Alternative methods to predict the nutritive value of broiler chicken diets

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Animal Science

at Massey University, Palmerston North, Manawatū,

New Zealand

Sivajanani Thiruchchenthuran

2024

ABSTRACT

Feed represents the major cost of poultry production accounting for up to 70% of the total. Accurate estimation of the chemical composition and nutritive value of the feed ingredients and diets is essential to reduce costs and optimise diets. Undersupply of nutrients will have a strong negative impact on the performance of birds and oversupply will have a negative impact on the environment as well as cost of production. Traditional methods of feed evaluation are time-consuming and costly. Both *in vivo* and *in vitro* techniques have several limitations. Therefore, there is a need for rapid and accurate analysis of feed samples for their chemical composition and nutritive value in real-time for optimal formulation of diets.

Prediction equations and Attenuated Total Reflectance Fourier Transform Mid-Infrared Spectroscopy (ATR-FT MIRS), however, could offer a much faster approach to predict the chemical composition and nutritive value of broiler diets. Therefore, this thesis aimed to develop and validate prediction equations to be applied in practical conditions and to investigate the potential of ATR-FT MIRS in predicting chemical composition and nutritive value of broiler diets. The first study (Chapter 3) was designed to develop prediction equations using stepwise regressions. Bootstrap was used to select the variables as well as to confirm the stability of the models. The results indicated that prediction models for coefficient of apparent ileal digestibility (CAID) and ileal digestible content (IDC) of nutrients could be developed using the stepwise regression and bootstrapping approach.

To ensure the practical application of each equation with greater confidence and to alert users to potential risks, Chapter 4 was designed to validate the proposed prediction models developed in Chapter 3. The validation revealed that some equations could not be used in all situations (CAID of nitrogen (N), fat, starch, and calcium (Ca) and IDC of Ca), and some could be used as a general guide (CAID and IDC of phosphorus (P)). The equations developed for CAID of

energy and dry matter (DM), and IDC of N, starch, energy, and DM could be used with good results in terms of R^2 and CCC in the validation study.

The 5th, 6th, and 7th Chapters investigated the potential use of ATR-FT MIRS in predicting the chemical composition and nutritive value of broiler diets using various approaches. Many of the wet chemical compounds in broiler diets, ileal digesta, and excreta were predicted well. The results also revealed that the nutritive value of broiler diets can be predicted from the diets itself, ileal digesta, and excreta spectra as well as the combination of diet and ileal digesta spectra or diet and excreta spectra.

Overall, the results of this thesis indicated that prediction equations and ATR-FT MIRS can be used as a rapid real-time technique to evaluate the nutritive value of broiler diets. The findings of this thesis regarding ATR- FT MIRS are novel and can be used for further update and development of MIRS calibration models, which could substantially reduce the cost of wet chemical analysis in future.

ACKNOWLEDGEMENTS

I would like to praise and thank the almighty God for his blessings and mercy during my PhD study.

First and foremost, I owe my deepest gratitude to my chief supervisor Professor Patrick C. H. Morel, for accepting me to do the PhD under his supervision and for his scholastic guidance, immense patience, correction of the manuscript, feedback and constant inspiration throughout this research work. Your guidance, encouragement, support, and valuable recommendations were significant to this PhD research. I am immensely grateful to my co-supervisors, Associate Professor Tim Wester, Professor Mark R. Waterland, Dr. Fifi Zaefarian, and Associate Professor M. Reza Abdollahi for their guidance and encouragement to complete my PhD. Thank you to Professor Mark R. Waterland for your valuable time in teaching me Python and all your expertise and guidance in spectroscopy. Your support and encouragement were extra special.

I want to express my sincere gratitude to Professor Nicolas Lopez-Villalobos for all the help and support you have given me regarding SAS.

I am enormously thankful for all the technical support from Edward James, Shaun de Malmanche and Kalwyn Pereka from the Poultry Research Unit. I sincerely appreciate the support and guidance during the research experiments conducted at the Poultry Research Unit.

I'm especially grateful to Dr. Sarmini Maheshwaran and her family for assisting me in getting this opportunity and guiding me throughout this journey. I'm always thankful to Associate Professor Thiagarajah Ramilan for your support in starting this journey, continuous encouragement, and for organizing the financial support AHEAD supplementary award given by Massey University. I'm very much thankful for Dr. Laura David for her support and guidance throughout this journey. I also wish to thank Gregory Coleman for helping me with the Python program and spectrometer scans. I am also grateful Dr. Paramsothy Jeyakumar for the support in this PhD journey.

I also gratefully acknowledge the funding provided by the Accelerating Higher Education Expansion and Development (AHEAD) project of the Sri Lankan Government under World Bank for my doctoral scholarship. The financial assistance from the Helen E Akers doctoral scholarship and Johannes August Anderson Postgraduate Scholarship are also greatly appreciated. My heartfelt gratitude to the Massey University Research Foundation for offering me a scholarship to conduct my research.

I take this opportunity to thank University of Jaffna, Sri Lanka for granting me study leave to pursue my PhD study and special thanks to my Animal Science Department staffs for their support and guidance in numerous ways.

I would like to extend my heartfelt thanks to all my PhD colleagues Asharp Godwin, Sagara, Dinesha, Wasim, James, Mande, Inthujaa, Shanika, Komahan, Than, Michelle, Mikel, Obright, Sarah, Mayaba, Juan, Nishamini, Venkatesh and Vania who have been a constant source of motivation and inspiration throughout my PhD journey. I am thankful for the support, laughter, coffee and lunch breaks, and shared moments that have made my PhD experience unforgettable.

I'm ever grateful to my best friends Tharsa, Saratha, Shankar, and Varthani for their endless support throughout this journey. Thank you for accompanying me through all the ups and downs. Your encouragement and friendship have made this experience more meaningful and enjoyable. Without you all it was impossible for me to complete this work.

Words can't describe how grateful I am to my aunt Valarmathy and uncle Jeyamohan for looking after me as their daughter. Your motivation and care during my difficult times made me recover quickly. I have never felt away from home because of you. Thank you for all your enormous support in all ways and the delicious foods. My gratitude also extends to all the Sri Lankan people in Palmerston North for their hospitality and moral support during my study.

Most importantly, none of this could have happened without my family. I value and respect the support and love of each member of my extended family who has always been there for me. I'm obliged to thank my mother Yasodara, father Santhiralingam, and sister Piriyanthi for their love, care, encouragement, and support always. I extend my thanks to my aunt Sahunthala and family, uncle Mahindan and family, and my mother-in-law and husband's family for their continuous support. I would like to offer my heartfelt thanks to my husband Thiruchchenthuran for all his understanding, motivation, support, and patience and for providing me with all financial help throughout this time. There are no words to describe all your sacrifices for me. Without you, I couldn't have come to this point. You left everything and sacrificed your own career to accompany me to New Zealand. I dedicate this thesis to you Senthu.

Looking back again, I appreciate everyone I met during these three years. Without you, this PhD journey would never have happened. As I write this acknowledgement, I am aware that no words can fully express the depth of my gratitude and appreciation for all those who have contributed to this journey.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIC	Akaike information criteria
AME	Apparent metabolisable energy
ATR	Attenuated total reflectance
ATR-FT MIRS	Attenuated total reflectance fourier transform mid-infrared spectroscopy
Ca	Calcium
CAID	Coefficient of apparent ileal digestibility
cATTD	Coefficient of apparent total tract digestibility
CCC	Concordance correlation coefficient
CF	Crude fibre
CV	Cross-validation
CoV	Coefficient of variation
DM	Dry matter
FTIR	Fourier transform infrared
GE	Gross energy
IDC	Ileal digestible content
MIRS	Mid-infrared spectroscopy
MSE	Mean square error
N	Nitrogen
NIRS	Near-infrared spectroscopy

NSP	non-starch polysaccharides
P	Phosphorus
PCA	Principal component analysis
PLSR	Partial least square regression
R^2	Coefficient of determination
RMSE	Root mean square error
RPE	Relative prediction error
RPD	Relative performance deviation
SD	Standard deviation
SE	Standard error
SG	Savitzky-Golay filtering
TiO_2	Titanium dioxide

CHAPTER 1

General Introduction

Currently, poultry meat is the most consumed meat worldwide and it is expected to increase by 16% in 2031, by then poultry meat will constitute 47% of the protein consumed from meat sources (OECD/FAO, 2022). To meet this demand, the poultry industry is likely to undergo a transformation towards precision feeding and management with the primary goal of matching nutrient intake with the nutrient requirement of birds (Zuidhof et al., 2023). This requires both mathematical characterisation of growth responses to nutrient intake and precise knowledge of available nutrients in feedstuffs. This will reduce the amount of feed and minimise the wastage of nutrients (Misiura et al., 2023; Zuidhof et al., 2023). Feed constitutes the major variable production cost, representing 70% of the total expenses in a broiler farm. Therefore, efficient management of feed formulation is essential to maximise profitability (Leeson and Summers, 2005; Mallick et al., 2020; Moss et al., 2021; Zampiga et al., 2021).

Least-cost diet formulation has been practiced for many years. Thus, knowledge of the nutrient compositions of feed ingredients is essential for optimum diet formulation. The classical method uses tables with nutrient values of different feedstuff for poultry (WPSA, NRC, INRA, Evonik, and Feedipedia etc.). These nutrient values are global averages of published values of nutritional information from various sources (Mateos et al., 2019; Zaefarian et al., 2021). Therefore, the nutritional value varies widely between and within ingredients. Factors such as cultivar, agronomic practices, harvest, storage, and processing parameters will influence the nutritive value of an ingredient (Knudsen et al., 2023). Therefore, it is essential to determine the specific chemical composition and nutritive value of ingredients that are being used when formulating a feed.

In recent years, feed formulation has been based on the available nutrient content, where the nutrients digested and metabolised by the animal are considered instead of the total nutrient content in the feeds (Van Barneveld et al., 2018; Cruz-Conesa, 2023). The *in vivo* assays for digestibilities, where excreta is collected for energy, and ileal content for protein and amino acids, have become the most preferred technique for measuring the availability of nutrients (Ravindran and Bryden, 1999).

In vivo method is the gold standard method to measure the direct animal response to a specific feedstuff (Zaefarian et al., 2021). The important steps in conducting a digestibility assay are formulation and mixing of diets, *ad libitum* feeding of birds for a pre-determined period, collection of ileal digesta or excreta, processing of digesta or excreta, and wet chemical analysis of digesta, excreta, and diet (Ravindran et al., 2017). Careful execution of these steps is essential and requires facilities for bird rearing and feed preparation, labour, and well-equipped laboratory facilities to analyse the diet, digesta, and excreta. Moreover, the welfare of the birds is questioned (Zaefarian et al., 2021). Another issue with those *in vivo* assays is that they are time-consuming, expensive, and the values are not readily available.

These limitations pave the way for the development of *in vitro* evaluation techniques. The *in vitro* method mimics the reactions along the digestion process in broilers. This technique is faster than the *in vivo* assays nevertheless requires enzymes and chemical reagents as well as expensive equipment. Moreover, a single *in vitro* method might not provide accurate predictions for all ingredients; hence, it is essential to focus on developing specific *in vitro* digestibility methods for each feedstuff and diet (Yegani et al., 2013).

The classical wet chemistry method is well-known for its accuracy and reliability in analysing the excreta, ileal digesta, and diet to calculate the nutritive value, but it requires significant investments in time and financial support for processing the samples. Moreover, the feed,

excreta, and ileal samples should be analysed with many replicates to minimise external effects. In addition to that, waiting for the results from the laboratory takes up valuable time and delayed results, making the data to be outdated for timely managerial decisions (Yakubu et al., 2022; Xu et al., 2023). Moreover, it also causes environmental pollution due to the use of solvents and the generation of chemical waste (Bastianelli et al., 2010). Due to these drawbacks, there is a need for reliable, faster, easier, and cheaper ways to determine the nutritive value of broiler diets. Predictive regression modelling and infrared (IR) spectroscopy are emerging as alternative faster and cheaper techniques, but they still require *in vivo*, or *in vitro* data for the initial development of prediction equations or the development of calibrations for IR spectroscopy. Once established with sufficient accuracy, both these methods could be used at no additional cost (Bastianelli, 2013).

Predictive models are widely known as informational tools to support rapid and economical assessment of feed (Baiz et al., 2020). It allows the use of simple chemical analysis of the diets to determine the nutritive values (Alvarenga et al., 2013b). These mathematical models are an integral part of smart poultry nutrition. Several researchers have proposed equations to predict energy and nutrient digestibility from chemical composition of feedstuffs for broilers (Nascimento et al., 2009; Mariano et al., 2013; Cerrate et al., 2019; Sheikhhasan et al., 2020b; Pedersen et al., 2021). Model validation is performed to determine whether predicted values from the model are likely to accurately predict responses on future subjects or subjects not used to develop the model. This could be done by internal and external validation strategies (Harrell et al., 2015; Steyerberg and Harrell, 2016).

One of the best ways for internal validation and variable selection is the use of bootstrap resampling (Harrell, 2015). The theory behind bootstrapping is that it replicates the process of sample generation from an underlying population by drawing samples with replacements from the original dataset (Steyerberg et al., 2001). However, to our knowledge, using bootstrapping

as an internal validation technique and for selection of variables to predict the nutritive value of poultry diets has not been reported in any studies. Even though internal validation could give valuable insight into the accuracy of regression models, external validation is essential to ensure the practical application of each equation with greater confidence and to alert users to risks, if any (Meloche et al., 2014). There were some validation studies carried out to ensure the accuracy of the proposed equations related to digestibility studies in broilers (Alvarenga et al., 2013b; Meloche et al., 2014; Alvarenga et al., 2015; Pedersen et al., 2021). Besides, many published reports relating to prediction equations presented limited information on how those equations were validated (Batal and Dale, 2006; Wu et al., 2019). Finding properly validated prediction equations to predict the nutrient digestibility of complex broiler diets based on the feed gross chemical composition needs to be further studied.

Infrared spectroscopy is a rapid, chemical-free, and non-destructive technique for the analysis of animal feeds. It does not need complicated sample preparation and requires only a small amount of sample for the analysis (Shi et al., 2019; Campbell et al., 2022; Kho et al., 2023). Thus, IR methods have been acknowledged as the most promising substitution for traditional analytical techniques (Kho et al., 2023). The IR spectrum is divided into the far-infrared, mid-infrared, and near-infrared ranges defined by wavelength, and hence the energy of the photons which in turn can induce different transitions between quantized vibrational energy states (Griffiths, 2006). Rapid vibrational spectroscopic methods combined with mathematical modelling are used in IR analysis of feed to extract chemo-structural data on feed components (Campbell et al., 2022).

Near-infrared spectroscopy (NIRS) has been used widely to predict gross chemical composition and digestibility of the feed and feedstuffs over recent years (Bastianelli et al., 2005; Bastianelli et al., 2010; Bastianelli, 2013; Bastianelli et al., 2013; Hell et al., 2016; Shi et al., 2019; Cruz-Conesa et al., 2022; Cruz-Conesa, 2023; Ramos Cruz et al., 2023; Zuidhof

et al., 2023). However, the potential use of mid-infrared spectroscopy (MIRS) in feed analysis is still at the research level and needs further investigation. The MIRS scans a wider spectral range than NIRS and yields information about fundamental vibrations, thus giving better insights into the molecular bands present in the sample and is not so convoluted by water signals. The samples could be directly related to their chemical composition (Belanche et al., 2013). Using MIRS, even a more complex and very similar structure could be differentiated (Hell et al., 2016).

The Attenuated Total Reflectance Fourier Transform (ATR-FT) technique has enabled the acquisition of MIR spectra of samples in reflectance mode (Cleland et al., 2018), while early MIRS depended on the dispersive technique (Abbas et al., 2020). This has significantly expanded the capabilities and applications of mid-infrared spectrometers, thus MIRS has now gained a significant advantage over NIRS (Cleland et al., 2018). In addition to that, this makes it possible to observe all frequencies simultaneously and to obtain a complete spectrum in a single scan. Moreover, it is highly reliable due to its simple design and allows increased optical throughput (Belanche et al., 2013; Abbas et al., 2020). The ATR-FT MIRS is anticipated to have sufficient quantitative accuracy and has the benefit of being able to identify specific nutrients (Wang, 2014). It has been successfully used to predict forage composition for ruminants (Cleland et al., 2018) and to predict chemical composition of feedstuffs for monogastrics (Qiao and Van Kempen, 2004; Ferreira et al., 2014; Hell et al., 2016; Ferreira et al., 2018; Shi et al., 2019).

Recently, accessing the nutritive value of diets using the faecal spectra of birds (digesta and excreta) or combining feed and faeces spectra has been gaining interest. Owing to the fact that digestion is an interactive process between feed and animal, integrating information about the feed and faeces will provide more insights into the digestion process (Bastianelli et al., 2013). However, no study has examined the potential of ATR-FT MIRS faecal spectra and the

combination of feed and faecal spectra to predict nutrient digestibility. Thus, integrating ATR-FT MIRS in real-time could reduce the massive workload in estimating the chemical composition and nutritive value of feeds and feedstuffs in laboratories using wet chemistry methods and guarantee the accuracy of diet formulation.

Research presented in this thesis was aligned with the need to develop a low-cost method to replace expensive wet chemical analysis involved in *in vivo* assays to predict the chemical composition and nutritive value of broiler feeds. Thus, the overall aim of this study is to examine prediction equations and ATR-FT MIRS as potential techniques to determine the nutritive value of broiler diets. To achieve this main aim, this thesis addressed the following specific objectives:

1. To develop and validate prediction equations to determine the nutritive value of broiler diets.
2. To explore the potential of ATR-FT MIRS in predicting the chemical composition and nutritive value of broiler diets.
3. To evaluate the usefulness and accuracy of ATR-FT MIRS calibrations based on spectra from ileal digesta or excreta to predict their chemical composition and nutritive value of broiler diets.
4. To study the different approaches based on ATR-FT MIRS predicted values for the determination of nutritive value of broiler diets.

The findings of this research will advance the understanding of the development of prediction equations using stepwise regression and bootstrapping and emphasize the need for external validation of the developed equations. Furthermore, the results of ATR-FT MIRS in the prediction of the nutritive value of broiler diets will provide a foundation for future research studies on developing real-time ATR-FT MIRS technology for rapid in-field screening.

Thesis structure

This thesis consists of eight Chapters including the general introduction (Chapter 1) providing an overview of the main research idea and objectives of the thesis, and a review of the literature (Chapter 2) highlighting the research gaps. Chapters 3-7 are research Chapters formatted as papers for publication. Therefore, there might be repetitions in concepts, analytical and statistical procedures, and references. Chapter 8 includes the general findings and limitations of this work and outline future research that could be designed based on the findings of this thesis. The overall outcome of this thesis is outlined in Figure 1.1.

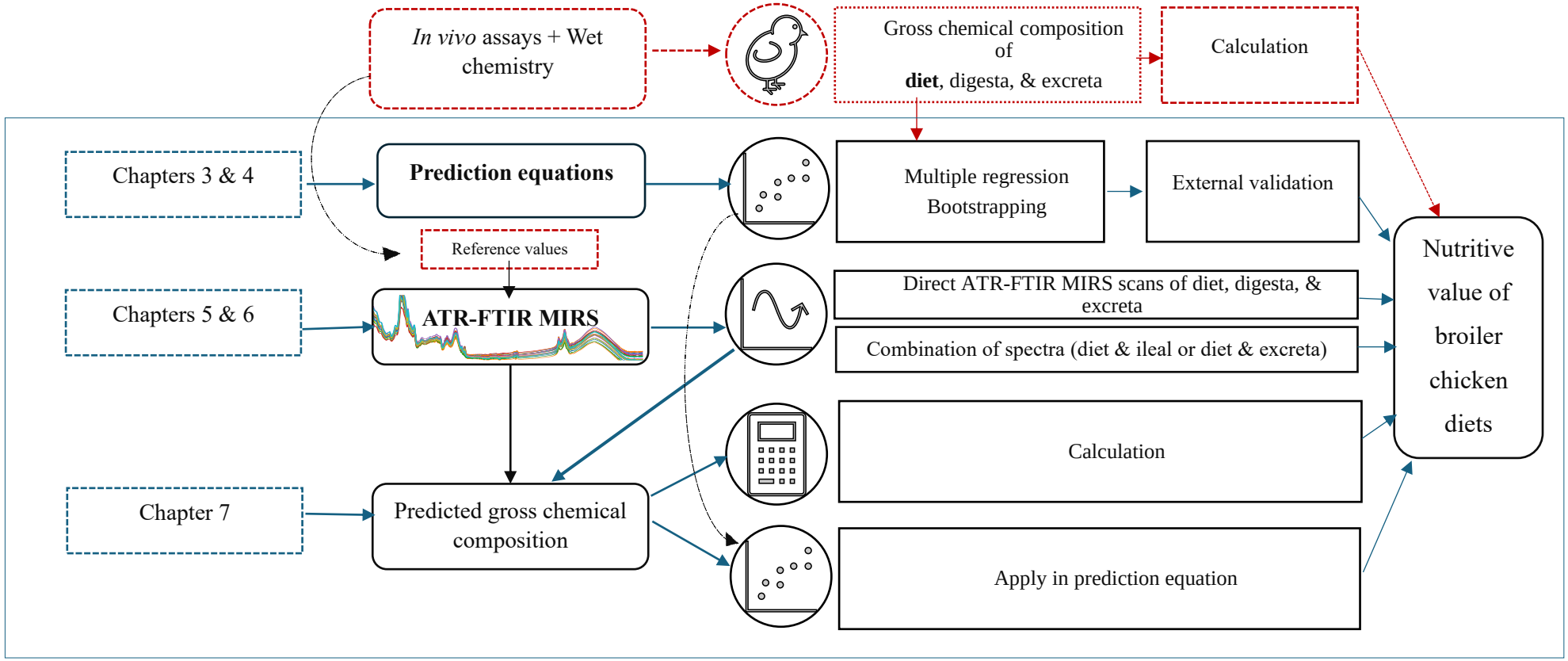


Figure 1.1. Schematic explanation of the methods for estimation of nutritive value of broiler diets in this study.

CHAPTER 2

Literature review

Feed evaluation is essential to produce reliable data on the chemical composition and nutritive value of various feed ingredients and diets (Zaefarian et al., 2021). Low nutrient content, as well as high nutrient content in the diet, may result in an increase in the intake of feed, decrease the animal performance, and increase the excretion of nutrients, thus impacting the profitability of the farm (Garnsworthy et al., 2000; Van der Klis and Fledderus, 2007). This literature review will cover the different methods used to determine the nutritive value of feed ingredients and diets as illustrated in Figure 2.1.

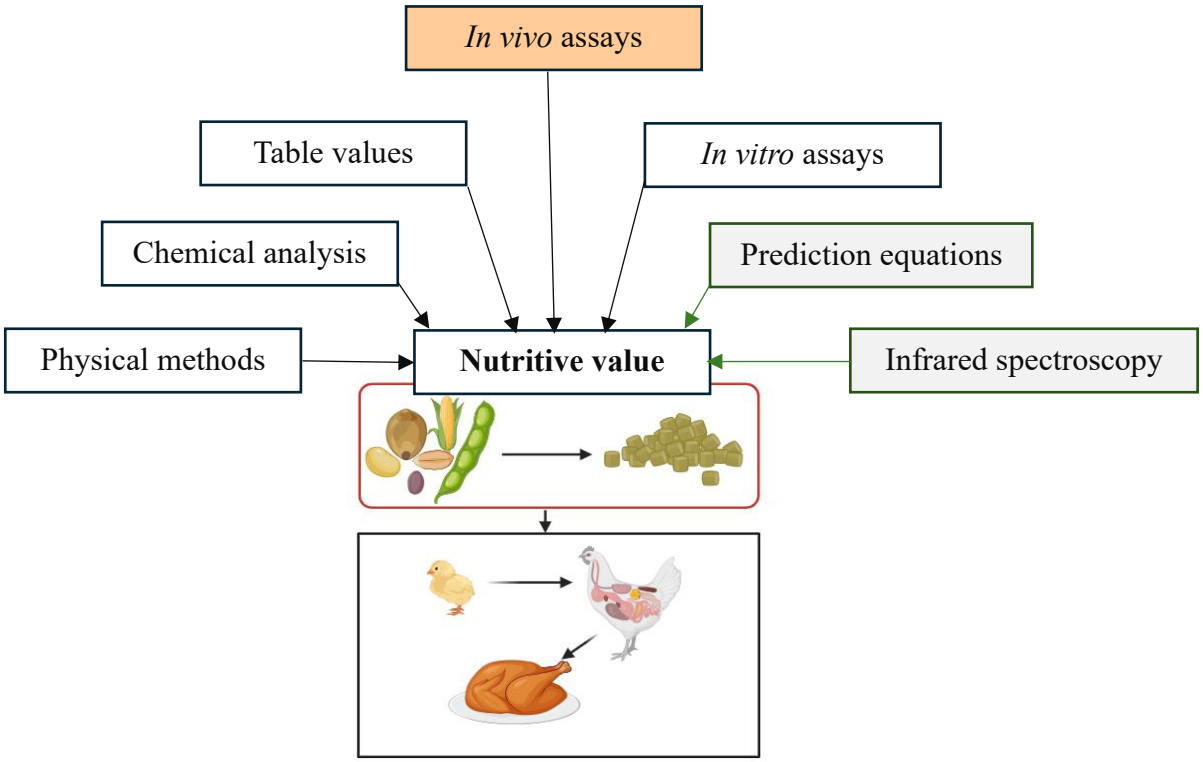


Figure 2.1. Methods of determination of nutritive value of feed ingredients and diets in broilers.

2.1. Measurement of nutritive value

The nutritive value represents how nutrients contained in an ingredient are utilised by the animal and it is the result of interaction between the ingredient and the animal consuming it. The most commonly used measurement of the nutritive value of feed ingredients is the digestibility of nutrients or energy (Choct, 2016). Digestibility data can offer an insight into the proper feeding of animals (Khan et al., 2003). Measuring digestibility of nutrients is crucial in monogastric animals as feed accounts for 70% of the total cost of production, indeed any gain in nutrient digestibility has the potential to lower these costs (Mallick et al., 2020; Zampiga et al., 2021). Different methods have been developed over the past decades to evaluate the nutrient content and digestibilities of raw material and complex diets. The gold standard method for determination of digestibility is the *in vivo* method.

2.2. Types of *in vivo* experiments

2.2.1. Total collection method

This method is carried out by measurement of feed intake and quantitative collation of excreta over a period of time. This procedure involves a 3 to 4 day adaptation period and a 3 to 4 day excreta collection period, this may sometimes differ between the institutions (Sales and Janssens, 2003). With these measurements, the digestibility of the component could be calculated as follows,

Digestibility (%) total collection (2.1)

$$= \left[\frac{(nutrient_{feed} \times feed\ intake) - (nutrient_{excreta} \times excreta\ output)}{(nutrient_{feed} \times feed\ intake)}\right] \times 100$$

Digestibility of amino acids (AA) is not often measured using total collection method because of the microbial activity and endogenous secretions in the hindgut. Moreover, excreta collection may not be accurate as there is contamination of faeces by urine and feathers etc.

and this measures metabolisability rather than digestibility since urine and faeces are excreted together in birds (Ravindran and Bryden, 1999; Sales and Janssens, 2003).

The metabolisable energy system is widely used for evaluating the energy requirements of birds, available energy of feed ingredients, and formulating complete poultry feeds. The available energy is calculated as the difference between dietary gross energy (GE) ingested and GE excreted in the faeces and urine and referred to as apparent metabolisable energy (AME) (Abdollahi et al., 2021).

$$\text{AME (MJ/kg diet)} = \frac{(GE_{diet} \times feed\ intake) - (GE_{excreta} \times excreta\ output)}{(Feed\ intake)} \quad (2.2)$$

2.2.2. Ileal digestibility assays

This requires the inclusion of indicator or marker in the feed and collection of representative digesta samples from the ileum. Ileal digestibility of nutrients may show a more accurate view of nutrient utilisation of broiler diets than the total tract utilisation due to the effect of microbial action in the large intestine and the void of urine and excreta in total tract measurements (Babatunde et al., 2020). Digesta are recovered from the distal part of the ileum and analysed in this method. According to the digesta collection techniques, ileal digestibility could be determined in two ways. The commonly used method is to slaughter the bird and direct collection of digesta, and the other method is to use a cannula inserted into the distal ileum (Ravindran and Bryden, 1999).

2.2.3. The indicator or marker method

The marker method is preferred when determining the ileal digestibility of nutrients as the total collection of ileal digesta is more complex than the excreta collection. The use of inert markers prevents the need for quantitative collection of excreta and eliminates the errors related to inaccurate feed intake and excreta output measurements (Smeets et al., 2015). The ratio of the

marker in the diet to the amount of marker in excreta or ileal digesta is used to calculate the digestibility (Scott and Boldaji, 1997). However, the precision relies on the accurate chemical analysis of the marker in the feed and excreta or ileal digesta assuming the marker is totally recovered (Kong and Adeola, 2014).

Digestibility (%) marker (2.3)

$$= \left[\frac{(\text{Nutrient /marker})_{diet} - (\text{Nutrient/marker})_{ileal/excreta}}{(\text{Nutrient/marker})_{diet}}\right] \times 100$$

where,

(Nutrient/marker) $_{diet}$ = ratio of diet component to the marker in the diet

(Nutrient/marker) $_{ileal/excreta}$ = ratio of the diet component to the marker in the ileal digesta or excreta

Titanium dioxide (TiO_2), chromic oxide (Cr_2O_3), and acid-insoluble ash (AIA) are the widely used markers in poultry experiments. The assumption is that the markers are completely indigestible, distributed evenly in the feed and faeces, which could be measured precisely at even lower concentrations and totally recovered (Kong and Adeola, 2014).

Excreta or ileal digesta sample collection processes can be used based on the components of interest. Ileal digesta samples are preferred for the AA digestibility measurements to avoid the interference of fermentation in the hindgut (Ravindran and Bryden, 1999). Normally, excreta samples will be collected to determine the total tract digestibility for energy, calcium (Ca), and phosphorus (P) (Zhang and Adeola, 2017). However, before conducting *in vivo* experiments, the methodology to determine the digestibility of nutrients and energy should be carefully chosen. Three different approaches are available for digestibility measurements such as direct, difference, and regression methods. The direct and difference methods are commonly used to measure the digestibility of nutrients and energy (Zhang and Adeola, 2017).

Direct method

This method is relatively easy and simple where test feed ingredient is formulated as the sole source of the component in the test diet. Only one diet is needed, and the determined dietary digestibility of the component is that of the test ingredient (Zhang and Adeola, 2017). It is impossible to apply the direct method for all of the ingredients if the test ingredients cannot be formulated to supply the component of interest alone in the diet.

Difference (Substitution) method

In this method a basal and a test diet are formulated, in which the basal diet is fed to a group of birds to determine the digestibility of the components, and another group is fed a test/assay diet with a known proportion of the basal diet replaced by the test ingredient (Zhang and Adeola, 2017). The test diets comprises of mixture (usually 50:50) of the basal and the test ingredient. It is assumed that there is no interaction between the basal diet and the test ingredient (Lemme et al., 2004). The digestibility of the nutrient in the test ingredient is then determined based on the difference in digestibility between the two assay diets and the concentration of the specific nutrient in the diet (David et al., 2023). The difference method is used to evaluate the test ingredients with poor palatability, high protein content, or high level of anti-nutritional factors (Khalil et al., 2021).

Regression method

The regression method is based on feeding a basal diet and assay diet with at least two levels of the basal diet replaced by the test ingredient (Khalil et al., 2021). It involves establishing a linear relationship between nutrient output and dietary nutrient input by formulating diets with graded concentrations of the nutrient from the test ingredient (David et al., 2023).

Even though *in vivo* methods measure the direct animal response to the variation in the feeds and evolved to be the best methods for determining the nutritional value of feed ingredients and diets, they also have obvious limitations (Zaefarian et al., 2021). When it comes to measuring the nutritional value of feedstuffs using animal experiments, there have been a lot of differences reported by different researchers. Furthermore, logistical constraints, the need for specialized expertise, ethical concerns related to the use of birds as well as the longer time and higher expenses associated with conducting animal research, are all factors that limit the use of animal studies in routine feed evaluation programs (Garnsworthy et al., 2000; Jha and Tiwari, 2016). In addition to that, *in vivo* assays involve feeding a large amount of feed to the birds and analysing multiple samples of feed, ileal digesta, and excreta. These analyses are traditionally carried out using slow and expensive wet chemistry methods. Moreover, wet chemical analysis produces a huge amount of chemical waste due to the use of solvents (Bastianelli et al., 2010). These constraints led to a strong thrust in developing alternative methods to determine the nutritive value of feeds.

2.3. Physical methods

The physical differences among the grains used for feeding livestock and poultry such as kernel density, hardness, and grinding resistance have an impact on performance (Moore et al., 2008). However, they are not thought to be reliable predictors of nutritional quality. Nevertheless, grain test weights have been used until now to test the grain quality as high-test weight grains have more starch-rich endosperm thus giving more calories (Zaefarian et al., 2021).

2.4. Table values

Poultry industries use different tables containing nutritive values of feedstuffs to formulate feeds. It is the easiest method of feed analysis used commonly under most practical situations where real time analyzing feed samples is not possible. However, the applicability of table

values is under question as there is a large difference among the institutions on the nutritive value of ingredients and most are average values from published resources worldwide (Mateos et al., 2019; Zaefarian et al., 2021). In accordance with this, Wiseman (2006) stated that the table values can be affected by many factors such as chemical composition of feedstuffs, the stage of the bird as well as the method by which the nutritive values are determined. Awareness of the procedures used is essential when using tables from various institutions for evaluation of feeds (Mateos et al., 2019). For e.g. discrepancies among the energy values among tables of different sources are shown in Table 2.1.

Table 2.1. Variability in energy content of high protein soybean meal and full fat soybeans from different institutions (as-fed basis).

				High protein soybean meal				Full fat soybeans			
Institution	Year	Country	Species	CP	NDF	EE	AMEn	CP	NDF	EE	AMEn
				%	%	%	Mcal/kg	%	%	%	Mcal/kg
WPSA	1989	Europe	Rooster	47.0	9.9	1.3	2.26	36.1	11.0	18.0	3.42
NRC	1994	USA	Poultry	47.0	9.0	0.9	2.37	37.0	10.3	18.0	3.30
INRA	2002	France	Broiler	47.2	8.9	1.5	2.32	34.8	11.0	17.9	3.35
NARO	2009	Japan	Poultry	47.0	11.1	1.6	2.47	36.9	7.9	18.9	3.41
Premier Atlas	2014	UK	Broiler	47.0	7.5	1.7	2.42	35.5	11.0	18.5	3.36
RPRI	2014	Russia	Poultry	47.0	12.1	1.4	2.55	35.5	11.6	17.6	3.38
CVB	2016	Netherland	Broiler	46.8	8.6	1.6	2.16	36.3	12.1	19.7	3.13
Evonik	2016	Germany	Poultry	47.5	10.7	2.1	2.34	35.6	12.6	19.6	3.28
Feedipedia	2017	France	Broiler	47.1	9.7	1.6	2.32	35.2	11.7	18.4	3.64
Fedna	2017	Spain	Poultry	47.0	8.8	1.7	2.32	37.0	11.3	19.2	3.42

Source: Mateos et al. (2019).

AMEn, nitrogen-corrected apparent metabolisable energy; CP, crude protein; CVB, Centraal Veevoeder Bureau; EE, ether extract; INRA, Institut National de la Recherche Agronomique; NARO, National Agriculture and Food Research Organization; NDF, neutral detergent fibre; NRS, National Research Council; RPRI, Russian Poultry Research Institute; WPSA, World's Poultry Science Association.

Other methods have been developed to overcome the drawbacks of the above mentioned methods. Such methods include *in vitro* evaluation techniques, predictive regression models, and the use of infrared (IR) spectroscopy.

2.5. *In vitro* assays

In vitro digestion models mimic the digestion process along the gastrointestinal tract of birds and could be an alternative to *in vivo* assays to evaluate feed quality (Jha and Mishra, 2021). The enzymes utilized should have similar specificities to those found in the digestive tract of poultry (Boisen and Eggum, 1991). Most current *in vitro* models for monogastrics are based on pigs and used to generate *in vitro* data for poultry as their digestive characteristics are similar to a great extent. However, there are noticeable differences in anatomy, with poultry having a crop and gizzard and pigs having a longer, well-developed hindgut. These variances cause variations in digesta transit time and pH along the digestive tract, affecting foregut digestion in chickens and hindgut fermentation in pigs (Zaefarian et al., 2021). It is essential to consider these differences to develop appropriate *in vitro* assays for poultry.

Problems exist in published *in vitro* digestion results for poultry such as the use of digestive enzymes of porcine, manual conduction of *in vitro* digestion procedures such as pH regulation, digestive enzyme injection, and separation of digested and undigested substance. Those may introduce errors and lead to non-repeatable procedures and imprecise results (Zhao et al., 2014). A single *in vitro* method might not provide accurate predictions for all ingredients; hence, it is essential to focus on developing specific *in vitro* digestibility methods for each feedstuff and diet (Yegani et al., 2013).

The major advantages of doing *in vitro* analyses are its rapid process, low cost, and lack of ethical issues (Bryan et al., 2018; Zaefarian et al., 2021; Sharma et al., 2022; Michels et al., 2023). Moreover, other influential factors are excluded in *in vitro* studies such as environment,

management, disease, and genotype (Zaefarian et al., 2021). Nevertheless, the *in vivo* conditions can never be completely reproduced under *in vitro* conditions, they can be set up to access the initial rate of hydrolysis or to give maximal digestibility values using the specific enzymes (Boisen and Eggum, 1991). Therefore, *in vitro* models should be developed to closely mimic the digestive process in the gastrointestinal tract of the birds as much as possible and the data should be validated by comparing the corresponding data obtained from the *in vivo* studies using the same samples (Zaefarian et al., 2021). Validated *in vitro* techniques may play a major role in providing data infrared spectroscopy calibrations (Yegani et al., 2013).

Enzyme specificity, enzyme activity, sample and particle size, and end-product separation technique are the major factors influencing *in vitro* digestibility (Boisen and Eggum, 1991). An effective *in vitro* method will exhibit a high degree of correlation with the *in vivo* estimations (Sakamoto et al., 1980; Clunies and Leeson, 1984; Boisen and Fernandez, 1995), hence, suitable statistical analysis ought to be employed to ascertain the precision and accuracy of prediction equations of *in vitro* data relative to *in vivo* results (Zaefarian et al., 2021).

The commonly used *in vitro* systems are 1-, 2- or 3-step digestion systems where different phases during digestion are stimulated as shown in Figure 2.2. The 1-step and 2-step models are commonly used in poultry while the 3-step model is more applicable to pigs.

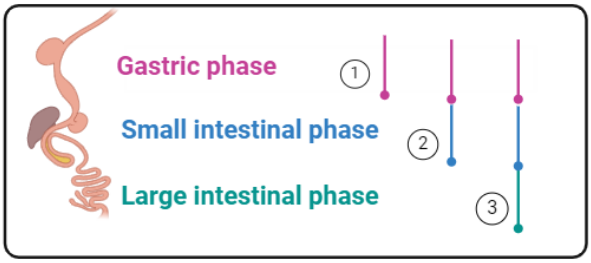


Figure 2.2. Illustration of 1-, 2-, and 3-step digestion systems.

Apart from the above-mentioned methods, several other *in vitro* assays are available such as pH-drop and pH-stat method, dialysis cell method, immobilized digestive enzyme assays

(IDEA), and computer-controlled methods (Thresher et al., 1989; Boisen and Eggum, 1991; Minekus et al., 1995; Butts et al., 2012). Even though the use of birds and laborious procedures as in *in vivo* assays are avoided in the *in vitro* system, it suffers from inadequacies and may require ingredient-specific techniques (Zaefarian et al., 2021). Furthermore, chemicals, equipment, and skilled technicians are necessary to conduct assays, thus increasing cost. Therefore, it cannot be used in routine feed evaluation. A detailed review of the *in vitro* procedures is available in previous publications (Boisen and Eggum, 1991; Farrell, 1999; Moughan, 1999; Zaefarian et al., 2021; Sharma et al., 2022).

2.6. Chemical analysis or wet chemistry methods

Chemical analysis of gross content of nutrients and energy is a very simple and direct method that is still widely used for feed evaluation (Zaefarian et al., 2021). However, this only provides details about the gross chemical composition of feedstuffs and diets and can't directly provide digestibility values. Wet chemistry methods are not only expensive requiring specialised equipment but also it takes a long time for the results to be available. Moreover, the use of hazardous chemicals for different analysis questions environmental safety (Jha and Tiwari, 2016). However, wet chemical analysis is crucial to develop prediction equations and to calibrate IR models.

2.7. Predictive regression models

In recent years, the use of prediction equations has been gaining more interest and is now used by most industries related to animal feed manufacture (Alvarenga et al., 2013a). Regression analysis is a statistical technique for modelling the relationship between variables. Prediction models in poultry nutrition relate the digestibility of the nutrients and energy values determined by the *in vivo* assays with the nutrient composition of the feed obtained by the wet chemistry analysis or *in vitro* digestibility values (Cruz-Conesa, 2023). The number of variables that form

the prediction equations and the methods by which the equations are obtained are responsible for the accuracy and reliability when comparing different equations. It is important to note that the selection of representative samples with a wide range of nutrient content is necessary to produce a model that is not only reliable and accurate but also useful to apply in commercial poultry settings (Meloche et al., 2013).

$$y = \beta o + \beta_1 x + \varepsilon \quad (2.4)$$

Equation 2.4 represents a simple linear regression model where x is referred to as the predictor or regressor variable, β_1 is the regression coefficient, and y is the response variable. The error term ε represents the variability that can't be explained by the model. If more than one regressor or predictor is involved, it is called a multiple linear regression model (Equation 2.5) (Montgomery et al., 2021).

$$y = \beta o + \beta_1 x_1 + \beta_2 x_2 + \ldots\ldots \beta_j x_j + \varepsilon \quad (2.5)$$

Several studies have shown that the application of appropriate mathematical models may release more precise information on the system output along with accurate prediction of performance (Mehri, 2013). The regression model-building process is described in Figure 2.3.

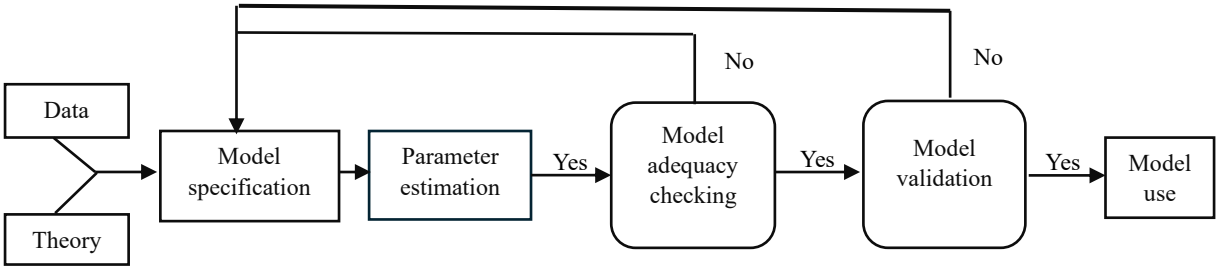


Figure 2.3. Regression model-building process adapted from Montgomery et al. (2021).

The most common problems encountered when fitting regression models are the correlation of the error terms, outliers, collinearity, high leverage points, and non-constant variance of error terms which were clearly explained by James et al. (2013).

2.7.1. Stepwise regression

Evaluation of all possible regressors or predictors can be a problem. Variable selection is the process of determining which predictors are related to the response to fit a single model involving only those predictors (James et al., 2013). Various methods have been developed for evaluating only a small number of subset regression models by either adding or deleting regressors one at a time. These methods are generally referred to as stepwise procedures (Montgomery et al., 2021). They are categorised into three broad divisions such as forward selection, backward elimination, and stepwise regression. Stepwise regression is a popular combination of forward selection and backward elimination.

The regressor variable with the highest simple correlation to the dependent variable is included first in the stepwise selection process. As each regressor is entered into the model, the partial correlation coefficients of the remaining candidate regressors are calculated to adjust for the effect of each selected variable on the dependent variable. Then a candidate regressor with the largest partial regression correlation coefficient enters the model. At each step, the regressors in the model are reevaluated for significance and may be removed if they exceed the criteria for entry. The process is repeated until no further candidate regressors meet the criteria for entry or elimination (Meloche et al., 2013; Wang et al., 2021a).

2.7.2. Evaluation of model fit

2.7.2.1. Coefficient of determination (R^2)

It was introduced by Wright (1921) and commonly indicated by R^2. Its original formulation quantifies how much the dependent variable is determined by the independent variables in terms of proportion of variables (Chicco et al., 2021). It is the most common method to evaluate the accuracy of the fit in multiple linear regression. Despite not being advised as the final model selection tool, it indicates how well the selected explanatory variables predict the response

(Renaud and Victoria-Feser, 2010). This qualitative measure lies between 0 and 1, where R^2 = 1 represents a perfect model fit and R^2 = 0 represents no fit (Harel, 2009).

The R^2 estimates the percentage of variance of the response variable explained with the explanatory variables and it can be calculated as,

$$R^2 = 1 - \frac{RSS}{TSS} \tag{2.6}$$

where, TSS is the total sum of squares and RSS is the residual sum of squares (Renaud and Victoria-Feser, 2010).

2.3.2.2. Adjusted coefficient of determination (adj. R^2)

The adj. R^2 estimates the amount of variance explained in the population (Miles, 2005; Karch, 2020). Since R^2 increases as variables are added to the model, the adj. R^2 will take the number of predictors in the regression model into consideration (Harel, 2009).

2.7.2.3. Root mean square error (RMSE)

Root mean square error is used for evaluating model fitting, model validation, model selection, model comparisons, and forecasting evaluations (Karunasingha, 2022). The RMSE is the square root of the mean square error (MSE). When presenting the RMSE, the underlying presumption is that the errors are unbiased and have a normal distribution. An important aspect of the error metrics used for model evaluation is their capability to discriminate among model results and RMSE usually is better at revealing model performance differences (Chai and Draxler, 2014). The RMSE is defined as the square root of the average square differences between predicted and measured values and can be calculated by the following equation.

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \tag{2.7}$$

where, $\hat{y}_i$ is the predicted value, y_i is the observed value, and n is the number of observations (Faber, 1999).

2.7.2.4. Akaike Information Criterion (AIC)

The Akaike Information Criterion is one of the data-driven methods to aid in the selection of good models. The criterion was introduced by Akaike (1973) in his seminal paper "Information Theory and an Extension of the Maximum Likelihood Principle." The traditional maximum likelihood framework, as applied to statistical modelling, provides a cogent paradigm for estimating the unknown parameters of a model having a specified dimension and structure. Akaike extended this paradigm by considering a setting in which the model size and structure are also unknown and must therefore be determined from the data. Thus, Akaike proposed and developed a framework wherein both model estimation and selection could be simultaneously achieved (Cavanaugh and Neath, 2019). It is used to compare models based on different probability distribution for outcome variable (Chakrabarti and Ghosh, 2011). The minimum AIC criterion chooses the candidate model with the smallest value of AIC.

2.7.3. Model validation

Many prediction equations appear to yield precise and accurate estimates, but this can be misleading unless the equations are tested with independent data (Sibbald, 1982). Therefore, it's very important to assess a model's validity before releasing any model to the end user. Model validation is performed to determine whether predicted values from the model are likely to accurately predict the responses on future subjects or subjects not used to develop the model (Harrell, 2015). The primary methods of validation are by using either internal or external techniques.

External validation using a new data set generated by an independent research team is the best strategy to validate prediction models. The user will have greater confidence in both the model and the model building process if the model gives an accurate prediction of the new data set (Montgomery et al., 2021). However, internal validation techniques can be performed when it is not practical to collect fresh test data to validate the model. The simplest internal validation technique is data-splitting, which keeps some of the original data and uses these observations to investigate the model's predictive performance. The data set is split into training and test data which corresponds to model development and model validation, respectively. This technique greatly reduces the sample size and when the process is repeated different predictive accuracy may be obtained. Therefore, different improvements in the data-splitting method are available to get more unbiased estimates of model performance without sacrificing the sample size (Harrell, 2015). Resampling is such a technique that repeatedly draws samples from a training dataset and refits a model of interest on each sample. The most commonly used resampling methods are the cross-validation and the bootstrap (James et al., 2013).

Each internal model validation strategy has pros and cons, and no one technique is consistently superior to another. Different researchers have different ideas on what approach is best for internal model validation. Before reaching a decision, several criteria need to be considered, including sample size, best indicators of a model's performance, and choice of models (Chowdhury and Turin, 2021).

2.7.4. The bootstrap

Bootstrap resampling which was first presented by Efron (1979), which is an unbiased way to validate models as it has an iterative resampling component. It is an efficient technique when only a few samples are available, and samples the data several times which makes it a more robust method for validation (Ramoelo et al., 2012; Rombach et al., 2019). The theory behind

bootstrapping is that it replicates the process of sample generation from an underlying population by drawing samples with replacements from the original dataset (Steyerberg et al., 2001).

It could be used as a variable selection technique to identify and remove unwanted variables from a larger number of initial candidate variables. When the same selection procedure as for the original data is used in an ideal validation study, then the same variables should be selected which is called 'replication stability' (Sauerbrei, 1999).

Recently, bootstrap resampling techniques have been promoted to evaluate the degree of stability of models resulting from stepwise procedures (Nunez et al., 2011). In stepwise regression, after a variable has been added to the model at each step of the variable selection process, it is possible to remove variables from the model. For instance, if the significance of a given predictor is above a specific threshold, it will be eliminated from the model. When a prespecified stopping rule has been satisfied, the iterative process will end (Austin and Tu, 2004a). If applied to regression analysis, bootstrapping provides variables that have a high degree of reliability (Brunelli, 2014).

Methods based on statistical models have been continually proposed for prediction of nutrient digestibility and digestible content of nutrients for broilers. However, only a few published reports are available that use bootstrap resampling for variable selection and validation of regression models in pigs (Castilho et al., 2015; Smith et al., 2015; Oliveira et al., 2019).

2.7.5. Prediction equations in poultry nutrition

To create the least expensive feed formulations, assess the potential inclusion of feed elements in diets, and lessen the environmental impact of animal production, reliable data on nutrient digestibility is crucial. Regression equations can be used as an alternate way of predicting the

digestibility of feedstuffs and diets for poultry because *in vivo* trials are time-consuming and expensive (Son et al., 2017). Regression studies showed that significant relationships existed between the chemical analysis and the digestion coefficients of the different nutrients.

Using prediction equations to determine the nutritive value of the feed has the advantage of eliminating the requirement for sacrificing birds and laboratory testing. However, for accurate prediction based on chemical composition, precise analytical methods must be used, although their validity may be limited by the presence of factors or processes that hinder nutrient digestion (Swiech, 2017).

The main drawback usually faced with the use of prediction equations is the lack of information on the wet chemical techniques used to analyse the major dietary components. Another problem is that the equations are derived from a lower number of samples and the values are generally applicable only within a specific range (Mateos et al., 2019). Indeed, a clear understanding and verification of prediction equations with the number of samples, confidence intervals, range of valid values, and the residual standard deviation of the equations before applying them in practical conditions is crucial to avoid over-prediction and underestimation of digestibility values.

Numerous studies have been published to predict digestibility in feedstuffs and diets for poultry by regression equations using the wet chemical composition of the feedstuffs and diets (NRC, 1994; Cerrate et al., 2019; Pedersen et al., 2021). Specifically, there are many studies available for the prediction of energy values in poultry feedstuffs (Alvarenga et al., 2011; Alvarenga et al., 2013a; Alvarenga et al., 2013b; Carré et al., 2013; Meloche et al., 2013; Yegani et al., 2013; Carre et al., 2014; Meloche et al., 2014; Alvarenga et al., 2015; Barzegar et al., 2019; Wu et al., 2019; Noblet et al., 2022). Some authors have published equations for the prediction of

ileal digestible content of AA in soybean (Sheikhhasan et al., 2020b) and in corn (Sheikhhasan et al., 2020a).

The best prediction equations for various digestibility parameters and AME for poultry from some of the previous studies are presented in Table 2.2. The fitness of the models could be accessed from the R^2, RMSE, and residual standard deviation (RSD) of prediction and validation statistics. Overall, the negative impact of different fibre fractions such as crude fibre (CF), hemicellulose (HC), and neutral detergent fibre (NDF), could be seen throughout Table 2.2 for prediction of energy as well as digestibility of nutrients. The most included variables for the prediction of nitrogen-corrected apparent metabolisable energy (AMEn) were fibre fractions, ether extract (EE), starch, ash, and crude protein contents. Moreover, a clear positive impact of EE, crude protein (CP), and GE on the prediction of AMEn could be evidenced (Batal and Dale, 2006; Losada et al., 2009; Silva et al., 2010; Meloche et al., 2013). A positive effect of starch and a negative effect of CF and NDF could be seen for the prediction of protein digestibility in broiler diets (Cerrate et al., 2019; Pedersen et al., 2021).

Even though there are many equations for predicting the energy values of feed ingredients available in many previous publications, there is a lack of equations for predicting the nutritive value of complex broiler diets. In addition to that, there is inadequate information about the validation strategies used, which questions the practical application of the equations under all conditions.

Table 2.2. Prediction equations for ileal digestibility of nutrients, digestible nutrient contents, and energy values using chemical composition data.

Feedstuff/diet	Parameter	Equation	n	R^2	RMSE	RSD	Regression technique	Validation method	R^2v	RMSEv	Reference
Wheat by-products	AMEn	3086 −165CF	15	0.77							(Dale, 1996)[1]
Sunflower seed meal	TMEn	2,816.8 −109.5HC	11	0.80		70.2	Stepwise				(Villamide and San Juan, 1998)[1]
DDGS	TMEn	2732.7 +36.4fat−76.3fibre +14.5protein −26.2ash	17	0.45			GLM procedure				(Batal and Dale, 2006)[1]
Starchy grains and cereal by-products	AMEn	3,697 (±52.9) −11.7 (±4.63) NDF +57.1 (±6.58) EE −177 (±29.7) ash	94	0.79		198	Stepwise				(Losada et al., 2009)[1]
Poultry offal meal	AMEn	−2315.69 + 31.44CP + 29.77MM + 0.77GE −49.36Ca	27	0.72			Backward elimination				(Silva et al., 2010)[1]
Protein and energetic concentrates	AMEn	4101.33 +5.63EE −23.30ash −2.49aNDFom +1.04ADFom	574	0.84		0.41	Stepwise	Method by Mayer et al. (1994)			(Nascimento et al., 2009)[2] (Alvarenga et al., 2011)
Corn co-products	AMEn	3,517 +46.02EE −82.7ash −33.27HC	15	0.89	191				0.74	335	(Rochell et al., 2011)[1]
	AMEn	−30.19NDF +0.81GE (kcal/kg) −12.26CP	15	0.87	96				0.78	380	
Reduced oil corn DDGS	AMEn	−12,282 +2.60GE+89.75CP +125.80starch −40.67TDF	15	0.90	99		Stepwise	External	0.80	488	(Meloche et al., 2013)[1]
	AMEn	−14,322+2.69GE+117.08CP +149.41starch −18.30NDF	15	0.92	90		Stepwise	External	0.71	502	
Wheat and triticale	AMEn	21.57−0.35NDF +1.52EE −0.13starch +0.10CP	8	0.99		0.05*	GLM procedure				(Yegani et al., 2013)[3]

Corn DDGS	AMEn	3,673 −121.35CF +51.29EE −121.08 ash	30	0.70	270		LASSO selection		0.62		(Meloche et al., 2014)[1]
Feedstuffs & diets	AMEn	4164.19 +51.01EE −197.663ash −35.69CF −20.59NDF	293	0.75				Method by Mayer et al. (1994)			(Alvarenga et al., 2015)[2]
Layer diets	AME	3033 +396EE +5.6starch −27.1ash	16			67	Stepwise				(Barzegar et al., 2019)[1]
	NE	0.781AME −11CP +16.4EE	16			93					
Broiler diets	IDC CP	−3.35 +0.782CP −0.121[CP−28.9]	45	0.59		2.2	Stepwise BIC, FS				(Cerrate et al., 2019)[4]
	CAID CP	0.92CP +1.66EE −0.81NDF +1.10starch	45	0.99		7.3					
	IDC fat	−0.641+0.889EE	45	0.99		0.4					
	CAID fat	2.019CP+0.524EE+0.994NDF	45	0.99		0.7					
	CAID starch	85.6 −0.48NDF	45	0.10		3.7					
	ME	54.3dCP +97.0dEE +46.1dCHO	40	0.99		60					
	NE	0.43ME +9.5CP +28.2EE +14.1starch −6.0NDF	80	0.99		140					
Broiler diets	IDE	0.73 +0.002starch −0.02CF −0.04phytate	56	0.77	0.05		PCA and PLS	External (n=34)	0.34	0.03	(Pedersen et al., 2021))[5]
	IDP	0.690 +0.001starch −0.011CF +0.003fat	56	0.42	0.06				0.31	0.03	

ADFom, acid detergent fibre exclusive of residual ash; AME, apparent metabolizable energy; AMEn, nitrogen-corrected apparent metabolisable energy; aNDFom, neutral detergent fibre assayed with heat stable amylase exclusive of residual ash; BIC, Bayesian information criteria; Ca, calcium; CAID, coefficient of apparent ileal digestibility; CF, crude fibre; CP, crude protein; dCP, digestible protein; dEE, digestible fat; dCHO, digestible dietary carbohydrate; DDGS, dried distiller grains with solubles; EE, ether extract; FS, forward selection; GE, gross energy; HC, hemicellulose; IDC, ileal digestible content; IDE, ileal digestibility of energy; IDP, ileal digestibility of protein; ME, metabolisable energy; MM, mineral matter; n, number of samples; NDF, neutral detergent fibre; NE, net energy; PCA, principal component analysis; PLS, partial least square regression; R^2, coefficient of determination; R^2v, coefficient of determination of validation; RMSE, root mean square error; RMSEv, root mean square error of validation; RSD, residual standard deviation; TMEn- nitrogen corrected total metabolisable energy; TDF, total dietary fibre.

[1] AMEn/TMEn (kcal/kg DM) and variable % DM; [2] AMEn = MJ/kg DM and variables % DM; [3] AMEn MJ/kg DM and variables g/kg DM; [4] all values expressed as % DM, ME/NE = kcal/kg DM; [5] independent variables unit % DM; * SEP, standard error of prediction.

2.8. Infrared spectroscopy

Infrared (IR) spectroscopy is an analytical technique that serves both quantitative and qualitative purposes. The infrared spectrum (12,500-10 cm^{-1}) is divided into far-infrared (400-10 cm^{-1}), mid-infrared (4000-400 cm^{-1}), and near-infrared (12,500-4000 cm^{-1}). It is not possible to use spectroscopy in the entire region as a single spectroscopy, therefore, far-infrared spectroscopy (FIRS), mid-infrared spectroscopy (MIRS), and near-infrared spectroscopy (NIRS) have been developed over the years (Ozaki, 2021). Infrared spectroscopy is based on the principle that the chemical bonds in organic molecules absorb or emit infrared light when their vibrational state changes (Van Kempen, 2001). Thus, it can provide a more rapid non-invasive and chemical-free technique for animal feed analysis (Campbell et al., 2022). Each type of spectroscopy has its own advantages and disadvantages (Griffiths, 2006).

The NIRS spectroscopy has been widely used in the animal nutrition field (Givens and Deaville, 1999) compared to MIRS. However, during the last decades it has become increasingly evident that MIRS has considerable potential for industries applications in the food, feed, and related industries (Ellis et al., 2012). The principles and use of NIRS could be referred by many previous publications which are not discussed in this Chapter (Cruz-Conesa, 2023). Further information on the uses of MIRS in other applications except animal nutrition studies could be referred to other publications (Karoui et al., 2010; Ellis et al., 2012; Bresolin and Dórea, 2020; Adiamo et al., 2021; Ozaki, 2021).

2.9. Mid-Infrared Spectroscopy (MIRS)

2.9.1. Principles of MIRS

Mid-infrared spectroscopy is one type of vibrational spectroscopy in the wavenumber range of 400-4000 cm^{-1}. Mid-infrared (MIR) region contains information about the fundamental

vibrations of molecules, and it is a highly sensitive method (Ozaki, 2021). This region contains information on chemical functional groups, thus allowing organic molecules to be identified and the structure and confirmation of molecules such as proteins, polysaccharides, and lipids to be characterised (Sun, 2009). Detailed study of the band assignments to MIR region are reported in previous publications (Sun, 2009; Karoui et al., 2010).

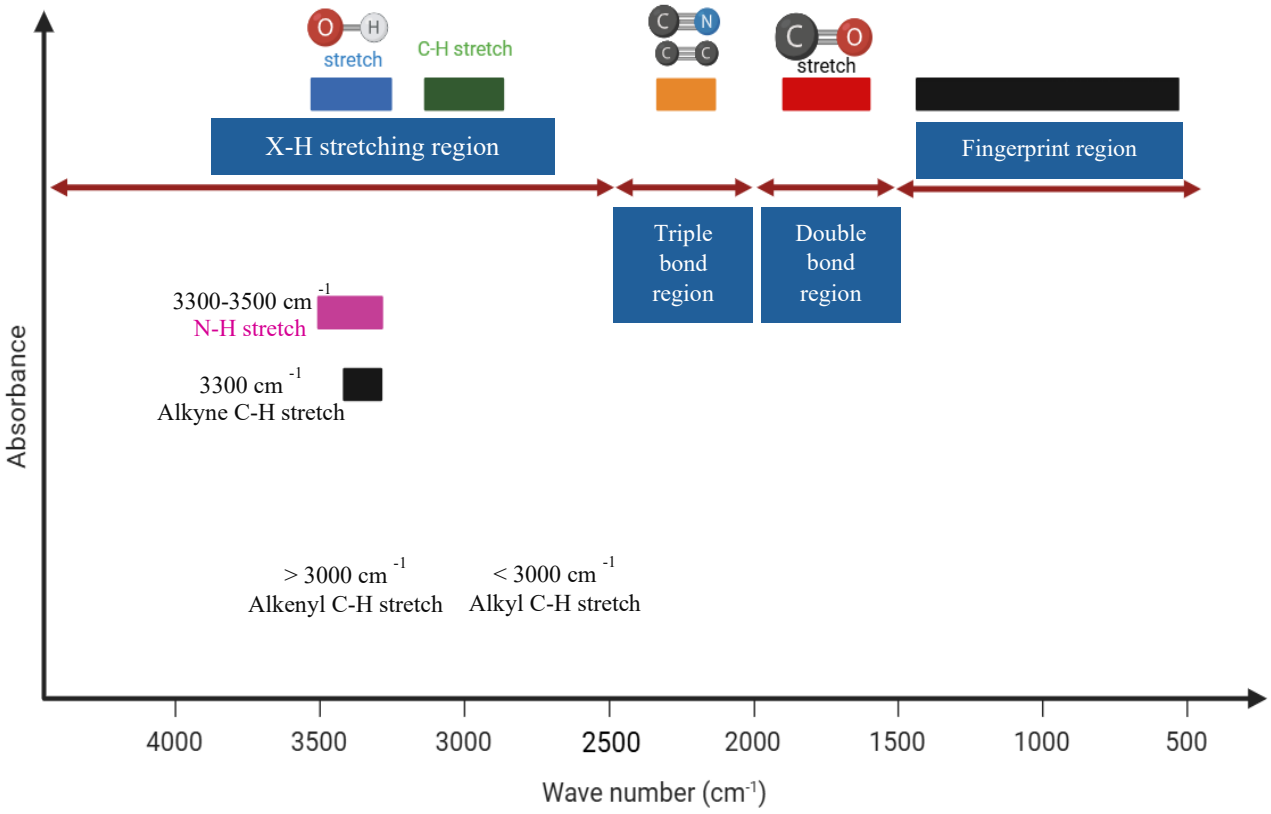


Figure 2.4. Typical mid-infrared absorption for various types of bonds adapted from Johnson et al. (2023b).

The MIR region can be divided into four broad regions (Figure 2.4): the X-H stretching region (4000-2500 cm^{-1}), the triple bond region (2500-200 cm^{-1}), the double bond region (200-1500 cm^{-1}), and the fingerprint region (1500-400 cm^{-1}) (Karoui et al., 2010). It could be further broken down to C-H_x stretching vibrations from fatty acids (3050-2800 cm^{-1}); C=O, N-H, and

C-N from proteins and peptides (1750-1500 cm^{-1}); C-O and C-O-C from polysaccharides (1200-900 cm^{-1}); and P-O vibrations from nucleic acids (1245-1220 and 1090-1085 cm^{-1}) (Ellis et al., 2012). The MIR spectral regime not only provides molecular sensitivity but benefits in cost, sample volume, and compactness from considerably decreasing system dimensions (Haas and Mizaikoff, 2016).

2.9.2. Major steps involved in the development of MIRS calibration models

The major steps involved in the calibration of MIRS models are shown in Figure 2.5 which will be discussed in detail.

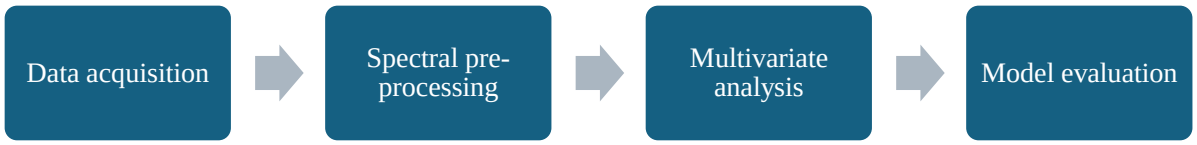


Figure 2.5. Schematic representation of mid-infrared spectral modelling.

2.9.2.1. Data acquisition

Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR)

Fourier transform infrared spectroscopy (FTIR) is an efficient, rapid, and non-destructive way of obtaining an infrared spectrum (Petit and Madejova, 2013; Dutta, 2017). It has replaced the conventional dispersive spectrometers given that modern computer technology allows the execution of fast Fourier transform algorithms in real-time (Haas and Mizaikoff, 2016). There are a variety of sample presentation methods available in IR spectroscopy to analyse a wide range of sample types (Johnson et al., 2023b). The traditional method is by transmittance which allows the IR light to enter one side of the sample while some wavelengths are absorbed, and the remaining light is measured as it exits the other side of the sample. In addition to this, there is a range of technologies available for analysing samples in various states and forms. These include attenuated total reflectance (ATR), infrared reflection absorption, and diffuse reflectance infrared fourier transform spectroscopy (DRIFTS) (Karoui et al., 2010; Haas and

Mizaikoff, 2016). In reflection mode, the IR light enters one side of the sample and interacts with the sample matrix by penetrating the sample (Johnson et al., 2023b). Reflection mode induces less heat compared to transmission mode (Agelet and Hurburgh, 2010) and offers flexibility for easy handling of solid samples and better repeatability (Johnson et al., 2023b).

ATR is one of the simpler non-invasive MIR techniques in which a solid or liquid sample is placed in intimate contact with the top horizontal surface of a crystal of high refractive index (made with germanium, diamond, zinc selenide etc.). It measures changes in intensity that occur in a totally internally reflected infrared beam when the beam comes into contact with the sample (Karoui et al., 2010) (Figure 2.6). ATR-FTIR probes the sample using an evanescent wave established at the sample-crystal interface. The evanescent wave propagates along the interface, but decays exponentially in the non-crystal side. The choice of crystal affects the penetration of the evanescent wave (Cleland, 2018). FTIR coupled with ATR offers the rapid and easy extraction of information on a small amount of samples and has been preferred across a wide range of applications (Sun, 2009; Haas and Mizaikoff, 2016; Cleland et al., 2018; Shi et al., 2019; Wang et al., 2021b). In recent times, the ATR-FTIR in the MIR region has been widely applied in routine chemical analysis.

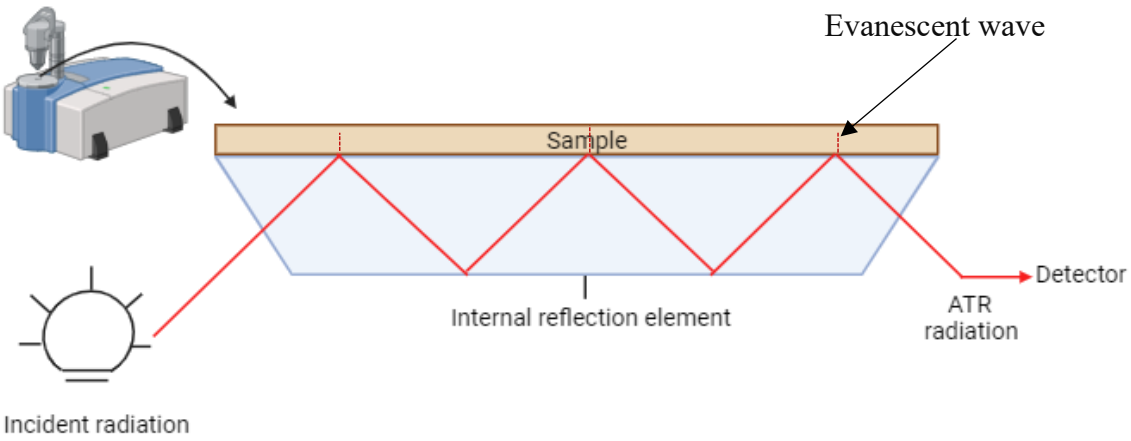


Figure 2.6. The ATR-FTIR technique adapted from Karoui et al. (2010).

2.9.2.2. Mid-infrared spectral pre-processing

The analysis of mid-infrared spectra usually requires pre-processing to minimise the effects of sources of spectral variability such as baseline shift, trend, noise, scattering etc. This may be introduced by many factors such as the particle size of the sample, chemical interference, data collection method, etc. Pre-treatments remove unwanted signals from high-dimensionality data before modelling. The main aim of pre-processing is to transform data in a way that the signals will better adhere to Beer's law as follows,

$$A = \varepsilon l c \tag{2.8}$$

where, A is the absorbance, ε is the molar absorptivity, l is the path length, and c is the concentration of the constituent of interest stating that absorbance and concentration are linearly correlated (Sun, 2009). Different pre-processing techniques are shown in Figure 2.7.

