EVALUATION OF BLACK SOLDIER FLY LARVAE *(HERMETIA ILLUCENS)* AS A PROTEIN SUPPLEMENT FOR BEEF STEERS CONSUMING LOW QUALITY KING RANCH BLUESTEM HAY

by

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ABSTRACT

Black Soldier Fly Larvae (BSFL; *Hermetia illucens*) have been identified as a potential feedstuff for livestock. Previous data indicate the land mass to produce one ton of soy protein, a conventional feedstuff, could produce 150 tons of insect protein. Further, production of BSFL is associated with 1.3-12 times the environmental benefit versus conventional feedstuffs. Accordingly, our objective was to conduct the first *in vivo* evaluation of BSFL in beef cattle. Six steers (603.3 ± 20.4 kg of BW, $n = 3$ and 404.3 ±17.5 kg of BW, $n = 3$) consuming King Ranch bluestem hay were used in two simultaneous 3 × 3 Latin squares. One of three treatments was provided each period: 1) a control with no supplement (CON), 2) a supplement comprised of conventional feed ingredients with whole cottonseed as the main protein source (CONV), and 3) a supplement with BSFL as the main protein source (BSFL). Three 14-d periods were conducted with 8-d to adapt to treatments, 5-d to measure intake and digestion, and 1-d to complete a ruminal fermentation profile. Dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and acid detergent insoluble ash were determined in forage, supplement, and fecal samples. Metabolic body weight (MBW) was body weight$^{0.75}$. Total tract digestibility was [1-(output of nutrient/intake of nutrient)] × 100. Treatment affected total digestible OM intake (TDOMI; $P \leq 0.01$); TDOMI for CON steers was 47.5 g /kg MBW/d which was significantly less ($P \leq 0.01$) than that of CONV or BSFL steers. However, CONV steers consumed significantly more TDOMI than BSFL steers ($P = 0.05$; 62.2 vs. 60.1 g/kg MBW/d, respectively). Treatment did not significantly affect digestibility of DM (*P*=0.74), OM (*P*=0.15), or NDF (*P*=0.53). BSFL stimulated TDOMI relative to CON but not to the same extent as CONV. The lack of treatment effect on digestibility was expected as we observed increased intake, indicating faster passage rate. Overall, this initial data indicates BSFL may be an effective protein supplement for beef cattle consuming low-quality forage.

Keywords: beef cattle, black soldier fly larvae, insect protein, protein supplementation

I. INTRODUCTION

The global population is expected to grow to 10 billion by 2050, placing pressure on agricultural producers to increase the edible output of their products by 70% (Barclay, 2019). Food fit for human consumption must be used for such and livestock producers will be encouraged to find new ways to meet food demands without jeopardizing the food supply and environmental quality for future generations. Livestock production is often criticized for reliance on natural resources; this criticism would likely intensify if production efforts using conventional methods that require significant natural resource inputs were increased. Accordingly, research that identifies novel feedstuffs to displace conventional feeds that are fit for human consumption and/or associated with environmental degradation is justified.

Sustainability is defined by the UN's FAO as "ensuring human rights and well-being without depleting or diminishing the capacity of earth's ecosystems to support life, or at the expense of others well-being". Insect protein, namely, Black Soldier Fly Larvae (BSFL), has come into view when considering novel feeding options for livestock production and has potential to meet these sustainability criteria when used as a protein source for livestock diets.

A. Black Soldier Fly Larvae

i. Industry Applications

BSFL have potential applications in several industries, some yet to be explored. The nutrient composition of BSFL varies in accordance with diet, but generally contains 39% fat on a dry matter basis (Newton et al., 2005, St. Hilaire et al., 2007). This fat can be extracted and converted to valuable products, including biodiesel. Generation of biodiesel from BSFL is an attractive option as there is a pressing need to identify additional sources of biofuel and relieve pressure on limited, depleting fossil fuel resources (Puppan, 2002; Demirbas, 2006). Alternatively, there is increasing interest in the use of extracted BSFL oil for skin care applications (Sea Berry Therapy). Perception of sustainability may be important to consumers and using this as an advertising point has likely contributed to the success of BSFL oil in skin care products. The use of BSFL oil in skin care has been the focus of recent research; Srisombat et al. (2020) reported the effective ability of BSFL oil to be used in aqueous based formulations for skin care as a lipid source.

