



Evaluation of Black Soldier Fly larvae (*Hermetia illucens*) as a protein supplement for beef steers consuming low-quality forage¹

Emma P. Fukuda,[†] Jodi R. Cox,[‡] Tryon A. Wickersham,^{†,‡} and Merritt L. Drewery^{†,2}

[†]Department of Agricultural Sciences, Texas State University, San Marcos, TX 78666, USA

[‡]Department of Animal Science, Texas A&M University, College Station, TX 77843, USA

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²Corresponding author: m_d553@txstate.edu

ABSTRACT

Black Soldier Fly larvae (BSFL; *Hermetia illucens*) has been the focus of recent feeding trials in poultry, swine, and fish; however, in vivo research has not yet been conducted in cattle. Accordingly, a study was conducted to evaluate the effects of BSFL as a protein supplement in beef steers. Six steers (603 ± 20 kg of BW, *n* = 3 and 404 ± 17 kg of BW, *n* = 3) consuming King Ranch bluestem hay (6.55% dry matter [DM] crude protein [CP]) ad libitum were used in two simultaneous 3 × 3 Latin squares. Steers were provided one of three treatments each period: 1) a control with no supplement (CON), 2) a supplement comprised of conventional feed ingredients with whole cottonseed and soybean meal as the main protein sources (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. DM, organic matter (OM), CP, neutral detergent fiber (NDF), and acid detergent insoluble ash were determined in forage, supplementation, ort, and fecal samples. Protein supplementation as CONV or BSFL stimulated forage OM intake ($P \leq 0.01$) relative to CON with a trend for a difference ($P = 0.08$) between the supplements such that CONV steers consumed more FOMI than BSFL steers. Treatment affected total digestible OM intake (TDOMI; $P \leq 0.01$); TDOMI for CON steers was 47.5 g/kg metabolic body weight (MBW) which was significantly less ($P \leq 0.01$) than that of CONV or BSFL steers. Steers supplemented with CONV consumed significantly more TDOMI than BSFL steers ($P = 0.05$; 62.2 vs. 60.1 g/kg MBW, respectively). Treatment did not significantly affect digestibility of DM, OM, or NDF ($P > 0.32$). There was also not a significant effect ($P \geq 0.17$) of treatment on ruminal ammonia-N, total volatile fatty acids, or ruminal pH. Overall, these data indicate that BSFL may be an effective protein supplement for beef cattle consuming low-quality forage.

Key words: beef cattle, BSFL, forage intake, insect protein, protein supplementation

INTRODUCTION

Insects are gaining attention as livestock feed because they efficiently convert nutrient-deplete substrates into nutrient-rich biomass (Wang and Shelomi, 2017), and their production is estimated to have a smaller environmental footprint than conventional feeds (Zanolli, 2014; Smetana et al., 2016; Allegretti et al., 2018). Specifically, Black Soldier Fly larvae (BSFL) have potential for large-scale utilization in feed applications due to their nutrient profile (47.0%–58.0% dry matter [DM] crude protein [CP], 39.0% DM fat; Newton et al., 2005; St. Hilaire et al., 2007; Kroeckel et al., 2012; Lock et al., 2016), scalability (English et al., 2021), and favorable feed efficiency (Oonincx et al., 2015). Previous research demonstrates that BSFL can be fed to poultry, swine, and fish as a replacement for conventional protein feeds (Newton et al., 1977; Bondari & Sheppard, 1980; Al-Qazzaz et al., 2016; Driemeyer, 2016; Cockcroft, 2018). Although preliminary in vitro work has evaluated the digestibility of BSFL for cattle (Jayanegara et al., 2017; Jayanegara et al., 2020), an in vivo feeding trial has not yet been conducted, to the best of our knowledge.

Protein supplements are often offered to cattle consuming low-quality forage (<7% CP) to increase intake and digestion

(Wickersham et al., 2004; Drewery et al., 2014). Our hypothesis was that BSFL would be a suitable protein supplement for cattle consuming low-quality forage. Accordingly, the objective of this study was to determine the effects of a BSFL-based supplement on forage utilization in steers consuming low-quality forage as compared to a supplement based on conventional feeds.

