NIRS Analysis of Feed Protein Materials

Presented by: Lujia HAN, Ph.D
China Agricultural University
hanlj@cau.edu.cn




Contents

- Background
- Materials and methods
- Results and discussion
- Conclusions

1 Background

- Feed contaminated with MBM is commonly accepted as the main transmission carrier of the prior responsible for BSE.
- In China, animal derived products are banned for feeding ruminants
  - Legal Text(*农牧发［2001］7号*)
- Potential risk of MBM still exist in feed market for commercial benefit reason
  - Illegal utilization of MBM in ruminant feed
  - Illegal adulteration of MBM in fishmeal
  - Illegal adulteration of Soybean meal in fishmeal

1 Background

- For the monitoring of a presence of animal protein, several methods can be applied for risk management
- To investigate the feasibility of using NIRS to analyze
  - MBM vs. ruminant feed
  - MBM vs. fishmeal
  - Fishmeal vs. soybean meal

2 Materials and Methods

2.1 Samples Collected

- Ruminant concentrate: **235**
  - Cattle 47
  - Calf 34
  - Milking cow 31
  - Dairy 48
  - Sheep & goat 47
  - Cattle & sheep 28
- MBM: **58**
  - Cattle 4
  - Sheep 3
  - Swine 12
  - Poultry 13
  - MBM (uncertain species) 26

2.1 Samples Collected

- Fishmeal: **201**
  - Imported 40
  - Domestic 161
- Soybean meal: **84**
  - Imported 32
  - Domestic 52

2.2 Samples Preparation

- All of the samples were crushed to pass a 0.5 mm sieve and mixed randomly
- Samples were artificial adulterated at different rate according to a factorial design of randomized blocks (Garrido-Varo et al. 2005)

MBM adulteration in ruminant concentrates for calibration

Ruminant feed

135 samples,

Cattle	31 kinds
Calf	18 kinds
Milking cattle	16 kinds
Dairy	32 kinds
Sheep	17 kinds
Lamb	7 kinds
Cattle and sheep	14 kinds

MBM

45 samples,

Cattle	4 kinds
Sheep	3 kinds
Swine	12 kinds
Poultry	13 kinds
MBM (uncertain species)	13 kinds

Mixed MBM at a rate of 0.5%-35%
（135 samples）

MBM adulteration in fishmeal for validation

Fishmeal

74 samples,

Imported 4 kinds

Domestic 70 kinds

MBM

37 samples,

Cattle	4 kinds
Sheep	3 kinds
Swine	10 kinds
Poultry	10 kinds
MBM (uncertain species)	10 kinds

Mixed MBM at a rate of 1%-33%
（74 samples）

2.3 NIRS Instruments

- **FOSS NIRSystems™ model 6500**
  - **Sample cell: Transport module (1/4 Full)**
  - **Scanning region: 400nm-2500nm (every 2nm)**

2.3 NIRS Instruments

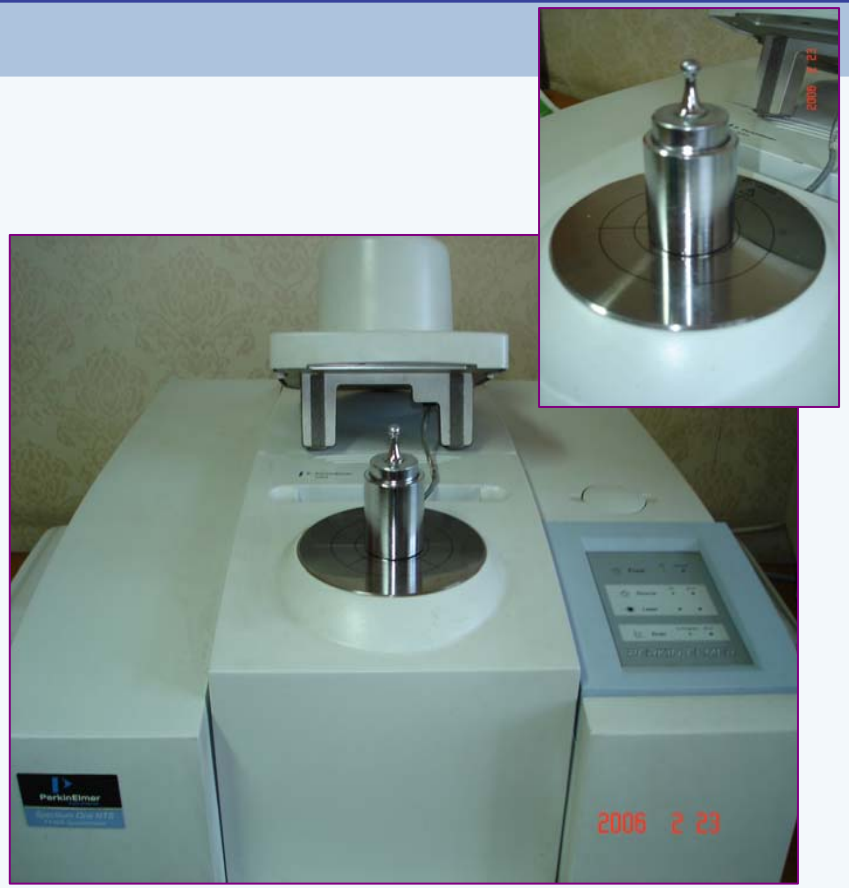
- Antaris (Thermo Nicolet, USA), InGaAs detectors
- Scanning region:10000cm^{-1}-4000cm^{-1}

  Scanning times：32

  Resolution：8cm^{-1}
- Software：TQ Analysis v6.0

2.3 NIRS Instruments

- Perkin Elmer, USA InGaAs detectors
- NIRS scanning：
  - Region ： 10000cm^{-1}-4000cm^{-1}
  - Scanning times：32
  - Resolution：8cm^{-1}
- Software：
  - SPECTRUMQUAN+5.0



SPECTRUM ONE NTS

2.4 NIRS scanning and data treaetment

- Considering packing density, each sample was scanned 3 times by 3 different people
- All samples were scanned in random sequence to prevent instrumental bias
- Software: Unscramber, Mathlab

