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Performance and Economic Evaluation of Low-Lignin Alfalfa 'Hi-Gest® 360' in Saskatchewan Canada

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Abstract: A three-year (2018–2020) study was conducted in Lanigan (Thin Black soil zone) and Saskatoon (Dark Brown soil zone), SK Canada to compare low-lignin alfalfa (*Medicago sativa*) cv. Hi-Gest® 360 (HiGest) with alfalfa- cv. AC Grazeland (Grazeland) in monoculture and binary mixtures with hybrid bromegrass (HBG; cv. AC Success) for forage yield, nutrient profile, and establishment costs. Field plots were seeded in August 2017. Stands were harvested at three maturity stages of alfalfa (1 = 10% bloom; 2 = 40% bloom; and 3 = 100% bloom). Compared to Grazeland, HiGest alfalfa yielded 25% less in Saskatoon in monoculture, but no difference was found in Lanigan. Averaged across two sites, HiGest had 8.6% less acid detergent lignin (6.51 vs. 5.95%) and 10.3% greater in vitro neutral detergent fiber digestibility (42.7 vs. 38.7% of neutral detergent fiber). In the binary mixtures, however, differences in yield and quality between the two alfalfas were negligible. The stand establishment costs averaged 300 Canadian dollar (CAD) ha^{-1}, 205 CAD ha^{-1}, 260 CAD ha^{-1}, and 303 CAD ha^{-1} for HiGest, Grazeland, Grazeland-HBG, and HiGest-HBG, respectively. Hi-Gest® 360 alfalfa could provide higher digestible nutrients when it was used as a monoculture and could maintain quality better into later maturing stages. However, the higher seed price, and no actual advantage in mixtures may delay its adoption.

Keywords: binary mixtures; low-lignin alfalfa; maturity; nutritive value

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1. Introduction

For the past several decades, plant breeders and geneticists have focused on reducing the overall lignin concentration in forage crops as a means of improving its nutritive value as the plant matures. While it is essential for normal plant growth, the deposition of lignin into plant cell walls can reduce the feeding value of alfalfa by negatively affecting rumen microbial degradation and the digestion of feed by intestinal enzymes [1]. Lignin is a complex structural polymer that is the second most abundant component of secondary plant cells walls [2], providing the strength and rigidity necessary for the plant to stand upright [3,4]. As a plant matures, lignin concentration increases, filling the space between cellulose, hemicellulose, and pectin molecules and forming cross-linkages with hemicellulose [3,5,6].

Reduction of lignin content in alfalfa has significant values to the beef and dairy industries as alfalfa is widely used for grazing and hay production due to its high nutrient content and high productivity [7]. Recently, Alforex Seeds (Woodland, CA, USA) released a low-lignin (LL) alfalfa cultivar Hi-Gest® 360 (HiGest), which is a product of conventional plant breeding with improved fiber digestibility, intake, and extent of digestion versus other conventional alfalfas. HiGest alfalfa plants are medium-tall with a dense canopy of fine stems and large leaves with a high leaf to stem ratio. HiGest LL alfalfa is a fall dormancy 3 cultivar and has a 1.5 winter survival rating, indicating its high adaptation to the Canadian prairie with cold winters. Low-lignin alfalfa is promising for backgrounding programs because of its high digestibility, which translates into an improved animal performance. However, field evaluations under western Canadian conditions are needed to determine the performance of this cultivar containing low-lignin, especially with regard to forage accumulation and nutritive value under different harvest frequencies. The objective of this study was to determine the forage value of the new low-lignin alfalfa for beef cattle in comparison with conventional alfalfa in monocultures and in binary mixtures at differing maturity stages in two soil zones in Saskatchewan. The items assessed included: (1) forage yield and lodging tolerance; (2) nutrient profiles; and (3) establishment costs.

2. Materials and Methods

2.1. Site Selection, Experimental Design, and Stand Establishment

The study was established in 2017 at the Agriculture and Agri-Food Canada (AAFC) Saskatoon Research and Development Centre, Saskatoon (lat 52°07′ N, long 106°38′ W) with Orthic Dark Brown Chernozem soils [8]. The second site was located at the Livestock and Forage Centre of Excellence Termuende Research Ranch (lat 51°51′ N, long 105°02′ W) in Lanigan, Saskatchewan. The soil type in Lanigan is a Chernozemic Black Oxbow soil [9]. Prior to seeding, soil cores were collected at 0–30 cm depth from 10 random locations in each site, composited, and analyzed at a commercial laboratory (ALS Saskatoon) to determine initial soil nutrient requirements. Available nitrate-N, sulfate-S, phosphate-P, and potassium in the soils were 55, 27, 120, and 670 kg ha^{-1}, respectively. The results indicated that no fertilizer was required for either site, based on the Government of Saskatchewan [10]. The AC Success hybrid bromegrass and AC Grazeland alfalfa seeds were purchased from DLF Pickseed (Lindsay, ON, Canada) and the Hi-Gest alfalfa seed was purchased from Treasure State Seeds (Fairfield, MT, USA). Forty-eight plots in each site were randomly assigned to 1 of 4 replicated (n = 4) treatments: two monoculture cultivars of alfalfa, cv. AC Grazeland and Hi-Gest, and two binary mixtures of the alfalfa cultivars with AC Success HBG at 3 maturity stages of alfalfa, 10% bloom, 40% bloom, and 100% bloom. AC Grazeland alfalfa and AC Success HBG were chosen in the study as they are the most commonly seeded forages in western Canada. Sites were managed according to the recommended practices for forage management in the region [10]. At both sites, field plots were arranged in a split-plot with Randomized Complete Block Design with four blocks using forages as main plots and cutting stage as sub-plots. Plot size was 1.2 m × 6 m.

At the Saskatoon site, germination tests were conducted prior to seeding for all species to calculate pure live seed (PLS). Seeding rates were 400 PLS/m^2 for alfalfa in a monoculture, 200 PLS/m^2 for alfalfa in binary mixtures, and 167/m^2 for HBG in binary mixtures. Plots were seeded on 24 July 2017, using pull-type 2019 Wintersteiger (Wintersteiger, Salt Lake, UT, USA). The site was a summer fallow prior to the seeding. Alfalfa and AC Success HBG seeds were mixed and seeded in the same row in the binary mixtures. All plots were uniformly mowed to a stubble height of 10 cm during the establishment year on 30 September 2017. In 2017, supplemental irrigation was applied due to lack of rainfall following seeding. After seeding, weeds were controlled by using a wheel hoe between rows and hand pulling pigweeds (*Amaranthus* sp.). The plots were well-established based on visual estimation in fall 2017.

At the Lanigan site, before the trial, the plot site had grown barley in the past 2 years. Before seeding, the plots were sprayed (using a quad mounted boomless sprayer with a single nozzle with glyphosate (Roundup; Monsanto, Creve Coeur, Greater St. Louis, MO, USA) at 1.7 L ha^{-1}, 0.78 L ha^{-1}, and 0.78 L ha^{-1} application rate on 6 and 28 June, and 26 July 2017, respectively. Plots were seeded on 1 August 2017, using a 6-row Hege cone seeder (Hege Equipment Inc., Colwich, KS, USA) at row spacing of 30 cm. Seeding rates were 20.5 kg ha^{-1} and 9.5 kg ha^{-1} for HiGest and Grazeland alfalfa monocultures, respectively; 10.5 kg ha^{-1} and 4.76 kg ha^{-1} for HiGest and Grazeland alfalfa in binary mixtures, respectively; and 15.95 kg ha^{-1} for AC Success HBG in binary mixtures. In each treatment, 11.2 kg ha^{-1} of 11-50-0 (Mono-ammonium phosphate) was also included to improve seed flow. Plots were not irrigated in 2017. The dry summer followed by colder winter compared to long-term averages at Lanigan resulted in very little alfalfa growth in the spring of 2018 in both monocultures and binary mixtures. Therefore, it was necessary to re-seed the plots in the second year. The alfalfa was re-seeded by hand on 13 June 2018. Prior to reseeding the alfalfa, plots were mowed. Reseeding rates were 14.5 kg ha^{-1} and 8.9 kg ha^{-1} for HiGest and Grazeland alfalfa monocultures, respectively; and 7.2 kg ha^{-1} and 4.44 kg ha^{-1} for HiGest and Grazeland alfalfa in binary mixtures, respectively. The plots were irrigated with 76 mm of water one month after reseeding due to drought. Additionally, the plots were weeded by hand while they were watered on 9, 11, and 12 July 2018.