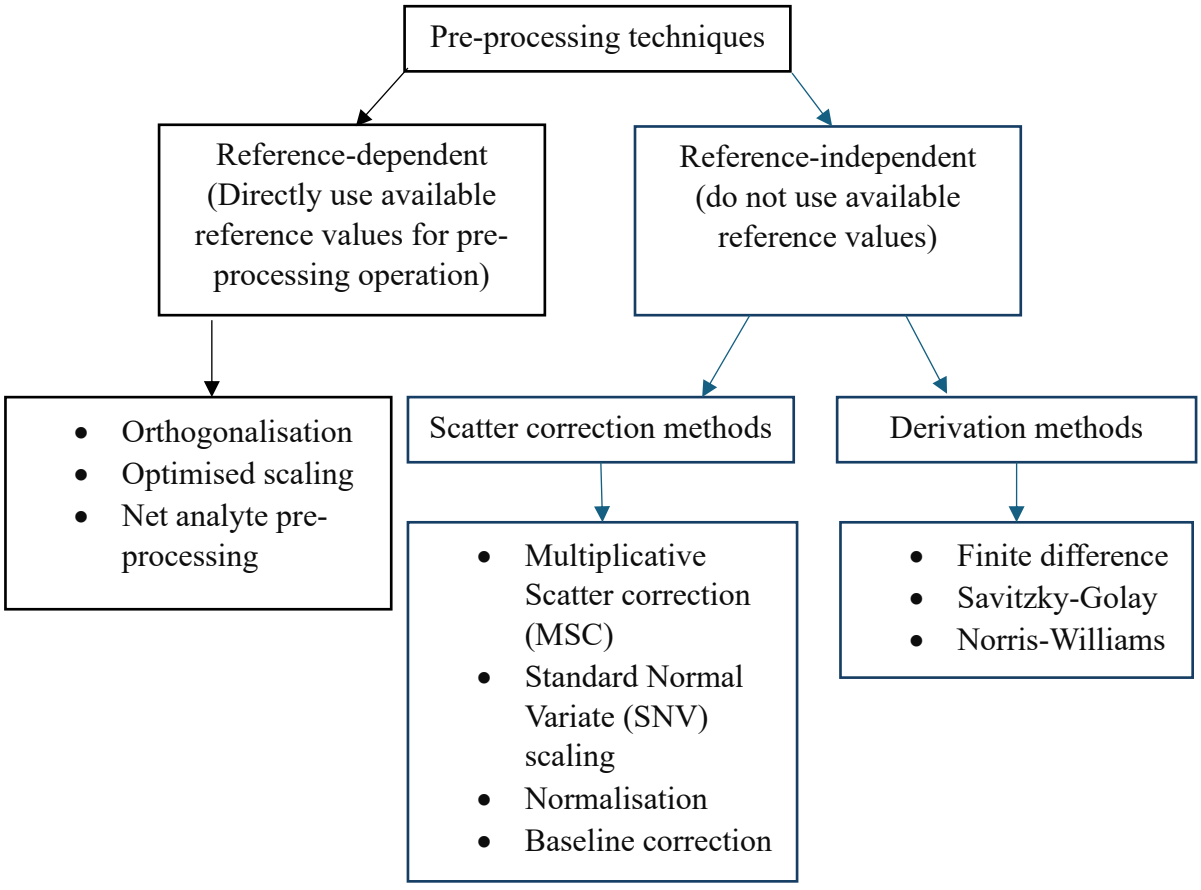


Figure 2.7. Spectral pre-processing methods adapted from Sun (2009).

Scatter correction methods are usually employed to minimise light scattering and derivations methods are used to minimise baseline variations. The derivative methods use a smoothing of spectra before calculating derivative to decrease the negative impact on signal-to-noise ratio. The first derivative removes the baseline, while the second eliminates the linear trend. The multiplicative scatter correction (MSC) removes the undesirable scatter effect by estimating the correction coefficients and correcting the recorded spectrum (Rinnan et al., 2009). The standard normal variate scaling (SNV) consists of mean centering and standard deviation scaling to give mean equal to 0 and a standard deviation equal to 1 (Cleland et al., 2018).

Savitzky-Golay (SG) (Savitzky and Golay, 1964) is a popular method for numerical derivation of a vector that includes a smoothing step. The SG uses a polynomial function of a specific degree to smooth a window of spectral points. Once the parameters of this polynomial are calculated, the derivative of any order of this function is applied to the central point of the window. The first derivative improves the resolution of spectra, and the second derivative gives a negative peak for each band and shoulder (De Marchi et al., 2014). This process is carried out for each point in the spectrum. The number of points used to calculate the polynomial window size and degree of the fitted polynomial need to be carefully selected (Rinnan et al., 2009). The SG filter could be used in combination with other scatter correction methods.

Reference-dependent methods comprise primarily those techniques that orthogonalise the data with respect to a reference of interest. Such methods are generally not applicable and will require special attention as the response variables are actively used in the modelling (Sun, 2009). The selection of pre-processing methods is not a straightforward task. The pre-processing methods are often combined and the optimum chemometric data pre-processing technique may vary depending on the sample matrix or analyte used. Apart from the above-mentioned methods, wavelet transform techniques are also utilised, including discrete wavelet transform (DWT) and wavelet packet transform algorithms, which involve smoothing,

dimension reduction, and noise removal. Detailed information about the data pre-processing techniques for IR spectroscopy is available in many previous works (Rinnan et al., 2009; Sun, 2009; Lee et al., 2017; Morais et al., 2020).

2.9.2.3. Data analysis methods

Spectral pre-processing is followed by the implementation of multivariate analysis. The well-known multivariate techniques used in IR analysis are principal component analysis (PCA) and partial least square regression (PLSR). The PCA is a technique for reducing the amount of data when there is a correlation present, by the projection of highly correlated variables into a small set of uncorrelated variables called the principal components. The PCA results are usually presented as scores and loading plots (Miller and Miller, 2005). It is carried out before multivariate analysis to find groups of samples and to detect outliers.

Multivariate calibration refers to the process of relating the analyte concentration or measured value of the physical or chemical property to a measured response (Lavine and Workman, 2010). The PLSR is employed to build the calibration model thereby correlating the pre-processed spectral data with the reference data. It is a variant of multiple linear regression method suited for high multicollinearity data such as IR spectra. The PLSR uses linear combinations of predictor variables called components (nc) or latent variables rather than the original variables. The linear combinations are generated such that they map onto the response variables and still capture the variation of the predictors (Miller and Miller, 2005). The latent variables in PLSR are developed simultaneously along with the calibration model, so that each latent variable is a linear combination of the original measurement variables rotated to ensure maximum correlation with the information provided by the property variable (Lavine and Workman, 2010).

Choosing nc is the key step in the calibration process. When a smaller nc is used than the optimal value, this can result in underfitting and lead to large errors. On the other hand, using a larger nc value can lead to overfitting thus leading to errors and too much noise (Sun, 2009). Cross-validation (CV) is one of the most widely used methods to find the optimum nc and to assess the goodness of fit of the model. The *k-fold* CV involves randomly dividing the set of observations into *k* groups or folds of approximately equal size. The first fold is used as a validation set and the method is fit on the remaining *k*-1 folds. This process is repeated *k* times with different groups of observations serving as a validation set each time. The mean squared error (MSE) is computed on the observations in the held-out fold and these values are then averaged (James et al., 2013). *Leave-one-out* CV (LOOCV) is a special case of *k-fold* CV where k is equal to the number of data points. This implies that each data point is used as a test set once, while the remaining points serve as the training set.

Other than PLSR, artificial neural networks (ANN), support vector machines (SVM), and deep learning are some machine learning approaches that have gained popularity in recent years. These methods extract the desired information and optimise model weighting by finding patterns within the data. When more data points are added to the dataset, the model could update over time to provide more accurate prediction results (Johnson et al., 2023b). The PLSR is widely used in the calibration of infrared spectroscopy and to determine the nutrient content of feedstuffs, compound feeds, and animal faeces (Cruz-Conesa, 2023).

2.9.2.4. Outlier removal

Any observation not fitting the model can be referred to as outliers which should be removed from the dataset before analysis. The outliers were spectra corresponding to the background noise detected in the experimental set-up while placing the sample in the ATR device. Outliers could be detected using several methods such as Mahalanobis distance, Hotelling's T^2, and Q

residuals. The Mahalanobis distance is used for spectral matching for detecting outliers during calibration or prediction and for detecting extrapolation of the model (Mark and Workman, 2018).

Hotelling T^2 value measures the sum of normalized squared scores which is the distance from the multivariate mean to the sample projection onto the principal component space. Q residuals is the sum of squares of each sample in the PCA error matrix, representing the residuals between a sample and its projection on to the principle components space (Morais et al., 2020). Hotelling's T^2 versus Q residuals is one of the popular visually intuitive techniques for outlier detection. A chart is created using the Hotelling T^2 values on the *x*-axis and Q residuals on the *y*-axis generating a scatter plot. Samples with high values in both Hotelling's T^2 and Q residuals are the outliers (Figure 2.8). This could also be used to investigate the possible sources for abnormalities in the samples.

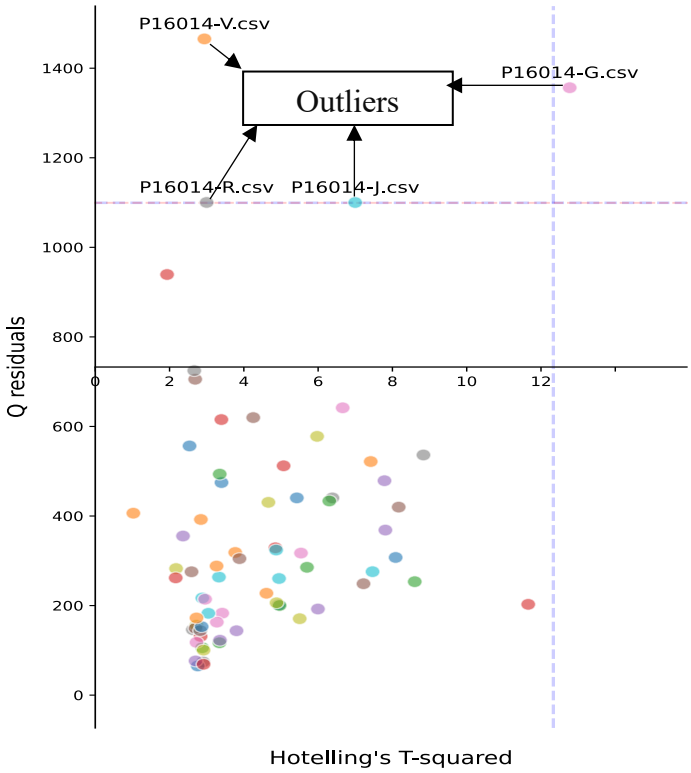


Figure 2.8. Example of using Hotelling's T^2 versus Q residuals plotting.

2.9.2.5. Assessment of calibration models

The purpose of calibration is to calculate the parameters within the model for a property, thus unknown samples could be accurately determined in the future with minimal deviation from the actual values. Therefore, the quality of the calibration models should be accessed. The most widely used statistics to calculate the accuracy and performance of the models are coefficient of determination (R^2), root mean square error of prediction (RMSEp), and relative performance deviation (RPD).

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(\hat{Y}i - Yi)^2}{\sum_{i=1}^{n}(\bar{Y} - \hat{Y}i)^2} \quad (2.9)$$

$\hat{Y}i$ is the predicted i^{th} value, Yi the observed i^{th} value $\bar{Y}$ is the mean of the actual value.

The R^2 can be interpreted as the proportion of the variance in the dependent variable that is predictable from the independent variables (Chicco et al., 2021) i.e. an estimate of the percentage of explained variance of the reference values.

$$\text{RMSEp} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \quad (2.10)$$

$$\text{SEp} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i - bias)^2}{n-1}} \quad (2.11)$$

$$\text{Bias} = \frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)}{n} \quad (2.12)$$

$$\text{RPD} = \frac{\text{SDm}}{\text{SEp}} \quad (2.13)$$

where, $\hat{Y}_i$ and Y_i are the predicted and observed (measured) values respectively, n is the number of data, SDm is the standard deviation of the measured value, and SEp is the standard error of prediction. Usually, a model is considered accurate if its RMSEp value does not exceed 1.4 times the standard error (SE) of the laboratory values (Sun, 2009).

The closer the R^2 to 1, and the lower the RMSEp the better the fit (Ferreira et al., 2014; Noel et al., 2022). More precisely $R^2 > 0.90$ is excellent, $R^2 = 0.70$-0.90 is good, and $R^2 < 0.70$ is insufficient. The RPD explains the ability of the model to predict future samples in relation to the initial variability of the data (Agelet and Hurburgh, 2010). The RPD value of < 2 is not suitable for prediction; values between 2.0-2.4 are acceptable for screening purposes or qualitative evaluation (e.g. high, medium, or low concentration of a particular nutrient); values between 2.5-2.9 are useful for quantification; and values ≥ 3 indicate high accuracy for quantitative analysis (Belanche et al., 2013; Lyons et al., 2016).

2.9.3. Advantages and disadvantages of MIRS

Significant research undertaken in recent years has shown that the MIR region is particularly information rich and MIR spectra are easier to interpret compared to the NIR spectra. It could detect chemical components at much lower levels in a mixture, as MIR is strongly absorbed by the sample (Cleland et al., 2018). Applying appropriate analysis methods provides higher performance of MIRS over NIRS in analysis of complex mixtures (Cleland et al., 2018) with the confident assignment of spectral bands to functional groups (Wang, 2014).

The FT-MIR with ATR has several advantages including better sensitivity, resolution, speed of analyses, and it doesn't need traditional sample preparation used for transmission FTIR spectroscopy. Indeed, the ATR technique requires minimal sample preparation and needs only a small quantity of samples. However, ATR allows a shallow penetration depth of 2-5 μm, which may be smaller than some particle sizes of components (Cleland et al., 2018). In addition to that, high surface homogeneity is needed for accurate measurements as the measurement is taken essentially at a contact surface for ATR (Karoui et al., 2010). Chemical reagents are not needed to prepare samples for ATR-FT MIRS as in wet chemistry methods, thus reducing the cost and the environmental pollution due to the use of various solvents.

The spectra could be recorded in less than a minute and could be used for the analysis of all parameters simultaneously. It reduces the huge time spent on laboratory analysis and the requirement of skilled labors. Another advantage of FTIR spectroscopy is that wavelength repeatability across the instrument is good which makes the transfer of calibrations from one laboratory to another possible (Van Kempen, 2001).

Another potential limitation of FTIR spectroscopy is that the absorption of water is very intense in the mid-infrared region which could be overcome by drying of samples, subtraction of the water signal, or using ATR as the sampling method (Ellis et al., 2012). Appropriate data pre-processing techniques and filtering reduce the contribution of the water signal to the sample variance.

Moreover, the development of calibration models for animal nutrition studies requires the reference data of chemical composition and digestibility data of feedstuffs and diets which should be obtained from standard wet chemistry data and *in vivo* or *in vitro* trials. This process is costly and requires skilled labor and time. However, once the models are established, they can be used over a long period of time. Nevertheless, after development, the calibration should be regularly updated which involves chemically analysing additional samples from time to time.

2.9.4. The use of MIRS in poultry nutrition

The mid-infrared region is popular for organic chemical analysis (Agelet and Hurburgh, 2010). It provides more detailed chemical information on sample composition than NIRS (Lyons et al., 2016). Earlier MIR spectra were collected with transmission methods which limited its wide application due to the precise requirement of sample thickness. In recent times to overcome this drawback, ATR has been preferred to transmission, thus providing a rapid, low-

cost, and non-destructive analysis with ease of application. In addition to that, the use of multivariate analysis to obtain spectral information aids in the wide applicability of MIRS.

Indeed, MIRS has been routinely used to predict milk composition, feed efficiency, methane emission, fertility, energy balance, and health status in the dairy and beef sector (Bresolin and Dórea, 2020) and to measure the chemical composition of grains, fruits, meat, and other agricultural products (Adiamo et al., 2021). During the past decade, it has been proven that ATR FT-MIRS could determine various properties of food products (Sun, 2009; Karoui et al., 2010; Ellis et al., 2012; Johnson et al., 2023b).

The animal feed industry has used the NIRS for many years with varying degrees of success to predict chemical composition of feedstuffs, compound feeds, and faeces, nutrient digestibility, as well as feed intake in ruminants and monogastrics (Van Kempen and Bodin, 1998; Pérez-Marín et al., 2004; Bastianelli et al., 2005; Agelet and Hurburgh, 2010; Bastianelli, 2013; Khaleduzzaman et al., 2017; Samadi et al., 2020; Evangelista et al., 2021; Nieto-Ortega et al., 2022; Noel et al., 2022). This will not be discussed in this literature review.

Owing to the benefits of ATR-FT MIRS, it could also be applied in the poultry feed industry as an additional tool to predict the chemical composition and digestibility using direct and indirect approaches. However, there is a lack of research on this aspect even though various other sectors have successfully utilised ATR-FT MIRS for routine application.

Mid-infrared spectroscopy has been applied successfully in ruminant nutrition for the prediction of forage composition (Cleland et al., 2018). In addition to that, MIRS has been used to predict the protein composition and its digestibility using undigested residue in pigs (Wang et al., 2013). The MIRS was also used to determine faecal composition, intake, and digestibility in sheep (Lyons et al., 2016). There are some studies published where MIRS has been used for the prediction of chemical composition of feedstuffs, for example, CP and moisture content

and protein digestibility of wheat (Shi and Yu, 2017; Shi et al., 2019), sugars in barley (Cozzolino et al., 2014a; Huang et al., 2016), nutrient composition of soybean (Ferreira et al., 2014) and wheat bran (Hell et al., 2016), the amino acid content in animal meals (Qiao and Van Kempen, 2004).

To date, only a few studies on the use of ATR-FT MIRS to analyse the chemical composition and nutritive value of compound broiler diets have been published. Mahesar et al. (2011) determined the fatty acid ratio in poultry feed lipids using MIRS. Moreover, no commercial ATR-FT MIRS calibrations are available to predict the gross chemical composition of poultry ileal digesta and excreta or digestibility of nutrients. Even though MIRS is widely applied in other fields, its application in the poultry industry is limited and needs to be investigated.

2.9.4.1. Prediction of chemical composition of feedstuffs and diets by MIRS

The present literature covers the studies available with MIRS only for the prediction of the chemical composition of feedstuffs and diets as numerous studies have been published on the use of NIRS in the prediction of the chemical composition of feedstuffs and compound feeds for monogastric animals (Cruz-Conesa, 2023). Table 2.3 summarises some recent publications on the use of MIRS to determine the chemical composition and digestibility of feedstuffs.

The main parameters investigated are moisture, CP, AA, fatty acids, fibre, and starch (Table 3). Different spectral pre-treatments have been used and most of the studies used ATR for sample presentation. Moisture content was successfully predicted in most of the cases. However, the R^2cv, R^2p (< 0.50) and RPD (< 2.0) for the prediction of moisture content in faba bean was low without any spectral pre-treatment (Johnson et al., 2023a). Shi et al. (2019) successfully predicted the CP content in wheat with R^2 calibration and prediction > 0.9 and RPD > 4.0 using the transmission mode MIRS. Ferreira et al. (2014) also obtained good results for the prediction of ash and protein content in soyabean (R^2 > 0.80), however, the prediction for moisture, and

lipids was comparatively low ($R^2 < 0.70$). Indeed, protein and AA obtained quality predictions in most cases. Mahesar et al. (2011) achieved the successful prediction of fatty acids in poultry feed lipids ($R^2c > 0.90$). Lipid content was better predicted in wheat bran, and compound feed lipids with high lipid content, than in barley seeds and soybean, which have comparatively a low lipid content. Usually, fibre components have high errors due to the reference values in which several chemical analysis procedures are used to determine fibre components. It should be noted that the accuracy of references used is one of the factors that could affect the accuracy of predictions (Prieto et al., 2009).

There was no literature available for the prediction of energy content in feedstuffs using MIRS. Energy is not a chemical aspect, but the energy content could be predicted by MIRS due to the correlation with fat and starch. The same could be applied for ash which could be predicted due to its correlation with other organic molecules. Minerals themselves do not absorb IR light. In cases, where a mineral correlates with an organic compound, quantification may be possible. In the mid-infrared spectrum, salts containing sulphate, phosphate or carbonate have different IR spectra depending on the cation present. No data are present on the minimum limits for the quantification of minerals (Van Kempen, 2001). There are no studies using MIRS for the prediction of mineral content in animal feeds. However, the prediction of the mineral contents especially Ca and P in poultry feeds using NIRS has been reported (Valdes et al., 1985; Aureli et al., 2017; Khaleduzzaman et al., 2017; Khaleduzzaman and Salim, 2020).

Table 2.3. Applications of MIRS calibration models to predict the chemical composition and digestibility of feedstuffs and diets for poultry and pig using the feed spectra.

Reference	Sample	n	Measurement mode	Pre-treatment	Parameter /Analyte	nc	R^2c	R^2cv	R^2p	$R^2v(E)$	RMSEc	RMSEcv	RMSEp	RPD
# (Qiao and Van Kempen, 2004)	Meat and bone meal	40	Reflectance	SG 1st deri	Cysteine%	7	0.84	-	-	-	-	-	0.04	-
		31			Histidine%	3	0.41	-	-	-	-	-	0.13	-
		43			Isoleucine%	6	0.61	-	-	-	-	-	0.13	-
		44			Leucine%	6	0.86	-	-	-	-	-	0.18	-
		42			Lysine%	4	0.61	-	-	-	-	-	0.27	-
		37			Methionine%	7	0.73	-	-	-	-	-	0.07	-
		42			Phenylalanine%	6	0.75	-	-	-	-	-	0.11	-
		50			Threonine%	9	0.59	-	-	-	-	-	0.19	-
		40			Valine%	6	0.74	-	-	-	-	-	0.14	-
#(Calderón et al., 2009)	Triticale and pea	194	Reflectance	MSC 1st deri	NDF g/kg	2	0.59	-	-	0.38	-	-	-	-
				MSC 1st deri	ADF g/kg	3	0.87	-	-	0.76	-	-	-	-
				MSC 1st deri	Total N g/kg	4	0.94	-	-	0.88	-	-	-	-
(Mahesar et al., 2011)	Poultry feed lipids	17	Reflectance	SG 1st deri	n-6	5	0.99	-	-	-	0.10	1.67	1.00	-
					n-3	3	0.99	-	-	-	0.02	0.08	0.06	-
					n-6:n-3	4	0.99	-	-	-	0.13	1.49	0.83	-
#(Cozzolino et al., 2014a)	Barley seeds	103	Reflectance	SNV/ SG 2nd deri	Palmitic%	4	0.45	-	-	0.30	-	-	-	1.0
					Stearic%	4	0.76	-	-	0.45	-	-	-	1.4
					Oleic%	4	0.75	-	-	0.54	-	-	-	1.7
					Total lipids%	6	0.89	-	-	0.60	-	-	-	1.8
*(Ferreira et al., 2014)	Soybean	40	Reflectance	1st deri MC	Moisture%	5	0.63	-	-	-	0.13	0.47	0.41	-

				SNV+MC	Ash%	7	0.87	-	-	-	0.06	0.41	0.25	-
				SNV+MC	Protein%	8	0.91	-	-	-	0.39	1.74	1.06	-
				SNV+MC	Lipid%	8	0.67	-	-	-	0.14	1.44	1.87	-
(Hell et al., 2016)	Wheat bran	44	Reflectance	None	Water%	10	0.91	-	-	0.79	0.84	1.10	-	-
			ATR-FTIR	MSC	Protein%	8	0.82	-	-	0.75	0.78	0.82	-	-
				Line subtraction	Ash%	10	0.95	-	-	0.85	0.27	0.41	-	-
				Offset correction	Starch%	10	0.82	-	-	0.82	2.38	2.32	-	-
				Min/max correction	Soluble DF%	9	0.66	-	-	0.60	0.76	0.72	-	-
				2nd derivative	Insoluble DF%	6	0.98	-	-	0.82	1.04	2.79	-	-
				MSC	Lipids%	8	0.94	-	-	0.88	0.20	0.25	-	-
*(Shi et al., 2019)	Wheat	48	Transmission	MSC	CP%	5	0.95	0.90	0.96	-	0.44	0.63	0.36	4.90
			FTIR	SNV	CP%	6	0.95	0.90	0.95	-	0.44	0.64	0.37	4.80
				MSC	InPD%	2	0.78	0.69	0.49	-	1.43	1.76	1.80	1.36
				SNV	InPD%	1	0.77	0.72	0.41	-	1.48	1.68	1.80	1.36
#(Karunakaran et al., 2020) [1]	Pea seeds	117	Transmission	VN	Protein%	25	0.99	-	-	-	0.69	-	0.88	-
			FTMIR		Fibre%	15	0.97	-	-	-	2.40	-	2.99	-
					Starch%	27	0.75	-	-	-	3.84	-	7.05	-
					Phytic acid µg/g	14	0.83	-	-	-	16.94	-	19.18	-
(Johnson et al., 2023a)	Faba bean	100	Reflectance	None	Moisture%	4	-	0.47	0.09	-	-	0.23	0.46	1.38
				SNV	Protein%	2	-	0.17	0.11	-	-	0.80	1.20	1.11

*DM basis; #as-fed basis; [1]correlation coefficient (r) instead of R^2

ADF, acid detergent fibre; CP, crude protein; deri, derivative; DF, dietary fibre; InPD, intestinal protein digestibility; MC, mean centering; MSC, multiplicative scatter correction; n, number of samples; nc, number of components; NDF, neutral detergent fibre; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; R^2v(E), coefficient of determination of evaluation data set; RMSEc, root mean square error of calibration; RMSEcv, root mean square error of cross-validation; RMSEp, root mean square error of prediction; RPD, relative prediction deviation; SG, Savitzky-Golay smoothing; SNV, standard normal variate; VN, vector normalisation.

2.9.4.2. Prediction of nutrient digestibility using feed MIRS

The gold standard method for estimating the digestibility of poultry feedstuff and diet is the *in vivo* method. However, it is impossible to perform *in vivo* evaluations rapidly due to the requirement of conducting an experiment with birds. In addition to that, the diet and digestion products (ileal digesta or excreta) collected from the experiment should be analysed for their chemical composition by laboratory wet chemical methods. This overall process is costly and time-consuming (Noel et al., 2022). Being able to predict the digestibility prediction directly from the feed spectra would allow us to replace the *in vivo* experiments completely.

There were many studies with NIRS available for the successful prediction of nutrient digestibility, especially AME using the feedstuffs or diets (Valdes et al., 1985; Valdes and Leeson, 1992; Black et al., 2009; Van Barneveld et al., 2018; Noel et al., 2021; Paternostre et al., 2021; Noel et al., 2022). However, the use of MIRS for this purpose still needs to be investigated. High-quality spectra is essential to investigate nutrient digestibility quantitatively. The ATR-FT MIRS could serve this purpose with signal to noise ratio of 50,000:1 (Wang, 2014) and with data of the best quality (Van Kempen, 2001). It is technically feasible to train and predict subjective parameters such as digestibility using IR and all interested parameters could be predicted at the same time as it uses the entire spectrum to correlate with many components. This direct method relies on the accuracy of *in vivo* assays and laboratory analysis of diets, digesta, and excreta.

2.9.4.3. Prediction of chemical composition of ileal digesta and excreta using MIRS

There are no published literature available regarding the prediction of chemical composition of ileal digesta and excreta using MIRS except the study by Lyons et al. (2016) who analysed the chemical composition of sheep faeces and a study by Reeves (2001a) who predicted mineral components in the dried poultry manure. Table 2.4 summarises published data on the faecal

composition of poultry and pigs, which have been analysed mainly by NIRS. The prediction of chemical composition of poultry ileal digesta and excreta could replace the costly and time-consuming wet chemical analysis required by *in vivo* assays. It is possible to predict most analytes in the excreta using MIRS. Moreover, it could also be possible to use the predicted values of ileal digesta and excreta to calculate the nutritive value of feedstuffs and diets without spending huge costs on the wet chemistry analysis.

2.9.4.4. Prediction of digestibility using faecal spectra

It is not possible to completely determine the digestion process with the feed spectra as digestion is the interactive process between a feed and an animal (Bastianelli et al., 2013). Indeed, it could be possible to predict the digestibility using the faecal spectra which integrate more information on animal part. This could avoid the use of indigestible markers and accurate measurement of feed intake and faecal output thereby avoiding huge work involved in *in vivo* experiments (Bastianelli et al., 2005).

As shown in Table 2.4, Bastianelli et al. (2015), Schiborra et al. (2015), Nirea et al. (2018), and Paternostre et al. (2021) predicted the digestibility using the pig faeces by NIRS. However, no available literature is available for the prediction of digestibility in poultry using faecal/excreta MIRS except for the study by Lyons et al. (2016) who predicted digestibility using sheep faeces by MIRS. Their study demonstrated that dry matter digestibility could be used for rough pasture screening to access the feed quality. However, the calibration equations for energy digestibility were unsuitable for accurate predictions, which may be due to the small number of observations in the data sets.

2.9.4.5. Predicting the digestibility using the combined spectra of feed and excreta

Digestion is the interaction between the feed and the animal. Feed has chemical and physical characteristics that confer potential digestibility, while the animal uses it more or less

effectively depending on species, age, genetic potential etc. Predicting digestibility from the feed cannot represent the entire digestion process while it is doubtful that the undigested residue in the faeces provides all the necessary information on the potential digestibility of feed (Coulibaly et al., 2013). Therefore, integrating information from both the feed spectra and faeces spectra should improve the prediction of digestibility as this combines information about the feed as well as the digestion product. In recent times, research has been carried out using combined spectra of feed and faeces to predict the nutritive value of diets for poultry and pigs using NIRS (Bastianelli et al., 2013; Coulibaly et al., 2013; Paternostre et al., 2021) (Table 2.5).

A combination of spectra could be carried out in different ways such as merging, subtracting, dividing, and averaging for each of the wavelengths (Paternostre et al., 2021). The absorbance (A) value of faeces could be merged, averaged, or subtracted from the corresponding absorbance values of feed. For example, if the data set contains 100 wavelengths and corresponding absorption values for feed and faeces,

Merging/concatenation $_{(i=1\ldots100)}$: A_{ifeed}, $A_{ifaeces}$ (2.14)

Subtracting $_{(i=1\ldots100)}$: $A_{ifeed} - A_{ifaeces}$ (2.15)

Averaging $_{(i=1\ldots100)}$: $\frac{A_{ifeed}+A_{ifaeces}}{2}$ (2.16)

Dividing $_{(i=1\ldots100)}$: $\frac{A_{ifeed}}{A_{ifaeces}}$ (2.17)

Pre-treatment of spectra could be done before or after combining, which may also impact the calibration outcomes (Paternostre et al., 2021). A concatenated NIR spectra from feed and faeces obtained by Bastianelli et al. (2013) is shown in Figure 2.9.

Coulibaly et al. (2013) obtained NIRS calibration results based on feed spectra alone with relatively low precision, especially for dry matter and protein digestibility. However, they showed that faeces spectra provided better calibration, particularly for the digestibility of

protein in broilers. The concatenation of feed and faeces spectra significantly improved the prediction accuracy for dry matter digestibility. They suggested that the combination offers more accurate prediction of digestibility. The same trend of calibration results was obtained by Bastianelli et al. (2013) using NIRS for the determination of AME and starch digestibility in broiler in which the standard error of cross-validation was reduced significantly, when using the concatenated spectra of feed and excreta compared to using the feed spectra alone. This approach could enhance understanding of digestibility itself and highlight the importance of faecal information as a practical tool for evaluating feed efficiency in poultry farming (Coulibaly et al., 2013).

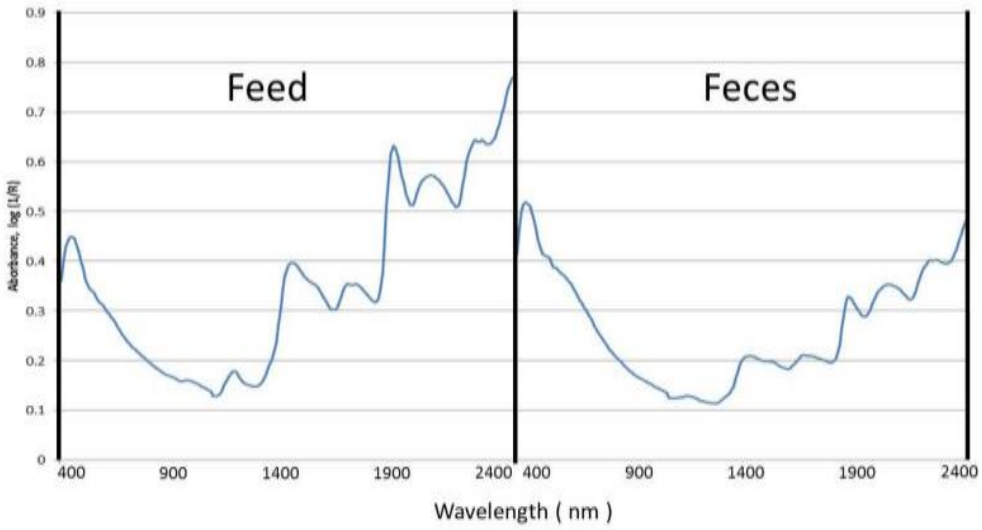


Figure 2.9. Average concatenated NIR spectrum of feed and faeces.
Source: Bastianelli et al. (2013).

Paternostre et al. (2021) showed the possibility of accurate prediction of net energy (NE) in pigs using the merged spectra and subtracted spectra of feed and faeces obtained by NIRS (R^2cv obtained for feed = 0.79, faeces = 0.59, merged feed and faeces then pre-treated together = 0.93, subtraction of faeces from feed and then pre-treated together = 0.89). They also highlighted that the pre-treatment before or after the concatenation, subtraction, or averaging

may influence the prediction accuracy. Similar results were obtained in prediction of digestibility in growing rabbits (Meineri et al., 2009) and in ruminants (Decruyenaere et al., 2009) (see Table 2.5). One drawback of this method is that the *in vivo* experiments could not be completely replaced, however, the cost of chemical analysis could be reduced to a huge extent. It should be noted that there are no previous studies available using the feed and faecal spectral combination to predict the digestibility in broilers using ATR-FT MIRS.

Figure 2.10 illustrates the entire process of implementing ATR-FT MIRS in feed mills and on farms to meet the requirements of birds.

Table 2.4. Calibration models to predict the chemical composition of faeces/excreta and prediction of digestibility using the faecal spectra by MIRS or NIRS.

Reference	Sample	n	Measurement mode	Pre-treatment	Parameter /Analyte	nc	R^2c	R^2cv	R^2p	$R^2v(E)$	RMSEc/ SEc	RMSEcv /SEcv	RMSEp/ SEp	RPD
*(Lyons et al., 2016)[1]	Sheep faeces	130	ATR-FT MIRS transmission	SG (1,4,4,1)	Ash g/kg		0.94	0.89			8.62	10.9		2.98
		130			NDF g/kg		0.88	0.85			25.6	30.7		2.49
		130			ADF g/kg		0.90	0.81			13.1	18.2		2.28
		128			Lignin g/kg		0.85	0.79			1.11	1.41		2.06
		129			DM g/kg		0.82	0.70			25.9	33.5		1.78
		128			N g/kg		0.83	0.70			0.83	1.13		1.77
		127			GE MJ/kg		0.79	0.60			0.15	0.22		1.82
		129			dDM		0.80	0.71			0.04	0.05		2.00
		129			dGE		0.90	0.66			0.03	0.03		1.67
*(Reeves, 2001a)[2]	Dried poultry manure	121	NIRS reflectance	MV, 2nd deri	Ca%	8	0.96			0.94	0.65	0.86		
				MSC, 2nd deri	Mg%	6	0.72			0.62	0.13	0.16		
				MV, 2nd deri	P as P_2O_5%	4	0.69			0.57	0.53	0.65		
			MIRS reflectance	MV, 2nd deri	Ca%	2	0.97			0.95	0.64	0.78		
				MV, 1st deri	Mg%	4	0.69			0.57	0.14	0.17		

				MV, 1st deri	P as P_2O_5%	7	0.82		0.64	0.40	0.57		
*(Smith et al., 2001) [3]	Poultry excreta	143	NIRS reflectance	SC + combinations	Moisture%			0.96	0.93		0.3	0.34	4.23
					N%			0.88	0.99		0.19	0.19	2.00
					Ca%			0.84	0.86		0.15	0.14	2.18
					P%			0.91	0.93		0.11	0.13	2.64
					GE kcal/kg			0.86	0.88		63.9	74.7	1.88
					Phytate P%			0.86	0.82		0.08	0.09	2.13
#(Xing et al., 2008)[4]	Layer manure	91	NIRS reflectance	Smooth+1st deri	Moisture g/kg	7	0.88	0.87	0.86	20.1	21.0	19.5	2.68
				SNV+ 1st deri	OM g/kg	7	0.88	0.86	0.89	6.32	6.98	6.17	2.91
				Smooth+1st deri	Total N g/kg	6	0.92	0.91	0.88	1.08	1.00	1.13	2.75
				NMSC +1st deri	Ammonium N g/kg	3	0.92	0.88	0.88	0.77	0.78	0.86	2.62
				NMSC +1st deri	Total P g/kg	4	0.81	0.78	0.80	0.60	0.60	0.56	2.01
				Smooth+SNV+1st deri	Total K g/kg	5	0.70	0.69	0.58	0.85	1.10	0.85	1.51
				SNV+1st deri	Cu mg/kg	2	0.51	0.50	0.48	2.60	2.98	2.96	1.38
				Smooth+SNV+1st deri	Fe mg/kg	5	0.77	0.74	0.55	0.12	0.12	0.15	1.47
				Smooth+SNV+1st deri	Mg mg/kg	5	0.74	0.69	0.60	0.60	0.54	0.67	1.51
				Smooth+SNV+1st deri	Na mg/kg	6	0.69	0.66	0.60	0.16	0.15	0.19	1.87
*(Bastianelli et al., 2010)[5]	Poultry excreta	229	NIRS reflectance	Normalisation, 1st & 2nd deri	Mineral matter%		0.96			0.53	0.59		4.3
		705			GE kJ/kg		0.99			133	150		8.9

		490			Starch%	1.00			0.52	0.58		13.7
		337			Crude fat%	0.99			0.38	0.42		9.5
		645			Total N%	0.95			0.14	0.16		4.2
		399			Uric acid N %	0.96			0.17	0.20		4.1
*(Bastianelli et al., 2015) [6]	Pig faeces	200	NIRS reflectance	SNV, MSC, SG no deri, 1st and 2nd deri	N%	0.88	0.85	0.60	0.08	0.09	0.13	
					ATTD DM%	0.64	0.58	0.60	0.97	1.04	1.08	
					ATTD OM%	0.80	0.69	0.66	0.79	0.97	1.04	
					ATTD N%	0.82	0.76	0.62	1.04	1.20	1.47	
					ATTD energy%	0.77	0.66	0.67	0.87	1.07	1.12	
					DE kJ/kg	0.77	0.66	0.67	167	203	213	
(De la Roza-Delgado et al., 2015)[7]	Poultry		NIRS	SNV+1st and 2nd deri	Gross calorific value cal/g	0.94	0.92	0.84	31.2	35.8	48.6	2.65
	Pig					0.97	0.94	0.85	30.3	46.4	53.6	3.51
	Poultry & pig	220				0.99	0.98	0.97	35.9	47.4	58.0	6.15
*(Schiborra et al., 2015)[8]	Pig faeces	202	NIRS reflectance	SNV & detrend + (2,4,4,1)	OM g/kg	0.86	0.73		10.0	13.7		1.9
					CP g/kg	0.84	0.74		11.8	15.0		2.0
					NDF g/kg	0.94	0.90		23.7	30.3		3.1
					ADF g/kg	0.96	0.94		15.3	20.8		3.9
					CF g/kg	0.92	0.90		12.9	14.4		3.1

					ATTD OM	0.87	0.77		0.02	0.02		2.1
					ATTD CP	0.89	0.82		0.02	0.02		2.4
					ATTD NDF	0.72	0.57		0.09	0.11		1.5
					ATTD ADF	0.80	0.61		0.07	0.10		1.6
					ATTD CF	0.77	0.71		0.08	0.09		1.9
*(Nirea et al., 2018)[9]	Pig faeces	198	NIRS	SG deri, MSC	OM g/kg	0.94	0.92	0.91	48.5	56.5	56.5	
					CP g/kg	0.89	0.89	0.89	18.1	18.8	17.9	
					GE MJ/kg	0.92	0.91	0.90	1.2	1.40	1.30	
					Fat g/kg	0.69	0.66	0.64	11.7	12.3	11.7	
					NDF g/kg	0.94	0.93		55.0	60.2		
					ATTD OM	0.94	0.91	0.94	5.5	6.7	5.5	
					ATTD CP	0.63	0.51	0.63	2.3	2.7	1.9	
					ATTD GE	0.88	0.85	0.87	2.3	2.6	2.5	
					ATTD fat	0.79	0.74	0.77	6.0	6.8	6.2	
					ATTD NDF	0.64	0.53		7.7	8.8		
*(Bedin et al., 2021) [10]	Poultry litter	160	NIRS reflectance	MC, SG	Carbon g/kg	0.93		0.93	1.34		1.30	3.78
					N g/kg	0.94		0.91	0.17		0.18	3.39
					P mg/kg	0.94		0.93	1276		1272	3.77
					K mg/kg	0.73		0.72	2550		2537	1.69

*(Paternostre et al., 2021) [11]	Pig faeces	310	NIRS reflectance	SNV, SG 1st deri	ATTD CP	11	0.79			0.02	0.03	2.0
					ATTD fat	9	0.48			0.04	0.05	1.3
					ATTD NSP	13	0.80			0.03	0.04	1.9
					ATTD CF	11	0.75			0.07	0.07	1.8
					ATTD OM	15	0.82			0.02	0.02	2.0
					NE MJ/kg	6	0.59			0.38	0.39	1.5
#(Cruz-Conesa et al., 2022) [12]	Poultry excreta	1025	NIRS reflectance	SNV, MSC, SG 1st and 2nd deri, OSC, GLSW	OM g/kg		0.97	0.95		13	14	4.6
					Protein g/kg		0.96	0.95		9.1	9.2	4.6
					Fat g/kg		0.96	0.94		3.2	4.0	3.8
					GE kcal/kg		0.94	0.93		46	49	3.7
					Uric acid g/kg		0.93	0.91		4.9	5.2	4.5
					P g/kg		0.94	0.93		0.6	0.7	3.9
	Broiler chicken	77			OM g/kg			0.74			7.7	1.9
		529			Protein g/kg			0.95			10	4.8
		154			Fat g/kg			0.96			4.1	4.9
		440			GE kcal/kg			0.90			38	3.1
		228			Uric acid g/kg			0.86			4.7	2.0
		178			P g/kg			0.93			0.8	3.8

	Laying hen	167			OM g/kg	0.87		20		2.7
		176			Protein g/kg	0.87		10		2.8
		50			Fat g/kg	0.34		5.0		1.2
		195			GE kcal/kg	0.73		80		1.9
		175			Uric acid g/kg	0.74		7.0		1.9
		133			P g/kg	0.76		1.0		1.9
*(Ramos Cruz et al., 2023) [13]	Pig ileal digesta	119	NIRS reflectance	SG 1st deri	Protein%	0.98	0.33	1.02	0.64	2.11
					Leucine%	0.95	0.04	0.12	0.10	1.70
					Lysine%	0.93	0.08	0.22	0.14	1.31
					Threonine%	0.67	0.21	0.23	0.19	1.57
					Lysine%	0.92	0.07	0.14	0.12	2.08
		222		SG 1st deri	Protein%	0.98	0.95	1.60	1.19	4.16
					Energy kcal/kg	0.94	60.8	106.3	95.3	2.53
					DM %	0.87	0.83	1.99	1.15	1.78
*(Bastianelli et al., 2005) [14]	Poultry faeces	200	NIRS reflectance	SNV, detrend, 2nd deri	GE MJ/kg	0.99	0.11	0.14		9.2
		190			dGE %	0.98	1.5	2.1		5.3

ADF, acid detergent fibre; ATTD, apparent total tract digestibility; Ca, calcium; CF, crude fibre; CP, crude protein; Cu, copper; deri, derivative; DM, dry matter; dDM, dry matter digestibility; deri, derivative; DE, digestible energy; dGE, gross energy digestibility; Fe, iron; GE, gross energy; GLSW, generalized least squares weighting; K, potassium; MC, mean centering; Mg, magnesium; MV, mean & variance scaling; MSC, multiplicative scatter correction; n, number of samples; N, nitrogen; Na, sodium; nc, number of components; NDF, neutral detergent fibre; NMSC, normal multiplicative scatter correction; NSP, non-starch polysaccharides; OM, organic matter; OSC, orthogonal

signal correction; P, phosphorus; RMSD, relative mean squared difference; $R^2v(E)$, coefficient of determination of evaluation data set; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; $R^2v(E)$, coefficient of determination of evaluation data set; RMSEc, root mean square error of calibration; RMSEcv, root mean square error of cross-validation; RMSEp, root mean square error of prediction; RPD, relative prediction deviation; SC, scatter corrections; SEc, standard error of calibration; SEcv, standard error of cross-validation; SEp, standard error of prediction; SG, Savitzky-Golay smoothing; SNV, standard normal variate.

*DM basis; #as-fed basis

[1] SEc, SEcv; [2] RMSED; [3] SEcv, SEp; [4] RMSEc, RMSEcv, RMSEp; [5] SEc, SEcv; [6] SEc, SEcv, SEp; [7] SEc, SEcv, SEp; [8] SEc, SEcv, SEp; [9]SEc, SEcv, SEp; [10] RMSEc, RMSEp; [11] SEc, SEcv; [12] Poultry excreta - RMSEc, RMSEp; broiler chicken set & laying hens set, SEc, SEp; [13] SEc SEcv, SEp; [14] SEc, SEcv.

Table 2.5. Calibration models to predict the digestibility of nutrients in different species using combined spectra of feed and faeces by NIRS.

Reference	Sample	n	Measurement mode	Pre-treatment	Parameter	Sample type	nc	R^2c	R^2cv	SEc	SEcv	RPD
(Bastianelli et al., 2013)[1]	Broiler feed+ excreta	916	Reflectance NIRS	Normalisation, Detrending, Smoothing, 2nd deri	AME kcal/kg	Feed	8	0.74		122	127	
		891				Faeces	13	0.80		108	117	
		882				Concat	13	0.95		52	57	
		267			dST%	Feed	2	0.33		2.75	2.76	
		260				Faeces	10	0.96		0.60	0.74	
		270				Concat	12	0.98		0.56	0.68	
(Coulibaly et al., 2013)[1]	Broiler feed+ excreta	931			dDM%	Feed	8	0.76		2.69	2.82	
		934				Faeces	15	0.87		2.00	2.26	
		937				Concat	13	0.95		1.29	1.35	
		519			dCP%	Feed	6	0.50		3.41	3.47	
		513				Faeces	7	0.73		2.42	2.47	
		525				Concat	9	0.75		2.33	2.50	
(Paternostre et al., 2021)[2]	Pig feed+ faeces	62	NIRS reflectance	SNV, SG 1st deri	ATTD CP	Feed	6	0.76		0.02	0.03	1.7
					ATTD fat		4	0.61		0.04	0.04	1.4
					ATTD NSP		4	0.61		0.04	0.05	1.3

	ATTD CF		5	0.72	0.06	0.08	1.4
	ATTD OM		4	0.73	0.02	0.02	1.6
	NE MJ/kg		4	0.79	0.27	0.31	1.9
310	ATTD CP	Faeces	11	0.79	0.02	0.03	2.0
	ATTD fat		9	0.48	0.04	0.05	1.3
	ATTD NSP		13	0.80	0.03	0.04	1.9
	ATTD CF		11	0.75	0.06	0.07	1.8
	ATTD OM		15	0.82	0.02	0.02	2.0
	NE MJ/kg		6	0.59	0.38	0.39	1.5
310	ATTD CP	Merged, pre-treated	10	0.86	0.02	0.02	2.39
	ATTD fat		11	0.80	0.03	0.03	1.94
	ATTD NSP		13	0.84	0.03	0.03	2.14
	ATTD CF		8	0.77	0.06	0.06	1.89
	ATTD OM		12	0.90	0.01	0.01	2.70
	NE MJ/kg		13	0.93	0.15	0.17	3.42
310	ATTD CP	Subtracted, pre-treated	12	0.84	0.02	0.02	2.21
	ATTD fat		9	0.75	0.03	0.03	1.78
	ATTD NSP		5	0.68	0.04	0.04	1.69
	ATTD CF		12	0.79	0.05	0.07	1.81

					ATTD OM		7	0.76	0.02	0.02	1.91
					NE MJ/kg		12	0.88	0.20	0.25	2.39
(Decruyenaere et al., 2009)[1]	Ruminants forage+ faeces	328	NIRS reflectance	SNVD 2nd deri	dOM	Forage		0.86	0.02	0.02	2.62
		951				Faeces		0.92	0.02	0.023	3.35
		943				Subtracted		0.90	0.02	0.02	3.10
		953				Concat		0.94	0.02	0.02	3.77
(Meineri et al., 2009)[1]	Rabbit feed+ faeces	46	NIRS reflectance	SNVD 1st deri	ATTD DM%	Feed		0.60		2.62	
		46			ATTD OM%			0.65		2.50	
		48			ATTD GE%			0.61		2.31	
		46			ATTD CP%			0.53		0.20	
		46			ATTD CF%			0.61		4.61	
		49			ATTD EE%			0.53		2.20	
		43			ATTD NFE%			0.86		1.43	
		46			ATTD ADF%			0.00		4.24	
		44			ATTD NDF%			0.00		3.89	
		59			ATTD DM%	Faeces		0.79		1.57	
		59			ATTD OM%			0.80		1.55	
		59			ATTD GE%			0.74		1.58	
		64			ATTD CP%			0.53		1.92	

56	ATTD CF%		0.82	2.99
59	ATTD EE%		0.52	2.20
58	ATTD NFE%		0.82	1.50
56	ATTD ADF%		0.45	2.78
58	ATTD NDF%		0.18	3.14
	ATTD DM%	Concat	0.76	1.74
	ATTD OM%		0.79	1.68
	ATTD GE%		0.83	1.49
	ATTD CP%		0.75	1.30
	ATTD CF%		0.82	3.06
	ATTD EE%		0.86	1.22
	ATTD NFE%		0.87	1.25
	ATTD ADF%		0.62	3.49
	ATTD NDF%		0.50	2.85

[1] DM basis; [2] Feed - as-fed basis, Faeces - freeze-dried basis.

ADF, acid detergent fibre; AME, apparent metabolisable energy; ATTD, apparent total tract digestibility; CF, crude fibre; Concat, concatenation; CP, crude protein; deri, derivative; DM, dry matter; dCP, protein digestibility; dDM, dry matter digestibility; dOM, organic matter digestibility; dST digestibility of starch; EE, ether extract; GE, gross energy; n, number of samples; nc, number of components; NSP, non-starch polysaccharides; OM, organic matter; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; RPD, relative prediction deviation; SEc, standard error of calibration; SEcv, standard error of cross-validation; SG, Savitzky-Golay smoothing; SNV, standard normal variate; SNVD, standard normal variate with detrends.

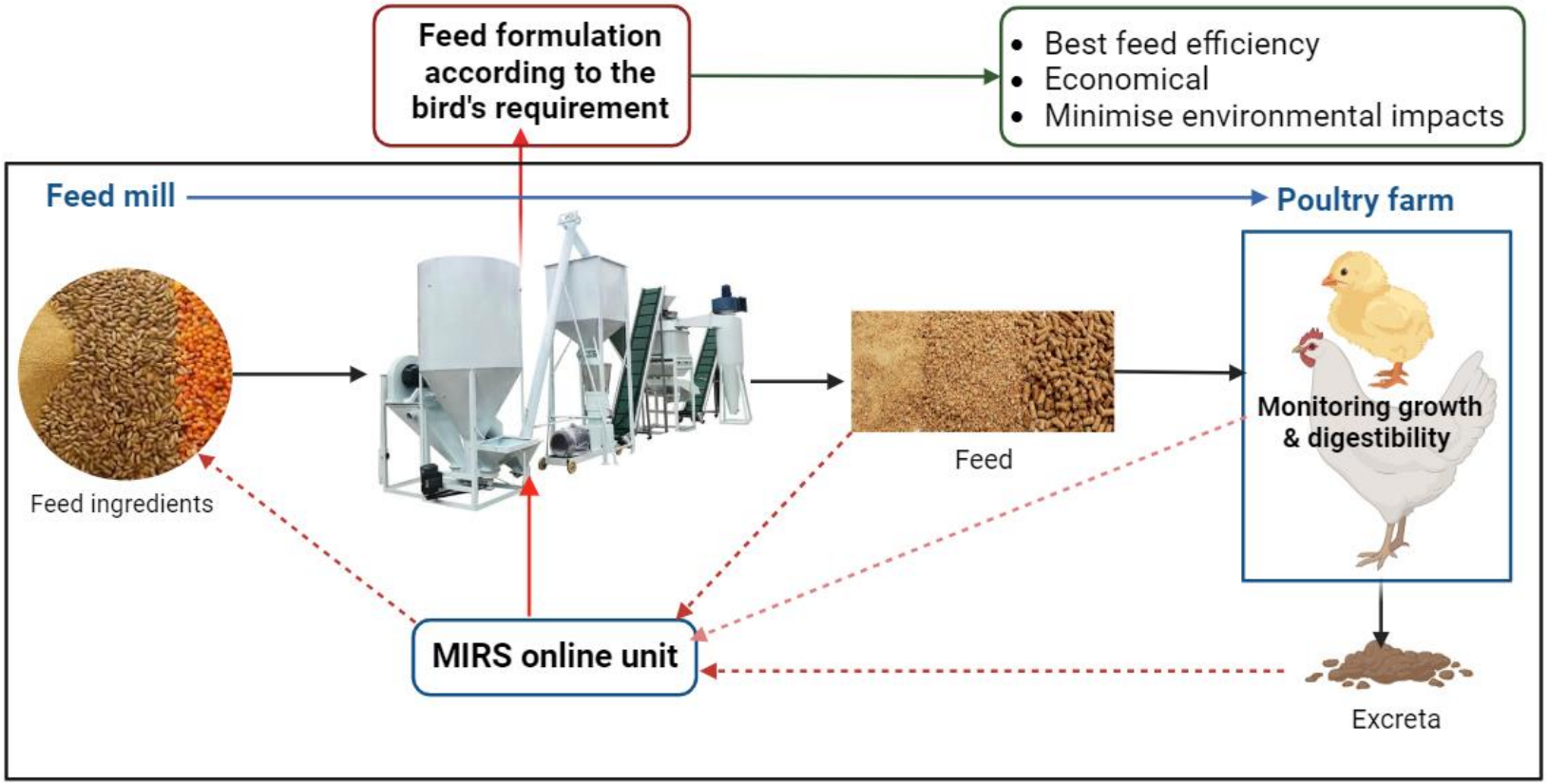


Figure 2.10. Schematic representation of using ATR-FT MIRS at feed mill to alter feed formulation according to the birds requirements monitored from the farm adapted from Knudsen et al. (2023). “Created with BioRender.com”.

2.10. Summary and implications

Literature evidence shows that there is a need for rapid and cheaper methods to estimate the chemical composition and nutritive value of feedstuffs and diets for an efficient feed formulation in broilers chicken. To achieve this, it is possible to modify the existing methods as well as to find additional feed evaluation systems. The application of prediction equations and ATR-FT MIRS calibration models are such techniques that could be applied routinely for rapid feed analysis without any tedious experimental procedures. It is well documented that energy values of feed ingredients could be predicted using the feed chemical composition data. However, there is a lack of equations for digestibility of nutrients in broiler diets which could be applied in all practical situations. This review also highlights insufficient external validation results of available prediction equations, emphasising the importance of formulating prediction equations with external validation to apply in a wide range of broiler diets.

In addition, while NIRS is widely used in poultry nutrition, the application of ATR-FT MIRS in the poultry industry remains relatively unknown. However, in the appropriate situation with the appropriate method, MIR promises to provide superior performance over NIR. Therefore, the objectives of this study are to derive prediction equations for the nutritive value of broiler diets with the most appropriate variables of chemical composition of diets and to externally validate them. Additionally, the study aims to explore the potential of ATR-FT MIRS for predicting chemical composition and nutritive value of broiler diets using different approaches.

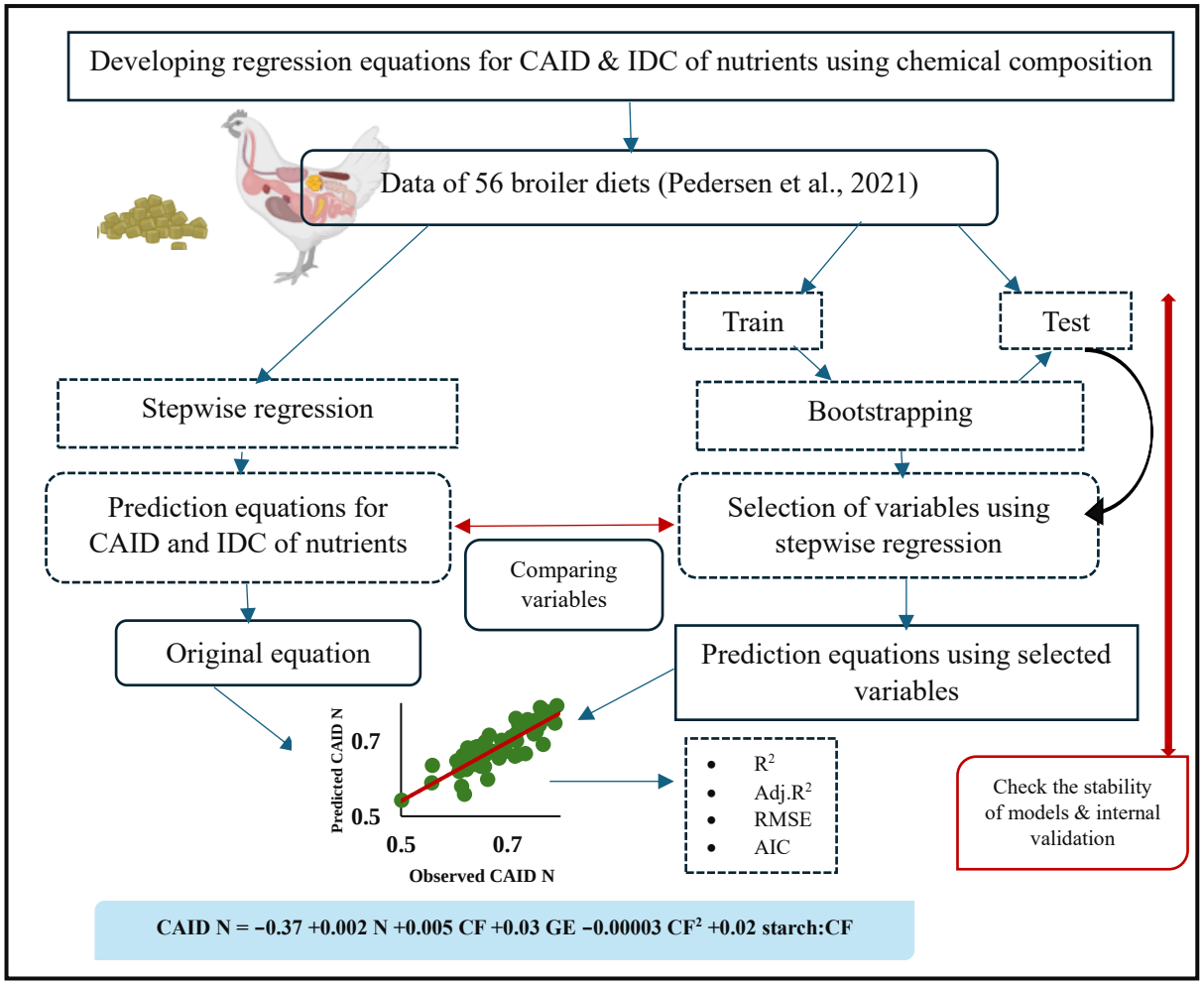
CHAPTER 3

Evaluation of equations for predicting ileal nutrient digestibility and digestible nutrient content of broiler diets based on their gross chemical composition

This Chapter has been published in
Thiruchchenthuran, S., Lopez-Villalobos, N., Zaefarian, F., Abdollahi, M.R., Wester, T.J., Pedersen, N.B., Storm, A.C., Cowieson, A.J., Morel, P.C.H., 2024. Evaluation of equations for predicting ileal nutrient digestibility and digestible nutrient content of broiler diets based on their gross chemical composition. Animal Feed Science and Technology, 115974. https://doi.org/10.1016/j.anifeedsci.2024.115974

The review of literature demonstrated that there is a need for rapid and cost-effective measurement of nutritive value of broiler diets. Therefore, this Chapter aims to develop prediction equations for CAID and IDC of nutrients using gross chemical composition of diets.

Graphical abstract



3.1. Abstract

The coefficient of apparent ileal digestibility (CAID) and ileal digestible contents (IDC) of nutrients of 56 diets using 10 feed ingredients were measured in broilers (21 to 24 days post-hatch). Diets contained varying inclusion levels of traditional and non-traditional ingredients and differed widely in chemical composition. The chemical composition and *in vivo* digestibility values were used to establish prediction equations for CAID and IDC of nutrients using stepwise multiple regression. The strength and accuracy of the developed equations were evaluated by root mean square error (RMSE), coefficient of determination (R^2), adjusted R^2 (adj. R^2), and Akaikie's Information Criteria (AIC). The bootstrap method was used to validate the choice of variables by stepwise selection method in the original equation based on their frequencies of selection. Selection of variables was validated if the variables that appear in the original stepwise model were selected in more than 30% of the 1000 bootstrap samples. A close agreement between the original equations and bootstrap resampling was observed for CAID of nitrogen (N) and energy and IDC of energy, starch, and calcium (Ca). Additionally, the original data was subjected to another run of stepwise regression analysis using the selected variables by bootstrapping. The initial regression showed that the CAID of N and energy was highly dependent on crude fibre (CF) and energy contents of the diets. The CAID of energy can be predicted (R^2 = 0.89 and RMSE = 0.035) by CF, gross energy (GE), CF^2, and starch-to-CF ratio (starch:CF). Calcium contents had a positive influence, while phosphorus (P) contents had a negative influence on the prediction of CAID of fat. The main variable to predict CAID and IDC of most nutrients was the dietary CF content. Based on the lowest RMSE and AIC, the selected variables for IDC of N were ash, N, fat, CF, CF^2, and starch:CF, while the best predictors for IDC of energy were CF, GE, CF^2, and starch:CF. The results of the original stepwise regression models and the stepwise regression with the selected variables from the bootstrap results for CAID of N, energy, fat, and DM, as well as IDC of energy, starch, and

Ca, were the same with no differences in R^2, Adj. R^2, RMSE, and AIC. This method can be useful for developing stable and reproducible models using stepwise regression. However, an external validation is needed to confirm the use of these equations in commercial settings.

Key words: bootstrapping, ileal digestibility, ileal digestible content, prediction equation, stepwise multiple regression, validation

3.2. Introduction

Advances in genetics, health, nutrition, and management practices have contributed to rapid growth of the poultry sector in recent years. These improvements raise the nutritional demands of birds that now require more complex diets. It is essential to accurately determine the chemical composition, digestible nutrients, and energy content of feedstuffs to formulate nutritionally balanced diets to fulfil birds' requirements (Alvarenga et al., 2015). Knowledge of the digestibility coefficients and requirement of digestible contents enables diet formulations closer to the requirements of the bird.

A rapid, inexpensive, and accurate method for estimation of the nutritive value of feedstuffs is a goal for animal production. Direct *in vivo* methods provide greater accuracy in feed evaluation, utilization of nutrients, and better prediction of bird performance. However, *in vivo* analysis is costly, time-consuming, and laborious (Zaefarian et al., 2021). Table values and prediction equations are used to quickly obtain digestibility values of feed ingredients that are used in feed formulation. However, errors in formulations may occur in using data from tables as these values represent an average of several previous studies in poultry (Mateos et al., 2019; Zaefarian et al., 2021).

In recent years, prediction equations have attained much interest and are used by most industries related to animal feed manufacturing (Mateos et al., 2019). Several researchers

(Cerrate et al., 2019; Sheikhhasan et al., 2020b; Pedersen et al., 2021) recently proposed equations to predict energy and nutrient digestibility from chemical composition of feedstuffs.

Alvarenga et al. (2013a) stated that to obtain the energy values of feed, it may be more appropriate to use prediction equations considering the chemical composition of the feed rather than performing *in vivo* assays for every raw material and utilising table values with numerous variations. In addition, predictions based on chemical composition were more accurate in terms of reflecting *in vivo* results (Yegani et al., 2013; Sheikhhasan et al., 2020a). However, more research and robust validation is warranted to develop more accurate equations. A proper validation procedure is necessary to assume that a prediction equation is effective.

Usually, mathematical models have good predictive power (coefficient of determination (R^2), root mean square error (RMSE)) using the original data set (Castilho et al., 2015). External validation using new data by an independent research team is the best approach to validate a model. However, it can be costly and slow, and disappointing results could often be avoided with rigorous internal validation performed earlier in the process. One such internal validation method is the bootstrap resampling technique (Steyerberg and Harrell, 2016).

Bootstrap resampling was first presented by Efron (1979). It is used mainly for estimation of parameters and their variability in a given model. Split-sample is a popular approach for internal validation in which a dataset is split into training (model development) and test (model validation) by a random process (Harrell, 2015). However, bootstrapping procedures are more useful than split-sample method and produce better results in terms of bias and variability (Steyerberg et al., 2001; Harrell, 2015). It is most appropriate in situations where the sample size is small and external validation data are not readily available (Chowdhury and Turin, 2021). The theory behind bootstrapping is that it replicates the process of sample generation from an underlying population by drawing samples with replacements from the original dataset

(Steyerberg et al., 2001). This method provides stable results in terms of less variance than other methods with a large number of repetitions. When the same selection procedure as for the original data is used in an ideal validation study, then (nearly) the same variables should be selected. This is sometimes called ‘replication stability’ (Sauerbrei, 1999).

Each internal model validation strategy has pros and cons, and no one technique is consistently superior to another. Different researchers have different ideas on what approach is best for internal model validation. Before reaching a decision, a number of criteria need to be considered, including sample size, best indicators of a model's performance, and choice of models (Chowdhury and Turin, 2021).

Recently, bootstrap resampling techniques have been promoted to evaluate the degree of stability of models resulting from stepwise procedures (Nunez et al., 2011). In stepwise regression, after a variable has been added to the model at each step of the variable selection process, it is possible to remove variables from the model. For instance, if the significance of a given predictor is above a specific threshold, it will be eliminated from the model. When a prespecified stopping rule has been satisfied, the iterative process will end (Austin and Tu, 2004a). If applied to regression analysis, bootstrapping provides variables that have a high degree of reliability (Brunelli, 2014).

Methods based on statistical models have been continually proposed for prediction of nutrient digestibility and digestible content of nutrients in feed ingredients. However, only a few published reports are available that use bootstrap resampling for validation of regression models (Castilho et al., 2015; Smith et al., 2015; Oliveira et al., 2019). Therefore, the objective of the current study was to formulate prediction equations to estimate ileal digestibility coefficients and digestible content of nutrients in broiler diets using stepwise multiple regression and application of bootstrap resampling as validation.

3.3. Materials and methods

A digestibility study conducted at Massey University to predict digestible nutrient content of poultry diets published by Pedersen et al. (2021). With the permission of authors, their data were used to develop prediction equations and then to validate them using a bootstrapping resampling technique. The original experimental design (Pedersen et al., 2021), stepwise regression, and bootstrapping are described below.

3.3.1. Dietary treatments

A total of 56 experimental diets was formulated based on 10 feed ingredients including maize, wheat, sorghum, soybean meal (SBM), canola meal (CM), palm kernel meal (PKM), full-fat soybeans (FFSB), meat and bone meal (MBM), wheat bran (WB), and wheat distillers dried grains with solubles (DDGS) (Table 3.1). A geometrically central diet was formulated by mixing equal proportions of each of the above 10 ingredients at the same level (100 g/kg). The inclusion level of each feed ingredient in different feed mixtures was either 20, 420, or 820 g/kg and all ingredients were included in all the diets. In dietary treatments based on cereal source, either 820 g/kg of one cereal source or 420 g/kg of two cereal sources were used. In dietary treatments based on protein source, either 820 g/kg of one protein source or 420 g/kg of two protein sources were used. By-product-based dietary treatments had 820 g/kg of either MBM, WB, or wheat DDGS or 420 g/kg of two by-product sources. All diets contained 5 g/kg of indigestible marker titanium dioxide (TiO_2) to determine apparent ileal nutrient digestibility. All diets were steam-conditioned at 60 ^{o}C for 30 s and pelleted through a pellet mill (Model Orbit 15; Richard Sizer Ltd., Kingston-Upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with a 3-mm hole and 35-mm thickness) (Pedersen et al., 2021).

Table 3.1. Major ingredients inclusion levels of 56 diets (g/kg) as per Pedersen et al. (2021).

Diets	Maize	Wheat	Sorghum	SBM	CM	MBM	WB	DDGS	FFSB	PKM
1	20	20	20	20	20	20	20	20	20	820
2	20	20	20	20	20	20	20	20	420	420
3	20	20	20	20	20	20	20	20	820	20
4	20	20	20	20	20	20	20	420	20	420
5	20	20	20	20	20	20	20	420	420	20
6	20	20	20	20	20	20	20	820	20	20
7	20	20	20	20	20	20	420	20	20	420
8	20	20	20	20	20	20	420	20	420	20
9	20	20	20	20	20	20	420	420	20	20
10	20	20	20	20	20	20	820	20	20	20
11	20	20	20	20	20	420	20	20	20	420
12	20	20	20	20	20	420	20	20	420	20
13	20	20	20	20	20	420	20	420	20	20
14	20	20	20	20	20	420	420	20	20	20
15	20	20	20	20	20	820	20	20	20	20
16	20	20	20	20	420	20	20	20	20	420
17	20	20	20	20	420	20	20	20	420	20
18	20	20	20	20	420	20	20	420	20	20
19	20	20	20	20	420	20	420	20	20	20
20	20	20	20	20	420	420	20	20	20	20
21	20	20	20	20	820	20	20	20	20	20
22	20	20	20	420	20	20	20	20	20	420
23	20	20	20	420	20	20	20	20	420	20
24	20	20	20	420	20	20	20	420	20	20
25	20	20	20	420	20	20	420	20	20	20
26	20	20	20	420	20	420	20	20	20	20
27	20	20	20	420	420	20	20	20	20	20
28	20	20	20	820	20	20	20	20	20	20
29	20	20	420	20	20	20	20	20	20	420
30	20	20	420	20	20	20	20	20	420	20
31	20	20	420	20	20	20	20	420	20	20
32	20	20	420	20	20	20	420	20	20	20
33	20	20	420	20	20	420	20	20	20	20
34	20	20	420	20	420	20	20	20	20	20
35	20	20	420	420	20	20	20	20	20	20
36	20	20	820	20	20	20	20	20	20	20
37	20	420	20	20	20	20	20	20	20	420
38	20	420	20	20	20	20	20	20	420	20
39	20	420	20	20	20	20	20	420	20	20
40	20	420	20	20	20	20	420	20	20	20
41	20	420	20	20	20	420	20	20	20	20

42	20	420	20	20	420	20	20	20	20	20
43	20	420	20	420	20	20	20	20	20	20
44	20	420	420	20	20	20	20	20	20	20
45	20	820	20	20	20	20	20	20	20	20
46	420	20	20	20	20	20	20	20	20	420
47	420	20	20	20	20	20	20	20	420	20
48	420	20	20	20	20	20	20	420	20	20
49	420	20	20	20	20	20	420	20	20	20
50	420	20	20	20	20	420	20	20	20	20
51	420	20	20	20	420	20	20	20	20	20
52	420	20	20	420	20	20	20	20	20	20
53	420	20	420	20	20	20	20	20	20	20
54	420	420	20	20	20	20	20	20	20	20
55	820	20	20	20	20	20	20	20	20	20
56	100	100	100	100	100	100	100	100	100	100

CM, canola meal; DDGS, wheat distillers dried grains with solubles; FFSB, full-fat soybeans; MBM, meat and bone meal; PKM, palm kernel meal; SBM, soybean meal; WB, wheat bran.

