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M.D. Casler

Correlated responses in forage yield and nutritional value from phenotypic recurrent selection for reduced fiber concentration in smooth bromegrass

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Abstract Selection for reduced fiber concentration in forage crops is considered to be an effective approach to improve the voluntary intake potential of forages. The purpose of this study was to determine the effectiveness of several modifications to phenotypic recurrent selection for reducing neutral detergent fiber (NDF) concentration and its effect on correlated variables of smooth bromegrass (*Bromus inermis* Leyss). The selection methods were based on differences in the growth stage sampled (vegetative vs heading), the method of determining NDF in the laboratory (wet-laboratory vs near-infrared reflectance spectroscopy), and the method of intercrossing selected individuals (in situ vs replicated polycross). Selection at the vegetative growth stage was most effective, probably, due to minimal sampling variation within plants. Polycrossing generally increased gains due to more effective interpollination, but increased cycle time by 50%, resulting in similar gains per year for in situ pollination vs polycrossing. Selection for reduced NDF did not generally affect the digestibility of the NDF fraction. Selection for reduced NDF led to reduced forage yield for all selection methods, due partly to a genetic correlation with NDF and partly to inbreeding depression. Three potential solutions were proposed to break the apparent association between reduced NDF and forage yield: increase effective population size, practice combined selection for both traits, and/or make chance hybrids between genetically divergent low-NDF strains. An empirical assessment will most likely be required to determine the best of these three potential solutions.

Key words Recurrent Selection · Fiber · Forage yield · Digestibility · Inbreeding · Forage crops · Pollination methods

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M.D. Casler
Department of Agronomy, University of Wisconsin-Madison, Madison, WI 53706-1597, USA
e-mail: mdcasler@facstaff.wisc.edu

Introduction

Most attempts to develop new cultivars with improved feeding value have focused on some measure of digestibility. There are numerous reports of realized gains from selection for digestibility and of animal gains associated with genetic increases in digestibility (Casler and Vogel 1999). However, most ruminant nutritionists consider voluntary intake to be a more important factor than digestibility in limiting animal performance (Fahey and Hussein 1999). Up to 70% of the variation in animal production can be attributed to variation in intake, while only 20% can be attributed to variation in digestibility (Crampton et al. 1960). Indeed, voluntary intake is limited by the rate of passage of digesta from the rumen, which is a function of digestibility and the rate of particle-size breakdown (Balch and Campling 1962). Thus, genetic improvements in digestibility may have indirectly led to improved animal performance through their effect on intake per se.

Intake can be more directly predicted in the laboratory and there have been some efforts to do so in forage breeding programs. Divergent selection for leaf shear strength in perennial ryegrass (*Lolium perenne* L.) shows a potential for improving the intake of ruminants. High and low shear-strength populations that differed by 32% showed a negative association with intake by sheep in two studies, although the effect on intake was not significant in a more recent study (MacKinnon et al. 1988; Inoue et al. 1993). Inoue et al. (1993) suggested that correlated responses toward longer leaves per unit dry weight for the low shear-strength population may have indirectly increased masticatory load, offsetting much of the benefit of low shear-strength leaves. Using smooth bromegrass (*Bromus inermis* Leyss.) leaves, Casler et al. (1996) developed a laboratory procedure to predict the extent of particle-size breakdown by rumination and comminution. The particle-size reduction index is heritable and some progress has been achieved (Culvenor and Casler 1999), but animal feeding and metabolic studies have not yet been initiated.

Table 1 Description of six phenotypic recurrent selection methods for reducing NDF (neutral detergent fiber) concentration and the variables that define their differences

Selection method	Defining variables			
	Harvest growth stage	Laboratory analysis method	Pollination method	No. of years per cycle
vNB2	Vegetative	NIRS	Open-pollination, selected plants only	2
hNU2	Heading	NIRS	Open-pollination, all plants	2
hWU2	Heading	Wet-laboratory	Open-pollination, all plants	2
vNP3	Vegetative	NIRS	Replicated polycross	3
hNP3	Heading	NIRS	Replicated polycross	3
hWP3	Heading	Wet-laboratory	Replicated polycross	3

Most efforts to improve the intake potential of forage crops are based on neutral detergent fiber (NDF) concentration as a selection criterion. This is generally considered to be the single laboratory measure most closely correlated with voluntary intake (Van Soest 1994). Genetic progress toward reduced NDF concentration has been reported for smooth bromegrass (Casler 1999), reed canarygrass (Surprenant et al. 1988), and maize (Wolf et al. 1993).

There have been two distinct and potentially serious correlated responses related to reduced NDF concentration. First, low NDF concentration is associated with increased susceptibility to European corn borer (*Ostrinia nubilalis* Hubner) in maize, *Zea mays* L. (Buendgeon et al. 1990). Although efforts to break this relationship have been unsuccessful (Ostrander and Coors 1997), it may not prevent the use of low-NDF silage maize. The relationship is observed primarily in the second stage of infection, which may be eliminated by an early silage harvest (Ostrander and Coors 1997). Second, in reed canarygrass (*Phalaris arundinacea* L.), divergent selection for NDF concentration led to positive and significant correlated responses in forage yield: 22 g m^{-2} change in yield for each 1 g kg^{-1} change in NDF (Surprenant et al. 1988). However, this relationship was not observed in three maize populations divergently selected for NDF concentration (Wolf et al. 1993). In the latter study, the phenotypic correlation between whole-plant yield and NDF ranged from r=0.02 to 0.30 and the phenotypic correlation between grain yield and whole-plant NDF ranged from r=-0.12 to 0.15.

