Institute of Animal Science
Department of Animal Nutrition
Prof. Dr. Markus Rodehutscord

Variability of amino acid digestibility of cereal grains in laying hens

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Dekan der Fakultät Agrarwissenschaften:	Prof. Dr. Ralf T. Vögele
Leitung des Kolloquiums:	Prof. Dr.-Ing. Stefan Böttinger
Berichterstatter, 1. Prüfer:	Prof. Dr. Markus Rodehutscord
Berichterstatter:	PD Dr. Holger Kluth
2. Prüfer:	Prof. Dr. Dr. h.c. Rainer Mosenthin
3. Prüfer:	apl. Prof. Dr. Michael A. Grashorn

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ABBREVIATIONS

Beneath abbreviations for the units defined by the international system of units and the symbols for chemical elements of the periodic table of elements the following abbreviations were used:

AA	Amino acids
ADF	Acid detergent fibre
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
AX	Arabinoxylans
BD	Basal diet
β-glucan	Mixed-linkage (1→3;1→4)-β-glucans
CP	Crude protein
Cys	Cysteine
DM	Dry matter
Glu	Glutamic acid
Gly	Glycine
HDP	Highly digestible protein
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
NDF	Neutral detergent fibre
NIRS	Near infrared reflectance spectroscopy
NSP	Non-starch polysaccharides
pc	Prececal
Phe	Phenylalanine
Pro	Proline
SAS	Statistical Analysis System
Ser	Serine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine

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1 General Introduction and Background

With an annual production of more than 2.5 billion tons, cereal grains are among the world's most important cultivated crops (FAOSTAT, 2016). A good part of cereal grains is used as food for man, and feed for farm animals. Intensive breeding activities have led to the development of numerous varieties of cereals with variable chemical compositions and physical properties (Pettersson and Åman, 1987; Hansen et al., 2004; Bryden et al., 2009; Rodehutscord et al., 2016; Smeets et al., 2016). However, the genetic background of plants as well as environmental factors like soil conditions, fertilization, precipitation, the growing season, and storage conditions affect the chemical composition and physical properties of cereal grains (McNab, 1991; Metayer et al., 1993; Coles et al., 1997; Kim et al., 2003; Siegert et al., 2016). Quantitatively, the predominant fraction of cereal grains is starch, due to which they are considered as a source of energy in poultry diets. With values generally less than 200 g/kg dry matter, the crude protein (**CP**) concentration of cereal grains is relatively low. However, when included in high levels in the diets of laying hens, cereal grains also contribute significantly to the amino acid (**AA**) supply to the birds.

Moreover, cereal grains were reported to contain significant and variable amounts of non-starch polysaccharides (**NSP**), with arabinoxylans (**AX**), mixed linkage (1→3;1→4)-β-glucans (**β-glucan**), and cellulose being the main polymers (Bach Knudsen, 1997; 2014). The polysaccharides, occasionally associated with lignin, are the principal components of the grain cell walls (Theander et al., 1993; Bach Knudsen, 1997). Chemically, NSP are an extremely heterogeneous group of components with considerable structural differences within and among the polymers (Fincher and Stone, 1986). The physicochemical properties of NSP are closely related to their chemical structure and the arrangement of the polymers within the cell walls (Fincher and Stone 1986; 2004). In poultry, NSP were shown to exhibit anti-nutritive properties. Thus, these have attracted increasing attention from the feed industry and the scientific community over the last decades. The soluble fraction of NSP was shown to increase digesta viscosity of the birds (Choct and Annison, 1992a), which negatively affects the digestion of nutrients, the litter quality, and consequently, bird performance (Choct and Annison, 1992b; Smits and Annison, 1996). The entrapping of nutrients within the polysaccharides, on the other hand, induces detrimental effects of the insoluble fraction of NSP. Hence, they are less accessible to digestive enzymes and their digestibility is limited (Annison and Choct, 1991). However, growing broiler chickens generally seem to be more sensitive to the anti-nutritive

properties of NSP than adult birds (Almirall et al., 1995). Nevertheless, a decrease in digesta viscosity (Mirzaie et al., 2012) and an increase in performance (Wyatt and Goodman, 1993; Pan et al., 1998) were also observed in adult laying hens when NSP-degrading enzymes were supplemented in cereal-based diets. Moreover, previous research has demonstrated that significant differences exist between the different poultry species regarding their digestive capacity and their susceptibility to anti-nutritive feed components (Huang et al., 2006; Kluth and Rodehutscord, 2006). Thus, poultry nutritionists generally agree that any data determined with one poultry species cannot be transferred to others (Adedokun et al., 2009).