2.2. Weather

Monthly average precipitation and average temperature of the growing season (Table 1) were obtained from Environment Canada (www.climate.weatheroffice.ec.gc.ca, accessed on 28 April 2022). Overall, the trial years were drier compared to long-term averages (LTA; 30-year) at both sites (during the trial years, the crops received only 79 and 73% of average precipitation for Saskatoon and Lanigan, respectively) in the growing season. Overall, these data suggested that the current study was conducted in an environment with comparable temperatures but with less precipitation relative to LTA. Specifically, in Lanigan, the summer of the establishment year (2017) was extremely dry compared to the LTA; during this time of the year, the crops received only 32.8% of average precipitation in the growing season. Thus, there were less favorable growing conditions for plants during experimental periods at both sites. As a result, the soil moisture was a limiting factor at either site. Therefore, the plots were watered after seeding (at Saskatoon) or after re-seeding (at Lanigan). At Lanigan, monthly average temperatures for Jan., Feb., and March 2018, were −12.7, −17.7, and −10.3 °C, respectively (data not shown). The 30-year average temperatures for Jan., Feb., and March were −16.30, −13.2, and −6.5 °C, respectively. Thus, Feb. and March of study years were on average −4.5 and −3.9 °C colder than LTA, respectively.

Table 1. Monthly mean air temperature and rainfall in Saskatoon and Lanigan, Saskatchewan during the growing seasons over 3 years (2017 to 2020).

Month	Monthly Mean Temperature (°C)					Monthly Rainfall (mm)				
	2017	2018	2019	2020	LTA †	2017	2018	2019	2020	LTA
Saskatoon										
May	12.1	14.3	9.7	11.1	11.8	46.3	35.0	4.4	42.1	36.5
June	16.1	17.3	16.0	15.3	16.1	30.9	19.9	84.8	106.9	63.6
July	19.6	18.7	17.8	18.9	19.0	25.5	31.1	67.6	52.1	53.8
August	17.8	17.1	15.4	18.0	18.2	25.2	17.2	20.3	16.2	44.4
Mean	16.4	16.8	14.7	15.8	16.3	127.9	103.2	177.1	217.3	198.3
Lanigan										
May	11.7	14.0	8.8	–	9.9	18.0	45.1	12.6	–	37.6
June	15.6	16.8	15.2	–	15.0	27.1	49.7	95.7	–	71.9
July	18.8	17.5	17.3	–	17.7	6.2	74.0	54.9	–	52.8
August	16.9	16.5	15.3	–	16.8	18.7	18.2	48.6	–	51.1
Mean	15.8	16.2	14.1	–	14.9	70.0	187.0	211.8	–	213.4

Note. Data were obtained from Environment Canada (www.climate.weatheroffice.ec.gc.ca) for Saskatoon (Climate ID 4057165; 52°17′ N, 106°72′ W) and Lanigan (Climate ID 4057165; Watrous East, Saskatchewan: 51°67′ N, 105°40′ W). † LTA, Long-term average from 1981 to 2010; Sum for precipitation (mm).

2.3. Data Collection

2.3.1. Forage Yield and Lodging

Data were collected from all treatment subplots within the whole plots assigned to that sampling period. Harvesting dates for each site were determined based on visual evaluation of alfalfa growth stage (Table 2). Just before harvesting by maturity stage, the lodging resistance score (LS) of alfalfa was visually scored in each plot according to Gungaabayar et al. [11], using 1–9 scale where 1 = completely upright and 9 = completely lodged.

Table 2. Crop harvest and alfalfa lodging evaluation date.

Year	Stage *	Sites	
		Saskatoon	Lanigan
2018	Stage 1	21 June 2018	-
	Stage 2	25 June 2018	-
	Stage 3	29 June 2018	-
	Second harvest	23 August 2018	-
2019	Stage 1	8 July 2019	27 June 2019
	Stage 2	12 July 2019	8 July 2019
	Stage 3	19 July 2019	29 July 2019
	Second harvest	23 August 2019	-
2020	Stage 1	26 June 2020	-
	Stage 2	3 July 2020	-
	Stage 3	10 July 2020	-
	Second harvest	26 August 2020	-

Note. * Forage harvested at 3 maturity stages of alfalfa: stage 1 = alfalfa 10% bloom; Stage 2 = 40% bloom; Stage 3 = 100% bloom.

Plots were harvested on three maturity stages of alfalfa (10% bloom; 40% bloom, and 100% bloom stage) over three consecutive production years (2018, 2019, 2020) at Saskatoon and one year (2019) at Lanigan. Forage yield clipping and forage samples were not collected during the seeding year (2017) but were collected from 2018 at Saskatoon and in 2019 at Lanigan. According to the western Canadian forage industry, stage 1 is approximately 1 wk before the point where the hay would be commercially harvested, stage 2 is at the point of commercial cutting, and stage 3 is approximately 1 wk after the commercial cutting stage. In the current study, the plots were cut identically with the above cutting times at Saskatoon. However, due to the slower development of blooming of alfalfa, the stage 2 and stage 3 cuttings occurred 11 and 31 days later, respectively, than stage 1 cutting at the Lanigan site.

At Saskatoon, plots were harvested by cutting the entire plot to a height of 5 cm using a WinterSteiger-forage harvester CiBus F with a digital scale (WinterSteiger, Salt Lake, UT, USA) to determine the total weight of fresh biomass. At Lanigan, forages were harvested using a Jari Mower. Plots were 6 rows with 0.30 m spacing, 1.5 m wide, 8 m long, and trimmed to 7 m before harvest. At harvest, a sub-sample (~2 kg) was taken from each plot in 3 paper bags, weighed fresh and dried at 55 °C in a forced-air oven for 72 h for forage dry matter yield (DMY) determination and used further for a nutrient profile analysis. Plot DMY was determined by multiplying the DM concentration by the plot fresh weight and expressed in DM Mg ha^{-1}. At Saskatoon, the second cut in the two-cut system was taken 23–26 August for 2018, 2019, and 2020 at the full bloom stage. Cumulative total DMY (TDMY) was determined by summing DMY of both the first and second cuts in the two-cut system at Saskatoon.

2.3.2. Nutrient Profile

Forage nutrient profile evaluation was done for all 3 years (in 2018, 2019, and 2020) for Saskatoon, and one year (in 2019) for Lanigan. Samples were analyzed for dry matter (DM; AOAC method # 930.15) and crude protein (CP; AOAC method # 984.13) contents according to the procedures of AOAC [12]. Crude protein was determined using a Leco FP-2000 nitrogen analyzer (Leco Corporation, St. Joseph, MI, USA), neutral detergent fiber with heat stable α-amylase (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed according to the procedures of Van Soest et al. [13] using an ANKOM Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA). In vitro neutral detergent fiber digestibility after 48h incubation ($NDFD_{48h}$) was analyzed as described in Tilley and Terry [14]. Forage total digestible nutrients (TDN) were determined according to

Weiss et al. [15]. Relative forage quality (RFQ) was estimated using the equations provided by Moore and Undersander (2002 [16]) to provide a relative measure of forage quality: with legumes RFQ = 1.0503 × RFV − 6.786; with binary mixture RFQ = 1.2464 × RFV − 14.721). Relative feed value (RFV) was calculated as RFV = (DDM × DMI)/1.29 [16], where DDM, digestible DM was calculated as DDM = 88.9 − (0.779 × ADF, % of DM) and DMI, dry matter intake was calculated as DMI = 120/NDF, % of DM. All data on forage yield and nutritive quality were reported on a DM basis. The Ca and P were analyzed using the dry ashing procedure (methods 927.02 and 965.17; AOAC, 2000, respectively) [12]. The Ca was determined using an atomic absorption spectrophotometer (Perkin Elmer, Model 2380, Nowalk, CT, USA), while P concentration was read at 410 nm on a spectrophotometer (Pharmacia, LKB-Ultraspec® III, Stockholm, Sweden).

