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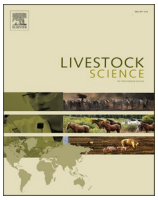

A rapid laboratory method for estimating the standardised precaecal digestible amino acids in pig feeds

Valérie Schumacher [a], Markus Rodehutscord [b], Karl-Heinz Südekum [a,*], Saskia Kehraus [a]

[a] Institute of Animal Science, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany
[b] Institute of Animal Science, University of Hohenheim, Emil-Wolff-Strasse 10, 70599 Stuttgart, Germany

HIGHLIGHTS

- Chemical analysis was suitable for prediction of precaecal digestible amino acids.
- Chemical analysis may partly replace invasive *in vivo* measurements on pigs.
- The method is rapid and does not involve multiple analytical steps.
- The method allows faster feed evaluation and improved ration planning.

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ABSTRACT

Amino acids (AA) are essential nutrients for diverse processes in the pig's body. The utilisation of AA depends on their digestibility and absorption. Therefore, methods to determine reliably the AA supply to pigs to sustain performance and animal health are critical for precise feed evaluation. The evaluation of AA supply has so far been based on *in vivo* determination of standardised precaecal digestible (pcd) AA (pcdAA) and *in vitro* estimates of pcdAA applying time-consuming and complex laboratory methods. The objective of this study was to develop and establish a rapid laboratory method for estimating pcdAA based on the determination of AA insoluble in neutral-detergent (ND) or acid-detergent (AD) (NDIAA, ADIAA). The laboratory method used the same procedure which was previously applied to estimate standardised pcd crude protein (pcdCP). The hypothesis was that the method was similarly suitable to estimate pcdAA. A sample pool of 74 feed ingredients (cereal grains, differently heat-treated legume grains) was available on which *in vivo* pcdAA were determined in cannulated pigs. Amino acids in feed ingredients and in ND or AD residues of feed ingredients were determined by an HPLC method. The concentrations (g/kg dry matter) of ND- and AD-soluble AA (NDSAA, ADSAA) were calculated by difference to total AA in feed. For the estimation of the concentrations of *in vivo* pcdAA for total AA and the entire dataset ($n = 74$), a linear relationship was established between the concentrations of NDSAA or ADSAA and the *in vivo* pcdAA: $y = 0.823$ (standard error [SE] 0.018) $x + 10.52$ (SE 4.420), where y represents the *in vivo* pcdAA (g/kg dry matter) and x represents the NDSAA (cereal grains) or ADSAA (protein feeds) value (g/kg dry matter). The coefficient of determination (R^2) of this equation was 0.968 and ranged from 0.895 to 0.984 for the 17 individual AA. This study shows that *in vivo* pcdAA values can be estimated following the same standardised and rapid laboratory procedure previously established for pcdCP, based on chemical analyses, namely determination of NDIAA and ADIAA, from which NDSAA and ADSAA values are calculated.

Glossary

AA	amino acid
AD	acid-detergent
ADF	AD fibre expressed inclusive of residual ash
ADIAA	AD-insoluble AA
ADSAA	AD-soluble AA
c	coarse
CI	95% confidence interval
CP	crude protein

* Corresponding author.
E-mail address: ksue@itw.uni-bonn.de (K.-H. Südekum).

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DM	dry matter
f	fine
FM	fish Meal
FFSB	full Fat Soy Bean
GLS	glucosinolates
ND	neutral-detergent
NDIAA	ND-insoluble AA
NDSAA	ND-soluble AA
pcd	standardised precaecal digestible
pcdAA	standardised precaecal digestible AA
pcDAA	fractional standardised precaecal digestibility of AA
pcdCP	standardised precaecal digestible CP
PeaP	pea Protein
RMSE	root mean square error
RSC	rapeseed Cake
RSM	rapeseed Meal
SBC	soybean cake
SBM	soybean meal
SPC	soy protein concentrate
SPI	soy protein isolate
SE	standard error
WG	wheat Gluten

1. Introduction

An ongoing challenge for animal nutrition is to compose and feed rations which provide the animals with an adequate quantity of amino acids (AA) to sustain performance and health. In pigs, protein digestion and AA absorption primarily occur in the small intestine, *i.e.* precaecally. Therefore, standardised precaecal (synonymously termed "ileal") digestible amino acids (pcdAA) are the key variable of AA evaluation of pig feeds (GfE, 2008). For the determination of *in vivo* pcdAA values, apparent digestible AA are corrected for basal endogenous AA losses, but specific losses, which depend on feed properties, such as content of fibre, lectin, tannins or protease inhibitor activity, are not considered (GfE, 2005; Adeola et al., 2016). *In vivo* methods are the most precise approaches, often called gold standard, for the evaluation of pcdAA, however they have some disadvantages. Animal trials can be difficult to perform, are time and cost intensive, have ethical limitations and are limited in their ability to cover the required high number of tests for new or differently treated feed ingredients (Brodkorb et al., 2019; Santos-Sánchez et al., 2024). Therefore, *in vitro* methods have been developed over the last decades to simulate digestion processes in a laboratory environment (Moughan et al. 2014). Santos-Sánchez et al. (2024) described and compared the scope and limitations of several *in vitro* methods and modifications to estimate the crude protein (CP) and AA digestibility in animals and humans. Only the study of Jezierny et al. (2010), which was based on a two-step incubation method with pepsin and pancreatin following the method of Boisen and Fernández (1995), was mentioned as being able to estimate pcdAA in pig feeds. Not cited by Santos-Sánchez et al. (2024), Eklund et al. (2015) and Rosenfelder-Kuon et al. (2020) were also using the two-step enzymatic method of Boisen and Fernández (1995) to estimate pcdAA. However, these studies used small sample pools which consisted of either cereal grains or protein feeds.

Recently, Schumacher et al. (2025) have demonstrated that pcdCP can be reliably estimated from the analysis of neutral-detergent insoluble (NDI) CP. Therefore, the aim of this study was to extend this method and estimate pcdAA based on the same sample pool used to estimate pcdCP which included different cereal grains, thermally treated protein feeds and other samples (Schumacher et al., 2025). The hypothesis was that the method developed and established to estimate pcdCP was similarly suitable to estimate pcdAA. The assumption was that insoluble AA determined as NDIAA or ADIAA are largely indigestible in the small intestine (Rezvani et al., 2012; Zeyner et al., 2015) and, therefore, soluble AA determined as ND-soluble (NDSAA) or AD-soluble (ADSAA) in the feed, represent the (potentially) digestible AA in the small intestine.

2. Materials and methods

2.1. Material

2.1.1. Samples

A large sample pool was accessible of 82 feed ingredients on which pcdAA had been determined *in vivo* in pigs at the University of Hohenheim, Stuttgart, Germany. The sample pool consisted of cereal (wheat, triticale, rye, barley) and legume (faba beans, peas, lupins) grains, each represented by several varieties, untreated and heat-processed rapeseed meal and cake, soybean products and miscellaneous samples (Table 1). Based on the results presented by Schumacher et al. (2025), the following feeds listed in Table 1 were excluded from the AA analyses: 3 full-fat soybeans (no. 49, 50, 57) with high trypsin inhibitor activity, resulting in low *in vivo* concentrations of pcdCP and pcdAA. Additionally, soy protein isolate (hydrolysed), pea protein, wheat gluten 1, wheat gluten 3 (hydrolysed) and fish meal 1 (no. 76, 77, 78, 80, 81) were also excluded due to the high *in vivo* digestibility and therefore expected very low amounts of AD residues for AA analyses which would reduce the accuracy of the AD determination and introduce variability not related to intrinsic feed characteristics. Ultimately, 74 feed ingredients were used for the analyses in this study. The pcdAA values for total and individual AA were then calculated by multiplying the fractional standardised precaecal digestibility of AA (pcDAA) by AA content. These values are shown in the Appendix Tables A1–A7.

2.1.2. Characterisation and selection for assigning samples to the neutral- or acid-detergent insoluble amino acid procedure

A condensed overview of anti-nutritional compounds compiled from the references given in Table 1 is presented in Schumacher et al. (2025; Appendix Tables A1–A4) which were used as a basis for the selection criteria of NDIAA or ADIAA (Table 2): Feed ingredients containing specific N compounds, *e.g.*, Maillard products or/and tannin-protein complexes, which are captured in the AD residue but not in the ND residue (Table 2), were analysed for ADIAA, whereas on all other samples NDIAA were analysed. Hence, NDIAA was analysed on all cereal grain samples and ADIAA on all other samples, which primarily can be referred to as protein feeds.

2.2. Methods

2.2.1. General analyses

The samples were milled through a 1-mm screen using an ultra-centrifugal mill at 18,000 rpm (ZM 200, Retsch GmbH & Co. KG, Haan, Germany). Dry matter (DM) was determined by oven-drying at 103 °C (Regulation (EG) 152/2009 Annex III, A). The ND or AD residues for NDIAA or ADIAA determination were isolated as specified in VDLUFA (2023) using Fibretherm (FT12; FibreBags ADF, 30 μm pore size; C. Gerhardt, Königswinter, Germany). The residues were dried at 103 °C, thereafter scraped out of the bags, ground with a coffee grinder (MX 32, Braun AG, Frankfurt, Germany) and collected in glass containers until a quantity of at least 2.3 g of the dried residue for AA analyses was reached in each case. Depending on feed ingredient, the boiling cycles had to be repeated 2 to 4 times to obtain this quantity. Afterwards, AA were analysed on the residue by HPLC according to Regulation (EG) 152/2009 (Annex III, F, G) by LUFA Nord-West, Oldenburg, Germany. Amino acids were separated on a PEEK column (for tryptophan, a Phenomenex Synergi Hydro RP column was used) with the following mobile phase: 6 ml acetic acid + 1800 ml H_20 + 100 ml trichloro-2-methyl-2-propanol, adjusted to pH 5 with ethanolamine and brought to 2 l volume with distilled water. Different buffers were used as eluents. After post-column ninhydrin derivatization, AA were detected by fluorescence at 280 nm excitation wave length and 356 nm emission

Table 1
The number, sample group, reference and pre-treatment of 82 feed ingredients of which 74 were assayed in this study (see Section 2.1.1. for exclusion reasons).

Number	Sample group	Sample	Reference	Pre-treatment*
1	Wheat	Skalmeje	[1]	Incubation
2		Tommi		
3		St. Tobak		
4		Event		
5		Mulan		
6		Tabasco		
7		Adler		
8		KWS Erasmus		
9	Triticale	Grenado	[2]	Incubation
10		Tarzan		
11		HYT Prime		
12		Massimo		
13		Cultivo		
14		SW Talentro		
15		Cando		
16		Agostino		
17	Rye	Conduct	[2]	Incubation
18		Visello		
19		Helltop		
20		Bellami		
21		Palazzo		
22		Dukato		
23		Guttino		
24		Dankowski Diament		
25	Barley	Yool	[3]	Incubation
26		Ack 2927		
27		Lomerit		
28		Campanille		
29		Canberra		
30		Antisette		
31		Metaxa		
32		Fridericus		
33	Pea	Santana	[4]	Incubation
34		Jutta		
35		Phönix		
36		Harnas		
37		Rocket		
38		Hardy		
39	Lupin	Probor	[4]	Incubation
40		Boregine		
41		Boruta		
42		Idefix		
43	Faba bean	Aurelia	[4]	Incubation
44		Divine		
45		Gloria		
46		Limbo		
47		Fuego		
48		Espresso		
49	Full-fat soybean/ soybean product	FFSB K0	[5]	Fat extraction
50		FFSB K1	[6]	Fat extraction
51		FFSB K2		/
52		FFSB K3		/
53		FFSB Z1		/
54		FFSB Z2		
55		FFSB Z3		
56		FFSB Z4		
57		FFSB		
58		FFSB (roasted)		
59		SBC		
60		SBM (Austria)		
61		SBM (GMO-free)		
62		SBM (standard)		
63	Rapeseed meal and cake	RSM48	[7]	/
64		RSM64	[6]	/
65		RSM76		/
66		RSM93		/
67		LOW-GLS RSM		/
68		RSC		Fat extraction
69		RSM		/
70	Various samples	Soybean (extruded)	[8]	Fat extraction
71		SBM (high protein)	[9]	/
72		SPC (A coarse)		/
73		SPC (A fine)		/
74		SPC (B coarse)		/
75		SPC (B fine)		/
76		SPI (hydrolysed)		/
77		PeaP		Fat extraction
78		WG1		/
79		WG2 (hydrolysed)		/
80		WG3 (hydrolysed)		/
81		FM1		Fat extraction
82		FM2 (extracted)		/

FFSB, Full-fat soybean; FM, Fish meal; GLS, Glucosinolates; PeaP, Pea protein; RSC, Rapeseed cake; RSM, Rapeseed meal; SBC, Soybean cake; SBM, Soybean meal; SPC, Soy protein concentrate; SPI, Soy protein isolate; WG, Wheat gluten; / no pre-treatment;
*Incubation: carried out according to McQueen and Nicholson (1979).
*Fat extraction: carried out according to Regulation (EG) 152/2009 Annex III, H 1.1 (without hydrochloric acid pre-treatment).
References: [1] Rosenfelder et al. (2015), [2] Strang et al. (2017), [3] Spindler et al. (2016), [4] Jezierny et al. (2010), [5] Kaewtapee et al. (2017a), [6] Kaewtapee et al. (2017b), [7] Eklund et al. (2015), [8] Urbaityte et al. (2009a), [9] Urbaityte et al. (2009b).

Table 2
The selection criteria Maillard products, condensed tannin-CP, isoelectric point protein and phytate-CP-complexes for neutral-detergent insoluble (NDIAA) or acid-detergent insoluble amino acids (ADIAA).

	Literature	NDIAA	ADIAA
Maillard products	Van Soest and Mason (1991) Licitra et al. (1996) Classen et al. (2004)	/	X
Condensed tannin-CP	Van Soest (1994)	/	X
Isoelectric point protein	Csonka et al. (1926); Csonka and Jones (1927) Morales et al. (2013)	X	X
Phytate-CP-complex	Morales et al. (2013)	X	X
		Cereal grains	**Protein feeds**

CP, Crude protein; ADIAA, Acid-detergent insoluble amino acid; NDIAA, Neutral-detergent insoluble amino acid; /, not included; X, included.

wave length.

2.2.2. Sample pre-treatment for removal of starch or fat

As listed in Table 1 and described in detail by Schumacher et al. (2025), starchy samples were pre-treated overnight with α-amylase (Termamyl 2X, Novozymes, Novo Industrials, Bagsværd, Denmark) in a buffer (pH 7) at 40 °C in a shaking (80 rpm) water bath (SW22, JULABO, Seelbach, Germany) according to McQueen and Nicholson (1979). Samples with fat content >80 g/kg DM (full-fat soybeans, soybean cake, rapeseed cake, samples no. 52–56, 58–59, 68, 70) were pre-treated to extract fat according to Regulation (EG) 152/2009 Annex III, H (1.1; without hydrochloric acid pre-treatment). The samples, weighed in

bags, were extracted in a Soxtherm device (Sox 404, C. Gerhardt) with petrolether (40–60 °C) for 1.5 h. The bags with pre-treated samples were afterwards used to isolate the ND and AD residue.

2.3. Calculations and statistical analysis

The concentrations of NDSAA or ADSAA of feeds were calculated as follows:

$$\text{NDSAA (g/kg DM)} = \text{AA (g/kg DM)} - \text{NDIAA (g/kg DM)} \quad (1)$$

and

$$\text{ADSAA (g/kg DM)} = \text{AA (g/kg DM)} - \text{ADIAA (g/kg DM)} \quad (2)$$

2.3.1. Regression analysis

The statistical data evaluation was carried out with the programme R 2.2 (R Foundation for Statistical Computing, Vienna, Austria). First, a raw data analysis was performed and means and standard deviations were calculated. Then, a linear model was applied for the regression analysis:

$$y = a\,x + b \quad (3)$$

where y= *in vivo* pcdAA (g/kg DM) and x= NDSAA or ADSAA (g/kg DM) as categorised above (2.1.2).

This model was applied to the entire dataset (n= 74) and separately to cereal grains at large (n= 38) and in different combinations of cereal grain species and separately to protein sources (n= 36). For the large subgroup of cereal grains in the dataset, an additional ANOVA, followed by a Tukey-Test to separate means for wheat, rye, barley and triticale, was carried out to further evaluate the data in accordance with the procedure reported for pcdCP by Schumacher et al. (2025). The relationship between residues and fitted values of data of all 74 samples for total, indispensible, dispensable and individual AA were plotted.

3. Results

3.1. All feed ingredients

3.1.1. Total amino acids

The entire dataset (n= 74) was used to display for total AA the relationship between *in vivo* pcdAA values and corresponding NDSAA values for cereal grains and ADSAA values for protein feeds (Fig. 1), resulting in the following linear equation to estimate pcdAA from NDIAA and ADIAA, respectively:

$$\begin{gathered} y = 0.823\ (\text{standard error [SE] } 0.018,\ \text{confidence interval [CI] } 0.788;\ 0.858)\ x + 10.52\ (\text{SE } 4.420,\ \text{CI } 1.709;\ 19.33) \\ R^2 = 0.968 \\ \text{Root mean square error (RMSE)} = 17.13 \\ y = \textit{in vivo}\ \text{pcdAA (g/kg DM) and } x = \text{NDSAA for cereal grains and ADSAA (g/kg DM) for protein feeds} \end{gathered} \quad (4)$$

Cereal grains were in the lower range and most of the protein feeds in the middle and upper range of values, with one particularly striking rapeseed meal value (RSM76).

The entire dataset (n= 74) was also used to evaluate the relationship between *in vivo* pcdAA values and corresponding NDSAA values for cereal grains and ADSAA values for protein feeds of indispensable AA

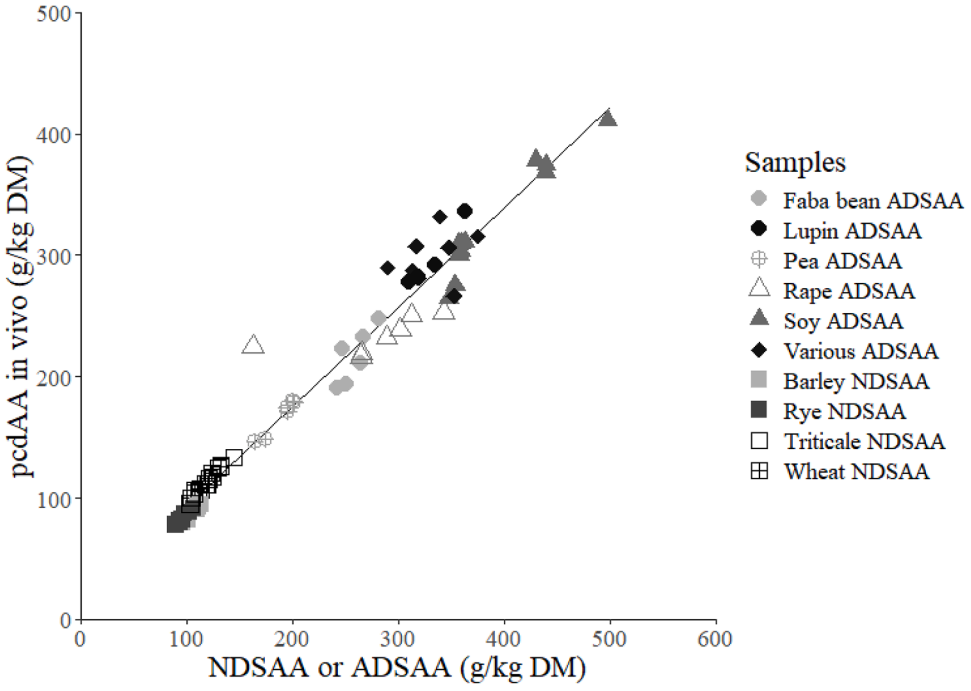

Fig. 1. Linear relationship between NDSAA and ADSAA data from the laboratory method (x) and *in vivo* pcdAA (y) for cereal grains and protein feed ingredients for all 74 samples and total AA (n= 17): y= 0.823 (SE 0.018, CI 0.788; 0.858) x+ 10.52 (SE 4.420, CI 1.709; 19.33).

