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Forage nutritive value and predicted fiber digestibility of Kernza intermediate wheatgrass in monoculture and in mixture with red clover during the first production year

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ABSTRACT

Kernza intermediate wheatgrass is the first perennial grain crop in the world and has been developed with conventional breeding to increase seed yield of forage intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey). When managed for dual-use (grain and forage), Kernza intermediate wheatgrass can produce grain, crop residue (straw) in the summer, and green forage in the spring and fall. Mixtures of this grass with legumes could increase forage yield and nutritive value and provide other environmental and economic benefits. Despite the growing interest in these dual-use production systems, forage nutritive value of Kernza intermediate wheatgrass forage in a dual-use system in the Upper Midwest is unknown. A replicated field experiment was established in two locations in southern Wisconsin (Arlington and Lancaster) with two treatments: Kernza intermediate wheatgrass grown in monoculture and mixture with red clover (*Trifolium pratense* L.). Forage samples were collected at late vegetative stage in the spring, at grain harvest in the summer, and at the end of the regrowth period in the fall. Forage nutritive value of the monoculture was greatest in the spring with 456, 249 and 225 g kg^{-1} for neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein (CP), respectively; lowest in the summer with 702, 427 and 51 g kg^{-1} NDF, ADF and CP, respectively, and intermediate in the fall with 590, 337 and 119 g kg^{-1} NDF, ADF and CP, respectively. Predicted total-tract neutral detergent fiber digestibility (ttNDFD) was 0.53 for the spring forage and averaged 0.37 for the summer and fall forage, with no differences between the mixture and monoculture. The relative forage quality (RFQ) for the monoculture was 175 for the spring forage, 65 for the summer residue, and 116 for the fall. Intercropping red clover with Kernza intermediate wheatgrass increased CP of the summer crop residue by 69%, and increased CP and RFQ of the fall forage by 49% and 11%, respectively, while reducing NDF and ADF of the fall forage by 25% and 18%, respectively. Therefore, Kernza intermediate wheatgrass forage is suitable for lactating beef cows, dairy cows, and growing heifers when harvested in the spring and fall, and it offers high potential for dual-use grain and forage systems.

Abbreviations: K_d, rate of NDF digestion; NDFD48, neutral detergent fiber digestibility following a 48-h in vitro digestion; NDFD240, neutral detergent fiber digestibility following a 240-h in vitro digestion; RFQ, relative forage quality; ttNDFD, total-tract neutral detergent fiber digestibility

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1. Introduction

Kernza is the first commercially available perennial grain in the world, developed through conventional breeding for increased seed production of intermediate wheatgrass (*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey) at The Land Institute (Salina, KS, USA, DeHaan et al., 2014). Kernza intermediate wheatgrass is generating a growing interest among farmers and processors for its environmental and economic benefits (DeHaan et al., 2014; Lubovski, 2016; Lanker et al., 2019) because it is a perennial cereal crop as compared to annual cereal crops such as corn, wheat, barley, or oats. While highly productive, annual cropping systems rely on annual tillage and replanting which are associated with environmental problems such as soil erosion and nutrient leaching (Ryan et al., 2018; Jungers et al., 2018a). Perennial crops therefore represent an effective alternative to improve the sustainability of the current agricultural system (DeHaan and Ismail, 2017; Ryan et al., 2018). While many years of breeding will be necessary to achieve grain yields similar to annual wheat (DeHaan et al., 2016), "forage" intermediate wheatgrass is already a preferred forage grass in the intermountain region and northern Great Plains of North America where it is used for hay, pasture, and erosion control in areas with 300 to 460 mm of annual rainfall (Ogle, 2011; Hybner and Jacobs, 2012; Jensen et al., 2017). Extensive selection has increased the forage yield and nutritive value of forage intermediate wheatgrass after it was imported from Eurasia a century ago, making it a preferred grass for environments too dry and cold for other high quality grasses such as smooth bromegrass (*Bromus inermis* L.) or orchardgrass (*Dactylis glomerata* L. - Jensen et al., 2016). In this paper we use the name "Kernza intermediate wheatgrass" to refer to the improved perennial grain (dual-use) crop and "forage intermediate wheatgrass" to refer to the previous materials used for forage only.

Managing Kernza intermediate wheatgrass for grain and forage has been proposed as an effective way to increase the annual productivity and profitability of this novel crop (Pugliese et al., 2019; Ryan et al., 2018). Farmers have identified the dual-use of Kernza for grain and forage as a relevant practice and demand information about forage nutritive value (Lanker et al., 2019). Management for dual-use consists in three annual harvests: a first spring forage harvest before stem elongation, a grain harvest at physiological maturity with removal of the crop residue (straw) and a third forage harvest in the fall. Intercropping forage legumes with this grass can provide several environmental and economic benefits such as increased forage yield and nutritive value, improved annual yield distribution, greater weed suppression, and biological N fixation potentially reducing the needs for N fertilizer (Picasso et al., 2008; Sleugh et al., 2000; Thilakarathna et al., 2016). Red clover is a promising legume to intercrop with Kernza intermediate wheatgrass because of its ease of establishment, yield potential and tolerance to grazing (Sheaffer and Seguin, 2003; Riday, 2008; Sheaffer et al., 2018).

