ISSN 0375－9202

東京農業大学農学集報

第37巻 第1号

平成4年8月

東京農業大学

ISSN 0375－9202

Journal of Agricultural Science, Tokyo Nogyo Daigaku
Vol. 37 No. 1 August, 1992

CONTENTS

Articles

Futoshi MIYAMOTO and Hideaki OHBA :
Observations of the Pteridophytes of Gunung (Mt.) Tahan, Malay Peninsula 1

Senkichiro HAYAKAWA, Akiko KAWABATA, Shinjiro CHIKUBU, Shigeo UMEDA,
Chiyoko TOKUE, Eiichiro SAKAGUCHI and Nobuhiro NAGASHIMA :
Studies on Storage and Quality of Milled Rice 30

Hozumi YOSHIDA, Naoharu MIZUNO and Shin OKAZAKI :
Change of Intensity and Effect on Crop Plants of Ultraviolet Radiation
(UV) in Abashiri Region 39

Sumimaro ITOH, Yoshio KURIHARA, Seizi SUKEMORI Sei-ichi TAKAHARA,
Kyushichi MIYAZAWA and Kei-ichiro SUGIMURA :
Antigenicity of Food Supplements Including Oyster Extract in Animal Models 46

Agung PURNOMOADI, Masahiro AMARI and Kenichi KAMEOKA :
Studies of Near Infrared Spectroscopy for Predicting Forage Quality
of Tropical Grasses 52

Tadashi OTANI, Mutsuyasu ITO, Misao MASHIMA and Masayuki NEMOTO :
Improving Cultivation Technics of Reed Canary Grass (*Phalaris arundinacea* L.) wards (1)
Relations Between Characteristic and Forage Quality in Reed Canary Grass 64

Wakanori AMAKI and Haruzo HIGUCHI :
Micropropagation of *Pteris ensiformis* 'Victoriae' 74

Hiromichi HAYASHI :
Viscoelastisty of Butter 83

TOKYO UNIVERSITY OF AGRICULTURE

学術雑誌編集委員会内規

第１条　学術雑誌編集委員会（以下委員会という）は図書館長及び同次長並びに大学の各学科，教養課程の各教室及び教職課程より選ばれた各１名の教務職員を以って組織する。

２　委員の任期は２年とし，重任を妨げない。

第２条　委員会の委員長は図書館長を以って之れに当てる。

第３条　委員会は委員長これを招集し，議長となり，会務を処理する。

第４条　委員会は紀要，農学集報及び一般教育学術集報の各編集委員会に分ける。

第５条　委員会に幹事１名をおき，図書館職員を以って之れに当てる。

附　　則

本内規は昭和42年11月１日より施行する。

校　閲　内　規　（昭和60年6月19日）

校閲者は原則として本学の大学院指導教授ならびにこれと同等の学外者からえらび２名以上とする。

農学集報編集委員

教授	長谷山　崇　彦	図書館長 教授	中　村　武　久
助教授	日　暮　賢　司	教授	進　士　五十八
教授	印　南　　敏	図書館次長	杉　本　秀　健
教授	井　上　勝　弘	教授	田　邊　　猛
教授	小　林　　正	図書館分館長 教授	若　菜　　繁
教授	小　松　善　雄	教授	渡　邉　誠　喜
教授	桑　原　　連	教授	山　本　　出
教授	右　田　一　雄	教授	柳　田　藤　治

（ABC順）

東京農業大学農学集報

第 37 巻 第 1 号

平成 4 年 8 月

目　次

論　文

宮本　太・大場秀章：
Observations of the Pteridophytes of Gunung (Mt.) Tahan, Malay Peninsula…… 1

早川千吉郎・川端晶子・竹生新治郎・梅田重夫・徳江千代子・坂口栄一郎・永島伸浩：
精米貯蔵法と米の品質に関する研究……30

吉田聡穣・水野直治・岡崎　眞：
網走地方の紫外線強度の変動とその作物への影響……39

伊藤澄層・栗原良雄・祐森誠司・高原征一・宮沢久七・杉村敬一郎：
Antigenicity of Food Supplements Including Oyster Extract in Animal Models……46

Agung Purnomoadi・甘利雅拡・亀岡喧一：
Studies of Near Infrared Spectroscopy for Predicting Forage
Quality of Tropical Grasses ……52

大谷　忠・伊東睦泰・真島　操・根本正之：
リードカナリーグラス（*Phalaris arundinacea* L.）の利用法の改善に関する研究（第 1 報）
リードカナリーグラスの生育習性と草質との関係……64

雨木若慶・樋口春三：
Micropropagation of *Pteris ensiformis* 'Victoriae' ……74

林　弘通：
バターの粘弾性について……83

Studies of Near Infrared Spectroscopy for Predicting Forage Quality of Tropical Grasses

Agung PURNOMOADI*, Masahiro AMARI**
and Kenichi KAMEOKA

(Received June 17, 1992)

Introduction

Near Infrared Reflectance Spectroscopy (NIRS) for forage has been widely and successfully used throughout the world, but the study for tropical grasses is still limited, especially in Asia including Japan and Indonesia.