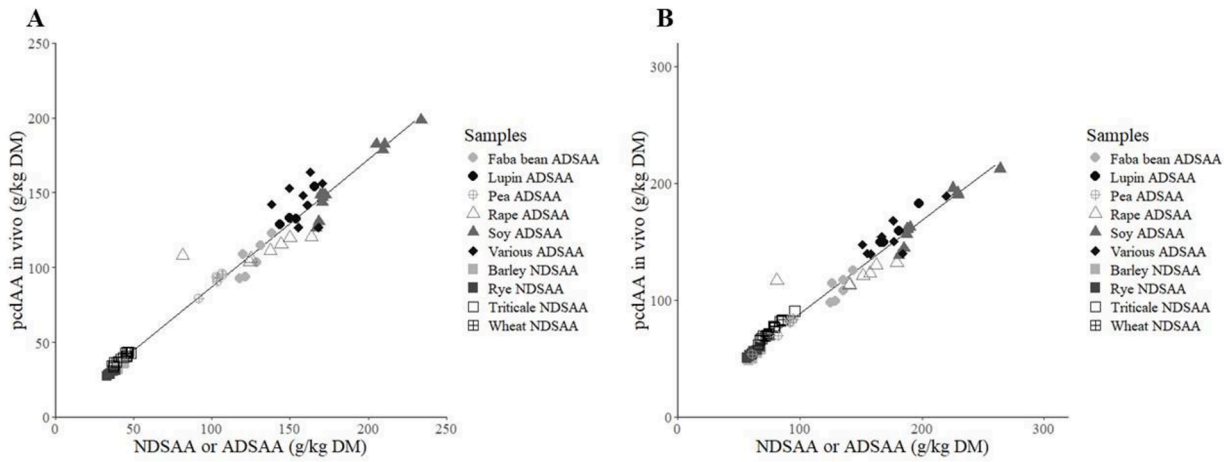

Fig. 2. (A). Linear relationship between NDSAA and ADSAA data from the laboratory method (x) and *in vivo* pcdAA (y) for cereal grains and protein feed ingredients for all 74 samples and indispensable AA (n =10): y= 0.853 (SE 0.017,0.819; 0.887) x + 1.754 (SE 2.021, CI -2.274; 5.783).
(B). Linear relationship between NDSAA and ADSAA data from the laboratory method (x) and *in vivo* pcdAA (y) for cereal grains and protein feed ingredients for all 74 samples and dispensable AA (*n*= 7): y= 0.797 (SE 0.019, CI 0.759; 0.834) x + 8.905 (SE 2.481, CI 3.958; 13.85).

(Fig. 2A) and dispensable AA (Fig. 2B), resulting in the following linear equation to estimate pcdAA from NDIAA and ADIAA, respectively:

Sum of indispensable AA:

$$\begin{gathered} y = 0.853\ (\text{SE}\ 0.017, 0.819;\ 0.887)\ x + 1.754\ (\text{SE}\ 2.021,\ \text{CI}\ -2.274;\ 5.783) \\ R^2 = 0.972 \\ \text{RMSE} = 8.610 \\ y = \textit{in vivo}\ \text{pcdAA}\ (\text{g/kg DM})\ \text{and}\ x = \text{NDSAA or ADSAA}\ (\text{g/kg DM}) \end{gathered} \tag{5}$$

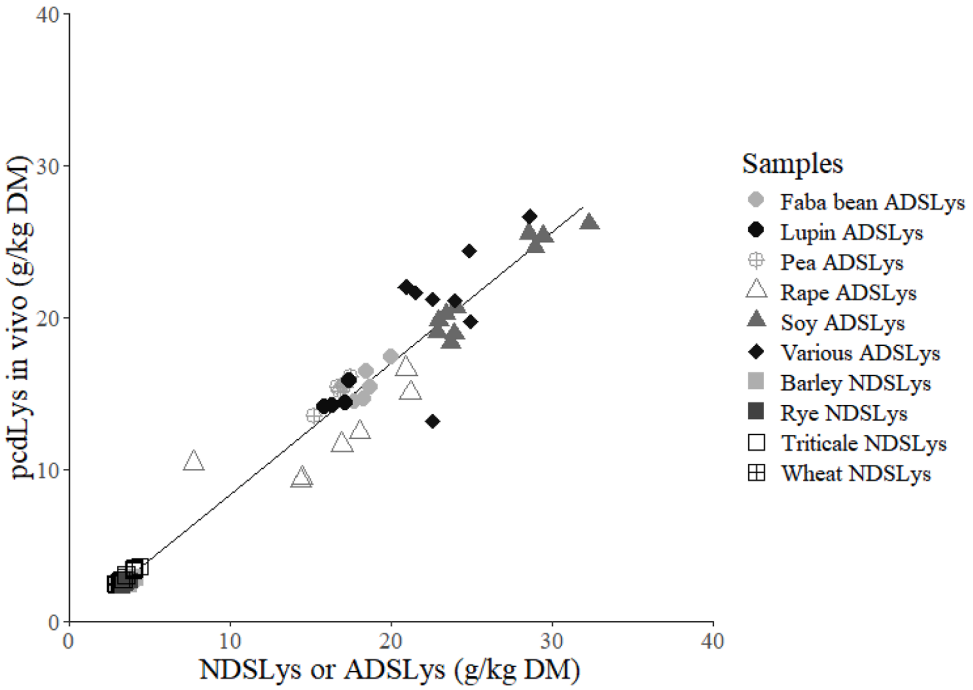

Fig. 3. Linear relationship between NDSLys and ADSLys data from the laboratory method (x) and *in vivo* pcdLys (y) for cereal grains and protein feed ingredients for all 74 samples: y= 0.863 (SE 0.019, CI 0.825;0.902) x - 0.272 (SE 0.311, CI -0.893; 0.348).

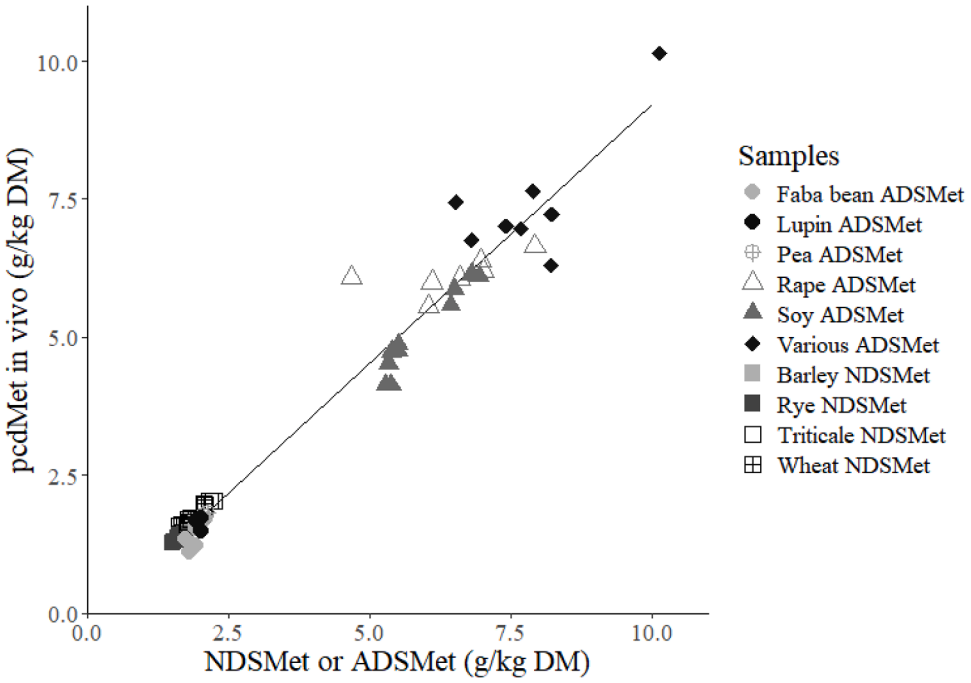

Fig. 4. Linear relationship between NDSMet and ADSMet data from the laboratory method (x) and *in vivo* pcdMet (y) for cereal grains and protein feed ingredients for all 74 samples: y= 0.936 (SE 0.019, CI 0.897; 0.975) x - 0.141 (SE 0.083, CI -0.306; 0.024). The encircled symbols represent four particularly striking values.

Sum of dispensable AA:

$$\begin{gathered} y = 0.797\ (\text{SE } 0.019,\ \text{CI } 0.759;\ 0.834)\ x + 8.905\ (\text{SE } 2.481,\ \text{CI } 3.958;\ 13.85) \\ R^2 = 0.962 \\ \text{RMSE} = 8.884 \\ y = \textit{in vivo}\ \text{pcdAA (g/kg DM) and } x = \text{NDSAA or ADSAA (g/kg DM)} \end{gathered} \quad (6)$$

For the group of indispensable AA, cereal grains were in the lower range and protein feeds in the upper range of the values (Fig. 2A). The value for rapeseed meal (RMS76) was again visually striking. Concerning the dispensable AA, cereal grains and peas were in the lower range of the values (Fig. 2B). The protein samples were in the middle and upper range of the values. For dispensable AA a striking value was also noticeable for the sample RSM76.

3.1.2. Individual amino acids

The entire dataset (n= 74) was also used to display, for each individual AA, the relationship between *in vivo* pcdAA values and corresponding NDSAA values for cereal grains and ADSAA values for protein feeds (Fig. 3 and Fig. 4), resulting in linear equations to estimate pcdAA from NDIAA or ADIAA, exemplified hereafter for Lys and Met:

Lys:

$$\begin{gathered} y = 0.863\ (\text{SE } 0.019,\ \text{CI } 0.825;0.902)\ x - 0.272\ (\text{SE } 0.311,\ \text{CI } -0.893;\ 0.348) \\ R^2 = 0.965 \\ \text{RMSE} = 1.505 \\ y = \textit{in vivo}\ \text{pcdLys (g/kg DM) and } x = \text{NDSLys or ADSLys (g/kg DM)} \end{gathered} \quad (7)$$

Met:

Table 3
Linear relationship between NDSAA and ADSAA data from the laboratory method (x) and *in vivo* AA (pcdAA) for all samples (cereal grains and protein feeds, *n*= 74): Regression equation (y= a x + b), standard error (SE), confidence interval (CI), coefficient of determination (R^2) and root mean square error (RMSE).

Amino acids	Samples		
	y =	R^2	RMSE
Indispensable AA			
Arginine	0.914 (SE 0.014, CI 0.886; 0.941) x + 0.287 (SE 0.282, CI -2.753; 0.850)	0.984	1.363
Histidine	0.845 (SE 0.018, CI 0.810; 0.880) x + 0.180 (SE 0.125, CI -0.069; 0.429)	0.970	0.496
Isoleucine	0.873 (SE 0.017, CI 0.840; 0.907) x + 0.011 (SE 0.191, CI -0.365; 0.396)	0.974	0.841
Leucine	0.854 (SE 0.016, CI 0.822; 0.885) x + 0.320 (SE 0.317, CI -0.311; 0.952)	0.976	1.317
Lysine	0.863 (SE 0.019, CI 0.825; 0.902) x – 0.272 (SE 0.311, CI -0.839; 0.348)	0.965	1.505
Methionine	0.936 (SE 0.019, CI 0.897; 0.975) x – 0.141 (SE 0.083, CI -0.306; 0.024)	0.970	0.394
Phenylalanine	0.818 (SE 0.024, CI 0.770; 0.866) x + 0.657 (SE 0.305, CI 0.049; 1.265)	0.941	1.227
Threonine	0.785 (SE 0.021, CI 0.742; 0.827) x + 0.214 (SE 0.227, CI -0.238; 0.667)	0.949	0.985
Tryptophan	0.784 (SE 0.025, CI 0.735; 0.834) x + 0.038 (SE 0.083, CI -0.128; 0.204)	0.932	0.364
Valine	0.817 (SE 0.021, CI 0.775; 0.860) x + 0.438 (SE 0.272, CI -0.103; 0.980)	0.953	1.132
Dispensable AA			
Alanine	0.811 (SE 0.023, CI 0.766; 0.857) x + 0.190 (SE 0.247, CI -0.302; 0.683)	0.946	1.016
Aspartic acid	0.822 (SE 0.016, CI 0.790; 0.855) x + 0.354 (SE 0.420, CI -0.481; 1.190)	0.973	2.155
Cysteine	0.721 (SE 0.028, CI 0.665; 0.776) x + 0.299 (SE 0.122, CI 0.056; 0.541)	0.904	0.417
Glutamic acid	0.818 (SE 0.018, CI 0.782; 0.853) x + 4.135 (SE 0.947, CI 2.248; 6.022)	0.967	3.160
Glycine	0.739 (SE 0.019, CI 0.700; 0.777) x + 0.491 (SE 0.218, CI 0.056; 0.925)	0.953	0.879
Proline	0.840 (SE 0.034, CI 0.772; 0.907) x + 1.187 (SE 0.595, CI 0.000; 2.374)	0.895	1.756
Serine	0.813 (SE 0.019, CI 0.775; 0.851) x + 0.533 (SE 0.248, CI 0.039; 1.028)	0.961	1.057

y= estimated pcdAA (g/kg DM) and x= NDSAA or ADSAA (g/kg DM)

$$\begin{gathered} y = 0.936\ (\text{SE } 0.019,\ \text{CI } 0.897;\ 0.975)\ x - 0.141\ (\text{SE } 0.083,\ \text{CI } -0.306;\ 0.024) \\ R^2 = 0.970 \\ \text{RMSE} = 0.394 \\ y = \textit{in vivo}\ \text{pcdMet (g/kg DM) and } x = \text{NDSMet or ADSMet (g/kg DM)} \end{gathered} \quad (8)$$

The regression equations and R^2 values for indispensable and dispensable AA are summarised in Table 3. The coefficients of determination were very high for almost all individual AA, both for indispensable and dispensable AA, with values between 0.895 for Pro and 0.984 for Arg.

For pcdLys, cereal grains were in the lower range and protein feeds in the middle and upper range of the values (Fig. 3). In contrast, values for pcdMet were in the upper range of values for rapeseed and rape products, soy and various samples and in the lower range for cereal grains and legume grains such as peas, faba beans and lupins (Fig. 4). The values for RSM76 and soy protein concentrate B fine (SPCBf) deviated remarkably from the regression line. A closer look at the lower range scatter plot with a different scaling showed that the values of the legumes are almost completely among those of the cereals (Fig. 5). There were noticeably high ADSMet values for three colour-flowering faba beans which had higher tannin contents than the white-flowering faba beans.

The illustration of the linear relationship between *in vivo* pcdAA values and corresponding NDSAA values for cereal grains and ADSAA values for protein feeds for all other AA can be found in the Appendix (Figs. A1–A15). For Met, Thr, Trp, His and Cys the values for RSM76 and soy protein concentrate B fine (SPCBf) were visually striking (Figs. 4, A1, A2, A6, A11). In addition, the sample RSM76 was visibly divergent for Val, Arg, Phe, Ala, Asp, Glu, Gly and Ser (Figs. A3, A5, A8, A9, A10, A12, A13, A15). In general, all linear regression equations had high coefficients of determination.

3.2. Cereal grains

3.2.1. Total amino acids

In accordance with *in vivo* pcdCP values in Schumacher et al. (2025), the *in vivo* pcdAA values of feed ingredients were grouped in cereal grains and protein feeds, just as forming clusters of wheat with triticale and barley with rye. The entire cereal grain dataset (*n*= 32) was used to display the relationship of the total AA between *in vivo* pcdAA values and corresponding NDSAA values for the two groups (Fig. 6), resulting in the following linear equation to estimate pcdAA from NDIAA:

Wheat and triticale:

$$\begin{gathered} y = 0.904\ (\text{SE } 0.045,\ \text{CI } 0.807;\ 1.001)\ x + 5.841\ (\text{SE } 5.389,\ \text{CI } -5.717;\ 17.40) \\ R^2 = 0.966 \\ \text{RMSE} = 1.992 \\ y = \textit{in vivo}\ \text{pcdAA (g/kg DM) and } x = \text{NDSAA (g/kg DM)} \end{gathered} \quad (9)$$

Barley and rye:

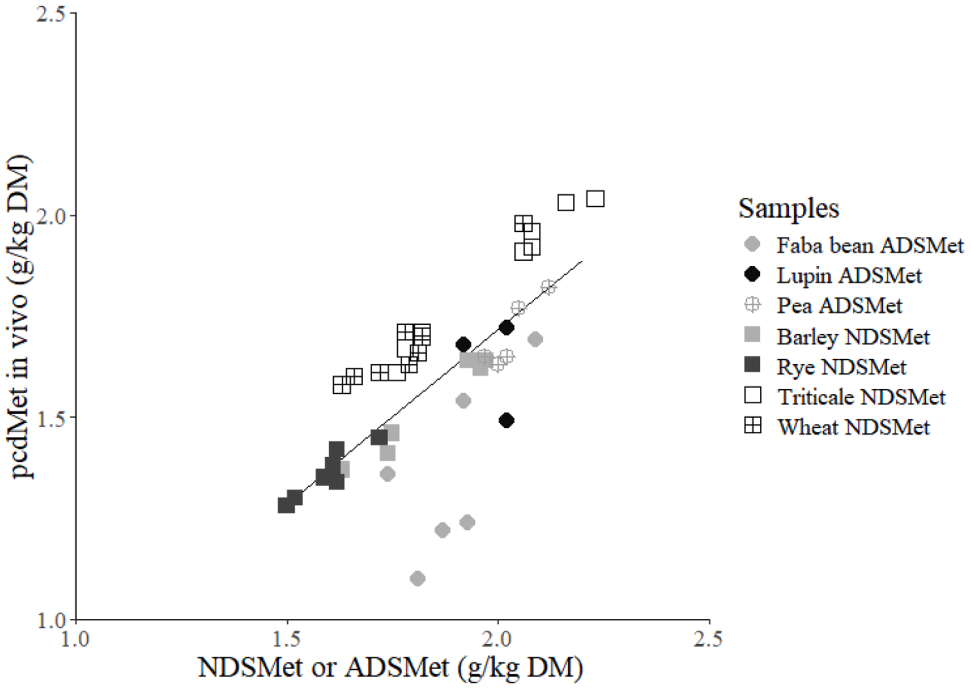

Fig. 5. Linear relationship between NDSAA and ADSAA data from the laboratory method (x) and *in vivo* pcdAA (y) for cereal grains and the legume grains faba bean, pea and lupin zoomed in from Fig. 4.

