AN *IN VIVO* EVALUATION OF DEFATTED BLACK SOLDIER FLY LARVAE (BSFL) FOR BEEF CATTLE CONSUMING A BASAL DIET OF FORAGE

by

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DEDICATION

To Papa, whose presence I feel every day. Thinking of you always.

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ABSTRACT

Increased food production demands have led to diminished natural resources and exacerbated pressure on livestock producers to increase output. Accordingly, there is a need to identify and investigate alternative livestock feeds that are economical and environmentally sustainable. Insect protein has been identified as an alternative to conventional protein sources, such as soybean meal (SBM), due to insect’s high feed efficiency and comparable protein concentrations. Specifically, black soldier fly larvae (BSFL) has received attention as livestock feed due to its scalability, nutritional value, and other characteristics. Recent feeding trials and economic analyses demonstrate promise for integrating BSFL into the cattle feeding industry. Data collected from this study will be integral to the insect rearing industry and provide a base for future *in vivo* studies investigating the feasibility of BSFL as cattle feed.

I. INTRODUCTION AND REVIEW OF LITERATURE

Introduction

A growing population and improved standard of living in developing countries has increased demand for accessible animal-derived proteins (FAO, 2021). To address this demand, the livestock industry is pressured to increase output while also decreasing the environmental footprint associated with production. Animal feeds have become more expensive due to declining resources exacerbating competition between human food and fuel and animal feed (Barragan-Fonseca et al., 2017). An approach to reducing land and water used for food production is to increase production of alternative sources of feed protein (Souza-Viela et al., 2019). Insect protein has recently been identified as a potential protein source (Wang and Shelomi, 2017).

Insects have comparable crude protein (CP) concentrations to conventional sources used in livestock feed, such as fish meal and soybean meal (SBM) (Khusro et al., 2012), and have favorable growth rates (van Huis, 2013). Insect production requires substantially less land and water than cattle production (van Huis et al., 2013) and insects recycle nutrients and emit less greenhouse gas and ammonia than cattle or pigs (Oonincx et al., 2016) which may alleviate existing pressure of food production on the environment. However, in Western cultures, insects are not readily accepted as human food. A survey by Higa et al. (2021) demonstrated that U.S. consumers had negative attitudes about eating black soldier fly larvae (BSFL) alone and were more willing to consume food products from livestock that had eaten insects rather than directly consuming insects. Previous research indicates BSFL is a potential alternative protein source for beef cattle (Fukuda et al., 2022). By identifying and implementing alternative protein sources for livestock production, milk and meat production systems potentially become more sustainable.

There are many insects being considered for livestock and animal feed. Crickets and mealworms are popular in the West but may not be the most environmentally sustainable insects to raise (Wang and Shelomi, 2017). Further, insects that are difficult to raise and harvest are expensive lowering their economic feasibility (Wang and Shelomi, 2017). Specifically, BSFL is the focus of research due to the ease of output collection, low-value feed substrate required, and nutritional value of resulting biomass.

Black soldier fly larvae (BSFL)

Specie characteristics. Black soldier flies (*Hermetia illucens*) are native throughout the central United States and South America but are now commonly found globally in tropical and temperate climates due to worldwide trade and transportation (Sheppard et al., 1994). Since they are not carriers of disease and adults do not sting or bite, they are not pests (Barragan-Fonseca et al., 2017). Black soldier flies are a holometabulous species - i.e., they undergo a complete metamorphosis starting with an egg then transforming into larvae, pupae, and, finally, an adult.

Since black soldier flies are not active in colder months, commercial rearing takes place in artificial indoor conditions (Zhang et al., 2010). Two weeks are required for BSFL to reach the prepupal stage at 30℃ (Furman et al., 1959) and leave the food substrate to pupate (Sheppard et al., 1994). Black soldier flies have an average life cycle of 40 d (Barragan-Fonseca et al., 2017). The larval development time is 24 d with larval molts occurring every 4 d (Boykin et al., 2021). In the final larval stage, also known as the prepupal stage, feeding slows and the cuticle turns black. Fourteen d are spent as a pupa followed by the shedding of the puparium to become an adult fly (Barragan-Fonseca et al., 2017). Food is not required by adult black soldier flies, but water, sugar, and honey increase their lifespan (Tomberlin and Sheppard, 2002; Rachmawati et al., 2010; Nakamura et al., 2016).

The unique ability of BSFL to self-harvest is known as “self-collection” (Diener et al., 2011). When transitioning from larvae to prepupae, the mouth of the larvae transforms into a hook which signals them to move to a dry environment for pupation (Newton et al., 2005; Diener et al., 2011). Producers rearing BSFL capitalize on this self-collection method by providing wet bedding and a moist food substrate to the BSFL, which forces them to seek a dry area when they reach the pupal phase (Khairuddin et al., 2022). Containment areas where BSFL are reared are lined with ramps allowing the larvae to move to a dry area and, at the end of the ramps, there are vessels where the larvae fall into for collection (Khairuddin et al., 2022). Insect producers often employ this self-collection method because it is not laborious and lowers production costs.

The CP of BSFL is between 37-63% on a dry matter (DM) basis (Barragan-Fonseca et al., 2017). The highest CP content has been demonstrated in five-day old larvae at 61% DM but at a higher moisture content (Rachmawati et al., 2010). Older larvae are easier to harvest, but average CP content drops (Newton et al., 1977). Prepupae, the final larval stage, are higher in CP and have lower chitin content than pupae, suggesting the best larval stage to use for livestock feed may be the prepupal phase, if the goal is to maximize CP yield (Makkar et al., 2014). The dietary essential amino acids in BSFL are greater than SBM, except for lysine (Makkar et al., 2014). Specifically, BSFL has a lysine content of 0.74% (Feedinamics, 2021) which is inferior to SBM, which has 3.38% lysine (NRC, 2001). However, when considering insects as livestock feed, it is important to note that ruminants, including cattle, have unique digestive anatomy eliminating a dietary requirement for essential amino acids. The mineral concentrations of BSFL are higher compared to other insects, including manganese, iron, phosphorous, zinc, copper, and calcium (Makkar et al., 2014). Finally, sodium is present in lower concentrations than other insects (Arango Gutiérrez, 2005).

Defatted BSFL. Ruminant animals (e.g., cattle) do not digest high amounts of fat well (Grummer, 1998). Given that BSFL contains high levels of CP and fat (30% DM), there is potential to extract oil from BSFL for conversion into high-value products, such as biodiesel (Li et al., 2011; Liew et al., 2022), although this process is not currently commercialized. The residual biomass, defatted BSFL, is higher in protein and other nutritional components. Oil derived from BSFL is a viable source for biodiesel production given its favorable fuel properties which are similar to biodiesel from rapeseed oil (Li et al., 2011) and biodiesel production would increase the sustainability of BSFL.

