

Anomalies in Feed Analyses NANP Symposium –Mystery of the missing DM/OM

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Organizing Committee; Fred Owens, Alex
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Feeds, byproducts, and diets have been assayed for nutrient content for over 130 Years; Evolution:

Weende Assays 1866

Proximate Analysis (Wet Chem)

Henneberg & Strohman in the German town of Weende

Grouped Nutrients by Chem compo:

Ash (500C)

Crude Protein (N x 6.25)

Crude fat (Ether extract)

Crude fiber (1.35% KOH & 1.25% HCl)??

NFE-Nitrogen Free Extract);Calculated: Added later

Calculated by difference

Wilhelm Henneberg



10 September 1825 – 22 November 1890

Feed Assay Process

1. Wet feed sample (Wt)

Oven Dried <70C; Freeze dried, microwave? → H₂O, VFA,

↑ charring?

2. Dry feed sample (Wt) *For sub-sampling*

Grinder → fines, airborne dust, residual water?

Any weight added from hydrolysis of starch, protein?

3. Ground sample (Wt) Wiley, Udy. Sharpness? Heat?

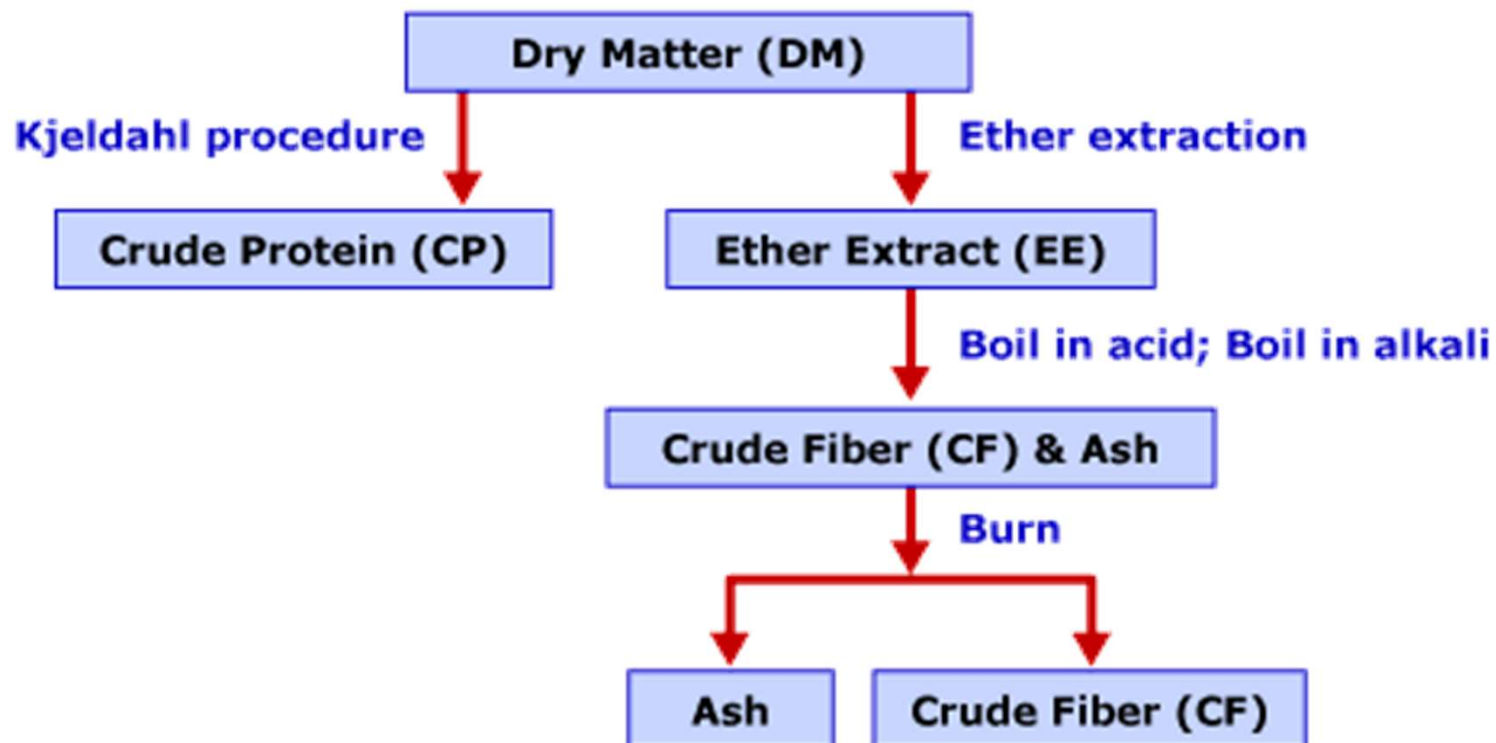
1mm, 6 mm grind or finer for NIR, NIT?

Assays, Ash, N, NDF, WSC of dried, ground sample.

Is % as a Fraction of dry or ground (hydrated) sample wt?

Could NIR assay for water & avoid need to dry samples?

Proximate Analysis



Proximate Principles

Fraction	Analysis	Main Contributory components
Moisture (M)	Oven-dry to constant weight at 100-105°C	Water (and volatile acids and bases)
Ash	Ignite in muffle oven at 450-550°C to remove organic compounds	Inorganic salts (minerals) (some sulphur and phosphorus from proteins and other organic compounds)
Crude protein (CP)	Kjeldahl digestion in sulphuric acid followed by distillation and NH_3 determination ($\text{CP} = \text{N} \times 6.25$)	Proteins, amino acids, amines, nucleic acids, nitrogenous glycosides, glycolipids, B-vitamins
Ether extract (EE)	Reflux extraction with petroleum ether (di-ethyl ether) (alternatively dichloromethane or hexane), sometimes following hydrolysis in strong acid	Fats, oils, waxes, sterols, lipid-soluble vitamins, organic acids, pigments (fatty acids present in polar lipids)
Crude fiber (CF)	Treatment of EE residue with boiling acid and boiling alkali	Cellulose, hemicellulose, lignin
Nitrogen-free extractives (NFE)	Estimated by difference $\text{NFE} = 100 - (\text{M} + \text{Ash} + \text{CP} + \text{EE} + \text{CF})$	Starch, sugars, pectins, fructans, (some CF, water-soluble vitamins, resins, organic acids)

Limitations of Proximate Analysis

Components	Supposed to contain	Contains	Missing	Excess
Water	Water	Volatile fatty acids Decomposed sugars	None	Volatile Fatty Acids, Decomposed sugars
CF	Fibrous matter	Cellulose Parts of lignin	Hemicellulose Part of lignin Acid Insoluble Ash	None
NFE	Soluble carbohydrates	Soluble CHO Hemicellulose Part of lignin Acid insoluble ash	None	Hemicellulose, Lignin, Acid Insoluble Ash
Ether Extract	Crude fat (Fats, oils, Fatty acids)	Free Fats, oils, Fatty acids, Chlorophyll, sterols, Anthocyanin, Carotenoids, Resins, Volatile oils, Lecithin Cholesterol, Alkali substances	Protein bound lipids, Fatty Acids formed soaps	Chlorophyll, Sterol, Anthocyanin, Carotenoids, Volatile Oils Resins Lecithin Cholesterol, Alkali Substances

Armidale feed analysis

Weende Method	Nitrogen-Free Extract				Crude Fibre		NFE	CF	Ash	Ether Ext.	Crude Protein	
Common Nomenclature	Carbohydrates											
			Polysaccharides									
				Non-starch Polysaccharides								
				True Fibre								
					Neutral Detergent Fibre							
						ADF (Wood)						
Armidale Method	Sugars	Oligosac- charides	Starch	Pectin	Hemi- cellulose	Cellulose	Lignin	Ash	Neutral Lipids	Polar Lipids	True Protein	NPNC
Sugar Units	1-2	3-12	>12		>12	>12	>12					
GE (kcal/g)	4.00	16.74	17.57	15.56	16.74	16.74	25.50	0.00	39.75	31.00	23.85	24.59
Potential Digestibility	1.00	0.90	1.00	0.70	0.50	0.10	0.00	0.00	1.00	1.00	1.00	1.00
Potential ME (MJ/g)	16.74	16.74	17.57	10.90	8.37	1.67	0.00	0.00	39.75	31.00	23.85	24.59
NE (based on ATP)	10.56	10.60	11.73	8.21	8.21	11.73	8.21	0.00	25.31	20.36	11.35	6.33

NPNC = Non-Protein Nitrogenous Compounds, the difference between Crude Protein and True Protein (~12%, TP = 0.88 x CP)

Pesti et al. (2024)

Armidale feed analysis

Recent Advances in Animal Nutrition:



COLLECTION | ANIMAL SCIENCE REFLECTIONS

<https://doi.org/10.1071/AN24176>

ANIMAL PRODUCTION SCIENCE

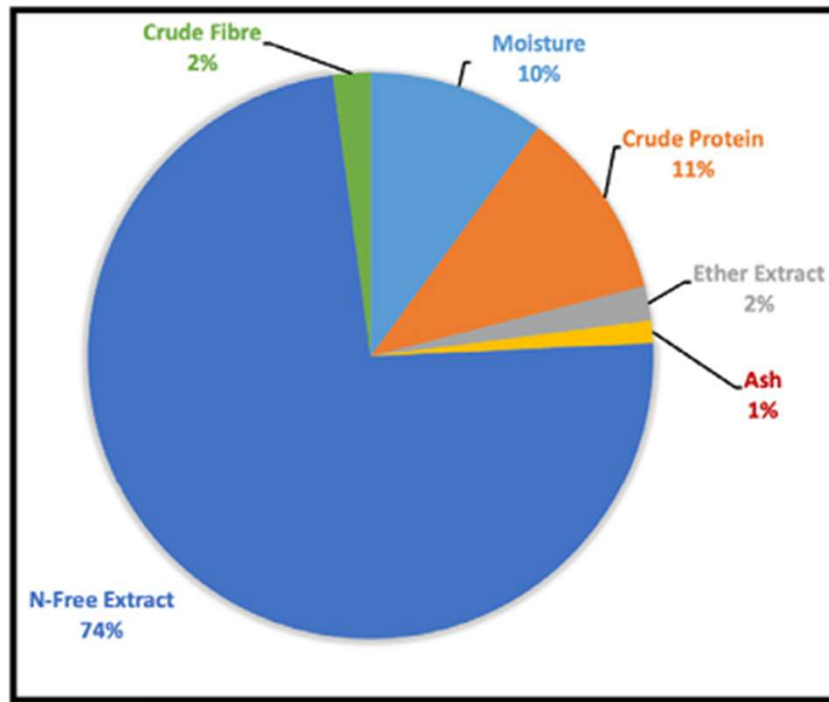
A new analytical procedure to replace the outdated Weende proximal feed ingredient analysis paradigm is long overdue

Gene M. Pesti ^{A,*} 

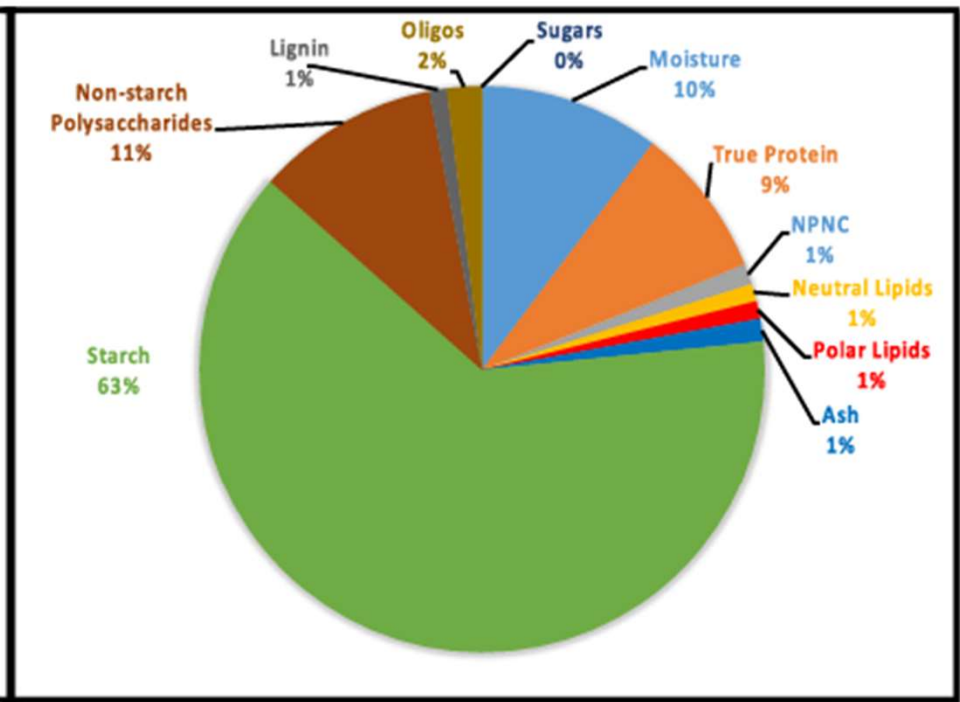
Armidale feed analysis

Comparison of Feed Composition Techniques for Wheat

True Fiber = Total NSP + Lignin = 12% vs. 2% CF



Weende Method



Armidale Method

Limitations - Moisture

- Formation of artifact lignin if dried $> 55^{\circ}\text{C}$ (e.g. 170°C for 7 hr)
 - Loss of more volatile acids, alcohols
- **Solutions**
 - Lower the drying temperature
 - 50°C until weight is stable vs 170°C for 7 hr
 - NIT, NIR substitution for oven drying
 - Freeze drying
 - Add base to convert volatile acids to stable salts
 - Direct moisture measurement via Toluene distillations; Fischer titration
 - Check for energy losses by bomb calorimetry with added primers

Limitations - Ash

- Loss of organic matter at 550°C; not 600°C?
- No measurement of individual minerals, cations
- Some ash comes from NDF
- Volatile loss of some minerals, conversion of sulfides, chlorides to oxides
- Still some carbonates remain at 550°C
- **Solutions**
 - ACP for individual minerals

Merits

- It forms the basis for
 - Description of feed composition tables
 - Purchasing feeds
 - Ration formulation
 - Various equations for energy estimations
- It is the starting point for specific analysis
- Provides 1st hand information about the potentiality of the feed to fulfill the required nutrient.

Merits Contd.

- Used for analysis of feeds, feces, urine, and body fluids
- No substitute till today except for fiber component
- Referred by Nutritionist, Chemist, Physiologists, Bacteriologists, dietitians, food processors, etc.
- Handy to handle
- The process is simple and economic.

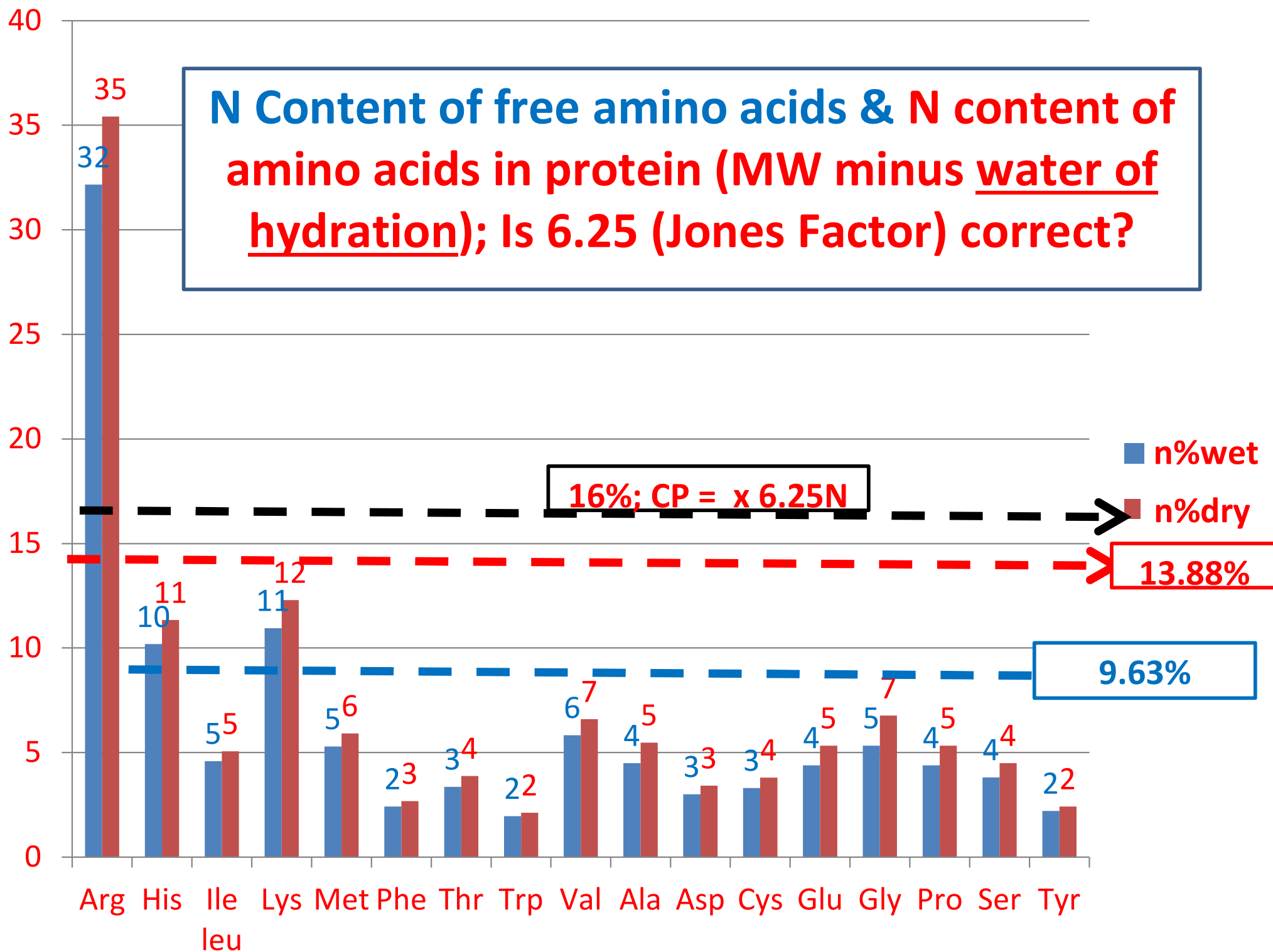
Johan Gustav Christoffer Thorsager Kjeldahl



16 August 1849 – 18 July 1900

Limitations – Crude Protein

- Classify NPNs (nucleic acids, ammonia, N in NDF as protein.
- Inaccuracy of Jones ($6.25 \times N$) factor for many proteins
- **Solutions**
 - Provide amino acid compositions for non-ruminants
 - Rumen amino acid escape, digestion site data based on in situ studies & data.