After fat extraction, the remaining larval biomass, referred to as defatted BSFL, has high protein content, approximately 47-58% on a dry matter basis (Kroeckel et al., 2012; Lock et al., 2016). Accordingly, there is growing interest for the application of whole and defatted BSFL in the animal feed industry, especially with regards to livestock production. In North America, AAFCO, an organization that sets regulatory standards for animal feeds, has approved whole and defatted BSFL as a feed for poultry and salmonids provided the BSFL are reared on feed grade material (e.g., dried distillers grains plus solubles).

ii. <u>Rearing and Harvesting Practices</u>

The BSFL industry is emerging and rearing and harvesting practices are not yet standardized due to a lack of research and consistent data on efficient and economically viable technology. However, one practice that seems to span all harvesting operations is "self-collection" (Diener et al., 2011). Self-collection is facilitated by the natural larval instinct to leave their food source and search for a dry place, displayed by **Figure 1**. This instinctual response is triggered by the physiological transformation of the larval mouth into a hook, signifying the larvae is moving into the next life stage, prepupae (Newton et al., 2005; Diener et al., 2011; Cockcroft, 2018). Newton et al. (2005) describes the technology used to capitalize on BSFL self-collection as a basin lined with a food source; the basin contains immature larvae and a food source with ramps on the sides that ultimately lead to a gutter and containers that collect BSFL via gravity. This design allows the migrating larvae to leave the basin in response to the above described physiological transformation and self-collect, minimizing the need for intensive human handling and intervention.

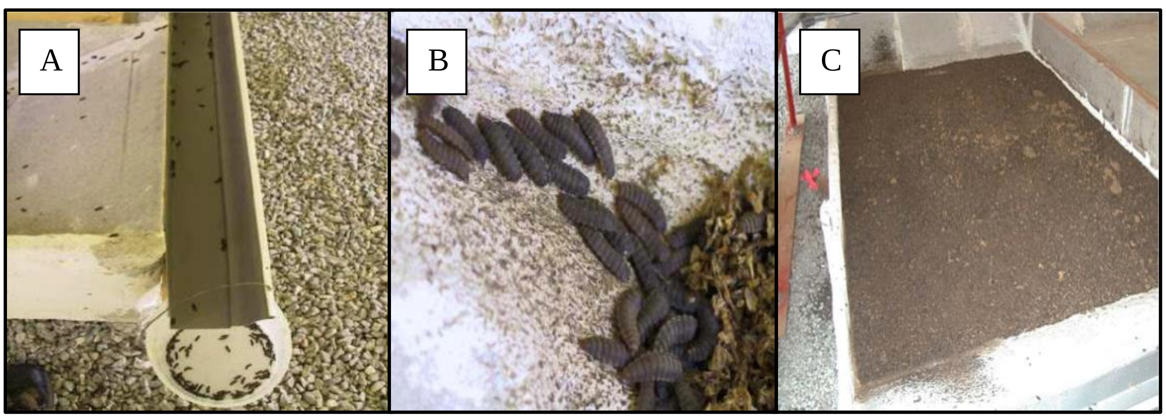

Figure 1: The harvesting process of Black Soldier Fly Larvae (BSFL)
A) BSFL climbing a ramp on the side of the basin; B) collection of BSFL after they fall into a gutter system; C) feed source (e.g., manure, compost) in the basin.
Pictures adapted from Newton et al., 2005.

iii. <u>BSFL as livestock feed</u>

Recently, there has been increased research on the use of insects as animal feed (Arnold van Huis, 2016; **Table 1**). Specifically, there has been a focus on BSFL as they are shown to be efficient converters of protein-deplete waste into protein rich biomass. Previous data indicate defatted BSFL have a protein content of 47-58% DM when reared on leftover food, grains, or organic waste (Kroeckel et al., 2012; Lock et al., 2016).

