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Yield and Forage Value of a Dual-Purpose *bmr-12* Sorghum Hybrid

Melinda K. Yerka
University of Nebraska-Lincoln, myerka@unl.edu

Andrea K. Pederson
University of Nebraska-Lincoln, awatson3@unl.edu

J. J. Toy
University of Nebraska-Lincoln, John.Toy@ars.usda.gov

Galen E. Erickson
University of Nebraska-Lincoln, gerickson4@unl.edu

Jeffrey F. Pedersen
University of Nebraska-Lincoln, jpedersen1@unl.edu

See next page for additional authors

Authors
Melinda K. Yerka, Andrea K. Pederson, J. J. Toy, Galen E. Erickson, Jeffrey F. Pedersen, and Rob B. Mitchell

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RESEARCH

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ABSTRACT

Grain sorghum [*Sorghum bicolor* (L.) Moench] is an important crop for rainfed production systems with 2.7 million ha grown in the United States in 2013. The *brown-midrib* (*bmr*) mutations, especially *bmr-12*, have resulted in low stover lignin and high fiber digestibility without reducing grain yield in some sorghum lines. However, the effect of the *bmr* trait on beef cattle (*Bos taurus*) performance when grazing crop residue is unknown. Our objectives were to validate previous small-plot results reporting no grain yield difference between near-isogenic *bmr-12* (BMR) and wild-type control (CON) A Wheatland × R Tx430 sorghum hybrids in a field-scale experiment and to determine if BMR stover enhances beef production in a grazing experiment. Four replicated paddocks (2.3 ha) were planted in 2006 and 2008 near Mead, NE. Crossbred yearling steers (240 ± 17 kg hd^{-1}) grazed (2.6 steers ha^{-1}) paddocks following grain harvest for 72 d in 2006 and 61 d in 2008. Forage was sampled 4, 30, and 60 d after grazing began. Grain yield of BMR was 6% less ($P = 0.01$) than CON with no difference in stover neutral detergent fiber (NDF) content, but BMR stover had higher in vitro NDF digestibility (IVNDFD) (31%; $P < 0.0001$), steer average daily gain (ADG; 0.18 kg hd^{-1} d^{-1}; $P = 0.001$), and body weight (BW) gain (29 kg ha^{-1}; $P = 0.002$), resulting in an estimated increase in net return of \$133.84 ha^{-1} due to BMR. Results suggest that the A Wheatland × R Tx430 *bmr-12* hybrid is an effective dual-purpose sorghum crop for both grain and beef production.

M.K. Yerka, J. J. Toy, J.F. Pedersen (retired), and R. Mitchell, USDA-ARS, Univ. of Nebraska, Lincoln, NE 68583; and A. Watson and G. Erickson, Dep. of Animal Science, Univ. of Nebraska, Lincoln, NE 68583. Received 18 June 2014. *Corresponding author (melinda.yerka@ars.usda.gov).

Abbreviations: ADF, acid detergent fiber; ADG, average daily gain; ADL, acid detergent lignin; *bmr, brown-midrib*; BMR, near-isogenic *bmr-12* hybrid; BW, body weight; CON, wild-type control; EPA, environmental protection agency; FW, final weight; IVNDFD, in vitro neutral detergent fiber digestibility; IW, initial weight; MP, midparent; NDF, neutral detergent fiber.

The *bmr* mutations associated with reduced lignin have been used in sorghum (Porter et al., 1977) and sudangrass [*Sorghum bicolor* (L.) Moench nothosubsp. *drummondii* (Steud.) de Wet ex Davidse] for more than three decades (Fritz et al., 1981). Currently, there are seven known *bmr* loci in sorghum (Saballos et al., 2008; Sattler et al., 2014), but *bmr-6* and *bmr-12* are the most widely used in breeding programs. The phenotype associated with *bmr-6* and *bmr-12* is due to mutations in the genes encoding enzymes that catalyze the last two steps of lignin biosynthesis, cinnamyl alcohol dehydrogenase 2, and caffeic *O*-methyltransferase, respectively, such that lignin content of *bmr* sorghums is reduced relative to the wild-type (Feltus and Vandenbrink, 2012; Sattler et al., 2010a).

