RALLF Forage Quality

preliminary report

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To do

Validate the predicted forage quality parameters with wet chemistry. When we predicted NDFD for IWG in FIG the values were reasonable (50%), but for alfalfa they are unreasonable (16%). We need to consult with a lab familiar with NIRS for both conventional and reduced lignin alfalfa.

Put in dates of harvests.

Look at predicted lignin from NIRS?

Investigate the 3rd cut in st paul 2022; was it somehow switched between 35 and 45 day intensity?

Expected results

Alfalfa varieties (HarvXtra) will have lower lignin content and higher NDFD than conventional varieties. The RFV should be higher for HarvXtra varieties.

Alfalfa with lower intervals between cuttings (35-day vs 45-day) will have higher forage quality (RFV, CP).

Discussion topics

RFV vs. RFQ

If we are only making comparisons among alfalfa cuttings, let's use RFV not RFQ. RFQ relies on NDFD, which is much harder to predict with NIRS. There is lot of variability in NDFD in wet chemsity due to variability in rumen fluid composition from donor cows, this variability extends into the NIRS database for NDFD, which reduces the accuracy of the NIRS predictions. RFQ is useful when comparing between mixtures of legumes and grasses, but since we are only making comparisons between alfalfa cuttings, RFV allows us to rely on just ADF and NDF, which NIRS performs better at predicting.

NIRS predictions

Do we trust the predicted values of the NIRS for this experiment?

Is the hay equation sufficient for making comparisons between HarvXtra vs. conventional alfalfa forage quality?

Why is the RFV prediction so high for alfalfa? Did the hail storm in May2022 impact forage quality? Perhaps people put more leaves in the forage quality subsample then stems, causing much higher RFV.

Why is neutral detergent digestibility so low? We need to be skeptical of RFQ so long as we are skeptical of NDFD.

Why is variance so low in the NIRS prediction? Is there an issue with how data is being read in or processed causing pseudoreplication?

Data availability

Click Me for Github Repository *https://github.com/jpukabeals/rallf*

Data we have

Table 1: NIRS data

year	site	intensity	cut	n	n_missing
2021	st paul	35-day	1	24	0
2022	st paul	35-day	1	24	0
2021	rosemount	35-day	1	24	0
2022	rosemount	35-day	1	0	24
2021	st paul	45-day	1	24	0
2022	st paul	45-day	1	24	0
2021	rosemount	45-day	1	24	0
2022	rosemount	45-day	1	0	24
2021	st paul	35-day	2	24	0
2022	st paul	35-day	2	24	0
2021	rosemount	35-day	2	24	0
2022	rosemount	35-day	2	24	0
2021	st paul	45-day	2	23	1
2022	st paul	45-day	2	24	0
2021	rosemount	45-day	2	24	0
2022	rosemount	45-day	2	24	0
2021	st paul	35-day	3	24	0
2022	st paul	35-day	3	24	0
2021	rosemount	35-day	3	24	0
2022	rosemount	35-day	3	24	0
2022	st paul	45-day	3	24	0
2022	rosemount	45-day	3	24	0
2022	st paul	35-day	4	24	0
2022	rosemount	35-day	4	24	0

Table 2: NIRS data

year	site	intensity	cut	n	n_missing
2022	rosemount	35-day	1	0	24
2022	rosemount	45-day	1	0	24
2021	st paul	45-day	2	23	1

As seen in tables, we have missing $24 + 24 + 1 = 85$ data points. The two sets of 24 data points are for the first cut at rosemount in 2022, which have not been located as of 15Mar2023.

Predicting forage quality parameters with NIRS

We are predicting alfalfa forage quality parameters using near infrared scanning with a generalist equation designed for all hay. Some labs use an alfalfa equation and some labs have different alfalfa equations for conventional vs. reduced lignin alfalfa. All predictions should be validated with wet chemistry, though NIRS predictions without recent validation can still be useful in estimating relative differences between treatments.

Soil contamination can also cause issues with forage quality predictions.

Expected forage quality parameters for alfalfa were easy to obtain for CP ADF NDF and RFV, they were not easy to obtain for RFQ. In general, I found that RFQ can range from 50 to 250, with most data between 100 and 200 and an average of 150.

Table 3: Expected forage quality ranges from Dairyland Labs

Parameter	Median	Range
CP	20	14-26
ADF	31	20-43
NDF	38	35-52
Lignin	6	4-8

Table 4: Expected forage quality ranges from UMN, SDSU, Univeristy of Georgia and Dairyland

Maturity	Stage	Grade	CP	ADF	NDF	RFV	RFQ
all	all		14-26	20-43	25-56	92-200	80-260
early maturity	bud	premium	20-22	27-29	34-36	170-185	180-220
early to average maturity	early bloom	good	18-20	29-32	36-40	150-170	140-180
late maturity	full bloom	fair	16-18	32-35	40-44	130-150	80-140

Table 5: Observed vs. expected forage quality in RALLF

Forage Quality Parameter	Observed	Expected	Expected Range
CP	20	20	14-26
ADF	24	30	20-43
NDF	33	40	25-56
RFV	205	150	92-200
RFQ	139	150	80-260

Summary tables

Table 6: Forage quality by rep

Rep	CP	ADF	NDF	NDFD	RFV	RFQ
1	20	24	34	16	201	136
2	21	24	33	16	208	141
3	21	24	33	16	206	140
4	20	24	33	16	207	141

Table 7: Forage quality by site

Site	CP	ADF	NDF	NDFD	RFV	RFQ
rosemount	20	26	36	19	186	127
st paul	21	23	31	14	222	150

