Evaluation of forage soybean, with and without pearl millet, as an alternative for beef replacement heifers¹

E. G. Taylor,* P. J. Gunn,† L. A. Horstman,‡ R. L. Atkinson,§² K. Herron,§ K. D. Johnson,# and R. P. Lemenager*

*Department of Animal Science, Purdue University, West Lafayette, IN 47907; †Department of Animal Science, Iowa State University, Ames 50011; ‡Veterinary Clinical Science, Purdue University, West Lafayette, IN 47907; §Department of Animal Science, Southern Illinois University, Carbondale 62901; and #Department of Agronomy, Purdue University, West Lafayette, IN 47907

ABSTRACT: Apparent ruminal digestibility of forage soybean-based silages, with and without pearl millet, was determined along with evaluation of silages on heifer performance and reproductive function. Fermenters were utilized in a Latin square design and randomly assigned to 1 of the following treatments: 1) control diet of alfalfa haylage (CON), 2) soybean silage (SB) or 3) soybean and pearl millet silage (SB×PM). All diets were formulated to meet or exceed nutrient requirements of replacement beef heifers targeted to gain 0.79 kg/d. These same diets were fed to 90 Angus-Simmental beef replacement heifers [body weight (BW) = 366 kg; body condition score (BCS) = 5.53; age $= 377 \pm 11$ d] 65 d prior to timed artificial insemination (TAI). Heifers were randomly allotted by breed, BCS and BW to 1 of the 3 treatments, with 3 reps/treatment. Diets were terminated 21 d post-TAI and heifers were commingled and placed on a common diet. Pubertal

status was determined by progesterone concentrations of 2 blood samples taken 10 d apart prior to both trial initiation as well as initiation of estrous synchronization. Ovulatory follicle diameter was determined at time of breeding by ultrasonography. Pregnancy diagnosis was accomplished 35 and 66 d post-TAI, respectively, to calculate TAI and end of season pregnancy rates. Neither SB nor SB×PM had an effect (P > 0.37) on apparent ruminal digestion of nutrients compared to the CON. Final BW (414 kg; $P \ge 0.10$) and BCS (5.28; P ≥ 0.26) for the heifers were similar among treatments. Likewise, there were no differences in TAI (48%; P >0.43) or overall breeding season (93%; P > 0.99) pregnancy rates. Ovulatory follicle diameters (11.7 mm) was not different (P > 0.19) among treatments. In summary, forage soybean-based silages, with and without pearl millet, was an acceptable alternative forage for developing replacement beef heifers.

Key words: alternative forage, beef heifer, reproduction, soybean

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INTRODUCTION

To maximize profitability in a cow-calf operation; growth rate, body energy reserves and target weight prior to breeding must be optimized to foster reproductive competence. Replacement heifers are developed to replace approximately 15 to 20% of a producer's herd and, therefore, represent a signifi-

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cant loss if they do not conceive in a timely manner and carry a calf to term. The expense associated with replacement heifer development, which is largely dictated by feed cost is one of the primary factors that dictate lifetime profitability. In addition, land conversion from forage to grain production has increased in the Midwest, leaving producers searching for feed alternatives. Soybean forage could have the potential to fill this void by double-cropping forage production following a grain crop such as wheat.

Some forage soybean cultivars were observed to have produced 13 tons ha⁻¹ with a June planting and 7.5 tons ha⁻¹ with a July planting (DM basis) with the late planting date simulating a double cropping system (Atkinson et al., unpublished data). Although forage

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²Corresponding author: ratkinsn@siu.edu

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yield is important to a livestock producer, forage quality needs to economically provide nutrients to allow optimal animal performance. Therefore, an assessment of forage quality is needed to determine if optimal animal performance can be obtained with minimal supplementation. Additionally, this assessment was done to compare the soybeans as a monoculture and when mixed with pearl millet to increase crop diversity. Pearl millet was selected to increase crop diversity because of similar growth rates, potential to increase yield and extend the time of harvest without introducing the potential for prussic acid.

