Org. Agr. (2022) 12:259–266
https://doi.org/10.1007/s13165-022-00388-0

Concept note: in vitro multi-enzyme approach to estimate crude protein and amino acid digestibility in grains for broilers

Stephanie Witten · Karen Aulrich

Received: 4 December 2020 / Accepted: 4 April 2022 / Published online: 20 April 2022

Abstract The knowledge of the precaecal digestibility of crude protein (CP) and amino acids (AA) has gained importance. However, since animal welfare is given high priority, animal digestibility experiments are recommended to be reduced or replaced. Thus, in vitro approaches are of interest for feed evaluation. The present study aimed for an adaption of a multi-enzyme assay. An in vitro assay to predict the precaecal digestibility of CP and AA in feedstuffs for swine was adapted to predict the standardised precaecal digestibility of CP and AA quickly in feedstuffs for broilers. In the adapted in vitro assay, the conditions of the gastro-intestinal tract of chicken were simulated in three steps, representing the crop, the stomach, and the small intestine. Samples were incubated at 41 °C for 30 min in a buffer solution at pH 6.0, for 135 min with pepsin at pH 2.6 and for 120 min with pancreatin at pH 6.4. The in vitro rate of CP disappearance of nine different cereal and legume grains was related to their standardised precaecal CP and AA digestibility previously determined in broiler chicken. Regressions between the in vitro rate of CP disappearance and the precaecal digestibility of CP for 42-day old broiler chicken had high coefficients of determination ($R^2 = 0.89$). The adapted method has the potential to predict the precaecal digestibility of CP and AA in feedstuffs for broilers. An extension of the calibration using further samples of single and mixed feed with known precaecal digestibility of CP and AA is recommended.

Keywords Ileal · Organic · Pancreatin · Pepsin

Abbreviations
AA Amino acid
CP Crude protein

Introduction

Over the last decades, the digestibility of crude protein (CP) and amino acids (AA) has gained importance in feed evaluation for monogastric animals (Dalibard and Paillard 1995; Ravindran and Bryden 1999; Perttilä et al. 2002). Since it is not allowed to add amino acids to organic feedstuffs, the knowledge of the digestibility of protein and amino acids can improve their supply from existing feedstuffs. In vivo approaches are diverse, time-consuming, costly, and problematic in terms of animal welfare, which is of special importance and growing interest. To improve the prediction of the precaecal CP and AA digestibility of different batches of feedstuffs without the need for animal trials, various in vitro approaches have been introduced. Their results must be replicable and correlated with the in vivo digestibility (Sibbald 1987; Butts et al. 2012) to be valuable. Boisen and

S. Witten (✉) · K. Aulrich
Institute of Organic Farming, Johann Heinrich von Thünen Institute, Trenthorst 32, 23847 Westerau, Germany
e-mail: stephanie.witten@thuenen.de

Fernández (1995) introduced a multi-enzyme assay to predict the precaecal CP and AA digestibility in swine. Since their approach is simple and realistic, it is used in scientific projects in its original or in modified forms (e. g., Pastuszewska et al. 2004, Jezierny et al. 2010). Furthermore, de Coca-Sinova et al. (2008) found a high correlation between the apparent digestibility of soybean meal in broiler chicks and the in vitro digestibility determined with the method of Boisen and Fernández (1995). This makes the approach promising in terms of the prediction of the precaecal CP and AA digestibility for broiler chicken. However, the method of Boisen and Fernández (1995) is rather time-consuming. The aim of the present study was to adapt the in vitro approach of Boisen and Fernández (1995) to predict the precaecal CP and AA digestibility of organic grains for broiler chicken and to shorten the analysis time.

Material and methods

Samples of different cereals and legumes were subjected to in vivo digestibility trials with broilers and to in vitro analyses to investigate the applicability of an adapted and shortened in vitro multi-enzyme approach.

Sample set

For the calibration of the approach, samples of nine organically cultivated cereal and legume grains (Table 1) with known precaecal digestibility of CP and AA in 42-day-old broiler chicken (Table 2) were available from a prior study (Ritteser 2015), in which the linear regression approach of Rodehutscord et al. (2004) was used. The samples were selected to determine the CP and AA digestibility of locally (in Southern Germany) cultivated feedstuffs for organic poultry and harvested in the years 2010 to 2012.