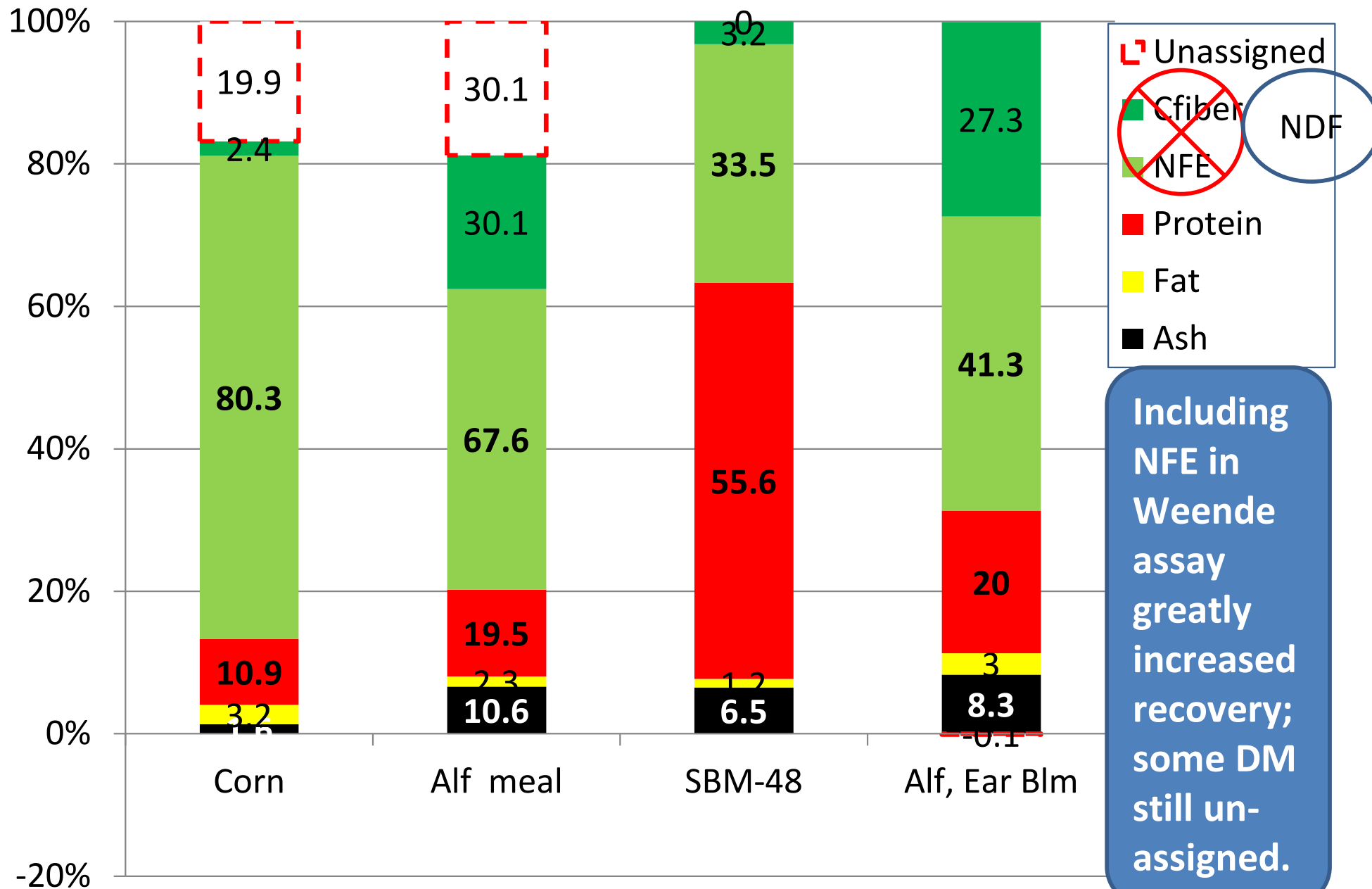
Calculating N:True Protein of feeds

As amino acids differ in N content, mixtures of amino acids in the protein of various feeds will differ in N content.

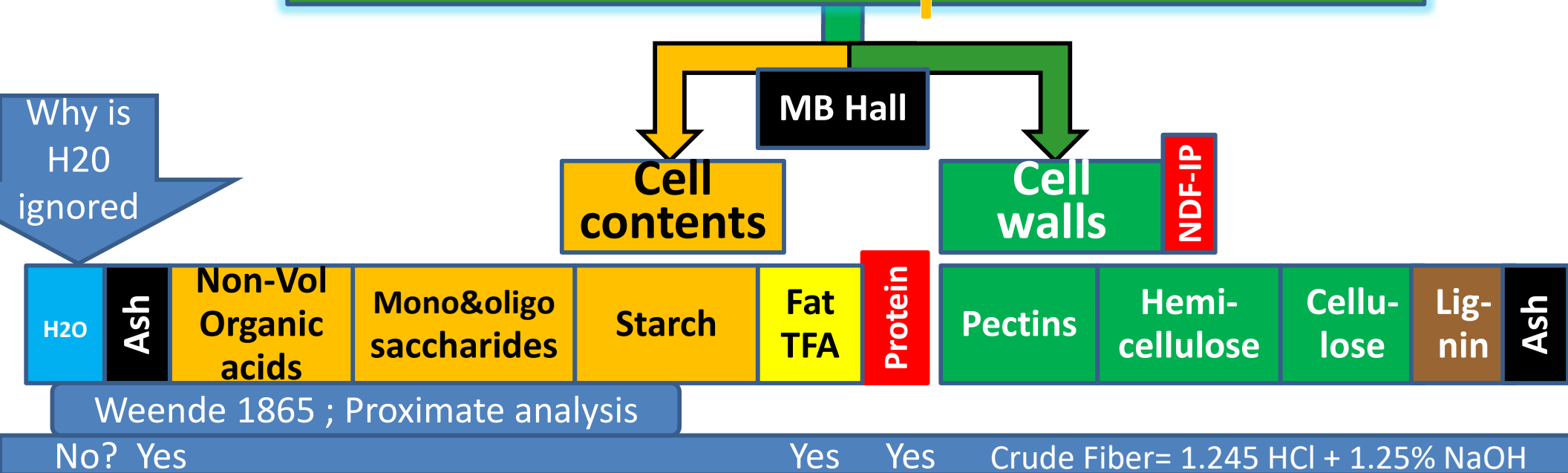
Feed	AA g/100 g DM (CP)	Sum N in AA	gN/100 gAA
Corn grain	8.79(8.8%)	6.33 g	17.4 g
Sorghum grain	10.6(11.6%)	11.64	18.0
Soybean meal	45.8(48.9%)	48.85	17.8
Casein	92.8(92.8%)	102.7	17.4
Meat meal	54.2(59.2%)	59.2	16.0

Protein content appears generally overestimated based on 16% N in protein, probably due to the presence of non-protein N in feeds from nucleic acids, amino sugars, NDF-N and ADF-N.

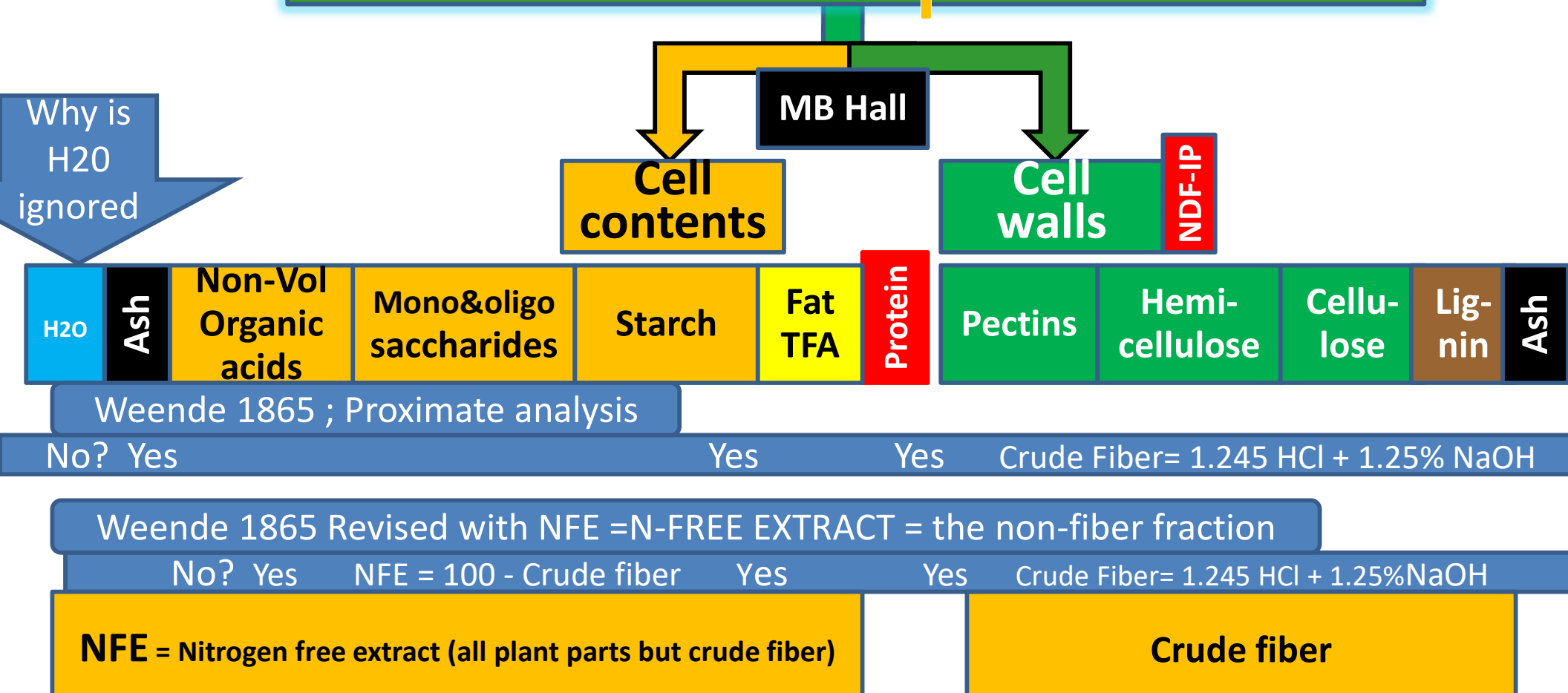
NRC Atlas 1971 **Weende** Analyses



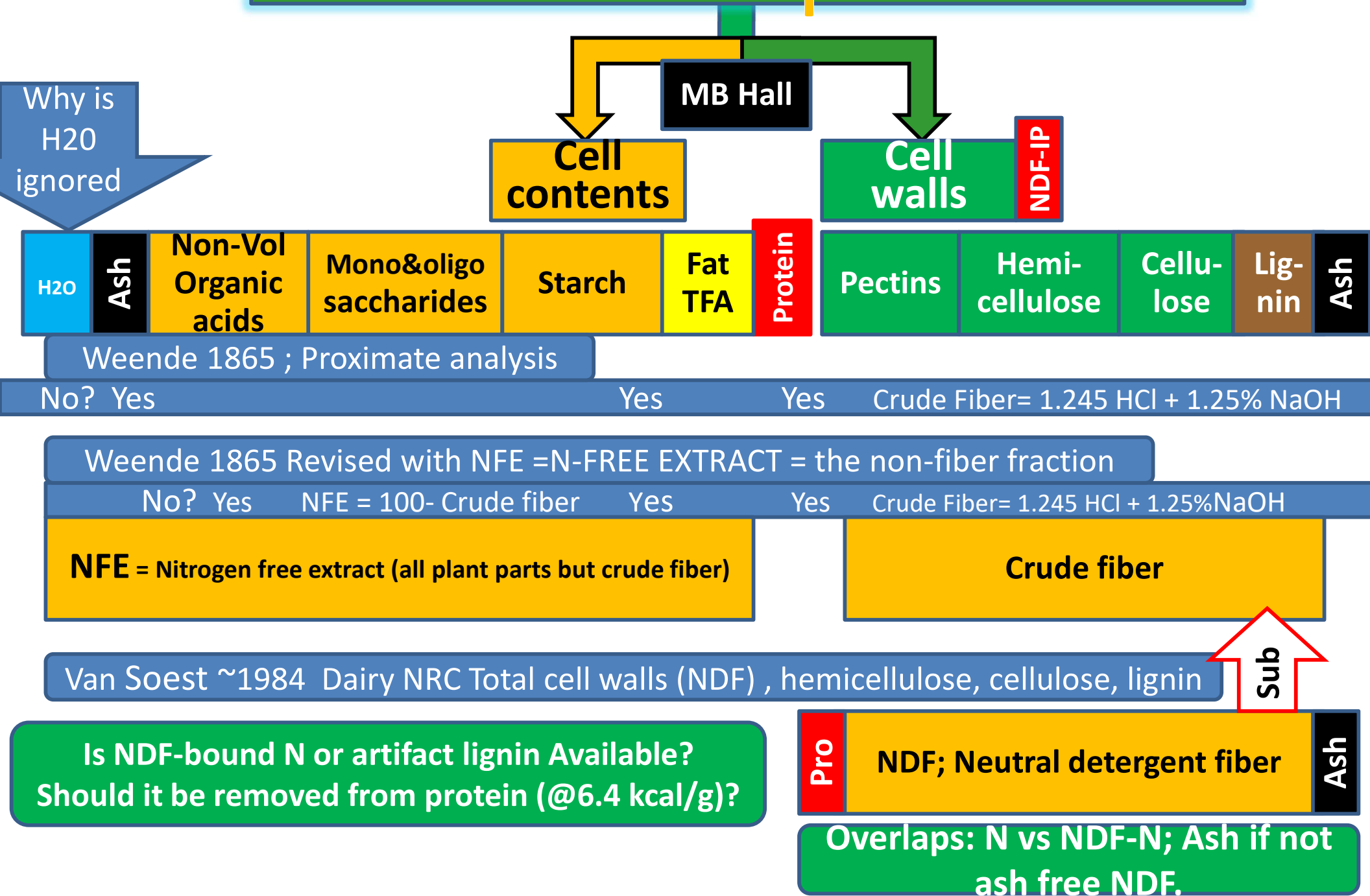
Plant Components



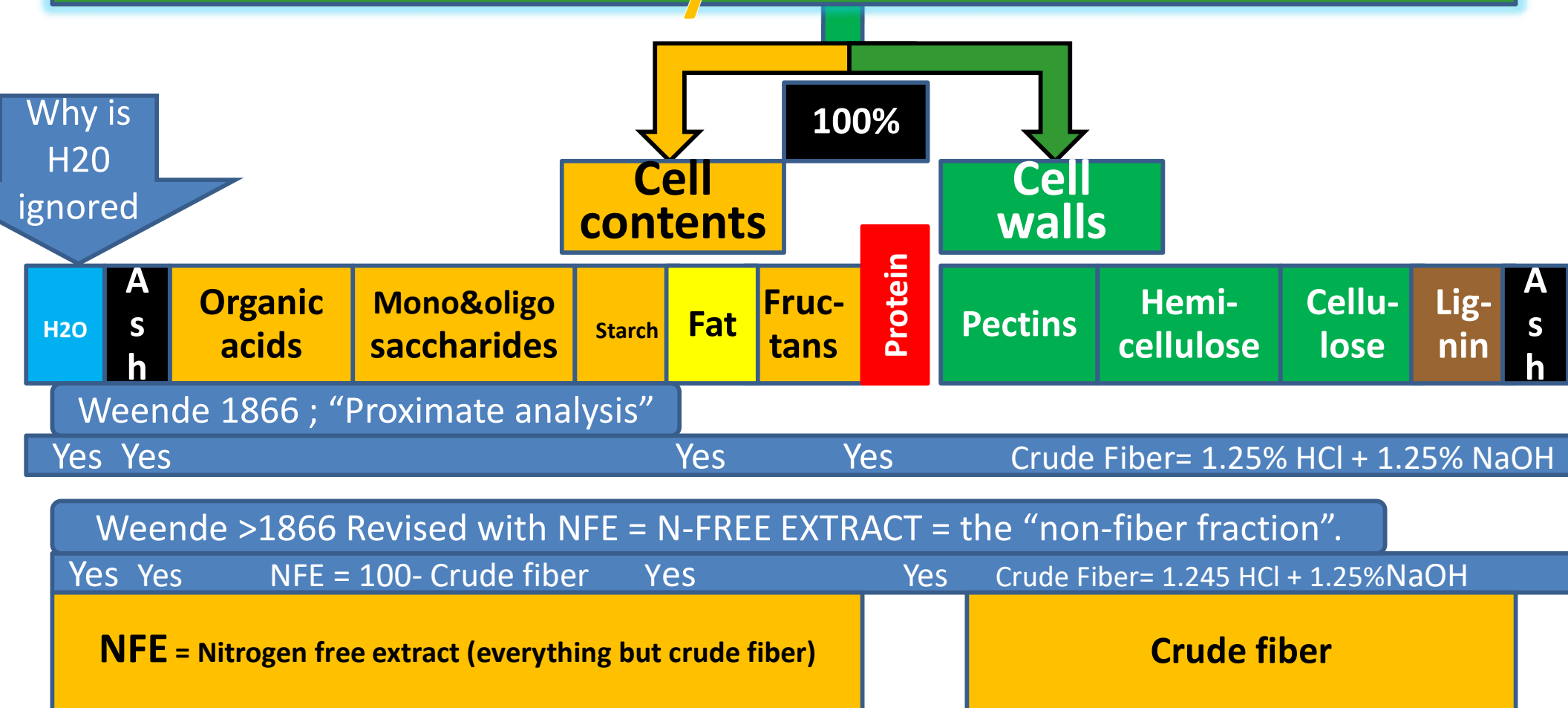
Plant Components



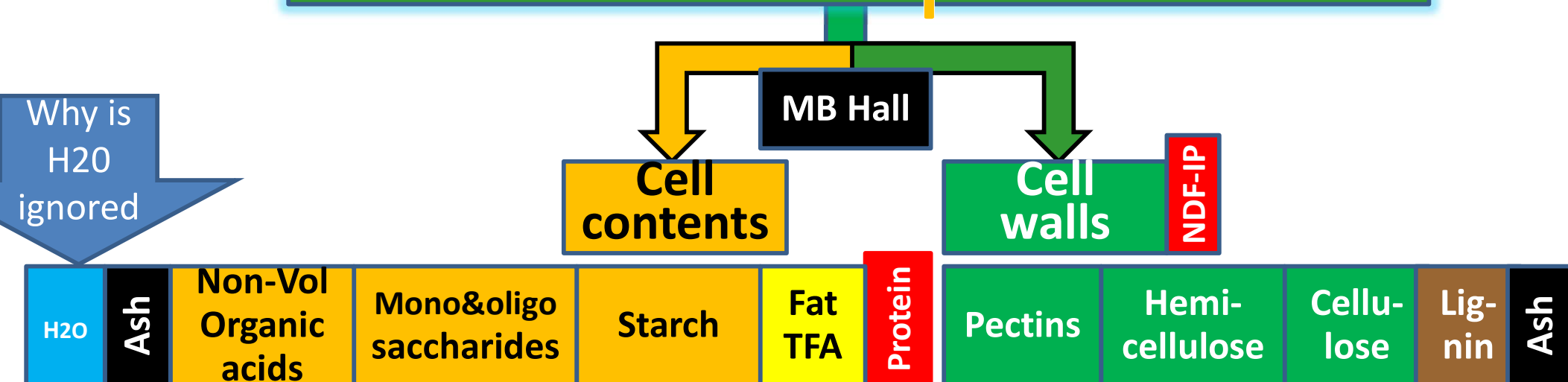
Plant Components



Feed Analysis- Evolution



Plant Components



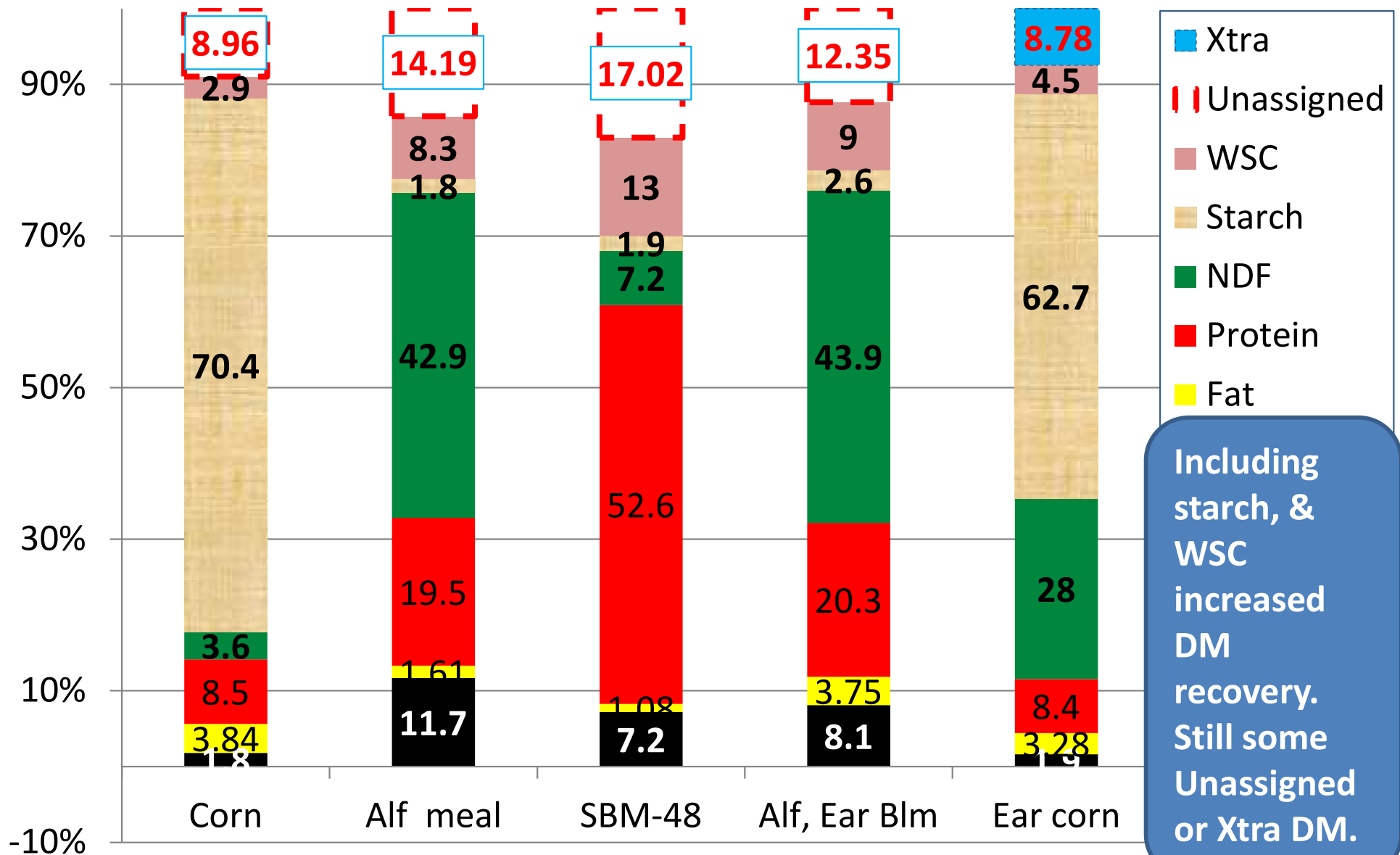
Assumption: Sum of assayed parts should equal 100% or total!

