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Developing prediction equations for digestibility of nutrients in faeces from individual Atlantic salmon (Salmo salar)

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DEVELOPING PREDICTION EQUATIONS FOR DIGESTIBILITY OF NUTRIENTS IN FAECES FROM INDIVIDUAL ATLANTIC SALMON

BY

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Kwarteng Appiah Augustine

ABSTRACT

The purpose of this study was to develop a robust and reliable approach to predict apparent digestibility coefficients of fat and protein of individual Atlantic salmon (*Salmo salar*), based on multivariate regression models from measured NIR (Near infrared Reflectance) spectra in their faeces.

The period of the experiment from rearing to faecal analysis was from July 2014 to March 2015. A total of 180 faecal samples of protein collected from 10 different experiments and 115 faecal samples of fat also all collected from 9 different experiments were used to calibrate the NIR instrument. Full cross-validation was used for the fat calibration, with 5 samples randomly selected and chemically analyzed for fat to complement the fat validation. 23 faecal samples from a different experiment were also used to validate the protein calibrations.

Faeces from 60 Atlantic salmon (*Salmo salar*) of different families were used for the prediction in the study. The developed equation had better prediction precision (R^2; 0.97, RESD; 0.19, and Bias; -0.002) for protein than for fat (R^2; 0.92, RESD; 0.29, and Bias; -0.02). In comparing with results of other studies performed in similar ways, the protein model had good prediction results (15.6, of data set A) and the fat (5.26, of data set A) as compared with other studies. However, detailed description explanation of the prediction (data set A) of individual is treated in parallel thesis.

KEYWORDS: Near infrared, root mean square error of cross-validation, partial least squares, coefficient of determination, extended multiplicative scatter correction, protein, fat, faeces.

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CHAPTER ONE

INTRODUCTION

As global demand for high quality Atlantic salmon (*Salmon salar*) increases, there is also the corresponding need for feed that provides macro (fat, protein, carbohydrates) and micro (minerals, vitamins) nutrients in their right proportions for growth and development. This require the assessment of the nutrient content in feed, and to what extent individual nutrients are absorbed and retained in the body of the fish (Morales et al. 1999).

Feed accounts for a large percentage (50-60%) of the total production cost of intensive aquaculture production (Hernández et al. 2007). Therefore, accurate determination of macro and micro nutrient digestibility within salmonid feeds is crucial to developing feeds that are both efficient at promoting growth and limiting environmental impact (Ward et al. 2005) and at the same time utilizing minimal but of low cost resources.

Digestibility, particularly of protein and energy, is an important quality criterion for fish feeds (Hatlen et al. 2015). To obtain the digestibility in fish, faeces may be collected by different methods, including careful stripping (Austreng 1978), dissection of the intestine or different ways of collection from the water outlet (Cho and Slinger 1979; Choubert et al. 1979, 1982; Glencross et al. 2007). Because the amount of feed ingested cannot be accurately measured and recorded as compared to other farm animals, the most useful method of determining apparent digestibility content (ADCs) of feed is indirectly, via the inclusion of markers in feed to quantify the amount of feed ingested and excreted (Austreng 1978; Cho and Slinger 1979; Hillestad et al 1999; Austreng et al. 2000).

In the past, chromium oxide was the marker of choice (Austreng 1978; Aksnes et al. 1996) for determining ADC, but recently various metal oxides, particularly yttrium oxide (Y_2O_3) and ytterbium oxide (Yb_2O_3) have been used (Sugiura et al. 1998a; Hillestad et al. 1999; Austreng et al. 2000; Carter et al. 2003). The effectiveness of Y_2O_3 (Sugiura et al. 1998a; Hillestad et al. 1999), for determining ingredient ADCs in salmonid feeds have been evaluated and accepted (Austreng et al. 2000; Carter et al. 2003; Hatlen et al 2015), but little information exists on how to predict nutrients (fat and protein) digestibility and yttrium in small enough samples to allow digestibility measurement for individual fish.

Until now ADCs have been obtained based on chemical analyses of nutrients and markers in the feed and faeces collected from several fish to obtain large enough sample size. In addition, chemical analyses of nutrients is costly and therefore put limits on the number of

the samples that can be analyzed as is the case when studying ADCs of families and in particular individuals in a selective breeding programme.

Near infrared reflectance spectroscopy (NIRS) upon discovery in the 1960's was first used for the rapid characterization of agricultural and food products (Niemoller and Behmer 2008). Today it's widely used in the agriculture and food industry for non-destructive qualitative and quantitative analysis of raw materials, processed materials, and finished products throughout the entire manufacturing process (Niemoller and Behmer 2008). NIRS is a non-destructive, rapid, economical, flexible and versatile technique. Sample presentation is simple and data (spectra) are collected very rapidly (Sánchez et al. 2013). In particular, the benefits associated with NIRS analysis of faeces are associated with decreasing cost, timeliness and convenience in handling unpleasant samples (Neumeister et al. 1998; Castrillo et al. 2005), and because it's not destructive the samples can be used for other analyses, saving time and cost in obtaining new samples. In the NIRS analysis, the sample is illuminated by NIRS light, and the absorbance at several wavelengths is measured.

Near infrared reflectance spectroscopy (NIRS) has been applied widely and numerous studies have also used NIR reflectance spectroscopy to examine the composition and characteristics of faeces (Dixon and Coates 2009). In agriculture, Meineri et al. (2009) used faecal NIR to predict chemical composition of faeces and digestibility of diets. The NIR spectroscopy is not only limited to the field of agriculture, but can also be used in the field of clinical biochemistry. In the clinical biochemistry, NIR spectroscopy has been used to analyze faecal fat, nitrogen and water with conventional methods and faecal energy content (Neucker et al. 2002).

Compared to other studies using NIR spectroscopy on faeces of other farm animals, the low amount of faeces obtained in fish poses a major challenge in using the method due to their aquatic environment which limits the amount of faeces obtained from single fish as most is leached into their aquatic environment. To the best of our knowledge, this is the first work dealing with the use of NIR in predicting the digestibility of individual Atlantic salmon. For this reason, the objective of the present study is to build a reliable and robust method for the prediction of ADCs of fat and protein of individual Atlantic salmon, based on multivariate regression models from measured NIR spectra of the respective faeces samples.

CHAPTER TWO

2.0 LITERATURE

2.1 DIGESTIBILITY, DIET FORMULATION AND FAECES

Feed stuff is of little value unless it can be digested and its content absorbed. Digestibility, particularly of protein and energy, is an important quality criterion for fish feeds (Hatlen et al. 2015). The increasing production of fish from intensive aquaculture as a result of diminishing wild catch corresponds with the increased demand for feed of high digestibility to meet the energy needs for growth and production. In meeting this requirement for high digestibility, the quantity ingested (total or nutrient) and faecal matter voided are determined, and the ratio gives the percentage digestibility of the feed and or the nutrients under consideration.

Information of the digestibility of the various feed ingredients is a basic requirement for formulating diets (Cho and Kaushik 1990) and to optimize the balance between nutrient requirements and cost of feed, and also provide a thorough assessment of the nutritive value of a particular protein source in a complete diet (Plakas and Katayama 1981). Nutrient and diet composition interactions is also important in assessing the digestibility of nutrients, and so the diet formulation. According to De Silva and Anderson (1995) factors such as the quality and quantity, energy source and content have significant effect on the requirement and metabolism of most nutrients. For example, thiamine (Vitamin B_{12}) availability is known to be influenced by fat and protein content of diets of the same caloric value (De Silva and Anderson 1995). Similarly, the metabolism of pyridoxine (vitamin B_6) is related to dietary protein or amino acid metabolism, and magnesium requirement is dependent on the calcium and phosphorus content of the diet (De Silva and Anderson 1995; Storebakken, personal communication 2013).

Faece from fish is made up of digestive enzymes, epithelial (stomach and intestinal) lesions and nutrients from indigested food (Storebakken, personal communication 2013). Fish faeces for scientific studies is collected by several methods as stripping (Austreng 1978), dissection of the intestine or different ways of collection from the water outlet (Cho and Slinger 1979; Choubert et al. 1979, 1982; Glencross et al. 2007). It is difficult to determine the digestibility of fish as such determinations are subject to many errors. Foremost amongst these is the leaching of faecal material and the difficulty in collecting all the faecal material, which tend to break up with time, a process facilitated by aeration and the movement of fish. Also, there are fine particles in suspension which is almost impossible to collect. Moreover,

the re-ingestion of faecal material and incomplete collection of all faecal matter is avoided. To minimize these errors in faeces collection and to obtain a representative value of faeces, each method selected should fit the anatomical characteristics of the species in research (AQN 250 Lecture 2013).

2.2 ELECTROMAGNETIC ENERGY

Electromagnetic energy (EM) is the form of energy that can be reflected or emitted in the form of electrical or electromagnetic waves that can travel through space. It includes radio waves, heat waves, x-rays, ultraviolet light, gamma rays etc. as shown in figure 2.1.

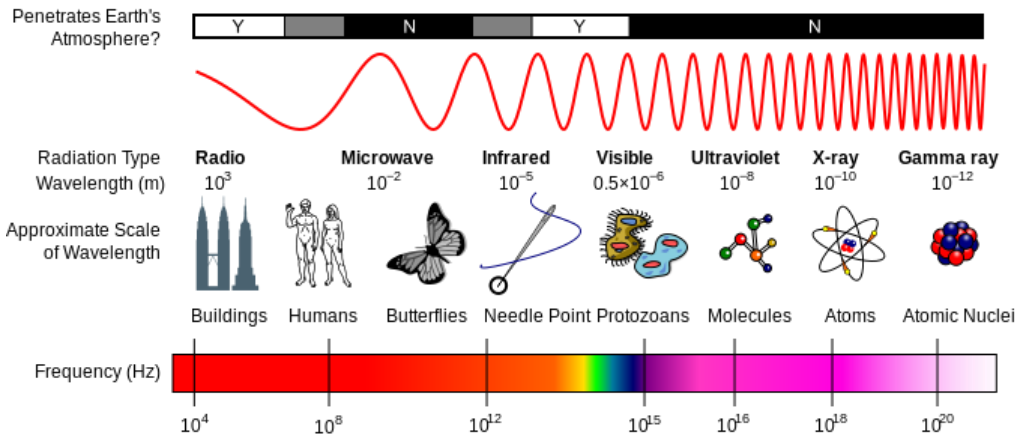


Figure 2.1. Graphical representation of the electromagnetic spectrum showing the wavelength (nm) and frequency (Hz) of some of the different energies (Wikimedia Commons, 2013).

When molecules are exposed to electromagnetic energy some are absorbed, not absorbed or re-emitted based on the wavelength of the energy received. Based on the intensity, number and frequency of these absorptions and or emissions, an insight can be gained into the structure of the material under consideration. This technique could be used to identify an unknown molecule by comparing its absorption to that of other molecules or could be used to gain further understanding of the physical properties of a known molecule (Ucdavis chemiwiki, N.D). One of the advantage of this method is that it can be done on unprepared products in gas, liquid or solid form (Li-Chan et al. 2011).

2.3 VIBRATIONAL SPECTROSCOPY

Vibrational spectroscopy is the science of measuring exactly which wavelengths of light are absorbed by a molecule (Ucdavis chemiwiki, N.D). It includes infrared (IR) energy which is based on transitions between quantized vibrational energy states of molecules (Li-Chan et al. 2011).

2.3.1 INFRARED ENERGY

Infrared (IR) refers to that part of the electromagnetic spectrum between the visible and microwave regions. The IR region is further divided into far-infrared (FIR, 200-10cm^{-1}), mid-infrared (MIR, 4000-200 cm^{-1}) and near-infrared (NIR, 12800-4000cm^{-1}, or 780nm-2500nm) (Ucdavis chemiwiki, N.D). When energy is absorbed by a molecule, molecular vibrations are induced and transitions occur from a ground vibrational state to an excited vibrational state as shown in the figure 2.2 below (Ucdavis chemiwiki, N.D).