Table 8: Forage quality by year

Year	CP	ADF	NDF	NDFD	RFV	RFQ
2021	21	22	32	14	215	141
2022	20	25	34	18	198	138

Table 9: Forage quality by cutting

Cut	CP	ADF	NDF	NDFD	RFV	RFQ
1	21	20	29	11	240	155
2	21	26	36	19	186	129
3	20	24	33	17	207	144
4	20	27	37	19	175	120

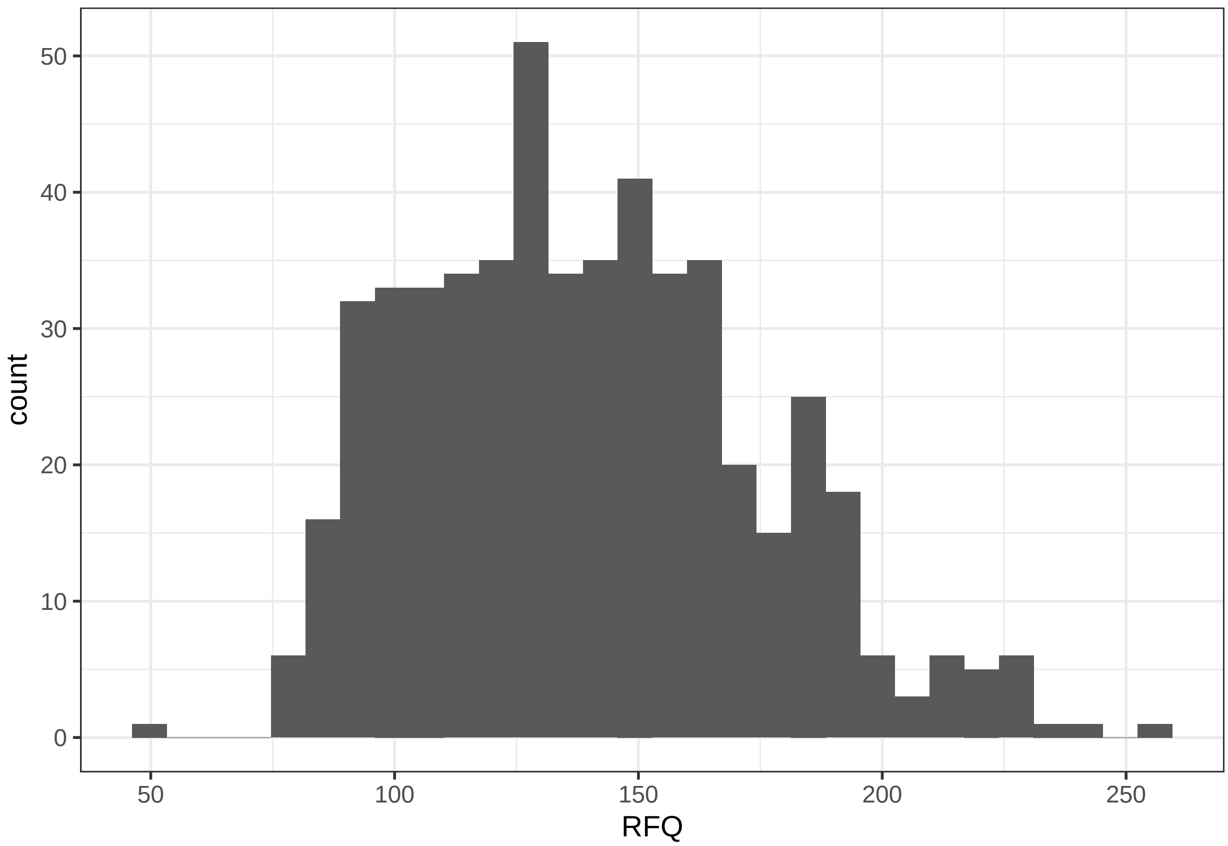
Table 10: Forage quality by harvest schedule

Intensity	CP	ADF	NDF	NDFD	RFV	RFQ
35-day	21	24	32	16	209	143
45-day	20	25	34	17	200	134

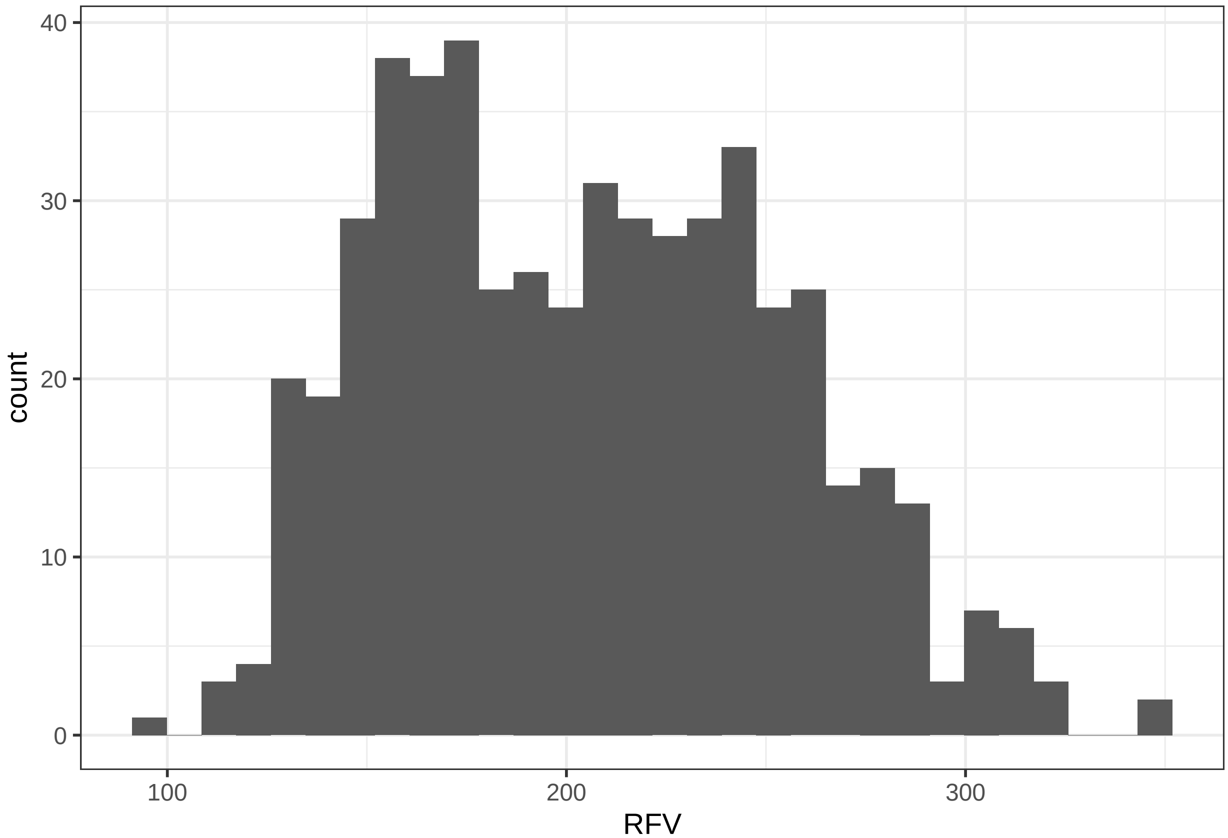
Table 11: Forage quality by variety

Variety	CP	ADF	NDF	NDFD	RFV	RFQ
HX3	21	23	32	14	215	143
HX4	20	24	33	16	204	137
HX5	20	24	34	16	200	133
RR3	20	24	33	17	208	144
RR4	20	25	34	18	202	140
RR5	20	24	33	17	204	140