3.3.2. Experimental design

A total of 2688, day-old male broiler chicks (Ross 308) were obtained from a commercial hatchery in 2 batches (1344 chickens per batch) and were fed a common starter diet from 1 to 21 days of age. These birds were used in two batches (3 replicates for each dietary treatment in every batch due to limits on housing) with a two-week interval between batches. In each batch on days 21, 1344 birds were allocated to 168 cages (8 chicks per cage). The 56 feed mixtures were then randomly assigned to 3 replicate cages each in each batch (6 replicate cages in total). Feed mixtures were fed in pelleted form from days 21-24.

3.3.3. Chemical analysis

The diets and digesta samples were analysed for DM, ash, titanium (Ti), N, starch, CF, fat, Ca, P, and GE. Dry matter was determined using standard procedures (Methods 930.15 and 925.10; AOAC, 2005). Ash was determined by standard procedures (Method 942.05; AOAC, 2016) using a muffle furnace at 550 ºC for 16 h. Samples were assayed for Ti on a UV spectrophotometer following the method of Short et al. (1996). Nitrogen was determined by

combustion (Method 968.06; AOAC, 2016) using a CNS-200 carbon, N, and sulphur auto analyser (LECO Corporation, St. Joseph, MI, USA). Total starch was determined using the assay procedure (Megazyme Total Starch Assay Procedure; Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable α-amylase and amyloglucosidase. Crude fibre was determined using standard procedures (Methods 962.09 and 978.10; AOAC, 2005). Fat was determined using the Soxhlet extraction procedure (Method 991.36; AOAC, 2005). Calcium and P were determined by colorimetric methods after combustion of the samples at 550 ºC and acid digestion in 6.0 M HCl using standard procedures (Method 968.08D; AOAC, 2005). Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid.

3.3.4. Calculations

The CAID of nutrients and energy was calculated using the following formula:

$$\text{CAID of nutrient} = \frac{(\text{Nutrient /Ti})_{diet} - (\text{Nutrient /Ti})_{ileal}}{(\text{Nutrient/Ti})_{diet}} \quad (3.1)$$

where,

(Nutrient/Ti) $_{diet}$ = ratio of nutrient to Ti in the diet, and (Nutrient/Ti) $_{ileal}$ = ratio of nutrient to Ti in the ileal digesta.

The IDC of nutrients and energy was calculated using the following formula:

$$\text{IDC} = \text{Gross composition of nutrient} \times \text{CAID of that nutrient} \quad (3.2)$$

3.3.5. Statistical analysis

3.3.5.1. Initial development of prediction equations for CAID and IDC of nutrients

Predictive multiple regression equations for CAID and IDC of N, energy, fat, starch, Ca, P, and DM were established by stepwise model selection procedure using the chemical composition

of the 56 broiler diets (ash, N, fat, starch, CF, Ca, P, and GE) and ratios between chemical composition in the diet (fat:CF and starch:CF) as well as non-linear relationship (CF^2). The primary goal of this study was to assess the contribution of each dietary component to nutrient digestibility in broilers. Variable selection was guided by prior knowledge of nutrient digestion in poultry. Dietary fibre is known to influence nutrient digestibility, with high fibre levels generally reducing digestibility, while moderate inclusion can enhance it (Mateos et al., 2012).

Therefore, both CF and CF^2 were retained in the model to facilitate the direct interpretation of fibre's effects. In contrast, other dietary components typically exhibit linear relationships with digestibility, and thus, their non-linear terms were not considered. In addition to this, the digestibility of fat and starch can be largely influenced by dietary fibre content. Therefore, the ratio of fat:CF and starch:CF were considered for a more comprehensive assessment of how fibre interacts with other dietary components in affecting nutrient digestibility.

The best model that fits the data was selected using AIC by comparing different models. All variables left in the model were significant at 0.15 level.

3.3.5.2. Bootstrapped variable selection and validation of stepwise regression equations

The bootstrap method was used to validate the choice of variables by the stepwise selection method in the original equation. The following method was used to determine inclusion or exclusion of variables (Royston and Sauerbrei, 2009). If a total of *B* bootstrap samples is used to explore variations among the possible models for the original dataset with *k* variables,

1. Draw a bootstrap sample of size n.
2. Apply the model selection procedure.
3. For each variable x_j (j = 1, ...,*k*), record whether x_j is selected in the model.
4. Repeat the steps above to a larger number, *B* number of times.
5. Summarize the results.

The outcome of this analysis will comprise a matrix consisting of *B* number of rows and *k* columns (Royston and Sauerbrei, 2009). This technique is dependent on the dataset because it relies on resampling observations from the dataset (Bertolini et al., 2022) (Figure 3.1).

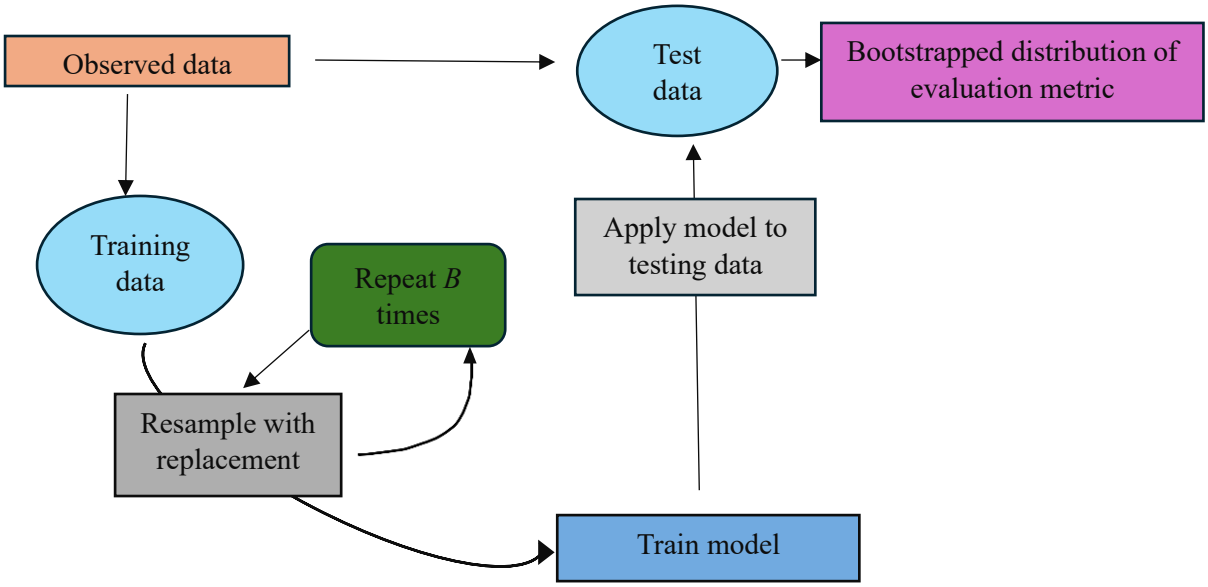


Figure 3.1. Bootstrap resampling method adapted from Bertolini et al. (2022).

Sixty percent of the original data set (56 diets) was assigned for variable selection by bootstrap resampling by drawing repeated 1000 bootstrap samples from the original data set. Thus, some cases in the original data set were duplicated in the bootstrap sample while others were not included at all. The remaining 40% was used to test the obtained results. As was done during the original equation development, stepwise multiple regression was used to select variables within each bootstrap sample. For each candidate variable, the proportion of bootstrap samples in which that variable was identified as an independent predictor of the outcome was determined. Then, variables were ordered according to the proportion of bootstrap samples in which they were selected as predictors of the outcome. A preliminary predictive model will consist of those variables identified as significant in all bootstrap samples (Austin and Tu, 2004b). The selection of variables was validated if the variables that appeared in the original stepwise model were selected in more than 30% of the bootstrap samples.

Another approach was used to rerun the stepwise regression analysis for original data with selected variables that appeared above 30% in 1000 bootstrap resamples. The final model was constructed using a stepwise regression procedure where top-ranked variables that were above 30% appearance in bootstrap samples were added to the model. This choice of cut points for percentage inclusion in a model and choice of section levels was arbitrary in this strategy, as explained by Sauerbrei and Schumacher (1992). Selection of prediction models in all instances was made using R^2, Adj. R^2, AIC, and RMSE values. Bootstrap resampling and stepwise multiple regression analyses were done using SAS software, version 9.4 package with various procedures (SAS, 2016).

3.4. Results

3.4.1. Chemical composition and nutrient digestibility coefficient of the diets

As expected, the analysed chemical composition of diets was quite variable as many traditional and non-traditional ingredients were used in varying amounts (Table 3.2). The N and GE contents of diets ranged from 22.3 to 78.1 g/kg DM and 17.9 to 24.0 MJ/kg, respectively. The digestibility coefficient of N varied between 0.466 and 0.806 (mean = 0.683), whereas the digestibility coefficient of energy ranged from 0.346 to 0.820 (mean = 0.611).

Table 3.2. Analysed chemical composition (g/kg DM), apparent ileal digestibility coefficient of nutrients, and ileal digestible nutrient content (g/kg DM) of the 56 diet mixtures used in the study as per Pedersen et al. (2021).

Variable (g/kg DM)	Mean	Standard deviation	Minimum	Maximum
Chemical Composition				
Ash	77.6	56.7	27.3	300
N	44.4	14.8	22.3	78.1
Fat	94.9	40.7	51.8	243
Starch	230	170	44.9	636
CF	68.1	29.6	26.6	145
Ca	15.4	22.4	3.31	105
P	11.2	9.02	4.18	47.0
GE (MJ/kg)	20.1	11.8	17.9	24.0
Digestibility Coefficient				
N	0.683	0.076	0.466	0.806
Fat	0.752	0.081	0.506	0.879
Starch	0.929	0.040	0.810	0.982
Ca	0.332	0.137	0.122	0.633
P	0.498	0.155	0.137	0.704
GE	0.611	0.100	0.346	0.820
DM	0.536	0.121	0.228	0.795
Digestible Content				
N	30.3	10.4	14.7	55.2
Fat	71.8	34.7	37.5	206
Starch	214	158	43.2	616
Ca	3.77	4.52	0.61	20.0
P	4.47	1.57	2.46	9.51
GE	12.3	2.13	7.00	16.7
DM	536	121	228	795

Ca, calcium; CF, crude fibre; DM, dry matter; GE, gross energy; N, nitrogen; P, phosphorus.

3.4.2. Prediction of coefficient of apparent ileal digestibility of N, energy, fat, starch, Ca, P, and DM

Prediction equations were calculated with an intercept and the best equation was selected as having the highest R^2 and minimum AIC values (Table 3.3). Prediction accuracy was improved for both CAID of N and energy when interactions between chemical composition and non-

linear relationship in the diet were considered, with the lowest AIC and RMSE values and highest R^2 values.

The CAID of N was predicted by dietary content of N, CF, GE, CF^2, and starch:CF ratio, where CF^2 had a negative relationship and N, CF, and GE content had a positive relationship, with an R^2 of 0.78 and AIC of −305 (Equation 1). For each additional unit of CF^2, digestibility of N decreased by 0.00003 units. High R^2 (0.87) and low AIC (−305) for prediction of CAID of energy was obtained when CF, GE, CF^2, and starch:CF comprised the equation (Equation 2).

The CAID of fat was negatively affected by dietary content of fat, P, and CF. For each increment of P content in the diet, CAID of fat was reduced by 0.06 units. The Ca and GE contents were positively correlated with CAID of fat (R^2 = 0.77 and AIC = −295; Equation 3). The best prediction was achieved for CAID of starch when fat, CF, and GE were included in the equation (Equation 4). For each additional MJ of GE, CAID of starch was reduced by 0.025 units and fat and CF contents were positively correlated with the CAID of starch.

The CAID of Ca (Equation 5) was predicted by N, starch, and GE content, CF^2, fat:CF and starch:CF. The CAID of P was influenced by content of N, starch, P, CF^2, and starch:CF. There was a negative relationship between CAID of P and dietary content of N, starch, P, and CF^2 (Equation 6). The CAID of DM depended on dietary content of fat, CF, and GE, and CF^2 and starch:CF. Fat content and CF^2 negatively correlated with CAID of DM, and other parameters positively correlated with CAID of DM. Prediction was more accurate (R^2 = 0.89) when CF^2 and starch:CF were included in the equation with a minimum AIC of −291 (Equation 7).

Table 3.3. Prediction equations of apparent ileal digestibility coefficients (CAID) of fat, starch, Ca, P, and DM based on chemical composition of the diets (g/kg DM) in broiler chickens.

No	Equation	RMSE	R^2	Adj.R^2	AIC
01	CAID N = −0.37 +0.002 N +0.005 CF +0.03 GE −0.00003 CF^2 +0.02 starch:CF	0.04	0.78	0.76	−305
02	CAID E = 0.14 +0.002 CF +0.021 GE −0.000021 CF^2 +0.013 starch:CF	0.04	0.87	0.86	−305
03	CAID fat = −1.78 −0.003 fat +0.03 Ca −0.06 P +0.15 GE −0.000003 CF^2	0.04	0.77	0.75	−295
04	CAID starch = 1.36 +0.0003 fat +0.0006 CF −0.025 GE	0.03	0.36	0.32	−319
05	CAID Ca = −0.228 −0.007 N −0.0008 starch +0.055 GE −0.00002 CF^2 −0.04 fat:CF +0.03 starch:CF	0.08	0.69	0.65	−218
06	CAID P = 1.32 −0.008 N −0.001 starch −0.016 P −0.000023 CF^2 +0.012 starch:CF	0.07	0.83	0.81	−238
07	CAID DM = −0.24 −0.0004 fat +0.004 CF +0.033 GE −0.00004 CF^2 +0.02 starch:CF	0.04	0.89	0.88	−291

Adj. R^2, adjusted R^2; AIC, Akaikie's Information Criteria; Ca, calcium; CAID, coefficient of apparent ileal digestibility; CF, crude fibre; CF^2, square value of crude fibre; DM, dry matter; Fat:CF, fat-to-crude fibre ratio; GE, gross energy; N, nitrogen; P, phosphorus; R^2, coefficient of determination; RMSE, root mean square error; Starch:CF, starch-to-crude fibre ratio.

3.4.3. Prediction of ileal digestible content of nutrients and energy

The IDC of N was positively affected by dietary N, fat, and starch:CF ratio, and negatively related to ash and CF^2 (Table 3.4; Equation 8). The IDC of energy was best predicted by CF, GE, CF^2, and starch:CF, with R^2 of 0.89 and AIC of 30 (Equation 9). Digestible fat was linearly influenced by dietary fat (Equation 10). Moreover, ash, starch, and CF^2 had a negative relationship with the IDC of fat.

The IDC of starch relied on dietary starch and GE content. For each MJ increment of GE, IDC of starch was reduced 0.25 g, and for each 1 g increment in dietary starch, IDC of starch increased by 0.92 g (Equation 11). The starch content of the diets varied from 44.9-636 g/kg DM while gross energy had the range between 17.9-24.0 MJ/kg. The best predictor of IDC of Ca was dietary content of Ca, and it was positively correlated with IDC of Ca (Equation 12).

Ash, N, starch, and Ca content and CF^2 were the best variables to predict IDC of P, with R^2 of 0.81 and AIC of 27 (Equation 13). Ash content of the diet was positively correlated with IDC of P, whereas N, starch, Ca, and CF^2 had a negative relationship with IDC of P. The prediction equation for IDC of DM was composed of fat, CF, GE content, CF^2, and starch:CF (Equation 14).

Table 3.4. Prediction equations of ileal digestible content (IDC) based on chemical composition (g/kg DM) in broiler chickens.

No	Equation	RMSE	R^2	Adj.R^2	AIC
08	IDC N = −10.17 −0.04 ash +0.8 N +0.03 fat +0.12 CF −0.0008 CF^2 +0.4 starch:CF	1.66	0.98	0.98	121
09	IDC E = −9.87 +0.03 CF +1.06 GE −0.0004 CF^2 +0.24 starch:CF	0.75	0.89	0.88	30.4
10	IDC fat = −75.76 −1.49 ash +0.48 N +0.61 fat −0.053 starch +3.46 Ca +7.23 GE −0.0005 CF^2	3.51	0.99	0.99	206
11	IDC starch = 52.41 +0.92 starch −2.5 GE	13.1	0.99	0.99	349
12	IDC Ca = 0.804 +0.193 Ca	1.30	0.92	0.92	88.9
13	IDC P = 5.71 +0.11 ash −0.1 N −0.005 starch −0.218 Ca −0.0002 CF^2	0.72	0.81	0.79	27.0
14	IDC DM = −237.47 −0.37 fat +3.89 CF +32.67 GE −0.04 CF^2 +18.2 starch:CF	42.3	0.89	0.88	483

Adj. R^2, adjusted R^2; AIC, Akaikie's Information Criteria; Ca, calcium; CF, crude fibre; CF^2, square value of crude fibre; DM, dry matter; GE, gross energy; IDC, ileal digestible content; N, nitrogen; P, phosphorus; R^2, coefficient of determination; RMSE, root mean square error; Starch:CF, starch-to-crude fibre ratio.

3.4.4. Bootstrap validation of stepwise regression analysis for CAID and IDC of nutrients

Generalizability and reproducibility of the variables in the stepwise regression, as determined using measures of goodness of fit for the 1000 bootstrap resampling of training (60% of the original data set) and test data (remaining 40% of the original data set), showed that there was a good agreement between the goodness of fit results of the training and test data (Table 3.5).

Table 3.6 shows the frequency of the variables entering the model for stepwise regression on 1000 bootstrap samples. The number of variables entered in the model varied from 2 to 9, 2 to 10, 2 to 8, and 1 to 9 for CAID of N, energy, Ca, and P, respectively. Eight variables were selected more than 30% (range, 31% to 85%), whereas three variables were selected infrequently (range, 9% to 17%) for CAID of N. Four variables were selected more than 30% (range, 43% to 89%) and seven variables were between 5% to 27% for CAID of energy. Similarly, five variables were selected for CAID of Ca and P whose frequency was above 30%.

The number of variables entered in the model varied from 3 to 9, 3 to 9, 1 to 10, and 1 to 9 for IDC of N, energy, Ca, and P, with mean number of variables of 4.4, 3.9, 1.5, and 4.2, respectively for the 1000 bootstrap resamples. Variables ash, N, fat, starch, P, and CF^2 were selected more than 30% of the time for IDC of N, whereas CF, GE, CF^2, and starch:CF were selected for IDC of energy. The only predictor selected for IDC of Ca was content of Ca, with 98.7% appearance rate in 1000 bootstrap samples. Starch was appeared in 100% bootstrap resamples for prediction of IDC of starch. Variables ash, N, Ca, GE, and CF^2 were selected more than 30% in 1000 bootstrap samples for predicting IDC of P. The mean value of parameter estimates of variables selected using bootstrap resampling for CAID and IDC of nutrients are shown in Table 3.7 at $P < 0.15$.

The comparison between the original equation and the bootstrap variable selection shows that most variables were the same in both. However, some variables in the original model were

omitted or some new variables were selected in bootstrap resampling except the models for CAID of energy and IDC of energy, starch, and Ca. Fat, Ca, and P contents which were selected above 30% in bootstrap resampling, did not appear in the original equation for CAID of N. Likewise, N content, which appeared above 30% in bootstrap resampling, was not included in the original equations for CAID of fat. At the same time, CF^2, which was selected only in 12% of the bootstrap resamples was included in the original equation for CAID of fat. For CAID and IDC of DM, ash content was not included in the original equation even though it was selected above 30% in the 1000 bootstrap resamples.

Table 3.5. Measures of goodness of fit for 1000 bootstrap resampling of 56 diets (60% training data and 40% test data).

	Training (60%)		Test (40%)	
	RMSE	R^2	RMSE	R^2
CAID N	0.04	0.76	0.04	0.62
CAID energy	0.04	0.87	0.04	0.82
CAID fat	0.05	0.70	0.04	0.59
CAID starch	0.03	0.37	0.02	0.24
CAID Ca	0.09	0.64	0.08	0.51
CAID P	0.07	0.82	0.08	0.72
CAID DM	0.04	0.89	0.05	0.84
IDC N	1.65	0.98	1.97	0.96
IDC energy	0.74	0.89	0.79	0.84
IDC fat	3.55	0.99	4.51	0.98
IDC starch	12.9	0.99	13.0	0.99
IDC Ca	1.23	0.92	1.17	0.92
IDC P	0.74	0.79	0.78	0.68
IDC DM	42.1	0.89	45.4	0.84

Ca, calcium; CAID, coefficient of apparent ileal digestibility; DM, dry matter; N, nitrogen; IDC, ileal digestible content; P, phosphorus; RMSE, root mean square error; R^2, coefficient of determination.

Table 3.6. Summary of appearance of predictors (%) in 1000 bootstrap resampling for coefficient of apparent leal digestibility (CAID) and ileal digestible content (IDC) of nutrients in broiler diets.

	CAID							IDC						
Predictors	N	Energy	Fat	Starch	Ca	P	DM	N	Energy	Fat	Starch	Ca	P	DM
Ash	8.8	26.8	17.8	5.4	15.6	24.5	51.5*	56.2*	5.9	41.1*	2.3	2.1	93.3*	49.8*
N	44.2*	14.8	56.7*	6.8	37.4*	48.0*	11.0	100*	9.4	72.2*	1.6	7.1	59.1*	11.0
Fat	30.7*	7.6	30.2*	23.5	9.2	27.0	6.8	61.6*	13.9	99.9*	0.7	1.9	18.0	5.9
Starch	9.8	12.9	12.0	9.6	44.1*	32.7*	19.0	32.4*	10.7	29.1	100*	1.5	26.0	15.3
CF	33.5*	42.5*	8.2	49.6*	12.1	24.3	53.2*	20.0	38.0*	12.3	4.4	5.8	13.6	50.5*
Ca	38.2*	24.1	78.1*	13.7	9.4	10.8	18.1	15.7	11.5	96.2*	3.4	98.7*	56.3*	16.4
P	40.7*	8.7	74.3*	7.2	12.6	79.0*	14.6	38.1*	13.5	69.1*	2.2	8.6	13.2	15.8
GE	39.7*	77.5*	49.6*	87.4*	82.3*	20.2	39.0*	18.1	93.1*	66.0*	37.1*	5.2	38.4*	37.4*
CF^2	85.3*	88.7*	11.7	31.2*	33.7*	58.9*	97.8*	55.4*	94.0*	19.2	0.6	10.4	52.9*	98.4*
Fat:CF	17.0	5.3	6.0	6.1	17.7	40.9*	8.2	23.6	8.7	17.6	1.7	2.9	23.6	6.4
Starch:CF	48.8*	78.4*	26.3	9.5	53.3*	24.2	85.2*	18.6	86.2*	6.9	13.7	2.4	25.3	86.6*
Predictors ≥ 30%	8	4	5	3	5	5	5	6	4	6	2	1	5	5
No. of variables entered														
Minimum	2	2	1	0	2	1	2	3	3	2	1	1	1	2
Maximum	9	10	11	9	8	9	10	9	9	10	9	10	9	8
Average	3.97	3.87	3.71	2.50	3.27	3.91	4.04	4.40	3.85	5.30	1.68	1.45	4.20	3.94

Ca, calcium; CF^2, square value of crude fibre; DM, dry matter; Fat:CF, fat-to-crude fibre ratio; GE, gross energy; N, nitrogen; P, phosphorus; Starch:CF, starch-to-crude fibre ratio; * (≥ 30%) selected for applying stepwise regression of the original diet data.

Table 3.7. Mean value of parameter estimates obtained using 1000 bootstrapping resamples of 56 broiler diets data for determining coefficient of apparent ileal digestibility of nutrients (CAID) and ileal digestible content of nutrients (IDC).

	Ash	N	Fat	Starch	CF	Ca	P	GE	CF^2	Fat:CF	Starch:CF
CAID N	0.004	0.003	0.001	0.0002	0.004	−0.003	−0.008	0.026	−0.00002	−0.016	0.015
CAID energy	−0.004	0.002	−0.002	−0.0001	0.002	0.010	−0.003	0.030	−0.00002	−0.011	0.013
CAID fat	−0.006	0.004	−0.003	−0.0001	0.001	0.022	−0.050	0.112	−0.00001	0.011	0.008
CAID starch	0.003	−0.001	0.0004	−0.0001	0.001	0.001	−0.016	−0.021	0	−0.009	−0.001
CAID Ca	0.003	−0.007	−0.002	0.00004	−0.001	0.015	−0.052	0.067	−0.00002	−0.060	0.024
CAID P	0.009	−0.009	−0.001	−0.001	−0.003	−0.014	−0.029	0.119	−0.00002	−0.007	0.018
CAID DM	−0.002	−0.001	−0.001	−0.0002	0.005	0.006	−0.006	0.028	−0.00003	0.003	0.016
IDC N	0.007	0.781	0.044	0.019	0.022	−0.103	−0.636	0.191	−0.0004	−1.130	0.426
IDC energy	−0.062	0.033	−0.014	0.004	0.046	0.200	−0.236	1.194	−0.0004	0.106	0.241
IDC fat	−1.213	0.405	0.663	−0.049	0.014	2.407	−4.345	9.737	−0.001	1.376	−0.047
IDC starch	1.335	−0.547	−0.030	0.923	0.265	0.145	−7.392	−3.441	−0.002	−3.237	−0.206
IDC Ca	−0.011	−0.040	−0.002	−0.004	0.025	0.206	−0.178	0.880	−0.0002	−1.411	−0.063
IDC P	0.090	−0.103	−0.029	−0.005	−0.004	−0.213	−0.150	1.212	−0.0002	−0.730	−0.061
IDC DM	−1.482	−0.095	−0.710	−0.129	4.666	6.305	−5.657	28.095	−0.029	0.278	15.005

Ca, calcium; CF^2, square value of crude fibre; DM, dry matter; Fat:CF, fat-to-crude fibre ratio; GE, gross energy; N, nitrogen; P, phosphorus; Starch:CF, starch-to-crude fibre ratio.

3.4.5. Stepwise regression models using selected variables by 1000 bootstrap resampling

Equations for prediction of CAID of N, energy, and DM were similar when regressed with the selected variables using bootstrapping (Table 3.8). The CF^2 was removed for CAID of fat and fat was removed for CAID of starch (Equations 17 and 18, respectively) compared to the original equations. The CAID of Ca was predicted by the variables N, GE, CF^2, and starch:CF (Equation 19), whereas CAID of P was predicted by content of P and CF^2 (Equation 20). Figure 3.2 (a and b) shows a close agreement between the predicted value and the observed value for CAID of N and energy, respectively, where the R^2 values obtained were 0.78 and 0.87 for CAID of N and energy, respectively. The calculated mean bias (MB) is 0 for both CAID of N and energy.

Equations for prediction of IDC of energy, starch, and Ca were similar when using all variables and selected variables with the same parameter estimates (Equations 23, 25, and 26, respectively; Table 3.9). Starch:CF was not selected as a predictor for prediction of IDC of N (Equation 22) and CF^2 was not selected for IDC of fat (Equation 24) when compared to the original equation. The equations developed using the selected variables for the IDC of N and energy showed good performance as the R^2 obtained was 0.98 and 0.89, respectively, with MB = 0 (Figure 3.3, a and b).

IDC of P was represented by content of ash, N, Ca, GE, and CF^2, where starch was removed, and GE was added (Equation 27) compared to the original equation (Equation 12). For IDC of DM, ash was replaced for the fat in the original equation (Equation 28). The R^2 and adj. R^2 decreased, while RMSE and AIC increased when using the selected variables from bootstrapping for CAID of fat, starch, Ca, and P. Similarly, R^2 and adj. R^2 decreased while RMSE and AIC increased for IDC of N, fat, P, and DM.

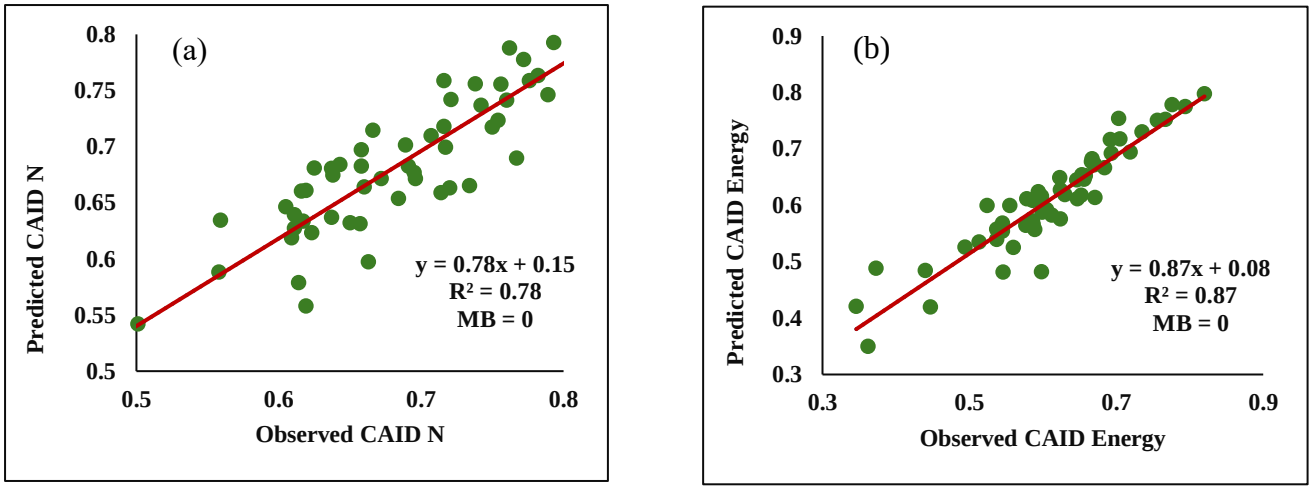


Figure 3.2. Predicted vs. observed values for coefficient of apparent ileal digestibility (CAID) of N (a) and energy (b) based on the gross chemical composition using selected predictors from bootstrapping.

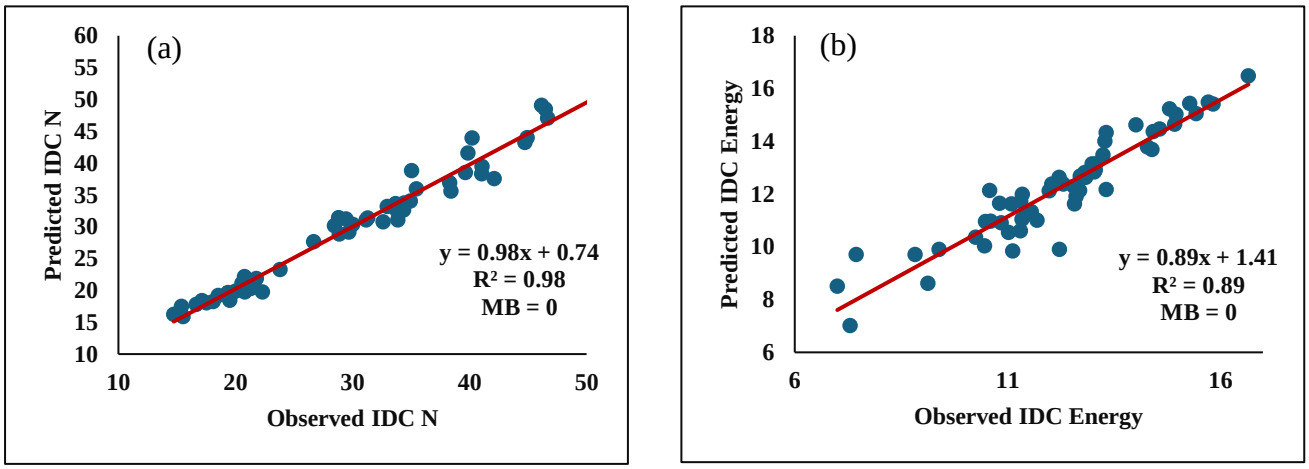


Figure 3.3. Predicted vs. observed values for ileal digestible content (IDC) of N (a) and energy (b) based on the gross chemical composition using selected predictors from bootstrapping.

Table 3.8. Prediction equations for coefficient of apparent ileal digestibility (CAID) of nutrients based on chemical composition (g/kg DM) in broiler chickens using selected predictors by bootstrapping.

No	Equation	RMSE	R^2	Adj.R^2	AIC
15	CAID N = −0.37 +0.002 N +0.005 CF +0.03 GE −0.00003 CF^2 +0.02 starch:CF *	0.04	0.78	0.76	−305
16	CAID E = 0.14 +0.002 CF +0.021 GE −0.000021 CF^2 +0.013 starch:CF *	0.04	0.87	0.86	−305
17	CAID fat = −1.74 −0.003 fat +0.03 Ca −0.06 P +0.15 GE	0.04	0.76	0.74	−293
18	CAID starch= 1.25 +0.0004 CF −0.02 GE	0.03	0.32	0.29	−318
19	CAID Ca = −0.47 −0.004 N +0.05 GE −0.000009 CF^2 +0.01 starch:CF	0.09	0.64	0.61	−212
20	CAID P = 0.71 −0.02 P −0.000008 CF^2	0.08	0.74	0.73	−221
21	CAID DM = −0.24 −0.0004 fat +0.004 CF +0.033 GE −0.00004 CF^2 +0.02 starch:CF *	0.04	0.89	0.88	−291

Adj. R^2, adjusted R^2; AIC, Akaikie's Information Criteria; Ca, calcium; CAID, coefficient of apparent ileal digestibility; CF, crude fibre; CF^2, square value of crude fibre; DM, dry matter; Fat:CF, fat-to-crude fibre ratio; GE, gross energy; N, nitrogen; P, phosphorus; R^2, coefficient of determination; RMSE, root mean square error; Starch:CF, starch-to-crude fibre ratio; *, same equation as original equation developed using all variables (56 diets).

Table 3.9. Prediction equations of ileal digestible content (IDC) based on chemical composition (g/kg DM) in broiler chickens using selected predictors by bootstrapping.

No	Equation	RMSE	R^2	Adj.R^2	AIC
22	IDC N = −4.7 −0.04 ash +0.8 N +0.02 fat +0.01 starch −0.0002 CF^2	1.71	0.98	0.97	124
23	IDC E = −9.87 +0.03 CF +1.06 GE −0.0004 CF^2 +0.24 starch:CF *	0.75	0.89	0.88	30.4
24	IDC fat= −94.27 −1.36 ash +0.57 N +0.64 fat −0.03 starch +3.2 Ca +7.2 GE	3.57	0.99	0.99	207
25	IDC starch = 52.41 +0.92 starch −2.5 GE*	13.1	0.99	0.99	349
26	IDC Ca = 0.804 +0.193 Ca*	1.30	0.92	0.92	88.9
27	IDC P = 3.77 +0.15 ash −0.1 N −0.3 Ca +0.3 GE −0.0001 CF^2	0.72	0.81	0.79	27.4
28	IDC DM = 543.7 −0.63 ash +2.2 CF +32.67 GE −0.03 CF^2 +11.1 starch:CF	43.8	0.88	0.87	486

Adj. R^2, adjusted R^2; AIC, Akaikie's Information Criteria; Ca, calcium; CF, crude fibre; CF^2, square value of crude fibre; DM, dry matter; GE, gross energy; IDC, ileal digestible content; N, nitrogen; P, phosphorus; R^2, coefficient of determination, RMSE, root mean square error; Starch:CF, starch-to-crude fibre ratio; *, same equation as original equation developed using all variables (56 diets).

3.5. Discussion

In the current study, the bootstrap approach was used primarily to select variables and construct prediction models for CAID and IDC of dietary nutrients for broilers. The frequency of the variables entering 1000 bootstrap models is used as a guide to validate variables selected in original stepwise regression. Selection based on a particular cut-off (i.e., > 30% frequency) is arbitrary. The data set used in the present study as per Pedersen et al. (2021) was very robust because it had a wide range of digestibility and chemical composition needed to develop the predictions with more complex diets. It is noteworthy that some variables selected were only responses to non-traditional ingredients viz. diets consisting of high levels of MBM had high amounts of Ca and P. This would not be applicable under practical situations as commercial diets consist of Ca and P in low concentrations and they are less variable (Pedersen et al., 2021). Therefore, the equations with Ca and P could be only considered as a general guide.

The variations in the stepwise regression models for sub-samples may have been caused by correlations between variables (Scalon et al., 1998; Steyerberg et al., 2001). The stepwise method can also be biased by an observation that has a significant impact on the inference process. The perfect model should have good stability, that is the variables selected and predictive ability of the model should be the same among different data sets from the same population (Scalon et al., 1998). With the bootstrapping, we estimated the whole distribution of important variables under consideration. Therefore, from a whole set of variables we can select only the important variables which are more stable.

In the current study, CF has been included as a variable along with its interactions to develop equations as it has been traditionally used in feed analysis because of its simplicity, feasibility, and historical usage. Prediction equations including CF as an important variable were reported in previous broiler studies (Campbell et al., 1986; Alvarenga et al., 2015; Pedersen et al., 2021). Moreover, variable components of an equation should come from simple analytical procedures

and generally CP, ash, fat, starch, and sometimes a fibre crieteria like CF are important parameters (Zaefarian et al., 2021).

According to Pedersen et al. (2021), ileal digestibility of protein in broiler diets can be predicted by dietary starch, CF, and fat contents. Starch and fat contents had a positive relationship, while CF had a negative relationship with the ileal digestibility of protein ($R^2 = 0.42$; $P < 0.05$). In the present study, CAID of N was predicted by Equation 1, with $R^2 = 0.78$ ($P < 0.15$). However, CF had a positive relationship and CF^2 had a negative relationship with CAID of N. Cerrate et al. (2019) reported that digestibility of protein in pigs and poultry reduced with increasing dietary fibre content and the negative effect was dependent upon the level and type of fibre. On the other hand, in the current study, fat content of the diet did not have an impact on digestibility of protein. This agrees with previous results by Honda et al. (2009) who found no effects on protein digestibility with chickens fed 3 to 10% crude fat.

Results of the present study showed that CAID of energy was best explained by dietary CF and GE content, CF^2, and starch:CF (Equations 2 and 16). Cerrate et al. (2019) stated that dietary ME can be predicted from digestible nutrients and crude dietary nutrients, where the interaction between dietary protein and fat was considered. A 0.02 unit increment in the CAID of energy was observed with a unit increase in dietary content of energy. Pedersen et al. (2021) proposed an equation for ileal digestibility of energy, where starch, CF, and phytate contents were necessary to achieve a significant prediction and CAID of energy was negatively affected by dietary CF and phytate. In contrast, the present study reported a positive relationship between dietary CF and CAID of energy (Equations 2 and 16).

The CAID of fat was predicted by dietary content of fat, Ca, P, GE, and CF^2 ($R^2 = 0.77$) (Equation 3) and the same predictors were selected when bootstrapping except for CF^2 (Equation 17). In bootstrap resampling, CF^2 appeared only 11.7% of the time and was, therefore, not included while developing the model with the selected variables. According to

Noblet and Perez (1993), digestibility coefficient of ether extract (EE) depended on dietary EE as well as the square value of EE (R^2 = 0.70). A negative correlation between CAID of fat and dietary content of P was observed in both equations. However, dietary Ca content had a positive effect on CAID of fat. This was in contrast to previous studies (Edwards et al., 1960; Griffith et al., 1961; Hakansson, 1974; Atteh and Leeson, 1983; Mutucumarana et al., 2014; Tancharoenrat and Ravindran, 2014) reported a decrease in digestibility of fat with an increase in dietary Ca level. High dietary Ca affects the utilisation of fat through the formation of Ca soaps (Atteh and Leeson, 1983). In the present study, however, Ca in the diets was only from the 10 ingredients and not from the major Ca sources such as limestone and dicalcium phosphate (DCP), which are known for the negative effects on fat and energy digestibility. Moreover, the concentrations of Ca and P were only high in 10 diets with high inclusion of meat and bone meal (55-105 g/kg DM).

The original equation for CAID of starch (Equation 4) with all predictors was composed of fat, CF, and GE content. However, fat content was not selected as a variable as it appeared only 23.5% of the time in the 1000 bootstrap resamples when the second stepwise regression was done (Equation 18). The CF and GE contents had a positive and negative relationship, respectively, with CAID of starch. Cerrate et al. (2019) reported that the digestibility coefficient for starch was negatively affected by neutral detergent fibre (NDF) content (R^2 = 0.10) in diets without enzyme and positively affected (R^2 = 0.03) with enzyme.

Precision of the equation for CAID of Ca was improved when CF^2, fat:CF, and starch:CF were considered. In contrast, the content of starch and fat:CF were eliminated from the model when variables from bootstrapping were included even though both had been selected more than 30% of the time in bootstrapping (Equation 19). The R^2 of Equation 19 was low compared to Equation 5. In both equations, CAID of Ca was not influenced by dietary Ca level. This agrees

with Mutucumarana et al. (2014) who suggested that Ca digestibility was not influenced by dietary Ca concentration.

Current results show that CAID of P was predicted by Equations 6 and 20. In both equations, a negative, curvilinear effect of CF was observed, and CAID of P decreased 0.02 units with every unit increase in dietary P. Even though N and starch contents were selected more than 30% of the time in bootstrap resamples, they were not included in Equation 20 as they had not met the 0.15 significance level for entry into the model. The CF was an important variable for prediction of CAID of DM. Results of the study by Mtei et al. (2019) demonstrated that CAID of DM, starch, fat, NDF, and GE were influenced by dietary fibre content, and it differed among different bird types (layers, broilers, and pullets).

In the current study, IDC of N increased linearly with dietary N content. In agreement with this, digestible protein in poultry (Tahir et al., 2008; Cerrate et al., 2019) and pigs (Noblet and Perez, 1993; Shi and Noblet, 1993) improved by adding dietary protein. The RMSE value of IDC of N increased when variables selected by bootstrapping were used with the removal of CF and starch:CF and the replacement of starch. Both equations also indicated a negative effect of ash on amount of N lost, where 0.04 g of N was lost per g increment in ash content of the diet.

The starch:CF, CF, and GE had a positive relationship, and CF^2 had a negative relationship for the prediction of IDC of energy. Furthermore, results from Noblet and Perez (1993) in pigs agree with the positive relationship of dietary GE content with IDC of energy. The IDC of fat increased by 0.6 units when there was a unit increase in dietary content of fat (partial R^2 of 0.94). In agreement with this, Wiseman and Salvador, (1991), Shi and Noblet (1993), and Cerrate et al. (2019) reported that fat digestibility improved linearly by adding dietary fat. According to Noblet and Perez (1993), digestible EE was predicted with either EE content alone ($R^2 = 0.97$) or with EE and NDF content ($R^2 = 0.97$), where EE content had a positive

relationship with digestible EE in both equations and NDF content had a negative relationship with digestible EE.

In the current study, the best predictors for IDC of starch were dietary content of starch and GE. Cerrate et al. (2019) stated that digestible content of starch could be estimated using dietary starch content. They reported a 0.94 decrement in digestible starch content for each unit increment in dietary starch ($R^2 = 0.94$). In the current study and that by Cerrate et al. (2019), digestible starch had a linear relationship with dietary starch content.

The best parameter to predict IDC of Ca was the dietary content of Ca. Consequently, dietary Ca content had a negative impact on IDC of P, with a positive effect of ash and a negative curvilinear effect of CF. As described earlier, these equations can be used as a general guide as the concentration of Ca and P were very high in diets containing high amounts of MBM.

In this study, we also found that the inclusion of CF and its interactions as a variable in prediction equations increased the R^2 value. Even though the CF represents a small portion of the total fibre present in the ingredients, the impact of CF can be seen in most of the developed equations. There is potential for further improvement of the developed equations by adding NDF, ADF, or non-starch polysaccharides (NSP). However, it is important to consider the cost for the analysis to best select the model with practical application.

The major limitation of this study was the diets used, which represented a wide range of chemical compositions due to varying inclusion level of ingredients. This ensured the development of better prediction equations assisting in finding the relationship between the variables in regression equations. However, compared to the diets used in the current study, commercial diets will be complete and nutritionally balanced. Therefore, in practice, the equations should be used with caution depending on the chemical composition of the feeds to

be studied. Moreover, further research is needed to assess the generalizability of our findings to commercial settings.

3.6. Conclusions

Prediction equations can be developed for CAID and IDC of nutrients in diets using chemical composition by including interactions between dietary chemical components. Measures of goodness of fit (R^2, Adj. R^2, RMSE, and AIC) of the original stepwise regression models and after the bootstrap exercise for estimated regression coefficients for CAID of N, energy, and DM, as well as IDC of energy, starch, and Ca, were the same with no differences in parameter estimates. This indicated the stability performance for the stepwise regression models among the bootstrap samples. Not much difference was observed between the performance of the original model and the model constructed with the selected variables using bootstrapping. However, this study gives a useful insight into the variable selection approach as stepwise selection is just a single model without any information about its stability and this might be useful to select the best variables when constructing prediction models with greater confidence. Rigorous analysis and external validation with a new data set should require ensuring the use of the equations in practical situations.

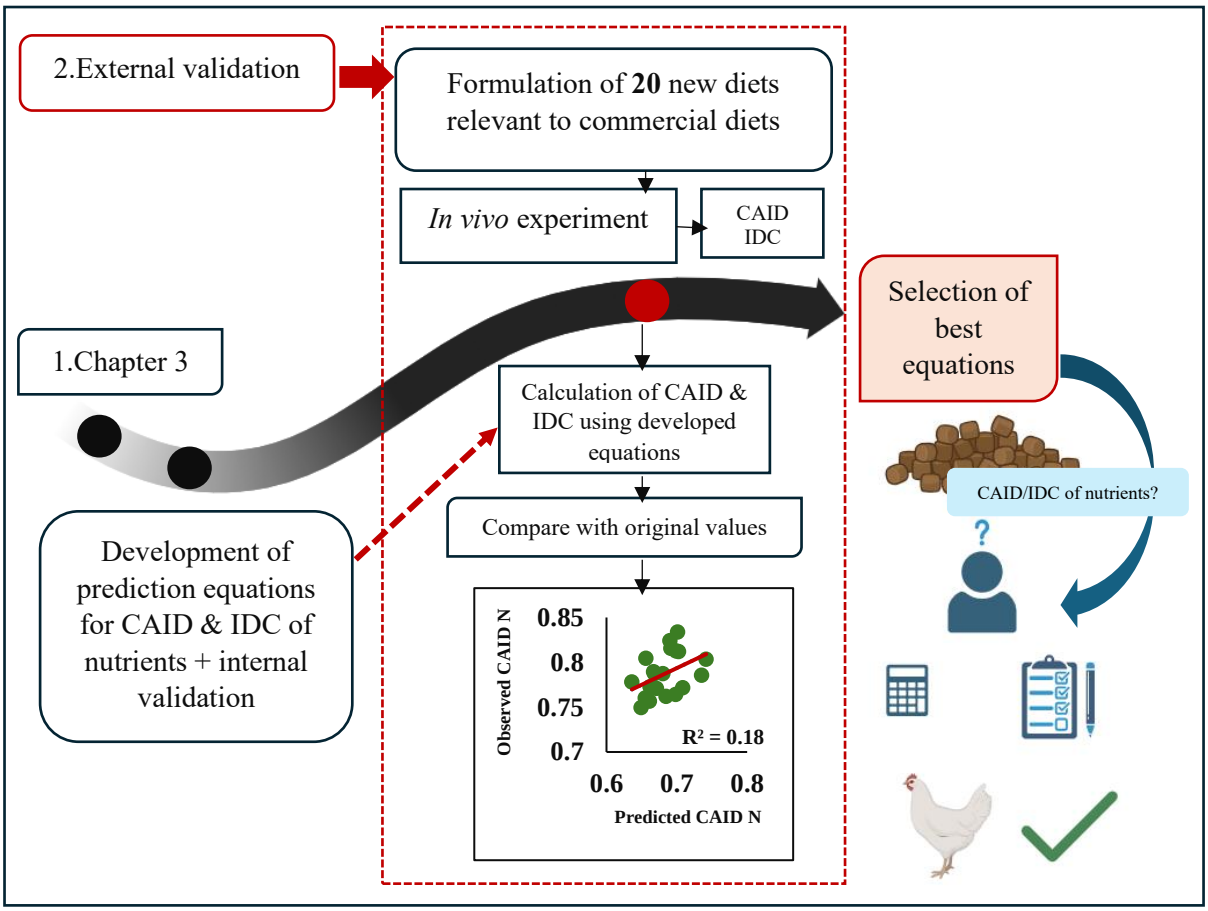
CHAPTER 4

Validation of prediction equations to estimate the nutritive value of broiler chicken diets based on their chemical composition

This Chapter has been submitted for publication:
Thiruchchenthuran, S., Zaefarian, F., Abdollahi, M.R., Wester, T.J ., Morel, P.C.H. 2024. Validation of prediction equations to estimate the nutritive value of broiler chicken diets based on their gross chemical composition. Submitted to Animal Feed Science and Technology.

External validation of developed prediction equation is essential for their practical application. Therefore, this Chapter was designed to externally validate the developed prediction equations in Chapter 3.

Graphical abstract



4.1. Abstract

An experiment was conducted to validate the accuracy of previously published prediction equations developed to estimate the coefficient of apparent ileal digestibility (CAID) and ileal digestible content (IDC) of nitrogen (N), fat, starch, calcium (Ca), phosphorus (P), energy, and dry matter (DM) in broilers using the chemical composition of diets. Twenty new diets were formulated to have a wide range of chemical characteristics relevant to commercial diets. The CAID of N, fat, starch, Ca, P, energy, and DM of the diets were determined in broiler growers fed *ad libitum* from 15 to 22 days post-hatch. The chemical composition and *in vivo* digestibility values were used to validate the prediction equations developed from a previous study. Comparison between the determined values and predicted values was used to assess the accuracy of prediction equations using the coefficient of determination (R^2), root mean square error of prediction (RMSEp**)**, concordance correlation coefficient (CCC), and mean bias (MB). The most accurate prediction was achieved in terms of R^2 and CCC for CAID of energy and DM (R^2: 0.57 and 0.66, CCC: 0.45 and 0.47, respectively) as well as for IDC of N, starch, energy, and DM (R^2: 0.90, 1.00, 0.65, and 0.66, CCC: 0.48, 0.97, 0.51, and 0.47, respectively). The R^2 and CCC values obtained for CAID of N, fat, starch, Ca, and P and IDC of Ca and P were not consistent with the expectation of predictive performance. The R^2 for IDC of fat was high (0.94), however, CCC was moderate (0.43). The determined MB values showed that some equations underpredicted (CAID and IDC of N, fat, starch, energy, and DM) and some overpredicted (CAID and IDC of Ca and P) the observed values of *in vivo* study. In conclusion, the equations obtained for CAID of energy and DM as well as IDC of N, starch, energy, and DM could be considered the best fit according to R^2 and CCC. Moreover, this study highlights the importance of validation with external data before applying each prediction equation to practical situations.

Key words: broiler, ileal digestible content, nutrient digestibility, prediction equation, validation

4.2. Introduction

The search for more accurate information about the nutritive value of feedstuffs and complete diets is important for sustainable poultry production (Alvarenga et al., 2013a). Chemical analysis, table values, prediction equations, infrared spectroscopy, *in vivo*, and *in vitro* assays are some of the methods used in the evaluation of feed for poultry (Pedersen et al., 2021). Among these, *in vivo* trials are the most accurate way to determine nutrient utilisation. On the other hand, *in vivo* techniques are costly and time-consuming. In recent years, prediction equations has been gaining more interest and are used by most animal feed industries (Mateos et al., 2019).

Predictive models are widely known as informational tools to support rapid and economical assessment of feed (Baiz et al., 2020). It allows the use of simple chemical analysis of the diets to determine the nutritive values (Alvarenga et al., 2013b). The precision of prediction equations depends greatly on the selection of an adequate number of test products with a sufficiently wide range of nutrient composition and the inclusion of relevant chemical analysis in the regression. Ideally, the variables of the equation should come from simple and routine analysis minimizing the cost and time (Alvarenga et al., 2013a).

Several previous studies have proposed equations mainly for predicting the energy values, digestibility of amino acids, and protein of feedstuffs or diets from their chemical composition (Nascimento et al., 2009; Losada et al., 2010; Alvarenga et al., 2011; Mariano et al., 2013; Cerrate et al., 2019; Walk and Rao, 2020; Pedersen et al., 2021). Nevertheless, there were some validation studies carried out to ensure the accuracy of proposed equations (Alvarenga et al., 2013b; Meloche et al., 2014; Alvarenga et al., 2015; Pedersen et al., 2021). Moreover, many

published reports relating to prediction equations presented limited information on how those equations were validated (Batal and Dale, 2006; Alvarenga et al., 2013a; Wu et al., 2019).

The most important final step in the development of regression equations is to validate them with a sample data set independent of the products used to generate the equations (Meloche, 2013). This will ensure the practical application of each equation with greater confidence and to alert users to risks, if any (Meloche et al., 2014). Prediction equations for determination of the coefficient of apparent ileal digestibility (CAID) and ileal digestible content (IDC) of nitrogen (N), fat, starch, calcium (Ca), phosphorus (P), energy, and dry matter (DM) were developed using 56 diets in Chapter 3 (Thiruchchenthuran et al., 2024). Therefore, the objective of the present study was to validate the developed prediction equations with unique data.

4.3. Materials and methods

4.3.1. Diets

Twenty broiler grower diets were formulated to represent a wide range of chemical compositions and with common ingredients used in commercial poultry feeding (Tables 4.1 and 4.2). A central diet was prepared with the standard nutritional requirements of Ross 308 broiler chickens (Ross, 2022). The remaining 19 diets were prepared such that they were in a range of 180 to 240 g/kg crude protein (CP) and 10 to 14 MJ/kg apparent metabolisable energy (AME).

All diets contained 5.0 g/kg titanium dioxide (TiO_2) as an indigestible marker for the determination of CAID of nutrients. All diets were steam conditioned at 60 ^{o}C for 30 s and pelleted through a pellet mill (Model Orbit 15; Richard Sizer Ltd., Kingston-Upon-Hull, UK) capable of manufacturing 180 kg of feed/h and equipped with a die ring with 3-mm hole and 35-mm thickness. Representative samples were collected for each diet after pelleting for the determination of nutrient content and pellet durability.

Table 4.1. Ingredient composition of the experimental diets (as-fed basis).

	Diets																			
Ingredients (g/kg)	A	B	C	D	E	F	G	H	I	J[a]	K	L	M	N	O	P	Q	R	S	T
Maize	254	565	-	358	300	260	300	108	250	560	400	543	400	350	240	503	408	553	554	337
Wheat	365	95.0	560	153	211	153	223	253	261	-	103	-	203	153	203	-	233	-	53.0	253
SBM	150	250	362	270	200	250	50.0	-	100	362	200	180	300	180	100	210	200	170	185	150
Canola meal	50.0	11.9	-	180	200	100	260	320	250	-	200	180	-	100	250	100	50.0	210	50.0	150
Wheat bran	50.0	-	-	-	-	128	40.0	150	50.0	-	31.4	-	-	110	100	48.0	-	-	35.0	-
MBM	50.0	-	-	-	-	40.0	50.0	110	30.0	-	30.0	50.0	40.0	50.0	20.0	60.0	20.0	-	20.0	-
Soybean oil	40.0	30.0	30.0	-	50.0	30.0	30.0	20.0	20.0	30.0	5.6	20.0	20.0	30.0	48.0	50.0	50.0	30.0	70.0	70.0
DCP	10.0	17.1	17.1	10.0	10.0	10.0	18.0	10.0	10.0	17.1	5.0	8.0	10.0	-	10.0	10.0	10.0	8.0	8.0	10.0
Limestone	10.0	9.5	9.5	10.0	10.0	10.0	10.0	10.0	10.0	9.5	7.0	-	8.0	8.0	10.0	-	10.0	10.0	-	5.0
L-Lysine HCl	4.0	4.1	4.1	5.0	5.0	5.0	5.0	5.0	5.0	4.1	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
DL- Methionine	3.5	3.5	3.5	3.0	3.0	3.0	3.0	3.0	3.0	3.5	2.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	5.0	5.0
L-Threonine	2.0	2.3	2.3	1.0	1.0	1.0	1.0	1.0	1.0	2.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	5.0	5.0
Salt	1.0	0.9	0.9	2.0	2.0	2.0	2.0	2.0	2.0	0.9	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Sodium bicarbonate	4.0	4.1	4.1	1.0	1.0	1.0	1.0	1.0	1.0	4.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
TiO_2	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

[a] Diet J was formulated to meet the Ross 308 strain recommendations for major nutrients (Ross 308 Nutritional Guide, 2022); [b] Supplied per kg of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co (cobalt sulfate), 0.3 mg; Cu (copper sulfate), 3.0 mg; Fe (iron sulfate), 25 mg; I (calcium iodate), 1 mg; Mn (manganese oxide), 125 mg; Mo (sodium molybdate) , 0.5 mg; Se (sodium selenite), 200 µg; Zn (zinc sulfate), 60 mg.

DCP, di calcium phosphate; MBM, meat and bone meal; SBM, soybean meal; TiO_2, titanium dioxide.

4.3.2. Birds and housing

Experimental procedures were conducted in accordance with the Massey University Animal Ethics Committee guidelines (MUAEC 22/39). A total 600, one-day old male Ross 308 broiler chicks were obtained from a commercial hatchery and fed a commercial starter diet from 1 to 11 d of age. Birds were initially placed on wood shavings over concrete floor pens. The temperature was maintained at 31 °C on d 1 and decreased by 3 °C per week to a final temperature of 22 °C at 21 d of age.

On d 12, birds were allocated to 100 battery cages and offered dietary treatments until d 22 in an environmentally controlled room with 20 h of fluorescent illumination per day. Each of the 20 diets was randomly assigned to five replicate cages, each housing six birds in a randomised complete block design. The space allocation per bird in grower cages was 640 cm^2. Cages with wired floors were equipped with feed troughs and nipple drinkers. Feed intake was monitored on a cage basis from d 15 to 22 post-hatch. Diets were offered *ad libitum* and water was freely available.

4.3.3. Pellet durability

Pellet durability was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Limited, Willow Park, North Walsham, Norfolk, UK). Clean pellet samples (5 replicates per diet; 100 g each) were rapidly circulated in an air stream around a perforated test chamber for 30 s. Fines were removed continuously through the perforations during the test cycle. After the test cycle, the subject pellets were ejected and weighed manually. The pellet durability index (PDI) was calculated as the ratio of the pellets not passing through the perforations after the test to whole pellets at the start (Abdollahi et al., 2010). The PDI was calculated to determine the quality of the pellets prepared as all prepared pellets differ in their composition.

4.3.4. Measurements of growth performance

Pen and cage served as experimental unit with weights of the birds in every unit recorded at the start (day 15) and end (day 22) of the experiment. The average daily gain was calculated from the weight gain of birds in each unit. Feed intake was calculated by subtracting the remaining feed from the offered feed in each unit during the experiment. Mortality was recorded daily. Feed intake and body weight gain were measured only for monitoring purposes as this experiment was not aimed at investigating the effects of diets on bird's performances.

4.3.5. Determination of apparent metabolisable energy (AME)

Feed intake and total excreta output of each cage were quantitatively measured from day 19 to 22 post-hatch. Daily collections from each cage were pooled, mixed in a blender, and sub-sampled. Each sub-sample was freeze-dried, ground to pass through a 0.5-mm sieve, and stored in airtight plastic containers at 4 ^{o}C until further analysis. The excreta samples were analysed for DM, gross energy (GE), ash, N, and titanium (Ti).

4.3.6. Determination of CAID of nutrients and GE

On d 23, all birds in the cages were euthanised by intravenous injection of sodium pentobarbitone (Provet NZ Pty Ltd., Auckland, New Zealand) and digesta were collected from the lower half of the ileum as described by Ravindran et al. (2007). Digesta from the birds within the cage were pooled, lyophilised (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve, and stored at 4 ^{o}C in airtight containers until laboratory analysis. The diets and digesta samples were analysed for DM, N, ash, GE, Ti, starch, fat, Ca, P, crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin.

4.3.7. Chemical analysis

Dry matter was determined using standard procedures (Methods 930.15 and 925.10; AOAC, 2016). Total N was determined by Dumas method (Method 968.06; AOAC, 2016). Crude protein content was calculated as N × 6.25. Ash was determined by the standard procedures (Method 942.05; AOAC, 2016) using a muffle furnace at 550 ^{o}C for 16 h. Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid. Samples were analysed for Ti on a UV spectrophotometer following the method of Short et al. (1996). Total starch was analysed using the assay procedure (Megazyme Total Starch Assay Procedure; Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable alpha-amylase and amyloglucosidase (Method 996.11; AOAC, 2016). Fat was determined using a Soxhlet extractor (Soxtec System HT 1043 Extraction Unit, Höganäs, Sweden) by hexane extraction method (Method 2003.06; AOAC, 2016).

Calcium and P concentrations were determined by colorimetric methods after combustion of the samples at 550 ^{o}C and acid digestion in 6.0 M HCl using standard procedures (Method 968.08D; AOAC, 2005). Crude fibre was measured using modified standard procedures (Methods 962.09 and 978.10; AOAC, 2016). The NDF, ADF, and lignin were analysed using Fibretec^{TM} (FOSS analytical AB, Höganäs, Sweden) following standard procedures (Methods 2002.04 and 973.18; AOAC, 2016).

4.3.8. Calculations and statistical analysis

The AME value of the diets by total excreta collection method was calculated using the following formula:

$$\text{AME (MJ/kg diet)} = \frac{[(\text{Feed intake} \times \text{GE}_{diet}) - (\text{Excreta output} \times \text{GE}_{excreta})]}{\text{Feed intake}} \quad (4.1)$$

The AME by marker method was calculated using the following formula:

$$\text{AME (MJ/kg diet)} = \text{GE}_{diet} - [\text{GE}_{excreta} \times \frac{Ti_{diet}}{Ti_{excreta}}] \quad (4.2)$$

The CAID was calculated using the following formula:

$$\text{CAID of nutrient} = \frac{(\text{Nutrient}/Ti)_{diet} - (\text{Nutrient}/\text{Ti})_{ileal}}{(\text{Nutrient}/\text{Ti})_{diet}} \quad (4.3)$$

where $(\text{Nutrient/Ti})_{diet}$ = ratio of diet component to Ti in the diet and $(\text{Nutrient/Ti})_{ileal}$ = ratio of the diet component to Ti in the ileal digesta.

The IDC was calculated by the following formula:

$$\text{IDC of nutrient} = \text{Nutrient in the diet} \times \text{CAID of that nutrient} \quad (4.4)$$

The prediction performance of the established prediction equations was estimated based on the difference between the predicted and observed values for each CAID and IDC of N, starch, fat, Ca, P, energy, and DM values. The accuracy of selected equations was evaluated based on the predicted coefficient of determination (R^2), root mean square error of prediction (RMSEp), concordance correlation coefficient (CCC), and mean bias (MB). Statistical significance was considered at $P \leq 0.15$. All the data analysis were carried out using SAS software, version 9.4 package (SAS, 2016). In addition, correlation analysis was performed to examine the relationship between the variables using the CORR procedures of SAS (2016).

The R^2 estimates the percentage of variance of the response variable explained with the explanatory variables and it can be calculated as,

$$R^2 = 1 - \frac{RSS}{TSS} \quad (4.5)$$

where TSS is the total sum of squares and RSS is the residual sum of squares (Renaud and Victoria-Feser, 2010).

The RMSEp can be explained as the square root of the average square differences between the predicted and measured values of the validation data set and can be calculated by the following equation.

$$\text{RMSEp} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \tag{4.6}$$

where, y_i is the observed value of the validation data set, $\hat{y}_i$ is the predicted value and n is the number of observations in the validation data set (Faber, 1999).

High value of R^2 and a low value of root mean quare error (RMSE) are considered a better model fit (Baiz et al., 2020).

The CCC evaluates the agreement between two readings by measuring the variation from the 45° line through the concordance line which is the origin. It is very simple to use and has desirable properties (Lin, 1989). It has a similar range to Pearson's, from 0 to 1. According to Altman (1990), it should be evaluated similarly to other correlation coefficients, such as Pearson's, with < 0.2 being considered poor and > 0.8 being considered outstanding. McBride (2005), however, recommended a different set of interpretation criteria where > 0.99 is almost perfect, 0.95 to 0.99 substantial, 0.90 to 0.95 is moderate, and < 0.90 is poor (Akoglu, 2018).

$$\text{CCC} = \frac{2\sigma_{PM}}{\sigma_P^2+\sigma_M^2+(\mu_P-\mu_M)^{2'}} \tag{4.7}$$

where, σ_{PM} is the covariance, σ^2_P and σ^2_M are the variances, μP and μM are the means, and P and M refer to the predicted and measured values (Marshall et al., 2023).

The MB compares the predicted means and the observed means of the evaluation data set. It shows whether the model is over-predicting or under-predicting the measured values and provides the uniformity of error prediction. Positive MB values imply overprediction, negative

values indicate underprediction, and zero implies a balanced distribution of the two (Mkhabela et al., 2011).

$$\mathrm{MB} = \frac{1}{n}\sum_{i=1}^{n}(\hat{y}_i - y_i) \tag{4.8}$$

where, y_i is the observed value, $\hat{y}_i$ is the predicted value and n is the number of observations. A t-test was used to test if the slopes were different from 1, to identify any slope bias.

4.4. Results

4.4.1. Chemical composition of the diets

The formulated 20 diets ranged in CP content and GE from 192 to 291 g/kg and 18.2 to 19.3 MJ/kg, respectively, on DM basis (Table 4.3). The fat content of the diets ranged from 30.8 to 99.2 g/kg, starch ranged from 240 to 461 g/kg, Ca content ranged from 6.18 to 24.7 g/kg, and P content ranged from 6.6 to 14.3 g/kg on DM basis. The AME content of the 20 diets measured by the total collection method ranged from 12.4 to 14.7 MJ/kg DM. The diets containing high-fat content had low PDI as observed in diets S and T, whose fat content was 94.8 and 99.2 g/kg DM, respectively. Diet D had 82.1% PDI, which was the highest among 20 diets and had the lowest fat content of 30.8 g/kg on DM basis.

Table 4.2. Calculated chemical composition of the experimental diets (g/kg as-fed basis).

Diet	DM	Ash	CP	Fat	Starch	CF	Ca	P	GE (MJ/kg)	AME (MJ/kg)	Lysine	Met+Cys	Threonine
A	883	40.8	190	67.2	396	31.0	12.3	7.60	16.8	12.6	11.4	9.30	8.24
B	881	25.6	184	57.6	424	27.2	8.50	6.30	16.6	12.9	12.1	9.10	8.80
C	881	33.2	243	47.7	340	29.8	8.90	6.80	16.3	12.0	15.2	10.50	10.7
D	877	37.9	242	27.6	326	43.6	8.20	6.40	16.2	11.1	16.1	11.00	9.97
E	882	35.1	219	74.2	323	42.9	8.20	6.10	17.3	12.3	14.5	10.40	9.04
F	881	48.1	236	59.1	290	42.1	11.9	8.30	16.8	11.5	15.5	10.30	9.35
G	882	47.4	201	60.5	337	46.5	15.5	10.1	16.5	11.5	12.7	10.20	8.14
H	882	70.9	233	55.6	255	56.4	20.1	12.4	16.3	10.3	13.9	11.20	9.07
I	880	44.3	214	48.7	331	48.3	11.5	7.90	16.4	11.3	13.6	10.50	8.66
J	881	30.3	220	57.6	365	28.3	8.70	6.60	16.8	12.6	14.7	9.90	10.3
K	878	45.3	232	37.2	329	45.0	9.00	6.90	16.4	11.2	15.2	9.80	9.52
L	879	47.2	220	54.4	352	40.0	9.10	7.90	16.7	12.0	14.5	10.30	9.03
M	880	40.3	221	48.9	383	27.1	10.5	7.00	16.6	12.4	14.8	9.40	8.69
N	880	46.8	212	61.4	344	39.5	9.60	6.50	16.9	12.0	13.8	9.70	8.38
O	882	42.4	209	75.1	300	50.7	10.4	7.70	17.1	11.7	13.5	10.40	8.55
P	883	48.2	215	83.0	337	35.4	10.2	8.50	17.2	12.7	14.3	9.70	8.66
Q	883	31.9	187	76.1	406	29.2	9.20	6.00	17.2	13.2	12.6	8.90	7.47
R	880	33.3	203	59.8	359	43.1	7.60	5.70	16.9	12.1	13.8	10.10	8.63
S	884	31.2	181	98.4	399	30.2	5.10	5.70	17.7	13.8	12.2	10.50	11.1
T	884	29.2	192	93.2	371	37.0	5.90	5.60	17.6	13.3	12.5	11.30	11.6
Mean	**881**	**40.5**	**213**	**62.2**	**348**	**38.7**	**10.0**	**7.30**	**16.8**	**12.1**	**13.9**	**10.13**	**9.19**
SD	1.89	10.2	19.2	17.5	41.4	8.63	3.28	1.65	0.42	0.85	1.27	0.66	1.05
Minimum	877	25.6	181	27.6	255	27.1	5.10	5.60	16.2	10.3	11.4	8.90	7.47
Maximum	884	70.9	243	98.4	424	56.4	20.1	12.4	17.7	13.8	16.1	11.30	11.6

AME, apparent metabolisable energy; Ca, calcium; CF, crude fibre; CP, crude protein; DM, dry matter; GE, gross energy; P, phosphorus; SD, standard deviation.

Table 4.3. Analysed nutrient composition (g/kg DM basis), AME (MJ/kg DM basis), feed intake, growth performance and pellet durability (%) of the experimental diets.

