Effects of monensin inclusion into increasing amount of concentrate on growth and physiological parameters of early-weaned beef calves consuming warm-season grasses

Joao M. B. Vendramini,^{*,1} Philippe Moriel,^{*} Reinaldo F. Cooke,^{†,⊙} John D. Arthington,^{*} Hiran Marcelo da Silva,^{*} Matheus B. Piccolo,^{*} Joao Marcelo D. Sanchez,^{*} Vinicius Gomes,^{*} and Pedro A. Mamede^{*}

^{*}IFAS/Range Cattle Research and Education Center, University of Florida, Ona, FL 33865; and [†]Department of Animal Science, Texas A&M University, College Station, TX 77840

ABSTRACT: Two experiments evaluated the effects of concentrate amount and monensin inclusion on growth and physiological parameters of early-weaned beef calves consuming warm-season grasses in drylot (Exp. 1) and pastures (Exp. 2). In both experiments, treatments consisted of two concentrate DM amounts (1 or 2% of BW) and two inclusion rates of monensin (0 or 20 mg of monensin/kg of total DM intake). In Exp. 1, 48 Angus × Brahman crossbred early-weaned (EW) beef calves (initial age = 90 ± 13 d; initial BW = 83 ± 12 kg) were distributed in 12 drylot pens (four calves per pen; three pens per treatment) and provided stargrass (*Cynodon nlemfuensis*) hay (9% CP and 52% IVDOM) at amounts to ensure 10% DM refusals for 56 d. In Exp. 2, 36 Angus × Brahman crossbred EW heifer calves (initial BW = 171 ± 15 kg) were randomly allocated into one of 12 bahiagrass (*Paspalum notatum*) pastures on a continuous and fixed stocking rate (1 ha and three heifers per pasture; three pastures per treatment) and received daily supplementation of their respective treatments for 84 d. In both experiments,

effects of monensin inclusion × concentrate amount were not detected for any variable ($P \geq 0.14$), but overall ADG and plasma IGF-1 concentrations were greater ($P \leq 0.05$), whereas fecal coccidia egg counts tended ($P = 0.09$; Exp. 1) or were less ($P = 0.05$; Exp. 2) for calves offered concentrate with vs. without monensin inclusion. Calves offered concentrate at 2% of BW had greater ($P \leq 0.05$) overall ADG (Exps. 1 and 2), herbage mass (Exp. 2 only), in vivo apparent digestibility, total DMI and plasma concentrations of glucose and IGF-1 (Exp. 1 only), less forage DM intake (Exp. 1 only), and no effects on fecal coccidia egg counts (Exps. 1 and 2) compared to calves offered concentrate at 1% of BW. Increasing concentrate amount is an effective management practice to increase ADG and decrease forage DMI in early-weaned calves consuming warm-season grasses, whereas the decrease in fecal coccidia egg count and additional ADG provide evidence that monensin should be supplied to early-weaned calves grazing warm-season pastures and receiving concentrate at 1% of BW or above.

Key words: beef cattle, monensin, ruminal fermentation parameters, supplementation, warm-season grass

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J. Anim. Sci. 2018.96:5112–5123

doi: 10.1093/jas/sky374

INTRODUCTION

Mild winters in the southern United States allow producers to raise early-weaned (EW) calves on pastures of cool- or warm-season grasses

¹Corresponding author: jv@ufl.edu

Received June 25, 2018.

Accepted September 20, 2018.

(Vendramini et al., 2006, 2007). Early-weaned calves need to be offered some concentrate amount to achieve similar performance of calves weaned at 8 mo of age (Arthington and Kalmbacher, 2003). Also, Vendramini et al. (2007) observed that EW calves grazing “Tifton 85” bermudagrass (*Cynodon* spp.) had linear increase in ADG with concentrate DM offered from 0% to 2% of BW.

Ionophores inclusion may induce positive responses in growth and feed efficiency in ruminants receiving diets with high concentrate inclusion (Duffield et al., 2012). Rouquette et al. (1980) compared the effects of monensin on beef calves grazing bermudagrass (*Cynodon dactylon*) and receiving 0.9 kg/d of concentrate. Calves receiving 200 mg/d of monensin had greater ADG compared to calves receiving concentrate only. In addition, calves are highly susceptible to coccidiosis from 4 to 8 mo of age and monensin has been an effective coccidiostatic used in dairy calf management (Stromberg et al., 1986). Although the use of monensin for dairy calves has been vastly reported in the literature, there is limited information about the potential benefits of monensin on EW beef calves grazing warm-season grass pastures and receiving concentrate supplementation. The hypothesis of the study was that monensin inclusion into the concentrate will decrease coccidiosis and improve growth performance of EW beef calves offered warm-season grasses, and that the magnitude of the increase on growth performance will be greater with the increasing concentrate amount due to greater substrate availability for propionic acid production. The objectives of this study were to test the effects of concentrate amount and monensin inclusion on coccidiosis infestation, growth and physiological parameters of EW calves offered warm-season grasses in drylot (Exp. 1) and pastures (Exp. 2).

MATERIALS AND METHODS

The experiments were conducted at the UF/IFAS Range Cattle Research and Education Center, Ona, FL (27° 26' N and 82° 55' W) from January to March 2017 (Exp. 1) and April to June 2014 (Exp. 2). The experimental procedures were approved by the University of Florida Institute of Food and Agriculture Sciences Animal Research Committee (protocol #201709703).