Throughout history there have been many reported reproductive failures in livestock species due to the estrogenic activity of feedstuffs. This has prompted several investigations into the specific factors that have resulted in these failures. While there are several possible estrogenic substances of plant origin that may be involved, it was reported that genistin is, overall, the highest isoflavon in the soybean (Franke et al., 1994; Magee and Matrone, 1958). It also has been reported that in comparison to other legumes, such as subterranean clover, the levels of genistin in soybean are relatively low (Pieterse and Andrews, 1956; Magee and Matrone, 1958). Although, the levels of the estrogenic factor in soybean are low, suggesting a negligible effect on reproductive outcomes (Magee and Matrone, 1958), there have been some reports of soybean having a detrimental response in rabbit reproduction (Kendall et al., 1950). Due to these findings and our current focus on cost effective replacement beef heifer development strategies, there is a need to evaluate the effect of forage soybean on reproductive outcomes. The objective of this study was to evaluate forage soybean as an alternative feed resource for developing replacement beef heifers, with or without pearl millet, on apparent ruminal digestibility and reproductive performance. We hypothesize that feeding forage soybean, with and without pearl millet, will have no detrimental effects on heifer growth performance, nor reproductive efficiency.

MATERIALS AND METHODS

This study was conducted at the Purdue Animal Sciences Research and Education Center near West Lafayette, IN, and at Southern Illinois University, Carbondale. All animals were handled in compliance with protocols approved by the Purdue Animal Care and Use Committee and SIU Animal Care and Use Committee. Both institutions follow the Guide for Care and Use of Agricultural Animals in Research and Teaching.

Forages

Forage cultivars used were Eagle Seeds (Weiner, AR) soybean 'Big Fellow', and Byron Seeds (Rockville,

IN) pearl millet 'Wonderleaf'. Soybean as a monoculture was seeded on June 22, 2012 and harvested on October 6, 2012. Dry matter yield was 5415 kg ha⁻¹. Soybean intercropped with pearl millet was seeded on May 19, 2012 and harvested on August 18, 2012 with a dry matter yield of 7023 kg ha⁻¹. Soybean and soybean plus pearl millet were planted in 18-cm row spacing. Pure live seeding rates were 495,050 seeds ha⁻¹ for SB and the soybean and pearl millet seeding rates were 247,500 seeds ha⁻¹ and 3.5 kg ha⁻¹, respectively. Seeding of the soybean and pearl millet were in alternating rows. All forages were harvested, ensiled and stored in an Ag-Bag (Miller-St. Nazianz, Inc. Company, St. Nazianz, WI) for a minimum of 4 mo prior to feeding. Samples were taken using a forage probe (Oakfield Hay Sampler, Fond du Lac, WI) through the plastic for nutrient analysis prior to trial initiation and diets were formulated according to results. The plastic area probed was sealed with recommended tape immediately after samples were taken.

Continuous Culture System

A dual-flow continuous culture apparatus (Stern and Hoover, 1990) was used. The temperature of the fermenter contents was maintained at $38 \pm 1.0^{\circ}$ C and the pH was recorded every 6 h. Fermenter inoculum was obtained from 2 ruminally cannulated Angus heifers (23 mo of age, body weight (BW) = 476 ± 22 kg). Whole ruminal contents were collected into pre-warmed coolers (10 L per heifer), immediately transported to the lab, and strained under pressure through 8 layers of cheesecloth. Strained ruminal fluid from each heifer was mixed at a 1:1 ratio and 1,200 mL of mixed fluid was added to each fermenter along with 300 mL of pre-warmed buffer (Weller and Pilgrim, 1974) without the addition of urea. Average fermenter volume was 1654 mL and the liquid dilution rate was 0.10 h⁻¹ using the pre-warmed buffer. The solids dilution rate was 0.055 h^{-1} , which produced a mean solids retention time of 20 h. The pH and temperature were determined prior to feeding the fermenters. Fresh rumen fluid was collected at the beginning of each period.

