Research Articles

Application of Near Infrared Spectroscopy for Determination of Nutrient Content in Fish Meal and Fish Meal Adulterated with Feather Meal

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Abstract

Near Infrared Spectroscopy (NIRS) was applied by reflectance mode for nutrient content in fish meal. The data pretreatment methods were Partial Least Squares (PLS) Regression and Multiple Linear Regression (MLR). In this study, PLS calibration equations were suitable for assessment of nutrient content of fish meal. The results shown the correlation coefficient (R) for crude protein, moisture, crude fiber and crude ash were 0.96, 0.97 0.84 and 0.97, respectively. They can be used as quality assurance (R 0.96-0.97). The standard error of prediction (SEP) for crude protein, moisture, crude fiber and crude ash were 1.55, 0.47, 0.15 and 1.69, respectively. The Ratio Performance Deviation (RPD) value of crude protein, moisture and crude ash by PLS were 3.27, 4.03 and 2.75, respectively which greater than 2.4. They can be used as screening purposes for estimation of protein, moisture and crude ash, except crude fiber (RPD =1.80). The verification of accuracy of PLS calibration by paired t-test for crude protein, moisture, crude fiber and crude ash in fish meal were shown the value of $t_{cal}<t_{crit}$, so it was indicated that they did not significant difference at 95% confidence. The NIRS method can be used as a screening method to monitor the quality of fish meal and fish meal adulterated with feather meal and to select samples which not complied with the specification of registration according to the feed quality control act B.E. 2525 to confirm the nutrient content by the reference method.

Keywords: NIRS, Nutrient content, Fish meal

การประยุกต์ใช้เทคนิค Near Infrared Spectroscopy เพื่อวิเคราะห์หาปริมาณสารอาหารในปลาป่นและปลาป่นปลอมปนขนไก่ป่น

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บทคัดย่อ

การใช้เทคนิค Near Infrared Spectroscopy (NIRS) ด้วยวิธีตรวจวัดแบบ Reflectance เพื่อวิเคราะห์หาปริมาณสารอาหาร ชนิดโปรตีน ความชื้น กาก และเถ้า ในปลาป่น และปรับแต่งสมการด้วย วิธี Partial Least Squares (PLS) regression และ Multiple Linear Regression (MLR) พบว่า สมการ PLS calibration equations มีความเหมาะสมสำหรับใช้ในการประเมินค่าโปรตีน ความชื้น กาก และเถ้า ซึ่งให้ค่า correlation coefficient (R) เท่ากับ 0.96 0.97 0.84 และ 0.97 และ Standard Error of Prediction (SEP) ดังนี้ 1.55 0.47 0.15 และ 1.69 ตามลำดับ ค่า Ratio Performance Deviation (RPD) ของ โปรตีน ความชื้น และเถ้า จากสมการ PLS calibration เท่ากับ 3.27 4.03 and 2.75 ตามลำดับ ซึ่งมีค่ามากกว่า 2.4 ยกเว้นค่ากาก (RPD = 1.8) จึงสามารถใช้สมการ PLS calibration เป็นวิธีคัดกรองตัวอย่างปลาป่นและทดสอบยืนยันความใช้ได้ของสมการ โดยเปรียบเทียบผลการวัด กับวิธีมาตรฐานพบว่าปริมาณโปรตีน ความชื้น เถ้าและกาก ให้ผลวิเคราะห์สอดคล้องกันทั้ง 4 รายการ และไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติที่ระดับความเชื่อมั่น 95% (paired t-test, $t_{cal} < t_{crit}$) และสามารถใช้เป็นวิธีทางเลือกในการทำนายค่าของปริมาณโปรตีน ความชื้นและเถ้าได้ วิธี NIRS สามารถใช้ตรวจวัดปริมาณโปรตีนในปลาป่นและปลาป่นปลอมปนขนไก่ป่น และสามารถใช้เป็นวิธีคัดกรอง และเฝ้าระวังคุณภาพปลาป่น ที่มีคุณภาพไม่เป็นไปตามที่ได้ขึ้นทะเบียนตามเกณฑ์มาตรฐานพระราชบัญญัติควบคุมคุณภาพอาหารสัตว์ พ.ศ. 2525 เพื่อนำไปตรวจยืนยันด้วยวิธีมาตรฐานในห้องปฏิบัติการต่อไป