Tropical grasses show some differences from temperate grasses in leaf anatomy, chemical composition, cell wall concentrations (AKIN and BURDICK, 1973), and physical property (AKIN *et al.*, 1975).

The NIRS analysis is a physical method, non-destructive measurement related to the energies absorbed from the incident radiation by molecular groups in the sample (MURRAY and WILLIAMS, 1990), and might be limited to successful prediction of tropical grasses due to all these differences.

This study has been done as prediction data from tropical forages by NIRS analysis in Asia, and for the reasons refered above. The aim of this study is to evaluate NIRS in prediction of nutrient content, such as moisture, protein, crude fiber, crude fat, ash, NFE, ADF, ADL, silica, organic matter, OCW, OCC, Oa, and Ob of tropical grasses compared with the previous study conducted in other forages.

Materials and Methods

A total of 85 tropical grass samples representing 8 species including Guinea grass (*Panicum maximum*), Napier grass (*Pennisetum purpureum*), Rhodes grass (*Chloris gayana*), Setaria (*Setaria sphacelata*), Brachiaria (*Brachiaria brizantha*), Pearl millet (*Pennisetum typhoides*) King grass/Pusa giant napier grass (*Pennisetum purpureum* × *P. typhoides*), Gatton panic (*Panicum* sp.), were obtained from Japan, Brunei, and Indonesia during the summer of 1990 (Table 1).

The samples from Japan (55) and Brunei) (7) were collected as grab samples of fresh materials and chopped (2 ~ 3 cm) and dried immediately at 60°C in a forced draft oven for 24 hours. However, the samples from Indonesia (23) were collected from farmer's field and chopped (1 ~ 2 cm) and dried under shelter until constant weight (3 to 5 days).

* Laboratory of Animal Feeding, Department of Zootechnical Science, Faculty of Agriculture, Tokyo University of Agriculture
** National Institute of Animal Industry, Tsukuba

Table 1. List of Samples

Country	Origin	Name of sample	Number
Japan	Okinawa[1]	Green panic	6
		Guinea grass	6
		Rhodes grass	6
		Setaria grass	6
	Okinawa[2]	Gatton panic	5
		Green panic	4
	Kyushu[3]	Pennisetum	7
		Gatton panic	8
		Pearl millet	7
Brunei	Brunei[4]	Napier grass	7
Indonesia	Semarang[5]	Napier grass	8
		Panicum	5
		King grass	6
		Brachiaria	2
		Setaria	2

1) Samples were raised from the field of Rukyu University at Iriomote Island, Okinawa, Japan.
2) Samples were raised from Okinawa Pref. Zootech. Exp. Sta.
3) Samples were raised from Kyushu National Agric. Exp. Sta.
4) Samples were raised from Mcfarm Sendirian Berhad, Brunei.
5) Samples were raised from farmer's field and Diponegoro Univ. farm, Semarang, Indonesia.

All samples were ground through a Willey mill (1 mm screen) and analyzed for moisture, crude protein (CP), crude fiber (CF), ether extract (EE), crude ash, acid detergent fiber (ADF), acid detergent lignin (ADL), silica (Si), organic cell wall (OCW), Oa (high digestible fraction in OCW), and Ob (low digestible fraction in OCW). All analysis was done by wet chemistry and NIR Spectroscopy.

Dry matter of samples were determined by drying for 2 hours at 135°C in a forced draft oven, and crude ash obtained by ashing the samples at 600°C for 2 hours. Crude protein was determined by the Kjeldahl method. Ether extracts were determined by drifting diethyl ether for 16 hours. Crude fiber was obtained after boiling sample material with dilute acid (1.25% H_2SO_4) and then with dilute alkali (1.25% NaOH). GOERING and VAN SOEST procedures conducted on ADF, and 72% sulfuric acid detergent lignin, and silica was obtained from the successive ADF and ADL analysis by ashing the last ADL residue.

Organic matter fraction (OCW and Ob), was adopted for enzymatic analysis by using glucoamylase for OCW determination and cellulase for Ob determination as described by ABE *et al.*, (1979). Oa (high digestible fraction in OCW) was found by subtracting Ob from OCW.

From 85 samples, 60 and 25 samples were selected randomly for calibration and prediction test by NIRS. Equations of prediction were used to calculate calibration sample test. NIRS instrument of Pacific Scientific (Neotec model FQA 51 A) was used for NIR analysis. This instrument was equipped with six filters for measuring reflectance at selected wavelengths in the general range of 1500 to 2400 nm properties of forage samples, with recording reflectance

Table 2. Standard Filter and Scanning Range FQA 51 A

Position number	Wavelength number	Filter in normal position (nm)[a]	Scanning range (nm)	Primary constituent
1	1-128	1720	1734-1718	None
2	129-256	1580	1501-1578	oil, protein
3	257-384	2100	1994-2197	moisture, protein, carbohydrate
4	358-512	2200	2108-2217	protein
5	513-640	2310	2243-2308	oil, carbohydrate
6	641-768	2360	2292-2358	oil, fiber

Source : Theory manual vol. 2, Pacific Scientific company, Gardner/Neotec division.
a) Normal position reached when the filter in perpendicular to the light source.

interval at 2 nm (see Table 2).

