Mathematical prediction of ileal energy and protein digestibility in broilers using multivariate data analysis

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ABSTRACT A proportional mixture design was used to systematically create a total of 56 diets using ten feed ingredients. Diets differed widely with regards to chemical characteristics and ingredient inclusion levels. Apparent ileal digestibility of energy and protein of the diets were determined in broiler growers fed *ad libitum* from 21 to 24 d post-hatch. The chemical composition and the *in vivo* digestibility values were used to establish prediction equations for energy and protein digestibility, using multivariate data analysis. Root mean square error as percentage of the observed means (**RMSE%**) and residual error were used to evaluate the strength and accuracy of the predictions and to compare predictions based on chemical characteristics with estimates based on table values. The estimates of ileal digestibility of energy from table values were relatively accurate (RMSE% = 5.15) and was comparable to those predicted based on the chemical composition of diets. Estimates of ileal digestibility of protein based on table values were less accurate (RMSE% = 8.21); however, the prediction was improved by multivariate regression (RMSE% = 5.46) based on chemical composition of diets. The best predictors for ileal energy digestibility were starch, crude fiber and phytate contents ($P < 0.01$) and the best predictors for crude protein digestibility were starch, CF and fat contents ($P < 0.05$). In conclusion, the ileal digestibility of energy can be accurately predicted using table values; however, the accuracy of prediction of the ileal digestibility of protein can be improved when chemical characteristics of the diet are considered.

Key words: broiler, nutrient digestibility, predictive equation, proportional mixture design, multivariate data analysis

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INTRODUCTION

Accurate estimation of the nutritive value of feed ingredients to optimize animal growth performance and reduce costs is fundamental in animal production (Farrell, 1999). Nutrient values of feed ingredients provided by recognized research institutions (WPSA, 1989; NRC, 1994; Sauvant et al., 2002; CVB, 2016; Evonik, 2016; "Feedipedia," 2017; FEDNA, 2017; Rostagno et al., 2017), along with chemical analysis of feed ingredients, facilitate the feed formulation process. However, the quality of feed ingredients varies with season and site of production, thus table values are only an approximation or can occasionally be non-existing for non-traditional feed ingredients (Mateos et al., 2019). Furthermore, digestibility coefficients are typically based on single ingredient evaluation and do not consider interactions between ingredients in a mixed diet (Ravindran et al., 2017). The presence of anti-nutritional compounds (such as phytate and some non-starch polysaccharides [**NSP**]) in one feed ingredient may affect not only the metabolizable energy of the single ingredient "per se" but can also affect the digestibility of other components of the diet. A single feed ingredient with relatively high phytate level can reduce the overall digestibility of protein and starch because of the possible direct and indirect complex formations between phytate and these compounds (Selle et al., 2012). Reduced energy, protein and lipid digestion have also been observed when diets contain high concentration of NSPs (Choct and Annison, 1992). These results can be related to overall increases in gut viscosity due to the

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aggregation of NSPs into large networks which reduce digestibility and transit time in the small intestine. The reduction in transit time also favors the intestinal bacteria, which can multiply, migrate to the upper part of the intestine and compete with the host animal for digestible nutrients (Bedford, 1995). Other feed-related compounds like different contaminants and toxins may also reduce the overall nutrient digestibility. However, feed additives such as enzymes, emulsifiers and organic acids can increase the overall nutrient digestibility of diets (Mateos et al., 2019). The interaction between feed ingredients and overall diet digestibility suggest that feed formulation based on single ingredients values might reduce our ability to reliably predict animal performance. It is suggested that there is a lack of additivity of apparent ileal digestibility **(AID)** values for some amino acids in mixed diets compared with standardized ileal digestibility values (Kong and Adeola, 2013; Cowieson et al., 2019). Digestibility predictions can therefore be improved using standardized ileal digestibility values, but for more complex diets containing by-product feeds such data do not exist.

Predictions of the inherent digestibility of nutrients are also important for the understanding of the efficacy of feed enzymes, where the effect of some enzymes is correlated with the inherent digestibility of the diet (Cowieson and Roos, 2014). A low inherent digestibility can result in a high feed enzyme efficacy. The inconsistency of feed enzymes might be reduced with a better understanding of the inherent digestibility of nutrients in poultry diets (Bao et al., 2013).

The objective of the current study was to establish a reliable prediction equation for ileal digestibility of energy (**IDE**) and ileal digestibility of protein (**IDP**). Multivariate analysis was used to find important chemical characteristics that described the *in vivo* digestibility of nutrient.