Crude protein and amino acid analyses

To calculate the in vitro rate of the CP and AA disappearance, the CP contents and the contents of 17 AA were determined in all samples and in the dried residuals. The CP analyses were conducted with the Dumas method according to VDLUFA (2012) (vario MAX CUBE, Elementar Analysensysteme GmbH, Hanau, Germany; N*6.25). Contents of AA in the

Table 1 Composition of feedstuffs available for the calibration of the in vitro method (g/kg 88% DM)

	Buck wheat	Millet	Naked barley	Naked oats	Spelt	Spring barley	Winter triticale	Winter wheat	Lentil rests
Crude protein	128.5	117.0	121.4	123.2	132.9	102.1	95.9	121.4	249.9
Lysine	7.1	1.8	4.3	5.0	3.6	4.0	3.3	3.0	13.7
Methionine	2.0	2.9	1.5	2.0	1.8	1.8	1.4	1.6	2.0
Cystine	2.8	1.8	2.0	3.5	2.6	2.6	2.0	2.5	2.5
Threonine	4.6	3.5	4.0	4.2	3.9	3.7	2.9	3.3	7.8
Tryptophan	1.7	1.7	1.5	1.7	1.4	1.1	0.9	1.2	2.0
Isoleucine	4.5	4.6	4.0	4.4	4.2	3.4	2.8	3.5	8.7
Leucine	7.7	14.7	8.1	8.8	8.4	6.7	5.6	7.3	15.7
Valine	5.8	5.5	5.8	6.0	5.5	4.9	4.0	4.6	10.2
Arginine	11.2	3.9	5.9	8.2	5.6	5.0	4.5	5.2	17.1
Histidine	3.9	2.7	3.3	3.3	3.8	3.3	2.6	3.3	7.4
Phenylalanine	5.5	6.6	6.2	6.2	5.8	4.8	3.9	5.1	10.5
Tyrosine	3.4	4.3	3.6	4.1	3.5	3.0	2.5	3.2	6.2
Alanine	5.2	12.4	4.8	5.7	4.6	4.6	3.6	3.9	9.1
Glycine	7.0	2.6	4.8	6.0	5.4	4.8	3.8	4.6	9.1
Serine	5.9	7.3	4.9	5.8	6.6	6.0	3.9	5.4	10.2
Proline	4.8	8.4	12.5	6.7	12.3	9.0	7.7	11.1	10.5
Aspartic acid	11.4	7.2	7.0	10.2	7.0	7.1	5.5	5.5	25.7
Glutamic acid	20.7	26.0	28.8	25.3	36.1	21.2	21.4	33.4	38.8

Table 2 Precaecal digestibility coefficients of the crude protein (CP) and amino acids of feedstuffs available for the calibration of the in vitro method (from Ritteser 2015)

	Buck wheat	Millet	Naked barley	Naked oats	Spelt	Spring barley	Winter triticale	Winter wheat	Lentil rests
Crude protein	0.71	0.80	0.64	0.91	0.86	0.78	0.85	0.86	0.86
Lysine	0.77	0.86	0.61	0.91	0.85	0.85	0.83	0.88	0.86
Methionine	0.79	0.91	0.79	0.99	0.90	0.88	0.93	0.94	0.90
Cystine	0.52	0.80	0.67	0.83	0.79	0.68	0.75	0.90	0.74
Threonine	0.71	0.76	0.61	0.99	0.82	0.81	0.76	0.78	0.80
Tryptophan	0.71	0.88	0.62	0.94	0.92	0.68	0.70	0.89	0.78
Isoleucine	0.71	0.80	0.67	0.96	0.81	0.77	0.75	0.85	0.84
Leucine	0.74	0.86	0.70	0.95	0.86	0.82	0.82	0.90	0.85
Valine	0.71	0.78	0.69	0.97	0.84	0.79	0.83	0.72	0.84
Arginine	0.83	0.87	0.72	0.93	0.87	0.81	0.80	0.86	0.89
Histidine	0.70	0.83	0.67	0.93	0.84	0.82	0.74	0.79	0.85
Phenylalanine	0.68	0.81	0.67	0.94	0.87	0.81	0.84	0.88	0.82
Tyrosine	0.75	0.86	0.69	0.85	0.81	0.84	0.77	0.88	0.83
Alanine	0.68	0.89	0.61	0.92	0.85	0.80	0.81	0.82	0.82
Glycine	0.68	0.76	0.57	0.89	0.85	0.76	0.74	0.78	0.80
Serine	0.75	0.85	0.66	0.89	0.85	0.85	0.83	0.88	0.80
Proline	0.67	0.81	0.77	0.97	0.92	0.78	0.89	0.92	0.81
Aspartic acid	0.73	0.81	0.51	0.92	0.73	0.74	0.67	0.77	0.83
Glutamic acid	0.75	0.86	0.72	0.95	0.90	0.94	0.85	0.92	0.86