Much of the sustainability of BSFL lies within the larval ability to recycle waste products into edible protein. BSFL are unique in that they can be reared on organic waste, compost, feed byproducts, and other nutrient sources. By AAFCO guidelines, however, BSFL can only be reared on feed grade material. This means that organic waste is a prohibited nutrient source for BSFL in the United States; this regulation likely stems from the potential of heavy metal contamination because heavy metals can be transferred between organisms, may be retained in the human body if consumed, and are associated with health risks, including cancer (Lopes et al., 2011). Previous data indicate cadmium is the only heavy metal retained in BSFL and lead and zinc do not accumulate in BSFL biomass despite their presence in the diet (Barragan-Fonseca et al., 2017). In relation to health risks, lead and zinc are categorized as higher risk heavy metals (Lopes et al., 2011). Thus, the fact that these heavy metals do not persist in BSFL reared on organic waste is promising and hints at the potential for BSFL reared and fed in the United States to become highly sustainable as regulations shift and adapt to emerging data.

As BSFL in the United States cannot currently be reared on organic waste, their environmental sustainability is slightly less than their physiological capacity allows; however, there are other factors of BSFL that contribute to their sustainability as a livestock feed, including the relatively low agricultural land and water inputs needed for production. Compared to soybean, which is a ubiquitous ingredient in livestock production, BSFL are produced at 150× greater quantities on the same amount of land (Zanolli, 2014). Further, production of 1 ton of soybean requires 1.6 million liters of water while it takes 0.1 million liters to produce the same amount of BSFL (ProNuvo, 2020). Water and agricultural lands are becoming increasingly scarce; of the world's water, only 2.5% is fresh and 69% of that is frozen (Shiklomanov, 2000), underlining a need for efficient use of water, among other natural resources.

BSFL can be incorporated as a feed ingredient in livestock production as defatted or full fat BSFL (Newton et al., 1977; Cockcroft, 2018). The addition of fat to the diet of animals greatly impacts intake and performance; this response is related to the digestive strategy of the animal. In feeding trials conducted in poultry and swine, both of which are monogastrics, full fat BSFL did not negatively impact intake or digestibility (Al-Qazzaz et al., 2016; Driemeyer, 2016; **Table 1**). However, previous data indicate high fat diets in cattle, which are ruminants, decrease the animal’s willingness to eat, resulting in lower dietary intake and decreased average daily gain (Doppenberg and Palmquist, 1991). Due to the specialized digestive strategy of ruminants and how fat affects their dietary intake and performance, BSFL would likely be better utilized by ruminants if fat were extracted prior to feeding.

Insect exoskeletons contain a complex carbohydrate called chitin. Monogastrics (e.g., fish, poultry, swine) have low capacity to digest this compound. Due to the digestive strategy of ruminants (e.g., cattle, sheep, goats), chitin can be fermented in the rumen, yielding volatile fatty acids which meet a majority of the energy demands of the animal. This was demonstrated by Kopecny et al. (1996) who identified a chitinolytic bacterial species in the rumen, implying that ruminants are more suitable utilizers of BSFL than monogastrics.

BSFL have been evaluated in ruminants as an *in vitro* study by Jayanegara et al. (2017). This research demonstrated that BSFL was of lower nutritional value than soybean due to the presence of complex chitin compounds. Further, they reported that feeding cattle BSFL instead of soybean lowered methane emissions (Jayanegara et al., 2017). Ultimately, these researchers suggested a need to process BSFL and mechanically remove chitinolytic compounds prior to feeding to allow the ruminant animal to more thoroughly utilize BSFL. A follow-up study by the same researchers demonstrated that, when chitin was extracted and converted to chitosan then added back to the BSFL, *in vitro* ruminal feed degradation and methane emissions were both decreased (Jayanegara et al., 2020). Overall, these data suggest that chitin effectively suppresses ruminal methanogenesis, which is beneficial from an energy utilization and environmental perspective, but negatively affects dietary utilization. More research is needed to validate these findings in an *in vivo* model.