MATERIALS AND METHODS

Models that can predict IDE and IDP in broilers, based on chemical characteristics of diets, were investigated in two broiler digestibility studies. The first assay was conducted to develop the calibration data set **(CDS).** The second assay was conducted to validate the predictive model and the data is referred to as the evaluation data set **(EDS).** Detailed description of the EDS study is presented in an earlier publication (Pedersen et al., 2021 unpublished data). Experimental procedures for both studies complied with Massey University Animal Ethics Committee guidelines. The same batch of feed ingredients were used in both studies, but the composition of the diets varied between the two studies. In the EDS study, the diets were more commercially relevant compared to the CDS study.

Birds and Diets

Proportional diets were created using proc print noobs (SAS Institute Inc., Cary, NC,) and diets were developed by the creation of 55 systematically arranged mixtures of 10 common feed ingredients. An additional geometrically central diet was created by mixing equal proportions of each of these ten ingredients (100 g/kg of each ingredient). The inclusion level of each feed ingredient in the different diets were either 20, 420 or 820g/kg and all ingredients were represented in all diets. As an illustration, examples of two diets are given below. 1) A diet with 820 g/kg wheat and 20 g/kg each of corn, sorghum, soybean meal, canola meal, full fat soybean meal, palm kernel meal, meat and bone meal, wheat bran and wheat distillers dried grains with solubles. 2) A diet with 420 g/kg wheat and 420 g/kg corn and 20 g/kg each of sorghum, soybean meal, canola meal, full fat soybean meal, palm kernel meal, meat and bone meal, wheat bran and wheat distillers dried grains with solubles.

All diets contained 5.0 g/kg titanium dioxide (TiO_2, Merck KGaA, Darmstadt, Germany) as an indigestible marker for the determination of apparent ileal nutrient digestibility. All diets were steam-conditioned at 60 °C for 30 seconds 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 apertures and a depth of 35 mm.

A total of 2688, day-old male Ross 308 broiler chicks were obtained from a commercial hatchery and fed a pre-experimental starter diet from 1 to 21 d of age. Birds were housed in an environmentally controlled room with 20 h of fluorescent illumination per day. 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. The pre-experimental diet was formulated to contain 12.7 MJ/kg AME, 225 g/kg crude protein (**CP**), 9 g/kg Ca, 4.5 g/kg available P, and 1.25 g/kg digestible lysine. On d 21, birds were allocated to 336 cages in electrically heated battery brooders and offered dietary treatments until d 24. Each of the 56 diets were randomly assigned to six replicate cages, each housing eight birds, in a randomized 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 cage basis, from d 21 to 24 post-hatch. Diets were offered *ad libitum* and water was freely available.

Ileal Digesta Collection

On d 24, six birds per cage were euthanized by intravenous injection (1 mL per 2 kg live weight) 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. (2005). The ileum was defined as the portion of the small intestine extending from the Meckel's diverticulum to a point ~40 mm proximal to the ileo-cecal junction. The ileum was then divided into two halves and the digesta was collected from the lower half towards the ileo-cecal junction. Digesta from birds within a cage were pooled, lyophilized (Model 0610, Cuddon Engineering, Blenheim, New Zealand), ground to pass through a 0.5-mm sieve and stored at 4 °C until laboratory analysis.

Chemical Analysis

Diets and digesta samples were analyzed for DM, TiO_2, CP, starch, fat, Ca and P, ash and gross energy. DM 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 °C for 16 hours. Nitrogen was determined by combustion (Method 968.06; AOAC, 2016) using a CNS-200 carbon, nitrogen and sulfur auto analyzer (LECO Corporation, St. Joseph, MI, USA). Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardized with benzoic acid. Samples were assayed for TiO_2 on a UV spectrophotometer following the method of (Short et al., 1996). 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. Fat was determined using the Soxhlet extraction procedure (Method 991.36; AOAC, 2005). The Ca and P concentration 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, 2005). Crude fiber (**CF**) was measured using standard procedures (Methods 962.09 and 978.10; AOAC, 2005). Phytate and NSP contents were determined for all ingredients and the feed matrix were used to calculate the phytate content of different diets. Phytate was analyzed on a high-performance ion chromatography system with an ICS5000 dual pump, VWD-3400RS absorbance detector and a TC-IC column oven (Dionex Corp., Sunnyvale, CA), according to the procedure described by Pontoppidan et al. (2007). Insoluble NSPs were determined with the Uppsala method (Theander et al., 1995) using gas chromatography (model 6850, Agilent, Santa Clara, CA). Viscosity was measured on a slurry of each diet using an MCR 302 rheometer (Anton Paar, Graz, Austria) equipped with a C-PTD200 peltier cooling element with a ST24-2D/2V/2V-30/109 stirrer. Diets were mixed in a slurry with a DM content of 24% the day before measuring viscosity and stored in a fridge, to make sure all samples had the same temperature. Samples were taken from the fridge and stored at room temperature for ten minutes, shaken ten times and viscosity was measured right after. Samples were placed in a CC27/T200/AL cup and viscosity was measured at 40°C, 200 rpm and recorded for 400 s. Amino acids were analyzed at Eurofins according to ISO 13903:2005 except for Tryptophan which was analyzed according to ISO 13904 (Eurofins Steins Laboratorium, Vejen, Denmark).