samples and the dried residuals after incubation were analysed according to Directive (EC) No 152/2009 (EC 2009) regarding sample preparation via oxidation and hydrolysis. The subsequent derivatisation and chromatography were performed according to Cohen and Michaud (1993). The analytical procedure including the subsequent derivatisation and chromatography was adapted and recently described in detail by Witten et al. (2020).

In vitro *technique*

The experiments were based on a multi-enzyme assay to predict the CP and AA digestibility of feedstuffs for pigs (Boisen and Fernández 1995). The adaptation aimed to shorten the analysis time and was based on a selection of literature reports on retention times in the digestive tract of chicken as well as dry matter content and pH value of the digesta. During the adaptation process, it became clear that the dry matter content throughout incubation greatly affects the analysis outcome. Thus, it is important to ensure that the amount of added fluid is an important point of standardisation of the assay.

Step 1

Samples were ground to pass a 0.5 mm sieve (Cyclotec 1093, Foss GmbH, Germany). A fourfold determination took place. Additionally, a selected wheat sample was used as control in a threefold determination in each series (the samples analysed on the same day). About 1.5 g of sample material were weighed to an accuracy of ± 1 mg in glass centrifuge tubes (45 ml) with screw caps. Into each tube 7.5 ml phosphate buffer (1 M, pH 6.0) and a magnetic rod (20 mm) were added. The tubes were closed with screw caps. Sample and buffer were mixed by gentle stirring (200 rotations/min) for 30 min in a stirring water bath with circulation thermostat (UNITHERM WAM 15, UniEquip, Planegg, Germany) at 41 °C to simulate the conditions of the crop.

Step 2

To simulate the digestion in the stomach, a pepsin solution containing 0.01 g pepsin/ml (Merck No. 7190 2000 FIP-U/g) was freshly prepared with HCl (0.2 M). Subsequently, 1.5 ml pepsin solution was

added and the pH value of the mixture was adjusted to pH 2.6 with HCl (5 M) and NaOH (5 M). The tubes were closed again and the samples were stirred for 135 min at 41 °C.

Step 3

To simulate the digestion in the small intestine, a pancreatin solution containing 0.05 g pancreatin/ml (Merck No. P1750, 4xUSP specifications) was freshly prepared with a phosphate buffer (0.2 M, pH 6.8) and filtered through a coarse filter. After adding 0.0375 ml NaOH (0.6 M), 1.5 ml pancreatin solution were added and the pH value of the mixture was adjusted to pH 6.4 with NaOH (5 M) and HCl (5 M). The tubes were closed again and the samples were stirred for 120 min at 41 °C. Afterwards, magnetic rods were removed with a magnetic stick and rinsed with demineralised water. Tubes were then centrifuged for 10 min with 3321*g at 4 °C (4000 rpm, HeraeusMultifuge 1S-R with swing-out rotor Sorvall®, Kendro Laboratory Products GmbH, Langenselbold, Germany). Following centrifugation, the supernatant was decanted and the residuals were gently dried at 40 °C in a vacuum oven (Vacutherm, Thermo Fisher Scientific Inc., Waltham, MA, USA) until weight constancy was reached (about 12 h). The dried samples were weighed separately and ground with mortar and pestle. The total volume of the replicates of the residuals was pooled into a mixed sample. The mixed sample was used to analyse CP and AA in the residual of the test feedstuffs in a twofold determination. The rate of CP and AA disappearance (RD) was calculated for each sample replicate using the following equation:

$$RD = 1 - (R/I)$$

with I = the content of the CP or a specific AA in the initial sample and R = content of the CP or a specific AA in the residual sample.