The objectives of the present study were to: (1) estimate the effect of selection for reduced NDF concentration in smooth bromegrass on sward-plot NDF concentration, in vitro digestibility, and forage yield, (2) determine the extent to which changes in forage yield resulting from selection for NDF concentration are due to correlated response or inbreeding, and (3) determine factors important in increasing the efficiency of phenotypic recurrent selection for low NDF concentration. The factors tested for the latter objective were: growth stage for evaluation of the selection unit (vegetative vs heading), laboratory method for determining NDF concentration (near-infrared reflectance spectroscopy vs wet-laboratory), and pollination method (open-pollination vs polycrossing of selected individuals).

Materials and methods

Phenotypic selection for reduced NDF concentration was practiced in the WB-RP_1 smooth bromegrass germplasm pool (Casler 1992). Plants were raised as seedlings in the greenhouse and transplanted to a Plano silt loam (fine-silty, mixed, mesic, Typic Argiudoll) near Arlington, Wis. Ten-week-old seedlings were transplanted to the field in mid-May and mowed twice during the establishment year. Weeds were controlled with herbicide (Casler 1992). All plants were fertilized with 112 kg N ha^{-1} in early spring of the year following establishment.

All selection nurseries were established with approximately 400 plants. Selection for reduced NDF concentration was practiced on 340–350 plants, allowing a small proportion of plants to be eliminated based on severe vigor or disease problems, if necessary. Nurseries were arranged into ten blocks of 40, in a 5×8 arrangement. Thirty-five plants from within each block were evaluated for NDF, and their samples were always processed together throughout the drying, grinding, and laboratory processes (Casler 1992). Cutting height was always 5 cm.

Samples were dried at 60°C and ground through a 1-mm screen of a Wiley-type mill and again through a 1-mm screen of a cyclone mill. The concentration of NDF was determined on 0.5-g samples using the procedure of Goering and Van Soest (1970), with the exception that sodium sulfite and decahydronaphthalene were excluded from the reflux solution. All NIRS predictions of NDF concentration, during the course of selection, were made using a Pacific Scientific scanning monochromator, model 51 A. Calibration and validation statistics are presented in Table 1. The selection criterion was computed as

$$t_{NDF}=(X_{ij}-M_j)/s_j,$$

where X_{ij}=NDF concentration of the i^{th} plant of the j^{th} block, M_j is the mean of the j^{th} block, and s_j is the standard deviation of the j^{th} block. This ensured that a large proportion of field and laboratory variation could be removed in the computation of the selection criterion (Casler 1992). Thirty five selections were made from each nursery, giving selection intensities ranging from 10 to 10.3%.

Selection methods

Notation for the names of the nurseries and selection methods is based on a 4-character code: ijkl, where i=growth stage (v=vegetative or h=heading), j=laboratory method (N=NIRS or W=wet-lab), k=gametic selection pressure/pollination method (B=biparental, U=uniparental, or P=replicated polycross), and l=number of years per cycle (Table 1). All nurseries and crossing blocks were established at least 100 m from each other and from other sources of smooth bromegrass to minimize the potential for pollen contamination.

In situ pollination: vNB2, hNU2, and hWU2

Three nurseries of WB-RP_1 were established in 1985 to begin the experiment (Table 1). In the vNB2 nursery, plants were harvested on the 10th of May 1986 when most plants were approximately 20–25 cm tall and consisted exclusively of leaves [stage 21 or 22 on the Simon and Park (1983) maturity scale]. Sample size was approximately 10 g of DM. A stratified random sample of 70 plants (seven plants per block) was analyzed for NDF concentration in duplicate and used to develop a calibration equation for the NIRS equipment and the subsequent prediction of NDF concentration for all 350 tissue samples. Validation statistics for each equation were computed on a random sample of 20 plants and were used to assist in selection of the calibration equation (Table 1). Values of t_{NDF} were computed for each plant, the 35 plants with the lowest values of t_{NDF}, irrespective of block, were tagged and all remaining plants were mowed prior to anthesis. Seed was harvested in July 1986, threshed, and cleaned independently from each plant, then bulked in equal quantities by mass.

The hNU2 and hWU2 nurseries were harvested just after all plants had reached the fully headed growth stage (stage 59 of Simon and Park 1983). Because this population did not possess genetic variation for relative maturity, this occurred on the same day for all plants within a nursery (Casler 1992). Samples consisted of a radial cross-section of 20–30 tillers. Samples from the hNU2 nursery were analyzed by NIRS as described for the vNB2 nursery (Table 1). Samples from the hWU2 nursery were analyzed for NDF by wet-laboratory methods. All plants in both nurseries were allowed to open-pollinate and set seed. Seed was harvested in July 1986, threshed, and cleaned for each plant. When the selected plants were identified following the laboratory analyses in winter 1986/87, their seed was bulked in equal quantities by mass and seed from all other plants was discarded.

Seed of each cycle-1 population was used to establish seedlings in the greenhouse for transplanting to the field in May 1987. Cycle 2 of the three selection methods was conducted in 1987 and 1988, and cycle 3 in 1989 and 1990. Remnant seed of each balanced bulk seed population was kept frozen to maintain maximum viability.