Crude Ash [% of DM] + Crude Fat[% of DM]+ Crude protein [% of DM]+Crude fiber, [% of SM] Should Sum to 100% DM of nutrients - equal 100%?

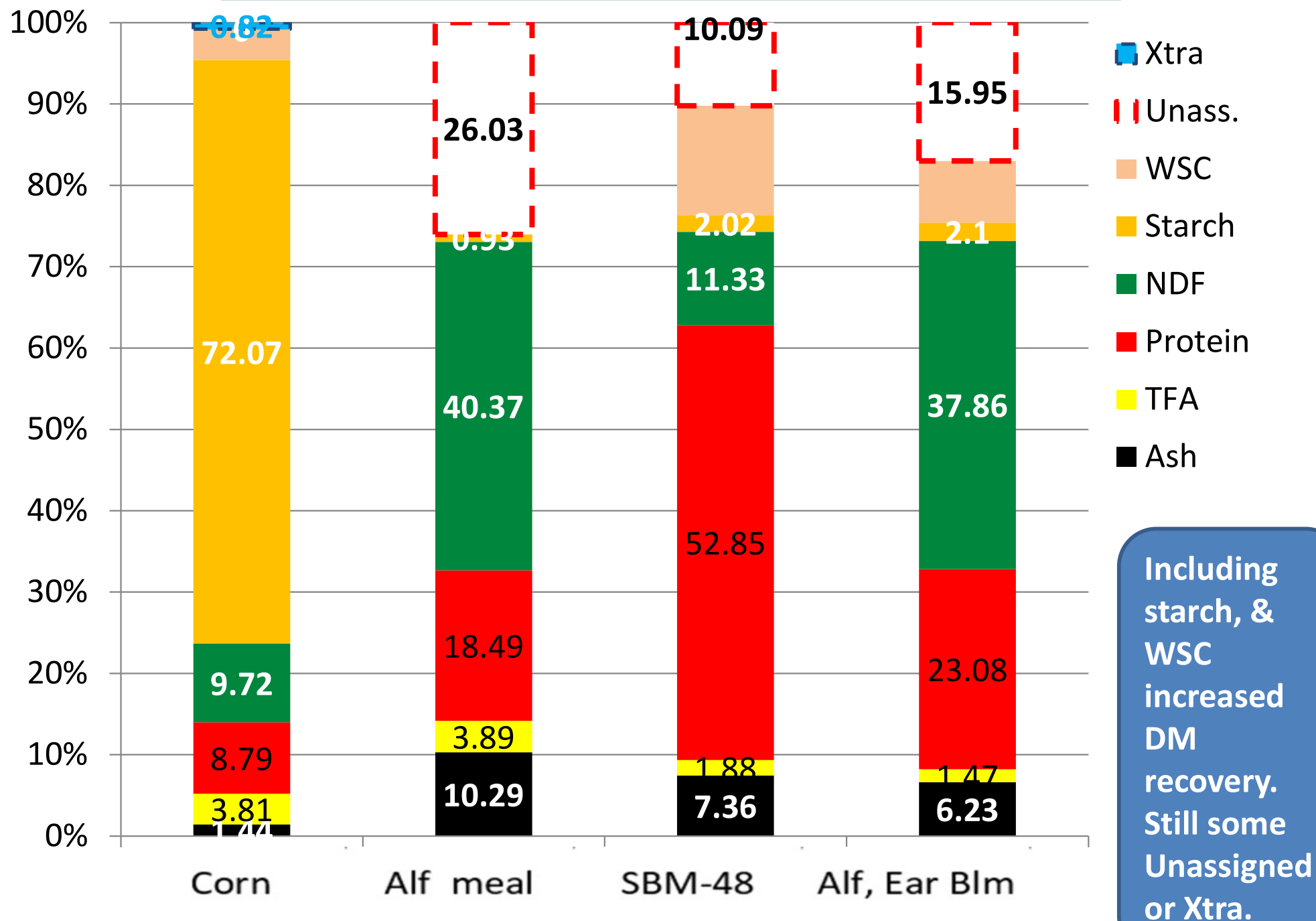
For most feeds, sum < 100%; Other cases > 100%. Why?

- 1. Some Components in > 1 category; 2. Assumptions: $N \times 6.25 = CP$; 3. Artifact lignin;**
- 4. Some Unassigned components (unidentified) – **NSP** Van Soest; **ROM** Dairy NRC 2021;**

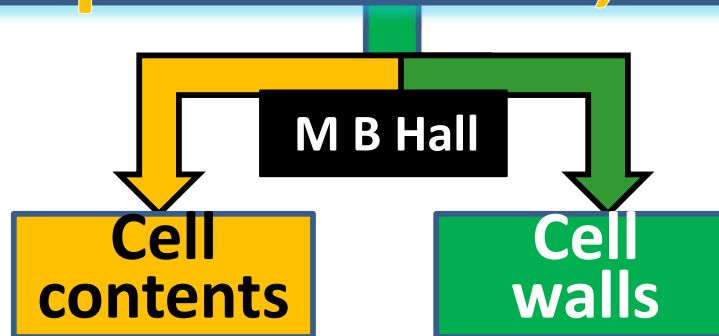
NRC Dairy 2021 Proximate Analyses



NRC Beef 2016 Proximate Analyses



Plant Components, % of DM



Ash	Organic acids	Mono & oligo saccharides	Starch % of DM	Fructans
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Pectins	Hemi-cellulose	Cellulose	Lignin
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Evolution of Analyses & Data

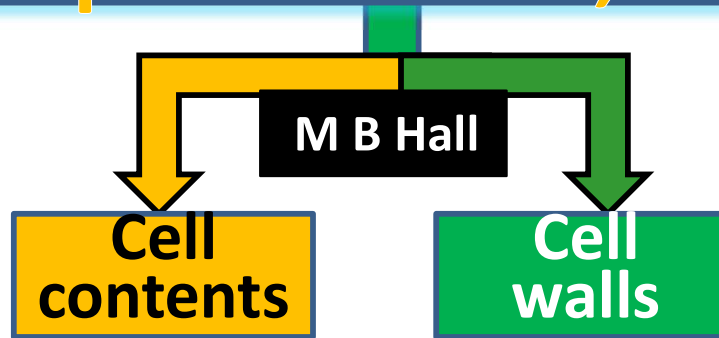
Weende 1866 Ash, Protein Nx6.25, Crude fiber (1.25% NaOH, 1.25% HCl)

Weende Revised Ash, Proteinx6.25, Crude fiber, NFE
(difference from 100%)

Detergent fiber 1989 NRC Dairy

Detergent fiber Revised Ash,Fat, Proteinx6.25(Jones Factor 1931; 50 ref, NPN?), FAT, NDF, ADF, Lignin, Starch,Sugars (WSC, ESC)

Plant Components, % of DM



Ash	Organic acids	Mono & oligo saccharides	Starch % of DM	Fructans	Proteins	Pectins	Hemi-cellulose	Cellulose	Lignin
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Sources of Data for Comparisons

Weende 1866 NRC Atlas of US and Canadian feeds, 1971

Weende Revised Detergent fiber

NRC Atlas of US and Canadian feeds, 1971

Detergent fiber NRC Dairy 1984

Detergent fiber Revised NASEM Beef 2016; NASEM Dairy 2021

Plant Components, % of DM

M B Hall

Cell
contents

Cell
walls

A
s
h

Organic
acids

Mono & oligo
saccharides

Starch
% of
DM

Fruc
tans

Prot
eins

Pectins

Hemi-
cellulose

Cellulose

Lig
nin

When Expressed as % of DM
The total should equal 100% of DM
for a feed. Does it? Overlaps?
Missing components?
Is undetected DM missing or
Unassigned?

Limitations – Crude Fiber

- Boiling in base solubilizes some lignin
- **Solutions**
 - Fully replace crude fiber assays with detergent fiber procedures (and feed tag info with detergent fiber fractions)

Limitations – Ether Extract

- Does not differentiate polar from neutral lipids for poultry
- Includes waxes, pigment of limited value, includes glycerol
- **Solutions**
 - Added assays of extracted components

Limitations – NFE

- Various soluble carbohydrates differ in value
- **Solutions**
 - Replace with direct assays for pectin, mono- & poly-saccharides by HPLC, NIR?

Why continue sampling & Assaying?

New feeds, cultivars, by-products e.g. BMR, hi lysine; amylases; Low fat Distiller's grains, low oligo soy:

**New procedures- NDF vs [Crude fiber + NFE];
NFE vs [starch & soluble CHO];**

- (Plant growth conditions – vary w/Year and location- temperature, moisture, day length,**
- Handling: Plant age, maturity, Harvest height, new processing methods, storage time, preservatives, inoculants.**

Evolution of Analysis

Year	Source	Feeds	Basis	Analytes
1956	Morrison	927	As Fed	DM, CP, EE, CF, NFE
1989	Dairy NRC	257	DM	DM, CP, EE, NFE
1971	NRC Atlas	6,152	As Fed	DM, CP, EE, Ash, NDF, Starch, Sol. CHO
2016	NRC Beef	168	DM	DM, CP, EE, Ash, NDF, Starch, Sol. CHO
2021	NRC Dairy	180	DM	DM, CP, EE, TriG, Ash, NDF, Starch, Sol. CHO
2025	Pesti	25	DM	DM, CP, N lipid, P Lipid, True CP, NPN, Mono-, Di-, & Oligosaccharide, Starch, Pectin, Lignin, Hemicellulose, Cellulose

Analytical changes since 1866

NIR vs. wet chemistry; Varies with data origin.

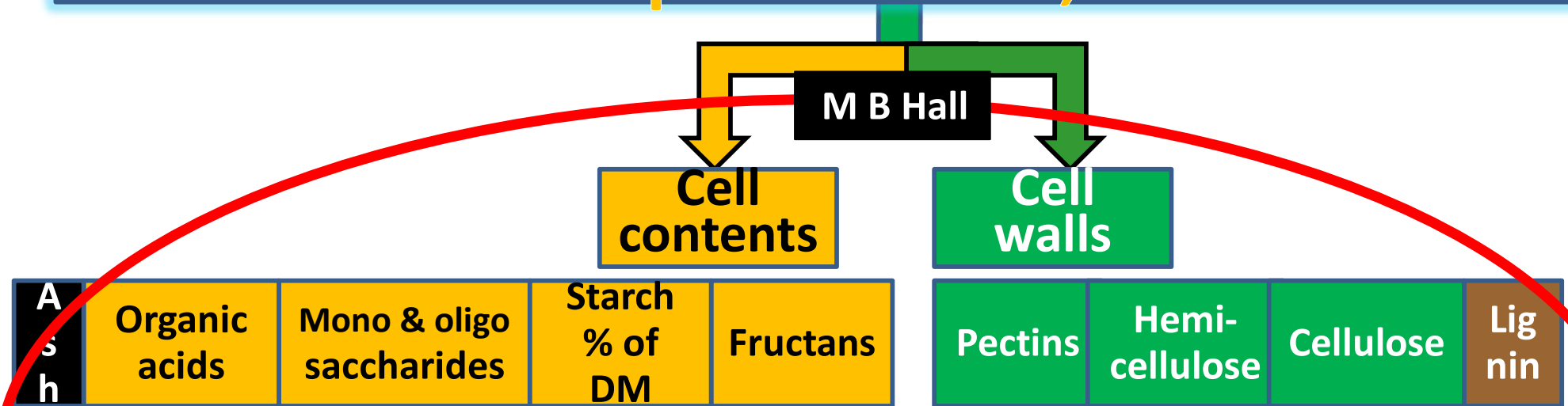
1. Detergent fiber analysis (Reed & Van Soest) FAO: Nutritive value of crop residues.
2. Van Soest, Robertson, & Lewis 1991 JDS 74:3583-3507 Advantages of detergent fiber assays versus crude fiber and NFE.
3. Starch
4. Sugars & soluble carbohydrates.
4. 5. Non-fiber polysaccharides, Residual OM

Does the **sum** of a feed's components (**ash, fat, protein, NDF, starch, WSC**) all expressed as the [% of feed DM] sum to 100%? If $< 100\%$, something must be missing; if the sum $> 100\%$, some part may be included in more than one nutrient class? (i.e. double-counting N crude protein or N-bound NDF)

Proximate Principle

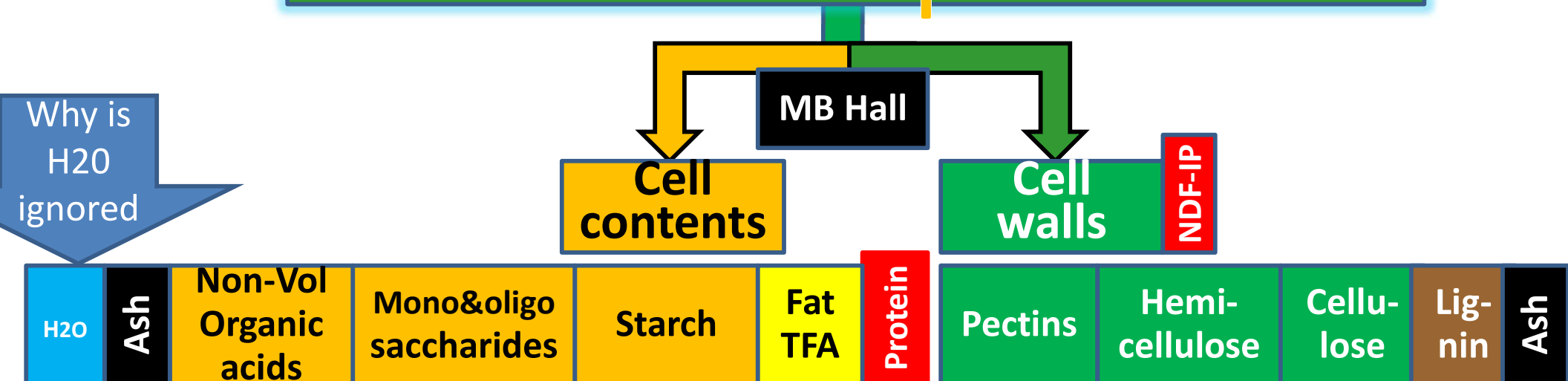
- Water or moisture
- Crude Protein (CP)
- Ether Extract (EE)
- Crude Fiber (CF)
- Nitrogen Free Extractives (NFE)
- Ash

Plant Components, % of DM



**When Expressed as % of DM
The total should equal 100% of DM
of any feed.
Does it?**

Plant Components

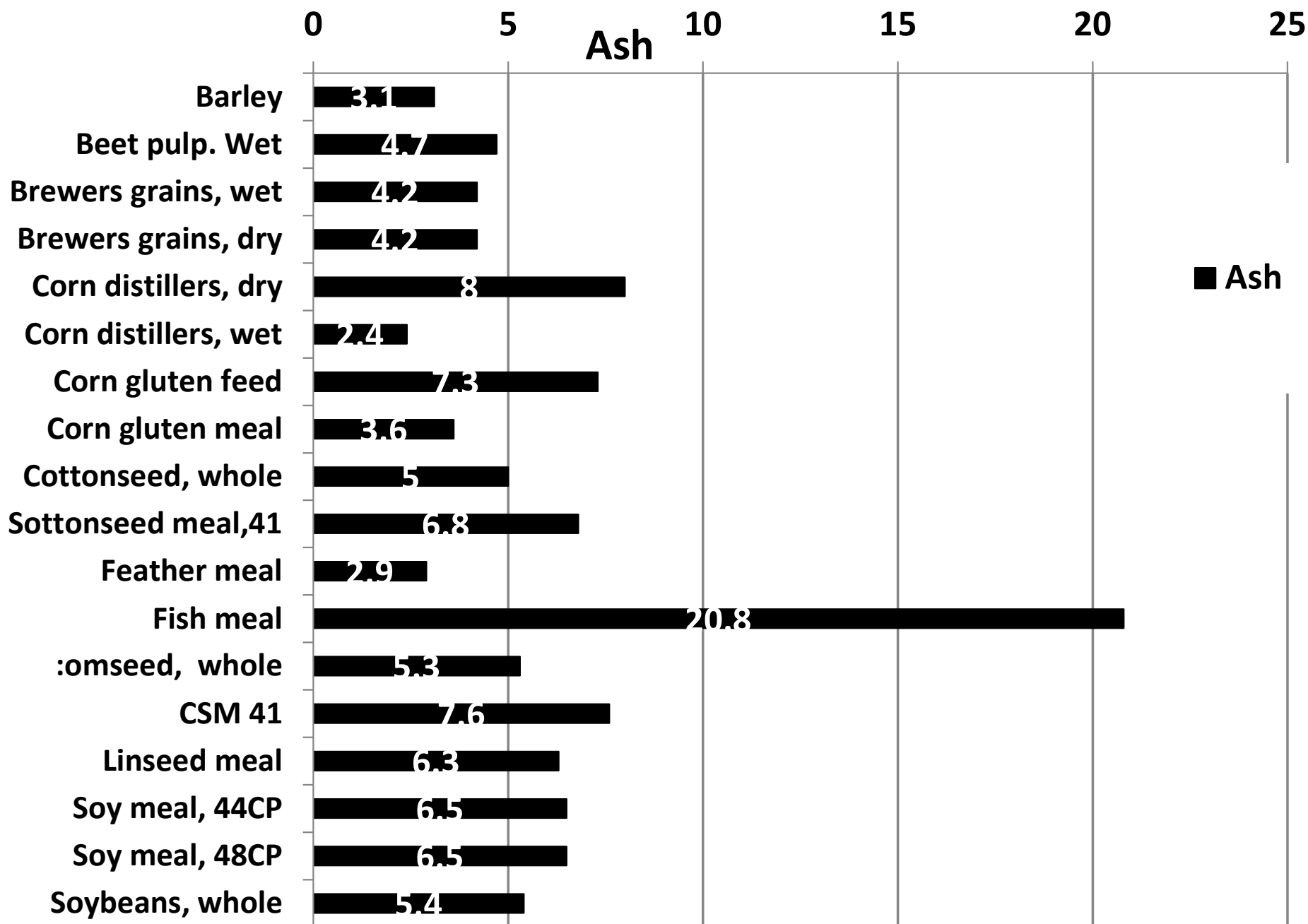


Are components independent or is there overlap (esp. NDF)?