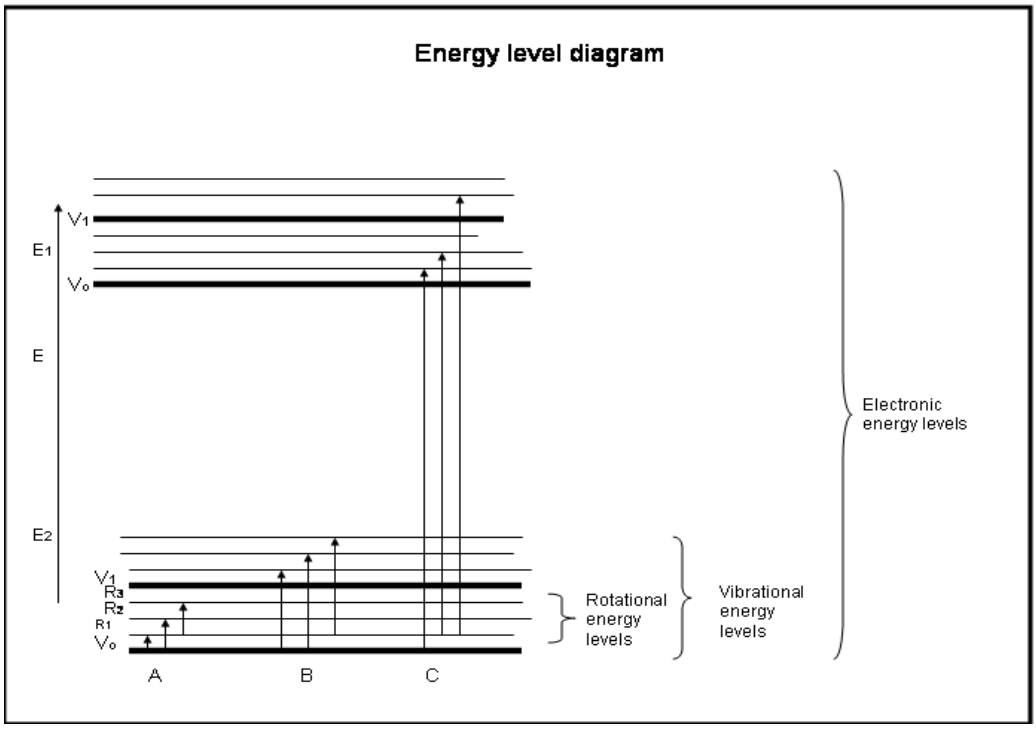


Figure 2.2: Energy level diagram. A: Rotational transitions, B: Rotational-Vibrational transitions, C: Rotational-Vibrational-Electronic Transitions, E_0: is the electronic ground state and E_1: Electronic excited state (Ucdavis chemiwiki, N.D).

Whereas MIR deals with the fundamental energy level of functional groups (Ucdavis chemiwiki, N.D), NIR deals with the corresponding vibrational overtones (Li-Chan et al. 2011).

2.3.2 NIR SPECTROSCOPY

NIR Spectroscopy is a type of vibrational spectroscopy that use the infrared region (780-2500nm) of the electromagnetic spectrum (Pasquini 2003). NIRs provides an alternative, non-destructive technology for measuring constituents of biological materials (Baye et al. 2006). Organic molecules have specific absorption patterns in the infrared region that can report the chemical composition of the materials being analyzed (Williams and Norris 2001). NIRs is mostly associated with the measurement of the overtones of C-H, O-H and N-H stretching vibrations (Li-Chan et al. 2011). Its overall objective is to probe a sample in order to acquire qualitative and or quantitative information coming from the interaction of near-infrared electromagnetic waves with its constituents (Pasquini 2003).

Near infrared spectra can be collected either from the reflectance (NIR) of a sample or transmittance (NIT) through a sample (Williams 1979; Delwiche 1995). The technique is rapid, robust and non-destructive, little or no sample preparation is required. The technique enables the use of fiber optical cables and several chemical components can be determined simultaneously with a single measurement. It has the advantage of being safe, efficient, more economical and environmentally friendly (Zhang et al. 2011). Due to these favourable features, the technique is used frequently within food analysis (both on-line and at-line in production sites) and the applications cover a broad range. It’s been used for predictions in soy, rapeseed meal, sunflower meal, peas, fish meal, meat meal products, and poultry meal (Fontaine, Hörr and Schirmer 2001).

NIR spectroscopy consist of several methods of sampling with transmittance, transflectance, diffuse reflectance and interactance as some of the available methods. (Figure 2.3).

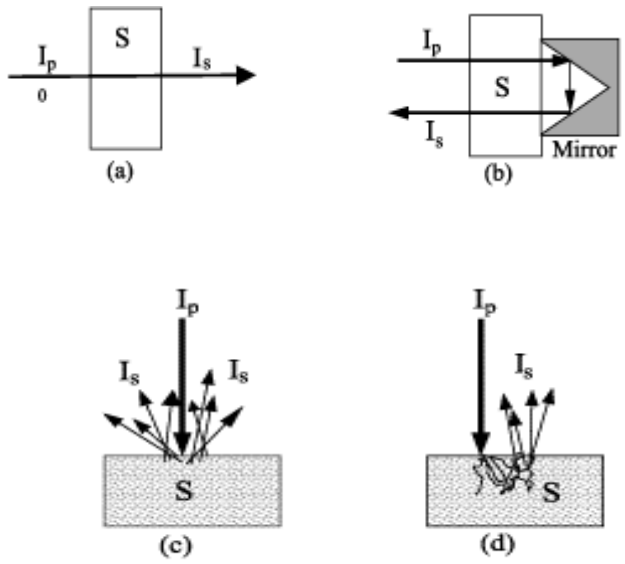


Figure 2.3. Modes of measurement employed in NIR spectroscopy. a: Transmittance b: Transflectance c: diffuse reflectance and d. interactance (Pasquini 2003).

The amount of radiation reflected from the sample in NIR spectroscopy is quantified as the reflectance (R) of the sample. The value is usually expressed as log(1/R) as shown in figure 2.5, which gives higher values at higher levels of absorbance (i.e. lower reflectance). There is an almost linear relationship between log (1/R) and the concentration of an absorbing component (Hruschka 1987). The log (1/R) curve is comparable to an absorption curve with peak values occurring at wavelengths which correspond to absorption bands in the sample (Norris et al. 1976).

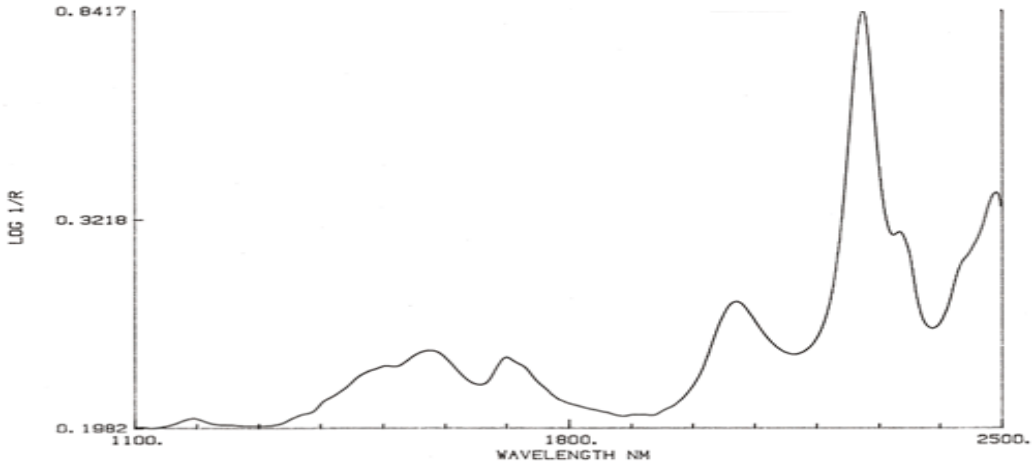


Figure 2.4. NIR plot indicating log (1/R) and wavelength (nm). (Im publications, 2015).

According to Dryden (2003), reflectance in the NIR spectrum represents the chemical structure of the sample. In particular it indicates the presence of chemical bonds and functional groups (e.g. C-H, O-H, N-H, and see Table 2.1).

The wavelength (spectral region) chosen for NIR spectroscopy should be such that, it is absorbed most by the sample under consideration with little interference by other constituents in the sample. That is, interference by other samples not under consideration but can be obtained at the chosen wavelength. For example, in Table 2.1 the chosen wavelength for peptide bonds (2140, 2180) can also be obtained in condensed tanins (2100-2200), so if peptide bonds are under consideration the interference from tanins should be minimized and peptide bonds maximized.

Table 2.1. Near infrared wavelengths and their association with chemical structures (from Barnes 1988; Osborne and Fearn 1986; Smith and Kelman 1997).

Wavelength (nm)	**Chemical entity**
1143	aromatic compounds, lignin
1496, 1668, 1976	amide bonds
1660 - 1670, 1720 - 1730, 2100 - 2200	condensed tannins
1772	ester bonds
1930	water
1960, 2180	protein
2140, 2180	peptide bonds
2088, 2410 - 2460	cellulose
2380	hemicellulose
2461	starch

2.4 MEASUREMENT OF THE ABSORBANCE OF RADIATION BY A SAMPLE

The Beer-Lambert law describes the relationship between the concentration of a solute and the amount of light absorbed by the solution:

$$Cx=A_X/e.l$$

where: C_X = concentration of the test solute
A_X = absorbance of the test solution
e = molar absorptivity of the test solute
l = path length travelled by the light through the solution

The important feature of this relationship is that it allows the measurement of C_X directly from A_X (Dryden 2003).

When infrared radiation is incident on the surface of a sample, some of it is reflected (specular reflectance) from the surface. Another proportion of the radiation enters the sample and may be absorbed within it. Radiation not absorbed may be transmitted through the sample or reflected from it (diffuse reflectance, Fig. 2.5).

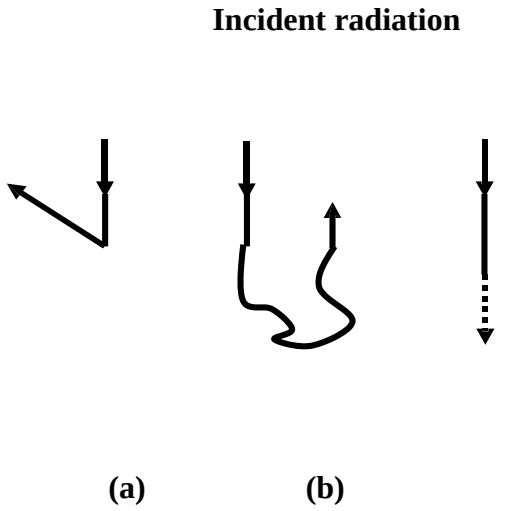


Fig. 2.5. Diagrammatic representation of specular (a) and diffuse (b) reflectance's, and absorption (c) of near infrared radiation from a sample (Givens et al. 1997).

While the Beer-Lambert law generally describes the relationship between radiation diffusely reflected from a solid sample and characteristics of that sample, the path length of diffusely reflected radiation cannot be predicted because it is scattered by random reflections, refractions and diffractions within the sample.

The variations within NIR diffuse reflectance spectra are mainly a result of (1) non-specific scatter of radiation, (2) variable path length, and (3) the chemical composition of the sample (Barnes et al. 1989).

As a result, the relationship between reflectance and analyte content cannot be described by any mathematical relationship (Givens et al. 1997). Thus while the characteristics of near infrared radiation reflected from a sample can be used to predict certain sample characteristics, each application of this type must be obtained by calibration.

2.5 DEVELOPING A CALIBRATION MODEL AND VALIDATION OF CALIBRATION MODEL

The steps involved in developing the calibration model from raw samples is summarized into these four steps (Williams and Norris 1987).

1. Response linearization: which aims to ensure the instrument responds linearly to chemical changes.

2. Optical correction, yielding quantitative information about the light scatter in each sample: This help eliminate near-infrared interferences as light scatter and remaining specular reflectance.

3. Data compression of regression factors (linear combination of the spectrum). This gives certain estimated parameters (near-infrared loading spectra) that defines how the values of the regression factors are to be calculated. It gives various diagnostic checks that reveal abnormalities in the data (measurement errors and extreme samples or wavelength) and it provides estimates of the ‘‘normal’’ range of variation and level of measurement noise in the near-infrared data, which helps in revealing abnormalities.

4. Calibration regression: The final step, which establishes linear regression relationships between the obtained regression factors and the chemical variables to be determined.