Among cereals, the grains of wheat (*Triticum spp*.) and corn (*Zea mays*) are most frequently used to feed laying hens. The grains of rye (*Secale cereale*) traditionally have minor importance, which can be mainly attributed to their relatively high concentration of NSP (Rodehutscord et al., 2016). Triticale (*xTriticosecale*), the cross between wheat and rye, was created to combine the advantages of both parental species, the winter hardiness and vigor of rye, and the yield potential and quality characteristics of wheat (Bushuk and Larter, 1980; Boros, 1999). However, like rye grain, triticale grain is also hardly used in layer diets today. This is probably due to the limited quantities of triticale grain available on the feed market (FAOSTAT, 2016). In future, the importance of rye and triticale as feed grains might increase as climatic conditions change and the use of adaptive crops becomes necessary. Furthermore, a rising demand for cereal grains could cause the expansion of cereal cropping to areas that seem to be unfavorable today.

The emission of nitrogen (**N**)-containing compounds from poultry enterprises is considered problematic in many respects. Excreted N results largely from an excessive supply of AA to laying hens (Latshaw and Zhao, 2011). Since feed costs usually represent a major part of the production costs, a reduction in any excess of CP supply is of fundamental economic importance. Moreover, the excreted N contributes to eutrophication of ecosystems and air pollution, and thus, is an environmental issue that is attracting increasing public attention (Aneja et al., 2009). Thus, the optimization of diets with regard to the efficiency of protein utilization is necessary for an efficient and sustainable production of eggs.

An approach to increase the efficiency of protein utilization is the consideration of AA digestibility during feed formulation. Nutritionists commonly accept that feed formulation based on digestible rather than total AA is preferable (Dalibard and Paillard, 1995; Rostagno et al., 1995). However, practical application of a digestible AA system by the feed industry requires data on the level and the variation of AA digestibility among and within the feed components used. Moreover, approaches are needed to rapidly, and more importantly, reliably,

predict AA digestibility under practical conditions to consider batch-specific differences during feed formulation. These predictions could be made either based on the chemical and physical characteristics of the grains or by using an *in vitro* system. The former includes the usage of appropriate regression equations, which at best combine the characteristics of the grains that can be determined without making an extensive analytical effort. The *in vitro* approach essentially simulates the digestive process to estimate AA digestibility. The *in vitro* system suggested by Boisen and Fernández (1995) for pigs is based on the determination of N solubility after pretreatment of the feed sample with pepsin and pancreatin. Jezierny et al. (2010) showed that this approach could predict AA digestibility in pigs with variable accuracy, depending on the feedstuff tested. However, it is still unknown whether this approach can be used to predict AA digestibility in poultry.

The AA digestibility of cereal grains in laying hens has been investigated only to a limited extent to date. A review of the literature leads to the conclusion that available data are insufficient to reflect the variability of AA digestibility. This is due to two reasons: Firstly, the individual studies dealt with only a few samples, and secondly, they applied different experimental approaches. Kluth and Rodehutscord (2009) observed that methodological details strongly influence the results of digestibility trials. Use of different methods also makes studies incomparable. Thus, there is an urgent need to standardize the methods used to determine AA digestibility in the different poultry species. Quantitative excreta collection of cecectomized laying hens is a suitable alternative to ileal sampling, which is commonly used for broiler chickens (Rezvani et al., 2008). This approach is advantageous because of the relatively low number of animals needed for the study. Moreover, the same birds can be used for several measurements.

However, as far as the author knows, neither were the approaches based on precaecal sampling, nor was excreta collection of cecectomized birds used to systematically investigate the AA digestibility of a large number of cereal samples in laying hens. A more profound overview of the available digestibility values in the literature is given in the four manuscripts included in this thesis and also in its general discussion. Hence, it shall not be elaborated here. This doctoral thesis aimed to generate a set of digestibility values for laying hens determined with a strictly standardized trial assay. Additionally, the researcher targeted to develop prediction equations based on chemical and physical characteristics of the grains. Furthermore, an established *in vitro* system, originally developed for pigs, was tested to prove its suitability to predict AA digestibility of cereal grains in laying hens.