Diet	DM	Ash	CP	Fat	Starch	CF	NDF	ADF	Lignin	Ca	P	GE (MJ/kg)	AME (MJ/kg) by total collection	AME (MJ/kg) by TiO_2 Marker	Feed Intake (g/bird)	BW gain (g/bird)	PDI (%)
A	896	73.7	216	70.3	417	36.9	150	45.7	16.1	14.2	8.71	18.8	14.2	13.6	1169	635	61.3
B	890	60.9	206	58.8	461	49.9	91.0	25.4	3.82	8.99	7.25	18.6	14.6	13.9	1226	604	66.9
C	900	70.2	275	48.8	325	34.3	97.0	35.9	9.70	9.79	7.92	18.8	13.5	13.3	1225	649	74.5
D	897	68.3	291	30.8	320	63.9	144.1	62.4	21.7	9.50	7.34	18.2	12.6	12.0	1180	557	82.1
E	901	67.1	242	84.0	337	57.7	152	68.4	24.6	9.81	7.75	18.7	13.3	12.2	1186	630	52.2
F	899	81.5	266	66.0	310	52.5	167	58.4	16.7	14.8	9.33	18.7	13.0	12.3	1159	640	72.5
G	898	82.5	242	65.2	333	64.3	183	73.0	24.1	16.9	11.2	18.6	13.0	11.6	1160	543	74.4
H	901	106.8	284	56.7	240	72.6	222	93.5	32.9	24.7	14.3	18.4	12.4	11.6	1134	557	79.3
I	895	75.8	254	68.9	321	68.0	146	66.4	23.0	14.1	9.50	18.5	12.8	11.7	1160	570	75.4
J	897	64.9	243	57.4	398	36.7	63.7	22.7	4.96	9.32	7.49	18.6	14.4	13.8	1215	657	70.2
K	895	69.6	273	36.0	339	48.3	138	60.2	19.2	10.8	8.17	18.4	12.9	12.7	1196	614	81.5
L	891	63.9	253	53.8	380	41.0	128	50.2	15.0	9.57	9.05	18.8	13.8	13.4	1204	621	69.2
M	894	69.4	246	43.0	411	29.4	102	33.9	8.61	11.8	8.25	18.4	14.0	12.8	1236	657	74.9
N	893	68.6	237	62.2	366	51.2	146	55.0	17.0	12.1	7.77	18.7	13.2	12.6	1179	629	65.1
O	889	73.4	243	79.2	310	63.0	187	81.0	26.0	13.0	8.69	19.2	13.3	13.0	1124	634	64.2
P	898	65.6	235	82.5	382	36.6	170	66.5	24.1	10.9	9.45	18.8	14.1	13.7	1091	630	49.6
Q	893	63.0	214	77.3	435	44.7	153	48.7	15.7	10.7	7.06	18.8	14.4	14.1	1186	617	53.9
R	886	60.1	236	64.2	380	49.2	161	74.3	26.6	8.56	6.78	18.8	13.8	13.6	1156	581	66.8
S	890	49.8	192	94.8	452	34.8	143	46.5	14.2	6.18	6.72	19.3	14.7	13.6	1148	544	19.5
T	892	53.6	214	99.2	394	49.5	184	72.8	31.8	6.62	6.60	19.0	13.8	13.4	1116	566	33.3
Mean	**895**	**69.4**	**243**	**65.0**	**366**	**49.2**	**146**	**57.0**	**18.8**	**11.6**	**8.47**	**18.7**	**13.6**	**13.0**	**1173**	**607**	**64.3**
SD	4.23	11.9	26.3	18.0	55.7	12.6	37.1	18.6	8.14	4.08	1.80	0.26	0.69	0.82	38.7	38.4	16.0
Minimum	886	49.8	192	30.8	240	29.4	63.7	22.7	3.82	6.18	6.60	18.2	12.4	11.6	1091	543	19.5
Maximum	901	106.8	291	99.2	461	72.6	222	93.5	32.9	24.7	14.3	19.3	14.7	14.1	1236	657	82.1

All data are mean values of 5 replicate cages per diet (6 birds per replicate cage)

AME, apparent metabolisable energy; ADF, acid detergent fibre; BW, body weight; Ca, calcium; CF, crude fibre; CP, crude protein; DM, dry matter; GE, gross energy; NDF, neutral detergent fibre; P, phosphorus; PDI, pellet durability index; SD, standard deviation; TiO_2, titanium dioxide.

4.4.2. Correlation (r) analysis

The GE was positively correlated to fat (r = 0.78, P < 0.001) and negatively correlated to N (r = −0.64, P = 0.003; Table 4.4). Interestingly, fat was not correlated with starch but negatively correlated to N (r = −0.71, P < 0.001), and starch was negatively correlated with N (r = −0.84, P < 0.001).

Table 4.4. Correlations between nutrient parameters of 20 diets used for the prediction of coefficient of apparent ileal digestibility (CAID) and ileal digestible content (IDC) of nutrients.

Item		Ash	N	Fat	Starch	CF	Ca	P	GE	CF^2	Fat:CF
N	r	0.63									
	P-value	0.003									
Fat	r	−0.33	−0.71								
	P-value	NS	0.0005								
Starch	r	−0.78	−0.84	0.28							
	P-value	<.001	<.001	NS							
CF	r	0.57	0.40	−0.03	−0.70						
	P-value	0.008	0.079	NS	0.0006						
Ca	r	0.97	0.46	−0.20	−0.67	0.56					
	P-value	<.001	0.042	NS	0.001	0.011					
P	r	0.92	0.47	−0.16	−0.67	0.50	0.93				
	P-value	<.001	0.035	NS	0.001	0.025	<.001				
GE	r	−0.46	−0.64	0.78	0.34	−0.29	−0.36	−0.30			
	P-value	0.043	0.003	<.001	0.141	NS	0.117	NS			
CF^2	r	0.63	0.43	-0.05	−0.72	0.99	0.61	0.56	−0.31		
	P-value	0.003	0.057	NS	0.0003	<.001	0.004	0.010	NS		
Fat:CF	r	−0.57	−0.76	0.74	0.64	−0.66	−0.45	−0.37	0.74	−0.65	
	P-value	0.009	0.001	0.0002	0.002	0.002	0.044	0.110	0.0002	0.002	
Starch:CF	r	−0.59	−0.56	0.10	0.82	−0.95	−0.52	−0.48	0.29	−0.93	0.70
	P-value	0.006	0.011	NS	<.001	<.001	0.018	0.032	NS	<.001	0.0005

Ca, calcium; CF, crude fibre; CF^2, square value of crude fibre; Fat:CF, fat-to-crude fibre ratio; GE, gross energy; N, nitrogen; NS, not significant; Starch:CF, starch-to-crude fibre ratio; P, phosphorus.

4.4.3. CAID and IDC of nutrients

The CAID of N, GE, Ca, P, and DM ranged from 0.75 to 0.83, 0.64 to 0.77, 0.11 to 0.53, 0.36 to 0.62, and 0.57 to 0.72, respectively, and varied to a greater extent (Table 4.5). The highest N and GE digestibility values were obtained for diet Q containing 408 g/kg maize, 233 g/kg

wheat, 200 g/kg soybean meal, and 50 g/kg canola meal. In comparison, the lowest N and GE digestibility values belonged to diet I containing 250 g/kg maize, 261 g/kg wheat, 100 g/kg soybean meal, 250 g/kg canola meal, and 50 g/kg wheat bran. Crude fat and starch were highly digestible in all the diets. The least digestible nutrient was CF, which averaged 14%. The IDC of N, Ca, P, and DM of the 20 diets was ranged from 24.7 to 37.4, 1.92 to 4.43, 3.34 to 5.18, and 566 to 722 g/kg DM, respectively, while GE ranged from 11.9 to 14.4 MJ/kg DM (Table 4.6).

Table 4.5. Coefficient of apparent ileal digestibility (CAID) of nutrients and energy in 20 diet mixtures.

Diet	N	Fat	Starch	CF	Ca	P	GE	DM
A	0.77	0.81	0.97	0.09	0.21	0.45	0.72	0.68
B	0.82	0.90	0.97	0.41	0.21	0.49	0.76	0.71
C	0.82	0.88	0.96	0.09	0.37	0.62	0.72	0.67
D	0.80	0.90	0.97	0.35	0.30	0.55	0.71	0.67
E	0.76	0.89	0.96	0.13	0.26	0.51	0.66	0.61
F	0.77	0.88	0.97	0.05	0.20	0.43	0.66	0.59
G	0.76	0.87	0.96	0.15	0.11	0.36	0.67	0.60
H	0.78	0.86	0.97	0.14	0.18	0.36	0.65	0.57
I	0.75	0.84	0.96	0.16	0.14	0.39	0.64	0.58
J	0.81	0.90	0.97	0.20	0.21	0.53	0.75	0.69
K	0.79	0.88	0.97	0.08	0.31	0.46	0.70	0.66
L	0.76	0.86	0.97	-0.01	0.30	0.46	0.72	0.67
M	0.79	0.85	0.97	-0.18	0.26	0.47	0.72	0.67
N	0.77	0.80	0.97	0.15	0.17	0.45	0.70	0.64
O	0.79	0.89	0.96	0.18	0.28	0.47	0.68	0.62
P	0.81	0.89	0.97	0.10	0.40	0.54	0.76	0.71
Q	0.83	0.90	0.97	0.34	0.29	0.51	0.77	0.72
R	0.79	0.88	0.95	0.11	0.25	0.49	0.70	0.65
S	0.80	0.86	0.95	0.13	0.53	0.58	0.74	0.71
T	0.76	0.81	0.94	0.17	0.46	0.55	0.68	0.64
Mean	**0.79**	**0.87**	**0.96**	**0.14**	**0.27**	**0.48**	**0.71**	**0.65**
SD	0.02	0.03	0.01	0.13	0.11	0.07	0.04	0.05
Minimum	0.75	0.80	0.94	-0.18	0.11	0.36	0.64	0.57
Maximum	0.83	0.90	0.97	0.41	0.53	0.62	0.77	0.72

CF, crude fibre; Ca, calcium; DM, dry matter; GE, gross energy; N, nitrogen; P, phosphorus; SD, standard deviation.

Table 4.6. Ileal digestible content (IDC) of nutrients (g/kg DM) and energy (MJ/kg) in 20 diet mixtures.

Diet	N	Fat	Starch	CF	Ca	P	GE	DM
A	26.7	57.0	405	3.32	2.92	3.94	13.6	678
B	26.9	53.0	449	20.5	1.92	3.52	14.0	707
C	36.3	42.9	314	3.08	3.62	4.89	13.5	671
D	37.4	27.7	312	22.4	2.82	4.01	13.0	670
E	29.4	74.8	322	7.50	2.53	3.97	12.3	609
F	32.9	58.1	300	2.62	2.94	3.98	12.4	593
G	29.5	56.8	321	9.65	1.93	4.03	12.2	601
H	35.4	48.7	232	10.2	4.43	5.18	11.9	566
I	30.4	57.9	308	10.9	1.97	3.75	11.9	581
J	31.5	51.7	387	7.34	1.94	4.01	13.9	693
K	34.5	31.7	328	3.86	3.36	3.77	12.9	656
L	31.0	46.3	367	−0.41	2.89	4.14	13.5	671
M	30.9	36.5	401	−5.29	3.12	3.89	13.2	666
N	29.3	49.8	354	7.68	1.99	3.53	13.0	644
O	30.6	70.5	297	11.4	3.59	4.08	13.1	617
P	30.5	73.4	371	3.66	4.30	5.11	14.2	715
Q	28.6	69.6	421	15.2	3.11	3.60	14.4	722
R	29.7	56.5	363	5.41	2.13	3.34	13.2	651
S	24.7	81.5	431	4.53	3.29	3.88	14.3	707
T	26.2	80.4	369	8.42	3.06	3.63	12.8	644
Mean	**30.6**	**56.2**	**353**	**7.59**	**2.89**	**4.01**	**13.2**	**653**
SD	3.37	15.3	53.9	6.55	0.76	0.50	0.75	45.9
Minimum	24.7	27.7	232	−5.29	1.92	3.34	11.9	566
Maximum	37.4	81.5	449	22.4	4.43	5.18	14.4	722

CF, crude fibre; Ca, calcium; DM, dry matter; GE, gross energy; N, nitrogen; P, phosphorus; SD, standard deviation.

4.4.4. Validation of developed prediction equations

The equations for predicting CAID of P, energy, and DM showed high CCC. The CCC values of CAID of N, fat, and starch were very low with lower R^2 and higher RMSEp. Compared to the equations for predicting CAID of nutrients, the equations for predicting IDC of nutrients showed close agreement between the predicted values and the observed values in the validation data set. The RMSEp for the validation set was higher for all equations compared to the RMSE observed for the original data except for the CAID of starch and IDC of starch and Ca. The R^2 value obtained for IDC of N, fat, starch, and energy in the validation study was 0.90, 0.94, 1.00, and 0.65, respectively. The corresponding CCC of IDC of N, fat, starch, and energy was 0.48, 0.43, 0.97, and 0.51, respectively, indicating a good agreement between the observed values of the *in vivo* study and predicted values by the equation. When comparing the MB of the validation data set, most equations (CAID and IDC of N, crude fat, starch, energy, and DM) systematically underpredicted the observed values, whereas the equations for CAID of Ca and P and IDC of P overpredicted the observed values ($P < 0.05$) (Table 4.7).

Relationship between the observed values and predicted values of CAID of N, and energy and IDC of N, energy, fat, and starch, based on the gross chemical composition in the validation data set (n = 20) were illustrated in Figure 4.1 (a) and 4.1 (b) and Figure 4.2 (a), 4.2 (b), 4.2 (c), and 4.2 (d), respectively.

Table 4.7. Validation of the developed equations using chemical composition for CAID of and IDC of nutrients (n = 20).

No.		Training set (56 diets)[1]			Validation set (20 diets)						
		R^2	RMSE	Mean	R^2	RMSEp	Mean observed	Mean predicted	CCC	Mean bias	Slope bias
01	CAID N	0.78	0.04	0.68	0.18	0.11	0.79	0.68	0.04	-0.10*	-0.63#
02	CAID crude fat	0.77	0.04	0.75	0.001	0.17	0.87	0.71	0.004	-0.16*	-0.98#
03	CAID starch	0.36	0.03	0.93	0.01	0.02	0.96	0.94	-0.03	-0.02*	-1.09#
04	CAID Ca	0.69	0.08	0.33	0.25	0.13	0.27	0.36	0.32	0.09*	-0.32
05	CAID P	0.83	0.07	0.50	0.53	0.09	0.48	0.56	0.41	0.07*	-0.11
06	CAID energy	0.87	0.04	0.61	0.57	0.06	0.71	0.66	0.45	-0.05*	-0.39#
07	CAID DM	0.89	0.04	0.54	0.66	0.06	0.65	0.60	0.47	-0.06*	-0.28#
08	IDC N	0.98	1.66	30.3	0.90	3.88	30.6	27.0	0.48	-3.63*	0.40#
09	IDC crude fat	0.99	3.51	71.8	0.94	22.5	56.2	34.1	0.43	- 22.1*	- 0.10
10	IDC starch	0.99	13.1	214	1.00	11.7	353	342	0.97	-11.0*	0.05#
11	IDC Ca	0.92	1.30	3.77	0.07	0.93	2.89	3.05	0.26	0.16	-0.75#
12	IDC P	0.81	0.72	4.47	0.40	0.91	4.01	4.84	0.20	0.82*	-0.04
13	IDC energy	0.89	0.75	12.3	0.65	1.04	13.2	12.3	0.51	-0.87*	-0.38#
14	IDC DM	0.89	42.3	536	0.66	63.8	653	597	0.47	-56.4*	-0.28#

Ca, calcium; CAID, coefficient of apparent ileal digestibility; CCC, concordance correlation coefficient; DM, dry matter; IDC, ileal digestible content; MB, mean bias; N, nitrogen; P, phosphorus; R^2, coefficient of determination of regression; RMSE, root mean square error; RMSEp, root mean square error predicted.
[1] Results of the equations in Chapter 3: Table 3.3: Equations 01-07, and Table 3.4: Equations 08-14; * MB was different from 0 at $P < 0.05$ (Paired t-test); # Slope was different from 1 at $P < 0.05$.

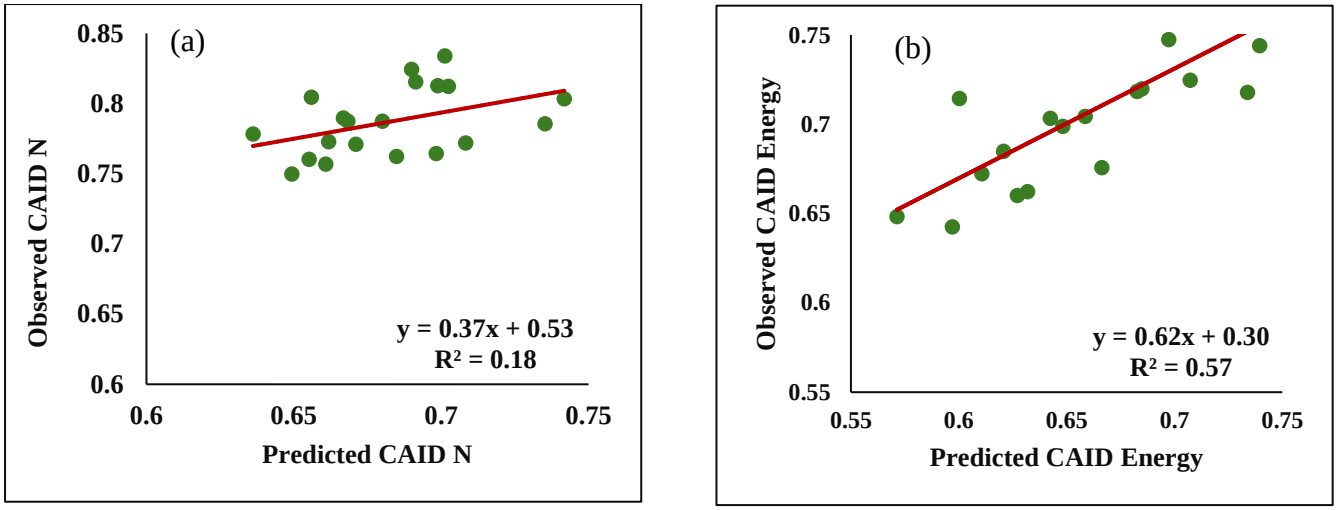


Figure 4.1. Relationship between predicted and observed values for coefficient of apparent ileal digestibility (CAID) of N (a) and energy (b) based on the gross chemical composition. (a) The slope was different from 1 (P = 0.004). (b) The slope was different from 1 (P = 0.007).

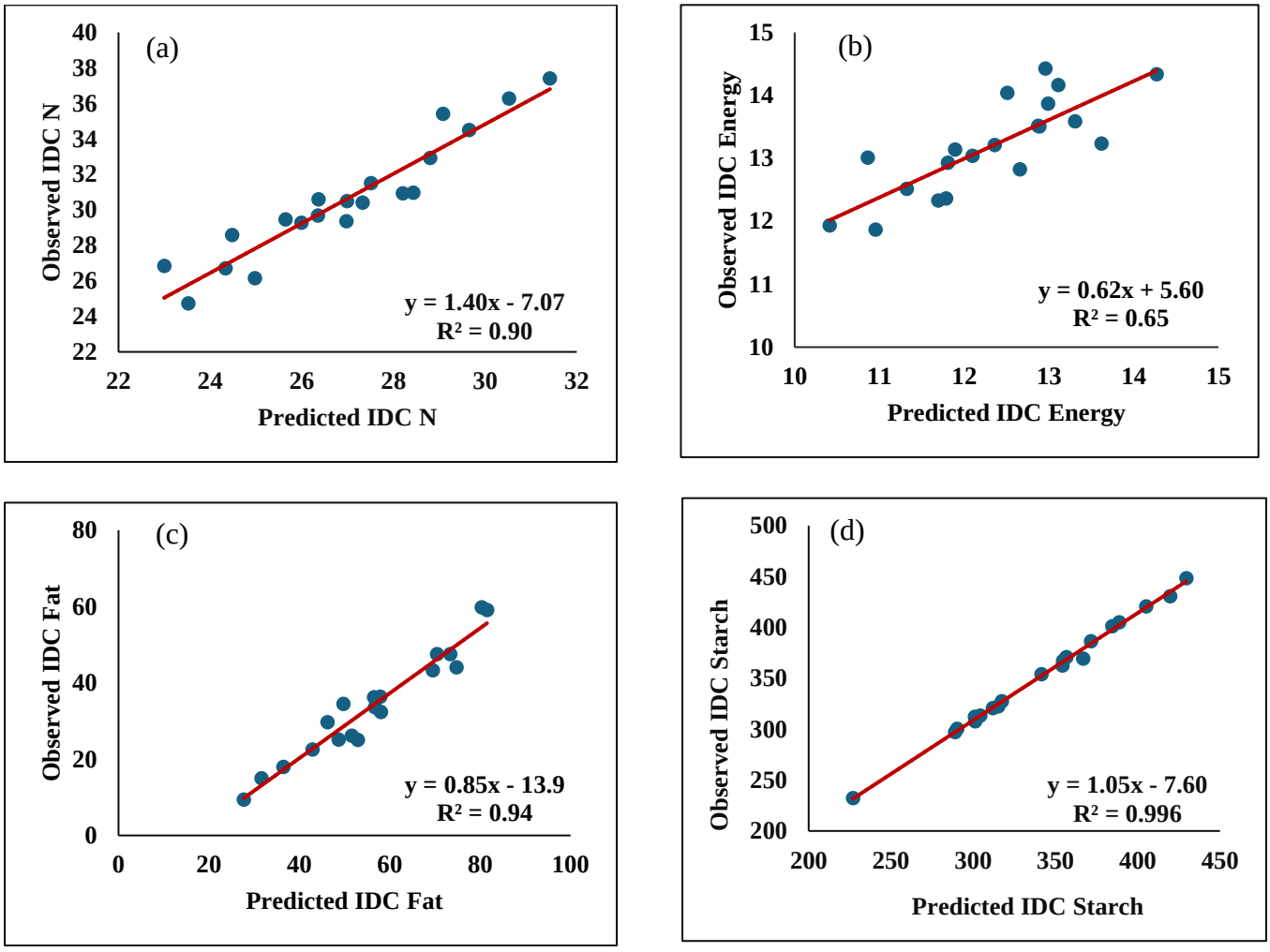


Figure 4.2. Relationship between predicted and observed values for ileal digestible content (IDC) of N (a), energy (b), fat (c), and starch (d) based on the gross chemical composition. (a) The slope was different from 1 (P = 0.002). (b) The slope was different from 1 (P = 0.002). (c) The slope was not different from 1 (P = 0.153). (d) The slope was different from 1 (P = 0.002).

4.5. Discussion

The use of prediction equations to estimate the apparent ileal digestibility values and IDC is desirable as the *in vivo* and *in vitro* methods are expensive and time-consuming. *In vivo* methods also question the bird's well-being. Therefore, the development of prediction equations becomes common for rapid determination of the energy values, and nutrient digestibility of feeds and complex diets for broilers. Several equations with different variables are available for estimating the energy values of feedstuffs in pigs (Noblet and Perez, 1993; Bulang and Rodehutscord, 2009; Urriola et al., 2014; Wang et al., 2021a; Lee et al., 2022), and broilers (Batal and Dale, 2006; Yegani and Korver, 2012; Meloche, 2013; Meloche et al., 2014; Baiz et al., 2020) as well as protein and amino acid digestibility for broilers (Ravindran et al., 2007; Szczurek, 2010; Ravindran et al., 2017) and pigs (Liu et al., 2015; Zeng et al., 2017; Ma et al., 2019). However, to the best of our knowledge, only a few reports (Cerrate et al., 2019; Pedersen et al., 2021) are available for the prediction of apparent ileal digestibility and IDC of nutrients for broilers using the complex diets having a wide range of digestibility and chemical compositions. The prediction equations available should be validated using a proper validation technique to be practically applied to the developed purpose.

This study, therefore, aimed to validate the original equations developed with 56 diet data using stepwise multiple regression in Chapter 3 (Tables 3.3 and 3.4). The first 14 equations were selected as the other set of equations derived using the selected variables from bootstrapping had not improved the goodness of fit and produced similar results (Annexture 4.7). Twenty newly formulated diets were used for validation as at least 15 to 20 new observations are

required and desirable to give a reliable assessment of prediction performance of models (Montgomery et al., 2021). It is noteworthy that these 20 diets were very similar to commercial complete diets. Despite this, the 56 diets used to develop the equations were incomplete and contained traditional and non-traditional ingredients. Hence, it is essential to do external validation to check the practical applicability of the equations and be aware of the risks, if any to the end users.

There is no consensus on the best strategy to check the precision of a prediction equation. Improved techniques must be developed through further research to assess the accuracy of prediction equations (Wang et al., 2021a). Different statistical techniques can be interpreted by finding out the difference between the predicted values and observed values in a validation data set such as R^2, RMSEp, CCC, and MB. The R^2 has been used as the main parameter to evaluate the performance of a model traditionally (Pedersen et al., 2007; Rochell et al., 2011; Meloche et al., 2013; Meloche et al., 2014).

The R^2 achieved for CAID of N and energy was 0.78 and 0.87, respectively, in the training dataset. However, in the current validation study, the model for CAID of energy managed to explain at least 57% of the variation but the model for CAID of N failed in this regard (R^2 = 18%). Pedersen et al. (2021) proposed a prediction equation for ileal digestibility of protein (IDP = $0.690 + 0.001 \times X_{starch\%} - 0.011 \times X_{CF\%} + 0.003 \times X_{fat\%}$) from chemical composition of 56 diets, where observed R^2 and RMSE were 0.42 and 0.06, respectively. Validation of this equation with 34 diets resulted in R^2 of 0.31 and RMSE of 0.06. A study by Cerrate et al. (2019) showed that the CP, ether extract (EE), NDF, and starch content were the best predictors to determine the digestibility coefficient of protein in broilers with R^2 of 0.99. Adding extra variables in the current equation for CAID of N may increase the prediction power of the equation.

The results obtained in the validation study showed that the CAID of fat, starch, and Ca could not be predicted accurately by the equations developed as expected because the obtained R^2 and CCC were very low. This could be because of the difference in the range of chemical compositions between the training data set and the validation data set. The training data set had a wide range of chemical compositions due to varying inclusion levels of traditional and non-traditional ingredients.

Cerrate et al. (2019) proposed an equation for predicting total tract digestibility of fat and digestible content of fat, which explained 99% of the variation, where the equation for the digestibility coefficient of fat consisted of CP, EE, and NDF as the predictors and the equation for digestible fat was explained by the variable EE only. However, these equations had not been validated. Moreover, Cerrate et al. (2019) found that digestible starch can be predicted by starch content ($R^2 = 0.94$) and the digestibility coefficient of starch can be predicted by using the NDF content ($R^2 = 0.10$). Similar results were obtained in the current study in terms of R^2, where CAID of starch was predicted by fat, CF, and GE with R^2 of 0.36 and IDC of starch was predicted by the starch content and GE, where R^2 obtained was 0.99. However, the validation study showed that the equation for CAID of starch could not predict the values as the R^2 obtained was very poor (0.01) and CCC was −0.03. It showed that the model was not able to explain the variation. This indicated that the model was unsatisfactory and there were factors contributing to the digestibility of starch that the model had failed to capture. Other parameters such as the non-starch polysaccharides (NSP) fraction and fibre fractions may need to be included to reformulate the model.

A significant decrease in the ileal digestibility of starch was observed when NSP were added to the diets with a clear correlation between the ileal digestibility of starch and the level of NSP (Pedersen et al., 2021). The soluble NSP increases the viscosity of the digesta, which reduces the mixing of feed with endogenous enzymes, thus reducing nutrient digestibility (Matthiesen

et al., 2021). Interestingly, the equation developed for IDC of starch successfully predicted the values with R^2 of 1 and CCC of 0.97 in the validation study. Therefore, this equation can be used with acceptable accuracy, where the starch and GE were the only predictors. The addition of GE content as a variable increased the R^2 and ensured a good result in the current validation study.

Satisfactory results were not obtained for prediction of CAID of Ca and IDC of Ca. The equations failed to successfully predict the results. The CCC for CAID and IDC of Ca were 0.32 and 0.26, respectively, which was not satisfactory. This may be because of the very low coefficient of variation (CoV) in the Ca content of the validation diets (CoV = 0.35) compared to Ca content in the training diets used to develop the equation (CoV = 1.46). The prediction error may be increased when applying prediction equations to diets with nutrient content irrelevant to the range used in model development (Meloche et al., 2014). Another reason for the lower prediction of CAID of Ca may be because of the high correlation between variables used as predictors (N was correlated with starch and GE negatively with r = −0.84 and r = −0.64, respectively). Sung and Kim (2021) also reported that highly correlated variables result in a decrease in the validity of regression coefficients as predictors. Similarly, CAID and IDC of P had lower R^2 in the validation set (0.53 and 0.40, respectively) compared to the training data set (0.83 and 0.81, respectively). The training data set (56 diets) had high concentrations of Ca and P from high content of meat and bone meal (Pedersen et al., 2021), whereas in commercial settings, Ca and P content will be lower and less variable.

Pedersen et al. (2021) proposed an equation for predicting the ileal digestibility of energy, where starch, CF, and phytate were the predictors. In that equation, the CF and phytate had a negative relationship, and starch had a positive relationship with the ileal digestibility of energy. The R^2 and RMSE obtained for ileal digestibility of energy in the study by Pedersen et al. (2021) were 0.77 and 0.05, respectively, whereas the validation study resulted in R^2 and

RMSE of 0.34 and 0.03, respectively. In contrast to the finding by Pedersen et al. (2021), in the present study using the same diets, the proposed equation for CAID of energy, significant predictors were CF, GE, square value of CF (CF^2), and starch-to-crude fibre ratio (starch:CF), where CF, GE, and starch:CF had a positive relationship, while CF^2 had a negative relationship. The R^2 and RMSE obtained were 0.87 and 0.04, respectively, while the validation study with the 20 diets (Table 4.7) showed that R^2 and RMSEp were 0.57 and 0.06, respectively, with CCC of 0.45, and this equation can be used as a general guide in practice.

The mean bias and slope bias represent the difference between the average of predicted and measured values and the consistency of prediction error across the range of data, respectively (Sung and Kim, 2021). Even though CAID of energy and DM and IDC of N, starch, energy, and DM had acceptable accuracy in terms of R^2 and CCC, they had significant mean and slope bias. Based on these results, none of the models can be considered fully acceptable. Bias correction, non- linear adjustments, and recalibration of the models using larger data could be used to further improve the models.

The reason for the inaccuracy of some equations may be some other causes such as inadequate sample size and multicollinearity among the regression variables. Moreover, the number of variables that make up the equations and the various calculation methods utilised to obtain the equations are factors that affect the accuracy and precision differences between the equations (Alvarenga et al., 2015). The strong interrelationship between variables used to develop the model makes it more difficult to eliminate their effects in model selection, which causes the removal of important variables (Leigh, 1988; Montgomery et al., 2021). In stepwise model selection, these related variables are no longer considered and are sometimes excluded from the final model (Montgomery et al., 2021). In addition, the quality of the prediction equation is mainly based on the accuracy of measurement of chemical compositions (Noblet and Perez, 1993).

One important limitation to consider is that the training data may have introduced a significant bias to the CAID values. Predicting CAID of nutrients was challenging and provides major relevance to the prediction of IDC values. The accuracy of prediction equations for IDC is greater than those of CAID when nutrient composition widely varies. In the case of energy and DM, the concentration of nutrients did not vary much, and the accuracy of the prediction equation did not differ much between CAID and IDC of energy and DM. Moreover, IDC values could correlate more with the CAID values in the validation step. Therefore, training data with a narrower range of composition similar to commercial diets may provide a different set of equations. It is essential to carefully select variables when developing prediction equations, utilising existing knowledge of nutrition and digestive interactions.

The relatively low R^2 in some equations indicates that these equations would serve only as a general guide. It suggests that the model could not adequately explain the variability in the data. The results of the present study indicate that some of the equations developed could not be used in all situations. Thus, it proves that validation with a new data set is required to ensure the applicability of the developed equations. Model improvement could be achieved by exploring alternative predictor variables, addressing issues like multicollinearity and sample size, and adding more interactions or non-linear relationships (Noblet and Perez, 1993; Smith et al., 2015; Pedersen et al., 2021).

Further studies can be developed by including more variables such as individual fibre fractions, NSP, amino acids, and starch components to check the impact of these variables on the CAID and the IDC of nutrients. Using these, the prediction equations can be fine-tuned ensuring more accuracy. However, the cost for analysis is also considered to best select the model with practical application. The analysis cost highly impacts the selection of variables to be included in the equations (Smith et al., 2015; Pedersen et al., 2021). Another factor is that inclusion of more variables may increase the R^2, hence, more awareness is needed when selecting the model

using R^2 as the primary measure of fit. The residual sum of squares decreases when predictors are added to the model causing an increase in R^2 (Montgomery et al., 2021). Adjusted R^2 can be used to overcome this problem (Meloche et al., 2014).

4.6. Conclusions

The present study validated equations for predicting the CAID and IDC of nutrients in complex diets for broilers based on the chemical composition of the diets. The CAID of energy and DM and IDC of N, starch, energy, and DM had acceptable accuracy in terms of R^2 and CCC. However, the overall findings including the bias showed that none of the equations were accurate. These equations can be further improved by adding other variables and by other methods or using digestible content of the diets for the development of equations. Further, this study showed that the validation of developed equations is essential to apply the equations to the external data with greater confidence.

4.7. Annexure

Table 4.1a

Validation of the original equations and equations developed with the selected variables by bootstrapping (Chapter 3).

	Training set (56 diets)		Validation set (20 diets)	
	R^2 (all variables)	R^2 (selected variables by bootstrapping)	R^2 (all variables)	R^2 (selected variables by bootstrapping)
CAID N	0.78	0.78	0.18	0.18
CAID fat	0.77	0.78	0.001	0.001
CAID starch	0.36	0.32	0.01	0.05
CAID Ca	0.69	0.64	0.25	0.30
CAID P	0.83	0.74	0.53	0.59
CAID energy	0.87	0.87	0.57	0.57
CAID DM	0.89	0.89	0.66	0.66
IDC N	0.98	0.98	0.90	0.91
IDC fat	0.99	0.99	0.94	0.94
IDC starch	0.99	0.99	1.00	1.00
IDC Ca	0.92	0.92	0.07	0.07
IDC P	0.81	0.81	0.40	0.30
IDC energy	0.89	0.89	0.65	0.65
IDC DM	0.89	0.89	0.66	0.64

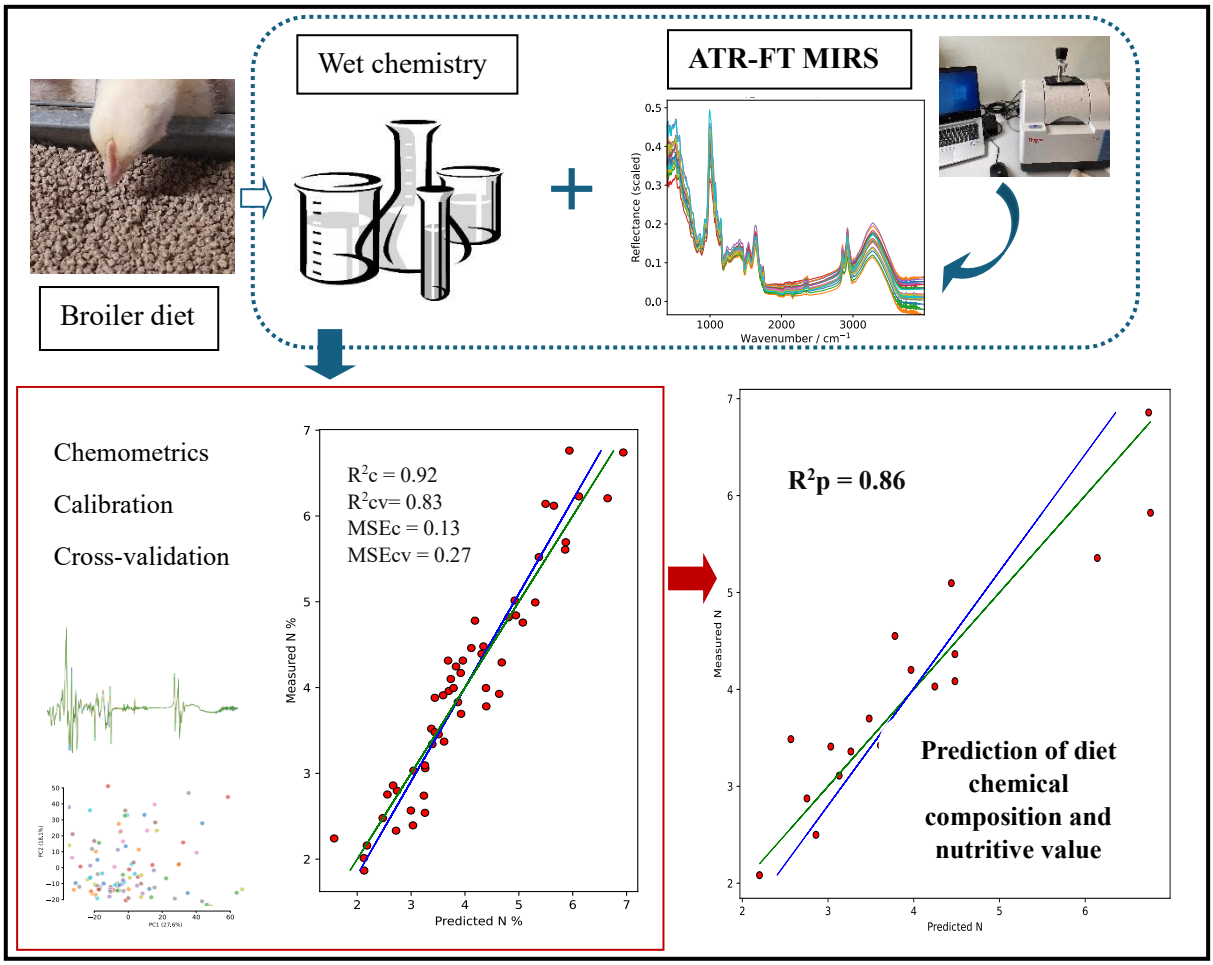
Ca, calcium; CAID, coefficient of apparent ileal digestibility; DM, dry matter; IDC, ileal digestible content; N, nitrogen; P, phosphorus; R^2, coefficient of determination of regression.

CHAPTER 5

The use of Attenuated Total Reflectance Fourier Transform Mid-Infrared Spectroscopy (ATR-FT MIRS) to determine the chemical composition, apparent ileal nutrient digestibility, and digestible nutrient content of broiler diets

This Chapter explores the other alternative method "ATR-FT MIRS" to develop models to predict the gross chemical composition of broiler diets directly and to examine the potential application of ATR-FT MIRS in direct prediction of the nutritive value (CAID and IDC of nutrients) of broiler diets.

Graphical abstract



5.1. Abstract

The potential of using Attenuated Total Reflectance Fourier Transform Mid-Infrared Spectroscopy (ATR-FT MIRS) for determination of gross chemical composition, coefficient of apparent ileal digestibility (CAID), and ileal digestible contents (IDC) of different broiler diets was evaluated. Mid-infrared (MIR) spectra of 76 diets were collected with a Thermofisher Scientific Nicolet iS5 Spectrometer at MIR range of 4000-400 cm^{-1} with 32 scans and at 2 cm^{-1} resolution. Data were analysed using principal component analysis (PCA) and modified partial least squares regression (PLSR) for the development of calibration equations. Prediction accuracy and performance were evaluated by coefficient of determination of prediction (R^2p) and relative performance deviation (RPD). Prediction was excellent for the gross chemical composition in terms of R^2 and RPD. Best models were generated for gross chemical composition of nitrogen (N), ash, dry matter (DM), calcium (Ca), phosphorus (P), and organic matter (OM) ($R^2p > 0.7$ and RPD > 3.0). The model developed for determination of crude fibre (CF) was useful for quantification ($R^2p = 0.87$ and RPD = 2.96), whereas the model of starch can be only used for screening purposes ($R^2p = 0.77$ and RPD = 2.44). Gross energy (GE) and fat models were not suitable for predictions since the RPD was < 2.0. Calibration equations developed for CAID of nutrients were poor ($R^2p < 0.60$ and RPD < 2.4) except for the CAID of fat which achieved satisfactory results ($R^2p = 0.73$, RPD = 2.41). Predictions for IDC of N, starch, and Ca was accurate ($R^2p > 0.7$, RPD > 3.0) while others were poor and unsuitable for prediction ($R^2p \leq 0.70$, RPD ≤ 1.8). Results from this study proved that ATR-FT MIRS can be used as a potential tool for determination of chemical composition of broiler feeds while more techniques like incorporating animal factor into the calibration process or processing of combined spectral data of feed and excreta samples are needed to improve the performance of ATR-FT MIRS for the prediction of CAID and IDC of nutrients.

Key words: ATR-FT MIRS, broilers, calibration, digestibility, ileal digesta, prediction

5.2. Introduction

Feed accounts for two-thirds or more of the cost of poultry production and also it is essential for the optimum performance (Noel et al., 2022). Knowing the chemical composition and nutritive values of a feedstuff or diet is important in formulating balanced diets for poultry (Khaleduzzaman et al., 2017) to achieve optimum performance. Traditionally, wet chemical analysis is used to gather information about the composition of a feedstuff or diet while the nutritive value is determined by the *in vivo* experiments using birds (Gizzi and Givens, 2004). However, due to logistical issues including the expenses and time needed as well as ethical problems, utilising an animal model for routine evaluation is not always feasible (Jha and Tiwari, 2016). Thus, *in vitro* models were developed based on wet chemical analysis to overcome the drawbacks of this gold standard *in vivo* method (Sharma et al., 2022). Even though this method is reasonable in terms of simplicity and accuracy, it is relatively slow and expensive (Givens and Deaville, 1999). Therefore, more rapid methods to estimate chemical composition and nutritive value have been developed.

Infrared spectroscopy (IR) is such a method to identify specific chemical bonds in composite substances. This technique has extensive application for the analysis of constituents of agricultural products, feeds, foods etc. (Ferreira et al., 2014; Shi et al., 2019). It provides a chemical-free and non-destructive technique for the analysis of animal feeds and does not need complicated sample preparation (Shi et al., 2019; Campbell et al., 2022). Moreover, it makes possible to evaluate the quality of feed materials and finished feeds in feed mills, including proximate analysis and energy values (Van Kempen, 2001). The infrared spectrum is divided into far-infrared (400-10 cm^{-1}), mid-infrared (4000-400 cm^{-1}), and near-infrared (13,333-4000 cm^{-1}). They differ in wavelength and transition between quantized vibrational energy states and each type of spectroscopy has advantages and disadvantages (Griffiths, 2006).

Near-infrared spectroscopy (NIRS) and mid-infrared spectroscopy (MIRS) are based on the absorption of radiation (Burns and Ciurczak, 2007) and their absorption techniques are illustrated in Figure 5.1. The NIRS is extensively used to predict the forage quality, feed composition, and digestibility of feeds based on C-H, N-H, and O-H absorptions from 400 to 2498 nm (Reeves, 2001b; Lyons et al., 2016). The near-infrared (NIR) region arises from overtones and combinations of fundamental vibrations and is sensitive to a variety of molecular interactions and chemical groups (Shi et al., 2019). A suitable sample preparation technique needs to be employed before NIR spectra are taken as methods to prepare dry samples such as freeze drying or oven drying can affect the NIR spectra (Alomar et al., 1999).

Mid-infrared (MIR) spectra contain information about the fundamental vibration of molecules and can identify very similar and complex structures thus used as a powerful technique for the identification of constituent molecules in the solid, liquid, and gaseous phases (Haas and Mizaikoff, 2016; Shi et al., 2019). Molecular information with trace to ultra-trace sensitivity, quick data acquisition rates, and high spectral resolution are available with MIRS for improved routine analysis (Haas and Mizaikoff, 2016).

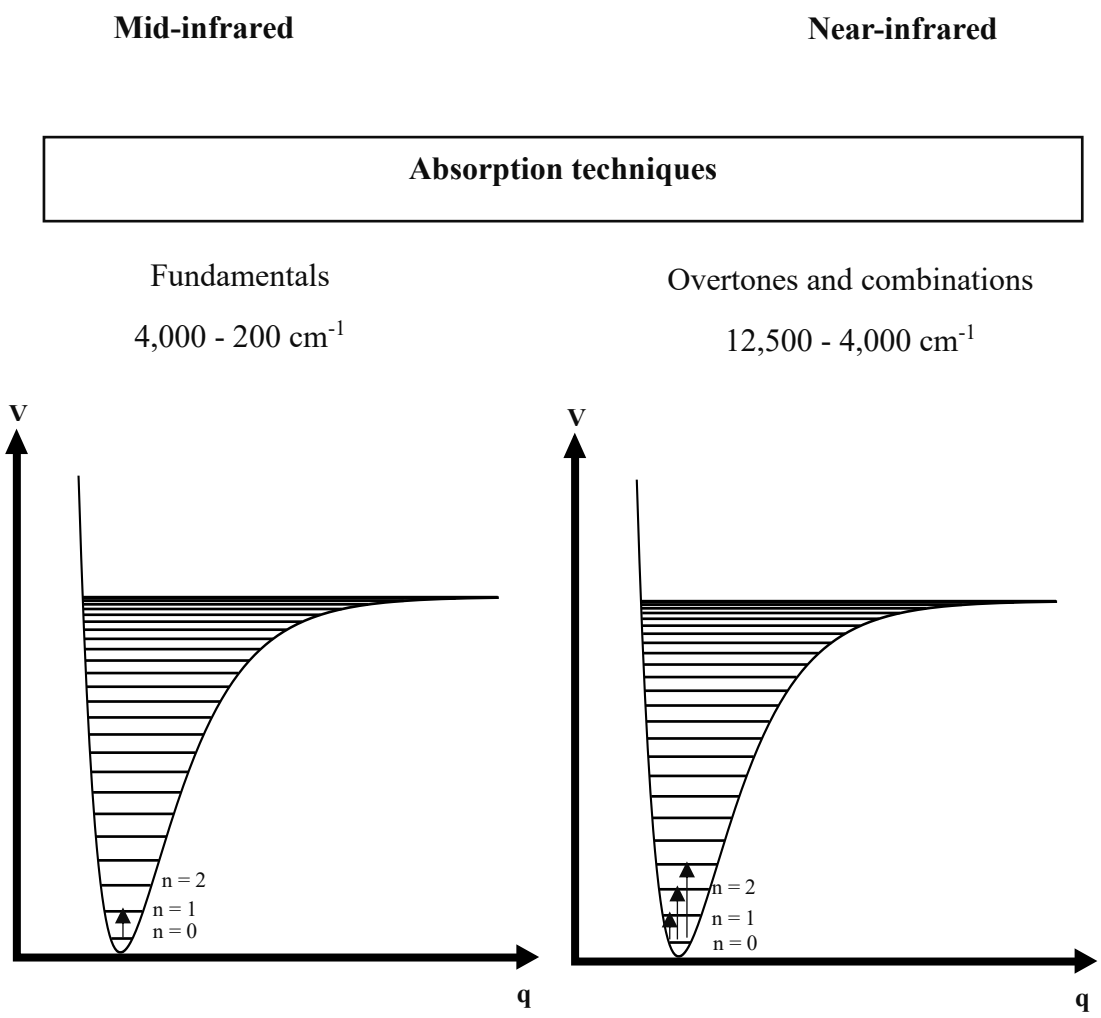


Figure 5.1. The principles of MIR and NIR spectroscopy, where V is the potential energy and q is the displacement coordinate.

The band positions, intensities, and forms of MIR spectra contain the chemical information about the samples. Peaks above 1500 cm^{-1} of MIR spectra can be assigned confidently to specific functional groups such as O-H, C-H, N-H, and C=O, etc. Thus, the MIR spectra can be interpreted easily (Mayo et al., 2004). While band positions provide information about the molecular structure of chemical compounds in a mixture, the intensities of the bands are related to the concentration of these compounds (Karoui et al., 2010). Therefore, multivariate mathematical approaches have been used with computer technology to extract this information from the spectra (Karoui et al., 2010). Principle component regression (PCR), and partial least

square regression (PLSR) could be used to establish prediction models and to predict the nutritional value of animal feeds rapidly and simultaneously (Samadi et al., 2020).

Unlike NIRS, an unusual broad line shape will be produced in MIRS for water compared to other bands in the region because of the high hydrogen bonding. This unusual shape can be sorted out using proper data pre-processing and filtering methods such as Standard Normal Variate (SNV) and Savitzky-Golay derivative to overcome the effects of water on the sample variance (Cleland et al., 2018).

The development of attenuated total reflectance (ATR) methods made possible the acquisition of the MIR spectra of biological samples in the reflectance mode (Lu and Rasco, 2012) instead of the traditional transmission mode (Cleland et al., 2018). Equipment such as fourier transform infrared spectroscopy (FTIR) is a rapid analytical technique to detect the molecular chemistry in different biological components (Yan et al., 2022). The FTIR technique equipped with ATR simplifies the sample-handling process (Van de Voort, 1992), thereby allowing minimal sample preparation and permit gathering quantitative information on small quantities of sample (Wang, 2014). Indeed, the ATR-FT technique in the MIR region has been utilised for analysis of liquid, pastes, and powder where transmission spectroscopy is not feasible due to intense water interference or scattering of absorption (Linker et al., 2005).

In addition, Khaleduzzaman et al. (2017) reported that the accuracy of the NIRS analysis primarily depends on the sample preparation. Wilman et al. (2000) added that NIR requires more samples for analysis than ATR-FTIR, which uses a smaller quantity. However, data obtained from the *in vitro* or *in vivo* assays, and laboratory analysis are required to calibrate both NIR and MIR spectral data (Bastianelli, 2013). From this chemical composition and nutritional values obtained, mathematical models will be established to relate the infrared

spectrum to the results of the laboratory measurements to predict the chemical composition or digestibility of the feedstuffs and diets.

Findings have demonstrated that MIR spectra-based calibrations are frequently, if not always, more accurate than NIR spectra-based calibrations (Reeves and Van Kessel, 2000; Reeves, 2001b). Another benefit of MIRS over NIRS is that it is rich in information with specific bands that are attributable to different chemical compounds or functional groups while NIR spectra may seem featureless and hard to interpret (Calderón et al., 2009).

The MIRS has been routinely used to predict milk composition, feed efficiency, methane emission, fertility, energy balance, and health status in dairy and beef sector (Bresolin and Dórea, 2020) and to measure chemical composition of grains, fruits, meat, and other agricultural products (Adiamo et al., 2021). Meanwhile, it has been used in prediction of protein digestibility of wheat (Shi et al., 2019), sugars in barley (Cozzolino et al., 2014b; Huang et al., 2016), protein molecular structures of canola meal and press cake (Theodoridou and Yu, 2013), nutrient composition of soyabean (Ferreira et al., 2014) and wheat bran (Hell et al., 2016), amino acid content in animal meals (Qiao and Van Kempen, 2004), and forage composition for ruminants (Cleland et al., 2018).

So far, there are not many studies where ATR-FT MIRS has been used to analyse the chemical composition and nutritive value of broiler diets except a study by Mahesar et al. (2011) to determine fatty acid ratio in poultry feed lipids. Therefore, the present study aimed to determine the efficacy of ATR-FT MIRS in predicting the gross chemical composition, coefficient of apparent ileal digestibility (CAID) and ileal digestible content (IDC) of broiler diets.

5.3. Materials and methods

5.3.1. Sample preparation

Gross chemical composition and nutrient digestibility data of 76 diets, obtained in digestibility studies at Massey University, were used for the analysis. Data from 56 diets were obtained from the study by Pedersen et al. (2021) and the remaining 20 diets were obtained from the study explained in Chapter 4. Experimental procedures for both *in vivo* studies were in accordance with Massey University Animal Ethics Committee guidelines. All diets represented a range of chemical compositions larger than those found in most practical situations and included most of the ingredients used in commercial poultry feeding. Representative samples were collected for each diet after pelleting for the determination of nutrient content. All diet samples were ground to pass through a 0.5 mm sieve and stored in airtight containers until analysis. Ileal digesta samples collected at the end of the experiment were pooled, lyophilized, ground to pass through a 0.5 mm sieve and stored in airtight containers until analysis.

5.3.2. Chemical analysis

The diets and digesta samples were analysed for DM, N, ash, GE, starch, fat, Ca, P, CF, and titanium (Ti) using the standard procedures as outlined in Chapter 3 and Chapter 4.

5.3.3. Calculation of CAID and IDC of nutrients

All diets contained 5.0 g/kg titanium dioxide (TiO_2) as an indigestible marker for the determination of coefficient of apparent ileal digestibility (CAID) of nutrients.

The CAID of nutrients was calculated using the following formula.

$$\text{CAID of nutrient} = \frac{[(\text{Nutrient/Ti})_{\text{diet}} - (\text{Nutrient/Ti})_{\text{ileal}}]}{(\text{Nutrient/Ti})_{\text{diet}}} \quad (5.1)$$

where,

(Nutrient/Ti)*diet* is the ratio of diet component to Ti in diet and (Nutrient/Ti)*ileal* is the ratio of the diet component to Ti in the ileal digesta.

The IDC was calculated by the following formula.

$$\text{IDC of nutrient} = \text{Nutrient in the diet} \times \text{CAID of that nutrient} \quad (5.2)$$

5.3.4. Spectral data collection using ATR-FT MIRS

Mid-infrared spectra were scanned at 400-4000 cm^{-1} with 32 scans per spectrum and a spectral resolution of 2 cm^{-1} using a Thermofisher Scientific Nicolet iS5 Spectrometer and recorded by the OMNIC^{TM} software. Background spectra were collected every 30 minutes without samples present on the crystal to minimise the effects of the water vapor and CO_2 present in the atmosphere. Three spectra were collected for each diet sample and the average of these spectra for each sample was used for the statistical analysis. The spectral files were exported as comma-separated value (csv) files for further analysis in Python (v3.11).

5.3.5. Spectral pre-treatment and calibration

Before being pre-treated all spectra were reduced to 1867 data points. Pre-treatment of the averaged spectra (3 replicates per diet) was carried out using Savitzky-Golay method to detect potential noises and to observe the detailed spectra along the wavelength region (Savitzky and Golay, 1964). A first-order derivative was utilised, followed by smoothing using a polynomial weighting. The smoothing polynomial was third order, and the window of smoothing was 11 units (11,3,1,0). Raw ATR-FT MIR spectra of 76 diets without pre-treatment and with pre-treatment are shown in Figures 5.2 (a) and (b), respectively.

Preliminary analysis of the dataset was done using principal component analysis (PCA). It is a valuable technique for dimensionality reduction of data, to identify variables of interest, and used to find batch or spectral baseline effects before conducting additional analyses (Grant-

Peters et al., 2022). Using the score and loading results shown in Figure 5.3, a modified partial least squares regression (PLSR) method was used to establish correlations between the collected ATR-FT MIRS spectra and the wet chemical analysis and to develop calibration models. A modification was made in the PLS algorithm, allowing one initial model calibration to identify and remove prediction outliers, after which the model was recalibrated on reduced calibration subset.

During the calibration stage, k-fold cross-validation was used to obtain the cross-validation parameters. Data was randomly split into 4 equal cross-validation groups and a model was trained on a subset of groups and predicted the target variable for the remaining group. This process is repeated for each group and the predicted values were concatenated. Cross-validation was applied to all models to obtain the number of principal components for each model.

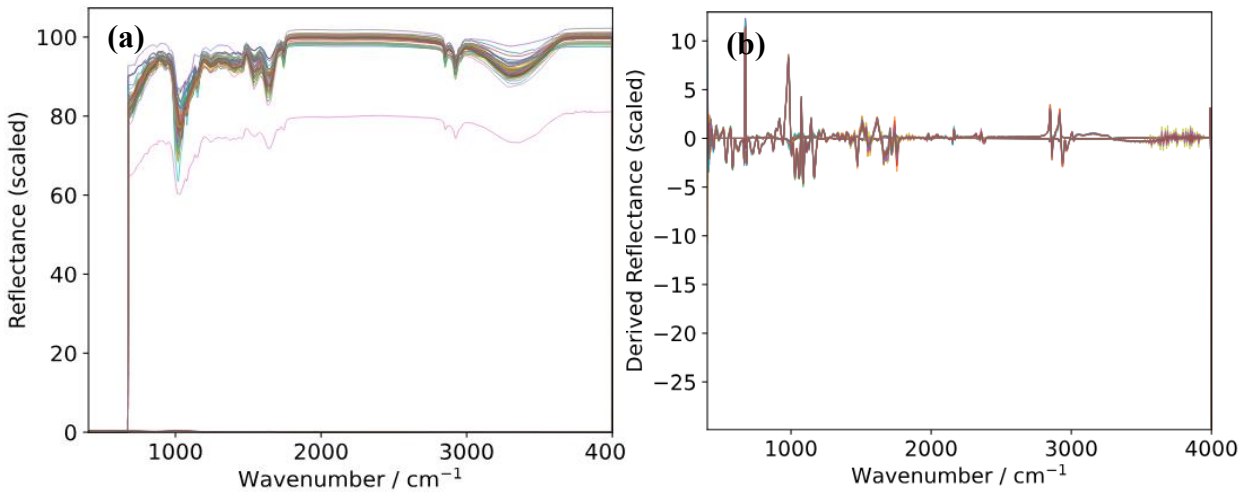


Figure 5.2. (a) Raw ATR-FT MIR spectra of 76 diets without pre-treatment, (b) ATR-FT MIR spectra of 76 diets after Savitsky-Golay filtering.

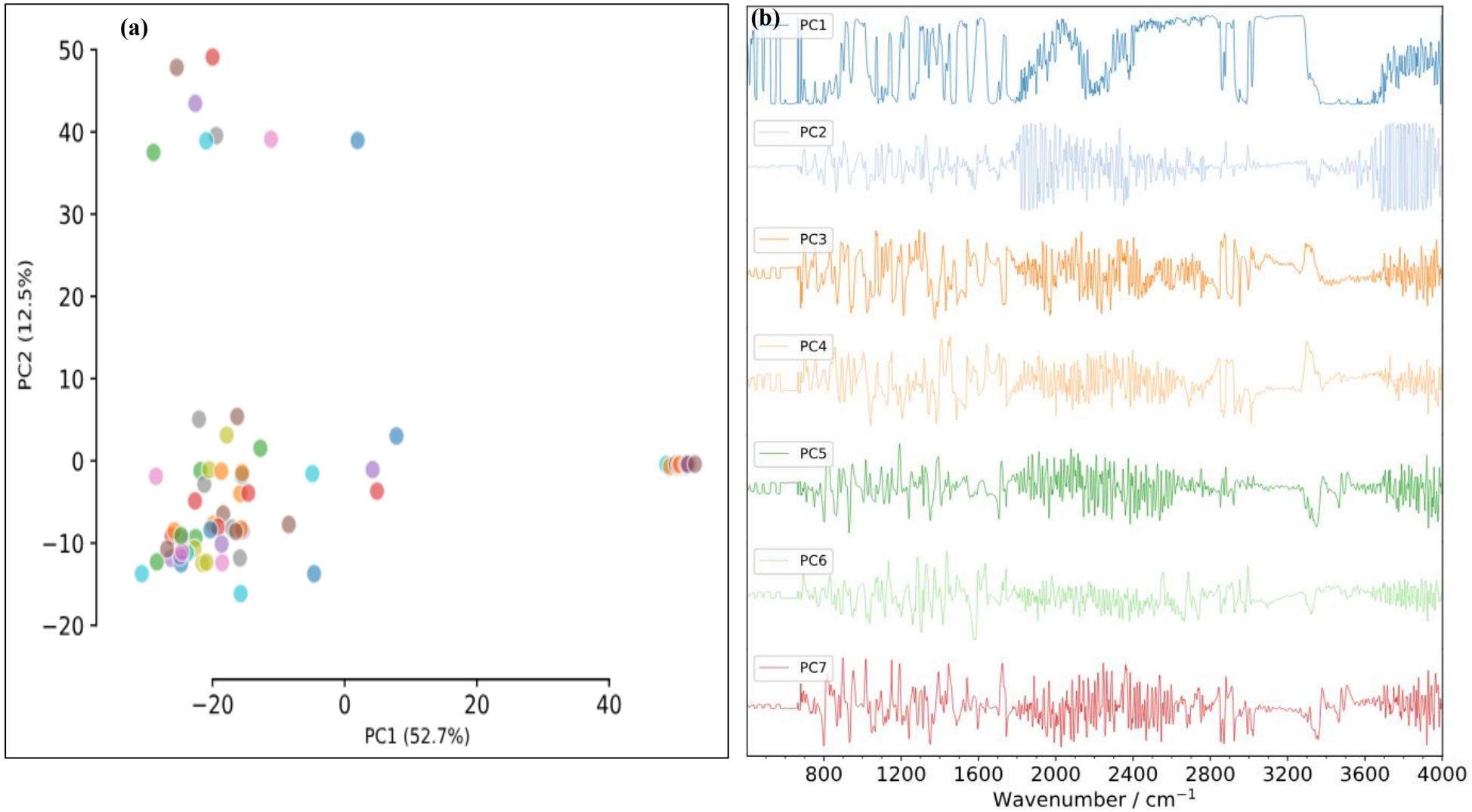


Figure 5. 3. (a) ATR-FT MIRS score plot, (b) ATR-FT MIRS loadings plot of 76 broiler diets.

5.3.6. Outlier removal

During the calibration process, the outliers were removed using Q-residuals and Hoteling's T-squared test. Q-residuals are derived from the error matrix and account for the variations in the data that are not explained by the model as built. Hotelling's T-squared looks at the variations within the model itself by setting a center and confidence interval. Mahalanobis distance and threshold distance were calculated at a confidence interval of 0.95. Calibration models were established for each analyte after outliers were removed.

5.3.7. Prediction

For the prediction, approximately 75% of the outlier removed sample set was used for training and the remaining 25% was used as a test set.

5.3.8. Statistics

The coefficient of determination of calibration and cross-validation (R^2c and R^2cv) of all samples and after outlier removed, mean square error of calibration and cross-validation (MSEc and MSEcv) for all samples and after outlier removed, coefficient of determination of prediction (R^2p), standard deviation of measured values (SDm), root mean square error of prediction (RMSEp) and relative performance deviation (RPD) were used to evaluate the performance of the models. The R^2 value closer to 1 and low value of MSE are a better fit (Ferreira et al., 2014; Noel et al., 2022). The RPD value of < 2 is not suitable for prediction; values between 2.0-2.4 are acceptable for screening purposes; values between 2.5-2.9 are useful for quantification; and values ≥ 3 indicate high accuracy for quantitative analysis (Lyons et al., 2016).

The optimum number of components (nc) in the regression model was determined based on the values of the lowest mean squared error (MSE) of cross-validation.

The RMSEp and RPD were calculated by the following equations.

$$\text{RMSEp} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \quad (5.3)$$

$$\text{SEp} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i - bias)^2}{n-1}} \quad (5.4)$$

$$\text{Bias} = \frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)}{n} \quad (5.5)$$

$$\text{RPD} = \frac{\text{SDm}}{\text{SEp}} \quad (5.6)$$

where, $\hat{y}_i$ and y_i are the predicted and observed (measured) values respectively, n is the number of data, and SDm is the standard deviation of the measured value and SEp is the standard error of prediction.

5.4. Results

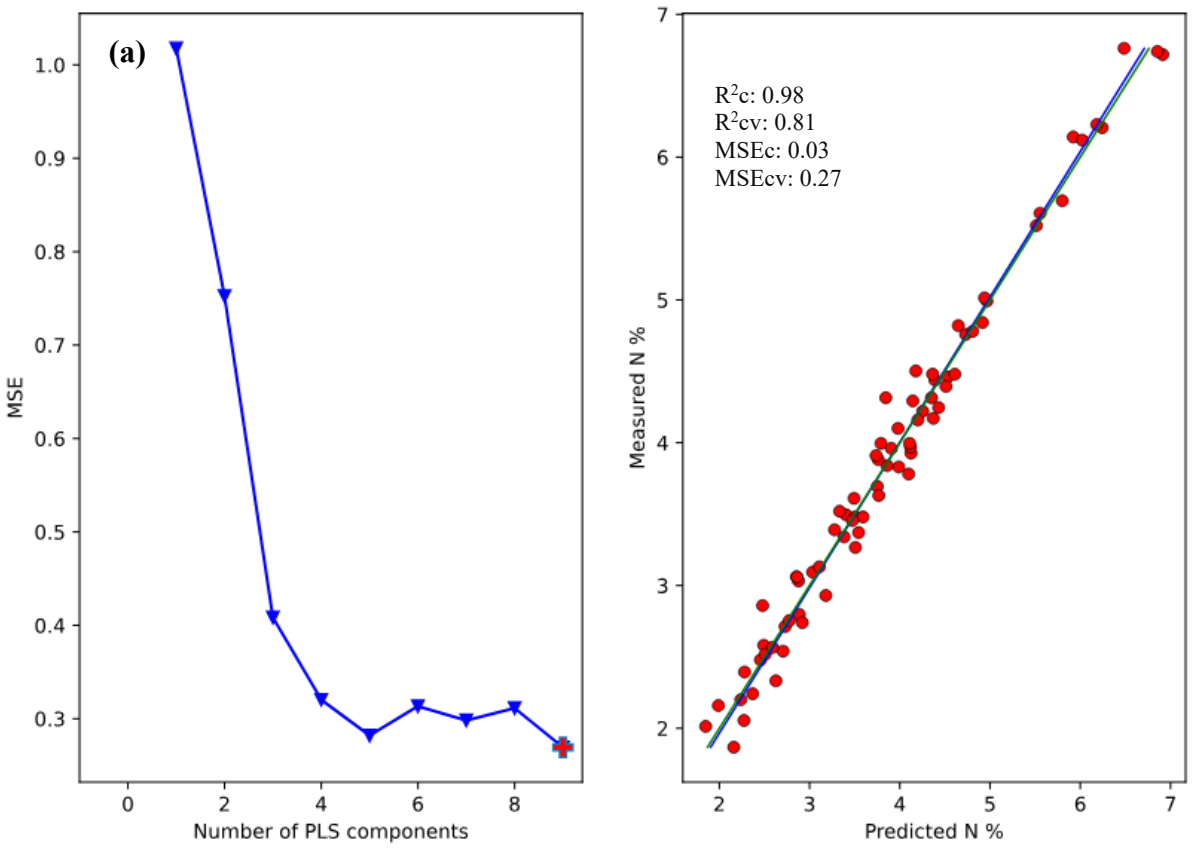
A total of 76 broiler diets were used to develop ATR-FT MIRS calibration, cross-validation, and prediction statistics for the gross chemical composition, CAID and IDC of nutrients. The descriptive statistics for gross chemical composition and nutritive value are listed in Tables 5.1 and 5.2, respectively. The statistics for each calibration model developed using ATR-FT MIRS are presented in Table 5.3 for the chemical constituents, Table 5.4 for the CAID of nutrients, and Table 5.5 for the IDC of nutrients.

The PCA scores plot (Figure 5.3 (a)) illustrates the distribution of broiler feed samples along first principal component (PC1) and second principal component (PC2), which together explained 65.2% of the total variance. The majority of the diet samples were closely clustered, indicating similar characteristics. The distinct subset of samples was separated along PC1, suggesting compositional differences between the two datasets. The PCA loading spectra

(Figure 5.3 (b)) revealed the wavenumber regions that contributed most significantly to the observed variance.

5.4.1. Prediction of chemical composition of broiler diets based on ATR-FT MIRS

The overall range of DM, N, GE, Ca, and P contents of broiler diets were 84.6 to 90.6%, 1.87 to 6.76%, 16.2 to 20.8 MJ/kg, 2.83 to 95.3 mg/g, and 3.54 to 42.5 mg/g, respectively (Table 5.1). The MIRS calibrations developed to estimate the diet chemical compositions of N, ash, DM, Ca, P, and OM had an excellent performance ($R^2p > 0.80$ and RPD > 3), while calibrations of CF had $R^2p = 0.87$ and RPD = 2.96. Starch obtained a moderate R^2p of 0.77 and RPD of 2.44. The calibrations for GE and fat were not up to the mark as their RPD was < 2 (Table 5.3).



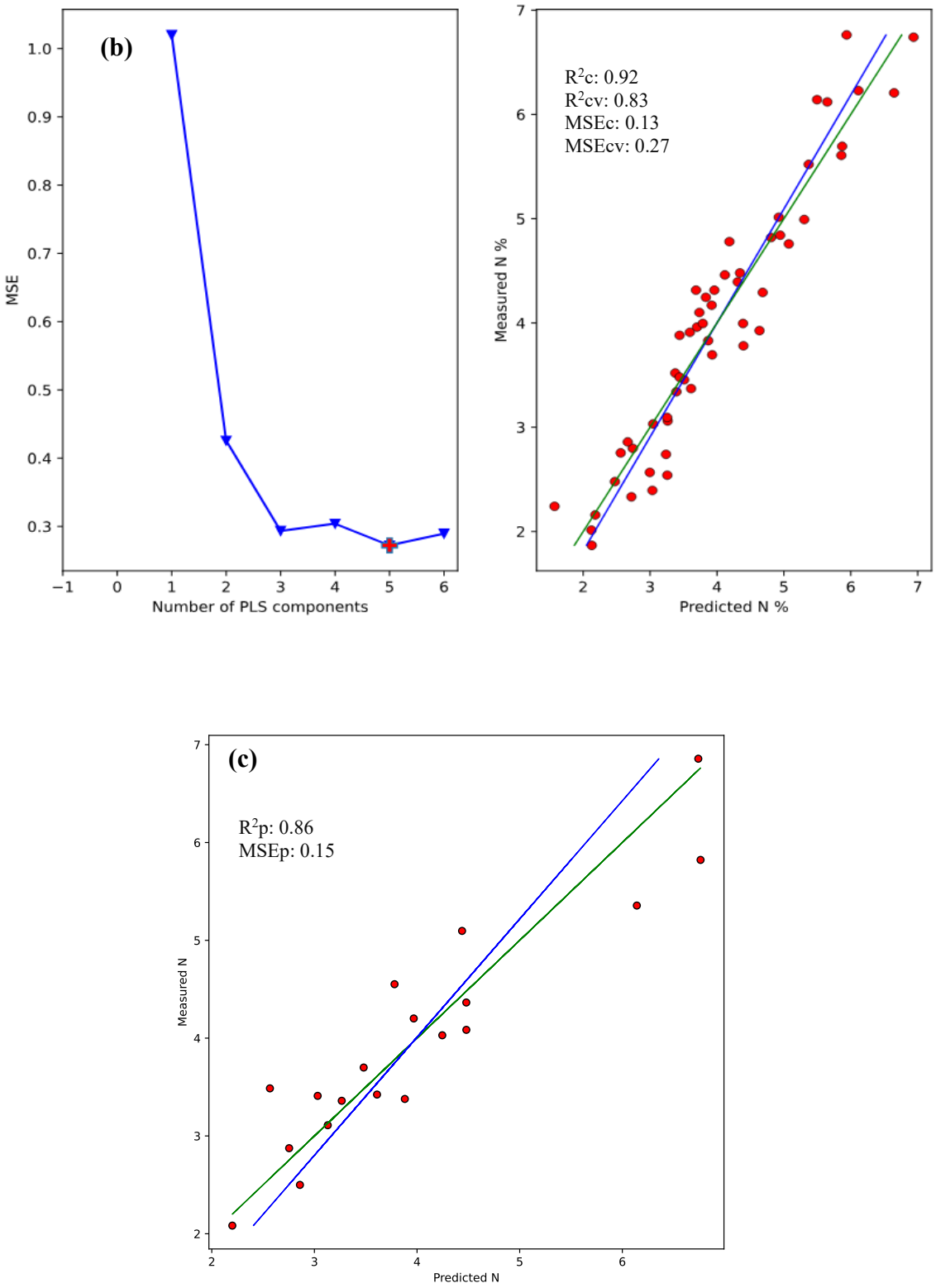


Figure 5.4. Example of plots obtained for the analyte N (a) Calibration for all samples (b) Training for prediction (c) Test outcome of prediction.