3 Results and Discussion

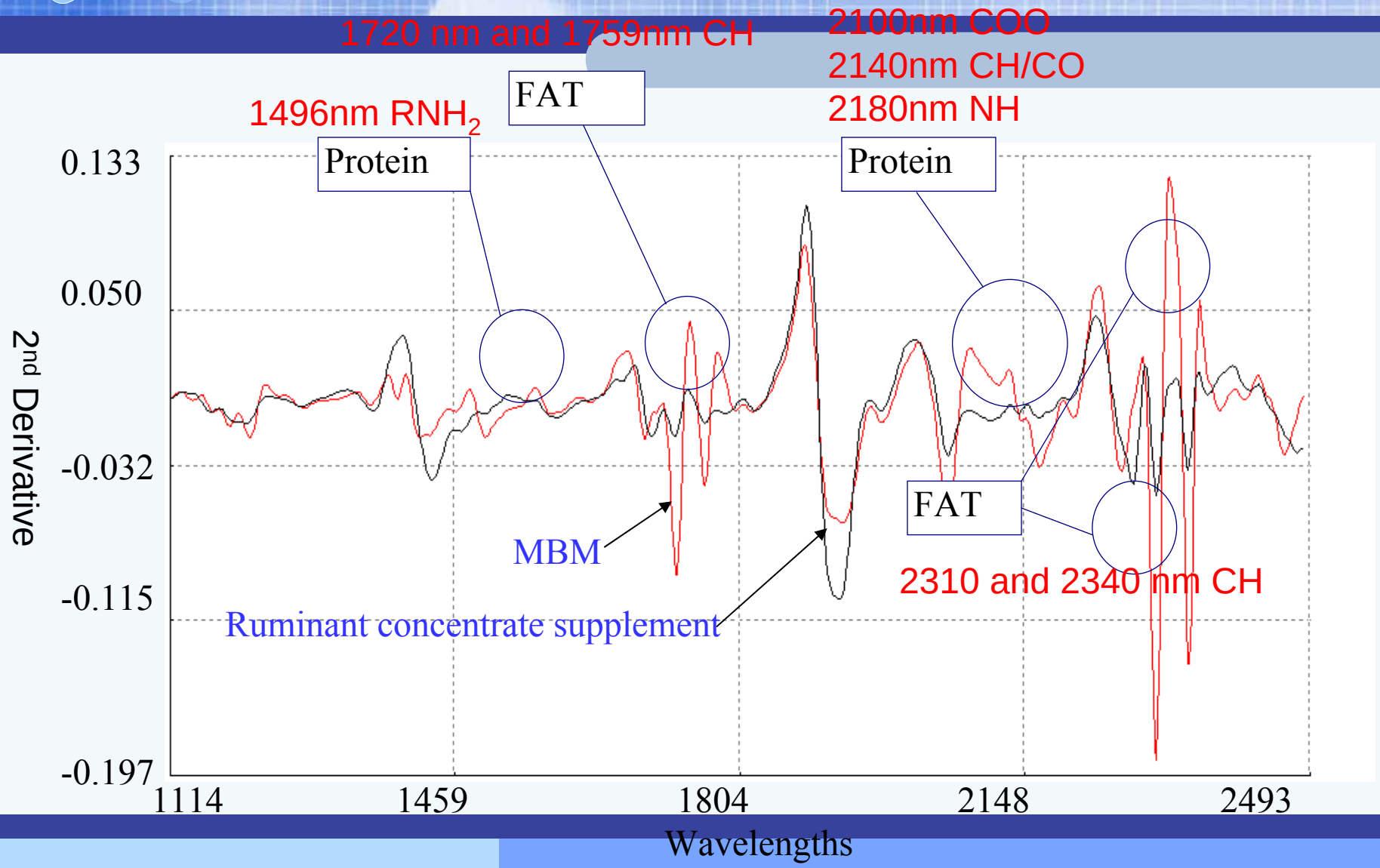
NIRS analysis of MBM in ruminant concentrate

Z. Yang, L. Han*, Q. Li, X. Fan. Journal of Animal and Feed Sciences, 2007,16(suppl.2)：442-447

Yang Zengling, Han Lujia*, Li Qingfei, et al. Spectroscopy and Spectral Analysis，2008，28（6）:1278-1282

Yang Zengling, Han Lujia*, Li Qingfei, et al. Transaction of CSAM, 2009(7)

The spectra difference of ruminant feed and MBM



Global H (GH) distances of 45 MBM samples

No.	GH	No.	GH	No.	GH	No.	GH	No.	GH
Cattle1	4.27	Swine3	3.53	Swine12	3.85	Poultry9	3.97	MBM5	3.77
Cattle2	4.49	Swine4	3.50	Poultry1	6.83	Poultry10	3.91	MBM6	3.64
Catttle3	4.48	Swine5	4.09	Poultry2	7.53	Poultry11	4.10	MBM7	5.17
Cattle4	4.88	Swine6	3.89	Poultry3	7.22	Poultry12	3.83	MBM8	4.05
Sheep1	4.34	Swine7	3.50	Poultry4	3.76	Poultry13	4.04	MBM9	3.56
Sheep2	4.89	Swine8	4.79	Poultry5	3.64	MBM1	5.70	MBM10	3.15
Sheep3	5.99	Swine9	4.01	Poultry6	4.37	MBM2	4.68	MBM11	5.06
Swine1	3.80	Swine10	4.14	Poultry7	4.05	MBM3	4.72	MBM12	4.68
Swine2	3.59	Swine11	4.90	Poultry8	3.89	MBM4	3.71	MBM13	7.01

The mean spectrum of 135 ruminant feed samples as the centre (GH=0)

Effect of mathematic pre-treatment and scatter correction on PLS discriminant equations

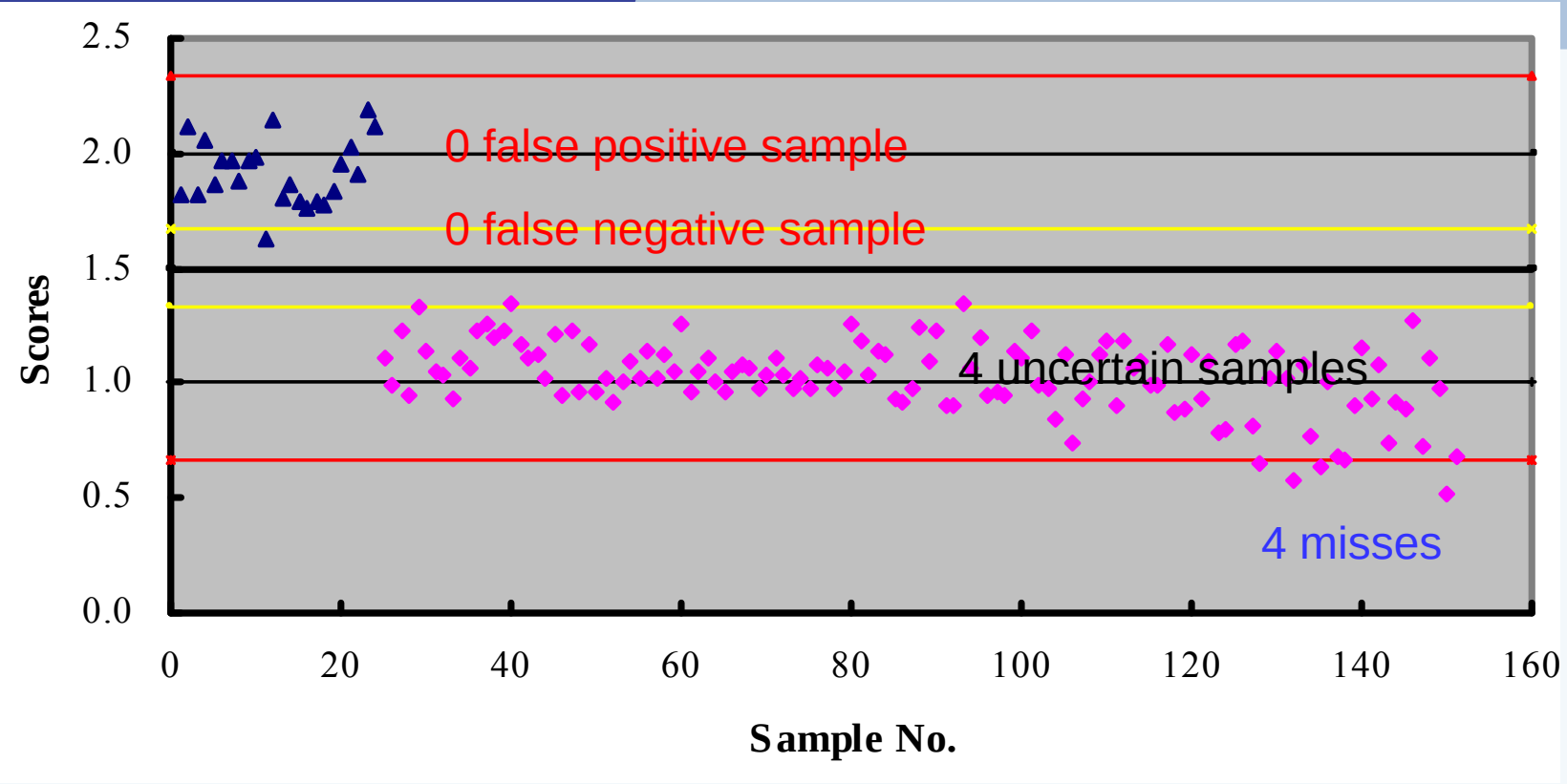
λ	Mathematic pretreatments	Scatter corrections	PLS terms used	Misclassified[a]	Uncertainty[a]	RSQ	SEC	1-VR	SECV
260	0,0,1,1	None	29	12	343	0.84	0.1983	0.82	0.2118
		SNVDT	30	18	321	0.83	0.2022	0.81	0.2179
		SNV	29	19	374	0.79	0.2257	0.76	0.2409
		DT	30	20	353	0.81	0.2155	0.78	0.2316
		Std MSC	29	16	340	0.82	0.2105	0.79	0.2240
		Wtd MSC	30	12	307	0.84	0.1961	0.82	0.2108
		Inv MSC	29	12	323	0.84	0.1994	0.81	0.2147
259	1,4,4,1	None	29	4	141	0.93	0.1314	0.91	0.1515
		SNVDT	28	1	130	0.93	0.1306	0.91	0.1501
		SNV	30	0	116	0.94	0.1242	0.91	0.1447
		DT	30	3	151	0.93	0.1334	0.90	0.1555
		Std MSC	30	0	118	0.94	0.1241	0.91	0.1448
		Wtd MSC	29	2	136	0.93	0.1291	0.91	0.1491
		Inv MSC	30	0	111	0.94	0.1248	0.92	0.1430
256	2,4,4,1	None	20	0	46	0.94	0.1184	0.91	0.1467
		SNVDT	28	0	34	0.96	0.1028	0.93	0.1332
		SNV	28	0	34	0.96	0.1030	0.93	0.1334
		DT	20	0	45	0.94	0.1183	0.91	0.1463
		Std MSC	28	0	35	0.96	0.1030	0.93	0.1334
		Wtd MSC	27	0	33	0.96	0.1032	0.93	0.1343
		Inv MSC	28	0	34	0.96	0.1031	0.93	0.1333