2.3.3. Nutrient Production and Ranking

Nutrient yield obtainable from each hectare field, crude protein yield (CPY) and total digestible nutrients yield (TDNY), was calculated by multiplying forage yield (Mg ha^{-1}) by nutrient concentration to allow a comparison of nutrient yield potential in animal feed production among the forage crops.

2.3.4. Economics

The costs to seed each treatment plot were scaled up to a cost per hectare unit (Canadian dollar; CAD ha^{-1}). A combination of published custom rates and suggested and actual retail prices (cropping inputs) were used to estimate the stand establishment costs for the 4 treatments at each site. Glyphosate was purchased for 6.40 CAD L^{-1} and applied by a custom operator at a rate of 12.35 CAD ha^{-1} per application. Plot-sized equipment (e.g., cone plot planter) was used to seed, but for the purposes of the economic analysis it was assumed that full-sized equipment at custom operator rates was used. Seeding was valued at 56.81 CAD ha^{-1} (24 CAD ac^{-1}) which falls within the published custom rate range for air seeding and air drills in the 2020–2021 Farm Machinery Custom and Rental Rate Guide [17]. Fertilizer (11-51-0) used for improved seed flow at Lanigan was valued at 685.47 CAD $tonne^{-1}$ (1.34 CAD kg^{-1} P) which is the January to June 2017 average price reported by Alberta Agriculture's Farm Input Prices database [18]. Actual treatment yields were multiplied by average prices for hay released by the Saskatchewan Forage Council each September [19] to generate revenue estimates for each treatment. The alfalfa monocultures were valued at the 5-year average (2015–2019) price for first cut alfalfa hay (152.74 CAD $tonne^{-1}$) and the binary mixtures were valued at the 5-year average price for alfalfa/grass mix hay (134.56 CAD $tonne^{-1}$) as reported in the Saskatchewan Forage Market Report published by the Saskatchewan Forage Council [19].

2.3.5. Statistical Analysis

Statistical analysis was performed using the GLIMMIX procedure in SAS version 9.4 [20]. Differences among environments, and mainly agrotechnical differences of stand establishment resulted in significant interactions between locations and cutting stages; therefore, data were analyzed by locations and reported separately. The replicate was considered a random effect; cutting treatment and cultivar were designated as fixed effects.

Therefore, the model used for the analysis was:

$$Y_{jk(i)} = \mu + F_i + V_{j(a)} + V_{j(t)} + M_{k(a)} + \mathrm{M}_{k(t)} + \mathrm{e}_{ijk}$$

where, $Y_{jk(i)}$ is an observation of the dependent variable for the forage (entry) j at maturity stage k in the forage i; μ is the population mean for the variable; F_i is the forage type i, $i = a, t$; a is for monoculture, and t is for binary mixtures with HBG; $V_{j(a)}$ is the effect of an alfalfa cultivar (Grazeland and HiGest) nested within monoculture; $V_{j(t)}$ is the effect of forage mixture (Grazeland-HBG and HiGest-HBG) nested within binary mixture; $M_{k(a)}$ is the effect of forage maturity nested within monoculture; $\mathrm{M}_{k(t)}$ is the effect of forage mixture nested within binary mixture; and e_{ijk} is the random error associated with the observation *jk(i)*. Treatment contrasts [20] (monoculture vs. binary; Grazeland alfalfa vs. HiGest alfalfa; Grazeland-HBG vs. HiGest-HBG; Stage 1 vs. Stage 2; Stage 1 vs. Stage 3; Stage 2 vs. Stage 3; Grazeland alfalfa stage 2 vs. HiGest alfalfa stage 3; Grazeland-HBG stage 2 vs. HiGest-HBG stage 3) were used to determine treatment differences. The year (for Saskatoon site) replications were included as random effects for statistical analysis. Individual plots comprised the experimental unit, and statistical significance was set at $p \leq 0.05$. For forage accumulation and forage nutritive values, statistical analysis indicated no significant interactions ($p > 0.05$) between cutting treatment and forage. Therefore, only the main effects of cutting stage and forage were reported. Comparison of traits by locations are not discussed greatly because they are not central to the objective of evaluating the cultivars included in this study. To assess the relationship between forage lignin concentration and nutrient profiles, Pearson's correlation coefficients were calculated between ADL and CP, NDF, ADF, RFQ, NDFD and TDN using the CORR procedure of SAS [20]). The correlation coefficients were classified as strong ($r > 0.6$), moderate ($0.6 > r > 0.4$), or weak ($r < 0.4$), respectively. Due to the nature of the data, a statistical analysis of economic evaluation was not performed.

3. Results and Discussion

3.1. Forage Yield and Lodging Resistance Score

Forage yield and lodging resistance score of Saskatoon and Lanigan sites are presented in Table 3. No significant interactions ($p > 0.05$) between cutting treatments, cultivars, and mixtures were found for forage yield, lodging resistance score, and nutritive value. Therefore, the main effects of cutting treatment and cultivar × mixtures were reported.

At the Saskatoon site, the 3-year average of the first-cut DMY was 1.70 ± 0.24 and 2.15 ± 0.28 Mg ha^{-1} for HiGest and Grazeland, respectively. However, the differences between first cut DMY among monocultures have not reached significant level ($p = 0.231$). Under second cut, Hi-Gest was numerically lower in TDMY (20% lower; 2.99 vs. 3.71 Mg ha^{-1}; $p = 0.114$) relative to AC Grazeland. In the monoculture plots, second cut DMY tended to be lower (1.32 vs. 1.55 Mg ha^{-1}; 15% lower; $p = 0.07$) in previously stage 3-cut than in previously stage 1-cut plots. There was no significant difference ($p > 0.05$) between HiGest-HBG and Grazeland-HBG ($p > 0.05$) in DMY, averaged at 2.13 ± 1.36 Mg ha^{-1}, 1.20 ± 0.493 Mg ha^{-1}, and 4.25 ± 1.88 Mg ha^{-1} for first cut, second cut, and TDMY, respectively. In the binary mixtures, second cut DMY did not differ due to maturity stage of the previous cutting and averaged 0.98 ± 0.120 Mg ha^{-1}. In both monoculture and binary mixtures, there was no difference ($p > 0.05$) due to alfalfa maturity. The Alfalfa-HBG binary mixture had greater ($p < 0.05$) DMY and TDMY relative to the alfalfa monoculture for first cut (4.16 vs. 1.92 Mg ha^{-1} and 5.15 vs. 3.35 Mg ha^{-1}, respectively). When analyzing the second cut, the binary mixture had lower ($p < 0.05$) DMY relative to alfalfa monoculture (0.98 vs. 1.42 Mg ha^{-1}) (Table 3). At Saskatoon, no differences in lodging were observed (data not shown) for the alfalfa.