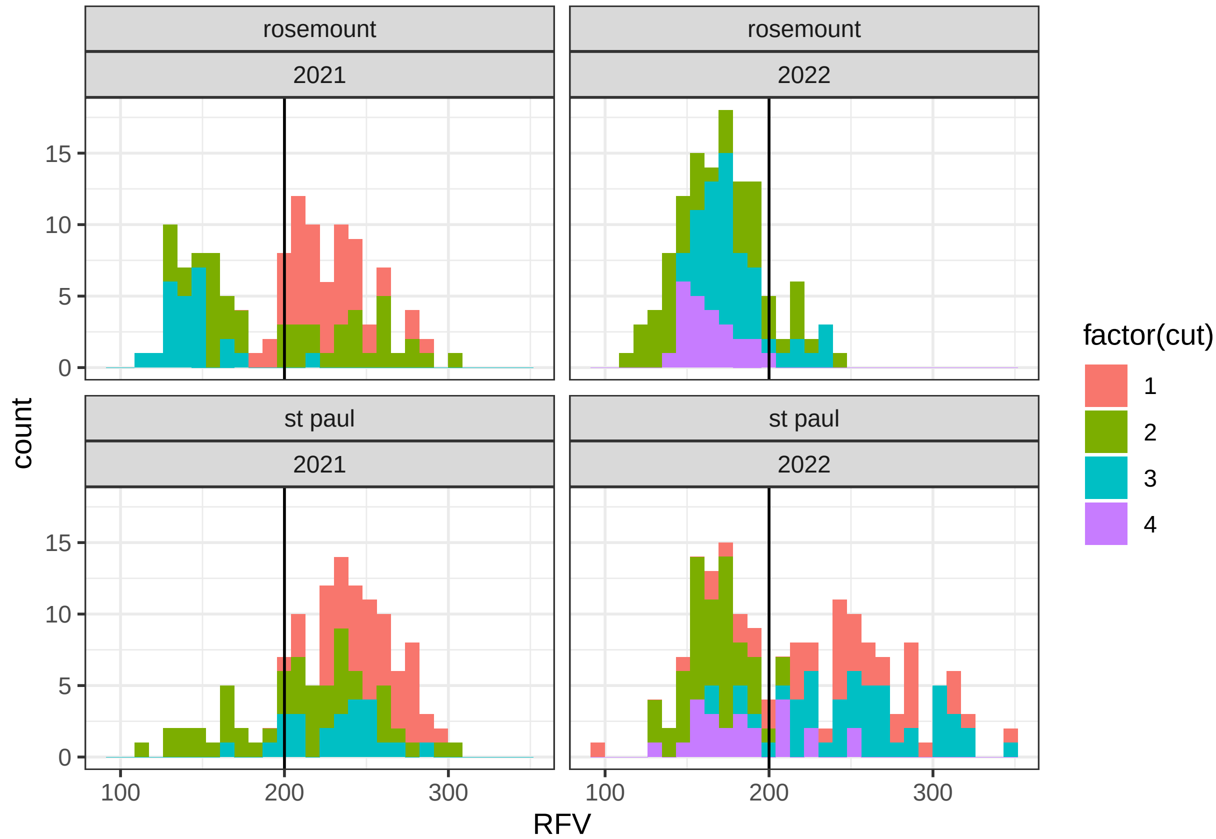
Histograms



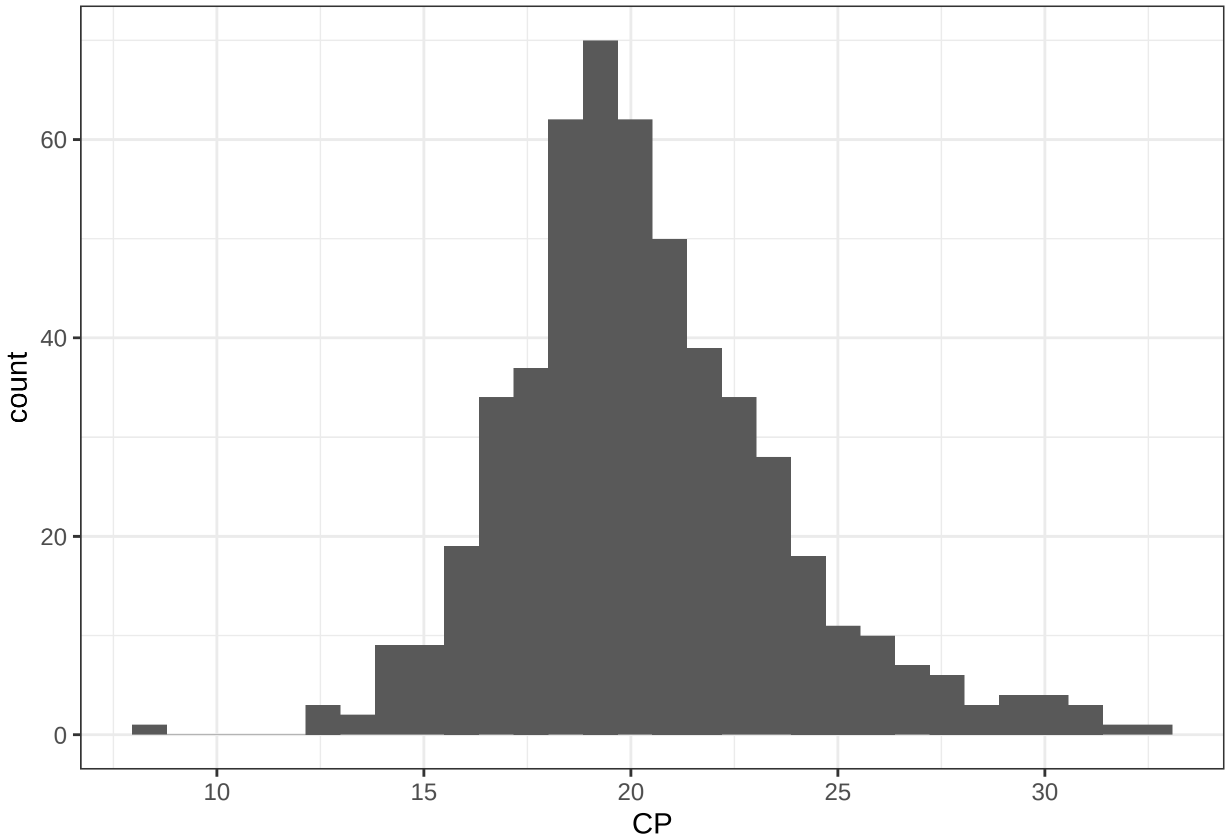
We expect an RFQ of around 150, we observed an RFQ of 139



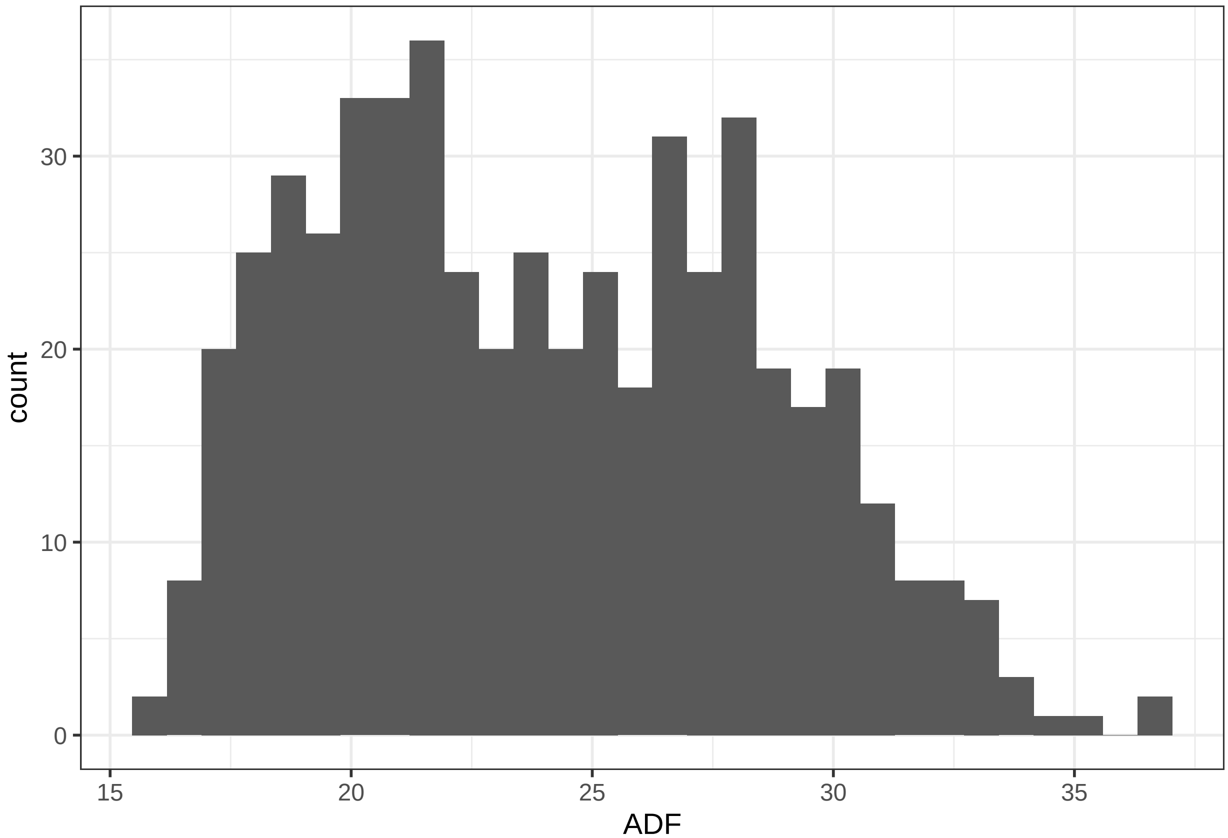
We expect an RFV of around 150, we observed an RFV of 205. Some samples were predicted to have an RFV greater than 300!