2 Overview of Own Work

The variability of AA digestibility of cereal grains in laying hens is widely unknown. The published studies investigated only a few samples and used different experimental approaches, which impaired the comparability of results. Therefore, the studies compiled in chapter 4 of this thesis were carried out to generate a comprehensive data set on AA digestibility values. A well-standardized assay was used to determine the data set. The study was part of the collaboration research project GrainUp (Rodehutscord et al., 2016). Twenty grain samples of triticale (chapter 4.1), rye (chapter 4.2), maize (chapter 4.3) and wheat (chapter 4.4) were investigated. Apart from maize, the grains were grown under standardized agronomic and environmental conditions. This revealed the genotypic differences in AA digestibility for the cereals. Moreover, a comprehensive analytical characterization of the grains enabled the author of this thesis to examine the relationships between AA digestibility and the chemical and physical properties of the grains.

3 General Discussion

The first subsection (chapter 3.1) of this chapter discusses the potential technical errors related to the housing of the birds and the sampling procedure. The second subsection (chapter 3.2) examines the assay- and feed-related factors that may affect AA digestibility. The consideration of the influencing factors will be expanded to other poultry species, too, to broaden the available data base. The third subsection (chapter 3.3) surveys the needs and the possibility of predicting AA digestibility in poultry using various approaches. The last subsection (chapter 3.4) draws conclusions from the present work and makes proposals for future research.

3.1 Possible sources of error

Amino acid digestibility measurements in poultry can be based on quantitative excreta collection or ileal digesta sampling using indigestible markers. Assays based on quantitative excreta collection imply that the feed consumption of the birds is quantified. However, what initially seems uncomplicated can produce several sources of error in practical use. For quantitative excreta collection, birds are usually housed in metabolism cages equipped with a wire mesh floor and trays to collect the droppings. Due to the movement of the birds and the moisture content in the excreta, part of it inevitably sticks to the cloaca, the feet of the birds, or the inside of the cages. In the trials presented herein, excreta were collected twice daily and those adhering to the perch or the wire mesh were scraped off gently to make those fall on the trays before collection. Nevertheless, small amounts of excreta must have remained in the cages or on the birds. However, the excreta were collected thoroughly to keep the losses as low as possible, and the quantities of lost excreta were probably similar for all the observations. Hence, the effect on treatment-specific differences is expected to be negligible.

Another source of sampling errors is impurities in the excreta collected on the trays. These impurities include feathers, flakes of shed skin, and feed pellets. Before being collected from the trays, the excreta were thoroughly cleaned of these impurities by hand. Feathers and flakes of shed skin were removed and discarded, whereas feed pellets, which only fell on the trays occasionally, were collected and added to the feed residues of the respective bird. The mixing of excreta with feed is particularly problematic because this causes an underestimation of AA consumed and, at the same time, an overestimation of AA excreted. In the present study, the

design of the feeding troughs and the fact that the experimental diets were pelleted almost completely prevented feed from falling on the trays. However, though impurities were removed as thoroughly as possible, those covered with droppings could not be separated. Yet, their influence is considered rather minor because excreta were collected twice daily. Hence, the amount of excreta on the trays was generally small and the droppings did not accumulate in one place. Furthermore, these impurities probably affected all observations to the same extent. Hence, it might not have influenced any differences between the treatments.

Moreover, with regard to possible N losses of the excreta during their retention time on the trays, a collection frequency twice daily, as used in the present study, is advantageous compared with excreta collection once daily. The microbial colonization of the excreta may cause a degradation of AA as these lie on the trays. Literature regarding the time effect on N losses is lacking, and the possible extent of AA degradation is hardly predictable. For the present thesis, excreta collection twice daily was reasonable to balance the workload manageable per day and the time of the excreta lay on the trays. Moreover, though the hens got used to the close contact with the personal staff during excreta collection, a higher collection frequency might disturb the feed intake and increase impurities in excreta due to an increase in the activity of the birds.

3.2 Factors affecting amino acid digestibility

The present work determined a considerable variation in AA digestibility within and among the four cereals studied (chapters 4.1–4.4). However, AA digestibility coefficients, which are determined by an assay, are affected by the crop's characteristics as well as the methodological details of the respective assay procedure. Thus, the influence of these two aspects will be discussed here. For the sake of completeness, it should also be mentioned that the preciseness of sampling and the accuracy of AA analysis are of fundamental importance in determining AA digestibility. However, the present chapter will not discuss these two aspects because their optimal execution is essential to obtain reliable digestibility values under the respective conditions of any assay procedure.

3.2.1 Assay-based factors

Several previous studies examined the AA digestibility of cereal grains in poultry. To give an overview of the available data-base, selected results and principle assay characteristics of these studies, as well as respective details from the own work, are summarized in Tables 1−3.