Other potential applications for BSFL oil include an alternative to conventional fat sources in animal feedstuffs (Li et al., 2016) and for skin care applications (Srisombat et al., 2020). Mai et al. (2019) suggests that BSFL oil could also be used as a suitable alternative to palm kernel and coconut oils in cosmetics. The fatty acid profile (Franco et al., 2022) of refined BSFL oil provides a saponification value that can be used in applications as an antimicrobial agent in soaps, shampoos, and hand washing detergents (Oyekunle and Omode, 2008). Further, BSFL could also replace palm kernel and coconut oils for glycine-acyl surfactant synthesis, which can be used in wetting agents, emulsifiers, and foaming agents (Verheyen et al., 2020).

With animal performance factored in, recent cost analyses indicate that BSFL can be economically integrated into aquaculture and poultry production (Oppong, 2017; Onsongo et al., 2018; Rawksi et al., 2021). Further, an economic analysis of BSFL as cattle feed indicates BSFL would be priced closely to conventional feeds, such as SBM (Drewery et al., 2022). Drewery et al. (2022) created a hedonic pricing model to estimate the market value of full-fat BSFL as cattle feed based on the total digestible nutrients (TDN, 109%) and CP (38%) in BSFL. The mean price of BSFL was estimated to be $312.30±4.90/short ton, which was closest to that of SBM, although slightly higher because of higher TDN in BSFL. Compared to whole cottonseed, which BSFL is nutritionally most similar to, BSFL was priced 1.474 times greater because of higher TDN and CP. Drewery et al. (2022) highlighted that TDN and CP are positive drivers of feed pricing. While a pricing model for defatted BSFL does not yet exist, we theorize that the market value that defatted BSFL could command would be comparable to conventional feeds.

Viability of commercially available defatted BSFL likely hinges on its scalability and establishing market streams for the extruded oil. As the commercial insect rearing industry is still emerging, especially in the U.S., economies of scale have not yet been realized and technology is not yet standardized. Therefore, it is unclear at what price-point BSFL and defatted BSFL can be produced and if production will be economically viable when products are marketed as livestock feed.

Waste mitigation. Globally, 33% of food produced is not consumed and wasted, creating both environmental and economic challenges (FAO, 2015). Sewage sludge, a byproduct from biological wastewater treatment plants, is also a major contributor to the accumulation of organic waste. The ability of sewage sludge to be reused or disposed in landfills is limited by the presence of heavy metals and other toxic components present in the waste (Mateo-Sagasta et al., 2015).

Animal manure is another contributor to the growing global issue of organic waste accumulation. Currently, animal manure is managed through land application and integration into soil; however, this can contribute to high levels of N and P in soil and, later, runoff pollution (Raksasat et al., 2020). Alternative management strategies such as compacting or composting are expensive and can also contribute to environmental pollution. The ability to feed on organic waste makes BSFL a sustainable waste management method. Converting waste into feed generates value while also reducing waste (Wang and Shelomi, 2017). Hen rations, pre- and post-consumer food wastes, and animal feces can be used to raise BSFL (Boykin and Mitchell, 2021). However, in the U.S., only BSFL raised on food or feed grade substrates have been approved for salmonids, poultry, and swine (AAFCO, 2021).

While BSFL are considered waste mitigators via their ability to be raised on organic wastes, rearing BSFL creates frass, which is the excrement of BSFL and is a waste product (Jasso, personal communication). Rearing BSFL could result in two to three times more frass produced than that of larval biomass (Ravi et al., 2020; Salomone et al., 2017). Existing research identifies potential uses of frass as fertilizer (Schmitt and de Vries, 2020), compost (Song et al., 2021; Jasso, personal communication), and as animal feed (Yildirim-Aksoy et al., 2020; Maggitt, personal communication). Most of this research focuses on the use of frass as fertilizer. Frass has been successful as a fertilizer given its ability to store C and N in the soil and diminish the concern for atmospheric loss of N and groundwater contamination via N fixation (Lovett et al., 2002). Frass is also safer for humans than chemical fertilizers which sometimes contain harmful substances such as carcinogens (Sharma and Singhvi, 2017). Perhaps with placement and marketing of frass as a fertilizer, compost, and/or animal feed, the economic viability of a defatted BSFL would increase given the potential for three marketable products to be created in production: the BSFL protein, the extruded oil, and the frass.

There is interest in and concern about the accumulation of heavy metals and microbial contaminants in BSFL given their preference for feeding on organic waste. Bessa et al. (2021) demonstrated that the substrate BSFL are reared on directly influences heavy metal accumulation in BSFL. Further, Diener (2010) identified cadmium, lead, and zinc in BSFL reared on organic waste. There are currently no regulations regarding the limits of heavy metals in BSFL that are safe for human consumption. However, Bessa et al. (2021) used contamination guidelines for crustaceans, fish, meat, and mealworms as a benchmark and reported that the heavy metal content of BSFL was far below the maximum levels for arsenic, cadmium, tin, mercury, and lead. In the U.S., AAFCO (2021) restricts BSFL entering the animal feed chain to be reared on feed grade or better substrates only. Therefore, heavy metal content of commercially reared BSFL in the U.S. would not be a concern given the lack of heavy metals in the feed substrate. However, this restriction set by AAFCO limits the sustainability of BSFL as livestock feed.

The pH (9.3) of the BSFL gut is relatively basic, which prevents bacteria like *E. coli* and *Salmonella* from surviving (Gold et al., 2018). However, accumulation of microbial contaminants can be further reduced with quality control implemented during rearing and harvesting (Diener, 2010). Blanching, a post-harvesting process, is an effective and economical tool to reduce microbial contaminant and heavy metal accumulation in BSFL. Bessa et al. (2021) demonstrated that blanching reduced microbial loads to a level safe for human consumption. Alternatively, high-pressure pasteurizing can also decontaminate BSFL, although not as effectively as blanching (Campbell et al., 2020). These quality control practices must be implemented with care to the resulting end-product; however, high-pressure pasteurization has reduced organoleptic attributes of BSFL (Bolumar et al., 2015).

BSFL as animal feed. Currently, BSFL is approved for commercial feeding in the U.S. for swine, poultry, some fish and, most recently, dogs (AAFCO, 2021). Further, BSFL digestibility studies have been conducted for swine (Newton et al., 1977; Driemeyer, 2016; Chia et al., 2021; Kar et al., 2021), poultry (Elwert et al., 2010; Al-Qazzaz et al., 2016; Cullere et al., 2016; Maurer et al., 2015; Cockroft, 2018; Heuel et al., 2022), fish (Bondari and Sheppard, 1987;

St-Hilaire et al., 2007; Sealey et al., 2011; Kroeckel et al., 2012; Lock et al., 2016; Dumas et al., 2018), exotic animals (Bodri and Cole, 2007; Dierenfeld and King, 2008) cats (Paßlack and Zentek, 2018), dogs (Kieronczyk et al., 2018; Freel et al., 2021) and cattle (Fukuda et al., 2022; Nekrasov et al., 2022). **Tables 1-5** include existing digestibility studies on the effect of dietary inclusion of defatted and full-fat BSFL.

