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Spring Yield and Silage Characteristics of Kura Clover, Winter Wheat, and in Mixtures

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ABSTRACT

Kura clover (*Trifolium ambiguum* M. Bieb.) grown in monoculture is difficult to harvest mechanically because of its decumbent growth habit, and legumes in general have poor ensiling characteristics. Our objectives were to assess forage yield, nutritive value, and silage characteristics of kura clover–winter wheat (*Triticum aestivum* L.) mixtures to determine usefulness as a silage crop. 'Cardinal' winter wheat was sown in monoculture and into an 'Endura' kura clover sward in autumn at two locations and in 2 yr, and harvested at wheat boot and milk stages the following spring. Sole kura clover was harvested at the same time as winter wheat and mixtures. In addition, two mixtures of kura clover with winter wheat were created at each harvest by blending desired proportions from crops grown in monoculture. The mixture of kura clover with winter wheat yielded 7.4 Mg dry matter (DM) ha^{-1} while sole kura clover yielded 3.4 Mg DM ha^{-1} and sole winter wheat 9.05 Mg DM ha^{-1}. However, kura clover yield in the binary mixture was similar to sole kura clover. Neutral detergent fiber and acid detergent fiber concentrations were lower in kura clover than winter wheat and intermediate in mixtures. Fermented mixtures had similar pH to sole winter wheat (pH 3.8) and lower than sole kura clover (pH 4.1). The proportion of total N recovered as nonprotein N in silage was 9% lower in kura clover and in mixtures than in winter wheat. Water-soluble carbohydrate (WSC) concentrations of wilted forage were higher in mixtures than sole kura clover (157 vs. 105 g kg^{-1} DM), and mixtures had lower WSC than sole winter wheat (198 g kg^{-1} DM). Lactate concentration was 13% higher in silage from mixtures than sole kura clover. Spring forage production of kura clover–winter wheat mixtures was greater than sole kura clover, possessed adequate nutritive value for high-producing livestock, and mixture silage characteristics were better than sole kura clover.

PRESERVING forage legume nutritive value as hay or silage is a challenge. Silage has the advantage of reducing weather risks and its adverse effects on the nutritional value of forage (Albrecht and Beauchemin, 2003). However, the ensiling of forage legumes often results in rapid and extensive degradation of proteins before and during fermentation (Albrecht and Muck, 1991). Legumes like alfalfa (*Medicago sativa* L.) and white clover (*Trifolium repens* L.) have high protein content, high buffering capacity, and low levels of WSC, making them susceptible to extensive proteolysis during fermentation. Some strategies reported to decrease proteolysis in legumes are ensiling at high DM concentration (Muck, 1987), increasing the level of soluble sugar in the plant by harvesting later in the day when plants have higher levels of WSC (Owens et al., 1999), applying organic acids to decrease pH faster (Davies et al., 1998), or rapid silo filling, good packing, and good sealing to minimize heating and maximize pH decline (Muck, 1987). Reduction of proteolysis in legume ensilage is important because it maintains the nutritional value of proteins, improving the value of the ration for dairy cows (*Bos taurus*) (Broderick, 1995).

Cereals have been studied widely for forage production and preservation as silage. Winter small grains harvested in early June at early heading stage can yield from 7.0 to 8.9 Mg DM ha^{-1} (Maloney et al., 1999). Silage quality of oat (*Avena sativa* L.), wheat, and barley (*Hordeum vulgare* L.), ensiled at milk and dough stages was reported acceptable after fermentation. The pH ranged from 3.8 to 4.1, and WSC were higher in milk-stage than dough-stage silages. Moreover, wheat had a lower buffering capacity and higher WSC content than oat and barley (Bergen et al., 1991).

Because of the difference in WSC concentration between grasses and legumes, research has been conducted to evaluate blends as a way to drop pH quickly and to reduce proteolysis. Mixing ryegrass (*Lolium multiflorum* Lam.) (250 g WSC kg^{-1} DM) with white clover (66 g WSC kg^{-1} DM) decreased silage pH by 2.21 units and increased lactic acid by 250% compared to sole white clover silage which had pH 5.75 and lactic acid concentration of 54 g kg^{-1} DM (Davies et al., 1998). In mixtures of red clover (*T. pratense* L.) and timothy (*Phleum pratense* L.), WSC concentration increased as the proportion of timothy increased, but because of the large amount of silage preservative (formic and orthophosphoric acids) used, pH was similar among treatments (Syrjala-qvist et al., 1984). These reports establish that mixing grass or cereals with a legume could increase WSC concentration, drop the pH faster, diminish proteolysis, and enhance the nutritive value of the silage.