MATERIALS AND METHODS

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 cannulated 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. Black Soldier Fly eggs were acquired from adult oviposition sites and fed a preformulated nursery diet that encourages egg hatch and growth for 5–7 d. After the nursery phase, larvae were mechanically sifted from the remaining diet and frass

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and placed in grow-out containers with a mixture of pre-consumer food waste and a preformulated diet. After 8–14 d, BSFL were mechanically sifted from the remaining grow-out diet and frass and dried. Once dried, the BSFL were mechanically ground into a fine powder.

Steers were housed in an enclosed barn in individual 2.1 × 1.5 m metabolism stalls. Steers were provided ad libitum access to fresh water and low-quality King Ranch bluestem hay (*Bothriochloa ischaemum* var *songarcia*, 6.55% CP; Table 1) at 130% of the previous 4-d average intake at 0730 h daily. Steers were also provided ad libitum access to trace mineral blocks (composition: ≥ 97% of NaCl, 1,000 ppm Ca, 1,500 ppm Fe, 2,500 ppm Zn, 3,000 ppm Mn, 90 ppm I, 150 ppm Cu, 25 ppm Co, and 10 ppm S; United Salt Corporation, Houston, TX) throughout the trial. During each period, one of three treatments was provided to each steer: 1) a control with no supplement (CON), 2) a supplement comprised of conventional feed ingredients with whole cottonseed and soybean meal as the main protein sources (CONV), and 3) a supplement with BSFL as the main protein source (BSFL). Treatments were formulated such that CONV and BSFL would be isonitrogenous; however, due to fluctuations in the nutritional value of ingredients, CONV contained 27.1% CP and BSFL contained 23.5% CP. Supplements were fed at 0730 h every day.

Three 14-d experimental periods were conducted; periods consisted of 8 d to adapt steers to treatments, 5 d to measure intake and digestion, and 1 d to characterize ruminal fermentation. Forage and supplements were sampled days 9 through 12. Orts were collected days 10 through 13. Collection of fecal grab samples occurred at a single timepoint every 8 h on days 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 production on a DM basis, which was then used to calculate digestibility. Collection of rumen fluid occurred at 0, 3, 6, 9, 12, and 18 h using a suction strainer

(19 mm diameter, 1.5 mm mesh; Raun and Burroughs, 1962). Rumen pH was determined using a portable pH meter with a combination electrode immediately after collection at each sampling time. Subsamples of rumen fluid were prepared and frozen at –20 °C for later determinations of volatile fatty acids (VFA) and ruminal ammonia-N. Prior to freezing, 8 mL of rumen fluid was combined with 2 mL of 25% *m*-phosphoric acid for analysis of VFA and 9 mL of rumen fluid was combined with 1 mL of 1 N HCl for analysis of ammonia-N.

Laboratory Analyses

Forage and supplement samples were composited across day by period. Ort and fecal samples were composited by steer across day within 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 a 1-mm screen. Samples were dried at 105 °C for 24 h and then weighed for determination of DM. Organic matter (OM) was determined as the loss in dry weight upon combustion for 8 h at 450 °C. Nitrogen was determined using Kjeldahl methods (AOAC, 2002): samples were digested in a sulfuric acid and sulfate salt mixture until conversion to ammonia sulfate which was distilled and quantified, and CP 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, and ADIA was determined by combusting Ankom bags containing ADF residues for 8 h at 450 °C in a muffle furnace. Gross energy (GE) was determined by direct calorimetry using a Parr 6300 Calorimeter (Parr Instrument Co., Moline, IL).

Rumen fluid samples were thawed and centrifuged at 20,000 × *g* for 14 min at room temperature. Volatile fatty acid concentrations were measured using a gas chromatograph with methods described by Vanzant and Cochran (1994). Ammonia-N concentrations were measured using a UV-vis spectrophotometer (Beckman Coulter, Brea, CA) with colorimetric procedures as described by Broderick and Kang (1980).