Previous research indicates BSFL can be successfully incorporated in swine diets (**Table 1**) due to its high concentration of CP (Makkar et al., 2014). Chia et al. (2021) fed four diets of full-fat BSFL, 25, 50, 75, and 100% as a replacement to fish meal in growing pigs in the finishing phase and found that higher dietary inclusion of BSFL (50-100%) resulted in higher body weight, feed conversion, and carcass yield than fish meal. Full-fat BSFL is well suited for pigs in the finisher phase given its high CP and fat content which provides the necessary protein and energy needed to support growth during this production phase (Crosbie et al., 2020; Nekrasov et al., 2018). Dietary inclusion of BSFL was effective among recently weaned pigs (Kar et al., 2021) when compared to SBM at isonitrogenous levels (160 g CP/kg as-fed basis). Supplementing BSFL encouraged the development of the intestinal microbiome and improved intestinal health in weaned pigs (Kar et al., 2021). Ultimately, BSFL can replace conventional proteins in the diets of growing and finishing pigs given its high CP and fat which is suitable for swine diets.

Table 1. Effect of dietary inclusion of defatted and full-fat black soldier fly larvae (BSFL) in swine			
Reference	**Animal**	**Dietary inclusion**	**Observations**
Newton et al., 1977	Pigs	0, 33% BSFL	BSFL was significantly less digestible and reduced N balance; BSFL significantly increased intake
Driemeyer, 2016	Piglets	0, 3.5% BSFL	No significant differences in intake, weight gain, or immunological blood parameters
Chia et al., 2021	Pigs	25, 50, 75, 100% BSFL	Higher levels of BSFL (50-100%) resulted in higher body weight, feed conversion, and carcass yield
Kar et al. 2021	Pigs	CP 160 g/kg (as-fed basis) BSFL	Inclusion of BSFL in the diet resulted in encouragement of the development of the intestinal microbiome and improved intestinal health

Researchers hypothesize that BSFL may hinder digestibility in poultry due to chitin, a complex carbohydrate found in the exoskeleton of insects (Tabata et al., 2017), however, previous feeding trials incorporating BSFL in the ration did not observe a depression in digestibility (**Table 2;** Elwert et al., 2010; Al-Qazzaz et al., 2016; Cullere et al., 2016). This may be attributed to the recent discovery of an acidic chitinase in the poultry gut that potentially degrades chitin (Tabata et al., 2017), but further research is needed to explore this hypothesis. Previous research has demonstrated that partial supplementation of BSFL in broiler diets did not affect production performance, feed efficiency, mortality, or carcass quality versus a conventional commercial diet (Arango Gutiérrez, 2005; Zhang et al., 2014; Cullere et al., 2016). According to the broiler mineral requirements proposed by the National Research Council (NRC, 1994), BSFL has a suitable mineral content for poultry nutrition. Heuel et al. (2022) fed partially defatted BSFL meal and BSFL fat to broilers and determined that BSFL may perform better than soybean protein; however, carcass fat from BSFL-fed broilers contained high levels of lauric acid, which may negatively affect meat quality.

Table 2. Effect of dietary inclusion of defatted and full-fat black soldier fly larvae (BSFL) in poultry

Elwert et al., 2010	Broiler chickens	0, 4.7, 5.4, 6.6% partly defatted BSFL; level of defatting was not standardized	No significant difference in weight gain or feed efficiency with higher inclusions of BSFL; significantly reduced performance at lower inclusion
Al-Qazzaz et al., 2016	Layer chickens	0, 1, and 5% BSFL	No significant difference in feed intake, weight gain, or hatchability
Cullere et al., 2016	Broiler quails	0, 10, 15% defatted BSFL	No significant difference in apparent dry matter digestibility, feed preference, productivity, mortality, or carcass attributes
Maurer et al., 2015	Layer chickens	0, 12, 24% partly defatted BSFL	No significant difference in feed intake, egg production, health, or mortality
Cockcroft, 2018	Broiler chickens	0, 15% BSFL; three types of BSFL: full-fat, defatted through extrusion, or defatted through dry- rendering	Processing affected response to BSFL; full-fat and extruded defatted significantly improved productivity
Heuel et al., 2022	Broiler chickens	200 g/kg (DM) partially defatted BSFL	Broilers grew similarly among all diets; high-quality BSFL can replace soybean proteins

As insects are a natural component of fish diets, there has been much and early interest in investigating BSFL for commercial aquaculture production (**Table 3**). Dumas et al. (2018) fed four diets containing varying levels of partially defatted BSFL (0, 7, 13, and 26%) to rainbow trout and found that, while BSFL decreased weight gain, it increased feed efficiency. Further, this study identified the maximum inclusion of BSFL in trout diets to be 13%. Digestibility and retention of hydroxyproline, an amino acid related to collagen production, also increased in trout supplemented BSFL (Dumas et al., 2018).

Table 3. Effect of dietary inclusion of defatted and full-fat black soldier fly larvae (BSFL) in aquaculture

Reference	**Animal**	**Dietary inclusion**	**Observations**
Bondari & Sheppard, 1987	Channel catfish	0 or 10% BSFL	BSFL significantly reduced weight gain in accelerated feeding but not relaxed feeding situations
St-Hilaire et al., 2007	Rainbow trout	0, 15, 35% BSFL	BSFL significantly reduced feed intake and weight gain but did not adverse effect feed efficiency
Sealey et al., 2011	Rainbow trout	0, 16%, 18%, 33%, 35% BSFL; two types of BSFL from different rearing methods	No significant difference in growth with one type of BSFL but reduced with the other; no significant difference in sensory measures
Kroeckel et al., 2012	Turbot	0, 17, 33, 49, 64, 76% defatted BSFL	Significantly decreased nutrient retention efficiency with increased BSFL
Lock et al., 2016	Atlantic salmon	0, 5, 10, 25% BSFL	No significant difference in sensory measures; BSFL decreased feed intake but increased feed efficiency
Dumas et al., 2018	Rainbow trout	0, 7, 13, 26% partly defatted BSFL	BSFL decreased weight gain but increased feed efficiency; no difference in mortality

Dietary inclusion of BSFL has been documented in exotic animals, including alligators and frogs (**Table 4**; Bodri and Cole, 2007; Dierenfeld and King, 2008), typically in live or dehydrated form (Valdes et al., 2022). Animals with alternative feeding strategies, such as chewing prey versus swallowing prey whole (Dierenfeld and King, 2008), may have altered digestibility and palatability when consuming BSFL. Dierenfeld and King (2008) theorized that animals which chew prey may have more adequate disruption to the exoskeleton and, thus, improved digestibility as compared to animals that swallow their prey whole.

