

# Effect of bambermycin and dietary distillers grains concentration on growth performance and carcass characteristics of finishing steers<sup>1</sup>

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**ABSTRACT:** An experiment was conducted comparing medicated feed additives (MFA) bambermycin or monensin sodium and tylosin phosphate in feedlot diets containing modified distillers grains (mDGS). Crossbred steers ( $n = 256$ ; initial full BW  $418 \pm 28.5$  kg) were allocated into three weight blocks in a randomized complete block design with a  $2 \times 2$  factorial arrangement of treatments. The factors were: MFA (bambermycin at 20 mg/steer daily or monensin + tylosin at 380 and 90 mg/steer daily, respectively) and mDGS inclusion (15% and 30% of diet DM). Twenty-four pens were utilized, resulting in six replications per treatment. Steers were fed a diet consisting of a 1:1 combination of dry-rolled and high-moisture corn with the roughage portion of the diet consisting of corn silage and corn stover. Steers in the heaviest two blocks were marketed after 104 d and steers in the lightest block were marketed after 126 d. Overall average daily gain (ADG) tended ( $P = 0.08$ ) to be

greater for bambermycin vs. monensin and tylosin, and was not affected ( $P = 0.17$ ) by mDGS level. Dry matter intake was lower ( $P \leq 0.05$ ) with 30% mDGS with monensin and tylosin than with any other treatment. Feed efficiency was not affected by mDGS, MFA, or their interaction ( $P \geq 0.30$ ). Marbling scores were greater ( $P = 0.01$ ) greater for bambermycin vs. monensin and tylosin, and tended ( $P = 0.08$ ) to be greater with 15% mDGS than with 30% mDGS. Carcasses from steers fed bambermycin had a greater percentage of USDA choice than those fed with monensin and tylosin ( $P = 0.01$ ). Liver abscess occurrence was not affected ( $P \geq 0.17$ ) by MFA, mDGS, or their interaction. Results from this experiment indicate that including bambermycin in feedlot diets containing 30% mDGS results in increased DMI when compared with including monensin and tylosin in 30% mDGS diet without the risk for increases in liver abscess occurrence.

**Key words:** bambermycin, beef cattle, distillers grains, feedlot

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## INTRODUCTION

In the last 10 yr, feedlot nutritionists have begun to routinely utilize corn-based ethanol by-products

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in diet formulations for feedlot cattle (Vasconcelos and Galyean, 2007; Samuelson et al., 2016). Corn and sorghum-based ethanol by-products are readily available to producers in the primary cattle feeding regions of the U.S. (i.e., Midwest and Texas Panhandle). Currently, the interaction between certain feed additives (i.e., bambermycin) and dietary distillers grain concentration is virtually unknown.

Bambermycin is an antimicrobial produced via various strains of *Streptomyces*. *Streptomyces*, being primarily active upon gram-positive bacteria, allows for increased fermentation of dietary substrates via gram-negative bacteria, subsequently resulting in a greater propionate production (Edrington et al., 2003). Bambermycin is different from most commonly used ionophores in that it is capable of altering ruminal protozoa populations and in turn can improve fiber digestion (Perry, 2002). In pasture and forage-based systems, beef cattle respond favorably to bambermycin supplementation (Beck et al., 2016). The influence bambermycin has on feedlot animal performance and carcass traits when concentrate based diets high in distillers grain are fed have yet to be determined. The objective of the current experiment was to determine the effects of bambermycin and monensin sodium/tylosin phosphate on feedlot animal performance and carcass characteristics when supplemented to diets containing different concentrations of modified distillers grains since no combination feeding clearance is approved with bambermycins.

## MATERIALS AND METHODS

### *Use of Animal Subjects*

The South Dakota State University Institutional Animal Care and Use Committee (IACUC) approved all procedures involving the use of animals in this experiment (IACUC 07-E020). The experiment was conducted at the South Dakota State University Southeast Research Farm (SERF) located near Beresford, SD.