- NDF- Contains some ash -unless it is ash-free NDF.
- NDF usually Contains some N, so not independent from protein. ($N \times 6.25$)
- Should N associated with NDF or ADF be considered part of total N in a feed, or due to low accessibility as a source of N, should N bound to fiber be deducted from total N because of its low digestibility? (Sniffen et al. 1992. NDF IP can equal 52 to 63% of crude protein in certain feed ingredients – Beet pulp; Distillers).

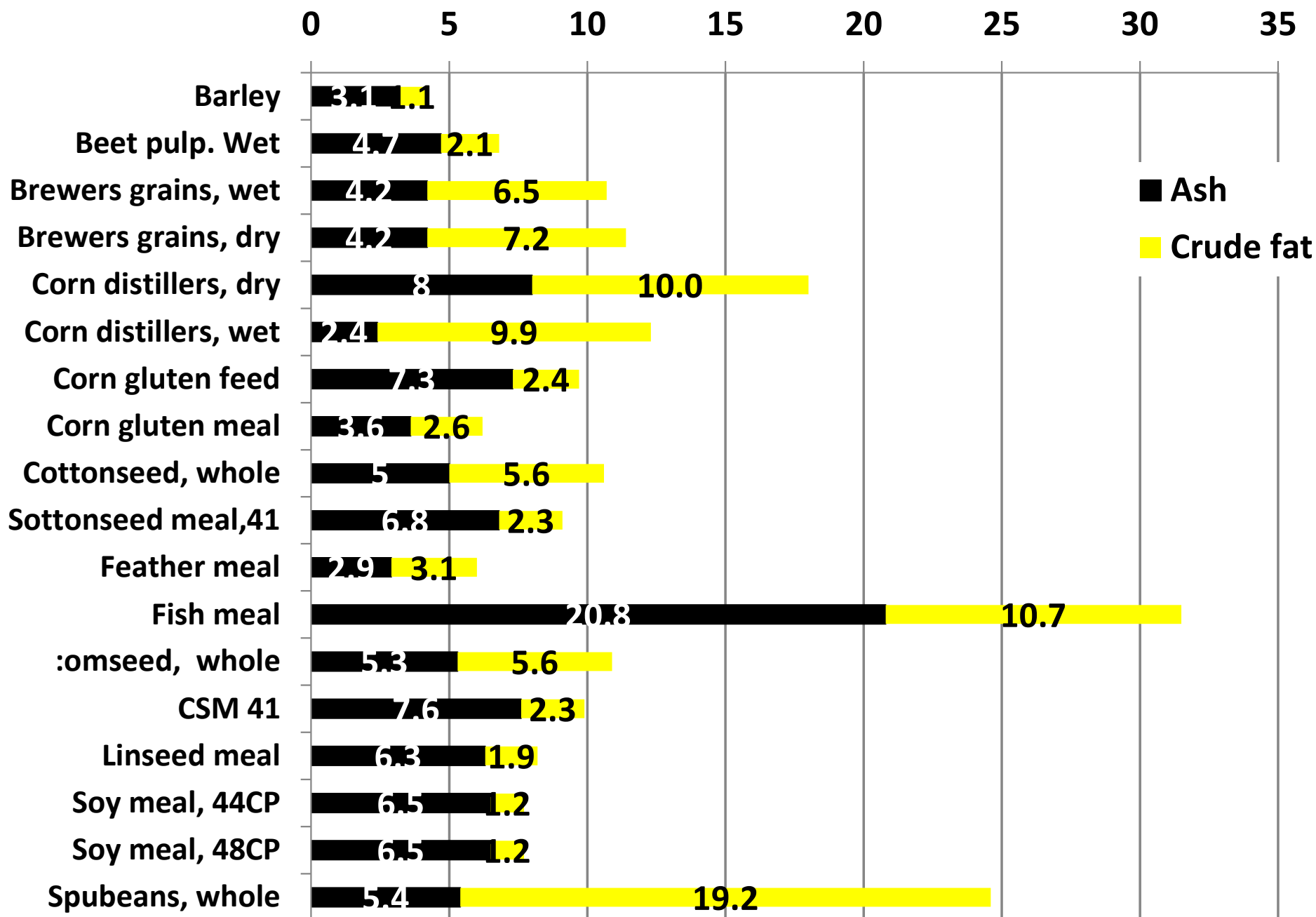
Grain Components, Composition (*Weende components*)

NRC Atlas of Feed Composition (1971) +ASH



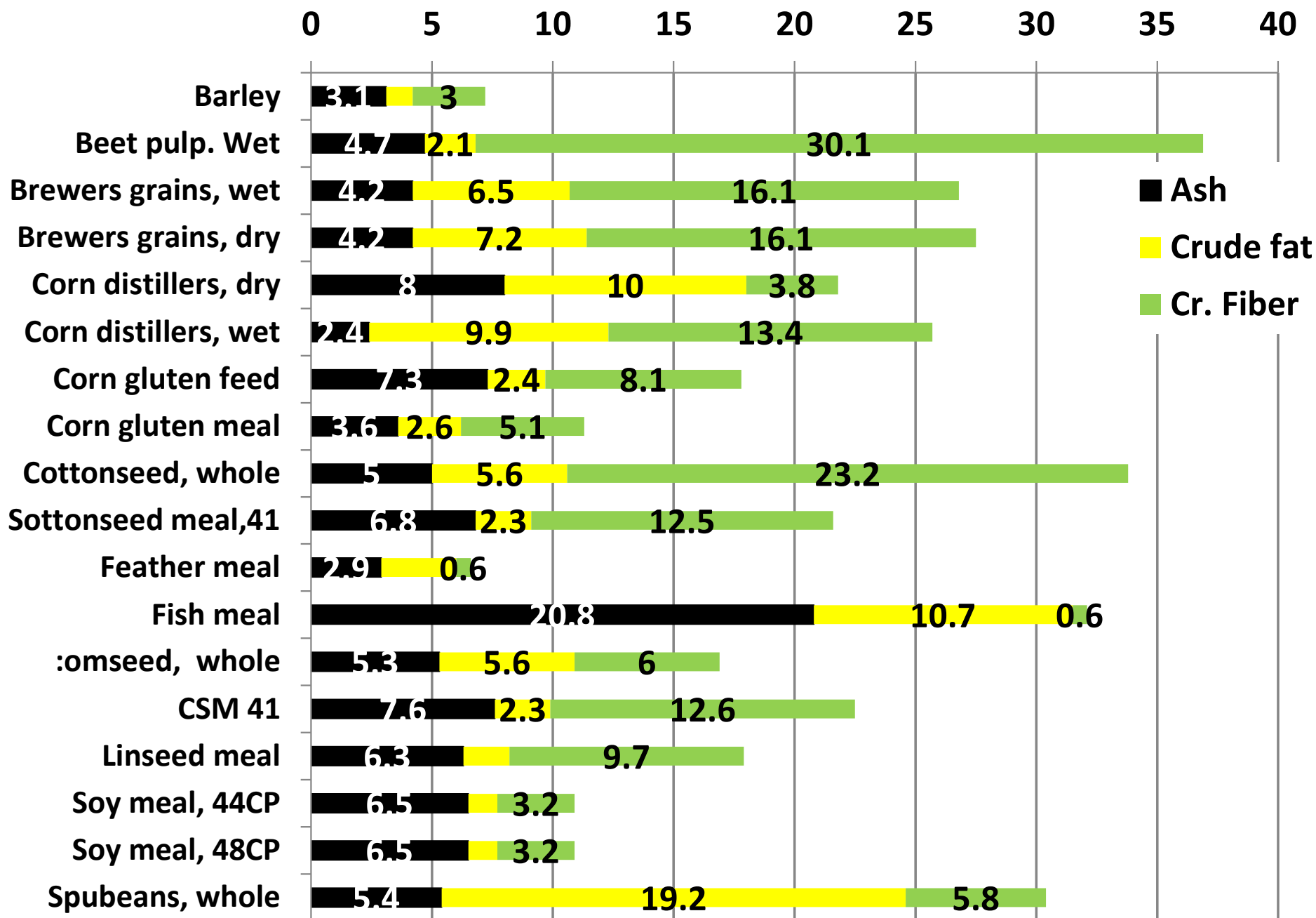
Protein Components, Composition (Weende components)

NRC Atlas of Feed Composition (1971) + ee



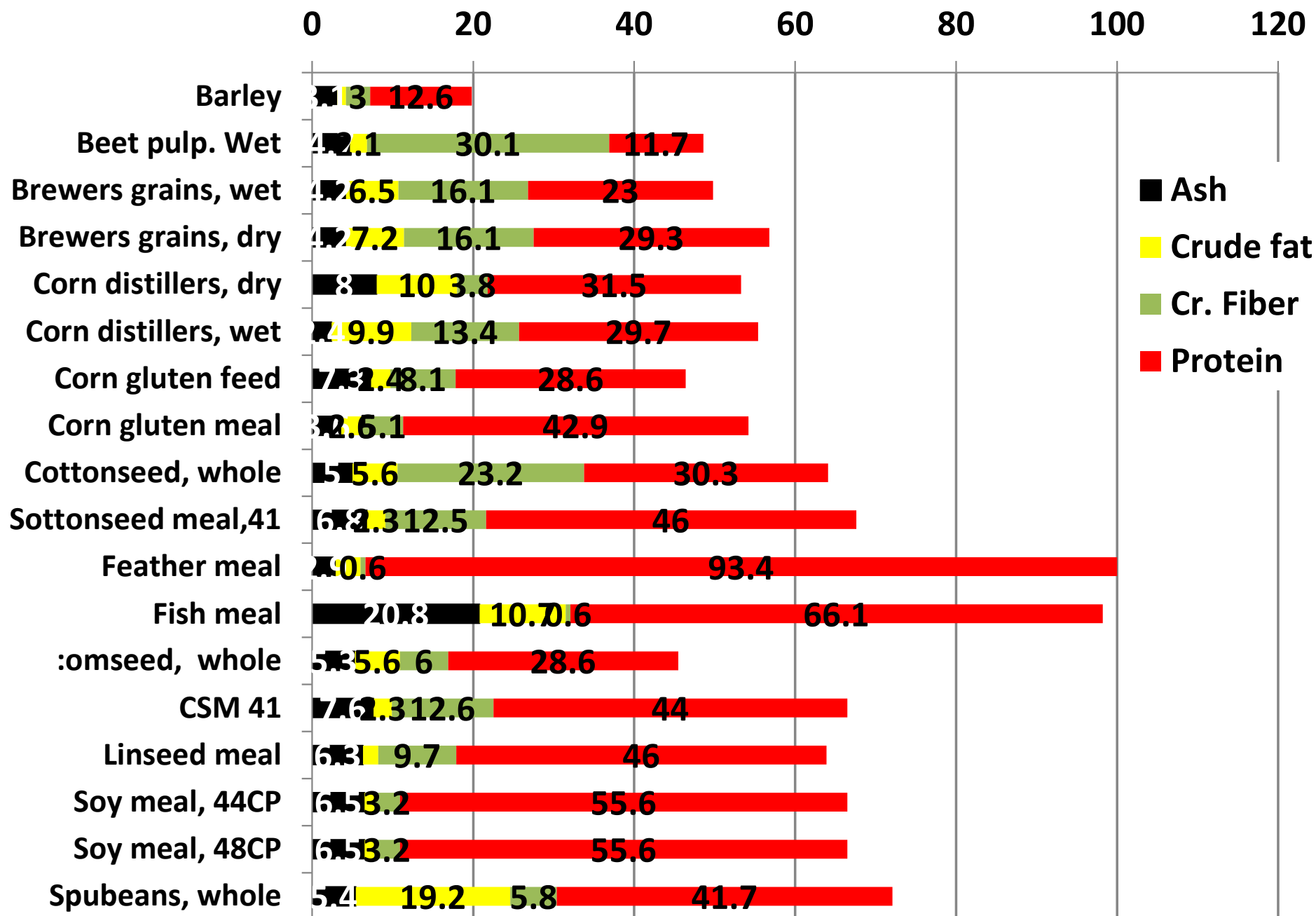
Grains Composition (Weende components)

NRC Atlas of Feed Composition (1971) =crude fiber



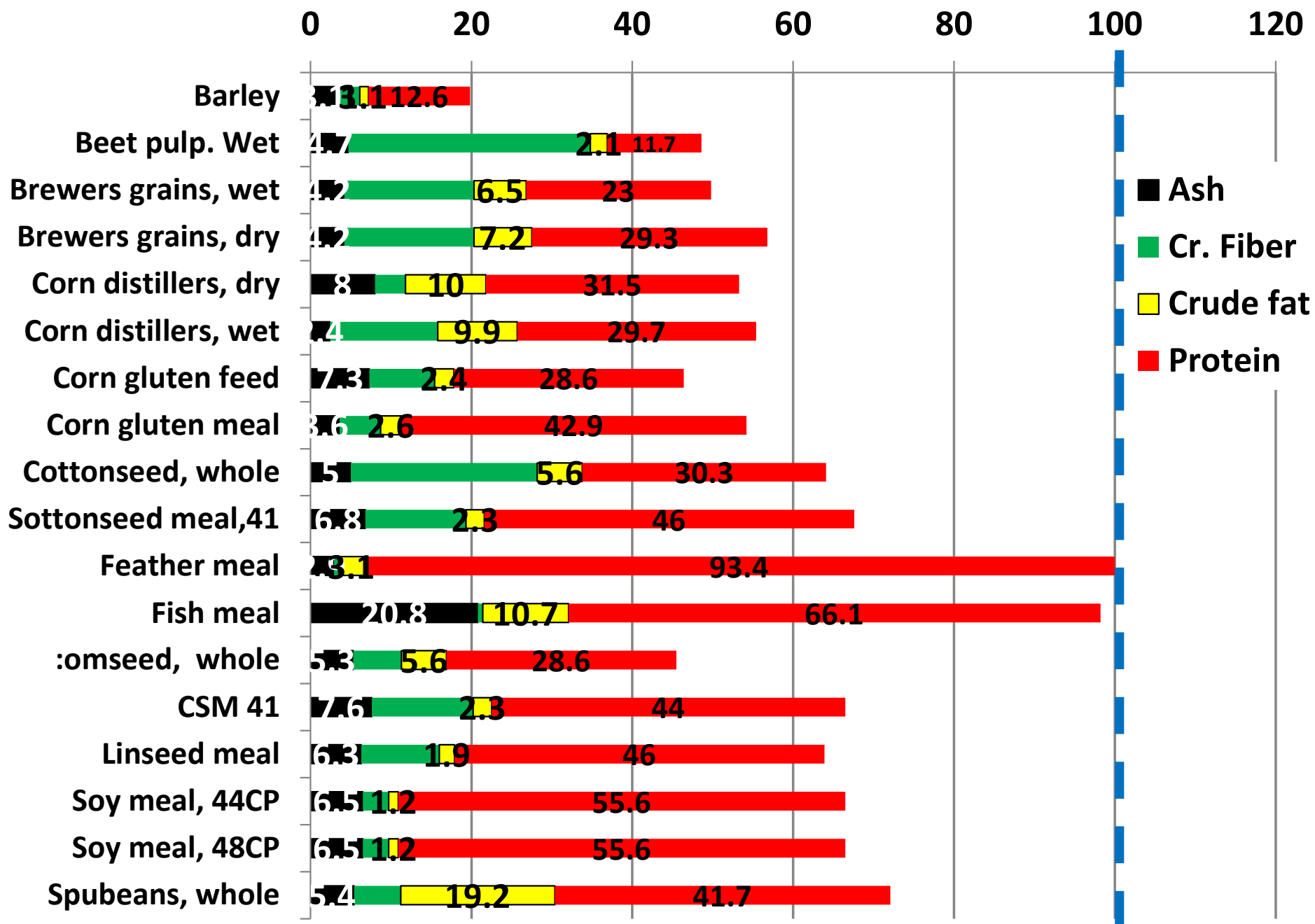
Grains, Composition (Weende components)

NRC Atlas of Feed Composition (1971) New



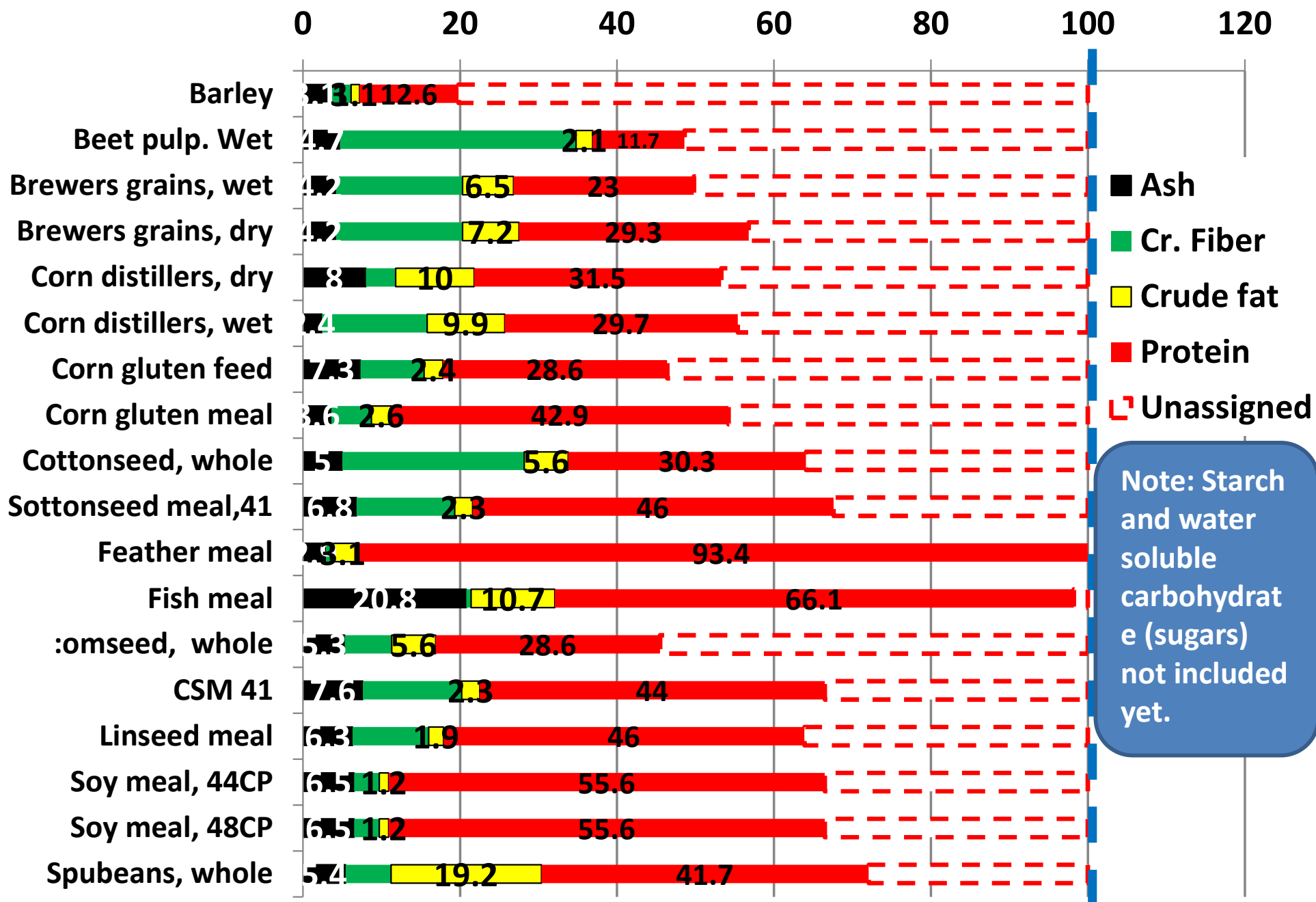
Grains Composition (Weende components)