Kura clover is gaining popularity as forage in the northern USA because of excellent persistence. Its nutritive value is greater than that of alfalfa and red clover (Allinson et al., 1985; Sheaffer and Marten, 1991), making it a potentially useful forage crop for dairy cows. While kura clover forage nutritive value changes little with maturity, its nutritive value must be preserved throughout storage. Ensiling is a feasible method to retain kura clover nutritional value. Recent research suggests kura clover ensiles well with a final pH of 4.08 and little effect of fermentation on forage quality (Seguin and Mustafa, 2003), but like alfalfa, low water-

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Abbreviations: DM, dry matter; KC, kura clover; NPN, nonprotein nitrogen; TCA, trichloroacetic acid; TN, total nitrogen; WSC, water-soluble carbohydrates; WW, winter wheat.

soluble carbohydrates and buffering capacity may induce significant proteolysis in kura clover before and during fermentation, resulting in high losses of protein through NPN formation. Kura clover has been successfully grown in mixture with winter grains (Contreras-Govea and Albrecht, 2005); however, nothing is known about silage characteristics of these mixtures. It is expected that mixtures of winter wheat with kura clover will have less silage NPN than sole kura clover because the high WSC in wheat will contribute to a more rapid drop in silage pH. The objectives of this study were to assess forage yield and nutritive value of kura clover–winter wheat mixtures, evaluate kura clover silage characteristics and determine if mixing with winter wheat improves its usefulness as a silage crop.

MATERIALS AND METHODS

The experiment was conducted at the University of Wisconsin Agricultural Research Stations at Arlington (43°18′ N, 89°21′ W) on Plano silt loam soils (fine-silty, mixed, mesic Typic Argiudolls) with pH 6.3 and 2.8% organic matter, and at Lancaster (42°50′ N, 90°47′ W) on Rozetta silt loam soils (fine-silty, mixed, mesic Typic Hapludalfs) with pH 6.9 and 2.5% organic matter, in 2000 and 2001.

Field Procedures

Cardinal winter wheat was sown in monoculture or into an established Endura kura clover sward at a seeding rate of 100 kg ha^{-1}. Before winter wheat was sown, four 36-m^2 sites were created for winter wheat monoculture. Kura clover in these areas was killed with clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) (0.094 kg a.e. ha^{-1}) plus glyphosate [*N*-(phosphonomethyl)glycine] (2.64 kg a.i. ha^{-1}). A seven-row no-till drill was used to plant winter wheat in late August (21 and 22) in 2000 and mid-September (12 and 14) in 2001. The entire kura clover sward was harvested to an 8-cm stubble height before wheat establishment in sole or intercrop treatments. Soil fertilization with P and K was based on soil test recommendations for alfalfa (Kelling et al., 1991). In 2001, 60 kg ha^{-1} N was applied at each location in the sole winter wheat treatment before sowing. Field treatments in 2000 consisted of: (i) sole winter wheat harvested at boot stage (WW-boot); (ii) sole kura clover harvested at the time of winter wheat at boot stage when kura clover was vegetative (KC-veg); (iii) sole winter wheat harvested at milk stage of kernel development (WW-milk), and (iv) sole kura clover harvested at the time of winter wheat at milk stage when kura clover was blooming (KC-bloom). All forages were harvested to leave an 8-cm stubble. The first year wet soil conditions precluded machine harvest for forage yield determination. Samples for ensiling and nutritive value analysis were hand harvested. Field treatments for 2001 were the same, but with two additional field mixtures of kura clover and winter wheat harvested at boot (Mix-boot) and milk stage (Mix-milk).

Harvest

Approximately 6 kg each of fresh kura clover and winter wheat were harvested for ensiling both years in late May (22–28) at wheat boot stage and in mid-June (11–24) at wheat milk stage. At the time of winter wheat harvest at boot and milk stages, kura clover was at vegetative and early bloom stages, respectively. In 2001, a 4.6-m^2 area was harvested for yield determination at each location from every plot with a flail mower on the same days that samples were collected for silage. A 500-g subsample was taken for DM determination. In addition, two 0.09-m^2 quadrates were hand clipped from binary mixtures to determine botanical composition.

Ensiling

In addition to the field treatments, four mixtures were created by blending winter wheat and kura clover grown in monoculture. These mixtures, blended on a DM basis were: 2/3 KC-veg + 1/3 WW-boot; 1/3 KC-veg + 2/3 WW-boot; 2/3 KC-bloom + 1/3 WW-milk and 1/3 KC-bloom + 2/3 WW-milk. A total of eight silage treatments were assessed the first year and 10 silage treatments the second year.