Table 4. Effect of dietary inclusion of defatted and full-fat black soldier fly larvae (BSFL) in exotic animals

Reference	**Animal**	**Dietary inclusion**	**Observations**
Bodri and Cole, 2007	Alligator hatchlings	0, 100% BSFL	BSFL significantly stunted weight and length but improved feed efficiency
Dierenfeld and King, 2008	Mountain chicken frogs	100% BSFL	BSFL was less digestible but can supply high levels of minerals without the need for additional Ca supplementation

Black soldier flies have become popular as a feed ingredient in dog and cat foods (**Table 5**; Valdes et al., 2022). Insect-based foods for dogs and cats are usually in pellet form or as treats such as cookies, bars, and dental sticks; however, there are also wet options such as pate and souffle (Valdes et al., 2022). Regarding the preference of different insect-containing dog foods, the smell of mealworms was preferred by male dogs while female dogs preferred the scent of Turkestan cockroaches; however, the black soldier fly and tropical house cricket were also “attractive” to dogs (Kieronczyk et al., 2018). Paßlack and Zentek (2018) evaluated two BSFL-containing diets, 22% BSFL and 35% BSFL, for adult cats and observed that 6-week supplementation of BSFL resulted in good tolerance and overall dietary acceptance; however, a small number of cats experienced low feed intake or diet rejection. Freel et al. (2021) examined defatted BSFL and BSFL fat as a food for adult beagle dogs with the diets including 1%, 2.5% and 5% fat and 5%, 10% and 20% insect meal. General health, acceptability, and fecal quality remained optimal throughout the 28-d study. While there have been many successes in including insects in pet food diets, barriers to commercialization still exist including high input costs (Veldkamp et al., 2012), food safety and allergenic risks (Jarett et al., 2019; Lei et al., 2019), and an overall lack of research on the inclusion of insect meals in dog and cat foods (Oonincx and de Boer, 2012).

Table 5. Evaluation of defatted and full-fat black soldier fly larvae (BSFL) for dogs and cats

Reference	**Animal**	**Dietary inclusion**	**Observations**
Kieronczyk et al., 2018	Adult dogs	100 g BSFL	Smell of BSFL was attractive to dogs
Paßlack and Zentek, 2018	Adult cats	22 and 35% BSFL	BSFL supplementation resulted in good tolerance and overall dietary acceptance; small number of cats experienced low feed intake or diet rejection
Freel at al., 2021	Adult dogs	1, 2.5, and 5% fat; 5, 10, and 20% defatted BSFL	General health, acceptability, and fecal quality were optimal

While BSFL has been well-evaluated in monogastric livestock and companion animals, there is a lack of research in ruminant animals, perhaps because they are herbivores. Jayanegara et al. (2017) conducted an *in vitro* study to evaluate ruminal digestibility of BSFL as compared to SBM; they concluded this substitution reduced ruminal methane production at the expense of digestibility, which is to be expected as a decrease in digestibility results from a decrease in fermentation and thus, methane. Similarly, Rea (2022) conducted an *in vitro* study to assess edible insects as potential alternative cattle feeds. They concluded that BSFL, mealworms, crickets, and grasshoppers have potential to replace traditional feeds. Pelleted, defatted BSFL had the highest CP among all samples (52 %) and another sample of BSFL had the highest fat content at 18.9%. Additionally, the mineral levels of the edible insects in this study were comparable to SBM and cottonseed meal (CSM). Two studies have assessed BSFL in cattle diets (**Table 6**; Fukuda et al., 2022; Nekrasov et al., 2022). Fukuda et al. (2022) concluded that full-fat BSFL stimulates forage intake and digestion for beef steers at the same rate as an isonitrogenous level of a blend of conventional proteins. Nekrasov et al. (2022) fed BSFL fat to lactating dairy cows and observed improved immunity, rumen digestion, metabolism, and milk productivity, with the most favorable outcomes observed at 100 g • hd^{-1} • d^{-1} of BSFL fat.

Table 6. Effect of dietary inclusion of defatted and full-fat black soldier fly larvae (BSFL) in cattle

Reference	**Animal**	**Dietary inclusion**	**Observations**
Fukuda et al., 2022	Beef steers	A supplement with full-fat BSFL as the main protein source (CP 23.5%)	BSFL stimulated forage intake and digestion at the same rate as a conventional feed
Nekrasov et al., 2022	Dairy cows	10 and 100 g/head/day BSFL fat	BSFL fat improved immunity, rumen digestion, metabolism, and milk productivity

As mentioned previously, insect exoskeletons, including that of BSFL, contain a complex carbohydrate called chitin. Chitin content of BSFL is comparable to that of other insects, ranging from <1-9% DM (Spranghers et al., 2017; Caligiani et al., 2017). Diener et al. (2010) and Kroeckel et al. (2012) reported that BSFL has a chitin content of 8.7-9.6%. Chitinolytic bacteria have been cultured from the rumen (Kopecny et al., 1996) demonstrating that rumen microbes digest chitin (Patton, 1971). Ruminant animals rely on microbial fermentation in the reticulorumen to digest the majority of their fibrous diet. This digestive strategy allows chitin to be fermented, yielding VFAs, an energy source for the animal. An *in vitro* study by Jayanegara et al. (2020) reported that chitosan, a derivative of chitin from BSFL, decreased ruminal feed degradation. Based on this data, and the hypothesis that chitin negatively affects digestion in ruminant livestock (Fukuda et al., 2022), the chitin content of BSFL may depress digestibility and, thus, pose a challenge for cattle consuming BSFL, but not to as great of an extent as it would for monogastric livestock.

Protein supplementation to low-quality forage

Beef cattle producers must balance economic viability with animal nutrient requirements. Producers on rangelands with poor forage quality due to maturity, soil fertility, and plant species must supplement low-quality forage with adequate protein (DelCurto et al., 2000). Protein should be supplemented to cattle when the CP of the basal forage is less than 7% (Campling, 1970). Protein supplementation for cattle consuming low-quality forage is critical due to the decreased digestibility of the forage which results in a decrease in the availability of N to the microbial population (Allden, 1981). Further, protein supplementation to low-quality forage should address seasonal nutrient deficiencies and precipitation fluctuations. Ultimately, protein supplementation to low-quality forage can prevent loss of body weight and body condition score (DelCurto et al., 2000).

Effect of protein supplementation on low-quality forage intake and digestibility. Supplementing protein to cattle consuming low-quality forage increases forage utilization (Drewery et al., 2014). Moore and Kunkle (1995) demonstrated that forage intake was positively correlated with forage CP content (**Figure 1**). When low-quality forage is consumed, N is the first limiting factor to microbial growth (Koster et al., 1996). As microbial N requirements are addressed through protein supplementation, rumen microbes proliferate, resulting in increased forage degradation, intake, and microbial N flow to the small intestine (Wickersham et al., 2008). When supplemental N is provided to cattle consuming low-quality forage, total tract organic matter digestibility (OMD) increases as a result of rumen microbial proliferation and increased fermentation (Olson et al., 1999; Klevesahl et al., 2003; Wickersham et al., 2008).