Table 1. Chemical composition of diets and supplement composition

Item	Hay	CONV	BSFL ¹
Chemical composition, %DM			
OM ²	89.6	95.0	92.8
NDF	75.7	41.0	39.5
ADF	47.4	28.9	23.8
CP	6.6	27.1	23.5
EE	–	10.0	12.6
Supplement composition, %			
Whole CS	–	56.0	–
BSFL	–	–	36.0
SBM	–	20.0	–
SH	–	12.0	41.0
WM	–	12.0	23.0

¹Values provided are for complete BSFL supplement. Nutrient values for BSFL as a single ingredient are (% DM): 89.7% OM, 14.1% NDF, 7.3% ADF, 38% CP, 34.9% EE, 47% rumen degradable protein (% CP), 1.85% Ca, 0.80% P, 1.22% K.

²OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; EE, ether extract; Whole CS, Whole Cottonseed; BSFL, Black Soldier Fly larvae; SBM, Soybean Meal; SH, Soyhulls; WM, Wheat Middlings.

Calculations

Calculations were performed in accordance with Boardman et al. (2020). Fecal production was calculated as

$$\text{Fecal production, kg} = \frac{\text{DMI} \times [\text{ADIA}_d]}{[\text{ADIA}_f]}$$

where
DMI, kg

$$[\text{ADIA}_d] = \text{Dietary ADIA concentration (\% DM)}$$

$$[\text{ADIA}_f] = \text{Fecal ADIA concentration (\% DM)}$$

Digestibility of DM, OM, and NDF was calculated as

$$\text{Digestibility}_x, (\%) = \frac{\text{Intake}_x - \text{Fecal}_x}{\text{Intake}_x} \times 100$$

where

$$\text{Intake}_x = \text{DMI (kg)} \times \text{dietary concentration of item } x \text{ (% DM)}$$

$$\text{Fecal}_x = \text{Fecal production (kg)} \times \text{fecal concentration of item } x \text{ (%DM)}$$

Metabolic body weight (MBW) was calculated as

$$\text{MBW} = \text{body weight}^{0.75} \text{ (NRC, 2016)}$$

Digestible energy (DE) was determined using calculations in NASEM (2016):

$$\text{DE (mCal/kg DM)} = \text{GE} \times \text{Digestibility}_{\text{GE}}$$

Statistical Analyses

Intake and digestion were analyzed using the MIXED procedure in SAS 9.4 (SAS Inst. Inc., Cary, NC). Fixed terms in the model included treatment, period, and square with steer as the random effect. Treatment means were calculated using the LSMEANS option, and the pdiff option was used to separate treatment means. Fermentation profile variables were analyzed using the MIXED procedure. Fixed terms in the model were treatment, period, hour, steer, square, and treatment \times hour with steer and treatment \times period \times steer included as random terms. The repeated term was hour, with treatment \times steer as the subject, and the specified covariance structure was compound symmetry based on the Bayesian Information Criterion. Treatment means were calculated using the LSMEANS option, and the pdiff option was used to separate treatment means. Statistical significance was determined for $P \leq 0.05$, and trends were determined for $P > 0.06$ and ≤ 0.10 .

RESULTS

Although treatments were formulated to be isonitrogenous, CONV was provided at 100 mg N/kg BW, whereas BSFL was provided at 88 mg N/kg BW due to variability in ingredient nutritive values. To adjust for differences in body size between squares, MBW was used for forage, supplement, and total N intake.

There was an effect of treatment on forage OM intake (FOMI; $P \leq 0.01$; Table 2). Relative to CONV, provision of CONV or BSFL stimulated FOMI ($P \leq 0.01$) with a trend for a difference between the supplements ($P = 0.08$). Control steers averaged 73.9 g FOMI/kg MBW, which was less than the 86.2 and 82.4 g FOMI/kg MBW observed for CONV and BSFL, respectively. As per the design, treatment affected supplement OM intake ($P \leq 0.01$) such that either supplement increased supplement OM intake relative to CONV ($P \leq 0.01$) with no difference between BSFL and CONV ($P = 0.93$). There was a treatment effect on total OM intake (TOMI) between CONV and each of the treatments ($P \leq 0.01$) and a trend ($P = 0.07$) for CONV to have greater TOMI than BSFL. Steers receiving CONV and BSFL consumed 95.8 g and 92.1 g TOMI/kg MBW, respectively, and those receiving CONV consumed 73.9 g TOMI/kg MBW. There was a treatment effect for TDOMI ($P \leq 0.01$); both BSFL and CONV stimulated TDOMI relative to CONV ($P \leq 0.01$) with a difference between the two supplements ($P = 0.05$). Steers on CONV consumed 47.5 g TDOMI/kg MBW, whereas steers on BSFL consumed 62.2 g TDOMI/kg MBW and those on CONV consumed 60.1 g TDOMI/kg MBW.