All fresh plant material was wilted in a forced air oven at 30°C to approximately 350 g kg^{-1} DM before processing. Wilting time ranged from 1 to 6 h, depending on moisture concentration of the forage. Once the target DM concentration was reached for each crop, forage was chopped with a small-scale stationary chopper, designed to chop plant material to particle size of approximately 1.0 cm, and the mixture treatments were made. After wilting, two 50-g samples from each treatment were collected and frozen at −20°C in sealed plastic bags for later analysis of pH, NPN, and sugar concentration. In addition, a 150-g sample was dried in a forced-air oven at 60°C for DM determination. This sample was ground to pass a 1.0-mm screen and used for neutral detergent fiber (NDF), acid detergent fiber (ADF), and total nitrogen (TN) determinations. Two 0.5-L glass jar mini-silos were used to ensile each treatment, following a method described by Muck (1987). About 250 g of chopped plant material was added to each jar after mixing in 2.5 mL of distilled water containing a minimum of 1×10^7 lactic acid bacteria (*Lactobacillus plantarum* and *Enterococcus faecium*; Pioneer Hi-Bred International, Inc., Johnston, IA). The glass jar was sealed with a rubber-lined lid and then stored at 25°C. After 100 d of fermentation, jars were frozen at −20°C until further analyses were performed.

Analyses of Wilted and Ensiled Forage

Forage pH was determined by placing a 20-g sample of wilted or ensiled frozen forage in a blender jar, diluting with deionized distilled water to 200 g, and blending for 30 s in a high-speed blender. The diluted sample was filtered through three layers of cheesecloth, and the pH was measured with a pH meter. Twenty-milliliter aliquots were taken from the ensiled and wilted forages and dispensed into separate 50-mL polypropylene centrifuge tubes. Five milliliters of 25% (w/v) trichloroacetic acid (TCA) were added to tubes containing wilted and ensiled samples, and allowed to stand for 1 h at room temperature to precipitate the protein from the solution. Tubes were centrifuged at 13000 × *g* for 20 min and the supernatant was decanted into 20-mL scintillation vials and stored at −20°C. The solutions with TCA were used for NPN determination with a Mitsubishi total N analyzer Model TN-05, equipped with auto-sampler model ASC-11 (Mitsubishi, Chemical Co. Tokyo, Japan). Oven-dried samples were analyzed for TN by rapid combustion (850°C) (LECO Model FP-528; LECO Corp., St. Joseph, MI).

A second 20-mL aliquot from ensiled forage was analyzed for organic acids. Fermentation products (succinate, lactate, acetate, propionate, butyrate, and ethanol) were determined using high performance liquid chromatography (Muck and Dickerson, 1988). The HPLC system consisted of a Shimadzu system controller (SCL-6A), pump (LC-6A), refractive index detector (RID-6A), and chromatopac (C-R6A) (Shimadzu Corp., Kyoto, Japan) with a Bio-Rad Aminex HPX-87H column (Bio-Rad Lab., Hercules, CA) heated to 42°C with a Fiatron TC-50 temperature controller (Fiatron Laboratory Systems, Oconomowoc, WI).

Wilted and ensiled samples were lyophilized before sugar analysis by a modification of the procedure developed by Li et al. (1996) using fructose as a standard. Anthrone reagent was added to an aliquot of water extract containing approximately 200-μg WSC, vortexed, boiled for 8 min, and after cooling light absorbance was read at 625 nm. Total sugars were expressed as fructose equivalents.

Neutral detergent fiber and ADF were determined by the batch procedures outlined by ANKOM Technology Corp. (Fairport, NY).

Statistical Analysis

Forage yield was analyzed as a randomized complete block design. Wilted and ensiled characteristics were analyzed as a completely randomized design with four replications. Because of the additional two treatments in 2001, years were analyzed individually. Location (Arlington and Lancaster) and the location × treatment interaction were included in the model. Analysis of variance was conducted to test statistical differences among treatments using the GLM Procedure of SAS (SAS Institute, 2001). Few environment × treatment interactions were detected, and interactions that were detected were small and not meaningful; therefore, data were analyzed across locations in both years. When the treatment effect was significant, means were separated using Fisher's protected LSD ($P = 0.05$).