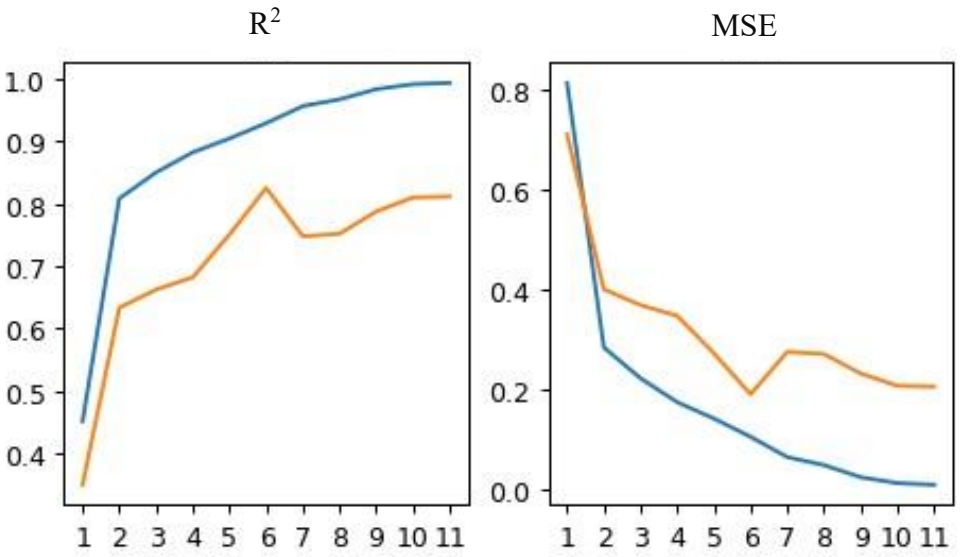


Figure 5.5. The R^2 and MSE vs number of components for the analyte N.

Figure 5.4 shows an example of the plots obtained for analyte N in multivariate PLSR where (a) calibration obtained with all 76 diet samples (b) calibrations obtained using training data after outlier removal and (c) test outcome of prediction. Figure 5.5 shows how the R^2 and MSE change with the number of components used for the prediction. These graphs were obtained for all the parameters analysed in ATR-FT MIRS to check for the overfitting of the results.

5.4.2. Prediction of coefficient of apparent ileal digestibility (CAID) and ileal digestible content (IDC) of broiler diets

Table 5.2 shows the descriptive statistics of CAID and IDC of 76 diets used in the study. The CAID of N varied from 0.466 to 0.834 with an average of 0.710 while CAID of GE varied from 0.346 to 0.820 with an average of 0.636. The statistics for the best calibration model developed for each CAID and IDC are presented in Table 5.4 and 5.5, respectively. Only the prediction of CAID of fat was satisfactory for screening with the R^2p of 0.73 and RPD = 2.41. Most parameters predicted well in calibration development but failed to predict the test data.

Calibration equation for CAID of N produced R^2p of 0.56 and RPD of 2.29 while CAID of GE obtained R^2p of 0.476 and RPD of 1.98 (Table 5.4). The IDC of N, starch, and Ca reported R^2p of 0.735, 0.811 and 0.730, respectively, with the RPD of 3.26, 3.31, and 3.18 (Table 5.5). The predictions were generally better for chemical compositions than the CAID and IDC of nutrients. However, the CAID of fat was more accurate than the prediction of chemical composition of fat.

Table 5.1. General statistics of analysed gross chemical composition of 76 diets (as-fed basis).

Analyte	Mean	SD	CoV	Min-Max
N%	3.86	1.21	31.4	1.87-6.76
GE MJ/kg	17.3	0.89	5.17	16.2-20.8
Ash%	6.65	4.44	66.7	2.31-27.2
Fat%	7.62	3.35	43.9	2.76-21.1
Starch%	23.3	13.9	59.8	4.07-53.8
DM%	87.6	1.70	1.94	84.6-90.6
CF%	5.53	2.40	43.3	2.41-13.0
Ca mg/g	12.8	17.4	136	2.83-95.3
P mg/g	9.27	7.11	76.6	3.54-42.5
OM%	81.0	3.82	4.71	63.4-84.9

Ca, calcium; CF, crude fibre; CoV, coefficient of variation (%); DM, dry matter; GE, gross energy; N, nitrogen; OM, organic matter; P, phosphorus; SD, standard deviation.

Table 5.2. General statistics for the coefficient of apparent ileal digestibility (CAID) and ileal digestible content (IDC) (% as-fed basis) of 76 diets.

Parameter	Mean	SD	CoV	Min-Max
CAID N	0.710	0.081	11.4	0.466-0.834
CAID fat	0.782	0.088	11.3	0.506-0.900
CAID starch	0.938	0.038	4.07	0.810-0.982
CAID Ca	0.316	0.132	41.6	0.114-0.633
CAID P	0.494	0.137	27.7	0.137-0.704
CAID energy	0.636	0.097	15.3	0.346-0.820
CAID DM	0.567	0.118	20.8	0.228-0.795
IDC N	2.72	0.83	30.5	1.36-4.78
IDC fat	5.93	2.73	46.1	2.48-17.9
IDC starch	21.91	13.17	60.1	3.78-52.1
IDC Ca	3.14	3.51	112	0.52-18.1
IDC P	3.82	1.25	32.7	2.15-8.60
IDC energy	11.0	1.63	14.9	6.09-14.4
IDC DM	56.7	11.8	20.8	22.8-79.5

Ca, calcium; CAID, coefficient of apparent ileal digestibility; CoV, coefficient of variation (%); DM, dry matter; GE, gross energy; IDC, ileal digestible content; N, nitrogen; P, phosphorus; SD, standard deviation.

Table 5.3. Coefficient of determination of calibration and cross-validation (R^2c and R^2cv), mean square error of calibration and cross-validation (MSEc and MSEcv), and number of partial least square model components (nc) for all diet samples, outlier removed samples, and training samples and coefficient of determination of prediction (R^2p), mean square error of prediction (MSEp), standard deviation of measured values (SDm), root mean square error of prediction (RMSEp), and relative performance deviation (RPD) for the test prediction of chemical composition of broiler diets (as-fed basis).

Analyte			R^2c	R^2cv	MSEc	MSEcv	nc		R^2p	MSEp	SDm	RMSEp	RPD
N%	All (n = 76)		0.98	0.81	0.03	0.27	9						
	After outliers (n = 72)		0.96	0.77	0.07	0.35	7						
	Prediction	Train	0.92	0.83	0.13	0.27	5	Test	0.86	0.15	1.22	0.37	3.27
GE MJ/kg	All (n = 76)		0.74	0.53	0.19	0.33	3						
	After outliers (n = 72)		0.73	0.56	0.19	0.32	3						
	Prediction	Train	0.74	0.58	0.13	0.21	3	Test	0.70	0.99	0.84	0.44	1.90
Ash%	All (n = 76)		0.98	0.83	0.32	3.39	9						
	After outliers (n = 73)		0.98	0.84	0.31	3.08	9						
	Prediction	Train	0.97	0.91	0.54	1.48	6	Test	0.87	2.27	4.39	1.00	4.38
Fat %	All (n = 76)		0.86	0.45	1.49	5.84	4						
	After outliers (n = 73)		0.69	0.51	3.44	5.30	4						
	Prediction	Train	0.65	0.43	3.26	5.22	3	Test	0.59	6.55	3.30	3.30	1.62
Starch%	All (n = 76)		0.88	0.75	21.7	45.2	4						
	After outliers (n = 72)		0.86	0.73	24.3	48.2	3						
	Prediction	Train	0.84	0.78	31.8	45.5	2	Test	0.77	23.6	13.4	5.47	2.44

DM%	All (n = 76)		0.94	0.67	0.17	0.98	7					
	After outliers (n = 72)		0.89	0.57	0.34	1.32	4					
	Prediction	Train	0.94	0.80	0.19	0.62	5	Test	0.75	0.73	1.74	0.57	3.05
CF%	All (n = 76)		0.93	0.54	0.32	2.25	9					
	After outliers (n = 72)		0.91	0.74	0.38	1.15	7					
	Prediction	Train	0.89	0.56	0.43	1.76	6	Test	0.87	0.69	2.10	0.71	2.96
Ca mg/g	All (n = 76)		0.98	0.84	4.74	48.5	9					
	After outliers (n = 72)		0.99	0.84	4.36	46.1	9					
	Prediction	Train	0.98	0.92	7.48	29.9	6	Test	0.85	15.9	17.2	3.09	5.56
P mg/g	All (n = 76)		0.98	0.83	0.81	8.68	9					
	After outliers (n = 73)		0.99	0.84	0.78	8.44	9					
	Prediction	Train	0.96	0.89	1.66	4.89	6	Test	0.95	3.97	7.19	1.51	4.76
OM%	All (n = 76)		0.92	0.78	1.15	3.07	5					
	After outliers (n = 72)		0.90	0.79	1.46	3.15	3					
	Prediction	Train	0.92	0.79	1.17	3.05	4	Test	0.89	1.71	3.86	1.14	3.39

Ca, calcium; CF, crude fibre; DM, dry matter; GE, gross energy; n, number of samples; N, nitrogen; OM, organic matter; P, phosphorus.

Table 5.4. Coefficient of determination of calibration and cross-validation (R^2c and R^2cv), mean square error of calibration and cross-validation (MSEc and MSEcv), and number of partial least square model components (nc) for all diet samples, outlier removed samples, and training samples and coefficient of determination of prediction (R^2p), mean square error of prediction (MSEp), standard deviation of measured values (SDm), root mean square error of prediction (RMSEp), and relative performance deviation (RPD) for the test prediction of coefficient of apparent ileal digestibility (CAID) of broiler diets.

Analyte			R^2_C	R^2_{CV}	MSE_C	MSE_{CV}	nc		R^2p	MSEp	SDm	RMSEp	RPD
CAID N	All (n = 76)		0.92	0.35	0.000	0.004	8						
	After outliers (n = 72)		0.63	0.36	0.002	0.004	3						
	Prediction	Train	0.87	0.36	0.001	0.004	6	Test	0.56	0.002	0.076	0.033	2.29
CAID energy	All (n = 76)		0.16	0.11	0.007	0.009	1						
	After outliers (n = 72)		0.79	0.19	0.002	0.007	5						
	Prediction	Train	0.87	0.48	0.001	0.004	6	Test	0.48	0.006	0.093	0.047	1.98
CAID fat	All (n = 76)		0.88	0.41	0.001	0.004	6						
	After outliers (n = 73)		0.64	0.36	0.001	0.002	3						
	Prediction	Train	0.89	0.60	0.001	0.002	6	Test	0.73	0.003	0.086	0.036	2.41
CAID starch	All (n = 76)		0.52	0.16	0.001	0.001	2						
	After outliers (n = 72)		0.51	0.16	0.001	0.001	2						
	Prediction	Train	0.85	0.30	0.000	0.001	6	Test	0.34	0.001	0.037	0.020	1.87
CAID Ca	All (n = 76)		0.85	0.07	0.002	0.016	8						
	After outliers (n = 72)		0.34	0.07	0.011	0.016	2						
	Prediction	Train	0.49	0.12	0.009	0.015	3	Test	0.16	0.014	0.131	0.102	1.29
CAID P	All (n = 76)		0.92	0.68	0.002	0.006	8						
	After outliers (n = 72)		0.92	0.66	0.002	0.007	8						
	Prediction	Train	0.75	0.45	0.005	0.009	3	Test	0.55	0.010	0.139	0.077	1.80
CAID DM	All (n = 76)		0.87	0.06	0.002	0.011	4						
	After outliers (n = 72)		0.87	0.34	0.002	0.008	6						
	Prediction	Train	0.91	0.57	0.001	0.006	6	Test	0.32	0.006	0.110	0.048	2.30

Ca, calcium; DM, dry matter; GE, gross energy; n, number of samples; N, nitrogen; P, phosphorus.

Table 5.5. Coefficient of determination of calibration and cross-validation (R^2c and R^2cv), mean square error of calibration and cross-validation (MSEc and MSEcv), and number of partial least square model components (nc) for all diet samples, outlier removed samples, and training samples and coefficient of determination of prediction (R^2p), mean square error of prediction (MSEp), standard deviation of measured values (SDm), root mean square error of prediction (RMSEp), and relative performance deviation (RPD) for the test prediction of ileal digestible content (IDC) of broiler diets (% as-fed basis).

Analyte			R^2c	R^2cv	MSEc	MSEcv	nc		R^2p	MSEp	SDm	RMSEp	RPD
IDC N	All (n = 76)		0.98	0.78	0.01	0.14	9						
	After outliers (n = 72)		0.95	0.76	0.04	0.16	7						
	Prediction	Train	0.93	0.66	0.05	0.25	6	Test	0.74	0.10	0.80	0.25	3.26
IDC energy	All (n = 76)		0.79	0.21	0.51	1.90	6						
	After outliers (n = 72)		0.82	0.18	0.38	1.72	7						
	Prediction	Train	0.80	0.12	0.40	1.74	6	Test	0.25	1.71	1.45	0.85	1.72
IDC fat	All (n = 76)		0.56	0.25	3.01	5.15	3						
	After outliers (n = 72)		0.49	0.24	2.58	3.87	3						
	Prediction	Train	0.41	0.24	2.84	3.63	2	Test	0.16	4.95	2.25	1.85	1.22
IDC starch	All (n = 76)		0.88	0.74	19.6	42.0	4						
	After outliers (n = 72)		0.86	0.72	21.8	45.4	3						
	Prediction	Train	0.94	0.81	9.07	30.1	6	Test	0.81	30.6	12.7	3.83	3.31
IDC Ca	All (n = 76)		0.97	0.54	0.32	5.56	9						
	After outliers (n = 72)		0.97	0.45	0.40	6.75	8						
	Prediction	Train	0.96	0.91	0.44	1.07	6	Test	0.73	3.45	3.49	1.10	3.18
IDC P	All (n = 76)		0.80	0.35	0.32	1.01	5						
	After outliers (n = 72)		0.82	0.37	0.30	1.01	5						
	Prediction	Train	0.73	0.49	0.42	0.79	3	Test	0.65	0.62	1.27	0.69	1.85
IDC DM	All (n = 76)		0.84	0.44	19.9	68.7	6						
	After outliers (n = 72)		0.93	0.19	7.34	86.5	8						
	Prediction	Train	0.90	0.63	9.45	33.7	6	Test	0.33	101	10.4	5.69	1.82

Ca, calcium; DM, dry matter; GE, gross energy; n, number of samples; N, nitrogen; P, phosphorus.

5.5. Discussion

The feed industry needs a quick and precise assessment of the nutritive value of feedstuffs for the optimal formulation of diets. In such instances, IR offers a rapid approach to predict the chemical composition as well as the nutritive value of feedstuffs compared to wet chemical methods, *in vivo* as well as *in vitro* methods (Khaleduzzaman et al., 2017; Khaleduzzaman and Salim, 2020). The ATR-FT MIRS has not been widely used in prediction for animal feeds compared to NIRS. To our knowledge, this is the first study stating the application of ATR-FT MIRS in prediction of chemical composition and nutritive value of broiler diets. A broad range of analytes was available with the 76 diets which was helpful to obtain calibration results and predict the performance of PLSR models based on ATR-FT MIRS. In that, the 56 diets were formulated to encompass a wide range of chemical composition utilising ten different feed ingredients. In contrast, the remaining 20 diets were prepared to closely resemble commercial broiler diets. This difference likely contributed to the separation observed along the PC1. The greater variation in the 56 diets resulted in a more dispersed distribution, while the 20 diets formed more tightly clustered group.

In addition to that, the ATR-FT MIR spectrometer used was easier to load samples, clean the surface, and scans were completed more quickly. Moreover, the spectra obtained were easier to interpret.

The appearance of MIR absorptions from vibrations of different functional groups in characteristic frequencies of IR facilitates easy band assignment and interpretation (Türker-Kaya and Huck, 2017). One major advantage of ATR-FT MIRS is the requirement of a small quantity of samples compared to wet chemistry methods (Karunakaran et al., 2020). However, it should be homogenous and fine ground to produce good spectra avoiding sample scattering by large particles (Divekar et al., 2017). Scattering effects are strongest when the particle size

and the wavelength of the radiation are similar. Therefore, particle size should be selected to be either several times larger or several times smaller than the wavelength.

Moreover, unlike wet chemistry, the protocol for MIRS is the same for all types of samples and the variability in the spectral features for replicate samples was minimal (Karunakaran et al., 2020).

The band positions determine the compounds present in the diet samples. Typical MIR spectra represent numerous absorbance peaks mainly divided into 4 regions generalised as the X-H stretching region (4000-2500 cm^{-1}), triple-bond region (2500-2000 cm^{-1}), the double bond region (2000-1500 cm^{-1}), and the fingerprint region (1500-600 cm^{-1}) which is a complex area showing many bands frequently overlapping each other (Türker-Kaya and Huck, 2017; Shi et al., 2019).

Calibrations are more selective in MIR than NIR as some of these bands can be well separated from others. Moreover, Savitzky-Golay treatment flattens nearly all the variation associated with the broad water centered at 3300 cm^{-1} and removes variation from unimportant regions and isolates peak of interest (Cleland et al., 2018) and PLSR reduces the negative effect of irrelevant spectra variations thus used in multivariate calibrations of IR spectra successfully (Shi et al., 2019).

According to the threshold values for RPD, the MIRS calibrations based on diet spectra could be considered reliable for N, ash, DM, Ca, P, and OM ($R^2p > 0.8$ and RPD > 3), while calibrations of CF are useful for quantification ($R^2p > 0.8$ and RPD = 2.96) and starch was acceptable for screening purposes (R^2 p = 0.77 and RPD = 2.44). The calibrations for GE and fat were not up to the mark since the RPD was < 2. The low RPD for GE could be explained by the small amount of variation in the dataset.

In a soybean study, Ferreira et al. (2014) obtained a MIRS calibration for protein with an R^2 of 0.91 while NIRS with R^2 of 0.88. Hell et al. (2016) reported a R^2 of 0.75 for MIRS calibration and R^2 of 0.66 for NIRS calibrations for wheat bran samples. Cleland et al. (2018) reported MIRS calibrations for protein with a coefficient of determination of validation (R^2v) of 0.72 and RPD of 2.33. In the present study, prediction of N was excellent with R^2c and R^2p of 0.91 and 0.87, respectively and RPD of 3.27 for the broiler diets as nitrogen bonds directly absorb energy in the MIR region. The inclusion of high protein sources (soybean meal, canola meal, and palm kernel meal) in the compound broiler diets may also account for the good prediction results. Hell et al. (2016) also stated that NH, OH, CO, and CN stretching, and NH and CN bending can be clearly observed and some of this band can be well separated from the rest of the spectrum in MIR.

The MIRS predictions of GE content showed lower coefficients of determination ($R^2c = 0.74$, $R^2p = 0.70$, and RPD = 1.90). Similar results were reported by Xiccato et al. (2003) for GE determination in rabbit feeds using NIRS (R^2c and R^2v 0.61 and 0.57, respectively). Lyons et al. (2016) also reported low R^2 values for sheep faecal determination of GE content using NIRS ($R^2c = 0.79$, $R^2v = 0.60$, and RPD = 1.82).

The variation in GE is mainly determined by fat content, however, MIRS prediction for fat content was also lower in the current study ($R^2p = 0.59$ and RPD = 1.62). Ferreira et al. (2014) recorded R^2 of 0.67 for lipids in soybean using MIRS compared to R^2 of 0.81 with NIRS. Even though some characteristic wavelengths associated with fat exist in the MIR region, the presence of many plant-based sources and by-products (maize, wheat, sorghum, soybean meal, soybean oil, and canola meal) in the diets may be the reason for the unsatisfactory prediction of fat in the present study. The fat content is more difficult to predict accurately in plant materials (Khaleduzzaman and Salim, 2020). When by-products of oil extraction are used in

the diets, their typical fat content will be low causing challenges in accurate prediction of fats (Chen et al., 2013).

However, Hell et al. (2016) reported R^2c of 0.94 and R^2v of 0.88 for predicting lipid content of wheat bran samples using MIRS where data pre-processing was done by multiplicative scatter correction. In addition to that, the fatty acid profile is nearly the same for wheat bran samples compared to our diets containing different compositions of various feed ingredients which vary in fatty acid profile. Compared to MIRS, fat content was well predicted by NIRS as reported in the studies by Xiccato et al. (2003) in rabbit feeds, Noel et al. (2022), and Paternostre et al. (2021) in pig feeds, and Khaleduzzaman et al. (2017) in commercial poultry diets because of the existence of characteristic wavelengths associated with fat in NIR region. On the other hand, Dowell et al. (2006) could not predict the lipids in wheat using NIRS.

Starch content showed acceptable predictions with R^2 of 0.77 and RMSEp of 5.47%. Hell et al. (2016) achieved an R^2v of 0.82 and RMSEp 2.32% for starch in wheat bran samples and the number of components required to model starch here was 10. However, the present study modelled starch with only 2 components which was far lower. Fewer components result in less chance of overfitting (Cleland et al., 2018).

The R^2 for DM was good in both calibration and prediction with RPD 3.05 even though the coefficient of variation (CoV) was low (0.02) compared to the other analytes. However, this stands in contrast to Cleland et al. (2018) who observed the worst prediction of DM of forage feed samples using MIRS (R^2c = 0.76, R^2cv = 0.50, and R^2external validation = 0.56; RPD = 1.16). Calibration of CF was useful for the quantification in the present study with RPD of 2.96. This was in agreement with the results obtained for commercial poultry diets using NIRS by Khaleduzzaman et al. (2017). Besides, the accuracy of predictions can be further improved by including more samples in the calibration data set.

No information has been reported in the literature about the application of MIRS for the prediction of the mineral composition of animal feeds. However, a study has been reported by Reeves (2001b) for the determination of minerals in dried poultry manure. Besides, few publications reported about the prediction of minerals using NIRS (Valdes et al., 1985; Khaleduzzaman et al., 2017; Khaleduzzaman and Salim, 2020). Minerals don't have a specific absorption band in the infrared spectroscopy region; however, predictions of Ca and P were excellent in the present study suggests the correlations may be due to the close association between minerals and organic compounds (Khaleduzzaman and Salim, 2020). The main source of minerals was provided by meat and bone meal, limestone, and dicalcium phosphate in the present study. Phosphorus is present as phosphate which does have a characteristic (and strong) IR spectrum. As a result, IR ought to anticipate P content accurately. Moreover, there is a stoichiometric relationship between Ca and P (via $CaHPO_4$) and Ca and carbonate in limestone. Therefore, IR is predicting Ca via its association with its anions in the mineral components. Similar observations were made with NIRS by Valdes et al. (1985) for poultry feeds. Moreover, Ca and P had a greater CoV which in turn supports that the better prediction models may be related to a wider range of variability. This agrees with those of Lastras et al. (2021) who reported that high variability results in better prediction models.

Ash content reflects the total amount of minerals found in feeds. Ash determination showed good predictability with R^2p of 0.87 and RPD of 4.38. Similar results were reported by Ferreira et al. (2014) ($R^2 = 0.87$). The number of components obtained in the present study was 6, whereas the study by Ferreira et al. (2014) had latent variables of 7 for ash. The results for ash with NIRS were poor in some studies (Ferreira et al., 2014) and excellent in some studies (Hell et al., 2016). Since the prediction of minerals was excellent, it reflected in the results of ash (Ca and P, represented nearly 33.11% of ashes). It in turn may be the reason for the high accuracy of OM which was calculated using the formula 100-ash. Besides, Xiccato et al. (2003)

reported that NIRS prediction was poor for OM in rabbit feeds with the R^2c of 0.35 and R^2v of 0.25.

It should be noted that if many components are chosen in a PLSR, model will try to account even the smaller changes in the dataset which results in overfitting (Khaleduzzaman et al., 2017). Besides, the number of components required to model each analyte in the current study was less than those obtained for NIRS in literature for animal feed studies (Xiccato et al., 2003; Khaleduzzaman et al., 2017; Khaleduzzaman and Salim, 2020; Paternostre et al., 2021; Noel et al., 2022) and in some MIRS related studies (Ferreira et al., 2014; Hell et al., 2016) suggest the advantage of ATR-FT MIRS in better prediction of unknown samples.

There is limited information available regarding the feasibility of applying MIRS for evaluating the CAID and IDC of nutrients for broilers. Digestibility calibration statistics for different components in pig feedstuffs were available using NIRS (Noel et al., 2021; Noel et al., 2022). It should be noted that the obtained MIRS models can be applied to samples similar to those of calibration sets. However, the models in the current study were developed across a wide range of chemical compositions. For different types of samples, new models must be developed according to their spectral data and physiochemical features (Shi et al., 2019).

The CAID and IDC achieved good predictions for calibration, but their cross-validation and prediction performance were very low. Prediction of the nutritive value is more complicated than the prediction of chemical composition of diets as the digestive response of animals in feeding will be involved (Xiccato et al., 2003). However, the models for predicting IDC of starch and Ca were satisfactory ($R^2p > 0.80$ and RPD > 2.0). Only the model for CAID of fat achieved $R^2p > 0.70$ and RPD > 2.0. The digestibility models developed with MIRS for comparisons with the present data are limited. On the other hand, similar studies using NIRS also failed to predict the digestibility of feeds accurately (Xiccato et al., 2003; Shi et al., 2019).

There could be several reasons for the poor prediction of digestibility. Primarily, digestibility is not a chemical parameter but it's a property of feed (Stuth et al., 2003; Shi et al., 2019). Previous studies also reported that the digestibility calibration statistics are usually poorer than chemical composition since they incorporate more errors of determination of both digestibility and chemical composition. Moreover, many factors affect the digestibility of nutrients in feeds such as interaction between nutrients and anti-nutritional factors which question the accuracy of reference digestibility values used (Shi et al., 2019). In addition, the *in vivo* nutritive value estimates may incorporate bird variations (Stuth et al., 2003). Consequently, Xiccato et al. (2003) stated that the inclusion of specific ingredients in diets at a high level may have associative effects on the *in vivo* results which can't be detected by infrared spectroscopy. On par with that, the present study incorporated specific ingredients at higher levels to achieve a wide range of nutritive parameters.

Coulibaly et al. (2013) reported that the NIRS calibrations developed using the combined spectra of feed and faeces predicted nutrient digestibility of diets for broilers more accurately than calibrations based on spectra from only feed or faeces while Paternostre et al. (2021) also showed combining NIRS spectral information from feed and faeces improve the prediction of digestibility and net energy of pig feed. Therefore, it can be applied and will be more useful when the MIRS spectral data of the excreta and ileal digesta of each sample is combined with the diet data.

The result of the present study shows that ATR-FT MIRS can be used as a useful tool for estimating the chemical composition of broiler diets with minimal sample processing by rich spectral features of the MIR region. However, methods should be refined for prediction of ileal digestibility and digestible content of diets.

5.6. Conclusions

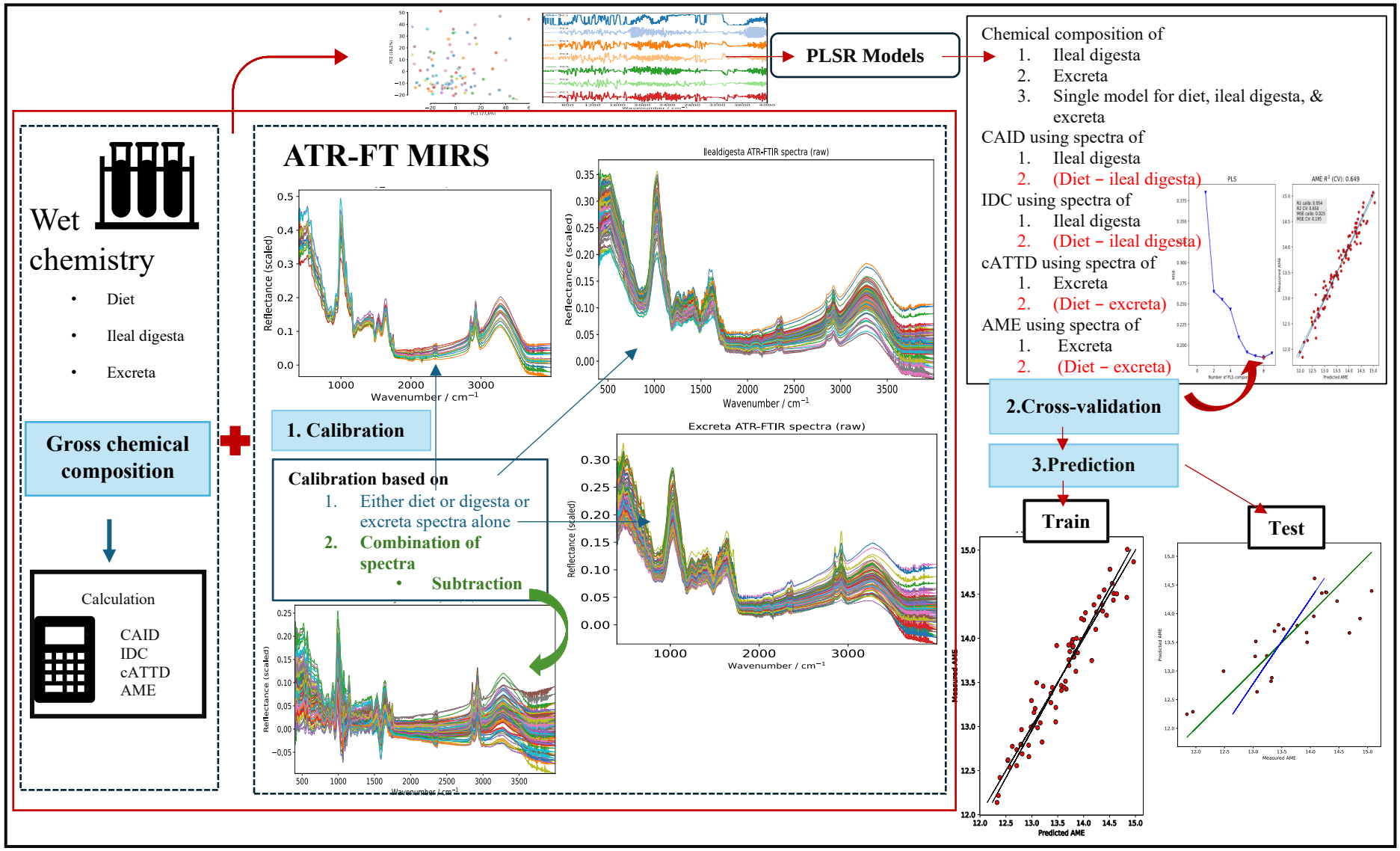
This is the first report on the use of ATR-FT MIRS to predict the chemical composition and nutritive value of broiler diets. The ATR-FT MIRS has proven to be a convenient and environmentally friendly method to rapidly predict the chemical composition of broiler diets. However, the models for CAID and IDC could not make an excellent prediction. More efforts are required to improve the accuracy of prediction of CAID and IDC of nutrients in broiler diets such as incorporating animal factors into the calibration process or processing of combined spectral data of diet and excreta samples.

CHAPTER 6

Prediction of the chemical composition of broiler excreta and ileal digesta using Attenuated Total Reflectance Fourier Transform Mid-Infrared Spectroscopy (ATR-FT MIRS) and its use for estimating ileal digestibility and apparent metabolisable energy

The results presented in Chapter 5 showed that ATR-FT MIRS could successfully predict the chemical composition of broiler chicken diets. Therefore, the next step is to examine if ATR-FT MIRS can predict the chemical composition of ileal digesta and excreta, as well as the nutritive value of the diets using the ileal digesta and excreta spectra alone or combined with the diet spectra.

Graphical abstract



6.1. Abstract

This study investigates the application of Attenuated Total Reflectance Fourier Transform (ATR-FT) Mid-Infrared Spectroscopy (MIRS) to predict the chemical composition of ileal digesta and excreta, as well as the coefficient of apparent ileal digestibility (CAID), ileal digestible content (IDC), coefficient of apparent total tract digestibility (cATTD), and apparent metabolisable energy (AME). Wet chemistry and spectral data from 100 ileal digesta and 100 excreta samples from broilers fed 20 different diets were used. The calibration equations were developed using principal component analysis (PCA) and partial least square regression (PLSR) techniques to predict the chemical composition of the ileal digesta and excreta, calibrations based on ileal digesta spectra were developed to predict the CAID and IDC of nutrients, calibrations based on excreta spectra were derived to predict the cATTD and AME. Calibrations based on combined spectra were also developed. This was done by subtracting the ileal digesta spectra from the diet spectra to predict CAID and IDC and by subtracting the excreta spectra from the diet spectra to predict cATTD and AME. Finally, a common calibration model was developed to predict the gross chemical composition across diets, ileal digesta, and excreta. Prediction accuracy and performance were accessed by coefficient of determination of prediction (R^2p) and relative performance deviation (RPD), respectively. Most of the chemical parameters in the excreta could be predicted successfully (nitrogen (N), gross energy (GE), ash, calcium (Ca), and phosphorus (P); $R^2p > 0.70$ and RPD > 2.4). Only the acid detergent fibre (ADF) in the ileal digesta was predicted accurately ($R^2p = 0.81$ and RPD = 3.08). The combination of diet spectra and ileal digesta spectra or diet spectra and excreta spectra resulted in an overall better estimation of CAID, IDC as well as cATTD than the prediction obtained by using the ileal digesta spectra or excreta spectra alone. Prediction of AME using the combined spectra could be used for quantification with R^2p of 0.82 and RPD of 2.86. Single models developed across diets, ileal digesta, and excreta to predict the gross chemical compositions

did not perform well except for the diets. Although a larger number of samples will be required to generate better calibrations, these results proved the ATR-FT MIRS technique is a potential non-destructive, cheaper, and faster method to predict the chemical composition of diet, ileal digesta, and excreta, as well as CAID, IDC, cATTD, and AME.

Key words: ATR-FT MIRS, broilers, digestibility, excreta, ileal digesta, prediction

6.2. Introduction

Faeces or excreta in chicken consist mainly of indigestible residues of the diets that are resistant to the digestion process and thus provide information regarding the nutritional value of the diets (Schiborra et al., 2015; Paternostre et al., 2021). The nutritive values of the diets are usually obtained through *in vivo* experiments requiring the measurement of nutrient intake (feed) and output (ileal digesta or excreta) (Paternostre et al., 2021). In those experiments, it is necessary to accurately measure the quantity and chemical composition of the feeds and faeces. Usually, wet chemical methods are used, thus resulting in high costs, time delays, and environmental concerns (Bastianelli et al., 2010).

Attenuated Total Reflectance Fourier Transform Mid-Infrared Spectroscopy (ATR-FT MIRS) is a fast non-destructive technique that could replace the costly, time-consuming wet chemical analysis methods. The mid-infrared spectrum (MIR) can be used to quickly predict various parameters simultaneously, at a low cost with no waste product generation. It provides better chemical information on sample composition compared to near-infrared spectroscopy (NIRS) due to its ability to detect fundamental molecular vibrations rather than overtones and combination bands (Lyons et al., 2016). The fingerprint region of MIR region contains the fundamental vibrations of many molecules including the amide groups of protein, esters, fatty acids, carbohydrates etc. (Kho et al., 2023).

The success of predicting the chemical composition of broiler diets by ATR-FT MIRS has been proved in the previous study (Chapter 5). Given that the estimation of chemical composition of diets by ATR-FT MIRS is easy, it is worth applying the same approach to estimate the chemical composition of excreta and ileal digesta. No commercial ATR-FT MIRS calibrations to predict the gross chemical composition of poultry ileal digesta and excreta or digestibility of nutrients are available. However, such calibrations have been reported in studies with other species: MIRS prediction of protein and its digestibility using undigested residue in pigs (Wang et al., 2013) and determination of faecal composition, intake, and digestibility in sheep (Lyons et al., 2016). In addition, many studies have been published on the analysis of faecal or excreta composition by NIRS in poultry or pigs (Reeves, 2001a; Reeves, 2001b; Smith et al., 2001; Xing et al., 2008; De la Roza-Delgado et al., 2015; Cruz-Conesa et al., 2022).

Prediction of digestibility based on the feed characteristics is usually carried out using infrared spectroscopy. However, this can't represent the whole digestion process as it involves both the feed and the animal (Coulibaly et al., 2013). In this context, the prediction of digestibility from faeces spectra could be more useful as it integrates more information on the animal part. The spectra contain both chemical and physical information on the excreta sample, thus maximizing the quantity of information related to the digestive process (Bastianelli et al., 2013; Bastianelli et al., 2015). However, this provides less information about the feed. Therefore, integrating information both from the feed spectra and faeces spectra may improve the prediction of digestibility.

Several authors found that the NIRS calibrations based on combined spectra from diets and faeces predicted the nutrient digestibility of diets more accurately than the calibrations based on spectra from diets or faeces/excreta only in poultry (Bastianelli et al., 2013; Coulibaly et al., 2013), in pigs (Paternostre et al., 2021), in rabbits (Meineri et al., 2009), and ruminants

(Decruyenaere et al., 2009). So far, no studies have been reported combining diet and excreta ATR-FT MIRS spectra to predict nutrient digestibility and AME of broiler diets.

The potential of ATR-FT MIRS for analysing the gross chemical composition of broiler diets is established in Chapter 5. Therefore, the present study aimed to evaluate the usefulness and accuracy of ATR-FT MIRS calibrations based on spectra from ileal digesta or excreta to predict their chemical composition, calibrations based on ileal digesta to predict the coefficient of apparent ileal digestibility (CAID) and ileal digestible content (IDC) of nutrients, and calibrations based on excreta to predict the coefficient of apparent total tract digestibility (cATTD) of nutrients and apparent metabolisable energy (AME). Finally, to evaluate calibrations based on the combined spectra of diet and ileal digesta to predict CAID and IDC or combined spectra of diet and excreta to predict cATTD and AME.

6.3. Material and methods

The twenty diets and the corresponding 100 freeze-dried and ground ileal digesta and excreta samples (20 diets × 5 cages) for spectral acquisition were obtained from the study described in Chapter 4. The apparent total tract digestibility was determined using the total collection method, apparent ileal digestibility was determined using the marker method, and AME was determined using both total collection and marker method (Chapter 4).

6.3.1. Spectra acquisition and data analysis

Spectral data collection using ATR-FT MIRS, spectral pre-treatment, calibrations, and predictions were carried out according to the methods described in Chapter 5 (illustrated in Figure 6.1). Before being pre-treated, all spectra were reduced to one-eighth of their size by taking the average of 8 consecutive values giving 1867 absorbance values for each spectrum.

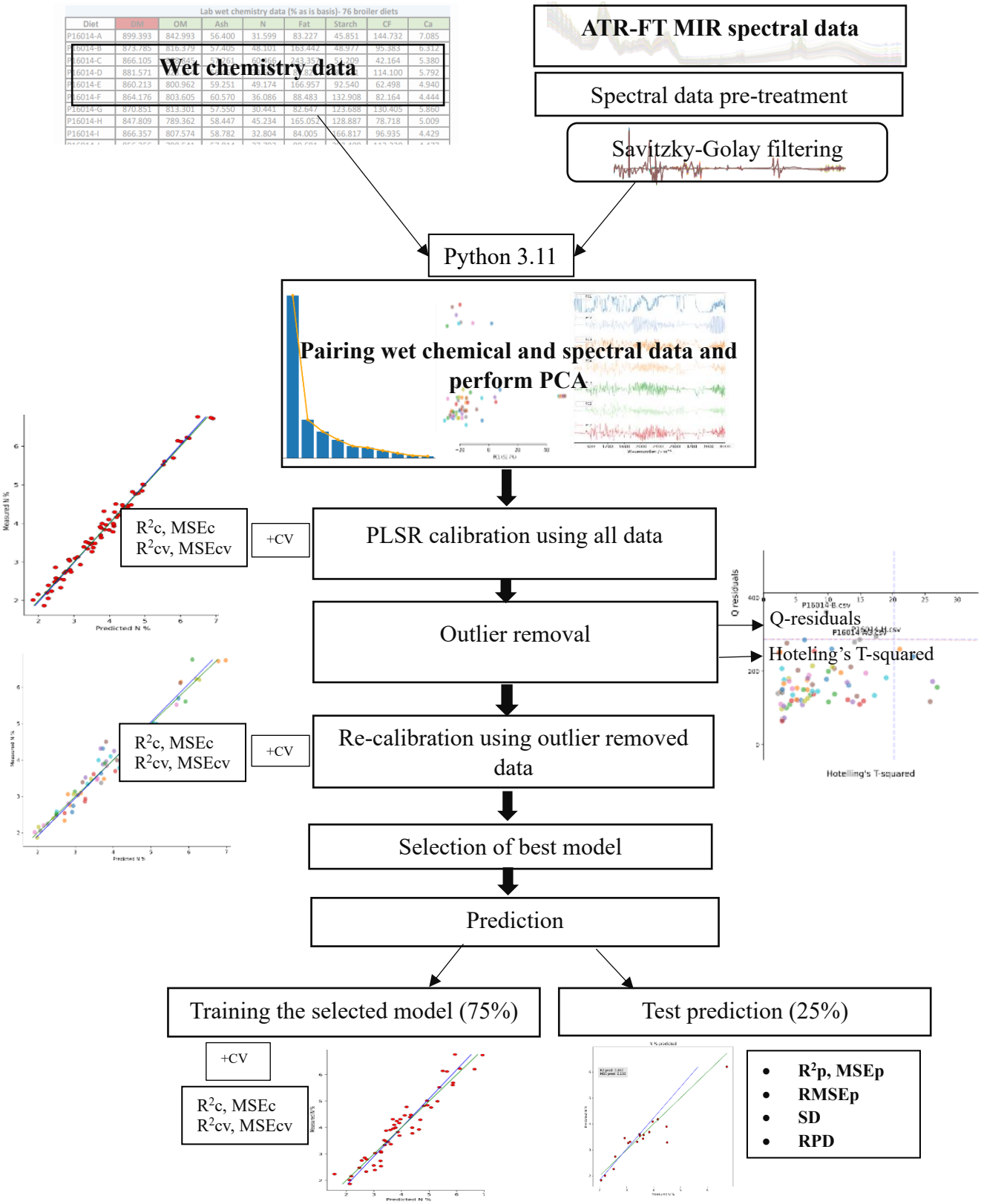


Figure 6.1. Overview of the ATR-FT MIRS calibration and prediction process as described in Chapter 5.

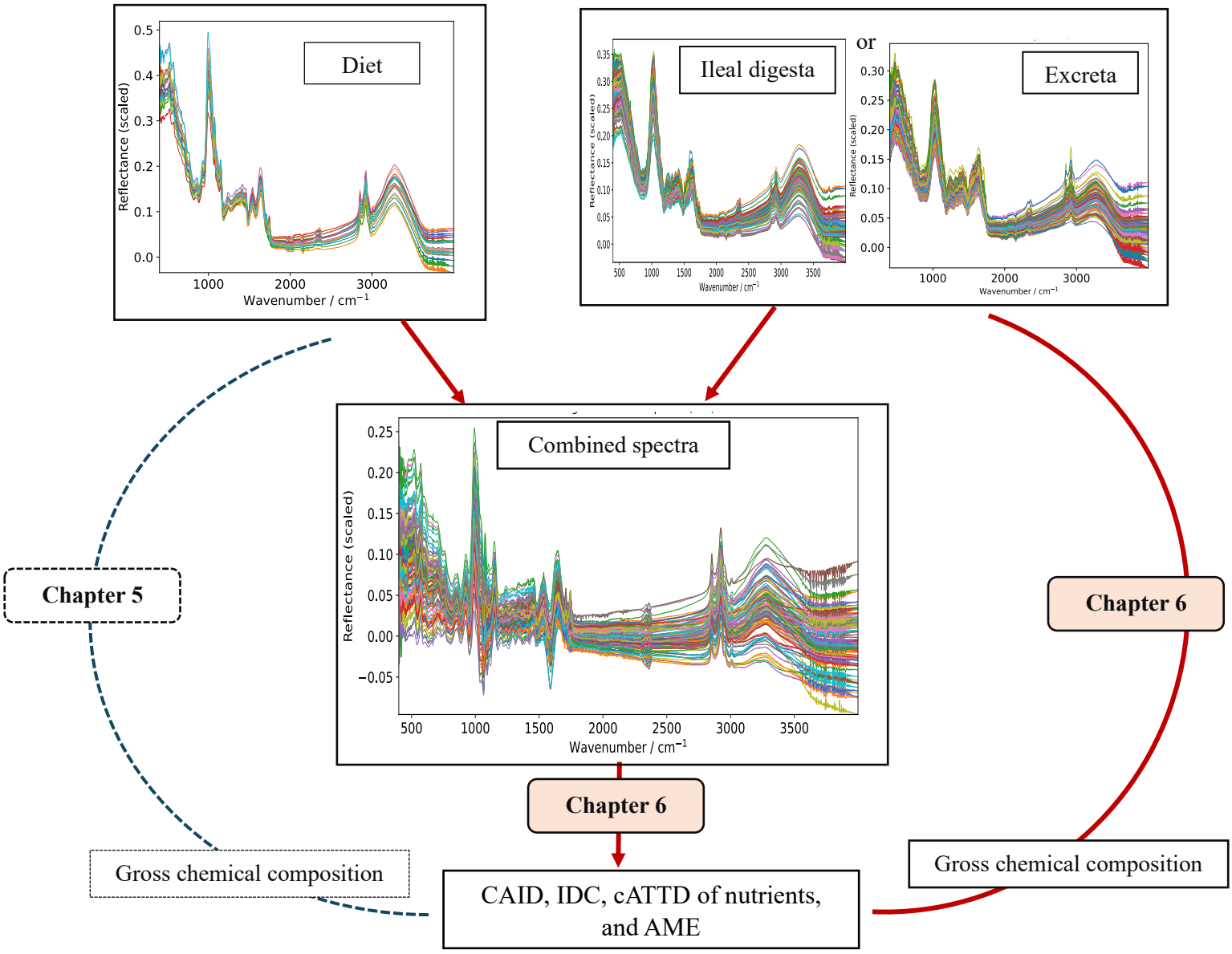


Figure 6.2. Methods used in the study for evaluation of chemical composition of ileal digesta and excreta, CAID, IDC, cATTD, and AME using ATR-FT MIRS.

6.3.2. Calibration based on ileal digesta and excreta

A total of 100 excreta and 100 ileal digesta samples representing 20 diets were utilised to determine their gross chemical composition, CAID, IDC, cATTD, and AME by ATR-FT MIRS. Figure 6.2 illustrates the overall technique used for prediction of chemical composition of

digestion products (ileal digesta and excreta), digestible content, digestibility, and AME of diets.

6.3.3. Calibrations based on combined spectra from diet and ileal digesta or excreta

The diet spectra and corresponding ileal digesta or excreta spectra were combined using subtraction. This method considers both the composition of the original diet, and the variation associated with the digestive process.

Subtracting: The reflectance value of the ileal digesta spectrum or excreta spectrum (1867 wavenumbers) was subtracted from the corresponding reflectance value of the diet spectrum and the subtracted spectrum was utilized to evaluate the CAID, IDC, cATTD, and AME.

Subtraction was done initially, and the pre-treatment (Savitzky-Golay) was applied to the subtracted spectra of diet and ileal digesta or diet and excreta.

6.3.4. Development of a single overall model to predict the gross chemical composition across diet, ileal digesta, and excreta

Finally, all the gross chemical composition data of the diet, excreta, and ileal digesta were combined and a common model was obtained using ATR-FT MIRS to study whether one overall calibration can be applied to predict the gross chemical composition of different sample types. Using the combined model, the gross chemical composition of diet, ileal digesta, and excreta (N%, GE MJ/kg, Ca mg/g, P mg/g, DM%, and Ash%) was predicted and compared with the measured values by wet chemistry. Means of the measured values and the predicted values, bias, coefficient of determination (R^2), concordance correlation coefficient (CCC), root mean square error (RMSE), and relative prediction error (RPE) were calculated. Additionally, a paired t-test was carried out to compare the measured and the predicted values. All these calculations were carried out to check whether a common model could be applied across all the samples type.

6.3.5. Statistics

The coefficient of determination of calibration and cross-validation (R^2c and R^2cv) of all samples and outlier removed samples, mean square error of calibration, and cross-validation (MSEc and MSEcv) for all samples and outlier removed samples, coefficient of determination of prediction (R^2p), standard deviation of measured values (SDm), root mean square error of prediction (RMSEp), and relative performance deviation (RPD) were used to evaluate the accuracy and performance of the models. Best equations were selected based on the highest R^2p and RPD. R^2 value closer to 1 and low value of MSE are a better fit (Ferreira et al., 2014; Noel et al., 2022). The RPD value of < 2 is not suitable for prediction; values between 2.0-2.4 are acceptable for screening purposes or qualitative evaluation (e.g., high, medium, or low concentration of a particular nutrient); values between 2.5-2.9 are useful for quantification; and values ≥ 3 indicate high accuracy for quantitative analysis (Belanche et al., 2013; Lyons et al., 2016).

RMSEp and RPD were calculated by the following equations.

$$\mathrm{RMSEp} = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \tag{6.1}$$

$$\mathrm{RPD} = \frac{\mathrm{SDm}}{\mathrm{SEp}} \tag{6.2}$$

where, $\hat{y}_i$ and y_i are the predicted and observed (measured) values respectively, n is the number of data, and SDm is the standard deviation of the measured values and SEp is the standard error of prediction.

The prediction accuracy and performance of the common model (section 6.3.4) established across the chemical composition of diets, ileal digesta, and excreta were estimated based on the difference between the predicted and observed values using R^2, RMSE, CCC, bias, and RPE.

The concordance correlation coefficient (CCC) evaluates the agreement between two readings by measuring the variation from the 45° line through the concordance line which is the origin

$$\text{CCC} = \frac{2\sigma_{PM}}{\sigma_P^2+\sigma_M^2+(\mu_P-\mu_M)^{2'}} \tag{6.3}$$

where, σ_{PM} is the covariance, σ^2_P and σ^2_M are the variances, μP and μM are the means, and P and M refer to the predicted and measured values.

Bias was calculated using the following formula:

$$\text{Bias} = \frac{1}{n}\sum_{i=1}^{n}(\hat{y}_i - y_i) \tag{6.4}$$

where, $\hat{y}_i$ is the predicted value, y_i is the observed value, and n is the number of observations.

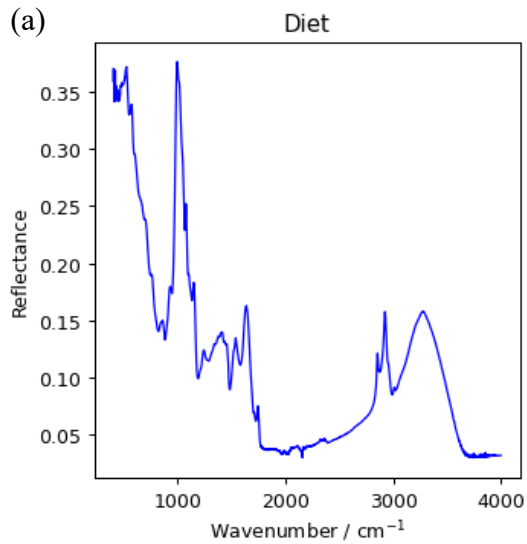
The RPE was calculated as the square root of mean square error divided by the mean of the measured values (μM) multiplied by 100 as indicated by the following equation.

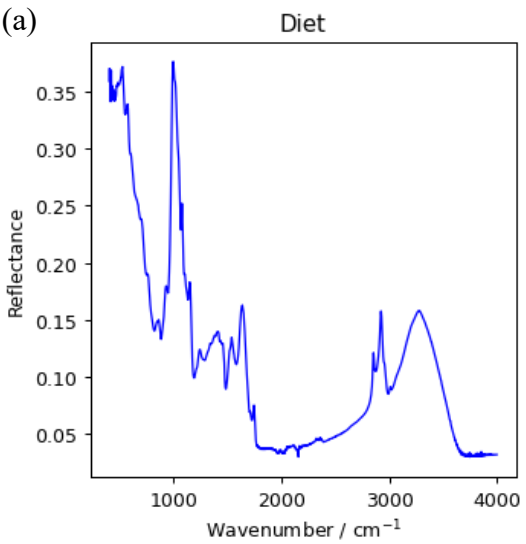
$$\text{RPE} = \frac{\sqrt{MSE}}{\mu M} \times 100 \tag{6.5}$$

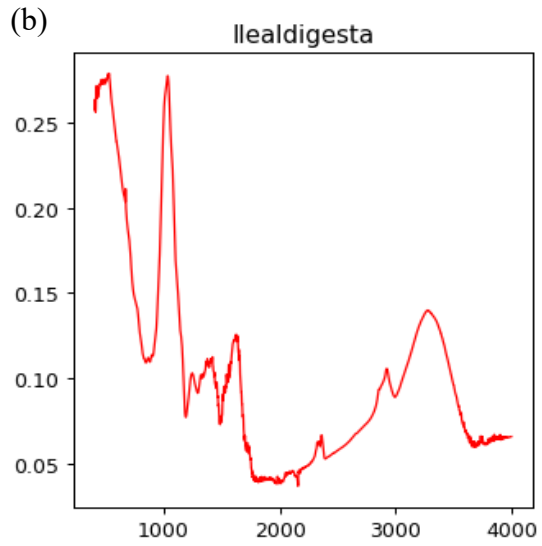
RPE < 10% is considered the best and between 10 and 20% is good for the prediction models.

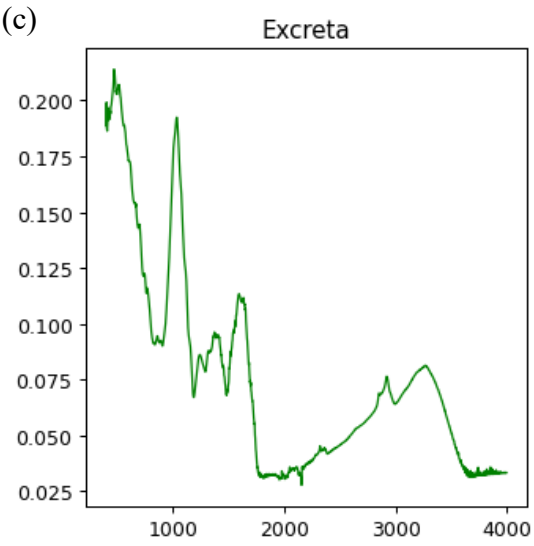
6.4. Results

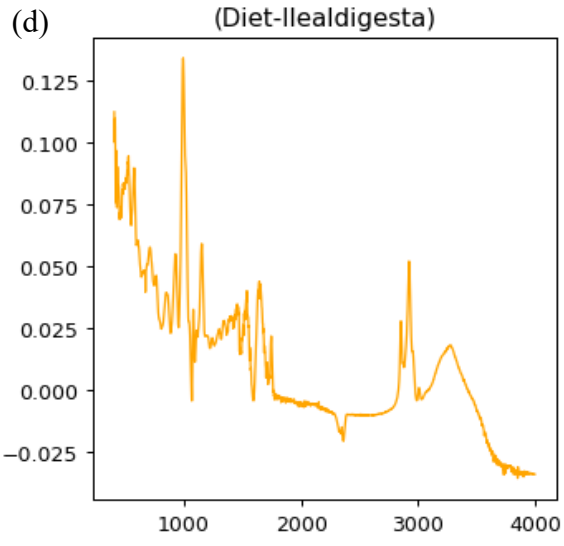
Figure 6.3 shows the mean spectra of a diet (a), its corresponding ileal digesta (b), and excreta (c) (average of 3 replicates) and the subtracted spectra of diet and ileal digesta (d) and diet and excreta (e). The spectral differences reflect the composition after digestion. Figure 6.3 (f) and 6.3 (g) shows the subtracted and pre-treated spectra of diet and ileal digesta and diet and excreta, respectively using Savitzky-Golay filtering (1st derivative).

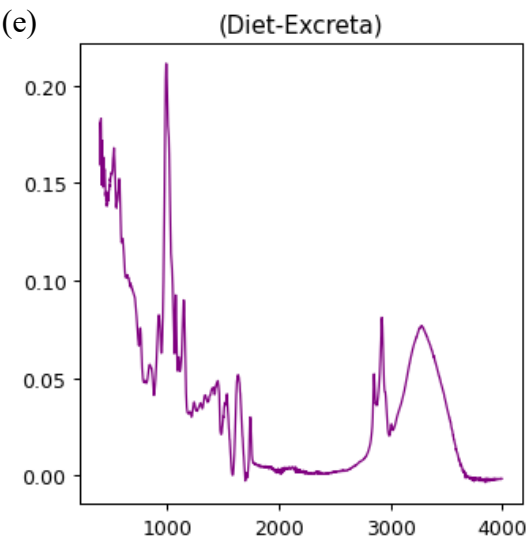

(a)
Diet
Reflectance
0.35
0.30
0.25
0.20
0.15
0.10
0.05
1000
2000
3000
4000
Wavenumber / cm^{-1}


(a)
Diet
Reflectance
0.35
0.30
0.25
0.20
0.15
0.10
0.05
1000
2000
3000
4000
Wavenumber / cm^{-1}


(b)
Ilealdigesta
0.25
0.20
0.15
0.10
0.05
1000
2000
3000
4000


(c)
Excreta
0.200
0.175
0.150
0.125
0.100
0.075
0.050
0.025
1000
2000
3000
4000


(d)
(Diet-Ilealdigesta)
0.125
0.100
0.075
0.050
0.025
0.000
−0.025
1000
2000
3000
4000


(e)
(Diet-Excreta)
0.20
0.15
0.10
0.05
0.00
1000
2000
3000
4000

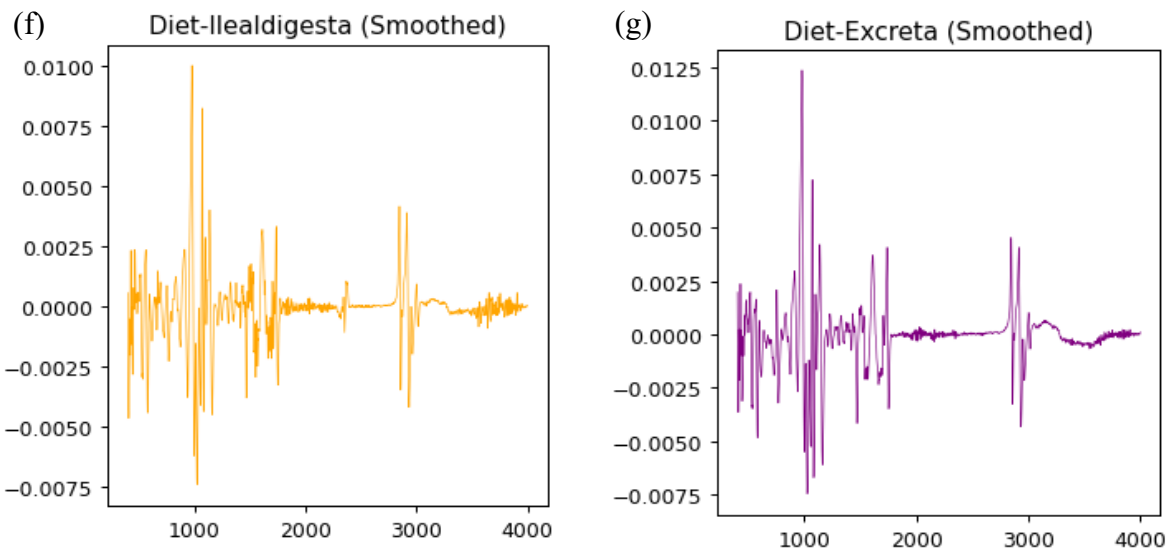


Figure 6.3. (a) Average raw spectra of a diet (b) average raw spectra of ileal digesta (c) average raw spectra of excreta (d) subtracted spectra of diet and ileal digesta (e) subtracted spectra of diet and excreta (f) subtracted and pre-treated spectra of diet and ileal digesta (g) subtracted and pre-treated spectra of diet and excreta (pre-treatment was done using Savitzky-Golay filtering).

Table 6.1 shows the descriptive statistics of the ileal digesta and excreta composition, CAID, IDC, cATTD of nutrients, and AME. The chemical composition and digestibility values varied considerably for ileal digesta and excreta. The N content of ileal digesta ranged from 0.03 to 3.05%, whereas excreta ranged from 3.45 to 5.44%. The GE content in the ileal digesta ranged from 14.3 to 17.3 MJ/kg and in the excreta from 14.7 to 18.5 MJ/kg. The Ca and P had high variations in both the ileal digesta and excreta. However, despite the variation in chemical composition, the standard deviation (SD) of digestibility coefficients were low. The highest coefficient of variation (CoV) was observed in gross chemical composition for fat (49.0%), lignin (41.0%), starch (39.0%), and Ca (36.0%) in ileal digesta and for Ca (35.0%) and P (21.0%) in excreta. The lowest CoV was obtained for the DM in both the ileal digesta and

excreta (1.00%). For the CAID of nutrients, the highest CoV was observed for CF (109%) and Ca (51.8%), the CAID of starch had the lowest CoV (1.20%). The CoV of AME by total collection was lower than by the TiO_2 method (5.40% vs 12.0%).

The relative standard error (SE) of the laboratory analysis was low for DM, N, GE, and Ti in both ileal digesta and excreta. The remaining parameters had higher SE as they may involve more complex wet chemical analysis. The highest error was obtained for fibre fractions and minerals (SE > 0.20).

Table 6.1. Descriptive statistics for the gross chemical composition of ileal digesta and excreta (% as-is basis), CAID of nutrients, IDC of nutrients (% as-is basis), AME (MJ/kg DM), and cATTD of nutrients.

Parameters	Mean	Min	Max	SD	CoV	SE
Ileal digesta n = 100						
DM	93.4	91.8	95.2	0.71	1.00	0.07
Ash	12.2	8.13	19.3	2.24	18.0	0.22
OM	81.2	74.9	85.3	2.26	3.00	0.23
N	2.31	0.03	3.05	0.35	15.0	0.03
Fat	2.47	0.46	6.54	1.22	49.0	0.12
Starch	3.76	1.08	8.85	1.46	39.0	0.15
CF	11.6	8.59	15.7	1.57	14.0	0.16
NDF	32.6	24.9	43.2	3.63	11.0	0.36
ADF	15.7	10.3	23.6	2.76	18.0	0.28
Lignin	5.05	0.82	11.8	2.08	41.0	0.21
Ti	1.59	1.09	2.27	0.27	17.0	0.03
GE	15.5	14.3	17.3	0.57	4.00	0.06
Ca	24.1	8.65	55.6	8.73	36.0	0.87
P	12.4	7.45	22.4	3.04	24.0	0.30
CAID ash	0.376	0.040	0.560	0.10	25.6	0.010
CAID N	0.787	0.686	0.852	0.04	4.50	0.004
CAID energy	0.706	0.539	0.794	0.05	6.70	0.005
CAID fat	0.868	0.651	0.953	0.05	5.60	0.005
CAID starch	0.964	0.925	0.992	0.01	1.20	0.001
CAID CF	0.143	−0.473	0.440	0.16	109	0.016
CAID Ca	0.272	−0.086	0.594	0.14	51.8	0.014
CAID P	0.484	0.207	0.640	0.08	17.0	0.008
CAID DM	0.653	0.479	0.750	0.06	8.60	0.006
IDC ash	2.29	0.29	3.62	0.56	24.6	0.06
IDC N	2.74	2.14	3.39	0.32	11.5	0.03
IDC energy	11.8	8.91	13.4	0.81	6.90	0.08
IDC fat	5.03	2.26	7.50	1.36	27.0	0.14
IDC starch	31.5	20.5	40.0	4.66	14.8	0.47
IDC CF	0.68	−1.24	2.52	0.72	105	0.07
IDC Ca	2.59	−0.93	8.18	1.32	51.1	0.13
IDC P	3.59	2.09	5.73	0.61	17.1	0.06
IDC DM	65.3	48.0	75.1	5.58	8.60	0.56
Excreta n = 100						
DM	95.9	94.6	97.0	0.47	1.00	0.05
Ash	14.4	10.9	18.7	1.73	12.0	0.17
OM	85.6	81.3	89.1	1.73	2.00	0.17
N	4.12	3.45	5.44	0.52	13.0	0.05
Ti	1.52	0.91	2.30	0.27	18.0	0.03
GE	15.9	14.7	18.5	0.59	4.00	0.06
Ca	22.8	8.43	46.1	8.01	35.0	0.80
P	14.6	10.3	22.9	3.13	21.0	0.31
AME (total collection)	13.6	11.8	15.1	0.73	5.40	0.07
AME (TiO_2)	13.4	10.8	18.8	1.62	12.0	0.16
cATTD DM	0.692	0.585	0.773	0.041	6.00	0.004
cATTD ash	0.333	0.251	0.492	0.038	11.5	0.004
cATTD N	0.661	0.567	0.751	0.042	6.30	0.004
cATTD energy	0.727	0.643	0.801	0.036	4.90	0.004
cATTD Ca	0.377	0.193	0.627	0.089	23.6	0.009
cATTD P	0.448	0.322	0.604	0.057	12.6	0.006
cATTD OM	0.718	0.618	0.799	0.039	5.50	0.004

ADF, acid detergent fibre; AME, apparent metabolisable energy; Ca, calcium; CAID, coefficient of apparent ileal digestibility; cATTD, coefficient of apparent total tract digestibility; CF, crude fibre; CoV, coefficient of variation (%); DM, dry matter; GE, gross energy; IDC, ileal digestible content; N, nitrogen; NDF, neutral detergent fibre; OM, organic matter; P, phosphorus; SD, standard deviation; SE, standard error; Ti, titanium.

6.4.1. Models for chemical composition, apparent ileal digestibility, and ileal digestible content based on the ileal digesta spectra alone or combined spectra of diet and ileal digesta using ATR-FT MIRS

Calibrations based on the ileal digesta for determination of its gross chemical composition resulted in R^2 higher than 0.70 and an RPD above 2.0 for most of the analytes (Table 6.2). In terms of RPD, the highest accuracy was achieved for the model to predict the ADF with R^2p of 0.81 and RPD of 3.08. The models for GE, fat, starch, and P were useful for quantifications as they had RPD between 2.4 and 3.0. The models for ash, CF, Ca, OM, NDF, and lignin can be used for screening purposes (RPD 2.0-2.4). The models for N, DM, and Ti were not satisfactory, which had the RPD below 2.0 and failed to predict the results accurately. Predictions of CAID based on ileal digesta spectra alone resulted in RPD between 1.0 and 2.0 with lower R^2p for all the parameters (Table 6.3), which were not satisfactory. Overall, the calibrations based on subtracted spectra (diet spectra−ileal digesta spectra) to predict the CAID of nutrients performed better than the calibrations based on the ileal digesta alone (Table 6.4). The CAID of energy had the highest RPD of 1.91.

Calibration equations for IDC of nutrients using the spectra of ileal digesta (Table 6.5) produced poor results in terms of R^2p and RPD for energy, fat, CF, Ca, P, and DM. Only IDC of N and starch reported R^2p of 0.57 and 0.59 with RPD of 2.45 and 2.34, respectively. Predictions developed for IDC of nutrients with the subtracted spectra are shown in Table 6.6. The IDC of starch and fat reported $R^2p > 0.80$ and RPD > 3.0, while IDC of N reported $R^2p = 0.80$ and RPD = 2.92. The RPD obtained for the other parameters such as IDC of energy, CF, Ca, P, and DM was < 2.0. For all the IDC of nutrients, the combined spectra of diet and ileal digesta produced better results compared to the prediction from the ileal spectra or diet spectra alone.

Table 6.2. Calibration and prediction statistics of the equations to predict the chemical composition of ileal digesta (as-is basis) and Ti (DM basis) using ATR-FT MIR spectra of ileal digesta.

Analyte			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
N%	All samples		0.69	0.38	0.04	0.07		4						
	After outliers		0.77	0.40	0.03	0.08	95	5						
	Prediction	Train	0.68	0.04	0.04	0.12		4	Test	0.66	0.04	0.35	0.20	1.75
GE MJ/kg	All		0.89	0.68	0.04	0.10		6						
	After outliers		0.89	0.65	0.04	0.11	95	6						
	Prediction	Train	0.84	0.50	0.04	0.14		5	Test	0.84	0.07	0.57	0.23	2.53
Ash%	All		0.84	0.75	0.80	1.23		4						
	After outliers		0.84	0.70	0.75	1.45	95	4						
	Prediction	Train	0.82	0.54	0.72	1.88		4	Test	0.82	1.21	2.18	0.92	2.37
Fat%	All		0.78	0.71	0.32	0.43		2						
	After outliers		0.89	0.73	0.17	0.41	95	5						
	Prediction	Train	0.86	0.72	0.23	0.46		4	Test	0.77	0.27	1.23	0.49	2.50
Starch%	All		0.90	0.81	0.21	0.40		5						
	After outliers		0.90	0.80	0.22	0.43	95	5						
	Prediction	Train	0.91	0.68	0.20	0.76		5	Test	0.75	0.44	1.48	0.51	2.92
DM%	All		0.60	0.07	0.20	0.46		4						
	After outliers		0.56	0.10	0.22	0.45	95	4						
	Prediction	Train	0.55	0.06	0.24	0.50		3	Test	0.05	0.38	0.71	0.53	1.34
CF%	All		0.85	0.64	0.36	0.88		5						
	After outliers		0.89	0.65	0.27	0.86	95	6						
	Prediction	Train	0.91	0.63	0.20	0.82		6	Test	0.63	1.18	1.57	0.67	2.33

Continued…

Analyte			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
Ca mg/g	All samples		0.83	0.73	12.6	20.4		4						
	After outliers		0.83	0.69	12.1	22.2	95	4						
	Prediction	Train	0.84	0.63	11.0	25.1		4	Test	0.74	21.3	8.48	3.70	2.29
P mg/g	All		0.85	0.72	1.39	2.54		4						
	After outliers		0.90	0.69	0.95	2.83	95	5						
	Prediction	Train	0.92	0.63	0.61	2.84		5	Test	0.76	3.09	3.01	1.12	2.70
OM%	All		0.82	0.69	0.93	1.59		4						
	After outliers		0.82	0.63	0.91	1.81	95	4						
	Prediction	Train	0.84	0.53	0.78	2.24		4	Test	0.71	1.49	2.22	0.99	2.25
NDF%	All		0.85	0.54	2.01	5.99		5						
	After outliers		0.85	0.52	2.02	6.33	95	5						
	Prediction	Train	0.79	0.42	2.66	7.34		4	Test	0.71	4.22	3.64	1.76	2.07
ADF%	All		0.97	0.68	0.20	2.39		9						
	After outliers		0.97	0.73	0.26	2.13	95	9						
	Prediction	Train	0.93	0.60	0.52	2.98		6	Test	0.81	1.71	2.80	0.91	3.08
Lignin%	All		0.73	0.20	1.17	3.42		4						
	After outliers		0.85	0.28	0.65	3.21	95	6						
	Prediction	Train	0.90	0.51	0.44	2.05		6	Test	0.44	2.88	2.11	1.03	2.04
Ti%	All		0.75	0.24	0.02	0.05		5						
	After outliers		0.87	0.28	0.01	0.05	95	7						
	Prediction	Train	0.78	0.11	0.02	0.07		5	Test	0.53	0.03	0.27	0.14	1.90

ADF, acid detergent fibre; Ca, calcium; CF, crude fibre; DM, dry matter; GE, gross energy; MSEc, mean square error calibration; MSEcv, mean square error cross-validation; MSEp, mean square error prediction; N, nitrogen; nc, number of components; NDF, neutral detergent fibre; OM, organic matter; P, phosphorus; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; RMSEp, root mean square error of prediction; RPD, relative performance to deviation; SDm, standard deviation of the measured values; Ti, titanium.