Polycrossing: vNP3, hNP3, and hWP3

In late April 1987, prior to the initiation of new growth, three clonal ramets of the 35 selected plants from each of the three 1985 nurseries were removed and transplanted to a replicated crossing block in a randomized complete block design with three replicates (Table 1). Plants were fertilized with 50 kg N ha^{-1} and well watered. Seed was harvested in July 1987, threshed, and cleaned for each clonal ramet and bulked in equal quantities by mass. Thus, in cycle 1, the three polycrossing methods shared the same sample of the source population and the same individual selections as the three *in situ* pollination methods. Cycle 2 of the polycrossing methods was conducted in 1988–90, independently of the in situ pollination methods.

Evaluation of selection progress

Sward plots of the 16 populations were seeded in replicated field plots in April or May 1992. In addition to the 16 populations described above (C0; C1, C2, and C3 for the three in situ methods; and C1 and C2 for the three polycross methods), two additional populations were included in this study. These populations were created by divergent selection for NDF concentration within WB-RP_1 in 1986 and 1987. The ten most extreme high and low individuals from the original hWU2 nursery were separately polycrossed to create these divergently selected populations.

Sward plots were established at Arlington, Lancaster, and Ashland, Wis. Soil types were: Plano silt loam (fine-silty, mixed, mesic, Typic Argiudoll) at Arlington, Fayette silt loam (fine-silty, mixed, mesic, Typic Hapludalf) at Lancaster, and Ontanogon silty clay loam (very fine, mixed, Glossic Eutroboralf) at Ashland. The experimental design was a randomized complete block with three replicates and a split-plot randomization restriction. Whole plots were selection methods (seven, including the divergent selection practiced within the original hWU2 nursery) and sub-plots were cycles. The base population, WB-RP_1, was included in every whole-plot to improve the accuracy of its mean estimation and the precision of selection response estimators. Plots were 0.9×3.0 m and were seeded at a rate of 20 kg pure live seed ha^{-1}. Plots were harvested two or three times per year in 1993–1995 at Arlington and Ashland, and in 1993–1994 at Lancaster. They were fertilized with 112 kg N ha^{-1} in early spring and following the first and second harvests. First harvest was made when plants had just reached the fully headed growth stage, second harvest was made in late July, and third harvest was made in October. Dry matter concentration was determined on each plot at each harvest using 300–500-g grab samples.

Dry matter samples for each harvest were dried at 60°C and ground through a 1-mm screen of a Wiley-type mill and again through a 1-mm screen of a cyclone mill. Near-infrared reflectance spectra (NIRS) were obtained on each sample with a Pacific Scientific 6500 scanning monochromator. A subset of 150 calibration samples were chosen by the NIRS software using a cluster analysis of reflectance spectra (Shenk and Westerhaus 1991). These samples were analyzed for NDF concentration using the procedure of Van Soest et al. (1991) with the exceptions that sodium sulfite and α-amylase were excluded. In vitro dry matter digestibility (IVDMD) was determined on calibration samples using triplicate 0.25-g subsamples and the two-stage direct-acidification procedure of Marten and Barnes (1980). In vitro NDF digestibility (IVFD) was determined on calibration samples using triplicate 0.25-g subsamples and the procedure described by Casler (1987). Values of NDF, IVDMD, and IVFD were predicted for all samples using a single calibration equation per variable (NDF: SE_{cal}= 7.3 g kg^{-1} and r^2_{cal}=0.88; IVFD: SE_{cal}=6.2 g kg^{-1} and r^2_{cal}=0.84; IVDMD: SE_{cal}=4.0 g kg^{-1} and r^2_{cal}=0.87).

Forage yield was expressed as the sum over three harvests within each year. Values of NDF, IVDMD, and IVFD were expressed as weighted seasonal means, where the value for each harvest was weighted by the forage yield at that harvest. Each variable was analyzed by analysis of variance for each individual location, using a repeated measures analysis and the Greenhouse-Geiser adjustment (Girden 1992). Populations were assumed to have fixed effects, while replicates and years were assumed to have random effects. Because there was little evidence for population×year interactions for all variables at all locations, plot means (over years) were computed for all further analyses.

Plot means over years for each variable were subjected to nearest-neighbor analysis (NNA) to adjust for spatial variation within each location (Brownie et al. 1993). Population means, adjusted for spatial variation, were used to compute a combined analysis of variance across locations for which the pooled within-location error mean square was obtained from the individual-location NNAs. Linear and non-linear selection responses and their interactions with locations and selection methods were computed using contrasts within each analysis of variance. Genotypic correlation coefficients and their standard errors were computed according to Mode and Robinson (1959).

Changes in forage yield resulting from recurrent selection for low NDF concentration may be due to a genetic correlation between NDF concentration and forage yield or to inbreeding depression. Two independent statistical methods were used to separate the effects of genetic correlation vs inbreeding depression on forage yield.

1. Plot NDF values were used as a covariate in an analysis of variance of forage yield, to adjust each population mean to a constant NDF concentration. Forage yield selection responses estimated from this analysis should be independent of NDF selection responses. This hypothesis was tested by measuring forage yield responses per se and the empirical relationship between selection responses for forage yield and NDF concentration.