Treatment did not affect ($P = 0.78$) dry matter digestibility (DMD) which was 60.5% for CONV, 61.2% for BSFL, and 60.2% for CONV. Organic matter digestibility (OMD) was not affected by treatment ($P = 0.88$) as steers on CONV had an OMD of 64.2%, and those supplemented with CONV and BSFL had an OMD of 64.6% and 64.0%, respectively. Similarly, treatment did not affect neutral

Table 2. The effect of a conventional supplement (CONV) or Black Soldier Fly larvae (BSFL) on intake and digestion in cattle consuming forage¹

	Treatment			SEM	P-value
	CONV	CONV	BSFL		
<i>n</i>	6	6	6		
OM intake, g/kg MBW ²					
Forage ³	73.9 ^a	86.2 ^b	82.4 ^b	0.05	< 0.01
Supplement	0.0 ^a	9.7 ^b	9.7 ^b	0.02	< 0.01
Total ⁴	73.9 ^a	95.9 ^b	92.0 ^b	0.05	< 0.01
Digestible	47.5 ^a	62.2 ^b	60.1 ^c	0.05	< 0.01
Total tract digestion, %					
DMD	60.5	61.2	60.2	1.39	0.78
OMD	64.2	64.6	64.0	1.36	0.88
NDFD	67.3	65.9	65.3	1.24	0.32
GE intake, Mcal	33.5 ^a	43.6 ^b	41.3 ^c	0.56	< 0.01
DE intake, Mcal	19.7 ^a	27.5 ^b	25.9 ^c	0.66	< 0.01

¹Observations with different subscripts are significantly different at $P \leq 0.05$.

²OM, 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.

³There was a trend for a difference between CONV and BSFL ($P = 0.08$) for forage OM intake.

⁴There was a trend for a difference between CONV and BSFL ($P = 0.07$) for total OM intake.

detergent fiber digestibility (NDFD; $P = 0.32$), ranging from 65.3% to 67.3%.

Total GE intake was affected by treatment ($P \leq 0.01$); CON steers consumed 32.5 Mcal which was less ($P \leq 0.01$) than steers supplemented with either CONV (43.6 Mcal) or BSFL (41.3 Mcal). There was a difference in GE intake between CONV and BSFL ($P \leq 0.01$). Total DE intake was affected by treatment ($P \leq 0.01$); this reflected a difference between CONV and BSFL ($P = 0.04$) in addition to a difference between CON and either supplement ($P \leq 0.01$). Total DE intake was 19.7, 27.5, and 25.9 Mcal for CON, CONV, and BSFL steers, respectively.

The treatment \times time interaction was not significant for either ruminal ammonia-N or total VFA ($P \geq 0.69$). A significant effect of time was observed for both ($P \leq 0.05$) resulting from the provision of supplement and hay each morning (Figures 1 and 2). Ruminal ammonia-N was 1.78 mM for BSFL, 1.84 mM for CON, and 2.21 mM for CONV ($P = 0.17$; Table 3). Total VFA were not affected by treatment ($P = 0.13$): 94.3 mM for CON, 97.7 mM for BSFL, and 97.8 mM for CONV. There also was not an effect of treatment on molar proportions of individual VFA ($P = 0.13$) or ruminal pH ($P = 0.69$).