The nutritive value and yield of forage intermediate wheatgrass have been documented for the growing conditions of the western United States and southern Canada where its annual forage productivity ranges between 2700 and 5000 kg ha^{-1}. Forage nutritive value at the late reproductive stage averages 100 g kg^{-1} CP, 394 g kg^{-1} ADF, 636 g kg^{-1} NDF and a neutral detergent fiber digestibility (NDFD) of 0.63 (Jensen et al., 2017), and 135 g kg^{-1} CP, 295 g kg^{-1} ADF, 601 g kg^{-1} NDF and 0.66 of NDFD when stockpiled into the winter (Zhao et al., 2008; Cattani and Asselin, 2018). In the Upper Midwest, where Kernza is grown for grain, clipping and grazing have shown a positive impact on grain yields and lodging (Pugliese et al., 2019) but the nutritive value of Kernza intermediate wheatgrass managed for dual-use remains unknown. Additionally, an in vitro assay, called ttNDFD has recently been developed to assess the rate and extent of fiber digestion in grasses and allows for objective inter-species comparison with other grasses and legumes (Combs, 2015). This procedure relies on 240 h in vitro digestion to quantify potentially digestible fiber and measurement of in vitro NDF digestion at 24, 30 and 48 h to determine the rate of digestion of potentially digestible NDF. The ttNDFD is then calculated by integrating rate of digestion of potentially digestible NDF with rate of passage of fiber to predict the in vivo digestion of NDF in dairy cow (Combs, 2015; Lopes et al., 2015). Given the potential for forage utilization, information on nutritive value and fiber digestibility in the different harvests are therefore needed for the development of Kernza intermediate wheatgrass as a dual-purpose, perennial grain crop for the Upper Midwest.

The first objective of this study was to characterize the forage nutritive value and fiber digestibility of Kernza intermediate wheatgrass managed for dual-use in three harvests during the first production year: vegetative initial growth (spring), crop residue after grain harvest (summer), and vegetative regrowth (fall). The second objective was to compare the forage nutritive value of Kernza intermediate wheatgrass grown in monoculture and in mixture with red clover.

2. Materials and methods

2.1. Experimental design and management

The experiment was established in the fall of 2016 at two locations on University of Wisconsin Agricultural Research Stations: Arlington (43°18′6.66″N, 89°19′41.55″W) and Lancaster (42°49′52.56″N, 90°48′1.78″W). Two treatments were applied: Kernza intermediate wheatgrass grown in monoculture and in mixture with red clover. The experimental design in each location was randomized complete blocks, with 5 replications in Arlington and 3 in Lancaster. In both locations, Kernza seed origin was from The Land Institute breeding Cycle 4. The seeding rate was 12.1 kg ha^{-1} of pure live seeds (PLS) and row-spacing was 38 cm. The row spacing was wider than what it is used for forage production (15 cm), and narrower than what is used for seed production (60–90 cm); previous research has not found differences in long term grain yields of Kernza between 15 and 75 cm of spacing (Tautges et al., 2018). In Arlington, plot size was 4.5 by 4 m separated by grass alleys mowed to a stubble height of 10 cm when they reached a height of 30 cm. Seeds were sown with a John Deer 1590 grain drill on 21 September 2016 after chisel-plowing the residues of a previous soybean crop. The mixture plots were broadcasted with 10.5 kg ha^{-1} PLS of FF 9615 red clover (LaCrosse Seeds) on 10

March 2017. In Lancaster, plot size was 0.06 to 0.2 ha, separated by grass alleys regularly mowed to a stubble height of 10 cm. Seeds were no-till drilled on 11 September 2016 into a 3-year-old alfalfa stand sprayed two weeks earlier with glyphosate. In the mixture plots in Lancaster, red clover was drilled between the Kernza intermediate wheatgrass rows on the same day at a density of 8.8 kg ha^{-1} PLS and with the same cultivar. No chemical or mechanical weed control was applied after planting at any location.

Soils were Plano silt loam with 2 to 6% slope (PaB – Web Soil Survey, 2018) at Arlington and Fayette silt loam with 6 to 12% slope (FaC2 – ibid.) at Lancaster. Soil samples were taken to a depth of 15 cm in October 2016 from both fields, dried for three days in a forced-air dryer at 50 °C and sent to the University of Wisconsin Soil and Forage Laboratory (Marshfield, WI). Soil test results indicated 57 ppm of P, 244 ppm of K and 35 g kg^{-1} of organic matter in Arlington and 19 ppm of P, 115 ppm of K and 26 g kg^{-1} of organic matter in Lancaster, with a similar pH of 6.8. Therefore, Arlington soils had greater nutrient and organic matter concentration than Lancaster, likely due to the repeated dairy manure applications on the Arlington field over the past 50 years (Bertram, 2018, pers. comm.). Monoculture and mixture plots were fertilized with urea (46-0-0) at a rate of 50 kg N ha^{-1} on 18 April in Arlington and 34 kg ha^{-1} on 10 April 2017 in Lancaster. The monoculture plots received a second application of urea equal to the first one at the beginning of stem elongation, on 26 May in Arlington and on 12 May 2017 in Lancaster.