Statistics

The calibration of the in vitro method was conducted by regression analyses (glm) with R 3.4.0 (R Core Team 2021). The in vitro rate of CP disappearance of the feedstuffs was related to the in vivo precaecal CP and AA digestibility of feedstuffs in broiler chicken. Furthermore, the in vitro rate of disappearance of each AA was related to its in vivo precaecal digestibility in broiler chicken. To assess the relationship, the coefficient of determination ($R^2 > 0.5$) and the root mean square error (RMSE < 0.05) were used.

Results

The in vitro rate of disappearance of the CP and AA of each feedstuff are summarised in Table 3. The relation of the in vitro rate of disappearance and the precaecal CP digestibility of the tested feedstuffs had a R^2 of 0.89 with a RMSE of 0.027 (Fig. 1). Since the residual of naked barley could not be separated from the fluid during centrifugation its in vitro rate of CP and AA disappearance could not be determined reliably. Therefore, its CP and AA digestibility would be overestimated and naked barley was excluded from the regression analysis.

The prediction of the AA digestibility for broiler chicken, with the exception of arginine, histidine, and tyrosine, was strongly related to the in vitro rate of either the CP or the associated AA disappearance (Table 4). For most AA the coefficient of determination (R^2) was higher for the regressions based on the AA disappearance or was comparable between the regressions based on the CP and AA rate of disappearance. However, the regression based on the CP disappearance had a considerably higher R^2 for lysine, methionine, cystine, and proline.

Discussion

In the present study, the CP and AA disappearance of an adapted in vitro assay was related to the precaecal CP and AA digestibility of buckwheat, lentil, and cereal grains in broiler chickens. In vitro assays typically cannot be used to simulate endogenous losses or microbial digestion, in vitro rates of disappearance display the standardised or true digestibility or even the bioavailability of CP and AA (Galibois et al. 1989) rather than the apparent digestibility. Since in the used approach correction for basal endogenous losses is implemented, the results of the present study compare to the standardised precaecal digestibility (Kong and Adeola 2014). Thus, the in vitro assay of the present study has the potential to be related to the results of the in vivo study and was indeed highly

Table 3 In vitro rate of disappearance of the crude protein (CP) and amino acids of feedstuffs available for the calibration of the in vitro method

	Buck wheat	Millet	Naked barley	Naked oats	Spelt	Spring barley	Winter triticale	Winter wheat	Lentil rests
Crude protein	0.57	0.68	0.69	0.80	0.74	0.68	0.73	0.78	0.70
Lysine	0.59	0.62	0.70	0.82	0.71	0.71	0.69	0.73	0.73
Methionine	0.53	0.69	0.62	0.81	0.71	0.75	0.67	0.78	0.60
Cystine	0.49	0.61	0.56	0.82	0.68	0.75	0.73	0.75	0.52
Threonine	0.59	0.68	0.71	0.81	0.66	0.71	0.69	0.71	0.70
Isoleucine	0.59	0.71	0.72	0.82	0.74	0.70	0.71	0.78	0.68
Leucine	0.58	0.75	0.73	0.83	0.74	0.71	0.73	0.78	0.70
Valine	0.58	0.70	0.73	0.82	0.74	0.71	0.71	0.77	0.69
Arginine	0.63	0.68	0.72	0.84	0.70	0.69	0.73	0.74	0.73
Histidine	0.34	0.71	0.71	0.85	0.67	0.80	0.84	0.80	0.88
Phenylalanine	0.58	0.72	0.73	0.83	0.76	0.71	0.73	0.80	0.67
Tyrosine	0.53	0.71	0.70	0.82	0.78	0.68	0.70	0.82	0.65
Alanine	0.58	0.75	0.70	0.82	0.71	0.69	0.68	0.74	0.69
Glycine	0.53	0.58	0.65	0.80	0.70	0.70	0.65	0.74	0.63
Serine	0.59	0.73	0.69	0.81	0.77	0.77	0.69	0.79	0.69
Proline	0.53	0.74	0.78	0.82	0.82	0.76	0.80	0.86	0.63
Aspartic acid	0.65	0.70	0.69	0.83	0.71	0.70	0.69	0.74	0.75
Glutamic acid	0.65	0.76	0.79	0.86	0.81	0.77	0.80	0.86	0.73