This instrument connected with N-400 computer terminal with NSAS (Near Infrared Spectral Analysis Software) program to compute stepwise regression equation from samples of known composition, and Hewlett-packard 7550 A graphics plotter to graph the results of multiple linear regression and occurrence of reflectance spectra. Wavelengths for calibration were selected by an optimum correlation for predicting each of the chemical analysis.

Result and Discussion

The results of chemical analysis of the 85 tropical grass samples which were used in NIRS analysis and separation for calibration test (60 samples) and prediction test (25 samples) are listed in Table 3.

Considering to this separation for calibration and prediction test, both calibration and prediction samples seem to show similarity in range and mean values. Notable exceptions were differences in mean values of crude protein and ether extract, where the calibration set was higher than the prediction set. In addition, the range of protein in calibration was wider than in prediction, but similar in ether extract. These sligh shifts are still generally right since mean values are close to mid values of population in range.

Near Infrared Reflectance Spectroscopy

The model spectra of tropical grass by FQA 51 A are shown in Fig. 1. Smooth line is log (1/R) and dot ted line is its second derivative. This figure appears to be divided into 6 segments by the vertical straight line in the point of certain wavelength. These segments appeared with the existence of six filters of each scanning range (see Table 2). The previous study of derivative technique to sharpen the detail in absorption spectra, resulted in the conclusion that the second derivative contains the best information relative to the composition (NORRIS *et al.*, 1976).

It had been demonstrated that the raw spectra of feed samples differed by particle size and moreover, even though the samples were ground in the same size screen, they also differed if different type grinders were used. (NORRIS *et al.* 1976 ; OSBORNE and FEARN, 1986)

It is known that the differences are eliminated by second differential calculus.

In this study, multiple linear regression analysis of second derivative reflectande was used to predict all chemical compounds in the samples.

Table 3. Ranges and Means of Chemical Composition of Samples Used for This Study (% DM), and Its Separation for Calibration and Prediction Test Samples

	Sample (85)		Calibration (60)		Prediction (25)	
	Range	Mean	Range	Mean	Range	Mean
Moisture	16.63- 7.32	9.28	11.64- 7.32	9.27	16.63- 7.44	9.29
C. Protein	19.26- 3.23	9.77	19.26- 3.23	10.21	15.90- 3.73	8.71
E. Extract	5.04- 0.98	2.09	5.04- 1.17	2.14	4.34- 0.98	1.97
C. Fiber	40.60-21.95	33.12	40.60-21.95	32.96	38.71-26.52	33.52
C. Ash	18.00- 4.95	10.69	16.52- 4.95	10.65	18.00- 4.99	10.79
NFE[1]	58.17-34.18	44.33	58.17-34.18	44.05	53.42-36.30	45.01
O. Matter	95.05-82.00	89.31	95.05-83.48	89.35	95.01-82.00	89.21
OCC[2]	26.71- 7.56	17.51	25.68- 7.56	17.30	26.71- 9.51	17.75
OCW[3]	85.86-55.29	72.17	85.86-61.66	72.04	84.57-55.29	72.47
Oa[4]	32.25- 4.01	17.68	29.35- 4.01	17.84	32.25- 8.15	17.33
Ob[5]	80.05-31.77	54.49	80.05-38.23	54.22	76.42-31.77	55.14
ADF[6]	53.78-28.44	40.36	53.78-28.44	40.34	48.09-32.70	40.40
ADL[7]	9.86- 2.91	5.57	9.86- 2.91	5.63	8.16- 3.00	5.43
Silica	7.90- 0.65	2.80	5.68- 0.66	2.80	7.90- 0.65	2.81

1) NFE : Nitrogen Free Extract.
2) OCC : Organic Cellular Contents.
3) OCW : Organic Cell Wall.
4) Oa : High digestible fiber fraction eliminated crude ash.
5) Ob : Low digestible fiber fraction eliminated crude ash.
6) ADF : Acid Detergent Fiber.
7) ADL : Acid Detergent Lignin.
( ) : Number of samples.

The correlation coefficient and standard error for the calibration test and the prediction test (Table 4) were of the same general magnitude. The prediction sample test was performed to ascertain the validation of the calibration test. There are no differences ($p<0.1$) between calibration to prediction by F-test, so that the results of calibration are valid.

Of all these measurements (Table 5), the third measurement (by using three wevelengths) gave the optimum results in whieh the confficient correlation was comparably highest and the standard error the lowest. Hence, this third measurement was used for the calculation of all chemical composition.