Comparison of visible and Herschel infrared region (400 to 1098 nm) and NIR region (1100-2498) in discrimination

Math treatment	Range	λ	PLS terms used	Misclassified [a]	Uncertainty [a]	RSQ	SEC	1-VR	SECV
1,4,4,1	Vis	86	30	38	399	0.74	0.2539	0.68	0.2784
	NIR	173	29	0	105	0.94	0.1204	0.92	0.1359
	Vis+NIR	259	29	4	141	0.93	0.1314	0.91	0.1515
2,4,4,1	Vis	84	10	29	320	0.74	0.2515	0.69	0.2744
	NIR	172	25	0	47	0.94	0.1162	0.92	0.1393
	Vis+NIR	256	20	0	46	0.94	0.1184	0.91	0.1467

- The visible region alone performed bad results and had little contribution significantly to the combined performance.
- The NIR region did produce a good calibration result which had the close result with combination visible and NIR region.
- The mathematic treatment 1,4,4,1 with none scatter correction was as the final spectra pretreatment according minimum SECV and SECV/SEC.

The validation of calibration equation on NIR region (1100 to 2500 nm)



The validation result on NIR region is better than on Visible and NIR region.

Effect of mathematic pre-treatment and scatter correction on MPLS quantified equations

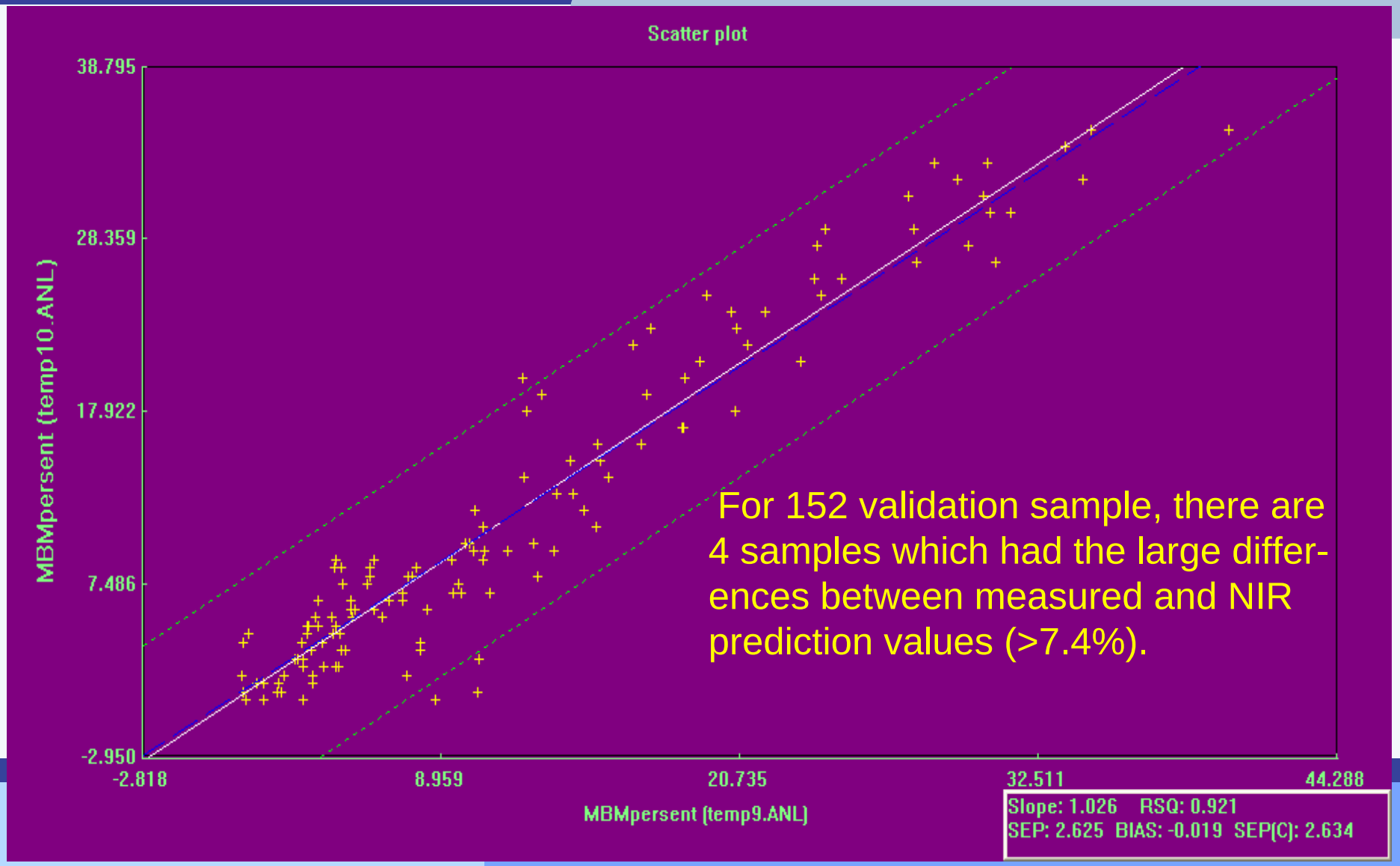
λ	Mathematic treatments	Scatter corrections	N	PLS terms	RSQ	SEC	1-VR	SECV	RPD
260	0,0,1,1	None	671	15	0.951	2.3639	0.9496	2.3961	4.46
		SNVDT	681	15	0.9455	2.5125	0.9439	2.548	4.23
		SNV	674	15	0.9506	2.3839	0.9486	2.4306	4.41
		DT	674	15	0.9422	2.5301	0.9413	2.5475	4.13
		Std MSC	677	15	0.9431	2.5541	0.9403	2.6151	4.10
		Wtd MSC	672	15	0.9445	2.5223	0.9424	2.5686	4.17
		Inv MSC	677	15	0.9485	2.4269	0.9457	2.4892	4.30
259	1,4,4,1	None	678	15	0.9685	1.8543	0.9659	1.9289	5.42
		SNVDT	670	16	0.9791	1.5243	0.9769	1.6015	6.59
		SNV	671	15	0.9766	1.6057	0.9735	1.7085	6.15
		DT	671	15	0.9706	1.7781	0.9654	1.9304	5.38
		Std MSC	676	15	0.9774	1.5812	0.9742	1.6874	6.23
		Wtd MSC	681	15	0.9767	1.6136	0.9747	1.6793	6.30
		Inv MSC	681	15	0.9755	1.657	0.973	1.7372	6.09
252	2,8,6,1	None	676	15	0.9759	1.6138	0.9723	1.7292	6.01
		SNVDT	682	15	0.9787	1.5432	0.9756	1.6493	6.40
		SNV	682	15	0.9786	1.5466	0.9757	1.6495	6.41
		DT	678	15	0.9761	1.6091	0.9725	1.7251	6.04
		Std MSC	681	16	0.9799	1.4964	0.9771	1.5974	6.61
		Wtd MSC	683	15	0.9796	1.5121	0.9764	1.6255	6.51
		Inv MSC	682	15	0.9786	1.5488	0.9756	1.6518	6.41