Table 6.3. Calibration and prediction statistics for the prediction of CAID of nutrients using spectra of ileal digesta.

Parameter			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
CAID N	All		0.78	0.31	0	0.001		6						
	After outliers		0.72	0.29	0	0.001	95	5						
	Prediction	Train	0.60	0.15	0	0.001		3	Test	0.28	0.001	0.04	0.03	1.42
CAID energy	All		0.69	0.13	0.001	0.002		5						
	After outliers		0.91	0.21	0.001	0.002	96	9						
	Prediction	Train	0.73	0.20	0.001	0.001		4	Test	0.04	0.002	0.05	0.03	1.55
CAID fat	All		0.64	0.41	0.001	0.001		4						
	After outliers		0.45	0.35	0.001	0.002	95	2						
	Prediction	Train	0.50	0.28	0.001	0.001		2	Test	0.26	0.002	0.05	0.04	1.33
CAID starch	All		0.76	0.49	0	0		5						
	After outliers		0.76	0.45	0	0	95	5						
	Prediction	Train	0.70	0.34	0	0		3	Test	0.61	0	0.01	0.01	1.75
CAID ash	All		0.65	0.22	0.003	0.007		4						
	After outliers		0.66	0.28	0.003	0.006	95	4						
	Prediction	Train	0.48	0.17	0.005	0.008		2	Test	0.12	0.007	0.10	0.07	1.28
CAID Ca	All		0.58	0.11	0.008	0.017		4						
	After outliers		0.38	0.20	0.012	0.016	95	2						
	Prediction	Train	0.43	0.14	0.011	0.017		2	Test	0.37	0.011	0.14	0.11	1.32
CAID P	All		0.68	0.32	0.002	0.005		4						
	After outliers		0.68	0.30	0.002	0.005	95	4						
	Prediction	Train	0.62	0.36	0.003	0.004		3	Test	0.24	0.004	0.08	0.06	1.47
CAID DM	All		0.67	0.13	0.001	0.003		4						
	After outliers		0.91	0.19	0.000	0.003	95	9						
	Prediction	Train	0.44	0.07	0.002	0.003		2	Test	0.14	0.002	0.06	0.04	1.29

Ca, calcium; CAID, coefficient of apparent ileal digestibility; DM, dry matter; MSEc, mean square error calibration; MSEcv, mean square error cross-validation; MSEp, mean square error prediction; N, nitrogen; nc, number of components; P, phosphorus; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; RMSEp, root mean square error of prediction; RPD, relative performance to deviation; SDm, standard deviation of the measured values.

Table 6.4. Calibration and prediction statistics for the prediction of CAID of nutrients using the subtracted spectra of diet and ileal digesta.

Parameter			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
CAID N	All		0.78	0.38	0	0.001		6						
	After outliers		0.81	0.35	0	0.001	95	6						
	Prediction	Train	0.72	0.41	0	0.001		4	Test	0.29	0.001	0.04	0.02	1.49
CAID energy	All		0.77	0.36	0	0.001		6						
	After outliers		0.85	0.31	0	0.002	95	7						
	Prediction	Train	0.79	0.26	0	0.002		5	Test	0.56	0.001	0.05	0.03	1.91
CAID fat	All		0.56	0.36	0.001	0.001		3						
	After outliers		0.64	0.34	0.001	0.002	95	4						
	Prediction	Train	0.64	0.20	1.00	0.002		3	Test	0.26	0.002	0.05	0.03	1.41
CAID starch	All		0.57	0.34	0	0		3						
	After outliers		0.66	0.37	0	0	95	4						
	Prediction	Train	0.75	0.16	0	0		3	Test	0.47	0	0.01	0.01	1.64
CAID ash	All		0.72	0.36	0.003	0.006		4						
	After outliers		0.72	0.31	0.003	0.006	95	4						
	Prediction	Train	0.77	0.36	0.002	0.005		5	Test	0.58	0.005	0.10	0.05	1.86
CAID Ca	All		0.62	0.15	0.007	0.017		4						
	After outliers		0.71	0.15	0.006	0.017	94	5						
	Prediction	Train	0.70	0.20	0.005	0.014		4	Test	0.56	0.012	0.14	0.08	1.71

Continued…

Parameter			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
CAID P	All		0.71	0.36	0.002	0.004		4						
	After outliers		0.74	0.40	0.002	0.004	95	4						
	Prediction	Train	0.73	0.51	0.002	0.003		4	Test	0.50	0.004	0.08	0.05	1.74
CAID DM	All		0.76	0.41	0.001	0.002		5						
	After outliers		0.79	0.39	0.001	0.002	95	5						
	Prediction	Train	0.74	0.34	0.001	0.002		4	Test	0.59	0.001	0.06	0.03	1.82
Ti (% DM)	All		0.71	0.41	0.021	0.043		4						
	After outliers		0.78	0.44	0.016	0.040	95	5						
	Prediction	Train	0.74	0.49	0.020	0.040		4	Test	0.41	0.029	0.27	0.15	1.79

Ca, calcium; CAID, coefficient of apparent ileal digestibility; DM, dry matter; MSEc, mean square error calibration; MSEcv, mean square error cross-validation; MSEp, mean square error prediction; N, nitrogen; nc, number of components; P, phosphorus; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; RMSEp, root mean square error of prediction; RPD, relative performance to deviation; SDm, standard deviation of the measured values; Ti, titanium.

6.4.2. Calibrations and predictions for chemical composition, apparent total tract digestibility, and apparent metabolisable energy based on excreta spectra alone or combined spectra of diet and excreta spectra using ATR-FT MIRS

The calibrations to predict the gross chemical composition of excreta (Table 6.7) were more accurate than the calibrations developed to predict the gross chemical composition of ileal digesta. The Ca and P had the $R^2p > 0.70$ and RPD > 3.0, while N, GE, and ash had the $R^2p >$ 0.70 and RPD between 2.5-3.0. The DM and TiO_2 could not be predicted (RPD < 2.0), whereas the prediction of OM could be used for screening purposes (RPD = 2.39). Statistical parameters for the prediction of cATTD from the excreta spectra are reported in Table 6.8. The cATTD of energy was well predicted (RPD = 3.16 and $R^2p = 0.80$). The AME (total collection) had the R^2c, R^2cv, and R^2p of 0.93, 0.44, and 0.70, respectively, with RPD of prediction 2.65. However, prediction of AME calculated using the marker achieved R^2c, R^2cv, and R^2p of 0.56, 0.36, and 0.14, respectively, with RPD 1.36. The attempt to predict the cATTD of nutrients using the combined spectra (diet spectra-excreta spectra) was successful with $R^2p = 0.60$-0.80 and RPD = 2.2-3.2 for most parameters except cATTD of N, Ca, and P (Table 6.9).

6.4.3. Comparison of predictive performance of a single overall model for prediction of gross chemical composition across diet, ileal digesta, and excreta

The overall predictions reported good results in terms of R^2 for all parameters (Table 6.10). The measured values versus predicted values of all samples are shown in Figure 6.4. The P-value of the t-test suggested that there was no significant difference between the observed and predicted values for most parameters except the P content of excreta. However, except for the diet, R^2 and CCC for the prediction of gross chemical composition of ileal digesta and excreta were not satisfactory when a single model combining the diet, ileal digesta, and excreta spectra was used for the prediction.

Table 6.5. Calibration and prediction statistics for the prediction of IDC of nutrients using spectra of ileal digesta (% as-is basis).

Parameter			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
IDC N	All		0.88	0.65	0.01	0.03		6						
	After outliers		0.88	0.60	0.01	0.04	95	6						
	Prediction	Train	0.90	0.55	0.01	0.04		6	Test	0.57	0.04	0.31	0.13	2.45
IDC energy	All		0.91	0.11	0.06	0.58		9						
	After outliers		0.67	0.13	0.22	0.58	95	4						
	Prediction	Train	0.65	0.01	0.22	0.62		4	Test	0.39	0.48	0.81	0.54	1.52
IDC fat	All		0.65	0.36	0.06	1.17		4						
	After outliers		0.66	0.40	0.65	1.15	95	4						
	Prediction	Train	0.62	0.25	0.72	1.43		3	Test	0.36	1.12	1.38	0.91	1.52
IDC starch	All		0.94	0.62	1.24	8.21		9						
	After outliers		0.94	0.60	1.21	8.64	95	9						
	Prediction	Train	0.88	0.56	2.57	9.73		6	Test	0.59	8.05	4.65	1.99	2.34
IDC CF	All		0.09	−0.05	0.47	0.54		1						
	After outliers		0.09	−0.08	0.48	0.57	94	1						
	Prediction	Train	0.12	−0.07	0.43	0.52		1	Test	0.003	0.66	0.73	0.70	1.04
IDC Ca	All		0.09	−0.36	1.58	2.36		1						
	After outliers		0.08	−0.27	1.34	1.85	95	1						
	Prediction	Train	0.08	−0.15	1.49	1.86		1	Test	−0.14	1.03	1.21	1.18	1.03
IDC P	All		0.10	−0.18	0.34	0.44		1						
	After outliers		0.11	−0.07	0.34	0.41	95	1						
	Prediction	Train	0.14	−0.004	0.36	0.42		1	Test	−0.05	0.30	0.62	0.59	1.05
IDC DM	All		0.67	0.13	10.2	26.8		4						
	After outliers		0.91	0.19	2.27	25.1	95	9						
	Prediction	Train	0.70	0.10	7.80	23.8		4	Test	0.16	34.8	5.55	3.82	1.45

Ca, calcium; CF, crude fibre; DM, dry matter; IDC, ileal digestible content; MSEc, mean square error calibration; MSEcv, mean square error cross-validation; MSEp, mean square error prediction; N, nitrogen; nc, number of components; P, phosphorus; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; RMSEp, root mean square error of prediction; RPD, relative performance to deviation; SDm, standard deviation of the measured values.

Table 6.6. Calibration and prediction statistics for the prediction of IDC of nutrients using the subtracted spectra of diet and ileal digesta (% as-is basis).

Parameter			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
IDC N	All		0.97	0.82	0.003	0.02		9						
	After outliers		0.98	0.84	0.002	0.02	95	9						
	Prediction	Train	0.92	0.76	0.01	0.02		5	Test	0.80	0.03	0.32	0.11	2.92
IDC energy	All		0.78	0.41	0.14	0.38		6						
	After outliers		0.76	0.35	0.16	0.42	95	5						
	Prediction	Train	0.86	0.38	0.09	0.39		6	Test	0.49	0.38	0.81	0.41	2.00
IDC fat	All		0.98	0.91	0.04	0.17		9						
	After outliers		0.98	0.89	0.04	0.20	96	9						
	Prediction	Train	0.96	0.82	0.08	0.33		6	Test	0.85	0.31	1.36	0.37	3.68
IDC starch	All		0.99	0.91	0.27	2.01		9						
	After outliers		0.99	0.92	0.22	1.75	95	9						
	Prediction	Train	0.96	0.76	0.80	5.06		6	Test	0.82	3.43	4.54	1.22	3.73
IDC CF	All		0.76	0.26	0.13	0.38		6						
	After outliers		0.75	0.20	0.13	0.42	95	6						
	Prediction	Train	0.79	0.06	0.11	0.52		6	Test	0.21	0.34	0.72	0.41	1.78
IDC Ca	All		0.08	−0.41	1.60	2.44		1						
	After outliers		0.08	−0.42	1.69	2.60	95	1						
	Prediction	Train	0.11	−0.25	1.48	2.00		1	Test	−0.16	2.36	1.35	1.31	1.03
IDC P	All		0.69	0.09	0.12	0.34		6						
	After outliers		0.70	0.11	0.11	0.34	95	6						
	Prediction	Train	0.41	0.11	0.20	0.29		2	Test	0.07	0.49	0.62	0.52	1.19
IDC DM	All		0.76	0.41	7.38	18.3		5						
	After outliers		0.79	0.39	6.51	18.7	95	5						
	Prediction	Train	0.82	0.58	4.18	9.80		5	Test	0.42	25.6	5.54	3.08	1.80

Ca, calcium; CF, crude fibre; DM, dry matter; IDC, ileal digestible content; MSEc, mean square error calibration; MSEcv, mean square error cross-validation; MSEp, mean square error prediction; N, nitrogen; nc, number of components; P, phosphorus; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; RMSEp, root mean square error of prediction; RPD, relative performance to deviation; SDm, standard deviation of the measured values.

Table 6.7. Calibration and prediction statistics of the equations to predict the chemical composition of excreta (as-is basis) and Ti (DM basis) using spectra of excreta.

Analyte			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
N%	All		0.88	0.79	0.03	0.06		3						
	After outliers		0.98	0.78	0.01	0.06	95	8						
	Prediction	Train	0.87	0.69	0.03	0.08		3	Test	0.75	0.06	0.50	0.20	2.50
GE MJ/kg	All		0.89	0.75	0.04	0.09		4						
	After outliers		0.87	0.71	0.04	0.09	94	4						
	Prediction	Train	0.93	0.66	0.02	0.09		5	Test	0.78	0.09	0.55	0.19	2.86
Ash%	All		0.89	0.72	0.31	0.83		5						
	After outliers		0.89	0.68	0.29	0.88	95	5						
	Prediction	Train	0.89	0.56	0.26	1.08		5	Test	0.82	0.64	1.65	0.60	2.77
DM%	All		0.16	−0.32	0.18	0.28		1						
	After outliers		0.18	−0.35	0.18	0.30	95	1						
	Prediction	Train	0.13	−0.16	0.16	0.22		1	Test	−0.10	0.35	0.47	0.46	1.02
Ca mg/g	All		0.92	0.80	5.09	12.4		5						
	After outliers		0.92	0.79	4.95	12.8	95	5						
	Prediction	Train	0.96	0.82	2.77	12.0		6	Test	0.72	13.8	7.87	2.37	3.32
P mg/g	All		0.95	0.78	0.45	2.14		7						
	After outliers		0.93	0.74	0.63	2.35	95	6						
	Prediction	Train	0.94	0.71	0.53	2.74		6	Test	0.73	2.03	3.03	0.96	3.16
OM%	All		0.89	0.72	0.31	0.83		5						
	After outliers		0.89	0.68	0.29	0.88	95	5						
	Prediction	Train	0.87	0.71	0.37	0.82		4	Test	0.65	0.79	1.65	0.69	2.39
Ti%	All		0.58	0.35	0.03	0.05		3						
	After outliers		0.54	0.34	0.03	0.05	95	2						
	Prediction	Train	0.57	0.30	0.03	0.06		2	Test	0.24	0.04	0.27	0.19	1.41

Ca, calcium; DM, dry matter; GE, gross energy; MSEc, mean square error calibration; MSEcv, mean square error cross-validation; MSEp, mean square error prediction; N, nitrogen; nc, number of components; OM, organic matter; P, phosphorus; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; RMSEp, root mean square error of prediction; RPD, relative performance to deviation; SDm, standard deviation of the measured values; Ti, titanium.

Table 6.8. Calibration and prediction statistics for the prediction of cATTD of nutrients and AME (MJ/kg DM) using spectra of excreta.

Parameter			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
cATTD N	All		0.88	0.60	0	0.001		5						
	After outliers		0.95	0.57	0	0.001	95	8						
	Prediction	Train	0.95	0.61	0	0.001		6	Test	0.62	0.001	0.04	0.02	2.86
cATTD OM	All		0.95	0.66	0	0.001		8						
	After outliers		0.94	0.61	0	0.001	95	7						
	Prediction	Train	0.90	0.62	0	0.001		5	Test	0.54	0.001	0.04	0.02	2.37
cATTD Ca	All		0.87	0.50	0.001	0.004		6						
	After outliers		0.92	0.54	0.001	0.003	95	7						
	Prediction	Train	0.79	0.42	0.002	0.005		4	Test	0.43	0.003	0.09	0.04	1.96
cATTD P	All		0.54	0.36	0.001	0.002		5						
	After outliers		0.66	0.33	0.001	0.002	95	3						
	Prediction	Train	0.57	0.26	0.001	0.003		2	Test	0.18	0.003	0.06	0.04	1.38
cATTD energy	All		0.80	0.56	0	0.001		9						
	After outliers		0.92	0.65	0	0.000	95	6						
	Prediction	Train	0.93	0.54	0	0.001		6	Test	0.80	0	0.04	0.01	3.16
cATTD DM	All		0.95	0.68	0	0.001		8						
	After outliers		0.95	0.61	0	0.001	95	8						
	Prediction	Train	0.93	0.70	0	0.001		6	Test	0.62	0	0.04	0.01	2.88
AME (total collection)	All		0.95	0.65	0.03	0.19		8						
	After outliers		0.94	0.59	0.03	0.22	95	7						
	Prediction	Train	0.93	0.44	0.03	0.27		6	Test	0.70	0.20	0.73	0.28	2.65
AME(marker)	All		0.57	0.30	0.40	0.65		3						
	After outliers		0.51	0.30	0.46	0.66	95	2						
	Prediction	Train	0.56	0.36	0.39	0.55		4	Test	0.14	0.89	0.97	0.72	1.36

AME, apparent metabolisable energy; Ca, calcium; cATTD, coefficient of apparent total tract digestibility; DM, dry matter; MSEc, mean square error calibration; MSEcv, mean square error cross-validation; MSEp, mean square error prediction; N, nitrogen; nc, number of components; OM, organic matter; P, phosphorus; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; RMSEp, root mean square error of prediction; RPD, relative performance to deviation; SDm, standard deviation of the measured values.

Table 6.9. Calibration and prediction statistics for the prediction of cATTD of nutrients and AME (MJ/kg DM) using the subtracted spectra of diet and excreta.

Parameter			R^2c	R^2cv	MSEc	MSEcv	No of spectra after filtering	nc		R^2p	MSEp	SDm	RMSEp	RPD
cATTD N	All		0.84	0.63	0	0.001		5						
	After outliers		0.78	0.62	0	0.001	95	3						
	Prediction	Train	0.90	0.70	0	0.000		5	Test	0.50	0.001	0.04	0.02	2.19
cATTD OM	All		0.96	0.78	0	0		8						
	After outliers		0.95	0.78	0	0	95	8						
	Prediction	Train	0.89	0.66	0	0.001		5	Test	0.81	0	0.04	0.01	2.82
cATTD Ca	All		0.72	0.49	0.002	0.004		4						
	After outliers		0.74	0.43	0.002	0.01	95	4						
	Prediction	Train	0.88	0.40	0.001	0.01		6	Test	0.60	0.004	0.09	0.04	2.20
cATTD P	All		0.93	0.42	0	0.002		9						
	After outliers		0.82	0.41	0.001	0.002	95	6						
	Prediction	Train	0.86	0.27	0	0.002		6	Test	0.31	0.002	0.06	0.03	1.9
cATTD energy	All		0.92	0.74	0	0		6						
	After outliers		0.94	0.75	0	0	95	7						
	Prediction	Train	0.92	0.66	0	0		6	Test	0.84	0	0.04	0.01	3.22
cATTD DM	All		0.96	0.79	0	0		8						
	After outliers		0.96	0.78	0	0	95	8						
	Prediction	Train	0.97	0.82	0	0		6	Test	0.61	0.001	0.04	0.01	3.03
AME (total collection)	All		0.97	0.78	0.02	0.12		9						
	After outliers		0.96	0.79	0.02	0.11	95	8						
	Prediction	Train	0.89	0.66	0.06	0.18		5	Test	0.82	0.10	0.73	0.26	2.86
AME (marker)	All		0.71	0.55	0.27	0.42	95	4						
	After outliers		0.73	0.55	0.26	0.42		4						
	Prediction	Train	0.76	0.48	0.22	0.49		4	Test	0.61	0.35	0.97	0.51	1.90

AME, apparent metabolisable energy; Ca, calcium; cATTD, coefficient of apparent total tract digestibility; DM, dry matter; MSEc, mean square error calibration; MSEcv, mean square error cross-validation; MSEp, mean square error prediction; N, nitrogen; nc, number of components; OM, organic matter; P, phosphorus; R^2c, coefficient of determination of calibration; R^2cv, coefficient of determination of cross-validation; R^2p, coefficient of determination of prediction; RMSEp, root mean square error of prediction; RPD, relative performance to deviation; SDm, standard deviation of the measured values.

Table 6.10. Goodness of fit statistics of predicted value of gross chemical composition (% as-is basis) by ATR-FT MIRS using single equation after removal of outliers vs measured value.

Analyte	Samples	n	Mean Measured	Mean Predicted	Bias	Paired T test P-value	R^2	CCC	RMSE	RPE %
N%	All	262	3.31	3.28	−0.03	0.26	0.86	0.92	0.41	12.3
	Feed	62	3.68	3.63	−0.05	0.45	0.83	0.91	0.50	13.5
	Excreta	100	4.09	4.05	−0.04	0.35	0.44	0.63	0.38	9.20
	Ileal digesta	100	2.30	2.29	−0.01	0.81	0.002	0.03	0.37	15.9
GE MJ/kg	All	262	16.0	16.0	−0.01	0.74	0.69	0.82	0.47	2.95
	Feed	62	17.1	17.0	−0.06	0.22	0.72	0.84	0.36	2.10
	Excreta	100	15.9	15.9	−0.04	0.48	0.26	0.40	0.54	3.41
	Ileal digesta	100	15.5	15.5	0.05	0.30	0.42	0.48	0.46	2.98
Ca mg/g	All	262	19.9	19.8	−0.09	0.82	0.69	0.81	6.12	30.7
	Feed	62	10.0	10.1	0.06	0.92	0.87	0.93	4.84	48.7
	Excreta	100	22.5	22.1	−0.42	0.50	0.49	0.50	6.13	27.2
	Ileal digesta	100	23.4	23.6	0.16	0.82	0.43	0.48	6.75	28.8
P mg/g	All	262	12.7	12.6	−0.11	0.45	0.77	0.87	2.37	18.7
	Feed	62	9.51	9.69	0.18	0.44	0.94	0.97	1.83	19.2
	Excreta	100	14.9	14.3	−0.54	0.04	0.41	0.40	2.64	17.8
	Ileal digesta	100	12.5	12.7	0.13	0.59	0.39	0.48	2.40	19.1
DM%	All	262	93.0	93.0	0.02	0.74	0.95	0.98	0.74	0.81
	Feed	62	87.7	87.8	0.09	0.30	0.84	0.91	0.71	0.81
	Excreta	100	93.4	93.4	0.04	0.62	0.001	0.02	0.78	0.84
	Ileal digesta	100	95.9	95.9	−0.06	0.41	0.006	−0.08	0.70	0.73
Ash%	All	262	11.8	11.8	0.03	0.79	0.86	0.93	1.51	12.9
	Feed	62	6.96	7.03	0.08	0.66	0.92	0.96	1.35	19.4
	Excreta	100	14.4	14.4	0.01	0.95	0.42	0.50	1.36	9.44
	Ileal digesta	100	12.2	12.2	0.01	0.96	0.40	0.49	1.74	14.3

Ca, calcium; CCC, concordance correlation coefficient; DM, dry matter; GE, gross energy; N, nitrogen; P, phosphorus; R^2, coefficient of determination; RMSE, root mean square error; RPE, relative prediction error.

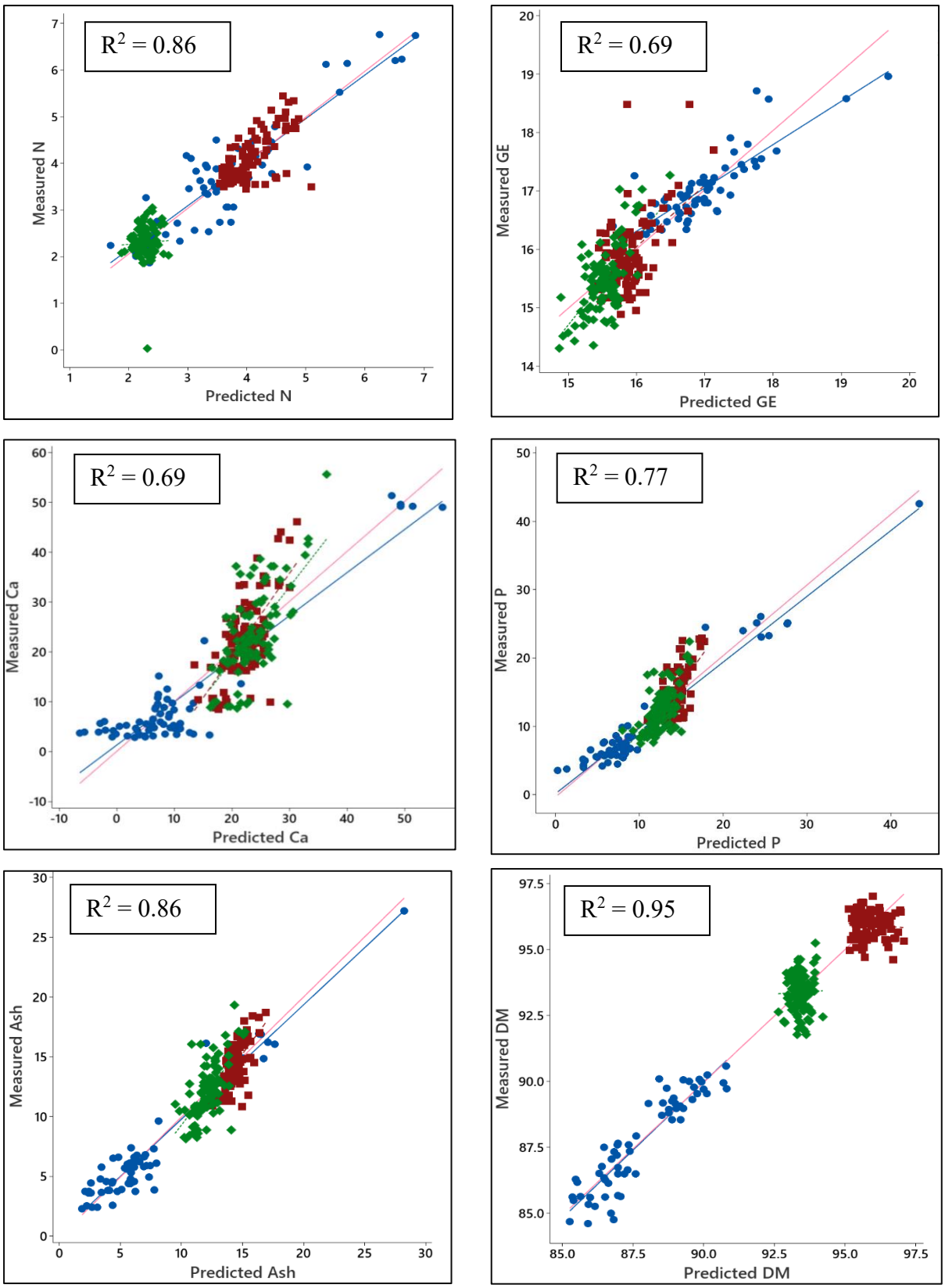


Figure 6.4. Measured vs Predicted values for N, GE, Ca, P, ash, and DM content in all samples using a single model (● diet, ■ ileal digesta, and ■ excreta).

6.5. Discussion

The results presented in this Chapter show that the ATR-FT MIRS can predict the chemical composition of ileal digesta and excreta, as well as that there is potential to predict the digestibility using combined spectra of diet and ileal digesta or excreta. Spectral data from scanning faeces (excreta in our case) often show slope or oscillatory baseline problems due to surface light scattering and therefore proper pre-processing of spectra with Savitzky-Golay derivative filtering was carried out to improve the accuracy of predictions (Kho et al., 2023). The 100 ileal digesta and excreta samples were representative of 20 diets, that had a large variation in their chemical composition. Using more data for calibration may produce more robust outcomes. Therefore, calibration results must be interpreted cautiously because of the relatively small dataset. The comparison with other studies is difficult as there are no existing references for the prediction of the chemical composition of excreta and ileal digesta and digestibility using ATR-FT MIRS. Therefore, in most instances, NIRS studies were cited.

6.5.1. Chemical composition of ileal digesta and excreta

According to the threshold values of RPD, the ATR-FT MIRS calibrations for the chemical composition of ileal digesta could be considered good for GE, fat, starch, and P (RPD between 2.5 to 3.0) and excellent for ADF (RPD = 3.08). The results obtained for N, DM, and Ti were poor (RPD < 2.0).

Ramos Cruz et al. (2023) obtained R^2c and RPD of 0.98 and 2.11, respectively, for crude protein (CP) content of ileal digesta using NIRS in pigs, whereas the value obtained in the current study for N was low with R^2p of 0.66 and RPD of 1.75. Comparatively, the prediction of N was better in excreta by ATR-FT MIRS than ileal digesta with the R^2c, R^2cv (train), and R^2p (test) of 0.87,0.69, and 0.75, respectively, and RPD of 2.50. Besides, Ramos Cruz et al. (2023) obtained R^2c of 0.98 and RPD of 4.16 for CP in excreta by NIRS while Bastianelli et al. (2010)

obtained R^2c of 0.95 and RPDcv of 4.2 with a larger number of poultry excreta samples using NIRS.

The prediction results for GE in ileal digesta and excreta (R^2p of 0.84 and 0.78 and RPD of 2.53 and 2.86, respectively) were better and could be used for quantitative predictions than those obtained for diets (R^2p = 0.70 and RPD = 1.90: Chapter 5). Further, Cruz-Conesa et al. (2022) developed models for CP and GE in excreta of broilers using NIRS with R^2cv of 0.95 and 0.90, respectively, and RPD of 4.8 and 3.1, respectively, which is high compared to the present study. This may be due to the lower number of samples used in the present study (100) compared to the number of samples used in the study by Cruz-Conesa et al. (2022) (306). Camp Montoro et al. (2023) conducted a similar study with pigs faeces using NIRS, the R^2cv obtained for CP and GE on DM basis were 0.95 and 0.75, respectively, with RPD of 4.31 and 2.18, respectively. Their R^2cv and RPD of GE prediction were similar to our study however, CP was better predicted in their study. Ramos Cruz et al. (2023) also predicted the GE content of faeces in pigs with R^2c of 0.94 and RPD of 2.52 using NIRS.

The R^2p and RPD for DM in ileal digesta and excreta were very poor thus the model can't be used for predictions. This may be due to the very low variation in the DM content in the ileal digesta and excreta (CoV = 1% for both) on account of freeze-drying of samples. Moreover, the samples were analysed after storage for more than 8 months of wet chemical analysis thus there may be a chance for rehydration with room moisture even though the samples were stored properly. Further, Jancewicz et al. (2016) also stated that there are possibilities for changes in chemical composition of samples during storage. Similar results were obtained by Garnsworthy et al. (2000) and Ramos Cruz et al. (2023) who proved that the properly stored samples became rehydrated with the ambient moisture and the samples may be easily excluded in the calibration range if the CoV of wet chemical analysis also narrow.

In the fibre fractions of ileal digesta, ADF was predicted accurately with R^2p, and RPD of 0.81 and 3.08, respectively, compared to NDF, lignin, and CF. This was on par with the study by Simoni et al. (2021) in beef cattle where NIRS predicted ADF accurately in the faeces. Also, in the present study, the CoV of ADF was low (18%) compared to lignin, which had the highest CoV among fibre fractions (41%).

The results for the prediction of starch in the ileal digesta could be used for quantification with the R^2p of 0.75 and RPD of 2.92. The attempts to predict the Ti were not successful in diet, excreta, and ileal digesta, as Ti may be spectrally inactive. Indeed, TiO_2 is added as a marker for digestion studies which is insoluble in water, hydrochloric acid, nitric acid, or dilute sulphuric acid and can't mix up with diet components (Sales and Janssens, 2003). Moreover, the estimation of minerals by infrared spectroscopy is dependent on the occurrence of those elements in organic or hydrated molecules or the correlation of minerals with organic molecules (Smith et al., 2001), thus explaining the poor prediction for Ti.

The Ca and P components in the excreta were predicted accurately compared to the Ca and P contents in ileal digesta. The current result for P was similar to those by Cruz-Conesa et al. (2022) who predicted the P content of excreta with R^2cv of 0.93 and RPDcv of 3.8 using the NIRS (n = 178). This may be because of the association of P with organic components.

The predictions obtained for ash in the ileal digesta (R^2p = 0.82 and RPD = 2.37) and excreta (R^2p = 0.82 and RPD = 2.77) could be used for screening and quantification, respectively. In addition to that, the predictions obtained for the OM in the ileal digesta, and excreta were similar with R^2p of 0.71 and 0.65 and RPD of 2.25 and 2.39, respectively. The OM was obtained by the calculation (100-ash), thus accumulating the errors of the separate analysis and these parameters don't represent a specific chemical compound (Paternostre et al., 2021). Moreover,

it is difficult to assign the spectral bands to the components of complicated samples such as faecal samples.

The optimal number of components (nc) of the PLSR models in the present study ranged from 1 (DM) to 6 (P) for the prediction of the chemical composition in excreta, which was low compared to the studies using NIRS (nc > 6) (Cruz-Conesa et al., 2022; Paternostre et al., 2021). No information was given regarding the number of components or factors used in most of the studies. Also, the maximum number of components fixed in the present study with 100 samples was 10. According to Paternostre et al. (2021), it is recommended to fix the maximum number to one-tenth of the total number of samples on the dataset as it may otherwise cause overfitted calibration.

6.5.2. Coefficient of apparent ileal digestibility, ileal digestible content, and coefficient of apparent total tract digestibility of nutrients using spectra of ileal digesta or excreta alone

Digestibility of the nutrients and the digestible energy are the major parameters that reflect the quality of the feeds. Usually, these values are obtained by *in vivo* assays. To our knowledge, there is no published literature for ATR-FT MIRS prediction of digestibility of nutrients in broiler diets. Only a few data are available for the estimation of the nutrient digestibility in complete feeds for poultry and they were obtained by NIRS (Bastianelli, 2013).

In the present study, calibrations derived to estimate the CAID using ileal digesta spectra were considered poor for all parameters. Even though the subtracted spectra (diet-ileal digesta) enhanced the prediction, satisfactory results were not obtained ($R^2p < 0.70$ and RPD < 2). Besides, lesser samples with lower variability may be the limitation in the present study, thus adding more samples from the various studies could enhance the prediction of CAID of nutrients. In contrast, the prediction of IDC of N, fat, and starch using combined spectra resulted in better predictions compared to the predictions by the ileal spectra alone.

Interestingly, the cATTD of nutrients were well predicted compared to CAID of nutrients by the ATR-FT MIRS. The cATTD of energy was well predicted in this work from the excreta spectra (R^2p = 0.80 and RPD = 3.16). In contrast to the present results, (Lyons et al., 2016) reported poor calibrations for gross energy digestibility with R^2cv = 0.66 and RPD = 1.67 using the NIR spectra of sheep faeces. Paternostre et al. (2021) reported R^2 of 0.79 and RPD of 2.0 for cATTD of CP with latent variables of 11 (n = 310). In contrast, in the present study R^2c, R^2cv and R^2p for cATTD of N was 0.95, 0.61 and 0.62, respectively, with RPD of 2.86 and the number of components used was only 6. In contrast to our study, Camp Montoro et al. (2023) obtained lower RPD and R^2 for cATTD of CP and GE (RPD between 1.62 and 1.70 and R^2 between 0.58 and 0.59, respectively) in pig faeces using NIRS. On the other hand, NIRS could not predict the total tract digestibility of nutrients in the faeces of beef cattle in a study by Simoni et al. (2021).

A few studies have developed NIRS calibration equations to estimate AME in complete poultry diets (Valdes and Leeson, 1992; Valdes and Leeson, 1994; Bastianelli et al., 2013). In a study with 891 excreta samples of poultry, Bastianelli et al. (2013) mentioned a NIRS calibration for AME with an R^2 of 0.80 (nc = 13). However, in the present study with 100 samples, prediction of AME resulted in an RPD of 2.65 and R^2p of 0.70 (nc = 6) which can be used for quantification. In contrast, Paternostre et al. (2021) obtained R^2 of 0.59 and RPD of 1.5 for prediction of net energy (NE) using NIRS in pig faeces. The prediction of AME based on total collection was more accurate than the use of marker method in the present study. As already explained, it was difficult to predict Ti which is inert with no specific absorption in the MIR region. This may have influenced the prediction of AME by TiO_2 method. In addition to that, the accuracy and repeatability of marker method can vary with the quantity and representability of the excreta sample as well as the accuracy of marker analysis in the diet and excreta (Noblet et al., 2022). Overall, the total collection method is considered more reliable than the marker

method for AME determination (Masood et al., 2011). The combination of spectra resulted in better prediction of AME, and this will be discussed in the next section. The cATTD of Ca and P could not be predicted by ATR-FT MIRS successfully even though the Ca and P in the diet and excreta were predicted with good accuracy.

The ATR-FT MIRS successfully predicted cATTD of many nutrients and AME with a lower number of components compared to the studies using NIRS (Paternostre et al., 2021; Simoni et al., 2021). Therefore, ATR-FT MIRS could be used as a potential technique for the prediction of total tract digestibility and AME.

6.5.3. Coefficient of apparent ileal digestibility, ileal digestible content, and coefficient of apparent total tract digestibility of nutrients using combined spectra of diet and ileal digesta or diet and excreta.

It is difficult to model the source of variation associated with the process of digestion when developing calibration equations using the diet spectra only. Hence, an improvement in the predictive ability of models that combine diets and excreta or ileal digesta spectra in comparison with the ileal or excreta spectra alone is observed. This can be explained by the fact that both the composition of the diet, as well as the digestion process are considered, when both spectra are combined.

We have used a subtraction technique for the prediction of CAID, IDC, cATTD of nutrients, and AME. Prediction of CAID of nutrients using the subtracted spectra of ileal digesta from the diet resulted in poor prediction of CAID of nutrients with R^2c 0.56-0.85, R^2cv 0.15-0.51 and R^2p 0.29-0.59 and RPD of 1.41-1.91, even though the accuracy and performance was increased compared to the predictions by ileal digesta spectra alone. However, the prediction of IDC of nutrients using the subtracted spectra (diet-ileal digesta) was better than using the ileal digesta spectra alone. The predictions achieved for IDC of starch and fat were accurate

with $R^2p > 0.80$ and RPD > 3.0 and the predictions for IDC of N could be used for screening purposes since the accuracy ($R^2p = 0.46$) was low but with good performance (RPD = 2.77).

The cATTD of nutrients were better predicted using the combined spectra of diet and excreta than the spectra of excreta alone. The prediction for cATTD of energy was most accurate ($R^2p = 0.84$ and RPD = 3.22) and cATTD of DM was good ($R^2p = 0.61$ and RPD = 3.21); those for OM could be used for quantification, whereas the cATTD of N and Ca could be used for screening purposes; and the calibration equation was poor for P. Besides, the calibration and prediction results derived for AME (total collection) were good with the R^2c, R^2cv, and R^2p of 0.89, 0.66, and 0.82, respectively, and RPD of 2.85.

A similar result was obtained by Paternostre et al. (2021) for the prediction of cATTD and NE in pigs using the subtracted NIRS spectra. However, they found that predicting digestibility using the spectra pre-treated separately and then subtracted improved the predictions. The present study used the subtracted spectra, which were then pre-treated together, which may have resulted in loss of information caused by external disruptions. Therefore, it will be more informative to do pre-treatment separately followed by subtraction to enhance the predictions.

The reliable results of prediction of digestibility using excreta rely on the principle that there is sufficient spectral information in the excreta regarding the composition of the diet ingested (Jancewicz et al., 2016). Thus, the main benefit of infrared technique is that it can predict the chemical characteristics of faeces and digestibility using faecal samples with minimal sample size if appropriate calibration equations are available. Moreover, this reduces the time spent in total collection of excreta and the time and cost of wet chemical analysis (Schiborra et al., 2015). Using a greater number of samples for calibration and prediction could enhance the accuracy thus, ATR-FT MIRS can be used as a potential instrument in accessing the AME and total tract digestibility of broiler diets.

Overall, the combination of spectra resulted in better results in predicting CAID, IDC, cATTD of nutrients, and AME than using the ileal digesta or excreta spectra alone. Interestingly, a concatenation that is the merging of diet and faecal spectra could also be utilised to get a more accurate prediction of digestibility using NIRS (Meineri et al., 2009; Bastianelli et al., 2013; Coulibaly et al., 2013; Paternostre et al., 2021). Bastianelli et al. (2013) obtained R^2 of 0.95 and 0.98 for AME and digestibility of starch, respectively, using concatenated spectrum of poultry feed and faeces spectra. This was more accurate with lower SE of cross-validation compared to the predictions using the feed spectra and faeces spectra alone. Paternostre et al. (2021) also observed that concatenated spectra improved the predictions of digestibility in pigs.

Therefore, it is recommended to focus on combined spectra for the prediction of digestibility rather than considering the single spectra as digestion is the combined interaction of feed and animal. Large-scale experimental procedures may be avoided once these calibrations are established. Utilising a larger set of data will eliminate the need for indigestible markers and precise measurements of feed intake and faecal output (Bastianelli et al., 2005).

6.5.4. Overall model across diet, ileal digesta, and excreta for the prediction of gross chemical composition

The results for predicting the chemical composition of diet, ileal digesta, and excreta using a common equation were not satisfactory. The main reason may be because of the inadequate number of samples and could also be due to the difference in physical and chemical composition between ileal digesta and excreta. The t-test for the bias showed that except for P in excreta, none of the calibrations had a significant bias. Nevertheless, the R^2, CCC, and RPE were not satisfactory except for all samples (262) and diet samples (62) for all the parameters. Therefore, a common equation cannot be used across all the samples using the current results. Cruz-Conesa et al. (2022) found that even though layers, broilers, and turkeys are different

species, their excretal spectra could be modelled together, and these models could be more widely applicable than those models that are specific to each species. However, in the present study, different samples within a species were utilised. Further analysis could be done with a greater number of samples to see whether it could improve the accuracy over single models.

6.6. Implications

Digestion is the result of the interaction between the feed and the animal and depends on the factors connected to these two. In addition to the chemical composition of the feed, digestibility is linked to other factors such as the presence of anti-nutritional factors, physical form, enzymes, treatments etc. (Bastianelli, 2013). When considering digestibility calibrations, a classification can be prepared in terms of high, medium, and low levels of digestibility, and this could serve in real-world settings to identify digestive issues early or enhance performances (Camp Montoro et al., 2023). As the estimation of the chemical composition of excreta or ileal digesta is more accurate than the estimation of digestibility, further research can be planned based on developing prediction equations for digestibility based on the chemical composition of excreta or ileal digesta predicted by ATR-FT MIRS (Camp Montoro et al., 2023).

The results of the current analysis gave promising results on the estimation of chemical composition and nutrient digestibility in broilers using ATR-FT MIRS, thus further investigation could be performed with a greater number of samples from a wide range of feed types, ileal, and faecal samples. The available reports based on NIRS suggested that using larger and more diverse datasets produced better calibration statistics and good prediction models than those with the small dataset (Lyons et al., 2016). In addition to that, an external validation with a separate set of samples is needed to confirm the application of ATR-FT MIRS

in all practical circumstances. Moreover, calibrations should be updated regularly with new information to make better models applicable in research and practice.

6.7. Conclusions

This study successfully demonstrated that ATR-FT MIRS is a cost-effective method to predict the chemical composition of ileal digesta and excreta as well as CAID, IDC, and cATTD of nutrients and AME with good results. It also showed the possibility of combining the results of diet and ileal digesta or excreta to better predict the digestibility of nutrients. This will be very useful avoiding the huge time and cost spent in the wet chemistry methods. For the results to be commercially applied, however, a larger dataset may be required to obtain a more robust calibration and to validate the results.

6.8. Annexure

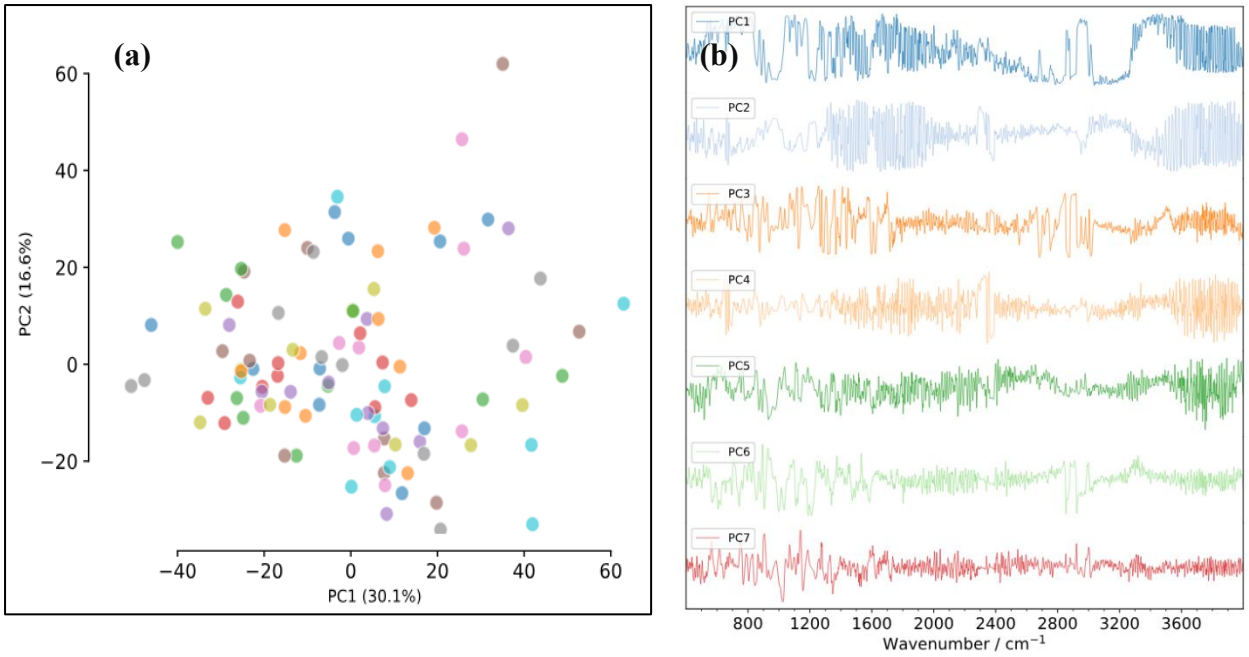


Figure 6.1a: (a) ATR-FT MIRS score plot and (b) loadings plot for all ileal digesta samples.

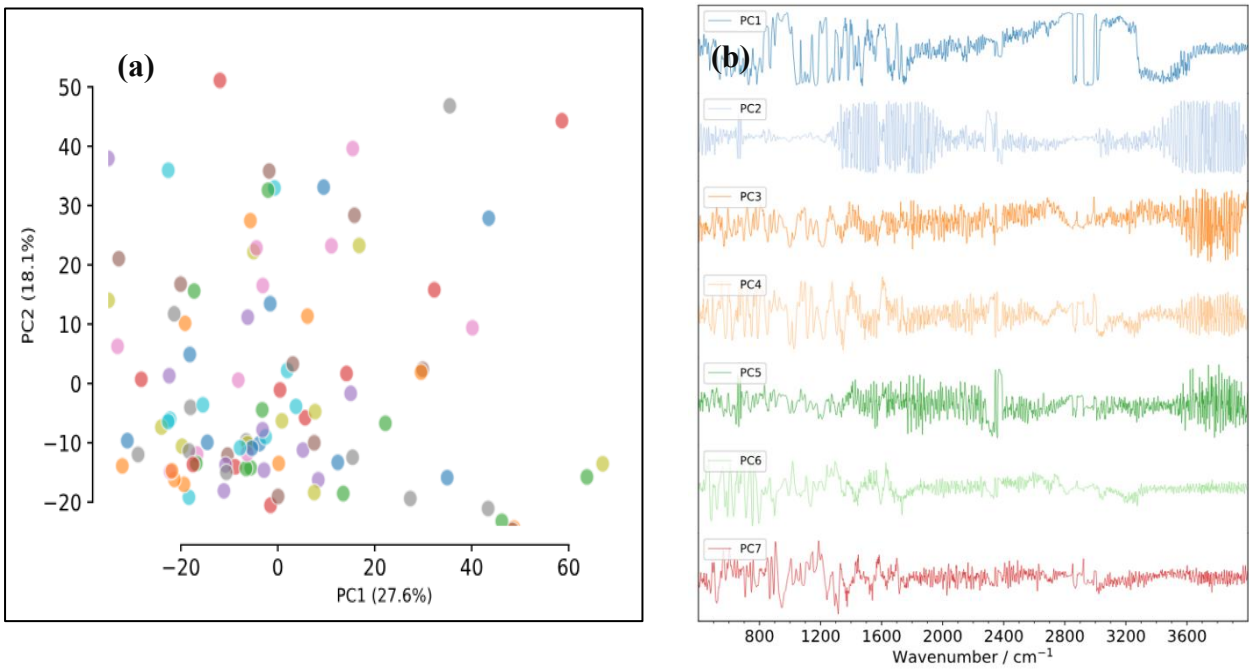


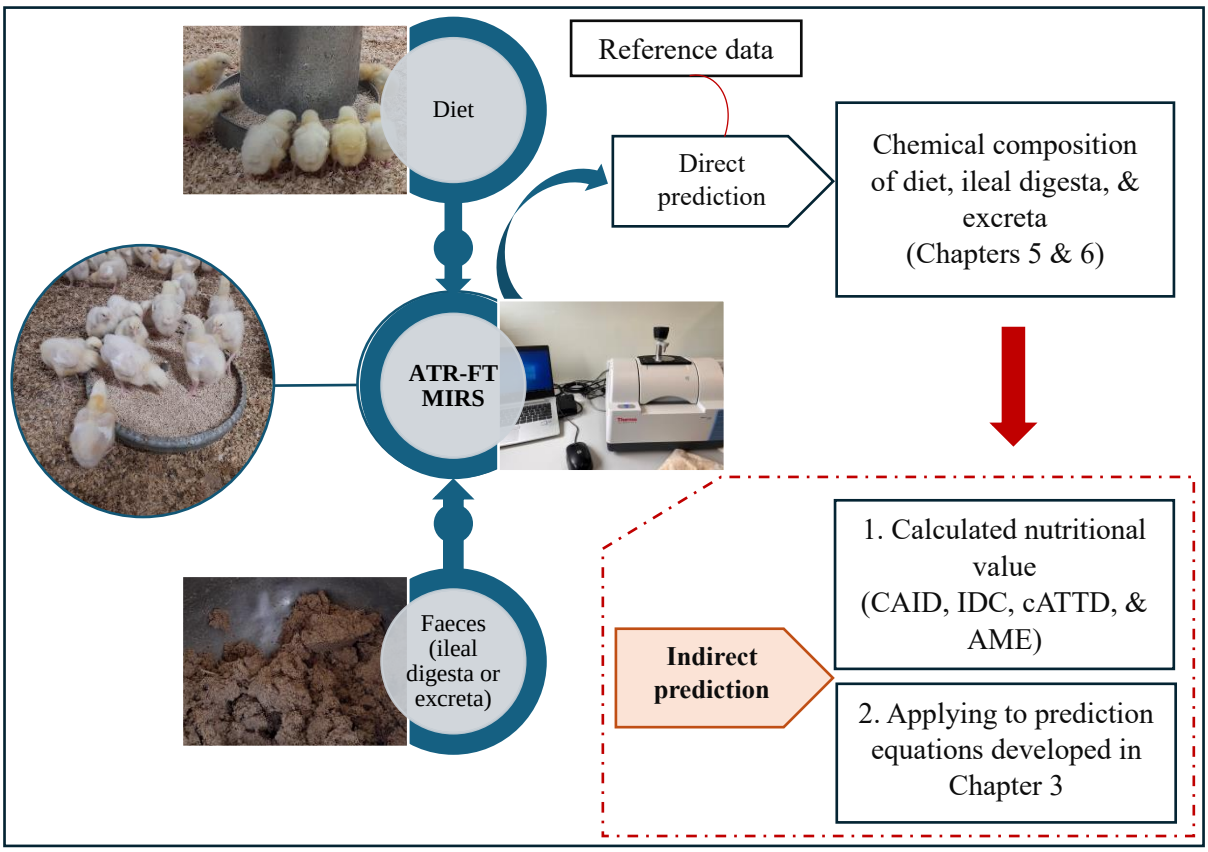
Figure 6.2a: (a) ATR-FT MIRS score plot and (b) loadings plot for all excreta samples.

CHAPTER 7

Estimation of the nutritive value of broiler diets based on the Attenuated Total Reflectance Fourier Transform Mid-Infrared Spectroscopy (ATR-FT MIRS) predicted chemical composition of diet, ileal digesta, and excreta

Chapters 5 and 6 demonstrated the potential application of ATR-FT MIRS in the prediction of the chemical composition of diet, ileal digesta, and excreta. Therefore, the final step is to use the predicted values for chemical composition by ATR-FT MIRS to either calculate the CAID, IDC, cATTD of nutrients, and AME or to use them in the regression equations derived in Chapter 3 and compare those predicted values with the actual calculated values using the wet chemistry data.

Graphical abstract



7.1. Abstract

Traditional wet chemistry methods for determining the gross chemical composition of diet, ileal digesta, and excreta are costly and require a lot of work and time. To overcome this, Attenuated Total Reflectance Fourier Transform (ATR-FT) Mid-Infrared Spectroscopy (MIRS) could be utilised as a successful technique to predict the chemical composition. Therefore, in this study, two different methods, involving ATR-FT MIRS predicted values of the gross chemical composition were explored to predict digestibility and apparent metabolisable energy (AME): 1) Calculation of coefficient apparent ileal digestibility (CAID) and ileal digestible content (IDC) of nitrogen (N), energy, calcium (Ca) and phosphorus (P), coefficient of apparent total tract digestibility (cATTD) of N and energy, and AME based on values of gross chemical composition of diet, ileal digesta, and excreta determined by ATR-FT MIRS and 2) Calculation of CAID and IDC of N and energy using ATR-FT MIRS predictions of diet gross composition in combination with previously developed regression equations (Chapter 3). Values obtained by both methods were then compared with the actual values calculated using the wet chemistry data. The accuracy of the prediction was evaluated based on the coefficient of determination (R^2), root mean square error (RMSE), concordance correlation coefficient (CCC), mean bias (MB), and relative prediction error (RPE). The first approach did perform well for the prediction of CAID, IDC, and cATTD of energy and AME with $R^2 > 0.90$, CCC > 0.90, and RPE < 10% with RMSE and MB closer to 0. Prediction of CAID of N and P was moderate (R^2 and CCC > 0.70) while predictions for CAID of Ca and IDC of Ca and P were unsuccessful (R^2 and CCC < 0.50, RPE > 20%). The second approach resulted in good prediction for IDC of N (R^2 and CCC > 0.80) while moderate accuracy was achieved for CAID and IDC of energy ($R^2 > 0.50$, CCC > 0.70, and RPE < 10%). The CAID of N did not yield good results (R^2 and CCC < 0.30). This study highlighted that ATR-FT MIRS is a cost-effective and efficient tool for accessing the chemical composition of diet, ileal digesta, and excreta of broilers and that it

can be used instead of costly wet chemical analysis to predict the nutritive value of broiler diets.

Key words: ATR-FT MIRS, apparent metabolisable energy, broilers, chemical composition, nutritive value

7.2. Introduction

Digestibility is an important factor affecting feed efficiency and is influenced by feed composition, genetics, and physiological phase of the animals as well as other environmental factors (Evangelista et al., 2021). The evaluation of nutrient digestibility is essential to establish the nutritive quality of compound feed and raw materials for animals. To obtain these values, *in vivo* assays are conducted where birds are fed experimental diets, and after a test period, the ileal digesta or excreta are collected and processed. The composition of the ileal digesta, excreta, and diet are then determined by wet chemistry (Ravindran et al., 2017). These analyses are currently done in the laboratory using costly methods requiring a considerable amount of work and time. Some analyses are criticized from the environmental point of view, for causing pollution by using solvents and generating chemical wastes (Bastianelli et al., 2010; Guerra et al., 2024). However, the use of ATR-FT MIRS is a promising alternative and efficient tool for cost-effective, rapid, and non-destructive way to predict the chemical composition preserving the wholeness of samples and allowing many samples to be analysed several times.

The potential of ATR-FT MIRS in the prediction of the gross chemical composition of the diet, ileal digesta, and excreta was demonstrated in the previous Chapters 5 and 6. However, the direct MIRS prediction of coefficient of apparent ileal digestibility (CAID) and ileal digestible content (IDC) were not accurate for most nutrients using diet or ileal digesta and combined spectra of diet and ileal digesta ($R^2p < 0.70$ and $RPD < 2.0$). Only the IDC of nitrogen (N), fat, and starch using combined spectra of diet and ileal digesta gave an adequate prediction. The

main reason may be the lower number of samples used in the prediction. However, the ATR-FT MIRS direct prediction of coefficient of apparent total tract digestibility (cATTD) of nutrients (N, organic matter (OM), calcium (Ca), energy, and dry matter (DM)) and apparent metabolisable energy (AME) was successful when combined spectra of diet and excreta were used. Nevertheless, it is possible to use another method of applying the ATR-FT MIRS predicted values of gross chemical composition to calculate the CAID and IDC or cATTD of nutrients and to compare them with the actual *in vivo* results obtained with the laboratory analysis to check the usability of ATR-FT MIRS to predict digestibility. Doing this will also result in a huge reduction in cost and time involved in the wet chemistry analysis, even though the *in vivo* experiment should still be conducted to collect the ileal and excreta samples. In addition to that, ATR-FT MIRS predicted values could be used in conjunction with prediction equations to estimate the digestibility of the nutrients.

Therefore, this study first focuses on calculating the digestibility values (CAID and IDC) with the gross chemical composition results predicted with the ATR-FT MIRS and compare them with the actual calculations performed using the wet chemistry data. This considered that the calibrations for chemical composition of diet, ileal digesta, and excreta were more accurate (Chapters 5 and 6) than the calibrations for the digestibility and digestible content of nutrients. The second approach was to use the ATR-FT MIRS predicted value of gross chemical composition of the diets in prediction equations formulated in Chapter 3 to estimate the CAID and IDC of N and energy and compare them with the values obtained in the *in vivo* study using wet chemistry.

7.3. Materials and methods

7.3.1. Reference values

Diet, excreta, and ileal digesta samples were analyzed in the laboratory using standard methods as explained in Chapter 4. Samples obtained after outlier removal in the spectral analysis were used for the calculations.

7.3.2. Calculation of CAID, IDC, cATTD of nutrients, and AME using ATR-FT MIRS predicted values of the gross chemical composition of diet, ileal digesta, and excreta

Table 7.1 illustrates the details of the analytes used for the calculations according to the prediction by ATR-FT MIRS (Chapters 5 and 6). It was impossible to predict the inorganic compound TiO_2 in all samples of diet, ileal digesta, and excreta as Ti does not absorb in the mid-infrared region and doesn't interact with the other organic compounds in the samples. Therefore, the wet chemistry values of Ti were used. In addition to Ti, the DM of ileal digesta and excreta also could not be well predicted using MIRS. Therefore, wet chemistry values were used. The predicted values of gross chemical composition by the ATR-FT MIRS were used in equations 7.1, 7.2, 7.3, and 7.4 together with the reference value of Ti for the calculations. The calculations were made on as-is basis.

Table 7.1. Details of the analyte values used for the calculations.

Analytes	Diet	Ileal digesta	Excreta
DM	MIRS predicted value	Wet chemistry value	Wet chemistry value
N	MIRS predicted value	MIRS predicted value	MIRS predicted value
GE	MIRS predicted value	MIRS predicted value	MIRS predicted value
Ca	MIRS predicted value	MIRS predicted value	----
P	MIRS predicted value	MIRS predicted value	----
Ti	Wet chemistry value	Wet chemistry value	Wet chemistry value

Ca, calcium; DM, dry matter; GE, gross energy; MIRS, mid-infrared spectroscopy; N, nitrogen; P, phosphorus; Ti, titanium.

7.3.3. Calculations

The AME value of the diets by total excreta collection method was calculated using the following formula:

$$\text{AME (MJ/kg diet)} = \frac{[(\text{Feed intake} \times \text{GE}_{\textit{diet by MIRS}}) - (\text{Excreta output} \times \text{GE}_{\textit{excreta by MIRS}})]}{\text{Feed intake}} \quad (7.1)$$

The AME by marker method was calculated using the following formula:

$$\text{AME (MJ/kg diet)} = \text{GE}_{\textit{diet by MIRS}} - [\text{GE}_{\textit{excreta by MIRS}} \times \frac{\text{Ti}_{\textit{diet wet chemistry}}}{\textit{Ti}_{\textit{excreta wet chemistry}}}] \quad (7.2)$$

The CAID was calculated using the following formula:

$$\text{CAID of nutrient} = \frac{(\text{Nutrient}/\text{Ti})_{\textit{diet}} - (\text{Nutrient}/\text{Ti})_{\textit{ileal}}}{(\text{Nutrient}/\text{Ti})_{\textit{diet}}} \quad (7.3)$$

where, $(Nutrient/Ti)_{diet}$ = ratio of diet component predicted by MIRS to Ti (wet chemistry) in the diet and $(Nutrient/Ti)_{ileal}$ = ratio of the diet component predicted by MIRS to Ti (wet chemistry) in the ileal digesta.

The IDC was calculated by the following formula:

$$\text{IDC of nutrient} = \text{Nutrient in the diet} \times \text{CAID of that nutrient (both predicted by MIRS)} \quad (7.4)$$

7.3.4. Calculation of CAID and IDC of N and energy using the ATR-FT MIRS predicted gross composition values of diets and developed regression equations (Chapter 3) and comparing them with the actual values calculated using the wet chemistry data

Further, the prediction equations developed in Chapter 3 were used to calculate the CAID and IDC of N and energy using the predicted gross chemical composition of diets by ATR-FT MIRS, and the results were compared with the actual digestibility values.

$$\text{CAID N} = -0.37 + 0.002\ \text{N} + 0.005\ \text{CF} + 0.03\ \text{GE} - 0.00003\ \text{CF}^2 + 0.02\ \text{starch:CF} \quad (7.5)$$

$$\text{CAID E} = 0.14 + 0.002\ \text{CF} + 0.021\ \text{GE} - 0.000021\ \text{CF}^2 + 0.013\ \text{starch:CF} \quad (7.6)$$

$$\text{IDC N} = -10.17 - 0.04\ \text{ash} + 0.8\ \text{N} + 0.03\ \text{fat} + 0.12\ \text{CF} - 0.0008\ \text{CF}^2 + 0.4\ \text{starch:CF} \quad (7.7)$$

$$\text{IDC E} = -9.87 + 0.03\ \text{CF} + 1.06\ \text{GE} - 0.0004\ \text{CF}^2 + 0.24\ \text{starch:CF} \quad (7.8)$$

7.3.5. Measures of goodness of fit

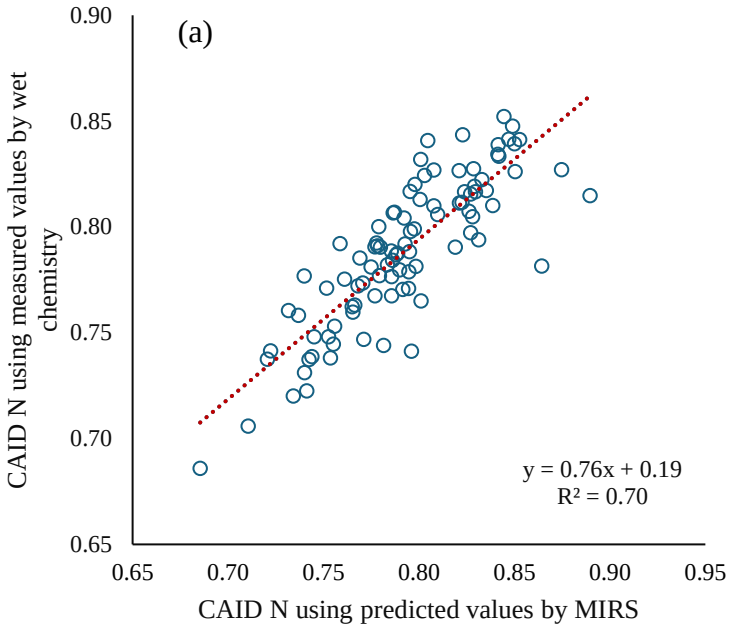
The accuracy of the prediction was evaluated based on the coefficient of determination (R^2), root mean square error of prediction (RMSE), concordance correlation coefficient (CCC), mean bias (MB), and relative prediction error (RPE) as described in Chapters 4 and 6. All the values were calculated using Excel.

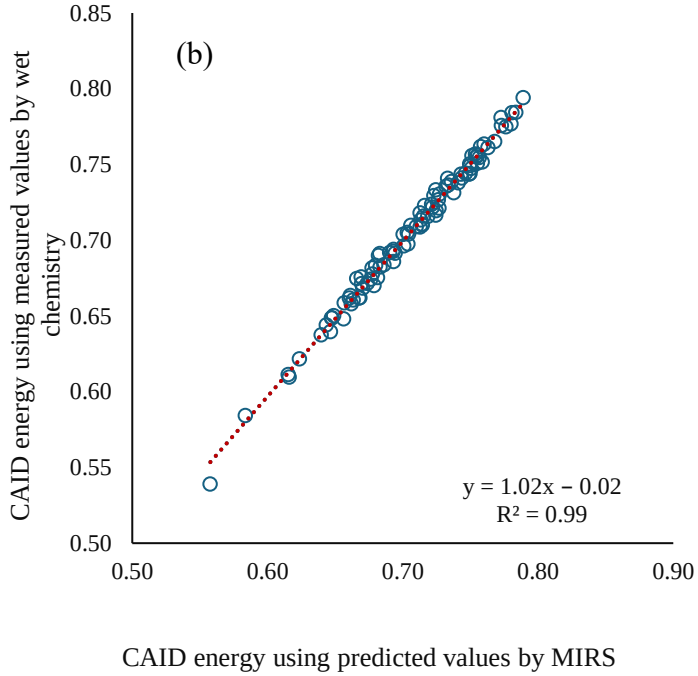
7.4. Results

7.4.1. Calculation of CAID, cATTD, and IDC of nutrients using the predicted gross composition values by ATR-FT MIRS and comparing them with the actual values calculated using the wet chemistry data

The outcome of the comparison of predicted values and observed values is illustrated in Figures 7.1, 7.2, and 7.3 with the measures of goodness of fit statistics in Tables 7.2 and 7.3. Best results were obtained for CAID and IDC of energy in terms of R^2 (> 0.90), CCC (> 0.90), and RPE (< 5%) with low RMSE and MB values, while the results for CAID of N were good (R^2 = 0.70, CCC = 0.83, and RPE = 2.71%). The CAID of P had good R^2 and CCC (> 0.70), however, the RPE was moderate (17.9%) while the attempt to predict CAID of Ca and IDC of N, Ca, and P, using the ATR-FT MIRS values failed (R^2 and CCC < 0.50; RPE > 20%). The MB results showed that IDC of Ca and P were overpredicted with very high RPE%.

The results for cATTD of N, energy, and AME using total collection and marker methods exhibited the highest precision as illustrated in Figure 7.3 with the measures of goodness of fit parameters in Table 7.3. These findings indicated the capability of the ATR-FT MIRS generated values to accurately predict the cATTD of N, energy, and AME with R^2 and CCC > 0.90 and RPE < 10% and MB and RMSE closer to 0.