คำสำคัญ : NIRS ปริมาณสารอาหาร ปลาป่น

Introduction

Fish meal is the major protein source which has been used in animal feed because of the suitable protein quality (Saeed et al., 2012).The percentage of protein contents in fish meal Class 1, 2 and 3 are not less than 60, 55 and 50 % respectively. At present, the increasing demand and uncertain availability of fish meal has prompted the nutritionist to study alternative source of protein supply as dietary protein in animal feed such as feather meal. Feather meal is the by-product from poultry production. It contains a complex protein (keratin), which can be broken down by hydrolysis to make them digestible (Arunlertaree and Moolthongnoi, 2008). The feather meal is not allowed to use in fish meal. It has been judged as adulterant according to the feed quality control act B.E. 2525. But it is allowed to use in poultry feed at the level 2-6%. The conventional methods used to evaluate the proximate chemical composition of fish meal are tedious, destructive and expensive. Near infrared spectroscopy (NIRS) is one of the techniques belonging to vibrational spectroscopy. Its radiation (750 to 2500 nm) interacts with organic matter, and the absorption spectrum is rich in chemical and physical information of organic molecules (Kuma et al., 2011 and De la Haba et al., 2007). In order to extract valuable information on the chemical properties of samples, it is necessary to mathematically process spectral data by chemometric tools (Blanco and Villarroya, 2002). The most important part in the development of an NIRS method is building the predicting model generally called calibration. NIR spectroscopy has several advantages over other analytical techniques: rapidity of analysis, no use of chemicals, minimal or no samples preparation, easily applicable in different work environments and nondestructive technique (Cozzolino et al., 2002; Lu et al., 2009 and Berzaghi and Riovanto, 2009). NIRS can be applied for quantitative measurement of chemical concentration in a matrix, and qualitative analysis to discriminate one material from another. From a single spectrum, numerous components within a matrix can be simultaneously quantified once calibration models have been developed (Bakeev, 2003 and Cozzolino et al., 2009). The recent study has focused on the application of NIRS for these purposes but just few studies have been determined the nutrients component in fish meal by NIRS (Saeed et al., 2012), especially fish meal in Thailand. The objective is to set the calibration model by NIRS with comparison between PLS and MLR and use to determination of crude protein, moisture, crude fiber and crude ash in fish meal and determination of crude protein of fish meal adulterated with feather meal.

Materials and methods

Apparatus

Near Infrared Spectroscopy (NIRFlex N500, Buchi, Flawil, Switzerland), Kjeltec analyzer (Tecator moldel 1030), Fibertec hot extractor (Foss model 2010), Fibertec cold extractor (Foss model 1021), Muffle furnace and Hot air oven Ultra centrifugal mill (Retsch ZM200, Germany), Analytical balance, capable of weighing to the nearest 0.0001 g. and Cooling bath.

Sample preparation

A total of 150 of fish meal samples Class 1, 2 and 3 were randomly collected from the feed mills. The fish meal samples were ground into 1 mm mesh at 25 ^{o}C. Eleven fish meal samples were mixed with feather meal at different concentration, 5%, 10% and 20% by weight. The samples were homogenized by trituration technique, mixing by hand for 30 minutes for each time and was added the fish meal samples in equal portion 5%, 10% and 20% by weight until get 100 grams.

Calibration equation and validation development

1. The powder fish meal about 20 grams were scanned in reflectance mode for three replications to collect the NIR spectral using NIRFlex N500 (Buchi, Flawil, Switzerland)) between 1100 nm to 2500 nm. range. One hundred samples for the calibration set were used to develop the calibration model. Fifty samples of

fish meal for the validation set were used to evaluated the developed model. The absorbance spectrum was recorded as the logarithm of the reciprocal of reflectance [log (1/R)]. These spectra were used for the calibration set of NIRS. The calibration set was used to develop the calibration model. The developed model was evaluated by using the fish meal samples included in the validation set which had not been previously used in the calibration procedure, in order to avoid overfitting of the calibration equation.