Experiment 1

Forty-eight Angus × Brahman crossbred calves (24 steers and 24 heifers) were weaned on January

5, 2017 (day 12; initial age = 90 ± 13 d; initial BW = 83 ± 12 kg). Calves were held in a drylot with access to long-stem stargrass (*Cynodon nlemfuensis*) hay ad libitum and 1 kg/d of preconditioning medicated concentrate (guaranteed analysis, as fed: 14% CP, 1.0% fat, 18% fiber, 0.75% Ca, 0.40% P, and 0.40% NaCl, Land O'Lakes Purina Feed LLC, Gray Summit, MO) for 12 d until the start of the experiment (day 0). On day 0, calves were stratified by sex, initial BW and age, and randomly allocated into one of 12 drylot pens (two steers and two heifers per pen). All calves remained in the drylot for 56 d (January 17 to March 14, 2017). Treatments were randomly assigned to pens (three pens per treatment), in a 2 × 2 factorial design, and consisted of two concentrate amounts (1 or 2% of BW; DM basis) and two inclusion rates of monensin (0 or 20 mg of monensin/kg of an estimated total DM intake of 2.5% of BW; NRC, 2016). Rumensin 90 (Elanco Animal Health, Greenfield, IN) was the commercial product used as a source of monensin and the proposed monensin amount was added daily to the concentrate at the time of feeding (0800 h).

Calves were offered ground stargrass hay (9% CP and 52% in vitro digestible organic matter; **IVDOM**) at amounts to ensure 10% of refusal DM. Hay was processed through a hay chopper (Balebuster 2100, Haybuster Jamestown, ND) to achieve approximately 5 cm of particle size and offered four times daily (0800, 1000, 1200, and 1400 h). Concentrate was hand-fed daily at 0800 h and consumed entirely (no concentrate refusals were observed from days 0 to 50). Chemical composition of concentrate is presented in Table 1. Calves were provided free choice access to a complete salt-based trace mineral mix (guaranteed analysis: 14% Ca, 9% P, 24% NaCl, 0.20% K, 0.30% Mg, 0.20% S, 0.005% Co, 0.15% Cu, 0.02% I, 0.05% Mn, 0.004% Se, 0.3% Zn, 0.08% F, and 82 IU/g of vitamin A; Lakeland Animal Nutrition, Lakeland, FL). Samples of the forage offered and refused were collected daily from days 0 to 50, and then dried at 60 °C for 96 h for DM calculations. Total DM intake was calculated for each pen using the cumulative DM intake of forage + concentrate, and then divided by: (i) total number of feeding days (reported as kilogram per day) and (ii) total sum of mean calf BW within each pen (reported as % of BW).

Animal measurements. Individual BW of all calves was collected on days 0, 28, and 56, following 16 h of feed and water withdrawn. Blood samples from

Table 1. Nutrient composition of concentrate supplement used in Exps. 1 and 2 (DM basis)¹

Item	Concentrate
CP, %	18.3
ADF, %	11.2
NDF, %	22.8
TDN ² , %	78.1
Ca, %	0.31
P, %	0.69
Mg, %	0.34
K, %	1.47
Fe, mg/kg	249
Zn, mg/kg	67
Cu, mg/kg	34
Mn, mg/kg	61

¹ Concentrate consisted of soybean hulls (40%), wheat middlings (22%), cottonseed meal (20%), cottonseed hulls (10%), molasses (6.25%), calcium carbonate (1.25%), and vitamin/mineral premix (0.50%).

² TDN was calculated as described by Weiss et al. (1992).

jugular vein were collected from all calves on days 0, 28, and 56. Blood samples were collected into 9 mL, Na-heparinized syringes (Luer Monovette, LH, Sarstedt, Inc., Newton, NC) and immediately placed on ice after collection. Blood was centrifuged at $2,000 \times g$ and 4 °C for 30 min, and plasma harvested and kept frozen at -80 °C until further analysis. Plasma concentrations of insulin were determined using Coat-A-Count solid-phase ¹²⁵I RIA kits (Siemens Healthcare Diagnostics, Los Angeles, CA) previously validated for bovine samples (Moriel et al., 2008). Plasma concentrations of glucose and plasma urea nitrogen (PUN) were determined using quantitative colorimetric kits (#G7521 and B7551, respectively; Pointe Scientific Inc., Canton, MI). Concentrations of IGF-1 were determined using a human-specific commercial ELISA kit (SG100; R&D Systems Inc., Minneapolis, MN) with 100% cross-reactivity with bovine IGF-1 and previously validated for bovine samples (Cooke et al., 2012). The intra- and inter-assay CV were 2.1% and 5.5% for insulin, 1.7% and 1.9% for glucose, 4.4% and 5.9% for PUN, 7.2% and 8.5 % for IGF-1. The minimum detectable concentration was 0.01 µIU/mL for insulin, and 0.056 ng/mL for IGF-1.