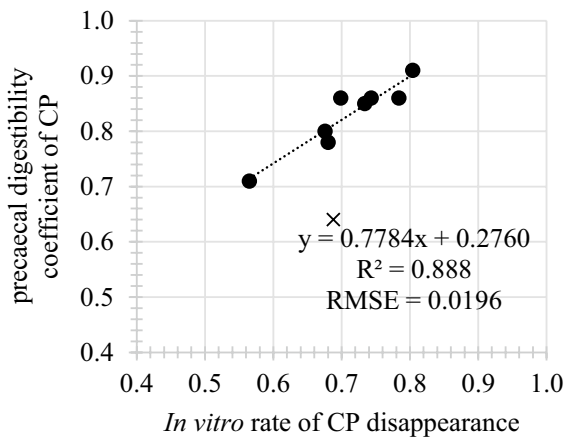

Fig. 1 Relation of the in vivo precaecal digestibility coefficient of CP in broilers with the rate of crude protein (CP) disappearance in vitro (x, sample of naked barley deemed as outlier and not included in regression)

correlated to the precaecal CP and AA digestibility in 42 days old broiler chickens. However, there were differences in the quality of the regression depending on the considered AA. Moreover, it had an impact if the CP disappearance or the AA disappearance was used as a basis for the regression. In the present study, the precaecal digestibility of arginine, histidine, and tyrosine could not be estimated reliably ($R^2 < 0.5$). The digestibility of threonine and asparagine could not be predicted using the rate of CP disappearance. However, the digestibility of cystine could exclusively be predicted using the rate of CP disappearance. Also for lysine, methionine, proline, valine, and phenylalanine, the coefficient of determination and RMSE were better, when the regression was based on the rate of CP disappearance rather than the rate of AA disappearance. Nevertheless, the digestibility of all other AA could best be predicted based on their own rate of disappearance. These results might be due to the small sample-set. The quality of the estimation of the digestibility of all AA probably increases when the underlying sample-set is expanded. Differences between the goodness of the estimations of the digestibility of different AA might be due to characteristics of the in vivo trial like to interactions between the feedstuffs and the animals. Characteristics of the feedstuff can, for example, affect absorption, endogenous losses, the microflora of the digestive tract, the viscosity of the digesta, and, consequently, the performance of the chick (Saki et al. 2010). Predictions of in vitro assays are not able to display interactions

Table 4 Regression equations, root mean square errors (RMSE) and coefficients of determination (R^2) of in vitro rate of disappearance (RD) of crude protein (CP) and amino acids (AA) of eight feedstuffs related to the precaecal AA digestibility on day 42