2.2. Forage and grain sampling

Forage samples were collected by hand-harvesting quadrat samples at the soil level. In Arlington, one 50-by-50 cm randomly placed quadrat per plot was harvested. In Lancaster, four to six 50-by-75 cm randomly placed quadrats were harvested per plot rotated perpendicularly to the rows to include two Kernza intermediate wheatgrass rows (in both treatments) and two red clover rows in the mixture plots. Plants from other species (weeds) were removed from the sample manually in the field. The two species were separated in the field and placed in individual bags. Samples were then placed in a forced-air dryer at 65 °C for five days. Dry matter yields per hectare were then extrapolated from the quadrat data on an area basis and one sample per plot was analyzed for nutritive value at each location. The spring forage samples were collected four days prior to stem elongation, on 26 April 2017 and 1 May 2017 in Arlington and Lancaster, respectively and no red clover was harvested in the early spring since it was emerging in Arlington and establishing in Lancaster. Forage was not removed from the whole plots in the spring, only quadrat samples were taken. Summer samples were collected on 29 and 28 July 2017 in Arlington and Lancaster, respectively, when the crops had reached physiological maturity (stage S4-5, Moore et al., 1991). The seed heads from these samples were cut 5 cm below the lowest seeds and threshed manually to estimate grain yield and the remaining biomass was weighed and considered as the crop residue. A day after summer data collection, the entire fields were harvested for grain, with small-plot combine in Arlington (Wintersteiger Elite, Ried im Innkreis, Austria) and farm-size combine in Lancaster (Case International AxialFlow 1688, Racine, WI, USA) at 50–70 cm height to harvest only the Kernza grain. The remaining forage was cut at a stubble height of 10 cm, baled and removed within a week of grain harvest. The fall forage samples were collected at the end of the growing season on 10 and 18 October 2017 in Arlington and Lancaster, respectively, according to the same protocol as the spring forage, and whole plot biomass was harvested and removed the following day.

2.3. Temperature and precipitation records

Temperatures and precipitation were recorded on the research farms and downloaded from the online database of the National Weather Service (NWS, 2018). The accumulation of heat units, expressed in growing degree-days (GDD), was calculated using daily average temperatures and the equation from McMaster and Wilhelm (1997), with a base temperature of 0 °C. The accumulation of GDD and precipitation was initiated at planting and paused for the winter between 30 November 2016 and 17 March 2017. These dates correspond to the point when average daily temperatures remained below and above the base temperature for 5 consecutive days in the fall and spring, respectively, as described by Jungers et al. (2018b). The accumulation ended at the time of the fall forage harvest for each location.

In the fall of 2016, precipitation was 139 and 116 mm in Arlington and Lancaster, respectively and the accumulation of heat-units reached 689 and 818 GDD, respectively. Temperatures were similar at both locations but the field in Lancaster was planted 7 days earlier, which provided it with an additional 109 GDD. During the 2017 growing season, which extended from 17 March to the fall forage harvest, precipitation was 664 mm in Arlington and 827 mm in Lancaster (Fig. 1), bringing the total accumulation to 767 mm and 908 mm; a 25 and 9% inferior value to the 30-years average for this time frame, respectively (NWS, 2018). In Arlington and Lancaster, a dry period marked the late spring at both locations with fewer than 3 mm of precipitation between 25 May and 12 June. Limited rainfall was again observed between 28 July and 5 October, where 21 mm of precipitation were recorded in Arlington and 70 mm in Lancaster. The accumulation of heat-units reached 3328 and 3472 GDD for Arlington and Lancaster, respectively, bringing the total degree-day accumulation to 4021 and 4290 GDD from planting to fall forage harvest (Fig. 1). The average temperatures during the growing season were similar to the 30-years average with 15.3 and 16.4 °C for Arlington and Lancaster, respectively.

2.4. Analysis of nutritive value parameters

Neutral detergent fiber, ADF and CP were analyzed using wet chemistry procedures and reported as a proportion of the DM for the following three crops: Kernza intermediate wheatgrass grown in monoculture, Kernza intermediate wheatgrass grown in mixture, and red clover. All samples were first ground with a Christy hammer mill (Christy-Turner Ltd, Ipswich, England) to pass a 1-mm screen. Total N was determined according to the Dumas combustion method (Method 990.03 - AOAC, 2000) and the analysis was conducted