RESULTS AND DISCUSSION

Forage Yield and Botanical Composition

Sole kura clover yielded 3.1 Mg DM ha^{-1} at the vegetative stage and 3.7 Mg DM ha^{-1} at bloom stage (Fig. 1), but these were not different ($P > 0.05$). Yield of the mixture of kura clover-winter wheat at milk stage (8.5 Mg DM ha^{-1}) was greater than the mixture at boot stage (6.3 Mg DM ha^{-1}) ($P < 0.05$) and both mixtures yielded more than sole kura clover ($P < 0.05$). Kura clover yield in the mixture (3.0–3.3 Mg DM ha^{-1}) was similar to sole kura clover yield, indicating that kura clover yield in mixtures was not affected by intercropping. Therefore, the additional yield of the mixture was the contribution of intercropped winter wheat. In contrast, when in mixture, winter wheat forage yield decreased by about 50% compared to sole winter wheat. Sole winter wheat harvested at milk stage had a DM yield of 10.7 Mg ha^{-1}, greater than kura clover and mixtures, while winter wheat at boot stage had a DM yield of 7.4 Mg ha^{-1}. Peterson et al. (1994) reported that kura clover harvested three times in a year produced 3.0 Mg DM ha^{-1} in each of the first two harvests and Edmisten et al. (1998) reported similar winter wheat forage yields when harvesting at maturity stages similar to ours. Overall, forage yields of kura clover–winter wheat mixtures were more than double those of sole kura clover.

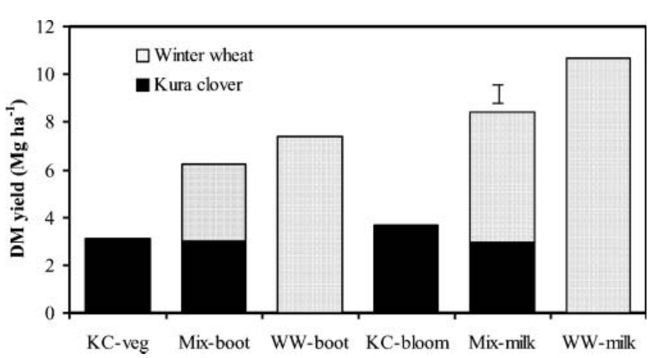

Fig. 1. Dry matter (DM) yield of winter wheat (WW) and kura clover (KC) and mixtures harvested at two maturity stages. Values are pooled means over two environments in 2001. Vertical bar represents LSD ($P = 0.05$) for comparing total yields.

Characteristics of Wilted and Ensiled Forage

The NDF and ADF concentrations of ensiled forage were generally 1 to 10% greater than those of wilted forage (Table 1). These increases in NDF and ADF concentrations of ensiled forages are associated with respiration and fermentation losses (McDonald et al., 1991). Averaged over maturity stages, ensiled kura clover had 49% less NDF and 28% lower ADF concentration than ensiled winter wheat (Table 1). In mixtures, the proportion of wheat affected NDF and ADF concentrations. Averaged over maturity stages, mixtures with 2/3 kura clover had 17% less NDF and 8% less ADF than mixtures with 1/3 kura clover. The two field mixture treatments harvested at the wheat boot (49% clover) or milk (44% clover) stage had similar NDF and ADF concentrations to those in the mixtures with 1/3 kura clover. Although monoculture winter wheat was the greatest yielding treatment evaluated, the nutritive value of mixtures was much greater than sole winter wheat, especially at the later stage of maturity.

Although DM concentrations of wilted forage differed among treatments ($P < 0.05$) (Tables 2 and 3), they were within the range recommended for silage (Muck, 1987). Dry matter differences between wilted and ensiled forage could be attributed to respiration losses as reported by Owens et al. (1999). One practical application of harvesting the mixture of kura clover with winter wheat would be a decrease in wilting time compared with sole kura clover, which could reduce the risk of losses caused by rain or other environmental factors that affect the nutritive value of the forage to be ensiled.

Wilted sole kura clover had lower pH than sole winter wheat ($P < 0.05$) with no differences between maturity stages (Tables 2 and 3). The proportion of kura clover and winter wheat in mixtures had an effect on pH. When kura clover made up 2/3 of mixtures, pH was lower than in mixtures with 1/3 kura clover; most of these cases being statistically significant ($P < 0.05$). In 2001 the field mixture at winter wheat boot stage had lower pH than that mixture at winter wheat milk stage (5.85 vs. 6.11), probably a consequence of differences in proportion of winter wheat or maturity of winter wheat (Fig. 1).