Reduced lignin content of ruminant feed has been associated with gains in animal performance. For example, dairy cows in early to mid lactation fed brown-midrib corn (*Zea mays* L.; *bm3*, orthologous to *bmr-12* in sorghum) silage during 28 d showed increased dry matter intake and higher milk production than

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cows fed isogenic wild-type controls (Oba and Allen, 1999). Silage from a *bm* corn hybrid improved total milk production after 180 d of lactation relative to a wild-type corn hybrid (Holt et al., 2013). Similar gains compared with the wild-type are well documented for forage quality, digestibility, and animal performance in other grass species (Aydin et al., 1999; Beck et al., 2013; Cherney et al., 1988, 1990; Oliver et al., 2005a, 2005b).

Lactational performance of dairy cows fed *bmr* sorghum silage (Aydin et al., 1999; Grant et al., 1995; Miron et al., 2007; Oliver et al., 2004) or *bmr* sorghum–sudangrass silage (Dann et al., 2008) is comparable to that of wild-type corn. Improved animal performance on corn silage in general compared with sorghum or sudangrass is due to the higher digestibility of corn grain when included in the silage. However, *bm* corn hybrids are widely associated with 10 to 20% reductions in grain and forage yield relative to isogenic wild-type controls (reviewed in Sattler et al., 2010a), whereas reduced grain and forage yields of some *bmr* sorghum hybrids have been overcome by recent breeding efforts (Oliver et al., 2005b). These efforts include the introgression of *bmr* alleles into improved varieties and the use of heterosis in hybrids (reviewed in Sattler et al., 2010a). In addition to having lower lignin content, the reduced water requirements of sorghum and sorghum–sudangrass hybrids relative to corn (Jagadish et al., 2014; Schittenhelm and Schroetter, 2014) make them desirable alternatives for reducing water use worldwide. Sorghum is planted later than corn (Grant et al., 1995) and may serve as an alternative crop where inclement weather has destroyed or prevented the timely establishment of higher-yielding, long-season corn varieties.

Although substantial research has been conducted on *bmr* silages to improve lactational performance of dairy cows, to date, none has focused on the grazing of *bmr* sorghum residues by beef cattle following grain harvest. Grazing crop residue is a common management tool for cow–calf operations producing feeder cattle for the beef industry. It would be useful for producers to know whether the *bmr* trait could provide similar gains for beef cattle as have been observed for dairy cattle. Lack of research on this topic is unfortunate, since the land area of corn and sorghum grain harvested in 2007 in the United States (the most recent period for which county-level national maps are available) was 34.9 and 2.7 million ha, respectively (Fig. 1A, B, USDA–National Agricultural Statistical Service, 2014). During that same time, 32.8 million head of beef cattle were raised, including within regions dominated by corn production in South Dakota, Nebraska, Iowa, Kansas, Oklahoma, and Texas (Fig. 1C, USDA- National Agricultural Statistical Service, 2014). In 2012, total corn and sorghum production in this area of the United States was 33.0 and 1.9 million ha, respectively, (FAO, 2012) and 35.8 million head of beef cattle were slaughtered (FAO, 2012).

Sorghum grain is now qualified by the United States Environmental Protection Agency (EPA) (Environmental Protection Agency, 2012) as an advanced biofuel. By EPA mandate, advanced biofuels must account for 50% of all biofuels produced in the United States by 2020 (Environmental Protection Agency, 2012). Thus, increases in grain sorghum acreage for biofuel may constitute a significant opportunity for producers interested in grazing cattle on the residue following grain harvest. Breeding dual-purpose sorghum hybrids for both ethanol conversion and animal feed may provide additional revenue opportunities (Lorenz et al., 2009a, 2009b; Oliver et al., 2005b). To address the potential dual use of sorghum for both grain and stover grazing, we previously reported no differences in grain yield, residue NDF, or acid detergent fiber (ADF) content between the common grain sorghum hybrid A Wheatland × R Tx430 and its near-isogenic *bmr-12* counterpart; *bmr-12*, however, was associated with lower acid detergent lignin (ADL) and greater IVNDFD (Oliver et al., 2005b). Our objectives were to validate the small-plot grain yield and stover quality results of Oliver et al. (2005b) with a field-based, commercial-scale grain yield experiment and to use the sorghum residue in a beef grazing experiment to determine whether the *bmr-12* gene improves animal performance relative to the wild-type.