The coefficient correlation for each chemical analysis was highly linear even though the samples included the variable grass in species, cutting time, and stage. This phenomenon indicated that NIR can predict chemical constituent without being influenced by species, cutting time or stage.

Moisture

WINDHAN *et al.* (1987) and LAW and TKACHUK (1977) stated that very strong absorbance of water appeared at 1930 nm. Unfortunately, FQA 51 A type has no scanning range at this absorbance (see Table 2). The first wavelength (2336 nm) appeared in cellulose absorbance (see Table 6), the second close to 2100 nm absorbance of starch and cellulose, ane the third close to 1580 nm absorbance of starch and glucose.

These appearances might be caused by the calibrated water existing in association with

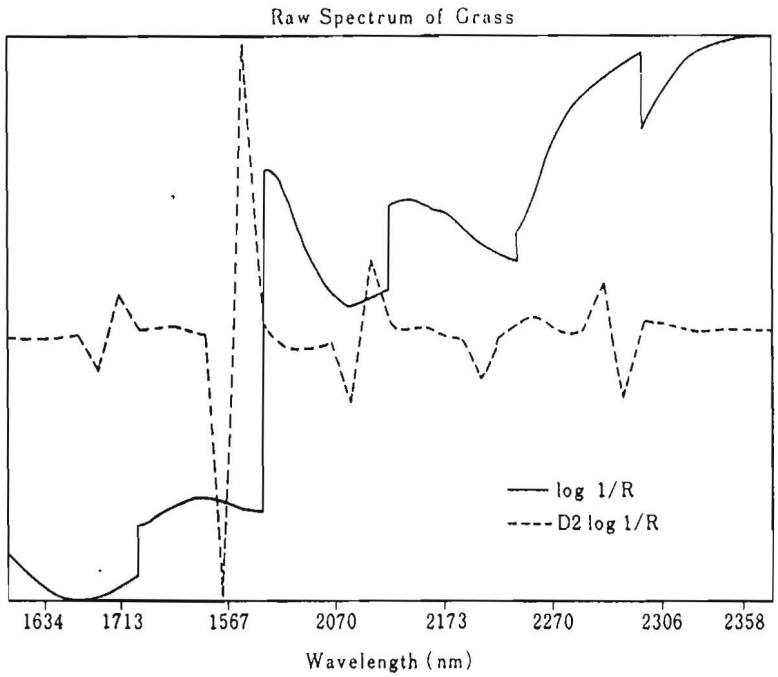

Fig. 1 Reflectance Spectra of Green Panic Grass in log (1/R) and Its Second Derivative.

Table 4. Statistical Results of Calibration and Prediction Test

	Calibration (60)		Prediction (25)	
	r	Se	r	Se
Moisture	0.89	0.47	0.96	0.48
Crude protein	0.96	1.33	0.94	1.44
Ether extract	0.93	0.32	0.92	0.31
Crude fiber	0.86	2.20	0.85	2.06
Crude ash	0.90	1.26	0.85	1.72
NFE[1)]	0.90	2.65	0.93	1.93
Organic matter	0.90	1.26	0.85	1.72
OCC[2)]	0.91	1.94	0.80	2.57
OCW[3)]	0.96	1.72	0.90	2.85
Oa[4)]	0.88	2.97	0.94	2.24
Ob[5)]	0.95	3.49	0.97	3.09
ADF[6)]	0.91	2.24	0.87	2.36
ADL[7)]	0.86	1.01	0.86	0.85
Silica	0.78	0.94	0.76	1.35

1-7), ( ) : See Table 3.

some organic constituents, such as starch or cellulose, as is known, water in sample exists in three forms, free, bound and absorbed.

Table 5. Wavelengths Selected and Statistical Results of The Calibration Set Samples

	Wavelength (nm)			Correlation coef.			Standard error		
	λ_1	λ_2	λ_3	r^a	r^b	r^c	Se^a	Se^b	Se^c
Moisture	2336	2121	1566	0.81	0.83	0.89	0.60	0.58	0.47
C. Protein	2157	2210	2289	0.95	0.96	0.96	1.39	1.36	1.33
C. Fat	2296	1709	2210	0.87	0.89	0.93	0.42	0.38	0.32
C. Fiber	2275	1709	2210	0.84	0.85	0.86	2.27	2.21	2.20
C. Ash	2087	2315	2243	0.87	0.89	0.90	1.42	1.32	1.26
NFE[1)]	2087	2308	1710	0.83	0.87	0.90	3.29	2.92	2.65
O. Matter	2087	2315	2243	0.87	0.89	0.90	1.42	1.32	1.26
OCC[2)]	2277	2289	1547	0.86	0.88	0.91	2.32	2.15	1.94
OCW[3)]	2280	1549	2326	0.93	0.95	0.96	2.32	1.94	1.72
Oa[4)]	2113	2336	1634	0.82	0.86	0.88	3.50	3.15	2.97
Ob[5)]	2301	1997	2331	0.93	0.94	0.95	3.91	3.68	3.49
ADF[6)]	2277	1501	1506	0.87	0.89	0.91	2.68	2.43	2.24
ADL[7)]	2280	2211	2308	0.83	0.84	0.86	1.10	1.07	1.01
Silica	2336	2123	1578	0.69	0.73	0.78	1.07	1.01	0.94

a) Statistical results obtained by using wavelength of λ_1.
b) Statistical results obtained by using wavelength of $\lambda_1+\lambda_2$.
c) Statistical results obtained by using wavelength of $\lambda_1+\lambda_2+\lambda_3$.
1-7), ( ) : See Table 3.