	From RD of CP	RMSE	R^2	From RD of AA	RMSE	R^2
Lysine	0.4689x + 0.5183	0.018	0.76	0.4910x + 0.5078	0.021	0.70
Methionine	0.7162x + 0.3964	0.018	0.89	0.4863x + 0.5679	0.032	0.65
Cystine	1.3611x—0.2153	0.046	0.82	0.6090x + 0.3446	0.083	0.40
Threonine	0.7617x + 0.2629	0.056	0.48	1.2132x—0.0398	0.038	0.76
Arginine	0.2683x + 0.6670	0.035	0.22	0.4433x + 0.5390	0.031	0.41
Isoleucine	0.7903x + 0.2501	0.044	0.62	0.9649x + 0.1212	0.038	0.72
Leucine	0.7384x + 0.3256	0.025	0.82	0.8182x + 0.2544	0.016	0.92
Valine	0.9917x + 0.1290	0.026	0.88	1.0624x + 0.0719	0.027	0.86
Histidine	0.5988x + 0.3873	0.050	0.41	0.2463x + 0.6314	0.052	0.37
Phenylalanine	0.9794x + 0.1357	0.013	0.97	0.9249x + 0.1622	0.019	0.93
Tyrosine	0.3500x + 0.5752	0.034	0.35	0.3116x + 0.6017	0.031	0.46
Serine	0.5220x + 0.4668	0.020	0.77	0.6060x + 0.3942	0.011	0.93
Proline	1.2567x—0.0462	0.020	0.95	0.7639x + 0.2770	0.044	0.76
Asparagine[1]	0.3787x + 0.5061	0.067	0.14	1.1858x—0.0772	0.039	0.70
Glutamine[1]	0.6957x + 0.3847	0.035	0.67	0.7867x + 0.2637	0.033	0.70
Alanine	0.7265x + 0.3078	0.043	0.59	1.0100x + 0.1090	0.020	0.92
Glycine	0.6886x + 0.2935	0.037	0.64	0.6150x + 0.3731	0.035	0.67

[1]Includes acidic form

between the animal and the feedstuff and thus mostly independent of the animal. However, in vitro methods, like the described assay, can be used as an indicator of in vivo measures and give valuable predictions, if the procedure is robust (Butts et al. 2012). Thus, measures must be taken to make the analysis results replicable and valid. In the method of Boisen and Fernández (1995) the sample was ground to pass a 1 mm sieve. The samples were transferred repeatedly from one vessel into another during the analysis. In the present study, during the whole analysis the same centrifuge tubes were used to avoid transfers and enhance the robustness. The use of the vacuum oven led to reliable results compared to other ovens, in which the material tended to burn. There was no difference in the rate of disappearance, when enzyme activity was stopped by decreasing pH value of liquid before centrifugation and oven drying. Furthermore, to increase the repeatability of the procedure, the sample material was ground to pass a 0.5 mm sieve and the amount of sample material used for the analysis was increased. The centrifugation was found not to be suitable for all feedstuffs. Thus, alternative methods should be tested.

By now, the equations used to predict the CP and AA digestibility in the described in vitro assay were based on a regression including in vivo results of eight different feedstuffs. Although the regressions mostly had satisfying coefficients of determination, which often exceeded 0.7, it is recommended to expand the number of feedstuffs used for method calibration to increase its validity. Furthermore, it would be of interest to relate the in vitro rate of CP and AA disappearance to the precaecal digestibility of a selection of samples originating from the same type of feedstuff (e.g. some different wheat samples) to validate the method. Since rapid, cheap, and simple analyses are required, it is desirable to use the in vitro CP disappearance also to predict the AA digestibility. However, shifts in the ranking of the precaecal digestibility of single AA cannot be illustrated sufficiently by this calculation. A further characterisation of the in vitro disappearance of the AA could be suitable to develop better predictions of the precaecal digestibility of the AA in broiler chicken.

Conclusions

The modified multi-enzyme assay to predict the precaecal CP and AA digestibility of feedstuffs for broiler chicken is promising regarding its use to predict precaecal CP and AA digestibility in feedstuffs for broiler chicken. However, it is recommended to further improve the validity of the multi-enzyme assay for the prediction of the precaecal digestibility

of CP and especially of AA using combined in vitro and in vivo studies.

Author contribution All authors contributed to the study conception and design.

Funding Open Access funding enabled and organized by Projekt DEAL. The project was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the Federal Programme for Ecological Farming and Other Forms of Sustainable Agriculture (Grant number 2811OE054).

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication All authors read and approved the final manuscript.

Conflict of interest There is no conflict of interest.