NRC Atlas of Feed Composition (1971) New

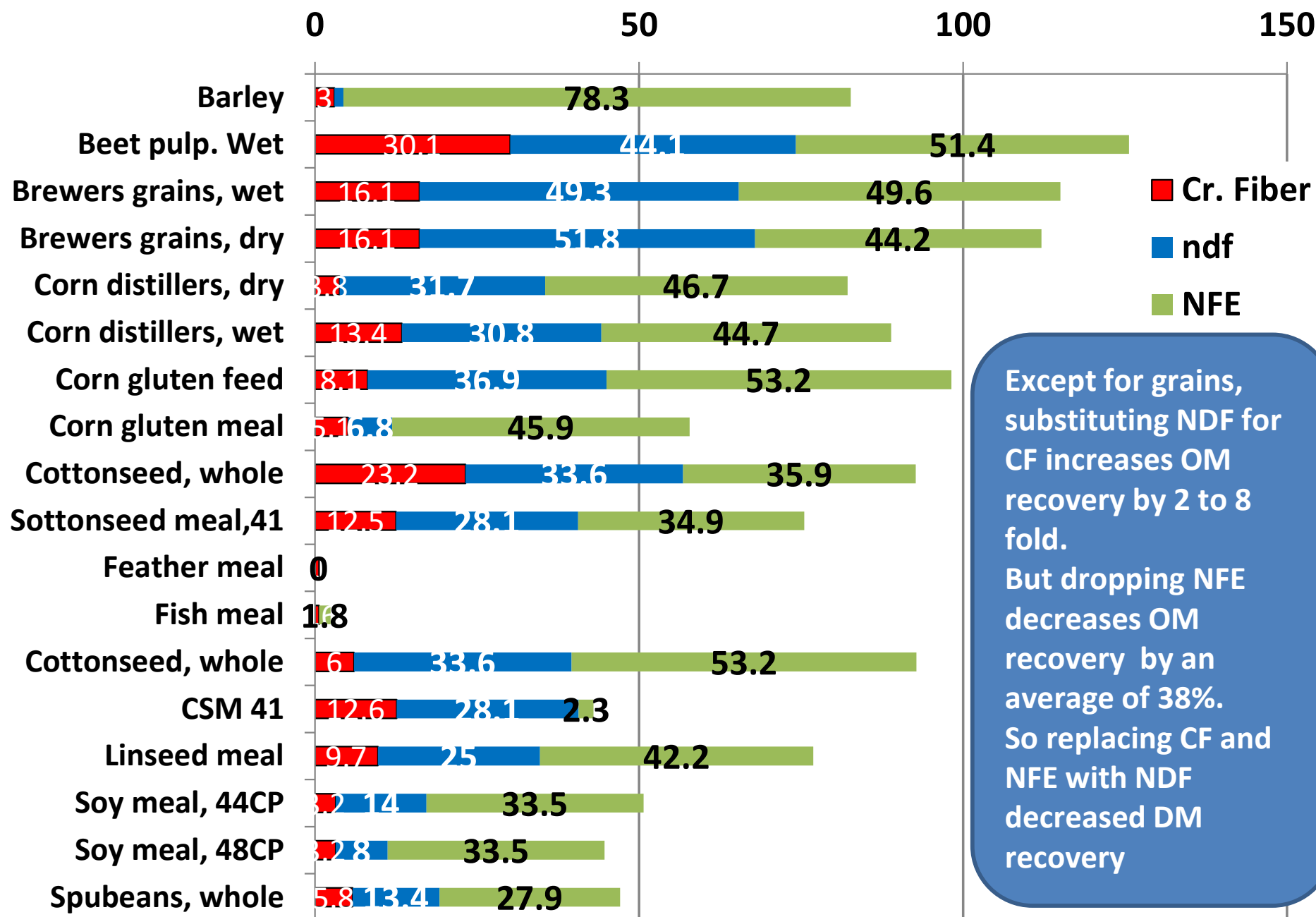


Grains (Weende components)

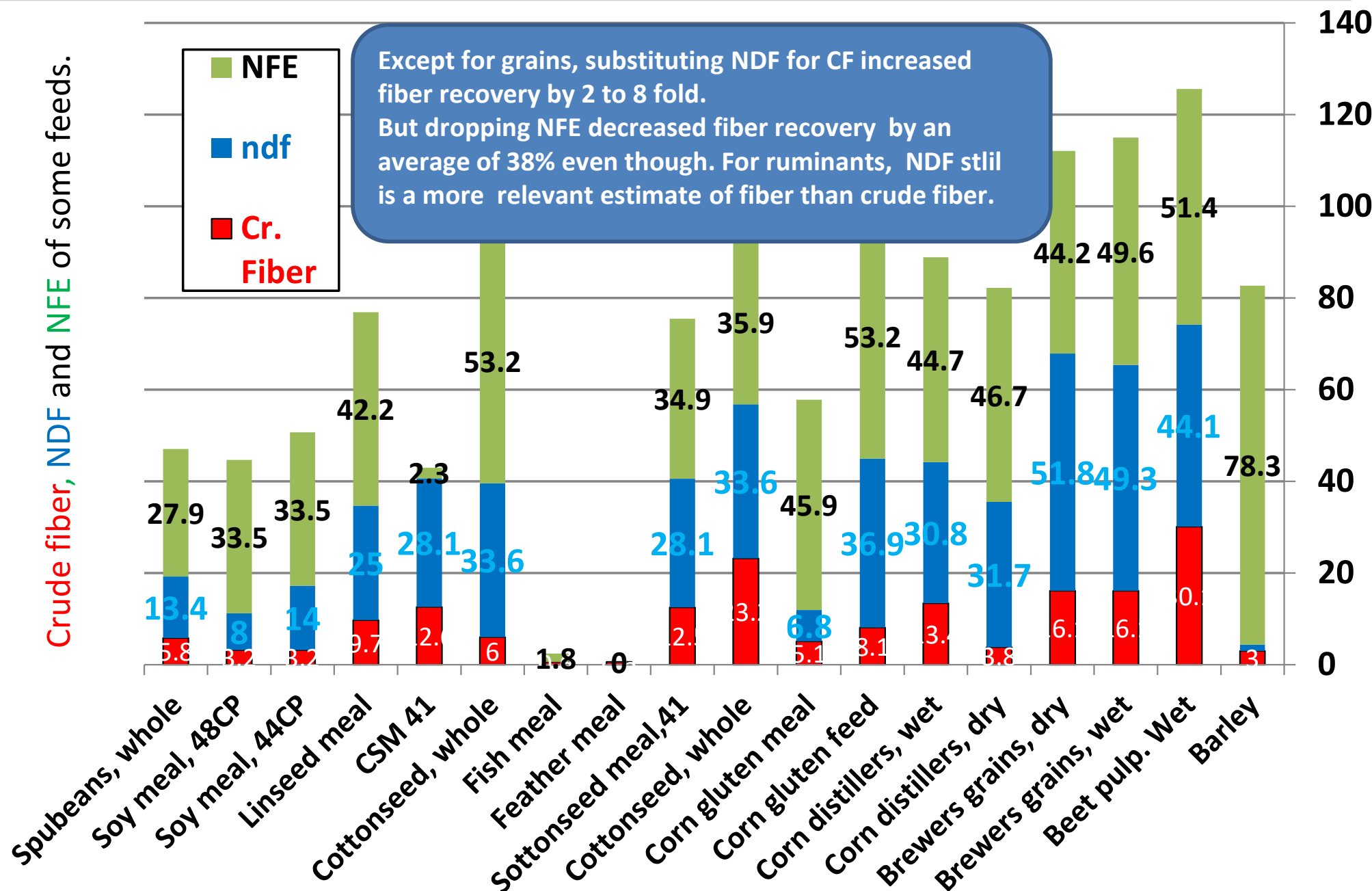
NRC Atlas of Feed Composition (1971) New



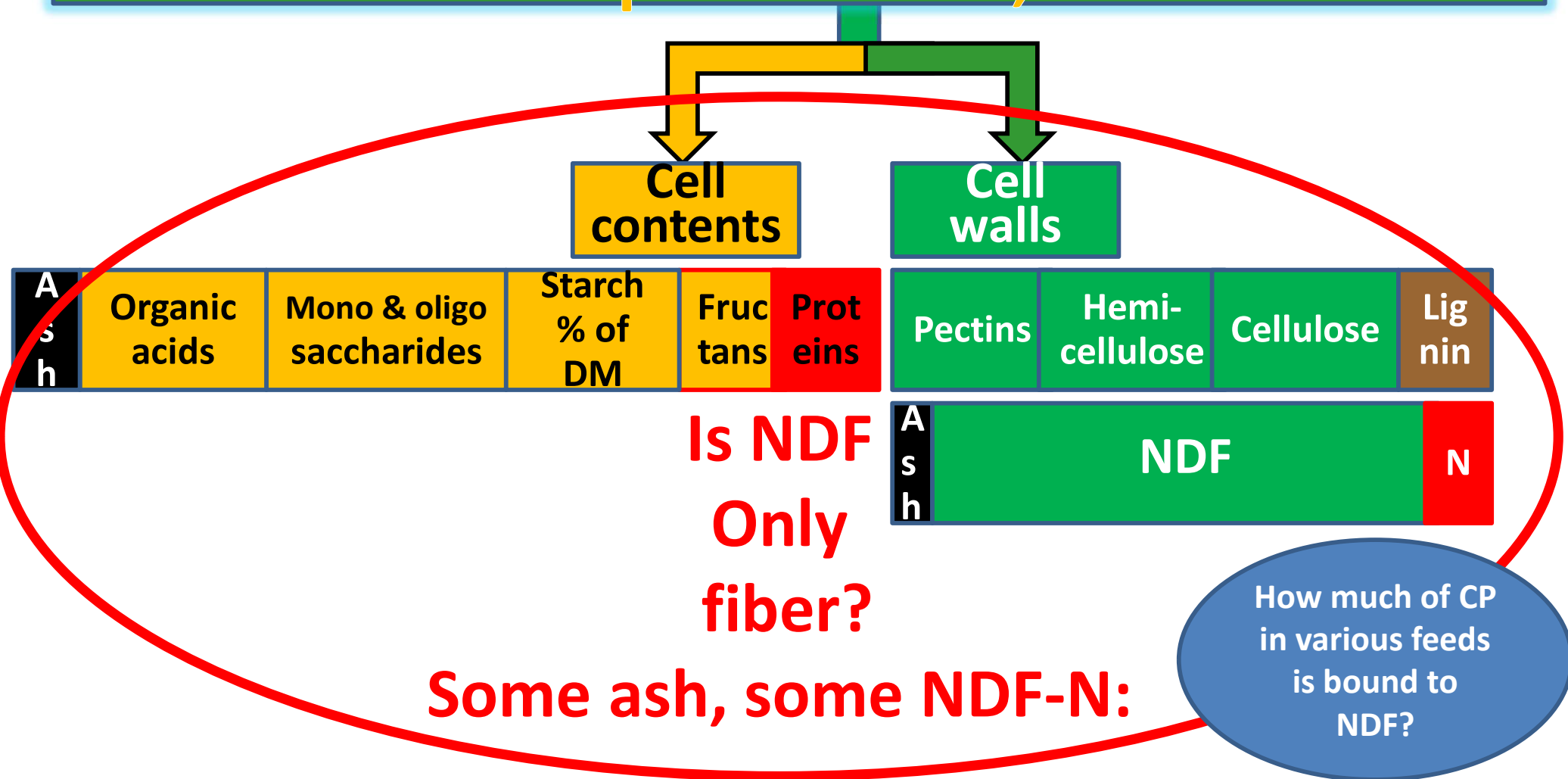
Crude Fiber vs. NDF and NFE Changes with NDF Substitution (1982 Dairy NRC, 2021 Dairy)



How does replacing [Crude Fiber and NFE] with NDF Change fiber recovery? (1982 Dairy NRC vs. 2021 Dairy)



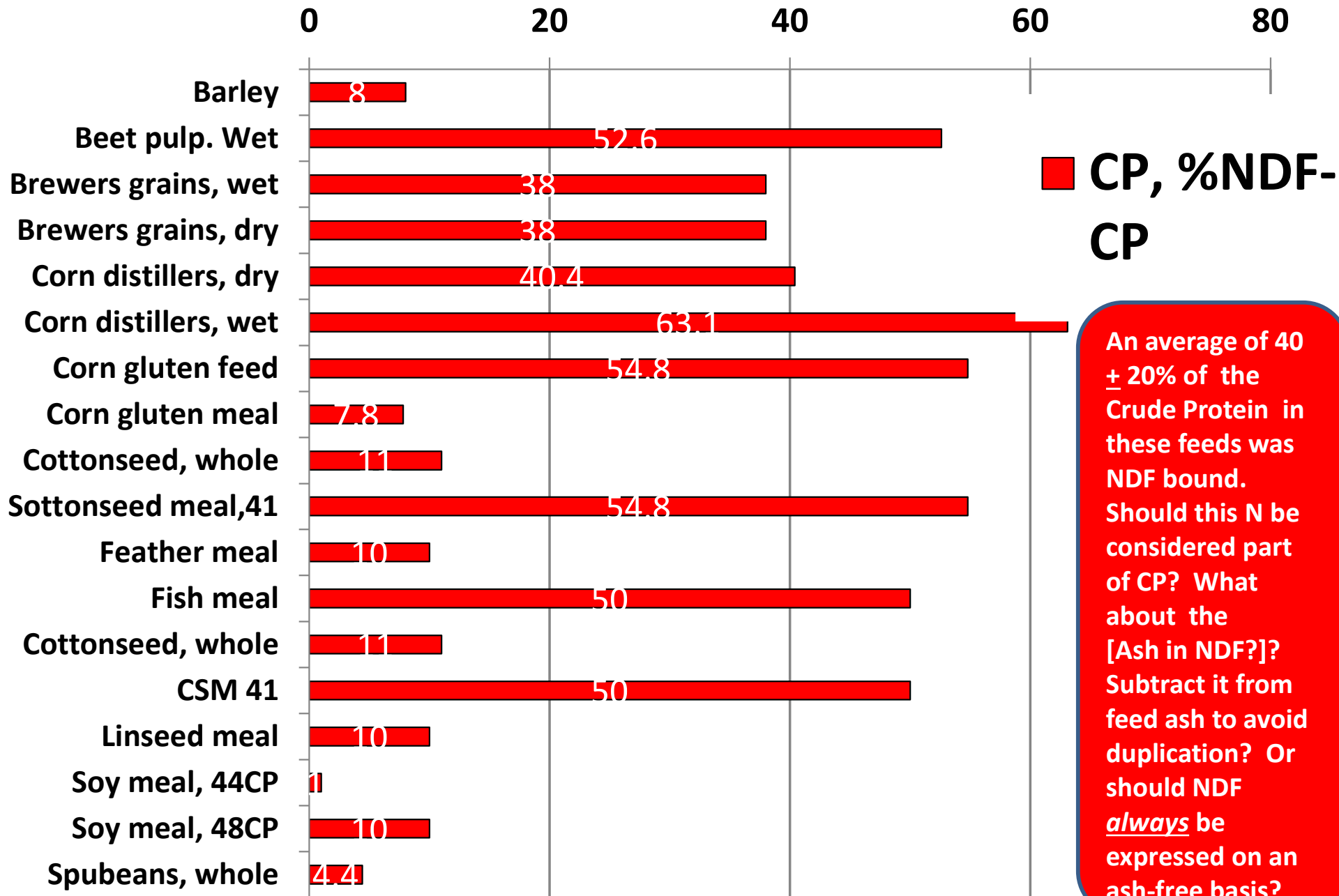
Plant Components, % of DM



If > 100% , some constituent(s) may be doubly-counted.
If < 100%, something may be either missing or under-assayed!

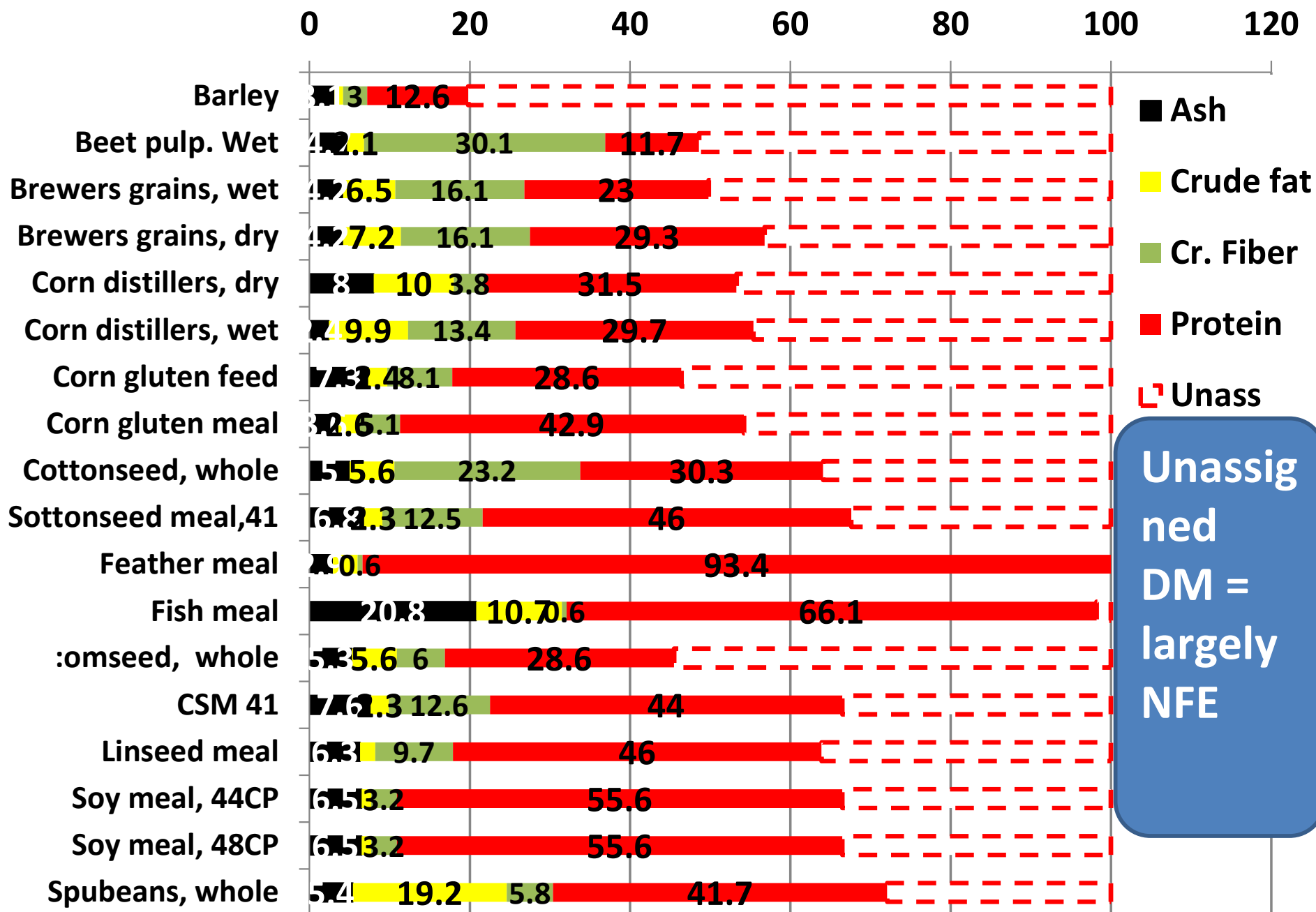
How much of Protein from various feeds is bound to NDF?