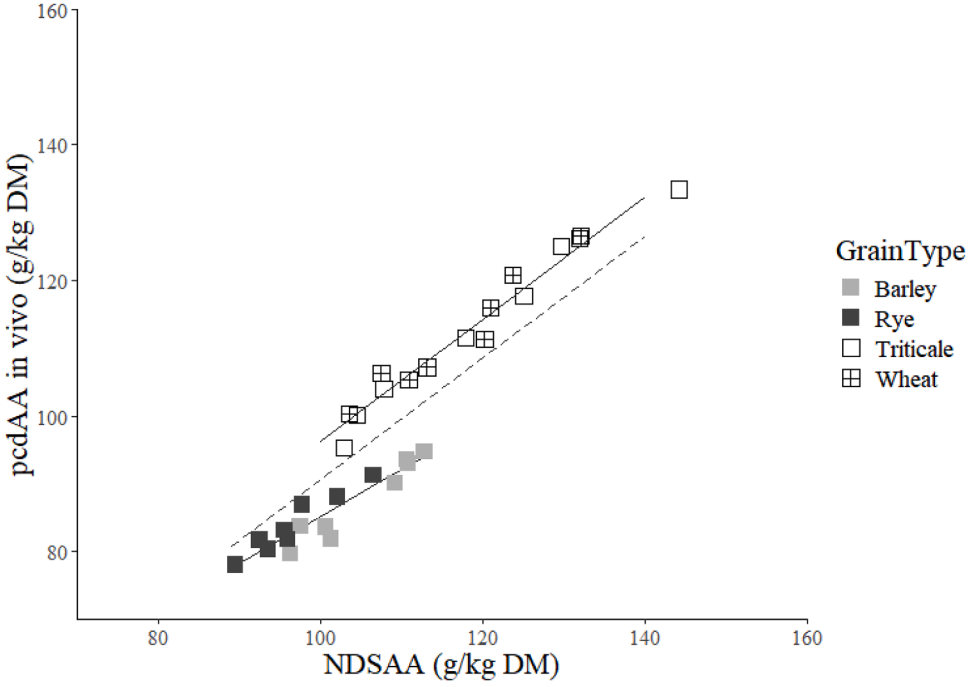

Fig. 6. Linear relationship between NDSAA data from the laboratory method (x) and *in vivo* pcdAA (y) for cereal grains for wheat, triticale, barley and rye with clustering of wheat with triticale and barley with rye. The dashed line shows the regression line for all cereal grain samples without clustering.

$$\begin{aligned} y &= 0.696\ (\text{SE } 0.069,\ \text{CI } 0.547;\ 0.844)\ x + 15.60\ (\text{SE } 6.978,\ \text{CI } 0.631;\ 30.56) \\ R^2 &= 0.879 \\ \text{RMSE} &= 1.813 \\ y &= \textit{in vivo}\ \text{pcdAA (g/kg DM) and } x = \text{NDSAA (g/kg DM)} \end{aligned} \tag{10}$$

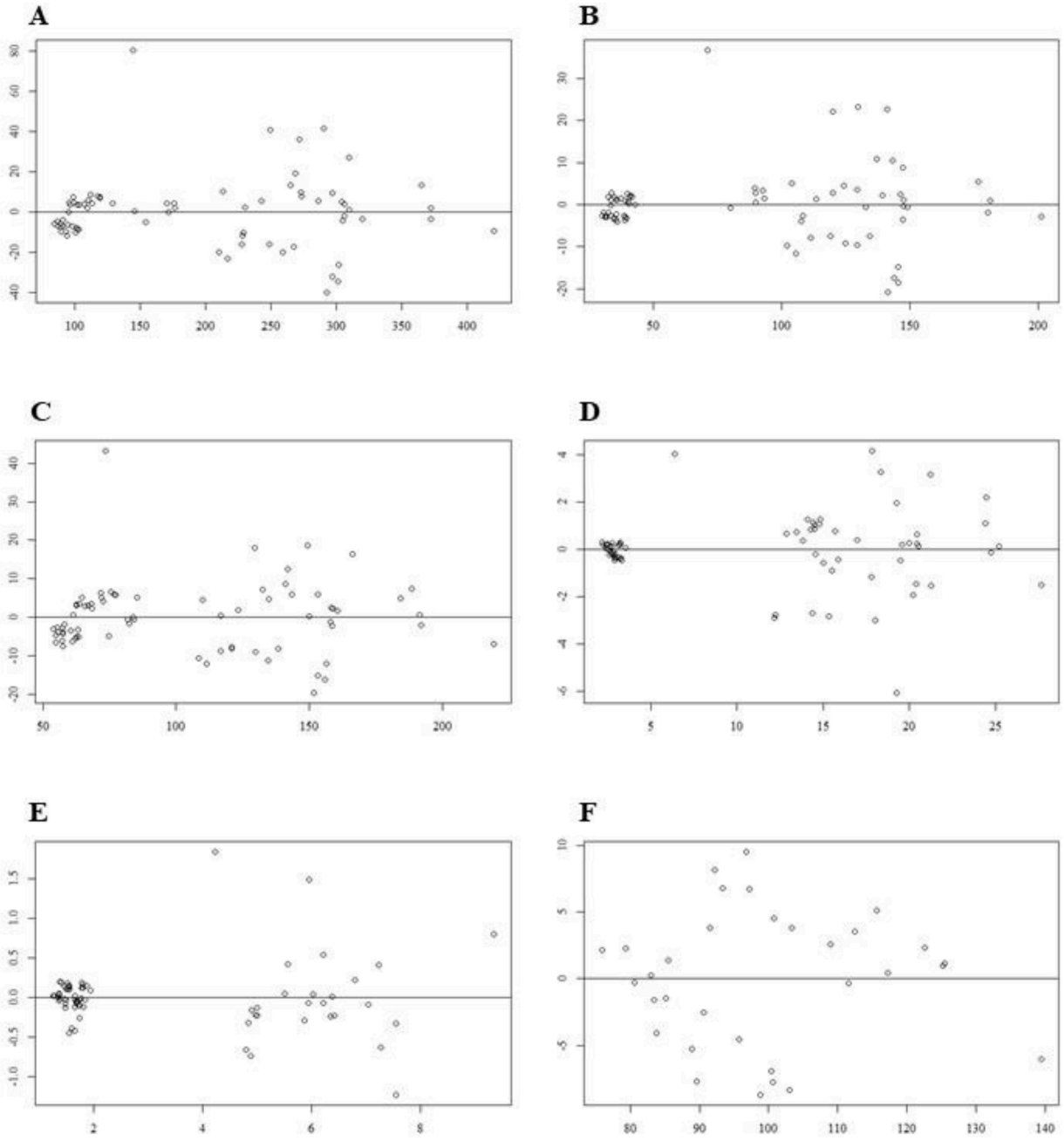

Fig. 7. Relationship between residues (y; g/kg DM) and fitted values (x; g/kg DM) of data of all 74 samples for all 17 amino acids (A), indispensable amino acids (B), dispensable amino acids (C), lysine (D), methionine (E) and for total amino acids for all cereal grains (F).

The relationship between residues and fitted values of data of all 74 samples for all 17 AA, indispensable and dispensable AA, Lys, Met and for total AA for all cereal grains are shown in Fig. 7.

The cereal grain dataset was also used to display the relationship between *in vivo* pcdAA values of indispensable and dispensable AA and the corresponding NDSAA values in the two cereal grain groups (Appendix Figs. A16 and A17), resulting in the linear regression equations to estimate pcdAA from NDSAA shown in Table A8. The R^2 values for cereal grains in general were very high, with 0.989 for the total AA, 0.980 for the indispensable AA and 0.993 for the dispensable AA. Comparing R^2 values of groups, the values for the total AA were higher for the wheat and triticale cluster (0.966 > 0.879). The values for indispensable AA were higher for the barley and rye cluster (0.969 > 0.933) and the values for dispensable AA were higher in the wheat and triticale cluster (0.979 > 0.775).

The relationships between residues and fitted values of data of all 74 samples for individual AA are shown in Figs. A18-A20.

3.2.2. *Individual amino acids*

The regression equations and R^2 values for the individual AA for the two cereal grain clusters are summarised in the Appendix Tables A9 and A10. The R^2 values of the wheat and triticale cluster were high for indispensable and dispensable AA, with values ranging between 0.817 for Gly and 0.999 for Trp. Similarly, the barley and rye cluster had high R^2 values for almost all AA, with values ranging between 0.730 for serine and 0.988 for leucine. Remarkably lower values were observed for the dispensable AA Gly (0.687) and Pro (0.402).

4. Discussion

4.1. General considerations

The main objective of this study was to investigate if the estimation of pcdAA could be based on the same rapid laboratory procedure developed and established for the estimation of pcdCP (Schumacher et al., 2025). Tedeschi et al. (2001) reported that NDSAA and NDIAA of various forages (different genotypes of grass and different legumes, among others soybeans and lucerne) had similar AA profiles. However, this was not observed for the untreated and differently heat-treated feed ingredients used in this research analysing NDIAA or ADIAA, which Tedeschi et al. (2001) already reported for AD residues. The discrepancy in the AA profiles of the AD-soluble and AD-insoluble fractions are attributable to complexes of proteins with anti-nutritional components like tannins and phytate, which are recovered in the AD-insoluble fraction and reflected in the ADIAA pattern (Table 2).

This study benefitted from a data on 74 feed ingredients, which were obtained from the same facilities, ensuring consistency in data generation and analytical characterisation and minimising assay effects on variation in pcdAA determination. Moreover, the specific chemical analyses for NDIAA and ADIAA were performed on the identical feed ingredients on which the *in vivo* pcdAA and general chemical characteristics had been determined using standardised and consistent methods.

4.2. Comparison with other methods

In vivo methods are the so called "gold standard", for the evaluation of AA digestibility of feed ingredients, however they pose several challenges and demanding features. Animal intervention trials can be difficult to perform as they require expensive and lengthy experiments, have ethical limitations and should adhere to policies on experimental animals which follow the principle of the 3R concept, namely replacement, reduction and refinement (Santos-Sánchez et al., 2024). Further, presumably *in vivo* experiments have insufficient capacity to cover the required high number of tests for new, differently processed and treated feed ingredients (Brodkorb et al., 2019; Santos-Sánchez et al., 2024). The pressure from consumers to reduce the number of experimental animals, due to environmental and animal welfare perspectives, is growing currently (Santos-Sánchez et al., 2024). Because of the aforementioned reasons, *in vitro* methods have been developed and used over the last decades to simulate protein digestion in the gastro-intestinal tract of several species (Santos-Sánchez et al., 2024). These methods show several advantages compared with *in vivo* methods. *In vitro* methods are rapid and more cost-effective, the variable effects of the animals are removed, they can simulate processes of different gastro-intestinal segments and ethical limitations and policies on experimental animals are no longer barriers (Moughan et al., 2014; Zaefarian et al., 2021). Also factors like livestock farming and management, environment, genotype and diseases do not affect the *in vitro* evaluation (Zaefarian et al., 2021). Santos-Sánchez et al. (2024) described and compared the scope and limitation of different *in vitro* methods and their modifications to estimate CP and AA digestibility in animals and humans; the majority of these approaches was based on the use of different digestive enzymes. Most studies determined apparent pcD, three studies reported standardised pcD in pig feed (Meunier et al., 2008; Jezierny et al., 2010; Salazar-Villanea et al., 2016). Jansman et al. (2002) described the lack of additivity of the apparent pcD for feed ingredients in mixed diets, because the apparent digestibility is influenced by the AA content in the feed (Furuya and Kaji, 1989; Jansman et al. 2002). In contrast, the standardised pcD is independent of the concentration of AA in the feed, because it is corrected for basal endogenous losses (Moter and Stein, 2004). Therefore, compared to the apparent pcD, the standardised pcD enables a more precise estimation of digestible AA in mixed feeds (Jansman et al., 2002).

Only in the study of Jezierny et al. (2010), which was based on the Boisen and Fernández (1995) two-step incubation method with pepsin and pancreatin, pcDAA in pig feed was estimated. Salazar-Villanea et al. (2016) also estimated standardised precaecal digestibility of CP and AA but did not show any results for AA. Another valuable reference is the INFOGEST digestion protocol, developed by a consortium for application to human nutrition (Minekus et al., 2014; Brodkorb et al., 2019), which represented a standardisation of the diverse *in vitro* methods to simulate, in a laboratory setting, digestion in the upper gastro-intestinal tract (oral, gastric, duodenal) (Minekus et al., 2014). Santos-Sánchez et al. (2024) quoted three adaptions of the method (number of feed ingredients in parentheses): Ariëns et al., 2021 (n= 8); Sousa et al., 2023 (n= 7); Martineau-Côté et al., 2024 (n= 5), which allowed to determine *in vitro* pcdAA.

The *in vitro* methods showed good comparability with the *in vivo* pcD of CP and AA in pigs (Santos-Sánchez et al., 2024). However, Santos-Sánchez et al. (2024) again pointed out explicitly that different digestive conditions like the selection of enzymes, pH value and digestion time, which were used in distinct ways by various authors, require optimisation and standardisation. This point also makes it difficult to compare results over the past 40 years and reinforces the statement that a harmonisation of *in vitro* methods is essential (Moughan et al. 2014).

The different *in vitro* methods characterized by Santos-Sánchez et al. (2024) were only applied to small sample numbers. In an extended literature search, more studies were identified in which also the two-step enzymatic method of Boisen and Fernández (1995) was employed to estimate pcDAA in pig feed (number of feed ingredients in parentheses), namely Boisen and Fernández (1995; n= 9), Pujol and Torrallardona (2007; n= 7), Jezierny et al., (2010; n= 17), Eklund et al., (2013; n = 16), Eklund et al. (2015; n = 6) and Rosenfelder-Kuon et al. (2020; n= 32). Although the total number of feeds investigated in the studies mentioned above (n= 87) outperformed the number of feeds in this study (n= 74), the major drawback appears that the specific procedures and analytical methods varied between laboratories (analytical steps and specifications of enzymes, incubation conditions such as duration and temperature) which hinders or even precludes to merge data into one large dataset for joint evaluation. Taken together, the portrayed situation further strengthens the need for a standardisation and harmonisation of *in vitro* methods both for application to animals and humans. This has – over the past 40 years – only been achieved for human nutrition studies by the INFOGEST digestion protocol (Minekus et al., 2014; Brodkorb et al., 2019). Unfortunately, the INFOGEST protocol is rather complex in structure and application and not practicable for routine use in (farm) animal nutrition studies. Therefore, there is still a need for laboratory methods with a tolerable degree of complexity and sufficient ease of handling. *In vitro* procedures with a satisfying degree of standardisation and reproducibility across laboratories have advantages compared with chemical methods because they basically allow to adjust the conditions (*e.g.*, pH, temperature, duration, type of enzymes and enzyme activity) both to different animal species and different segments with the gastro-intestinal tract of a given species.

The determination of NDSAA and ADSAA is based on procedures for isolating ND and AD residues which have been developed, refined and standardised over decades mainly by Van Soest and co-workers (see, *e.g.*, Van Soest et al., 1991). These procedures have been applied to analytical schemes for CP fractionation (Licitra et al., 1996) and integrated into agricultural chemical analysis handbooks such as VDLUFA (2023). Analytical quality assurance is routinely performed, *e.g.*, in ring tests following international standards (VDLUFA, 2023). An advantage is that AD and ND residues can be determined by instrumental analysis. This facilitates processing of large sample sets which currently appears unfeasible for *in vitro* incubations. The AA determination on ND and AD residues follows the approved routine method for feed AA analysis (Regulation (EG) 152/2009 Annex III, F, G) and does not require new or modified procedures. Therefore, consistent with the procedures characterised by Schumacher et al. (2025) for NDICP and ADICP, the

determination of NDIAA or ADIAA appears robust and reliable.

In general, data in the present study showed a close relationship between NDSAA and ADSAA data from the laboratory method and *in vivo* pcdAA, which is reflected in the close alignment of the vast majority of data to the regression line for total AA, indispensible and dispensible AA and also individual AA. For only two feed ingredients, notable exceptions from the general observation occurred, one thermally treated rapeseed meal and one soy protein concentrate. The rapeseed meal (RSM76) had a residence time of 76 min in the desolventizer/toaster with unsaturated steam (Eklund et al., 2015), and all AA except Lys, Ile, Leu and Pro showed a remarkable distance from the regression line. This is surprising because other RSM samples treated similarly with desolventizer/toaster residence times from 48 to 93 min were not notably distant from the regression line, therefore it can be assumed that the particular values of RSM76 were not due to a too short or too long thermal treatment, which in the latter case would have increased the risk of heat damage with the formation of Maillard products. Hence, no explanation for the divergent values of this specific sample can be given.

The other exception from the close alignment of data to the regression line was soy protein concentrate B fine (SPCBf), which – for the indispensible AA Thr and Trp – had *in vivo* pcd values above the regression line, *i.e.*, ADSThr and ADSTrp values were low. The first assumption was that this could be explained with fine grinding, as the same sample treated similarly, but with coarser grinding, soy protein concentrate B coarse (SPCBc) did not show the same distinct feature. For ND and AD residues, Mertens (1992) mentioned that in analyses, finer particles can be washed out of the bag during processing. Also, there is a larger surface for the solvent to attack in finer milled sample material. This, however, would have resulted in overestimated ADSAA values, in contrast to the observed low ADSAA values. Again, it remains unclear why the values of this specific soy protein concentrate were in contrast to expectations, and moreover, no rationale is obvious why only two AA were affected.

The results of this study suggest that the estimation of pcdAA based on procedures for isolating ND and AD residues can be carried out reliably using equations developed for the entire dataset which consisted mainly of ceral grains and protein feeds. These equations are recommended currently for routine analysis of feed ingredients. Specific equations for particular groups of feed ingredients may improve the quality of pcdAA estimation which was exemplarily shown for cereal grains. To avoid bias, however, it appears advisable to use the general equations until specific estimates for other particular groups of feed ingredients have been developed. This was not possible in this study due to limited sample size.

5. Conclusions

In vivo determined standardised pcdAA of cereal grains and protein feeds was predicted with high accuracy by chemical procedures. The procedure encompassed the determination of NDIAA on cereal grains and ADIAA on other feedstuffs, mainly protein feeds. This procedure can be used in any laboratory equipped for standard feedstuff analysis, including AA. The hypothesis could thus be maintained that the method developed and established to estimate pcdCP (Schumacher et al., 2025) is similarly suitable to estimate *in vivo* determined pcdAA of cereal grains and protein feeds. However, independent validation of the established regressions was impossible because no independent *in vivo* data set could be identified. An extension of the database of *in vivo* pcdAA values may yield predictions for specific types of feeds which would aid in further improving the accuracy of the prediction of pcdAA without compromising robustness. The applicability of the method to poultry feeds applying the same rapid laboratory method reported in this study is also conceivable and currently thoroughly examined.

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CRediT authorship contribution statement

Valérie Schumacher: Writing – original draft, Investigation, Formal analysis, Data curation. **Markus Rodehutscord:** Writing – review & editing. **Karl-Heinz Südekum:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Saskia Kehraus:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors have no conflict of interest.

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Appendix

Table A1
The number, sample group, sample, amino acid content (AA; [g/kg DM]), fractional standardised precaecal digestibility of AA (pcDAA; [g/g AA]) and calculated standardised precaecal digestible AA concentration (pcdAA; [g/kg DM]) *in vivo* of the 74 assayed feedstuffs for the indispensable AAs arginine, histidine and isoleucine.

			Arginine			Histidine			Isoleucine		
Number	Sample group	Sample	AA (g/kg DM)	pcDArg (g/g AA)	pcdArg *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDHis (g/g AA)	pcdHis *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDIso (g/g AA)	pcdIso *in vivo* * (g/kg DM)
1	Wheat	Skalmeje	6.27	0.85	5.33	3.21	0.86	2.76	4.05	0.86	3.48
2		Tommi	6.37	0.84	5.35	3.42	0.86	2.94	4.33	0.86	3.72
3		St. Tobak	6.29	0.86	5.41	3.28	0.87	2.85	4.08	0.86	3.51
4		Event	6.40	0.88	5.63	7.57	0.88	6.66	4.03	0.88	3.55
5		Mulan	6.65	0.85	5.65	3.38	0.86	2.91	4.04	0.84	3.39
6		Tabasco	6.01	0.86	5.17	3.25	0.86	2.80	3.46	0.86	2.98
7		Adler	7.18	0.86	6.17	3.83	0.87	3.33	4.74	0.88	4.17
8		KWS Erasmus	6.32	0.85	5.37	3.22	0.86	2.77	3.91	0.87	3.40
9	Triticale	Grenado	6.03	0.84	5.07	2.95	0.83	2.45	3.71	0.81	3.01

(*continued on next page*)

Table A1 (*continued*)