Calculation of Apparent Ileal Digestibility

All data were expressed on a DM basis and coefficient of AID of nutrients was calculated using the following equation:

$$\textit{Coefficient of AID} = \frac{(Nutrient/TiO_2)_{diet} - (Nutrient/TiO_2)_{ileal}}{(Nutrient/TiO_2)_{diet}} \quad (1)$$

Where $(Nutrient/TiO_2)_{diet}$ =ratio of nutrient to TiO_2 in the diet, and $(Nutrient/\ TiO_2)_{ileal}$ =ratio of nutrient to TiO_2 in the ileal digesta.

Calculation of Nutrient Digestibility of Feed Mixtures

The nutrient digestibility of feed mixtures was calculated using published feed table values for energy and CP digestibility for each feed ingredients (Table 1).

Multivariate Analysis

Principle component analysis (**PCA**) was used to identify trends in the chemical composition of the diets and the variables (Hotelling, 1933). Partial least squares regression (**PLS**) was used to establish a linear model based on multivariate data, which enabled the prediction of *in vivo* digestibility based on the values from multiple chemical characteristics (Wold et al., 1983).

All data were centered and auto-scaled to correct for the differences in variance between different types of measurements. The prediction model was made using dispersion cross validation of 10 segments repeated four times. The variable important in projection scores were used to select important variables. The prediction model was developed using data from the calibration data set and the model was validated with the evaluation data

Table 1. Digestibility coefficients of energy and protein in different feed ingredients used for the calculation of ileal digestibility of energy and protein of feed mixtures.

Sources	Evonik (2016)1 DEC	CVB (2016)2 DCCP
Ingredient		
Corn	0.848	0.850
Wheat	0.800	0.850
Sorghum	0.831	0.710
Soybean meal	0.557	0.850
FFSB	0.65	0.87
Canola meal	0.439	0.730
Palm kernel meal	0.311	0.300 (pigs)*
Meat and bone meal	0.622	0.730 (layers)*
Wheat bran	0.514	0.710
Wheat DDGS	0.639	0.660 (pigs)*

[1]Digestibility coefficients of energy from Evonik (Evonik, 2016).
[2]Digestibility coefficients of protein from CVB (CVB, 2016). Abbreviations: DCCP, digestibility coefficient of crude protein (digestible crude protein/crude protein); DDGS, distillers dried grains with solubles; DEC, digestible energy coefficient (AMEn/GE); FFSB, Full fat soybeans.
*Values for broilers were not available.

set. The PCA and PLS analysis were done using R 3.6.1 (R Core Team, 2019), *mdatools* package (Kucheryavskiy, 2020).

Statistical Analysis

The prediction equations were evaluated with two statistical methods. Standard regression was used to determine the strength of linear relationships through analysis of residuals. The fit of predicted data to observed data was evaluated with the root mean square error as percentage of observed mean (**RMSE%**) and mean square error (**MSE**) decomposed into mean bias, slope bias and dispersion bias (Bibby and Toutenburg, 1977).

RESULTS

Chemical Characteristics of Diets

Diets were created to represent a wide range of chemical composition using varying inclusion levels of number of traditional and nontraditional ingredients. The chemical composition of feed ingredients is shown in Table 2. As expected, the chemical composition of diets was variable for all criteria. The mean, minimum and maximum values from chemical analysis of the 56 diets are shown in

Table 3. The gross energy, CP, CF content of diets ranged from 17.87 to 24.03 kJ/g, 148.1 to 496.0 g/kg and 26.6 to 144.7 g/kg, respectively. The lowest and highest energy contents of diets was obtained with 820 g/kg diet of meat and bone meal, and 820 g/kg diet of full fat soya bean meal, respectively. The lowest and highest protein contents of diets was obtained with 820 g/kg diet corn and 820 g/kg diet soybean meal, respectively. The insoluble NSP sugar content of diets ranged from 5.19 to 69.1, 6.67 to 112, 9.19 to 334, 2.46 to 37.5 and 9.26 to 93.3 g/kg for arabinose, xylose, mannose, galactose and glucose, respectively.