Fermenter Diets and Feeding

All silages were lyophilized and ground through a 2-mm screen in a Thomas Wiley Mill (Thomas-Scientific; Philadelphia, PA) prior to mixing and feeding. Fermenters were fed 26.83 g 3 times daily at 0700, 1300, and 1900 to prevent stirring problems for a daily total of 80.5 g. Fermenters were utilized in a 3×3 Latin square design and randomly assigned to 1 of the following treatments per period: 1) 76.1% alfalfa silage, 16.1% corn stover, 6.7% dry distiller's grains (CON); 2) 75.1% forage soybean silage, 6.5% corn stover, 17.3% soyhulls (SB); and 3) 63.5% forage soybean, pearl millet mixed silage, 15.2% corn stover, 20.2% soyhulls (SB×PM). All diets contained 1.1% of a vitamin-mineral premix and were balanced to meet or exceed NRC (2000) requirements for a developing heifer and to contain 13.9% crude protein (CP; Table 1). After dietary analysis, composition of the rations contained 12.8 to 14.6% CP. Donor animals were fed a diet of alfalfa/ grass hay mix and distiller's grains plus solubles for the entire duration of the study to mimic the control diet.

Fermenter Sampling and Analyses

Each fermentation period was 10 d, with a 7 d adaptation period followed by a 3 d sampling period. During sampling periods, effluent was continuously collected and held at 4°C in a cold water bath to minimize bacterial fermentation. Total effluent from each 24 h within the 3 d sampling period was mixed, and a 1-L subsample was composited each day and stored at -20° C, resulting in 3 L of total composited effluent. Composited effluent was lyophilized (VirTis BenchTop, SP Industries, Inc., Gardiner, NY) prior to analysis. An additional 450-g subsample of wet effluent was collected and processed for bacterial isolation (Merchen and Satter, 1983), without the addition of saline in the last step, and the microbes were analyzed for DM (AOAC, 1990). This allowed for the estimation of microbial DM outflow which was used to extrapolate g of microbe DM per g of effluent.

Feed and ruminal contents were analyzed for DM, CP, NDF, and ADF. The DM was determined by oven drying 1 g of sample, in triplicate, at 105°C for 24 h (AOAC, 1990). Crude protein was calculated as N × 6.25, and N content was determined by combustion (LECO Model FP-528 Nitrogen analyzer; LECO Corp., St. Joseph, MI) using EDTA as a calibration standard. Neutral and acid detergent fiber (Goering and Van Soest, 1970; as modified by Ankom Technology, Fairport, NY) contents of feed and effluent were determined using an ANKOM²⁰⁰ fiber analyzer (ANKOM Technology), with α-amylase used during the NDF procedure.

Animals and Diets

Angus-Simmental crossbred heifers (n = 90; BW = 366 kg ± 25; body condition score (BCS) = 5.53 ± 0.35 ; Age = 377 d ± 11) were used in a complete randomized block design study to evaluate the effects of feeding an ensiled forage soybean or soybean × pearl millet forage on heifer BW, BCS, follicular growth, and pregnancy.

Heifers were stratified by percentage of Angus, BCS and BW, and randomly assigned to 1 of 3 treatments with 3 replications per treatment (10 heifers/ replicate). Dietary treatments were: 1) alfalfa haylage **Table 1.** Formulated (actual) dietary feed ingredients,