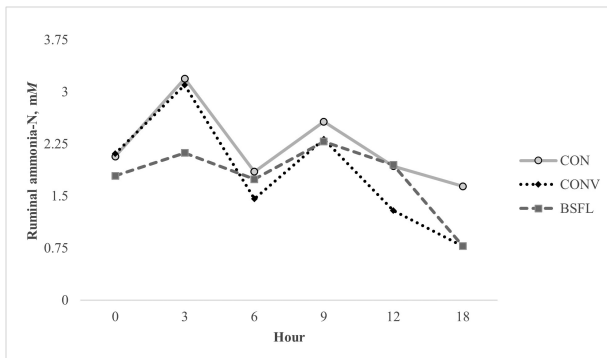


Figure 1. Ruminal ammonia-N concentrations of steers fed low-quality forage supplemented with a conventional feed or Black Soldier Fly larvae (BSFL). No effect of treatment ($P = 0.17$) or treatment \times time ($P = 0.89$). Effect of time ($P = 0.05$). SEM \pm 0.33.

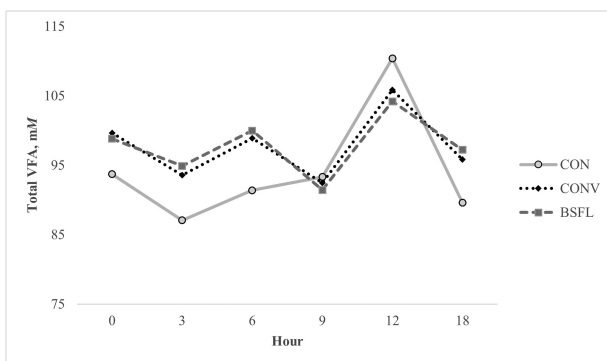


Figure 2. Total volatile fatty acid (VFA) concentrations of steers fed low-quality forage supplemented with a conventional feed or Black Soldier Fly larvae (BSFL). No effect of treatment ($P = 0.13$) or treatment \times time ($P = 0.69$). Effect of time ($P \leq 0.01$). SEM \pm 1.37.

DISCUSSION

The objective of this study was to evaluate a novel protein supplement, BSFL, in beef steers consuming low-quality forage and compare its ability to stimulate intake and digestion versus a protein supplement based on conventional ingredients (e.g., whole cottonseed and soybean meal, CONV).

Provision of a protein supplement as BSFL or CONV stimulated forage intake to a similar degree and significantly more than CON, indicating the basal forage diet was deficient in N (Beaty et al., 1994; Mathis et al., 1999; Olson et al., 1999; Mathis et al., 2000; Bohnert et al., 2011). There was not a significant difference between CONV or BSFL for FOMI, although there was a trend for CONV to stimulate FOMI to a greater extent than BSFL. Both supplements alleviated the depression in FOMI that was associated with N deficiency inherent in low-quality forage, as expected given previous research (Wickersham et al., 2008; Drewery et al., 2014; Drewery et al., 2021).

The increase in FOMI we observed was comparable to Olson et al. (1999) but less than that of Wickersham et al. (2008), who supplemented a similar amount of N to cattle consuming low-quality forage. The disparity in our response versus that of Wickersham et al. (2008) may lie in the CP content of the basal forage, as the CP of our forage was 6.6% DM and theirs was 4.9% DM. As the CP content of the basal forage approaches 7% DM, there are diminishing returns in providing supplemental protein to stimulate forage intake, and once forage CP reaches 7% DM, additional supplemental protein generally does not further increase forage intake (Moore and Kunkle, 1995).

As with FOMI, we observed that protein supplementation significantly enhanced TDOMI of cattle consuming low-quality forage versus no supplement (CON), in alignment with previous research (Wickersham et al., 2008; Drewery et al., 2014; Drewery et al., 2021). However, there was a significant difference between the supplements with CONV stimulating TDOMI to a greater extent than BSFL; this resulted from the combination of a trend for greater TOMI and numerically greater OMD for CONV versus BSFL. Although this could have been a consequence of fluctuations in ingredient nutritive values and, thus, the supplements not being provided isonitrogenously (100 mg N/kg BW of CONV versus 88 mg N/kg BW of BSFL), we hypothesize that this finding may also be related to the site of protein degradability and/or chitin content of BSFL. Specifically, previous research indicates that rumen degradable protein (RDP) supplementation dramatically stimulates FOMI and TDOMI relative to no protein supplementation (Köster et al., 1996). In a comparison of RDP and rumen undegradable protein (RUP), Bandyk et al. (2001) reported that ruminal infusion of protein, RDP, numerically increased TDOMI relative to an isonitrogenous post-ruminal infusion, RUP. In our study, the main protein source of the BSFL supplement was BSFL, which has a protein degradability of 47% of CP. This RDP fraction is less than that of whole cottonseed (68% of CP as RDP) or SBM (65% of CP as RDP; NRC, 2000), the main protein sources of the CONV supplement. The difference in protein degradability between supplements could have contributed to our observed difference between treatments for TDOMI. Future work should further characterize protein degradation of BSFL.