Rectal fecal samples from all calves were collected twice daily (0800 and 1500 h) from days 51 to 54 to determine in vivo apparent DM digestibility using the indigestible NDF (iNDF) procedure. Fecal samples were pooled from all calves within each pen and across all fecal collection days (days 51 to 54). Concentrations of iNDF in the forage, concentrate, and feces were determined as described

by Cole et al. (2011) with modifications proposed by Krizsan and Huhtanen (2013). Samples were weighed (0.5 g) into F57 filter bags, and then incubated at 39 °C using a 4:1 ratio of McDougall's buffer:rumen fluid in a Daisy^{II} incubator (ANKOM Technology Corp., Macedon, NY) for 288 h. After incubation, samples were rinsed and analyzed for NDF concentration using the method of Van Soest et al. (1991) adapted for an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp.). Heat-stable α-amylase and sodium sulfite were used in the NDF assay and the results are presented inclusive of residual ash. Total feces output was calculated as expressed in Eq. (1). Apparent DM digestibility was calculated as indicated in Eq. (2):

$$\text{fecal output (kg)} = \left[\frac{\left(\text{iNDF concentration in feed} \times \text{DM intake (kg)} \right)}{\text{iNDF concentration in feces (\%)}} \right] \quad (1)$$

$$\begin{aligned} \text{apparent DM digestibility (\%)} \\ = 100 - \left[\left(\frac{\text{fecal output (kg)}}{\text{DM intake (kg)}} \right) \times 100 \right] \end{aligned} \quad (2)$$

Rectal fecal samples were also collected from all calves on days 0 and 56, and then sealed in plastic bags, identified, and sent in an insulated container with ice to a commercial laboratory (Myers Parasitology Services, Magnolia, KY) for analysis of fecal coccidia egg count using the Modified Wisconsin Sugar Flotation Technique (Cox and Todd, 1962). The laboratory staff was blinded to treatment assignments. Coccidia egg counts (observed egg count + 1) of each calf were log transformed before statistical analyses, and reported as log₁₀ (Martins et al., 2016).

Experiment 2

Soil at the research site was Eaugallie sand (sandy, siliceous, hyperthermic Alfic Alaquod). Before the initiation of the study, mean soil pH was 6.1 and Melich-1 extractable P, K, Mg, and Ca in the Ap 1 horizon (0 to 15 cm depth) were 52, 56, 198, and 1,100 mg/kg, respectively.

Thirty-six Angus × Brahman crossbred heifer calves were weaned at approximately 90 d of age and grazed “Jumbo” annual ryegrass from January to April 2014. In April (day 0), heifers were stratified by initial BW (171 ± 15 kg), and randomly allocated into one of 12 bahiagrass (*Paspalum notatum*) pastures on a continuous and fixed stocking rate (1 ha and three heifers per pasture). Treatments

were randomly assigned to pastures (three pastures per treatment), in a 2×2 factorial design, and consisted of two concentrate amounts (1 or 2% of BW; DM basis) and two inclusion rates of monensin (0 or 20 mg of monensin/kg of the expected total dry matter intake of 2.5% of BW). Heifers remained on their respective pasture and treatment assignments for 84 d. Rumensin 90 (Elanco Animal Health) was the commercial product used as a source of monensin and the proposed inclusion rate of monensin was added daily to the concentrate (similar concentrate as in Exp. 1; Table 1) immediately before feeding (0800 h). Heifers had ad libitum access to a complete salt-based trace mineral mix described previously in Exp. 1.

Pasture sampling. Herbage mass (HM) and hand-plucked samples of pastures to assess nutritive value (CP and IVDOM) were collected at initiation of grazing and every 14 d for 84 d. The double sampling technique was used to determine HM according to Gonzalez et al. (1990). The indirect measure was the settling height of a 0.25-m² aluminum disk, and the direct measure involved hand clipping all herbage to 2.5 cm above soil level using an electric clipper. Every 28 d, two double samples were taken from each of the 12 experimental units for a total of 24 double samples per date. Sites for double sampling were chosen to represent the range of HM present on the pastures. At each site, the disk settling height was determined and the forage under the disk was clipped at ground level. Clipped forage was dried at 60 °C for 72 h in a forced air-oven and weighed. Indirect measures (disk heights) were taken every 14 d at 20 sites per pasture. Sites were selected by walking a fixed number of steps between each drop of the disk to ensure that all sections of the pasture were represented. The average disk height of the 20 indirect measures was entered into the regression equation developed from double sampling to predict HM. The average r^2 values for the equations were from 0.72 to 0.84. Herbage allowance (HA) was calculated for each pasture as the average HM (mean across two sampling dates within each 28 d period) divided by the average total heifer BW during that period (Sollenberger et al., 2005).

Twenty hand-plucked samples were randomly taken at the average forage height from each pasture. Herbage was composited across sites, dried at 60 °C for 48 h in a forced-air oven to constant weight and ground to pass a 1-mm stainless steel screen in a Wiley mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedesboro,

NJ). Samples were analyzed for IVDOM using the two-stage technique described by Tilley and Terry (1963) and modified by Moore and Mott (1974). Nitrogen concentration was determined using a micro-Kjeldahl method, a modification of the aluminum block digestion technique described by Gallaher et al. (1975). Crude protein was determined by multiplying N concentration by 6.25.

Animal measurements. Heifers were weighed individually at 0730 h every 28 d from days 0 to 84, following a 16-h period of feed and water withdrawn. Blood samples from jugular vein were collected from all heifers every 28 d from days 0 to 84. Blood samples were collected into 9 mL, Na-heparinized syringes (Luer Monovette, LH, Sarstedt, Inc.) and immediately placed on ice after collection. Blood was centrifuged at $2,000 \times g$ and 4 ° for 30 min, and plasma harvested and kept frozen at -80 °C until further analysis. Plasma samples were then analyzed for the same physiological parameters as described in Exp. 1. In addition, rectal fecal samples were collected from all heifer calves every 28 d from days 0 to 84 to determine coccidia count using the procedure described in Exp. 1.