MATERIALS AND METHODS

Plant Materials and Field Cultural Practices

The wild-type control grain sorghum hybrid A Wheatland × R Tx430 and its near-isogenic *bmr-12* counterpart (Oliver et al., 2005b; Sattler et al., 2010b) were used for grain yield and animal performance experiments to facilitate the direct comparison of wild-type and *bmr-12* genetic backgrounds. Hybrid treatments (CON or BMR) were randomly assigned to 2.3-ha paddocks arranged in a randomized complete block design with four replicates grown on a Sharpsburg silty clay loam soil (fine, montmorillonitic, mesic Typic Argiudoll) at the University of Nebraska Agricultural Research and Development Center near Mead, NE (41.14° N, 96.29° E; 369 m elevation). Fields were planted on 19 May of both 2006 and 2008 with a grain drill following no-till soybean [*Glycine max* (L.) Merr]. For the 2006 season, 130 kg ha^{-1} N was applied the previous fall. Preemergent herbicides were applied as 0.60 kg ai ha^{-1} atrazine (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine), 0.40 kg ai ha^{-1} *S*-metolachlor ((*RS*)-2-Chloro-*N*-(2-ethyl-6-methyl-phenyl)-*N*-(1-methoxypropan-2-yl)acetamide), 1.10 kg ae ha^{-1} glyphosate (*N*-(phosphonomethyl)glycine, isopropylamine salt), and 2.2 kg ha^{-1} ammonium sulfate. For the 2008 season, 101 kg ha^{-1} N was applied the previous autumn and 0.60 kg ai ha^{-1} atrazine, 0.40 kg ai ha^{-1} *S*-metolachlor, 0.28 kg ae ha^{-1} glyphosate, and 0.35 kg ae ha^{-1} 2,4-D (2,4-Dichlorophenoxyacetic acid) were applied preemergence. Postemergence herbicides included 0.053 kg ai ha^{-1} halosulfuron-methyl (methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methylpyrazole-4-carboxylate), 0.12 kg ae ha^{-1} 2,4-D (2,4-Dichlorophenoxyacetic