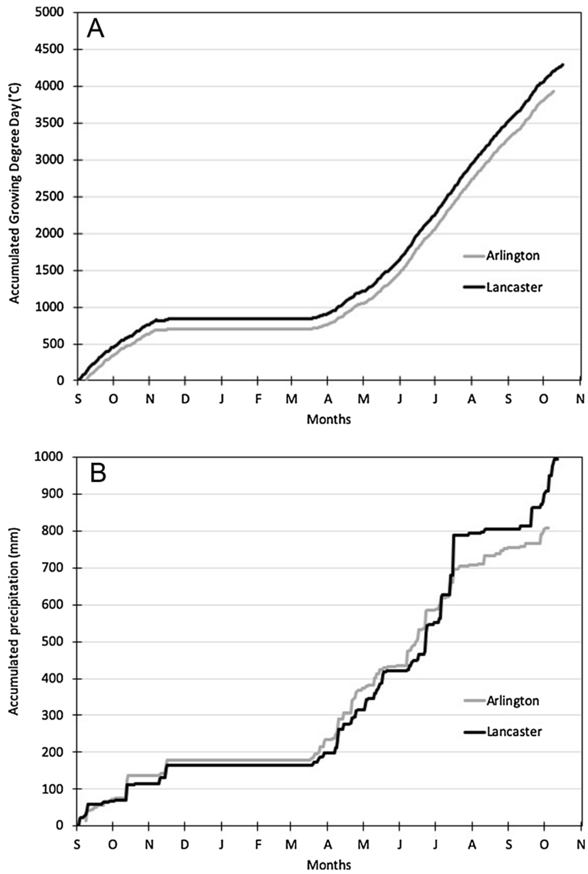

Fig. 1. Growing degree-day (GDD - A) and precipitation (B) accumulated in the fall of 2016 and during the 2017 growing season, paused during the winter months, at Arlington (ARL) and Lancaster (LAN).

in a LECO FP-528 (LECO Corporation, St-Joseph, MI). Crude protein was calculated as N x 6.25. Neutral detergent fiber and ADF were analyzed sequentially in an Ankom 2000 Fiber Analyzer (Ankom technology, Macedon, NY) according to the procedure of Robertson and Van Soest (1981) and modified by Hintz et al. (1996) to include sodium sulfite during refluxing. For the grass-legume mixture samples, NDF, ADF, and CP concentration of the mixture forage was calculated as the weighted average of Kernza intermediate wheatgrass and red clover based on their respective DM proportion of the total quadrat weight.

Fiber digestibility and the rate of digestion were characterized with 24, 30, 48 and 240 -hs in vitro NDF digestions using rumen fluid. The NDF residue remaining after 240 h in vitro digestion is a measure of indigestible NDF. The rate of digestion of potentially digestible NDF is estimated from the loss of potentially digestible NDF as measured from the disappearance from the 24, 30, 48 -h in vitro digestions. A prediction of in vivo total tract NDF digestion (ttNDFD) in a lactating dairy cow is modeled according to the procedure described by Combs (2015) and Lopes et al. (2015). The crops analyzed were Kernza intermediate wheatgrass grown in monoculture, Kernza intermediate wheatgrass grown in mixture, and the Kernza intermediate wheatgrass-red clover mixture. Two samples per treatment were used due to space limitation in the water baths. Each sample was replicated three times and statistical analyzes were conducted with the mean of three subsamples.

Relative forage quality (RFQ), an index that compares the nutritive value of forages relative to that of fresh full-bloom alfalfa (RFQ = 100), was calculated according to the equations from Moore and Undersander (2001) for grass and grass-legume hay. It relies on NDF, NDFD, CP, fatty acids, and ash.

2.5. Statistical analyses

Statistical analyses were conducted with RStudio (2016). Treatment effects on nutritive value parameters and seasonal forage DM yield were tested with an analysis of variance (ANOVA) and considered significant at $p < 0.05$. The effects of crop, harvest season, and location, as well as their respective two and three-way interactions were included in the following linear mixed model and analyzed as a split-plot design with crop as whole plot and harvest season as split-plot: Yijkl = μ + Ci + Lj + Sk + Bl(j) + C*Lij + C*Sik + L*Sik + C*L*Sijk + Eijkl, where Yijkl = seasonal forage DM yield or nutritive value parameters; μ = the overall mean; Ci = the effect of crop (i.e., Kernza intermediate wheatgrass in monoculture, in mixture, red clover, or forage mixture); Li = effect of location; Sk = effect of harvest season (i.e., spring, summer, and fall); Bl(j) = effect of blocks nested within location

Table 1
P-values from the analysis of variance for the effect of crop (Kernza intermediate wheatgrass monoculture, Kernza intermediate wheatgrass from mixture, red clover from mixture, and calculated mixture of Kernza intermediate wheatgrass and red clover based on dry matter (DM) yields), location (Arlington and Lancaster, WI), harvest season (spring, summer, and fall) and their respective interaction on neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), rate of NDF digestion (K_d), neutral detergent fiber digestibility after 48 and 240 h of in vitro digestion (NDFD48 and NDFD240), predicted total-tract neutral detergent fiber digestibility (ttNDFD), relative forage quality (RFQ), season forage DM yield, grain yield and annual forage DM yield. P values < 0.05 are highlited in bold.