A review shows that previously published literature described considerable differences in AA digestibility within and among the cereal grains. This is generally in accordance with the results of the present work (chapter 4.1−4.4). However, no consistent pattern of AA digestibility across the different assays for the same cereal species is observed. A possible explanation, therefore, might be that previous studies on AA digestibility of cereal grains were conducted using a multitude of assays and approaches, each different from the other in terms of the sampled poultry species, age and surgical modification of the birds, site of sampling, feeding procedure, and consideration of endogenous AA losses.

Table 1. Selected results and principle assay characteristics of studies examining the amino acid digestibility of triticale and rye grain in poultry

Reference	Age	Modification	Site of sampling	Feeding regime	Correction endogenous excretions[#]	Cereal samples	Digestibility (%)		
							Lys	Met	Thr
Triticale:									
Laying hens									
Present thesis	45–64 wk	Cecectomy	Excreta	Substitution for corn starch	Lin. reg.	20	68–80	77–86	68–78
Siegert et al. (2016)	36–50 wk	Cecectomy	Excreta	Substitution for corn starch	Lin. reg.	6	65–74	71–82	68–79
Gruhn et al. (1991)	–	Colostomy	Feces	*Ad libitum*, sole source of protein	–	6	69–76	75–84	69–74
McNab and Shannon (1975)	52 wk	Colostomy	Feces	*Ad libitum*, sole source of protein	Lin. reg	1	80	94	78
Broilers									
Bryden et al. (2009)	42 d	–	Ileum	*Ad libitum*, sole source of protein	–	3	69–78	79–88	61–71
Perttilä et al. (2005)	35 d	–	Ileum	*Ad libitum*, sole source of protein	–	1	89	88	78
Ravindran et al. (2005)	42 d	–	Ileum	*Ad libitum*, sole source of protein	–	4	75	86	68
Hew et al. (1999)	40 d	–	Ileum	*Ad libitum*, sole source of protein	–	1	75	86	71
Johnson and Eason (1988)	36 d	–	Ileum	*Ad libitum*, sole source of protein	–	9	85–95	87–94	81–90
Rye:									
Laying hens									
Present thesis	32–55 wk	Cecectomy	Excreta	Substitution for corn starch	Lin. reg.	20	35–59	57–75	34–54
McNab and Shannon (1975)	52 wk	Colostomy	Feces	*Ad libitum*, sole source of protein	Lin. reg.	1	66	74	72

[#]Lin. reg.: linear regression

Table 2. Selected results and principle assay characteristics of studies examining the amino acid digestibility of corn grain in poultry

Reference	Age	Modification	Site of sampling	Feeding regime	Correction endogenous excretions[#]	Corn samples	Digestibility (%)		
							Lys	Met	Thr
Laying hens									
Present thesis	55–74 wk	Cecectomy	Excreta	Substitution for corn starch	Lin. reg.	20	64–85	86–94	72–89
Adedokun et al. (2015)	50 wk	–	Ileum	*Ad libitum*, sole source of protein	N-free	3	83–93	90–93	80–87
Adedokun et al. (2009)	37 wk	–	Ileum	*Ad libitum*, sole source of protein	N-free	1	75	88	69
Huang et al. (2007)	60 wk	–	Ileum	*Ad libitum*, sole source of protein	–	1	76	89	62
Huang et al. (2006)	56 wk	–	Ileum	*Ad libitum*, sole source of protein	–	1	75	87	61
McNab and Shannon (1974)	52 wk	Colostomy	Feces	*Ad libitum*, sole source of protein	Lin. reg.	1	76	92	78
Roosters									
Kim et al. (2012)	–	Cecectomy	Excreta	Force-fed (30 g)	Starved	1	75	93	91
Adedokun et al. (2009)	104 wk	Cecectomy	Excreta	Force-fed (30 g)	Starved	1	88	94	85
Vasan et al. (2008)	25 wk	–	Excreta	Force-fed (100g)	N-free	1	83	94	89
	25 wk	Cecetomy	Excreta	Force-fed (100 g)	N-free	1	89	95	89
Garcia et al. (2007)	33 wk	Cecectomy	Excreta	Force-fed (35 g)	Starved	1	92	92	82
Huang et al. (2006)	45 wk	–	Ileum	*Ad libitum*, sole source of protein	–	1	73	92	63
Green et al. (1987)	–	Cecectomy	Excreta	Force-fed (50 g)	N-free	1	80	91	86
Sibbald (1979)	adult	–	Excreta	Force-fed (10, 20, 30 g)	Starved	1	95–97	93–98	92–98
Likuski and Dorrell (1978)	–	–	Excreta	Force-fed (25 g)	Starved	4	96	98	92
Broilers									
Adedokun et al. (2015)	21 d	–	Ileum	*Ad libitum*, sole source of protein	N-free	3	92–93	95	89–91
Iyayi and Adeola (2014)	26 d	–	Ileum	*Ad libitum*, sole source of protein	N-free	1	80	88	75
Kim et al. (2012)	22 d	–	Ileum	Force-fed (10g)	N-free	1	95	101	80
	21 d	–	Ileum	*Ad libitum*, sole source of protein	N-free	1	74	89	77
Adedokun et al. (2009)	21 d	–	Ileum	*Ad libitum*, sole source of protein	N-free	1	86	95	84
Bryden et al. (2009)	42 d	–	Ileum	*Ad libitum*, sole source of protein	–	8	78–85	87–93	60–73
Adedokun et al. (2008)	5 d	–	Ileum	*Ad libitum*, sole source of protein	N-free	1	73	79	70
	21 d	–	Ileum	*Ad libitum*, sole source of protein	N-free	1	86	95	84
	5 d	–	Ileum	*Ad libitum*, sole source of protein	HDP	1	83	84	74
	21 d	–	Ileum	*Ad libitum*, sole source of protein	HDP	1	99	104	93
Garcia et al. (2007)	7 d	–	Ileum	*Ad libitum*, sole source of protein	HDP	1	80	84	75
	21 d	–	Ileum	*Ad libitum*, sole source of protein	HDP	1	87	89	84