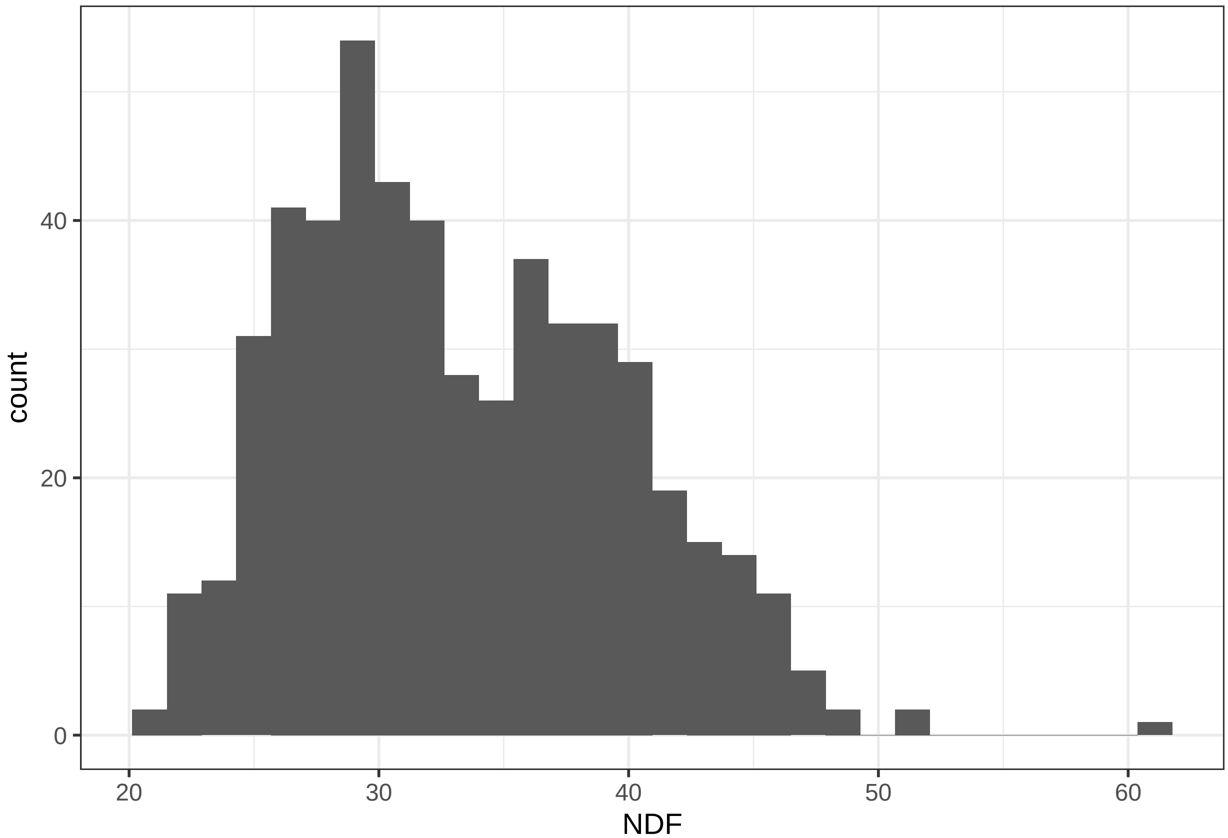
With the exception of Rosemount in 2022, all site-years had multiple cuts with predicted RFV over 200. First cuts tended to have the highest quality, which is expected.



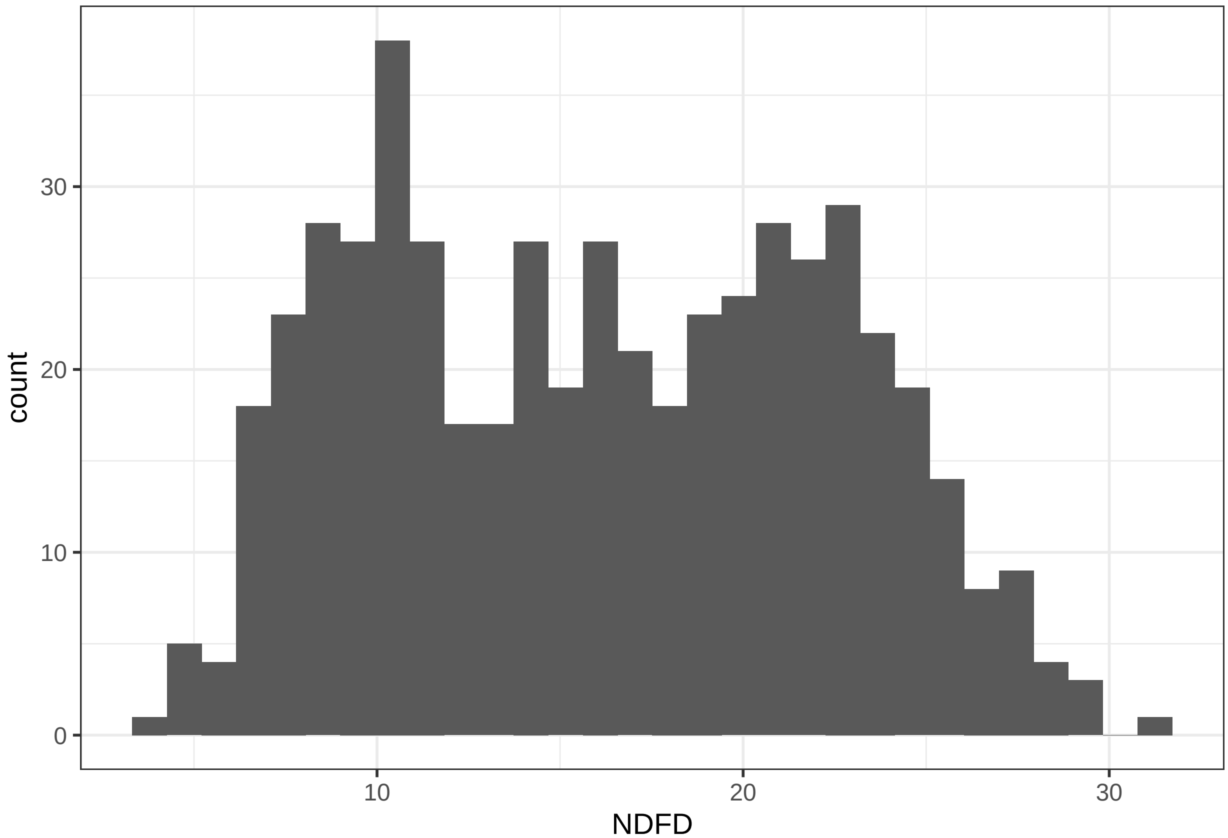
We expect a crude protein of around 20%, we observed a CP of 20



