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

S. Thiruchchenthuran [a,b], F. Zaefarian [a,c], M.R. Abdollahi [a,d], T.J. Wester [a], P.C. H. Morel [a,*]

[a] School of Agriculture and Environment, Massey University, Private Bag 11 222, Palmerston North 4442, New Zealand
[b] Department of Animal Science, Faculty of Agriculture, University of Jaffna, Kilinochchi 44000, Sri Lanka
[c] Adisseo France S.A.S. European Laboratory of Innovation Science & Expertise Saint Fons, 69190 France
[d] A2Z Poultry Feed DynamikZ, Villeurbanne, France

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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), crude 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, crude 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, 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, crude 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 crude 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, crude fat, starch, energy, and DM) and some overpredicted (CAID of Ca and P and IDC of 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

Abbreviations: ADFom, acid detergent fibre expressed exclusive of residual ash; ADG, average daily gain; AME, apparent metabolisable energy; ANDFom, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; CAID, coefficient of apparent ileal digestibility; Ca, calcium; CCC, concordance correlation coefficient; CF, crude fibre; CF^2, square value of crude fibre; CP, crude protein; CV, coefficient of variation; DM, dry matter; Crude fat:CF, Crude fat-to-crude fibre ratio; GE, gross energy; IDC, ileal digestible content; MB, mean bias; N, nitrogen; NDF, neutral detergent fibre; NSP, non-starch polysaccharides; P, phosphorus; PDI, pellet durability index; RMSE, root mean square error, RMSEp, root mean square error of prediction; R^2, coefficient of determination of regression; Starch:CF, starch-to-crude fibre ratio; TiO_2, titanium dioxide.

* Corresponding author.
E-mail address: Morel@massey.ac.nz (P.C.H. Morel).

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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.

1. 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, near-infrared reflectance spectroscopy, *in vivo* data, and *in vitro* digestive procedures are some of the methods used in the evaluation of feed for poultry (Zaefarian 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., 2021b). Nevertheless, there were some validation studies carried out to ensure the accuracy of proposed equations (Alvarenga et al., 2013b, 2015; Meloche et al., 2014; Pedersen et al., 2021b). 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), crude fat, starch, calcium (Ca), phosphorus (P), energy, and dry matter (DM) were developed using 56 diets in our previous study (Thiruchchenthuran et al., 2024). Therefore, the objective of the present study was to validate the developed prediction equations with unique data.

2. Materials and methods

2.1. 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 1 and 2). A central diet was prepared with the standard nutritional requirements of Ross 308 broiler chickens (Ross 308 Nutritional Guide, 2022). The remaining 19 diets were prepared such that they were in a range of 180–240 g/kg (as-fed basis) crude protein (CP) and 10–14 MJ/kg (as-fed basis) 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 °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.

2.2. Birds and housing

Experimental procedures were conducted in accordance with the Massey University Animal Ethics Committee guidelines (MUAEC 22/39). A total of 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 days of age. Birds were initially placed on wood shavings over concrete floor pens. The temperature was maintained at 31 °C on day 1 and decreased by 3 °C per week to a final temperature of 22 °C at 21 days of age.

On day 12, birds were allocated to 100 battery cages and offered dietary treatments until day 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. 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 day 15–22 post-hatch. Diets were offered *ad libitum* and water was freely available.

2.3. 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

Table 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

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

[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.

Table 2
Calculated chemical composition of the experimental diets (g/kg as fed basis).

Diet	DM	Crude ash	CP	Crude 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.

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.

2.4. 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.

2.5. Determination of apparent metabolisable energy (AME)

Feed intake and total excreta output of each cage were quantitatively measured from day 19–22 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 °C until further analysis. The excreta samples were analysed for DM, gross energy (GE), ash, N, and Ti.

2.6. Determination of CAID of GE and nutrients

On day 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 °C in airtight containers until laboratory analysis. The diets and digesta samples were analysed for DM, N, ash, GE, Ti, starch, crude fat, Ca, P, crude fibre (CF), neutral detergent fibre (aNDFom), acid detergent fibre (ADFom), and lignin.

2.7. Chemical analysis

The DM 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). The CP 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 °C for 16 h. The GE was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid. Samples were analysed for titanium (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). Crude fat was determined using a Soxhlet extractor (Soxtec System HT 1043 Extraction Unit, Höganäs, Sweden) by hexane extraction method (Method 2003.06; AOAC, 2016).