Number	Sample group	Sample	Arginine			Histidine			Isoleucine		
			AA (g/kg DM)	pcDArg (g/g AA)	pcdArg *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDHis (g/g AA)	pcdHis *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDIso (g/g AA)	pcdIso *in vivo* * (g/kg DM)
10		Tarzan	7.13	0.88	6.27	3.44	0.86	2.96	4.31	0.85	3.66
11		HYT Prime	7.72	0.85	6.56	3.54	0.83	2.94	4.89	0.83	4.06
12		Massimo	6.93	0.86	5.96	3.34	0.85	2.84	4.29	0.85	3.65
13		Cultivo	7.72	0.86	6.64	3.83	0.90	3.45	4.72	0.84	3.96
14		SW Talentro	7.57	0.86	6.51	3.73	0.85	3.17	4.61	0.83	3.83
15		Cando	6.04	0.85	5.13	3.01	0.85	2.56	3.74	0.83	3.10
16		Agostino	6.61	0.83	5.49	3.13	0.83	2.60	4.04	0.83	3.35
17	Rye	Conduct	5.95	0.79	4.70	2.89	0.77	2.23	3.52	0.74	2.60
18		Visello	5.96	0.78	4.65	2.94	0.75	2.21	3.11	0.72	2.24
19		Helltop	6.11	0.78	4.77	2.93	0.76	2.23	3.72	0.74	2.75
20		Bellami	5.93	0.75	4.45	3.04	0.74	2.25	3.18	0.70	2.23
21		Palazzo	5.84	0.76	4.44	2.83	0.73	2.07	3.39	0.72	2.44
22		Dukato	5.74	0.76	4.36	2.88	0.74	2.13	3.42	0.73	2.50
23		Guttino	5.68	0.76	4.32	2.78	0.74	2.06	3.11	0.71	2.21
24		Dankowski Diament	6.34	0.76	4.82	3.13	0.73	2.28	3.74	0.71	2.66
25	Barley	Yool	5.70	0.79	4.50	2.50	0.77	1.93	3.90	0.74	2.89
26		Ack 2927	6.10	0.80	4.88	2.60	0.79	2.05	3.80	0.78	2.96
27		Lomerit	5.80	0.78	4.52	2.60	0.78	2.03	4.20	0.77	3.23
28		Campanille	6.00	0.77	4.62	2.60	0.76	1.98	4.30	0.72	3.10
29		Canberra	6.50	0.79	5.14	2.60	0.78	2.03	4.20	0.77	3.23
30		Antisette	6.20	0.82	5.08	2.60	0.79	2.05	4.50	0.77	3.47
31		Metaxa	6.30	0.81	5.10	2.60	0.79	2.05	4.20	0.76	3.19
32		Fridericus	6.50	0.79	5.14	2.90	0.79	2.29	4.40	0.75	3.30
33	Pea	Santana	22.8	0.90	20.5	6.40	0.84	5.38	10.8	0.84	9.07
34		Jutta	25.2	0.91	22.9	6.30	0.82	5.17	10.2	0.83	8.47
35		Phönix	20.8	0.88	18.3	6.30	0.83	5.23	10.7	0.81	8.67
36		Harnas	23.2	0.90	20.9	6.30	0.82	5.17	10.3	0.82	8.45
37		Rocket	23.2	0.89	20.7	5.70	0.80	4.56	10.6	0.80	8.48
38		Hardy	19.0	0.88	16.7	5.60	0.78	4.37	9.40	0.78	7.33
39	Lupin	Probor	48.8	0.97	47.3	10.3	0.91	9.37	15.4	0.92	14.2
40		Boregine	38.9	0.95	37.0	9.20	0.88	8.10	13.6	0.88	12.0
41		Boruta	41.2	0.94	38.7	9.40	0.88	8.27	14.1	0.88	12.4
42		Idefix	41.1	0.93	38.2	9.50	0.87	8.27	15.1	0.85	12.8
43	Faba bean	Aurelia	30.6	0.91	27.9	8.00	0.85	6.80	12.7	0.85	10.8
44		Divine	30.8	0.89	27.4	7.50	0.81	6.08	11.8	0.82	9.68
45		Gloria	32.8	0.90	29.5	8.50	0.84	7.14	13.9	0.85	11.8
46		Limbo	29.7	0.86	25.5	8.20	0.76	6.23	12.3	0.75	9.23
47		Fuego	24.9	0.84	20.9	7.70	0.73	5.62	11.8	0.76	8.97
48		Espresso	25.2	0.84	21.2	7.60	0.73	5.55	11.5	0.74	8.51
51	Full-fat soybean/ soybean product	FFSB K2	28.6	0.82	23.5	10.3	0.77	7.92	17.4	0.73	12.7
52		FFSB K3	28.6	0.88	25.2	10.2	0.83	8.47	17.6	0.81	14.3
53		FFSB Z1	28.8	0.90	25.9	10.3	0.85	8.76	17.5	0.84	14.7
54		FFSB Z2	28.1	0.92	25.9	10.1	0.85	8.58	17.9	0.85	15.2
55		FFSB Z3	27.7	0.92	25.5	10.0	0.86	8.60	17.6	0.87	15.3
56		FFSB Z4	27.9	0.91	25.4	10.2	0.84	8.58	17.7	0.86	15.2
58		FFSB (roasted)	28.5	0.80	22.8	10.2	0.74	7.55	17.3	0.71	12.3
59		SBC	34.7	0.93	32.3	12.2	0.88	10.7	20.7	0.87	18.0
60		SBM (Austria)	34.7	0.90	31.2	12.7	0.86	10.9	21.2	0.86	18.2
61		SBM (GMO-free)	40.5	0.89	36.1	13.9	0.83	11.5	24.5	0.83	20.3
62		SBM (standard)	35.2	0.91	32.0	12.3	0.85	10.5	21.3	0.85	18.1
63	Rapeseed meal and cake	RSM48	22.1	0.82	18.1	10.1	0.75	7.58	15.3	0.71	10.9
64		RSM64	21.5	0.81	17.4	10.0	0.73	7.30	15.3	0.71	10.9
65		RSM76	20.6	0.80	16.5	10.0	0.71	7.10	15.2	0.70	10.6
66		RSM93	20.6	0.79	16.3	10.0	0.69	6.90	15.0	0.68	10.2
67		LOW-GLS RSM	20.9	0.79	16.5	10.0	0.71	7.10	14.6	0.70	10.2
68		RSC	22.6	0.86	19.4	9.80	0.83	8.13	14.5	0.74	10.7
69		RSM	24.5	0.80	19.6	10.4	0.77	8.01	15.6	0.70	10.9
70		Soybean (extruded)	20.8	0.82	17.1	9.80	0.78	7.64	17.0	0.72	12.2
71	Various samples	SBM (high protein)	20.6	0.89	18.3	9.80	0.86	8.43	17.3	0.81	14.0
72		SPC (A coarse)	19.7	0.95	18.7	9.50	0.91	8.65	17.1	0.88	15.1
73		SPC (A fine)	19.5	0.93	18.1	9.30	0.89	8.28	16.7	0.87	14.5
74		SPC (B coarse)	22.0	0.96	21.1	10.3	0.89	9.17	18.5	0.90	16.7
75		SPC (B fine)	20.1	0.94	18.9	9.70	0.89	8.63	17.5	0.90	15.8

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Table A1 (*continued*)

Number	Sample group	Sample	Arginine			Histidine			Isoleucine		
			AA (g/kg DM)	pcDArg (g/g AA)	pcdArg *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDHis (g/g AA)	pcdHis *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDIso (g/g AA)	pcdIso *in vivo* * (g/kg DM)
79		WG2 (hydrolysed)	13.4	0.87	11.7	8.80	0.85	7.48	15.8	0.84	13.3
82		FM2 (extracted)	18.8	0.89	16.7	9.90	0.83	8.22	18.6	0.82	15.3

FFSB, Full-fat soybean; FM, Fish meal; GLS, Glucosinolates; PeaP, Pea protein; RSC, Rapeseed cake; RSM, Rapeseed meal; SBC, Soybean cake; SBM, Soybean meal; SPC, Soy protein concentrate; SPI, Soy protein isolate; WG, Wheat gluten, / no pre-treatment;
The samples 49, 50, 57, 76, 77, 78, 80 and 81 were not used for the determination of pcdAA.
* The precaecal digestible AA was calculated from AA content and pcDAA reported in the particular reference.

Table A2
The number, amino acid content (AA; [g/kg DM]), fractional standardised precaecal digestibility of AA (pcDAA; [g/g AA]) and calculated standardised precaecal digestible AA concentration (pcdAA; [g/kg DM]) *in vivo* of the 74 assayed feedstuffs for the indispensable amino acids leucine, lysine and methionine.

Number	Leucine			Lysine			Methionine		
	AA (g/kg DM)	pcDLeu (g/g AA)	pcdLeu *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDLys (g/g AA)	pcdLys *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDMet (g/g AA)	pcdMet *in vivo* * (g/kg DM)
1	8.60	0.86	7.40	3.50	0.71	2.49	1.86	0.85	1.58
2	9.35	0.86	8.04	3.60	0.69	2.48	2.02	0.84	1.70
3	8.72	0.86	7.50	3.60	0.69	2.48	1.95	0.85	1.66
4	9.01	0.88	7.93	3.55	0.73	2.59	1.96	0.87	1.71
5	8.90	0.85	7.57	3.76	0.69	2.59	1.92	0.85	1.63
6	8.07	0.85	6.86	3.43	0.71	2.44	1.86	0.86	1.60
7	10.1	0.87	8.76	4.01	0.73	2.93	2.25	0.88	1.98
8	8.44	0.86	7.26	3.59	0.74	2.66	1.87	0.86	1.61
9	7.59	0.83	6.30	3.86	0.72	2.78	1.92	0.84	1.61
10	9.12	0.86	7.84	4.46	0.77	3.43	2.21	0.87	1.92
11	9.57	0.84	8.04	4.87	0.74	3.60	2.40	0.85	2.04
12	8.89	0.85	7.56	4.46	0.75	3.35	2.22	0.86	1.91
13	9.86	0.85	8.38	4.70	0.73	3.43	2.39	0.85	2.03
14	9.80	0.84	8.23	4.74	0.75	3.56	2.31	0.85	1.96
15	7.89	0.85	6.71	3.77	0.73	2.75	1.96	0.85	1.67
16	8.31	0.89	7.40	4.20	0.73	3.07	2.04	0.84	1.71
17	7.22	0.76	5.49	4.23	0.65	2.75	1.82	0.78	1.42
18	6.96	0.75	5.22	4.11	0.64	2.63	1.71	0.75	1.28
19	7.48	0.75	5.61	4.25	0.64	2.72	1.81	0.76	1.38
20	7.12	0.73	5.20	4.13	0.61	2.52	1.73	0.75	1.30
21	7.05	0.73	5.15	4.07	0.62	2.52	1.81	0.74	1.34
22	7.09	0.74	5.25	4.16	0.63	2.62	1.80	0.75	1.35
23	4.74	0.73	3.46	3.94	0.60	2.36	1.71	0.75	1.28
24	7.80	0.73	5.69	4.43	0.60	2.66	1.93	0.75	1.45
25	7.70	0.76	5.85	4.00	0.64	2.56	1.80	0.76	1.37
26	8.00	0.79	6.32	4.50	0.65	2.93	1.80	0.79	1.42
27	7.70	0.77	5.93	4.00	0.61	2.44	1.90	0.77	1.46
28	8.10	0.75	6.08	4.10	0.62	2.54	1.90	0.74	1.41
29	8.90	0.77	6.85	4.50	0.63	2.84	2.10	0.78	1.64
30	8.70	0.78	6.79	4.10	0.66	2.71	2.10	0.78	1.64
31	8.90	0.78	6.94	4.30	0.65	2.80	2.10	0.78	1.64
32	8.90	0.78	6.94	4.40	0.64	2.82	2.10	0.77	1.62
33	18.1	0.82	14.8	18.5	0.87	16.1	2.30	0.79	1.82
34	17.8	0.81	14.4	17.8	0.85	15.1	2.30	0.79	1.82
35	17.7	0.80	14.2	18.1	0.85	15.4	2.20	0.75	1.65
36	18.0	0.81	14.6	18.1	0.85	15.4	2.30	0.77	1.77
37	18.1	0.80	14.5	18.5	0.84	15.5	2.20	0.75	1.65
38	15.7	0.77	12.1	16.3	0.83	13.5	2.20	0.74	1.63
39	26.6	0.91	24.2	17.6	0.90	15.8	2.00	0.84	1.68
40	23.3	0.87	20.3	16.1	0.88	14.2	2.10	0.82	1.72
41	24.3	0.87	21.1	16.5	0.86	14.2	2.10	0.82	1.72
42	24.7	0.84	20.8	17.3	0.83	14.4	2.10	0.71	1.49
43	22.4	0.84	18.8	18.9	0.87	16.4	2.00	0.77	1.54
44	22.0	0.82	18.0	18.5	0.84	15.5	2.00	0.68	1.36
45	24.1	0.84	20.2	20.7	0.84	17.4	2.20	0.77	1.69
46	22.3	0.76	17.0	19.3	0.80	15.4	2.00	0.61	1.22
47	20.8	0.75	15.6	18.5	0.79	14.6	2.00	0.62	1.24
48	20.6	0.74	15.2	18.3	0.79	14.5	1.90	0.58	1.10
51	29.8	0.73	21.8	24.6	0.77	18.9	5.52	0.75	4.14
52	30.0	0.81	24.3	24.6	0.84	20.7	5.52	0.82	4.53
53	30.1	0.84	25.3	24.3	0.85	20.7	5.61	0.85	4.77
54	29.8	0.85	25.3	23.8	0.85	20.2	5.59	0.85	4.75
55	29.4	0.87	25.6	23.3	0.85	19.8	5.60	0.87	4.87
56	29.6	0.86	25.5	23.2	0.82	19.0	5.51	0.86	4.74

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Table A2 (*continued*)

Number	Leucine			Lysine			Methionine		
	AA (g/kg DM)	pcDLeu (g/g AA)	pcdLeu *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDLys (g/g AA)	pcdLys *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDMet (g/g AA)	pcdMet *in vivo* * (g/kg DM)
58	29.1	0.71	20.7	24.4	0.75	18.3	5.60	0.74	4.14
59	35.1	0.87	30.5	29.0	0.88	25.5	6.60	0.89	5.87
60	35.9	0.85	30.5	29.8	0.85	25.3	6.90	0.89	6.14
61	41.3	0.82	33.9	32.7	0.80	26.2	7.02	0.87	6.11
62	35.9	0.84	30.1	29.3	0.84	24.6	6.50	0.86	5.59
63	27.0	0.75	20.3	19.5	0.64	12.5	7.60	0.84	6.38
64	26.7	0.74	19.8	18.8	0.62	11.7	7.40	0.82	6.07
65	26.8	0.72	19.3	17.7	0.59	10.4	7.60	0.80	6.08
66	26.8	0.71	19.0	17.2	0.54	9.29	7.04	0.79	5.56
67	26.6	0.72	19.2	17.2	0.55	9.46	7.40	0.81	5.99
68	25.2	0.78	19.7	21.6	0.77	16.6	7.20	0.86	6.19
69	27.9	0.72	20.1	21.8	0.69	15.0	8.10	0.82	6.64
70	30.6	0.73	22.3	25.3	0.78	25.3	8.30	0.76	6.31
71	30.5	0.82	25.0	25.1	0.84	25.1	8.00	0.87	6.96
72	30.4	0.88	26.8	23.3	0.91	23.3	7.80	0.90	7.02
73	29.5	0.87	25.7	24.3	0.89	24.3	7.60	0.89	6.76
74	32.7	0.90	29.4	26.8	0.91	26.8	8.40	0.91	7.64
75	30.9	0.90	27.8	25.3	0.87	25.3	8.10	0.92	7.45
79	29.8	0.86	25.6	22.7	0.58	22.7	8.30	0.87	7.22
82	33.6	0.85	28.6	31.0	0.86	31.0	11.4	0.89	10.2

* The precaecal digestible AA was calculated by AA content and pcDAA given in reference.

Table A3
The number, amino acid content (AA; [g/kg DM]), fractional standardised precaecal digestibility of AA (pcDAA; [g/g AA]) and calculated standardised precaecal digestible AA concentration (pcdAA; [g/kg DM]) *in vivo* of the 74 assayed feedstuffs for the indispensable amino acids phenylalanine, threonine and tryptophan.

Number	Phenylalanine			Threonine			Tryptophan		
	AA (g/kg DM)	pcDPhe (g/g AA)	pcdPhe *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDThr (g/g AA)	pcdThr *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDTrp (g/g AA)	pcdTrp *in vivo* * (g/kg DM)
1	5.97	0.86	5.13	3.66	0.80	2.93	1.38	0.81	1.12
2	6.62	0.87	5.76	3.94	0.78	3.07	1.56	0.80	1.25
3	6.02	0.87	5.24	3.73	0.78	2.91	1.50	0.83	1.25
4	6.43	0.89	5.72	3.91	0.82	3.21	1.51	0.85	1.28
5	5.96	0.85	5.07	3.83	0.78	2.99	5.56	0.81	4.50
6	5.49	0.86	4.72	3.60	0.78	2.81	1.49	0.81	1.21
7	6.86	0.87	5.97	4.36	0.81	3.53	1.69	0.83	1.40
8	6.01	0.86	5.17	3.62	0.79	2.86	1.44	0.81	1.17
9	5.32	0.83	4.42	3.68	0.73	2.69	1.35	0.80	1.08
10	6.50	0.86	5.59	4.33	0.77	3.33	1.50	0.82	1.23
11	6.73	0.84	5.65	4.59	0.76	3.49	1.68	0.81	1.36
12	6.14	0.85	5.22	4.25	0.77	3.27	1.50	0.83	1.25
13	7.16	0.84	6.01	4.68	0.75	3.51	1.62	0.80	1.30
14	6.97	0.85	5.92	4.55	0.74	3.37	1.48	0.80	1.18
15	5.60	0.85	4.76	3.70	0.75	2.78	1.38	0.81	1.12
16	5.94	0.83	4.93	3.93	0.75	2.95	1.27	0.79	1.00
17	5.49	0.80	4.39	3.76	0.66	2.48	1.19	0.67	0.80
18	5.20	0.78	4.06	3.67	0.64	2.35	1.13	0.65	0.73
19	5.74	0.77	4.42	3.83	0.65	2.49	1.21	0.66	0.80
20	5.42	0.76	4.12	3.69	0.62	2.29	1.12	0.63	0.71
21	5.23	0.77	4.03	3.72	0.64	2.38	1.14	0.65	0.74
22	5.34	0.78	4.17	3.72	0.63	2.34	1.19	0.65	0.77
23	4.99	0.78	3.89	5.80	0.64	3.71	1.12	0.65	0.73
24	6.03	0.78	4.70	4.05	0.62	2.51	1.30	0.63	0.82
25	5.70	0.75	4.28	3.90	0.71	2.77	1.50	0.68	1.02
26	5.60	0.77	4.31	4.10	0.72	2.95	1.50	0.72	1.08
27	5.70	0.76	4.33	3.90	0.71	2.77	1.40	0.69	0.97
28	5.70	0.72	4.10	3.90	0.68	2.65	1.40	0.68	0.95
29	6.40	0.77	4.93	4.20	0.70	2.94	1.60	0.69	1.10
30	6.80	0.78	5.30	4.20	0.72	3.02	1.60	0.72	1.15
31	6.90	0.79	5.45	4.20	0.72	3.02	1.60	0.72	1.15
32	6.50	0.79	5.14	4.30	0.72	3.10	1.60	0.72	1.15
33	12.0	0.83	9.96	9.10	0.77	7.01	2.40	0.69	1.66
34	11.6	0.81	9.40	9.30	0.77	7.16	2.40	0.69	1.66
35	12.0	0.81	9.72	8.90	0.76	6.76	2.30	0.67	1.54
36	11.6	0.82	9.51	9.40	0.75	7.05	2.40	0.69	1.66
37	12.2	0.82	10.0	8.90	0.75	6.68	2.20	0.65	1.43
38	10.6	0.79	8.37	8.40	0.72	6.05	2.20	0.62	1.36
39	15.7	0.91	14.3	13.1	0.88	11.5	3.20	0.88	2.82
40	13.5	0.88	11.9	11.8	0.83	9.79	3.10	0.80	2.48
41	14.1	0.87	12.3	12.3	0.83	10.2	3.00	0.79	2.37
42	14.9	0.84	12.5	12.5	0.78	9.75	3.20	0.77	2.46

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Table A3 (*continued*)

Number	AA (g/kg DM)	Phenylalanine pcDPhe (g/g AA)	pcdPhe *in vivo* * (g/kg DM)	AA (g/kg DM)	Threonine pcDThr (g/g AA)	pcdThr *in vivo* * (g/kg DM)	AA (g/kg DM)	Tryptophan pcDTrp (g/g AA)	pcdTrp *in vivo* * (g/kg DM)
43	13.0	0.84	10.9	10.5	0.80	8.40	2.70	0.69	1.86
44	12.7	0.82	10.4	10.8	0.78	8.42	2.60	0.66	1.72
45	14.0	0.85	11.9	11.1	0.80	8.88	2.70	0.71	1.92
46	13.0	0.76	9.88	10.8	0.71	7.67	2.70	0.54	1.46
47	12.5	0.75	9.38	10.2	0.68	6.94	2.60	0.53	1.38
48	12.4	0.74	9.18	9.9	0.67	6.63	2.50	0.53	1.33
51	19.7	0.73	14.4	15.6	0.68	10.6	5.20	0.68	3.54
52	19.6	0.80	15.7	15.6	0.76	11.9	5.20	0.78	4.06
53	19.8	0.83	16.4	15.8	0.79	12.5	5.21	0.81	4.22
54	19.6	0.85	16.7	15.4	0.79	12.2	5.19	0.82	4.26
55	19.3	0.86	16.6	15.4	0.80	12.3	5.08	0.83	4.22
56	19.6	0.85	16.7	15.5	0.79	12.3	5.19	0.81	4.20
58	19.7	0.73	14.4	15.5	0.68	10.5	5.20	0.66	3.43
59	23.7	0.86	20.4	18.7	0.81	15.2	6.30	0.85	5.36
60	23.8	0.84	20.0	19.1	0.80	15.3	6.80	0.86	5.85
61	28.1	0.82	23.0	21.0	0.76	16.0	7.20	0.76	5.47
62	24.0	0.84	20.2	18.8	0.78	14.7	6.60	0.76	5.02
63	15.3	0.75	11.5	17.6	0.66	11.6	5.10	0.68	3.47
64	15.0	0.74	11.1	17.1	0.63	10.8	5.00	0.67	3.35
65	15.2	0.72	10.9	17.3	0.61	10.6	5.10	0.64	3.26
66	15.1	0.70	10.6	17.5	0.60	10.5	5.10	0.63	3.21
67	15.1	0.72	10.9	17.5	0.61	10.7	5.10	0.64	3.26
68	14.7	0.75	11.0	16.0	0.69	11.0	5.10	0.71	3.62
69	16.5	0.70	11.6	18.0	0.64	11.5	5.60	0.66	3.70
70	19.1	0.69	13.2	16.1	0.67	10.8	4.70	0.71	3.34
71	19.0	0.80	15.2	16.5	0.76	12.5	4.70	0.82	3.85
72	18.2	0.88	16.0	16.4	0.83	13.6	4.40	0.85	3.74
73	18.1	0.84	15.2	15.2	0.78	11.9	4.30	0.83	3.57
74	20.1	0.89	17.9	17.3	0.84	14.5	4.70	0.84	3.95
75	18.8	0.90	16.9	16.0	0.83	13.3	4.70	0.86	4.04
79	19.3	0.89	17.2	16.6	0.74	12.3	3.90	0.77	3.00
82	19.0	0.76	14.4	17.4	0.81	14.1	4.50	0.74	3.33

* The precaecal digestible AA was calculated by AA content and pcDAA given in reference.