Prior to the PCA and PLS analyses, the distribution of variables among different feed ingredients were examined. Since the prediction equation should be applicable to commonly used diets, there was no interest in including variables that only described one feed ingredient if that ingredient was nontraditional. Mannose was not used because it was found in high concentrations only in palm kernel meal and would not be representative of commercially used diets. The concentration of Ca and P were high in diets with high content of meat and bone meal. Under normal conditions, Ca and P concentrations will be present at low concentrations and less variable; these two variables were therefore not used.

Table 2. Chemical composition of feed ingredients (g/kg DM).

Ingredient	CP	Fat	Starch	CF	Ash	Ca	P	GE kJ/g
Corn	96.9	52.7	722	27.3	13.7	0.61	2.27	18.6
Wheat	129	40.3	627	29.6	17.6	0.57	2.85	18.4
Sorghum	121	52.1	604	31.2	17.2	0.15	3.42	18.7
Soybean meal	530	50.0	20.8	39.6	74.5	3.98	7.84	19.8
FFSB	423	269	6.3	34.1	49.8	2.68	4.79	24.0
Canola meal	416	67.4	4.3	121	74.5	6.72	10.79	20.0
Palm kernel meal meal meal	182	79.9	1.9	162	50.8	5.00	6.17	20.1
Meat and bone meal	493	150	1.2	16.3	344	123	54.0	16.9
Wheat bran	177	74.4	190	121	50.8	1.58	8.03	19.3
Wheat DDGS	218	84.8	106	83.7	54.8	1.58	8.02	20.4

Abbreviations: CF, crude fiber; DDGS, distillers dried grains with soluble; FFSB, Full fat soybeans; GE, Gross energy.

Table 3. Analyzed chemical characteristics of the 56 diet mixtures.

Chemical composition (g/kg)	Mean	SE	Minimum	Maximum
CP	286	12.6	148	496
Fat	94.9	5.44	51.8	243
Starch	230	22.8	44.9	636
CF	68.1	3.95	26.6	145
Ca	15.4	2.99	3.31	105
P	11.2	1.21	4.18	47.0
Phytate	1.49	0.077	0.297	2.83
GE kJ/g	20.1	0.16	17.9	24.0
Insoluble NSP				
Arabinose	25.9	1.76	5.19	69.1
Xylose	33.4	2.98	6.67	112
Mannose	45.9	9.49	9.19	334
Galactose	12.3	1.13	2.46	37.5
Glucose	46.3	2.44	9.26	93.3
Amino acids				
Trp	3.10	0.167	1.20	6.60
Gly	15.8	0.144	5.90	57.0
Ala	13.2	0.723	5.86	31.1
Ile	9.11	0.508	4.17	19.3
Leu	18.2	0.808	9.46	33.6
His	5.76	0.295	2.96	11.2
Phe	10.7	0.552	5.64	22.3
Arg	16.6	0.902	6.42	30.4
Ser	11.5	0.571	5.76	22.2
Glu	41.6	1.75	21.0	77.0
Pro	15.6	0.786	6.98	33.8
Asp	21.5	1.34	8.85	49.1
Val	11.4	0.544	5.48	20.6
Cys	3.79	0.166	2.06	6.81
Met	3.87	0.179	2.06	6.81
Lys	11.9	0.814	4.18	25.3
Tyr	8.59	0.440	4.51	18.0
Thr	9.16	0.482	4.37	17.3

Abbreviations: CF, crude fiber; GE, Gross Energy; NSP, non-starch polysaccharides.

Nutrient and Energy Utilization

Feed intake over the 3-day assay period for all diets varied from 356.3 to 518.0 g DM/bird with a mean of 443.7 g/bird DM (SE = 5.51; df = 55). Diets with lower feed intake tended to have higher IDE and IDP. Diets with 820 /kg diet of palm kernel meal, wheat bran or the combination of these 2 feed ingredients had low feed intake, IDE and IDP. Data from the current study were considered to represent a sufficiently wide array of digestibility values. The ileal digestibility of energy, CP and DM of the geometrically central diet was 0.630, 0.689, and 0.554 respectively. The digestibility for the rest of the diet mixtures ranged from 0.346 to 0.820,

Table 4. Apparent ileal digestibility coefficient of nutrients and energy in 56 diet mixtures.

	Mean	SE	Minimum	Maximum
Crude protein	0.683	0.010	0.466	0.806
Energy	0.611	0.013	0.346	0.820
DM	0.538	0.016	0.228	0.795

0.466 to 0.806 and 0.228 to 0.795 for energy, CP and DM, respectively. The mean, minimum and maximum AID of nutrients and energy in diets are shown in Table 4. The lowest energy digestibility value was obtained for the diet with 420 g/kg diet of wheat bran plus 420 g/kg diet of palm kernel meal and the highest for the diet with 820 g/kg corn mixture. The lowest and highest CP digestibility values belonged to diets containing 820 g/kg of palm kernel meal and 820 g/kg diet of wheat, respectively.