Table 2 Linear responses to selection, measured as NDF (neutral detergent fiber) concentration, following each of six phenotypic recurrent selection methods for reduced NDF concentration described in Table 1

Selection method	Growth stage				Seasonal weighted mean	
	Reproductive		Regrowth			
	g kg^{-1} cycle^{-1} (% cycle^{-1})					
vNB2	–8.5[b]	(–1.4)	–9.0[b]	(–1.8)	–9.3[b]	(–1.6)
hNU2	–4.2	(–0.7)	–2.0	(–0.4)	–2.4	(–0.4)
hWU2	–5.0[a]	(–0.8)	–2.3	(–0.4)	–4.0	(–0.7)
vNP3	–13.8[b]	(–2.3)	–5.5[a]	(–1.1)	–12.6[b]	(–2.2)
hNP3	–3.8	(–0.6)	–6.4[b]	(–1.3)	–4.8	(–0.8)
hWP3	–8.7[a]	(–1.5)	–0.9	(–0.2)	–7.6[a]	(–1.3)
Mean	–7.4[b]	(–1.2)	–4.3[a]	(–0.9)	–6.8[b]	(–1.2)
Selection growth stage						
Vegetative	–11.2[a]	(–1.9)	–7.2	(–1.5)	–11.0[a]	(–1.9)
Heading	–5.4	(–0.9)	–2.9	(–0.6)	–4.7	(–0.8)
Laboratory analysis method						
NIRS (hN__)	–4.0	(–0.7)	–4.2	(–0.8)	–3.6[a]	(–0.6)
Wet-laboratory (HW__)	–6.9	(–1.2)	–1.6	(–0.3)	–5.8	(–1.0)
Intercrossing method						
Open-pollination	–5.9	(–1.0)	–4.4	(–0.9)	–5.2[a]	(–0.9)
Polycrossing	–8.8	(–1.5)	–4.3	(–0.9)	–8.4	(–1.4)

[a,b] Linear response significantly different from zero, or means within a pair were significantly different at $P<0.05$ or 0.01

2. The forage yield means of the Cycle-1 divergent selections for NDF concentration and the original population were partitioned into two single-degree-of-freedom contrast effects: divergence=(C1High–C1Low)/2, a measure of response to selection for low NDF per se, and asymmetry=(C1High+C1Low)/2–C0, a measure of the effect of inbreeding. The asymmetry contrast is based on the facts that selection intensity and population size were constant in the high and low directions, and that a uniform reduction in forage yield in both directions would be due entirely to the effects of inbreeding.

Theoretical inbreeding coefficients of each cycle were computed according to Han and Casler (1999), assuming $F=0$ for the C0 population.

Results and discussion

There was significant spatial variation, which the experimental design could not account for, at each location. Nearest-neighbor analysis had relative efficiencies ranging from 115 to 149% compared to the split-plot analysis. Therefore, all population means were adjusted for spatial variation using nearest neighbor analysis.

Population×location interactions were significant ($P<0.05$) for many NDF selection responses, but not for the other variables. These interactions for NDF usually involved changes in the magnitude of selection responses among locations for a given selection method (see Table 1). They rarely involved changes in the direction of selection responses among locations or in the ranking of selection responses for the six selection methods. Therefore, because results and conclusions were similar across locations, all results are presented as means over locations. This lack of biologically meaningful genotype x location interaction is similar to observations from numerous previous studies on forage nutritive value traits of smooth bromegrass (Casler and Vogel 1999).

Neutral detergent fiber (NDF) concentration

Selection for reduced NDF concentration, using unreplicated spaced plants sampled at a single point in time, resulted in an average reduction of –6.8 g kg^{-1} cycle^{-1} in the NDF concentration of sward plots (Table 2). This response was almost double for reproductive growth (–7.4 g kg^{-1} cycle^{-1}) compared to regrowth (–4.3 g kg^{-1} cycle^{-1}). These responses are about 40% as high as the progress achieved in a single cycle of selection in reed canarygrass, *P. arundinacea* L. (Surprenant et al. 1988).

Selection for reduced NDF concentration was more effective when conducted at the vegetative growth stage, with responses 107, 127, and 134% greater than at the heading growth stage for reproductive growth, regrowth, and seasonal growth, respectively (Table 2). Realized heritability was approximately twice as high at the vegetative growth stage compared to the heading growth stage, most likely because of morphological heterogeneity and extreme sampling variation at heading (Casler 1999). The higher realized heritability of the vegetative growth stage apparently gave the vegetative growth stage a considerable advantage over the heading growth stage.

The greater efficiency of selection at the vegetative growth stage, regardless of the evaluation growth stage, suggests extremely high genetic correlations between growth stages for smooth bromegrass sward plots. This is similar to observations made from previous experi-

ments (Reich and Casler 1985; Ehlke et al. 1986). However, it is contradictory to results from spaced-plant evaluations of these selected populations, which suggest that selection for a particular target growth stage was most effective when conducted at that growth stage (Casler 1999). The dense tillering and intergenotypic competition unique to sward plots appeared to moderate changes in genotypic expression among growth stages, maintaining relatively constant population ranking and high genotypic correlations among growth stages as observed empirically for sward plots (Reich and Casler 1985; Ehlke et al. 1986).

