyl-D-glucosamine, and is indigestible by monogastrics (Newton et al., 1977). Ruminants can digest chitin to an extent, but it can cause digestibility issues when present in large amounts (Jayanegara et al., 2017). It is recommended that edible insect products be processed to remove excess chitin to increase digestibility and protein availability (Jayanegara et al., 2020).

Throughout all samples, IVTD and $IVTD_{DM}$ were in line with data collected in similar studies (Jayanegara et al., 2017; 2020). These values were also comparable to the $IVTD_{DM}$ averages of soybean and cottonseed meals (77.5% and 56.6%, respectively); see Table 6 (Mabjeesh et al. 2000). All samples had higher $IVTD_{DM}$ averages compared to cottonseed meal; averages for samples 2 and 5 above 60%, for samples 1, 6, 7, 9, 10, 11, and 12 above 70%, and for samples 3, 8, 13, and 14 above 80% $IVTD_{DM}$. This data implies that the ruminal digestibility of the edible insect species tested would be viable in cattle feed, as the cattle could digest and obtain nutrients from said feed.

By comparison, the average NDF and ADF values for soybean meal are 10.3% and 7.0%, respectively (National Research Council, 2000). The average for cottonseed meal is moderately higher, with an NDF of 28.9% and an ADF of 17.9% (National Research Council, 2000). The tested edible insect NDF and ADF values from our study are closer in significance to the cottonseed meal averages. Samples 8, 9, 10, and 14 had NDF values no greater than 25%. The NDF values of samples 3, 4, 5, 7, and 11 were within + 13% of the average for cottonseed meal. Samples 2, 12, and 13 all had a greater than + 20% difference from the cottonseed average. All samples, excluding sample 5, had ADF values of 25% or lower, which falls under the average ADF value for cottonseed meal, but above the average for soybean meal. These averages suggest that edible insect protein products may be more suitable, from a digestible standpoint, to replace cottonseed meal in cattle diets.

VI. CONCLUSION

In conclusion, this *in vitro* study expands upon previous research on edible insect species as an alternative protein source for large ruminant feed. Based on our data, house cricket (*Acheta Domesticus*), banded cricket (*Gryllodes sigillatus*), grasshopper (*Locusta migratoria*), mealworm (*Tenebrio molitor*), and black soldier fly larvae (*Hermetia illucens*) have potential as a protein supplement for cattle. These insect species are widely cultivated, available, and can potentially be integrated into livestock systems. Palatability and willingness to eat feeds containing edible insects is a little-studied aspect of utilizing this novel protein source. Many defatted insect products come in forms that are foreign to large ruminants, which can reduce feed intake. We recommend that additional studies be conducted *in vivo*.

Data from previous studies corroborate the data collected. Toral et al. (2022) utilized three species of mealworm and house cricket meals in Merino ewes successfully. The *in situ* study achieved similar ruminal nitrogen degradation with these insect protein-based diets to that of soybean meal feeds (Toral et al., 2022). The *in vivo* study by Fukuda et al. (2022) using BSFL as a protein supplementation in steers fed low-quality forage suggested that BSFL is a viable protein alternative for large ruminants (Fukuda et al., 2022). Together, this suggests that insect protein can be used successfully in place of traditional protein supplements such as cottonseed or soybean meal.

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