References

Boisen S, Fernández JA (1995) Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by in vitro analyses. Anim Feed Sci Tech 51:29–43. https://doi.org/10.1016/0377-8401(94)00686-4

Butts CA, Monro JA, Moughan PJ (2012) In vitro determination of dietary protein and amino acid digestibility for humans. Br J Nutr 108:S282–S287. https://doi.org/10.1017/S0007114512002310

Cohen SA, Michaud DP (1993) Synthesis of a fluorescent derivatizing reagent, 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate, and its application for the analysis of hydrolysate amino acids via High-Performance Liquid Chromatography. Anal Biochem 211:279–287. https://doi.org/10.1006/abio.1993.1270

Dalibard P, Paillard E (1995) Use of the digestible amino acid concept in formulating diets for poultry. Anim Feed Sci Tech 53:189–204. https://doi.org/10.1016/0377-8401(95)02010-W

de Coca-Sinova A, Valencia DG, Jiménez-Moreno E, Lázaro R, Mateos GG (2008) Apparent ileal digestibility of energy, nitrogen, and amino acids of soybean meals of different origin in broilers. Poult Sci 87:2613–2623. https://doi.org/10.3382/ps.2008-00182

Galibois I, Savoie L, Simoes Nunes C, Rérat A (1989) Relation between in vitro and in vivo assessment of amino acid availability. Reprod Nutr Dev 29:495–507

Jezierny D, Mosenthin R, Sauer N, Eklund M (2010) In vitro prediction of standardised ileal crude protein and amino acid digestibilities in grain legumes for growing pigs. Animal 4:1987–1996. https://doi.org/10.1017/S1751731110001114

Kong C, Adeola O (2014) Evaluation of amino acid and energy utilization in feedstuff for swine and poultry diets. Asian Austral J Anim 27:917–925. https://doi.org/10.5713/ajas.2014.r.02

Pastuszewska B, Vitjazkova M, Swiech E, Taciak M (2004) Composition and in vitro digestibility of raw versus cooked white- and colour-flowered peas. Nahr/food 48:221–225. https://doi.org/10.1002/food.200300417

Perttilä S, Valaja J, Partanen K, Jalava T, Venäläinen E (2002) Apparent ileal digestibility of amino acids in protein feedstuffs and diet formulation based on total vs digestible Lysine for poultry. Anim Feed Sci Tech 98:203–218. https://doi.org/10.1016/S0377-8401(02)00031-7

Ravindran V, Bryden WL (1999) Amino acid availability in poultry - in vitro and in vivo measurements. Aust J Agric Res 50:889–908. https://doi.org/10.1071/Ar98174

Rodehutscord M, Kapocius M, Timmler R, Dieckmann A (2004) Linear regression approach to study amino acid digestibility in broiler chickens. Br Poult Sci 45:85–92. https://doi.org/10.1080/00071660410001668905

Saki AA, Hemati Matin HR, Tabatabai MM, Zamani P, Harsini RN (2010) Microflora population, intestinal condition and performance of broilers in response to various rates of pectin and cellulose in the diet. Arch Geflügelk 74:183–188

Sibbald IR (1987) Estimation of bioavailable amino-acids in Feedingstuffs for poultry and pigs - a review with emphasis on balance experiments. Can J Anim Sci 67:221–301

Witten S, Böhm H, Aulrich K (2020) Effect of variety and environment on the contents of crude nutrients and amino acids in organically produced cereal and legume grains. Org Agricult 10:199–219. https://doi.org/10.1007/s13165-019-00261-7

EC (2009). Commission Regulation, EC) No 152/2009 of 27 January 2009 Laying Down the Methods of Sampling and Analysis for the Official Control of Feed.

R Core Team (2021) R: A language and environment for statistical computing. R Version 4.1.2 "Bird Hippie". R Foundation for Statistical Computing, Vienna, Austria. https://www.Rproject.org/. Accessed 13 Jan 2022

Ritteser C (2015) Bestimmung präcecaler Verdaulichkeitskoeffizienten für heimische Energie- und Proteinfuttermittel für die Bio-Hühnermast [Estimation of ileal nutrient digestibility of native energy and protein feeding stuffs for organic broilers]. Dissertation, University of Hohenheim.

VDLUFA (2012) VDLUFA Methodenbuch Band III Die Chemische Untersuchung von Futtermitteln einschl. 1.-8. Ergänzungslieferung [VDLUFA Method Book Volume III The Chemical Analyses of Feedstuffs incl. 1st-8th Supplemental Delivery], VDLUFA Verlag, Darmstadt.