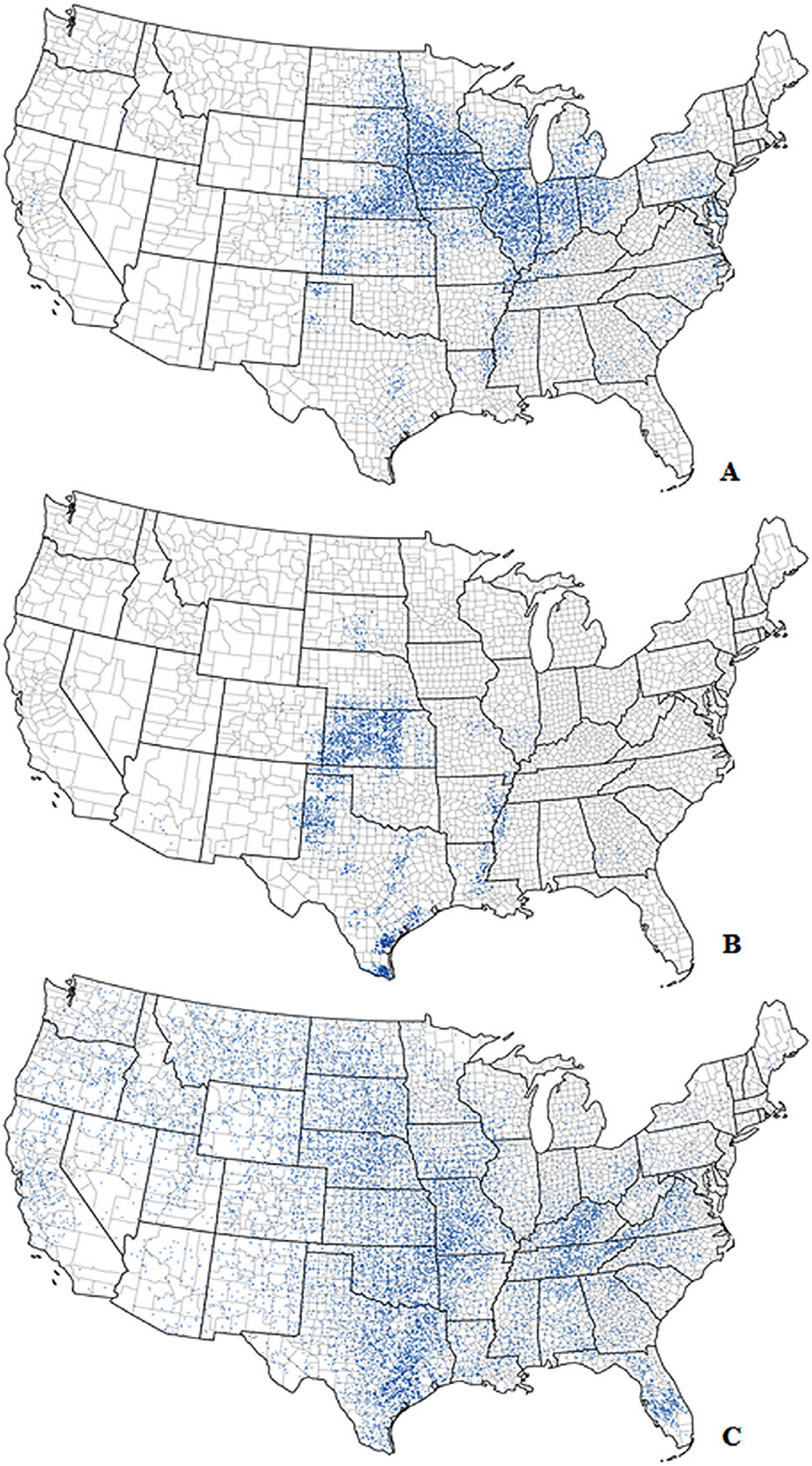

Figure 1. Areas of corn grain (A), sorghum grain (B), and beef cattle (C) production in the United States in 2007. Source: U.S. Department of Agriculture, National Agricultural Statistics Service (USDA-NASS).

acid), 0.25% nonionic surfactant (v/v), and 2.2 kg ha^{-1} ammonium sulfate. No irrigation was applied either year. Precipitation was monitored throughout the growing season for both years using data from the High Plains Regional Climate Center, University of Nebraska, Lincoln (www.hprcc.unl.edu/index.php, accessed 29 July 2013).

Grain Yield and Forage Quality Experiment

Grain was harvested from each paddock with a commercial combine in October of each year, weighed in a commercial grain cart, and yields were adjusted to 145 g kg^{-1} water content. In 2006, residue samples were collected on Days 4, 30, and 60 after grazing was initiated. In 2008, residue samples were collected on Days 1, 31, and 60. For ease of discussion, sample dates are hereafter referred to as Days 4, 30, and 60 for both years. Stover samples in each paddock included all crop residue (leaves plus stems) collected to a 10-cm stubble height from 91 cm within one randomly-chosen row in the grazed portions of each paddock and from 91 cm within one randomly-chosen row in nongrazed exclosures for comparison of forage quality between grazed and nongrazed residue types. The exclosures served as a control for residue quality over time. Residue samples were separated into stem and leaf tissues and dried to constant mass in a 60°C forced air oven. When dry, samples were ground through a 1-mm screen, subsampled, and analyzed in duplicates for NDF and analyzed in triplicates for IVNDFD. Samples were biologically replicated by paddock (four paddocks of each hybrid per year), plant tissue (leaves or stems), residue type (grazed and nongrazed), and over time with the three dates of sample collection.