### *Animals and Initial Processing*

Two hundred and fifty-six crossbred steers (initial full BW = 418 kg [SD 28.5 kg]) obtained from local South Dakota auction barns were used in this feedlot experiment conducted from October 2007 to February 2008. All cattle were processed within 24 to 48 h of arrival to the SERF. At initial processing, all steers were weighed on a legal for trade scale (readability  $\pm$  0.454 kg), administered vaccinations against viral infectious bovine rhinotracheitis (IBR), bovine virus diarrhea (BVD) Types 1 and 2, parainfluenza 3 (PI3), and bovine respiratory syncytial virus (BRSV) (Bovishield Gold 5, Zoetis, Parsippany, NJ) and clostridial diseases (Vision 7, Merck Animal Health, Madison, NJ) as well as application of a parasiticide (Cydectin, Bayer Animal Health, Shawnee, KS) according to label directions, and a uniquely numbered identification tag in the ear. All steers were

administered a terminal implant (120 mg TBA and 24 mg E<sub>2</sub>; Revalor-S, Merck Animal Health) on day 28 of the experiment (November, 13, 2007). A total of six animals were removed during the course of experiment for reasons not related to dietary treatment.

### *Experimental Design and Treatments*

A randomized complete block design was used to evaluate animal performance and carcass traits. Treatments were arranged in a 2  $\times$  2 factorial with the factors of medicated feed additive (MFA; bambermycin or monensin sodium and tylosin phosphate) and modified distillers grains inclusion (mDGS; 15% of 30% diet DM). The four resulting treatment combinations consisted of: Bambermycin at 20 mg/hd d<sup>-1</sup> (Gainpro, Huevapharma, Peachtree City, GA) and mDGS at 15% inclusion diet DM (BAM15). Bambermycin at 20 mg/hd d<sup>-1</sup> (Gainpro, Huevapharma, Peachtree City, GA) and mDGS at 30% inclusion diet DM (BAM30). Monensin sodium and tylosin phosphate at 380 mg/hd d<sup>-1</sup> and 90 mg/hd d<sup>-1</sup>, respectively (Rumensin and Tylan, Elanco, Greenfield, IN) and mDGS at 15% inclusion diet DM (MT15). Monensin sodium and tylosin phosphate at 380 mg/hd d<sup>-1</sup> and 90 mg/hd d<sup>-1</sup>, respectively (Rumensin and Tylan, Elanco, Greenfield, IN) and mDGS at 30% inclusion diet DM (MT30).

### *Study Initiation*

All information related to timing of events can be found in Table 1. Consecutive individual full BW measurements were captured at the initiation of the experiment. The average of these two BW measurements was used as the initial BW. Steers were blocked by BW into three weight blocks. Within each block steers were assigned to pens. Treatment was then randomly assigned to pen within a block, resulting in 6 pen replicates per treatment. For each treatment, 2 pen replicates were housed in concrete surfaced pens, partially covered by a pole barn, with 4.9 m of bunk space and 26.2 square m of pen space per steer ( $n = 8$  steers/pen). The other 4 pen replicates for each treatment were housed in open-lot, soil-surfaced pens, these pens that had 6.1 m of bunk space, a 3.0 m concrete bunk apron, and 149 m<sup>2</sup> of pen space per steer ( $n = 12$  steers/pen).

### *Diet and Intake Management*

Steers were gradually acclimated to the final finishing diet using a 5-step process. The final finishing

diets (Table 2) were fortified with vitamins and minerals to meet or exceed nutrient requirements for finishing beef steers (NRC, 1996).

Feed bunks were evaluated at approximately 0730 h daily to estimate orts and adjust feed calls to ensure ad libitum access to feed. The bunk management approach was to achieve  $\leq 0.454$  kg of dry orts in the bunk at the time of feeding each day (Thompson et al., 2016). Diets were mixed in a 56-cubic m feed wagon with a scale accuracy of 1.0 kg (Rotomix 184-10 wagon mixer; Rotomix, Dodge City, KS), and delivered once daily beginning at 0900 hours.

**Table 1.** Timeline (date with days on feed in parentheses) for experiment initiation, implant dates, and end of finishing period for each block of cattle

Cattle block	Initial BW	Implant	End finishing period
Light	10/14/2007 and 10/15/2007	11/13/2007 (day 28)	2/19/2008 (day 126)
Intermediate	10/14/2007 and 10/15/2007	11/13/2007 (day 28)	1/28/2008 (day 104)
Heavy	10/14/2007 and 10/15/2007	11/13/2007 (day 28)	1/28/2008 (day 104)

Feedstuff samples were taken weekly and analyzed for DM (AOAC, 1990), CP (AOAC, 1984), NDF and ADF (Goering and Soest, 1970), and ash (AOAC, 1990). Orts were collected, weighed, and dried in a forced air oven at 100°C for 24 h in order to determine DM content if carryover feed went out of condition, or was present on weigh days. If carryover feed was present on weigh days, the residual feed was removed prior to the collection of BW measurements. The DMI of each pen was adjusted to reflect the total DM delivered to each pen after subtracting the quantity of dry orts for each interim period.