2. Crude protein was analysed by Kjeldahl method (ISO 5983-2:2005). Moisture content was measured by oven drying the sample at 103±°C for 4 h, ISO 6496:1999 (E).Crude fiber was loss on ignition of dried residue remaining after digestion of sample with 1.25% H_2SO_4 (w/v) and 1.25% NaOH(w/v) analysed by AOAC official method 978.10 (2012) .Crude ash was obtained after incineration at 550 °C for 3 h, ISO 5984: 2002 (E). Each sample analysis was performed in dulplicate and used the average value as the reference value. The accuracy and precision of nutrient content analysis in Feed Quality Control Laboratory Division was shown in Table 1

3. Building the PLS and MLR calibration models for fish meal total (Class 1, 2 and 3). The calibration equations were developed by the data preprocessing techniques with two data pretreatment methods. Partial least squares (PLS) regression was first derivative BCAP (Buhler Chemical Analytic Package) (db1) and 2nd Savitzky-Golay 9 Points (dg2). Multiple linear regression (MLR) with 4 data pretreatments methods were tested, first derivative BCAP (db1), average 3 points (sa3), Savitzky-Golay 9 points gap2 (sg9g2) and smooth Savitzky-Gplay 9 points (sg9). Multiplicative Scatter Correction (MSC) and first or second order derivatives were applied to the spectra to reduce the noise and light scattering effects.

Calibration set (C-set): From all spectra within a project only the spectra which are in the C-set used for the calculation of the calibration.

Validation set (V-set): From all spectra within a project only the spectra which are in the V-set used for an internal validation of the calibration.

The difference between the reference value and the predicted value by the NIR model were expressed as Bias *(é) (< 0.6SEC)* and can be defined as:

$$é = \frac{1}{n}(\Sigma_{t=1}^{n} ei)$$ where n = number of sample

ei = residual as define as:

ei = yi-$\bar{y}$i where yi = reference value

$\bar{y}$i = predicted value

The verify accuracy of calibration was performed bias checking by paired-t test at $P \leq 0.05$ level.

4. Selection of suitable calibration to predict the nutrient contents in fish meal. The calibration statistics were calculated include correlation coefficient (R), Standard Error of calibration (SEC), Standard Error of Prediction (SEP) calibration1.5 SEC and SEP validation <1.3 SEC) and Ratio Performance Deviation (RPD). (Table 2.and Table 3.)

Verification of calibration equation

1. Twenty fish meal samples from the feed mill were tested by using NIRS method Comparison of crude protein, moisture, crude ash and crude fiber contents from NIRS method with reference methods.

2. Thirty three samples of fish meal mixed with feather meal were determined crude protein by using NIRS method and Kjeldahl method (ISO5983-2:2005).

Results and discussion

The statistic characteristics of the calibration and the validation set in fish meal shown the average, standard deviation and range of the calibration for crude protein were 63.27%, 6.97 and 42.86-73.61 respectively. The average, standard deviation and range of the validation for crude protein were 65.07%, 3.68 and 56.67-71.65% respectively. The statistic characteristics of the calibration and the validation set for moisture, crude fiber and crude ash were shown as in Table 4.

The calibration results for determination nutrient content of fish meal were shown in Table 5. Statistical parameters of the PLS calibration models such as correlation coefficient (R), standard error of calibration (SEC) and standard error of prediction (SEP) were 0.96, 1.65 and 1.55 for crude protein (Figure 3a); 0.97, 0.59 and 0.47 for moisture (Figure 4a); 0.84, 0.17 and 0.15 for crude fiber (Figure 5a) and 0.97, 1.21 and 1.69 for crude ash (Figure 6a), respectively.The PLS calibration models of moisture and crude ash(R=0.97)and crude protein (R= 0.96) can be used as quality assurance.

Statistical parameters of the MLR calibration models such as R, SEC and SEP for crude protein, moisture, crude fiber and crude ash were 0.96, 1.65 and 1.66 (Figure 3b); 0.96, 0.60 and 0.60 (Figure 4b); 0.81, 0.19 and 0.16 (Figure 5b); 0.90, 2.13 and 2.01 (Figure 6b), respectively.