Estimation of Energy and Protein Digestibility Based on Tabulated Values

Estimated IDE from tabulated values, when evaluated with *in vivo* IDE for the CDS, had a RMSE% of the observed mean of 8.08. Decomposition of MSE showed that the mean, slope and dispersion bias were 2.40, 3.52, and 94.10%, respectively (Table 5). Residuals for the tabulated estimates are graphically presented in Figure 1. Evaluating the estimates with *in vivo* IDE from the EDS decreased the RMSE% of the observed mean to 5.15. Decomposition of MSE showed that the mean, slope and dispersion bias were 34.47, 1.95, and 60.58%, respectively (Table 5). The residuals show that the estimates were higher than the observed values of IDE in the EDS (Figure 2).

Comparing the estimated IDP from tabulated values with *in vivo* IDP from the CDS gave a RMSE% of the observed mean of 9.08 and decomposition of the MSE showed 24.94, 1.59, and 73.47% mean, slope and dispersion bias, respectively. The RMSE% of the observed mean decreased to 8.21 when the IDP from tabulated values were compared with EDS. Decomposing the MSE showed 67.56, 1.05, and 31.38% mean, slope and dispersion bias, respectively (Table 5). Table values were found to underpredict the digestibility of the CDS and overpredict the digestibility of the EDS, which are graphically shown in the residual plots in Figures 3 and 4.

Prediction of Energy and Protein Digestibility Based on Chemical Composition of the Diets

The PCA was conducted to visualize the ability of the variables to differentiate the diets with different chemical properties. The score plot described 82% of the variation of the chemical properties among the diets and showed small groupings of diets, but they were generally spread out in the scores plot (data not shown). The loadings plot strongly indicates correlations between some of the variables, indicating that the number of variables can be reduced (data not shown).

Based on variable important for projection scores for variable selection from the PLS analysis, the following equations are proposed for the prediction of IDE and IDP.

$$IDE = 0.728 + 0.002 * X_{Starch\%} - 0.016 * X_{CF\%} - 0.038 * X_{Phytate\%} \quad (2)$$

Starch, CF and phytate content were important variables used to predict IDE, the p-values were $P < 0.01$. IDE was negatively affected by the dietary CF and phytate. A negative correlation between IDE and CF (r^2=0.67) were observed (data not shown).

$$IDP = 0.690 + 0.001 * X_{Starch\%} - 0.011 * X_{CF\%} + 0.003 * X_{Fat\%} \quad (3)$$

Starch, CF and fat content were important variables used to predict IDP, the p-values were $P < 0.05$. IDP was negatively affected by dietary CF.

Prediction of IDE was evaluated with the EDS and showed a RMSE% of the observed mean of 4.72. Decomposition of MSE showed 18.22, 1.32, and 80.45% mean, slope and dispersion bias, respectively (Table 6). The prediction based on the chemical composition of diet mixtures overpredicted the IDE, which can be seen in the residuals plot (Figure 5).

The IDP prediction, based on the EDS, yielded a RMSE% of the observed mean of 5.46 and the decomposition of MSE showed 21.53, 3.83, and 74.64% mean, slope and dispersion bias, respectively (Table 6). The predictions were lower than observed values, which is graphically shown in the residuals plot in Figure 6.

Table 5. Correlation, root mean square error of prediction and mean square error of prediction decomposed into mean bias, slope and dispersion bias after fitting the tabulated model to observed data.

							% of MSE		
Study	Item	n	Observed mean	R^2	RMSE	RMSE % of observed mean	Mean bias	Slope bias	Dispersion bias
CDS	IDE	56	0.61	0.77	0.05	8.08	2.40	3.52	94.10
CDS	IDP	56	0.68	0.42	0.06	9.80	24.94	1.59	73.47
EDS	IDE	34	0.67	0.34	0.03	5.15	37.47	1.95	60.58
EDS	IDP	34	0.72	0.31	0.06	8.21	67.56	1.05	31.38

Abbreviation: CDS, Calibration data set; EDS, Evaluation data set; IDE, Ileal digestibility of energy; IDP, Ileal digestibility of protein; MSE, mean square error of prediction; RMSE, root mean square error of prediction.