The pH of ensiled forage was from 1.52 to 2.61 units below that of wilted forage, and it was lower in winter wheat than kura clover ($P < 0.05$) (Tables 2 and 3). In ensiled sole kura clover the pH ranged from 4.02 to 4.26, similar to that reported for kura clover by Seguin and Mustafa (2003), while in ensiled sole winter wheat the pH

Table 1. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) of wilted and ensiled winter wheat (WW) harvested at two maturity stages, and kura clover (KC) in monoculture and in mixture with WW. Values are pooled means over two locations in 2000 and 2001.

	2000				2001			
	Wilted		Ensiled		Wilted		Ensiled	
Treatment	NDF	ADF	NDF	ADF	NDF	ADF	NDF	ADF
	g kg^{-1} DM							
KC-veg	253	204	264	221	229	170	221	180
WW-boot	542	293	542	318	424	249	488	278
KC + 1/3 WW-boot	345	240	357	254	352	218	320	218
KC + 2/3 WW-boot	437	265	453	287	359	221	415	252
Mix-boot	–	–	–	–	333	212	353	236
KC-bloom	302	244	306	252	282	234	301	247
WW-milk	479	270	523	308	557	320	597	349
KC + 1/3 WW-milk	363	257	386	278	391	261	392	279
KC + 2/3 WW-milk	436	262	452	285	464	285	505	319
Mix-milk	–	–	–	–	490	309	515	337
LSD†	22	16	21	7	41	16	14	7

† Fisher's protected LSD (0.05) for comparing any two treatment means within a column.

ranged from 3.63 to 3.90. The effect of maturity stage on pH of sole kura clover and sole winter wheat silage was inconsistent. In 2000 vegetative kura clover pH was lower than flowering kura clover (4.02 vs. 4.26) ($P <$ 0.05) with no differences between winter wheat at boot and milk stages (3.64 vs. 3.63). But, in 2001 vegetative and flowering kura clover were not different (4.21 vs. 4.16) ($P > 0.05$), while boot-stage winter wheat was greater than milk-stage winter wheat (3.90 vs. 3.67) ($P <$ 0.05). The pH of mixtures of kura clover-winter wheat silage tended to be lower when winter wheat made up a greater proportion of the mixture. Probably the lower pH of mixtures was associated with lower TN and greater WSC concentration of the winter wheat. In 2001 the mixture Mix-boot had greater pH than Mix-milk (3.92 vs. 3.81) ($P < 0.05$) probably a result of a lower proportion of winter wheat at boot than milk stage (Fig. 1). Syrjala-qvist et al. (1984) reported a trend for lower pH (3.86 vs. 3.98) in red clover-timothy than sole red clover, and Cussen et al. (1995) reported a slight decrease in pH (3.60 vs. 3.52) when the proportion of ryegrass in mixture with white clover increased.

In mixtures with greater kura clover proportions, TN of wilted forage ranged from 28 to 39 g kg^{-1} DM (Tables 2 and 3). Conversely with lower kura clover proportions, TN ranged from 24 to 34 g kg^{-1} DM. A similar trend in TN concentration of mixtures was observed in ensiled forage. When the proportion of kura clover was greater, TN ranged from 37 to 41 g kg^{-1} DM compared to when the kura clover proportion was lower, where TN ranged from 23 to 34 g kg^{-1}. In 2001 the TN of the wilted and ensiled forage of field mixtures at both maturities was intermediate to those of sole kura clover and sole winter wheat. The field mixture harvested when wheat was in the milk stage had a greater proportion of grass and lower TN than when harvested wheat was at the boot stage ($P < 0.05$).

Nonprotein Nitrogen Concentration in Wilted and Ensiled Forage

Differences in NPN of wilted and ensiled forage (on a TN basis) were inconsistent among treatments and between years (Tables 2 and 3). This inconsistency is not surprising because several factors, including DM concentration, buffering capacity, WSC concentration, and harvest time have been shown to affect NPN formation (Muck, 1987). Ohshima and McDonald (1978) stated that plant enzymes increase NPN during wilting and ensiling periods. Papadopoulos and McKersie (1983) found that NPN formation was greater in legumes than grasses and different between first and second cut, with no clear indication of the factors that had the greatest impact on proteolysis. McKersie (1985) reported that

Table 2. Dry matter (DM), pH, total nitrogen (TN), nonprotein nitrogen (NPN), and water-soluble carbohydrates (WSC) of wilted, and ensiled winter wheat (WW) harvested at two maturity stages, and kura clover (KC) in monoculture and in mixture with WW. Values are pooled means over two locations in 2000.