We expect an ADF of around 30%, we observed an ADF of 24



We expect an NDF of around 40%, we observed an NDF of 33



For neutral detergent digestibility over 48 hours, we expect values near 50%, we predicted values of 16

Analysis

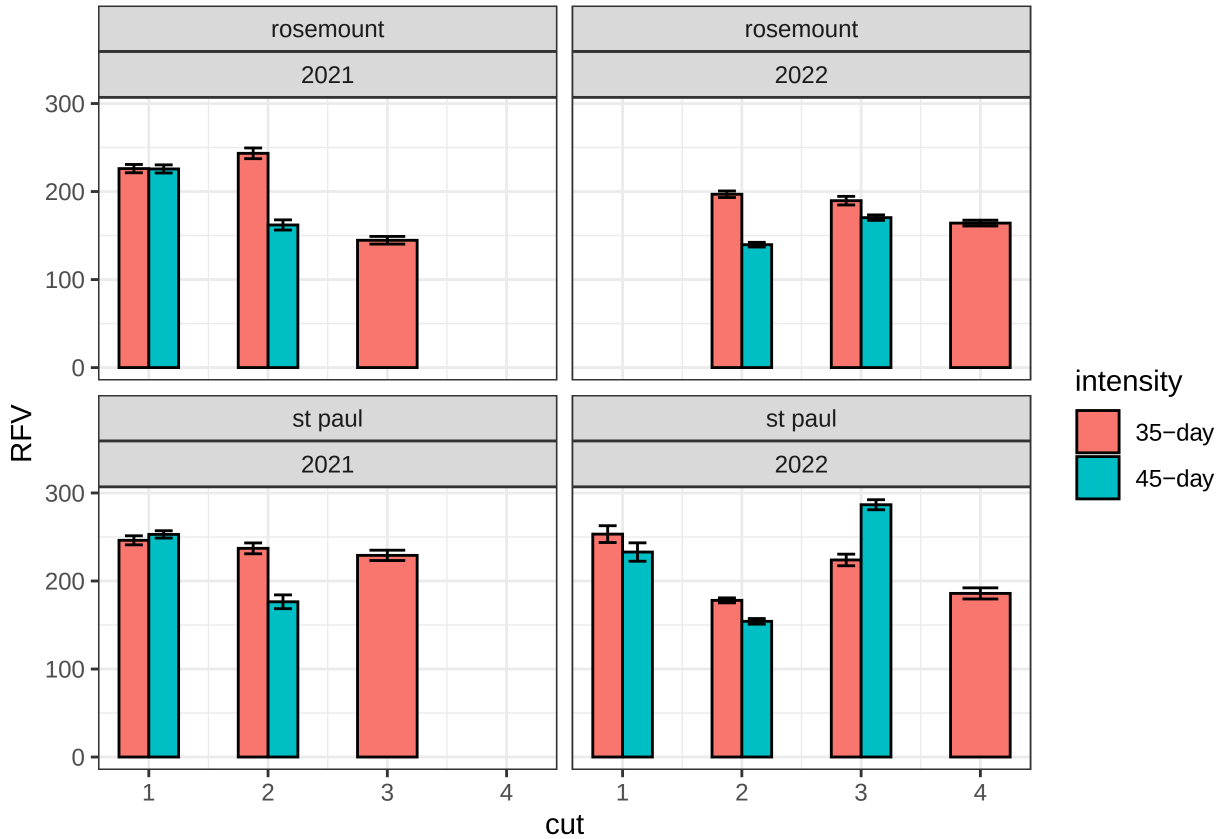
Relative feed value

Since relative feed value and relative forage quality are strongly correlated, the results of the analysis tend to be the same.

```r
dat1 %>%
  lm(
    rfv~site*year*variety*intensity*cut,.
  ) %>%
  anova() %>%
  filter(`Pr(>F)` < 0.05)
```

```
Analysis of Variance Table

Response: rfv
                    Df Sum Sq Mean Sq  F value    Pr(>F)    
site                 1 162579  162579 113.3777 < 2.2e-16 ***
year                 1  50422   50422  35.1625 6.226e-09 ***
intensity            1  10497   10497   7.3204  0.007088 ** 
cut                  1  78499   78499  54.7433 7.246e-13 ***
site:intensity       1  11603   11603   8.0915  0.004659 ** 
site:cut             1  25041   25041  17.4629 3.549e-05 ***
year:cut             1  51679   51679  36.0394 4.108e-09 ***
intensity:cut        1  10174   10174   7.0948  0.008021 ** 
site:year:intensity  1  28985   28985  20.2132 8.916e-06 ***
site:year:cut        1   8230    8230   5.7393  0.017015 *  
year:intensity:cut   1 108115  108115  75.3965 < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

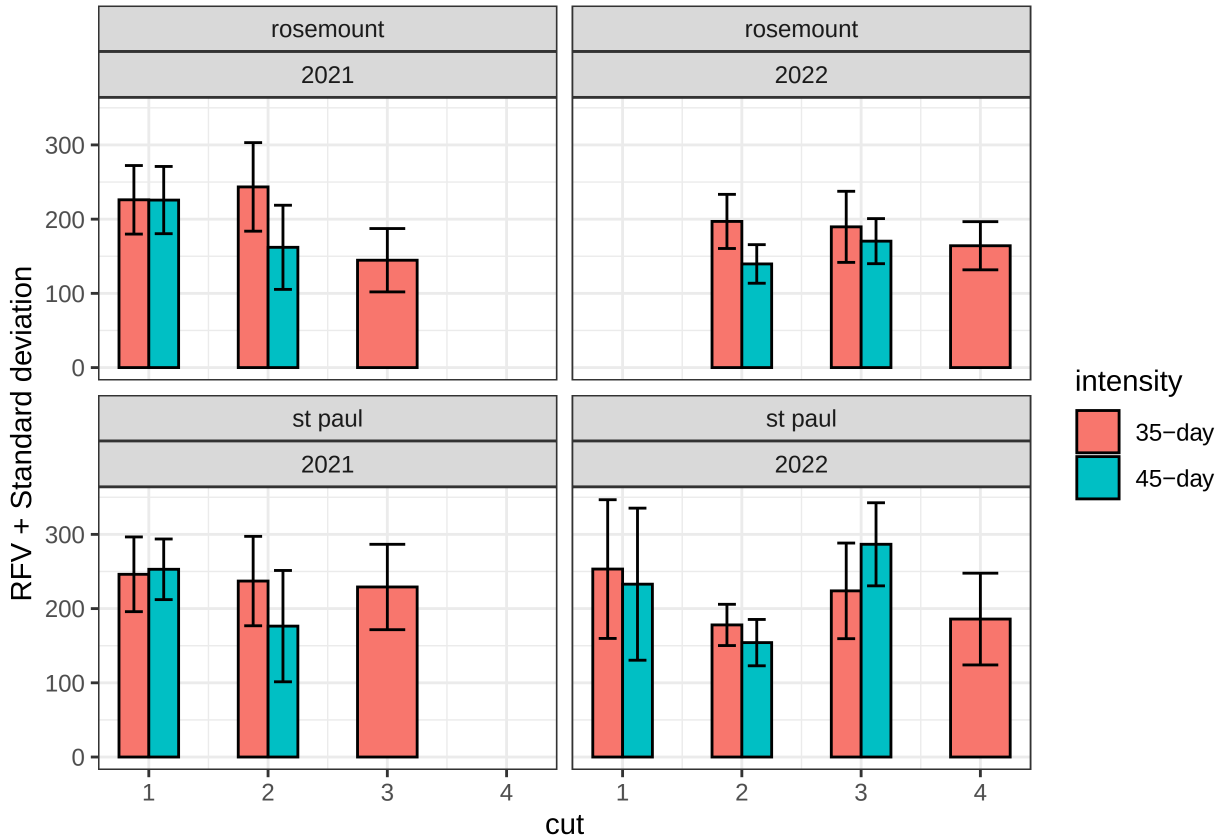
```r
# year:intensity:cut
# site:year:cut
# site:year:intensity