### Production Data

Individual BW measures were captured in the morning before the daily feed delivery (~0730 h). No feed or water was withheld prior to any BW measurements. Cumulative performance was calculated using full initial BW and final BW pencil shrunk 4% from day 104 (Intermediate and Heavy blocks) and day 126 (Light block), and also by assuming a constant dressing percentage of 63% applied to HCW (Smith et al., 2019).

**Table 2.** Composition of finishing diets (DM basis)

Item, % of DM	15 mDGS		30 mDGS	
	Bambermycin	Monensin/tylosin	Bambermycin	Monensin/tylosin
Dry-rolled corn	31.6	31.6	25.2	25.2
High-moisture corn	33.3	33.3	26.6	26.6
Corn silage	9.8	9.8	9.8	9.8
Modified distillers Grains with solubles	14.4	14.4	29.1	29.1
Corn stover	3.0	3.0	3.0	3.0
Soybean meal	1.6	1.6	0.0	0.0
Liquid supplement <sup>a</sup>	4.3	4.3	4.3	4.3
Pelleted supplement <sup>b</sup>	2.0	2.0	2.0	2.0
Formulated nutrient composition, DM basis				
NEm, Mcal/45.4 kg	95	95	97	97
NEg, Mcal/45.4 kg	63	63	64	64
Crude protein, %	13.0	13.0	15.2	15.2
NDF, %	19.60	19.60	25.00	25.00
ADF, %	8.79	8.79	10.90	10.90
Ca, %	0.70	0.70	0.72	0.72
P, %	0.38	0.38	0.45	0.45
K, %	0.93	0.93	1.01	1.01
S, %	0.26	0.26	0.37	0.37
Bambermycin, g/ton <sup>c</sup>	1.74	-	1.74	-
Monensin, mg/kg <sup>c</sup>	-	36.00	-	36.00
Tylosin, mg/kg <sup>c</sup>	-	8.55	-	8.55

<sup>a</sup>Liquid supplement was formulated to contain 10% Ca, 8.4% K, 7.2% NaCl, 475 mg/kg of Zn, 121 mg/kg of Cu, 428 mg/kg of Fe, 1.9 mg/kg of Co, 25,000 IU of vitamin A, 6,300 IU of vitamin D, and 350 IU of vitamin E. <sup>b</sup>Soybean meal based pelleted supplement for inclusion of medicated feed additives.

<sup>c</sup>Formulated using an estimated 10.4 kg DMI/hd d<sup>-1</sup>.

### Carcass Evaluation

When treatment blinded personnel estimated that the pens within each block had ~1.27 cm of 12th rib fat, all pens within each block were transported 96 km to a commercial abattoir (Tyson Fresh Meats, Dakota City, NE). Cattle in the intermediate and heavy block were harvested on January 28, 2008 (104 d-on-feed) and cattle from the light block were harvested on February 19, 2008 (126 d-on-feed). All carcass measurements were collected by trained personnel. During the harvest process extra trim, and fat and hide pulls of soft tissue  $\geq 6.8$  kg were noted, and liver abscess incidence and HCW were recorded. Following a 24 h chilling period other individual carcass measurements including 12<sup>th</sup> rib fat depth (BF), LM area, KPH %, marbling score and USDA quality grade were collected. The percentage of empty body fat (EBF) was estimated using the equation described by (Guiroy et al., 2002). Yield grade was calculated by using the USDA regression equation (USDA, 1997). A 4% pencil shrink was applied to final BW for calculation of dressing percentage.

### Statistical Analysis

Live performance and continuous carcass data were analyzed as a randomized complete block design using the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Categorical data (i.e., USDA quality grade, yield grade, and liver abscess incidence) were analyzed as multinomial proportions using the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc.). For all analyses the model included MFA, mDGS and their interaction, with pen serving as the experimental unit and block a random variable. If a significant preliminary *F*-test was detected, data were separated and denoted to be different using the pairwise comparisons PDIF and LINES option of GLIMMIX in SAS 9.4. Differences were considered significant at  $P \leq 0.05$  and trends discussed at  $P \geq 0.05$  and  $\leq 0.10$ .