Ruminants are also likely better utilizers of BSFL because they lack a dietary essential amino acid requirement. The amino acid profile of BSFL does not contain adequate concentrations of essential amino acids to meet dietary requirements of monogastrics (Newton et al., 1977), indicating growth would be stunted if amino acids were not supplemented to the basal diet. Compared to soybean, BSFL have higher concentrations of alanine, histidine, methionine, and tryptophan, but lower arginine (Barragan-Fonseca et al., 2017). Overall, methionine, cystine, and threonine would need to be supplemented or met through other dietary ingredients for monogastrics consuming BSFL (Makkar et al., 2014). The amino acid profile of BSFL does not pose an issue for ruminants because ruminal microbes synthesize amino acids from the nitrogen supplied to them, eliminating dietary amino acid requirements.

Table 1. Effect of dietary inclusion of defatted and full-fat Black Soldier Fly Larvae (BSFL)			
Reference	**Animal**	**Dietary inclusion**	**Observations**
Aquaculture			
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 significantly decreased weight gain but increased feed efficiency; no difference in mortality
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.,	Broiler quail	0, 10, 15% defatted	No significant difference

2016		BSFL	in apparent dry matter digestibility, feed preference, productivity, mortality, or carcass attributes
Maurer et al., 2016	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
Pigs			
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
Alligators			
Bodri and Cole, 2007	Hatchlings	0, 100% BSFL	BSFL significantly stunted weight and length but improved feed efficiency

Table 1 presents a summary of the existing research on BSFL inclusion in animal diets. These data indicate BSFL can be included in animal diets up to 25% (replacing up to 100% of conventional protein) without significant adverse effects. However, these data are limited to monogastrics which, for the aforementioned reasons, are likely less efficient utilizers of BSFL as compared to ruminants. Thus, there is potential for BSFL to be included at high percentages in the diets of ruminants.

B. Ruminant Digestive Physiology

i. Low Quality Forage

Cattle production systems rely heavily on forage as a dietary component as this is the natural diet of most ruminants. The importance of forage to ruminants is reflected by their physiology as they evolved a specialized dietary strategy that increases their ability to utilize fibrous feedstuff as compared to monogastrics such as poultry and swine. In many forages, nutrient deficiencies are common in certain seasons or with certain soil conditions. As demonstrated by Wilson et al. (1983), cool season forages have superior overall nutritional value, dry matter digestibility, and cell wall content than warm season forages. Bohnert et al. (2011) reported higher crude protein (CP) content in low-quality cool season forages as compared to low-quality warm season forages. These studies suggest that, during the warm season or in other conditions that decrease the nutritional value of forage, protein must be supplemented to ruminants to compensate for deficiencies in the basal forage diet. Forages can also experience nutrient deficiencies due to energy limiting situations such as poor management of fertilizer and soil health, as well as water limitations due to drought. Research on protein supplementation and, more importantly, novel proteinaceous feedstuffs, is important to offer producers a cost-effective management strategy to maximize utilization of forage and, thus, animal performance.

ii. Protein Supplementation

There have been numerous studies on protein supplementation for cattle fed low quality forage, which is defined as forage containing less than 7% CP (Koster et al., 1996; Mathis et al., 1999; Drewery et al., 2014). Protein is key to the health and success of cattle as the ruminal microbes utilize N intrinsic to protein to meet host amino acid requirements. Supplementing proteinaceous feed (e.g., soybean meal, cottonseed meal) to a N deficient ruminant increases forage intake and utilization, as well as ruminal ammonia-N concentrations (Caton et al., 1988; Guthrie and Wagner, 1988; Koster et al., 1996). Nitrogen is used to produce microbial crude protein, which meets nutritional demands of the host animal.

iii. Macronutrients in Ruminants

Carbohydrates and protein have similar and extreme importance for ruminants as they have interconnected functions. Carbohydrate fermentation provides an energy source for ruminal microbes to grow, creating microbial crude protein and providing ruminants with an endogenous N source that meets essential amino acids requirements.