At the Lanigan site, Hi-Gest exhibited similar ($p = 0.797$; 2.98 ± 0.405 Mg ha^{-1}) DMY and lodging scores (LS) ($p = 0.723$; 2.1 ± 0.23) to Grazeland (Table 3) in 2019. The lack of differences detected among alfalfa cultivars in forage accumulation at Lanigan might have been influenced in part by several factors, including but not limited to the re-seeding that took place in the year following establishment, and large variation among cultivars, indicated by greater standard errors. Likewise, Grazeland-HBG and HiGest-HBG mixtures did not vary ($p = 0.622$; 9.12 ± 0.647 kg ha^{-1}) in DMY and lodging resistance score ($p = 0.164$; 2.1 ± 0.14). As alfalfa matured (stage 1 to 3), DMY and LS increased ($p < 0.05$) (DMY: 1.94, 2.97, and 4.02 Mg ha^{-1}; LS: 1.0, 2.3, and 3.1; for stages 1, 2, and 3, respectively). The DMY tended to be greater ($p = 0.09$; 35.4% more) in HiGest at stage 3, than in Grazeland at stage 2 (4.24 Mg ha^{-1} vs. 3.13 Mg ha^{-1}). In binary mixtures, forages at maturity stage 1 produced lower DMY (6.58 Mg ha^{-1}) than those at maturity stage 2 (9.86 Mg ha^{-1}) and stage 3 (10.65 Mg ha^{-1}), whereas forages in stage 2 and stage 3 did not differ ($p = 0.119$) in DMY. In binary mixtures, with advancing morphological development (stage 1 to 3), LS increased ($p < 0.05$) and averaged 1.0, 2.1, and 3.9 for stage 1, stage 2, and stage 3, respectively. Monoculture alfalfa were also lower ($p < 0.05$) in DMY, but were similar in LS ($p = 0.531$) compared to their binary mixtures. Although not significant ($p = 0.217$), HiGest-HBG at stage 3 yielded numerically greater (11% more) than Grazeland-HBG at stage 2 (10.65 Mg ha^{-1} vs. 9.58 Mg ha^{-1} DMY). Alfalfa cultivars in monocultures and in binary mixtures were not different ($p = 0.531$) in LS (2.2 ± 0.23). Likewise, no differences ($p = 0.182$) were observed between HiGest at stage 3 and Grazeland at stage 2 in LS, however, HiGest-HBG at stage 3 had greater LS ($p = 0.014$) than Grazeland-HBG at stage 2. Overall, the results of the current study indicate that Hi-Gest differed very little from Grazeland in DMY in monocultures and binary mixtures at Lanigan (Black soil zone), although only 1 year of production was studied there; hence further research evaluating several years of data is needed for definite conclusions to be drawn. Nevertheless, this study demonstrated that HiGest alfalfa can be persisted well both in a monoculture and in binary mixtures in Saskatoon (Dark Brown soil zones) and Lanigan (Black soil zones) of Saskatchewan.

Table 3. Effects of cultivar, maturity, and their interaction on yield (DMY, Mg ha^{-1}) of alfalfa entries in monoculture and in binary mixtures with AC Success hybrid bromegrass (HBG) at Saskatoon and Lanigan, Saskatchewan.

Forage	Stage *	Sites				
		Saskatoon			Lanigan	
		DMY	2nd Cut DMY	TDMY	DMY	Lodging †
	Monoculture					
Grazeland	Stage 1	2.13	1.71	3.84	1.87	1.0
	Stage 2	2.13	1.62	3.75	3.13	2.0
	Stage 3	2.19	1.35	3.54	3.81	3.3
HiGest	Stage 1	1.56	1.39	2.95	2.01	1.0
	Stage 2	1.72	1.18	2.90	2.82	2.5
	Stage 3	1.81	1.29	3.10	4.24	3.0
	SEM	0.462	0.144	0.566	0.405	0.28
	Binary					
Grazeland-HBG	Stage 1	4.09	1.13	5.22	6.97	1.0
	Stage 2	4.19	1.03	5.22	9.58	2.3
	Stage 3	4.41	0.98	5.39	11.21	4.0
HiGest-HBG	Stage 1	3.76	0.93	4.69	6.18	1.0
	Stage 2	4.35	0.97	5.32	10.13	2.0
	Stage 3	4.18	0.85	5.03	10.65	3.8
	SEM	0.424	0.120	0.397	0.647	0.14
Treatment contrasts				*p*-value		
Monoculture vs. Binary		<0.001	<0.001	<0.001	<0.001	0.531
Grazeland vs. HiGest		0.231	0.011	0.011	0.797	0.723
Grazeland-HBG vs. HiGest-HBG		0.700	0.180	0.180	0.622	0.164
In Monoculture						
Stage 1 vs. Stage 2		0.863	0.238	0.238	0.022	<0.001
Stage 1 vs. Stage 3		0.743	0.078	0.078	<0.001	<0.001
Stage 2 vs. Stage 3		0.876	0.549	0.549	0.020	0.007
In Binary Culture						
Stage 1 vs. Stage 2		0.421	0.797	0.797	<0.001	<0.001
Stage 1 vs. Stage 3		0.389	0.329	0.329	<0.001	<0.001
Stage 2 vs. Stage 3		0.954	0.470	0.470	0.119	<0.001

Note. * Forage harvested at 3 maturity stages of alfalfa: stage 1 = alfalfa 10% bloom; Stage 2 = 40% bloom; Stage 3 = 100% bloom. See Table 2 for the harvest dates. † Lodging score using 1–9 scale with 1 = completely upright and 9 = completely lodged.

3.2. Nutrient Profile

Forage nutrient profiles at Saskatoon are presented in Table 4. HiGest had greater ($p < 0.05$) TDN (2.6% more, 68.4 vs. 66.6%, DM basis), relative forage quality (RFQ) index (14.9% greater; 170 vs. 148), as well as in vitro neutral detergent fiber digestibility after a

48-h incubation ($NDFD_{48h}$: 13.5% greater; 42.9 vs. 37.8%), but had lower ADF (9.1% lower; 26.3 vs. 28.7%) and NDF (5.4% more; 34.7 vs. 36.6%) compared to AC Grazeland.

Table 4. Forage nutrient profile (% DM basis unless otherwise stated) of low-lignin and conventional alfalfa cultivar in monoculture and binary mixtures with AC Success hybrid bromegrass (HBG) at Saskatoon, Saskatchewan over 3 years.

Forage	Stage *	CP	NDF	ADF	ADL	TDN	$NDFD_{48h}$	RFQ	Ca	P
Monoculture										
Grazeland	Stage 1	20.4	35.6	27.9	6.4	67.2	39.0	157.7	2.65	0.18
	Stage 2	18.9	36.4	28.5	6.3	66.7	38.9	152.9	2.62	0.16
	Stage 3	18.9	37.8	29.7	6.8	65.8	35.6	132.1	2.59	0.15
HiGest	Stage 1	21.2	34.0	25.3	5.4	69.2	46.5	183.9	2.71	0.18
	Stage 2	19.5	35.0	26.9	5.9	68.0	41.4	161.3	2.71	0.17
	Stage 3	20.2	35.1	26.7	6.3	68.1	40.9	165.8	2.58	0.16
	SEM	0.81	1.15	0.98	0.40	0.76	2.25	9.17	0.09	0.011
Binary mixtures										
Grazeland-HBG	Stage 1	14.1	60.3	35.4	4.5	61.3	53.2	109.2	0.48	0.15
	Stage 2	12.1	61.1	36.5	4.5	60.5	49.6	99.7	0.55	0.13
	Stage 3	10.4	60.8	36.2	4.6	60.7	47.2	94.7	0.46	0.11
HiGest-HBG	Stage 1	13.2	60.7	35.8	4.6	61.0	53.6	106.7	0.50	0.15
	Stage 2	12.6	61.9	34.9	4.5	61.7	50.3	99.6	0.41	0.12
	Stage 3	10.3	59.6	34.6	4.4	61.9	45.6	99.9	0.41	0.12
	SEM	0.60	1.01	0.67	0.17	0.52	2.02	5.83	0.080	0.012
Treatment contrasts						*p*-value				
Monoculture vs. Binary		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Grazeland vs. HiGest		0.183	0.048	0.004	0.057	0.004	0.002	0.002	0.540	0.679
Grazeland-HBG vs. HiGest-HBG		0.752	0.999	0.106	0.793	0.106	0.863	0.812	0.398	1.000
Monoculture										
Stage 1 vs. Stage 2		0.053	0.438	0.286	0.593	0.287	0.178	0.115	0.881	0.249
Stage 1 vs. Stage 3		0.137	0.151	0.114	0.116	0.114	0.021	0.014	0.313	0.054
Stage 2 vs. Stage 3		0.641	0.502	0.599	0.294	0.600	0.321	0.350	0.389	0.426
Binary mixtures										
Stage 1 vs. Stage 2		0.023	0.328	0.882	0.842	0.882	0.006	0.065	0.913	0.053
Stage 1 vs. Stage 3		<0.001	0.778	0.772	0.771	0.773	<0.001	0.019	0.500	0.003
Stage 2 vs. Stage 3		0.001	0.209	0.662	0.927	0.661	0.005	0.594	0.572	0.274