2.5.1 DEVELOPING A CALIBRATION MODEL

Calibration is needed for data set to be usable for quantitative purposes using NIR spectroscopy. The calibration turns the wavelength and the corresponding intensities into precise and relevant information which can be used for quantification *(*Martens and Næs 1989).

NIR instruments determine protein and other components by measuring log (1/R) values, which must then be related to the amount of the component as determined by the corresponding values obtained from a reference method (Williams and Norris 1987). Establishing this relationship by using a set of samples of known composition is called calibration, whereas using the relationship to determine the amount of a component in a new sample is called prediction (Williams and Norris 1987).

The relationship between the log (1/R) values and the reference method values is expressed as an approximation, and always involves some form of regression equation. The regression equation has regression constants (the Y-intercept and regression coefficients), independent variables, and one dependent variable (the reference value). The independent variables are mathematical combinations of log (1/R) at various wavelengths. These combinations can be so complicated that they're better thought of as a series of steps, so we use the term ‘‘data treatment’’ to mean any mathematical treatment that combines log (1/R) values into independent variables for use in a regression equation (Williams and Norris 1987).

Each data treatment has data treatment constants (such as the derivative parameters, or the amount of smoothing). Developing a calibration model involves testing different data treatments, data treatment constants, or sets of wavelengths. Calibration means finding the regression constants that go into the approximation once the form of approximation, the data treatment constants, and the wavelengths have been decided upon.

There are two main approaches to calibration, namely, univariate calibration and multivariate calibration. In univariate calibration, only one independent measurement

variable is used and the calibration finds the linear relationship between these variables and a given reference value (Næs et al. 2002*)*. Multivariate calibration on the other hand takes into account several measurement variables in the calibration (Næs et al. 2002*)*. Multivariate calibration take all independent variables into account, and create a multivariate prediction model or equation which often has a much higher reliability than a model based on a single variable (Næs et al. 2002).

2.5.1.1 PRINCIAL COMPONENT ANALYSIS (PCA)

Data collected from spectroscopic experiments are usually sorted in an X-matrix with n objects and p variables as shown in the equation below:

$$X=(X\ [n,\ p])$$

In NIR spectroscopy, n is the number of samples and p is the number of wavelengths used for each sample (Esbensen, 2009). Since spectroscopic data often contain several hundreds of wavelengths in each spectra, many of these variables are co-varying and thus do not contain independent information (Næs et al. 2002). PCA compresses this spectral data into principal components that gives an interpretable overview on the main information (or variations) of the data (Esbensen, 2009). The form of a PCA model can be expressed as

$$X=TP^{I}+E$$

Here, T denotes the score values, P the loading matrix, and E is the residual matrix representing any noise or unexplained variation (Martens and Næs 1989). The first component explains the direction of the highest variation of the data, and the second component explains the direction of the next highest variation, and so on. PCA removes any collinearity in the spectroscopic data, and shows more clearly any outliers that might be in the data.

2.5.1.2 PARTIAL LEAST SQUARES (PLS) REGRESSION

NIR spectra has so many data points in the wavelength dimension such that it creates collinearity problems of the spectra. PLS regression often can be an effective way to describe latent relationship among spectral variables unlike straight forward general inverse techniques like multiple linear regression (MLR) (Ritthiruangdej et al. 2011). PLSR is a

method for relating the variations of one or several response or reference variables (Y-variables) to the variations of for instance the spectral data (X-variables). In PLSR the information of the X-data (spectra) is projected onto a small number of underlying variables called PLSR components. The Y-data are actively used in this projection procedure and the overall procedure is frequently used for calibration of NIR instruments (CAMO PROCESS AS, OSLO, NORWAY).

In PLSR, one of the most important points lies in the fact that spectral data matrix X is decomposed into a linear combination of scores *T* and loading *P* matrices. For example, the decomposition of *X* is carried out by repeatedly regressing the score along the given concentration vector *y* (Næs et al. 2002). PLS model essentially yields a regression vector *b*, which is often referred to as PLS model (Næs et al. 2002). Consequently it becomes

$$y=Xb + e$$

Here, *X* is the observed spectra, *y* denotes the concentration of corresponding analyte and *e* means residual (Næs et al. 2002). The regression vector *b* carries the information most relevant to the determination of the concentration of the analyte and less sensitivity to the overall effect of interference, i.e., chemical components which are not associated with the actual quantity of *y* (Næs et al. 2002).

2.5.2 PREDICTION AND VALIDATION OF CALIBRATION MODEL (PREDICTION ABILITY)

Once the NIR instrument has been calibrated against the reference values, it can be used to determine (predict) the percentage of a constituent in different samples (unknown) or measure some physical quantity of these samples (Williams and Norris 1987).

Comparison of NIR predicted and reference values on a new set of samples provides a basis for calculation of the true measurement error called the validation (Williams and Norris 1987). This is done to test the strength of the model created with PLSR.

Validation is done using data from other samples acquired in the same manner as the data in the model. Normally, calibration and initial validation are performed simultaneously by splitting the data into one calibration set and one validation set (where the samples are

treated as unknowns), or by performing cross-validation (Segtnan et al. 2009). Either test-set validation or full cross validation method is used.

In the test-set validation, data collection is repeated using new samples (Esbensen 2009). However, this is costly, time consuming and more samples may not be available for creating an entire new data set of validation (Esbensen 2009).

With full cross validation (leave-one-out), one sample from the model is removed and estimated with the remainder of the model, and the result is compared with the reference value. In the next step, data is replaced and a different sample is removed and used to test the model in the same manner. This continues until all samples have been kept out once (Stone 1974). Giving an average calibration equation to be used for future predictions (Segtnan et al. 2009).

There are a few validation criteria for prediction performance measure in NIR spectroscopy such as standard error of cross-validation (SECV), root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP), and root mean square error of cross-validation (RMSECV) etc. (Næs et al. 2002). However, RMSEP, SECV, Bias, R^2 and RMSECV were used in this experiment.

R^2 is a dimensionless number between 0-1.00 that say something about the correlation between the *x* and *y* values (Esbensen 2009). It should be as close as possible to 1, and the closer R^2 is to 1.00, the more of the variation in the y-values can be explained by the measured x-values (Esbensen 2009). The RMSECV and RMSEP are virtually the same, however, RMSECV is reported when a full cross-validation is used. They on the other hand, say something about the error expected from any predictions made using the model (Esbensen 2009). RMSECV/RMSEP and Bias are expressed as

$$\text{RMSEP/CV} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2} \qquad \text{And}$$

$$\text{BIAS} = \frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)$$

Here, *i* denotes the samples from 1 to N, y_i the reference value, and $\hat{y}_i$ the predicted value.

2.6 USING ESTABLISHED PREDICTION EQUATION ON NEW SAMPLES

Prediction consists of predicting the chemical composition of a sample from near-infrared measurements using calibration equation (Williams and Norris 1987). Before using the prediction on new samples, the samples (spectral data) must pass through the same processes as during the calibration process. That is, measurements from the NIR instrument are submitted to response linearization (Williams and Norris 1987). Secondly, the data are passed through the optical correction step, yielding quantitative information about the light scatter in each sample (Williams and Norris 1987). Thirdly, the values of the regression factors, which are linear combinations of the spectrum, are computed in the data compression step. This step may also give various warning diagnostics for samples that appear abnormal (Williams and Norris 1987). Fourth, the desired chemical variables are determined linearly from the computed regression factors, using chemical loadings found in the calibration (Williams and Norris 1987).

2.7 SOURCES OF ERROR IN NIR SPECTROSCOPY

Errors are defined as the estimated difference between the predicted value and true or observed value (e.g. chemical analyzed) value. Though NIR spectroscopy is rapid and technically simple to carry out, there are nearly 40 sources of error (Williams 1987). These errors are dependent on the interaction of many sources, and may be categorized as sampling error, reference value error and NIR method error.

2.7.1 SAMPLING ERROR

Sampling error is caused by lack of homogeneity in the material being sampled (Williams and Norris 1987). Williams (1987) listed sources of error (summarized in table 2.2) and described ways in which these may be controlled.

Table 2.2. Procedural sources of error in NIR spectroscopy (selected from Williams 1987)

Instrument factors	**Sample factors**
Instrument noise	variation in water content
Stray light	bulk density, texture, packaging characteristics
Non-linearity of signal	sample temperature
Static electricity	sub sampling procedures
Instrument temperature control	mean particle size
Fluctuations in power supply	mixing after preparation
Instrument geometry	sample storage
Cell window characteristics	

According to Williams and Norris (1987), although it is rarely practicable to measure accurately all the kinds of sampling error in a particular application, it is useful to know where they occur and the approximate contribution of each source to the total sampling error. This can result in savings by indicating where to repeat the sampling.

2.7.2 REFERENCE VALUE ERROR

Reference value errors arise from using different sub samples to conduct the NIR spectroscopy and reference analysis, and from random and systematic errors in the method used to obtain the reference value (Hruschka 1987; Sorensen 2002). The difference between the errors measured by the NIR instrument and that measured by the reference value should be identified and removed because they inflate the errors associated with the prediction equations (Williams and Norris 1987). These may include errors relating to the chemistry of the reference determination such as the loss of N from refractory substances in the Kjeldahl N determination or use of an inappropriate factor to convert N to protein (Dryden 2003).

2.7.3 NIR METHOD ERROR

NIR method errors are caused by spectral measurement errors, lack of intrinsic correlation between spectral and reference methods data, and poor choice of data (Williams and Norris 1987). Noise (any disturbance, especially a randomly distributed and persistent disturbance

that influences the quality and clarity of the electronic signal) associated with NIR instrument markedly affect its efficiency. This noise include that which affects the instrument output on a standard background and that caused by interaction between instrument and sample. These instrumental noise can be caused by temperature, stray light (light energy that reaches the detector from wavelengths other than those at which the device is designed to take reading or from sources other than the sample) etc.

Errors associated with wavelength selection is also critical to NIR analysis, as this affects the regression coefficients of the calibration. For example, according to Williams and Norris (1987), as a wavelength becomes farther away from the optimum point, the associated regression coefficient changes and tends to compensate for the wavelength difference. Above a certain deviation from optimum, the regression coefficient no longer compensates for the deviation and the accuracy of determination and measurement suffer.

Also, the accumulation of electrical charge (static electricity) affects the orientation of particles of ground material (example is faeces) in sample cells. It makes particles of dust attracted and become closely associated with the surface, making it difficult to clean and allowing sample to sample contamination (Williams and Norris 1987). These static electrical charges makes samples (e.g. fibrous particles) strongly oriented at the cell surface, affecting the intensity and pattern of the diffuse reflectance signal and, as a result, the precision and accuracy of the NIR analysis.

Sample cells of most NIR instruments interpose an optical glass or quartz window between the sample and the detector to ensure uniformity of the surface (Williams and Norris 1987). According to Williams and Norris (1987) the thickness and refractive index of the window can affect the accuracy of the testing because they are rarely completely planar or of uniform thickness.

2.8 NIR SPECTROSCOPY OF FAT, PROTEIN AND FAECES

Due to the high cost of feed in aquaculture production coupled with the decreasing sources of feed materials, knowledge of the chemical composition of faeces is important in determining the animal requirements and the efficiencies of feed conversion. Subsequently, it is needed for feed formulation to provide the right amount of nutrient needed for growth whilst making little use of the already limited feed sources. Existing chemical procedures are time consuming and expensive (Maja et al., 2010) compared to NIR spectroscopy. In preparing faecal samples for NIR analysis, it's particularly important to homogenize faecal samples before NIR analysis to ensure efficient scattering of light and detection from the analyzer.

Because it's non-destructive (preserving samples to be used for other analysis), timeliness in obtaining samples and results, flexibility and economical in saving cost of obtaining new samples. It's been widely applied in the prediction of fat and protein in the food industry.

In using NIR spectroscopy to predict protein and fat contents in samples, the wavelengths at which the overtones for protein and fat are observed is chosen to obtain the desired objective. For example, in the food industry, Maja (2010) used NIR spectroscopy to predict the chemical composition of different raw meat and meat products. In the study, 294 muscle and meat products were analyzed within the wavelength range of 400-2500nm. They obtained coefficient of determination in prediction (R^2_p) for intramuscular fat and protein as 0.94-0.99 and 0.87-0.96 respectively.

Bázár et al. (2010) also used NIR spectroscopy (wavelengths from 1100-2500nm) to predict protein and intramuscular fat content of rabbit hind leg meat and found R^2 of 0.99 and 0.97 for fat and protein respectively.