Comparison of visible and Herschel infrared region (400 to 1098 nm) and NIR region (1100-2498) in quantification

Pre-treatment	Range	λ	N	PLS terms	RSQ	SEC	1-VR	SECV	RPD
1,4,4,1 SNV+DT	Vis	86	683	12	0.8079	4.6166	0.7905	4.8178	2.19
	NIR	173	665	16	0. 9776	1.5631	0.9761	1.6137	6.47
	Vis+NIR	259	670	16	0.9791	1.5243	0.9769	1.6015	6.59
2,8,6,1 Std MSC	Vis	82	679	11	0.8204	4.4540	0.8037	4.6527	2.26
	NIR	170	672	16	0.9792	1.5192	0.9776	1.5775	6.68
	Vis+NIR	252	681	16	0.9799	1.4964	0.9771	1.5974	6.61

- The visible region alone performed bad results and had no contribution significantly to the combined performance.
- The NIR region did produce a very good calibration result which was close result with combination visible and NIR region.
- The mathematic treatment 2,8,6,1 with standard MSC scatter correction was as the final spectra pretreatment according minimum SECV and maximum RPD.

The validation of calibration equation on NIR region (1100 to 2500 nm)

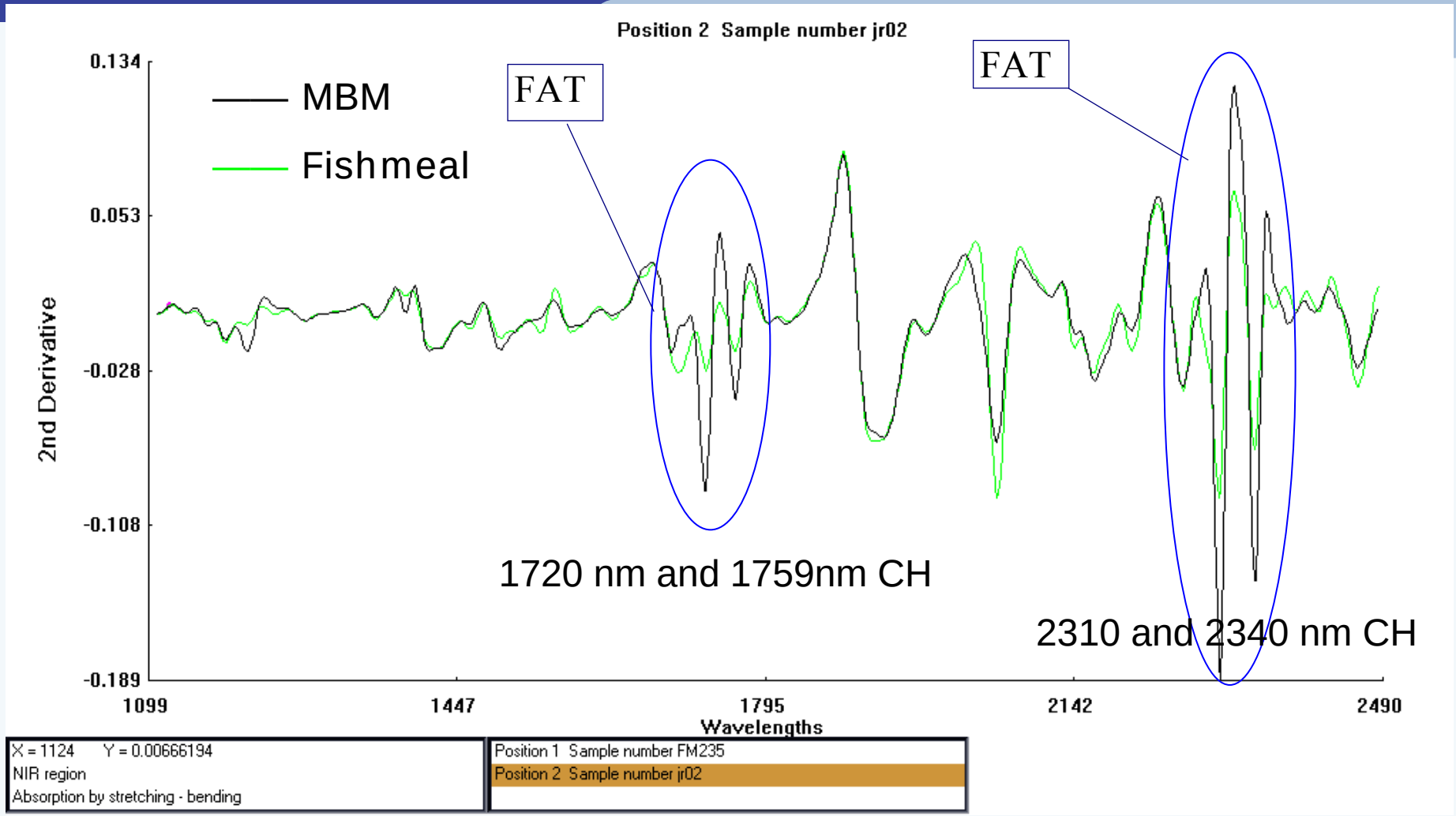


For 152 validation sample, there are 4 samples which had the large differences between measured and NIR prediction values (>7.4%).

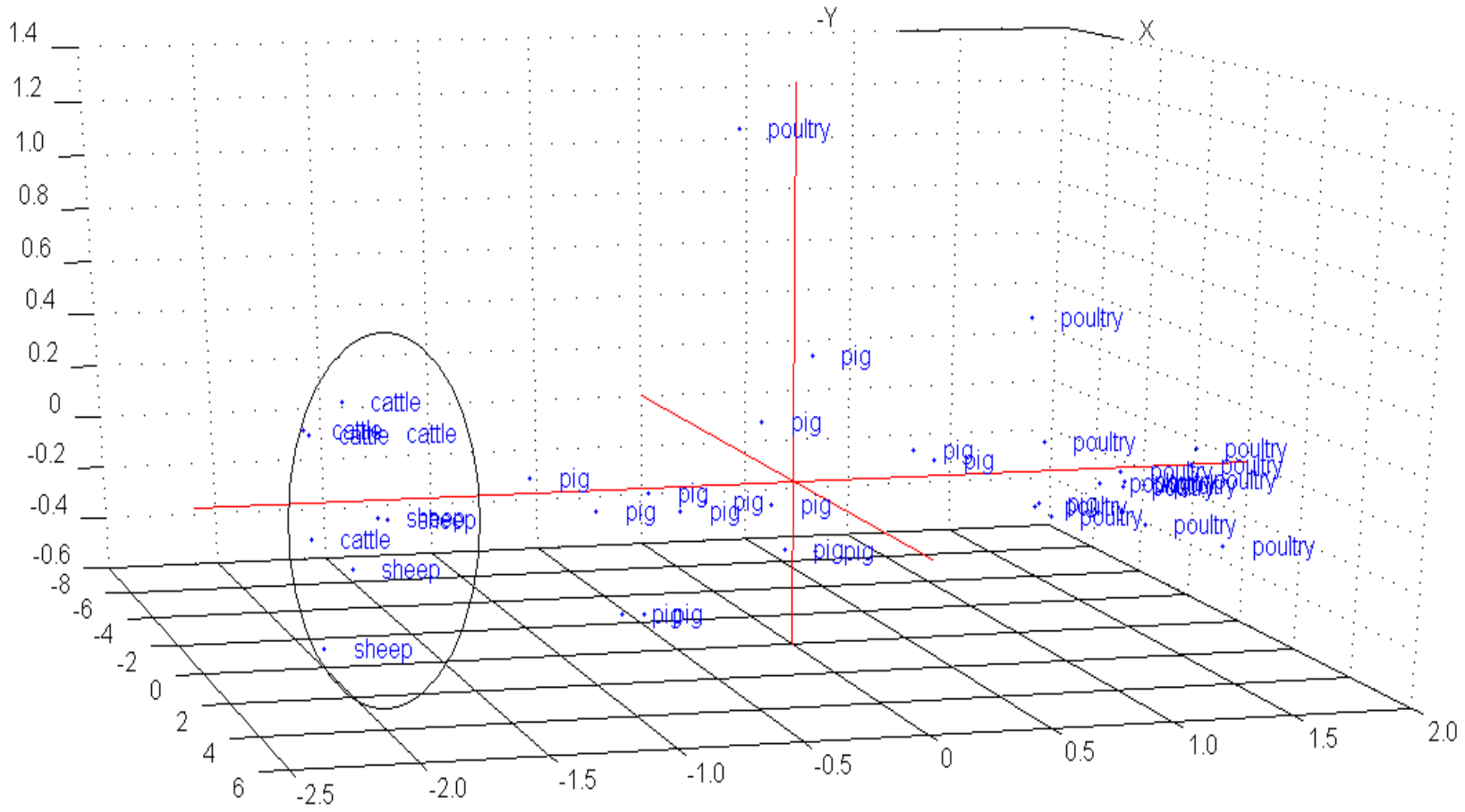
Detection and quantification of MBM in fishmeal by NIRS