## RESULTS AND DISCUSSION

### Animal Performance

Animal performance variables can be found in Table 3. There was no interaction ( $P \geq 0.10$ ) between MFA and mDGS detected for any of the performance variables, except for DMI ( $P = 0.05$ ; Table 3 and Figure 1). Initial BW measurements did not differ between MFA ( $P = 0.92$ ) or level of mDGS inclusion ( $P = 0.92$ ). The main effect of MFA tended

( $P = 0.10$ ) to increase final shrunk BW measurements by 1.4% for BAM steers over MT, but did not influence ( $P = 0.13$ ) carcass adjusted final BW. The main effect of mDGS did not influence ( $P \geq 0.18$ ) final or carcass adjusted BW in the present experiment. The type of MFA used influenced final BW of these steers which is consistent with others who have compared differing MFA relative to monensin (Thompson et al., 2016). It has also been reported that BAM feeding results in greater DMI with moderate effects on gain, and that subsequently increases G:F (Lemos et al., 2016). In the present experiment, cattle fed BAM had greater intakes and gain, with no influence on overall G:F. While dietary distillers grain concentration did not influence final BW measures; others have reported increased final BW in steers when greater dietary concentrations of distillers grains were fed to beef steers (Hales et al., 2014).

There was an interaction detected ( $P = 0.05$ ) between MFA and mDGS for DMI. Steers in BAM15, BAM30 and MT15 had greater ( $P \leq 0.05$ ) DMI compared to steers in MT30. DMI was improved in the present experiment when BAM was included in diets containing 15% or 30% mDGS compared with steers fed MT with 30% inclusion of mDGS. Others have reported no differences in intake when steers were fed 0% or 30% inclusion of distillers grains in feedlot finishing diets (Hales et al., 2014). The interaction of MFA and mDGS in the present experiment on DMI could be due to differences in S consumption due to greater mDGS inclusion. Bambermycin has been demonstrated to reduce hydrogen sulfide accumulation compared to monensin using in vitro models (Kung et al., 2000). The increased intake in BAM30 vs. MT30 is likely a function of differences in hydrogen sulfide accumulation in the rumen, although this is speculation, as this was not actually measured in these steers. Differing MFA tended ( $P \leq 0.09$ ) to increase shrunk and carcass adjusted ADG by 5.8% and 5.9%, respectively for BAM steers. The main effect of mDGS did not influence ( $P \geq 0.17$ ) final shrunk or carcass adjusted ADG in these steers. The use of MFA and level of dietary mDGS did not impact ( $P \geq 0.31$ ) G:F of steers in the present experiment.

### Carcass Traits

Influence of bambermycin on carcass traits of finishing beef cattle are limited (Lemos et al., 2016) and the influence of distillers grain inclusion on carcass traits of beef steers are well



**Table 3.** Effect of medicated feed additive (MFA) and dietary concentration of modified distillers grains (mDGS) on growth of feedlot steers

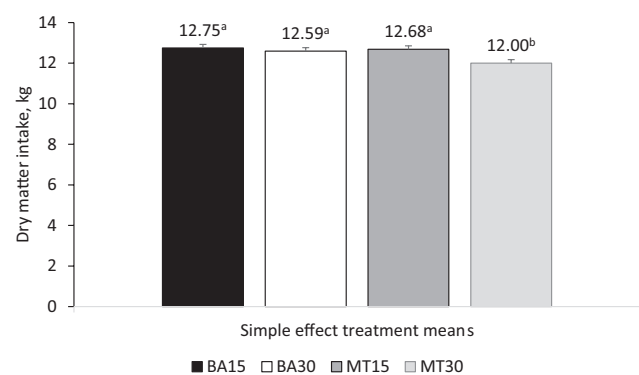
Item	MFA		mDGS		SEM <sup>2</sup>	P-values		
	BAM <sup>1</sup>	MT <sup>1</sup>	15	30		MFA	mDGS	MFA × mDGS
Pens	12	12	12	12	-	-	-	-
Steers	125	125	126	124	-	-	-	-
Initial BW, kg	418	418	418	418	17.4	0.92	0.92	0.30
DMI, kg	12.67	12.34	12.72	12.26	0.619	0.01	0.01	0.05
Live basis								
Final BW <sup>3</sup> , kg	600	591	599	592	17.8	0.10	0.18	0.10
ADG, kg	1.63	1.54	1.62	1.55	0.108	0.08	0.17	0.14
G:F	0.129	0.125	0.127	0.126	0.0035	0.32	0.81	0.30
Carcass adjusted basis								
Final BW <sup>4</sup> , kg	578	570	575	573	13.7	0.13	0.78	0.22
ADG, kg	1.43	1.35	1.40	1.38	0.043	0.09	0.65	0.26
G:F	0.113	0.110	0.113	0.110	0.0031	0.31	0.41	0.54