 dietary nutrient composition, and calculated dry matter

 intake of yearling beef heifer diets

		Treatment ¹		
Item	CON ²	SB ³	SB×PM ⁴	
Ingredient, formulated %5				
(delivered kg of DM/d ⁶)				
Alfalfa haylage	76.1(7.28)			
Soybean silage		75.1(6.31)		
Soybean × pearl millet silage			63.4(5.24)	
Corn stover	16.3(1.45)	6.5(.66)	15.2(1.33)	
Dried distiller's grains with solubles	6.7(.57)			
Soybean hulls		17.2(1.5)	20.3(1.56)	
Mineral Supplement ⁷	1.11(0.10)	1.11(0.11)	1.11(0.09)	
Formulated Nutrient, (delive	red)			
Intake ⁸				
CP, g/d	1365(1295)	1368(1170)	1371(1135)	
NE _m , Mcal/d	7.74(7.86)	7.74(7.74)	7.74(7.74)	
NE _g , Mcal/d	2.92(2.48)	2.93(1.94)	2.92(1.62)	
Ca, g/d	119(114)	87(76)	84(69)	
P, g/d	30(28)	30(27)	29(24)	
Mg, g/d	23(22)	36(31)	37(31)	
K, g/d	149(142)	184(158)	225(187)	
NDF, kg/d ⁹	(3.84)	(3.95)	(4.27)	

 1 CON = control, alfalfa haylage; SB = soybean silage; SB×PM = soybean and pearl millet silage.

²Alfalfa forage analysis provided on DM basis: 43.6% DM, 24.59% Adjusted CP, 34.35% ADF, 39.05% NDF.

³Soybean forage analysis provided on DM basis: 94.7% DM, 19.5% Adjusted CP, 39.9% ADF, 53% NDF.

 4 Soybean \times Pearl millet analysis provided on DM basis: 91.0% DM, 17% Adjusted CP, 36.4% ADF, 58.8% NDF.

⁵The formulated diet fed to the heifers also was used to feed the fermenters.

⁶DMI was measured for CON, SB and SB×PM total mixed rations delivered. ⁷Mineral delivered contained: 11% Ca, 5% P, 2%, 2% K, 19.5% Salt, 1500 ppm Cu, 3000 ppm Mn, 3700 ppm Zn, 27ppm Se, 40ppm Co, 660 kIU/kg Vitamin A, 66 IU/kg Vitamin E.

⁸Calculated dietary chemical composition based on analysis of individual dietary feed ingredients.

⁹NDF concentration of total mixed ration determined by wet chemistry methods.

(CON), 2) soybean silage (SB), and 3) soybean × pearl millet silage (SB×PM). Diets and their chemical composition are described in Table 1.

All diets were formulated to be isocaloric, isonitrogenous, and either meet or exceed all other nutrient requirements (NRC, 2000), meet 100% DMI capabilities and to obtain a target gain of 0.79 kg and mean target weight of 65% of mature BW prior to initiation of breeding (Table 1). Ingredient composition of feed stuffs used to formulate diets were obtained by wet chemistry methods (AOAC, 1990) before trial initiation (Dairy One, Ithaca, NY). Feed samples were collected from the total mixed ration (TMR) mixer beginning, middle and at the

end of the study and frozen at -20°C. Upon completion of the study, samples were composited, mixed, and dried in a forced-air oven at 60°C. Dried forage samples were ground through a 1-mm screen in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA). Samples were analyzed for NDF content (Goering and Van Soest, 1970; as modified by Ankom Technolgy) using an ANKOM²⁰⁰ fiber analyzer (Ankom Technology, Macedon, NY), with a-amylase used during the NDF procedure. Heifers were housed in 9 mounded lots $(15 \times 46 \text{ m})$ with concrete feeding apron, concrete feed bunks (0.9 m per head), and frost-proof automatic waterers. All dietary treatments were fed as a TMR beginning 65 d prior to timed-artificial insemination (TAI) and were fed ad libitum daily at 0800 and orts were weighed weekly to determine actual intake. Daily feed delivery adjustments were made based on daily bunk scores and weekly ingredient DM analysis.

At the beginning of the trial d-0, BW and BCS (1 = emaciated, 9 = obese; Wagner et al., 1988) was determined by the average of 2 preprandial measurements taken on consecutive days. A single day preprandial midpoint BW and BCS was also taken. An experienced single investigator was responsible for determining BCS throughout the study. Also, a single day preprandial BW and BCS were obtained on d-62 at termination of estrous synchronization, and were used for final BW and BCS determination. At TAI, d-65, heifers were commingled within treatment and remained on their treatment diets 21 d post-TAI, and then placed on a common pasture of orchard grass-alfalfa mix.