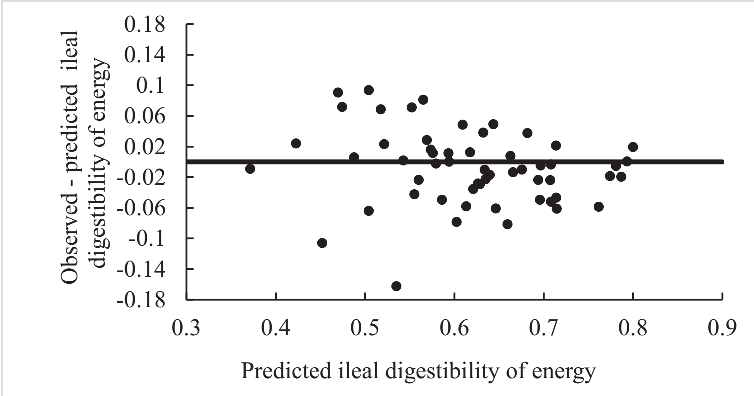

Figure 1. Residuals vs. predicted values for ileal digestibility of energy for the 56 diets based on tabulated values (calibration data set). The horizontal line represents Observed - predicted, y = 0.

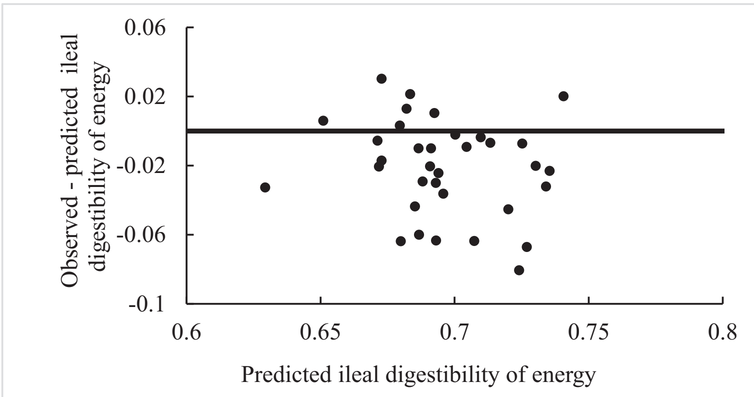

Figure 2. Residuals vs. predicted values for ileal digestibility of energy for the 34 diets based on tabulated values (evaluation data set). The horizontal line represents Observed - predicted, y = 0.

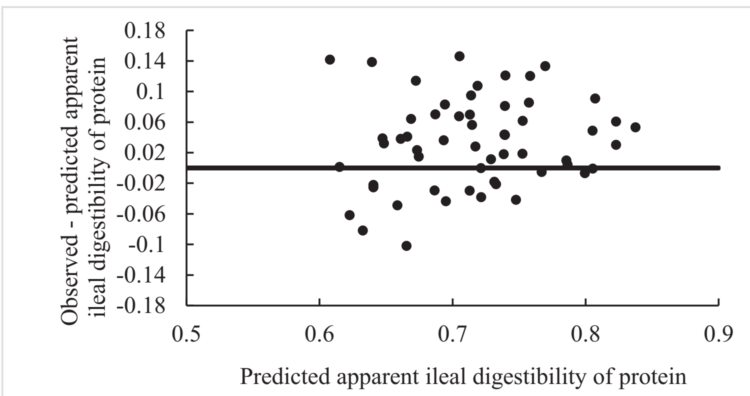

Figure 3. Residuals vs. predicted values for apparent ileal digestibility of protein for the 56 diets based on tabulated values (calibration data set). The horizontal line represents Observed - predicted, y = 0.

DISCUSSION

Accurate prediction of inherent digestibility of energy and CP of feed ingredients is important for both feed formulation and feed enzyme use. In the current paper, a proportional mixture design was used to make a broad and complex landscape of diet digestibility and chemical composition. The variation recorded in the feed intake of different diet mixtures is in accordance with those of Abdollahi et al. (2018) who showed an increase in feed intake, when nutrient density was decreased. The present results suggest that the dataset was robust and had the complexity and broad landscape of digestibility and chemical composition needed to make predictions with more complex diets.

In poultry, several prediction equations have been proposed to calculate nitrogen corrected apparent

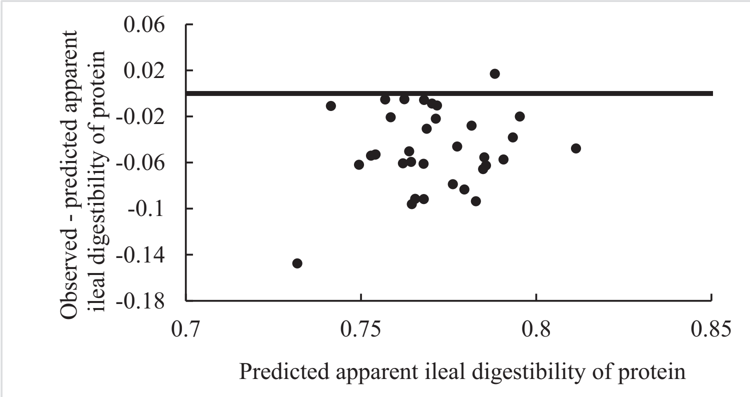

Figure 4. Residuals vs. predicted values for apparent ileal digestibility of protein for the 34 diets based on tabulated values (evaluation data set). The horizontal line represents Observed - predicted, y = 0.