Table 6. Chemical Assignments of Some Observed Near Infrared Absorption Bands

Wavelength (nm)	Structure	Reference	Wavelength (nm)	Structure	Reference
1500	NH	11, 15	2092	Fibre	8
1510	protein	12, 11, 15	2100	starch	12, 15
1520	$CONH_2$, urea	12, 15	2100	starch, cellulose	8
1528	starch	15	2110	$CONH_2$, CONHR	15
1530	RNH_2	8, 15	2150	$CONH_2$	15
1533	C=H	15	2160	CONHR	15
1540	starch	11, 15	2200	-CHO	8, 15
1570	-CONH-	11, 15	2242	amino acid	15
1580	starch, glucose	15	2270	cellulose	12
1620	$=CH_2$	8, 15	2276	starch	15
1645	R-CH-CH	15	2280	starch	12
	O		2294	amino acid	15
1705	CH_3	12, 11, 15	2300	protein	12
1725	CH_2	12, 11, 15	2310	oil	12
1990	urea	12	2330	starch	12
2000	starch	15	2335	cellulose	12
2080	sucrose, starch	15	2336	cellulose	15

Protein

Crude protein (CP) absorbance occurred at 2157, 2210 and 2289 nm for first, second and third measurements respectively. With reference to Table 6, all of these wavelengths are

proper to protein or amino acids. The correlation coefficient of CP (0.96) is highest and standard error (1.33) is lowest.

This result is still lower than that reported by NORRIS (1976) which found 0.99 for coefficient correlation with 0.74% standard error, but it is most notable that NORRIS did this by using eight wavelengths while this study uses just three wavelengths. Eliminating two samples did not relatively improve the correlation coefficient of CP calibration and standard error.

The high result of CP caused by all fuctional groups composed of CP (-OH, -CH- and -NH-) can be calibrated completely by NIR.

Ether Extract

The major NIR absorption bands in fat or oil are due to the long chain fatty acid moiety, which give rise to a CH_2 second overtone at 1200 nm, CH_2 first overtones at 1734 and 1765 nm, and CH_2 stretch-bond combinations at 2310 and 2345 nm (OSBORNE and FEARN, 1986). The absorbances which occurred in this study appear separate to these stated wavelengths.

With reference to Table 6, the first wavelength (2296 nm) is very close to 2294 nm wavelength properties of protein, the second (1709 nm) close to CH_3 structure (1705 nm) and the 2276 nm wavelength is the property of starch in the combination O-H and C-C stretch bonding vibration. These combinations of bonding vibration might be similar or close together with the strongest functional groups contributing to ether extract vibration.

Crude Fiber

As shown in Table 6, the first wavelength (2275 nm) is lying very close to wavelength properties of starch in 2276 nm, the second (1709 nm) close to 1705 nm properties of CH_3, and the third (2210 nm) close to 2200 nm properties of -CHO.

Crude fiber was defined as the coarse fibrous portion of plants, such as cellulose, partially digestible and relatively low in nutritional value (HEATH *et al.*, 1985). Chemically, cellulose itself is an anhydride of beta-D glucose units. In addition, the glucose and starch molecules are very similar, so that the results in CF calibration may lead to the conclusion that CF stay in the proper wavelengths.

The data from chemical analysis of CF may contain small amounts of lignin and protein included in computation.

Ash and Organic Matter

Ash and organic matter (OM) calibration give the same wavelength, correlation coefficient and standard error value as well. Organic matter, which is computed by subtracting ash from DM, makes a strong relation between ash and OM in value. The first wavelength (2087 nm), is close to 2080 nm properties of sucrose and starch, the second (2315 nm) is close to 2310 nm, properties of CH_2 and oil, and the third (2243 nm) is very close to 2242 nm properties of protein.

Ash itself, defined as inorganic matter, contained most mineral in parts. SHENK *et al.* (1979) stated that minerals do not have reflectance spectra in the portion of IR spectrum, so that, ash has no properties of specific wavelength either. The computation might be done by computing with relation to another form of organic matter. By the explanation above, ash spectra might be OM spectra. It is clearer if we focus attention on the wavelength spectra

itself, which occurred at sucrose, starch, oil and protein. These constituents are the basis of organic matter in the plant.

Nitrogen Free Extract

Nitrogen free extract is obtained by subtraction of CF from the total carbohydrate analysis in the proximate system of feed analyshs (HEATH *et al*, 1985), and in practics done by subtracting CP, ash, CF and EE from dry matter. VAN SOEST (1967) stated that NFE contain hemicellulose and lignin in small amount.