[#]Lin. reg.: linear regression; N-free: nitrogen free diet; HDP: highly digestible protein

continues

Table 2. continued

Reference	Age	Modification	Site of sampling	Feeding regime	Correction endogenous excretions[#]	Corn samples	Digestibility (%)		
							Lys	Met	Thr
Broilers									
Huang et al. (2005)	14 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	69	87	61
	28 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	77	91	67
	42 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	80	90	69
Perttilä et al. (2005)	35 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	84	90	73
Ravindran et al. (2005)	42 d	−	Ileum	*Ad libitum*, sole source of protein	−	8	79	87	68
Turkeys									
Adedokun et al. (2008)	5 d	−	Ileum	*Ad libitum*, sole source of protein	N-free	1	63	77	66
	21 d	−	Ileum	*Ad libitum*, sole source of protein	N-free	1	91	94	88
	5 d	−	Ileum	*Ad libitum*, sole source of protein	HDP	1	63	75	62
	21 d	−	Ileum	*Ad libitum*, sole source of protein	HDP	1	86	92	78
Ducks									
Kong and Adeola (2010)	19 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	78	86	62
Hong et al. (2002)	11 wk	−	Excreta	Force-fed (50g)	Dextrose	1	78	93	84

[#]N-free: nitrogen free diet; HDP: highly digestible protein

Table 3. Selected results and principle assay characteristics of studies examining the amino acid digestibility of wheat grain in poultry

Reference	Age	Modification	Site of sampling	Feeding regime	Correction endogenous excretions[#]	Wheat samples	Digestibility (%)		
							Lys	Met	Thr
Laying hens									
Present thesis	55–76 wk	Cecectomy	Excreta	Substitution for corn starch	Lin. reg.	20	69–87	70–93	71–88
Huang et al. (2007)	60 wk	−	Ileum	*Ad libitum*., sole source of protein	−	1	62	79	57
Huang et al. (2006)	56 wk	−	Ileum	*Ad libitum*, sole source of protein	−	1	69	83	67
McNab and Shannon (1974)	52 wk	Colostomy	Feces	*Ad libitum*, sole source of protein	Lin. reg.	1	81	90	72
Roosters									
Garcia et al. (2007)	33 wk	Cecectomy	Excreta	Force-fed (35 g)	Starved	1	91	87	78
Huang et al. (2006)	45 wk	−	Ileum	*Ad libitum*, sole source of protein	−	1	69	82	66
Green et al. (1987)	−	Cecectomy	Excreta	Force-fed (50 g)	N-free	1	80	90	85
Sibbald (1979)	Adult	−	Excreta	Force-fed (10, 20, 30 g)	Starved	1	91	88–93	88–93
Broilers									
Bandegan et al. (2011)	21 d	−	Ileum	*Ad libitum*, sole source of protein	N-free	6	83–85	91–92	84–88
Bryden et al. (2009)	42 d	−	Ileum	*Ad libitum*, sole source of protein	−	27	64–86	74–92	61–87
Kluth et al. (2009)	21 d	−	Ileum	Substitution for corn starch	Lin. reg	1	82	86	76
Garcia et al. (2007)	7 d	−	Ileum	*Ad libitum*, sole source of protein	HDP	1	58	65	55
	21 d	−	Ileum	*Ad libitum*, sole source of protein	HDP	1	77	78	81
Huang et al. (2006)	49 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	76	85	72
Huang et al. (2005)	14 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	68	81	63
	28 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	58	76	61
	42 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	64	75	59
Perttilä et al. (2005)	35 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	87	85	78
Ravindran et al. (2005)	42 d	−	Ileum	*Ad libitum*, sole source of protein	−	8	79	91	73
Hew et al. (1999)	40 d	−	Ileum	*Ad libitum*, sole source of protein	−	2	80	89–91	78
Short et al. (1999)	21 d	−	Ileum	*Ad libitum*, sole source of protein	Lin. reg.	4	51–79	72–89	57–81
Johnson and Eason (1988)	36 d	−	Ileum	*Ad libitum*, sole source of protein	−	2	97	95–96	91–92
Ducks									
Kong and Adeola (2010)	19 d	−	Ileum	*Ad libitum*, sole source of protein	−	1	77	85	66