Note. * Stage: Forage harvested at three maturity stages of alfalfa: 1 = 10% bloom; 2 = 40% bloom; and 3 = 100% bloom). See Table 2 for the harvest dates. CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; $NDFD_{48h}$, in vitro neutral detergent fiber digestibility after 48 h incubation; RFQ, relative forage quality; TDN, total digestible nutrients.

In the first cut forage, HiGest also was numerically lower in ADL (10.2% lower; 5.9 vs. 6.5%; $p = 0.57$) relative to Grazeland (Table 3). HiGest did not differ ($p > 0.05$) from Grazeland for CP (avg. 19.9 ± 0.33% DM), Ca (2.6 ± 0.04% DM), and P (0.17 ± 0.01%, DM).

However, at maturity stage 3, alfalfa samples had lower $NDFD_{48h}$ (11.8% lower; 38.2 vs. 42.7%; SEM = 1.80; $p = 0.021$), RFQ (14.8% lower; 149 vs. 171; SEM = 6.9; $p = 0.014$) than those at maturity stage 1. Otherwise, stages 1, 2, or 3 did not differ ($p > 0.005$) between each other. Differences in nutrient parameters among binary mixtures were minimal ($p > 0.05$) and inconsistent. As alfalfa growth stage advanced (stages 1 to 3), CP, as well as $NDFD_{48h}$ decreased ($p < 0.05$) (CP: 13.7, 12.3, 10.4; $NDFD_{48h}$: 53.4, 49.9, 46.4% NDF; for stages 1, 2, and 3, respectively).

In binary mixtures, forages harvested in stage 1 were greater ($p < 0.05$) in P relative to those harvested in stage 3. Expectedly, a moderate correlation was detected between ADL concentration and forage yield both in monoculture ($r^2 = 0.37$; $p < 0.01$) and in binary mixtures ($r^2 = 0.15$; $p < 0.01$).

Ten percent bloom is an important time to cut alfalfa to maintain quality in conventional crop production. As evident from Table 4, HiGest the stage 3 forage was similar with the Grazeland stage 1 in CP (i.e., HiGest maintains quality for longer time). Moreover, HiGest harvested in all three stages (10, 40, and 100% bloom) had greater RFQ (from 2.3 to 16.7% greater) relative to Grazeland harvested at 10% bloom. Later findings with similar patterns were also observed in TDN and $NDFD_{48h}$. Therefore, this increased forage quality of Hi-Gest is widening the harvest window and lengthening the time period when alfalfa can be harvested by livestock producers. Thus, increased harvest flexibility is one of the important advantages to using low-lignin HiGest alfalfa.

At the Lanigan site, HiGest was similar ($p > 0.05$) in all measured nutrient profile parameters with Grazeland (Table 5). For monocultures, the maturity stage at harvest influenced ($p < 0.01$) nutrient parameters; forages harvested at maturity stage 3 had lower $NDFD_{48h}$ (15.0% less; 38.1 vs. 43.8% DM), RFQ (21.7% less; 115 vs. 147), and P (14.3% less; 0.28 vs. 0.32% DM;), but had greater NDF (13.4% more; 45.7 vs. 40.3;) and ADL (36.4% more; 7.5 vs. 5.5% DM;) than those at maturity stage 1. As alfalfa maturity advanced (stages 1 to 3), CP, $NDFD_{48h}$, as well as TDN concentration decreased ($p \leq 0.05$) (CP: 23.1, 21.5, 20.0; $NDFD_{48h}$: 43.8, 43.2, 38.15; TDN: 65.5, 62.9, 60.8% DM for stages 1, 2, and 3, respectively; Table 5), which was in agreement with the findings of Llamas-Lamas and Combs [21] and Balde et al. [22]. On the contrary, ADF had increased ($p < 0.05$) (30.0, 33.4, 36.1% of DM for stages 1, 2, and 3, respectively) with the maturity of alfalfa. However, the Ca concentration was not affected ($p > 0.05$) by the stage of maturity of alfalfa.

Table 5. Forage nutrient profile (% DM basis, unless otherwise stated) of low-lignin and conventional alfalfa cultivar in monoculture and binary mixtures with AC Success hybrid bromegrass at Lanigan, Saskatchewan in 2019.

Forage	Stage *	CP	NDF	ADF	ADL	TDN	$NDFD_{48h}$	RFQ	Ca	P
Monoculture										
Grazeland	Stage 1	23.5	39.5	30.5	5.6	65.1	42.8	147.5	1.73	0.34
	Stage 2	21.0	44.0	34.0	6.4	62.4	44.2	135.1	1.62	0.30
	Stage 3	19.4	46.3	36.0	7.5	60.9	37.4	112.5	1.46	0.27
HiGest	Stage 1	22.8	41.1	29.5	5.5	65.9	44.7	147.2	1.64	0.31
	Stage 2	22.1	40.3	32.8	5.5	63.3	42.2	142.3	1.72	0.31
	Stage 3	20.7	45.1	36.1	7.5	60.8	38.9	118.0	1.65	0.29
	SEM	0.69	1.70	1.19	0.33	0.93	3.53	10.98	0.12	0.01
Binary mixtures										
Grazeland-HBG	Stage 1	13.0	66.3	38.5	4.1	58.9	59.4	115.0	0.27	0.25
	Stage 2	9.5	66.4	39.6	5.0	58.0	49.4	90.7	0.22	0.19
	Stage 3	10.8	63.5	37.0	5.7	60.1	42.4	81.0	0.30	0.18
HiGest-HBG	Stage 1	12.6	66.6	38.1	4.4	59.2	59.3	114.0	0.26	0.24
	Stage 2	12.5	66.4	38.6	4.6	58.9	51.2	95.3	0.25	0.21
	Stage 3	12.1	62.9	37.0	5.2	60.1	43.5	84.8	0.32	0.18
	SEM	1.24	1.04	0.91	0.23	0.71	2.38	6.64	0.01	0.01
Treatment contrasts						*p*-value				
Monoculture vs. Binary		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	0.001
Grazeland vs. HiGest		0.309	0.423	0.478	0.239	0.478	0.831	0.620	0.470	0.275
Grazeland-HBG vs. HiGest-HBG		0.207	0.848	0.531	0.323	0.531	0.635	0.658	0.169	0.812
Monoculture										
Stage 1 vs. Stage 2		0.011	0.222	0.283	0.011	0.843	0.843	0.398	0.892	0.086
Stage 1 vs. Stage 3		<0.001	<0.001	0.006	<0.001	0.053	0.053	0.006	0.255	<0.001
Stage 2 vs. Stage 3		0.039	<0.001	0.055	0.039	0.077	0.077	0.032	0.312	0.007
Binary mixtures										
Stage 1 vs. Stage 2		0.397	0.032	0.925	0.397	0.002	0.002	0.006	0.030	0.002
Stage 1 vs. Stage 3		0.173	<0.001	<0.002	0.174	<0.001	<0.001	0.000	0.013	<0.002
Stage 2 vs. Stage 3		0.036	0.015	0.002	0.036	0.007	0.007	0.149	0.000	0.104

Note. * Forage harvested at three maturity stages of alfalfa: Stage 1 = alfalfa 10% bloom; Stage 2 = 40% bloom; Stage 3 = 100% bloom. Forage was harvested on 27 June, 8 July, and 29 July 2019; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; $NDFD_{48h}$, in vitro neutral detergent fiber digestibility after 48-h incubation; RFQ, relative forage quality; TDN, total digestible nutrients.