Supplemental protein is available to the ruminant in two forms: degradable intake protein (DIP) or undegradable intake protein (UIP). DIP is utilized in the rumen, the first compartment of the ruminant stomach located in the foregut of the gastrointestinal tract. DIP is degraded by ruminal microbes from feed protein to peptides and then to amino acids; this step releases volatile fatty acids as well as a carbon skeleton and ATP. Once broken into amino acids, the N is then converted into feed ammonia and either enters the ruminal ammonia pool to be utilized by rumen microbes and the animal or is sent to the liver if in excess (Koster et al., 1996). This eliminates the ruminant's requirement for dietary amino acids because the microbes break feed protein down to ammonia then effectively rebuild it to create the amino acids needed. This facet of the ruminant digestive system enables ruminants to be the most efficient utilizers of low quality feedstuffs because they can convert low quality protein into high quality protein that is fit for human consumption (e.g., beef, milk). Conversely, UIP, which is primarily digested by mammalian enzymes synthesized and secreted into the abomasum and small intestine, has roles in N recycling, the process of absorbing and metabolizing dietary protein then distributing it back into the ruminal ammonia pool. This also increases the efficiency of feed utilization in ruminants because, instead of disposing excess nutrients as urinary waste when they are not immediately absorbed, those nutrients can be re-routed to re-enter the rumen as endogenous urea to be re-incorporated in the ruminal ammonia pool. Overall, UIP plays a critical role in providing energy to the ruminant, but DIP is a more efficient nutrient source as it is digested in the first location of the digestive tract, the rumen (Wickersham et al., 2004).

II. STUDY OBJECTIVES

The overarching goal of our study is to increase sustainability of beef cattle production by identifying a novel protein source to displace conventional feedstuffs that are fit for human consumption and/or have a larger environmental footprint. To meet this goal, our study addresses the following objectives:

1) Assess the ability of BSFL to stimulate intake and digestion in cattle consuming low-quality forage.
2) Contrast the effects of BSFL on intake and digestibility versus that of a conventional protein supplement.

III. METHODS

A. Data and Sample Collection

These procedures were approved by the Institutional Animal Care and Use Committee at Texas State University (6753) and Texas A&M University (2019-0445).

Six ruminally fistulated steers (603.3 ± 20.4 kg of BW, $n = 3$ and 404.3 ±17.5 kg of BW, $n = 3$) were used in two simultaneous 3 × 3 Latin squares to evaluate the suitability of BSFL (*Hermetia illucens*) as a protein supplement. Steers were housed in an enclosed barn in 2.1 × 1.5 m individual metabolism stalls. Steers were provided *ad libitum* access to fresh water and low quality, King Ranch bluestem hay (6.55% CP; **Table 2**) at 130% of the previous 4-d average intake at 0730 h daily. During each period, one of three treatments was provided to each steer: 1) a control (CON) with no supplement, 2) a supplement comprised of conventional feed ingredients with whole cottonseed as the main protein source (CONV), and 3) a supplement with BSFL as the main protein source (BSFL). Supplement compositions are fully outlined in Table 2. Treatments were designed such that CONV and BSFL would be isonitrogenous; however, due to fluctuations in the nutritional value of ingredients, CONV contained 27.1% protein and BSFL, 23.5% (DM basis). Treatments were supplied to the animal isonitrogenously (100 mg N/kg BW) such that we provided slightly more BSFL than CONV. Supplements were fed at 0730 h every day, with forage orts collected prior to feeding and weighed for calculation of the amount of forage to feed to meet *ad libitum* requirements.

Table 2. Chemical composition of diets and supplement composition			
Item	Hay	CONV	BSFL
	- - - Chemical composition, g/kg DM - - -		
OM[1]	895.8	950.1	927.6
NDF	756.8	410.1	395.4
ADF	473.7	289.3	237.9
CP	65.5	270.7	235.3
	- - - Supplement composition, g/kg - - -		
Whole CS		560	--
BSFL		--	360
SBM		200	--
SH		120	410
WM		120	230

[1]OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; CP = crude protein; Whole CS = Whole Cottonseed; BSFL = Black Soldier Fly Larvae; SBM = Soybean Meal; SH = Soyhulls; WM = Wheat Middlings

Three 14-d experimental periods were conducted; periods consisted of 8-d to adapt steers to treatments and 5-d to measure intake and digestion, and 1-d to collect samples for future analyses (data not shown). Forage and supplements were sampled d 9 through 12. Orts samples and intake measurements were collected d 10 through d 13 before feeding forage at 0730 h. Collection of fecal samples occurred every 8 h on d 10 through 13 with sampling time advancing 2 h every day to obtain samples that represented every even hour of an entire day. Acid detergent insoluble ash (ADIA) served as an internal marker to calculate fecal output on a DM basis, which was then used to calculate digestibility.