NIR spectroscopy has never been used to predict protein and fat content in faeces in individual Atlantic salmon.

CHAPTER THRE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION OF THE EXPERIMENT

The work of this study was divided into three parts, namely, the experimental setup to faecal acquisition (Data set A), establishing the prediction equation (Data set B (from the protein samples) and Data set C (from the fat samples)) and using the prediction equation to predict protein and fat contents of the experimental faeces samples (Data set A). The whole process is summarized in the figure 3.1 below.

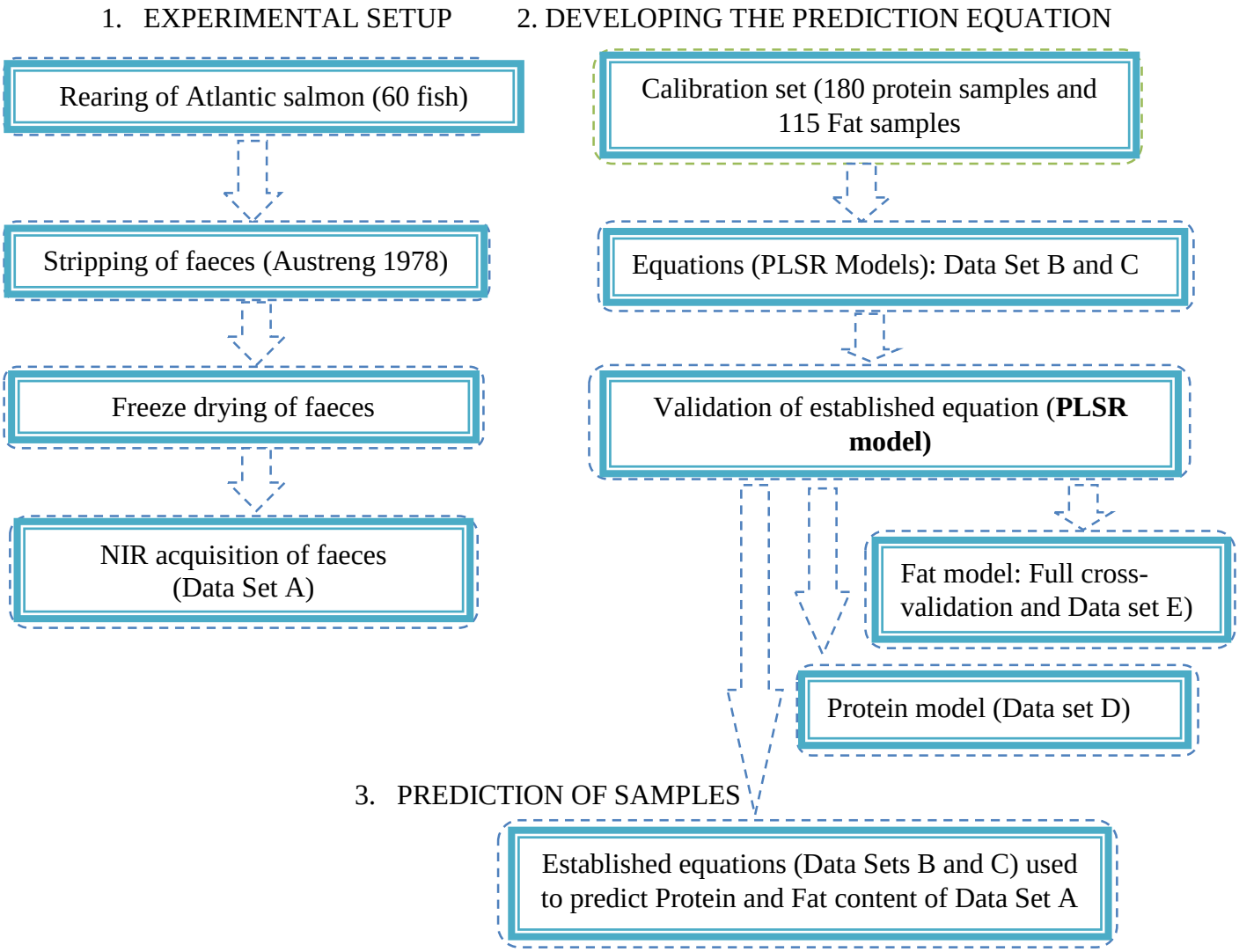


Figure 3.1: Chart showing the description of the study. Data set A (Experimental faeces samples), Data set B (180 samples of protein), Data set C (115 samples of fat), Data set D (23 additional samples for protein validation) and Data set E (Randomly selected 5 samples to complement the fat validation).

3.2 MATERIALS

3.2.1 DIET AND CHEMICAL COMPOSITION OF FEED

Dietary composition of compound feeds used in the experiment is shown in the table 3.1 while the chemical composition is shown in table 3.2.

Table 3.1: Dietary composition of the diet used in the experiment

Diet name	Control (%)
Norse Nat LT	38.53
SPC 16/13	12.00
Fish oil (herring) O1/13	10.00
Rapeseed oil O1/11	12.00
Horse beans 53/13	5.45
Wheat 3/14	8.00
Sun seed meal 88/12	3.33
Wheat gluten 36/13	5.00
Betafin T 4/13	1.00
Soy lecithin T21/13	1.00
Vitamin mix T3/13	2.00
Mineral mix T1/14	0.52
Monosodium phosphate T49/10 (24% P)	1.00
Carop. Pink (10%) T 35/10	0.01
Yttrium oxide T20/13	0.150

Table 3.2: Chemical composition of the diet used in the experiment

CHEMICAL ANALYSIS	PERCENTAGE (%)
Ash	7.49049
Dry Matter (DM)	95.197
Nitrogen (7.05*6.25)	44.0625
Energy	23.38
Crude fat	28.1
Y_2O_3	0.09798

3.2.2 EXPERIMENTAL SET UP

60 salmon (*Salmo salar*) from a total of 434 salmon breed full-sib families from Nofima, Sunndalsøra, of which prediction equations were developed for were reared in the same tank during the experimental period. They were reared for 56 days under the same management conditions of water temperature, oxygen content, pH etc.

The fish were fed *ad libitum* extruded pelleted feed made from the feed plant at Nofima, Bergen.

Dry Matter (DM), Crude Protein (CP), Crude Fiber (CF) and ash were determined in diet samples and CP, CF and ash in dried faeces were treated in parallel thesis.

3.2.3 FAECAL SAMPLING:

At sampling, fish were netted at random using a sweep net and anaesthetized in Finquel (Finquel vet. 1000mg/g, Scan Aqua AS, Årnes, Norway). Faeces samples were obtained by the stripping method (Austreng 1978). Fish were stripped three times during the experimental period (02.10.2014, 12.10.2014 and 21.10.2014). Weight of the stripped faeces was recorded and placed in a desiccant and were freeze dried at -40^{o}C for 1-7days before being sent to the laboratory (Nofima, Ås, Norway) where it was grounded into powder for the spectroscopic analysis. Materials involved in the sampling and preparation are shown in the picture below (Figure 3.2).

a.  b.  c. 

Figure 3.2: Materials used in the faecal collection and sampling a: Finquel anaesthetizer, b: Marel Scale and c: Desiccant

3.2.4 NIR CALIBRATION

The NIR instrument was calibrated using reference freeze-dried fish faeces sample containing known chemical analyzed protein crude protein (Nx6.25, combustion according to the Dumas principle, ISO 16634-1) and fat (Folch et al., 1957, after acid hydrolysis) values obtained from multiple feeding experiments performed at Nofima, Sunndalsøra.

For the protein equation, a total of 180 samples were from 10 different feeding experiments, while for fat a total of 115 samples were obtained from 9 different feeding experiments.

The NIR spectra of faeces samples were obtained using NIR Systems Model XDS Rapid Content Analyzer module (Foss NIR Systems, Silver Spring, MD, USA), Nofima.

Validation of predicted protein was done using 23 additional samples from a different experiment also performed at Nofima, Sunndalsøra. Full cross-validation was used for fat. Because we had the least samples (115) for developing the fat equation and to get an indication of the quality of the equation, five samples were randomly chosen from the experimental samples and chemically analyzed for fat content to check the prediction ability of the developed fat equation.

3.2.5 ANALYTICAL SOFTWARE

Spectral data were collated into Vision spectral analysis software for windows (Copyright 2006 FOSS NIRsystems, Inc. www.foss.dk) and imported into Unscramble X (Version 10.3) statistical analysis software (CAMO PROCESS AS, OSLO, NORWAY) for data processing and equation development.

Reference data were treated in Microsoft excel software (2007) and imported into Unscramble X where it was analyzed against the spectral data.

3.3 METHODS

3.3.1 NIR SPECTRA ACQUISITION

All NIR spectra were recorded with NIR Systems Model XDS Rapid Content Analyzer module (Foss NIR Systems, Silver Spring, MD, USA) equipped with a quartz halogen lamp and a PbS detector where spectral data was taken. The DCM (data collection method) had a spot-size of 9.5mm.

In this process, a quarter teaspoon of faeces was loaded unto the optic vial and the magnetic interference neutralized using METTLER TOLEDO (HAUG GmbH & CO. KG). It was then placed in the NIR instrument and spectral measurement taken. Each sample measurement was repeated three times and the corresponding spectra averaged. All measurements were done at room temperature (22°C). Samples were analyzed in the period from January 2015 until March 2015. Materials involved in this process are shown in the picture below (Figure 3.3).

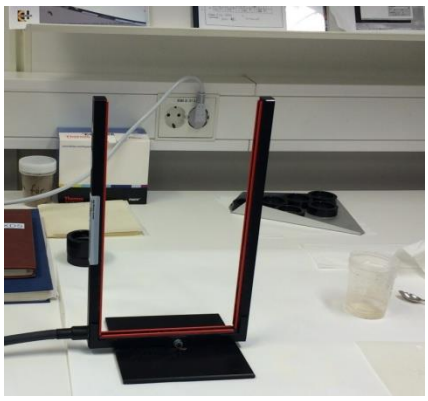
a.  b. 

Figure 3.3: Materials used in the spectra acquisition a: Toledo static electricity neutralizer and b: NIR system.

3.3.2 DATA ANALYSIS

Pre-processing of spectra was done to transform the data into forms easy to analyze (Esbensen 2009) and for the relationship between the measurements and the given reference

value. All NIR spectra were pre-processed the same way prior to regression analysis. First, all replicate spectra (3 replicates) were averaged and imported into Unscramble.

The spectra were then subjected to scatter correction based on the method of Extended Multiplicative Signal Correction (EMSC). Pre-processed NIR spectra covering the spectral region 1150 – 2450 nm were used to develop multivariate regression models based on partial least-squares regression (PLSR) to obtain the calibration equation.

Validation was done to test the strength of the model created with PLSR. The optimum number of PLSR components was determined using full cross-validation (leave-one-out-validation). The reference value, y_i, and the predicted value, $\hat{y}_i$, of every sample were used to calculate the prediction error of the cross-validated calibration model, expressed as the root mean square error of cross-validation (RMSECV). The RMSECV value is defined in the following way:

$$\text{RMSECV} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}$$

Where *i* denotes the samples from 1 to N. Both the RMSECV and the multivariate correlation coefficient (R^2) between reference and predicted values, the ratio of RMSECV and the standard deviation (SD) for the chemical analyzed ('true') values for the same samples (RESD) were used to evaluate the performance of the regression models. Bias and RMSEP were used to check the protein validation. RESD, Bias and RMSEP are defined as;

$$\text{RESD} = (\text{RMSECV})/\text{SD}$$

$$\text{BIAS} = \frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)$$

$$\text{RMSEP} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}$$

Here, *i* denotes the samples from 1 to N, y_i the reference value, and $\hat{y}_i$ the predicted value.

CHAPTER FOUR

4.0 RESULTS

4.1 CALIBRATION DATA SETS B AND C

180 samples of chemical analyzed crude protein and 115 samples of chemical analyzed fat were used to calibrate the equation. The minimum, maximum, mean and standard deviation were calculated. The results are shown in table 4.1.

Table 4.1: Calibration data statistics showing the parameters under consideration, number of samples used, minimum, maximum, mean and standard deviation (STD).