The consistency of selection responses across years, locations, and growth stages suggests that the use of unreplicated selection units is extremely efficient for the NDF concentration of smooth bromegrass. Ehlke et al. (1986) used a 3-year mean IVDMD as their selection criterion in WBHD smooth bromegrass. When expressed as a percentage of the population mean, realized gains ranged from 0.2 to 2.5% cycle^{-1} for Ehlke et al. (1986) and 0.2 to 2.3% cycle^{-1} for the current study. The similarity of responses for these two studies indirectly suggests that sampling for multiple years during selection may not be cost-effective in smooth bromegrass. Exceptions to this generalization would be traits with known or suspected low heritability, such as lignin and ferulic acid concentration (Jung and Casler 1990), or unknown heritability, such as a particle-size reduction index (Casler et al. 1996).

There were no large differences in selection response between NIRS and wet-laboratory selection methods at the heading growth stage (Table 2). The NIRS procedure tended to give lower responses, except for the polycross methods measured on regrowth. These results are similar to those observed on spaced plants (Casler 1999) and can generally be explained by the 1-r^2 reduction in phenotypic variance associated with the multiple regression prediction of NDF from reflectance spectra. Because selection response is partly a function of the phenotypic standard deviation, reduced phenotypic variation can reduce selection responses (Falconer and Mackay 1996).

Overall, NIRS selection was only 62% as effective as wet-laboratory selection at the heading growth stage, a difference that was remarkably consistent for both open-pollination and polycrossing methods of intermating (Table 2). The fact that this trend was not observed for regrowth is irrelevant because regrowth made up 46% of the average annual smooth bromegrass hay crop and because selection at the heading growth stage was relatively ineffective at reducing regrowth NDF per se. Thus, if selection is to be practiced at the heading growth stage, it should be done using strictly wet-laboratory techniques or an improved NIRS technique. Recent developments in software, hardware, and calibration and validation methods may improve the efficiency of NIRS-based selection methods relative to those used in this study between 1985 and 1990. Nevertheless, calibration and validation statistics, as well as genetic correlation coefficients between NIRS and wet-laboratory estimates of NDF, generally indicated a good agreement between NIRS and wet-laboratory methods (Casler 1999). Thus, the apparent disadvantage of NIRS at the heading growth stage may derive simply from its inherent loss of phenotypic variance, which is equal to 1-r^2 where r=the simple correlation coefficient between NIRS and wet-laboratory NDF concentration, and/or a greater sensitivity of NIRS to the extreme morphological variability at the heading growth stage. The NIRS selection method at the heading growth stage could probably be made more efficient by replication of selection units in either space or time.

The three polycrossing methods were included to measure the effect of replicated polycrossing vs open-pollination because of the known lack of random pollination in smooth bromegrass (Hittle 1954; Knowles 1969). Polycrossing resulted in a 49% increase in selection response for reproductive growth, mixed results for regrowth, and a 41% increase in selection response overall (Table 2). The improvements due to polycrossing for the vNP3 vs vNB2 methods were due to more complete pollination among selected individuals within polycross blocks vs in situ crossing blocks. For the heading-stage selection methods, quantitative genetic theory would predict polycrossing to be twice as effective as open-pollination due to selection practiced among both female and male gametes. Over all harvests, polycrossing averaged 94% greater in selection response than open-pollination without selection of males, nearly equal to its theoretical advantage. However, because the polycrossing methods required an additional year per cycle, for a 50% increase in time, they were not always cost effective. On a per-year basis, vNP3 was 11% less effective than vNB2, but hNP3 and hWP3 were 33 and 25% more effective, respectively, than hNU2 and hWU2. Thus, selection of male gametes, which offers a theoretical doubling of selection response, was cost effective, while more effective interpollination of selections was not quite cost effective. Nevertheless, for this slight reduction in selection response, the vNP3 method will most likely be more effective in longer-term selection experiments than the vNB2 method. Furthermore, the vNB2 method has one major operational disadvantage: it requires considerable field and laboratory work during May, which is typically the busiest month for other activities in the breeding program.

In vitro digestibility

Selection for reduced NDF concentration led to an overall increase in IVFD of 5.8 g kg^{-1} cycle^{-1} for reproductive growth (P<0.01) and 3.6 g kg^{-1} cycle^{-1} overall (P<0.05), but no change in IVFD of regrowth (Table 3). For individual selection methods, only vNP3 had a significant (P<0.05) correlated selection response for IVFD, with a 15.6 g kg^{-1} cycle^{-1} increase for reproductive growth and a 12.6 g kg^{-1} cycle^{-1} increase overall (Table 3). For this selection method, the increase in IVFD occurred in nearly a 1:1 ratio to the decrease in

Table 3 Linear responses to selection, measured as in vitro fiber digestibility (IVFD), following each of the six phenotypic recurrent selection methods for reduced NDF (neutral detergent fiber) concentration