B. Laboratory Analyses

Ort and fecal samples were composited by steer across day within period. Forage and supplement samples were composited across day by period. Forage, supplement, ort, and fecal samples were dried in a forced air oven at 55°C for 96 h, allowed to air equilibrate, and weighed for partial DM analysis. Forage, supplement, ort, and fecal samples were then ground with a Wiley mill to pass through a 1-mm screen. Samples were dried at 105°C for 24 h and allowed to air equilibrate for 20 minutes in a desiccator, then weighed for determination of DM. Organic matter (OM) was determined as the loss in dry weight on combustion for 8 h at 450°C. CP content of the forage and supplement was calculated as 6.25 × Kjeldahl N. Analysis for neutral detergent fiber (NDF) was conducted using an Ankom Fiber Analyzer with sodium sulfite and amylase omitted and without correction for residual ash (Ankom Technology Corp. Macedon, NY). Acid detergent fiber (ADF) was also determined using an Ankom Fiber Analyzer as well as acid detergent insoluble ash (ADIA) by combusting Ankom bags containing ADF residues for 8 h at 450°C in a muffle furnace. Energy values were determined by direct calorimetry using a Parr 6300 Calorimeter (Parr Instrument Co., Moline, IL)

C. Calculations

Digestion was calculated as: [1- (output of nutrient/intake of nutrient)] × 100. Metabolic body weight (MBW) was calculated as: body weight$^{0.75}$ (NRC, 2016). Gross energy (GE) and digestible energy (DE) were determined using calculations in the NRC (2016).

D. Statistical Analyses

Intake and digestion were analyzed using the MIXED procedure in SAS 9.4 (SAS Inst. Inc., Carry, NC). Terms in the model included treatment, period, and square with steer as the random effect. Treatments means were calculated using the LSMEANS option. Statistical significance was determined for $P \leq 0.05$ and trends were determined for $P \geq 0.06$ and ≤ 0.10.

IV. RESULTS

There was a significant effect of treatment on forage OM intake ($P \leq 0.01$; **Table 3**). Relative to CON, provision of CONV ($P \leq 0.01$) or BSFL ($P \leq 0.01$) stimulated forage OM intake with a trend for a difference between the supplements ($P = 0.08$). Control steers consumed 73.88 g forage OM/kg MBW/d, which increased to 86.21 and 82.40 g/kg MBW/d for CONV and BSFL, respectively.

Treatment significantly affected supplement OM intake ($P \leq 0.01$) such that either supplement increased supplement OM intake relative to CON ($P \leq 0.01$) and with no significant difference between BSFL and CONV ($P = 0.93$). Steers consuming CONV treatment had an intake of 9.67 g supplement OM/kg MBW/d while those consuming BSFL had an intake of 9.66 g supplement OM/kg MBW/d. As CON was a negative control, steers receiving that treatment had no supplement OM intake.

There was a treatment effect on total OM intake (TOMI; $P \leq 0.01$) driven by a statistical trend between CONV and BSFL ($P = 0.07$), and a significant difference between CON and each of the two supplements ($P \leq 0.01$). Steers receiving CONV and BSFL consumed 95.88 g and 92.06 g total OM/kg MBW/d, respectively, and those receiving CON had an intake of 73.88 g total OM/kg MBW/d.

There was a significant treatment effect for TDOMI ($P \leq 0.01$); both BSFL and CONV significantly stimulated TDOMI relative to CON ($P \leq 0.01$) with a significant difference between the two supplements ($P = 0.05$). Steers receiving CON consumed 47.46 g total digestible OM/kg MBW/d while steers receiving CONV had an intake of 62.24 g total digestible OM/kg MBW/d and those receiving BSFL consumed 60.07 g total digestible OM/kg MBW/d.