	NDF	ADF	CP	K_d	NDFD 48	NDFD 240	ttNDFD	RFQ	Seasonal forage DM yield	Grain yield
Crop (C)	< **0.01**	< **0.01**	< **0.01**	0.79	0.83	< **0.01**	0.36	**0.02**	< **0.01**	< **0.01**
Location (L)	**0.01**	0.37	0.14	0.63	0.79	0.52	0.76	**0.03**	0.26	0.13
Season (S)	< **0.01**	< **0.01**	< **0.01**	**0.02**	< **0.01**	< **0.01**	< **0.01**	< **0.01**	< **0.01**	
C*L	0.22	< **0.01**	< **0.01**	0.68	0.21	0.07	0.15	0.82	**0.02**	0.67
C*S	< **0.01**	< **0.01**	< **0.01**	0.43	0.17	0.05	0.08	**0.02**	< **0.01**	
L*S	< **0.01**	< **0.01**	< **0.01**	0.60	0.89	0.52	0.95	< **0.01**	< **0.01**	
C*S*L	0.33	0.28	0.18	0.88	0.88	0.77	0.81	0.08	0.07	

(random); C*Lij, C*Sik, L*Sik and C*L*Sijk = effect of the two and three-ways interactions and Eijkl = random residual. For grain yield we used a model for a randomized complete block design: Yijl = μ + Ci + Lj + Bl(j) + C*Lij + Eijl, where Yijl = grain yield; μ = the overall mean; Ci = the effect of crop (i.e., Kernza intermediate wheatgrass in monoculture or mixture); Li = effect of location; Bl(j) = effect of blocks nested within location (random); C*Lij, = effect of the two-way interaction and Eijl = random residual. When significant, differences among treatments were further investigated with a Tukey test and considered significant at $p < 0.05$.

3. Results

3.1. Neutral detergent fiber, acid detergent fiber, and crude protein

No three-way interaction was significant (Table 1). An interaction was detected between crop and harvest season for most parameters (Table 1) and results are therefore presented by harvest season and crop (Table 2). A location-by-harvest season

Table 2
Means and standard errors of means (SEM) for neutral detergent fiber (NDF), acid detergent fiber (ADF), crude protein (CP), neutral detergent fiber digestibility after 48 and 240 h of in vitro digestion (NDFD48 and NDFD240), predicted total-tract neutral detergent fiber digestibility (ttNDFD) and rate of NDF digestion (K_d) of first-year Kernza intermediate wheatgrass managed for dual-use (forage harvest in the spring and fall, grain and forage harvest in the summer) and grown in monoculture and mixture with red clover. Crop includes Kernza intermediate wheatgrass (IWG) from monoculture, IWG from mixture, red clover from mixture, and their calculated mixture of both species based on DM yields. Means were calculated over two Wisconsin locations (Arlington and Lancaster); means with the same letter within each parameter are not different at alpha = 0.05. ttNDFD was calculated based on the equation: ttNDFD = {(1-NDFD240)*[K_d/(K_d+K_p)]}/0.9, and K_p was assumed to be 2.67% hr^{-1} (Lopes et al., 2013).

		Harvest season						
Parameter	Crop	Spring		Summer		Fall		SEM
NDF g kg^{-1} DM	IWG monoculture	456	ef	702	ab	590	cd	12
	IWG from mixture	–		716	a	543	d	
	Red clover from mixture	–		483	e	393	g	
	IWG/clover mixture	–		637	bc	443	f	
ADF g kg^{-1} DM	IWG monoculture	249	d	427	ab	337	c	7
	IWG from mixture	–		438	a	314	c	
	Red clover from mixture	–		332	c	257	d	
	IWG/clover mixture	–		412	b	276	d	
CP g kg^{-1} DM	IWG monoculture	225	a	51	f	119	d	6
	IWG from mixture	–		48	f	161	c	
	Red clover from mixture	–		159	c	187	b	
	IWG/clover mixture	–		86	e	177	bc	
NDFD48 g g^{-1} NDF	IWG monoculture	0.63	a	0.35	bc	0.48	b	0.03
	IWG from mixture	–		0.33	c	0.47	bc	
	IWG/clover mixture	–		0.38	bc	0.40	bc	
NDFD240 g g^{-1} NDF	IWG monoculture	0.88	a	0.56	cd	0.69	bc	0.03
	IWG from mixture	–		0.52	d	0.75	ab	
	IWG/clover mixture	–		0.52	d	0.56	cd	
K_d of NDF (% h^{-1})	IWG monoculture	2.72	a	1.23	b	1.59	b	0.1
	IWG from mixture	–		1.11	b	1.53	b	
	IWG/clover mixture	–		1.26	b	1.07	b	
ttNDFD	IWG monoculture	0.53	a	0.41	b	0.40	b	0.02
	IWG from mixture	–		0.33	b	0.39	b	
	IWG/clover mixture	–		0.35	b	0.33	b	