As shown in Table 5, the lack of significant difference ($p = 0.831$) in $NDFD_{48h}$ among the alfalfa cultivars in the current study could potentially be the reason for the absence of any cultivar × maturity interaction. HiGest at stage 3 was similar ($p > 0.05$) to Grazeland at stage 2 in eight out of nine nutrient profile parameters. Nevertheless, HiGest at stage 3 was greater in ADL (16.7% greater; $p = 0.02$; 7.5 vs. 6.4% DM) and lower in P, as well as in $NDFD_{48h}$ (12.0% less; $p = 0.008$; 38.9 vs. 44.2% NDF) than Grazeland at stage 2 (Table 5). Thus, delaying HiGest alfalfa harvest has increased forage mass, although forage quality was maintained. Differences ($p > 0.05$) were not observed in nutrient profiles among binary mixtures (Table 5). In the binary mixtures, as plant maturity advanced (stage 1 to 3), $NDFD_{48h}$ decreased ($p < 0.05$), however, ADL (Table 5) concentrations were increased ($p < 0.05$). The results of the current study showed that binary mixtures with AC Success HBG had lower energy density (52% less RFQ) relative to the alfalfa monoculture. The ADL concentration of HiGest was 98.8%, 86.7%, and 99.2% (avg. 94.9%) of that of Grazeland alfalfa (check cultivar) for the stage 1, stage 2, and stage 3, respectively. In agreement with the current study, others, [23,24], also reported that low lignin alfalfa decreased lignin concentrations ranging from 4 to 12% compared to control cultivars. The ADL concentrations of HiGest-HBG were 106.3%, 93.7%, and 90.9% of (avg. 96.7%) that of Grazeland-HBG for the stage 1, stage 2, and stage 3, respectively (Table 5). The ADL concentrations for reference cultivars (cv. Grazeland) were comparable to previous findings by others [25,26]. Likewise, the cultivar description by Alforex (2021) stated that the whole plant lignin of Hi-Gest® 360 alfalfa is lower (by 7–10%) than non-selected elite commercial cultivar, which was supported by the results in the current study.

As a plant grows, the deposition of lignin is necessary to provide the strength and rigidity for a plant to stand upright [3,4]. Reductions in lignin are generally associated with negative impacts on plant growth, development, lodging tolerance, and/or productivity [27]. However, as noted in the previous section in the current study, HiGest did not differ in lodging resistance relative to Grazeland in monocultures or in binary mixtures with HBG. The HiGest-HBG at stage 3 did not differ ($p > 0.05$) from Grazeland-HBG at stage 2 in eight of nine measured nutrient profiles parameters (data not shown). However, HiGest-HBG at stage 3 was greater in Ca ($p = 0.014$; 1.7 vs. 1.6% DM) relative Grazeland-HBG at stage 2 (data not shown). Thus, delaying HiGest alfalfa harvest increased forage yield and maintained quality. Alfalfa monocultures had lower ($p < 0.05$) ADF, NDF, and $NDFD_{48h}$, but had higher CP, TDN, ADL, and RFQ than binary mixtures (Grazeland-HBG and HiGest-HBG), as was mostly expected. The difference in NDF concentration between alfalfa and alfalfa-HBG (42.7 vs. 65.3% DM) can be accounted for by the difference between NDF and ADF of these two mixtures, which is primarily hemicellulose (9.6 vs. 27.2%, data not shown), which was in agreement with the findings of Elizalde et al. [28]. Likewise, Hoffman et al. [29] also reported higher NDF in grasses (i.e., timothy, orchardgrass, perennial ryegrass, quackgrass, and bromegrass) compared with legumes (i.e., alfalfa, red clover, and birdsfoot trefoil).

Also seen in the current study, averaged by two sites, an increase of 10% $NDFD_{48h}$ for HiGest compared with Grazeland was observed, which concurred with Guo et al. [4] who observed an increase of eight percent NDFD for one of these transgenic reduced lignin lines compared with its isogenic counterpart. In the current study, in both HiGest and HiGest-HBG binary mixtures, the ADL concentration of the forages was relatively lower and consistent up to stage 2 and increased rapidly thereafter (Table 5). Furthermore, for Grazeland monoculture or Grazeland-HBG binary mixtures, ADL concentration gradually increased as plant maturity advanced. However, an opposite pattern (decreased) with lignin was observed on CP, TDN, and RFQ, as well as on P. In agreement with the current study, Hall et al. [30] and Yu et al. [7] also reported declines in CP concentrations with advancing morphological development across multiple harvests. The greater reduction in RFQ (from 142.3 to 118; by 20.5%) from stage 2 to stage 3 for HiGest likely suggests that the ideal harvest stage for Hi-Gest for hay is stage 2.

Alfalfa is generally harvested at the 40% bloom stage of maturity in western Canada, balancing yield and nutrient quality [31]. Crude protein concentrations differed among cutting stages at both sites (Tables 4 and 5). Across sites, CP concentrations for both alfalfa cultivars ranged from 18.9 to 23.5% DM and were comparable to previously reported values by others [26,30,32]. Concurring with the current study, previous studies examining reduced lignin alfalfa experimental lines have, also, found similar CP concentrations for LL alfalfa compared to reference alfalfa cultivars [2,33,34]. To the best of our knowledge, this is the first study comparing forage accumulation between low-lignin HiGest and reference alfalfa cultivars under diverse cutting schedules in monocultures and binary mixtures in western Canada; therefore, no data for comparison was available. Overall, the reduction in ADL was present across both sites. Significant differences ($p < 0.05$) in ADL were observed between stage 1 and stage 3, or between stage 2 and stage 3.

According to Van Soest [35], legumes tend to have lower ADF and NDF concentrations compared to grass species. As plants mature over the growing season, the nutritive value of both annual and perennial forages declines [36] as a result of a simultaneous decrease in CP and increase in NDF concentrations and decrease in fiber digestibility. This decline in nutritive value of forages was evident in the current study.

However, Ca concentrations of the binary mixtures in Saskatoon averaged 0.47% greater compared to those in Lanigan (0.27% DM) which was likely, as we speculated, due to higher proportions of alfalfa at the Saskatoon site since alfalfa has greater Ca concentrations compared to grasses [37].

3.3. Nutrient Production

Foraged total digestible nutrients (TDNY) and protein produced (CPY) on a per hectare basis of forages (Table 6) and related ranking data are presented in Table 6. HiGest was not different statistically ($p > 0.05$) in the CPY and TDNY relative to AC Grazeland at both sites in both monoculture and binary mixtures (Table 6). However, HiGest CPY (0.34 vs. 0.41 Mg ha^{-1}; 16% less), and TDNY (1.14 vs. 1.40 Mg ha^{-1}; 18% less) were numerically lower at Saskatoon relative to AC Grazeland alfalfa (check cultivar). The latter was primarily the reflection of DMY differences among alfalfa cultivars. Meanhile, at Lanigan, little difference was observed between HiGest and AC Grazeland in CPY and TDNY which averaged 0.63 Mg ha^{-1} and 1.86 Mg ha^{-1}, respectively (Table 6).