Fraction of N as NDF-N, % (Sniffen 1992)



Grains Composition (Weende components)

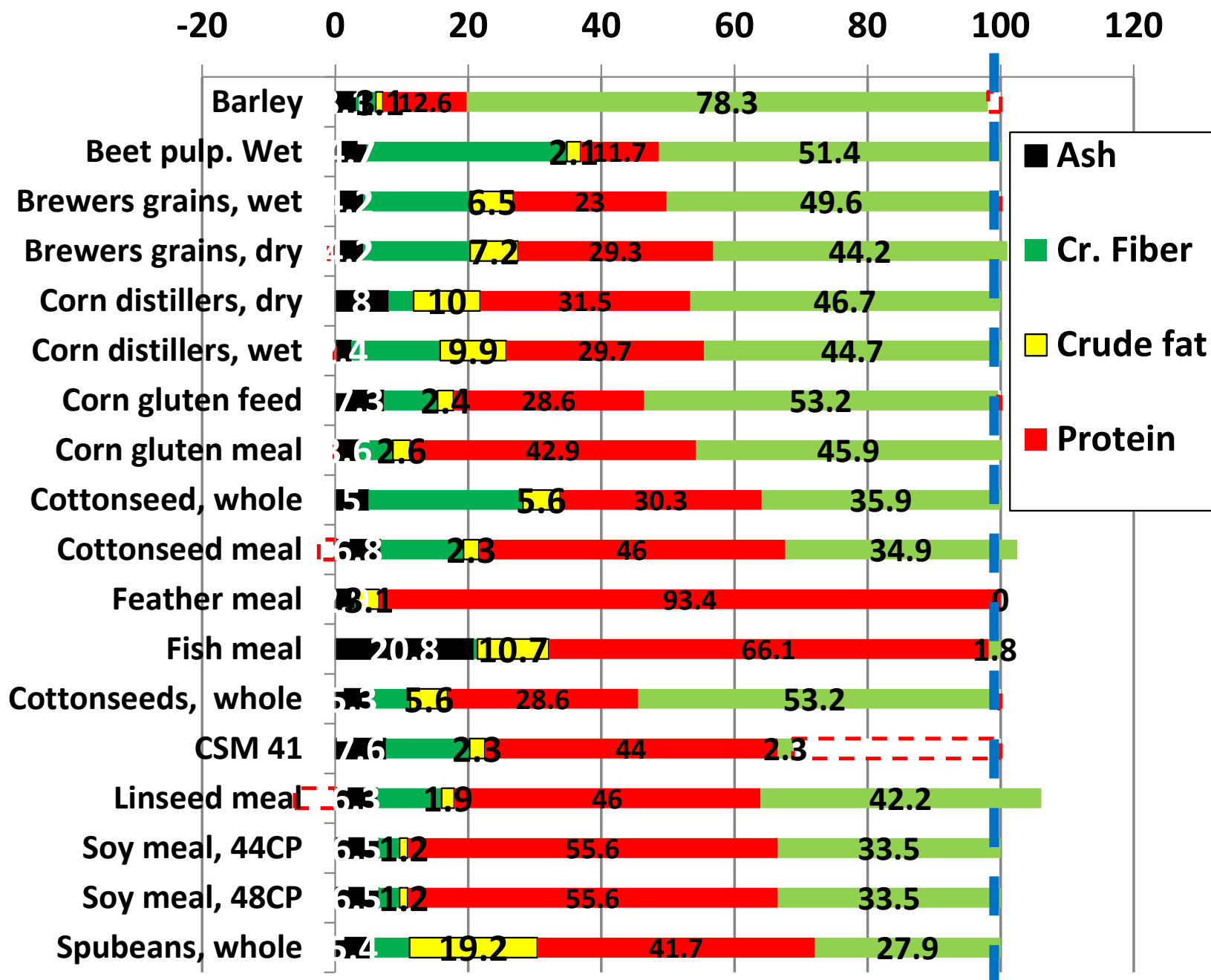
NRC Atlas of Feed Composition (1971) +CP



Grains, Composition (Weende components)

NRC Atlas of Feed Composition (1971) +NFE

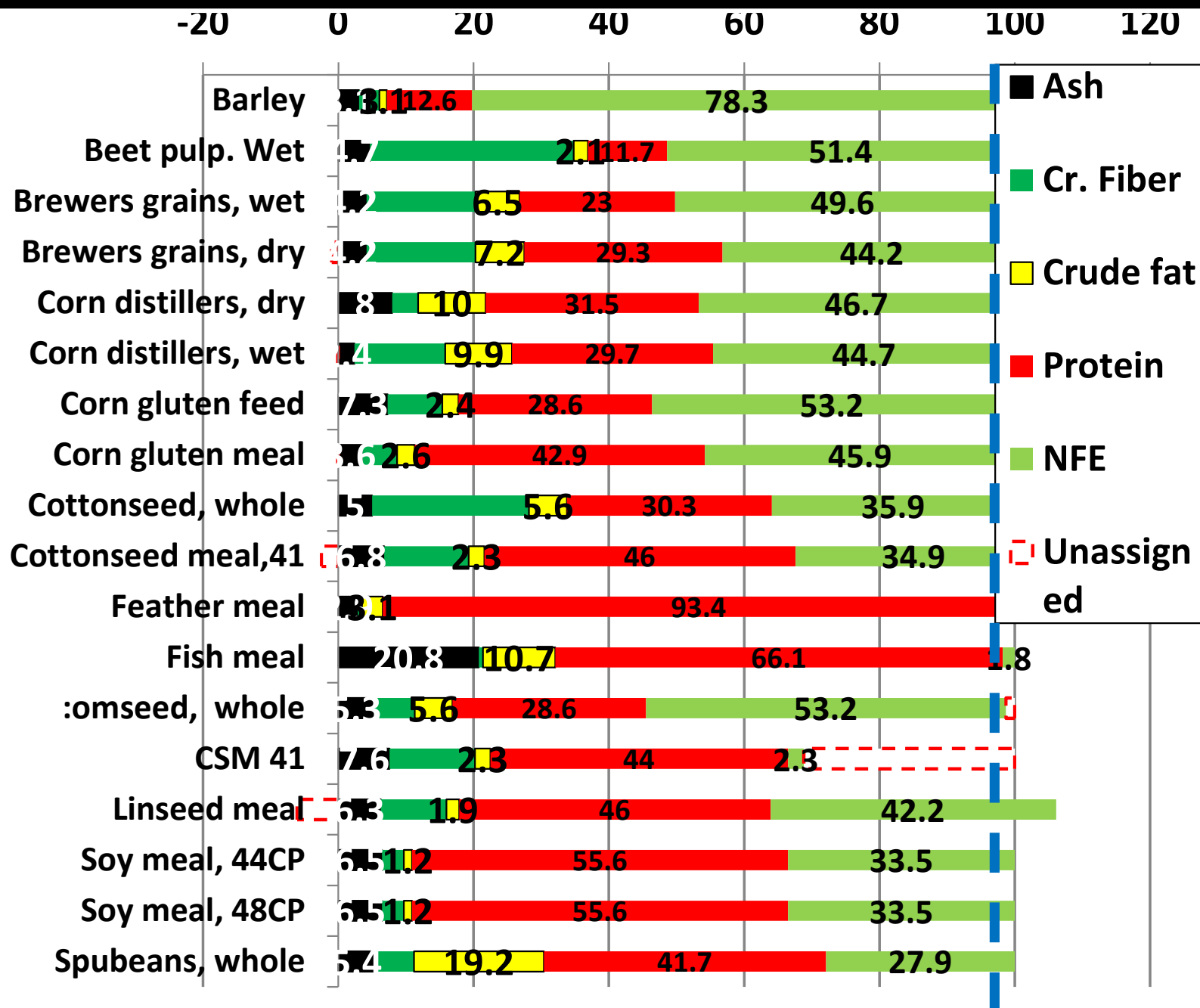
Unassigned
Mean
=+1.4% of
OM.
Range=
-6.3%
(Linseed)
to 31+%
of CSM.



Grain Components, Composition (Weende components)

NRC Atlas of Feed Composition (1971) +NFE FROM Sniffen et al 1992

Unassigned
Mean
=+1.4%
of OM.
Range=
-6.3%
(Linseed)
to
31+%
of CSM.

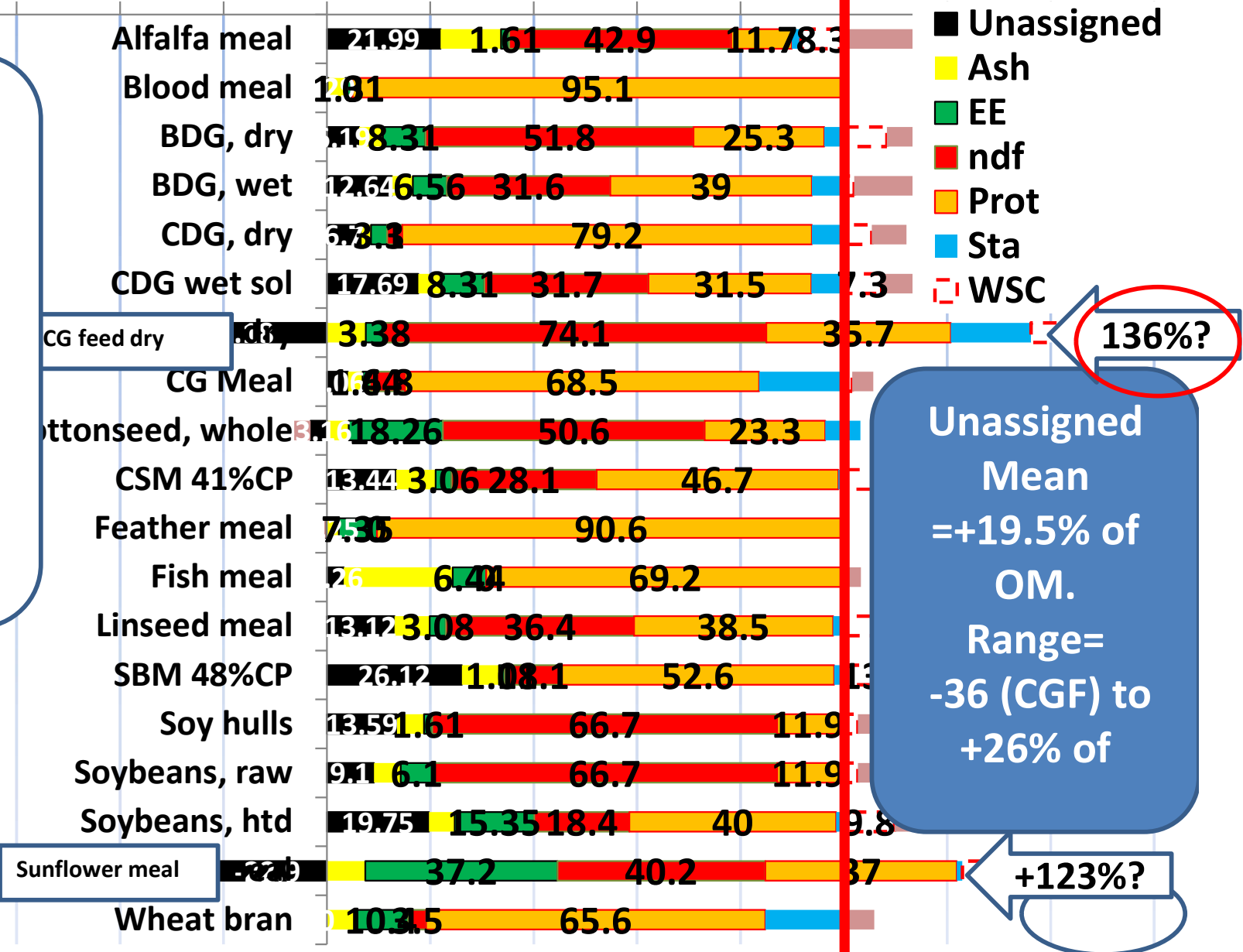


Protein Concentrates (Components)

NASEM Dairy (2021)

-100 -80 -60 -40 -20 0 20 40 60 80 100 120 140 160

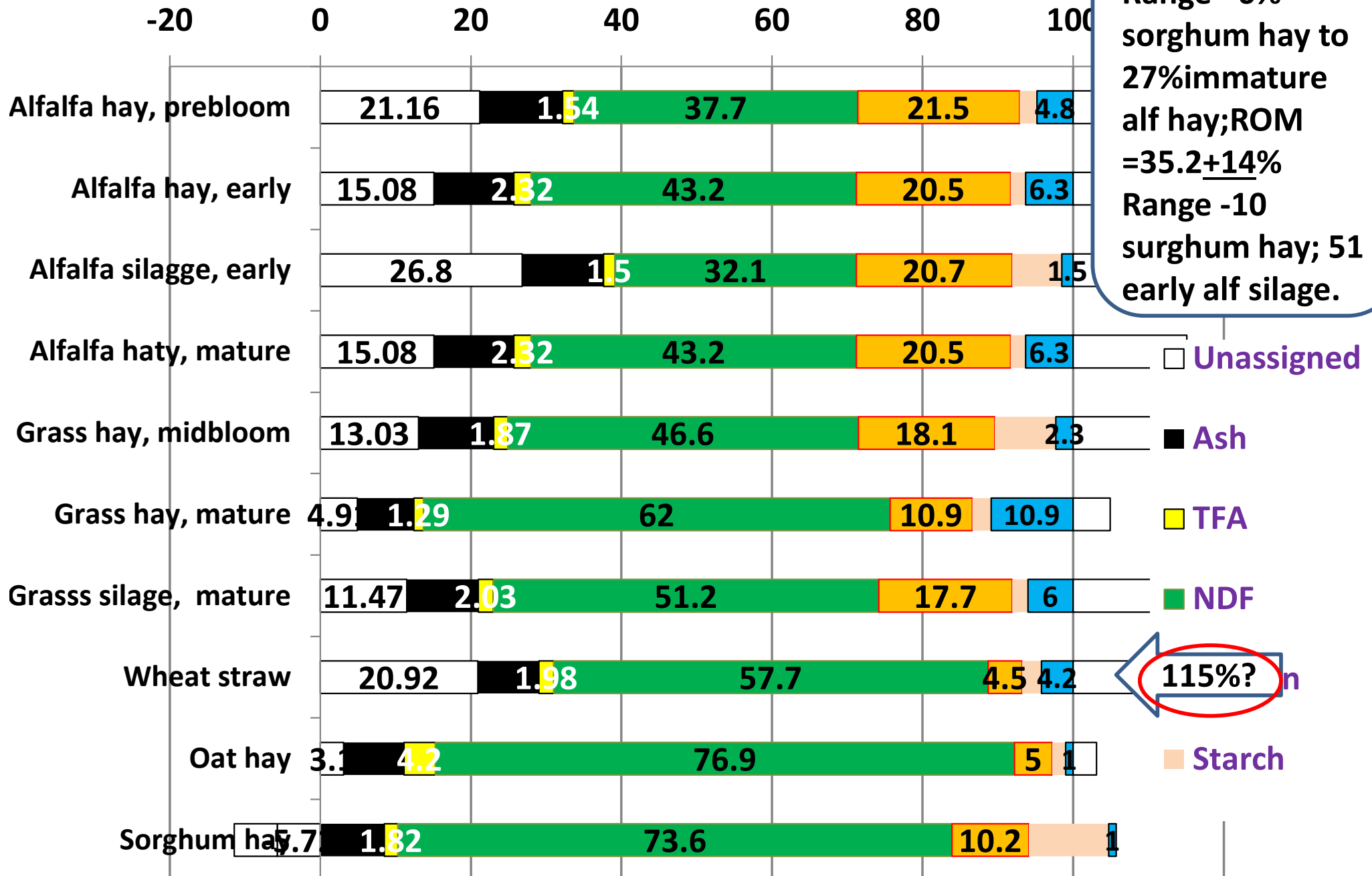
Unassigned DM
Mean =
5.8±10.2%.
Range -36% Dry
CGF; 28 for CGM;
-23 for Sunflower
meal
ROM = 53.5±27%
Range -4 for dry
CGF; 95 for blood
meal.



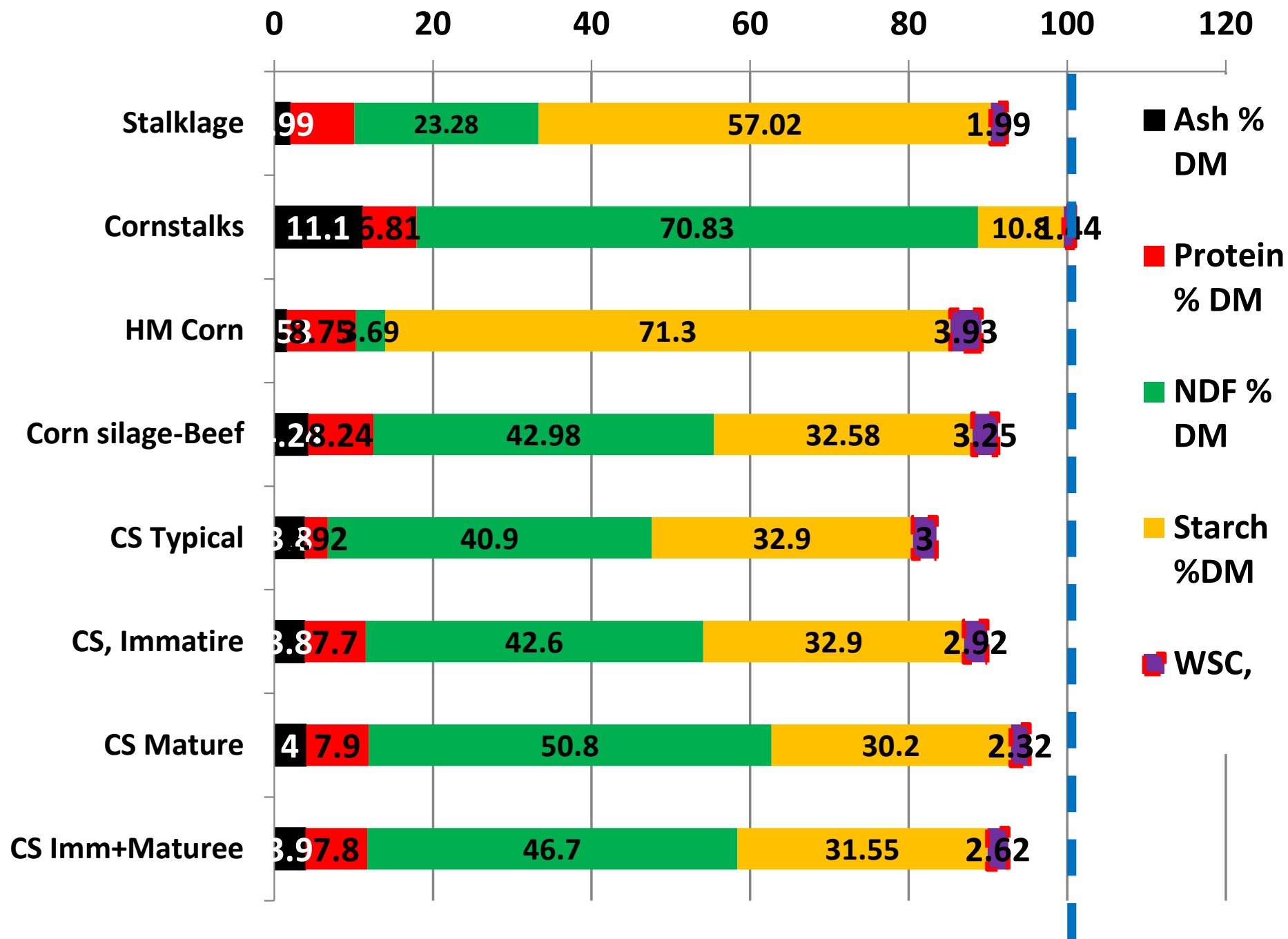
Hays and Silages: Feed Composition

NASEM DAIRY 2021

Unassigned DM
Mean =
12.6+14%.
Range -6%
sorghum hay to
27%immature
alf hay;ROM
=35.2+14%
Range -10
surghum hay; 51
early alf silage.