[#]Lin reg.: linear regression; N-free: nitrogen free diet; HDP: highly digestible protein

As numerous authors have reviewed, all the approaches have their advantages and limitations (McNab, 1979; Papadopoulos, 1985; Sibbald, 1987; McNab, 1994; Ravindran and Bryden, 1999; Parsons, 2002; Lemme et al., 2004; Kluth and Rodehutscord, 2009). However, there is growing evidence that the methodological influence on the outcome of the digestibility assays is considerable. By means of four examples, the following section will elucidate this.

To explore the possibility of transferring AA digestibility values from one species to another, several authors have compared different poultry species with regard to their capacity to digest AA (Johns et al., 1986; Huang et al., 2006; Kluth and Rodehutscord, 2006; Adedokun et al., 2015). Poultry nutritionists generally accept that the transfer of digestibility values from one species to another is invalid, and that the digestibility coefficients should be determined for each poultry species separately. Moreover, the fact that different experimental approaches were used for the different poultry species lessened the success potential of these comparisons (Rodehutscord, 2015). The comparison of AA digestibility values determined in the present work (chapter 4.4) with values determined in broiler chickens for the same wheat samples by another GrainUp consortium project (Bormann and Kluth, 2013) showed that differences exist even between different types of birds within the same poultry species. Bormann and Kluth (2013) also used the regression approach to determine AA digestibility but sampled the broiler chickens at the terminal ileum. Selected results from this comparison are displayed in Figure 1 for the wheat samples that were investigated in both studies with both types of birds.

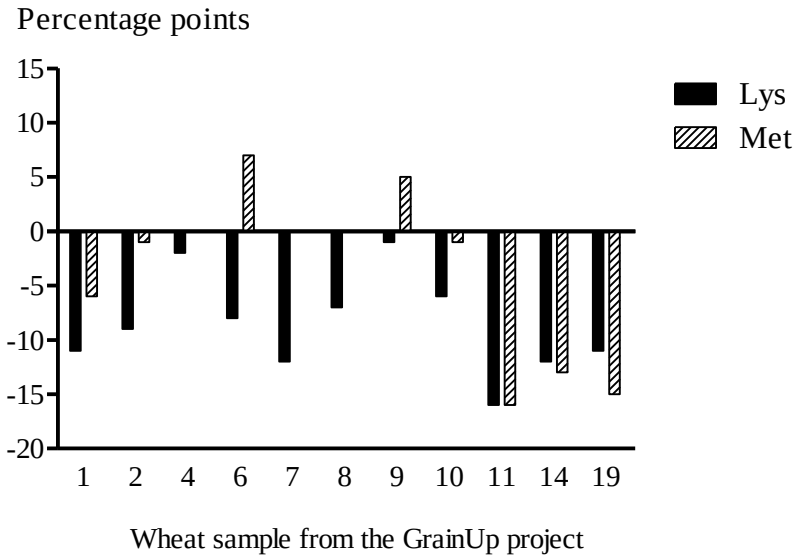

Figure 1. Differences in the digestibility of amino acids of 11 wheat samples determined in laying hens and broiler chickens (laying hens minus broiler chickens, data from chapter 4.4 and Bormann and Kluth [2013])

Differences, expressed as percentage points, between laying hens and broiler chickens were inconsistent between different wheat samples for the same AA (e.g. digestibility of Met in wheat samples 6 and 11) and for the same wheat sample among AA (e.g. wheat sample 6). Moreover, differences in AA digestibility showed no consistency between the two types of birds. However, though Bormann and Kluth (2013) and the author of the present thesis (chapter 4.4) used a similar experimental approach, a methodological influence on the determined digestibility values, and thus differences between the studies cannot be excluded. Nevertheless, it is clear that the application of conversion factors to transfer data from broilers to laying hens and vice versa seems impossible.