At Lanigan, TDNY increased ($p < 0.05$) with the maturity of alfalfa. In the monoculture, TDNY was 1273, 1858, and 2.45 Mg ha^{-1}, and in binary mixtures 3.88, 5.76, and 6.56 Mg ha^{-1} for stages 1, 2, and 3, respectively. In Saskatoon, stage 2 and stage 3 cutting occurred 7 and 14 days later than stage 1 cutting. However, at Lanigan, stage 2 and stage 3 cutting occurred 11 and 31 days later than stage 1 cutting, which may cause clear distinctive character in forage nutrient yields in this site. Overall, in Saskatoon, CPY and TDNY of HiGest were 83.6% and 81.7% (avg. 94.9%) of AC Grazeland alfalfa, respectively. CPY and TDNY of HiGest-HBG were 95.3% and 98.3%, respectively. In Lanigan, CPY and TDNY of HiGest were 100.4% and 103.5% (avg. 94.9%) of AC Grazeland alfalfa, respectively. CPY and TDNY of HiGest-HBG was 103.2% and 100.5%, respectively. An important difference between grasses and legumes is that while tissues in legumes with thick lignified walls can be only marginally digested by rumen microbes, similar thick lignified walls of grass tissues are extensively degraded, albeit slowly [5,38]. In agreement with the statement above, the current study has shown that NDFD was greater in binary mixtures relative to monocultures of alfalfa at both sites.

Table 6. Forage nutrient yield (Mg ha^{-1} DM) of low-lignin and conventional alfalfa cultivars in monoculture and binary mixtures with AC Success hybrid bromegrass (HBG) at Saskatoon and Lanigan, Saskatchewan.

Forage	Stage *	Saskatoon		Lanigan	
		CPY	TDNY	CPY	TDNY
Monoculture					
Grazeland	Stage 1	0.422	1.406	0.441	1.219
	Stage 2	0.406	1.393	0.658	1.943
	Stage 3	0.396	1.401	0.738	2.326
HiGest	Stage 1	0.330	1.083	0.465	1.328
	Stage 2	0.340	1.145	0.618	1.773
	Stage 3	0.353	1.201	0.876	2.570
	SEM	0.875	0.296	0.919	0.2544
Binary mixtures					
Grazeland-HBG	Stage 1	0.580	2.515	0.921	4.103
	Stage 2	0.504	2.521	0.919	5.563
	Stage 3	0.438	2.670	0.120	6.733
HiGest-HBG	Stage 1	0.493	2.305	0.788	3.651
	Stage 2	0.542	2.695	1.245	5.952
	Stage 3	0.416	2.578	1.276	6.382
	SEM	0.507	0.260	0.1346	0.3563
Treatment contrasts		*p*-value			
Monoculture vs. Binary		0.003	<0.001	<0.001	<0.001
Grazeland vs. HiGest		0.354	0.291	0.596	0.773
Grazeland-HBG vs. HiGest-HBG		0.555	0.842	0.435	0.641
Monoculture					
Stage 1 vs. Stage 2		0.972	0.933	0.062	0.036
Stage 1 vs. Stage 3		0.987	0.848	0.002	<0.001
Stage 2 vs. Stage 3		0.986	0.915	0.086	0.035
Binary mixtures					
Stage 1 vs. Stage 2		0.783	0.449	0.112	<0.001
Stage 1 vs. Stage 3		0.029	0.413	0.012	<0.001
Stage 2 vs. Stage 3		0.054	0.951	0.259	0.040

Note. * Forage harvested at three maturity stages of alfalfa: Stage 1 = alfalfa 10% bloom; Stage 2 = 40% bloom; Stage 3 = 100% bloom. See Table 2 for the harvest dates. CPY, crude protein yield; TDNY, total digestible nutrients yield.

3.4. Relationship between Forage Lignin and Yield and Nutrient Profiles

Pearson correlation analysis between the DMY and ADL concentration of forage pooled at both sites is presented in Table 7. Expectedly, ADL concentration was moderately and positively correlated with DMY in both Grazeland ($r = 0.49$; $p < 0.001$) and HiGest ($r = 0.42$; $p < 0.001$) alfalfa. For HiGest, ADL concentration moderately correlated with ADF ($r = 0.47$; $p < 0.001$), NDF ($r = 0.43$; $p < 0.01$), RFQ ($r = -0.52$; $p < 0.001$), $NDFD_{48h}$ ($r = -0.51$; $p < 0.001$), and TDN ($r = -0.47$; $p < 0.001$). It can be assumed that the lower value of ADL in HiGest, the magnitude of r value between ADL concentration and DMY as well as nutrient profiles was lower in HiGest relative to Grazeland excluding the r value between $NDFD_{48h}$ and ADL, which was numerically greater in HiGest. A moderate correlation was also observed between ADL and DMY in both Grazeland-HBG ($r = 0.57$; $p < 0.001$) and HiGest-HBG ($r = 0.43$; $p < 0.01$). However, very weak or no correlation was also observed between ADL and nutrient profiles in both Grazeland-HBG and HiGest-HBG. Changes in lignin concentration account for most of the improvements in digestibility rather than the effects of lignin composition/structure as strong negative correlations were reported for lignin concentrations with digestibility [39–41]. Summarizing all four forages on both sites through all years, each percentage unit increase in lignin concentration, the main factor hindering cell wall digestion, decreased cell wall in vitro $NDFD_{48h}$ degradability by 3.8 percentage units ($NDFD_{48h}$, % of NDF = 65.80 − 3.766 × ADL, % of DM; $r^2 = 0.38$, n = 192, $p < 0.001$; data not shown). In a similar fashion with the current study, others, [42], have also documented that the lignin concentration predicted NDF in degradability with reasonable accuracy. Likewise, a 1-unit increase in forage NDF digestibility is associated with 0.17 and 0.25 kg d^{-1} increases in DMI and milk production, respectively [43].

Table 7. Pearson's correlation analysis between acid detergent lignin (ADL) and dry matter yield (DMY) and nutrient profile of forages of low-lignin and conventional alfalfa cultivars in monoculture and binary mixtures with AC Success hybrid bromegrass (HBG) grown in Saskatoon and Lanigan, SK, Canada.

Forage	Correlations (*r*) with ADL † Concentration						
	DMY	CP	NDF	ADF	RFQ	$NDFD_{48h}$	TDN
Monoculture	0.47 ***	−0.47 ***	0.52 ***	0.57 ***	−0.62 ***	−0.49 ***	−0.57 ***
Grazeland	0.49 ***	−0.59 ***	0.57 ***	0.62 ***	−0.68 ***	−0.39	−0.62 ***
HiGest	0.42 **	−0.31 *	0.43 **	0.47 ***	−0.52 ***	−0.51 ***	−0.47 ***
Binary mixtures	0.50 ***	−0.21 *	0.07	0.28 **	−0.20 *	−0.18	−0.28 **
Grazeland-HBG	0.57 ***	−0.27	0.02	0.23	−0.11	−0.13	−0.23
HiGest-HBG	0.43 **	−0.13	0.12	0.32 *	−0.30 *	−0.23	−0.32 *
All	−0.13	0.38 ***	−0.49 ***	−0.19 *	0.17 *	−0.60 ***	0.19 *

Note. † ADL, acid detergent lignin; Grazeland, AC Grazeland alfalfa; HiGest, Hi-Gest® 360 alfalfa; Grazeland-HBG, AC Grazeland alfalfa with AC Success hybrid bromegrass; HiGest-HBG, Hi-Gest® 360 in mixture with AC Success hybrid bromegrass; DMY, dry matter yield; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; RFQ, relative forage quality; $NDFD_{48h}$, in vitro neutral detergent fiber digestibility after 48-h incubation; TDN, total digestible nutrients; Correlations significantly different from 0 at *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Forages were harvested at three maturity stages of alfalfa (10% bloom; 40% bloom, and 100% bloom stage) over three consecutive production years (2018, 2019, and 2020) in Saskatoon and one year (2019) in Lanigan. See Table 2 for the harvest dates.