# no effect from variety
```



Similar pattern across years and sites; RFV is similar for the first cut and then the second cut the RFV is lower for the 45-day cuts than the 35-day cuts. The third cut at St Paul in 2022 is unexpected, what could explain this?

Why is variance so low? Standard error bars are barely visible.

Let's look at standard deviation instead of standard error



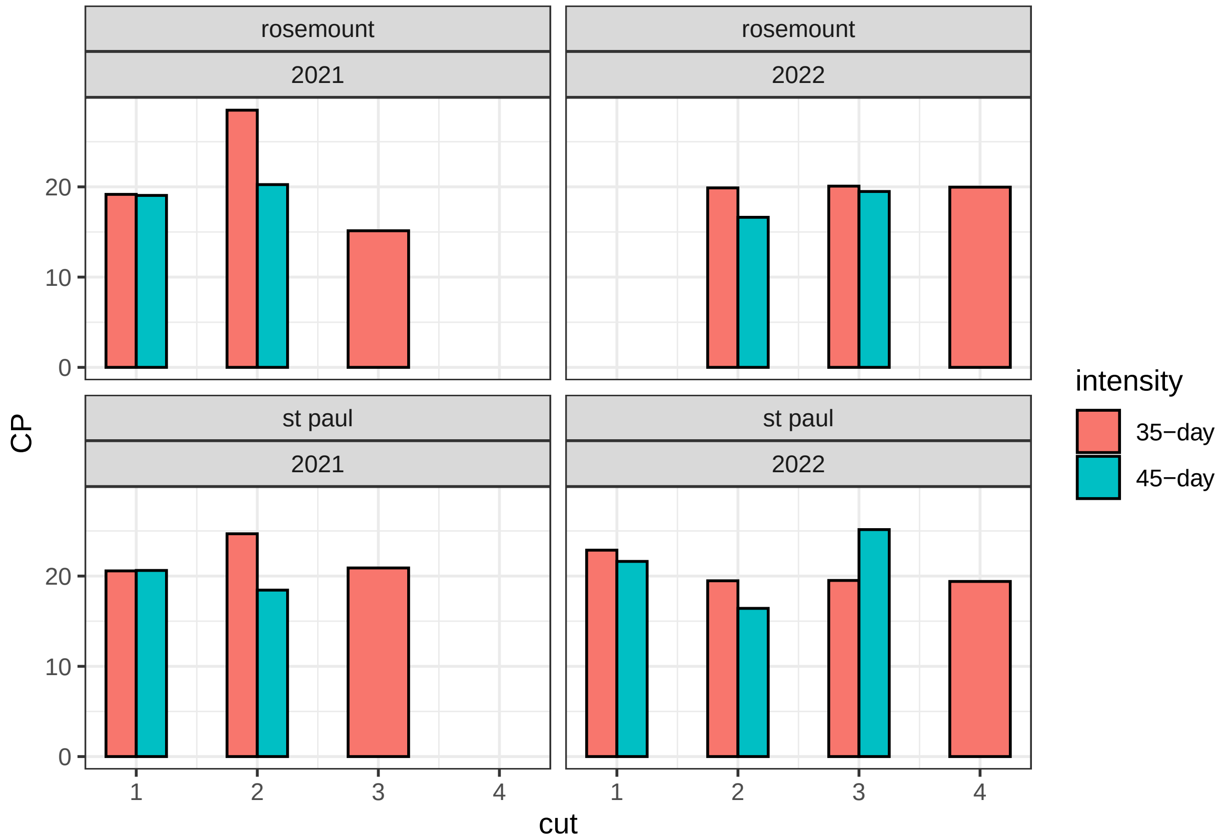
There is simply a large difference between standard error and standard deviation, likely because n() is larger than usual (n=24) so standard error really reduces the visual representation of variance.

Crude protein

```
dat1 %>%
  lm(
    CP~site*year*variety*intensity*cut,.
  ) %>%
  anova() %>%
  filter(`Pr(>F)` < 0.05)
```

```
Analysis of Variance Table

Response: CP
                        Df  Sum Sq Mean Sq F value    Pr(>F)
site                     1 130.903 130.903 12.7804 0.0003899 ***
year                     1  81.138  81.138  7.9218 0.0051081 **
intensity                1 136.190 136.190 13.2966 0.0002984 ***
year:intensity           1  65.898  65.898  6.4338 0.0115487 *
year:cut                 1  51.652  51.652  5.0429 0.0252331 *
intensity:cut            1 199.703 199.703 19.4976 1.275e-05 ***
site:year:intensity      1 180.747 180.747 17.6468 3.234e-05 ***
site:year:cut            1  78.997  78.997  7.7127 0.0057227 **
year:intensity:cut       1 106.183 106.183 10.3670 0.0013800 **
site:year:intensity:cut  1  87.771  87.771  8.5693 0.0035998 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# 4-way interaction