(a)
CAID N using measured values by wet chemistry
0.90
0.85
0.80
0.75
0.70
0.65
y = 0.76x + 0.19
R² = 0.70
0.65
0.70
0.75
0.80
0.85
0.90
0.95
CAID N using predicted values by MIRS


(b)
CAID energy using measured values by wet chemistry
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
y = 1.02x – 0.02
R² = 0.99
0.50
0.60
0.70
0.80
0.90
CAID energy using predicted values by MIRS

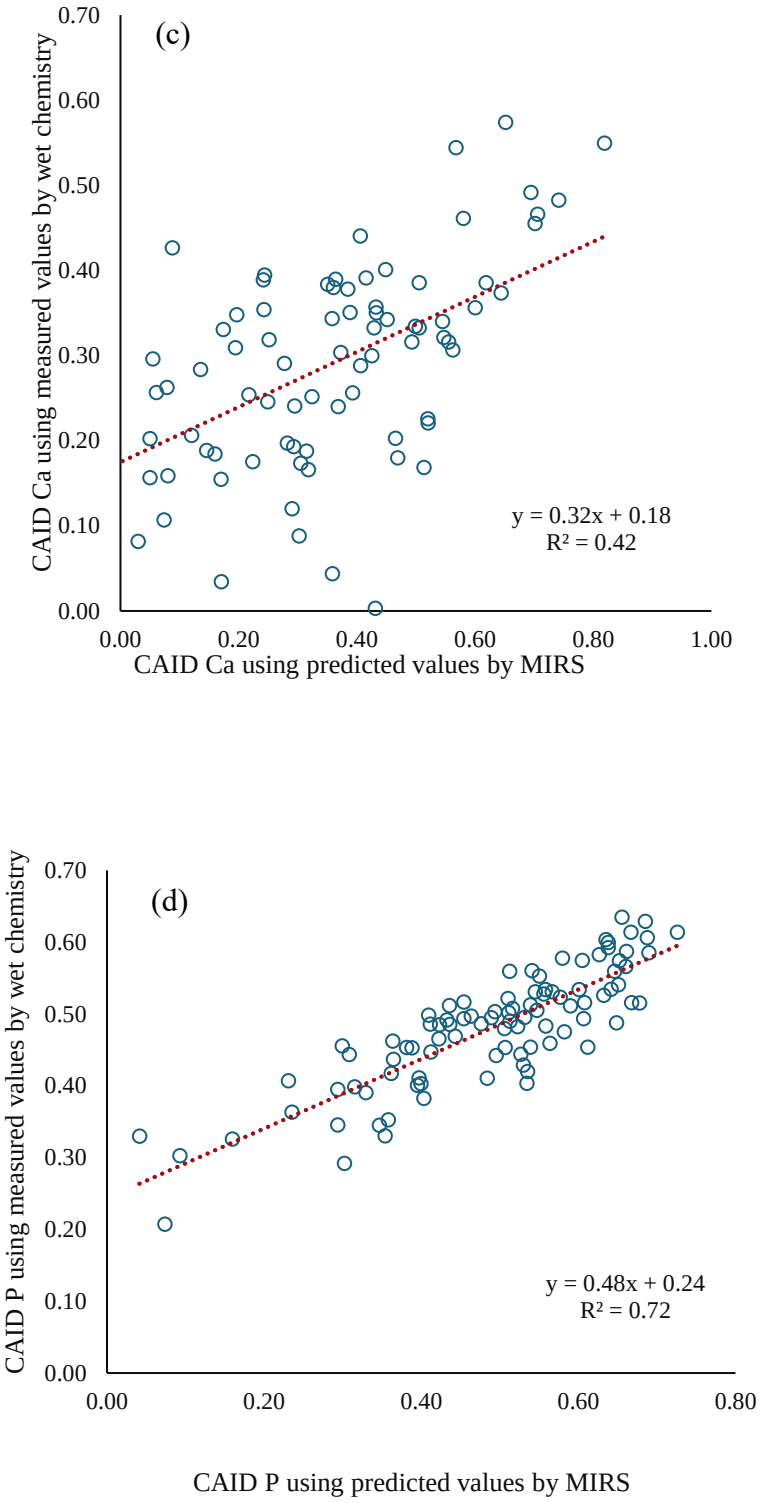
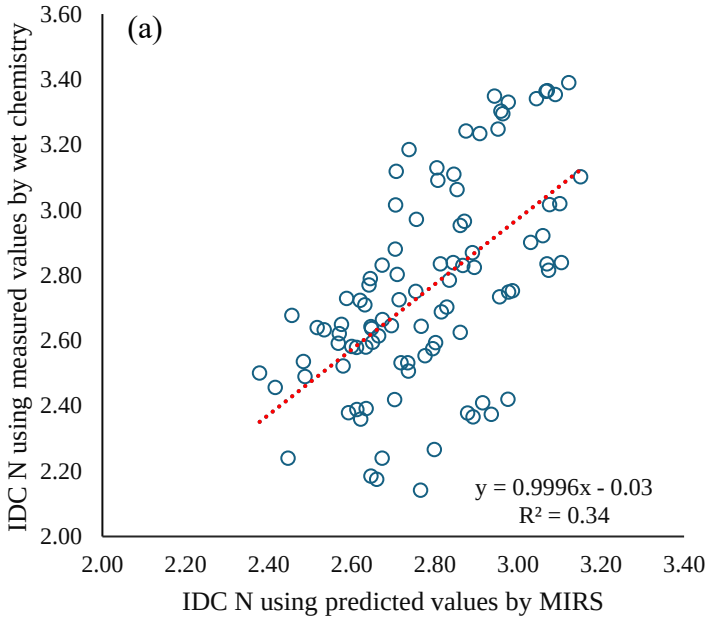
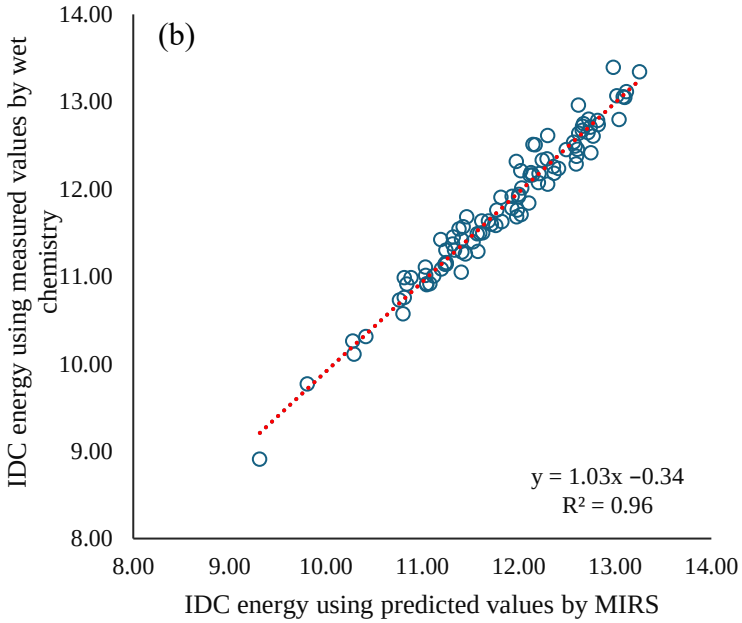
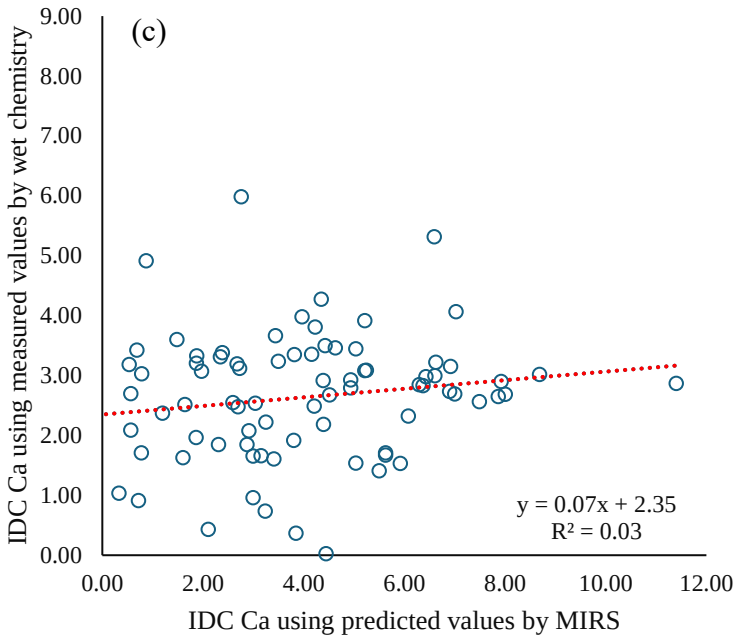


Figure 7.1. Comparison of calculated coefficient of apparent ileal digestibility (CAID) of N (a), energy (b), Ca (c), and P (d) using the wet chemistry chemical composition data (*y*-axis) with the calculations using the ATR-FT MIRS predicted chemical composition values (*x*-axis).


(a)
IDC N using measured values by wet chemistry
3.60
3.40
3.20
3.00
2.80
2.60
2.40
2.20
2.00
y = 0.9996x - 0.03
R² = 0.34
2.00
2.20
2.40
2.60
2.80
3.00
3.20
3.40
IDC N using predicted values by MIRS


(b)
IDC energy using measured values by wet chemistry
14.00
13.00
12.00
11.00
10.00
9.00
8.00
y = 1.03x −0.34
R² = 0.96
8.00
9.00
10.00
11.00
12.00
13.00
14.00
IDC energy using predicted values by MIRS



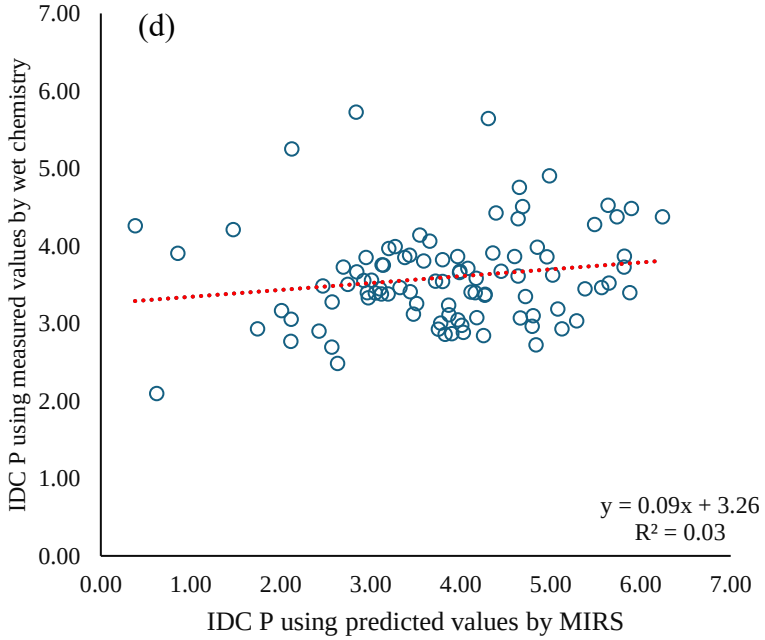


Figure 7.2. Comparison of calculated ileal digestible content (IDC) of N (a), energy (b), Ca (c), and P (d) (% as-fed basis) using the wet chemistry chemical composition data (*y*-axis) with the calculations using the ATR-FT MIRS predicted composition values (*x*-axis).

Table 7.2. Comparison of CAID and IDC (% as-is basis) of nutrients using the predicted gross composition values by ATR-FT MIRS with the actual values calculated using the wet chemistry data.

Parameters	Mean [a]	Mean [b]	R^2	CCC	RMSE	MB	RPE%
CAID N (n = 95)	0.793	0.789	0.71	0.83	0.02	0.004	2.71
CAID energy (n = 95)	0.707	0.706	0.99	1.0	0.01	0.001	0.66
CIAD Ca (n = 95)	0.274	0.264	0.42	0.52	0.21	0.01	79.8
CAID P (n = 95)	0.489	0.481	0.72	0.73	0.09	0.01	17.9
IDC N (n = 95)	2.782	2.755	0.34	0.51	0.26	0.03	9.26
IDC energy (n = 95)	11.851	11.810	0.96	0.98	0.17	0.04	1.44
IDC Ca (n = 95)	3.007	2.564	0.03	0.12	3.27	0.44	127
IDC P (n = 95)	3.822	3.594	0.03	0.14	1.27	0.23	35.4

[a], calculated using predicted values by ATR-FT MIRS; [b], calculated using values measured by wet chemistry; Ca, calcium; CAID, coefficient of apparent ileal digestibility; CCC, concordance correlation coefficient; IDC, ileal digestible content; MB, mean bias; N, nitrogen; P, phosphorus; RMSE, root mean square error of prediction; RPE, relative prediction error.

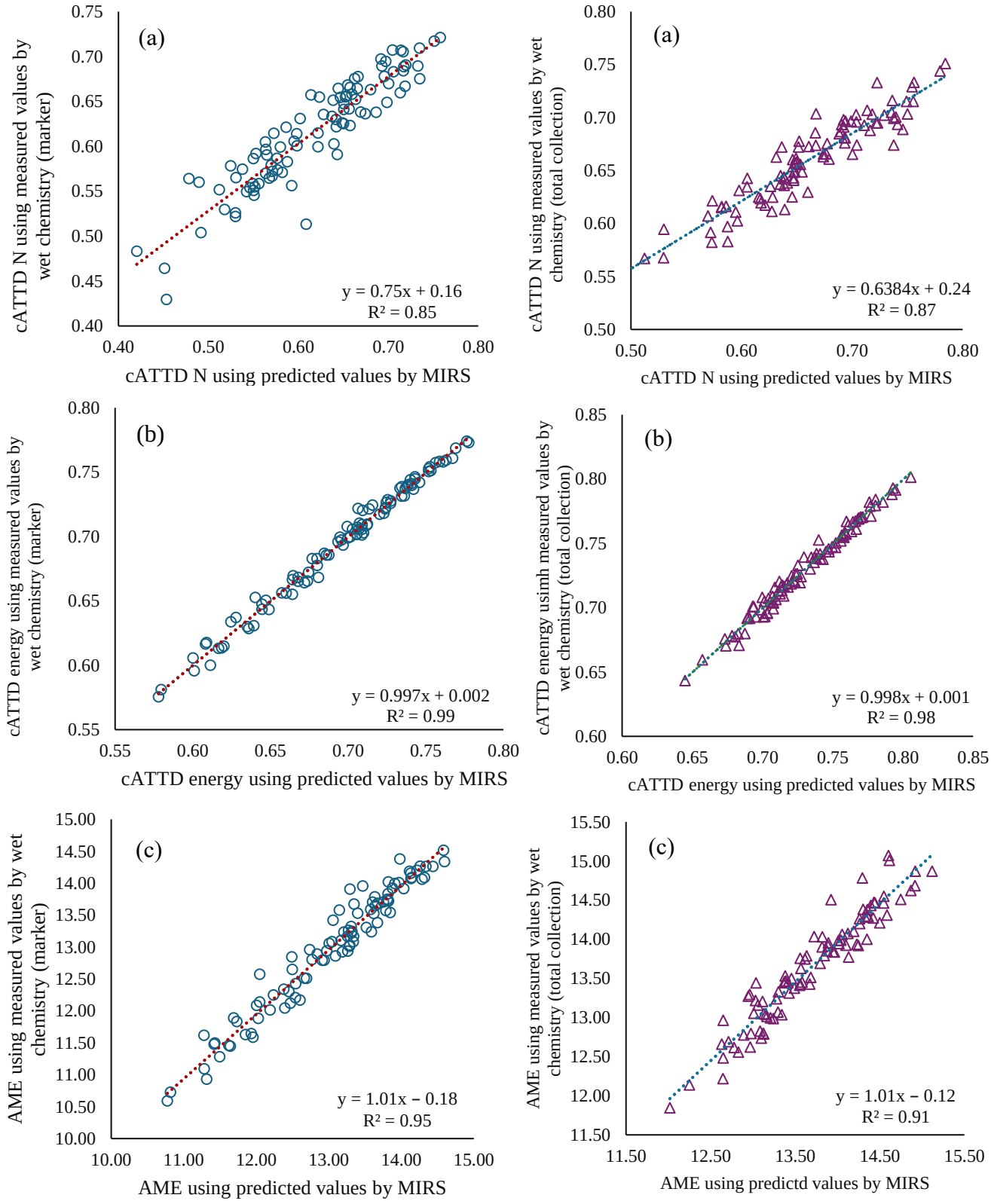


Figure 7.3. Comparison of calculated cATTD of N (a), energy (b), and AME (MJ/kg DM) (c) using the wet chemistry data (marker method -○- and total collection method -△-) (*y*-axis) with the calculations using the predicted values by ATR-FT MIRS (*x*-axis).

Table 7.3. Comparison of cATTD of nutrients and AME (MJ/kg DM) (marker and total collection methods) using the predicted gross composition values by ATR-FT MIRS with the actual values calculated using the wet chemistry data.

Parameters	Method	Mean [a]	Mean [b]	R^2	CCC	RMSE	MB	RPE%
cATTD N	Marker	0.618	0.617	0.85	0.90	0.03	0.002	4.84
(n = 95)	Total collection	0.661	0.660	0.87	0.87	0.03	0.001	3.96
cATTD E	Marker	0.696	0.695	0.99	0.99	0.01	0.001	0.75
(n = 94)	Total collection	0.730	0.730	0.98	0.99	0.01	0.000	0.62
AME	Marker	13.034	12.991	0.95	0.97	0.22	0.044	1.68
(n = 94)	Total collection	13.688	13.641	0.91	0.95	0.21	0.046	1.54

[a], calculated using predicted values by MIRS; [b], calculated using values measured by wet chemistry; AME, apparent metabolisable energy; cATTD, coefficient of apparent total tract digestibility; CCC, concordance correlation coefficient; E, energy; MB, mean bias; N, nitrogen; RMSE, root mean square error of prediction; RPE, relative prediction error.

7.4.2. Calculation of CAID and IDC of N and energy using the predicted gross composition values by ATR-FT MIRS and developed regression equations (Chapter 3) and comparing them with the actual values calculated using the wet chemistry data

Figure 7.4 shows the scatter plots comparing the predicted values by the regression equation using ATR-FT MIRS data to the actual values calculated using wet chemistry data while Table 7.4 shows the goodness of fit statistics of the above values. The prediction of CAID of energy and IDC of N resulted in good agreement with R^2 of 0.63 and 0.81, respectively. The CCC was > 0.70 for CAID of energy, and IDC of N and energy. The mean bias showed that the equations underpredicted the CAID of N and IDC of energy, whereas the CAID of energy and IDC of N were overpredicted.

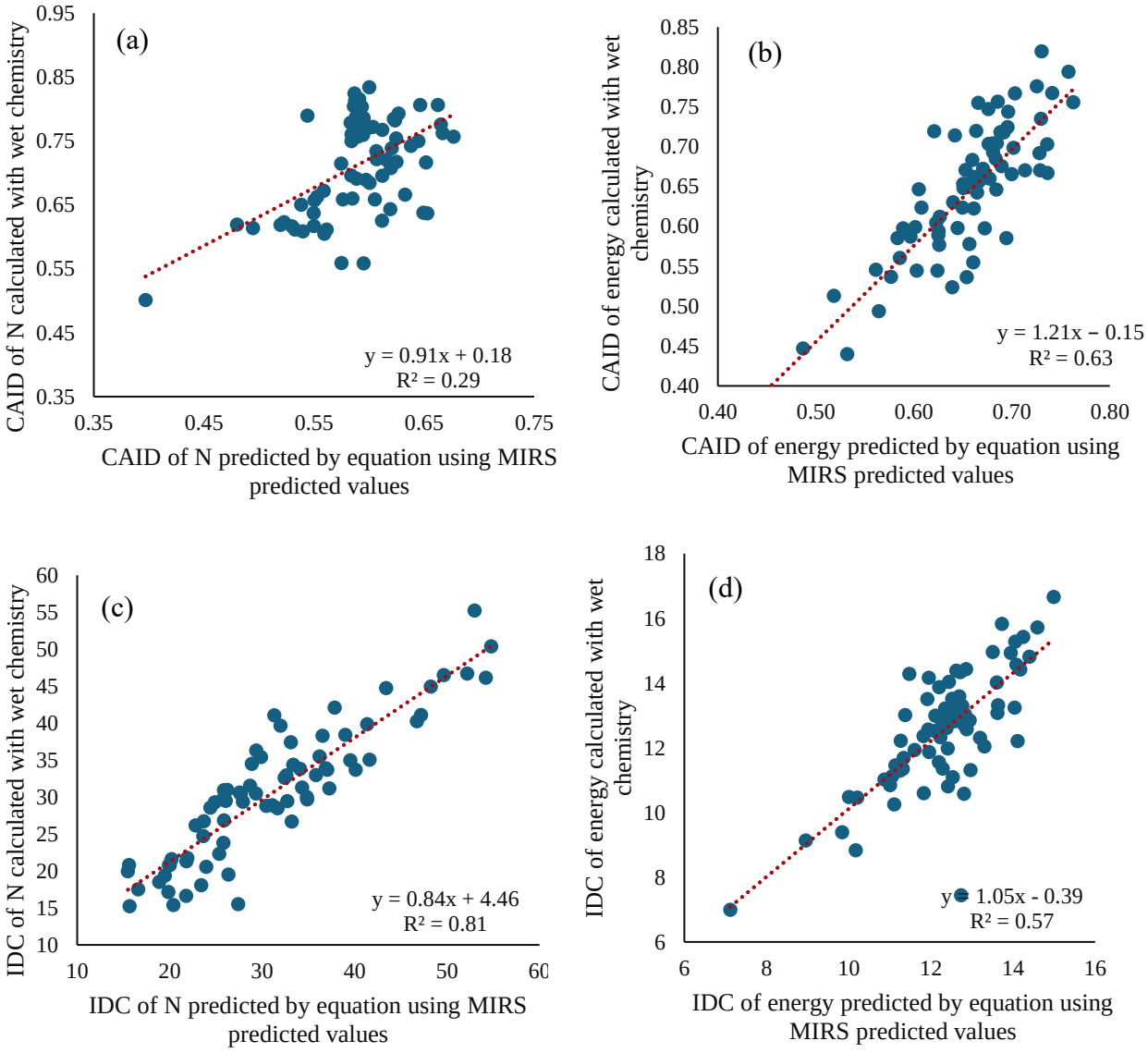


Figure 7.4. Scatter plots comparing CAID of N (a), CAID of energy (b), IDC of N (c) and IDC of energy (d) calculated using the regression equations with ATR-FT MIRS predicted gross chemical composition (*x*-axis) and the actual values calculated with wet chemistry (*y*-axis).

Table 7.4. Comparison of CAID and IDC of N and energy using the predicted gross composition values by ATR-FT MIRS and regression equations with the actual values calculated using the wet chemistry data

Parameters (n = 74)	Mean [a]	Mean [b]	R^2	CCC	RMSE	MB	RPE%
CAID N	0.591	0.713	0.29	0.16	0.14	−0.12	19.4
CAID energy	0.654	0.640	0.63	0.72	0.06	0.01	9.19
IDC N (g/kg DM)	31.073	30.527	0.81	0.89	4.26	0.55	14.0
IDC energy (g/kg DM)	12.340	12.568	0.57	0.71	1.21	−0.23	9.64

Abbreviations: [a] Mean predicted by prediction equation; [b] Mean actual data; CAID, coefficient of apparent ileal digestibility; CCC, concordance correlation coefficient; IDC, ileal digestible content; MB, mean bias; N, nitrogen; RMSE, root mean square error of prediction; RPE, relative prediction error.

7.5. Discussion

The most common method for calculating the nutrient content of a diet is to use tables that have the chemical composition and the digestibility values of the feed ingredients. However, as each batch of ingredients has unique properties, table values may not be entirely accurate and are often highly variable (Mateos et al., 2019; Paternostre et al., 2021). Furthermore, there may not be table values for non-common feed ingredients or under-utilised feed ingredients. The nutritive values of these non-traditional feedstuffs are not well understood due to the lack of analytical facilities (Ravindran, 2013). Mathematical models or prediction equations are simple, inexpensive, and a rapid method of estimating nutritive values of diets and feedstuffs compared to direct methods (*in vivo* assay) (Swiech, 2017). However, the nutrient content of each sample must be analysed by wet chemistry, which is costly and time-consuming. These wet chemistry values could be replaced by the ATR-FT MIRS predicted values, thus the cost

and the time spent on the analysis could be minimised. Mateos et al. (2019) also stated that the use of prediction equations based on the gross chemical composition values obtained using near-infrared spectroscopy (NIRS) is promising for the estimation of energy contents of the feedstuffs and feeds.

To the best of our knowledge, there are no reports on the indirect use of ATR-FT MIRS predicted values to calculate digestibility in broilers. There is just one report on this strategy to predict digestibility in monogastrics using NIRS by Cruz-Conesa (2023). However, few studies are available using NIRS in conjunction with prediction equations to estimate the nutritive value of feeds for cattle (De Boever et al., 1996), sheep (Kneebone and Dryden, 2014), and pigs (Ferreira et al., 2018).

The estimates of R^2 and CCC were closer to 1, and RPE < 10% for CAID and IDC of energy, CAID of N, cATTD of N, energy, and AME in the present study using the calculation method. The closer the R^2 value is to 1, the more accurate the prediction results are (Mariano et al., 2013), whereas the RPE < 10% is considered the best and between 10% to 20% is good (Marshall et al., 2023). The CCC values closer to 1 indicate that the actual and predicted values are in agreement with low biases in the mean and regression line of the actual on predicted values (Lin, 1989).

Energy is the main factor considered in the feed evaluations which decide the cost of the feed (Zaefarian et al., 2021). Therefore, an accurate estimation of the available energy content of ingredients and diets is crucial for economic feed formulations (Abdollahi et al., 2021). Apparent metabolisable energy (AME) is the most determined energy form in poultry (Kong and Adeola, 2014). The current analysis showed that the CAID, IDC of energy as well as cATTD of energy and AME (total collection and marker methods) could be accurately calculated using the predicted chemical composition values from ATR-FT MIRS with R^2 >

0.90 and CCC > 0.85 and a very low mean bias (< 0.005) and RPE (< 10%). As explained in the previous Chapter, it is also quicker and cost-effective to directly predict the cATTD of energy and AME values using ATR-FT MIRS. With the success of the present study, the analysis of N, energy, P, and DM in diet and N and energy in the ileal digesta and excreta by wet chemistry methods would no longer be needed.

Cruz-Conesa (2023) used the predicted GE and P values of broiler diets by NIRS together with reference TiO_2 values to calculate AME and CAID of P, respectively, and obtained good results. Moreover, the AME result reported by Cruz-Conesa (2023) was similar to the present study (R^2cv = 0.98 and R^2 = 0.97, respectively), whereas CAID of P had comparatively higher accuracy (R^2cv of 0.82) than the present study (R^2 = 0.72).

Results obtained in the present study showed that ATR-FT MIRS predicted values could be used for the calculations of digestibility when the wet chemistry methods are expensive. However, *in vivo* experiments should be conducted to get the ileal digesta and excreta samples. Besides, this method could replace the costly and time-consuming wet chemical analysis for determining the nutrient content of diet, ileal digesta, and excreta with no generation of chemical waste. Moreover, a single MIRS spectrum could be used to predict many parameters at the same time (Bastianelli et al., 2010). It is essential to carefully monitor the performance of models using wet chemistry and compare those results with the results predicted by MIRS (Mazabel et al., 2020). It is also important to note that the accuracy and precision of MIRS predictions are linked to the quality of the wet chemical analysis as calibration of MIRS relies on wet chemical data (Xu et al., 2023).

This study has also shown that ATR-FT MIRS predicted diet gross composition could be used in prediction equations to find the nutritive values of the diets. The equations for CAID of energy and IDC of N were effective in predicting when associated with the MIRS predicted

values with $R^2 > 0.60$ and CCC > 0.70. The equations for CAID of energy and IDC of N and energy gave acceptable results upon validation with new data compared to CAID of N (Chapter 4). This may be a reason for the low accuracy of CAID of N predicted with the values from MIRS compared to others. The variables used in the equations were predicted well by the MIRS ($R^2 > 0.70$ and RPD > 2) except fat ($R^2 = 0.59$ and RPD = 1.62). Ferreira et al. (2018) used previously published prediction equations to estimate the ME values of corn for pigs using the predicted values by NIRS and laboratory values. They concluded that some equations by Noblet and Perez (1993) and Morgan et al. (1987) were adequate using NIRS compared to the laboratory tests.

The laboratory analyses are costly and take time to process the samples. Moreover, the feeds and faecal samples should be analysed with many replicates to increase precision and to detect errors in the analytical process. In addition to that, waiting for the results from the laboratory takes up valuable time and delays results, thus making timely managerial decisions impossible (Yakubu et al., 2022; Xu et al., 2023). Thus, the current study has highlighted that different approaches could be applied to predict the digestibility of broiler diets using ATR-FT MIRS.

A limitation of the present study was the use of calibrations developed from 20 diets and their corresponding ileal digesta and excreta samples, thus results should not be more generalised. Even though there was a large variation between diets, including a greater number of samples may increase the prediction accuracy and performance. Further research could be focused on including more robust calibrations and validations for evaluating chemical compositions of diet and ileal digesta or excreta and using them to develop prediction equations for estimation of the nutritive value of the diets (Camp Montoro et al., 2023).

7.6. Conclusions

It will be cost-effective to use ATR-FT MIRS predicted values of gross chemical composition of diet, ileal digesta, and excreta in calculating the CAID of N, energy, and P, IDC of energy, cATTD of N and energy and AME instead of using the time-consuming and costly wet chemical analysis. It could also be possible to use the ATR-FT MIRS predicted diet gross composition values in prediction equations to get a quicker estimation of the digestibility of nutrients.

CHAPTER 8

General Discussion

8.1. Need for alternative methods to determine the chemical composition and nutritive value of broiler chicken diets

Poultry production is one of the largest sources of animal protein supply for human consumption across the world (Govoni et al., 2021). Advances in feed formulation for broilers is essential to ensure the bird gets optimal nutrients for growth and development. As feeds represent about 70% of the total production cost, accurate feed formulation is crucial to minimise nutrient wastage, thus reducing the feed inputs required. Nutrient requirements of the birds are dynamic, changing with age, stage, level of production, reproductive status, health status, and environmental conditions (Zuidhof et al., 2023). Meeting nutrient requirements in real-time will optimise nutrient intake which in turn will reduce the cost of feeding and minimise the excretion of excess nutrients such as nitrogen and phosphorus (Andretta et al., 2016). When dietary intake of nutrients is high compared to the bird's requirement, excess energy may be stored as fat, and amino acids may be deaminated (Moss et al., 2021). Feed optimisation is only possible when both nutrient requirements and nutrient availability from the feedstuffs or diets are known and matched (Mallick et al., 2020; Zuidhof et al., 2023).

It is important to note that the current methods for formulating diets using table values for the chemical composition of feedstuffs and their digestibilities are not entirely accurate for individual feedstuff batches (Van Barneveld et al., 2018; Zaefarian et al., 2021). For example, Mallick et al. (2020) formulated starter and finisher diets for broilers using linear programming (least-cost diet formulation) and stated that the main drawback was the sourcing of nutrient composition data from many different sources such as textbooks and tables, which displayed a wide variation in the nutrient composition for a same ingredient. Moreover, Moss et al. (2021)

stated that nutritionists still refer to the book values, which limits accurate ingredient characterisation, particularly when the nutritive value of feed ingredients is highly variable due to differences in management, cultivar, year of production, and environmental conditions. The most precise determination of an ingredient nutritive value is by *in vivo* trial, under controlled experimental conditions (Ravindran and Bryden, 1999; Yegani et al., 2013). *In vivo* experiments involve the accurate determination of the chemical composition of feeds, ileal digesta, and excreta by wet chemical analysis which is generally based on gravimetric, colorimetric, and chromatographic techniques (Tamaki and Mazza, 2011). These chemical analytical techniques have several drawbacks including being expensive, labor-intensive, time-consuming, and causing environmental pollution by producing hazardous wastes that must be processed properly (Bastianelli et al., 2010; Raccary et al., 2022). Another approach involves estimating nutrient digestibility using *in vitro* techniques that mimic the digestion process along the gastrointestinal tract of birds. However, this too requires lengthy chemical analysis and equipment. These drawbacks hinder a routine and rapid feed evaluation at the feed mill or farm level (Tamaki and Mazza, 2011; Jha and Tiwari, 2016).

Therefore, it is essential to find simple alternative ways to the costly wet chemical methods to determine the chemical composition and nutritive value of broiler feeds with similar accuracy as the traditional *in vivo* methods. Prediction equations based on gross chemical composition of diets to estimate the nutritive value have been reported in several studies, however only a few were validated in independent trials. A successfully validated model should ideally be used to predict new samples for a longer period. Moreover, the potential of ATR-FT MIRS in determining the nutritive value of broiler diets has not been studied yet. The use of ATR-FT MIRS could be suggested as an additional tool rather than as a replacement for current methods. Each method has its own advantages and disadvantages, thus clearly defining or recommending the best method to be applied in all conditions is difficult. However, based on the cost and time

spent, prediction equations and infrared spectroscopy could be effectively used. Therefore, this study was conducted to develop prediction models using multiple regression based on the gross chemical composition and using ATR-FT MIRS with the following specific objectives:

1) To develop and validate prediction equations based on wet chemical analysis to determine the nutritive value of broiler diets (Chapters 3 and 4).

2) To explore the potential of MIRS in predicting the gross chemical composition and nutritive value of broiler diets (Chapter 5).

3) To evaluate the usefulness and accuracy of ATR-FT MIRS calibrations based on spectra from ileal digesta or excreta to predict their chemical composition and nutrient digestibility (Chapter 6).

4) To study the different approaches based on ATR-FT MIRS predicted values for the determination of the nutritive value of broiler diets (Chapter 7).

In this present Chapter, key findings from the research work, limitations, and future research are discussed.

8.2. Key findings of this study

8.2.1. Prediction equations for estimation of nutritive value of broiler diets

The use of prediction equations is gaining interest and is used by animal feed industries as an alternative way to replace animal trials (Son et al., 2017). Regression studies have shown that significant relationships existed between the gross chemical analysis and the digestion content of different nutrients in the animals (Noblet and Perez, 1993). Plenty of regression equations are available to predict the energy components (AME, DE, and NE), apparent ileal digestibility of protein, and amino acids of the feedstuffs and diets while some are available for prediction of apparent ileal digestibility of Ca and P in specific feed ingredients (NRC, 1994). The recent

renewed interest in real-time analysis of the nutritive value and limitation in the availability of equations to apply for a wide range of complex broiler diets, necessitates the development of more up-to-date equations for digestible nutrient content and digestibility. Therefore, Chapter 3 was designed to develop prediction equations for determining CAID and IDC of nutrients in complex broiler diets. This Chapter included 56 diets from a study by Pedersen et al. (2021), which were formulated with 10 feed ingredients, thus providing a wide range of values for chemical composition, CAID, and IDC of nutrients.

The results indicated that the CAID of N was predicted with R^2 of 0.78. The CF had a positive relationship and CF^2 had a negative relationship with CAID of N. The CAID of energy was best explained by dietary CF and GE content, CF^2, and starch:CF ($R^2 = 0.89$). Moreover, the IDC of N increased linearly with dietary N content. The starch:CF, CF, and GE had a positive relationship, and CF^2 had a negative relationship for the prediction of IDC of energy. The best predictors for IDC of starch were the dietary content of starch and GE. We also found that the inclusion of CF and its interactions with fat or starch as a variable in the prediction equations increased the R^2 values. Even though the CF represents a small portion of the total fibre present in the ingredients, the impact of CF can be seen in most of the developed equations (see Tables 3.3 and 3.4: Chapter 3). This was in accordance with many previous studies where the impact of at least one type of fibre measurement (CF, ADF, NDF, NSP or HC) could be always observed in regression equations when predicting digestibility in monogastric animals (Noblet and Perez, 1993; Alvarenga et al., 2011; Yegani et al., 2013; Meloche et al., 2014; Cerrate et al., 2019; Pedersen et al., 2021)

8.2.2. Use of bootstrap as a variable selection method

The use of bootstrap resampling technique has gained interest in assessing the degree of stability of models resulting from stepwise regression procedures (Nunez et al., 2011). In

stepwise regression, variables are added and removed from the model at each step of the variable selection process. When applied to regression analysis, bootstrapping helps identify variables that have a high degree of reliability (Brunelli, 2014). The bootstrap approach was used primarily to select variables and construct prediction models for CAID and IDC of dietary nutrients for broilers in Chapter 3. Of the 56 diets, 60% was assigned for variable selection and the remaining 40% was used to test the obtained results. The total number of combinations obtained was 2.14×10^{15} and we have used 1000 times resampling in this study. The frequency of the variables entering 1000 bootstrap models was used as a guide to validate variables selected in original stepwise regression and selection based on a > 30% cut-off frequency was arbitrary (Sauerbrei and Schumacher, 1992). We estimated the whole distribution of important variables under consideration using bootstrapping. Therefore, from a whole set of variables we could select only the important variables which are more stable.

There were no differences in the goodness of fit between the original and bootstrap models for CAID of N, energy, and DM, as well as IDC of energy, starch, and Ca (see Tables 3.8 and 3.9). This indicated the stability performance for the stepwise regression models among the bootstrap samples. However, there wasn't much difference between the performance (R^2 and RMSE) of the original model and the model constructed with the selected variables using bootstrapping. Besides, these results give a useful insight into the variable selection approach as stepwise selection is just a single model without any information about its stability, thus bootstrapping is useful to select the best variables when constructing prediction models with greater confidence.

8.2.3. Importance of external validation of predictive models

Even though the diets used in Chapter 3 (Pedersen et al., 2021) had a wide range of chemical composition and nutritive value, the major limitation was the varying inclusion level of

different ingredients. However, compared to these diets, commercial diets will be complete, nutritionally balanced, and will have a smaller range of ingredient inclusion levels. Therefore, in practice, the prediction equations developed in Chapter 3 should be used with caution depending on the chemical composition of the diets to be studied. To ensure the practical applicability of developed equations, a follow-up study (Chapter 4) was designed to carry out an external validation with 20 newly formulated broiler diets, which were representative of commercial conditions. This validation revealed that some equations could not be used in all situations (CAID N, fat, starch, and Ca and IDC of Ca), and some could be used as a general guide (CAID and IDC of P). Equations developed for CAID energy and DM and IDC of N, starch, energy, and DM had acceptable accuracy in terms of R^2 and CCC (Chapter 4: Table 4.7). However, the overall findings including the bias showed that none of the equations were accurate. These findings proved that external validation is a crucial step before the implementation of prediction equations at a commercial level.

8.2.4. The ATR-FT MIRS can directly predict the chemical composition of the broiler chicken diets, ileal digesta, and excreta

Mid-infrared spectroscopy has not been widely used in the prediction of gross composition in animal feeds compared to NIRS. Some studies have evaluated single feed ingredients using MIRS such as wheat (Shi et al., 2019), wheat bran (Hell et al., 2016), soybean (Ferreira et al., 2014), and forages (Cleland et al., 2018). To our knowledge, this is the first study stating the application of ATR-FT MIRS in the prediction of chemical composition and nutritive value of broiler diets. Therefore, an exact comparison with other scientific articles published in the literature was not possible.

One hypothesis behind Chapters 5 and 6 was that ATR-FT MIRS can be used to predict the gross chemical composition of diets, ileal digesta, and excreta. Seventy-six diets, 100 ileal

digesta, and 100 excreta samples have been used to build the MIRS calibration models to predict the gross chemical composition of the diets, ileal digesta, and excreta, respectively. Savitzky-Golay derivative was applied as a pre-treatment to the averaged spectra to eliminate the random effects due to sample presentation on the ATR crystal. The PLSR was carried out separately for diets, ileal digesta, and excreta to build the calibration models by correlating the pre-treated MIRS spectral data with the reported wet chemical composition values. Four-fold cross-validation was applied to check the robustness of each model. The optimum number of PLSR components was determined based on the values of MSE of cross-validation. Table 8.1 shows the summary of the prediction of chemical composition of diets, ileal digesta, and excreta by ATR-FT MIRS in this thesis. The R^2 value closer to 1 indicates better fit (Ferreira et al., 2014). RPD value of < 2 is not suitable for prediction (**1**); values between 2.0-2.4 are acceptable for screening purposes (**2**); values between 2.5-2.9 are useful for quantification (**3**); and values ≥ 3 indicate high accuracy for quantitative analysis (**4**) (Lyons et al., 2016) (see Table 8.1).

It was possible to accurately predict N, Ca, P, DM, ash, and OM of the diets while the prediction of starch could be used for quantification. The predictions for fat and GE were not accurate enough (Cruz-Conesa, 2023). Even though the number of diet samples used in the study was 76, they had a wide range of values for chemical composition, thus aiding in the prediction of most components. The compound diets are spectrally complex due to the use of a wide variety and proportions of ingredients as each raw material has its own characteristic spectral pattern. Therefore, to develop good calibration, samples with large variations must be used (Givens et al., 1997). Following that, new models could be developed as more samples become available.

Meanwhile, for the ileal digesta, the ADF content could be accurately predicted and the predictions for GE, P, starch, and fat could be used for quantification. In the excreta, accurate

results were obtained in the prediction of Ca and P while the prediction for N, GE, and ash could be used for quantification.

It must be noted that the number of components required to model each analyte in the current study ($nc \leq 6$) was less than those obtained for NIRS in literature for animal feed studies by Xiccato et al. (2003) ($nc \leq 9$), Khaleduzzaman et al. (2017) and Khaleduzzaman and Salim (2020) ($nc \leq 11$), and Paternostre et al. (2021) ($nc \leq 15$) and in some MIRS related studies Ferreira et al. (2014) ($nc \leq 9$) and Hell et al. (2016) ($nc \leq 10$). If many components are chosen in a PLSR, the model will try to account for even the smaller changes in the dataset, which results in overfitting (Khaleduzzaman et al., 2017).

The models developed in Chapters 5 and 6 provide evidence that ATR-FT MIRS can replace wet chemistry methods for the determination of the gross chemical composition of samples generated in *in vivo* assays to some extent. This substitution of wet chemistry by ATR-FT MIRS could offer significant cost reduction in digestibility studies. This further enables a better formulation of broiler diets. It was also found that the models developed on the whole dataset (diets, ileal digesta, and excreta) to predict the chemical composition didn't work well compared to the individual models developed for diet, or ileal digesta or excreta (Chapter 6).

Table 8.1. Summary of prediction results obtained for gross chemical composition using ATR-FT MIRS (as-is basis unless otherwise specified).

Analyte	Diet (n=76)				Ileal digesta (n=100)				Excreta (n=100)			
	R^2c	R^2p	nc	RPD	R^2c	R^2p	nc	RPD	R^2c	R^2p	nc	RPD
N%	0.92	0.86	5	3.27[4]	0.68	0.66	4	1.75[1]	0.87	0.75	3	2.50[3]
GE MJ/kg	0.74	0.70	3	1.90[1]	0.84	0.84	5	2.53[3]	0.93	0.78	5	2.86[3]
Ca mg/g	0.98	0.85	6	5.56[4]	0.84	0.74	4	2.29[2]	0.96	0.72	6	3.32[4]
P mg/g	0.96	0.95	6	4.76[4]	0.92	0.76	5	2.70[3]	0.94	0.73	6	3.16[4]
Starch%	0.84	0.77	2	2.44[2]	0.91	0.75	5	2.92[3]		-		
Fat%	0.65	0.59	3	1.62[1]	0.86	0.77	4	2.50[3]		-		
DM%	0.94	0.75	5	3.05[4]	0.55	0.05	3	1.34[1]	0.13	−0.10	1	1.02[1]
CF%	0.89	0.87	6	2.96[3]	0.91	0.63	6	2.34[2]		-		
NDF%		-			0.79	0.71	4	2.07[2]		-		
ADF%		-			0.93	0.81	6	3.08[4]		-		
Lignin%		-			0.90	0.44	6	2.04[2]		-		
Ash%	0.97	0.87	6	4.38[4]	0.82	0.82	4	2.37[2]	0.89	0.82	5	2.77[3]
OM%	0.92	0.89	4	3.39[4]	0.84	0.71	4	2.25[2]	0.87	0.65	4	2.39[2]
Ti%*		-			0.78	0.53	5	1.90[1]	0.57	0.24	2	1.41[1]

* DM basis

ADF, acid detergent fibre; Ca, calcium; CF, crude fibre; DM, dry matter; GE, gross energy; N, nitrogen; nc, number of components; NDF, neutral detergent fibre; OM, organic matter; P, phosphorus; R^2c, coefficient of determination of calibration; R^2p, coefficient of determination of prediction; RPD, relative performance deviation; Ti, titanium.

It should be noted that for most parameters predicted with NIRS or MIRS in literature, R^2c or R^2cv is reported and but information on the R^2 for test prediction (R^2p) or external validation (R^2v) are scarce. This make the comparison of different studies difficult and sometimes the accuracy of prediction is overestimated or underestimated (see Tables 2.3, 2.4, and 2.5; Chapter 2).

8.2.5. Direct prediction of nutritive value of broiler chicken diets using ATR-FT MIRS

As it is not possible to routinely perform *in vivo* evaluations to determine the nutritive value of feed ingredients and diets due to the time and cost, it is essential to develop alternative quick and reliable methods. This allows for accurate feed formulation and control of complete feeds in real-time (Knudsen et al., 2023). The calibration models developed using mid-infrared spectroscopy could be imported to online instruments. This could be installed in feed processing plants for the adjustment of feed composition to obtain a uniform end product (see Figure 2.9) (Van Barneveld et al., 2018; Knudsen et al., 2023). For this purpose, ATR-FT MIRS has the potential in addition to NIRS to be used as the online-tool tool for measuring not only chemical composition but also nutritive value of the broiler diets. This hypothesis has been verified by Chapters 5 and 6 in the present study. The ATR-FT MIRS showed potential as a technique to predict the CAID, IDC, and cATTD of nutrients and AME in broiler diets.

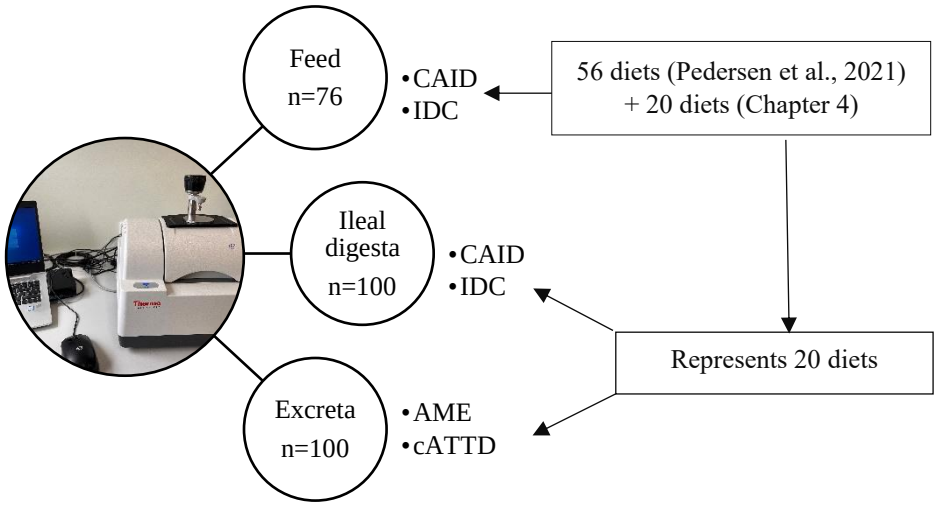


Figure 8.1. Measurements of CAID, IDC, cATTD, and AME by ATR-FT MIRS calibrations of diets, ileal digesta, and excreta

Digestibility predictions using the diet spectra would offer the benefit of avoiding the use of animals. However, only the prediction of CAID of fat was satisfactory for screening with the R^2p of 0.73 and RPD = 2.41. Most parameters predicted well in calibration development but

failed to predict the test data. However, IDC of N, starch, and Ca obtained good calibration and prediction results and, therefore, could be successfully predicted using the diet spectra (R^2p > 0.70 and RPD > 3.0). It should be noted that the models for AME and cATTD of nutrients were not developed using the diet spectra due to the use of different datasets in which one set was without this information (only the 20 diets data set had the AME values). However, the current results for the digestibility of most nutrients suggested that there is potential for further improvement and AME and cATTD of nutrients could also be predicted using the diet spectra. Therefore, we strongly believe that adding more samples and enhancing statistical techniques in future may give more reliable predictions for the measurement of nutritive value from the diet spectra.

Another approach was used in this study (Chapter 6) to check whether it could be possible to predict the CAID and IDC of nutrients using the spectra of ileal digesta and cATTD using the spectra of excreta. The reliable results for prediction of digestibility using excreta or ileal digesta rely on the principle that there is sufficient spectral information in the ileal digesta or excreta regarding the composition of the diet ingested (Jancewicz et al., 2016). However, satisfactory results were not obtained (RPD < 2) for the prediction of CAID and IDC using the spectra of ileal digesta, except IDC of N and starch whose results could be used for screening purposes. This may be due to the smaller number of samples representing only 20 diets. Besides, the cATTD of energy was accurately predicted using the excreta spectra. In addition to that, the predictions obtained for the cATTD of N and DM could be used for quantification. It was also possible to predict the AME using the excreta spectra (R^2p = 0.82 and RPD = 2.86). This could simplify the *in vivo* assays, thus avoiding the use of indigestible markers and exact measurement of feed intake and excreta output (Bastianelli et al., 2005).

Overall, results of Chapters 5 and 6 could not be compared as the number of samples used for diet, ileal digesta, and excreta were different (Figure 8.1). However, the results showed that it

is possible to predict the nutritive value of broiler diets using the diets, ileal digesta, and excreta. The datasets used had a large variation helping in the development of the model even though the number of samples was limited. More samples undoubtedly would contribute to improve the prediction accuracy.

8.2.6. Combined spectra of diet and ileal digesta or diet and excreta could be used to predict the nutritive value of broiler chicken diets

The source of variation associated with the digestion process could not be modelled when developing calibrations if only the diet spectra are used (Cruz-Conesa, 2023). Instead, the faecal spectra could be utilised to predict digestibility, thus integrating more information on the animal part of the digestion process. However, there will be no information about the diet. The combination of both diet and excreta spectra assumes that the composition of diet is considered as well as how the animal has digested the diet (Bastianelli et al., 2013). This could be carried out in different ways such as merging, subtracting, dividing, and averaging (Paternostre et al., 2021). We aimed to test one of the combination methods of spectra "subtraction", to predict the nutritive value of the broiler diets (Chapter 6).

The use of subtracted spectra of diet and ileal digesta or diet and excreta resulted in better prediction of CAID and cATTD for most of the parameters compared to the predictions by either diet or the faecal spectra alone. Prediction of CAID of nutrients using the subtracted spectra of ileal digesta from the diet resulted in poor prediction of CAID of nutrients with R^2c 0.56-0.85, R^2cv 0.15-0.51 and R^2p 0.29-0.59 and RPD of 1.41-1.91. Nevertheless, the accuracy and performance were increased compared to the predictions by the ileal digesta spectra alone. However, the prediction of IDC of nutrients using the subtracted spectra (diet-ileal digesta) was better than using the ileal digesta spectra alone. The predictions achieved for IDC of starch and fat were accurate with $R^2p > 0.80$ and RPD > 3 and the predictions for IDC of N could be used

for screening purposes since the accuracy (R^2p = 0.46) was low but with good performance (RPD = 2.77).

The cATTD of nutrients were well predicted using the combined spectra of diet and excreta than the spectra of excreta alone. The prediction for cATTD of energy was most accurate (R^2p = 0.84 and RPD = 3.22) and cATTD of DM was good (R^2p = 0.61 and RPD = 3.21); those for OM could be used for quantification, whereas the cATTD of N and Ca could be used for screening purposes; and the prediction was poor for P. Besides, the calibration and prediction results derived for AME by total collection were good with the R^2c, R^2cv, and R^2p of 0.89, 0.66, and 0.82, respectively, and RPD of 2.85. Indeed, integrating information from diet spectra and digestion products spectra improve the prediction of the nutritive value.

It can also be noted that the overall predictions of cATTD of N and energy were better than CAID of N and energy (see Tables 8.2 and 8.3). This may be because the sampling of ileal digesta is more variable than the sampling of excreta (Noel et al., 2021). Moreover, ileal digestibility was determined by the marker method while total tract digestibility was determined using the total collection method. It was difficult to predict Ti using MIRS which is inert with no specific absorption in the MIR region. Taken as a whole, the developed models for cATTD of nutrients can be considered good to give useful estimates on the total tract digestibility.

8.2.7. Indirect strategies also could be used to predict the CAID, IDC, and cATTD of broiler chicken diets based on ATR-FT MIRS

Throughout Chapter 7 we aimed to demonstrate two indirect approaches for determining the nutritive value of broiler diets using ATR-FT MIRS. This thesis has shown that MIRS could predict most of the gross chemical composition parameters in diets, ileal digesta, and excreta. By complementing the predictions of chemical compositions in diets, ileal digesta, and excreta,

it is possible to calculate the CAID, IDC, and cATTD of broiler diets without the need to conduct wet chemistry analysis to determine every gross chemical composition. For example, the GE values predicted by ATR-FT MIRS can be used in the standard equation for calculation of CAID and IDC of energy and AME (see Chapter 4). However, in the present study, attempt to predict the Ti failed as this compound does not absorb in the MIR region, we have used the Ti wet chemistry values instead.

Additionally, we could use the predicted values of chemical compositions of diets by MIRS and the prediction equations developed in Chapter 3 to successfully predict the nutritive value (for example CAID and IDC of energy (Table 8.2)). Since there are numerous equations available in the literature to predict nutritive value of single ingredients and compound diets, this strategy would be more beneficial. Furthermore, formulating prediction equations using the ATR-FT MIRS predicted values of gross chemical composition would also be possible when more robust calibrations are available for the prediction of gross chemical composition (Camp Montoro et al., 2023). Both these approaches can drastically reduce the time and money associated with using wet chemistry to analyse the chemical composition of multiple samples.

Table 8.2. Coefficient of determination (R^2) obtained for prediction of CAID and IDC (% as-is basis unless otherwise specified) by different methods in this thesis.

	Prediction equation and validation *		Bootstrap prediction equation & validation *		MIRS diet			MIRS ileal digesta			MIRS combined diet and ileal digesta (subtraction)			a	b *
No. of samples	56	20	56	20	76			95			95			74	74
	Train	Validation	Train	Validation	c	cv	p	c	cv	p	c	cv	p		
CAID N	0.78	0.18	0.78	0.18	0.87	0.36	0.56	0.60	0.15	0.28	0.71	0.41	0.29	0.70	0.29
CAID energy	0.87	0.57	0.87	0.57	0.87	0.48	0.48	0.73	0.20	0.04	0.79	0.26	0.56	0.99	0.63
CAID Ca	0.69	0.25	0.64	0.30	0.49	0.12	0.16	0.43	0.14	0.37	0.69	0.20	0.56	0.42	-
CAID P	0.83	0.53	0.74	0.59	0.75	0.45	0.55	0.62	0.36	0.24	0.73	0.51	0.50	0.72	-
CAID starch	0.36	0.01	0.32	0.05	0.85	0.30	0.34	0.70	0.34	0.61	0.75	0.16	0.47	-	-
CAID fat	0.77	0.001	0.78	0.001	0.89	0.60	0.73	0.50	0.28	0.26	0.64	0.20	0.26	-	-
CAID DM	0.89	0.66	0.89	0.66	0.91	0.57	0.32	0.44	0.07	0.14	0.74	0.34	0.59	-	-
IDC N	0.98	0.90	0.98	0.91	0.93	0.66	0.74	0.90	0.55	0.57	0.92	0.76	0.80	0.34	0.80
IDC energy	0.89	0.65	0.89	0.65	0.80	0.12	0.25	0.65	0.01	0.39	0.86	0.38	0.49	0.96	0.57
IDC Ca	0.92	0.07	0.92	0.07	0.96	0.91	0.73	0.08	−0.15	−0.14	0.11	−0.24	−0.16	0.03	-
IDC P	0.81	0.40	0.81	0.30	0.73	0.49	0.65	0.14	−0.01	−0.05	0.41	0.11	0.07	0.03	-
IDC starch	0.99	1.00	0.99	1.00	0.94	0.91	0.81	0.88	0.56	0.59	0.96	0.76	0.82	-	-
IDC fat	0.99	0.94	0.99	0.94	0.41	0.24	0.16	0.62	0.25	0.36	0.96	0.82	0.85	-	-
IDC DM	0.89	0.66	0.89	0.64	0.90	0.63	0.33	0.70	0.10	0.16	0.82	0.58	0.42	-	-

a, calculation using predicted values by MIRS; b, predicted by the regression equation developed in Chapter 3 using MIRS predicted values; *, IDC (g/kg DM basis); c, calibration; Ca, calcium; CAID, coefficient of apparent ileal digestibility; cv, cross-validation; DM, dry matter; IDC, ileal digestible content; N, nitrogen; P, phosphorus; p, prediction.

Table 8.3. Coefficient of determination (R^2) obtained for the prediction of cATTD and AME (MJ/kg DM) by the different methods used in this study.

	MIRS excreta			MIRS combined diet and excreta (subtraction)			a
	c	cv	p	c	cv	p	
cATTD N (n=95)	0.95	0.61	0.62	0.90	0.70	0.50	0.85
cATTD energy (n=94)	0.93	0.54	0.80	0.92	0.66	0.84	0.99
AME (n=94)	0.93	0.44	0.70	0.89	0.66	0.82	0.95

a, calculation using MIRS predicted values; AME, apparent metabolisable energy (by total collection); c, calibration; cv, cross-validation; cATTD, coefficient of apparent total tract digestibility; N, nitrogen; p, prediction.

The highlights of the thesis:

1. Stepwise variable selection for developing prediction equations could be done using bootstrap resampling and external validation is crucial for successful application of prediction equations.
2. Calibration models were developed using ATR-FT MIRS to predict the gross chemical composition of broiler chicken diets, ileal digesta, and excreta.
3. The cATTD of energy and AME could be successfully predicted using ATR-FT MIRS spectra of excreta and combined spectra of diet and excreta.
4. Several strategies could be utilised to calculate the nutritive value of broiler diets at low cost by replacing wet chemistry values with the values predicted by ATR-FT MIRS.

8.3. Limitations of this study and future research

For the variable selection approach (Chapter 3), we have used a combination of stepwise regression and bootstrapping. However, there are many statistical methods available for variable selection such as shrinkage (ridge regression and LASSO) and dimension reduction (principal components and partial least squares) etc. All variable selection approaches are well defined by James et al. (2013). Moreover, in recent times, machine learning approaches like deep learning (neural network) (Ahmadi et al., 2008; Ahmadi and Rodehutscord, 2017) and Gaussian process regression (GPR) (Baiz et al., 2020) have been used to create best-fit models. Further research that evaluates these methods as they are or in combination with bootstrapping or other methods for selecting the best models would be useful. The dataset used in Chapter 3 had a wide range of chemical composition and nutritive value due to the varying inclusion levels of different traditional and non-traditional ingredients. However, commercial broiler diets should be complete and nutritionally balanced. Further works applying similar regression methods that include larger datasets made of commercial feeds would be beneficial to develop robust equations. For long-term use of equations with greater confidence, external validation is necessary.

The results of the predictions for CAID and IDC of nutrients could not be compared across Chapters in this thesis as the number of samples used for diets, ileal digesta, and excreta were different (see Table 8.2). Due to the unavailability of data on AME and cATTD of nutrients in a dataset, we could not predict them using the diet spectra. However, in future, it will be more useful to predict AME using diet MIRS spectra and if it is successful, there will be no need for *in vivo* experiments, which consequently reduces the use of birds.

The sample size used to develop the models for chemical composition of ileal digesta and excreta and for prediction of nutritive value using the faecal samples was 100 individual birds

fed 20 different broiler diets similar to commercial broiler diets (Chapter 6). This has taken into account the variations between birds and diet. Even though there was a wide range of chemical composition, it will be worth using more faecal samples representing more wider range of chemical composition. Another limitation was that we haven't performed external validation for the developed ATR-FT MIRS models with new data. As this was a preliminary study on the use of ATR-FT MIRS to assess the chemical composition and nutritive value of broiler diets, we aimed to first check whether ATR-FT MIRS could be utilised for this purpose. Further external validation is recommended as the next step to develop the real-time MIRS technology for rapid on-farm screening. Owing to these facts, a database could be created containing spectral as well as wet chemistry details for broiler diets, ileal digesta, and excreta samples and this database could be updated from time to time for calibrations. As the absorption depends on the nature of the molecular bands of the sample, it is related to its chemical composition. Therefore, this technique requires calibrations for each type of target sample according to their spectral and physiochemical features which is quite a lot of work. This is relatively complex for the digestibility of different nutrients as it requires a large database of samples that have been evaluated *in vivo* (Noel et al., 2022). However, once the calibrations are established and validated, they could be used for a long time, while updating them when new information are available (Belkhanchi et al., 2023). Calibrations could be also developed for the composition and digestibility of individual AA and NSP in the broiler diets and feedstuffs in the future. However, given the wet chemical analysis cost for AA and NSP, developing such calibration will be expensive.

It is important to have accurate reference measurements to develop accurate MIRS calibrations. Indeed, measurements done using standardised protocols in the same laboratory would be more beneficial and it is also highly recommended to scan the samples at the same time as the wet chemistry measurements are made (Noel et al., 2021). However, in the current study, all

samples were scanned using ATR-FT MIRS eight months after the wet chemistry analysis. The samples were initially stored at 4 ^{o}C in airtight containers for wet chemistry analysis and then at room temperature. Therefore, in future studies, samples could be analysed simultaneously using MIRS and wet chemistry to check if the storage time has an impact on the chemical compositions of the samples, thus in the accuracy of MIRS predictions.

Savitzky-Goaly derivative (Savitzky and Golay, 1964) was only applied as a pre-treatment to the averaged spectra to remove the random effects due to sample presentation on the ATR-crystal and to remove the varying components unrelated to the changes in the chemical composition (Chapters 5 & 6). Different mathematical pre-treatments and their combinations such as multiplicative scatter correction (MSC), standard normal variate (SNV), discrete wavelet transform (DWT), vector normalisation (VN) etc. (Chapter 2: section 2.9.2.2) could be applied to identify which technique is more appropriate to improve the spectral data quality (Cleland et al., 2018; Shi et al., 2019).

There are a few studies using NIRS reporting on the combination of spectra from diets and faeces to predict the nutritive value (Meineri et al., 2009; Bastianelli et al., 2013; Coulibaly et al., 2013; Paternostre et al., 2021), however, no studies are reported for MIRS. Different combinations could be evaluated such as averaging, subtracting, and merging the spectra. However, we have only used the subtraction method to predict the nutritive value of broiler diets (Chapter 6). Thus, it will be worth investigating each method separately with different pre-treatment strategies (Paternostre et al., 2021). This may enhance the robustness of the prediction models for the estimation of nutritive value while revealing the information contained in the diets and ileal digesta or excreta in a single scan. We have used freeze-dried and ground samples of excreta to predict the chemical composition and nutritive value of the diets. Further research should also explore the possibility of using either fresh samples or freeze-dried and non-ground samples to determine how sample preparation affect the accuracy

of predictions for chemical composition or nutritive value and if different spectral pre-treatment strategies are needed. If the results from fresh or freeze-dried and non-ground samples are similar to the freeze-dried and ground samples, we could avoid the extensive processing required for excreta samples. Moreover, if fresh samples are used, it could also be possible for early detection of health problems related to the digestion process (Camp Montoro et al., 2023).

8.4. Conclusions

Prediction equations and ATR-FT MIRS offer the benefits of rapid, easier, and cheaper ways to determine the nutritive value of broiler diets.

This study demonstrated that the developed prediction equations based on the gross chemical composition for estimating the nutritive value of broiler chicken diets could accurately predict certain IDC values but were less effective for CAID. Although bootstrapping proved useful for variable selection and internal validation, external validation results indicated limited predictive accuracy, necessitating further refinement of the equations. Future research should focus on enhancing these models to ensure their applicability across diverse diet samples.

It could be highlighted that this is the first study applying ATR-FT MIRS to analyse compound broiler diets. The chemical composition of diets, ileal digesta, and excreta was successfully predicted using the ATR-FT MIRS method. This underscores its potential as a reliable tool for routine analysis when the calibration models are updated. However, direct prediction of CAID from diet spectra was not feasible. The findings suggest that combining spectra of diet and ileal digesta or diet and excreta offers a more effective approach for predicting CAID and cATTD, respectively. Alternatively, integrating chemical composition data determined by ATR-FT MIRS from diets, ileal digesta, and excreta into standard equations can provide accurate estimates of CAID, IDC, and cATTD.

If the prediction models established using ATR-FT MIRS in this thesis are updated with larger data and validated extensively, there is a possibility to reduce the huge cost and time spent in the laboratory for wet chemistry analysis of broiler diets, ileal digesta, and excreta samples. In addition to that, the nutritive value could be predicted directly using the ATR-FT MIRS spectra of diet, ileal digesta, and excreta alone or in combination. This can lead to the development of an additional online tool for rapidly estimating the chemical composition and nutritive value of diets and feedstuffs ensuring accurate formulation of diets for broilers.

REFERENCES

Abbas, O., Pissard, A. & Baeten, V. 2020. Near-infrared, mid-infrared, and Raman spectroscopy. Chemical Analysis of Food, 77-134. https://doi.org/10.1016/B978-0-12-813266-1.00003-6

Abdollahi, M.R., Ravindran, V., Wester, T.J., Ravindran, G. & Thomas, D.V. 2010. Influence of conditioning temperature on performance, apparent metabolisable energy, ileal digestibility of starch and nitrogen and the quality of pellets, in broiler starters fed maize- and sorghum-based diets. Animal Feed Science and Technology, 162, 106-115. https://doi.org/10.1016/j.anifeedsci.2010.08.017

Abdollahi, M.R., Wiltafsky-Martin, M. & Ravindran, V. 2021. Application of apparent metabolizable energy versus nitrogen-corrected apparent metabolizable energy in poultry feed formulations: a continuing conundrum. Animals, 11, 2174. https://doi.org/10.3390/ani11082174

Adiamo, O.Q., Sultanbawa, Y. & Cozzolino, D. 2021. Mid-infrared spectroscopy as a rapid tool to qualitatively predict the effects of species, regions and roasting on the nutritional composition of Australian acacia seed species. Molecules, 26, 1879. https://doi.org/10.3390/molecules26071879

Agelet, L.E. & Hurburgh, C.R. 2010. A tutorial on near infrared spectroscopy and its calibration. Critical Reviews in Analytical Chemistry, 40, 246-260. https://doi.org/10.1080/10408347.2010.515468

Ahmadi, H., Golian, A., Mottaghitalab, M. & Nariman-Zadeh, N. 2008. Prediction model for true metabolizable energy of feather meal and poultry offal meal using group method of data handling-type neural network. Poultry Science, 87, 1909-1912. https://doi.org/10.3382/ps.2007-00507

Ahmadi, H. & Rodehutscord, M. 2017. Application of artificial neural network and support vector machines in predicting metabolizable energy in compound feeds for pigs. Frontiers in Nutrition, 4, 27. https://doi.org/10.3389/fnut.2017.00027

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. In B. N. Petrov & F. Csáki (Eds.), 2nd International symposium on information theory. Budapest, Hungary. Akadémia Kiadó, 267-281.

Akoglu, H. 2018. User's guide to correlation coefficients. Turkish Journal of Emergency Medicine, 18, 91-93. https://doi.org/10.1016/j.tjem.2018.08.001

Alomar, D., Montero, R. & Fuchslocher, R. 1999. Effect of freezing and grinding method on near-infrared reflectance (NIR) spectra variation and chemical composition of fresh silage. Animal Feed Science and Technology, 78, 57-63. https://doi.org/10.1016/S0377-8401(98)00268-5

Altman, D.G. 1990. Practical statistics for medical research. 1st edition. Chapman and Hall/CRC, New York, 1-624. https://doi.org/10.1201/9780429258589

Alvarenga, R.R., Zangeronimo, M.G., Pereira, L., Wolp, R. & Almeida, E. 2013a. Formulation of diets for poultry: the importance of prediction equations to estimate the energy values. Archivos de Zootecnia, 62, 1-11. http://www.redalyc.org/articulo.oa?id=49558826001

Alvarenga, R.R., Rodrigues, P.B., Zangeronimo, M.G., Freitas, R.T. F., Lima, R.R., Bertechini, A.G. & Fassani, E.J. 2011. Energetic values of feedstuffs for broilers determined with *in vivo* assays and prediction equations. Animal Feed Science and Technology, 168, 257-266. https://doi.org/10.1016/j.anifeedsci.2011.04.092

Alvarenga, R.R., Rodrigues, P.B., Zangeronimo, M.G., Makiyama, L., Oliveira, E.C., Freitas, R.T.F., Lima, R.R. & Bernardino, V.M. 2013b. Validation of prediction equations to estimate the energy values of feedstuffs for broilers: performance and carcass yield. Asian-Australasian Journal of Animal Sciences, 26, 1474-1483. https://doi.org/10.5713/ajas.2013.13136

Alvarenga, R.R., Rodrigues, P.B., Zangeronimo, M.G., Oliveira, E.C., Mariano, F.C.M., Lima, E.M.C., Garcia, A.A., Naves, L.P. & Nardelli, N.B.S. 2015. Validation of prediction equations of energy values of a single ingredient or their combinations in male broilers. Asian-Australasian Journal of Animal Sciences, 28, 1335-1344. https://doi.org/10.5713/ajas.14.0339

Andretta, I., Pomar, C., Rivest, J., Pomar, J. & Radünz, J. 2016. Precision feeding can significantly reduce lysine intake and nitrogen excretion without compromising the performance of growing pigs. Animal, 10, 1137-1147. https://doi.org/10.1017/S1751731115003067

AOAC. 2005. Official Methods of Analysis. 18th edition. Association of Official Analytical Chemists,Washington DC, USA.

AOAC. 2016. Official Methods of Analysis. 20th edition. Association of Official Analytical Chemists, Washington DC, USA.

Atteh, J. & Leeson, S. 1983. Effects of dietary fatty acids and calcium levels on performance and mineral metabolism of broiler chickens. Poultry Science, 62, 2412-2419.

https://doi.org/10.3382/ps.0622412

Aureli, R., Ueberschlag, Q., Klein, F., Noël, C. & Guggenbuhl, P. 2017. Use of near infrared reflectance spectroscopy to predict phytate phosphorus, total phosphorus, and crude protein of common poultry feed ingredients. Poultry Science, 96, 160-168. https://doi.org/10.3382/ps/pew214

Austin, P.C. & Tu, J.V. 2004a. Automated variable selection methods for logistic regression produced unstable models for predicting acute myocardial infarction mortality. Journal of Clinical Epidemiology, 57, 1138-1146. https://doi.org/10.1016/j.jclinepi.2004.04.003

Austin, P.C. & Tu, J.V. 2004b. Bootstrap methods for developing predictive models. The American Statistician, 58, 131-137. https://doi.org/10.1198/0003130043277

Babatunde, O., Osho, S., Park, C. & Adeola, O. 2020. Additivity of apparent and standardized ileal digestibility of phosphorus in mixed diets containing corn and soybean meal fed to broiler chickens. Poultry Science, 99, 6907-6913. https://doi.org/10.1016/j.psj.2020.09.022

Baiz, A.A., Ahmadi, H., Shariatmadari, F. & Karimi Torshizi, M.A.K. 2020. A Gaussian process regression model to predict energy contents of corn for poultry. Poultry Science, 99, 5838-5843. https://doi.org/10.1016/j.psj.2020.07.044

Barzegar, S., Wu, S., Noblet, J., Choct, M. & Swick, R. 2019. Energy efficiency and net energy prediction of feed in laying hens. Poultry Science, 98, 5746-5758. https://doi.org/10.3382/ps/pez362

Bastianelli, D. 2013. NIRS as a tool to assess digestibility in feeds and feedstuffs. Proceedings of the International congress on advancements in poultry production in the Middle East and African countries, Antalya, Turkey, 21-25. https://agritrop.cirad.fr/571607/

Bastianelli, D., Bonnal, L., Jaguelin-Peyraud, Y. & Noblet, J. 2015. Predicting feed digestibility from NIRS analysis of pig faeces. Animal, 9, 781-786. https://doi.org/10.1017/S1751731114003097

Bastianelli, D., Bonnal, L., Juin, H., Mignon-Grasteau, S., Davrieux, F. & Carré, B. 2010. Prediction of the chemical composition of poultry excreta by near infrared spectroscopy. Journal of Near Infrared Spectroscopy, 18, 69-77. https://doi.org/10.1255/jnirs.864

Bastianelli, D., Carré, B., Mignon-Grasteau, S., Bonnal, L. & Davrieux, F. 2005. Direct prediction of energy digestibility from poultry faeces using near infrared spectroscopy.