Statistical parameters of the PLS calibration was better than the MLR calibration. Therefore, the PLS calibration models are suitable for determination of nutrient contents of fish meal. This result was corresponding to the previous result of Saeed et al., 2012.

The ratio between the Standard Deviation (SD) and SEP of validation set (known as Ratio Performance Deviation (RPD)) shows how good the calibration and prediction will work for analytical purposes (Table 5). The RPD value of crude protein, moisture and crude ash by PLS were 3.27, 4.03 and 2.75, respectively which greater than 2.4 (Williams and Sobering, 1993 and Williams, 2004), so it was valid for estimation of crude protein, moisture and crude ash (Saeed et al., 2012), except crude fiber (RPD=1.80). The RPD of crude protein in experiment showed in the present study (RPD=3.27) was better than the reported by Cozzolino et al., 2002 for fish meal (RPD= 2.77), probably as consequence of higher range of the reference data in the current research. Furthermore, the results are in broad agreement with other studied applying the NIR spectroscopy to assess the crude protein in animal feeds and surimi better than those showed by Xiccato et al., 2004, in sea bass meal (RPD < 2). This phenomenon may due to wide variations of chemical characteristics in the set of sea bass which included great difference in fish rearing systems and weight. The RPD statistic of moisture (RPD=4.03) was better than the previous study of Saeed et al., 2012 (RPD= 2.28) while RPD of crude protein lower than the reported by them. This may cause from the adulteration of fish meal and bone meal in, so should be considered the source and history of fish meal. In comparison with our results, Cozzolino et al., 2002, Xiccato et al., 2004 and Uddin et al., 2006 provided less reliable calibrations for moisture content prediction (RPD < 4) when analyzing fish meal, European sea bass and surimi samples, respectively. This results probably due to wider ranges of moisture content. However, some features including the species of fish used to produce the fish meal. And also, the seasonality of the fish and modify in the steps of the manufacturing process during the period of sampling, which could have endangered the prediction. (Cozzolino et al., 2005)

The verification of accuracy of PLS calibration by paired t-test for crude protein, moisture, crude fiber and crude ash in fish meal were presented in Table 6. The results were shown the value of $t_{cal}<t_{crit}$, so it was indicated that did not significant difference at 95% confidence between NIRS, crude protein and Kjeldahl (ISO 5983-2:2005); NIRS, moisture and ISO 6496: 1999 (E); NIRS, crude fiber and AOAC official method 978.10 (2012); NIRS, crude ash and ISO 5984:2002 (E).This PLS calibration can be used as alternative method for good estimation of nutrient content in fish meal.

The NIRS spectra of fish meal were appeared lower than feather meal (Figure 1). The spectra of crude protein were located at 1510 nm, 2050-2060 nm and 2180 nm which corresponded to N-H stretch first overtone, N-H stretch combination and N-H bend second overtone, respectively. These wavelengths are typical of protein absorption whose chemical structure is based on N-H bonds (Saeed et al., 2012). However, it was

difficult to discriminate the spectra of fish meal adulterated with feather meal at 5%, 10 % and 20 % (Figure 2).It should be studied how to discriminate fish meal adulterated with feather meal.

The crude protein in fish meal determinated by Kjeldahl method did not significantly differ from NIRS method at 95% confidence (Table 7). Furthermore, both methods predict crude protein in fish meal mixed feather meal with concentration of 5%, 10% and 20% did not significantly differ from Kjeldahl method due to the value of tcal<tcrit at 95% confidence.However, the trend of predicted values of crude protein were lower than the Kjeldahl method. This phenomenon may occur due to the crude protein in feather meal adulterated in fish meal was extracted by Kjeldahl method which led to the high crude protein. The NIRS method found only the crude protein in fish meal that not include crude protein in feather meal.

However, the NIRS method can select the samples that not complied with the specification of registration in crude protein, moisture and crude ash. It can reduce the number of samples for confirmation by reference method. The NIRS method can be used for quantity improving of the routine analysis in feed quality control laboratory.