Statistical Analysis

Experiment 1. Data were analyzed using MIXED procedure of SAS (SAS Institute Inc., 2006; version 9.4). Pen was considered the experimental unit, and pen (concentrate amount $\times$ monensin) considered the random variable in all statistical analyses, using Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Calf ADG, DMI, and apparent digestibility were tested for fixed effects of concentrate amount, monensin, and resulting interaction, using calf sex and calf(pen) as random effects. The DM intake was presented as the average of two periods of collection. Calf BW, plasma measurements, and fecal coccidia egg count were analyzed as repeated measures and tested for fixed effects of concentrate amount, monensin, day of the study, and resulting interactions, using calf sex as random effect and calf(pen) as subject. Plasma and fecal measurements collected on days 0 were included as covariates in each respective analysis but removed from the model when $P > 0.10$. Proper covariance structure for each repeated measure analysis was selected based on the lowest Akaike information criterion. Compound symmetry covariance structure was used for statistical analyses of calf BW, plasma, and fecal measurements. Means were

reported as least squares means and were separated using PDIFF. Treatments were considered different when $P \leq 0.05$ and tendencies when $P > 0.05 < 0.10$.

Experiment 2. Data were analyzed using MIXED procedure of SAS (SAS Institute Inc., 2006; version 9.4). Pasture was considered the experimental unit, and pasture (concentrate amount $\times$ monensin) considered the random variable in all statistical analyses, using Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Heifer ADG was tested for fixed effects of concentrate amount, monensin, and resulting interaction, using heifer(pen) as random effect. Heifer BW, plasma measurements, and fecal coccidia egg count were analyzed as repeated measures and tested for fixed effects of concentrate amount, monensin, day of the study, and resulting interactions, using heifer(pen) as subject. Plasma and fecal measurements collected on day 0 were included as covariates in each respective analysis but removed from the model when $P > 0.10$. Compound symmetry covariance structure was used for statistical analyses of heifer BW, plasma, and fecal measurements. Means were reported as least squares means and were separated using PDIFF. Treatments were considered different when $P \leq 0.05$ and tendencies when $P > 0.05 < 0.10$.

RESULTS

Experiment 1

Calf BW on day 0 did not differ among treatments ($P \geq 0.94$) but were included as covariate ($P < 0.0001$) in the statistical analyses of calf BW. Effect of monensin inclusion $\times$ concentrate amount $\times$ day of the study was not detected for calf BW ($P = 0.31$). Effects of monensin inclusion $\times$ day of the study and concentrate amount $\times$ day of the study, but not monensin inclusion $\times$ concentrate amount $\times$ day of the study ($P = 0.31$), were detected for calf BW ($P \leq 0.0007$). Body weight of calves on days 28 and 56 was greater ($P \leq 0.03$) for calves offered concentrate at 2% vs. 1% (Figure 1a) and for calves offered concentrate with vs. without monensin inclusion (Figure 1b). Effect of monensin inclusion $\times$ concentrate amount was not detected for calf ADG from days 0 to 28, 28 to 56, and 0 to 56 ($P \geq 0.15$). Calf ADG from days 0 to 28, 28 to 56, and 0 to 56 was greater for calves offered concentrate at 2% vs. 1% of BW ($P < 0.01$), and calves offered concentrate with vs. without monensin ($P \leq 0.04$; Table 2).

Figure 1. Body weight of early-weaned calves offered free choice access to long stem stargrass hay and provided, in a 2×2 factorial design, two concentrate DM amounts (1% and 2% of BW) added or not with monensin (20 mg/kg of total dry matter intake) in a drylot facility for 56 d (Exp. 1; four calves per pen; three pens per treatment). Calf BW on days 28 and 56 were covariate adjusted for BW obtained on day 0 ($P < 0.0001$). Effects of concentrate amount $\times$ day of the study (Figure 1a) and monensin inclusion $\times$ day of the study (Figure 1b) were detected for calf BW ($P \leq 0.0007$). ^{a-b}Within day, bars without a common superscript differ ($P \leq 0.05$).

Effects of concentrate amount were detected for total DM intake ($P < 0.001$), forage DM intake ($P < 0.001$), and in vivo apparent digestibility ($P = 0.001$; Table 2). Calves offered concentrate at 2% of BW had greater total DM intake and lesser forage DM intake compared to calves offered concentrate at 1% of BW. The in vivo apparent digestibility was greater for calves offered concentrate at 2% vs. 1% BW. Effects of monensin inclusion were not detected ($P \geq 0.19$) for total DM intake, forage DM intake, and in vivo apparent digestibility (Table 2).

Plasma concentrations of glucose, insulin, and IGF-1 on day 0 did not differ among treatments ($P \geq 0.44$) but were included as covariate ($P \leq 0.03$) in each respective statistical analysis. Except for effects of day of the study ($P < 0.001$), effects of monensin inclusion $\times$ concentrate amount $\times$ day of the study, monensin inclusion $\times$ day of the study, concentrate amount $\times$ day of the study, monensin inclusion $\times$ concentrate amount, and main effects of concentrate amount and monensin inclusion were not detected

for plasma concentrations of blood urea nitrogen (BUN) ($P \geq 0.11$; Table 2).

Effects of monensin inclusion $\times$ concentrate amount $\times$ day of the study, monensin inclusion $\times$ day of the study, concentrate amount $\times$ day of the study, monensin inclusion $\times$ concentrate amount, and main effects of monensin inclusion were not detected for plasma concentrations of glucose ($P \geq 0.62$). However, effects of day of the study and concentrate amount were detected ($P \leq 0.05$) for plasma concentrations of glucose, which was greater ($P = 0.05$) for calves offered concentrate at 2% vs. 1% of BW (Table 2).