Table A4
The number, amino acid content (AA; [g/kg DM]), fractional standardised precaecal digestibility of AA (pcDAA; [g/g AA]) and calculated standardised precaecal digestible AA concentration (pcdAA; [g/kg DM]) *in vivo* of the 74 assayed feedstuffs for the indispensable amino acid valine.

Valine			
Number	AA (g/kg DM)	pcDVal (g/g AA)	pcdVal *in vivo* * (g/kg DM)
1	5.06	0.85	4.30
2	5.26	0.84	4.42
3	5.02	0.85	4.27
4	5.07	0.88	4.46
5	5.22	0.84	4.38
6	4.47	0.85	3.80
7	5.86	0.86	5.04
8	4.84	0.85	4.11
9	4.87	0.80	3.90
10	5.61	0.84	4.71
11	6.31	0.81	5.11
12	5.71	0.83	4.74
13	5.99	0.82	4.91
14	6.10	0.82	5.00
15	4.93	0.82	4.04
16	5.22	0.81	4.23
17	4.95	0.74	3.66
18	4.64	0.72	3.34
19	5.15	0.73	3.76
20	4.75	0.74	3.52
21	4.74	0.72	3.41
22	4.80	0.71	3.41
23	4.61	0.71	3.27
24	5.17	0.71	3.67
25	5.50	0.76	4.18
26	5.70	0.79	4.50
27	5.90	0.77	4.54
28	5.90	0.75	4.43
29	6.10	0.78	4.76
30	6.20	0.78	4.84

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Table A4 (*continued*)

Valine			
Number	AA (g/kg DM)	pcDVal (g/g AA)	pcdVal *in vivo* * (g/kg DM)
31	5.90	0.78	4.60
32	6.30	0.77	4.85
33	12.0	0.80	9.60
34	11.1	0.78	8.63
35	11.7	0.78	9.13
36	11.7	0.78	9.13
37	11.8	0.78	9.20
38	10.6	0.74	7.84
39	14.4	0.88	12.7
40	13.6	0.84	11.4
41	13.9	0.84	11.7
42	14.4	0.81	11.7
43	14.0	0.82	11.5
44	13.1	0.79	10.4
45	14.9	0.82	12.2
46	13.6	0.72	9.79
47	13.1	0.71	9.30
48	13.0	0.71	9.23
51	18.4	0.72	13.2
52	18.5	0.80	14.8
53	18.5	0.82	15.2
54	19.0	0.83	15.8
55	18.6	0.85	15.8
56	18.8	0.83	15.6
58	18.0	0.69	12.4
59	21.7	0.85	18.4
60	22.3	0.84	18.7
61	25.0	0.80	20.0
62	22.0	0.81	17.8
63	19.7	0.68	13.4
64	19.6	0.67	13.1
65	19.7	0.67	13.2
66	19.1	0.64	12.2
67	18.6	0.67	12.5
68	18.6	0.73	13.6
69	20.1	0.67	13.5
70	19.9	0.71	14.1
71	20.1	0.81	16.3
72	19.9	0.87	17.3
73	19.4	0.85	16.5
74	21.4	0.89	19.0
75	20.2	0.90	18.2
79	18.9	0.84	15.9
82	23.0	0.81	18.6

* The precaecal digestible AA was calculated by AA content and pcDAA given in reference

Table A5
The number, amino acid content (AA; [g/kg DM]), fractional standardised precaecal digestibility of AA (pcDAA; [g/g AA]) and calculated standardised precaecal digestible AA concentration (pcdAA; [g/kg DM]) *in vivo* of the 74 assayed feedstuffs for the dispensable amino acids alanine, aspartic acid and cysteine.

	Alanine			Aspartic acid			Cysteine		
Number	AA (g/kg DM)	pcDAla (g/g AA)	pcdAla *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDAsp (g/g AA)	pcdAsp *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDCys (g/g AA)	pcdCys *in vivo* * (g/kg DM)
1	4.41	0.75	3.31	6.48	0.77	4.99	2.81	0.88	2.47
2	4.64	0.75	3.48	6.66	0.75	5.00	3.07	0.88	2.70
3	4.47	0.75	3.35	6.34	0.75	4.76	2.93	0.88	2.58
4	4.57	0.78	3.56	6.43	0.79	5.08	3.00	0.90	2.70
5	4.63	0.74	3.43	6.79	0.75	5.09	2.94	0.88	2.59
6	4.33	0.75	3.25	6.28	0.75	4.71	2.87	0.88	2.53
7	5.18	0.78	4.04	7.38	0.78	5.76	3.42	0.89	3.04
8	4.46	0.77	3.43	6.81	0.79	5.38	2.75	0.88	2.42
9	4.59	0.74	3.40	7.88	0.78	6.15	2.60	0.85	2.21
10	5.37	0.79	4.24	8.68	0.81	7.03	3.17	0.87	2.76
11	5.70	0.76	4.33	9.09	0.78	7.09	3.25	0.85	2.76
12	5.19	0.78	4.05	7.94	0.79	6.27	3.11	0.87	2.71
13	5.66	0.76	4.30	9.23	0.78	7.20	3.47	0.86	2.98
14	5.61	0.77	4.32	9.04	0.79	7.14	3.26	0.86	2.80
15	4.47	0.76	3.40	7.29	0.78	5.69	2.88	0.87	2.51
16	4.84	0.76	3.68	7.58	0.78	5.91	2.81	0.85	2.39
17	4.67	0.66	3.08	8.10	0.71	5.75	2.53	0.79	2.00
18	4.60	0.63	2.90	7.86	0.69	5.42	2.38	0.77	1.83

(*continued on next page*)

Table A5 (*continued*)

	Alanine			Aspartic acid			Cysteine		
Number	AA (g/kg DM)	pcDAla (g/g AA)	pcdAla *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDAsp (g/g AA)	pcdAsp *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDCys (g/g AA)	pcdCys *in vivo* * (g/kg DM)
19	4.76	0.64	3.05	7.97	0.69	5.50	2.57	0.79	2.03
20	4.69	0.62	2.91	8.02	0.68	5.45	2.34	0.76	1.78
21	4.60	0.64	2.94	7.86	0.69	5.42	2.45	0.79	1.94
22	4.68	0.64	3.00	8.13	0.70	5.69	2.47	0.76	1.88
23	4.52	0.62	2.80	7.62	0.68	5.18	2.38	0.78	1.86
24	4.86	0.61	2.96	8.29	0.66	5.47	2.66	0.76	2.02
25	4.70	0.66	3.10	7.10	0.69	4.90	2.50	0.79	1.98
26	4.90	0.67	3.28	7.20	0.69	4.97	2.70	0.80	2.16
27	4.70	0.63	2.96	6.70	0.66	4.42	2.50	0.80	2.00
28	4.70	0.62	2.91	7.10	0.66	4.69	2.40	0.79	1.90
29	4.90	0.64	3.14	7.40	0.67	4.96	3.00	0.80	2.40
30	4.80	0.67	3.22	7.10	0.70	4.97	2.60	0.81	2.11
31	4.90	0.67	3.28	7.20	0.69	4.97	2.90	0.82	2.38
32	5.00	0.65	3.25	7.50	0.68	5.10	2.70	0.81	2.19
33	10.8	0.79	8.53	10.8	0.85	9.18	3.60	0.71	2.56
34	10.7	0.78	8.35	10.7	0.84	8.99	3.60	0.71	2.56
35	10.5	0.77	8.09	10.5	0.83	8.72	3.50	0.69	2.42
36	10.7	0.77	8.24	10.7	0.83	8.88	3.60	0.66	2.38
37	10.4	0.75	7.80	10.4	0.81	8.42	3.00	0.62	1.86
38	9.80	0.73	7.15	9.80	0.80	7.84	3.40	0.63	2.14
39	12.5	0.86	10.8	39.3	0.91	35.8	5.40	0.87	4.70
40	11.4	0.82	9.35	33.1	0.88	29.1	5.20	0.83	4.32
41	12.2	0.83	10.1	35.1	0.88	30.9	5.20	0.81	4.21
42	12.0	0.76	9.12	37.4	0.86	32.2	4.50	0.78	3.51
43	12.4	0.81	10.0	33.4	0.86	28.7	3.80	0.65	2.47
44	11.8	0.78	9.20	32.3	0.83	26.8	3.50	0.63	2.21
45	13.0	0.80	10.4	36.6	0.86	31.5	4.00	0.64	2.56
46	12.4	0.72	8.93	33.6	0.79	26.5	3.80	0.51	1.94
47	11.8	0.70	8.26	31.0	0.77	23.9	3.60	0.52	1.87
48	11.6	0.70	8.12	31.1	0.77	24.0	3.40	0.48	1.63
51	16.8	0.71	11.9	44.2	0.74	32.7	6.30	0.67	4.22
52	16.9	0.79	13.4	44.2	0.81	35.8	6.19	0.75	4.64
53	16.9	0.81	13.7	44.5	0.81	36.0	6.01	0.76	4.57
54	16.9	0.82	13.8	43.9	0.80	35.1	5.91	0.73	4.31
55	16.6	0.83	13.8	43.5	0.80	34.8	5.72	0.74	4.23
56	16.7	0.82	13.7	43.8	0.76	33.3	5.59	0.69	3.86
58	16.4	0.69	11.3	44.0	0.73	32.1	6.10	0.64	3.90
59	19.8	0.82	16.2	52.9	0.86	45.5	7.20	0.79	5.69
60	20.2	0.82	16.6	54.0	0.84	45.4	7.70	0.76	5.85
61	22.8	0.77	17.6	62.7	0.79	49.5	7.70	0.72	5.54
62	19.9	0.78	15.5	54.3	0.82	44.5	6.80	0.74	5.03
63	17.1	0.72	12.3	28.3	0.67	19.0	9.10	0.70	6.37
64	16.8	0.71	11.9	27.7	0.65	18.0	8.90	0.68	6.05
65	17.0	0.69	11.7	27.9	0.63	17.6	8.80	0.61	5.37
66	17.0	0.67	11.4	27.6	0.61	16.8	8.70	0.59	5.13
67	16.8	0.68	11.4	27.4	0.62	17.0	8.30	0.60	4.98
68	15.5	0.75	11.6	27.0	0.74	20.0	9.30	0.74	6.88
69	17.1	0.71	12.1	30.0	0.64	19.2	9.10	0.70	6.37
70	13.4	0.73	9.78	33.9	0.74	25.1	4.70	0.58	2.73
71	13.5	0.81	10.9	34.2	0.81	27.7	4.70	0.72	3.38
72	13.5	0.86	11.6	34.2	0.85	29.1	4.40	0.76	3.34
73	13.0	0.85	11.1	32.5	0.85	27.6	4.40	0.74	3.26
74	14.5	0.87	12.6	37.2	0.85	31.6	5.20	0.75	3.90
75	13.5	0.88	11.9	34.3	0.83	28.5	4.80	0.64	3.07
79	10.8	0.77	8.31	19.1	0.63	13.2	5.40	0.83	4.48
82	17.6	0.85	15.0	32.7	0.80	26.2	4.50	0.40	1.80

* The precaecal digestible AA was calculated by AA content and pcDAA given in reference.

Table A6
The number, amino acid content (AA; [g/kg DM]), fractional standardised precaecal digestibility of AA (pcDAA; [g/g AA]) and calculated standardised precaecal digestible AA concentration (pcdAA; [g/kg DM]) *in vivo* of the 74 assayed feedstuffs for the dispensable amino acids glutamic acid, glycine and proline.

Number	Glutamic acid			Glycine			Proline		
	AA (g/kg DM)	pcDGlu (g/g AA)	pcdGlu *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDGly (g/g AA)	pcdGly *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDPro (g/g AA)	pcdPro *in vivo* * (g/kg DM)
1	37.5	0.95	35.6	5.14	0.79	4.06	14.3	0.97	13.8
2	42.3	0.95	40.2	5.57	0.79	4.40	15.8	0.97	15.3
3	37.6	0.95	35.8	5.11	0.80	4.09	14.5	0.96	13.9
4	41.8	0.96	40.1	5.65	0.84	4.75	15.9	0.98	15.6
5	37.6	0.94	35.3	5.72	0.77	4.40	14.7	0.96	14.1
6	35.0	0.95	33.2	5.08	0.77	3.91	13.4	0.96	12.9
7	44.5	0.95	42.3	6.21	0.80	4.97	17.1	0.96	16.4
8	36.3	0.95	34.5	5.12	0.78	3.99	14.2	0.97	13.8
9	31.2	0.92	28.7	5.11	0.71	3.63	12.9	1.03	13.3
10	37.7	0.94	35.4	5.65	0.81	4.58	16.1	1.05	16.9
11	52.3	0.93	48.6	6.25	0.75	4.69	16.8	1.03	17.3
12	35.5	0.93	33.0	5.73	0.79	4.53	15.0	1.05	15.8
13	41.4	0.93	38.5	6.22	0.78	4.85	17.9	1.04	18.6
14	41.7	0.93	38.8	6.24	0.80	4.99	17.3	1.05	18.2
15	33.2	0.93	30.9	4.80	0.76	3.65	13.6	1.06	14.5
16	33.9	0.92	31.2	5.22	0.78	4.07	14.2	1.05	14.9
17	28.0	0.87	24.3	4.93	0.65	3.20	13.4	1.04	14.0
18	26.6	0.86	22.9	4.83	0.61	2.95	12.7	1.03	13.1
19	29.6	0.87	25.8	5.03	0.60	3.02	14.6	0.94	13.7
20	27.7	0.86	23.8	4.91	0.57	2.80	13.5	0.93	12.5
21	26.6	0.85	22.6	4.81	0.58	2.79	12.4	1.00	12.4
22	27.3	0.86	23.5	4.80	0.61	2.93	13.2	1.02	13.5
23	25.5	0.85	21.7	4.73	0.62	2.93	12.0	1.04	12.5
24	31.0	0.86	26.7	5.28	0.59	3.12	15.3	1.01	15.5
25	26.3	0.84	22.1	4.80	0.65	3.12	11.7	0.80	9.36
26	26.1	0.88	23.0	5.10	0.65	3.32	11.5	0.83	9.55
27	28.4	0.87	24.7	4.70	0.63	2.96	12.5	0.83	10.4
28	28.6	0.83	23.7	4.60	0.63	2.90	12.8	0.78	9.98
29	29.6	0.86	25.5	5.10	0.62	3.16	13.4	0.84	11.3
30	31.9	0.86	27.4	4.80	0.67	3.22	14.6	0.83	12.1
31	30.9	0.86	26.6	5.00	0.67	3.35	14.4	0.84	12.1
32	31.8	0.87	27.7	5.50	0.68	3.74	14.5	0.83	12.0
33	42.2	0.89	37.6	10.9	0.75	8.18	10.4	0.81	8.42
34	42.4	0.89	37.7	10.7	0.74	7.92	10.5	0.81	8.51
35	40.8	0.88	35.9	10.8	0.74	7.99	10.6	0.81	8.59
36	42.0	0.86	36.1	10.7	0.74	7.92	10.2	0.80	8.16
37	12.5	0.84	10.5	10.4	0.72	7.49	10.3	0.79	8.14
38	36.6	0.84	30.7	9.80	0.71	6.96	9.10	0.78	7.10
39	89.4	0.94	84.0	15.8	0.85	13.4	16.4	0.95	15.6
40	74.1	0.94	69.7	14.3	0.82	11.7	13.2	0.87	11.5
41	74.1	0.91	67.4	14.3	0.81	11.6	13.2	0.85	11.2
42	80.4	0.92	74.0	15.4	0.81	12.5	14.7	0.85	12.5
43	49.8	0.89	44.3	13.0	0.73	9.49	12.4	0.83	10.3
44	50.5	0.88	44.4	12.5	0.74	9.25	13.0	0.77	10.0
45	53.6	0.87	46.6	13.8	0.73	10.1	13.0	0.82	10.7
46	50.9	0.84	42.8	12.8	0.63	8.06	12.0	0.69	8.28
47	47.3	0.84	39.7	12.3	0.64	7.87	11.4	0.65	7.41
48	47.4	0.83	39.3	11.9	0.59	7.02	11.4	0.67	7.64
51	68.9	0.77	53.1	16.7	0.69	11.5	19.6	0.85	16.6
52	69.1	0.82	56.7	16.8	0.77	12.9	19.3	0.91	17.6
53	69.6	0.85	59.2	16.7	0.79	13.2	20.2	0.94	19.0
54	69.0	0.86	59.3	16.9	0.80	13.5	19.8	0.95	18.8
55	68.5	0.86	58.9	16.6	0.81	13.4	20.1	0.96	19.3
56	69.0	0.84	57.9	16.5	0.78	12.9	20.1	0.94	18.9
58	68.8	0.77	53.0	15.7	0.67	10.5	18.0	0.75	13.5
59	83.3	0.87	72.5	19.5	0.80	15.6	22.4	0.89	19.9
60	83.8	0.83	69.6	19.6	0.76	14.9	22.6	0.87	19.7
61	98.8	0.81	80.0	22.0	0.71	15.6	26.0	0.84	21.8
62	85.0	0.84	71.4	19.9	0.72	14.3	22.4	0.87	19.5
63	60.3	0.79	47.6	20.1	0.65	13.1	22.4	0.62	13.9
64	59.5	0.78	46.4	19.8	0.63	12.5	23.5	0.65	15.3
65	59.9	0.75	44.9	20.1	0.62	12.5	24.0	0.63	15.1
66	59.6	0.73	43.5	20.0	0.60	12.0	22.8	0.59	13.5
67	59.3	0.75	44.5	19.7	0.60	11.8	22.2	0.60	13.3
68	68.3	0.79	54.0	17.9	0.71	12.7	21.0	0.76	16.0
69	62.3	0.84	52.3	19.6	0.68	13.3	23.1	0.68	15.7
70	72.2	0.77	55.6	11.3	0.71	8.02	32.6	0.74	24.1
71	71.7	0.85	60.9	11.3	0.80	9.04	31.7	0.82	26.0

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Table A6 (*continued*)

Number	Glutamic acid			Glycine			Proline		
	AA (g/kg DM)	pcDGlu (g/g AA)	pcdGlu *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDGly (g/g AA)	pcdGly *in vivo* * (g/kg DM)	AA (g/kg DM)	pcDPro (g/g AA)	pcdPro *in vivo* * (g/kg DM)
72	71.8	0.85	61.0	11.1	0.85	9.44	27.1	0.87	23.6
73	69.0	0.82	56.6	10.6	0.82	8.69	28.3	0.86	24.3
74	76.1	0.86	65.4	11.9	0.86	10.2	28.9	0.87	25.1
75	71.6	0.85	60.9	11.0	0.85	9.35	27.5	0.84	23.1
79	113	0.86	97.5	10.3	0.86	8.86	41.8	0.93	38.9
82	69.1	0.80	55.3	14.2	0.80	11.4	32.4	0.79	25.6

* The precaecal digestible AA was calculated by AA content and pcDAA given in reference.

Table A7
The number, amino acid content (AA; [g/kg DM]), fractional standardised precaecal digestibility of AA (pcDAA; [g/g AA]) and calculated standardised precaecal digestible AA concentration (pcdAA; [g/kg DM]) *in vivo* of the 74 assayed feedstuffs for the dispensable amino acid serine.