Furthermore, in perennial ryegrass (*Lolium perenne* L.), a 5 to 6% increase in digestibility was estimated to increase milk production by up to 27% [44]. Thus, each percentage unit increase in lignin concentration in forage cell walls can severely constrain DMI and milk production. Moreover, several studies have confirmed the positive effect of feeding forage with increased NDF digestibility on DMI and productivity of dairy cattle [45]. It is well known that forage quality may be affected by cultivar [46], soil type, climatic conditions [47], as well as harvest time or maturity stage [7,48] among other factors. As plants mature, leaf proportions decrease, stem proportions increase, stem cell wall concentrations increase, and whole plant nutritive value decreases [49–51].

Hall et al. [30] concluded that the selection for greater nutritive value did not inadvertently result in the selection for delayed morphological development. As the lodging tolerance score indicated in the case of Hi-Gest® 360 alfalfa, our results demonstrated the genetic selection for lower ADL concentrations and greater NDFD had essentially no difference in morphological development than the non-LL cultivar. As expected, the results of the current study indicate that binary mixtures had decreased energy density (less lignin, CP and higher ADF, NDF) compared to alfalfa monocultures.

Guo et al. [4] examined the lignin concentration and NDFD of six independent transgenic alfalfa lines with reduced lignin concentration compared with control lines and reported a range from 13 to 29% in lignin concentration, which was in agreement with the results of the current study. Furthermore, they observed an increase of 8% in vitro NDFD for one of these reduced lignin lines compared with its isogenic counterpart. In agreements with the latter, HiGest alfalfa showed increased NDF digestibility relative to AC Grazeland alfalfa cultivar in the current study. Small decreases in the lignin concentration of forages can be expected to improve the fiber digestibility at any plant maturity stage [24]. Overall, the result of the current study indicated that the Hi-Gest® 360 alfalfa could be harvested from 7 to 14 days later and still maintain slightly greater than or similar nutrient quality concentrations and similar (at the Lanigan) or lower (at the Saskatoon) forage yields as the AC Grazeland alfalfa checks harvested earlier.

3.5. Economics

The stand establishment costs for monoculture and binary treatments are presented in Table 8. At the Lanigan site, glyphosate was applied three times (total of 3.24 L ha^{-1}) for a product cost of 20.74 CAD ha^{-1} and an application cost of 37.05 CAD ha^{-1} (12.35 CAD ha^{-1} per application). Seed costs were calculated from actual seeding rates which varied by treatment and were based on germination tests and seed weight (per 100 seeds), and the actual price paid for the seed. For HiGest alfalfa, the seed was purchased for 16.41 CAD kg^{-1} (7.46 CAD lb^{-1}) for a total cost of 147.22 CAD ha^{-1}. The AC Success seed was purchased for 10.89 CAD kg^{-1} (4.95 CAD lb^{-1}). The seed cost for the HiGest-HBG binary treatment was 122.25 CAD ha^{-1}. AC Grazeland alfalfa seed was valued at 11.66 CAD kg^{-1} (5.30 CAD lb^{-1}), for a total cost of 104.59 CAD ha^{-1}. The total seed cost for Grazeland-HBG was 111.60 CAD ha^{-1}. In each treatment, 11.2 kg ha^{-1} of P (11-51-0) was added to improve seed flow. Phosphorus was valued at 685.47 CAD tonne^{-1} based on published prices for 11-51-0 fertilizer for January to June 2017 by Alberta Agriculture and Forestry [18]. The inclusion of fertilizer for seed flow costed 7.66 CAD ha^{-1} (3.10 CAD ac^{-1}). Stand total establishment costs varied by treatment from a high of 269.47 CAD ha^{-1} for HiGest monoculture to a low of 226.85 CAD ha^{-1} for Grazeland monoculture.

Table 8. Establishment costs for monoculture and binary treatments at the Lanigan and Saskatoon, SK, Canada *.

Item	Grazeland	HiGest	Grazeland-HBG	HiGest-HBG
		CAD ha^{-1}		
Lanigan				
Glyphosate	20.74	20.74	20.74	20.74
Spraying	37.05	37.05	37.05	37.05
Seeding	56.81	56.81	56.81	56.81
Seed				
Legume	104.59	147.22	26.12	36.76
Grass	–	–	85.49	85.49
Fertilizer †	7.66	7.66	7.66	7.66
Total stand establishment costs	226.85	269.47	233.86	244.51
Saskatoon				
Cultivating	22.48	22.48	22.48	22.48
Seeding	56.81	56.81	56.81	56.81
Seed				
Legume	103.77	251.1	51.3	126.37
Grass	–	–	155.73	155.73
Total stand establishment costs	183.06	330.39	286.32	361.39

Note. * The study site was established in 2017 in both sites, and re-seeded at the Lanigan in 2018. † Mainly for improved seed flow.

At the Saskatoon site, rototilling was used for weed control on the Saskatoon plots which has been equated with cultivating (Table 8). The suggested custom rate for cultivating is 22.48 CAD ha^{-1} in the 2016–2017 Farm Machinery Custom and Rental Rate Guide [17]. Seed costs for Saskatoon varied from Lanigan due to differing seeding rates. HiGest was seeded at a 72% higher seeding rate than Grazeland resulting in higher seed costs for treatments containing HiGest. The HiGest monoculture seed cost 251.10 CAD ha^{-1} (15.3 kg ha^{-1} $\times$ 16.41 CAD kg^{-1}) at Saskatoon. The AC Grazeland seed cost 103.77 CAD ha^{-1} (8.9 kg ha^{-1} $\times$ 11.66 CAD kg^{-1}). The HiGest-HBG treatment had 282.10 CAD ha^{-1} seed costs and Grazeland-HBG was 207.03 CAD ha^{-1}. There was no fertilizer or herbicide applied on the Saskatoon plots. At the Saskatoon site, the Grazeland monoculture and binary treatments had 45% and 21% lower establishment costs, respectively. The difference is due to the higher seeding rates and seed prices for HiGest. Establishment costs for the Saskatoon treatment plots from lowest to highest were: Grazeland (183.06 CAD ha^{-1}), HBG-Grazeland (286.32 CAD ha^{-1}), HiGest (330.39 CAD ha^{-1}), and HBG-HiGest (361.39 CAD ha^{-1}).

4. Conclusions

The current study showed that Hi-Gest® 360 alfalfa established well, suggesting it could be a viable alternative alfalfa cultivar for both Dark Brown and Black soil zones. However, during establishment, irrigation is necessary in dryer-than-usual years. HiGest alfalfa as a monoculture yielded less (in the Dark Brown soil zone) or similar (in the Black soil zone) compared to reference alfalfa cultivar (AC Grazeland), while it had higher nutritive value than Grazeland. In binary mixtures with AC Success hybrid bromegrass, the difference in yield and quality between the two alfalfas was minimal. If nutritive quality was the major goal of a producer, then Hi-Gest® 360 alfalfa or Hi-Gest® 360 alfalfa mixed with HBG (cv. AC Success) would be the top choice. However, if forage yield was the goal, then conventional alfalfa such as AC Grazeland would be a suitable forage for the Saskatoon site. The findings of this study suggest that it may be possible to delay harvest up two weeks to achieve the same nutritive value or higher CP with the same yield or 11% (in binary) to 35.4% (in monoculture) greater yield as from conventional alfalfa cultivars that are cut earlier. The stand establishment costs were greater for HiGest than for Grazeland which may delay its adoption. A large-scale research study (over multiple years and evaluating animal performance) needs to be performed to understand how this new low-lignin alfalfa cultivar can perform in Canadian prairies.

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