The Ca and P concentrations were determined by colorimetric methods after combustion of the samples at 550 °C and acid digestion in 6.0 M HCl using standard procedures (Method 968.08D; AOAC, 2016). The crude fibre was measured using modified standard procedures (Methods 962.09 and 978.10; AOAC, 2016). The aNDFom, ADFom, and lignin were analysed using Fibretec™ (FOSS analytical AB, Höganäs, Sweden) following standard procedures (Methods 2002.04 and 973.18; AOAC, 2016).

2.8. Calculations and statistical analysis

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

$$\text{AME}\ (\text{MJ}/\text{kg diet}) = \frac{[(\text{Feed intake} \times \text{GE}_{diet}) - (\text{Excreta output} \times \text{GE}_{excreta})]}{\text{Feed intake}} \quad (1)$$

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

$$\text{AME}\ (\text{MJ}/\text{kg diet}) = \text{GE}_{diet} - [\text{GE}_{excreta} \times \frac{\text{TiO}_{2\,diet}}{\text{TiO}_{2\,excreta}}] \quad (2)$$

The CAID was calculated using the following formula:

$$\text{CAID of nutrient} = \frac{(\text{Nutrient}/\text{TiO}_2)_{diet} - (\text{Nutrient}/\text{TiO}_2)_{ileal}}{(\text{Nutrient}/\text{TiO}_2)_{diet}} \quad (3)$$

where $(\text{Nutrient}/\text{TiO}_2)_{diet}$ = ratio of diet component to TiO_2 in the diet and $(\text{Nutrient}/\text{TiO}_2)_{ileal}$ = ratio of the diet component to TiO_2 in the ileal digesta.

The IDC was calculated by the following formula:

IDC of nutrient = Nutrient in the diet × CAID of that nutrient (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, crude 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 (SAS, 2016), version 9.4 package. 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,

$$R^2 = 1 - \frac{RSS}{TSS} \tag{5}$$

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.

$$\text{RMSEp} = \sqrt{\frac{\sum_{i=1}^{n}(P-M)^2}{\text{n}}} \tag{6}$$

where M is the measured value of the validation data set, P 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 square 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 (2019), however, recommended a different set of interpretation criteria where > 0.99 is almost perfect, 0.95–0.99 substantial, 0.90–0.95 is moderate, and < 0.90 is poor (Akoglu, 2018).

$$\text{CCC} = \frac{2\sigma_{PM}}{\sigma_P^2 + \sigma_M^2 + (\mu_P - \mu_M)^2}, \tag{7}$$

where σ_{PM} is the covariance, $\sigma^2{}_P$ and $\sigma^2{}_M$ are the variances, μ_P and μ_M 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 measured 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 over-prediction, negative values indicate underprediction, and zero implies a balanced distribution of the two (Mkhabela et al., 2011).

$$\text{MB} = \frac{1}{n}\sum_{i=1}^{n}(P-M) \tag{8}$$

where M is the measured value, P 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.

3. Results

3.1. Chemical composition of the diets

The formulated 20 diets ranged in CP content and GE from 192 to 291 g/kg and 18.2–19.3 MJ/kg, respectively, on DM basis (Table 3). The crude 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-crude fat content had low PDI as observed in diets S and T, whose crude 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 crude fat content of 30.8 g/kg on DM basis.

3.2. Correlation (r) analysis

The GE of the 20 diets was positively correlated to crude fat ($r = 0.78$, $P < 0.001$, Table 4) and negatively correlated to N ($r = -0.64$, $P = 0.003$). Interestingly, crude 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 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	Crude ash	CP	Crude fat	Starch	CF	aNDFom	ADFom	Lignin	Ca	P	GE (MJ/kg)	AME (MJ/kg) by total collection	AME (MJ/kg) by Ti 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 expressed exclusive of residual ash; BW = body weight; Ca = calcium; CF = crude fibre; CP = crude protein; DM = dry matter; GE = gross energy; aNDF = neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash; P = phosphorus; PDI = pellet durability index; SD = standard deviation; Ti = titanium.