interaction was also detected for most parameters, which we attributed to the difference in planting timing of the red clover and growing degree-day accumulation in the fall. In Lancaster, the fall-planted red clover was harvested at the ripe seed pod stage, while in Arlington, the frost-seeded red clover reached the bud stage at the time of the summer harvest, so forage nutritive value was greater in Arlington than Lancaster in summer. Additionally, the GDD accumulation in the fall 2016 was 110 GDD greater than in Lancaster, where there was a southwest-facing slope and residual N left by the previous three years-old alfalfa stand, which led to a stem elongation and forage harvest 25 days earlier in Lancaster than Arlington. After summer defoliation, both the Kernza intermediate wheatgrass and red clover regrew from the same starting point and were harvested at equal maturity stage in the fall when no location effect was detected. Treatment differences between crops were however consistent between both locations and results were therefore averaged between locations.

Neutral detergent fiber was different between seasons and was lowest in the spring, intermediate in the fall, and greatest in the summer (Table 2). A similar concentration between the monoculture and mixture was observed in the summer with an average concentration of 670 g kg^{-1}. In the fall, however, NDF of the mixture was lower than the monoculture with 443 versus 590 g kg^{-1}. This effect was driven by the low NDF concentration in the red clover which represented 60% of the biomass.

Acid detergent fiber responded similarly than NDF to crop and harvest season. It was lowest in the spring, intermediate in the fall, and greatest in the summer with 249, 337, and 427 g kg^{-1} DM for the monoculture forage (Table 2). In the summer, ADF was similar in the monoculture and mixture and averaged 420 g kg^{-1}. At this time, ADF of the red clover was lower with 332 g kg^{-1} but did not contribute to a significant decrease contrary to what was observed for NDF. In the fall, ADF was lower for the mixture than the monoculture with 276 versus 337 g kg^{-1}. This difference is explained by the low concentration of the red clover with 257 g kg^{-1} as no differences were detected between the Kernza intermediate wheatgrass in the monoculture or mixture.

Crude protein was similarly affected by season and its concentration decreased from 225 g kg^{-1} in the spring to 119 in the fall, and 51 in the summer for the monoculture forage (Table 2). In the summer, CP concentration of the red clover was three-times superior to that of the Kernza intermediate wheatgrass with 159 g kg^{-1} and explained a 69% increase in the mixture forage with 86 g kg^{-1} CP. A similar difference was observed in the fall, but with greater magnitude. Crude protein concentration of the mixture was 177 g kg^{-1} versus 119 g kg^{-1} for the monoculture, a difference supported by the high CP concentration in the red clover with 187 g kg^{-1} and its important yield contribution (Table 3). Also it is noteworthy that Kernza intermediate wheatgrass CP concentration in the mixture was 35% higher than in the monoculture in the fall (Table 2).

3.2. Fiber digestibility and relative forage quality

Fiber digestibility was affected by season and no two-way interactions were found with crop or location. Neutral detergent fiber digestibility after 48 h of in vitro digestion was 0.63 for the monoculture in the spring and averaged 0.40 in summer and fall (no differences were detected for the monoculture and mixture, Table 2). Neutral detergent fiber digestibility after 240 h of in vitro digestion (NDFD240) was also greatest for the monoculture in the spring with 0.88, lowest in the summer (averaging 0.53 with no differences between monoculture and mixture), and intermediate in the fall (Table 2). The rate of digestion of NDF was highest in the spring, and averaged 1.30% h^{-1} in summer and fall (no differences between monoculture and mixture, Table 2). Predicted total tract neutral detergent fiber digestibility averaged 0.53 in the spring and was lower in the summer and fall, with no differences between the mixture and monoculture averaging 0.37 (Table 2).

There was a significant crop by harvest season interaction for RFQ. The RFQ was 175 in the spring for the monoculture forage (Table 3). The monoculture and mixture had a similar RFQ in the summer of 65, and greater values were observed for the mixture in the fall with 129 in the mixture versus 116 in the monoculture. Even though red clover was 32% of the forage DM in the summer, it was too mature and dry to improve the RFQ of the summer crop residue mixture. However, in the fall regrowth harvest time, red clover contributed 60% of the DM and significantly increased the RFQ of the forage mixture (Table 3).

Table 3
Relative forage quality (RFQ), grain yield, forage yield, and % of red clover in the mixture from the first year of Kernza intermediate wheatgrass grown in monoculture and mixture with red clover and sampled in the spring (before elongation) in the summer (at grain harvest) and at the late fall regrowth. Results are averaged between two Wisconsin locations (Arlington and Lancaster). Means with the same letter within each parameter and harvest season are not different at alpha = 0.05.