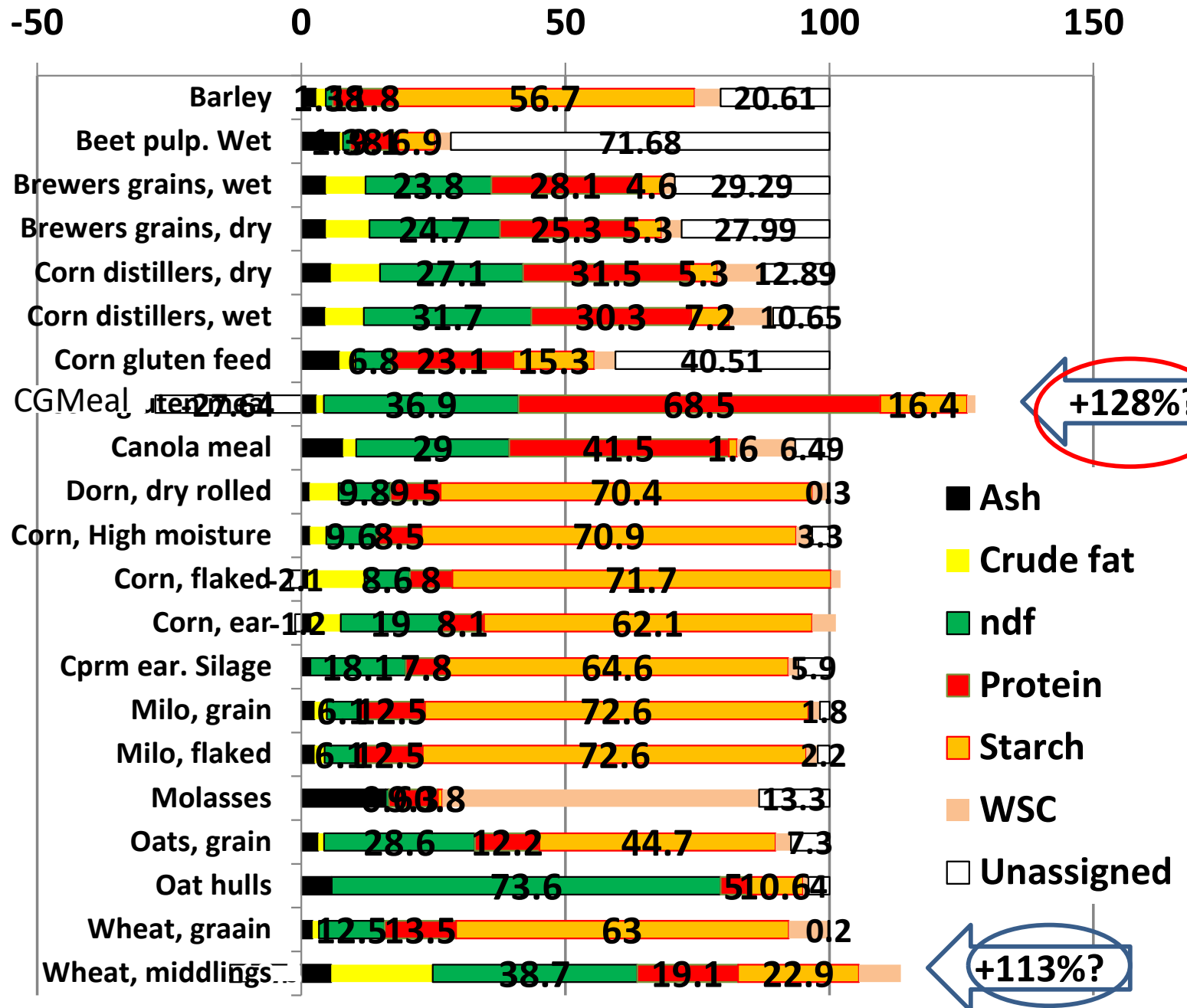
Silage Composition (Dairy NRC 2021)



GRAINS: Feed Compositions

NASEM DAIRY 2021

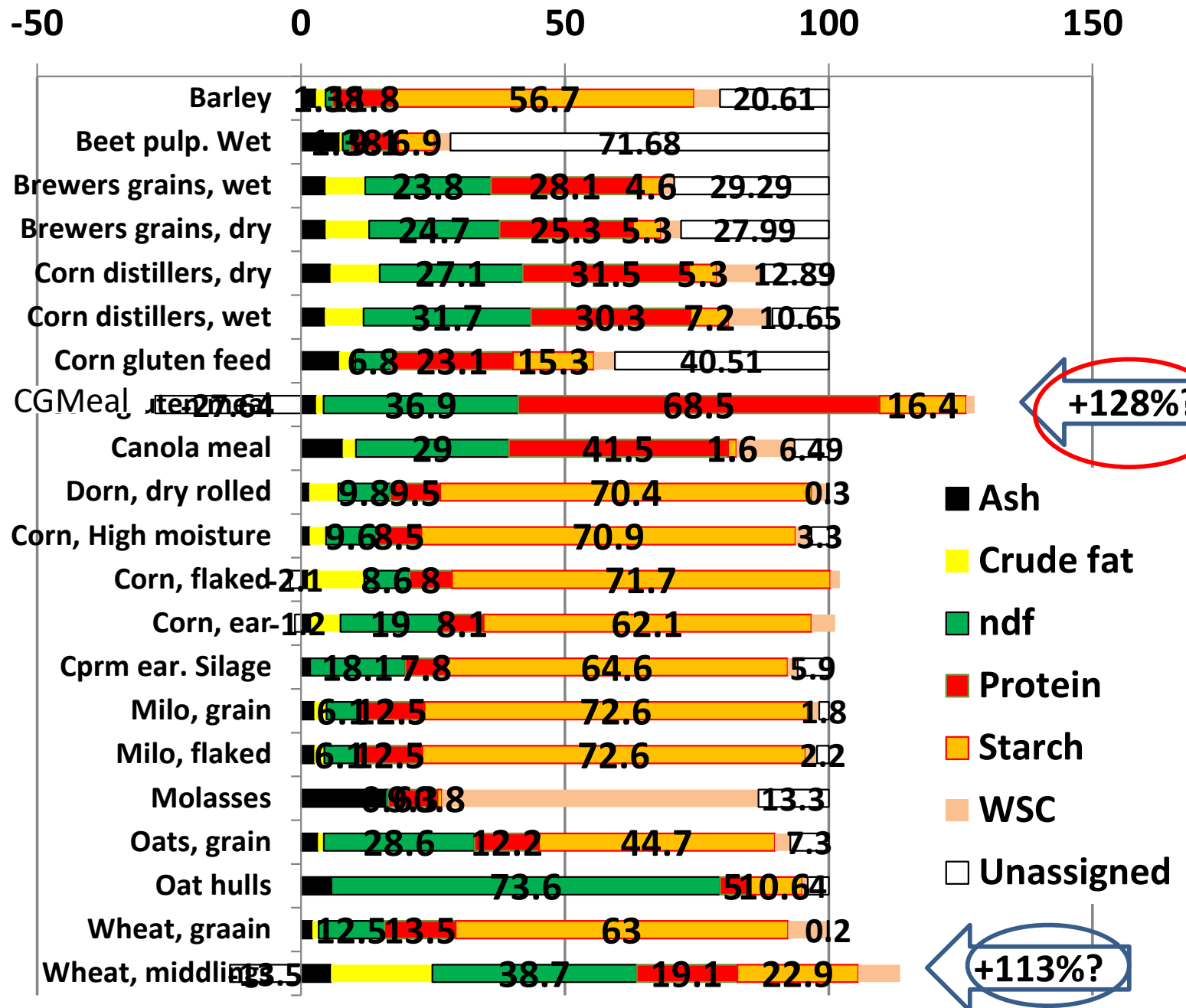
Unassigned DM
Mean = 10.2 ± 21
Range -28 for
CGM; 71.7% for
wet beet pulp.
ROM = $37.25 \pm 25\%$
Range 8.8 for
flaked corn; 84 for
wet beet pulp.



GRAINS: Feed Compositions

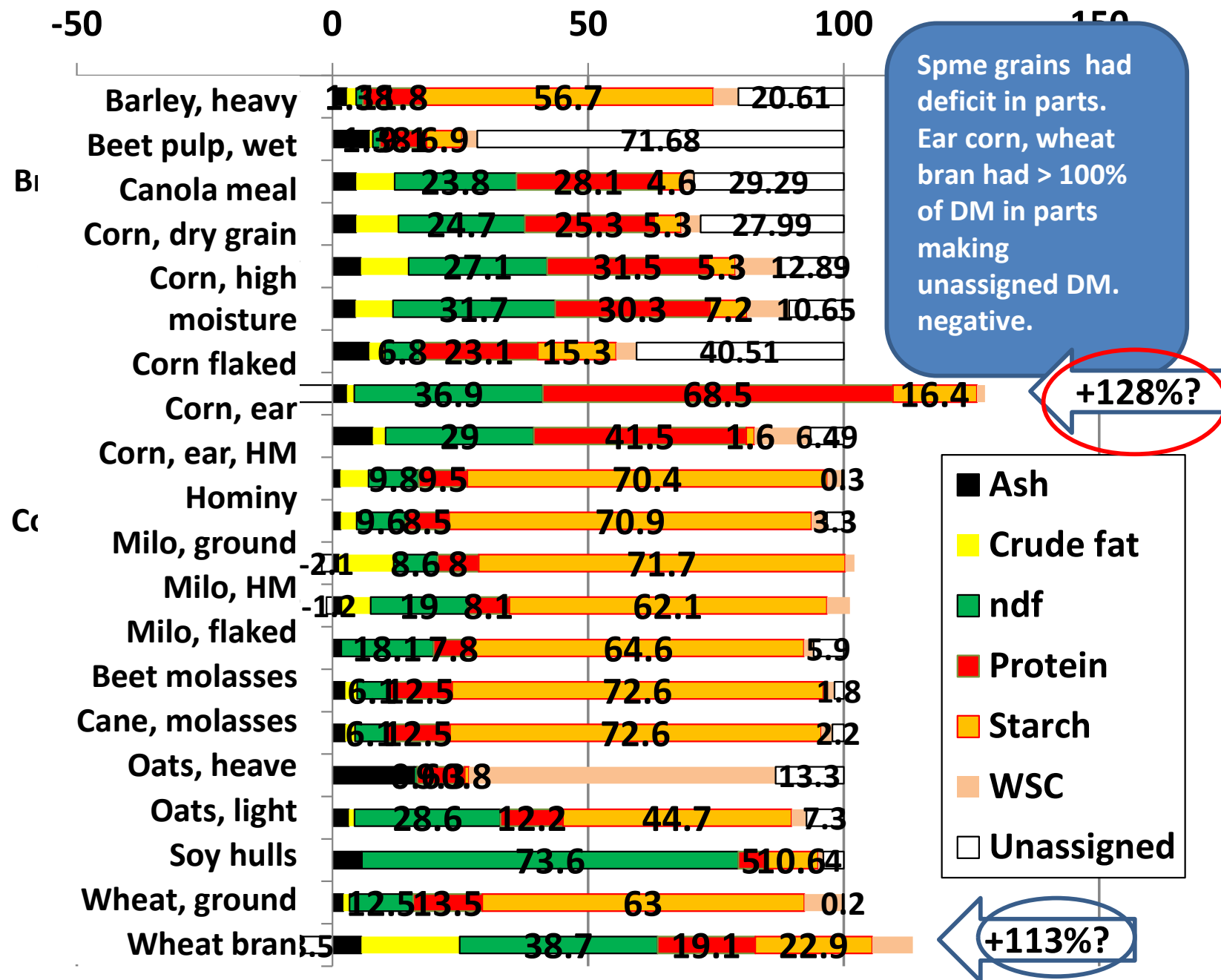
NASEM DAIRY 2021

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GrainsHays and Silages: Feed Compositions

NASEM DAIRY 2021



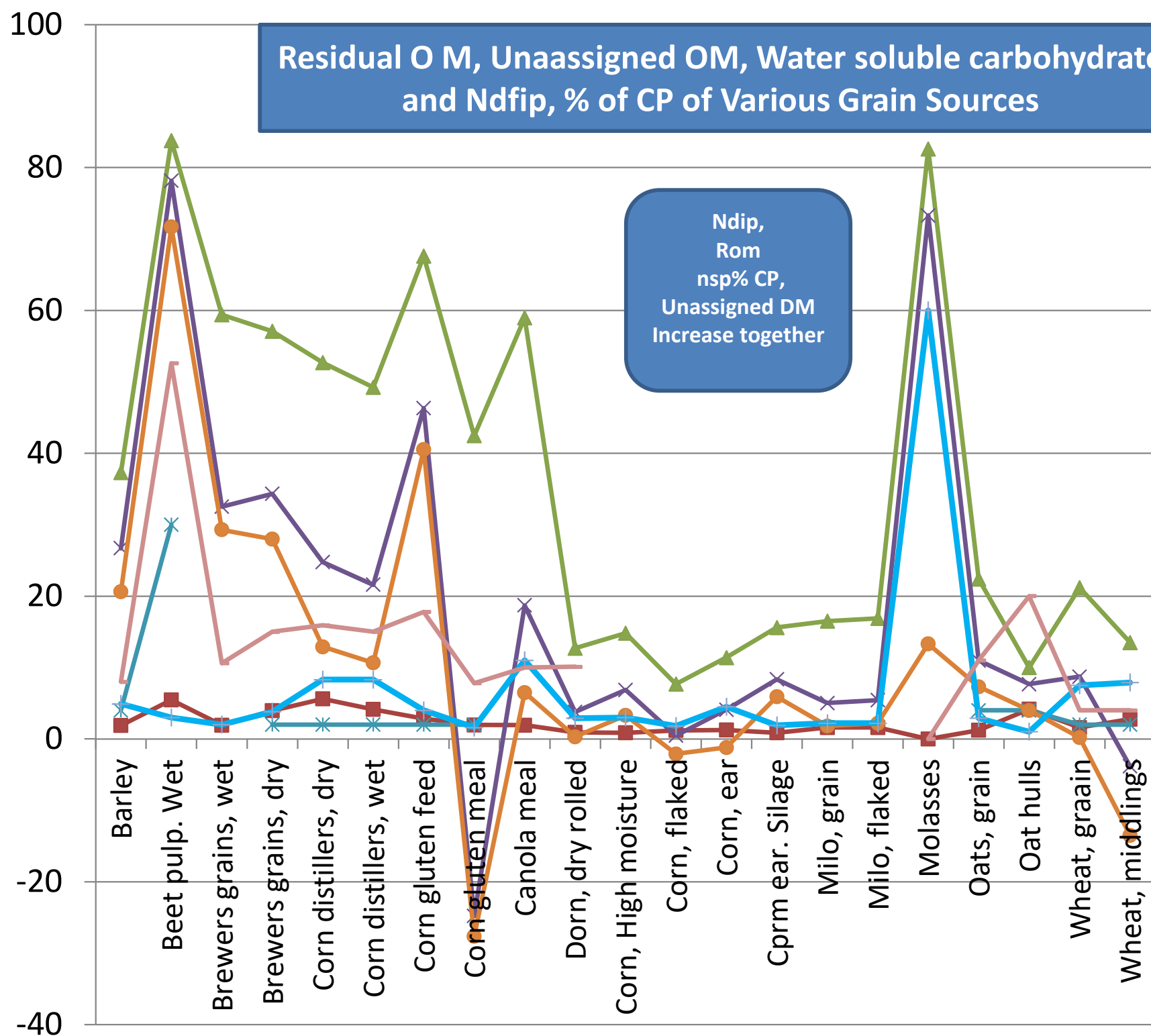
What corrections for unassigned DM have been proposed? NFE, ROM & NSC

1. ROM: Describes all unassigned OM as ROM (Residual organic matter) as discussed in NRC EM Dairy 2021 to theoretically include water soluble carbohydrates, fermentation products and short chain fatty acids, glycerol, tannins, waxes, pectins, beta-glucans, pigments and oligosaccharides but subtracts ash and NDF-N from ash and N to avoid overlap of components; assigns ROM a gross energy value of 3.9 mcal/kg and a digestibility of 96% based on apparent digestibility of non-NDF Omas cited by Van Soest text . Presumably all WSC and starch are handled are included in ROM and not considered independent. Yet separate digestibility values for starch for dairy are summarized and tabulated.
2. NSC: Include a value called non-structural carbohydrate or polysaccharides (NSC or NSP) that a) substitutes ash-free NDF for NDF and b) adjusts NDF to subtract overlapping NDF or ADF-bound N. Suggested formula for NSC (Sniffen et al. 1992) is $NSC = 100 - [ndf - ndfip] + ash + fat$; theoretically NSC would include WSC and starch that might be considered independently and could be assayed separately; sources and processed starch sources are now assigned separate digestibilities.