Effect of monensin inclusion $\times$ concentrate amount $\times$ day of the study was not detected for plasma concentrations of IGF-1 ($P = 0.40$). Effects of concentrate amount $\times$ day of the study and monensin inclusion $\times$ day of the study were detected for plasma concentrations of IGF-1 ($P \leq 0.04$). Plasma concentrations of IGF-1 were greater ($P < 0.001$) on days 28 and 56 for calves offered concentrate at 2% vs. 1% of BW (Figure 1a). Plasma concentrations of IGF-1 on day 28 did not differ between treatments ($P = 0.23$) but was greater for calves fed concentrate added with vs. without monensin ($P = 0.02$; Figure 2b).

Effects of monensin inclusion $\times$ concentrate amount and concentrate amount were not detected

for coccidia egg counts ($P \geq 0.61$). However, calves provided monensin tended ($P = 0.09$) to have less coccidia egg counts compared to calves offered concentrate without monensin (Table 2).

Experiment 2

Heifer BW on day 0 did not differ among treatments ($P \geq 0.94$) but were included as covariate ($P < 0.0001$) in the statistical analyses of heifer BW. Effects of monensin inclusion $\times$ concentrate amount $\times$ day of the study were not detected for heifer BW ($P = 0.52$). Effects of concentrate amount $\times$ day of the study (Figure 3a) and monensin inclusion $\times$ day of the study (Figure 3b) were detected for heifer BW from days 0 to 84 ($P < 0.01$). Heifers offered concentrate at 2% of BW was heavier on days 56 and 84 ($P < 0.01$), but not day 28 ($P = 0.56$) compared to heifers receiving concentrate at 1% of BW (Figure 3a). Heifers offered concentrate with monensin was heavier on days 56 and 84 ($P < 0.01$), but not day 28 ($P = 0.14$) compared to heifers offered concentrate without monensin (Figure 3b).

Effects of monensin inclusion $\times$ concentrate amount were not detected for any heifer ADG variable ($P \geq 0.14$). Except for ADG from days 0 to

Table 2. Growth, intake, in vivo apparent digestibility, plasma measurements, and fecal coccidia egg count of early-weaned calves offered free choice access to long-stem stargrass hay in drylot and provided, in a 2×2 factorial design, two concentrate DM amounts (1% and 2% of BW) added or not with monensin (20 mg/kg of total DM intake) for 56 d (four calves per pen; three pens per treatment).

Item	Concentrate amount		Monensin		SEM	P-value		
	1% BW	2% BW	0 mg/kg	20 mg/kg		Concentrate amount	Monensin	Concentrate amount $\times$ monensin
ADG ¹ , kg/d								
Days 0 to 28	0.33	0.56	0.33	0.56	0.023	<0.01	0.04	0.18
Days 28 to 56	0.18	0.54	0.32	0.40	0.021	<0.01	<0.01	0.43
Days 0 to 56	0.26	0.55	0.36	0.44	0.016	<0.01	<0.01	0.15
Plasma BUN ² , mg/dL	12.5	13.0	13.3	12.2	0.46	0.44	0.11	0.80
Plasma glucose ² , mg/dL	73.5	81.7	76.6	78.6	2.8	0.05	0.62	0.86
Plasma IGF-1 ² , ng/mL	39.7	59.7	46.2	53.0	2.4	<0.01	0.06	0.83
Coccidia egg count on day 56 ³ , log ₁₀ of eggs per gram of feces	1.16	1.06	1.39	0.82	0.21	0.74	0.09	0.61
Forage DMI, % of BW	1.5	0.9	1.2	1.2	0.09	<0.01	0.50	0.40
Total DMI, % of BW	2.3	2.6	2.5	2.5	0.09	<0.01	0.43	0.26
In vivo apparent digestibility, %	56	62	58	60	0.8	<0.01	0.19	0.28

¹ Calculated using calf shrunk body weight collected every 28 d, after 16 h of feed and water withdrawn.

² Plasma concentrations of glucose, insulin, and IGF-1 on day 0 did not differ among treatments ($P \geq 0.44$) but were included as covariate ($P \leq 0.03$) in each respective statistical analysis. Means represent the average plasma concentration of each respective parameter collected on days 0, 28, and 56.

³ Coccidia egg counts (observed egg count + 1) of each calf were log transformed before statistical analyses, and reported as log₁₀ (Martins et al., 2016). All calves were negative for coccidia egg presence on day 0.

Figure 2. Plasma concentrations of IGF-1 (ng/mL) of early-weaned calves offered free choice access to long stem stargrass hay and provided, in a 2×2 factorial design, two concentrate DM amounts (1% and 2% of BW) added or not with monensin (20 mg/kg of total dry matter intake) in a drylot facility for 56 d (Exp. 1; four calves per pen; three pens per treatment). Plasma concentrations of IGF-1 on days 28 and 56 were covariate-adjusted for plasma concentrations of IGF-1 obtained on day 0 ($P < 0.0001$). Effects of concentrate amount $\times$ day of the study (Figure 2a) and monensin inclusion $\times$ day of the study (Figure 2b) were detected for plasma concentrations of IGF-1 ($P \leq 0.04$). ^{a,b}Within day, bars without a common superscript differ ($P \leq 0.05$).