Table 6. Correlation, root mean square error of prediction (RMSE) and mean square error of prediction (MSE) decomposed into mean bias, slope and dispersion bias after fitting chemical composition model to observed data.

							% of MSE		
Study	Item	N	Observed mean	R^2	RMSE	RMSE % of observed mean	Mean bias	Slope bias	Dispersion bias
EDS	IDE	34	0.67	0.26	0.03	4.72	18.22	1.32	80.45
EDS	IDP	34	0.72	0.27	0.04	5.46	21.53	3.83	74.64

Abbreviation: EDS, Evaluation data set; IDE, Ileal digestibility of energy; IDP, Ileal digestibility of protein; MSE, mean square error of prediction; RMSE, root mean square error of prediction.

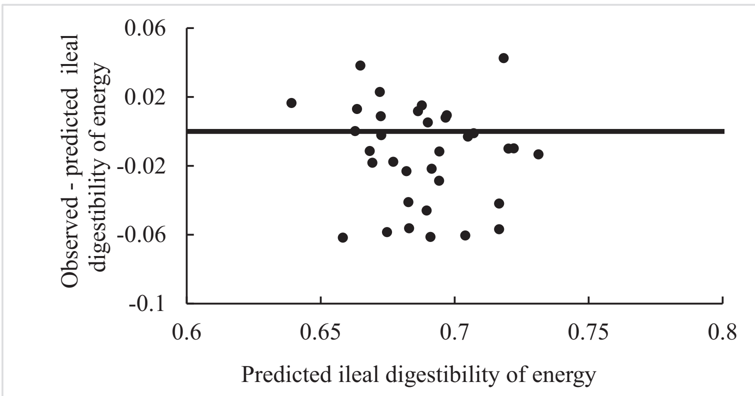

Figure 5. Residuals vs. predicted values for ileal digestibility of energy (IDE) for the 34 diets based on chemical composition (evaluation data set). The horizontal line represents Observed - predicted, y = 0.

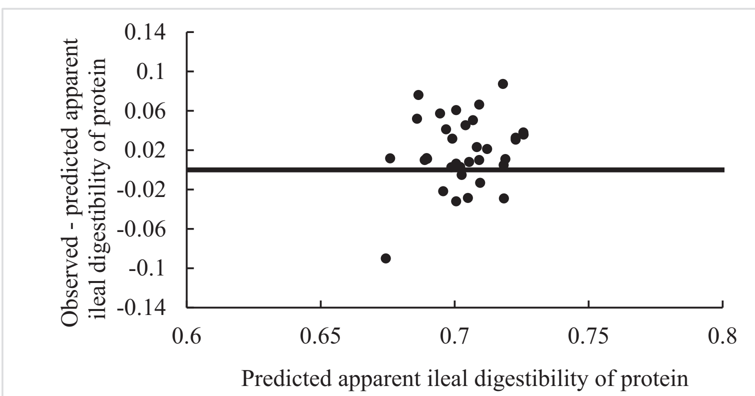

Figure 6. Residuals vs. predicted values for apparent ileal digestibility of protein (IDP) for the 34 diets based on chemical composition (Evaluation data set). The horizontal line represents Observed -predicted, y = 0.

metabolizable energy (Robbins and Firman, 2006; Silva et al., 2010; Alvarenga et al., 2011). An example of prediction equation to determine AMEn content in poultry diets is: 39.78*CP + 69.68 * Ether extract + 35.4*Nitrogen free extract (Rostagno et al., 2005). Another example of prediction equation proposed by Janssen, (1989) which was specific for soybean meal (AMEn = 37.5 CP + 46.39 EE + 14.9 NFE). Ball et al. (2013) showed that inclusion of multiple predictors increased the prediction of protein and starch digestibility, but the additional analytical cost of inclusion would limit such approach from a commercial persepctive. In the current paper, the number of predictors was kept to a minimum to make the prediction relevant to commercial settings.

The above mentioned predictions were not based on complex diets, but on simple diets. In the current study, matrices of complex diet mixtures were used to enable preductions for IDE and IDP that could be used across a wide range of diets.