# no effect from variety
```



Similar pattern across years and sites, same as RFQ; CP is similar for the first cut and then the second cut the CP is lower for the 45-day cuts than the 35-day cuts.

Same as with RFQ, summarizing with standard error shows much less variation around means than standard deviation.

Diving deeper

Relative feed value and ADF

RFV is the only parameter where we are outside of our expected range. We expect around 150 and we are averaging 200. We have some RFV values over 300.

This may be due to an issue with the equation

Let's look at the RFV equation I used

```
function (nir_tidy_data)
{
    nir_tidy_data %>% mutate(DM = drymatter, CP = protein, NDF = ndf,
        NDFD = ndf48h, ADF = adf, EE = 2.05, FA = EE - 1, Ash = 100 -
            DM, NFC = 100 - ((0.93 * NDF) + CP + EE + Ash), NDFn = NDF *
            0.93, NDFDp = 22.7 + 0.664 * NDFD, TDN.legume = (NFC *
            0.98) + (CP * 0.93) + (FA * 0.97 * 2.25) + (NDFn *
            NDFD/100) - 7, DMI.legume = (120/NDF) + (NDFD - 45) *
            0.374/1350 * 100, rfq.legume = DMI.legume * TDN.legume/1.23,
        DDM.rfv = 88.9 - (0.779 * ADF), DMI.rfv = 120/NDF, rfv.sdsu = DDM.rfv *
            DMI.rfv/1.29)
}
```

So I used …

Digestible dry matter
$DDM.rfv = 88.9 - (0.779 * ADF)$

Dry matter intake
$DMI.rfv = 120/NDF$

Relative Forage Value
$RFV = (88.9 - (0.779 * ADF)) * (120/NDF)/1.29$

These equations are correct.

Let's look at the samples with the highest RFV values

Table 12: The samples with the highest estiamted relative feed value

site	year	intensity	cut	variety	rep	CP	ADF	NDF	NDFD	rfv	rfq.legume
st paul	2022	45-day	3	RR5	2	29	16	20	5	350	253
st paul	2022	35-day	1	HX3	2	24	16	21	6	343	241
st paul	2022	45-day	3	RR4	4	27	18	22	8	322	235
st paul	2022	45-day	1	HX5	2	23	16	22	9	320	218
st paul	2022	45-day	3	RR4	3	25	17	22	9	319	231

The forage samples with the highest RFV are from st paul in 2022. The ADF is 16% and the NDF is 20%. A forage sample with these values would have a relative feed value of 355, so the equation is not incorrect. The question is whether alfalfa can be this high quality.

Can an alfalfa sample be 16% ADF and 20% NDF?

It's notable that the crude protein is also very high, 29%, where we would normally expect 20%. This supports the idea that this was young vegetative leafy alfalfa.

It seems very possible that our predicted ADF and NDF values simply need a bias adjustment. If this is the case, they can still be used to determine relative differences between treatments, but will need a wet chemistry calibration before they can be used to determine the actual ADF or NDF values.

NDFD

Neutral detergent fiber digestibility estimates the amount of digestion that will occur within the rumen and this varies by ruminant. The most common durations are 24 hours for cattle, 30 hours for sheep goats and horses, 48 hours for swine. There are also very long digestions (72, 120 or 240), which are meant to determine how much fiber is indigestible.

We predict neutral detergent fiber digestibility over 48 hours because this is used for the RFQ equation (Undersander and Moore, 2002). We expect an NDFD range of 30-50% for alfalfa based on data from Dairyland and Forage Genetics International, but NDFD can range up to 70%(Hoffman et al., 2001).

Table 13: Expected lignin and NDFD values of different varieties and harvest schedules

Variety	Intensity	Lignin (%)	NDFD (%)
HarvXtra	45-day	5.0	40
HarvXtra	35-day	4.5	49
Conventional	45-day	6.5	35
Conventional	35-day	5.7	43

Table 14: Predicted NDFD values did not match with expected values

Variety	Intensity	NDFD_expected	NDFD_observed
HarvXtra	45-day	40	15.8
HarvXtra	35-day	49	14.9
Conventional	45-day	35	17.2
Conventional	35-day	43	17.0

We observed around 15% NDFD, which very low.

We expected as time between cuttings decreased (35-day harvest schedule), NDFD would increase, however we observed a decrease in NDFD for both the HarvXtra and Conventional varieties

We expected greater NDFD in HarvXtra vs conventional, but observed lower NDFD.

One possible explanation is that our NDFD prediction needs a new equation for alfalfa NDFD as it differs from grass NDFD.

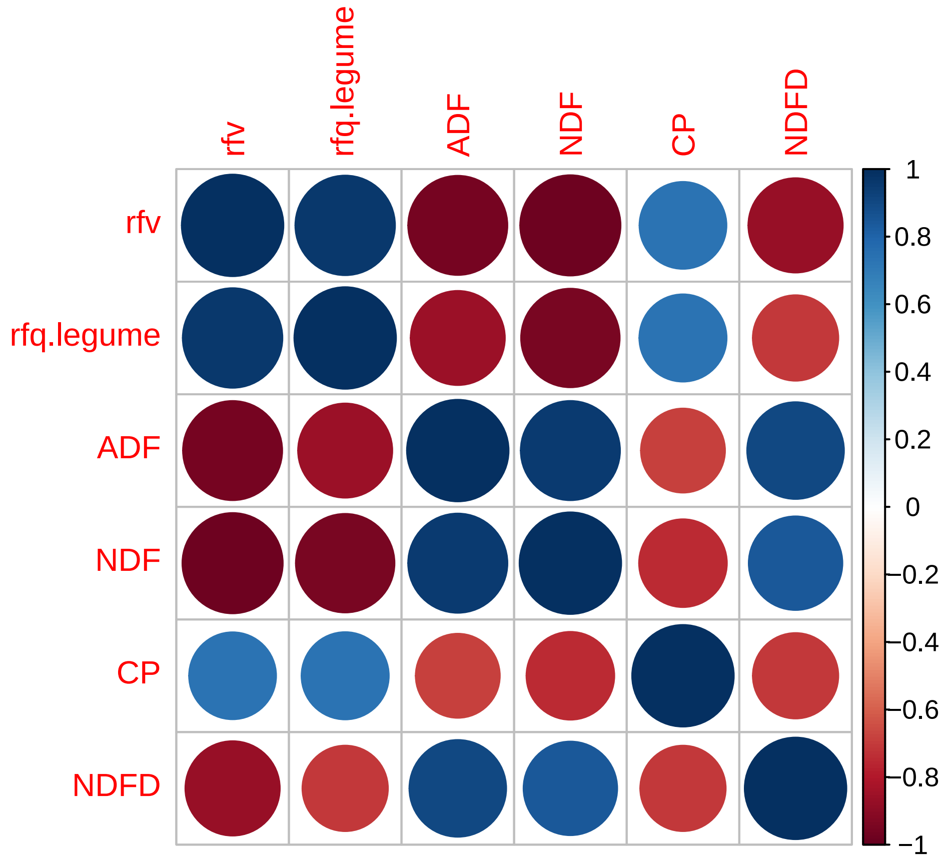
Another explanation is that we need a different equation for HarvXtra alfalfa vs. conventional alfalfa.

The predicted NIRS values for NDFD should be viewed with skepticism, and it likely cannot be fixed with a bias.

Table 15: Neutral detergent digestibility ranged from 4 to 31%. This is very low and should be validated with wet chemistry

max(NDFD)	min(NDFD)	median(NDFD)
32	4	16

Does the low NDFD values make the RFQ values lower, causing the RFQ and RFV to not be well correlated?



Yes, RFV and RFQ are strongly positively correlated as they should be.

Other forage quality parameters are correlated as expected with the exception of NDFD.

As forages mature, CP should decrease and NDFD should decrease, therefore they should be positively correlated, but instead they are negatively correlated. Furthermore, as NDFD increases RFQ should increase, but they are also negatively correlated.

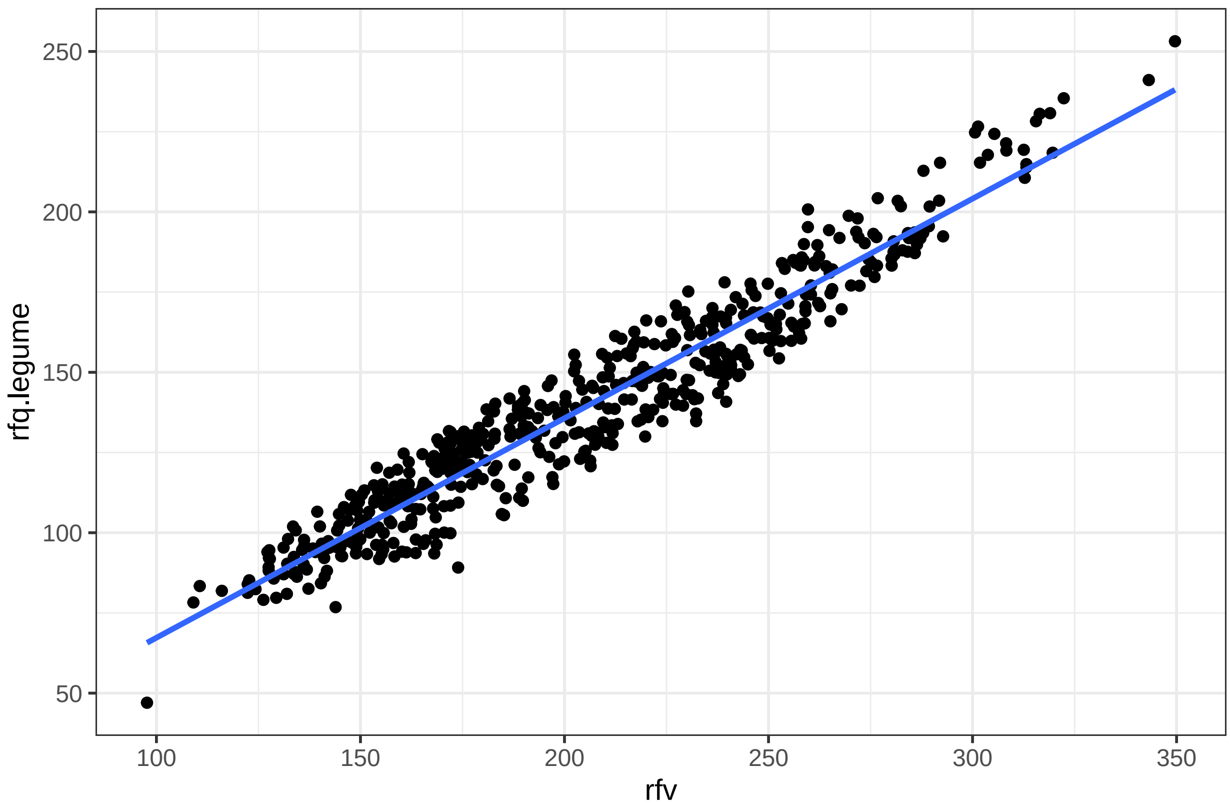
RFQ

RFQ should be positively correlated with RFV and have a close relationship (slope near 1)

$$RFQ = 1.2 * RFV - 32.4$$

(Undersander and Moore, 2002)