Selection method	Growth stage				Seasonal weighted mean	
	Reproductive		Regrowth			
	g kg^{-1} cycle^{-1} (% cycle^{-1})					
vNB2	4.9	(0.8)	–3.0	(–0.6)	4.9	(0.9)
hNU2	7.4	(1.3)	–1.2	(–0.2)	3.7	(0.6)
hWU2	5.8	(1.0)	–5.5	(–1.0)	2.6	(0.5)
vNP3	15.6[a]	(2.7)	5.6	(1.0)	12.6[a]	(2.2)
hNP3	5.3	(0.9)	4.6	(0.9)	5.7	(1.0)
hWP3	–4.0	(–0.7)	–11.2	(–2.1)	–7.8	(–1.4)
Mean	5.8[a]	(1.0)	–1.8	(–0.3)	3.6[a]	(0.6)
Selection growth stage						
Vegetative	10.2	(1.8)	1.3	(0.2)	8.8	(1.6)
Heading	3.6	(0.6)	–3.3	(–0.6)	1.1	(0.2)
Laboratory analysis method						
NIRS (hN__)	6.3	(1.1)	1.7	(0.3)	4.7	(0.8)
Wet-laboratory (HW__)	0.9	(0.2)	–8.3	(–1.6)	–2.6	(–0.4)
Intercrossing method						
Open-pollination	6.0	(1.0)	–3.2	(–0.6)	3.7	(0.7)
Polycrossing	5.6	(1.0)	–0.4	(–0.1)	3.5	(0.6)

[a] Linear response significantly different from zero, or means within a pair were significantly different at $P<0.05$

NDF concentration (Table 2). Thus, genetically reduced NDF concentration may lead to greater availability of that fiber to digestion by ruminant microbes. However, because this correlated response was observed in only one of four selection methods for which NDF responded to selection, it does not appear to be a general mechanism for increasing IVFD.

Selection for reduced NDF concentration led to an increase in IVDMD for all six selection methods, ranging from 3.7 to 9.1 g kg^{-1} cycle^{-1} for reproductive growth and from 3.0 to 7.5 g kg^{-1} cycle^{-1} overall (Table 4). Polycrossing and wet-laboratory evaluation led to greater responses in IVDMD than open-pollination and NIRS, respectively. Only one of the six selection methods showed a signficant response for regrowth.

On average, each 1-unit reduction in NDF concentration led to a 0.7-unit increase in IVDMD. Combined with the general lack of response in IVFD, this suggests that the increases in IVDMD were an artifact of dilution. Low-fiber populations were higher in IVDMD simply because they contained less relatively indigestible fiber, not because of any changes in the digestibility of that fiber. These results suggest that NDF concentration and digestibilty are independent genetic traits. Any improvement in voluntary intake due to this selection program should result from increased bulk density of the forage, increased ease of mastication and/or comminution, and/or more rapid particle size breakdown associated with reduced NDF concentration (Van Soest 1994; Culvenor and Casler 1999). While the ruminant may not derive more energy per unit of dry matter from these reduced-fiber forages, it should be able to increase its consumption. These hypotheses must be tested in feeding trials.

Forage yield

The simple correlation coefficient between NDF concentration and forage yield was low (r=–0.02). Therefore, the analysis of covariance of forage yield, using NDF as the covariate, resulted in very little adjustment to population means and gave nearly identical results to the forage yield analysis of variance per se. The phenotypic correlation coefficient between NDF concentration and forage yield changed very little following adjustment of forage yields by NDF (from r=0.53 for unadjusted yield to r=0.47 for adjusted yield). Indeed, the genotypic correlation coefficient between unadjusted forage yield and NDF concentration was 0.92±0.05, indicating a strong genetic relationship between the two variables (excluding all environmental and population×environment interaction effects). Unfortunately, the high genotypic correlation coefficient does not help one to understand the nature of this apparent relationship. Forage yield was expected to decrease with selection for reduced NDF, either by correlated response, inbreeding, or both. This high genotypic correlation simply tells us that our expectation was realized. Adjusted mean forage yield was used in all subsequent analyses.

Recurrent selection resulted in an average reduction of forage yield of –0.15 Mg ha^{-1} cycle^{-1} (Table 5). This overall response was significant ($P<0.01$) as were the responses for four of six selection methods ($P<0.05$). All six methods led to a numerical reduction in forage yield, ranging from –0.05 to –0.22 Mg ha^{-1} cycle^{-1} (–0.6 to –2.5% cycle^{-1}). Variation in forage yield selection response among the six methods was generally not related to theoretical inbreeding rates. Furthermore, because

Table 4. Linear responses to selection, measured as in vitro dry matter digestibility (IVDMD), following each of the six phenotypic recurrent selection methods for reduced NDF (neutral detergent fiber) concentration

Selection method	Growth stage				Seasonal weighted mean	
	Reproductive		Regrowth			
	g kg^{-1} cycle^{-1} (% cycle^{-1})					
vNB2	3.8[a]	(0.6)	1.3	(0.2)	3.4[b]	(0.5)
hNU2	4.6[b]	(0.7)	1.2	(0.2)	3.2[a]	(0.5)
hWU2	3.7[a]	(0.6)	0.9	(0.1)	3.0[a]	(0.5)
vNP3	7.9[b]	(1.2)	–1.0	(–0.2)	5.7[b]	(0.9)
hNP3	5.7[a]	(0.9)	3.5	(0.5)	5.2[a]	(0.8)
hWP3	9.1[b]	(1.4)	4.4	(0.7)	7.5[b]	(1.2)
Mean	5.8[b]	(0.9)	1.7	(0.3)	4.6[b]	(0.7)
Selection growth stage						
Vegetative	5.8	(0.9)	0.1	(0.0)	4.6	(0.7)
Heading	5.8	(0.9)	2.5	(0.4)	4.7	(0.7)
Laboratory analysis method						
NIRS (hN__)	4.1[a]	(0.6)	1.0	(0.1)	3.1[a]	(0.5)
Wet-laboratory (HW__)	7.4	(1.1)	4.0	(0.6)	6.3	(1.0)
Intercrossing method						
Open-pollination	4.0[a]	(0.6)	1.1	(0.1)	3.2[a]	(0.5)
Polycrossing	7.6	(1.2)	2.3	(0.4)	6.1	(1.0)