Table 3. The effect of a conventional feedstuff or Black Soldier Fly Larvae (BSFL) on intake and digestion in cattle consuming forage[1]	Treatment				
	CON	CONV	BSFL	SEM	*P*-value
n	6	6	6		
OM intake, g/kg MBW[2]					
Forage	73.88_{a}	86.21_{b}	82.40_{b}	0.05	< 0.01
Supplement	0.00_{a}	9.67_{b}	9.66_{b}	0.02	< 0.01
Total	73.88_{a}	95.88_{b}	92.06_{b}	0.05	< 0.01
Digestible	47.46_{a}	62.24_{b}	60.07_{c}	0.05	< 0.01
Total tract digestion, g/kg					
DMD	605.01_{a}	613.38_{a}	615.53_{a}	13.9	0.74
OMD	642.33_{a}	612.71_{a}	621.44_{a}	13.9	0.15
NDFD	672.58_{a}	659.16_{a}	660.85_{a}	12.4	0.53
GE intake, mCal/d	32.45_{a}	43.61_{b}	41.27_{c}	0.56	< 0.01
DE intake, mCal/d	19.73_{a}	27.52_{b}	25.91_{c}	0.66	< 0.01

[1]Observations with different subscripts are significantly different at $P \leq 0.05$
[2]OM = organic matter; MBW = metabolic body weight; DMD = dry matter digestibility; OMD = organic matter digestibility; NDFD = neutral detergent fiber digestibility; GE = gross energy; DE = digestible energy

Treatment did not significantly affect ($P = 0.74$) dry matter digestibility (DMD). DMD for CON steers was 605.01 g/kg while steers receiving CONV had a DMD of 613.38 g/kg and BSFL steers had a DMD of 615.53 g/kg. Organic matter digestibility (OMD) was also not significantly affected by treatment ($P = 0.15$), as steers consuming CON had an OMD of 642.33 g/kg, and those consuming CONV and BSFL had an OMD of 612.71 and 621.44 g/kg, respectively. Similarly, treatment did not affect neutral detergent fiber digestibility (NDFD; $P = 0.53$), with NDFD of CON, CONV, and BSFL being 672.58, 659.16, and 660.85 g/kg, respectively

Total GE intake was significantly affected by treatment ($P \leq 0.01$); CON steers consumed 32.45 mCal GE/d and steers consuming CONV or BSFL had a GE intake of 43.61 or 41.27 mCal/d, respectively. Relative to CON, supplementation of BSFL or

CONV ($P \leq 0.01$) significantly stimulated total GE intake with a statistical difference between the supplements ($P \leq 0.01$). DE intake was significantly affected by treatment ($P \leq 0.01$); this treatment effect reflected a statistical difference between CONV and BSFL ($P = 0.04$), and a statistical difference between CON and either supplement ($P \leq 0.01$). DE intake was 19.73, 27.52, and 25.91 mCal/d for CON, CONV, and BSFL steers, respectively.

V. DISCUSSION

This experiment evaluated the effects of BSFL supplementation on intake and digestibility in beef steers consuming a basal diet of low–quality forage. To achieve this, BSFL and a conventional protein supplement (CONV) were provided to beef steers at isonitrogenous amounts and compared a negative control, no supplemental protein (CON).

We used MBW to account for differences in intake between our squares (NRC, 2016). One 3 × 3 Latin Square had smaller steers (*n* = 3; 404.3 ±17.5 kg BW) and the other, larger steers (*n* = 3; 603.3 ± 20.4 kg BW). Indeed, we observed significant effects of square on our findings (data not shown), justifying the inclusion of square in our statistical model and the expression of intake on a MBW basis.

Provision of protein as BSFL or CONV stimulated intake, which is consistent with previous observations (Beaty et. Al, 1994; Mathis et. al., 1999, Bohnert et al., 2011). The increase we observed in FOMI in accordance with protein supplementation indicated that the basal forage diet caused a N deficiency in steers not receiving supplemental protein (Mathis et al., 2000). There was not a statistically significant difference between CONV or BSFL for FOMI, although there was a trend for CONV to stimulate FOMI to a greater extent than BSFL, indicating both alleviated the depression in FOMI that is associated with N deficiency. Similar data were observed for TOMI.