```
Call:
lm(formula = rfq.legume ~ rfv, data = dat)

Coefficients:
(Intercept)          rfv
    -1.1377       0.6842
```



RFQ = 0.7*RFV −1.4

We observe a relationship of $RFV = 0.7 * RFV - 1.4$

This indicates there is an issue with how we are estimating some of the forage quality parameters that comprise RFV and RFQ

ADF NDF underestimation We observed low ADF and NDF values, but they were within an acceptable range. The RFV prediction of 205, however, was outside the expected range of 100-200. When we compared our NIRS prediction with Europe, we estimated lower ADF and NDF from the same sorghum sudangrass. If we simply increased ADF by 6.25% and NDF by 8.3333333%, the RFV goes from 205 to 187 and is within the acceptable range.

While increasing the ADF and NDF values brings down the RFV to an acceptable range, it also decreases the RFQ from 139 to 121. The relationship of $RFQ = 0.7 * RFV - 1.4$ remains…

NDFD underestimation Our NDFD range is also low. We expect a range of 30 to 70 Undersander et al. (2016), but we observe a range of 4 to 31 with a mean of 16. We should have a mean of 50.

Let's see how the RFV-RFQ relationship changes when we shift the NDFD mean to 50 $NDFD * 3.125$ and keep our adjustment of ADF $ADF * 1.0625$ and NDF $NDF * 1.083$.

With an RFV of 187, we would expect an RFQ of 192 and we predict 200. Much better! By multiplying NDFD by 3.125, we are still not fixing the issue of NDFD not being positively correlated with CP, indicating a larger problem with it's prediction. More importantly, the linear relationship is ever worse now at $RFQ = 0.34 * RFV + 193$

At what NDFD value could we achieve the slope closest to 1.2*RFV while keeping the ADF and NDF adjustment?

We cannot achieve a slope of $RFQ = 1.2RFV$ by simply adjusting NDFD.

What would it take? It's not clear but NDFD needs to be addressed. Simply multiplying NDFD is too crude and we likely need to improve our equation for NDFD prediction. Adjustments of ADF and NDF shift both RFQ and RFV, adjustments of CP and EE do not seem to change relationship greatly.

Another step that could be taken would be to note the mahalanoibis distances for NDFD prediction for RALLF. We would expect them to be above 5, indicating a warning.

Extractable ether Extractable ether in our equation is set at 2.05 as a constant. But according to Undersander et al. (2016), extractable ether ranges from 1 to 4 and averages at 3 for alfalfa/grass forage.

Adjusting extractable ether does not have a large effect on predicted RFQ or RFV, so it doesn't really matter. Just a heads up.

References

Hoffman, P.C., R.D. Shaver, D.K. Combs, D.J. Undersander, L.M. Bauman, et al. 2001. Understanding NDF Digestibility of Forages. Focus on Forage 3(10): 3–5.

Undersander, D., D. Combs, and R. Shaver. 2016. Milk2016 (ALFALFA-GRASS): Index Combining Yield and Quality. University of Wisconsin-Extension 2016: 1–3. https://fyi.uwex.edu/forage/milk2016-combining-yield-and-quality-into-a-single-term/.

Undersander, D., and J.E. Moore. 2002. Relative forage quality. Focus on Forage 12(6): 1–3. http://www.foragelab.com/Media/Relative_Forage_Quality.pdf.