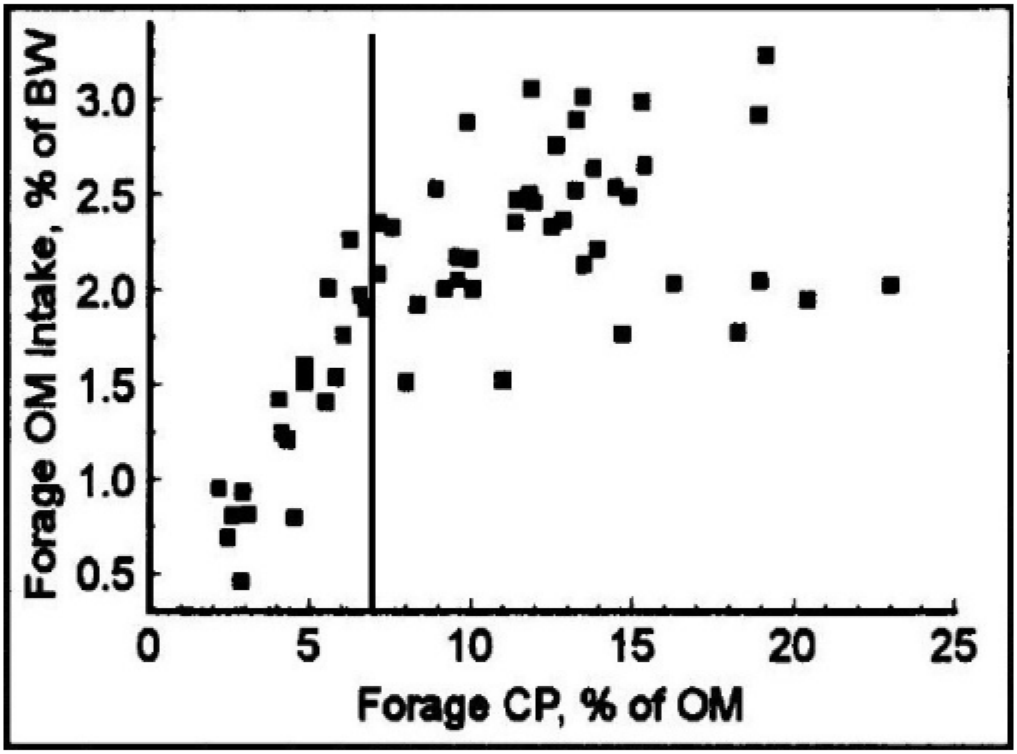

Figure 1. The relationship between increased forage crude protein and forage OM intake (Moore and Kunkle, 1995)

High-protein feeds, such as SBM and CSM, are commonly used as a source of protein due to their CP content of 50 and 45%, respectively (DelCurto et al., 2000). Additionally, their energy densities are similar to those of cereal grains and, thus, they can provide significant energy to the basal diet (DelCurto et al., 2000). Other oilseed supplements include canola, sunflower, rapeseed, and crambe meals. However, oilseed feeds are often expensive; thus, identifying alternative protein supplements would be beneficial to cattle producers.

Degradable versus undegradable intake protein. Feed CP can be divided into rumen degradable protein (RDP) and rumen undegradable protein (RUP; Bohnert et al., 2011). Ruminal microbes degrade RDP in the rumen whereas RUP is enzymatically digested and absorbed in the abomasum and small intestine (NRC, 1985). The ability of ruminants to utilize low-quality forage is dependent on cellulolytic activity of microbes in the rumen. These microbes rely on RDP to meet their nutritional needs (Allison, 1969), allowing them to proliferate.

Ultimately, RDP is more beneficial than RUP when supplemented to low-quality forage. Bandyk et al. (2001) compared RDP to RUP and found that, when protein was ruminally infused (i.e., RDP), total digestible organic matter intake (TDOMI) increased compared to an isonitrogenous amount of post-ruminally infused protein (i.e., RUP). Further, RDP administration resulted in an approximate two-fold increase in forage intake compared to RUP administration (Bandyk et al., 2001).

Infrequent supplementation of RDP can create an excess of N which is transported to the liver and metabolized to urea N which can be excreted via urine or recycled back to the gastrointestinal tract (Leng and Nolan, 1984). Amino acids and peptides are liberated from RUP and absorbed in the small intestine for the host animal to directly meet their nutritional needs. Accordingly, RUP may be better suited for infrequent supplementation as compared to RDP (Leng and Nolan, 1984).

II. BLACK SOLDIER FLY LARVAE (BSFL) AS AN ALTERNATIVE PROTEIN SOURCE FOR BEEF CATTLE CONSUMING FORAGE: PREFERENCE, INTAKE, AND DIGESTION

Abstract

Livestock producers have been challenged to increase output to feed a growing population while also minimizing the environmental footprint of production. Increasing interest in identifying alternative protein sources that do not compete with the human food sector, are sustainable, and can be fed to livestock has motivated research into black soldier fly larvae (BSFL) as a potential feed. Compared to traditional feeds, such as soybean meal (SBM), BSFL has comparable protein and requires less land and water to produce. The objective of our experiment was to assess the viability of defatted BSFL as an alternative protein source for beef cattle consuming forage. Two experiments were conducted using ruminally cannulated steers fed low-quality forage (<7% CP) in 5 × 5 Latin square designs. Experiment 1 was designed to determine if cattle readily consume BSFL as a protein supplement and included five 5-d periods with 3-d for washout and 2-d for measurement of supplement intake and preference. There were five treatments delivered in addition to a basal forage diet: 100% SBM; 75% SBM + 25% BSFL; 50% SBM + 50% BSFL; 25% SBM + 75% BSFL; 100% BSFL. Supplement intake did not differ between treatments (P=0.45) nor was there a treatment × day interaction (P≥0.45) for supplement or forage intake or a treatment effect for hay (P=0.65) intake. Experiment 2 determined the effect of graded BSFL supplementation on forage intake and digestion and included five 14-d periods with 8-d for treatment adaptation, 5-d for measurement of intake and digestion, and 1-d for determination of ruminal fermentation. There were four treatments of supplemental BSFL provided at graded N levels: 0, 50, 100, or 150 mg N/kg BW and one level of SBM at 100 mg N/kg BW. Graded levels of BSFL supplementation linearly increased forage organic matter intake (FOMI; P=0.04), total organic matter intake (TOMI; P<0.01), total digestible organic matter intake (TDOMI; $P<0.01$), dry matter digestibility (DMD; $P=0.01$), and organic matter digestibility (OMD; $P=0.02$). In comparing isonitrogenous levels of BSFL and SBM, there were no significant differences ($P\geq0.17$) in any measures of intake or digestibility between the supplements. Our data indicate that, at isonitrogenous levels, BSFL performs as well as SBM. Therefore, BSFL can replace SBM as a sustainable protein option in beef cattle diets without sacrificing forage intake or digestibility.

Introduction

To alleviate environmental strains created by crop production and human expansion, there is interest in identifying alternative feed sources for livestock (van Huis et al., 2013). Black soldier fly larvae (BSFL) has previously been identified as a viable protein source for swine, poultry, aquaculture, exotic animals, dogs, cats, and most recently, cattle (Boykin et al., 2021; Makkar et al., 2014; Zhang et al., 2014; Cullere et al., 2016; Fukuda et al., 2022). Currently, BSFL is commercially approved for swine, poultry, aquaculture, and dogs in the U.S. (AAFCO, 2021). Black soldier flies have the capacity to be reared on organic waste that would otherwise be disposed of, making them a sustainable option for waste mitigation. However, only BSFL raised on food or feed grade substrates are commercially approved as animal feed in the U.S. (AAFCO, 2021). Compared to traditional feeds, such as soybean meal (SBM), BSFL requires significantly less land and water; provided the same land and water inputs, 130× BSFL protein can be produced as compared to SBM protein (Matthews, 2021).