Number	Serine		
	AA (g/kg DM)	pcDSer (g/g AA)	pcdSer *in vivo* * (g/kg DM)
1	6.18	0.90	5.56
2	6.89	0.89	6.13
3	6.35	0.89	5.65
4	6.84	0.91	6.22
5	6.40	0.89	5.70
6	6.13	0.89	5.46
7	7.39	0.90	6.65
8	6.11	0.89	5.44
9	5.64	0.82	4.62
10	6.79	0.85	5.77
11	6.84	0.84	5.75
12	6.38	0.85	5.42
13	7.32	0.84	6.15
14	7.21	0.84	6.06
15	5.85	0.84	4.91
16	6.08	0.83	5.05
17	5.36	0.75	4.02
18	5.31	0.74	3.93
19	5.45	0.74	4.03
20	5.48	0.72	3.95
21	5.20	0.72	3.74
22	5.29	0.72	3.81
23	5.11	0.73	3.73
24	5.89	0.72	4.24
25	4.90	0.78	3.82
26	5.10	0.80	4.08
27	5.00	0.79	3.95
28	5.10	0.77	3.93
29	5.50	0.78	4.29
30	5.50	0.80	4.40
31	5.40	0.80	4.32
32	5.50	0.79	4.35
33	11.4	0.84	9.58
34	11.6	0.83	9.63
35	11.1	0.82	9.10
36	11.7	0.82	9.59
37	11.7	0.80	9.36
38	10.3	0.75	7.73
39	19.8	0.93	18.4
40	16.1	0.87	14.0
41	16.1	0.87	14.0
42	18.0	0.86	15.5
43	14.2	0.85	12.1
44	14.7	0.85	12.5
45	15.5	0.86	13.3
46	14.9	0.77	11.5
47	13.6	0.77	10.5
48	13.3	0.77	10.2
51	20.0	0.72	14.4
52	19.9	0.79	15.7
53	20.2	0.82	16.6
54	19.4	0.83	16.1
55	19.5	0.84	16.4
56	19.4	0.82	15.9

(*continued on next page*)

Table A7 (*continued*)

	Serine		
Number	AA (g/kg DM)	pcDSer (g/g AA)	pcdSer *in vivo* * (g/kg DM)
58	19.4	0.71	13.8
59	23.9	0.85	20.3
60	24.1	0.84	20.2
61	27.4	0.81	22.2
62	24.1	0.82	19.8
63	16.4	0.67	11.0
64	16.2	0.66	10.7
65	16.0	0.61	9.76
66	16.7	0.62	10.4
67	16.7	0.62	10.4
68	17.4	0.66	11.5
69	15.3	0.70	10.7
70	19.7	0.73	14.4
71	1.90	0.80	1.52
72	2.00	0.89	1.78
73	18.8	0.86	16.2
74	21.1	0.90	19.0
75	19.7	0.90	17.7
79	20.1	0.89	17.9
82	19.8	0.76	15.0

* The precaecal digestible AA was calculated by AA content and pcDAA given in reference.

Table A8
The regression equation (y= a x + b), coefficient of determination (R^2) and root mean square error (RMSE) for pcdAA for the cereal grains (n= 32) and group wheat and triticale and barley and rye (each N= 8).

Amino acids (g/kg DM)	Samples Y =	R^2	RMSE
	Cereal grains wheat, triticale, barley, rye		
Total AA	0.896 (SE 0.035, CI 0.825; 0.967) x – 6.466 (SE 3.699, CI -14.06; 1.122)	0.989	1.648
Indispensable AA	0.844 (SE 0.043, CI 0.756; 0.931) x – 1.062 (SE 1.793, CI -4.742; 2.618)	0.980	0.678
Dispensable AA	0.918 (SE 0.030, CI 0.855; 0.980) x – 4.612 (SE 1.964, CI -8.643; -0.582)	0.993	0.992
	Group wheat and triticale		
Total AA	0.904 (SE 0.045, CI 0.807; 1.001) x + 5.841 (SE 5.389, CI -5.717; 17.40)	0.966	1.992
Indispensable AA	0.835 (SE 0.060, CI 0.707; 0.963) x + 3.854 (SE 2.525, CI -1.563; 9.270)	0.933	0.879
Dispensable AA	0.932 (SE 0.037, CI 0.853; 1.010) x + 2.725 (SE 2.806, CI -3.294; 8.744)	0.979	1.108
	Group barley and rye		
Total AA	0.696 (SE 0.070, CI 0.547; 0.844) x + 15.60 (SE 6.978, CI 0.631; 30.56)	0.879	1.813
Indispensable AA	0.775 (SE 0.037, CI 0.696; 0.855) x + 1.831 (SE 1.438, CI -1.252; 4.915)	0.969	0.511
Dispensable AA	0.745 (SE 0.107, CI 0.515; 0.975) x + 7.610 (SE 6.690, CI -6.736; 21.96)	0.775	1.568

Table A9
The regression equation (y= a x + b), coefficient of determination (R^2) and root mean square error (RMSE) for pcdAA for the group wheat and triticale (each N= 8).

Amino acids (g/kg DM)	Samples y =	R^2	RMSE
Indispensable AA			
Arginine	0.882 (SE 0.057, CI 0.761; 1.003) x + 0.580 (SE 0.330, CI -0.127; 1.289)	0.964	0.128
Histidine	0.904 (SE 0.021, CI 0.858; 0.950) x + 0.125 (SE 0.074, CI -0.034; 0.285)	0.992	0.083
Isoleucine	0.849 (SE 0.065, CI 0.710; 0.988) x + 0.333 (SE 0.247, CI -0.196; 0.863)	0.925	0.093
Leucine	0.860 (SE 0.069, CI 0.712; 1.008) x + 0.624 (SE 0.563, CI -0.584; 1.832)	0.917	0.179
Lysine	0.871 (SE 0.600, CI 0.742; 1.000) x - 0.130 (SE 0.212, CI -0.584; 0.324)	0.938	0.103
Methionine	0.886 (SE 0.046, CI 0.787; 0.986) x + 0.095 (SE 0.088, CI -0.094; 0.284)	0.963	0.032
Phenylalanine	0.901 (SE 0.055, CI 0.784; 1.019) x + 0.150 (SE 0.316, CI -0.527; 0.828)	0.951	0.105
Threonine	0.727 (SE 0.077, CI 0.563; 0.892) x + 0.481 (SE 0.278, CI -0.115; 1.077)	0.865	0.100
Tryptophan	0.803 (SE 0.006, CI 0.791; 0.816) x + 0.121 (SE 0.011, CI 0.097; 0.145)	0.999	0.022
Valine	0.754 (SE 0.065, CI 0.615; 0.893) x + 0.901 (SE 0.309, CI 0.239; 1.563)	0.906	0.123
Dispensable AA			
Alanine	0.826 (SE 0.060, CI 0.697; 0.954) x + 0.215 (SE 0.257, CI -0.336; 0.766)	0.931	0.104
Aspartic acid	0.860 (SE 0.040, CI 0.773; 0.946) x + 0.109 (SE 0.270, CI -0.470; 0.689)	0.970	0.150
Cysteine	0.895 (SE 0.057, CI 0.774; 1.016) x + 0.097 (SE 0.161, CI -0.247; 0.442)	0.947	0.048
Glutamic acid	0.959 (SE 0.035, CI 0.883; 1.034) x + 0.825 (SE 1.318, CI -2.001; 3.651)	0.981	0.652
Glycine	0.816 (SE 0.103, CI 0.594; 1.037) x + 0.374 (SE 0.506, CI -0.710; 1.459)	0.817	0.612
Proline	1.109 (SE 0.111, CI 0.871; 1.347) x - 0.944 (SE 1.634, CI -4.449; 2.560)	0.877	0.599
Serine	0.931 (SE 0.108, CI 0.700; 1.161) x + 0.053 (SE 0.651, CI -1.343; 1.450)	0.842	0.201

y= estimated pcdAA (g/kg DM) and x= NDSAA (g/kg DM).

Table A10
The regression equation (y= a x + b), coefficient of determination (R^2) and root mean square error (RMSE) for pcdAA for the group barley and rye (each N= 8).

Amino acids (g/kg DM)	Samples		
	y =	R^2	RMSE
Indispensable AA			
Arginine	0.695 (SE 0.071, CI 0.544; 0.846) x + 1.019 (SE 0.376, CI 0.213; 1.825)	0.874	0.097
Histidine	0.777 (SE 0.069, CI 0.629; 0.925) x + 0.167 (SE 0.174, CI -0.206; 0.540)	0.900	0.035
Isoleucine	0.773 (SE 0.033, CI 0.702; 0.843) x + 0.179 (SE 0.114, CI -0.065; 0.423)	0.975	0.064
Leucine	0.791 (SE 0.029, CI 0742; 0.840) x + 0.342 (SE 0.160, CI -0.001; 0.684)	0.988	0.094
Lysine	0.532 (SE 0.097, CI 0.323; 0.741) x + 0.678 (SE 0.361, CI -0.967; 1.453)	0.681	0.084
Methionine	0.755 (SE 0.043, CI 0.664; 0.847) x + 0.151 (SE 0.073, CI -0.006; 0.308)	0.975	0.025
Phenylalanine	0.728 (SE 0.057, CI 0.606; 0.849) x + 0.606 (SE 0.055, CI -0.044; 1.257)	0.922	0.131
Threonine	0.696 (SE 0.061, CI 0.564; 0.829) x + 0.202 (SE 0.226, CI -0.282; 0.686)	0.902	0.115
Tryptophan	0.766 (SE 0.029, CI 0.704; 0.829) x - 0.022 (SE 0.036, CI -0.099; 0.056)	0.980	0.022
Valine	0.827 (SE 0.026, CI 0.771; 0.884) x + 0.078 (SE 0.127, CI -0.195; 0.351)	0.986	0.067
Dispensable AA			
Alanine	0.545 (SE 0.096, CI 0.340; 0.750) x + 0.790 (SE 0.397, CI -0.060; 1.640)	0.699	0.080
Aspartic acid	0.977 (SE 0.107, CI 0.749; 1.206) x - 1.427 (SE 0.721, CI -2.973; 0.119)	0.857	0.135
Cysteine	0.896 (SE 0.042, CI 0.807; 0.986) x - 0.129 (SE 0.101, CI -0.345; 0.087)	0.970	0.030
Glutamic acid	0.836 (SE 0.050, CI 0.728; 0.944) x + 1.877 (SE 1.368, CI -1.056; 4.810)	0.952	0.406
Glycine	0.640 (SE 0.115, CI 0.392; 0.887) x + 0.329 (SE 0.499, CI -0.742; 1.401)	0.687	0.131
Proline	0.921 (SE 0.301, CI 0.276; 1.566) x - 0.399 (SE 3.838, CI -7.833; 8.632)	0.402	1.264
Serine	0.783 (SE 0.127, CI 0511; 1056) x + 0.239 (SE 0.617, CI -1.084; 1.564)	0.730	0.111

y= estimated pcdAA (g/kg DM) and x= NDSAA (g/kg DM).

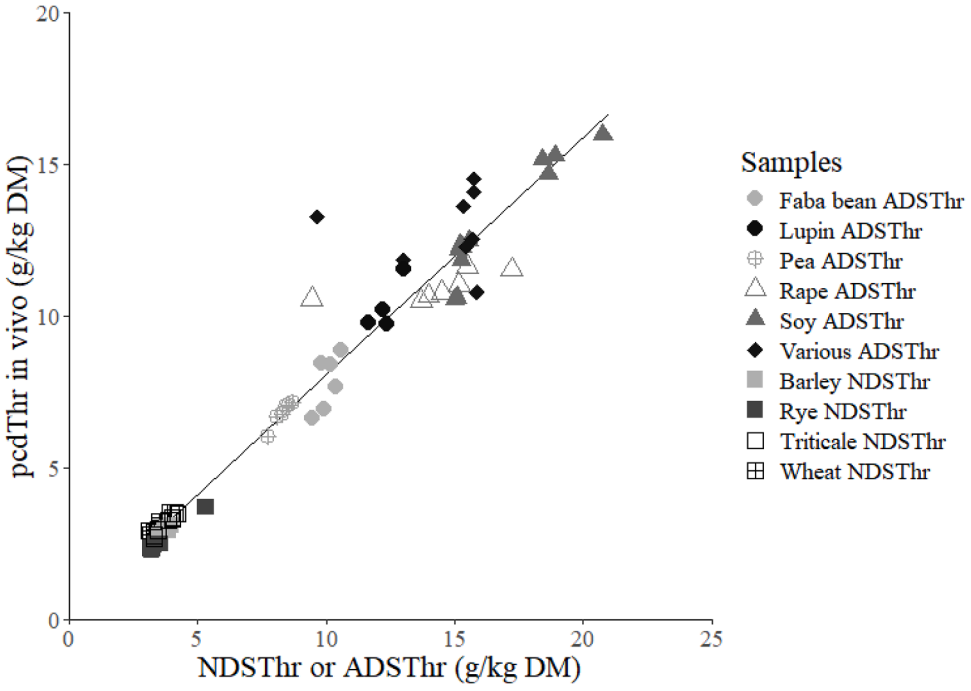

Fig. A1. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for threonine (Thr). With the regression equation y= 0.785 (SE 0.021, CI 0.742; 0.827) x + 0.214 (SE 0.227, CI -0.238; 0.667), R^2= 0.949, RMSE= 0.985.

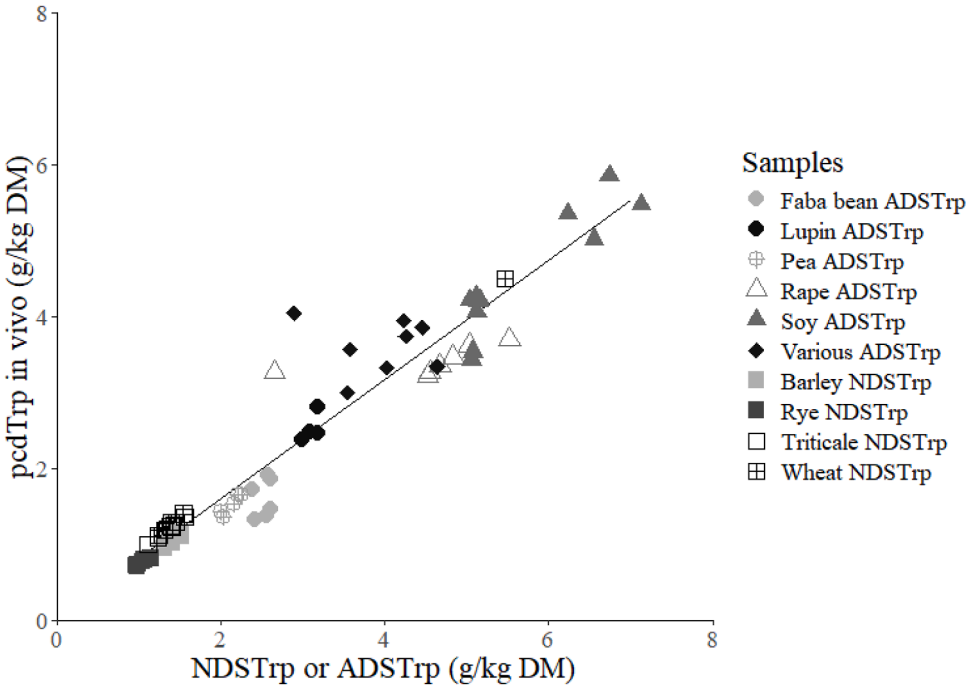

Fig. A2. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in* vivo pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for tryptophan (Trp). With the regression equation y= 0.784 (SE 0.025, CI 0.735; 0.834) x + 0.038 (SE 0.083, CI -0.128; 0.204), R^2= 0.932, RMSE= 0.364.

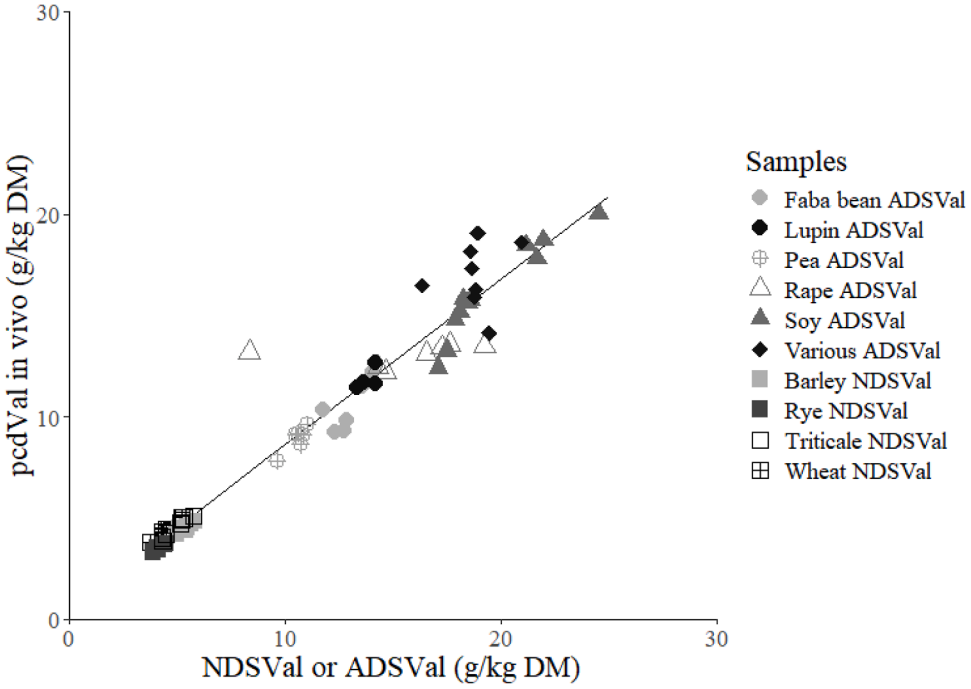

Fig. A3. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for valine (Val). With the regression equation y= 0.817 (SE 0.021, CI 0.775; 0.860) x + 0.438 (SE 0.272, CI -0.103; 0.980), R^2= 0.953, RMSE= 1.132.

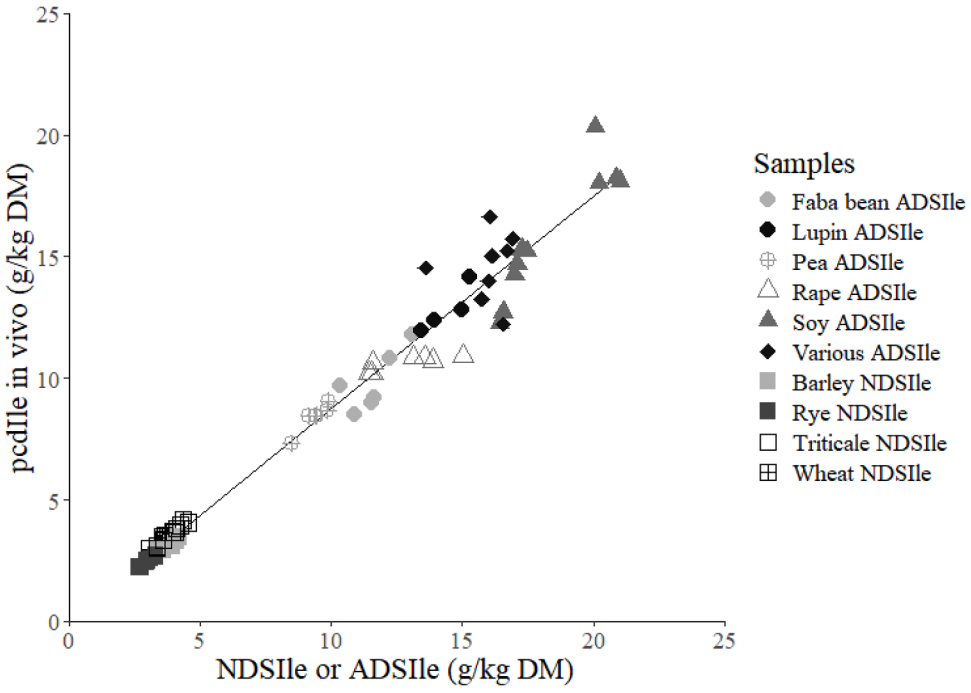

Fig. A4. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for isoleucine (Ile). With the regression equation y= 0.873 (SE 0.017, CI 0.840; 0.907) x + 0.011 (SE 0.191, CI -0.365; 0.396), R^2= 0.974, RMSE= 0.841.

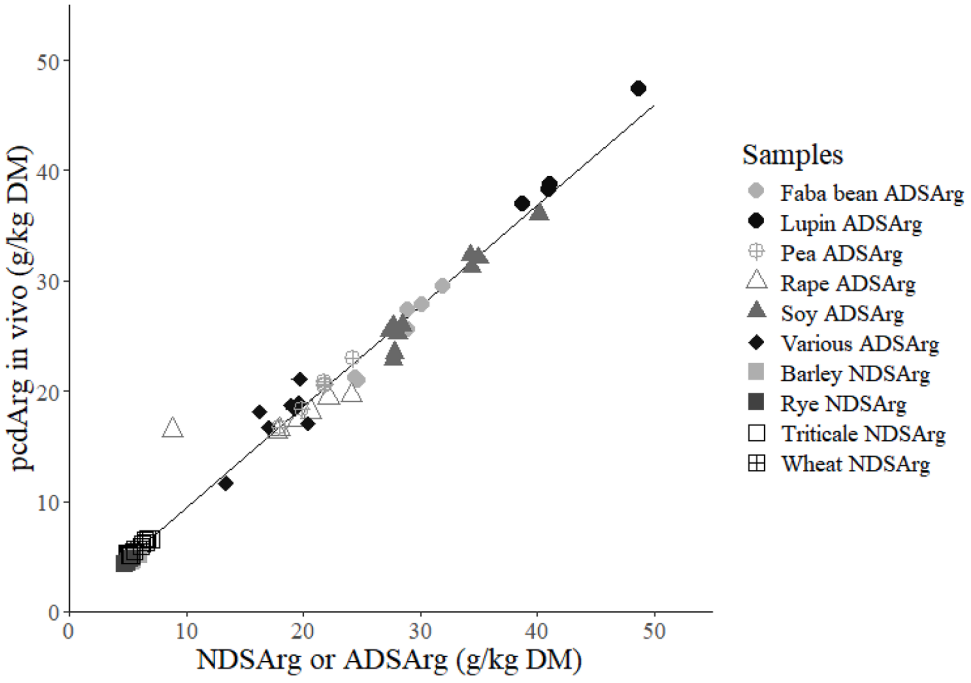

Fig. A5. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in* vivo pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for arginine (Arg). With the regression equation y= 0.914 (SE 0.014, CI 0.886; 0.941) x + 0.287 (SE 0.282, CI -2.753; 0.850), R^2= 0.984, RMSE= 1.363.