Secondly, Kim et al. (2012), who compared the prececal (pc) AA digestibility of a corn sample in three-week-old broilers using two methods of feeding, demonstrated the methodological influence on the outcome of AA digestibility studies (Table 2). These authors found the digestibility of Lys and Met to be respectively 21 and 12 percentage points higher when boiler chickens were precision-fed 10 g of the corn sample instead of receiving a complete diet in which the corn sample supplied the entire CP. In the same study, cecectomized roosters were also precision-fed 30 g of the corn sample. The digestibility of Lys and Met determined in roosters was intermediate between those determined in the two broiler assays, but closer to the chickens that were not force-fed. Thus, in the study of Kim et al. (2012), the variability of AA digestibility between different feeding procedures for the same sample was equal to (Lys) or even higher (Met) than the differences in AA digestibility between the 20 corn samples examined in the present work.

Thirdly, Kluth et al. (2005) demonstrated the necessity of a strictly standardized procedure for digestibility trials with poultry. They investigated the net disappearance of AA in three sections of the region between Meckel's diverticulum and 2 cm prior to the ileo-caeco-colonic junction in broiler chickens. As these authors noted, previous studies on this topic had sampled different sections of this region of the small intestine without considering the fact that the net disappearance of AA might not be complete at all sites. However, Kluth et al. (2005) clearly demonstrated with several experimental diets that significant differences in the net disappearance exist among the three subsections. Depending on the experimental diet, the digestibility of Lys was 3 to 11 percentage points higher in the terminal compared with the proximal section. To standardize AA digestibility trials even further, Kluth et al. (2005) consequently suggested that only the medial and terminal parts of the section between Meckel's diverticulum and 2 cm prior to the ileo-caeco-colonic junction should be sampled.

Finally, the study of Adedokun et al. (2008) is suitable to clarify the influence of methodological details on the determined AA digestibility. These authors examined the pc AA digestibility of one corn sample in broiler chickens at the age of 5 and 21 days, and used two different methods (N-free vs. highly digestible protein) to correct the digestibility coefficients for endogenous AA. Their results throw up mainly two conclusions. On the one hand, and this is not surprising, the AA digestibility is underestimated when endogenous AA are not considered. On the other hand, the determined digestibility of Lys and Met differed depending on the method used to correct endogenous AA flow. At the age of 5 days, Adedokun et al. (2008) determined the digestibility of Lys and Met to be 10 and 5 percentage points higher when a highly digestible protein was used to determine endogenous AA flow compared to a N-free diet. Differences between the two methods became even greater as the birds grew older. At 21 days of age, the method based on highly digestible protein provided digestibility values that were 13 and 9 percentage points higher for Lys and Met, respectively.

These four examples demonstrate the influence of methodological details on the outcome of digestibility trials. The variation in AA digestibility between different approaches can be as high, as the differences between different samples determined with the same approach. Thus, it is impossible to differentiate between the assay-based and sample-based factors that contributed to the variation in the published AA digestibility values. Such differentiation requires the investigation of a larger set of samples using the same assay. Moreover, methodological differences must be considered while interpreting the results of studies that use different assays. Ultimately, this means that a comparison of AA digestibility values across different assays is invalid and the interpretation of published data (Tables 1−3) should be restricted to values that were determined within one assay using the same experimental approach. However, the vast majority of published studies examined only a few cereal samples; so, the possible extent of variation in AA digestibility is still unknown.