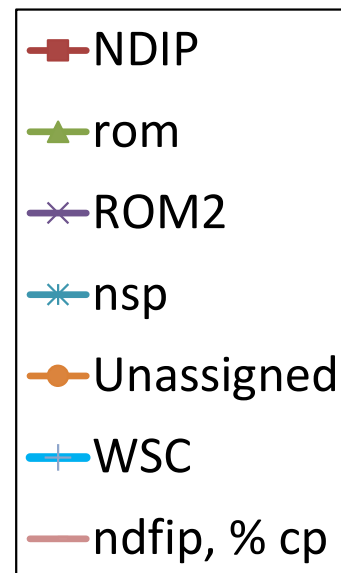
Residual O M, Unassigned OM, Water soluble carbohydrate and Ndfip, % of CP of Various Grain Sources

Ndip,
Rom
nsp% CP,
Unassigned DM
Increase together

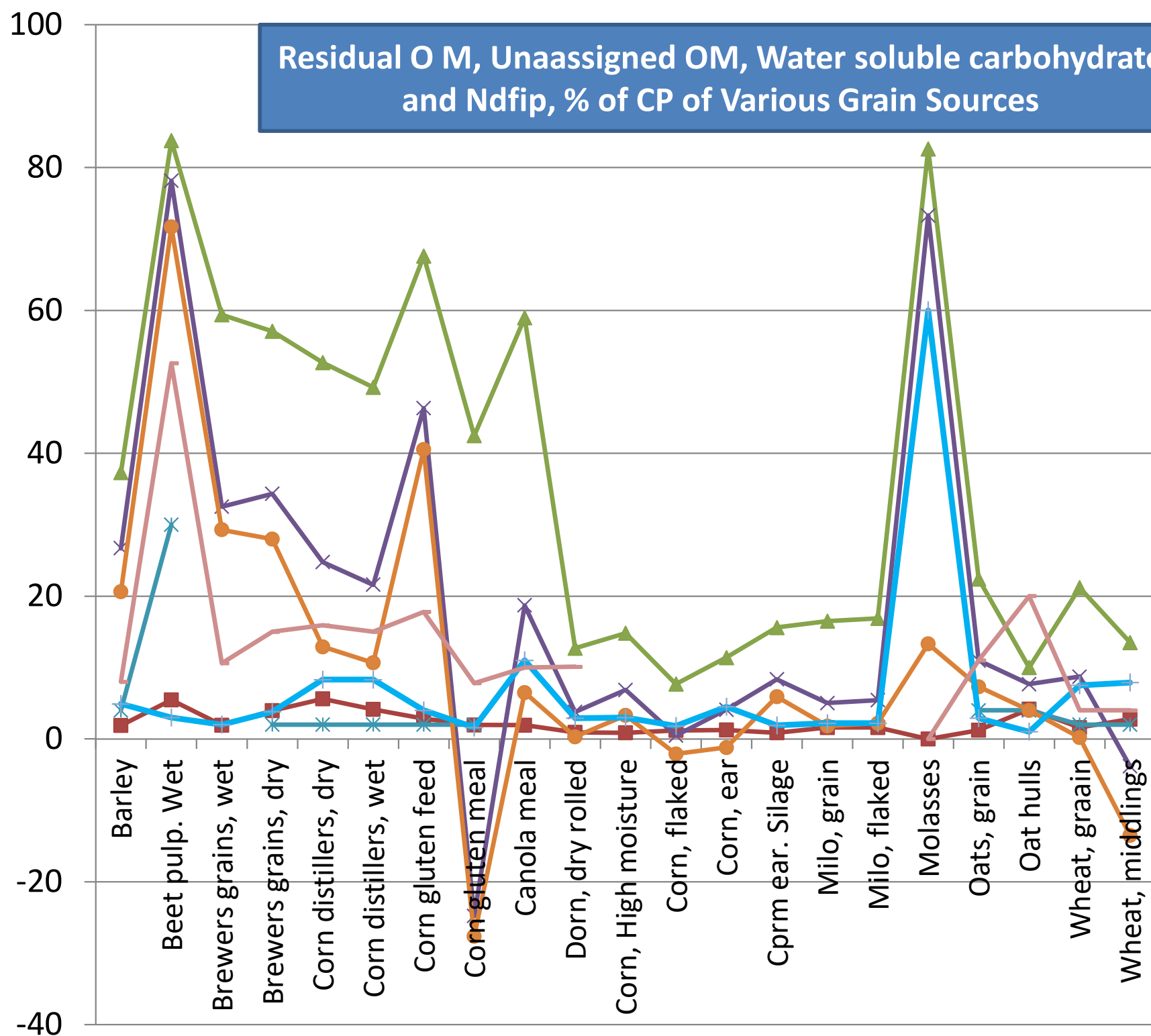
- NDIP
- rom
- ROM2
- nsp
- Unassigned
- WSC
- ndfip, % cp

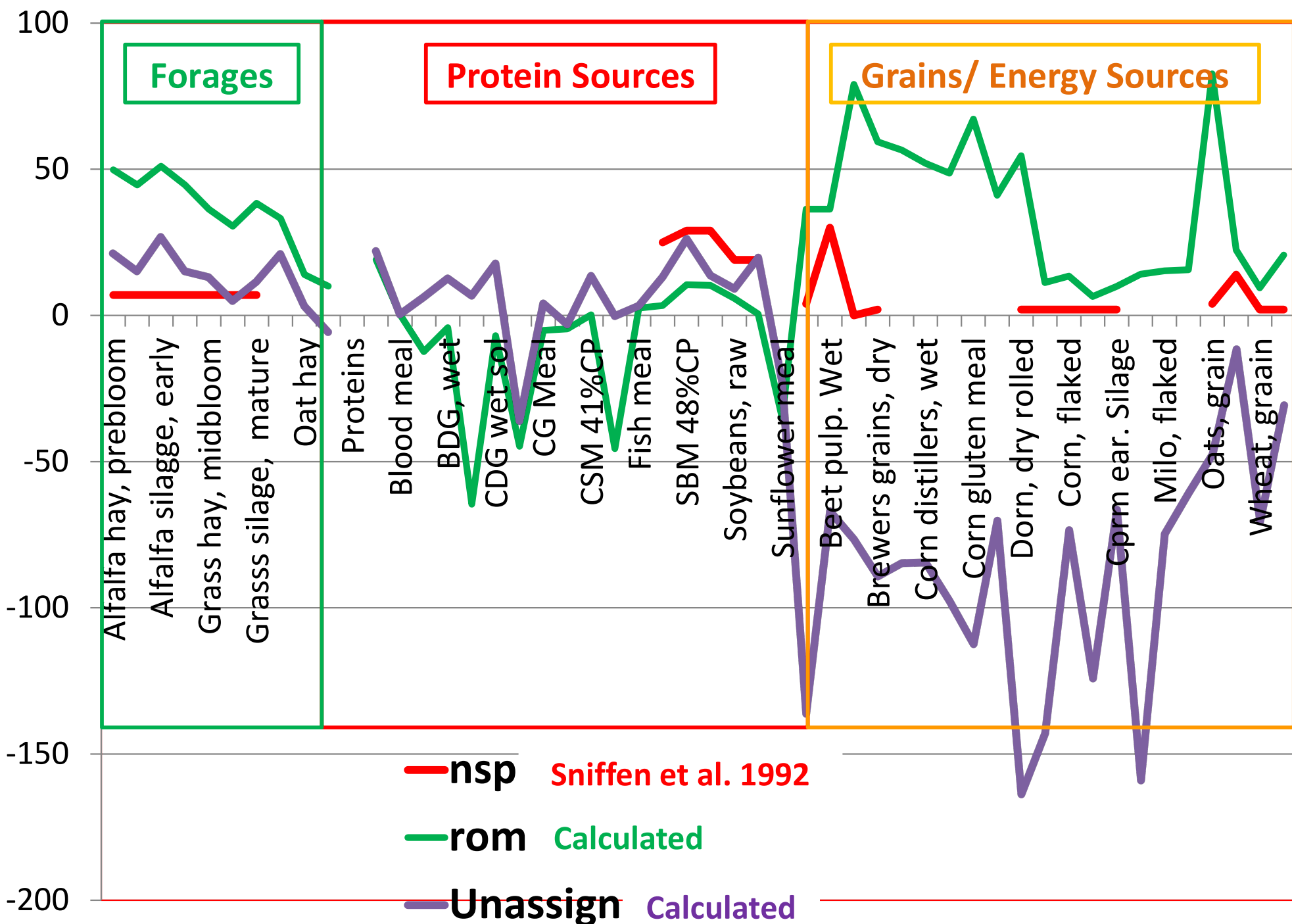


Residual O M, Unassigned OM, Water soluble carbohydrate and Ndfip, % of CP of Various Grain Sources

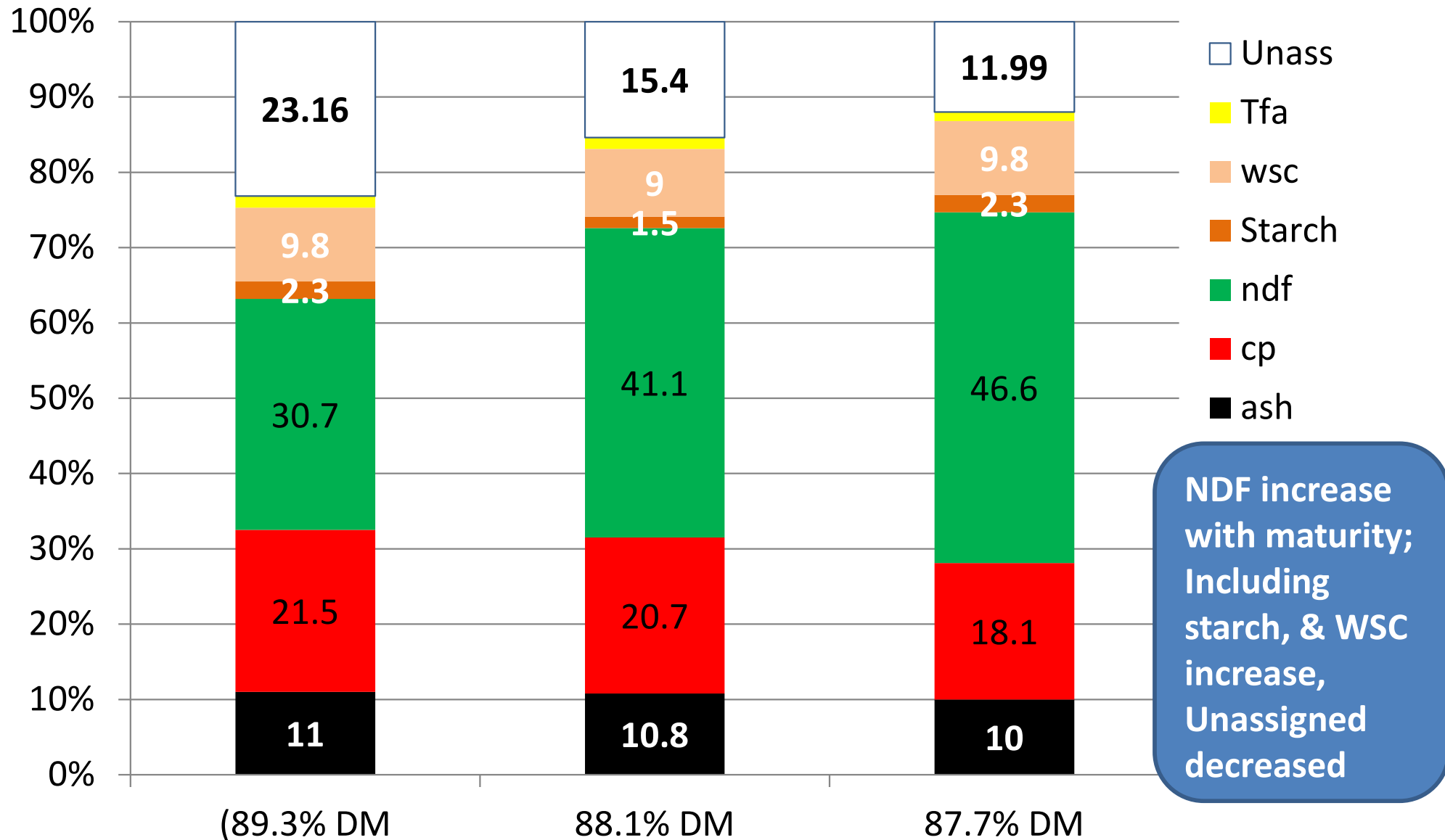


Unassigned Increased with ROM, NSP, NDIP but feeds differed markedly.



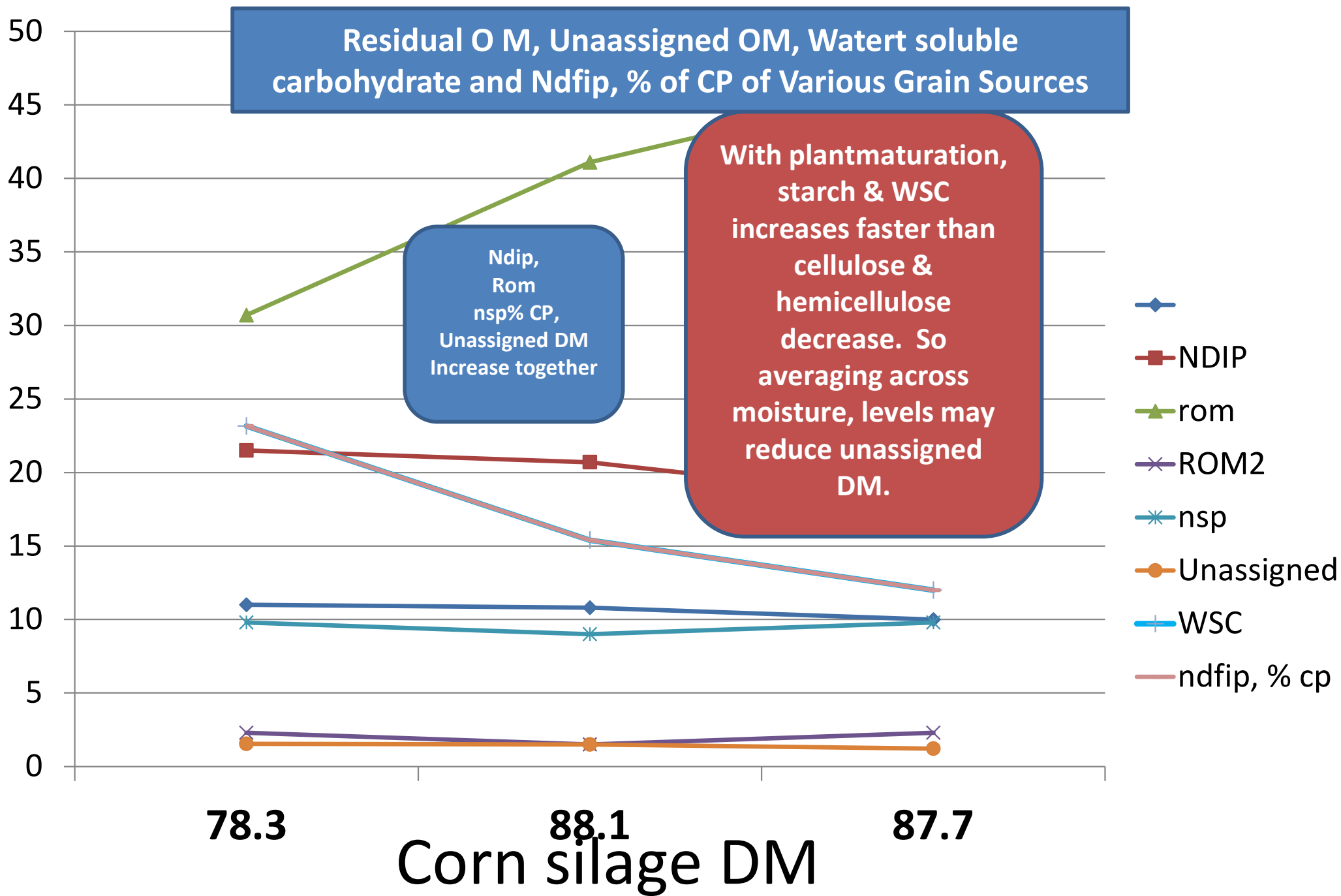


NRC 2021 Proximate Analyses . Alfalfa Hay, Immature; Mid-mature; Mature;



Unassigned DM by Feed Group

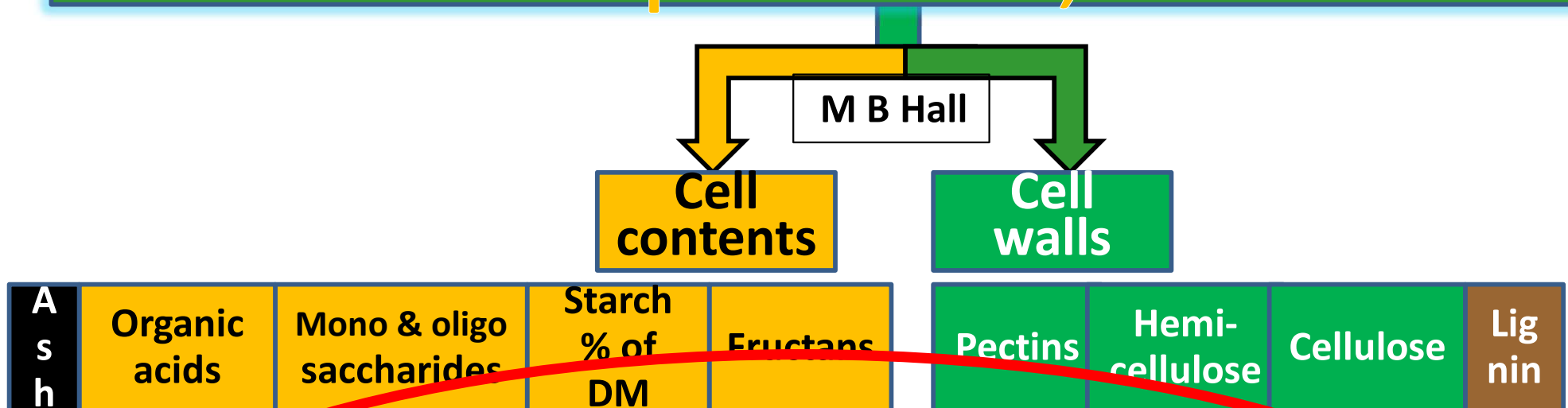
Data set	Weende (NDF)	Dairy 21	Dairy 21	Dairy 21
Group	Grains	Grains	Proteins	Hay/Silages
Average	39.15	10.18	5.85	12.58
StDev	18.41	20.3	36.18	9.67
Min	0	-27.64	-36.2	-5.72
Feed	Feathers	CGM	CGF	Sorg hay
Max	80.2	71.68	26.12	26.8
Feed	Barley	Wet beet pulp	SBM 48	Alf sil. Early
Feeds, N	19	22	19	11



Recommended Changes in Assays

1. Define, describe, include numbers for feeds in assay tables for NSC, ROM, Unassigned DM.
2. Avoid double counting
 1. Report NDF only on ash-free basis. [doublecounting]
 2. Report CP as CP – NDF bound N.
3. Develop, & report results for pectins, B glucans.
4. Searchable assays by assay date, NIR vs wet chem, location as being developed currently.
5. Develop and publish all assay methods (AOAC).
6. Separate NDF fractions for estimating relationships.
 1. Responses to Lignin differ from hemi & cellulose.

Plant Components, % of DM



Recommended New Analyses

Detergent fiber Recommendations:

Include ROM and NSP values in tables.

Avoid overlaps: Report NDF on an Ash free basis; Protein= (N-NDF-N)x5.6 (Marriotti2008), Add pectins, & Beta glucan assays

Locating Unassigned DM/OM

Weende & NFE

Van Soest et al. 1991

Dairy NASEM, 2021

DM
basis

100 - ash

100 - ash - urea

100 - ash - urea

OM
basis

- Crude Prot = $N \times 6.25$

- Crude Prot = $N \times 6.25$

- Crude Prot = $N \times 6.25$

- Crude Fat

- Crude Fat

- Total fatty acids

- Crude Fiber??

- NDF

- [NDF-lignin-
NDF

NFE = *Nitrogen
free extract*

- NSC = *Non-
structural CHO*

- Starch

- Starch

ROM = *Residual
organic matter*

- WSC= sugars or -
ESC=Oligosacch

- WSC or - ESC

- NDFSF= *NDF
Soluble fiber*

- Glycerol

= Pectins, tannins

- NDFSF

Diet Formulation

Feed-centric

Least cost energy

Animal-centric

Addition

- Butyrate
- Essential AAs

Delete

- Gluten
- Lactate
- Oligosaccharides
- Prolamins

Summary

- Recommended to include mean values for ROM and NSNSC within tabular feed tables
 - Index of the extent of unidentified substances
 - Degree that E values and digestibility may be altered to compensate for missing/excess DM or OM.
- Nutritional values are imperative to diet formulation
 - Discrepancies in actual vs estimated values may be underestimated and pose significant performance and economic detriments.