Table 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		Crude ash	N	Crude fat	Starch	CF	Ca	P	GE	CF^2	Crude fat:CF
N	r	0.63									
	P-value	0.003									
Crude fat	r	−0.33	−0.71								
	P-value	NS	0.0005								
Starch	r	−0.78	−0.84	0.28							
	P-value	< 0.001	< 0.001	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	< 0.001	0.042	NS	0.001	0.011					
P	r	0.92	0.47	−0.16	−0.67	0.50	0.93				
	P-value	< 0.001	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	< 0.001	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	< 0.001	0.004	0.010	NS		
Crude 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	< 0.001	< 0.001	0.018	0.032	NS	< 0.001	0.0005

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

3.3. CAID and IDC of nutrients

The CAID of N, GE, Ca, P, and DM ranged from 0.75 to 0.83, 0.64–0.77, 0.11–0.53, 0.36–0.62, and 0.57–0.72, respectively, and varied to a greater extent (Table 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, 1.92–4.43, 3.34–5.18, and 566–722 g/kg DM, respectively, while GE ranged from 11.9 to 14.4 MJ/kg DM (Table 6).

Table 5
Coefficient of apparent ileal digestibility (CAID) of nutrients and energy in 20 diet mixtures.

Diet	N	Crude 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 6
Ileal digestible content (IDC) of nutrients (g/kg DM) and energy (MJ/kg) in 20 diet mixtures.

Diet	N	Crude 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.

3.4. Validation of developed prediction equations

The equations for predicting CAID of P, energy, and DM showed high CCC (Table 7). The CCC values of CAID of N, crude 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, crude 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, crude 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 ($P < 0.05$). The slope was not different from 1 for CAID of Ca and P and for IDC of crude fat and P.

Table 7
Validation of the developed equations using chemical composition for CAID of and IDC of nutrients (n = 20).

		Training set (56 diets)[1]			Validation set (20 diets)						
No.		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 published by Thiruchchenthuran et al. (2024); Table 3: Equations 01–07, and Table 4: Equations 08–14.

* Mean bias was different from 0 at $P < 0.05$ (Paired *t*-test).

\# Slope was different from 1 at $P < 0.05$.

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 Fig. 1a and Fig. 1b and Fig. 2a, 2b, 2c, and 2d, respectively.

4. 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 estimation the energy values of feedstuffs in pigs (Noblet and Perez, 1993; Bulang and Rodehutscord, 2009; Urriola et al., 2014; Wang et al., 2021; Lee et al., 2022), and broilers (Batal and Dale, 2006; Yegani and Korver, 2012; Meloche, 2013; Meloche et al., 2013, 2014; Baiz et al., 2020) as well as protein and amino acid digestibility for broilers (Ravindran et al., 2007, 2017; Szczurek, 2010) 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., 2021b) 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 by Thiruchchenthuran et al. (2024). 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. Twenty newly formulated diets were used for validation as at least 15–20 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., 2021). 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, 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. (2021b) 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 56 diets' chemical composition, 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 crude 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

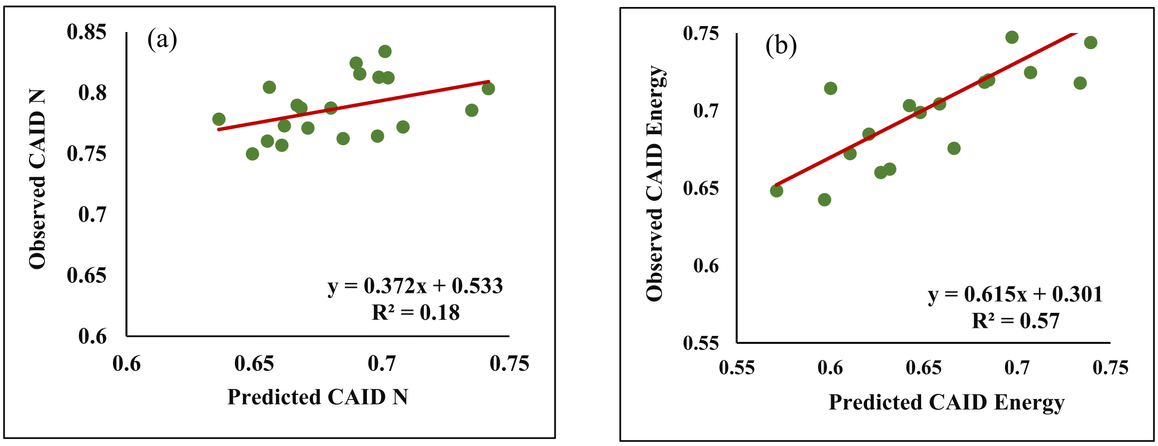

Fig. 1. Predicted vs. observed values for coefficient of apparent ileal digestibility (CAID) of N (a) and energy (b) based on the gross chemical composition. A *t*-test was used to test if the slopes were different from 1. (a) The slope was different from 1 (P = 0.004). (b) The slope was different from 1 (P = 0.007).