		Harvest season		
Parameter	Crop	Spring	Summer	Fall
RFQ	IWG monoculture	175	65	116 b
	IWG/clover mixture		65	129 a
Grain yield (kg ha^{-1})	IWG monoculture		1089 a	
	IWG/clover mixture		789 b	
Forage yield (kg ha^{-1})	IWG monoculture	1409	6141 b	1394 b
	IWG/clover mixture		7422 a	3035 a
Red clover in DM (%)	IWG/clover mixture	0	32	60

3.3. Forage and grain yields

No three-way interaction was detected for seasonal forage yields, but a significant two-way interaction was found between location and season ($p < 0.01$ – Table 1). The Kernza intermediate wheatgrass monoculture had similar forage yields than the Kernza intermediate wheatgrass-clover mixture in summer in Arlington (6243 kg ha^{-1} on average) but in Lancaster the mixture had higher yields (8421 kg ha^{-1}) than the monoculture (6124 kg ha^{-1}). In the fall forage yields were higher for the mixture than the monoculture in both locations. This interaction we attribute to the different planting dates of the red clover and different environmental conditions during the establishment and the early spring. The greater GDD accumulation in the first fall, south-western slope exposure and residual N from the previous alfalfa crop allowed for greater forage production in Lancaster in the summer. Forage yields were however similar between locations in the spring and fall and the same yields were recorded for red clover in the summer at both locations, despite different planting dates, and results were therefore averaged between locations. The amount of Kernza intermediate wheatgrass biomass before stem elongation averaged 1409 kg ha^{-1}of DM and no forage was removed. In the summer, forage yields were greater for the mixture than the monoculture with 7422 kg ha^{-1}versus 6141 kg ha^{-1}, and the red clover accounted for 32% of the DM in the mixture. Forage yields were greater for the mixture in the fall with 3035 kg ha^{-1} versus 1394 kg ha^{-1} for the monoculture, when the red clover produced 1803 kg ha^{-1} of DM and accounted for 60% of the biomass. Fall forage harvest represented 19% and 29% of the annual harvested forage, for the monoculture and the mixture, respectively. No crop-by-location interaction ($p = 0.67$) nor location effect ($p = 0.13$) were detected for the first-year grain yields. They were greater for the monoculture with 1089 kg ha^{-1} versus 789 kg ha^{-1} in the mixture ($p < 0.01$), a 27% decrease (Table 3).

4. Discussion

The nutritive value of Kernza intermediate wheatgrass observed in this study was similar to what was reported at the same morphological stages in other environments. For instance, the crop residue had the same nutritive value as what Nelson et al. (1989) reported for forage intermediate wheatgrass harvested at the dough stage in central Oklahoma with 49 g kg^{-1} CP, 744 g kg^{-1} NDF and 444 g kg^{-1} ADF and the fall regrowth showed similar values as those reported by Coleman et al. (2010) in western Washington with 100 g kg^{-1} CP, 575 g kg^{-1} NDF and 316 g kg^{-1} ADF. Jensen et al. (2017) also reported CP of forage intermediate wheatgrass in a study comparing the evolution of this parameter from May to November on a two-weeks interval in Utah and reported 201 g kg^{-1} at the late vegetative stage, a concentration similar to the 225 g kg^{-1} observed in our study.

The nutritive value of Kernza intermediate wheatgrass was within the same range as what is commonly observed for cool-season grasses in the Upper Midwest. In the spring for instance, Brink et al. (2010) reported 478, 497 and 487 g kg^{-1} of NDF for meadow fescue (*Schedonorus pratensis* (Huds.) P. Beauv.), tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort.) and orchardgrass harvested in Lancaster, respectively, a concentration similar to what was observed with Kernza intermediate wheatgrass in this study. Crude protein of Kernza intermediate wheatgrass managed for dual-use was similar to what is observed in dual-purpose winter-wheat systems grazed before elongation in the Central Plains, where this annual cereal shows CP concentration ranging between 201 and 331 g kg^{-1} based on soil, nutrient, and weather conditions (Lyon et al., 2001; Begna et al., 2017). Crude Protein concentration of Kernza intermediate wheatgrass in its first spring was however 74% greater than what was reported by Brink et al. (2010) before fertilization on established stands of fescues and orchardgrass. Neutral detergent fiber and CP of Kernza intermediate wheatgrass in the fall were also similar to the values reported by Hedtcke et al. (2002) for orchardgrass, smooth bromegrass and tall fescue stockpiled until mid-October in Arlington and Lancaster. Neutral detergent fiber and CP concentration for these grasses averaged 597 and 114 g kg^{-1}, respectively. The crop residue harvested after grain harvest was more nutritious than what is commonly observed with wheat straw as the stems are still green when the plants are cut. As a consequence, the crop residue contained 13% less NDF and 30% more CP than wheat straw (NRC, 2000).