Conclusion

NIRS method can be used for nutrient contents determination of fish meal when compare to the Kjeldahl method (ISO 5983-2:2005), ISO 6496:1999 (E), and ISO 5984:2002 (E). The correlation coefficient (R) for crude protein, moisture, crude fiber and crude ash were 0.96, 0.97 0.84 and 0.97, respectively. PLS calibration method of fish meal can be used to monitor crude protein, moisture and crude ash content in fish meal. It can reduce the number of samples that analyzed by conventional method for nutrient contents according to the feed quality control act B.E. 2525. The determination of crude protein in fish meal adulterated with feather meal by NIRS was lower than the Kjeldahl method. This research is an opportunity to help the farmers, not be exploited by manufacturers and reduce the unfavorable trade. Therefore, NIRS can be used as a tool for the rapid determination of nutrient contents in monitoring the quality of fish meal.

Table 1 Accuracy and precision of nutrient contents analysis in animal feed by reference method of Feed Quality Control Laboratory Division. Bureau of Quality Control of Livestock Products.

Items	Method validation			% Relative measurement uncertainty
	Working range (%)	Accuracy % Recovery	Precision (Repeatability) 2.8 x Sr %*	
Protein: ISO 5983-2:2005	0.8 - 87.0	Poultry feed = 99.31 Pig feed = 100.4 Dairy feed = 100.9	1.30**	1.46
Moisture: ISO 6496:1999 (E)	0.1 - 27.0	Fish meal = 97.15	0.20	0.97
Fiber: AOAC official method 978.10(2012)	0.4 - 27.0	Poultry feed = 97.07 Dairy feed = 102.7	0.10	2.45
Ash: ISO 5984:2002 (E)	0.7 - 96.5	Wheat flour = 101.7	0.27	0.51

* Repeatability limit = HORRAT (Horwitz,s Ratio) <2

**Repeatability limit (2.8 x Sr) % crude protein was equal to the reference value of fish meal (ISO 5983-2:2005)

Table 2 Criteria of correlation coefficient (R)

Value of R	Interpretation
Up to ± 0.5	Not usable in NIRS calibration
± 0.51 - 0.70	Poor correlation, research the reasons
± 0.71 - 0.80	Rough screening
± 0.81 - 0.90	Screening and approximate calibration
± 0.91 - 0.95	Usable with caution for most applications, including research
± 0.96 - 0.98	Usable in most application, including quality assurance
≥ ± 0.99	Usable in any application

Table 3 Criteria of Ratio Performance Deviation (RPD).

RPD Value	Classification	Application
0.0 - 2.3	Very poor	Not recommended
2.4 - 3.0	Poor	Rough screening
3.1 - 4.9	Fair	Screening
5.0 - 6.4	Good	Quality control
6.5 - 8.0	Very good	Process control
≥ 8.1	Excellent	Any application

Table 4 Statistical characteristics of the calibration and the validation in fish meal

Items	Crude Protein		Moisture		Crude Fiber		Crude Ash	
	C-set	V-set	C-set	V-set	C-set	V-set	C-set	V-set
Mean	63.27%	65.07%	7.15%	7.21%	0.97%	1.12%	22.79%	21.75%
Standard deviation	6.97	3.68	2.48	2.39	0.28	0.29	4.80	3.94
Minimum content, % mass fraction	42.86%	56.67%	2.28%	2.96%	0.14%	0.19%	12.87%	14.19%
Maximum content, % mass fraction	73.61%	71.65%	17.09%	13.57%	1.35%	1.19%	39.22%	33.30%
Number of samples, *N*	82	38	100	50	57	27	98	48

C-set = Calibration set, V-set = Validation set

Table 5 PLS equation and MLR equation results for predicting % nutrients of fish meal

Nutritents	Calibration types	Wavelength region (nm)	R	SEC	SEP	RPD	SD	Pretreatment
Protein	PLS	1100 - 2500	0.96	1.65	1.55	3.27	5.07	db1
	MLR	1445 - 2350	0.96	1.65	1.66	3.01	5.00	db1
Moisture	PLS	1100 - 2500	0.97	0.59	0.47	4.03	1.89	db1
	MLR	1445 - 2078	0.96	0.60	0.60	3.98	2.39	sa3
Fiber	PLS	1100 - 2500	0.84	0.17	0.15	1.80	0.27	db1
	MLR	1362 - 2332	0.81	0.19	0.16	1.50	0.24	sg9g2
Ash	PLS	1100 - 2500	0.97	1.21	1.69	2.75	4.65	dg2
	MLR	1178 - 2168	0.90	2.13	2.01	2.14	4.30	sg9