Livestock producers feeding low-quality forage (<7% crude protein, CP) to cattle must supplement protein to maintain animal performance. Protein supplementation is critical as low-quality forage does not provide enough N to address microbial requirements and promote microbial proliferation and VFA production (Scott and Hibberd, 1990; Koster et al., 1996;

Wickersham et al., 2008). Ultimately, a N deficiency reduces energy availability to the animal, reducing animal performance.

In a previous *in vivo* evaluation of full-fat BSFL for beef steers consuming low-quality forage, a BSFL-containing supplement stimulated forage organic matter intake (FOMI) to a similar extent, but total digestible organic matter intake (TDOMI) to a lesser extent, than a mixed supplement based on conventional protein sources (Fukuda et al., 2022). The observed depression in TDOMI for the BSFL-containing supplement was attributed to the chitin and/or fat content of BSFL (Fukuda et al., 2022), given that ruminant animals do not tolerate high-fat diets well (Grummer, 1998) and BSFL has a high chitin content (Jayanegara et al., 2017).

The purpose of this project was to examine defatted BSFL as an alternative protein source for cattle consuming low-quality forage (<7% CP). To build on the previous research of Fukuda et al. (2022), the objectives of our study were to: 1) evaluate steer acceptance and preference of defatted BSFL versus SBM; and 2) examine low-quality forage utilization in steers supplemented with graded levels of defatted BSFL compared to an isonitrogenous level of a conventional protein source, SBM.

We hypothesized that cattle will readily consume BSFL as a stand-alone and blended supplement and there will be no preference differences when compared to SBM. We also expected defatted BSFL fed to cattle consuming a basal diet of low-quality forage to elicit similar forage utilization responses as a conventional protein source.

Materials and methods

The experimental design and procedures were approved by the Institutional Animal Care and Use Committee at Texas State University (#7726). Two experiments were conducted to evaluate the potential for BSFL to be fed as an alternative protein source to beef cattle consuming low-quality forage. The BSFL used in our study was processed by River Road Research. Fat extrusion of BSFL was conducted via a liquid CO_2 extraction and pelleted prior to incorporation into steer diets.

Experiment 1: Preference. Experiment 1 utilized five steers in a 5 × 5 Latin square to determine if cattle accept and/or prefer BSFL as a stand-alone or blended protein supplement, as compared to SBM. Periods were as follows: a 3-d washout period where steers were supplemented only cottonseed meal (CSM) and a 2-d experimental period where treatments were offered. A basal diet of forage (**Table 7;** 3.5% CP, 81% NDF) offered at 130% of the previous 5-d consumption was consumed by the steers. Dietary treatments were: 100% SBM; 75% SBM : 25% BSFL; 50% SBM : 50% BSFL; 25% SBM : 75% BSFL; 100% BSFL.

Steers were housed individually in a partially enclosed barn. A feed bunk was provided to each steer in each pen. Steers were provided CSM in the feed bunk during the washout period. The treatments were offered on d 4 and 5. Initially, 1.36 kg of each treatment was offered. Bunks were monitored in 10-min increments and treatments were added in 0.45 kg increments as necessary once the initial treatment was completely consumed to ensure availability of feed did not limit intake. After 1 h, 10-min disappearance and refusals were recorded.

Hay and supplement samples were dried at 55°C in a forced-air oven for 96 h, allowed to air-equilibrate, and weighed to determine partial dry matter (PDM). Samples were then composited across days by period and ground with a Wiley Mill to pass a 1-mm screen. Ground samples were dried at 105°C for DM determination. Organic matter (OM) was determined as the loss in dry weight upon combustion for 8 h at 450°C. Nitrogen was measured through Dumas combustion and CP was calculated as N × 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analysis were performed using an Ankom Fiber analyzer with Na sulfite and amylase omitted and without correction for residual ash.

Experiment 2: Intake and digestion. Experiment 2 was designed as described by Drewery et al. (2014). Five steers (283 ± 35.6 kg BW) were fitted with ruminal cannulas and used in a 5 × 5 Latin square to determine intake and digestion as a result of protein supplementation to a low-quality forage diet of King Ranch bluestem hay (*Bothriochloa ischaemum*) (**Table 9**; 5.3% CP, 80% NDF). There were four treatments of supplemental defatted BSFL provided at graded N levels: 0, 50, 100, or 150 mg N/kg BW (CON, 50B, 100B, and 150B, respectively) and one level of a conventional protein supplement, SBM, at 100 mg N/kg BW (100S). Level of protein supplementation was based on previous literature where this range maximized low-quality forage utilization by cattle (Koster et al., 1996; Wickersham et al., 2008; Drewery et al., 2014).

Steers were housed individually in a partially enclosed barn. Low-quality forage was provided at 130% of the previous 5-d average consumption. The five experimental periods were set up as follows: 8-d for treatment adaptation, 5-d for measurement of intake and digestibility, and 1-d for determination of ruminal fermentation parameters.

Observations on d 9 through 13 were used to calculate intake and digestion. Hay and supplement samples were collected d 9 to 12, feed refusals (orts) were collected d 10 to 13, and fecal grab samples were collected every 8 hours on d 10 to 13. Fecal sampling time increased by 2 h every day to obtain a representative sample. To calculate digestibility, acid detergent insoluble ash (ADIA) was used as an internal marker to calculate fecal production on a DM basis. On d 14, a rumen fermentation profile was conducted.

Prior to feeding (0 h) and at 4 h intervals for 20 h after feeding, rumen fluid was collected using a suction strainer (Raun and Burroughs, 1962). The pH of each sample at the time of sampling was measured using a pH meter. Subsamples of ruminal fluid were prepared and frozen at -20°C for volatile fatty acid (VFA) and ammonia N concentration determinations. Eight mL of rumen fluid was combined with 2 mL of 25% *m*-phosphoric acid for volatile fatty acid (VFA) analysis and 9 mL of rumen fluid was combined with 1 mL of 1 N HCL for ammonia-N determination. Rumen fluid samples were centrifuged at 20,000 × *g* for 20 minutes after thawing. Measurement of VFA concentrations was conducted by gas chromatography. Measurement of ammonia N was conducted by colorimetric procedures using a UV-vis.

Forage and supplement samples were composited across day by period. Hay refusals and fecal samples were composited by steer across day within period. Supplement, forage, hay refusals, and fecal samples were analyzed for PDM, DM, OM, NDF, ADF, and CP as described in Experiment 1. All samples were analyzed for ADIA determination by combusting ADF residues for 8 h at 450°C.

Formulas

Digestibility was calculated as: [1-(output of nutrient/intake of nutrient)] × 100. Daily fecal production was estimated as, kg = [DMI × ($ADIA_d$)] / ($ADIA_f$) where DMI = dry matter intake, $ADIA_d$ = dietary ADIA concentration (% DM), and $ADIA_f$ = fecal ADIA concentration (% DM).