	Wilted					Ensiled				
Treatment	DM	TN	WSC†	NPN	pH	DM	TN	WSC	NPN	pH
	g kg^{-1} DM			g kg^{-1} TN		g kg^{-1} DM			g kg^{-1} TN	
KC-veg	331	43	124	249	5.66	284	45	27	754	4.02
WW-boot	331	23	192	220	6.10	271	21	106	893	3.64
KC + 1/3 WW-boot	341	28	152	254	5.65	272	37	37	667	3.87
KC + 2/3 WW-boot	355	24	180	251	5.77	277	29	65	629	3.75
KC-bloom	308	37	97	366	5.69	286	42	13	544	4.26
WW-milk	360	17	236	237	6.15	362	14	181	605	3.63
KC + 1/3 WW-milk	308	31	137	275	5.65	304	32	49	555	3.88
KC + 2/3 WW-milk	335	26	200	250	5.81	304	23	113	596	3.77
LSD‡	26	6	19	77	0.06	19	1	10	117	0.05

† Water-soluble carbohydrates expressed on a fructose equivalent basis.
‡ Fisher's protected LSD (0.05) for comparing any two treatment means within a column.

Table 3. Dry matter (DM), pH, total nitrogen (TN), nonprotein nitrogen (NPN), and water-soluble carbohydrates (WSC) of wilted and ensiled winter wheat (WW) harvested at two maturity stages, and kura clover (KC) in monoculture and in mixture with WW. Values are pooled means over two locations in 2001.

	Wilted					Ensiled				
Treatment	DM	TN	WSC†	NPN	pH	DM	TN	WSC	NPN	pH
	$g\ kg^{-1}$ DM			$g\ kg^{-1}$ TN		$g\ kg^{-1}$ DM			$g\ kg^{-1}$ TN	
KC-veg	299	45	103	345	5.73	312	47	15	533	4.21
WW-boot	346	29	203	326	6.28	349	29	77	604	3.90
KC + 1/3 WW-boot	319	39	140	301	5.93	316	41	24	545	3.99
KC + 2/3 WW-boot	335	34	169	291	5.91	295	34	55	549	4.05
Mix-boot	301	34	185	252	5.85	295	35	58	613	3.92
KC-bloom	263	43	93	332	5.78	249	45	11	504	4.16
WW-milk	285	20	162	267	6.32	279	21	59	576	3.67
KC + 1/3 WW-milk	263	37	130	227	5.89	259	37	16	496	3.86
KC + 2/3 WW-milk	271	29	145	233	6.06	260	28	36	624	3.80
Mix-milk	278	23	166	304	6.11	270	24	47	578	3.81
LSD‡	27	2	17	81	0.07	27	2	16	84	0.07

† Water-soluble carbohydrates expressed in a fructose equivalent basis.
‡ Fisher's protected LSD (0.05) for comparing any two treatment means within a column.

proteolysis was strongly dependent on two factors: the rate of pH drop and proteinase activity in the plant that could be affected by the growth environment and crop management. In our study, NPN $g\ kg^{-1}$ TN tended to be greater in kura clover than in winter wheat after the wilting period; but after ensiling, NPN was 13% greater in sole winter wheat than sole kura clover. Nonprotein N $g\ kg^{-1}$ TN in mixtures was 3 to 10% greater than in sole kura clover silage, except in 2000 when the mixture at boot stage had 17% lower NPN than vegetative kura clover. Our expectation was that mixing winter wheat with kura clover would contribute to silage with lower NPN $g\ kg^{-1}$ TN than sole kura clover because addition of winter wheat would reduce the pH more rapidly. Although the pH was lower in mixtures than in sole kura clover silages, apparently the rate of pH drop was not rapid enough to markedly reduce proteolysis, as suggested by McKersie (1985). Although the fraction of TN in the form of NPN tended to be greater in winter wheat and mixture silage than in kura clover silage, the absolute amount of NPN expressed on a DM basis was the greatest in sole kura clover silage. The NPN concentration on a TN basis of sole kura clover silage was similar to previous reports for alfalfa but greater than red clover (Owens et al., 1999), while the NPN concentration of sole winter wheat was similar to NPN of wheat, oat, and barley ensiled at milk and dough stages in earlier research (Bergen et al., 1991).

Water-Soluble Carbohydrate Concentrations in Wilted and Ensiled Forage

Water-soluble carbohydrate concentration of wilted forage, averaged over all treatments and both years, was always greater in winter wheat than in kura clover (Tables 2 and 3). The WSC concentration of sole kura clover was in the range reported for red clover and alfalfa (42–127 $g\ kg^{-1}$ DM) (Owens et al., 1999), while the WSC of sole winter wheat was similar to that reported for wheat at milk stage (189 $g\ kg^{-1}$ DM) (Bergen et al., 1991).