The first wavelength of NFE appear in 2087 nm close to 2080 nm sucrose and starch absorbance (refers to table 6). The second (2308 nm) close to 2310 nm of oil and CH_2 absorbance. The third wavelength (1710 nm) close to 1705 nm properties of CH_3. This case is completely similar with ash and OM, which are the measurements given by the various constituents of which NFE was composed or associated with. These absorbances of starch and cellulose give contribution to NFE of which the main constituent is carbohydrate.

Organic Cell Fraction

Organic Cell fraction such as OCC, OCW, Oa and Ob, theoretically, will give strong absorbance in the same area. The compositions of these fractions exist in tight relation.

-organic cellular contents

Considering the composition of OCC, which includes mono, oligo, poly saccharides, fructosans, organic acids, soluble protein, lipids and others (ABE, 1988), we can predict that strong absorbance will be shown by sugar or protein group. The results are 2277 nm, 2289 nm, and 1547 nm for the first, second, and third wavelength, respectively. The first wavelength 2277 nm is very close to 2276 nm wavelength properties of starch absorbance, and 2289 nm close with 2290 nm spectra of cellulose reported by NORRIS *et al.* (1976). The Third wavelength is 1547 nm close to 1549 nm starch absorbance (Table 6).

According to these results, we concluded that the absorbance was occurred in cellulose and starch -the OCC main composed of- region. Differences between occurred absorbance and the absorbance in Table 6, might be caused by noise of the instrument which read the spectra in 2 nm interval. The third wavelength resulted in the highest correlation coefficient by 0.91 and standard error of 1.94%. The results of r value are lower, but standard error is better than that reported by AMARI *et al.* (1987) with 0.95 and 2.46% for hay and 0.92 and 2.32% for grass silage of temperate grass.

-organic cell wall

Organic Cell Wall, which includes cellulose, hemicellulose, lignin, and insoluble protein (ABE, 1988) give strong absorbance at 2280 nm, 1549 nm, 2326 nm for the first, second and third wavelength, repectively. As shown in Table 6, these wavelengths are close to the absorbance of starch (2280 nm, 1540 nm, 2322 nm, and 2230 nm). The nearest absorbances of cellulose were listed in 2270 nm, 2335 nm, and 2336 nm. These results indicated that there is a strong relation between starch and cellulose to OCW.

The first wavelength of OCC and OCW are very close to the first wavelength of OCC and OCW reported by AMARI *et al.* (1987), occuring in 2279 nm and 2281 nm, only 1-2 nm difference. It was strongly indicated the absorbance wavelength in 2277 nm and 2281 nm are properties of OCC and OCW, respectively.

equal to that reported by COELHO *et al.* (1988).

Silica

The silica absorbance occurs in 2336 nm, 2123 nm, and 1578 nm. The first wavelength is properties of cellulose, the second wavelength lying between 2100 nm of starch and cellulose absorbance, 2110 nm of $CONH_2$ and CONHR absorbance, and 2132 nm of amino acid absorbance.

As stated by SHENK *et al.* (1979), silica as well as mineral do not have reflectance spectra in NIR portion. Therefore, the calibration of Si might be done in association with some organic constituent in the samples such as cellulose or amino acids.

The Si calibration, up to now, still has a low correlation coefficient, because calibration is not directly to the Si, but must compensate to other constituent which Si is associated with.

Conclusion

In general, all of the NIRS analysis resulted in relatively high correlation coefficient between chemical analysis and NIRS analysis, even though the samples included variable species, cutting time, stage and origin.

All samples used in this study were ground through Willey mill 1-mm screen. Although reported that grinding through Willey mill gives less accuracy (WINDHAN *et al.*, 1987), this study was able to show the results have accuracy enough for chemical analysis prediction.

This study showed that NIRS has high capability to calibrate tropical grasses quality, and showing no limitation due to some differences between temperate and tropical grasses.

Summary

Near infrared reflectance spectroscopy (NIRS) has been widely used for chemical analysis of temperate grasses and legumes, but study for tropical grasses is still limited.

Tropical grasses which have some differences in physical anatomy, cell wall concentration and cell wall matrix, might be limited for successful prediction due to all these differences.

For the reasons given above, the prediction data of tropical forages by NIRS in Asia including Japan and Indonesia is still limited, therefore this study has been done. The aim of this study is to evaluate NIRS prediction of chemical composition such as moisture, protein, crude fiber, crude fat, ash, NFE, ADF, ADL, silica, organic matter, OCW, OCC, Oa, Ob, of tropical grasses compared to the previous study conducted in other forages.

A total of 85 tropical grass samples representing various species and stages were obtained from Japan (Okinawa and Kyushu, 55), Brunei (7) and Indonesia (Semarang, 23) during the summer of 1990. All these samples were conducted for chemical analysis and in application of NIRS.