28 ($P = 0.46$), ADG from days 28 to 56, 56 to 84, 0 to 84 were greater ($P \leq 0.01$) for heifers offered concentrate at 2 vs. 1% of BW (Table 3). Except for ADG from days 28 to 56 ($P = 0.16$), ADG from days 0 to 28, 56 to 84, 0 to 84 was greater ($P \leq 0.05$) for heifers offered concentrate with vs. without monensin (Table 3).

There was a trend that pastures grazed by heifers offered concentrate at 2% of BW had greater ($P = 0.09$) HM and HA ($P = 0.06$) compared to those grazed by heifers fed concentrate at 1% of BW (Table 3). Effects of concentrate amount $\times$ monensin inclusion and monensin inclusion were not detected ($P \geq 0.71$) for HM and HA. Effects of concentrate amount $\times$ monensin inclusion, concentrate amount, and monensin inclusion were not detected ($P \geq 0.17$) for bahiagrass concentrations of CP and IVDOM (Table 3). However, there was a significant decrease ($P < 0.01$) in concentrations of CP and IVDOM from days 0 to 84 (Table 4).

Figure 3. Body weight of early-weaned heifers grazing bahiagrass pastures (three heifers per pastures) and provided, in a 2×2 factorial design, two concentrate DM amounts (1% and 2% of BW) added or not with monensin (20 mg/kg of total dry matter intake) for 84 d (Exp. 2; three pastures per treatment). Body weight of heifers on days 28, 56, and 84 were covariate-adjusted for heifer BW obtained on day 0 ($P < 0.0001$). Effects of concentrate amount $\times$ day of the study (Figure 3a) and monensin inclusion $\times$ day of the study (Figure 3b) were detected for heifer BW from days 0 to 84 ($P < 0.01$). ^{a,b} Within day, bars without a common superscript differ ($P \leq 0.05$).

Plasma concentrations of glucose, insulin, and IGF-1 on day 0 did not differ among treatments ($P \geq 0.86$) but were included as covariate ($P \leq 0.03$) in each respective statistical analysis. Except for effects of day of the study ($P < 0.001$), effects of monensin inclusion $\times$ concentrate amount $\times$ day of the study, monensin inclusion $\times$ day of the study, concentrate amount $\times$ day of the study, monensin inclusion $\times$ concentrate amount, and main effects of concentrate amount and monensin inclusion were not detected for plasma concentrations of BUN, glucose, and insulin ($P \geq 0.12$; Table 3).

Effects of monensin inclusion $\times$ concentrate amount $\times$ day of the study, concentrate amount $\times$ day of the study, and main effects of concentrate amount were not detected for plasma concentrations of IGF-1 ($P \geq 0.14$; Table 3). Effects of monensin inclusion $\times$ day of the study were detected ($P = 0.04$) for plasma concentrations of IGF-1, which were greater ($P \leq 0.04$) for heifers offered concentrate with monensin on days 28, 56, and 84 compared to heifers offered concentrate without monensin (Figure 4).

Table 3. Growth, plasma measurements, and forage production parameters of early-weaned heifers grazing bahiagrass pastures (three heifers per pasture) and provided, in a 2×2 factorial design, two concentrate DM amounts (1% and 2% of BW) added or not with monensin (20 mg/kg of total DM intake) for 84 d (three pastures per treatment).

Item	Concentrate amount		Monensin		SEM	P-value		
	1% BW	2% BW	0 mg/kg	20 mg/kg		Concentrate amount	Monensin	Concentrate amount $\times$ monensin
ADG ¹ , kg/d								
Days 0 to 28	0.99	1.04	0.93	1.10	0.045	0.46	0.03	0.46
Days 28 to 56	0.85	1.21	0.95	1.12	0.080	0.01	0.16	0.72
Days 56 to 84	0.69	0.92	0.73	0.88	0.047	<0.01	0.05	0.14
Days 0 to 84	0.85	1.06	0.87	1.03	0.042	<0.01	0.02	0.52
Plasma BUN ² , mg/dL	20.8	21.3	21.5	20.6	0.71	0.57	0.42	0.66
Plasma glucose ² , mg/dL	71.9	75.8	72.3	75.3	1.80	0.19	0.31	0.43
Plasma insulin ² , μ IU/mL	2.02	2.81	2.25	2.58	0.519	0.31	0.67	0.60
Plasma IGF-1 ² , ng/mL	155	171	149	176	6.7	0.14	<0.01	0.30
Coccidia egg count on day 84 ³ , log ₁₀ of eggs per gram of feces	0.45	0.39	0.70	0.14	0.192	0.83	0.05	0.22
Herbage mass (HM) ⁴ , kg/ha	3,700	4,400	4,100	4,100	200	0.09	0.75	0.71
Herbage allowance (HA) ⁴ , kg DM/kg BW	8.0	10.0	9.4	9.6	0.4	0.06	0.69	0.90
CP, %	14.0	14.0	14.7	13.9	0.3	0.25	0.17	0.65
IVDOM, %	48.5	48.8	48.5	48.9	0.52	0.64	0.59	0.78

¹ Calculated using heifer shrunk body weight collected every 28 d, after 16 h of feed and water withdrawn.

² Except for plasma insulin ($P = 0.55$), plasma concentrations of glucose, insulin, and IGF-1 on day 0 did not differ among treatments ($P \geq 0.86$) but were included as covariate ($P \leq 0.03$) in each respective statistical analysis. Means represent the average plasma concentration of each respective parameter collected on days 0, 28, 56, and 84.