	Calibration data statistics				
Parameter	No. of samples	Min	Max	Mean	STD
Protein	180	8.8	38.4	21.9	8.4
Fat	115	5.2	12.6	7.3	2.4

As shown in table 4.1, there is a high gap between the minimum, maximum, mean and standard deviation of both protein and fat data. And this is to be expected as the samples were collected from different experiments (10 experiments for protein and 9 experiments for fat) significantly spanning the variation range of the data sets. This is a positive sign for developing the model (equation), as a much greater variation will assure a more robust model.

The figures below (4.1.1, 4.1.2 and 4.1.3) shows a line plot of the averaged NIR spectra of the calibration data sets (Data set B and data set C) and the pre-processed spectra of the calibration data set used in the PLSR analysis after estimated multiple scatter correction (EMSC) respectively. For each faecal sample, three replicates of measurements were made within the wavelength 1105-2450nm respectively. The spectra absorbance peaks are associated with the main constituents of the faeces.

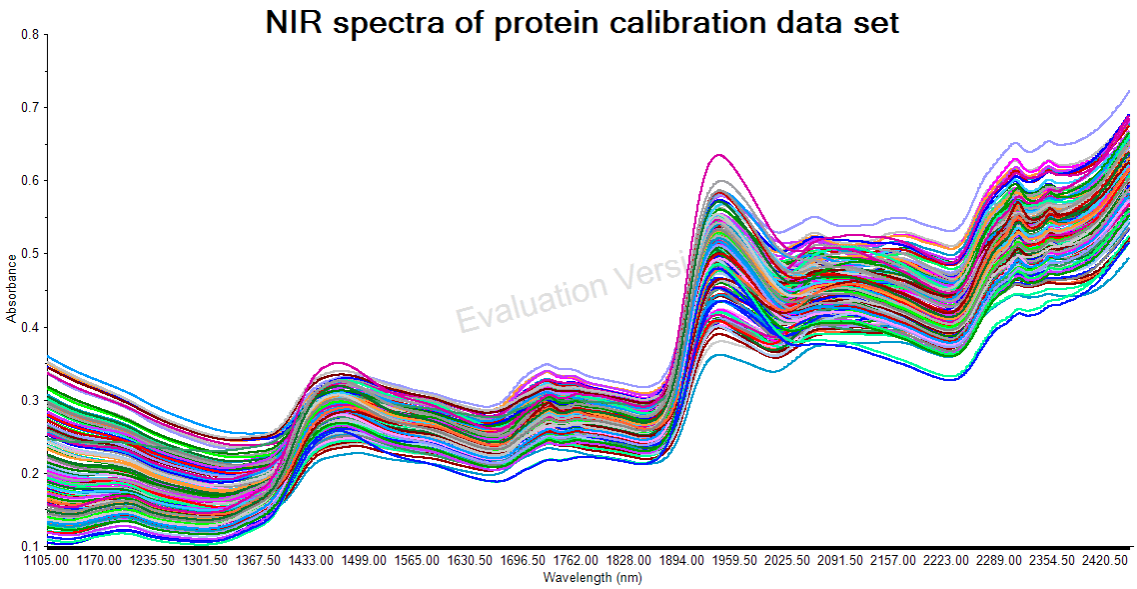


Figure 4.1.1: NIR spectra of protein calibration data (Data set B).

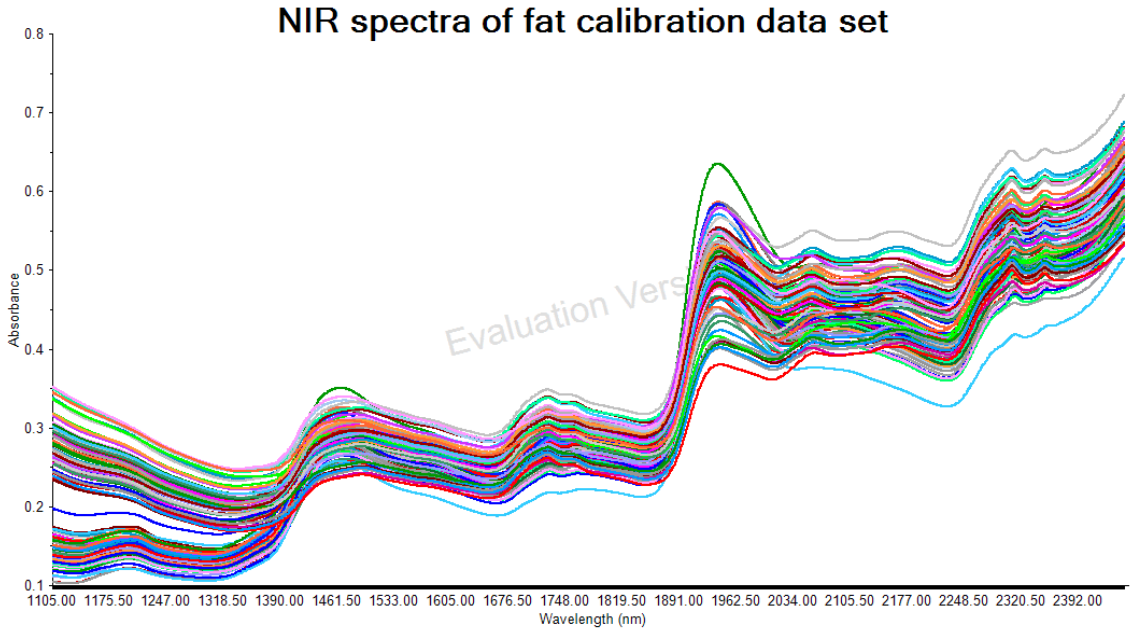


Figure 4.1.2: NIR spectra of fat calibration data (Data set C).

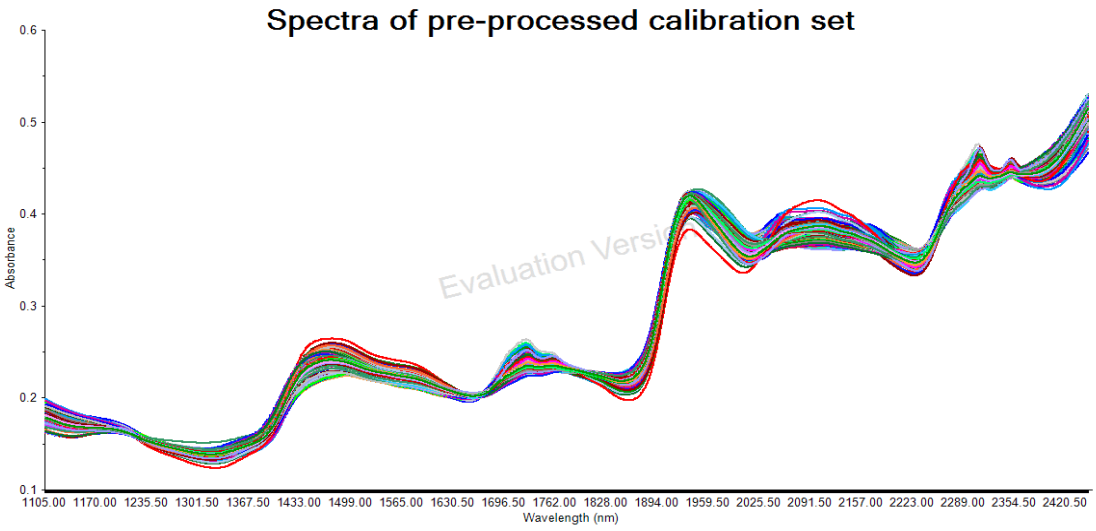


Figure 4.1.3: Spectra of pre-processed calibration data set

4.2: PREDICTION DATA SET A

Three replicates of measurements were made within the wavelength 1105-2450nm respectively. The spectra absorbance peaks are associated with the main constituents of the faeces. The figure below shows the NIR spectra of data set A after scatter correction.

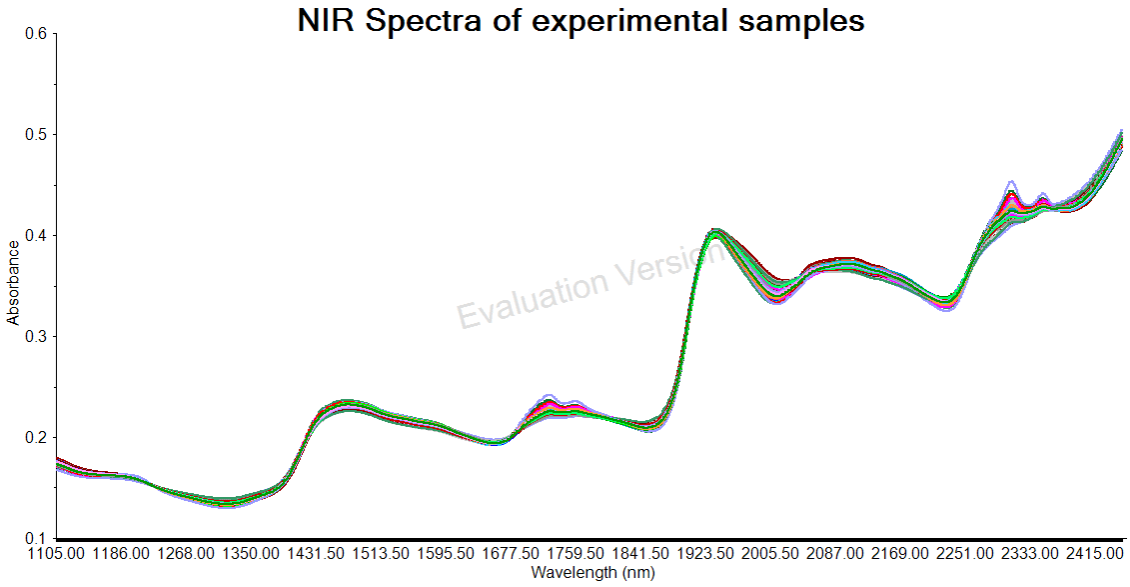


Figure 4.2: NIR spectra of experimental samples (Data set A).

4.3: PREDICTED VS TRUE VALUES FOR THE CALIBRATION DATA SETS B AND C

PLSR OF PROTEIN

PLSR and Regression coefficient analysis of protein is shown in the figure 4.3.1 and figure 4.3.1.1 below. Pre-processed spectra were connected to the reference using PLSR.