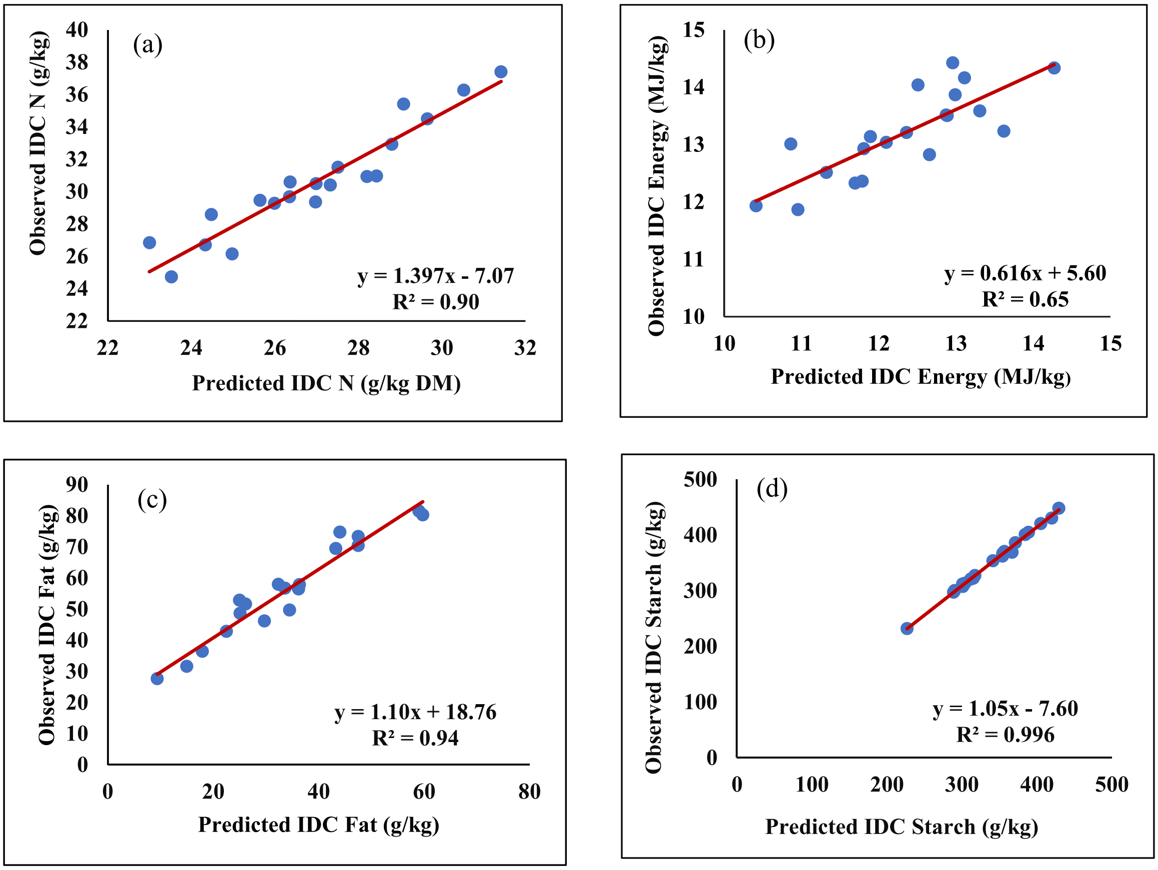

Fig. 2. Predicted vs. observed values for ileal digestible content (IDC) of N (a), energy (b), fat (c), and starch (d) based on the gross chemical composition. A *t*-test was used to test if the slopes were different from 1. (a) The slope was different from 1 (P = 0.002). (b) The slope was different 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).

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 crude 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. Thus, it 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., 2021a). 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 (CV) in the Ca content of the validation diets (CV = 0.35) compared to Ca content in the training diets used to develop the equation (CV = 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., 2021b) whereas in commercial settings, Ca and P content will be lower and less variable.

Pedersen et al. (2021b) 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. (2021b) 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. (2021b), 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 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 couldn't 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., 2021b).

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., 2021b). 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).

In conclusion, 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.

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CRediT authorship contribution statement

Thiruchchenthuran S.: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Morel P.C.H.:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Abdollahi M.R.:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition. **Zaefarian F.:** Writing – review & editing, Supervision, Methodology, Investigation. **Wester T.J.:** Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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