R = the correlation coefficient, SEC = standard error of calibration, SEP = standard error of prediction, SD = Standard Deviation, Unit = %, Ratio Performance Deviation (RPD) = SD/SEP,

Table 6 The Verification of PLS calibration for nutrient contents* in fish meal

Sample No.	Classes of fish meal	Moisture			Crude protein			Crude ash			Crude fiber		
		ISO 6496:1999 (E)	NIRS	é	ISO 5983-2 :2005	NIRS	é	ISO 5984:2002 (E)	NIRS	é	AOAC 978.10 (2012)	NIRS	é
1	1	9.84	9.84	0.00	63.25	63.20	0.05	17.32	17.37	0.05	0.33	0.33	0.00
2	1	8.34	8.34	0.00	60.80	60.77	0.03	20.95	20.98	0.03	0.36	0.36	0.00
3	1	7.96	7.70	0.26	64.26	63.61	0.65	19.75	19.15	0.60	0.19	0.20	0.01
4	1	6.89	7.60	0.71	68.79	63.35	5.44	16.40	18.93	2.53	0.28	0.20	0.08
5	2	8.13	8.13	0.00	59.28	59.30	0.02	20.80	20.80	0.00	0.14	0.14	0.00
6	2	8.66	8.67	0.01	58.56	58.58	0.02	19.13	19.10	0.03	0.26	0.26	0.00
7	2	4.86	5.38	-0.52	61.44	60.73	0.71	20.53	21.63	-1.10	0.38	0.46	-0.08
8	2	6.74	6.73	0.01	57.40	57.37	0.03	24.63	24.66	0.03	0.17	0.17	0.00
9	3	9.38	9.39	0.01	64.28	64.32	0.04	18.75	18.68	0.07	0.33	0.32	0.01
10	3	8.36	8.37	0.01	59.81	59.85	0.04	20.12	20.06	0.06	0.22	0.22	0.00
11	3	5.86	5.87	0.01	57.28	57.31	0.03	28.49	28.46	0.03	0.12	0.12	0.00
12	3	6.95	6.95	0.00	61.42	61.40	0.02	21.19	21.21	0.02	0.18	0.18	0.00
13	3	7.50	7.50	0.00	57.31	57.28	0.03	25.91	25.93	0.02	0.10	0.10	0.00
14	3	5.19	5.19	0.00	56.98	57.00	0.02	25.09	25.07	0.02	0.60	0.60	0.00
15	3	8.54	8.06	0.48	56.04	57.98	-1.94	29.5	29.70	-0.20	0.61	0.97	-0.36
16	3	8.87	8.27	0.60	55.52	57.28	-1.76	29.12	29.41	-0.29	0.58	0.96	-0.38
17	3	8.01	7.78	0.23	65.6	65.91	-0.31	22.83	21.73	1.10	0.32	0.62	-0.30
18	3	7.16	7.22	-0.06	69.44	69.58	-0.14	18.48	17.28	1.20	0.18	0.27	-0.09
19	3	6.4	6.10	0.30	56.29	55.59	0.70	25.45	26.61	-1.16	0.54	0.71	-0.17
20	3	7.34	6.52	0.82	54.74	54.14	0.60	23.78	24.55	-0.77	0.19	0.68	-0.49
Mean		7.38	7.30	0.16	60.25	60.03	0.23	22.78	22.94	0.12	0.30	0.40	-0.10
SD		1.26	1.16	0.32	4.52	3.99	1.48	3.92	3.95	0.86	0.17	0.29	0.17
F		1.12			1.27			0.98			0.36		
Fc		2.17			2.17			0.46			0.46		
t_{cal}		0.17			0.15			-0.12			-1.26		
t_{crit}		2.02			2.02			2.02			2.02		

é is the Bias or systematic error, é = nutrient content (ISO5983-2:2005) - nutrient content (NIRS), nutrient contents* = moisture, crude protein, crude ash and crude fiber