In the equations suggested in the current study, CF had negative effects on the IDE and IDP. These results are consistent with previous studies (Lodhi et al., 1976; Kluth and Rodehutscord, 2009; Cerrate et al., 2019), wherein dietary fiber was found to reduce protein digestibility in poultry. The negative effect of fiber on the endogenous loss of nitrogen has been shown to be dependent upon the level and type of fiber in pigs (Schulze et al., 1995). Kluth and Rodehutscord (2009) reported significant increases in the ileal endogenous flow of protein and amino acids in broilers with s increasing levels of cellulose.

A negative correlation between CF and IDE was observed ($r^2 = 0.67$). This observation agrees with Jiménez-Moreno et al. (2011), who showed that nutrient digestibility was impaired when the amount of fiber was increased in the diet. In the current study, several fibrous feed ingredients like palm kernel meal, canola meal and wheat bran were included in diet mixtures. A high inclusion level of these ingredients into complex diets is therefore suggested to decrease digestibility of both IDE and IDP based on the current results.

The IDE was negatively affected by the phytate content, which may be explained by the complexing of starch and lipids with phytate (Selle et al., 2012). Low starch digestibility was reported by Thompson and Yoon (1984) as a result of phytate-mineral-starch complex formation in the intestine. Knowledge from the literature and the results from this study indicates that phytate content is an important variable for the prediction of IDE. It is suggested that phytate from feed ingredients with a high phytate content like soybean meal, canola meal and wheat, might influence the overall energy digestion of the diet.,

Fat was found to be an important variable for the prediction of IDP. Diets with a high content of full fat soybeans (the ingredient with the highest fat content) had a high digestibility of CP, in agreement with the table value of CVB (2016). However, improved protein digestibility might be related more to the amount of full fat soybeans in the diet, not too fat per se.

Starch was an important variable for the predictions of both IDE and IDP. A high content of starch was observed in wheat, corn and sorghum and IDE was positively correlated with the cereal content in the diets. IDE values above 0.703 were determined for diets with 860 g/kg diet cereals. Such a correlation, however, was not observed for the IDP. Starch as an important predictor for protein digestion was also suggested by Ball et al. (2013). Starch is present in all cereal-based diets and is therefore a representable variable to include in prediction equations.

The comparison of predictions based on chemical characteristics and estimates for IDE and IDP based on table values (CVB, 2016; Evonik, 2016) for commercially relevant diets showed that the accuracy of IDE prediction was similar. The RMSE% for the prediction of IDE from tabulated values and chemical composition were 5.15 vs. 4.72, respectively, suggesting that both models were overpredicting the IDE. It appears that the IDE can be estimated based on tabulated values, since the inclusion of chemical properties does not improve the prediction. On the other hand, digestibility estimates for energy based on tabulated values for the more complex diets (CDS showed that estimates were not so accurate (RMSE%, 8.08); hence predictions based on chemical composition are more useful for diets with a high content of by-product feeds. Furthermore, the dispersion bias from predictions based on chemical composition, is preferred over mean and slope bias, since it can be eliminated by testing larger sample sizes. Prediction of IDP was improved from 8.21 RMSE% using tabulated values to 5.46 RMSE% with the inclusion of chemical composition for the prediction. Most of the bias for the estimates of IDP based on tabulated values was mean bias (67.56%).

It is well known that AID values of ingredients lack additivity when combined in feed mixtures because of the contribution of endogenous loss of protein (Cowieson et al., 2019). The endogenous loss of protein can be divided into two components: basal and specific. The basal loss is dependent on the DM intake and is unrelated to the dietary composition whereas the specific loss is related to the dietary composition and needs to be measured for each feed ingredient. When protein digestibility is based on AID values the basal endogenous loss of nitrogen is included resulting in underpredictions (Ravindran et al., 2017). However, this was not the case for the prediction of crude protein digestibility in the EDS, where the residual plot shows that the table values are overpredicting the digestibility. It is suggested that mean bias is related to the specific loss of nitrogen in diets which might increase in complex diets due to interactions amongst feed ingredients. However, bias from the prediction based on chemical composition was mainly dispersion (74.64%) followed by mean bias (21.53%), showing underprediction of IDP.

To reduce the mean bias and improve the prediction of IDP, other variables can be analyzed, or *in vitro* models can be used to add an extra parameter to the equation. The cost and complexity of adding more variables to the prediction equations need to be considered in relation to the additional value it generates.

In conclusion, the present study provides equations for predicting the IDE and IDP in complex diets for broilers based on the chemical characteristics of the diets. The best predictors for IDE were starch, CF and phytate contents, and the best predictors for IDP were starch, CF and fat contents. The predictions of IDE based on chemical characteristics and table values were similar and it appears that the table values are enough to predict the IDE. The prediction of IDP was improved when the chemical characteristics were included in the equation. There is potential for further improvement by addition of other variables or by other methods like *in vitro* models.

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