Table 3. Ruminal fermentation profile of steers fed low-quality forage supplemented with a conventional feed (CONV) or Black Soldier Fly larvae (BSFL)

	Treatment			SEM	P-Value
	CON	CONV	BSFL		
<i>n</i>	6	6	6		
Ammonia-N, mM	1.84	2.21	1.78	0.33	0.17
Total VFA ¹ , mM	94.3	97.8	97.7	1.37	0.13
Molar proportions					
Acetate	76.2	76.0	76.1	0.17	0.74
Propionate	14.0	14.1	14.0	0.08	0.59
Butyrate	8.0	7.9	8.0	0.13	0.85
Isobutyrate	0.61	0.63	0.68	0.02	0.10
Valerate	0.49	0.52	0.49	0.03	0.69
Isovalerate	0.79	0.86	0.80	0.03	0.28
pH	6.46	6.55	6.51	0.07	0.63

¹VFA, volatile fatty acid.

In our study, BSFL and CONV did not affect OMD relative to CON, in line with other research in which forage with a similar CP content to ours (6.6% DM) was fed as the basal diet and supplementation of 100 mg N/kg BW of protein as defatted algae or cottonseed meal did not affect OMD relative to no supplementation (Drewery et al., 2021). In previous research (Jayanegara et al., 2017; Jayanegara et al., 2020), when BSFL or chitin derived from BSFL was incubated with grasses and forages in an *in vitro* ruminal fermentation system, DMD and OMD were depressed, a consequence the authors attributed to the ether extract and/or chitin content of BSFL (Jayanegara et al., 2017). Although BSFL did not stimulate digestion in the current study, it also did not depress it, in conflict with this previous *in vitro* work. Rather, our data are in line with early work which evaluated the DMD of crab meal, a chitinous feed, in pre- and post-weaned dairy bull calves consuming a basal diet of hay and grains; it was reported that the dietary presence of crab meal did not significantly depress DMD (Patton, 1971). Indeed, research conducted by Kopecny et al. (1996) identified a chitinolytic bacterium in the rumen, indicating potential for chitin to be digested via ruminal fermentation. The chitin content of BSFL (9.6% DM; Kroeckel et al., 2012) is commonly cited as a factor limiting inclusion of BSFL in non-cattle livestock rations (Kroeckel et al., 2012; Makkar et al., 2014). Further work should be conducted on the impact of the chitin in BSFL on digestibility with a specific focus on cattle and impact on the ruminal microbiome. Furthermore, research evaluating defatted BSFL as a protein supplement for cattle should be conducted.

The total VFA concentrations for our protein supplements were comparable to that of Drewery et al. (2021) who fed hay with an identical CP content to our basal forage and supplemented the same amount of protein (100 mg N/kg BW) as in our study. Interestingly, they observed a novel protein supplement, defatted algae, numerically depressed total VFA relative to cottonseed meal or no supplement; this was hypothesized to be a consequence of the salt content of the algae, causing increased water intake and a dilution effect in the rumen (Drewery et al., 2021). It is promising that the novel protein supplement in our study, BSFL, did not cause a similar depression in total VFA concentrations or other

ruminal fermentation parameters that would indicate a disturbance in rumen physiology and/or microbial ecology associated with diet.

This study is the first *in vivo* feeding trial evaluating the suitability of BSFL as a protein supplement for cattle. Cumulatively, our data indicate that BSFL can be fed to cattle consuming low-quality forage to enhance forage intake without sacrificing digestibility or ruminal fermentation. We recommend further work evaluating BSFL and defatted BSFL in cattle diets with an emphasis on determining the site of protein degradation, evaluating the impact of chitin on digestibility, and characterizing the impact on the ruminal microbial population.

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