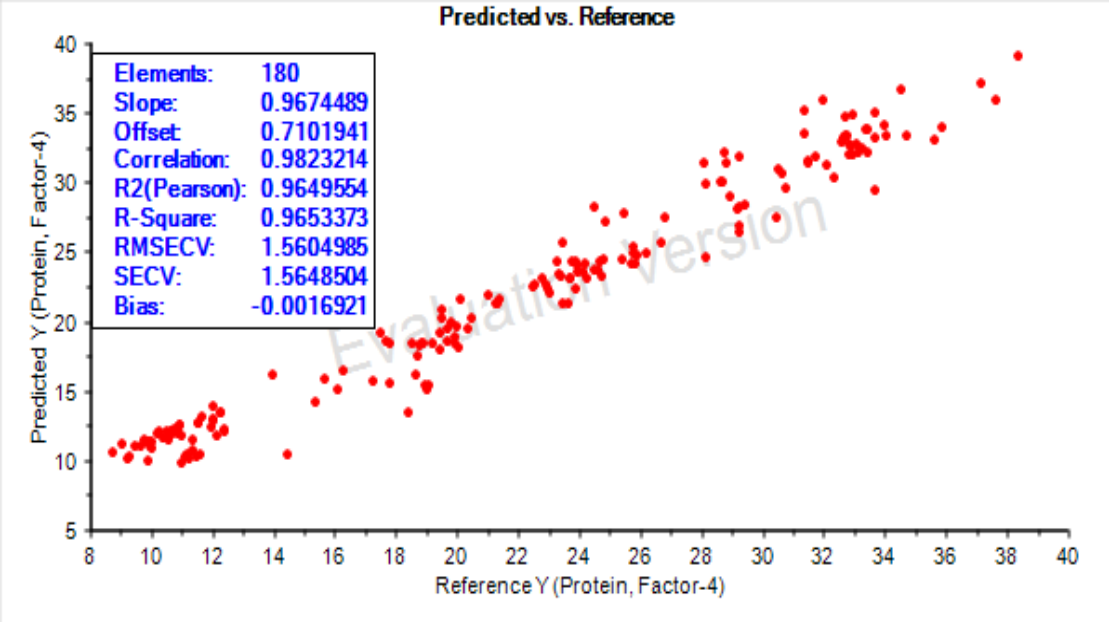


Figure 4.3.1: Predicted vs reference plot for protein

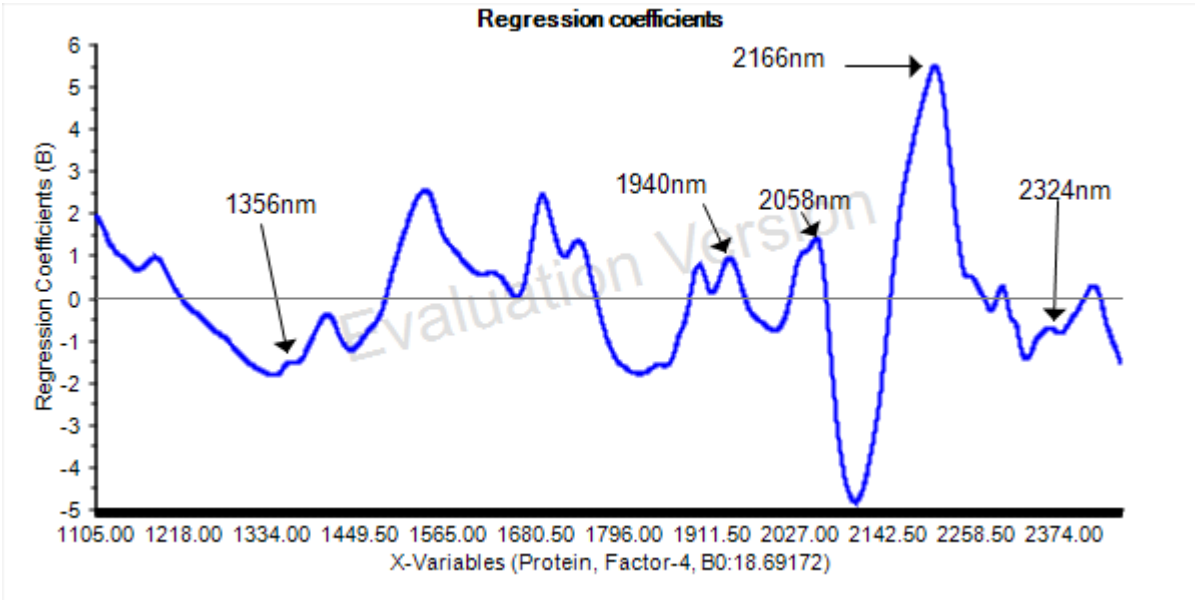


Figure 4.3.1.1: Regression coefficient for the PLSR model for protein

PLSR OF FAT

These figures below (figure 4.3.2 and figure 4.3.2.1) also show the PLSR analysis and regression coefficient of fat. Pre-processed spectra was also connected to the reference using PLS regression.

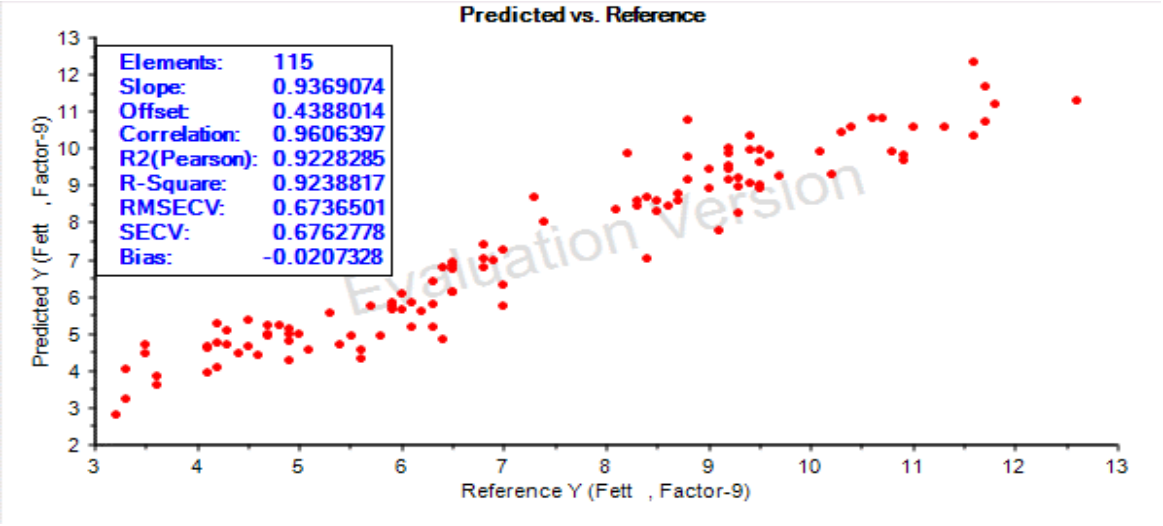


Figure 4.3.2: Predicted vs reference plot for fat

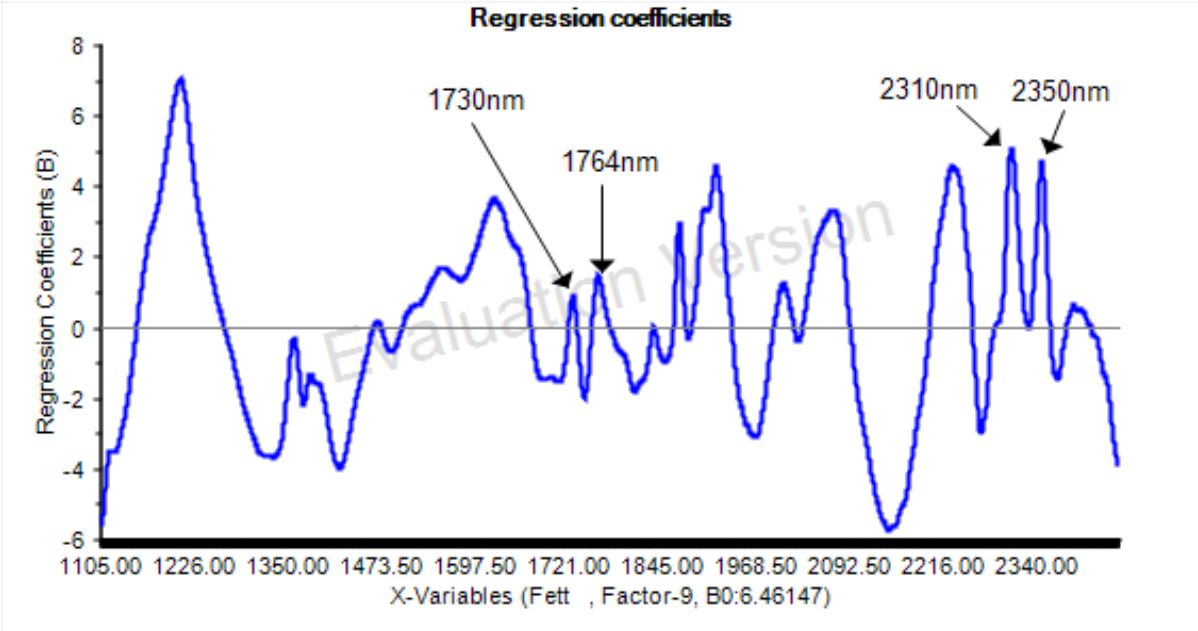


Figure 4.3.2.1: Regression coefficient for the PLSR model for fat

Table 4.3: Calibration results statistics showing the coefficient of determination (R^2), root mean square error of cross validation (RMSECV), factors (number of PLS factors), ratio of RMSECV or RMSEP and Standard deviation (RESD) and Bias.

	Calibration results				
Parameter	R^2	RMSECV	factors	RESD	Bias
Protein	0.97	1.6	4	0.19	-0.002
Fat	0.92	0.7	9	0.29	-0.02

Regression coefficient relating to functional groups outside the absorption bands for protein and were observed and this could be attributed to variations in particle size, moisture (from sample handling) and different analytical groups (Williams 2001). Bands associated with protein (1940nm, 2058nm and 2166nm) and fat (1730nm, 1764nm, 2310nm and 2350nm) as reported by Decaruyenaere et al. (2001) and Núñez-Sánchez et al. (2012) were also identified. Also, the wavelength of protein from faecal spectra (2321nm and 1356nm) was observed (Norris et al. 1996; Showers 1997; Givens and Deauville 1999). Wavelength of fat as observed by Mathias et al. 1987 (2100nm and 2139nm) for percent fat in fish carcasses was also identified. From the figures 4.3.1.1 and 4.3.2.1, the wavelengths between 2134.5-2236.5nm and 1105-1220nm seems to be the most important region for protein and fat absorbance's respectively.

4.4 VALIDATION DATA SETS D AND E

PROTEIN

In testing for the strength of the model created with PLS, 23 samples of known protein content (reference) were taken to validate the reference test (equation) for proteins. Three replicates were made for each sample of protein and the mean, standard deviation, RMSEP and were calculated and the results shown in table 4.4.1. The result of the predicted and the reference protein is shown in figure 4.4.1.

Table 4.4.1: Validation test of protein (data set D) showing the minimum, maximum, mean, standard deviation (STD), RMSEP and Bias.

Sample	No. of samples	Min.	Max.	Mean	STD	RMSEP	Bias
Predicted	23	14.77	25.05	19.85	2.69	1.82	-0.72
Reference	23	16.06	22.69	19.12	2.03		

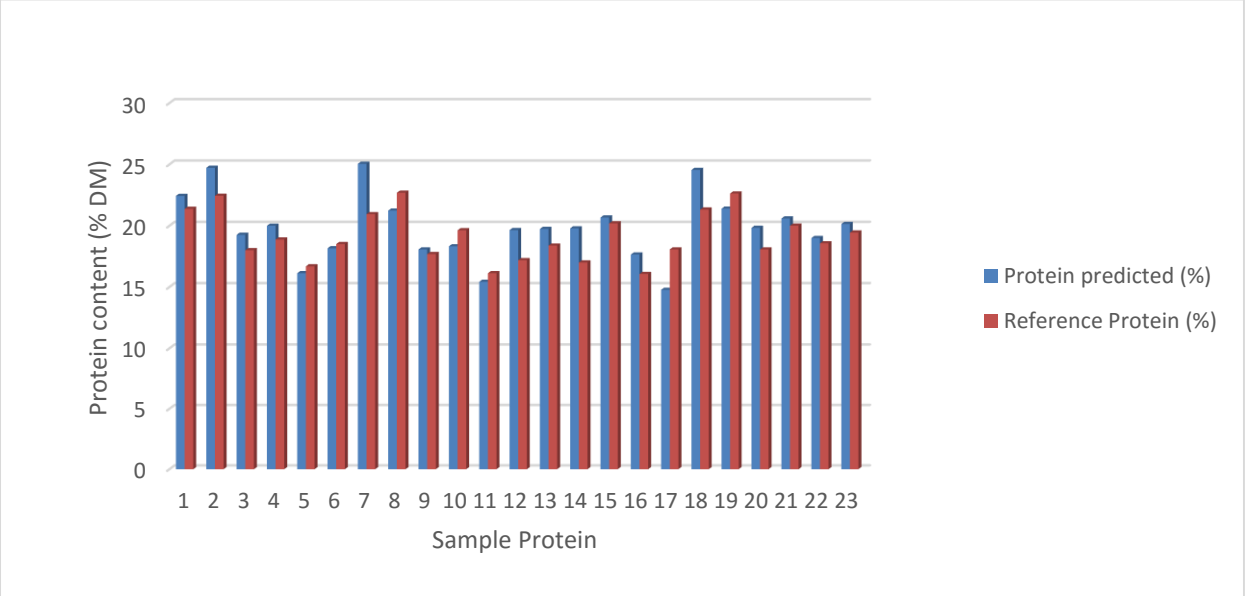


Figure 4.4.1: Chart showing the validation protein test

.

FAT

Full cross-validation was used for fat. Because there wasn't much samples for the fat calibration compared to the protein calibration, five samples were selected at random and chemically analyzed for fat to compare the predicted results with. The results of the predicted experimental samples (5 selected) and its corresponding chemically analyzed content are shown in table 4.4.2 and figure 4.4.2.

Table 4.4.2: Predicted and chemically analyzed selected samples for fat validation. Showing number of samples used, minimum, maximum, mean and standard deviation (STD).