In wilted forage, the proportion of kura clover and winter wheat had an effect on the concentration of WSC of mixtures (in most cases $P < 0.05$). When kura clover made up two-thirds of the mixture, WSC ranged from 130 to 152 $g\ kg^{-1}$ DM. When kura clover made up one-third of the mixture, WSC ranged from 145 to 200 $g\ kg^{-1}$ DM. In 2001, the WSC concentrations of the field mixture treatments Mix-boot and Mix-milk were similar to mixtures with one-third kura clover. Other studies have also reported that WSC concentration decreased when the proportion of the legume increased in the mixture (Syrjala-qvist et al., 1984; Cussen et al., 1995). Overall, mixing kura clover with winter wheat increased the concentration of WSC compared to sole kura clover. Although a greater WSC concentration of the mixtures resulted in lower silage pH than sole kura clover, it did not result in lower NPN formation as would be expected.

The unfermented or residual WSC concentrations in sole kura clover silage were lower than residual WSC in sole winter wheat silage ($P < 0.05$) (Tables 2 and 3). Likewise, the proportion of each species affected WSC in the mixtures. Mixtures with two-thirds kura clover had lower WSC concentrations than mixtures with one-third kura clover ($P < 0.05$). Residual WSC concentrations found in sole kura clover, sole winter wheat, and mixture silages are similar to those reported by Owens et al. (1999) in alfalfa (7–15 $g\ kg^{-1}$ DM), Bergen et al. (1991) in wheat (114 $g\ kg^{-1}$ DM), and Cussen et al. (1995) in mixture of ryegrass-white clover (33–57 $g\ kg^{-1}$). During wilting and fermentation a greater proportion of initial WSC was consumed in sole kura clover (78–88%) compared to sole winter wheat (23–62%). Probably the greater buffering capacity of sole kura clover compared to winter wheat resulted in longer fermentation and thus a greater utilization of WSC compared with winter wheat. Likewise, in the mixtures of kura clover with winter wheat, a greater proportion of kura clover in the mixture resulted in greater utilization of WSC (64–88%) than in mixtures with a low proportion of kura clover (44–75%). Similar utilization of WSC was reported by Owens et al. (1999) in red clover and alfalfa (73–94%), Bergen et al. (1991) in winter wheat (40%), and Cussen et al. (1995) in mixtures of ryegrass with white clover (64–74%). The data show that pH stabilized at a lower level and more WSC was consumed during fermentation in the mixtures compared to sole kura clover. However, silage made from mixtures had greater amounts of residual WSC potentially available for rumen fermentation than silage made from sole kura clover.

Fermentation Products of Ensiled Forage

Propionate and butyrate were not detected in this study, indicating minimal clostridial fermentation (McDonald et al., 1991). Ethanol concentration in all treatments was low and not biologically significant. The concentration of lactate in ensiled forage was greater in sole kura clover than in sole winter wheat in 2000 ($P < 0.05$) but not in 2001 ($P > 0.05$) (Tables 4 and 5). Sole kura clover lactate concentrations were greater than those reported by Seguin and Mustafa (2003), likely due to greater WSC concentrations and thus extended fermentation. The proportion of kura clover in mixtures had an effect on the concentration of lactate. In 2000, mixtures with two-thirds kura clover contained a lactate concentration of 129 g kg^{-1} DM, while lactate in mixtures with one-third kura clover contained 106 g kg^{-1} DM ($P < 0.05$) (Table 4). In 2001, the proportion of kura clover in mixtures had no effect on lactate concentration ($P > 0.05$) (Table 5). Dissimilarities in forage composition between years could explain differences in lactate concentrations: WSC concentrations tended to be lower in 2001 than 2000, and the pH of wilted forages tended to be greater in 2001 than in 2000. Substrate limitation may have led to lower net lactate formation in 2001, with some of this lactate used to produce acetate and succinate (Lindgren et al., 1990).

There was a tendency for greater lactate concentrations in mixtures compared to sole kura clover or winter wheat silages (Tables 4 and 5). The higher pH in sole kura clover compared to mixture silages (Tables 2 and 3) suggests that sole kura clover fermentation was limited by substrate, so mixtures logically would contain more fermentation products. Winter wheat would be expected to have lower buffering capacity than kura clover, based on the literature (McDonald et al., 1991), and consequently less acid would be required to get to the same pH in wheat than kura clover silage. Thus the mixtures would require more acid to get to the same pH as sole wheat. Because the mixtures had similar or slightly higher pH compared to sole wheat, greater lactate in mixtures would be expected and was observed.