Puberty

Puberty was determined by collection of blood samples analyzed for progesterone on 2 separate 10-d intervals. The first sample collection period was taken on d-10 and d-0 relative to trial initiation, and the second sample collection period was d-46 and d-56 prior to the initiation of estrous synchronization. Blood samples were collected via coccygeal venipuncture in 6 mL EDTA tubes (BD VacutinerTM; Becton-Dicknson, Franklin Lakes, NJ) and placed on ice for no more than 4 h until processed. Samples were centrifuged at $1,750 \times g$ for 25 min at 4°C. Plasma was transferred to polystyrene tubes and frozen at -20°C for subsequent progesterone analysis. Plasma progesterone concentrations were determined using a commercially available RIA kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA). Heifers with a progesterone concentration ≥ 2 ng/ml at either time point or ≥ 1 ng/ml at both time points in a sampling period were determined to be pubertal for that period. Across 4 assays, the average intraassay coefficient of variation (CV) was 0.5% and the

inter-assay CV for pooled plasma samples containing 0.5 ng/ml and 6.8 ng/ml of progesterone were 0.37% and 2.2%, respectively. In addition, reproductive tract scores (RTS) were performed using rectal palpation by a board certified theriogenologist on d-47 according to the procedure described by (Anderson et al., 1991).

Estrous Synchronization, Breeding, and Luteal Function

On d-56 of the study, all heifers were enrolled in a 5-d Co-Synch + CIDR protocol to synchronize ovulation (Bridges et al., 2012). At protocol initiation, all heifers were administered an intravaginal progesterone insert (CIDR Zoetis Animal Health, Florham Park, NJ) concurrent with the administration of 100 µg of GnRH (Cystorelin, Merial Animal Health, Duluth, GA). Five days after protocol initiation, the CIDR was removed and 2 separate 25-mg injections of prostaglandin $F_{2\alpha}$ (PG; Lutalyse, Zoetis Animal Health, Florham Park, NJ) were simultaneously given. At CIDR removal, all heifers were tail painted (Tell Tail; FIL, 132 Mount Maunganui, New Zealand) to assist in estrus detection during the 72 h period immediately following PG administration. Heifers exhibiting estrus within 60 h following PG administration, were artificially inseminated (AI) 12 h post-standing heat. Heifers not exhibiting behavioral estrus were TAI bred at 72 h following PG administration. At breeding, all heifers were evaluated by trans-rectal ultrasonography (Variable MHz Linear Array Transducer, MicroMacxx-Sonosite, Bonchell, WA) to measure the diameter of dominant follicle. Both ovaries were scanned for follicles, with the largest follicle being determined as dominant. The dominant follicle was measured using the caliper function of the ultrasound (mm in diameter) and recorded. Both breeding and ultrasonography were conducted by 2 trained technicians. On d-7 post-AI, blood samples were collected and processed for progesterone concentrations, as described above, to indirectly measure corpora luteua function. Progesterone concentrations were analyzed using the same RIA kit and procedure previously described.

Statistical Analysis

All digestion data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) for a Latin square design. The model included treatment and period with fermenter specified in the RANDOM statement of SAS. The following contrasts were used to test treatment effects; 1) CON diet vs. the average of diets containing soybeans, and 2) SB diet vs. SB×PM diet. A *P*-value \leq 0.05 was identified as significant and a *P*-value of 0.06 to 0.1 was considered a tendency.