Statistical analysis

For Experiment 1, 1 h disappearance of BSFL, SBM, and mixed supplements were analyzed using the MIXED procedure in SAS v9.4. Terms in the model included treatment, period, day, and treatment × day, with steer and treatment × period × steer as the random terms. The repeated term was day with treatment × steer as the subject and the specified covariance structure was selected based on the Bayesian Information Criterion. The LSMEANS option was used to calculate treatment means.

For Experiment 2, analysis of intake and digestion was conducted with the MIXED procedure in SAS v9.4. Terms in the model were treatment and period with steer as a random effect. Analysis of rumen fermentation parameters was conducted using the MIXED procedure. Terms in the model included treatment, period, hour, and hour × treatment with steer and treatment × period × steer included as random terms. The repeated term was hour with treatment × steer as the subject. Calculation of treatment means was performed with the LSMEANS option and separated using linear and quadratic polynomial orthogonal contrasts for graded levels of BSFL and using contrasts between isonitrogenous levels of BSFL (100B) and SBM (100S).

Results

Experiment 1: Preference. In experiment 1, steers were offered one of five experimental treatments to a basal diet of forage to determine if cattle readily consume and prefer BSFL as a protein supplement.

Intake of BSFL, SBM, and mixed supplements are presented in **Table 8**. Steers consumed an average of 1.53, 1.16, 1.54, 1.30, and 0.73 kg/d of 100% SBM, 75% SBM : 25% BSFL, 50% SBM : 50% BSFL, 25% SBM : 75% BSFL, and 100% BSFL, respectively. There was not a treatment × day interaction ($P \geq 0.45$) on supplement or forage intake. The effect of treatment on supplement intake was not statistically significant ($P=0.45$); however, there were numerical differences between consumption of the 100% BSFL treatment versus the supplements containing SBM that may have biological and practical value. Period ($P=0.38$) did not significantly affect supplement intake although there was an effect of day ($P=0.04$) with steers consuming an average of 0.24 kg more supplement on d 5 versus 4. There was not a treatment ($P=0.65$) effect on forage intake, although there were effects of period ($P<0.01$) and day ($P \leq 0.01$) with steers consuming the most forage in period 5 and on d 4.

Table 7. Chemical composition of forage, black soldier fly larvae (BSFL), and soybean meal (SBM) in Experiment 1

Item	Hay	BSFL	SBM
	---------------%DM----------------		
Organic matter	90.0	89.0	94.0
Crude protein	3.5	50.9	49.1
Neutral detergent fiber	81.0	61.0	43.0
Acid detergent fiber	49.0	36.0	27.0

Table 8. Intake of black soldier fly larvae (BSFL), soybean meal (SBM), and mixed supplements by beef steers (*n*=5) consuming forage

Treatment[1]	Supplement intake, kg/d[1]	Hay intake, kg/d[2]
100% SBM	1.53	4.85
75% SBM:25% BSFL	1.16	4.55
50% SBM:50% BSFL	1.54	4.54
25% SBM:75% BSFL	1.30	4.64
100% BSFL	0.73	4.75

[1]SEM ± 0.41; P=0.45; [2]SEM ± 0.34; P=0.65

Experiment 2: Intake and digestion. In experiment 2, steers were offered graded levels of defatted BSFL (CON, 50B, 100B, 150B) to evaluate the effect on supplementation on intake and digestion. An isonitrogenous level of SBM (100S) was also offered. Chemical composition of hay and supplements are presented in **Table 9**. Hay offered was 5.26% CP whereas the CP of BSFL and SBM were 66.31 and 49.35% CP, respectively.

Level of BSFL supplementation. Increased supplementation of BSFL, from 0 to 150 mg N/kg BW, as per the design, linearly increased (P<0.01; **Table 10**) supplement organic matter intake (SOMI) to a peak of 0.43 kg/d. Forage organic matter intake (FOMI) also linearly increased (P=0.04) in response to graded levels of supplemental BSFL, from 1.98 kg/d to 2.25 kg/d for CON and 150B, respectively. Total organic matter intake (TOMI) linearly increased (P<0.01) with increasing supplementation of BSFL from 1.98 kg/d for CON to 2.66 kg/d for 150B. Total digestible organic matter intake (TDOMI) also linearly increased (P<0.01) as BSFL supplementation increased, from 1.09 kg/d for CON to 1.62 kg/d for 150B.

Organic matter digestibility (OMD) linearly increased (*P*=0.02) from 54.9% for CON to 61.0% for steers supplemented 150B. There was not a linear (*P*=0.57) nor quadratic (*P*=0.18) response of neutral detergent fiber digestibility (NDFD) for steers supplemented increasing levels of BSFL. Overall NDFD mean across BSFL treatments was 60.37%.

Comparison of BSFL and SBM. In comparing isonitrogenous (100 mg N/kg BW) levels of BSFL (100B) and SBM (100S), SOMI was similar (*P*=0.58) for steers supplemented with either 100B or 100S (0.27 kg/d and 0.29 kg/d, respectively). Similarly, FOMI was not significantly affected by protein source (*P*=0.45). TOMI was similar (*P*=0.41) for steers supplemented with either 100B or 100S (2.51 kg/d and 2.65 kg/d, respectively). TDOMI was not significantly different (*P*=0.17) between protein sources, 0.79 and 0.87 kg/d for 100B and 100S, respectively. Further, OMD and NDFD were not different (*P*≥0.48) between isonitrogenous levels of BSFL and SBM.

Table 9. Chemical composition of forage, black soldier fly larvae (BSFL), and soybean meal (SBM) in Experiment 2

Item	Hay	BSFL	SBM
	---------------%DM----------------		
Organic matter	86.0	88.0	91.0
Crude protein	5.3	66.3	49.4
Neutral detergent fiber	80.0	57.0	36.0
Acid detergent fiber	64.0	32.0	25.0
Acid detergent insoluble ash	7.0	2.0	3.0

Table 10. Effect of black soldier fly larvae (BSFL) or soybean meal (SBM) on intake and digestion (Exp 2) in beef steers (n = 5) consuming low-quality forage

	Treatment[1]						Contrast *P*-values		
	CON	50B	100B	150B	100S	SEM	Linear	Quadratic	100B vs 100S
Organic matter intake, kg/d									
Supplement	0.00	0.13	0.27	0.40	0.29	0.02	<0.01	0.93	0.58
Forage	1.98	2.01	2.24	2.25	2.36	0.11	0.04	0.96	0.45
Total	1.98	2.14	2.51	2.66	2.65	0.11	<0.01	0.97	0.41
Digestible	1.09	1.20	1.41	1.62	1.52	0.04	<0.01	0.10	0.18
Total tract digestion (%)									
Organic matter	54.8	56.3	56.4	61.0	57.5	1.62	0.02	0.30	0.53
Neutral detergent fiber	60.9	59.3	59.3	62.3	61.2	1.77	0.57	0.18	0.48

[1]CON = 0 mg N/kg BW; 50B = 50 mg N/kg BW BSFL; 100B = 100 mg N/kg BW BSFL; 150B = 150 mg N/kg BW BSFL; 100S = 100 mg N/kg BW SBM