Proceedings of 12th International conference on near infrared spectroscopy, Auckland, New Zealand. IM Publications, 626-629. https://publications.cirad.fr/une_notice.php?dk=531003

Bastianelli, D., Coulibaly, I., Vilariño, M., Chartrin, P., Bouvarel, I., Hogrel, P., Davrieux, F. & Mahaut, B. 2013. Combining spectra from feeds and faeces for NIRS prediction of digestibility in poultry. Proceedings of 16th International conference on near infrared spectroscopy, la Grande-Motte, France. IRSTEA, 677. https://publications.cirad.fr/une_notice.php?dk=571969

Batal, A. & Dale, N. 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. Journal of Applied Poultry Research, 15, 89-93. https://doi.org/10.1093/japr/15.1.89

Bedin, F.C.B., Faust, M.V., Guarneri, G.A., Assmann, T.S., Lafay, C.B.B., Soares, L.F., de Oliveira, P.A.V. & dos Santos-Tonial, L.M. 2021. NIR associated to PLS and SVM for fast and non-destructive determination of C, N, P, and K contents in poultry litter. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 245, 118834. https://doi.org/10.1016/j.saa.2020.118834

Belanche, A., Weisbjerg, M., Allison, G., Newbold, C. & Moorby, J. 2013. Estimation of feed crude protein concentration and rumen degradability by Fourier-transform infrared spectroscopy. Journal of Dairy Science, 96, 7867-7880. https://doi.org/10.3168/jds.2013-7127

Belkhanchi, H., Ziat, Y., Hammi, M. & Ifguis, O. 2023. Formulation, optimization of a poultry feed and analysis of spectrometry, biochemical composition and energy facts. South African Journal of Chemical Engineering, 44, 31-41. https://hdl.handle.net/10520/ejc-chemeng-v44-n1-a6

Bertolini, R., Finch, S.J. & Nehm, R.H. 2022. Quantifying variability in predictions of student performance: Examining the impact of bootstrap resampling in data pipelines. Computers and Education: Artificial Intelligence, 3, 100067. https://doi.org/10.1016/j.caeai.2022.100067

Black, J.L., Hughes, R., Nielsen, S., Tredrea, A. & Flinn, P. 2009. Near infrared reflectance analysis of grains to estimate nutritional value for chickens. Australian Poultry Science Symposium, Sydney, New South Wales, Australia, 31-34.

Boisen, S. & Eggum, B. 1991. Critical evaluation of *in vitro* methods for estimating digestibility in simple-stomach animals. Nutrition Research Reviews, 4, 141-162. https://doi.org/10.1079/NRR19910012

Boisen, S. & Fernandez, J. 1995. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by *in vitro* analyses. Animal Feed Science and Technology, 51, 29-43. https://doi.org/10.1016/0377-8401(94)00686-4

Bresolin, T. & Dórea, J.R. 2020. Infrared spectrometry as a high-throughput phenotyping technology to predict complex traits in livestock systems. Frontiers in Genetics, 11, 923. https://doi.org/10.3389/fgene.2020.00923

Brunelli, A. 2014. A synopsis of resampling techniques. Journal of Thoracic Disease, 6, 1879-1882. https://doi.org/10.3978%2Fj.issn.2072-1439.2014.09.09

Bryan, D.D., Abbott, D.A. & Classen, H.L. 2018. Development of an *in vitro* protein digestibility assay mimicking the chicken digestive tract. Animal Nutrition, 4, 401-409. https://doi.org/10.1016/j.aninu.2018.04.007

Bulang, M. & Rodehutscord, M. 2009. Development of equations for predicting metabolisable energy concentrations in compound feeds for pigs. Archives of Animal Nutrition, 63, 442-454. https://doi.org/10.1080/17450390903217317

Burns, D.A. & Ciurczak, E.W. 2007. Handbook of near-infrared analysis. 3rd edition. CRC press, Boca Raton, 1-834. https://doi.org/10.1201/9781420007374

Butts, C.A., Monro, J.A. & Moughan, P.J. 2012. *In vitro* determination of dietary protein and amino acid digestibility for humans. British Journal of Nutrition, 108, 282-287. https://doi.org/10.1017/S0007114512002310

Calderón, F.J., Vigil, M.F., Reeves III, J.B. & Poss, D.J. 2009. Mid-infrared and near-infrared calibrations for nutritional parameters of triticale (*Triticosecale*) and pea (*Pisum sativum*). Journal of Agricultural and Food Chemistry, 57, 5136-5142. https://doi.org/10.1021/jf803936x

Camp Montoro, J., Solà-Oriol, D., Muns, R., Gasa, J., Llanes, N. & Garcia Manzanilla, E. 2023. Predicting chemical composition and apparent total tract digestibility on freeze-dried not ground faeces using near-infrared spectroscopy in pigs. Animals, 13, 2090. https://doi.org/10.3390/ani13132090

Campbell, G., Salmon, R. & Classen, H. 1986. Prediction of metabolizable energy of broiler diets from chemical analysis. Poultry Science, 65, 2126-2134. https://doi.org/10.3382/ps.0652126

Campbell, M., Ortuño, J., Koidis, A. & Theodoridou, K. 2022. The use of near-infrared and mid-infrared spectroscopy to rapidly measure the nutrient composition and the *in vitro* rumen dry matter digestibility of brown seaweeds. Animal Feed Science and Technology, 285, 115239. https://doi.org/10.1016/j.anifeedsci.2022.115239

Carre, B., Lessire, M. & Juin, H. 2014. Prediction of the net energy value of broiler diets. Animal, 8, 1395-1401. https://doi.org/10.1017/S175173111400130X

Carré, B., Lessire, M. & Juin, H. 2013. Prediction of metabolisable energy value of broiler diets and water excretion from dietary chemical analyses. Animal, 7, 1246-1258. https://doi.org/10.1017/S1751731113000359

Castilho, R.A., Pozza, P.C., Oliveira, N.T.E., Sangali, C.P., Langer, C.N. & Nunes, R.V. 2015. Equations to predict the metabolizable energy of meat and bone meal for growing pigs. Ciencia e Agrotecnologia, 39, 565-573. https://doi.org/10.1590/S1413-70542015000600003

Cavanaugh, J.E. & Neath, A.A. 2019. The Akaike information criterion: Background, derivation, properties, application, interpretation, and refinements. Wiley Interdisciplinary Reviews: Computational Statistics, 11, e1460. https://doi.org/10.1002/wics.1460

Cerrate, S., Ekmay, R., England, J. & Coon, C. 2019. Predicting nutrient digestibility and energy value for broilers. Poultry Science, 98, 3994-4007. https://doi.org/10.3382/ps/pez142

Chai, T. & Draxler, R.R. 2014. Root mean square error (RMSE) or mean absolute error (MAE)?-Arguments against avoiding RMSE in the literature. Geoscientific Model Development, 7, 1247-1250. https://doi.org/10.5194/gmd-7-1247-2014

Chakrabarti, A. & Ghosh, J.K. 2011. AIC, BIC and recent advances in model selection. Philosophy of Statistics, 7, 583-605. https://doi.org/10.1016/B978-0-444-51862-0.50018-6

Chen, L., Yang, Z. & Han, L. 2013. A review on the use of near-infrared spectroscopy for analyzing feed protein materials. Applied Spectroscopy Reviews, 48, 509-522. https://doi.org/10.1080/05704928.2012.756403

Chicco, D., Warrens, M.J. & Jurman, G. 2021. The coefficient of determination R-squared is more informative than SMAPE, MAE, MAPE, MSE and RMSE in regression analysis evaluation. Peerj Computer Science, 7, e623. https://doi.org/10.7717/peerj-cs.623

Choct, M. 2016. Measurements of nutrients and nutritive value. Nutrition experiments in pigs and poultry: A practical guide. CABI Wallingford, UK, 74-98. https://doi.org/10.1079/9781780647005.0074

Chowdhury, M.Z. & Turin, T.C. 2021. Validating prediction models for use in clinical practice: Concept, steps, and procedures focusing on hypertension risk prediction. Hypertension Journal, 7, 21-29. http://doi.org/10.15713/ins.johtn.0221

Cleland, J.D, 2018. ATR-FTIR chemometrics for biological samples. Master of Science in Nanoscience thesis, Massey University, Manawatū, New Zealand. 1-242.

Cleland, J.D., Johnson, E., Morel, P.C.H., Kenyon, P.R. & Waterland, M.R. 2018. Mid-infrared reflectance spectroscopy as a tool for forage feed composition prediction. Animal Feed Science and Technology, 241, 102-111. https://doi.org/10.1016/j.anifeedsci.2018.04.022

Clunies, M. & Leeson, S. 1984. *In vitro* estimation of dry matter and crude protein digestibility. Poultry Science, 63, 89-96. https://doi.org/10.3382/ps.0630089

Coulibaly, I., Métayer, J.P., Chartrin, P., Mahaut, B., Bouvarel, I., Hogrel, P. & Bastianelli, D. 2013. La combinaison des informations issues des aliments et des fientes améliore la prédiction par SPIR de la digestibilité chez le poulet: JRA-JRFG 2013-165, La Rochelle, France, 640-644. https://publications.cirad.fr/une_notice.php?dk=572538

Cozzolino, D., Roumeliotis, S. & Eglinton, J. 2014a. Evaluation of the use of attenuated total reflectance mid infrared spectroscopy to determine fatty acids in intact seeds of barley (*Hordeum vulgare*). LWT-Food Science and Technology, 56, 478-483. https://doi.org/10.1016/j.lwt.2013.11.019

Cozzolino, D., Roumeliotis, S. & Eglinton, J. 2014b. Feasibility study on the use of attenuated total reflectance MIR spectroscopy to measure the fructan content in barley. Analytical Methods, 6, 7710-7715. https://doi.org/10.1039/c4ay01560f

Cruz-Conesa, A. 2023. Novel applications of NIR spectroscopy for the optimization of monogastric animal diets. PhD thesis, Universitat Rovira i Virgili, Spain.

Cruz-Conesa, A., Ferré, J., Pérez-Vendrell, A.M., Callao, M.P. & Ruisánchez, I. 2022. Use of visible-near infrared spectroscopy to predict nutrient composition of poultry excreta. Animal Feed Science and Technology, 283, 115169. https://doi.org/10.1016/j.anifeedsci.2021.115169

Dale, N. 1996. The metabolizable energy of wheat by-products. Journal of Applied Poultry Research, 5, 105-108. https://doi.org/10.1093/japr/5.2.105

David, L.S., Anwar, M.N., Abdollahi, M.R., Bedford, M.R. & Ravindran, V. 2023. Calcium nutrition of broilers: Current perspectives and challenges, Animals, 13, 1590. https://doi.org/10.3390/ani13101590

De Boever, J., Cottyn, B., De Brabander, D., Vanacker, J. & Boucqué, C.V. 1996. Prediction of the feeding value of grass silages by chemical parameters, *in vitro* digestibility and near-infrared reflectance spectroscopy. Animal Feed Science and Technology, 60, 103-115. https://doi.org/10.1016/0377-8401(95)00914-0

De la Roza-Delgado, B., Modroño, S., Vicente, F., Martínez-Fernández, A. & Soldado, A. 2015. Suitability of faecal near-infrared reflectance spectroscopy (NIRS) predictions for estimating gross calorific value. Spanish Journal of Agricultural Research, 13, e0203-e0203. https://doi.org/10.5424/sjar/2015131-6959

De Marchi, M., Toffanin, V., Cassandro, M. & Penasa, M. 2014. Invited review: Mid-infrared spectroscopy as phenotyping tool for milk traits. Journal of Dairy Science, 97, 1171-1186. https://doi.org/10.3168/jds.2013-6799

Decruyenaere, V., Lecomte, P., Demarquilly, C., Aufrere, J., Dardenne, P., Stilmant, D. & Buldgen, A. 2009. Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. Animal Feed Science and Technology, 148, 138-156. https://doi.org/10.1016/j.anifeedsci.2008.03.007

Divekar, M.T., Karunakaran, C., Lahlali, R., Kumar, S., Chelladurai, V., Liu, X., Borondics, F., Shanmugasundaram, S. & Jayas, D.S. 2017. Effect of microwave treatment on the cooking and macronutrient qualities of pulses. International Journal of Food Properties, 20, 409-422. https://doi.org/10.1080/10942912.2016.1163578

Dowell, F., Maghirang, E., Xie, F., Lookhart, G., Pierce, R., Seabourn, B., Bean, S., Wilson, J. & Chung, O. 2006. Predicting wheat quality characteristics and functionality using near-infrared spectroscopy. Cereal Chemistry, 83, 529-536. https://doi.org/10.1094/CC-83-0529

Dutta, A. 2017. Fourier transform infrared spectroscopy. Spectroscopic Methods for Nanomaterials Characterization, 73-93. https://doi.org/10.1016/B978-0-323-46140-5.00004-2

Edwards, H., Dunahoo, W.S., Carmon, J. & Fuller, H.L. 1960. Effect of protein, energy and fat content of the ration on calcium utilization. Poultry Science, 39, 1389-1394. https://doi.org/10.3382/ps.0391389

Efron, B. 1979. Bootstrap Methods: Another Look at the Jackknife. The Annals of Statistics, 7, 1-26. https://doi.org/10.1007/978-1-4612-4380-9_41

Ellis, D.I., Brewster, V.L., Dunn, W.B., Allwood, J.W., Golovanov, A.P. & Goodacre, R. 2012. Fingerprinting food: Current technologies for the detection of food adulteration and contamination. Chemical Society Reviews, 41, 5706-5727. https://doi.org/10.1039/C2CS35138B

Evangelista, C., Basiricò, L. & Bernabucci, U. 2021. An overview on the use of near infrared spectroscopy (NIRS) on farms for the management of dairy cows. Agriculture, 11, 296. https://doi.org/10.3390/agriculture11040296

Faber, N.K.M. 1999. Estimating the uncertainty in estimates of root mean square error of prediction: application to determining the size of an adequate test set in multivariate calibration. Chemometrics and Intelligent Laboratory Systems, 49, 79-89. https://doi.org/10.1016/S0169-7439(99)00027-1

Farrell, D. 1999. *In vivo* and *in vitro* techniques for the assessment of the energy content of feed grains for poultry: A review. Australian Journal of Agricultural Research, 50, 881-888. https://doi.org/10.1071/AR98173

Ferreira, D., Galão, O., Pallone, J. & Poppi, R. 2014. Comparison and application of near-infrared (NIR) and mid-infrared (MIR) spectroscopy for determination of quality parameters in soybean samples. Food Control, 35, 227-232. https://doi.org/10.1016/j.foodcont.2013.07.010

Ferreira, S.L., Vasconcellos, R.S., Rossi, R.M., Paula, V.R.C., Fachinello, M.R., Huepa, L.M.D. & Pozza, P.C. 2018. Using near infrared spectroscopy to predict metabolizable energy of corn for pigs. Scientia Agricola, 75, 486-493. http://dx.doi.org/10.1590/1678-992X-2016-0509

Garnsworthy, P., Wiseman, J. & Fegeros, K. 2000. Prediction of chemical, nutritive and agronomic characteristics of wheat by near infrared spectroscopy. The Journal of Agricultural Science, 135, 409-417. https://doi.org/10.1017/S0021859699008382

Givens, D., De Boever, J. & Deaville, E. 1997. The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans. Nutrition Research Reviews, 10, 83-114. https://doi.org/10.1079/NRR19970006

Givens, D. & Deaville, E. 1999. The current and future role of near infrared reflectance spectroscopy in animal nutrition: A review. Australian Journal of Agricultural Research, 50, 1131-1145. https://doi.org/10.1071/AR98014

Gizzi, G. & Givens, D. 2004. Variability in feed composition and its impact on animal production. In : Asssessing quality and safety of animal feeds. FAO Animal Production and Health Paper, 160, 36-52. https://www.fao.org/4/y5159e/y5159e04.htm

Govoni, C., Chiarelli, D.D., Luciano, A., Ottoboni, M., Perpelek, S.N., Pinotti, L. & Rulli, M.C. 2021. Global assessment of natural resources for chicken production. Advances in Water Resources, 154, 103987. https://doi.org/10.1016/j.advwatres.2021.103987

Grant-Peters, M., Rich-Griffin, C., Grant-Peters, J.E., Cinque, G. & Dendrou, C.A. 2022. Photizo: an open-source library for cross-sample analysis of FTIR spectroscopy data. Bioinformatics, 38, 3490-3492. https://doi.org/10.1093/bioinformatics/btac346

Griffith, F., Grainger, R. & Begin, J.J. 1961. The effect of dietary fat and cellulose on apparent calcium digestibility in growing chickens. Poultry Science, 40, 1492-1497. https://doi.org/10.3382/ps.0401492

Griffiths, P.R. 2006. Introduction to vibrational spectroscopy. Handbook of vibrational spectroscopy, John Wiley & Sons Inc. https://doi.org/10.1002/9780470027325.s8901

Guerra, A., Simoni, M., Longobardi, V., Goi, A., Mantovani, G., Danese, T., Neglia, G., De Marchi, M. & Righi, F. 2024. Effectiveness of near-infrared spectroscopy to predict the chemical composition of faeces and total-tract apparent nutrients digestibility estimated with uNDF or AIA in lactating buffaloes' faeces. Journal of Dairy Science, 107, 5653-5666. https://doi.org/10.3168/jds.2023-24511

Haas, J. & Mizaikoff, B. 2016. Advances in mid-infrared spectroscopy for chemical analysis. Annual Review of Analytical Chemistry, 9, 45-68. https://doi.org/10.1146/annurev-anchem-071015-041507

Hakansson, J. 1974. Factors affecting the digestibility of fats and fatty acids in chicks and hens. Swedish Journal of Agricultural Research. 4, 33-47.

Harel, O. 2009. The estimation of R^2 and adjusted R^2 in incomplete data sets using multiple imputation. Journal of Applied Statistics, 36, 1109-1118. https://doi.org/10.1080/02664760802553000

Harrell, F.E. 2015. General aspects of fitting regression models. Regression modeling strategies: with applications to linear models, logistic and ordinal regression, and survival analysis, New York, Springer, 1-572. https://doi.org/10.1007/978-3-319-19425-7

Hell, J., Prückler, M., Danner, L., Henniges, U., Apprich, S., Rosenau, T., Kneifel, W. & Böhmdorfer, S. 2016. A comparison between near-infrared (NIR) and mid-infrared (ATR-FTIR) spectroscopy for the multivariate determination of compositional properties in wheat bran samples. Food Control, 60, 365-369. https://doi.org/10.1016/j.foodcont.2015.08.003

Honda, K., Kamisoyama, H., Isshiki, Y. & Hasegawa, S. 2009. Effects of dietary fat levels on nutrient digestibility at different sites of chicken intestines. The Journal of Poultry Science, 46, 291-295. https://doi.org/10.2141/jpsa.46.291

Huang, Y., Carragher, J. & Cozzolino, D. 2016. Measurement of fructose, glucose, maltose and sucrose in barley malt using attenuated total reflectance mid-infrared spectroscopy. Food Analytical Methods, 9, 1079-1085. https://doi.org/10.1007/s12161-015-0286-4

James, G., Witten, D., Hastie, T. & Tibshirani, R. 2013. An introduction to statistical learning, Springer, 1-607. https://doi.org/10.1007/978-3-031-38747-0

Jancewicz, L.J., Swift, M.L., Penner, G., Beauchemin, K., Koenig, K., Chibisa, G., He, M., McKinnon, J., Yang, W.Z. & McAllister, T.A. 2016. Development of near-infrared spectroscopy calibrations to estimate faecal composition and nutrient digestibility in beef cattle. Canadian Journal of Animal Science, 97, 51-64. https://doi.org/10.1139/cjas-2016-0107

Jha, R. & Mishra, P. 2021. Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: A review. Journal of Animal Science and Biotechnology, 12, 1-16. https://doi.org/10.1186/s40104-021-00576-0

Jha, R. & Tiwari, U.P. 2016. Rapid techniques for feed evaluation: scope and limitations. Proceedings of the 13th New Zealand Poultry Industry Conference, 84-102.

Johnson, J.B., Walsh, K.B. & Naiker, M. 2023a. Assessment of bioactive compounds in faba bean using infrared spectroscopy. Legume Science, 5, e203. https://doi.org/10.1002/leg3.203

Johnson, J.B., Walsh, K B., Naiker, M. & Ameer, K. 2023b. The use of infrared spectroscopy for the quantification of bioactive compounds in food: A review. Molecules, 28, 3215. https://doi.org/10.3390/molecules28073215

Karch, J. 2020. Improving on adjusted R-squared. Collabra: Psychology, 6, 45. https://doi.org/10.1525/collabra.343

Karoui, R., Downey, G. & Blecker, C. 2010. Mid-infrared spectroscopy coupled with chemometrics: A tool for the analysis of intact food systems and the exploration of their molecular structure-quality relationships - A review. Chemical Reviews, 110, 6144-6168. https://doi.org/10.1021/cr100090k

Karunakaran, C., Vijayan, P., Stobbs, J., Bamrah, R.K., Arganosa, G. & Warkentin, T.D. 2020. High throughput nutritional profiling of pea seeds using Fourier transform mid-infrared spectroscopy. Food Chemistry, 309, 125585.

https://doi.org/10.1016/j.foodchem.2019.125585

Karunasingha, D.S.K. 2022. Root mean square error or mean absolute error? Use their ratio as well. Information Sciences, 585, 609-629. https://doi.org/10.1016/j.ins.2021.11.036

Khaleduzzaman, A., Mamun, M. & Salim, H. 2017. Development of local calibrations for the nutritional evaluation of commercial poultry diets by using near infrared reflectance spectroscopy. Journal of Applied Animal Research, 45, 8-14. https://doi.org/10.1080/09712119.2015.1091332

Khaleduzzaman, A. & Salim, H. 2020. Development of local calibrations for the nutritional evaluation of fish meal and meat & bone meal by using near-infrared reflectance spectroscopy. Journal of Applied Animal Research, 48, 257-263. https://doi.org/10.1080/09712119.2020.1776715

Khalil, M.M., Abdollahi, M.R., Zaefarian, F. & Ravindran, V. 2021. Influence of feed form on the apparent metabolisable energy of feed ingredients for broiler chickens. Animal Feed Science and Technology, 271, 114754. https://doi.org/10.1016/j.anifeedsci.2020.114754

Khan, M.A., Mahr-Un-Nisa, M.U.N. & Sarwar, M. 2003. Techniques measuring digestibility for the nutritional evaluation of feeds. International Journal of Agriculture and Biology. 5, 91-94.

Kho, E.A., Fernandes, J.N., Tilbrook, A.J., Fox, G.P., Sikulu-Lord, M. ., Kotze, A.C., Beasley, A.M., James, P.J., Tolleson, D.R. & Cozzolino, D. 2023. State of the art and the future of faecal analysis using infrared spectroscopy. Applied Spectroscopy Reviews, 58, 755-785. https://doi.org/10.1080/05704928.2022.2143795

Kneebone, D. & Dryden, G.M. 2014. Prediction of diet quality for sheep from faecal characteristics: Comparison of near-infrared spectroscopy and conventional chemistry predictive models. Animal Production Science, 55, 1-10. https://doi.org/10.1071/AN13252

Knudsen, K.E.B., Noel, S. & Jørgensen, H. 2023. Assessment of the nutritive value of individual feeds and diets by novel technologies. In Smart Livestock Nutrition; Springer International Publishing: Cham, Switzerland, 71-101. https://doi.org/10.1007/978-3-031-22584-0

Kong, C. & Adeola, O. 2014. Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian-Australasian Journal of Animal Sciences, 27, 917-925. https://doi.org/10.5713%2Fajas.2014.r.02

Lastras, C., Revilla, I., González-Martín, M. & Vivar-Quintana, A. 2021. Prediction of fatty acid and mineral composition of lentils using near infrared spectroscopy. Journal of Food Composition and Analysis, 102, 104023. https://doi.org/10.1016/j.jfca.2021.104023

Lavine, B. & Workman, J. 2010. Chemometrics. Analytical Chemistry, 82, 4699-4711. https://doi.org/10.1021/ac101202z

Lee, L.C., Liong, C.Y. & Jemain, A.A. 2017. A contemporary review on data preprocessing (DP) practice strategy in ATR-FTIR spectrum. Chemometrics and Intelligent Laboratory Systems, 163, 64-75. https://doi.org/10.1016/j.chemolab.2017.02.008

Lee, S.A., Ahn, J.Y. & Kim, B.G. 2022. Digestible and metabolizable energy concentrations in cereal grains and byproduct ingredients fed to growing pigs. Animal Feed Science and Technology, 292, 115408. https://doi.org/10.1016/j.anifeedsci.2022.115408

Leeson, S. & Summers, J. 2005. Commercial poultry nutrition. 3rd edition. Nottingham University Press, UK, 1-398.

Leigh, J.P. 1988. Assessing the importance of an independent variable in multiple regression: Is stepwise unwise? Journal of Clinical Epidemiology, 41, 669-677. https://doi.org/10.1016/0895-4356(88)90119-9

Lemme, A., Ravindran, V. & Bryden, W. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poultry Science Journal, 60, 423-438. https://doi.org/10.1079/WPS200426

Lin, L.I.K. 1989. A concordance correlation coefficient to evaluate reproducibility. Biometrics, 255-268. https://doi.org/10.2307/2532051

Linker, R., Shmulevich, I., Kenny, A. & Shaviv, A. 2005. Soil identification and chemometrics for direct determination of nitrate in soils using FTIR-ATR mid-infrared spectroscopy. Chemosphere, 61, 652-658. https://doi.org/10.1016/j.chemosphere.2005.03.034

Liu, J., Li, Q., Zeng, Z., Li, P., Xu, X., Wang, H., Zhang, S. & Piao, X. 2015. Determination and prediction of the amino acid digestibility of sunflower seed meals in growing pigs. Asian-Australasian Journal of Animal Sciences, 28, 86-94. https://doi.org/10.5713%2Fajas.14.0109

Losada, B., García-Rebollar, P., Álvarez, C., Cachaldora, P., Ibáñez, M.A., Méndez, J. & De Blas, J.C. 2010. The prediction of apparent metabolisable energy content of oil seeds and oil seed by-products for poultry from its chemical components, *in vitro* analysis or near-infrared reflectance spectroscopy. Animal Feed Science and Technology, 160, 62-72. https://doi.org/10.1016/j.anifeedsci.2010.06.012

Losada, B., Rebollar, P.G., Cachaldora, P., Álvarez, C. & de Blas, J.C. 2009. A comparison of the prediction of apparent metabolisable energy content of starchy grains and cereal by-products for poultry from its chemical components, *in vitro* analysis or near-infrared reflectance spectroscopy. Spanish Journal of Agricultural Research, 813-823.

Lu, X. & Rasco, B. A. 2012. Determination of antioxidant content and antioxidant activity in foods using infrared spectroscopy and chemometrics: A review. Critical Reviews in Food Science and Nutrition, 52, 853-875. https://doi.org/10.1080/10408398.2010.511322

Lyons, G., Sharma, S., Aubry, A., Carmichael, E. & Annett, R. 2016. A preliminary evaluation of the use of mid infrared spectroscopy to develop calibration equations for determining faecal composition, intake and digestibility in sheep. Animal Feed Science and Technology, 221, 44-53. https://doi.org/10.1016/j.anifeedsci.2016.08.014

Ma, X., Zhang, S., Shang, Q., Long, S. & Piao, X. 2019. Determination and prediction of the apparent and standardized ileal amino acid digestibility in cottonseed meals fed to growing pigs. Animal Science Journal, 90, 655-666. https://doi.org/10.1111/asj.13195

Mahesar, S., Sherazi, S., Kandhro, A., Bhanger, M., Khaskheli, A. & Talpur, M. 2011. Evaluation of important fatty acid ratios in poultry feed lipids by ATR FTIR spectroscopy. Vibrational Spectroscopy, 57, 177-181. https://doi.org/10.1016/j.vibspec.2011.06.009

Mallick, P., Muduli, K., Biswal, J.N. & Pumwa, J. 2020. Broiler poultry feed cost optimization using linear programming technique. Journal of Operations and Strategic Planning, 3, 31-57. https://doi.org/10.1177/2516600X19896910

Mariano, F.C., Paixão, C.A., Lima, R.R., Alvarenga, R.R., Rodrigues, P.B. & Nascimento, G.A. 2013. Prediction of the energy values of feedstuffs for broilers using meta-analysis and neural networks. Animal, 7, 1440-1445. https://doi.org/10.1017/S1751731113000712

Mark, H. & Workman, J. 2018. Chapter 75-The statistics of spectral searches. Chemometrics in Spectroscopy, 507-511. https://doi.org/10.1016/C2015-0-04023-0

Marshall, A.C., Lopez-Villalobos, N., Loveday, S.M., Ellis, A. & McNabb, W. 2023. Modelling lactation curves for dairy sheep in a New Zealand flock. Animals, 13, 349. https://doi.org/10.3390/ani13030349

Masood, W., Khan, S.H., Bhatti, S.A. & Parveen, A. 2011. Comparison of sample source (excreta or ileal digesta) and age of broiler chick on measurement of apparent metabolisable energy of local feed ingredients. Journal of Applied Animal Research, 39, 359-366. https://doi.org/10.1080/09712119.2011.621534

Mateos, G.G., Cámara, L., Fondevila, G. & Lázaro, R.P. 2019. Critical review of the procedures used for estimation of the energy content of diets and ingredients in poultry. Journal of Applied Poultry Research, 28, 506-525. https://doi.org/10.3382/japr/pfy025

Matthiesen, C.F., Pettersson, D., Smith, A., Pedersen, N.R. & Storm, A.C. 2021. Exogenous xylanase improves broiler production efficiency by increasing proximal small intestine digestion of crude protein and starch in wheat-based diets of various viscosities. Animal Feed Science and Technology, 272, 114739. https://doi.org/10.1016/j.anifeedsci.2020.114739

Mayo, D.W., Miller, F.A. & Hannah, R. W. 2004. Course notes on the interpretation of infrared and Raman spectra, John Wiley & Sons, 1-559. https://doi.org/10.1002/0471690082

Mazabel, J., Worthington, M., Castiblanco, V., Peters, M. & Arango, J. 2020. Using near infrared reflectance spectroscopy for estimating nutritional quality of *Brachiaria humidicola* in breeding selections. Agrosystems, Geosciences & Environment, 3, e20070. https://doi.org/10.1002/agg2.20070

McBride, G. 2005. A proposal for strength-of-agreement criteria for Lin's concordance correlation coefficient. NIWA Client Report; National Institute of Water & Atmospheric Research: Hamilton, New Zealand. http://www.medcalc.org/download/pdf/McBride2005.pdf.

Mehri, M. 2013. A comparison of neural network models, fuzzy logic, and multiple linear regression for prediction of hatchability. Poultry Science, 92, 1138-1142. https://doi.org/10.3382/ps.2012-02827

Meineri, G., Giorgio Peiretti, P. & Masoero, G. 2009. Appraisal of ingestion and digestibility in growing rabbits using near infrared reflectance spectroscopy (NIRS) of feeds and faeces. Italian Journal of Animal Science, 8, 75-82. https://doi.org/10.4081/ijas.2009.75

Meloche, K.J., Kerr, B.J., Shurson, G.C. & Dozier III, W.A. 2013. Apparent metabolizable energy and prediction equations for reduced-oil corn distillers dried grains with solubles in broiler chicks from 10 to 18 days of age. Poultry science, 92, 3176-3183. https://doi.org/10.3382/ps.2013-03290

Meloche, K. J. 2013. The development and validation of prediction equations for the apparent metabolizable energy of distillers dried grains with solubles in broilers. Master of Science thesis, Auburn University, USA. http://hdl.handle.net/10415/3859

Meloche, K.J., Kerr, B.J., Billor, N., Shurson, G.C. & Dozier III, W.A. 2014. Validation of prediction equations for apparent metabolizable energy of corn distillers dried grains with solubles in broiler chicks. Poultry Science, 93, 1428-1439. https://doi.org/10.3382/ps.2013-03712

Michels, D., Verkempinck, S.H., Panozzo, A., Vermeulen, K., Hendrickx, M.E., Thijs, L. & Grauwet, T. 2023. Importance of adapted digestion conditions to simulate *in vitro* lipid digestion of broilers in different life stages. Animal Nutrition, 12, 151-158. https://doi.org/10.1016/j.aninu.2022.09.008

Miles, J. 2005. R-squared, adjusted R-squared. Encyclopedia of Statistics in Behavioral Science. https://doi.org/10.1002/0470013192.bsa526

Miller, J.N. & Miller, J.C. 2005. Statistics and Chemometrics for Analytical Chemistry. 5th edition. Pearson/Prentice Hall: Harlow, England. 1-276. https://doi.org/10.1198/tech.2004.s248

Minekus, M., Marteau, P., Havenaar, R. & Veld, J.H.H. 1995. A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. Alternatives to Laboratory Animals, 23, 197-209. https://doi.org/10.1177/026119299502300205

Misiura, M.M., Filipe, J.A. & Kyriazakis, I. 2023. Mathematical and statistical approaches to the challenge of forecasting animal performance for the purposes of precision livestock feeding. In Smart Livestock Nutrition; Springer International Publishing: Cham, Switzerland, 141-167. https://doi.org/10.1007/978-3-031-22584-0

Mkhabela, M., Bullock, P., Raj, S., Wang, S. & Yang, Y. 2011. Crop yield forecasting on the Canadian Prairies using MODIS NDVI data. Agricultural and Forest Meteorology, 151, 385-393. https://doi.org/10.1016/j.agrformet.2010.11.012

Montgomery, D.C., Peck, E.A. & Vining, G.G. 2021. Introduction to linear regression analysis, John Wiley & Sons, Hoboken, NJ, USA, 1-821.

Moore, S., Stalder, K., Beitz, D., Stahl, C., Fithian, W. & Bregendahl, K. 2008. The correlation of chemical and physical corn kernel traits with production performance in broiler chickens and laying hens. Poultry Science, 87, 665-676. https://doi.org/10.3382/ps.2007-00184

Morais, C.L., Lima, K.M., Singh, M. & Martin, F.L. 2020. Tutorial: Multivariate classification for vibrational spectroscopy in biological samples. Nature Protocols, 15, 2143-2162. https://doi.org/10.1038/s41596-020-0322-8

Morgan, C., Whittemore, C., Phillips, P. & Crooks, P. 1987. The prediction of the energy value of compounded pig foods from chemical analysis. Animal Feed Science and Technology, 17, 81-107. https://doi.org/10.1016/0377-8401(87)90007-1

Moss, A.F., Chrystal, P.V., Cadogan, D.J., Wilkinson, S.J., Crowley, T.M. & Choct, M. 2021. Precision feeding and precision nutrition: A paradigm shift in broiler feed formulation? Animal Bioscience, 34, 354-362. https://doi.org/10.5713%2Fab.21.0034

Moughan, P.J. 1999. *In vitro* techniques for the assessment of the nutritive value of feed grains for pigs: A review. Australian Journal of Agricultural Research, 50, 871-880. https://doi.org/10.1071/AR98172

Mtei, A., Abdollahi, M., Schreurs, N., Girish, C. & Ravindran, V. 2019. Dietary inclusion of fibrous ingredients and bird type influence apparent ileal digestibility of nutrients and energy utilization. Poultry Science, 98, 6702-6712. https://doi.org/10.3382/ps/pez383

Mutucumarana, R.K., Ravindran, V., Ravindran, G. & Cowieson, A.J. 2014. Influence of dietary calcium concentration on the digestion of nutrients along the intestinal tract of broiler chickens. The Journal of Poultry Science, 51, 392-401. https://doi.org/10.2141/jpsa.0140022

Nascimento, G.A.J., Rodrigues, P.B., Freitas, R.T.F., Bertechini, A.G., Lima, R.R. & Pucci, L.E. 2009. Prediction equations to estimate the energy values of plant origin concentrate feeds for poultry utilizing the meta-analysis. Revista Brasileira de Zootecnia, 38, 1265-1271. https://doi.org/10.1590/S1516-35982009000700015

Nieto-Ortega, B., Arroyo, J.J., Walk, C., Castañares, N., Canet, E. & Smith, A. 2022. Near infrared reflectance spectroscopy as a tool to predict non-starch polysaccharide composition and starch digestibility profiles in common monogastric cereal feed ingredients. Animal Feed Science and Technology, 285, 115214. https://doi.org/10.1016/j.anifeedsci.2022.115214

Nirea, K.G., Pérez de Nanclares, M., Skugor, A., Afseth, N K., Meuwissen, T.H., Hansen, J.Ø., Mydland, L.T. & Øverland, M. 2018. Assessment of faecal near-infrared spectroscopy to predict faeces chemical composition and apparent total-tract digestibility of nutrients in pigs. Journal of Animal Science, 96, 2826-2837. https://doi.org/10.1093/jas/sky182

Noblet, J. & Pérez, J.M. 1993. Prediction of digestibility of nutrients and energy values of pig diets from chemical analysis. Journal of Animal Science, 71, 3389-3398. https://doi.org/10.2527/1993.71123389x

Noblet, J., Wu, S.B. & Choct, M. 2022. Methodologies for energy evaluation of pig and poultry feeds: A review. Animal Nutrition, 8, 185-203.

https://doi.org/10.1016/j.aninu.2021.06.015

Noel, S.J., Jørgensen, H.J.H. & Knudsen, K.E.B. 2021. Prediction of protein and amino acid composition and digestibility in individual feedstuffs and mixed diets for pigs using near-infrared spectroscopy. Animal Nutrition, 7, 1242-1252. https://doi.org/10.1016/j.aninu.2021.07.004

Noel, S.J., Jørgensen, H.J.H. & Knudsen, K.E.B. 2022. The use of near-infrared spectroscopy (NIRS) to determine the energy value of individual feedstuffs and mixed diets for pigs. Animal Feed Science and Technology, 283, 115156. https://doi.org/10.1016/j.anifeedsci.2021.115156

NRC. 1994. Nutrient requirements of poultry: 9th Revised edition. Washington, DC, The National Academies Press. https://doi.org/10.17226/2114

Nunez, E., Steyerberg, E.W. & Nunez, J. 2011. Regression modeling strategies. Revista Española de Cardiología (English Edition), 64, 501-507. https://doi.org/10.1016/j.rec.2011.01.017

OECD/FAO. 2022. OECD-FAO Agricultural outlook 2022-2031. OECD publishing, Paris, France. https://doi.org/10.1787/f1b0b29c-en

Oliveira, N.T.E., Pozza, P.C., Castilha, L.D., Pasquetti, T.J. & Langer, C.N. 2019. Metabolisable energy prediction in energy feedstuffs and evaluation of the stepwise validation procedure using bootstrapping. Revista Ciencia Agronomica, 50, 131-139. https://doi.org/10.5935/1806-6690.20190016

Ozaki, Y. 2021. Infrared spectroscopy - Mid-infrared, near-infrared, and far-infrared/terahertz spectroscopy. Analytical Sciences, 37, 1193-1212. https://doi.org/10.2116/analsci.20r008

Paternostre, L., Baeten, V., Ampe, B., Millet, S. & De Boever, J. 2021. The usefulness of NIRS calibrations based on feed and faeces spectra to predict nutrient content, digestibility and net energy of pig feeds. Animal Feed Science and Technology, 281, 115091. https://doi.org/10.1016/j.anifeedsci.2021.115091

Pedersen, C., Boersma, M. & Stein, H. 2007. Digestibility of energy and phosphorus in ten samples of distillers dried grains with solubles fed to growing pigs. Journal of Animal Science, 85, 1168-1176. https://doi.org/10.2527/jas.2006-252

Pedersen, N.B., Zaefarian, F., Storm, A.C., Ravindran, V. & Cowieson, A.J. 2021. Mathematical prediction of ileal energy and protein digestibility in broilers using multivariate data analysis. Poultry Science, 100, 101106. https://doi.org/10.1016/j.psj.2021.101106

Pérez-Marín, D.C., Garrido-Varo, A., Guerrero-Ginel, J. & Gómez-Cabrera, A. 2004. Near-infrared reflectance spectroscopy (NIRS) for the mandatory labelling of compound feedingstuffs: chemical composition and open-declaration. Animal Feed Science and Technology, 116, 333-349. https://doi.org/10.1016/j.anifeedsci.2004.05.002

Petit, S. & Madejova, J. 2013. Fourier transform infrared spectroscopy. Developments in Clay Science, 5, 213-231. https://doi.org/10.1016/S0167-9244(97)80013-3

Prieto, N., Roehe, R., Lavín, P., Batten, G. & Andrés, S. 2009. Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. Meat Science, 83, 175-186. https://doi.org/10.1016/j.meatsci.2009.04.016

Qiao, Y. & Van Kempen, T. 2004. Comparison of Raman, mid, and near infrared spectroscopy for predicting the amino acid content in animal meals. Journal of Animal Science, 82, 2596-2600. https://doi.org/10.2527/2004.8292596x

Raccary, B., Loubet, P., Peres, C. & Sonnemann, G. 2022. Evaluating the environmental impacts of analytical chemistry methods: From a critical review towards a proposal using a life cycle approach. TrAC Trends in Analytical Chemistry, 147, 116525. https://doi.org/10.1016/j.trac.2022.116525

Ramoelo, A., Skidmore, A.K., Cho, M.A., Schlerf, M., Mathieu, R. & Heitkönig, I.M.A. 2012. Regional estimation of savanna grass nitrogen using the red-edge band of the spaceborne RapidEye sensor. International Journal of Applied Earth Observation and Geoinformation, 19, 151-162. https://doi.org/10.1016/j.jag.2012.05.009

Ramos Cruz, R., Basurto Gutiérrez, R., Ramírez Rodríguez, E., Reis de Souza, T.C. & Mariscal Landín, G. 2023. Prediction of the chemical composition of pig faeces and ileal digesta by near-infrared reflectance spectroscopy (NIRS). Revista mexicana de ciencias pecuarias, 14, 488-504. https://doi.org/10.22319/rmcp.v14i3.6175

Ravindran, V. & Bryden, W.L. 1999. Amino acid availability in poultry - *In vitro* and *in vivo* measurements. Australian Journal of Agricultural Research, 50, 889-908. https://doi.org/10.1071/AR98174

Ravindran, V. 2013. Poultry feed availability and nutrition in developing countries. Poultry Development Review, 2, 60-63.

Ravindran, V., Hew, L.I., Ravindran, G. & Bryden, W.L. 2007. Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. Animal Science, 81, 85-97. https://doi.org/10.1079/ASC42240085

Ravindran, V., Adeola, O., Rodehutscord, M., Kluth, H., Van der Klis, J., Van Eerden, E. & Helmbrecht, A. 2017. Determination of ileal digestibility of amino acids in raw

materials for broiler chickens - Results of collaborative studies and assay recommendations. Animal Feed Science and Technology, 225, 62-72. https://doi.org/10.1016/j.anifeedsci.2017.01.006

Reeves, J. & Van Kessel, J. 2000. Near-infrared spectroscopic determination of carbon, total nitrogen, and ammonium-N in dairy manures. Journal of Dairy Science, 83, 1829-1836. https://doi.org/10.3168/jds.S0022-0302(00)75053-3

Reeves, J.B. 2001a. Near-infrared diffuse reflectance spectroscopy for the analysis of poultry manures. Journal of Agricultural and Food Chemistry, 49, 2193-2197. https://doi.org/10.1021/jf0013961

Reeves, J.B. 2001b. Near- versus mid-infrared diffuse reflectance spectroscopy for determination of minerals in dried poultry manure. Poultry Science, 80, 1437-1443. https://doi.org/10.1093/ps/80.10.1437

Renaud, O. & Victoria-Feser, M.P. 2010. A robust coefficient of determination for regression. Journal of Statistical Planning and Inference, 140, 1852-1862. https://doi.org/10.1016/j.jspi.2010.01.008

Rinnan, Å., Van Den Berg, F. & Engelsen, S.B. 2009. Review of the most common pre-processing techniques for near-infrared spectra. TrAC Trends in Analytical Chemistry, 28, 1201-1222. https://doi.org/10.1016/j.trac.2009.07.007

Rochell, S.J., Kerr, B J. & Dozier, III W.A. 2011. Energy determination of corn co-products fed to broiler chicks from 15 to 24 days of age, and use of composition analysis to predict nitrogen-corrected apparent metabolizable energy. Poultry Science, 90, 1999-2007. https://doi.org/10.3382/ps.2011-01468

Rombach, M., Sudekum, K.H., Munger, A. & Schori, F. 2019. Herbage dry matter intake estimation of grazing dairy cows based on animal, behavioral, environmental, and feed variables. Journal of Dairy Science, 102, 2985-2999. https://doi.org/10.3168/jds.2018-14834

Ross. 2022. Ross 308 Broiler: Nutrient specifications. Ross Breeders Limited, Newbridge, Midlothian, Scotland, UK.

Royston, P. & Sauerbrei, W. 2009. Bootstrap assessment of the stability of multivariable models. The Stata Journal, 9, 547-570. https://doi.org/10.1177/1536867X0900900403

Sakamoto, K., Asano, T., Furuya, S. & Takahashi, S. 1980. Estimation of *in vivo* digestibility with the laying hen by an *in vitro* method using the intestinal fluid of the pig. British Journal of Nutrition, 43, 389-391. https://doi.org/10.1079/BJN19800103

Sales, J. & Janssens, G. 2003. The use of markers to determine energy metabolizability and nutrient digestibility in avian species. World's Poultry Science Journal, 59, 314-327. https://doi.org/10.1079/WPS20030019

Samadi, S., Wajizah, S. & Munawar, A.A. 2020. Near infrared spectroscopy (NIRS) data analysis for a rapid and simultaneous prediction of feed nutritive parameters. Data in Brief, 29, 105211. https://doi.org/10.1016/j.dib.2020.105211

SAS. 2016. SAS software, version 9.4. SAS Institute Cary, NC, USA.

Sauerbrei, W. 1999. The use of resampling methods to simplify regression models in medical statistics. Journal of the Royal Statistical Society Series C: Applied Statistics, 48, 313-329. https://doi.org/10.1111/1467-9876.00155

Sauerbrei, W. & Schumacher, M. 1992. A bootstrap resampling procedure for model building: Application to the Cox regression model. Statistics in Medicine, 11, 2093-2109. https://doi.org/10.1002/sim.4780111607

Savitzky, A. & Golay, M.J. 1964. Smoothing and differentiation of data by simplified least squares procedures. Analytical Chemistry, 36, 1627-1639. https://doi.org/10.1021/ac60214a047

Scalon, J.D., Freire, S.M. & Cunha, T.A. 1998. Validation of models for predicting the use of health technologies. Medical Decision Making, 18, 311-319. https://doi.org/10.1177/0272989X9801800309

Schiborra, A., Bulang, M., Berk, A., Susenbeth, A. & Schlecht, E. 2015. Using faecal near-infrared spectroscopy (FNIRS) to estimate nutrient digestibility and chemical composition of diets and faeces of growing pigs. Animal Feed Science and Technology, 210, 234-242. https://doi.org/10.1016/j.anifeedsci.2015.10.011

Scott, T. & Boldaji, F. 1997. Comparison of inert markers [chromic oxide or insoluble ash (Celite)] for determining apparent metabolizable energy of wheat-or barley-based broiler diets with or without enzymes. Poultry Science, 76, 594-598. https://doi.org/10.1093/ps/76.4.594

Sharma, N.K., Wu, S.B., Morgan, N.K. & Crowley, T.M. 2022. Artificial gut and the applications in poultry: A review. Animal Nutrition. 10, 156-166. https://doi.org/10.1016/j.aninu.2021.12.010

Sheikhhasan, B.S., Moravej, H., Ghaziani, F., Esteve-Garcia, E. & Kim, W.K. 2020a. Relationship between chemical composition and standardized ileal digestible amino acid contents of corn grain in broiler chickens. Poultry Science, 99, 4496-4504. https://doi.org/10.1016/j.psj.2020.06.013

Sheikhhasan, B.S., Moravej, H., Shivazad, M., Ghaziani, F., Esteve-Garcia, E. & Kim, W.K. 2020b. Prediction of the total and standardized ileal digestible amino acid contents from the chemical composition of soybean meals of different origin in broilers. Poultry Science, 99, 4947-4957. https://doi.org/10.1016/j.psj.2020.06.033

Shi, H. & Yu, P. 2017. Comparison of grating-based near-infrared (NIR) and Fourier transform mid-infrared (ATR-FT/MIR) spectroscopy based on spectral preprocessing and wavelength selection for the determination of crude protein and moisture content in wheat. Food Control, 82, 57-65. https://doi.org/10.1016/j.foodcont.2017.06.015

Shi, H., Lei, Y., Prates, L.L. & Yu, P. 2019. Evaluation of near-infrared (NIR) and Fourier transform mid-infrared (ATR-FT/MIR) spectroscopy techniques combined with chemometrics for the determination of crude protein and intestinal protein digestibility of wheat. Food Chemistry, 272, 507-513. https://doi.org/10.1016/j.foodchem.2018.08.075

Shi, X.S. & Noblet, J. 1993. Contribution of the hindgut to digestion of diets in growing pigs and adult sows: effect of diet composition. Livestock Production Science, 34, 237-252. https://doi.org/10.1016/0301-6226(93)90110-4

Short, F.J., Gorton, P., Wiseman, J. & Boorman, K. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology, 59, 215-221. https://doi.org/10.1016/0377-8401(95)00916-7

Sibbald, I. 1982. Measurement of bioavailable energy in poultry feedingstuffs: A review. Canadian Journal of Animal Science, 62, 983-1048. https://doi.org/10.4141/cjas82-123

Silva, E.P., Rabello, C.B.V., Albino, L.F.T., Ludke, J.V., Lima, M.B.D. & Dutra Junior, W.M. 2010. Prediction of metabolizable energy values in poultry offal meal for broiler chickens. Revista Brasileira de Zootecnia, 39, 2237-2245. https://doi.org/10.1590/S1516-35982010001000020

Simoni, M., Goi, A., De Marchi, M. & Righi, F. 2021. The use of visible/near-infrared spectroscopy to predict fibre fractions, fibre-bound nitrogen and total-tract apparent nutrients digestibility in beef cattle diets and faeces. Italian Journal of Animal Science, 20, 814-825. https://doi.org/10.1080/1828051X.2021.1924884

Smeets, N., Nuyens, F., Van Campenhout, L., Delezie, E., Pannecoucque, J. & Niewold, T. 2015. Relationship between wheat characteristics and nutrient digestibility in broilers: comparison between total collection and marker (titanium dioxide) technique. Poultry Science, 94, 1584-1591. https://doi.org/10.3382/ps/pev116

Smith, B., Hassen, A., Hinds, M., Rice, D., Jones, D., Sauber, T., Iiams, C., Sevenich, D., Allen, R. & Owens, F., McNaughton, J., Parsons, C. 2015. Predicting the digestible energy of corn determined with growing swine from nutrient composition and cross-species measurements. Journal of Animal Science, 93, 1025-1038. https://doi.org/10.2527/jas.2014-7807

Smith, T., Pesti, G., Bakalli, R., Kilburn, J. & Edwards Jr, H. 2001. The use of near-infrared reflectance spectroscopy to predict the moisture, nitrogen, calcium, total phosphorus, gross energy, and phytate phosphorus contents of broiler excreta. Poultry Science, 80, 314-319. https://doi.org/10.1093/ps/80.3.314

Son, A.R., Park, C.S. & Kim, B.G. 2017. Determination and prediction of digestible and metabolizable energy concentrations in byproduct feed ingredients fed to growing pigs. Asian-Australasian Journal of Animal Siences, 30, 546-553. https://doi.org/10.5713/ajas.16.0607

Steyerberg, E.W. & Harrell Jr, F.E. 2016. Prediction models need appropriate internal, internal-external, and external validation. Journal of Clinical Epidemiology, 69, 245-247. https://doi.org/10.1016/j.jclinepi.2015.04.005

Steyerberg, E.W., Harrell Jr, F.E., Borsboom, G.J., Eijkemans, M., Vergouwe, Y. & Habbema, J.D.F. 2001. Internal validation of predictive models: Efficiency of some procedures for logistic regression analysis. Journal of Clinical Epidemiology, 54, 774-781. https://doi.org/10.1016/S0895-4356(01)00341-9

Stuth, J., Jama, A. & Tolleson, D. 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. Field Crops Research, 84, 45-56. https://doi.org/10.1016/S0378-4290(03)00140-0

Sun, D.W. 2009. Infrared spectroscopy for food quality analysis and control, Academic press, 1-448. https://doi.org/10.1016/b978-0-12-374136-3.X0001-6

Sung, J.Y. & Kim, B.G. 2021. Prediction equations for digestible and metabolizable energy concentrations in feed ingredients and diets for pigs based on chemical composition. Animal Bioscience, 34, 306-311. https://doi.org/10.5713/ajas.20.0293

Swiech, E. 2017. Alternative prediction methods of protein and energy evaluation of pig feeds. Journal of Animal Science and Biotechnology, 8, 1-14. https://doi.org/10.1186/s40104-017-0171-7

Szczurek, W. 2010. Practical validation of efficacy of the standardized ileal digestible amino acid values in diet formulation for broiler chickens. Journal of Animal and Feed Sciences, 19, 590-598. https://doi.org/10.22358/jafs/66325/2010

Tahir, M., Saleh, F., Ohtsuka, A. & Hayashi, K. 2008. An effective combination of carbohydrases that enables reduction of dietary protein in broilers: Importance of hemicellulase. Poultry Science, 87, 713-718. https://doi.org/10.3382/ps.2007-00340

Tamaki, Y. & Mazza, G. 2011. Rapid determination of carbohydrates, ash, and extractives contents of straw using attenuated total reflectance Fourier transform mid-infrared spectroscopy. Journal of Agricultural and Food Chemistry, 59, 6346-6352. https://doi.org/10.1021/jf200078h

Tancharoenrat, P. & Ravindran, V. 2014. Influence of tallow and calcium concentrations on the performance and energy and nutrient utilization in broiler starters. Poultry Science, 93, 1453-1462. https://doi.org/10.3382/ps.2013-03817

Theodoridou, K. & Yu, P. 2013. Application potential of ATR-FT/IR molecular spectroscopy in animal nutrition: revelation of protein molecular structures of canola meal and presscake, as affected by heat-processing methods, in relationship with their protein digestive behavior and utilization for dairy cattle. Journal of Agricultural and Food Chemistry, 61, 5449-5458. https://doi.org/10.1021/jf400301y

Thiruchchenthuran, S., Lopez-Villalobos, N., Zaefarian, F., Abdollahi, M.R., Wester, T.J., Pedersen, N.B., Storm, A.C., Cowieson, A.J. & Morel, P.C.H. 2024. Evaluation of equations for predicting ileal nutrient digestibility and digestible nutrient content of broiler diets based on their gross chemical composition. Animal Feed Science and Technology, 115974. https://doi.org/10.1016/j.anifeedsci.2024.115974

Thresher, W.C., Swaisgood, H.E. & Catignani, G.L. 1989. Digestibilities of the protein in various foods as determined *in vitro* by an immobilized digestive enzyme assay (IDEA). Plant Foods for Human Nutrition, 39, 59-65. https://doi.org/10.1007/BF01092402

Türker-Kaya, S. & Huck, C.W. 2017. A review of mid-infrared and near-infrared imaging: principles, concepts and applications in plant tissue analysis. Molecules, 22, 168. https://doi.org/10.3390/molecules22010168

Urriola, P.E., Li, M., Kerr, B.J. & Shurson, G.C. 2014. Evaluation of prediction equations to estimate gross, digestible, and metabolizable energy content of maize dried distillers grains with solubles (DDGS) for swine based on chemical composition. Animal Feed Science and Technology, 198, 196-202. https://doi.org/10.1016/j.anifeedsci.2014.09.006

Valdes, E. & Leeson, S. 1992. Near infrared reflectance analysis as a method to measure metabolizable energy in complete poultry feeds. Poultry Science, 71, 1179-1187. https://doi.org/10.3382/ps.0711179

Valdes, E. & Leeson, S. 1994. Measurement of metabolizable energy, gross energy, and moisture in feed grade fats by near infrared reflectance spectroscopy. Poultry Science, 73, 163-171. https://doi.org/10.3382/ps.0730163

Valdes, E., Young, L., Leeson, S., McMillan, I., Portela, F. & Winch, J. 1985. Application of near infrared reflectance spectroscopy to analyses of poultry feeds. Poultry Science, 64, 2136-2142. https://doi.org/10.3382/ps.0642136

Van Barneveld, R., Graham, H. & Diffey, S. 2018. Predicting the nutritional quality of feed ingredients for pigs using near-infrared spectroscopy (NIRS) and chemical analysis. Animal Production Science, 58, 709-718. https://doi.org/10.1071/AN17144

Van de Voort, F. 1992. Fourier transform infrared spectroscopy applied to food analysis. Food Research International, 25, 397-403. https://doi.org/10.1016/0963-9969(92)90115-L

Van der Klis, J. & Fledderus, J. 2007. Evaluation of raw materials for poultry: What's up. Proceedings of the 16th European Symposium on Poultry Nutrition. World Poultry Science Association, Strasbourg, France.

Van Kempen, T. 2001. Infrared technology in animal production. World's Poultry Science Journal, 57, 29-48. https://doi.org/10.1079/WPS20010004

Van Kempen, T. & Bodin, J.C. 1998. Near-infrared reflectance spectroscopy (NIRS) appears to be superior to nitrogen-based regression as a rapid tool in predicting the poultry digestible amino acid content of commonly used feedstuffs. Animal Feed Science and Technology, 76, 139-147. https://doi.org/10.1016/S0377-8401(98)00207-7

Villamide, M. & San Juan, L. 1998. Effect of chemical composition of sunflower seed meal on its true metabolizable energy and amino acid digestibility. Poultry Science, 77, 1884-1892. https://doi.org/10.1093/ps/77.12.1884

Walk, C.L. & Rao, S.V.R. 2020. Increasing dietary phytate has a significant anti-nutrient effect on apparent ileal amino acid digestibility and digestible amino acid intake requiring increasing doses of phytase as evidenced by prediction equations in broilers. Poultry Science, 99, 290-300. https://doi.org/10.3382/ps/pez489

Wang, L.F., Swift, M.L. & Zijlstra, R.T. 2013. A novel approach for a functional group to predict protein in undigested residue and protein digestibility by mid-infrared spectroscopy. Applied Spectroscopy, 67, 1343-1347. https://doi.org/10.1366/13-07161

Wang, L. 2014. Mid-infrared spectroscopy estimates nutrient digestibility in pigs to improve *in vitro* digestion models. PhD thesis, University of Alberta, Canada. https://doi.org/10.7939/R3KQ3H

Wang, L., Hu, Q., Li, P., Lai, C., Li, D., Zang, J. & Ni, S. 2021a. Development and validation of equations for predicting the metabolizable energy value of double-low rapeseed cake for growing pigs. Animals, 11, 1168. https://doi.org/10.3390/ani11041168

Wang, R., Wei, X., Wang, H., Zhao, L., Zeng, C., Wang, B., Zhang, W., Liu, L. & Xu, Y. 2021b. Development of attenuated total reflectance mid-infrared (ATR-MIR) and near-infrared (NIR) spectroscopy for the determination of resistant starch content in wheat grains. Journal of Analytical Methods in Chemistry, e5599388. https://doi.org/10.1155/2021/5599388

Wilman, D., Field, M., Lister, S. & Givens, D. 2000. The use of near infrared spectroscopy to investigate the composition of silages and the rate and extent of cell-wall degradation. Animal Feed Science and Technology, 88, 139-151. https://doi.org/10.1016/S0377-8401(00)00224-8

Wiseman, J. 2006. Variations in starch digestibility in non-ruminants. Animal Feed Science and Technology, 130, 66-77. https://doi.org/10.1016/j.anifeedsci.2006.01.018

Wiseman, J. & Salvador, F. 1991. The influence of free fatty acid content and degree of saturation on the apparent metabolizable energy value of fats fed to broilers. Poultry Science, 70, 573-582. https://doi.org/10.3382/ps.0700573

Wright, S. 1921. Correlation and causation. Journal of Agriculturural Research, 20, 557-585.

Wu, S.B., Swick, R.A., Noblet, J., Rodgers, N., Cadogan, D. & Choct, M. 2019. Net energy prediction and energy efficiency of feed for broiler chickens. Poultry Science, 98, 1222-1234. https://doi.org/10.3382/ps/pey442

Xiccato, G., Trocino, A., De Boever, J., Maertens, L., Carabaño, R., Pascual, J., Perez, J., Gidenne, T. & Falcao-E-Cunha, L. 2003. Prediction of chemical composition, nutritive value and ingredient composition of European compound feeds for rabbits by near infrared reflectance spectroscopy (NIRS). Animal Feed Science and Technology, 104, 153-168. https://doi.org/10.1016/S0377-8401(02)00294-8

Xing, L., Chen, L. & Han, L. 2008. Rapid analysis of layer manure using near-infrared reflectance spectroscopy. Poultry Science, 87, 1281-1286. https://doi.org/10.3382/ps.2007-00464

Xu, Y., Chen, T., Zhang, H., Nuermaimaiti, Y., Zhang, S., Wang, F., Xiao, J., Liu, S., Shao, W. & Cao, Z. 2023. Application of near-infrared reflectance spectroscopy for predicting chemical composition of faeces in holstein dairy cows and calves. Animals, 14, 52. https://doi.org/10.3390/ani14010052

Yakubu, H.G., Kovacs, Z., Toth, T. & Bazar, G. 2022. The recent advances of near-infrared spectroscopy in dairy production-A review. Critical Reviews in Food Science and Nutrition, 62, 810-831. https://doi.org/10.1080/10408398.2020.1829540

Yan, M., Guevara-Oquendo, V.H. & Yu, P. 2022. Using Mid-IR spectroscopy (ATR-FTIR) as a fast analytical tool to reveal association between protein spectral profiles and metabolizable protein supply, protein rumen degradation characteristics and estimated intestinal protein digestion before and after rumen incubation of faba bean partitions and faba bean silage. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 273, 121022. https://doi.org/10.1016/j.saa.2022.121022

Yegani, M. & Korver, D.R. 2012. Review: Prediction of variation in energetic value of wheat for poultry. Canadian Journal of Animal Science, 92, 261-273. https://doi.org/10.4141/cjas2011-114

Yegani, M., Swift, M.L., Zijlstra, R.T. & Korver, D.R. 2013. Prediction of energetic value of wheat and triticale in broiler chicks: A chick bioassay and an *in vitro* digestibility technique. Animal Feed Science and Technology, 183, 40-50. https://doi.org/10.1016/j.anifeedsci.2013.03.010

Zaefarian, F., Cowieson, A.J., Pontoppidan, K., Abdollahi, M.R. & Ravindran, V. 2021. Trends in feed evaluation for poultry with emphasis on *in vitro* techniques. Animal Nutrition, 7, 268-281. https://doi.org/10.1016/j.aninu.2020.08.006

Zampiga, M., Calini, F. & Sirri, F. 2021. Importance of feed efficiency for sustainable intensification of chicken meat production: implications and role for amino acids, feed enzymes and organic trace minerals. World's Poultry Science Journal, 77, 639-659. https://doi.org/10.1080/00439339.2021.1959277

Zeng, Z., Shurson, G. & Urriola, P. 2017. Prediction of the concentration of standardized ileal digestible amino acids and safety margins among sources of distillers dried grains with solubles for growing pigs: A meta-analysis approach. Animal Feed Science and Technology, 231, 150-159. https://doi.org/10.1016/j.anifeedsci.2017.07.010

Zhang, F. & Adeola, O. 2017. Techniques for evaluating digestibility of energy, amino acids, phosphorus, and calcium in feed ingredients for pigs. Animal Nutrition, 3, 344-352. https://doi.org/10.1016/j.aninu.2017.06.008

Zhao, F., Ren, L., Mi, B., Tan, H., Zhao, J., Li, H., Zhang, H. & Zhang, Z. 2014. Developing a computer-controlled simulated digestion system to predict the concentration of metabolizable energy of feedstuffs for rooster. Journal of Animal Science, 92, 1537-1547. https://doi.org/10.2527/jas.2013-6636

Zuidhof, M.J., Afrouziyeh, M., Van der Klein, S.A. & You, J. 2023. Smart poultry nutrition. In Smart Livestock Nutrition; Springer International Publishing: Cham, Switzerland, 201-225. https://doi.org/10.1007/978-3-031-22584-0

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Sivajanani Thiruchchenthuran
Name and title of main supervisor:	Professor Patrick C.H Morel
In which chapter is the manuscript/published work?	Chapter 3

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

S. Thiruchchenthuran:Conceptualization ,Methodology ,Software ,Validation ,Formal analysis ,Investigation ,Data Curation ,Writing - Original Draft ,Writing - Review & Editin / P. C. H. Morel :Conceptualization ,Methodology ,Formal analysis ,Investigation ,Writing - Review & Editing ,Supervision ,Project administration / N. Lopez-Villalobos:Methodology ,Software ,Formal analysis ,Writing - Review & Editing / F. Zaefarian and M.R Abdollahi:Writing - Review & Editing ,Supervision ,Funding acquisition / T. J. Wester:Writing - Review & Editing ,Supervision / N. B. Pedersen, A.C. Storm, A.J. Cowieson Writing - Review & Editing ,Funding acquisition

Please select one of the following three options:

- (●) **The manuscript/published work is published or in press**

  Please provide the full reference of the research output:
  Thiruchchenthuran, S., Lopez-Villalobos, N., Zaefarian, F., Abdollahi, M.R., Wester, T.J., Pedersen, N.B., Storm, A.C., Cowieson, A.J., Morel, P.C.H., 2024. Evaluation of equations for predicting ileal nutrient digestibility and digestible nutrient content of broiler diets based on their gross chemical composition. Animal Feed Science and Technology, 115974. https://doi.org/10.1016/j.anifeedsci.2024.115974

- ( ) **The manuscript is currently under review for publication**

  Please provide the name of the journal:

- ( ) **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	Sivajanani Thiruchchenthuran Digitally signed by Sivajanani Thiruchchenthuran Date: 2024.11.15 13:07:32 +13'00'	Main supervisor's signature:	Patrick Morel Digitally signed by Patrick Morel DN: cn=Patrick Morel, c=NZ, o=Massey University, ou=SAE, email=p.c.morel@massey.ac.nz Date: 2024.11.25 11:24:10 +13'00'

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Sivajanani Thiruchchenthuran
Name and title of main supervisor:	Professor Patrick C.H Morel
In which chapter is the manuscript/published work?	Chapter 4

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

S. Thiruchchenthuran: Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing/ P. C. H. Morel : Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition/ F. Zaefarian:Methodology, Investigation, Writing - Review & Editing, Supervision/ M. R. Abdollahi: Methodology, Investigation, Writing - Review & Editing, Supervision, Funding acquisition/ T. J. Wester: Writing - Review & Editing, Supervision

Please select one of the following three options:

○	**The manuscript/published work is published or in press** Please provide the full reference of the research output:
◉	**The manuscript is currently under review for publication** Please provide the name of the journal: Animal Feed Science and Technology
○	**It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	Sivajanani Thiruchchenthuran Digitally signed by Sivajanani Thiruchchenthuran Date: 2024.11.20 10:39:40 +13'00'	Main supervisor's signature:	Patrick Morel Digitally signed by Patrick Morel DN: cn=Patrick Morel, c=NZ, o=Massey University, ou=SAE, email=p.c.morel@massey.ac.nz Date: 2024.11.25 11:28:35 +13'00'

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Sivajanani Thiruchchenthuran
Name and title of main supervisor:	Professor Patrick C.H Morel
In which chapter is the manuscript/published work?	Chapter 5

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

S. Thiruchchenthuran: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft,
M.Waterland:Methodology, Software, Formal analysis, Investigation, Writing - Review & Editing,Resources
P. C. H. Morel : Conceptualization, Methodology, Investigation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition,Resources
T. J. Wester: Supervision,

Please select one of the following three options:

○	**The manuscript/published work is published or in press** Please provide the full reference of the research output:
○	**The manuscript is currently under review for publication** Please provide the name of the journal:
◉	**It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	Sivajanani Thiruchchenthuran Digitally signed by Sivajanani Thiruchchenthuran Date: 2024.11.20 10:40:10 +13'00'	Main supervisor's signature:	Patrick Morel Digitally signed by Patrick Morel DN: cn=Patrick Morel, c=NZ, o=Massey University, ou=SAE, email=p.c.morel@massey.ac.nz Date: 2024.11.25 12:57:40 +13'00'

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Sivajanani Thiruchchenthuran
Name and title of main supervisor:	Professor Patrick C.H Morel
In which chapter is the manuscript/published work?	Chapter 6

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

S. Thiruchchenthuran: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft,
M.Waterland:Methodology, Software, Formal analysis, Investigation, Writing - Review & Editing,Resources
P. C. H. Morel : Conceptualization, Methodology, Investigation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition,Resources
T. J. Wester: Supervision,

Please select one of the following three options:

- ○ **The manuscript/published work is published or in press**
  Please provide the full reference of the research output:
- ○ **The manuscript is currently under review for publication**
  Please provide the name of the journal:
- ◉ **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	Sivajanani Thiruchchenthuran Digitally signed by Sivajanani Thiruchchenthuran Date: 2024.11.20 10:40:33 +13'00'	Main supervisor's signature:	Patrick Morel Digitally signed by Patrick Morel DN: cn=Patrick Morel, c=NZ, o=Massey University, ou=SAE, email=p.c.morel@massey.ac.nz Date: 2024.11.25 12:58:11 +13'00'

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

Student name:	Sivajanani Thiruchchenthuran
Name and title of main supervisor:	Professor Patrick C.H Morel
In which chapter is the manuscript/published work?	Chapter 7

Describe the contribution that the student and members of the supervisory team have made to the manuscript/published work:[1]

S. Thiruchchenthuran: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft,
M.Waterland:Methodology, Software, Formal analysis, Investigation, Writing - Review & Editing,Resources
P. C. H. Morel : Conceptualization, Methodology, Investigation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition,Resources
T. J. Wester: Supervision,

Please select one of the following three options:

- ○ **The manuscript/published work is published or in press**
  Please provide the full reference of the research output:
- ○ **The manuscript is currently under review for publication**
  Please provide the name of the journal:
- ◉ **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

Student's signature:	Sivajanani Thiruchchenthuran Digitally signed by Sivajanani Thiruchchenthuran Date: 2024.11.20 10:41:07 +13'00'	Main supervisor's signature:	Patrick Morel Digitally signed by Patrick Morel DN: cn=Patrick Morel, c=NZ, o=Massey University, ou=SAE, email=p.c.morel@massey.ac.nz Date: 2024.11.25 12:58:31 +13'00'

This form should be placed at the beginning of each relevant thesis chapter.

[1] Refer to the Massey University Publishing and Authorship guidelines (OneMassey for staff, Stream for students) and/ or Contributor Roles Taxonomy (CRediT) guidelines for guidance.