The NDF content was determined following the methods of Van Soest et al. (1991). The IVNDFD was determined according to the methods of Tilley and Terry (1963) using McDougall's buffering solution (McDougall, 1948) with the addition of 1 g L^{-1} urea. Five empty tubes (no sample) were also incubated with 30 mL of McDougall's buffer and rumen fluid to quantify undigested particles and microorganisms present in the rumen fluid. The IVNDFD was calculated from the weight of undigested residue on the filters, less the blank tube residue amount. Neutral detergent fiber and IVNDFD were determined for samples collected on Days 4, 30, and 60.

bmr-12 Grazing Experiment

Following grain harvest, BMR or CON residues were grazed by crossbred yearling steers (239 ± 17 kg hd^{-1}; 24 steers per hybrid treatment per year) in late November or early December in each year. Steers were randomly assigned to paddocks and stocked at 2.6 steers ha^{-1} (6 steers paddock^{-1}). Paddocks of BMR or CON were assigned to random locations within the experiment in each year such that paddock within year was the experimental unit. Steers grazed for 72 d in 2006 and 61 d in 2008. Before the start of the trial, steers were limit fed at 2% of body weight a diet of 25% alfalfa, 25% grass hay, and 50% wet corn gluten feed for 5 d and then weighed on two consecutive days to minimize variation due to gut fill and obtain an accurate initial weight (IW), as described by Watson et al. (2013). Following grazing, steers were again limit fed the same diet at a projected two percent of body weight for 5 d and weighed on two consecutive days to obtain final weight (FW) from which ADG was calculated as kg hd^{-1} d^{-1} and BW gain was calculated as kg ha^{-1}. During the course of the grazing period, steers were supplemented daily at 1.1 kg hd^{-1} with 93.8% dry distillers grains, 4.7% limestone, 0.8% tallow, 0.1% Rumensin-80, 0.3% beef trace mineral, 0.2% selenium, and 0.1% vitamin A-D-E. Mean net gains ($ ha^{-1}) for the duration of the experiment were calculated on the basis of the Nebraska USDA cattle auction sale price report (USDA-Agricultural Marketing Service, 2014) for medium and large feeder steers.

Statistical Analysis

Sorghum grain yield, IW, FW, ADG, and BW gain data were analyzed in a split-plot design using mixed models in the MIXED procedure of SAS, version 9.3 (SAS Institute, 2012). Year, hybrid, and year × hybrid interaction were fixed effects and replicate was a random effect. Neutral detergent fiber and IVNDFD data were analyzed using a repeated measures mixed model to account for changes in residue quality over sampling date. A first-order antedependence covariance structure across days was included to account for the sequential nature of sampling, and the correlation between date and decreased residue quality due to weathering. For all analyses, normality was assessed with a Shapiro-Wilke test and Q-Q plots in PROC UNIVARIATE. Homogeneity of variance was assessed with a Levene's test in PROC GLM at $P < 0.05$. Least square means for fixed effects were separated at $P < 0.05$ with Fisher's Protected LSD test.

RESULTS AND DISCUSSION

Grain Yield

Total precipitation for the period of May to October was lower (471 mm) in 2006 and higher (761 mm) in 2008 than the long-term average (515 mm; Fig. 2), but year-by-treatment (hybrid) interactions for grain yield were not significant (data not shown) such that data were pooled across years. Pooled grain yields were 6% lower ($P = 0.01$) for BMR (7192 kg ha^{-1}) than for CON (7666 kg ha^{-1}). Grain yield of CON and BMR hybrids has been evaluated previously in two small-plot experiments. Oliver et al. (2005b) demonstrated a 1% decrease in BMR grain yield (7549 and 7629 kg ha^{-1} for BMR and CON, respectively) with marginal significance ($P = 0.05$). Sattler et al. (2010b) compared CON and BMR with near-isogenic hybrid counterparts in *bmr6* or stacked *bmr6 bmr 12* (double mutant) backgrounds and showed no differences in yield among any hybrids, which ranged from 7700 (*bmr6 bmr12*) to 8100 (CON) kg ha^{-1}. While yield in our study was comparable to that reported in Oliver et al. (2005b) and in Sattler et al. (2010b), the lower yield that we observed in BMR relative to CON was probably due to the limited number of environments tested in the current study or the much larger scale of the experiment and its associated sample sizes, which would lead to a more accurate estimation of mean values. Yield drag was not due to lodging or field emergence differences between hybrids, as none were observed, and reduced lignin conferred by *bmr-12* is not associated with increased infection by the common sorghum grain pathogens *Fusarium* or *Alternaria* spp. in Wheatland or Tx430 backgrounds (Funnell and Pedersen, 2006).