The increase in FOMI, expressed as g/kg MBW, we observed with provision of 100 mg N/kg BW was similar to that observed by Olson et al. (1999) but less than that of Wickersham et al. (2008), who supplemented a similar level of DIP to cattle consuming low quality forage. The disparity in our response versus that of Wickersham et al. (2008)

may lie in the N content of the basal forage, as the CP content of our forage was 6.6% and theirs was 4.9% CP. As the CP content of the basal forage increases to 7%, there are diminishing returns in providing supplemental protein to stimulate forage intake and, once forage CP reaches 7%, additional supplemental protein does not further increase forage intake (Moore and Kunkle, 1995). Further, the protein degradability characteristics (e.g., DIP versus UIP) of BSFL are unknown, which could also explain the disparity between our results and those of Wickersham et al. (2008), who supplemented 100% DIP. Provision of DIP results in an additional 34% increase in FOMI as compared to UIP when supplemented to cattle consuming low quality forage (Bandyk et al., 2001), underlining the site of protein degradation affects intake when forage CP is limiting. Future work should characterize the protein degradability characteristics of BSFL.

Both supplements in our study contained fat, as whole cottonseed was used in the CONV supplement and whole BSFL was included in the BSFL supplement. High fat content has previously been demonstrated to reduce the ability of steers to effectively utilize protein, which reduced dietary intake (Grummer, 1987). It would be justified, then, to conduct a similar trial with defatted BSFL and conventional feed ingredients that are low in fat content to observe if FOMI is stimulated to a greater extent than observed here.

The trend for a difference between CONV and BSFL in measures of FOMI and TOMI was more pronounced and reached statistical significance for TDOMI. The CON supplement stimulated TDOMI to a greater extent than BSFL and both supplements effectively stimulated TDOMI relative to CON. This observation may be related to the chitin content of BSFL. Chitinolytic bacteria have been identified in the rumen of cattle fed chitin from crab shells, indicating ruminants may be able to utilize chitin (Kopecny, 1996). However, in an *in vitro* study, Jayanegara et al. (2017) observed decreased ruminal DMD and OMD when BSFL was incubated with Napier grass, as compared to Napier grass alone or Napier grass and SBM. This was hypothesized to be related to the chitin and ether extract content of BSFL, effectively lowering its nutritional value to the ruminant animal. In a follow-up study, Jayanegara et al. (2020) extracted chitin from BSFL and converted it to chitosan then evaluated digestibility using an *in vitro* model; BSFL containing chitosan depressed ruminal feed degradation similarly to the previous study (Jayanegara et al., 2017). Further research on the ability of ruminants to digest chitin in whole and defatted BSFL should be conducted.

Total tract OMD often increases when supplemental N is provided to cattle consuming low quality forage (Olson et al., 1999; Klevesahl et al., 2003; Wickersham et al., 2008). However, other studies (Köster et al., 1996; Drewery et al., 2014) observed depressions in OMD at levels of supplemental N that effectively enhanced FOMI and TDOMI. As intake increases, rate of passage increases, decreasing ruminal retention times. Thus, there is less opportunity for rumination and microbial fermentation of the diet when intake is stimulated. It is plausible that, although provision of CONV or BSFL likely increased digestibility per unit of feed, the overall effects were cancelled out due to increased rate of passage. This hypothesis is in line with Egan (1965) who observed an 11% increase in DM intake and no effect on ruminal digestibility when urea was supplemented.

In conclusion, this preliminary *in vivo* evaluation of BSFL demonstrates that it increases forage intake and does not inhibit digestibility, indicating it may be a suitable protein supplement for cattle consuming low quality forage. Ultimately, before BSFL is adopted by the beef industry, more research needs to be performed. We propose a carcass quality study of cattle consuming BSFL to ensure dietary inclusion does not negatively affect the organoleptic attributes of meat. Further, this study should be replicated by other researchers and adapted to include defatted BSFL to confirm our results and build a base of literature.

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