Table 2. Effects of yearling beef heifer diet on apparent

 ruminal digestibility of rations

	Treatment ¹				Contrast P-value1	
Digestibility, % of intake	CON	SB	SB×PM	SEM	1 vs 2,3	2 vs 3
DM, apparent ruminal	38.3	35.8	32.6	3.43	0.39	0.55
DM, corrected for microbial DM	67.3	63.7	62.9	1.92	0.38	0.55
NDF	62.3	56.1	52.4	4.41	0.21	0.59
ADF	62.4	57.2	56.6	4.45	0.37	0.94
СР	62.4	66.0	69.0	4.60	0.42	0.67

 1 CON = control (1); SB = soybean silage (2); SB×PM = soybean and pearl millet silage (3).

Table 3. Effect of yearling beef heifer diet on dry matter

 intake and growth performance

		Treatment ¹			Contrast P-value1	
Item	CON	SB	SB×PM	SEM	1 vs 2,3	2 vs 3
BW, kg						
Initial	366	367	365	3.90	0.98	0.76
Final	422	413	408	6.07	0.16	0.55
BCS ²						
Initial	5.53	5.56	5.49	0.02	0.69	0.07
Final	5.33	5.22	5.28	0.04	0.26	0.37
DMI, kg/d						
Overall	9.39	8.58	8.24	0.18	0.01	0.22
ADG, kg						
Overall	0.87	0.71	0.65	0.04	0.01	0.38
G:F, kg/kg						
Overall	0.09	0.08	0.08	0.01	0.06	0.55

 1 CON = control (1); SB = soybean silage (2); SB×PM = soybean and pearl millet silage (3).

²Body condition score on a scale of 1 to 9 (1 = emaciated, 9 = obese; Wagner et al., 1988).

Performance and reproductive data were analyzed with pen as the experimental unit. Differences among treatments were analyzed using the MIXED procedure of SAS. The following contrasts were used to test treatment effects; 1) CON diet vs. the average of diets containing soybeans, and 2) SB diet vs. SB×PM diet. For dependent variables, the model included the fixed main effect of treatment. Ovary side, sire and tech were initially included in the model as covariates for pregnancy, but were removed due to insignificance. For all variables analyzed, a *P*-value ≤ 0.05 was identified as significant and a *P*-value of 0.06 to 0.1 was considered a tendency.

RESULTS

Apparent Ruminal Digestibility

Apparent ruminal digestibility was not affected by forage source. There were no differences in DM, NDF, ADF, or CP digestibility ($P \ge 0.21$, Table 2).

Growth Performance

Initial heifer BW (366 ± 25 ; $P \ge 0.70$) and BCS (5.53 ± 0.35 ; $P \ge 0.07$) did not differ among treatments (Table 3). Likewise, final BW ($P \ge 0.16$) and BCS ($P \ge 0.26$) were similar among treatments. Overall, heifers fed the diets containing soybean forage consumed 10% less (P = 0.001) DMI than control fed heifers, but the DMI of the soybean containing diets were not different (P = 0.22) from each other. Similarly, ADG of the heifers fed the soybean diets were not different from each other (P = 0.38), but heifers fed the soybean containing diets gained 21.4% slower (P = 0.01) than control heifers. Feed efficiency tended to be higher in the CON when compared to the SB and SB×PM diets ($P \ge 0.06$).

Reproductive Performance

The proportion of pubertal heifers prior to initiation of treatments was not different ($P \ge 0.76$; Table 4) among treatments. Reproductive tract scores ($P \ge 0.18$) were similar among treatments. However, the proportion of heifers exhibiting estrus prior to estrous synchronization tended to be lower in the SBXPM treatment ($P \ge 0.09$; Table 4). Of those heifers that exhibited estrus prior to TAI, there were no differences in the interval between prostaglandin administration and estrus ($P \ge 0.46$). Ovulatory follicle diameter at time of AI ($P \ge 0.19$) and progesterone analysis on d-7 post AI ($P \ge 0.51$) were similar among treatments. There also were no differences in TAI ($P \ge 0.43$), AI pregnancy ($P \ge 0.25$), resorbed pregnancies ($P \ge 0.40$) or overall breeding season (P >0.99) pregnancy rates among treatments.