	No. of samples	Minimum	Maximum	Mean	S. D
Chemical	5	6.1	8.7	7.2	0.95
Predicted	5	4.7	12.4	8.3	2.78

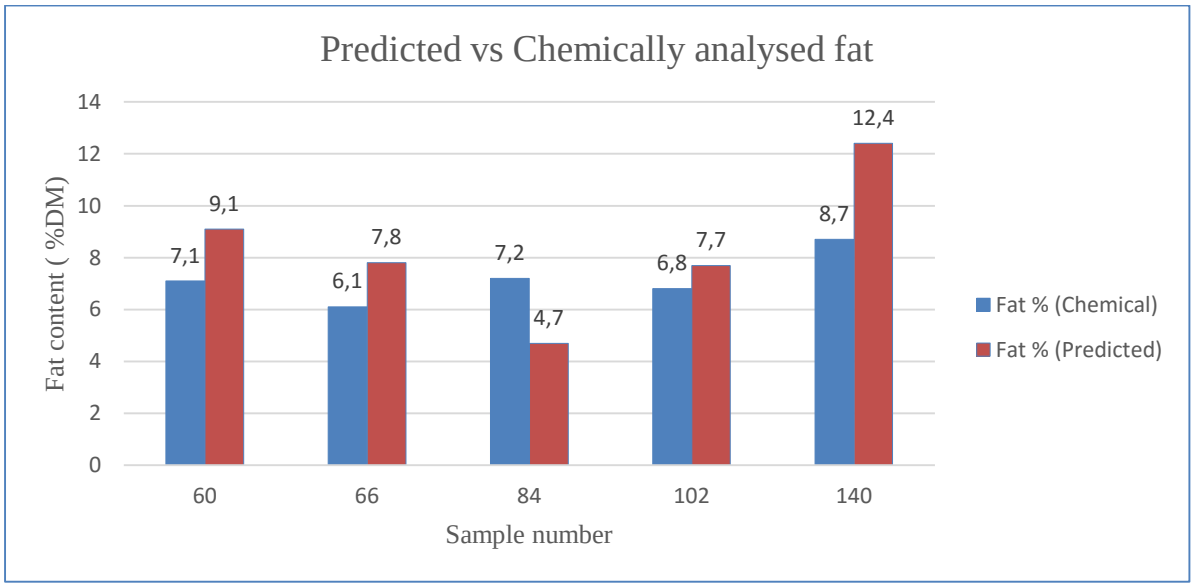


Figure 4.4.2: Chart showing chemically analyzed and predicted fat content of selected samples used in comparing the fat validation.

4.5 PREDICTION EQUATION (DATA SET B AND C) PREDICTION OF PREDICTION SET (DATA SET A).

The created PLSR model (equation) was used to predict protein and fat content of the faeces of the individual Atlantic salmon from the experiment. The results are shown in table 4.5 and in figure 4.5 and figure 4.5.1 for easy representation in a chart graph.

Table 4.5: Prediction results for protein and fat showing the minimum, maximum, mean and standard deviation (STD) of individual Atlantic salmon.

Parameter	Minimum	Maximum	Average	STD
Protein	13.71	17.4	15.64	0.80
Fat	3.31	9.74	5.26	0.91

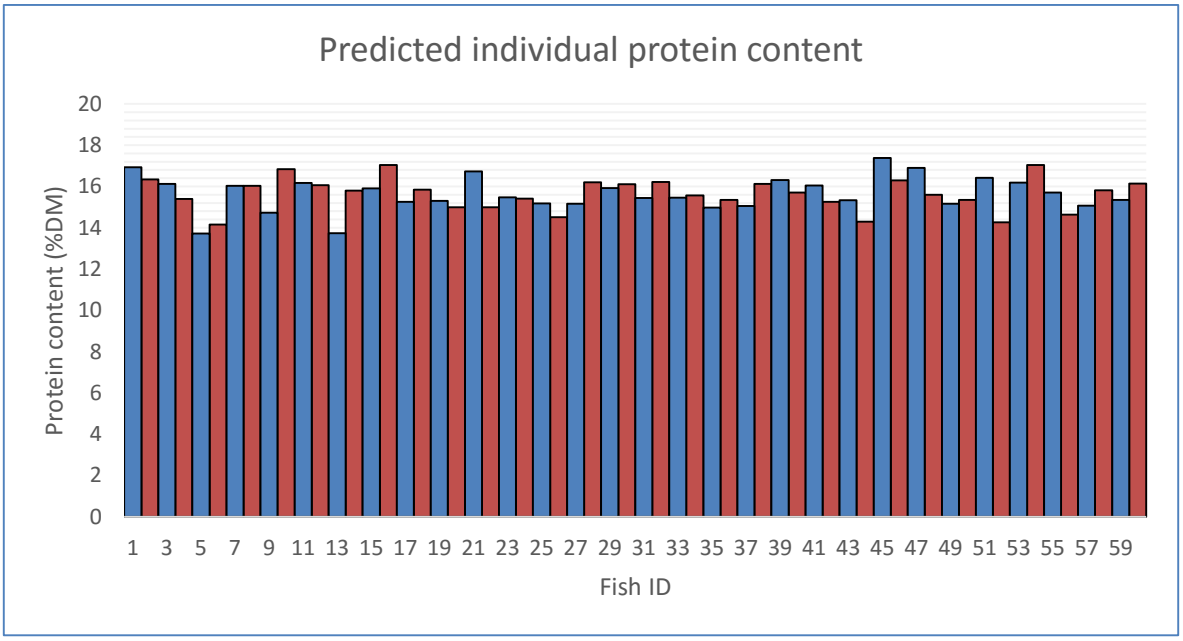


Figure 4.5: Chart showing the average predicted protein content of individual Atlantic salmon. Even numbered fish ID numbers are coloured red for easy identification and consistency of numbers.

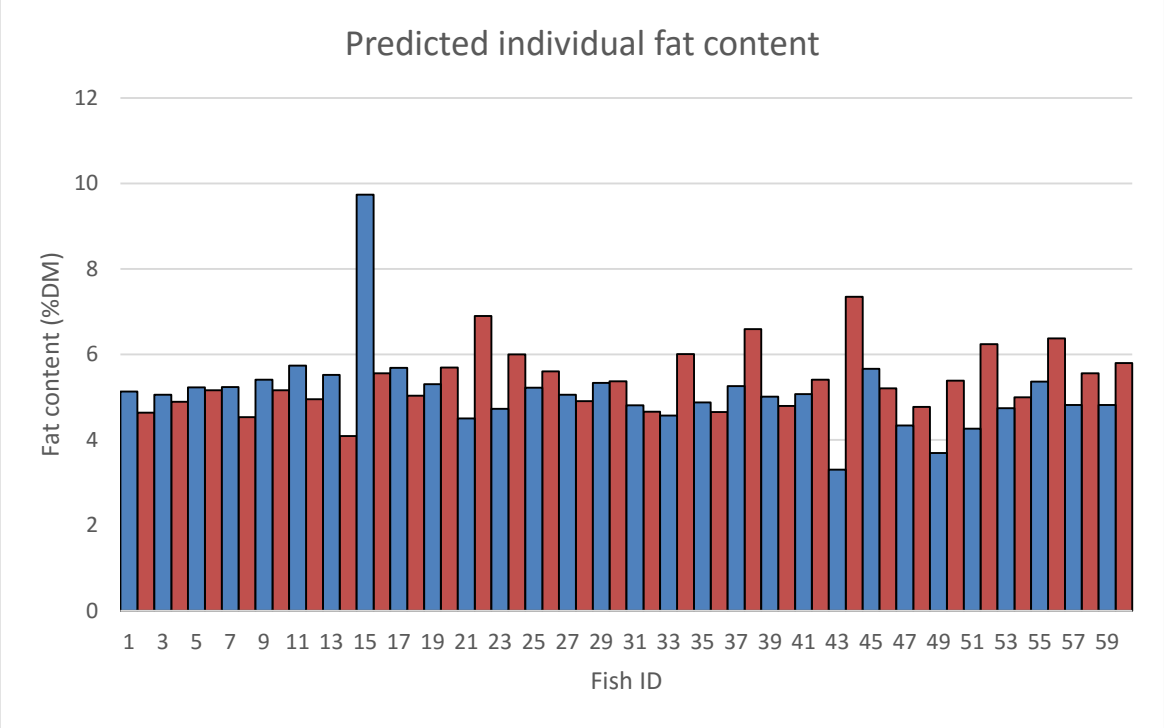


Figure 4.5.1: Chart showing the average predicted fat content of individual Atlantic salmon. Even numbered fish ID numbers are coloured red for easy identification and consistency of numbers.

CHAPTER FIVE

DISCUSSION

5.1 NIR SPECTRA

All the spectra (figures 4.1.1-4.2) had bands corresponding to fatty acids or protein content which are located around 1209nm and is related to the C-H second overtone, around 1500nm due to N-H stretch, first overtone, at 1727nm and 1764nm associated with the C-H first overtone, at 2308nm and 2348nm due to the C-H combinations (Ritthiruangdej et al. 2011; Osborne, Fearn and Thindle 1993; Williams 2001).

The highest absorption is at wavelengths 1930-2415nm. Consequently, the spectral features observed in this study are very similar to those reported for Ruminants by Decaruyenaere et al. 1993, for non-ruminants by Ritthiruangdej et al. 2011 and for fish carcasses by Mathias et al. 1987.

The highest absorption is at wavelengths 1930-2415nm for data set A. Consequently, the spectral features observed in this study are very similar to those reported for Ruminants by Decaruyenaere et al. 1993, for non-ruminants by Ritthiruangdej et al. 2011 and for fish carcasses by Mathias et al. 1987.

5.2 PARTIAL LEAST SQUARE REGRESSION MODEL

For the results of the model created with PLS as shown in table 4.3. 4 PLS factors was used for protein and 9 PLS factors used for fat to avoid over-fitting of the model created.

For NIRs equations to be considered acceptable, it should have an R^2>0.80. The protein and fat models had high correlation rates. But, the prediction equation is better for protein (R^2=0.97) than for fat (R^2=0.92) and low bias (-0.002, protein and -0.02, fat) indicating a high precision though protein had a high RMSECV. The high RMSECV of protein can be attributed to the large number of samples used, increasing the errors associated with the cross-validation. However, for a very illustrative statistics protein had low (0.19) ratio of RMSECV and standard deviation than fat (0.29). The lower the value of the ratio of RMSECV and standard deviation, the better the prediction equation, as also illustrated by their respective R^2's.

5.3 VALIDATION SET

PROTEIN

Just as with the calibration data (Table 4.3), the variation range for the predicted protein is good comparing favourably with the already known samples (reference) with minimal bias between the predicted and reference. And this confirms the good prediction performance for the protein model. However, it had a slightly higher RMSEP (1.82) which could have arisen from sample preparation and handling and other functional groups interlocking at the same wavelengths.

FAT

The chemically analyzed fat didn't compare favourably with its corresponding prediction with the model as illustrated by the large difference in standard deviation further iterating a weaker fat model. However, it should be noted that the uncertainty of the chemical analysis is very high as a result of the purity of reagents used, assumed stoichiometry, measurement conditions, operator effect etc. all affecting the chemical results.

5.3 PREDICTION RESULTS OF PREDICTION DATA SET

Because, to our knowledge, there was no previous work on faecal NIRs to directly predict the digestibility of individual salmon to compare the results with, the prediction results were compared to other studies using near infrared spectroscopy on faeces. The average prediction for protein was above that obtained by Sánchez et al. 2013 (11.82) and Li et al 2007 (11.97, 12.04) for rabbit and sheep faecal samples respectively. Also, the average prediction for fat compared favourably with that obtained by Hernandez-Martinez et al. 2013 (5.29). However detailed explanation of the observed variations as well as the digestibility of the predicted contents is treated in parallel thesis.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS FOR FUTURE WORKS

The objective of this study was to develop prediction equation for protein and fat in individual Atlantic salmon using NIR spectroscopy.

The statistics of the equations are satisfactory, however, the equation for protein showed high precision of prediction and therefore useful for predicting protein of individual Atlantic salmon. The large variation in the calibration data (table 4.1) is good for developing prediction equations as it spans the range of several wavelengths. Consequently, the source of the data from several experiments makes the developed equation robust with a very high predictive ability as it spans several wavelengths with a high probability of finding the wavelength of the constituents under consideration.