The coefficient correlation for each Weende proximate chemical analysis was relatively high, and especially for organic cell fraction, correlation coefficient showed comparably high, even though compared with another study in temperate grass. Analysis of fibrous component (ADF, OCW, Ob), ADL and silica found the same magnitude with another previous study.

This study concluded that NIRS has a capability to calibrate tropical grass, but any further study needs a proper statistical program to reduce bias from particle size.

Compared to AMARI *et al.* (1987) who found the same r value of OCC and OCW by 0.96 and 2.21% and 2.19% standard error, the result of this study is lower in OCC and similar in OCW, but standard error results are significantly better. Therefore, these results strongly indicated that NIRS has the capability to calibrate both of OCC and OCW.

-organic a fraction (Oa and b fraction (Ob)

Oa -high digestible fraction in OCW-, is a part of non-lignified Cell Wall, while Ob is lignified portion of CW (ABE, 1988). Three spectra of Oa are 2113 nm, 2336 nm, 1634 nm, that of close to 2100 nm absorbance of starch and cellulose, 2336 nm cellulose absorbance, and to 1620 nm = CH_2 absorbance. The contribution of cellulose absorbance, is clearly showed.

The absorbance of Ob occurs in 2301 nm, 1997 nm, and 2331 nm for the first, second and third wavelength. The first wavelength is very close to 2300 nm protein absorbance, the second between 1990 nm and 2000 nm, properties of urea and starch absorbance, respectively. The third wavelength lies between 2330 nm of starch and 2335-2336 nm of cellulose absorbance.

The absorbance of Oa and Ob signed that contribution of cellulose absorbance is very strong. Considering the r value of Oa and Ob, the r of Oa increased gradually, and in Ob increased by 0.01 in each wavelength. This occurence lead us to think that measurement to second wavelength might be enough for Ob.

Coefficient correlation of Oa (0.88) and Ob (0.91) are comparably high. These results are similar in r value compared to AMARI *et al.* (1987) which reported r value of 0.79 and 0.94.

Acid Detergent Fiber

Acid Detergent Fiber (ADF) has become most widely accepted as an estimate of forage plant fiber for routine laboratory analysis. Beside contained cellulose and lignin, ADF also includes hemicellulose, cutin, silica, fiber-bound protein, and some pectin.

Calibration by NIRS resulted in a strong absorbance in 2277 nm, 1501 nm and 1506 nm for the first, second and third calibration, respectively. This first wavelength is close to that of reported by COELHO *et al.* (1988) and AMARI *et al.* (1987) in which the first wavelength occured in 2270 nm and 2281 nm. Refers to the Table 6, the first absorbance might indicate to 2276 nm of starch. The second and the third lies between 1490 nm of cellulose, 1500 nm of NH, and 1510 nm of protein absorbance.

All of these calibrations showed that cellulose and protein absorbance give an important contribution to ADF absorbance. Coefficient correlation of ADF (0.91) is relatively high, but is still lower compared to NORRIS *et al.* (1976), AMARI *et al.* (1987), and COELHO *et al.* (1988).

Lignin

Lignin determined by 72% sulfuric acid treatment for ADF extract, was named ADLignin. The absorbance in calibration occurs in 2280 nm, 2211 nm, and 2308 nm. When compared to another occurence absorbance, the first wavelength is close to the first of ADF, the second close to the second of protein, and the third close to the second of NFE absorbance.

Structure of lignin is complex and incompletely known, and there are variations in the relationship between indigestible lignin and partly digestible cellulose, depending upon environmental conditions (BARNES and MARTEN, 1979). These conditions might inform the fact that absorbance in ADL is calibrated in association with cellulose or carbohydrates.

Correlation coefficient of lignin is relatively low to another chemical composition, but

Acknowledgement

The authors are thankful to Dr. shosei HOSHINO, Professor of Ryukyu University, Iriomote, Okinawa, Dr. Sumimaro ITO, Assoc. Professor of Tokyo Univ. of Agriculture, for providing the grass samples for this study, and to Dr. Yoshio KURIHARA, Assoc. Professor of Tokyo Univ. of Agriculture for his support during the conducting of the chemical analysis for this study.