Yang Z., Han L., Liu X., Li Q. Animal Feed Science and Technology. 2008, 147（4）：357-367

The spectra difference of fishmeal and MBM



The plot of principal component score



Global H (GH) distances of 37 MBM samples

No.	GH	No.	GH	No.	GH	No.	GH	No.	GH
Cattle1	2.783	Swine2	2.923	Swine10	3.914	Poultry8	2.628	MBM6	3.874
Cattle2	2.763	Swine3	2.506	Poultry1	4.799	Poultry9	2.292	MBM6	4.831
Catttle3	2.906	Swine4	2.588	Poultry2	4.907	Poultry10	2.338	MBM8	2.367
Cattle4	3.157	Swine5	2.637	Poultry3	2.958	MBM1	4.410	MBM9	2.155
Sheep1	2.803	Swine6	2.762	Poultry4	3.026	MBM2	3.320	MBM10	4.123
Sheep2	2.764	Swine7	2.880	Poultry5	2.372	MBM3	4.203		
Sheep3	5.752	Swine8	2.355	Poultry6	2.555	MBM4	3.774		
Swine1	2.338	Swine9	5.065	Poultry7	3.392	MBM5	3.822		

111 fishmeal samples as the centre (GH=0)

There were only 16 MBM samples which had GH it is greater than 3. there were 21 MBM samples could not be discriminated with fishmeal only by GH.

Effect of mathematic pre-treatment and scatter correction on MPLS discrimination equations

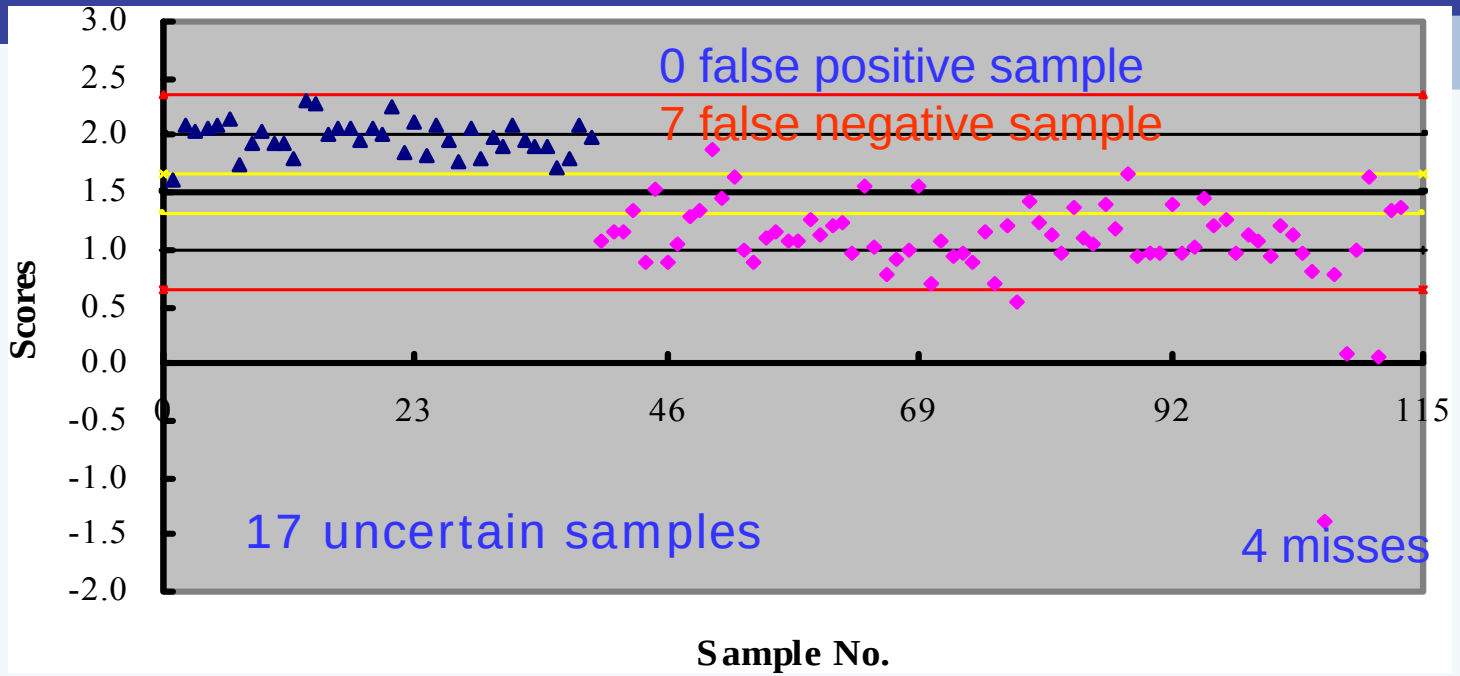
λ	Mathematic treatments	Scatter corrections	PLS terms used	Misclassified	Uncertainty	RSQ	SEC	1-VR	SECV
260	0,0,1,1	None	30	0	160	0.91	0.1463	0.90	0.1585
		SNVDT	24	1	157	0.89	0.1670	0.87	0.1807
		SNV	28	1	172	0.90	0.1543	0.89	0.1658
		DT	27	1	143	0.90	0.1591	0.88	0.1709
		Std MSC	27	0	131	0.90	0.1567	0.89	0.1670
		Wtd MSC	25	2	167	0.87	0.1773	0.86	0.1869
		Inv MSC	27	0	135	0.90	0.1540	0.89	0.1669
259	1,4,4,1	None	22	1	72	0.92	0.1419	0.90	0.1587
		SNVDT	20	1	47	0.93	0.134	0.91	0.1498
		SNV	21	1	52	0.93	0.1354	0.91	0.1510
		DT	22	1	51	0.93	0.1356	0.91	0.1534
		Std MSC	21	1	54	0.93	0.1358	0.91	0.1512
		Wtd MSC	18	1	70	0.91	0.1477	0.89	0.1632
		Inv MSC	18	1	67	0.91	0.1473	0.90	0.1606
252	2,8,6,1	None	27	1	31	0.94	0.1191	0.91	0.1452
		SNVDT	22	1	23	0.94	0.1228	0.91	0.1484
		SNV	27	1	26	0.95	0.1117	0.92	0.1397
		DT	26	1	27	0.94	0.1208	0.91	0.1470
		Std MSC	27	1	25	0.95	0.1119	0.92	0.1400
		Wtd MSC	17	1	39	0.92	0.1393	0.90	0.1573
		Inv MSC	19	1	27	0.94	0.1177	0.92	0.1451

Comparison of visible and Herschel infrared region (400 to 1098 nm) and NIR region (1100-2498) in discrimination

Math treatment	Range	λ	PLS terms used	Misclassified	Uncertainty	RSQ	SEC	1-VR	SECV
1,4,4,1 None	Vis	86	24	20	234	0.82	0.2119	0.77	0.2370
	NIR	173	29	1	47	0.95	0.1147	0.93	0.1353
	Vis+NIR	259	22	1	72	0.92	0.1419	0.90	0.1587
2,8,6,1 SNV	Vis	82	27	17	147	0.84	0.2004	0.79	0.2271
	NIR	170	19	1	64	0.91	0.1472	0.90	0.1592
	Vis+NIR	252	27	1	26	0.95	0.1117	0.92	0.1397

- The visible region alone performed bad results and had little contribution significantly to the combined performance.
- The NIR region did produce a very good calibration result which had the close result with combination visible and NIR region.
- For NIR region, the mathematic treatment was1,4,4,1 with none scatter correction. For Vis+NIR region, it was 2,8,6,1 with SNV scatter correction.