The good prediction results obtained for the protein (Table 4.5) further validates the potential of the developed equation for prediction in individual Atlantic salmon.

With this study serving as a foundation for future faecal predictions in individual Atlantic salmon, I recommend further works be done using the same or similar method to develop new equation for fat to better compare the developed equation with. Also, to use fish of the same families to access the prediction results also within families.

CHAPTER SEVEN

7.0 REFERENCES

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CHAPTER EIGHT

8.0 APPENDICES

Table 8.1: Table showing the predicted and reference (validation) protein

	Protein predicted (%)	Reference Protein (%)
307.b1	22.42417	21.375
307b1	24.71613	22.4375
308.b1	19.25731	18
308b1	19.99459	18.875
309.b1	16.12472	16.6875
309b1	18.15656	18.5
311.b1	25.04253	20.9375
311b1	21.2302	22.6875
312.b1	18.06159	17.6875
312b1	18.31725	19.625
313.b1	15.42277	16.125
314.b1	19.63698	17.1875
314b1	19.7338	18.375
315.b1	19.77802	17
315b1	20.66568	20.1875
316.b1	17.65016	16.0625
316b1	14.76538	18.0625
317.b1	24.5435	21.3125
317b1	21.38691	22.625

318.b1	19.82629	18.0625
318b1	20.58947	20
319.b1	18.993	18.5625
319b1	20.13437	19.4375

Table 8.2: Table showing the prediction results of averaged NIR samples for protein and fat content of faeces.

Sample ID	Protein *Predicted*	Fat *Predicted*		Sample ID	Protein *Predicted*	Fat *Predicted*
1	16.29447	4.6403		27	15.34291	6.3358
2	16.79815	4.2632		28	14.91126	5.7517
3	15.01933	5.865		29	15.8203	6.057
4	15.61309	3.8035		30	17.00941	5.2467
5	17.62198	3.9685		31	15.04215	5.0284
6	14.96263	5.2622		32	16.32381	5.0928
7	16.28295	5.5169		33	16.82598	5.5372
8	15.23842	5.5944		34	16.78425	5.2832
9	17.22538	5.9827		35	14.96218	5.7322
10	15.62425	5.2004		36	15.61623	4.2652
11	16.47167	6.3702		37	17.02757	5.3461
12	15.02166	5.5469		38	15.80364	5.192
13	15.59468	5.4545		39	16.0761	5.5432
14	14.60761	6.2864		40	15.66051	5.4799
15	16.41623	4.595		41	13.84982	5.6666

16	16.34821	6.9204		42	17.23637	4.7666
17	15.91954	5.7475		43	15.64416	5.3407
18	15.31604	5.0711		44	17.12207	5.1561
19	15.34681	4.7884		45	14.31061	6.5209
20	16.98812	5.2865		46	14.21144	5.2483
21	17.56816	5.6637		47	14.85427	5.7655
22	15.61249	5.2258		48	15.45566	5.1641
23	15.16419	3.6913		49	10.73997	5.1141
24	16.58562	4.1611		50	17.03703	4.9471
25	15.05275	7.7201		51	16.19741	5.5814
26	15.62337	6.5761		52	16.40339	5.7868
Sample ID	Protein *Predicted*	Fat *Predicted*		Sample ID	Protein *Predicted*	Fat *Predicted*
53	15.16493	5.0613		82	15.58202	4.0314
54	15.76699	5.7687		83	14.91834	4.5987
55	15.39905	4.8231		84	16.28123	4.7468
56	15.80315	4.9596		85	14.48049	5.0022
57	15.10229	5.6777		86	15.32943	4.3271
58	15.60404	5.1677		87	13.01869	6.634
59	14.86071	6.077		88	14.25455	5.0382
60	16.99891	9.1179		89	15.56142	5.1935
61	15.02083	4.9234		90	15.51433	4.7937
62	16.39461	3.6655		91	16.0923	5.1407
63	15.21621	6.0203		92	15.6549	4.2907

64	14.6831	5.3835		93	15.23449	4.4758
65	15.78904	5.3421		94	15.83729	5.6982
66	16.98036	7.7744		95	13.57823	4.7911
67	15.63179	4.5544		96	15.46441	4.5133
68	15.88293	5.003		97	14.72019	5.5443
69	13.72932	5.4668		98	17.85735	5.2758
70	17.43812	3.2085		99	15.83101	4.7695
71	13.95299	4.76		100	14.71721	5.304
72	17.51303	4.4066		101	14.35806	4.2738
73	15.63796	4.3643		102	14.61303	7.7122
74	14.06629	4.3603		103	15.09476	4.2801
75	14.53893	5.6958		104	13.56696	6.18
76	15.53518	5.3934		405	17.29992	5.2751
77	14.67498	4.6157		106	14.54087	4.4626
78	14.83868	4.1379		107	14.30013	4.5781
79	14.97043	3.5388		108	15.26267	5.171
80	15.33167	5.6765		109	15.23573	4.1521
81	15.30532	4.2156		110	15.73446	4.67
Sample ID	Protein *Predicted*	Fat *Predicted*		Sample ID	Protein *Predicted*	Fat *Predicted*
111	15.91753	5.7653		141	15.9063	5.6029
112	15.31582	6.3579		142	18.76843	3.9461
113	13.62536	4.2855		143	16.61989	5.3106

114	15.08791	5.7514		144	15.78176	4.8826
115	14.30715	6.446		145	16.27584	5.2611
116	15.02917	5.2035		146	15.77682	6.0759
117	16.49677	5.3986		147	17.57863	4.3864
118	16.52819	4.5394		148	17.5519	5.3229
119	17.18195	5.6712		149	16.23173	6.0553
120	16.54337	4.5599		150	15.21444	4.7916
121	17.64654	4.584		151	16.234	4.2367
122	14.26487	8.8469		152	15.34289	4.9842
123	18.16652	4.1228		153	15.05055	5.1836
124	16.39437	4.1849		155	15.67785	5.9032
125	16.49784	6.1094		156	17.23634	3.5369
127	14.31045	7.3238		157	16.17658	5.717
128	13.20737	8.8513		158	15.71122	5.197
128	17.52579	4.366		159	14.70447	3.7969
130	16.24381	6.0373		160	14.18527	5.9992
131	16.63609	4.8484		161	16.30571	5.421
132	15.71708	5.3705		162	16.13836	5.9084
133	15.15955	2.8718		163	15.95242	5.1956
134	15.25464	5.5564		164	11.05383	4.4965
135	14.19917	5.9884		165	14.92229	6.3889
136	16.34302	4.2766		166	16.07507	4.6131
137	17.14201	5.4613		167	15.58014	5.6218

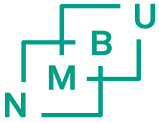
138	15.23386	1.4209		168	15.04357	6.9151
139	17.08615	5.2295		169	15.24209	5.3074
140	16.0901	12.3915		170	18.09166	4.4151
Sample ID	Protein *Predicted*	Fat *Predicted*				
171	17.09249	5.7487				
172	16.49868	5.8884				
173	15.03315	5.841				

Table 8.5: Table showing the predicted protein and fat content of individual Atlantic salmon.

PROTEIN (% Dry Matter)			**Average DM Protein (%)**	**FAT**			**Average Fat**
15.91954	17.29992	17.57863	16.933	5.748	5.275	4.386	5.136
17.23637	15.46441	E	16.350	4.767	4.513	E	4.640
15.60404	15.63179	17.14201	16.126	5.168	4.554	5.461	5.061
15.45566	13.62536	17.08615	15.389	5.164	4.285	5.230	4.893
13.84982	13.57823	Died	13.714	5.667	4.791	Died	5.229
16.40339	15.02917	11.05383	14.162	5.787	5.203	4.497	5.162
15.76699	15.91753	16.39437	16.026	5.769	5.765	4.185	5.240
15.31604	15.23573	17.52579	16.026	5.071	4.152	4.366	4.530
14.96263	14.30013	14.92229	14.728	5.262	4.578	6.389	5.410
16.47167	15.88293	18.16652	16.840	6.370	5.003	4.123	5.165
15.34291	16.0923	17.09249	16.176	6.336	5.141	5.749	5.742
17.12207	15.32943	15.71708	16.056	5.156	4.327	5.371	4.951
10.73997	14.6831	15.77682	13.733	5.114	5.383	6.076	5.524

15.61623	14.54087	17.23634	15.798	4.265	4.463	3.537	4.088
16.99891	14.61303	16.0901	15.901	9.118	7.712	12.391	9.741
17.02757	17.85735	16.23173	17.039	5.346	5.276	6.055	5.559
14.85427	15.33167	15.58014	15.255	5.765	5.677	5.622	5.688
14.96218	16.39461	16.17658	15.844	5.732	3.665	5.717	5.038
15.02166	15.53518	15.34289	15.300	5.547	5.393	4.984	5.308
16.0761	14.72019	14.19917	14.998	5.543	5.544	5.988	5.692
15.80364	15.63796	18.76843	16.737	5.192	4.364	3.946	4.501
16.34821	14.30715	14.31045	14.989	6.920	6.446	7.324	6.897
15.39905	15.23449	15.78176	15.472	4.823	4.476	4.883	4.727
14.86071	15.21621	16.13836	15.405	6.077	6.020	5.908	6.002
16.79815	13.56696	Died	15.183	4.263	6.180	Died	5.222
15.64416	13.72932	14.18527	14.520	5.341	5.467	5.999	5.602
15.16493	Died	Died	15.165	5.061	Died	Died	5.061
17.00941	15.6549	15.95242	16.206	5.247	4.291	5.196	4.911
15.34681	E	16.49868	15.923	4.788	E	5.888	5.338
16.28295	15.78904	16.27584	16.116	5.517	5.342	5.261	5.373
15.62425	14.48049	16.23399	15.446	5.200	5.002	4.237	4.813
16.29447	15.83101	16.54337	16.223	4.640	4.769	4.560	4.657
16.58562	15.08791	14.70447	15.459	4.161	5.751	3.797	4.570
14.60761	15.83729	16.24381	15.563	6.286	5.698	6.037	6.007
15.66051	14.06629	15.21444	14.980	5.480	4.360	4.792	4.877
15.61309	13.95299	16.49677	15.354	3.803	4.760	5.399	4.654
15.10229	15.02083	15.05055	15.058	5.678	4.923	5.184	5.262
15.05275	16.98036	16.34302	16.125	7.720	7.774	4.277	6.590
15.59468	15.26267	18.09166	16.316	5.455	5.171	4.415	5.014
17.03703	14.83868	15.24209	15.706	4.947	4.138	5.307	4.797
15.01933	15.58202	17.5519	16.051	5.865	4.031	5.323	5.073
15.8203	14.67498	15.25464	15.250	6.057	4.616	5.556	5.410
15.80315	14.97043	15.23386	15.336	4.960	3.539	1.421	3.306
15.62337	13.01869	14.26487	14.302	6.576	6.634	8.847	7.352
17.56816	E	17.18195	17.375	5.664	E	5.671	5.667

16.82598	15.73446	16.30571	16.289	5.537	4.670	5.421	5.209
15.61249	17.43812	17.64654	16.899	5.226	3.208	4.584	4.339
16.32381	15.31582	15.15955	15.600	5.093	6.358	2.872	4.774
15.16419	E	E	15.164	3.691	E	E	3.691
16.78425	14.25455	15.03315	15.357	5.283	5.038	5.841	5.387
17.62198	15.09476	16.52819	16.415	3.968	4.280	4.539	4.263
15.23842	14.35806	13.20737	14.268	5.594	4.274	8.851	6.240
16.41623	15.51433	16.63609	16.189	4.595	4.794	4.848	4.746
16.98812	17.51303	16.61989	17.040	5.287	4.407	5.311	5.001
14.91126	16.28123	15.9063	15.700	5.752	4.747	5.603	5.367
14.31061	14.53893	15.04357	14.631	6.521	5.696	6.915	6.377
14.21144	14.91834	16.07507	15.068	5.248	4.599	4.613	4.820
16.19741	15.56142	15.67785	15.812	5.581	5.193	5.903	5.559
15.04215	15.30532	15.71122	15.353	5.028	4.216	5.197	4.814
17.22538	14.71721	16.49784	16.147	5.983	5.304	6.109	5.799



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