³ Covariate adjusted to coccidia egg count collected on day 0 ($P = 0.05$). Coccidia egg counts (observed egg count + 1) of each calf were log transformed before statistical analyses, and reported as log₁₀ (Martins et al., 2016).

⁴ HM and hand-plucked samples of pastures to assess nutritive value (CP and IVDOM) were collected at initiation of grazing and every 14 d for 84 d. HA was calculated for each pasture as the average HM (mean across two sampling dates within each 28-d period) divided by the average total heifer BW during that period (Sollenberger et al., 2005).

Table 4. Effects of day of the study on forage response variables in Exp. 2 (grazing trial)

Item ¹	Interval			SE
	Days 0 to 28	Days 28 to 56	Days 56 to 84	
HM, kg/ha	3,700 ^c	4,400 ^b	4,900 ^a	200
HA, kg DM/kg BW	8.9 ^b	9.7 ^a	9.9 ^a	0.3
CP, %	17.1	13.9	11.8	0.25
IVDOM, %	53.2	49.7	42.7	0.64

^{a,b,c} In a row followed by different small case letters are different ($P \leq 0.05$).

¹ HM and hand-plucked samples of pastures to assess nutritive value (CP and IVDOM) were collected at initiation of grazing and every 14 d for 84 d. HA was calculated for each pasture as the average HM (mean across two sampling dates within each 28-d period) divided by the average total heifer BW during that period (Sollenberger et al., 2005).

Effects of monensin inclusion $\times$ concentrate amount and concentrate amount were not detected for fecal coccidia egg counts ($P \geq 0.22$; Table 3). Heifers offered concentrate with monensin had less ($P = 0.05$) fecal coccidia egg counts compared to heifers offered concentrate without monensin (Table 3).

Figure 4. Plasma concentrations of IGF-1 (ng/mL) of early-weaned heifers grazing bahiagrass pastures (three heifers per pastures) and provided, in a 2×2 factorial design, two concentrate DM amounts (1% and 2% of BW) added or not with monensin (20 mg/kg of total dry matter intake) for 84 d (Exp. 2; three pastures per treatment). Plasma concentrations of IGF-1 on days 28, 56, and 84 were covariate-adjusted for plasma concentrations of IGF-1 obtained on day 0 ($P < 0.0001$). Effects of monensin inclusion $\times$ day of the study were detected for plasma concentrations of IGF-1 ($P = 0.04$). ^{a,b} Within day, bars without a common superscript differ ($P \leq 0.05$).

DISCUSSION

Bergen and Bates (1984) stated that monensin acts on the flux of ions through the membranes

dissipating cation and proton gradients and interfering with the uptake of solutes and the primary transport system in the cells. This process selects Gram-negative bacteria in the rumen and potentially increases the proportion of propionic acid. In addition, monensin may also have the potential to decrease methanogenesis and increase fermentation efficiency in the rumen (Russell, 1987). Rowe et al. (1991) suggested that there is less likelihood of a positive response to ionophore in animals grazing low-quality pastures due to its decreased concentration of nonstructural carbohydrates and limited intake, leading to a decrease in precursors for propionate production in the rumen. In agreement, Vendramini et al. (2015) observed no increase in ADG when monensin was added to the diet of heifers grazing bahiagrass pastures and daily supplemented with 0.4 kg of concentrate. Vendramini et al. (2015) stated that the limited amount of concentrate did not provide sufficient quantity of nonstructural carbohydrates (precursors for propionic acid production) to achieve positive results on growth induced by monensin supplementation. In contrast, the results from both experiments described herein suggest that when greater amount of concentrate were offered (1% and 2% of BW), calves receiving monensin were likely more efficient in producing propionic acid. However, contrary to our hypothesis, the monensin-induced increment on growth performance did not depend on concentrate amount, indicating that for EW calves offered warm-season grasses, supplemental carbohydrate substrate amount was not a limiting factor in calves offered concentrate at 1% and 2% of BW. Hence, data will be discussed separately for the effects of concentrate amount and monensin inclusion.

Calves offered concentrate at 2% of BW had greater concentrate intake and lesser forage intake than calves offered concentrate at 1% BW, which resulted in greater ADG due to greater energy and protein intake. In Exp. 1, the concentrate had greater digestibility than the forage and there was a substitution effect of forage by concentrate. In Exp. 2, pastures grazed by heifer calves provided supplementation at 2% of BW tended to have greater HM and HA, possibly due to decreased forage intake. Vendramini et al. (2007) observed a linear increase in ADG of EW calves receiving concentrate from 0% to 2% of BW and grazing Tifton 85 bermudagrass (*Cynodon* spp.) pastures. The same authors also used variable stocking rates and observed that there was an increase in stocking rate from 11.1 to 13.1 animal units (450 kg BW) as concentrate amount increased from 1% to 2%

BW, primarily due to the substitution of forage by supplement. Early-weaned calves consuming warm-season grasses had a total DMI of approximately 2.5% BW (Exp. 1), and therefore, forage was a minor portion of the diet of calves receiving concentrate at 2% of BW. Despite greater HA for the 2% BW treatment, the levels of HA observed in this study were significantly greater compared to the 0.5 kg DM/kg BW levels indicated as the HA levels that could limit forage intake for EW calves supplemented on warm-season grass pastures (Vendramini and Arthington, 2008).