DISCUSSION

The ability of a producer to double-crop forage has become increasingly popular with recent land conversions in the Midwest U.S. from perennial pasture to cereal grain production. Alternative feeding strategies are being evaluated that would allow land to be used for both grain and forage production in the same calendar year to efficiently and sustainably meet animal nutrient requirements. This study suggests that the digestibility of forage soybean is similar to the digestibility of alfalfa. In contrast to the current study, however, Vargas-Bello-Pérez et al. (2008) observed that in situ soluble DM and NDF fractions were greater for alfalfa silage compared to forage soybean silage when bags were incubated in lactating Holstein cows. In that study, forage soybeans were not harvested for silage until full-pod stage and when the lower leaves started to become yellow. When alfalfa silage and soybean silages were fed to ruminally cannulated lactating dairy cows, total tract digestibility of DM, OM CP, NDF, and gross energy were similar (Vargas-Bello-Pérez et al.,

Table 4. Effect of yearling beef heifer diet on reproductive performance

		Treatment ¹			Contrast P-value ¹	
Item	CON	SB	SBXPM	SEM	1 vs 2,3	2 vs 3
Puberty initial ² , %	63.3	66.7	66.7	0.09	0.76	1.00
Puberty synch ³ , %	100.0	96.7	86.7	0.06	0.10	0.09
Tract score ⁴ ,	4.5	4.2	4.2	0.15	0.18	0.88
Estrus ⁵ , %	63.3	46.6	43.3	0.16	0.38	0.89
Estrus interval to estrus ⁶ , h	59.2	54.2	55.2	4.62	0.46	0.88
Follicle diameter, mm ⁷	11.2	12.1	11.9	0.44	0.19	0.70
Progesterone ⁸ , ng/ml	3.9	3.8	3.7	0.29	0.51	0.86
TAI, ⁹ %	36.6	50.0	56.6	0.16	0.43	0.78
AI pregnancy ¹⁰ ,%	53.3	50.0	66.6	0.09	0.67	0.25
Season Pregnancy ¹¹ , %	93.3	93.3	93.3	0.05	1.00	1.00

¹CON = control; SB = soybean silage; SBxPM = soybean and pearl Millet silage

²Percent of heifers determined to be pubertal prior to initiation of treatments.

³Percent of heifers determined to be pubertal prior to initiation of estrous synchronization.

⁴Reproductive tract scores; based on Anderson et al., (1991).

⁵Proportion of heifers that exhibited estrus within 72 h after prostaglandin F_{2a} in the 5 d CO-Synch + CIDR protocol.

 6 Of heifers exhibiting standing estrus within 72 h after prostaglandin F_{2a}, defined as the interval from PG to initiation of standing estrus.

⁷Diameter of dominant follicle at time of breeding.

⁸Progesterone concentration d-7 post-AI breeding.

 9 Proportion of heifers that did not exhibit estrus within 60 h after prostaglandin F_{2a} that were timed-artificially inseminated.

¹⁰Number of confirmed pregnant heifers at 35 d post AI/number of heifers inseminated.

¹¹Number of confirmed pregnant heifers at 66 d end of season pregnancy diagnosis.

2008). Additionally, cows fed soybean silage consumed less DM, CP, and NDF compared to cows fed alfalfa silage. Vargas-Bello-Pérez et al. (2008) concluded that feeding forage soybean silage reduced the DMI compared with alfalfa silage, likely because of an increased forage NDF content and its reduced ruminal degradability and that more research was needed to determine the optimal stage of soybean development at harvest. In the current study, both NDF and ADF concentrations were greater for SB silage compared to CON, but the forage soybeans were harvested for silage at 15 wk of growth, which was late bloom stage for a group 7 forage soybean. The stage of maturity at harvest for soybean is likely the reason why there was no differences in apparent digestibility of DM, NDF, ADF, or CP observed in the current study.