[a,b] Linear response significantly different from zero, or means within a pair were significantly different at $P<0.05$ or 0.01

Table 5 Theoretical inbreeding coefficients and linear responses to selection, measured as forage yield, following each of the six phenotypic recurrent selection methods for reduced NDF (neutral detergent fiber) concentration

Selection method	Theoretical inbreeding for cycle			Selection response	
	1	2	3		
				Mg ha^{-1} cycle^{-1} (% cycle^{-1})	
vNB2	0.0143	0.0284	0.0422	–0.22[b]	(–2.5)
hNU2	0.0015	0.0061	0.0107	–0.14[b]	(–1.6)
hWU2	0.0015	0.0061	0.0107	–0.21[b]	(–2.5)
vNP3	0.0143	0.0284	0.0422	–0.18[a]	(–2.2)
hNP3	0.0143	0.0284	0.0422	–0.05	(–0.6)
hWP3	0.0143	0.0284	0.0422	–0.09	(–1.0)
Mean				–0.15[b]	(–1.7)
Selection growth stage					
Vegetative				–0.20[c]	(–2.4)
Heading				–0.12	(–1.4)
Laboratory analysis method					
NIRS (hN__)				–0.09	(–1.1)
Wet-laboratory (HW__)				–0.15	(–1.8)
Intercrossing method					
Open-pollination				–0.19[c]	(–2.2)
Polycrossing				–0.11	(–1.3)

[a,b,c] Linear response significantly different from zero, or means within a pair were significantly different at $P<0.10$, 0.05, or 0.01.

population means for forage yield were theoretically adjusted to a constant NDF concentration, these selection responses should be independent of selection responses for NDF concentration per se. A plot of overall selection responses for forage yield and NDF concentration revealed this assumption to be nearly true (Fig. 1). Thus, little of the variation in forage yield selection responses can be attributed to correlation with NDF selection responses (i.e., a genetic correlation between forage yield and NDF concentration). The high genotypic correlation observed for these populations appeared to be at least partly due to the effects of inbreeding coincidentally reducing forage yield for all selection methods. However, average selection responses for forage yield were not re-

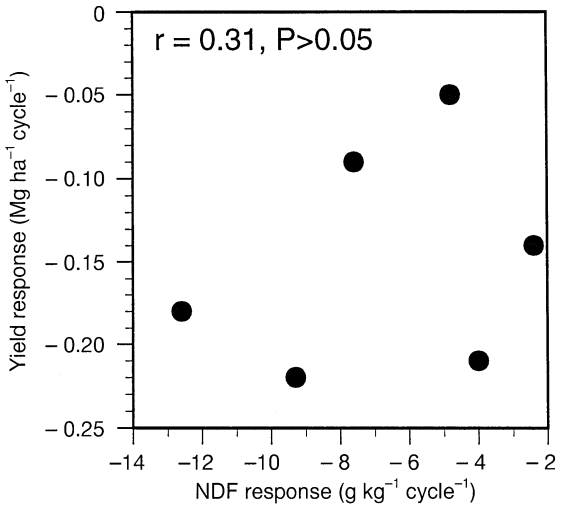

Fig. 1 Scatterplot of observed selection responses for mean NDF (neutral detergent fiber) concentration and forage yield for six selection methods

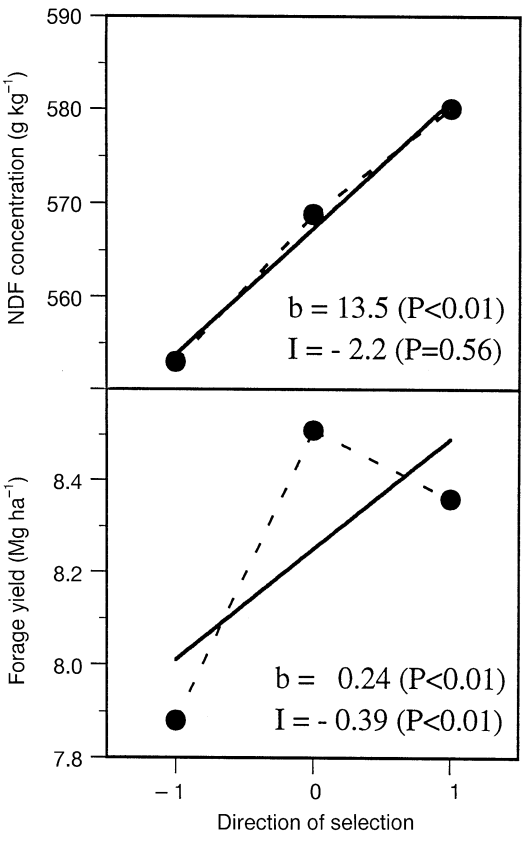

Fig. 2 Effect of one cycle of divergent selection for high (+1) or low (–1) NDF (neutral detergent fiber) concentration on NDF and forage yield. Selection response is partitioned into two single-degree-of-freedom components: b=selection per se (*solid lines*) and I=inbreeding (*dashed lines*)

lated to theoretical inbreeding coefficients (Table 5). Because differences in theoretical inbreeding rate could explain little of the variation in forage yield selection responses among methods, there is most likely a true genetic correlation between NDF and forage yield. However, this correlation is probably considerably lower than the 0.92 observed in this study.