Fiber digestibility of Kernza intermediate wheatgrass assessed with the traditional 48 h procedure was lower than for other cool-season grasses grown in Wisconsin. It was 0.63 in the spring and is lower than what Brink et al. (2010) reported for meadow fescue, tall fescue, and orchardgrass harvested at the vegetative stage with an average digestibility of 0.78. It is also lower than the values reported by Jensen et al. (2017) in Utah with 0.75. However, ttNDFD is a more relevant indicator of the fiber digestibility, and it is therefore recommended over the traditional procedure (Combs, 2015). Total tract NDFD of Kernza intermediate wheatgrass was similar to what has been observed for other cool-season grasses and ruminant forages in the Upper Midwest with an average of 0.42 in the summer and fall compared to 0.38 for tall fescue silage (Verbeten et al., 2011), 0.36-0.38 for corn silage (Verbeten et al., 2011; Bender et al., 2016b) and 0.43-0.45 for alfalfa silage (Lopes et al., 2013; Bender et al., 2016b). Wheat straw, in comparison, has lower ttNDFD with 0.33 (Verbeten et al., 2011). The rate of NDF disappearance (K_d) observed in this study is similar to corn silage with 2.1% h^{-1} and lower than tall fescue hay with 2.9% h^{-1} (Bender et al., 2016a, b).

Intercropping red clover with Kernza intermediate wheatgrass increased the fall forage yield by 118% and the annual forage yield by 39% despite a 50% reduction in N fertilizer. It also increased the nutritive value of the fall forage. The greater CP concentration for the Kernza intermediate wheatgrass grown in mixture indicates a significant N transfer from the legume to the grass, as documented by Suter et al. (2015) in an experiment across the European continent. These results also support the findings of Zemenchik et al. (2001) who showed that grass-legume mixtures outyielded grass monocultures fertilized with less than 182 kg ha^{-1} of N, in the same environments. The decrease in first-year Kernza grain yields shows a competition between Kernza intermediate wheatgrass and red clover and is consistent with the 40% reduction in Kernza grain yield reported by Tautges et al. (2018) for second-year stands intercropped with alfalfa in Minnesota. Although maximal grain production was achieved in the monoculture in the first production year, seed yields are known to decline in the following years; substantially reducing the profitability of a stand managed for seed only. In this regard, intercropping Kernza intermediate wheatgrass with red clover - or other forage legumes - and managing the stand for dual-use can represent a valuable investment with long-term benefits. Forage legumes such as red clover or alfalfa grown in association with Kernza have also shown potential to increase seed yield of three and four-year-old stands (Tautges et al., 2018). They are also well known to reduce the need for synthetic N fertilizer through biological N fixation and mutual stimulation (Huss-Danell et al., 2007; Nyfeler et al., 2011), reduce weed invasion in hayfields (Sheaffer et al., 1990; Picasso et al., 2008) and increase biomass productivity (Picasso et al., 2008).

The bulk of the annual biomass production occurs when forage from the reproductive growth is baled after grain harvest. In the present study, crop residue yields represented 81% and 71% of the annual harvested forage in the monoculture and mixture, respectively. Due to the late summer harvest, these yields are greater than those observed with meadow and tall fescue managed in a three-cut system or forage-oat harvested at the late heading stage for silage, with 7790, 9200, and 8700 kg ha^{-1}, respectively (Brink et al., 2010; Favre et al., 2019). They are however similar to those reported by Jungers et al. (2018b) with the same line (TLI-C4) in St-Paul, MN. Forage yields of Kernza intermediate wheatgrass in the fall are also similar to other cool-season grasses stockpiled until mid-October (Riesterer et al., 2002).

These findings indicate that Kernza intermediate wheatgrass managed for dual-use in Wisconsin could be suitable for lactating dairy cattle when utilized in the early spring before stem elongation. In the fall, forage quality is greater than full-bloom alfalfa and is therefore adequate for lactating beef cows and dairy heifers when grown in monoculture, and it can be utilized for dairy cattle at the end of the lactation if intercropped with red clover. With its high NDF concentration and low CP concentration, the crop residue from a Kernza grain crop could be fed to non-lactating beef cows given proper supplementation; research is needed to evaluate this alternative. Another potential use for the crop residue consists in replacing straw in high-starch dairy diets to maintain proper rumen function and prevent acidosis (Mazzenga et al., 2009; Li et al., 2014). Bedding and cellulosic biofuel are two other options that deserve attention.

5. Conclusion

Intercropping red clover with Kernza intermediate wheatgrass consistently increased the nutritive value of the summer and fall forage of a first-year stand and tripled the amount of available forage in the fall, following grain harvest. Kernza intermediate wheatgrass forage nutritive value is similar to other cool-season grasses commonly grown in the humid climate of the Upper Midwestern US. The nutritive value of Kernza intermediate wheatgrass harvested in the spring and fall makes it suitable for lactating beef cows, dairy cows and growing heifers. Kernza crop residue can potentially be fed to dry cows mixed with other higher value forages into mixed rations, which should be investigated in the future. These results indicate that utilizing forage and crop residue from Kernza intermediate wheatgrass can greatly increase the profitability of this new crop by increasing the productivity of the stand without increasing inputs, and therefore show very positive perspectives to be managed for dual-use. More research is however needed to determine the impacts of forage harvest on grain yields, forage yields, and stand longevity. The trade-off between forage and grain production in stands intercropped with red clover mixture must be assessed over multiple years and other forage legumes deserve to be tested to assess their compatibility with Kernza intermediate wheatgrass managed for dual-use.

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Declaration of Competing Interest

None.

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