<sup>1</sup>Bambermycin at 20 mg/hd d<sup>-1</sup> (BAM); monensin sodium at 380 mg/hd d<sup>-1</sup> and tylosin phosphate at 90 mg/hd d<sup>-1</sup> (MT) and mDGS at 15 or 30% inclusion diet DM.

<sup>2</sup>Pooled SEM.

<sup>3</sup>Calculated as BW × 0.96.

<sup>4</sup>Calculated as HCW/0.63.



**Figure 1.** Simple effect means for medicated feed additive and dietary concentration of modified distillers grains on DMI of feedlot steers (Pooled standard error of the mean = 0.173;  $n = 6$  pens/treatment). Treatments were: Bambermycin at 20 mg/hd d<sup>-1</sup> with 15% inclusion (DM basis) of mDGS (BAM15); Bambermycin at 20 mg/hd d<sup>-1</sup> with 30% inclusion (DM basis) of mDGS (BAM30); Monensin sodium at 380 mg/hd d<sup>-1</sup> and tylosin phosphate at 90 mg/hd d<sup>-1</sup> with 15% inclusion (DM basis) of mDGS (MT15); Monensin sodium at 380 mg/hd d<sup>-1</sup> and tylosin phosphate at 90 mg/hd d<sup>-1</sup> with 30% inclusion (DM basis) of mDGS (MT30).

documented in the literature (Buckner et al., 2008; Leibovich et al., 2009; Koger et al., 2010; Mello et al., 2012; Hales et al., 2014; Opheim et al., 2016). There was no interaction ( $P \geq 0.10$ ) between MFA and mDGS detected for any carcass trait variables in Table 4, therefore only main effect means are presented.

In the present experiment, BAM had no influence ( $P = 0.11$ ) on HCW, which is consistent with others (Lemos et al., 2016). Others have reported increased HCW when laidlomycin propionate and chlortetracycline are compared to monensin sodium and tylosin

phosphate in finishing beef cattle (Thompson et al., 2016) and this has been attributed to greater DMI for the laidlomycin propionate and chlortetracycline steers. Steers supplemented with BAM had increased ( $P = 0.04$ ) BF accumulation compared with MT steers ( $1.14$  vs.  $1.04 \pm 0.055$  cm) and greater dietary concentrations of mDGS tended ( $P = 0.10$ ) to increase BF ( $1.05$  vs.  $1.13 \pm 0.055$  cm). Steers supplemented with BAM had greater ( $P = 0.01$ ) marbling scores compared with MT steers ( $553$  vs.  $520 \pm 11.7$ ) and greater levels of mDGS tended ( $P = 0.08$ ) to decrease marbling scores ( $545$  vs.  $528 \pm 11.7$ ). Steers from BAM had greater ( $P = 0.01$ ) estimated EBF(%) compared with MT steers ( $29.69$  vs.  $28.88 \pm 0.269$ ). There was a tendency ( $P = 0.08$ ) for greater dietary concentrations of mDGS to reduce the percentage of carcasses grading USDA prime. The BAM steers had a greater ( $P \leq 0.01$ ) percentage of carcasses grading USDA Choice ( $76.8$  vs.  $61.6 \pm 4.10$ ) and lower occurrence of USDA Select carcasses ( $20.8$  vs.  $36.8 \pm 3.99$ ) compared with MT steers. There was a tendency ( $P \leq 0.10$ ) for MT carcasses to have a greater occurrence of USDA yield grade 1 carcasses and fewer USDA yield grade 4 carcasses compared with BAM. Greater dietary concentrations of mDGS resulted in increased ( $P = 0.01$ ) incidence of A0 liver scores ( $1.6$  vs.  $8.1 \pm 1.90$ ) for 15% vs. 30% mDGS inclusion, respectively. Hot carcass weight, LM area, KPH%, calculated yield grade, and percentage of carcasses with overall liver abscess occurrence were not influenced ( $P \geq 0.17$ ) by the main effects of MFA or mDGS.