Table 7 The determination of protein content in fish meal and fish meal adulterated with feather meal by ISO 5983-2:2005 (Kjeldahl method) and NIRS Technique

Sample no.	% protein content							
	fish meal 100%		fish meal mixed 5% feather meal		fish meal mixed 10% feather meal		fish meal mixed 20% feather meal	
	ISO 5983-2:2005	NIRS	ISO 5983-2:2005	NIRS	ISO 5983-2:2005	NIRS	ISO 5983-2:2005	NIRS
1	67.82	66.34	67.37	66.54	68.8	66.26	71.02	66.98
2	68.56	67.25	67.57	65.71	68.35	66.12	70.78	67.37
3	72.2	70.57	72.39	70.1	73.24	70.66	74.36	69.98
4	74.74	71.39	74.2	71.15	74.66	71.39	76.34	71.42
5	71.88	69.57	72.03	68.91	72.55	68.68	74	69.34
6	70.25	69.56	70.2	67.87	70.56	67.35	71.84	67.88
7	54.81	55.89	54.89	55.2	55.6	56.45	57.98	59.18
8	63.53	62.36	63.4	62.75	62.89	62.4	63.62	63.89
9	57.66	57.88	57.07	57.86	58.37	58.76	59.25	60.35
10	66.87	66.35	66.26	64.05	64.79	64.84	64.99	65.51
11	67.6	65.3	67.83	65.26	68.83	65.88	67.33	66.37
Sum	735.92	722.46	733.21	715.4	738.64	718.79	751.51	728.27
Mean	66.9	65.68	66.66	65.04	67.15	65.34	68.32	66.21
SD	6.10	5.08	6.12	4.93	6.13	4.62	6.18	3.81
F	1.44		1.54		1.76		2.63	
F_c	2.98		2.98		2.98		2.98	
t_{cal}	0.51		0.68		0.78		0.96	
t_{crit}	2.09		2.09		2.09		2.09	

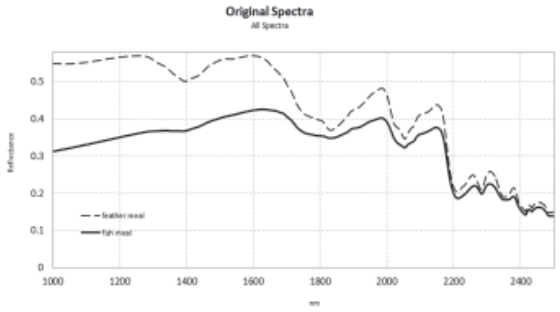


Figure 1 Raw NIR spectra of fish meal (-) and feather meal (- -)

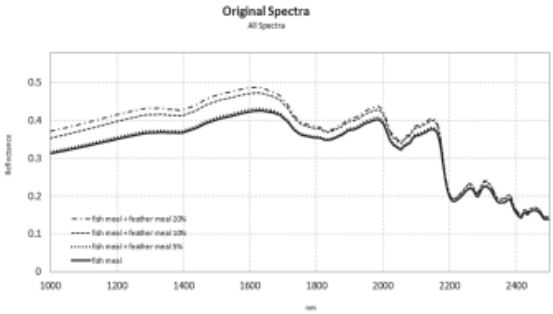
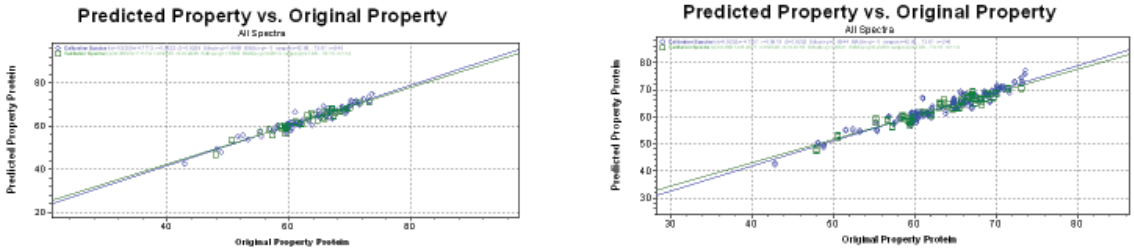
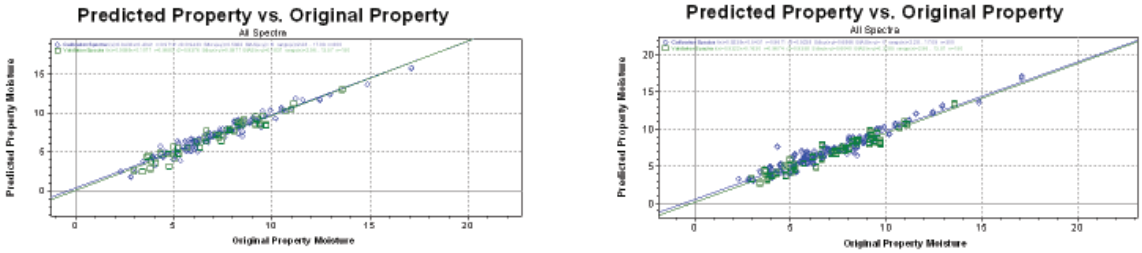