Table 4. Concentration of organic acids in silage made from winter wheat (WW), kura clover (KC), and mixtures at two maturity stages. Values are pooled means over two locations in 2000.

Treatment	Succinate	Lactate	Acetate	Ethanol
	g kg^{-1} DM			
KC-veg	1.6	110	8.9	2.3
WW-boot	1.0	90	3.6	4.3
KC + 1/3 WW-boot	1.6	125	9.6	3.1
KC + 2/3 WW-boot	1.3	108	7.5	3.5
KC-bloom	3.9	108	20.5	2.1
WW-milk	1.2	77	3.9	4.1
KC + 1/3 WW-milk	2.8	133	11.9	3.0
KC + 2/3 WW-milk	1.9	104	7.5	4.2
LSD†	0.5	10	2.0	0.6

† Fisher's protected LSD (0.05) for comparing treatment means within columns.

Table 5. Concentration of organic acids in silage made from winter wheat (WW), kura clover (KC), and mixtures at two maturity stages. Values are pooled means over two locations in 2001.

Treatment	Succinate	Lactate	Acetate	Ethanol
	g kg^{-1} DM			
KC-veg	4.9	101	18.4	2.3
WW-boot	2.5	96	5.5	4.5
KC + 1/3 WW-boot	3.6	117	11.6	3.9
KC + 2/3 WW-boot	2.8	111	7.8	5.2
Mix-boot	2.6	124	9.4	3.7
KC-bloom	3.5	93	19.2	3.9
WW-milk	3.0	98	7.8	5.1
KC + 1/3 WW-milk	3.2	110	13.2	4.7
KC + 2/3 WW-milk	3.1	114	10.4	5.0
Mix-milk	2.9	120	9.9	4.3
LSD†	0.6	9	1.7	0.8

† Fisher's protected LSD (0.05) for comparing treatment means within columns.

Acetate and succinate concentrations demonstrated patterns among treatments that were similar to lactate (Tables 4 and 5). Acetate and succinate concentrations were greater in sole kura clover silage than in sole winter wheat ($P < 0.05$). There was a trend that mixtures with two-thirds kura clover had greater acetate and succinate concentrations than mixtures with one-third kura clover. Likewise, acetate and succinate concentrations tended to be lower in mixtures than in sole kura clover. These results agree with those reported by Cussen et al. (1995), who found acetate concentration was greater in a mixture of red clover-ryegrass compared to sole ryegrass silage.

A possible explanation for the greater concentration of acetate and succinate in kura clover silage is that kura clover had lower WSC concentrations and most likely contained greater concentrations of organic acids than did winter wheat (McDonald et al., 1991). Under substrate limitation, lactic acid bacteria could metabolize lactate to produce acetate and succinate (Lindgren et al., 1990) and increase acetate and succinate in kura clover relative to winter wheat silage. Greater acetate and succinate concentrations in silage made from flowering kura clover than from vegetative kura clover indicates that substrate limitation was greater in flowering clover. Acetate and succinate concentrations in sole kura clover silage were lower than those reported for alfalfa (Owens et al., 1999), which had lower WSC than kura clover. Finally, the mixtures of kura clover with winter wheat had greater WSC and lower concentrations of acetate and succinate than sole kura clover.

CONCLUSIONS

Spring forage yield of kura clover–winter wheat mixtures was more than double that of sole kura clover. These data demonstrate that winter wheat can be sown into established kura clover in early autumn and harvested the following spring with greater forage yield than sole kura clover. Although mixtures contained less crude protein and more fiber than sole kura clover, nutritive value was suitable for inclusion in rations for high producing dairy cattle. In addition, silage characteristics were enhanced in the mixtures of kura clover with winter wheat compared to sole kura clover. Overall, sole kura clover, sole winter wheat, and mixtures made excellent silage, but silage made from mixtures had lower pH (3.90 vs. 4.14), 50% greater WSC concentration, and 13% greater lactate concentrations than sole kura clover. The expected decrease in proteolysis associated with greater fermentable substrate in mixtures compared to sole kura clover was not observed. Our experience has demonstrated that sole kura clover is difficult to harvest for hay or silage with farm-scale equipment because of a somewhat decumbent growth habit. Kura clover grown with winter wheat stands more upright, allowing improved harvest efficiency. When sole kura clover is grown for use as a living mulch for corn silage production (Zemenchik et al., 2000; Affeldt et al., 2004), sowing winter wheat into kura clover after corn silage removal is an attractive option for production of early season, high nutritive value silage the following year.

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