The validation of calibration equation on Vis+NIR region (400 to 2500 nm)

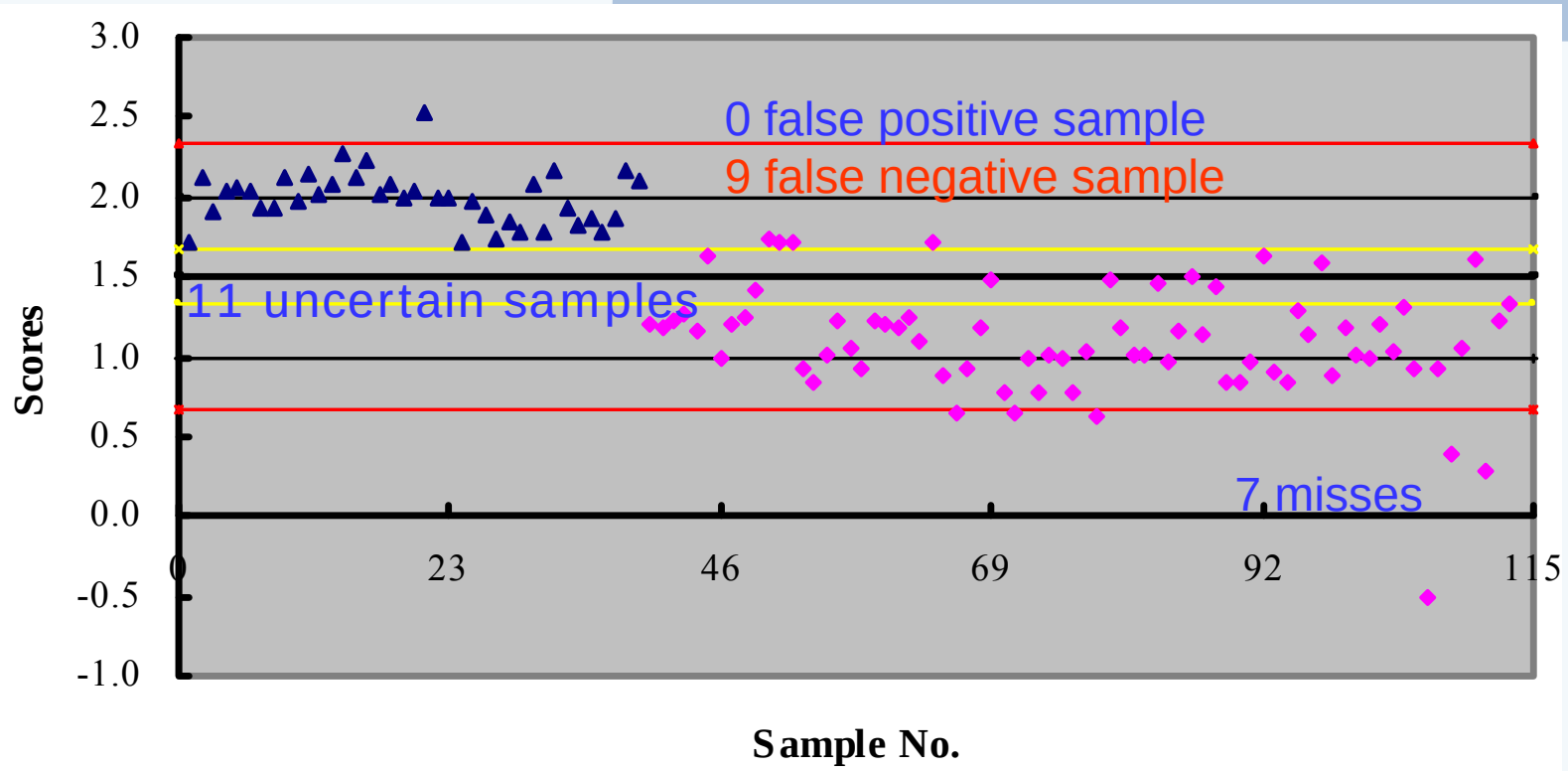


◆Figure showed the score plots. MBM-free fishmeal group together in one cluster around a score of 2.0, while samples containing different levels of MBM cluster around 1.0.

◆The samples between two yellow lines were uncertainty samples. It is uncertain which group these samples should belong to. The samples outside the red lines were as the misses, but, it is clear which group these samples belong to.

◆There were 7 false negative samples.

The validation of calibration equation on NIR region (1100 to 2500 nm)



The validation result on NIR region is close with on Visible and NIR region.

There are 9 false negative samples.

Effect of mathematic pre-treatment and scatter correction on MPLS quantified equations