Figure 2 Raw NIR spectra of fish meal (-), fish meal mixed 5% feather meal (▪▪▪▪▪▪), fish meal mixed 10% feather meal (- -) and fish meal mixed 20% feather meal (—▪—)



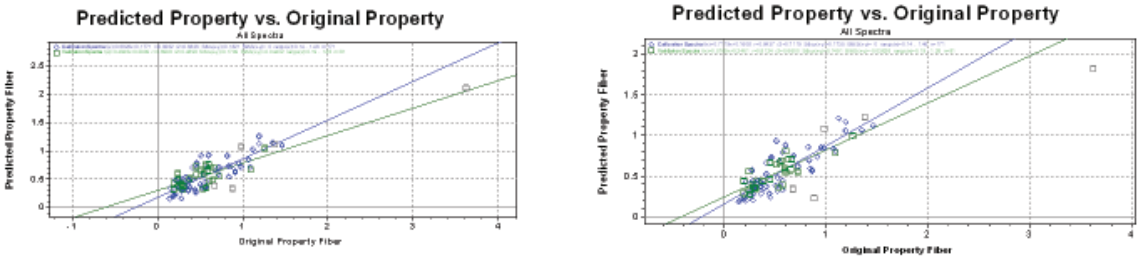
(a) **(b)**

Figure 3 NIRS predicted data against reference data for protein by PLS (a) and MLR (b)



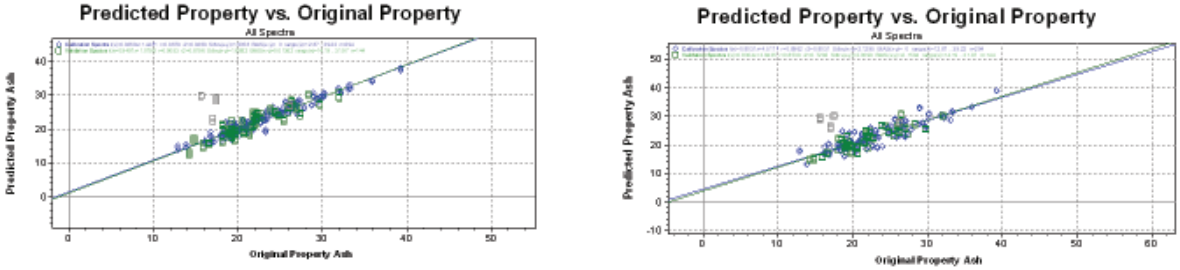
(a) **(b)**

Figure 4 NIRS predicted data against reference data for moisture by PLS (a) and MLR (b)



(a) **(b)**

Figure 5 NIRS predicted data against reference data for fiber by PLS (a) and MLR (b)



(a) **(b)**

Figure 6 NIRS predicted data against reference data for Ash by PLS (a) and MLS (b)

Acknowledgements

The authors wish to thanks Dr.Pairoj Thumrongopas, Director of Bureau of Quality Control of Livestock Products, Department of Livestock Development for supporting the research and thanks to Miss.Rattakan Srisiri and Miss.Chalermporn Poomma for their assistant.

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