References

1) ABE, A., 1988. Feed Analyses Based on Carbohydrates and Their Application to the Nutritive Value of Feeds. Memoirs of National Institute of Animal Industry No. 2. National Institute of Animal Industry. Ministry of Agriculture, Forestry & Fisheries. Tsukuba. Japan. 6～8 (In Japanese).
2) ABE, A., S. HORII, and K. KAMEOKA., 1979. Application of Enzymatic Analysis with Glucoamylase, Pronase and Cellulase to Various Feeds for Cattle. *J. Animal Sci.* 48 (6) : 1483～1490.
3) AKIN, D.E., F.E. BARTON, II and D. BURDICK., 1975. Scanning Electron Microscopy of Coastal Bermuda Grass and Kentucky-31 Tall Fescue Extracted with Neutral and Acid Detergents. *J. Agric. and Food Chem.* 25 : 924.
4) AKIN, D.E. and D. BURDICK., 1973. Microanatomical Differences of Warm Season Grasses Revealed by Light and Electron Microscopy. *Agron. J.* 65 : 825～828.
5) AMARI, M., A. ABE, R. TANO, S. MASAKI, S. SERIZAWA, and T. KOGA., 1987. Prediction of Chemical Composition and Nutritive Value of Forages by Near Infrared Reflectance Spectroscopy, 1. Prediction of chemical composition. *J. Japanese Society Grassland Sci* (3) : 219～226 (In Japanese with English Summary).
6) BARNES, R.F. and G.C. MARTEN., 1979. Recent Developments in Predicting Forage Quality. *J. Anim. Sci.* 48 (6) : 1554.
7) COELHO, M., F.G. HEMBRY, F.E. BARTON and A.M. SAXTON., 1988. A Comparison of Microbial, Enzymatic, Chemical and Near- infrared Reflectance Spectroscopy Methods in Forage Evaluation. *Anim. Feed Sci. Technol.* 20 : 249～231.
8) GODDU, R.F. 1960. Adv. Anal. Chemistry Instrument, vol 1. Interscience, N.Y. 347.
9) GOERING, H.K. and P.J. VAN SOEST., 1970. Forage Fiber Analyses (apparatus, reagents, procedures, and some applications). USDA Handbook 379. U.S. Goverment Printing Office, Washington, D.C.
10) HEATH, M.E., R.F. BARNES and D.S. METCALFE (Editors). 1985. Forages, The Science of Grassland Agriculture. Fourth Edition. Iowa University Press, Ames, Iowa. USA. 619～630.
11) LAW, D.P. and R. TKACHUK., 1977. Near Infrared Diffuse Reflectance Spectroscopy of Wheat and Wheat Components. *Cereal Chem.*, 54 : 256～266.
12) MARK, H. Unpublish results.
13) MURRAY, I,, and P.C. WILLIAMS., 1990. Chemical Principles of Near-Infrared Technology in : WILLIAMS, P.C. and K.H. NORRIS (Editors) Near-Infrared Technology in Agricultural and Food Industries. American Association of Cereal Chemists, Inc. St. Paul, Minnaesota. USA.
14) NORRIS, K.H., R.F. BARNES, J.E. MOORE and J.S. SHENK. 1976. Predicting Forage Quality by Infrared Reflectance Spectroscopy. *J. Anim. Sci.* 43 (4) : 889-897.
15) OSBORNE, B.G., and T. FEARN., 1986. Near Infrared Spectroscopy in Food Analysis. Longman Scientific & Technical. Longman Group UK Limited, Longman House, Burnt Mill, Harlow. Essex CM 20 2 JE, England.
16) SHENK, J.S., M.O. WESTERHAUS, and M.R. HOOVER., 1979. Analysis of Forages by Infrared Reflectance. *J. Dairy Sci.* 62 (5) : 807～812.
17) SHENK, J.S., NORRIS, K.H., BARNES, R.F. and FISSEL, G.W. 1977. 13th Int. Grassland.

Congress, Leipzig, GDR. 454～463.
18) VAN SOEST, P.J., 1967. Development of a Comprehensive System of Feed Analyses and Its Application to Forage. *J. Anim. Sci.* 26 : 119.
19) WINDHAN, W.R., J.A. ROBERTSON and R.G. LEFFLER., 1987. A Comparison of Methods for Moisture Determination of Forages for Near Infrared Reflectance Spectroscopy Calibration and Validation. *Crop Sci.* 27 : 777～783.

(平成4年3月25日受付)

摘　　要

近赤外分析による粗飼料成分分析法の暖地型牧草への適用†

Agung PURNOMOADI*・甘利雅拡**・亀岡暄一*

西表島，インドネシア，ブルネイなどから暖地型牧草計85点（8草種を含む）を集め，近赤外分析による粗飼料成分分析の精度について検討をおこなった。すなわち，これらの飼料のうち，60点を検量線の作成用に，25点を検量線の検定用に用いた。

なお，分析項目としては，水分，粗蛋白質，粗脂肪，粗繊維，NFF，粗灰分，有機物，OCW，OCC，Oa，Ob，ADF，ADL-リグニン，Siの14成分とした。

また，各成分の推定精度は，相関係数と標準誤差によって検討した。この結果，粗蛋白質，OCWおよびObは相関係数が0.95と非常に高い相関が得られた。しかし，Siでは0.78と相関が低かった。その他の成分は，0.86～0.93と一応満足出来る値であった。

本研究によって，暖地型牧草についても，草種の細分化や，検量線作成用の試料点数を増加させることによって，近赤外分析法による粗飼料の成分推定値が実用に供しうることが可能であることが示された。

† 平成4年6月17日受理
* 東京農業大学農学部畜産学科家畜飼養学研究室
** 農林水産省畜産試験場栄養部飼料鑑定研究室