The ADG results corroborated with the increase in plasma concentrations of glucose and IGF-1 of calves receiving greater concentrate amount. The increase in plasma concentrations of glucose and IGF-1 was expected due to greater DM intake and dietary energy density (Cappellozza et al., 2014a,b). Likewise, plasma IGF-1 concentrations were greater for Holstein heifers with greater dietary energy intake and gaining 1.2 vs. 0.8 kg/d (Radcliff et al., 2004). Systemic IGF-1 has been positively correlated with muscle skeletal growth (Jiang and Ge, 2014). In addition, cattle somatotrophic axis is functional at birth (Granz et al., 1997) and gradually increases its response to nutritional manipulations as age increases (Velayudhan et al., 2007). Hence, the greater plasma concentrations of IGF-1 of heifers in Exp. 2 were likely a result of the differences in dietary energy intake and also age compared to calves in Exp. 1.

It has been observed that monensin may decrease DMI in diets with greater proportion of concentrate (Oscar et al., 1987; Poos et al., 1979) due to changes in rumen fermentation (Muntifering et al. 1980) and slower liquid and solid turnover rates (Lemenager et al. 1978); however, Vagnoni et al. (1995) and Vendramini et al. (2015) observed no difference in DMI on steers receiving bermudagrass and bahiagrass, respectively. Thus, the lack of monensin effects on total DM and forage DM intake (Exp. 1) may suggest that the positive effect on ADG was possibly due to greater rumen fermentation efficiency, propionic acid production, and lesser methane production.

Concentrations of BUN are positively associated with intake of rumen-degradable protein, ruminal ammonia concentration, and ruminal protein:energy ratio (Hammond, 1997). Monensin did not affect plasma concentrations of BUN in Exps. 1 and 2 but it has been observed that monensin may result in greater plasma BUN concentrations due to improved utilization of N (Poos et al., 1979; Muntifering et al., 1980; Vendramini et al.,

2015) associated with decreased proteolysis of dietary protein and altered site of protein digestion (Poos et al., 1979). Despite the lack of monensin effects, plasma concentrations of BUN were within (Exp. 1) or above (Exp. 2) the optimal concentration of BUN (11 and 15 mg/dL; Byers and Moxon, 1980), indicating that rumen-degradable protein and thus CP were consumed in adequate amounts in Exp. 1 and in excess in Exp. 2.

Monensin supplementation to cattle can enhance plasma concentrations of IGF-1 indirectly by stimulating the synthesis of glucose, leading to a boost release of insulin and IGF-1. Plasma concentrations of glucose in both experiments (and plasma insulin concentrations in Exp. 2) did not differ between calves fed supplements with or without monensin inclusion, which was unexpected but can potentially be explained by the influence of timing of blood collection. Plasma concentrations of glucose and insulin peak between 1 and 2 h after concentrate feeding (Moriel et al., 2008), whereas blood samples were collected immediately before feeding in both studies. Hence, peak concentrations of plasma glucose and insulin were missed. Nonetheless, plasma concentrations of IGF-1 were increased in Exp. 1 and approached a trend to be greater in Exp. 2 when calves were fed supplements added with monensin.

In addition to greater propionic acid production and efficiency of ruminal fermentation, monensin can enhance cattle growth performance by other mechanisms, such as controlling coccidiosis. Coccidiosis is an important parasitic disease of young ruminant livestock caused by the protozoan parasite of the genus *Eimeria* and that can lead to subclinical production losses (poor growth) and clinical disease (Keeton and Navarre, 2018). Accumulation of high concentration of contamination are most common in crowded areas where animals congregate, and feces accumulate, such as feedlots, drylots, heavily stocked pastures, and around watering and feeding areas (Keeton and Navarre, 2018). Cattle usually become immune to coccidia by 1 yr of age but serve as a reservoir to younger animals (Keeton and Navarre, 2018). Hence, coccidiosis can be an important disease with detrimental impacts on performance of EW calves. It is expected that forage and water are the main sources of coccidia on early-weaned calves and although calves receiving concentrate at 1% BW likely had greater forage intake, there was no effect of concentrate amount on coccidia egg count, which may indicate that the water may have been the main source of coccidia.

Monensin is often utilized in cattle to control coccidia levels in the body (Stromberg et al., 1986; Hurst et al., 2018). Ionophore residues are excreted unmetabolized by treated animals, and the manure produced from these animals may be applied on croplands as fertilizers, leach into the groundwater or enter surface water through runoff (Hurst et al., 2018). In Exps. 1 and 2, calves offered supplements added with monensin had less coccidia levels in the feces, which is likely a result of ionophore controlling levels of coccidia in the body as well as decreasing ground level contamination of drylot (Exp. 1) and pasture (Exp. 2). Hence, the lower coccidia contamination of monensin-fed calves likely partially contributed to the greater growth performance compared to nonfed monensin calves.

Overall Conclusions

Contrary to our hypothesis, the benefits of monensin inclusion did not depend on concentrate amount. Increasing the concentrate amount is an effective management practice to enhance ADG and decrease forage DMI in early-weaned calves offered warm-season grasses. The reduction in forage DMI may allow greater stocking rates and gain per area, and the concentrate amount should be based on concentrate cost and calves' market price. The supplementation of monensin for early-weaned calves increased plasma concentrations of glucose and IGF-1, suppressed fecal levels of coccidia, and resulted in greater ADG compared to no monensin inclusion, regardless of the concentrate amount being offered (1% vs. 2% of BW). The decrease in fecal coccidia egg count and additional ADG provide sufficient evidence that monensin should be supplied to early-weaned calves grazing warm-season pastures and receiving concentrate above 1% of BW.

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