**Table 4.** Effect of medicated feed additive (MFA) and level of modified distillers grains (mDGS) inclusion on carcass traits of feedlot steers

Item	MFA		mDGS		SEM <sup>2</sup>	P-values		
	BAM <sup>1</sup>	MT <sup>1</sup>	15	30		MFA	mDGS	MFA × mDGS
Final BW, kg <sup>3</sup>	600	591	599	592	17.8	0.10	0.18	0.10
HCW, kg	364	359	362	361	8.5	0.11	0.75	0.22
Dress, %	60.83	60.75	60.52	61.07	0.700	0.77	0.06	0.40
BF, cm	1.14	1.04	1.05	1.13	0.055	0.04	0.10	0.67
LM area, cm <sup>2</sup>	80.75	79.32	79.33	80.74	1.924	0.30	0.30	0.26
Marbling <sup>4</sup>	553	520	545	528	11.7	0.01	0.08	0.19
KPH, %	2.32	2.34	2.31	2.35	0.141	0.63	0.50	0.82
Calculated yield grade	3.06	3.03	2.99	3.10	0.127	0.66	0.20	0.93
EBF, % <sup>5</sup>	29.69	28.88	29.28	29.29	0.269	0.01	0.97	0.62
Prime, % <sup>6</sup>	0.8	1.6	2.4	0.0	0.97	0.55	0.08	0.55
Choice, % <sup>6</sup>	76.8	61.6	71.4	67.1	4.10	0.01	0.46	0.17
Select, % <sup>6</sup>	20.8	36.8	25.4	32.1	3.99	0.01	0.24	0.12
No roll, % <sup>6</sup>	1.6	0.0	0.8	0.8	0.79	0.16	0.97	0.97
YG1, % <sup>6,7</sup>	1.0	4.4	2.0	3.4	1.37	0.10	0.41	0.98
YG2, % <sup>6,7</sup>	40.2	42.8	41.7	41.3	4.43	0.70	0.98	0.74
YG3, % <sup>6,7</sup>	53.8	51.6	52.7	52.6	4.52	0.71	0.99	0.72
YG4, % <sup>6,7</sup>	5.0	1.2	3.6	2.7	1.48	0.06	0.74	0.66
Liver abscess, % <sup>6</sup>	15.2	11.2	10.3	16.1	3.04	0.34	0.17	0.93
Liver a-, % <sup>6</sup>	6.3	4.8	7.1	4.0	2.07	0.60	0.29	0.30
Liver a0, % <sup>6</sup>	6.5	3.2	1.6	8.1	1.90	0.21	0.01	0.21
Liver a+, % <sup>6</sup>	2.4	3.2	1.6	4.0	1.48	0.71	0.25	0.73

<sup>1</sup>Bambermycin at 20 mg/hd d<sup>-1</sup> (BAM); monensin sodium at 380 mg/hd d<sup>-1</sup> and tylosin phosphate at 90 mg/hd d<sup>-1</sup> (MT) and mDGS at 15% or 30% inclusion diet DM.

<sup>2</sup>Pooled SEM.

<sup>3</sup>Final shrunk BW = Final live BW \*0.96

<sup>4</sup>400 = small<sup>0</sup>.

<sup>5</sup>Empty Body Fat % according to equations described by [Guiroy et al. \(2002; Journal of Animal Science 80: 1791\)](#).

<sup>6</sup>Calculated using distributions for multinomial proportions with the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC).

<sup>7</sup>YG = yield grade, calculated using the USDA regression equation.

Greater BF accumulation, marbling scores, and EBF% in BAM vs. MT at equal days on feed are likely due to greater DMI by BAM steers compared with MT steers. It has been reported that feeding diets containing distillers grains compared with dry-rolled-corn based diets without distillers grains resulted in greater BF accumulation and USDA yield grades in beef steers ([Koger et al., 2010](#)), and [Hales et al. \(2014\)](#) has demonstrated that greater dietary concentrations of distillers grains increased BF accumulation and dressing percentage of steers.

## CONCLUSIONS

Results from this experiment indicate that including bambermycin in feedlot diets containing 30% mDGS increased DMI when compared with including monensin and tylosin in 30% mDGS diet. Bambermycin is a viable alternative to monensin

and tylosin as a MFA in feedlot diets containing mDGS.

*Conflict of interest statement:* None declared.

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