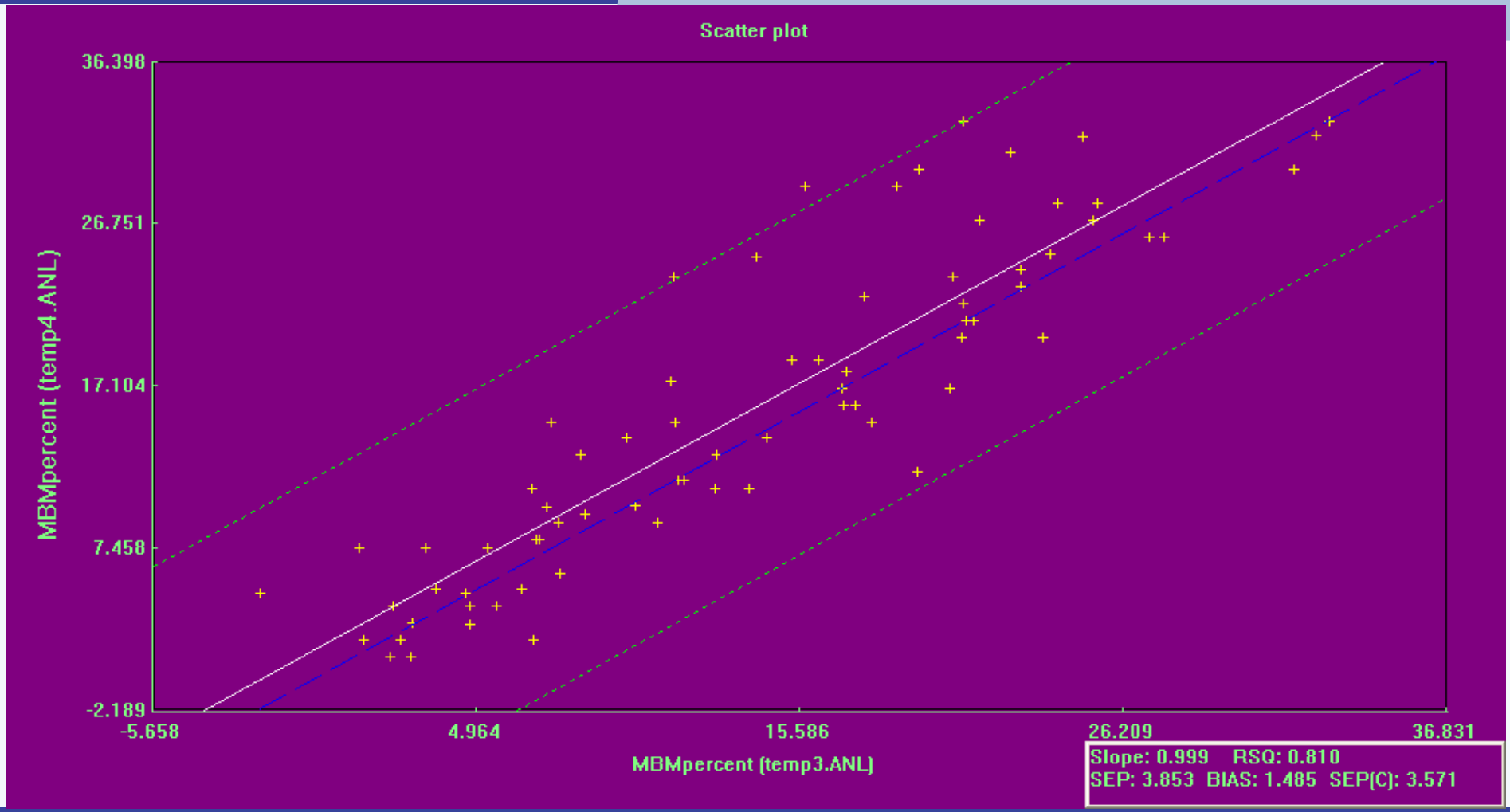
λ	Mathematic treatments	Scatter corrections	N	PLS terms	RSQ	SEC	1-VR	SECV	RPD
260	0,0,1,1	None	563	15	0.91	3.023	0.91	3.062	3.38
		SNVDT	571	15	0.93	2.756	0.93	2.706	3.92
		SNV	573	15	0.93	2.769	0.93	2.739	3.87
		DT	561	15	0.93	2.803	0.88	3.691	2.84
		Std MSC	576	15	0.93	2.777	0.93	2.849	3.73
		Wtd MSC	573	15	0.93	2.858	0.92	2.903	3.65
		Inv MSC	573	15	0.93	2.745	0.93	2.786	3.84
259	1,4,4,1	None	563	15	0.96	2.015	0.96	2.198	4.81
		SNVDT	562	15	0.97	1.946	0.96	2.056	5.17
		SNV	559	15	0.97	1.907	0.96	2.026	5.23
		DT	557	15	0.97	1.897	0.96	2.040	5.22
		Std MSC	559	15	0.97	1.961	0.96	2.075	5.10
		Wtd MSC	562	15	0.97	1.939	0.96	2.073	5.12
		Inv MSC	559	15	0.97	1.917	0.96	2.031	5.22
256	2,4,4,1	None	571	15	0.97	1.944	0.96	2.271	4.73
		SNVDT	569	15	0.97	1.807	0.96	2.116	5.09
		SNV	575	15	0.97	1.868	0.96	2.189	4.93
		DT	568	15	0.97	1.911	0.96	2.225	4.80
		Std MSC	568	15	0.97	1.794	0.96	2.107	5.11
		Wtd MSC	568	15	0.97	1.835	0.96	2.154	5.01
		Inv MSC	569	15	0.97	1.801	0.96	2.119	5.08

Comparison of visible and Herschel infrared region (400 to 1098 nm) and NIR region (1100-2498) in quantification

Pre-treatment	Range	λ	N	PLS terms	RSQ	SEC	1-VR	SECV	RPD
1,4,4,1 SNV	Vis	86	564	14	0.74	5.321	0.711	5.634	1.86
	NIR	173	561	16	0. 95	2.353	0.95	2.469	4.33
	Vis+NIR	259	559	15	0.97	1.907	0.96	2.026	5.23

- The visible region alone performed bad results and had little contribution significantly to the combined performance.
- The calibration on Vis+NIR region with RPD of 5.23 was better than on NIR region.

The validation of calibration equation on Vis+NIR region (400 to 2500 nm)

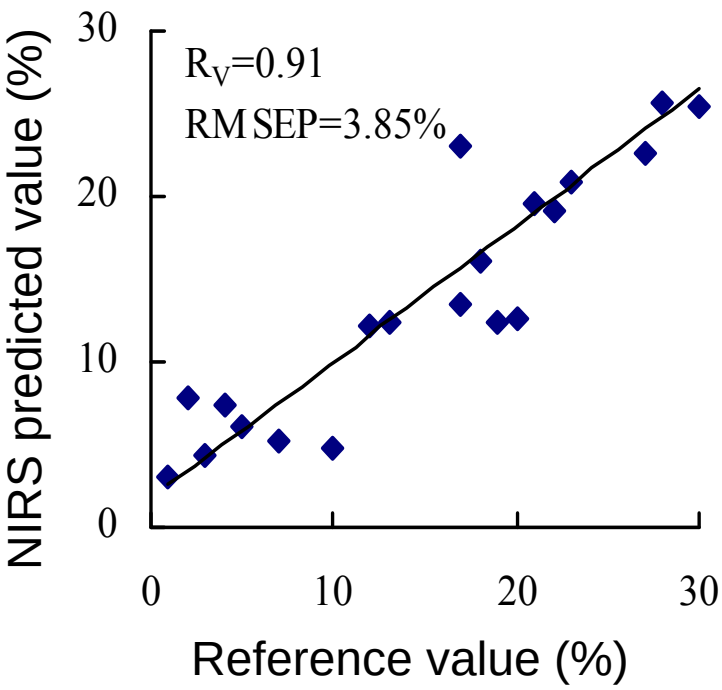


Validation results

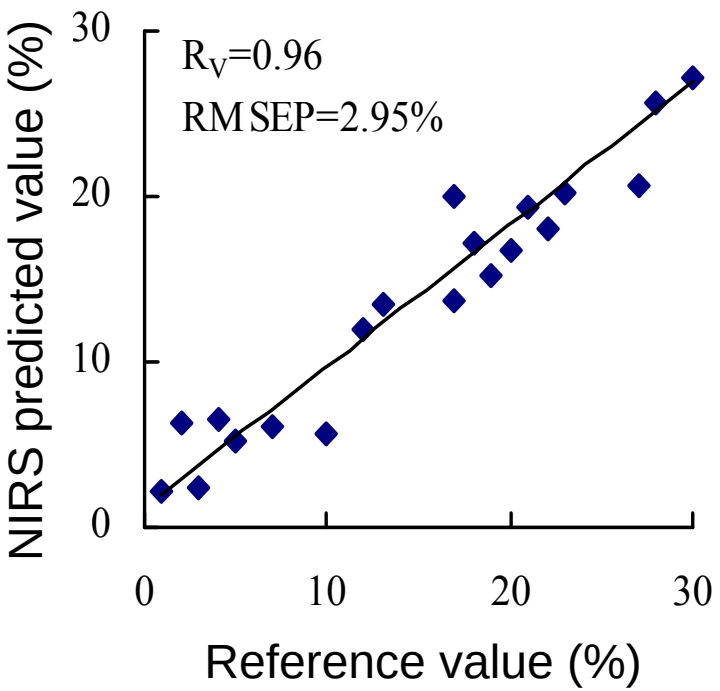
Range	r^2	SEP	SEP(C)	Slope	Bias
Vis+NIR	0.889	3.853	3.571	1.009	1.485

- The validation resulted was not good. There were larger Bias and SEP.
- For this validation set, there were 11 false positive samples and 1 negative sample.