Discussion

Two experiments were conducted to evaluate the viability of BSFL as an alternative protein source for beef cattle consuming forage. In Experiment 1, we assessed the acceptance and preference of beef steers for BSFL, SBM, or mixed supplements provided to a basal forage diet. While not statistically significant, steers consumed less of the 100% BSFL treatment offered, indicating decreased preference relative to mixed BSFL and SBM supplements or 100% SBM. Day affected supplement intake; steers consumed more supplement on the second versus first day of offering. The effect on day was likely due to the steers requiring more time to acclimate to and familiarize themselves with their new diet, as the pelleted form of BSFL was novel compared to SBM or forage. Perhaps the inclusion of an additional experimental day would have allowed steers more time to acclimate to the novel form of the BSFL supplement; this is supported by previous research indicating that repeated provision of novel food would decrease the sense of novelty (Herskin et al., 2003). Drewery et al. (2021) observed a treatment × day interaction for steers supplemented post-extraction algal residue (PEAR) as a stand-alone supplement or in blends with carrier ingredients to steers consuming forage. The introduction of PEAR, a novel feed, resulted in reduced feed consumption over three days, but the conventional supplement did not.

The sense of novelty steers experienced when exposed to the new supplement each period may be attributed to several factors, such as neophobia and palatability (Herskin et al., 2003). Previous learning and post-ingestive feedback impact diet selection (Provenza, 1995). Keunen et al. (2002) fed long alfalfa hay or alfalfa pellets to dairy cows and reported that cows preferred long alfalfa hay, theorizing this may be because the cows had been fed hay daily which may have swayed their preference and perception of the long alfalfa hay as a “comfort” food over the novel alfalfa pellets (Keunen et al., 2002). Since steers in our study were fed CSM, which was in meal form, in the 3-d washout period prior to the experimental diet being offered, their preference for SBM, also in meal form, over pelleted BSFL may have stemmed from familiarity and previous knowledge of the effects of a ground feed versus pelleted. Additional research could be conducted on the preference of BSFL as a meal versus pelleted when offered a conventional protein in the same form. Further, we recommend that future preference studies involving novel feeds or novel forms of feeds be conducted over at least three, rather than two, days for cattle to become comfortable and achieve a stabilized level of intake.

Supplementing protein to low-quality forage (<7% CP) is a common management practice to increase forage utilization by beef cattle (Wickersham et al., 2008). As forage CP approaches then surpasses 7%, the impact of protein supplementation is less pronounced in measurements of intake and digestion (Mathis et al., 2000) because the N demands of the rumen microbes have been met. When 100 mg N/kg BW of BSFL was supplemented in Experiment 2, forage OM intake increased to a similar extent as when an isonitrogenous level of SBM was provided, indicating BSFL promoted forage utilization as effectively as a conventional supplement. This finding aligns with previous research conducted by Drewery et al. (2021) where PEAR supplemented at 100 mg N/kg BW stimulated forage intake at the same rate as an isonitrogenous level of CSM for beef steers consuming *ad libitum* hay (CP 5.26%).

TDOMI in the current study also increased by 49% from CON steers to steers consuming 150 mg N/kg BW of BSFL. This observed increase in TDOMI in accordance with provision of protein agrees with other research supplementing protein to low-quality forage (Koster et al., 1996; Olson et al., 1999; Wickersham et al., 2008; Drewery et al., 2021). Our TDOMI observation is in contrast with previous research by Fukuda et al. (2022) where steers fed a conventional feed containing supplement consumed more TDOMI than steers supplemented BSFL. This depression in TDOMI was attributed to the chitin content of BSFL. Further, TDOMI in our study was higher than previous research by Mathis et al. (2000) where TDOMI increased by 18% for beef steers consuming 5.9% CP brome grass and receiving supplemental rumen degradable protein at 131 mg N/kg BW.

Increased supplementation of BSFL resulted in increased OMD and NDFD as N was increasingly provided to ruminal microbes to supplement the N deficient basal forage diet. Jayanegara et al. (2017) reported a depression in *in vitro* OMD when BSFL was evaluated with forage, which they attributed to the chitin content of BSFL. In our study, the ability of BSFL to stimulate digestibility did not surpass that of the conventional protein (i.e., SBM), but also did not depress it, in alignment with previous *in vivo* work by Fukuda et al. (2022).

For our study, BSFL was offered in graded levels from 0 to 150 mg N/kg BW to maximize protein supplementation. According to previous research (Koster et al., 1996), protein supplementation at 150 mg N/kg BW would exceed the N demands of the rumen microbial population, which would result in a plateau effect for FOMI. However, when supplementation of BSFL increased from 0 to 150 mg N/kg BW, FOMI linearly increased instead of the expected quadratic response, indicating we did not reach the threshold for defatted BSFL to stimulate forage intake. This is in contrast to Martin and Hibberd (1990) and Drewery et al. (2021) who observed quadratic responses in FOMI to protein supplementation. However, our linear response in FOMI in accordance with increased protein supplementation aligns with Wickersham et al. (2008) who demonstrated increases in FOMI when steers were supplemented graded levels of casein as rumen degradable protein with the maximal response at 177 mg N/kg BW. Future research supplementing graded levels of defatted BSFL to low-quality forage should include a level of protein supplementation beyond 150 mg N/kg BW to identify the threshold of N supplementation to the microbial population.

Data collected from this project are valuable to the emerging and growing industry of commercial BSFL rearing and will encourage further investigations into the viability of BSFL in ruminant diets. As large-scale insect rearing operations become feasible, companies may consider creating standardized rearing protocols to maximize nutritional properties of BSFL, such as CP, for ruminants as the environmental conditions in which the insects are reared affects the nutritional qualities of BSFL (Sealey et al., 2011). Additionally, standardized protocols for defatting BSFL are warranted given the success of defatted BSFL in our study. Identifying a specific market and creating standardized protocols to tailor product characteristics to the beef cattle industry can enhance production efficiency and encourage economic success.

Study limitations include offering steers different forms of feed (i.e., ground SBM versus pelleted BSFL) when assessing preference in Experiment 1 which may have affected our results. Further, in Experiment 2, we did not achieve the threshold of supplemental N resulting a linear increase in FOMI as opposed to a quadratic response, which warrants further research to include higher levels of protein supplementation than offered in our study.

Conclusion

Previous research in the swine, poultry, and aquaculture industries have set a promising precedent for the integration of BSFL into the beef cattle sector. Cumulatively, our data indicates that defatted BSFL can be integrated into the beef cattle industry as an alternative protein source for beef cattle consuming low-quality forage. We anticipate that the commercial viability of BSFL will rely on the creation of standardized insect rearing and processing protocols to maximize product value for livestock. We recommend future work evaluating preference of BSFL to use both a ground BSFL and a ground conventional protein to ensure preference of feed is not impeded by its medium. Additionally, we recommend future digestibility trials using defatted BSFL to include higher levels of protein supplementation beyond 150 mg N/kg BW in order to achieve the threshold of supplemental N in stimulating FOMI.

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