Oliver et al. (2005b) observed heterosis between the wild-type A Wheatland and R Tx430 parents used for production of the CON hybrid, with 6068 and 6776 kg ha^{-1}, respectively, in the parents (19% midparent [MP] heterosis in CON, calculated as $[F_1 - MP]/MP \times 100$). However, much greater heterosis was observed between *bmr-12* A Wheatland and R Tx430 parents used for production of

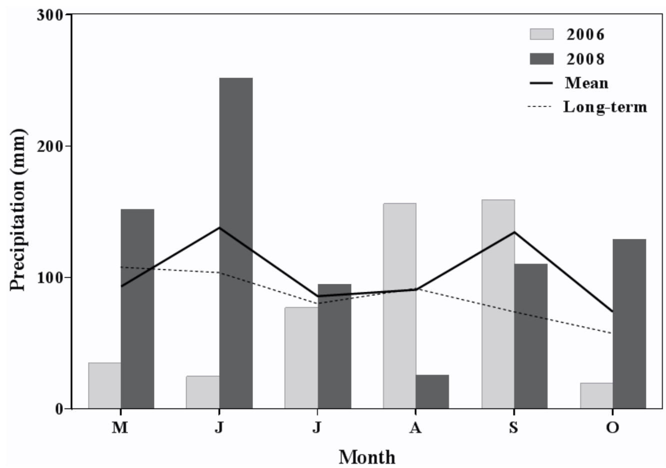

Figure. 2. Monthly precipitation received at Mead, NE in 2006 and 2008 (High Plains Regional Climate Center, University of Nebraska, Lincoln, www.hprcc.unl.edu/index.php, accessed 29 July 2013). Monthly (May to October, 2006 and 2008) mean precipitation is represented by the solid line and the long-term (1969 to 2012) mean is represented by the dashed line.

Table 1. Effect of *bmr-12* grain sorghum residue on the performance of yearling crossbred steers grazing for an average of 72 d in 2006 and 61 d in 2008. Means are pooled across years.

Characteristic	BMR†	CON‡	SEM
	kg hd^{-1}		
Pre-grazing live weight	239 a§	240 a	0.84
Post-grazing live weight	280 a	271 b	2.00
	kg hd^{-1} d^{-1}		
Average daily gain	0.63 a	0.45 b	0.03
	kg ha^{-1}		
Body weight gain	110 a	81 b	5.17
	$ ha^{-1}¶		
Mean net return	179.22	45.38	

† Treatment in which steers grazed a near-isogenic *bmr-12* grain sorghum hybrid (BMR).

‡ Control (CON) treatment in which steers grazed a near-isogenic wild-type grain sorghum hybrid.

§ Least squares means within row followed by the same letter do not differ at P = 0.05 according to Fisher's Protected LSD test.

¶ Pre-grazing steer live weight value ($5.54 kg^{-1}) subtracted from post-grazing steer live weight value ($4.97 kg^{-1}) using May 2014 local weight-specific live weight market prices (Mitchell et al., 2005).

BMR, with 4498 and 4322 kg ha^{-1}, respectively, in the parents (71% MP heterosis in the BMR hybrid). Therefore, our grain yield results are similar to those of Oliver et al. (2005b) in that heterosis in the *bmr-12* A Wheatland × R Tx430 hybrid background overcame much of the yield loss associated with the *bmr* trait in the *bmr-12* parent. The compensating effect of heterosis was further confirmed by Sattler et al. (2010b), who compared CON and BMR to a near-isogenic *bmr-6 bmr-12* counterpart. The ADF and ADL were reduced in the double mutant and crude protein and IVDMD of the crop residue were higher with no reduction in grain yield, residue yield, or grain crude protein.

In order for sorghum varieties or hybrids to qualify as a dual-purpose crop and for associated loans and loan deficiency payments, they must have a grain yield potential that is at least 80% of commercial grain hybrids (USDA, 2002). The relatively small reduction in grain yield we observed in BMR relative to CON at a commercial scale easily meets this threshold and may pose a real economic opportunity for the beef industry, particularly in areas where ethanol plants accept sorghum grain as a feedstock and the cost of irrigation may favor sorghum production over corn.