Results of GA for PLS quantitative models



Before GA



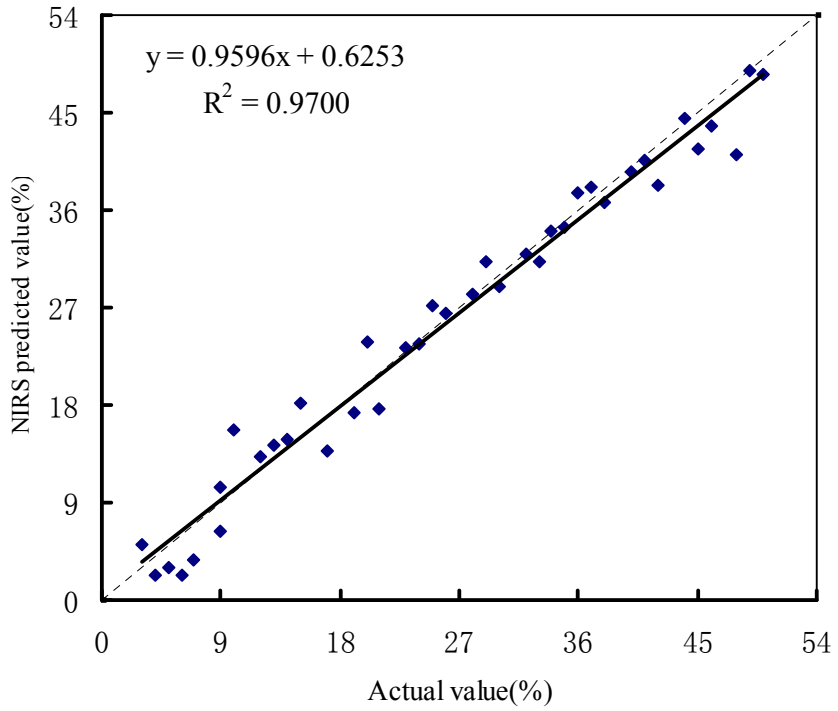
After GA

Quantitative analysis of soybean meal in fish meal by NIRS

•Shi GT, Han LJ, Yang Zengling. Spectroscopy and Spectral Analysis，2009，29（3）

Calibration result

PLS Factors	R^2	SEC	1-VR	SECV	RSD/%	RPD
10	0.97	2.20	0.95	3.19	8.30	6.5



The scatter plot of predicted versus actual value for external validation

NIRS calibration transfer for quantitative analysis of fish meal adulterated with MBM or soybean meal

•Zhan X.M. Han L., Liu X. OpticalTransaction，2009

NIRS apparatus

Master Instrument

- Instrument: Antaris NIRSystems (Thermo Nicolet, USA)
- Sample Cell: cup of quartz window of 5 cm diameter
- Scanning range: 10000-4000cm^{-1}
- Resolution: 8 cm^{-1}.

Slave Instrument

- Instrument: FOSS 6500 NIRSystems (FOSS, USA)
- Sample Cell: ¼ full cup
- Scanning range: 1100-2500 nm
- Resolution: 2nm

Calibration transfer techniques

- Orthogonal signal correction（OSC）
  - transfer set： 1~64
  - OSC factors： 1~5
- Slope/bias correction
  - transfer set： 1~64
- Directed standardization (DS)
  - transfer set： 1~64
- Piecewise Directed Standardization （PDS）
  - transfer set： 1~64
  - PDS windows size： 3~19

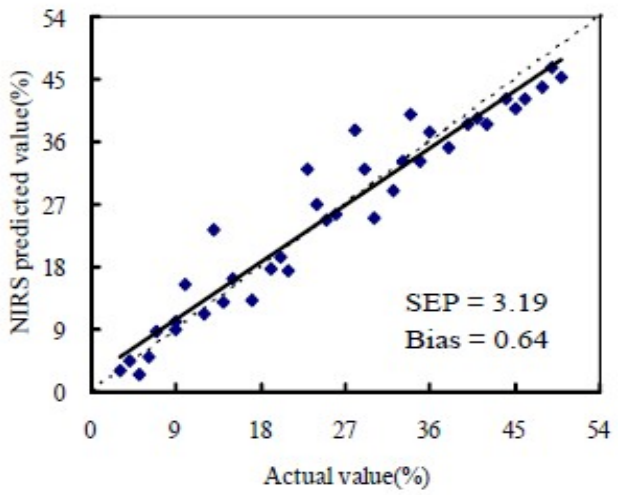
Calibration transfer results
(Fishmeal vs. MBM)

Predictions after calibration transfer

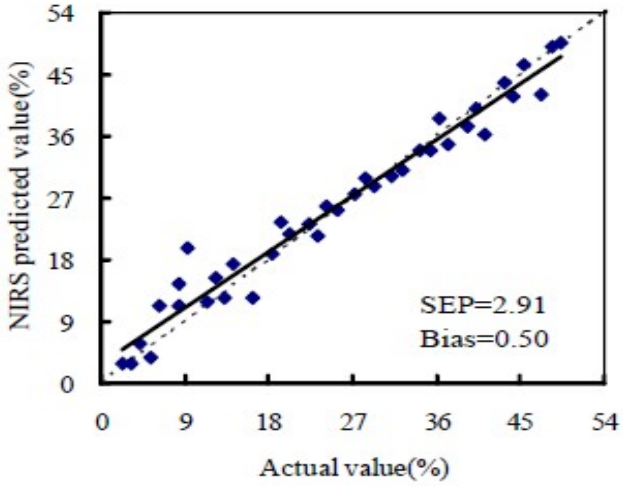
Calibration transfer techniques	Transfer set	parameter	R_V	RMSEP（%）	Bias（%）
Original model	-	-	0.914	3.854	-1.001
OSC	30	3（factors）	0.889	4.357	-1.079
Slop/Bias	64	-	0.863	4.677	-1.153
DS	55	-	0.915	3.855	0.500
PDS	8	9（window size）	0.881	4.350	0.302

The number of validation set was 21

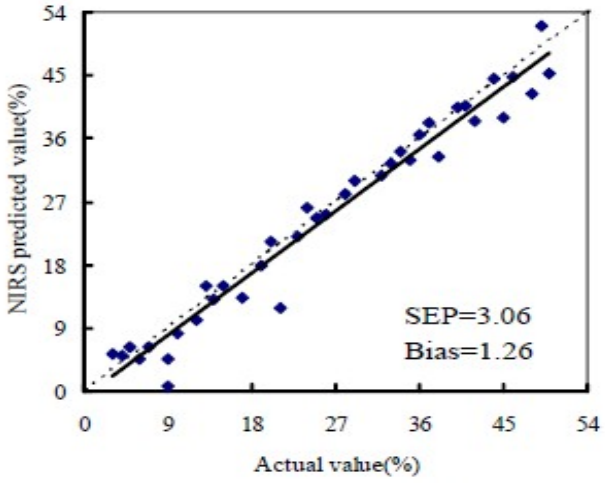
Calibration transfer results
(Fishmeal VS. soybean meal)



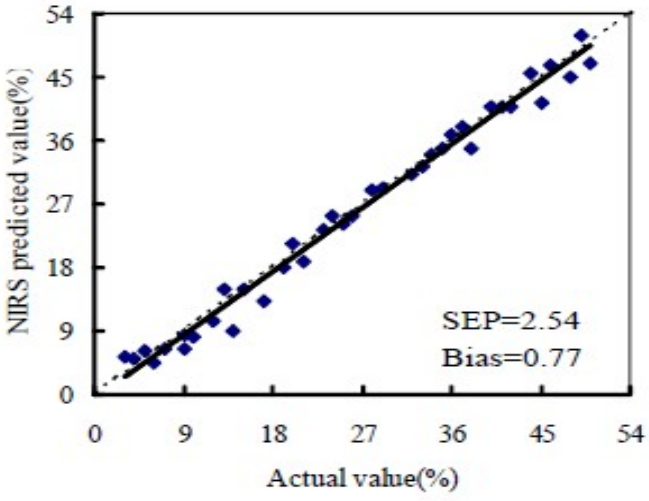
a. Slope/bias corection



b. Local centring



c. Direct standardization



d. Piecewise direct standardization

4 Conclusions

- For the analysis of MBM, NIRS is a rapid technique for large scale screening applications with relatively higher LOD.
- Further work is needed
  - enlarge the spectral libraries with authenticated samples in order to increase the robustness and applicability of the qualitative and quantitative NIR models.
  - use of chemometrics methods to improve the prediction of NIRS models

Thanks for your attention!

- Special thanks to
  - Dr. Yang Zengling, Dr. Liu Xian, Dr. Li Qiongfei,
  - Mr. Shi Guangtao, Ms Zhan Xiaomei