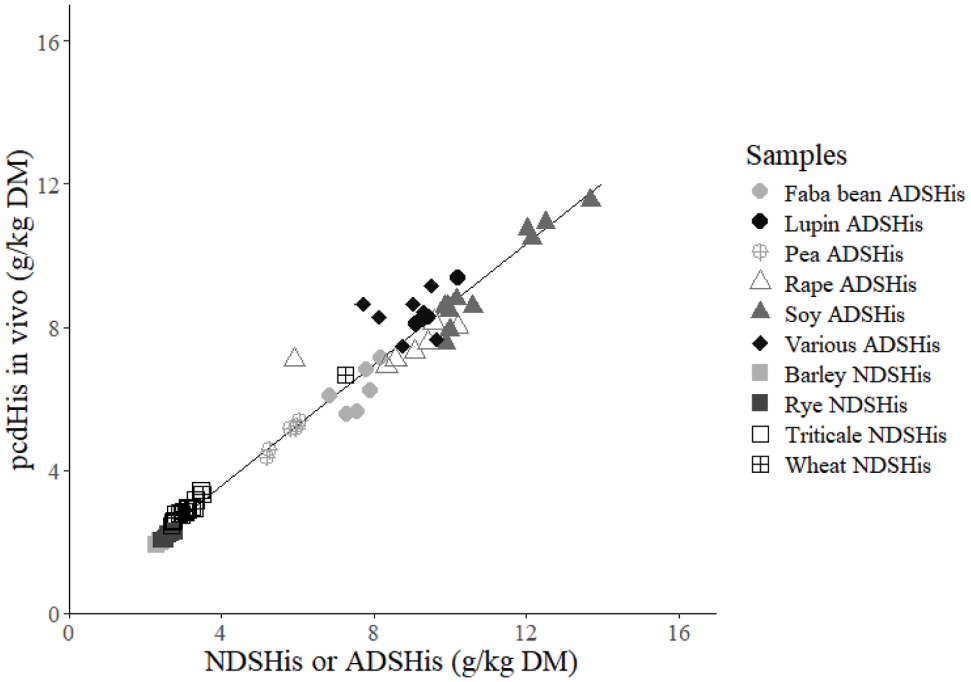

Fig. A6. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for histidine (His). With the regression equation y= 0.845 (SE 0.018, CI 0.810; 0.880) x + 0.180 (SE 0.125, CI -0.069; 0.429), R^2= 0.970, RMSE= 0.496.

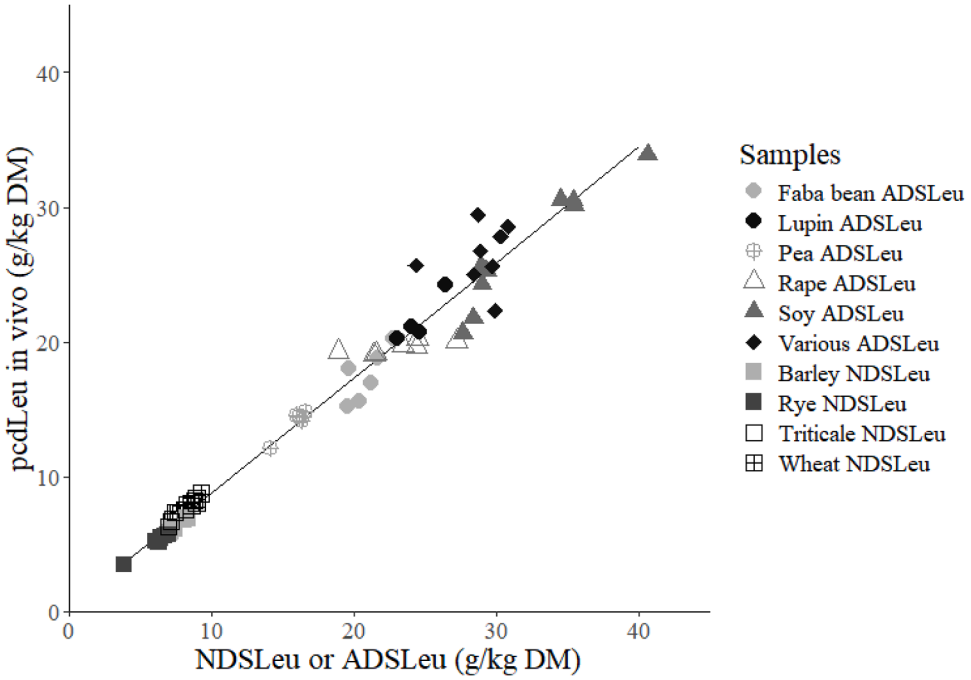

Fig. A7. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for leucine (Leu). With the regression equation y= 0.854 (SE 0.016, CI 0.822; 0.885) x + 0.320 (SE 0.317, CI -0.311; 0.952), R^2= 0.976, RMSE= 1.317.

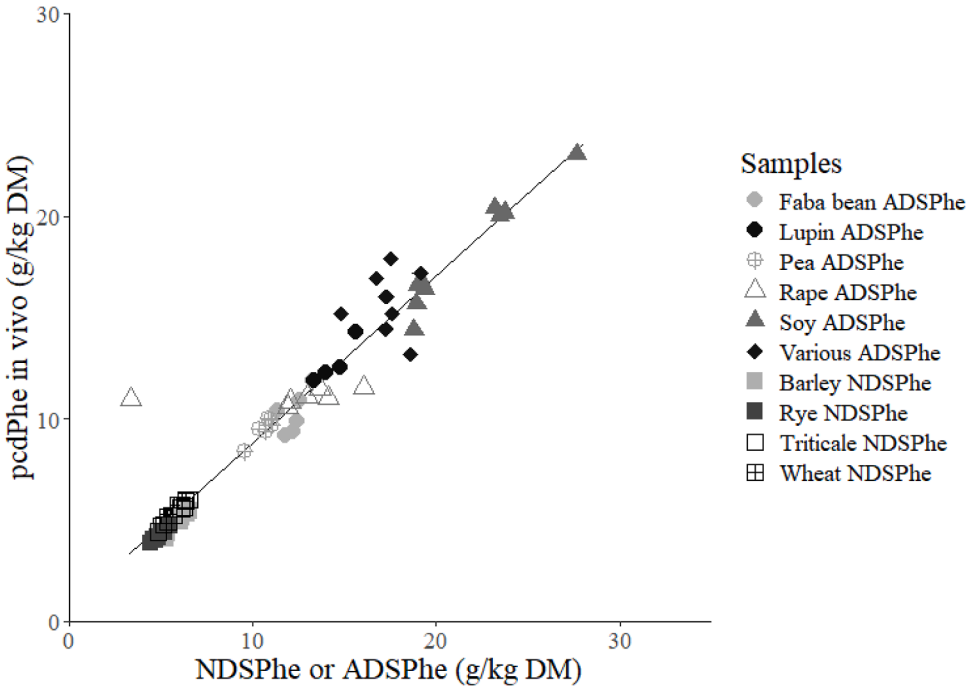

Fig. A8. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for phenylalanine (Phe). With the regression equation y= 0.818 (SE 0.024, CI 0.770; 0.866) x + 0.657 (SE 0.305, CI 0.049; 1.265), R^2= 0.941, RMSE= 1.227.

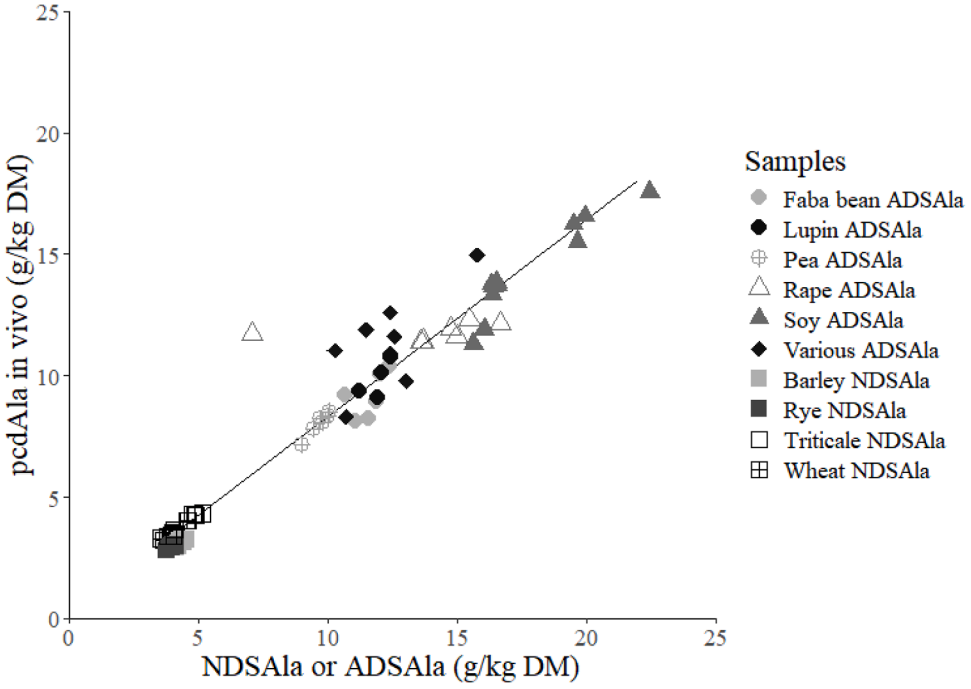

Fig. A9. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for alanine (Ala). With the regression equation y= 0.811 (SE 0.023, CI 0.766; 0.857) x + 0.190 (SE 0.247, CI -0.302; 0.683), R^2= 0.946, RMSE= 1.016.

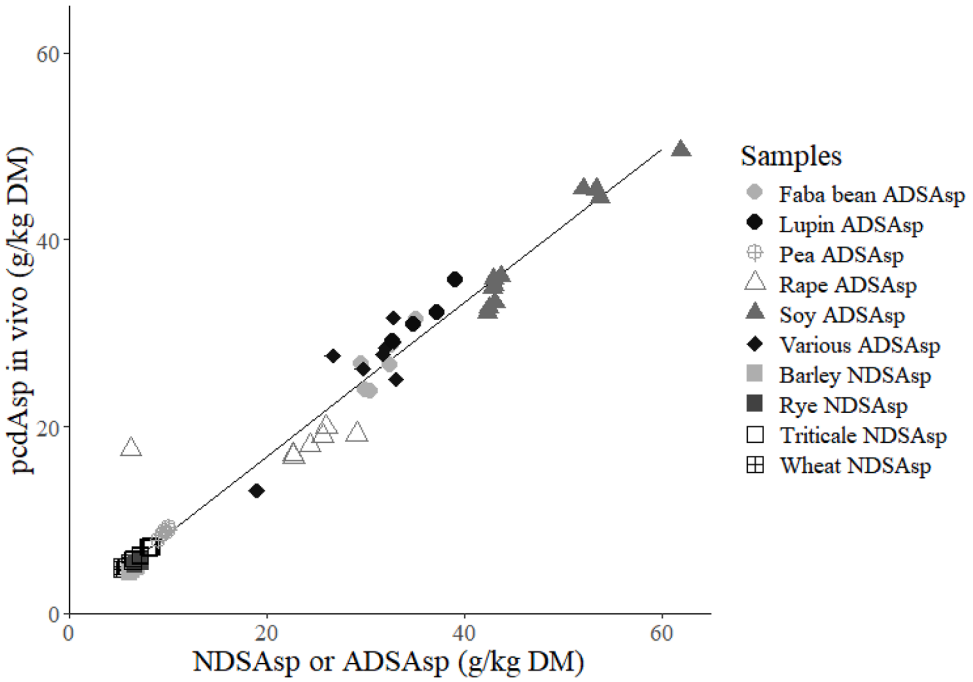

Fig. A10. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for aspartic acid (Asp). With the regression equation y= 0.822 (SE 0.016, CI 0.790; 0.855) x + 0.354 (SE 0.420, CI -0.481; 1.190), R^2= 0.973, RMSE= 2.155.

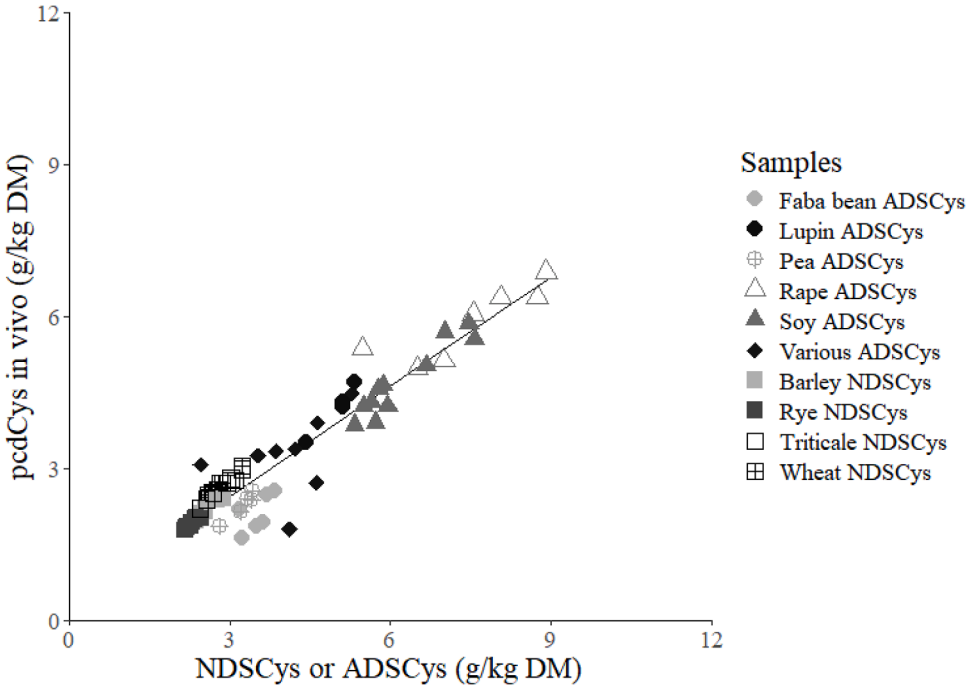

Fig. A11. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for cysteine (Cys). With the regression equation y= 0.721 (SE 0.028, CI 0.665; 0.776) x + 0.299 (SE 0.122, CI 0.056; 0.541), R^2= 0.904, RMSE= 0.417.

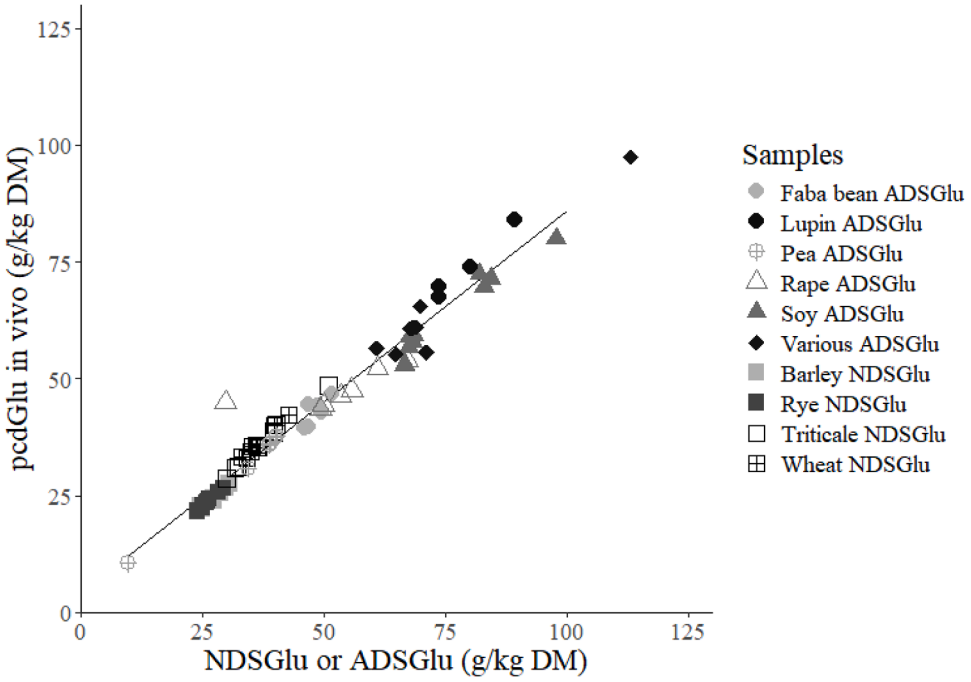

Fig. A12. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for glutamic acid (Glu). With the regression equation y= 0.818 (SE 0.018, CI 0.782; 0.853) x + 4.135 (SE 0.947, CI 2.248; 6.022), R^2= 0.967, RMSE= 3.160.

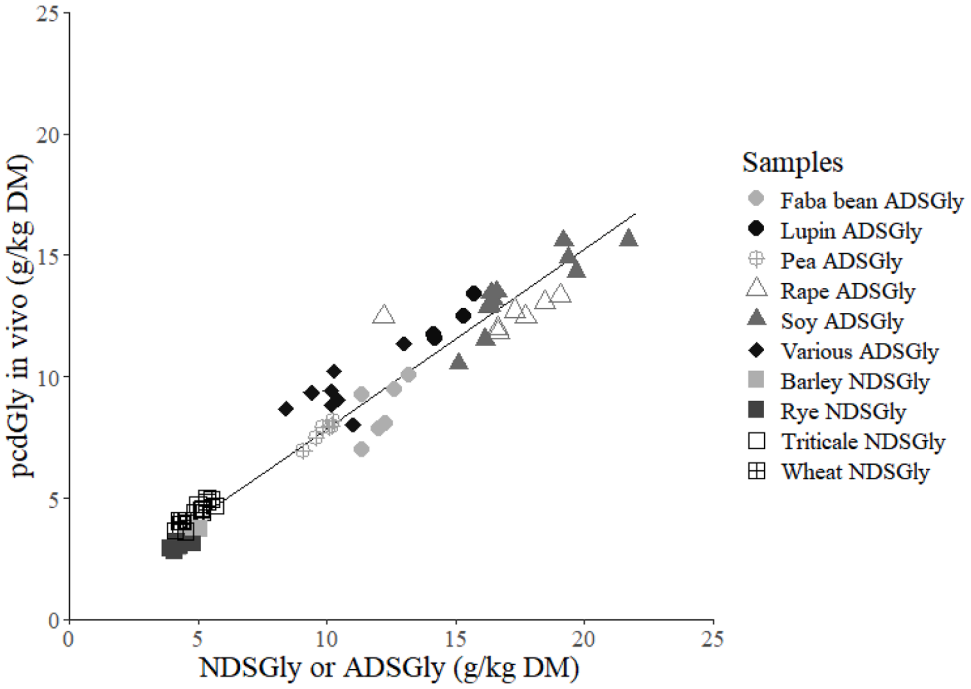

Fig. A13. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for glycine (Gly). With the regression equation y= 0.739 (SE 0.019, CI 0.700; 0.777) x + 0.491 (SE 0.218, CI 0.056; 0.925), R^2= 0.953, RMSE= 0.879.