Animal Performance

The effect of year on ADG and BW gain was not significant (data not shown) such that steer performance data were pooled across years (Table 1). Initial weights averaged 240 kg and did not differ among hybrid treatments at the beginning of the experiment. Steer ADG (kg hd^{-1} d^{-1}) and total BW gain (kg ha^{-1}) differed between hybrids (both $P < 0.01$) following grazing. Steers grazing the BMR residue gained more weight (0.63 kg hd^{-1} d^{-1}, 110 kg ha^{-1}) than CON (0.45 kg hd^{-1} d^{-1}, 81 kg ha^{-1}), an increase of 0.18 kg hd^{-1} d^{-1} and 29 kg ha^{-1}. On the basis of an initial average steer live weight of 239 kg (BMR) and 240 kg (CON) at a market value of $5.54 kg^{-1} and a final average steer live weight of 280 kg (BMR) and 271 kg (CON) at a market value of $4.97 kg^{-1}, the net return per hectare from grazing was $179.22 and $45.38 for BMR and CON, respectively, according to the calculations used in Mitchell et al. (2005), an increased value of $133.84 ha^{-1} for BMR. These observations are consistent with previous studies (Aydin et al., 1999; Beck et al., 2013; Cherney et al., 1988, 1990; Holt et al., 2013; Oba and Allen, 1999; Oliver et al., 2005b) reporting increased digestibility or animal performance on *bmr* genotypes relative to the wild-type. Corn silage tends to enhance performance of dairy cows more than sorghum or other grass species when the cob and grain are included (Aydin et al., 1999; Dann et al., 2008; Grant et al., 1995; Miron et al., 2007; Oliver et al., 2004), but those fractions would be removed in a dual-purpose cropping system where cattle graze crop residue. It would be interesting to test the effects of *bm* corn residue on animal performance compared with *bmr* sorghum. Lorenz et al. (2009a) in particular noted that corn biomass bred for improved lignocellulosic conversion to ethanol also improved ruminant digestibility.

Neutral Detergent Fiber

Neutral detergent fiber data were pooled over years and are presented in Fig. 3A. No hybrid effect on NDF was observed, but significant differences existed between stems and leaves, between grazed and nongrazed residues, and across days. When pooled over hybrid, grazing status, and day, NDF averaged 75.9 and 70.9% for stems and leaves, respectively ($P < 0.0001$). The NDF content of stems in particular increased from Day 4 (74.2%) to Day 60 (77.8%, $P < 0.0001$), presumably due to weathering