Development of the replacement beef heifer is recognized as a top priority for the industry due to the lasting effects she will have in the herd. Not only puberty attainment and first service conception rate, but also lifetime productivity may be affected during the replacement heifer development period. There has been considerable debate as to what an acceptable target weight should be for a beef heifer entering the breeding season (65%, Patterson et al., 1989; 55%, Funston and Deutscher, 2004). The mature cow weight in this herd was 648 kg \pm 34 kg, with the heifers in this study obtaining a mean of 65, 63.7, and 63% of their mature weight prior to breeding, respectively, for CON, SB, and SBxPM treatments. Although the heifers fed soybean diets didn't achieve 65% of mature body weight they still fell within the general recommended guidelines of

being 60 to 66% of mature body weight (Patterson et al., 1992). It was concluded that all treatments performed adequately and all animals obtained an acceptable target weight for a developing replacement beef heifer.

While diets were formulated to be similar, there were differences in the DMI of the 3 treatments. As eluded from previous studies, an animal's response related to DMI may be dictated by the quality of forage being fed, specifically the NDF concentration of that forage (Mertens, 1994; Vargas-Bello-Pérez et al., 2008). The DMI is lower for a low-quality forage with a higher NDF, than a high-quality forage with a lower NDF (Mertens, 1994; Varel and Kreikemeier, 1999).

Predicted intake for the heifers when formulating the diets was 9.68 kg of DM/d. The actual DMI for the CON diet was 9.37 kg of DM/d (5% lower than predicted), while the SB diet was 8.58 kg of DM/d (13% lower than predicted) and the SB×PM diet was 8.22 kg of DM/d (16.6% lower than predicted). Analysis of TMR diets in the current study resulted in NDF concentrations of; 41.0%, 46.0%, and 52.7% in the CON, SB and SB×PM diets, respectively. The SB treatment was 12.2% higher in NDF than the CON treatment and the resulting DMI was 8.6% lower. The SB×PM treatment was 14.6% higher in NDF concentration than the SB treatment, resulting in a 4% difference in DMI. Using the average pre-prandial BW mean for each treatment in this study, the NDF values of each diet were compared to DMI. Neutral detergent fiber intake expressed as a percent of BW was calculated to be, 0.97, 1.01 and 1.10%, respectively, for the CON, SB, and SB×PM treatments. This resulted in an average

NDF intake of 1.03% of BW across treatments. Results in the current study suggest that heifers will consume a near constant 1% of their BW in NDF per d. Due to the fluctuation in NDF among diets, it could be concluded that differences in dietary NDF concentration likely caused the observed differences in DMI across treatments. Average daily gain differed across treatments and could be directly associated with the differences in DMI. These data and the data from previous studies (Mertens, 1994; Vargas-Bello-Pérez et al., 2008) strongly suggests a need to utilize NDF concentration of a forage-based TMR when estimating DMI for formulating replacement beef heifer diets to make sure that nutrient requirements are met.

Reproductively, heifers performed similarly and adequately on all dietary treatments. Although it has been suggested previously that estrogenic properties of the soybean may impede reproductive outcomes there were no differences in attainment of puberty, reproductive tract scores, proportion of heifers exhibiting estrus, or interval to estrus observed in this study when soybean forage was included in the diet. Though, there was a tendency for the SBXPM treatment to have a lower number of heifers exhibiting estrous prior to estrous synchronization, one could argue this has little, if any, biological significance, as no differences were seen in TAI or season long pregnancy rates. Because estrogen is naturally occurring in the ovary, and is important for signaling of follicular growth, it is not clear if exogenous or excess estrogen could be detrimental to this process (Jefferson, 2010). Since follicular size did not differ among treatments, this strongly suggests that dietary estrogens did not have a negative nor a positive effect. While it is acknowledged that using pen-averaged pregnancy data hinders statistical power for such binary traits there were no differences found in TAI or breeding season pregnancy rates. In conclusion, forage soybean appears to be an acceptable alternative forage in diets designed for replacement heifer development. Based on these data, it may be recommended that when forage-based rations are developed for replacement heifers, a 1% of BW NDF intake be used to more accurately estimate total diet DMI.

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