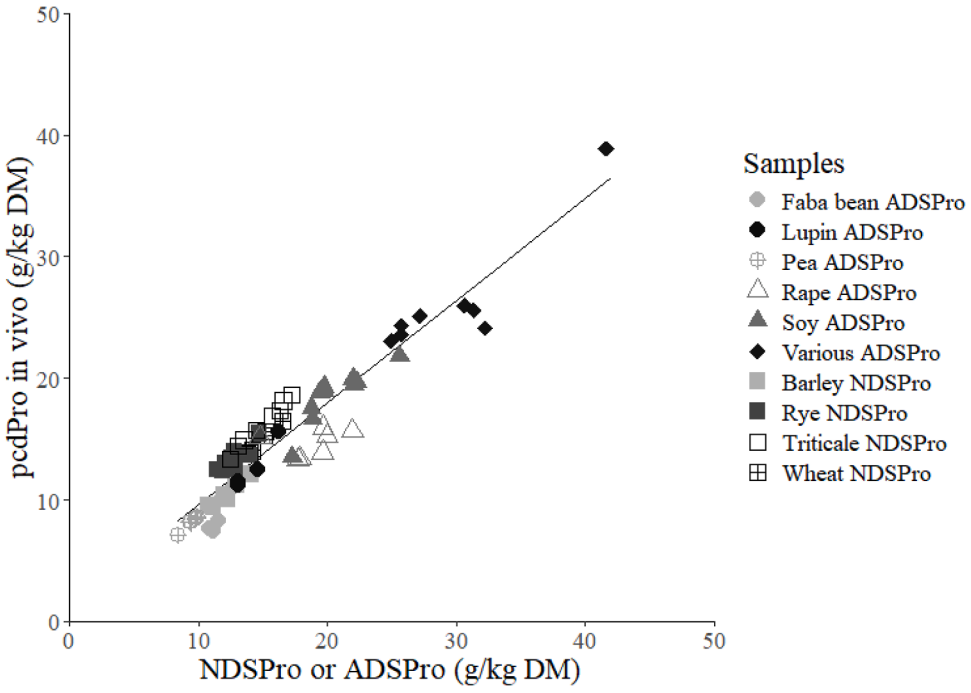

Fig. A14. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for proline (Pro). With the regression equation y= 0.840 (SE 0.034, CI 0.772; 0.907) x + 1.187 (SE 0.595, CI 0.000; 2.374), R^2= 0.895, RMSE= 1.756.

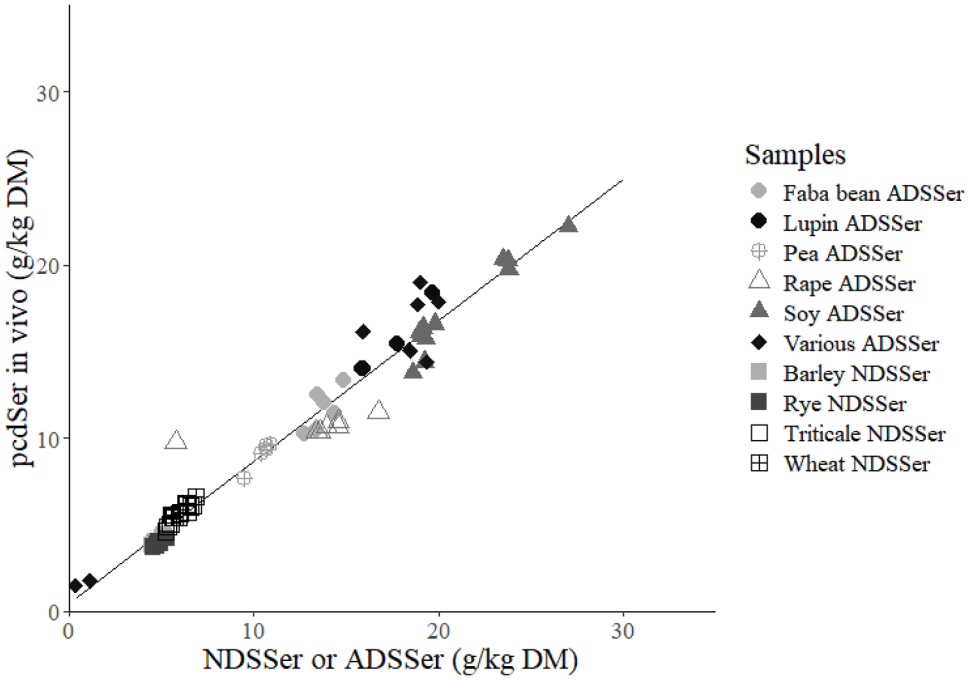

Fig. A15. Linear regression of NDSAA and ADSAA data (X) from the laboratory method and *in vivo* pcdAA (Y) for cereal grains and protein feed ingredients from the reference of all 74 samples for serine (Ser). With the regression equation y= 0.813 (SE 0.019, CI 0.775; 0.851) x + 0.533 (SE 0.248, CI 0.039; 1.028), R^2= 0.961, RMSE= 1.057.

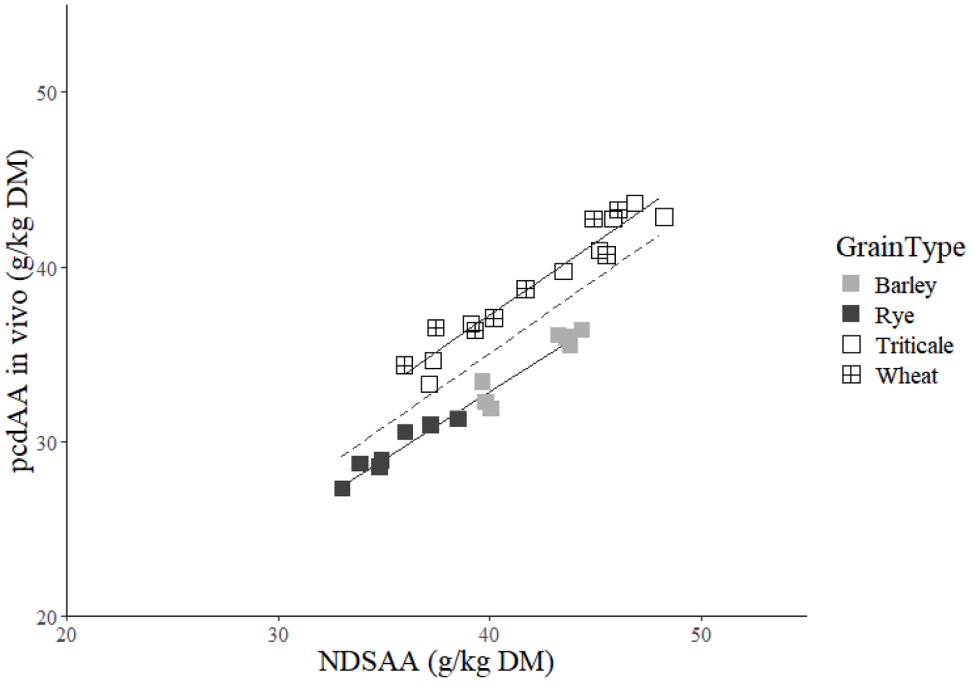

Fig. A16. Relationship between NDSAA data from the laboratory method (X) and *in vivo* pcdAA (Y) for cereal grains for wheat, triticale, barley and rye with grouping according to wheat/triticale and barley/rye for 10 indispensable AA. The dashed line shows the regression line over all cereal grain samples. See text and table S8 for details.

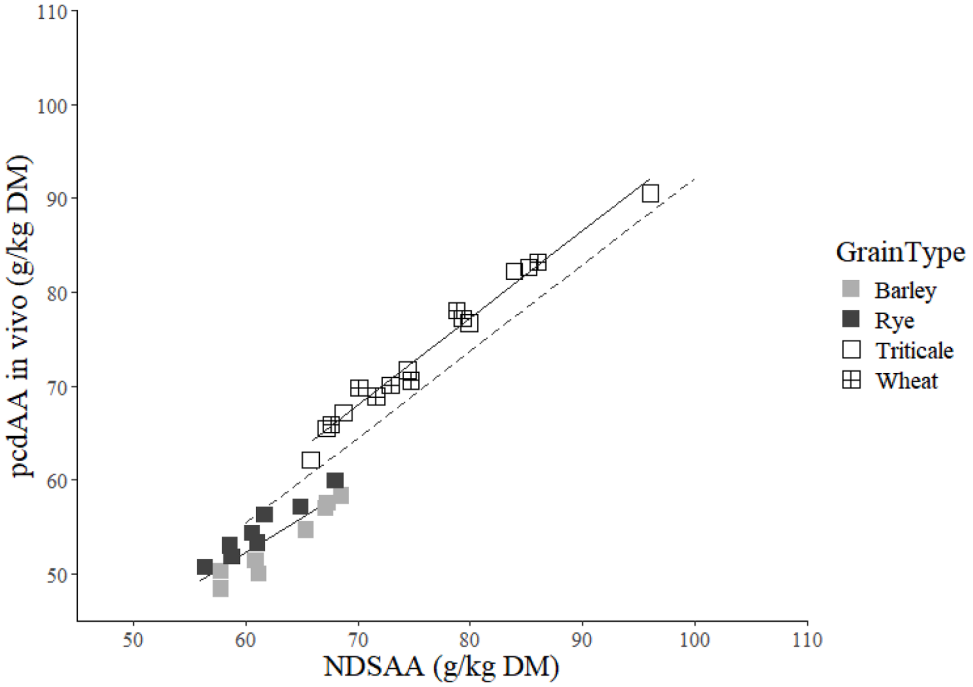

Fig. A17. Relationship between NDSAA data from the laboratory method (X) and *in vivo* pcdAA (Y) for cereal grains for wheat, triticale, barley and rye with grouping according to wheat/triticale and barley/rye for 7 dispensable AA. The dashed line shows the regression line over all cereal grain samples. See text and table S8 for details.

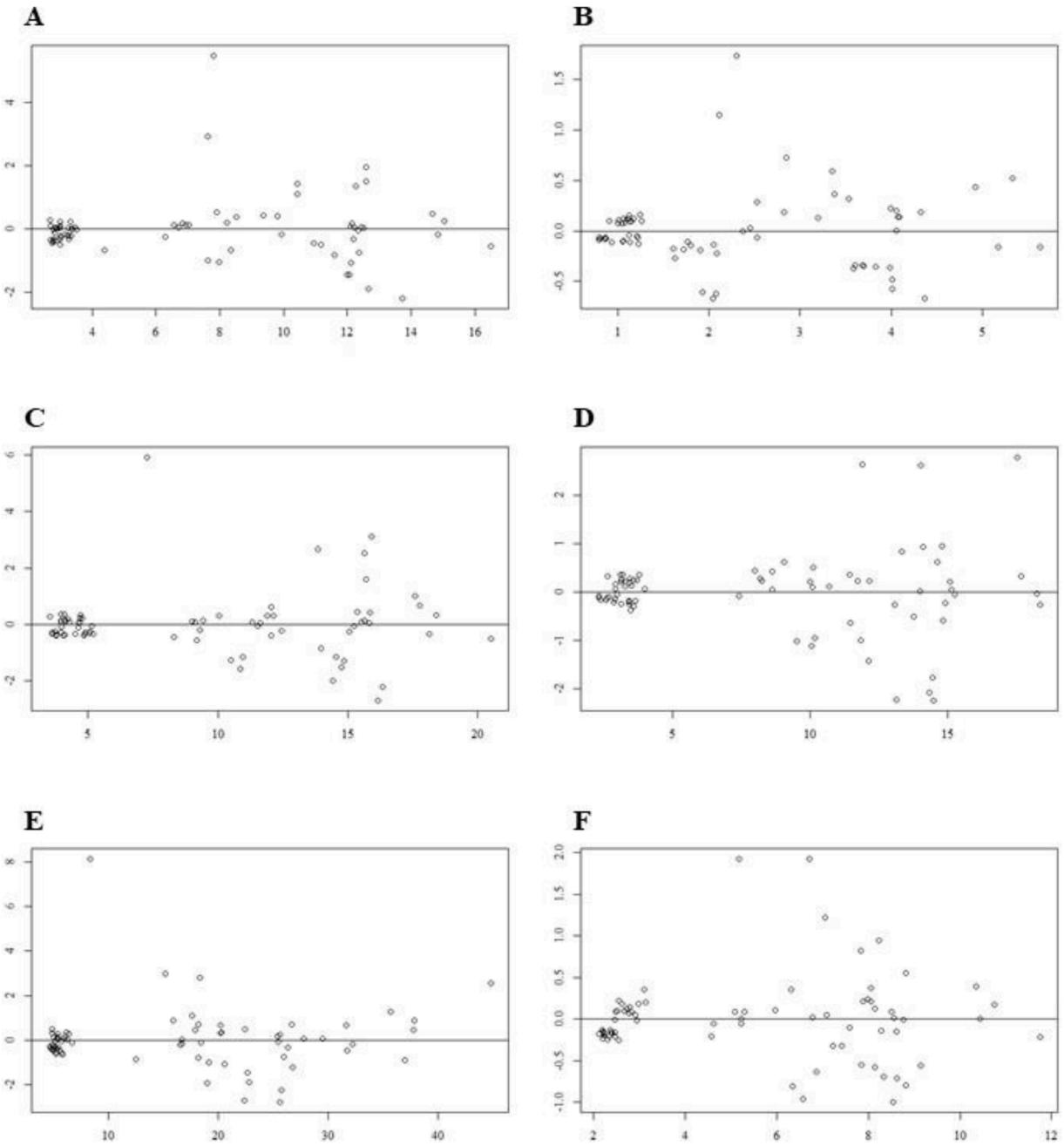

Fig. A18. Relationship between residues (y; g/kg DM) and fitted values (x; g/kg DM) of data of all 74 samples for the amino acids threonine (A), tryptophan (B), valine (C), isoleucine (D), arginine (E) and histidine (F).

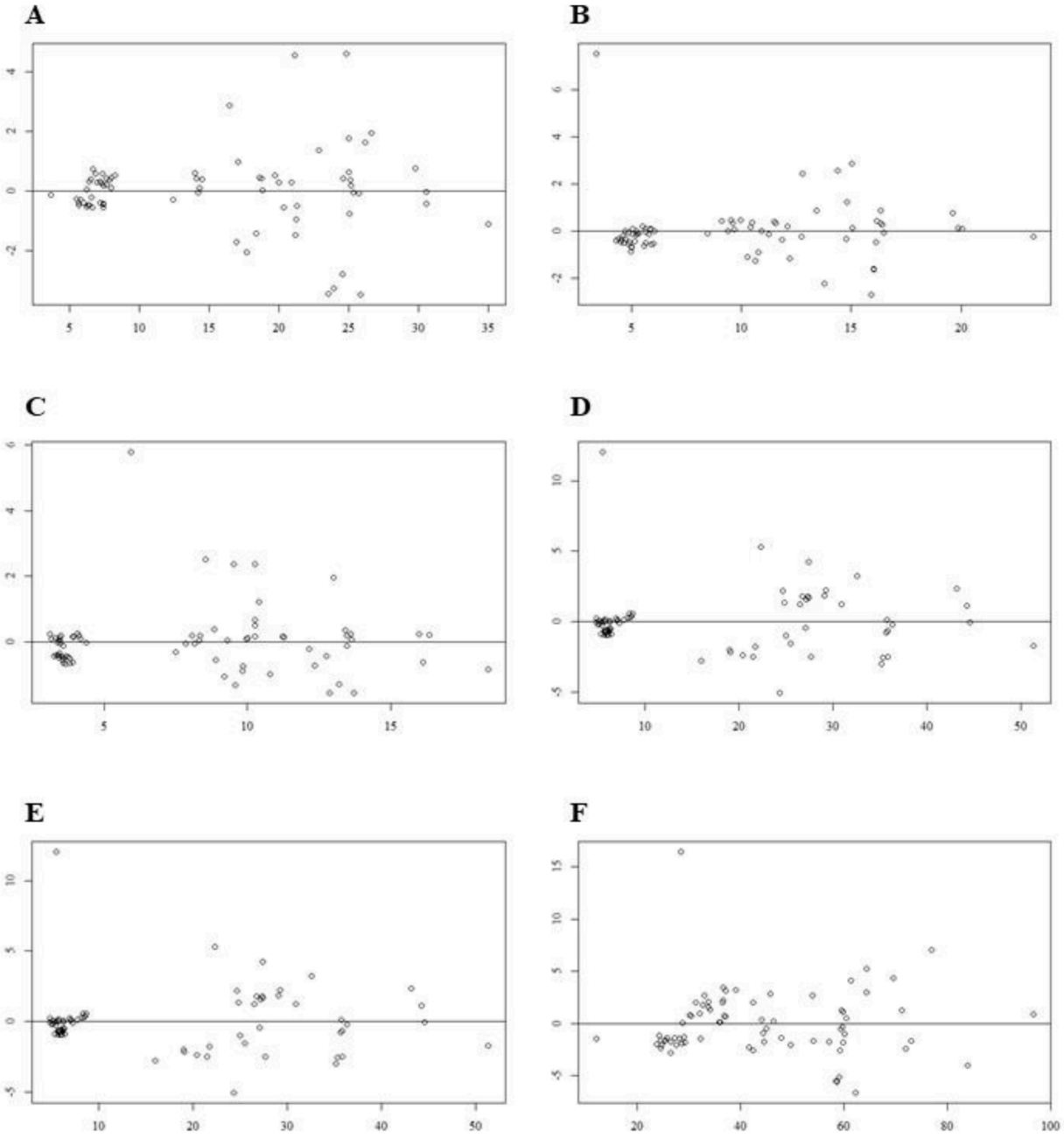

Fig. A19. Relationship between residues (y; g/kg DM) and fitted values (x; g/kg DM) of data of all 74 samples for the amino acids leucine(A), phenylalanine (B), alanine (C), aspartic acid (D), cysteine (E) and glutamic acid (F).

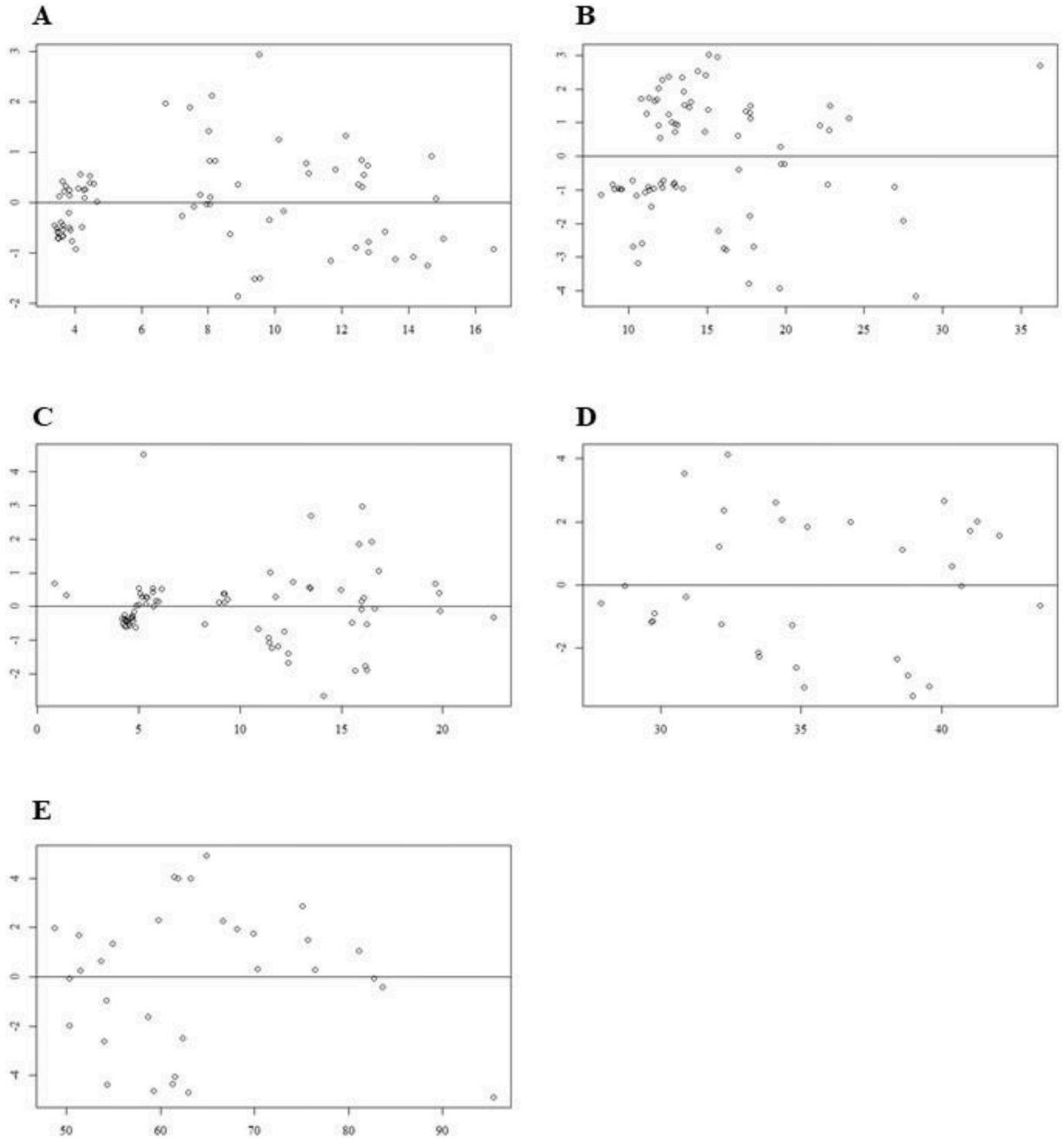

Fig. A20. Relationship between residues (y; g/kg DM) and fitted values (x; g/kg DM) of data of all 74 samples for the amino acids glycine (A), proline (B), serin (C) and all cereal grains for indispensable amino acids (D) and dispensable amino acids (E).

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