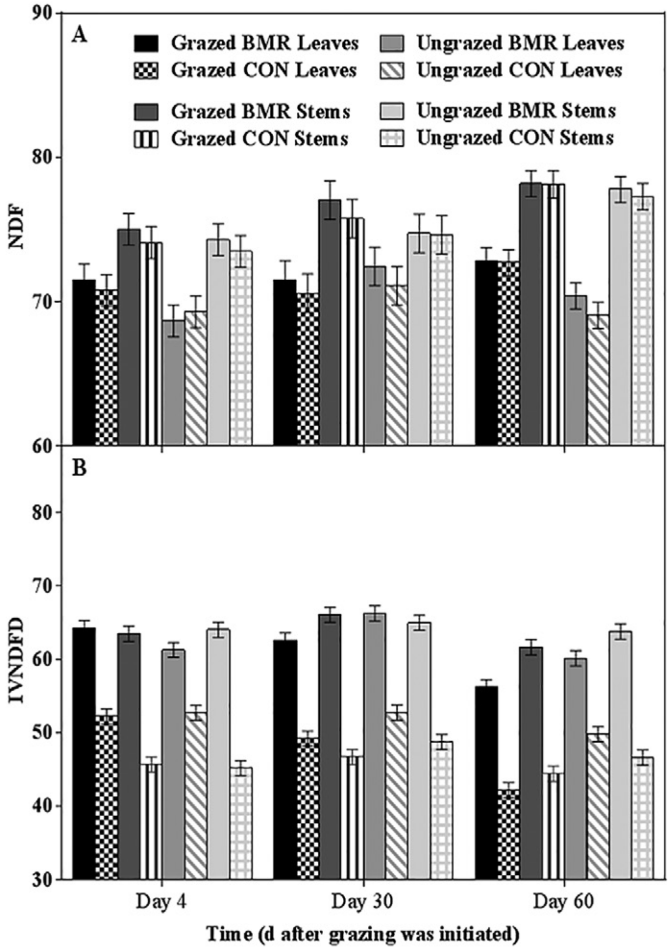

Figure 3. Neutral detergent fiber (NDF, A) and in vitro neutral detergent fiber digestibility (IVNDFD, B) of near-isogenic *bmr-12* (BMR) and wild-type (CON) grain sorghum residue from grazed and nongrazed areas of paddocks over time. Experiments were conducted near Mead, NE in 2006 and 2008. Data were pooled across years.

effects and leaching of cell solubles. The NDF content of leaves increased marginally from Day 4 (70.1%) to Day 60 (71.3%, $P = 0.05$). When pooled over hybrid, type, and day, grazed residue had higher NDF content (74.0%) than nongrazed residue (72.8%, $P = 0.02$), probably due to animal preference for (and removal of) higher-quality, lower-NDF material.

In Vitro Neutral Detergent Fiber Digestibility

The IVNDFD data were pooled over years and are presented in Fig. 3B. A strong hybrid effect was observed such that IVNDFD in BMR was consistently higher than in CON (31%; $P < 0.0001$). When pooled over days and grazing status, BMR stem IVNDFD (64.4%) was 4% greater than that of leaves (61.9%, $P = 0.04$), but CON stem IVNDFD (46.4%) was 7% lower than that of leaves (50.0%, $P < 0.0001$). Thus *bmr-12* resulted in a 39% increase in stem IVNDFD ($P < 0.0001$) over the wild-type and leaf IVNDFD increased 24% ($P < 0.0001$). Leaf IVNDFD decreased 7% from 63.0% on Day 4 to 58.3% on Day 60 in BMR ($P = 0.002$) and 12% from 52.6% on Day 4 to 46.2% on Day 60 in CON ($P < 0.0001$). Stem IVNDFD did not differ over days for BMR or for CON. The overall effect of grazing was significant ($P = 0.005$) and also reflected animal preference for higher quality forage. Grazed BMR leaf IVNDFD (56.4%) was 6% less but did not significantly differ from that of nongrazed leaves on Day 60 (60.3%, $P = 0.11$), but grazed CON leaf IVNDFD (42.4%) was 15% less than that of nongrazed leaves on Day 60 (50.1%, $P = 0.003$). A possible explanation for these results is that BMR IVNDFD was less variable than CON such that animals were unable to select higher quality forage. However, standard error of mean grazed BMR leaf IVNDFD increased slightly from Day 4 ($\pm$ 1.02) to Day 60 ($\pm$ 1.03) and was comparable to that of CON ($\pm$ 1.02 on Day 4 and $\pm$ 1.04 on Day 60) (Fig. 3B). Thus, our results are intriguing and suggest that BMR residue is of sufficient quality or palatability that animals were less selective in consuming it than they were with CON.

CONCLUSIONS

Previous studies had revealed similar NDF content but greater IVNDFD in BMR relative to CON (Oliver et al., 2005b), and results of the current study support this observation. In the present study, a larger decrease was observed in BMR grain yield relative to CON than reported by Oliver et al. (2005b) or Sattler et al. (2010b), but BMR yield was nevertheless well above the 80% threshold necessary to qualify as a dual crop (USDA, 2002). Animal performance, as determined by ADG and BW gain, was improved on BMR relative to CON, resulting in an estimated increase in net return of \$133.84 ha^{-1} due to BMR. It will be of further interest to study animal performance on the *bmr-6 bmr-12* double mutant residue, in which two alleles act additively to provide further reductions in lignin content (Dien et al., 2009), resulting in even higher digestibility than BMR (Sattler et al., 2010b). Our results suggest that grazing *bmr-12* hybrid grain sorghum residue is a promising option to increase animal performance of feeder cattle in cow-calf operations in areas too dry for corn, under irrigated systems where decreased water use is desired, or where sorghum grain is targeted for the biofuel industry.

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