The divergent selections made in Cycle 1 allowed an independent assessment of the relative importance of correlated response vs inbreeding effects on forage yield (Fig. 2). For NDF concentration, the selection criterion, the effect of selection was 6.1-times greater than the effect of inbreeding, which was not significant (P>0.05). Furthermore, the linear selection response, due to change in allele frequency, accounted for 99% of the variation among the three populations. Conversely, for forage yield, the effect of inbreeding was 1.6-times greater than the effect of selection per se and both were significant (P<0.01). The effect of inbreeding accounted for 47% of the variation among the three populations. The theoretical inbreeding coefficient of the divergent populations was $F={}^{1}/_{2(10)}$=0.05 compared to an average inbreeding depression of 4.6%. This nearly 1:1 ratio of inbreeding depression to theoretical inbreeding is similar to the average inbreeding depression rates of Wilsie et al. (1952) and the most severely affected clones of Hawk and Wilsie (1952) for smooth bromegrass.

The importance of inbreeding in regulating forage yield responses raises a fundamental issue in breeding for reduced fiber concentration. Must reduced fiber concentration be accompanied by reduced forage yield? The results of this study imply that there is a much smaller causal genetic relationship between forage yield and NDF concentration than empirical correlations would suggest. Thus, it should be possible to reduce NDF concentration without reducing forage yield or else with a considerably smaller reduction that the average of –0.15 Mg ha^{-1} cycle^{-1} observed herein. How might this be accomplished? First and most obvious would be to increase the effective population size during selection, so reducing F. The disadvantage of this approach is that it either becomes more expensive to maintain a high selection intensity or less efficient if selection intensity is relaxed.

A second approach would be to practice simultaneous selection for low NDF concentration and high forage yield. This would involve considerably more work in smooth bromegrass because forage yield is not amenable to selection based on spaced plants (Carpenter and Casler 1990). Furthermore, positive genetic correlations between sward-plot forage yield and NDF concentration in a random-mating population suggest an association between these two traits that may not be present in spaced plants (Casler et al. 1990). These authors speculated that lack of correlated responses of forage yield to selection for reduced NDF concentration on spaced plants, observed by Carpenter and Casler (1990), may have been due simply to the low heritability of spaced-plant forage yield. Many breeding programs routinely incorporate relatively intense selection for forage yield or vigor into their selection protocols for high nutritive-value traits (Coors et al. 1986; Surprenant et al.

1988; Hopkins et al. 1993). For alfalfa (*Medicago sativa* L.) and switchgrass (*Panicum virgatum* L.), the lack of forage yield responses to selection for high forage nutritive value may be due to this selection pressure (Coors et al. 1986; Hopkins et al. 1993). This suggests that concomitant selection pressure for yield or vigor may, in the short-term, counteract the effects of inbreeding. If there is a true genetic correlation between forage yield and NDF, simultaneous selection pressure for high vigor and low NDF will reduce realized gains for reduced NDF compared to selection ignoring vigor (Hopkins et al. 1993). In reed canarygrass, one cycle of selection for low NDF led to a mean NDF reduction of 2.1% and a mean forage yield reduction of 6.0% (Surprenant et al. 1988). Simultaneous selection pressure for high forage yield was ineffective in the low-NDF direction, but increased forage yield by 9.4% in the high-NDF direction. Thus, in this reed canarygrass population, forage yield and NDF appeared to have a moderate to high positive genetic correlation, which appeared to be more important than inbreeding in regulating selection responses.

Finally, chance hybrids or strain crosses may be the most practical effective solution. This would involve simultaneous selection for reduced NDF concentration in genetically diverse populations, followed by strain crossing of the low-fiber populations. If the original populations represent different germplasms or origins, there is a good likelihood of forage yield restoration due to chance hybridization and heterosis (Bingham et al. 1994). The greater unknown becomes: what happens to NDF concentration in the hybrid population? If NDF is not affected by inbreeding per se, as was shown for the divergently selected populations, then it should not be affected by heterosis per se. Hybridization by strain crossing should restore hybrid vigor for forage yield without affecting NDF concentration if the parent populations share similar low-NDF genes. Conversely, if parent populations possess different low-NDF genes, then strain crossing has the potential to further reduce NDF concentration per se by transgressive segregation for low NDF due to new combinations of low-NDF genes.

This proposed method should not be considered lightly. At most, inbreeding appears to account for half of the variation among populations in forage yield, with genetic correlation between NDF and forage yield accounting for an equivalent amount. Thus, strain crossing to restore hybrid vigor may partially negate the progress made in selecting for low NDF, due to the apparent moderate and positive genetic correlation between NDF and forage yield. Strain crossing should not be attempted until sufficient progress has been achieved that any increase in NDF due to heterosis does not negate the progress achieved during recurrent selection. At the present time, it is impossible to predict either the likelihood or the magnitude of these potential responses.

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