To the best of the author's knowledge, only one Australian study conducted by Bryden et al. (2009), systematically investigated a greater number of samples from different cereal species within one assay in broiler chickens. Their results indicated that a considerable variation exists between different samples of the same cereal grain, even when determined with the same assay procedure. In triticale (n = 3), corn (n = 8), and wheat (n = 27) grains, the authors found the digestibility of Lys to vary by 9, 7, and 22 percentage points, respectively. Bryden et al. (2009) did not examine rye grain, probably because this crop has no quantitative importance in Australia (FAOSTAT, 2016). The present study (chapter 4.4) found a similar variation with 18 percentage point differences, in the digestibility of Lys in wheat. However,

the digestibility of Lys in triticale and corn varied more in the present study, with differences of 12 (chapter 4.1) and 21 (chapter 4.3) percentage points being recorded between extreme samples. It is likely that the higher number of samples examined in the present thesis might have contributed to the higher variability in AA digestibility. However, comprehensive studies of cereal grains conducted on laying hens were lacking until now. The results of the present work clearly demonstrate that the AA digestibility in laying hens varies for cereal grains, while the findings generally confirm the observations made by Bryden et al. (2009) for broiler chickens. Moreover, since the four cereal grain species of the present thesis were investigated using a strictly standardized approach, the level and variation between the grain species can be compared. Nevertheless, some methodological aspects of the assay procedure used in the present thesis can be relevant for the obtained results. The following section will discuss those.

Rezvani et al. (2008) investigated the possibility of using quantitative excreta collection of cecectomized laying hens as an alternative to measurements made at the terminal ileum. These authors concluded that this approach is suitable when the regression method, as suggested by Rodehutscord et al. (2004), is used. Furthermore, Rezvani et al. (2008) showed that compared with ileal sampling, the standard error of the estimate was lower when excreta were collected quantitatively. The effort of the surgical removal of the ceca is justified by the possibility to use the same laying hens repeatedly in several trials. In the present thesis, the excreta collection was extended over 19, 23, 19, and 21 weeks for the 20 samples of triticale, rye, corn, and wheat, respectively. Moreover, the same group of birds (animal group 2, Table 4) was used to examine the 20 samples of triticale, rye, and corn (chapters 4.1−4.3). The experimental period for these grains lasted 42 weeks. The effect of the bird's age on AA digestibility discussed in literature is controversial. Especially in young broiler chickens, the effect of age was found to differ among studies. In the study of Fonolla et al. (1981), the apparent digestibility of crude protein decreased in broiler chicks between 21 and 52 days of age. Zurprizal et al. (1992) made a similar observation. They determined a decreased AA digestibility in broiler chickens at 6 weeks of age compared with 4 weeks of age, when the birds were fed on a rapeseed meal and soybean meal-based diet. In contrast, the results of Wallis and Balnave (1984) and Adedokun et al. (2008) indicated that the AA digestibility increased with the age of broiler chickens. In other studies, the effect of age was inconsistent within the same investigation (Ten Doeschate et al., 1993) or depending on the feedstuff tested (Firman, 1992). A possible explanation for these conflicting results might be the variability in endogenous AA flow determined in growing broiler chickens (Adedokun et al., 2007; Ravindran and Hendriks, 2004). The effect of age on AA digestibility was rarely studied in adult laying hens, which have

a more developed gastrointestinal tract. Rezvani et al. (2007) determined the AA digestibility in cecectomized laying hens at 27, 40, and 57 weeks of age and found an increasing AA digestibility as the birds grew older. For eight out of 15 AA, the digestibility was significantly higher at the age of 57 weeks compared to 27 or 40 weeks. Moreover, significant differences between the two groups of younger birds were detected for two AA. Furthermore, the AA digestibility for six AA differed significantly among the birds that were 40 and 57 weeks of age with the largest difference in Cys (5.8 percentage points). The laying hens used in the present thesis were, at least partly, sampled in the age period, in which significant age effects were proved by Rezvani et al. (2007). Nevertheless, the potential influence of a bird's age on the determined AA digestibility coefficients is considered rather small in the present thesis. This assessment comes from on the fact, that the experimental design was arranged as Latin Squares, and the cereal samples tested within one Latin Square were fed to one bird in each age period. Moreover, as described in the four manuscripts (chapter 4.1−4.4), each of the 16 Latin Squares contained a basal diet (**BD**) in addition to the five cereal diets. Thus, the AA digestibility of the BD can be further considered as a reference value. As summarized in Table 4, the AA digestibility of the BD was constant across the four cereal species tested. Moreover, the four Latin Squares for each cereal species were distributed across two runs, and the AA digestibility of the BD was statistically compared between the two runs of each cereal species. For all comparisons, no significant differences in AA digestibility were detected between the respective runs ($P > 0.05$). In this context, it is noteworthy that the four BD had the same composition, but were mixed separately for the trials investigating the triticale, rye, corn and wheat grains, respectively. Furthermore, due to the duration of the trials, the laying hens were replaced in the course of the experiment so that two groups of birds were used. Therefore, the consistency in AA digestibility is very high, and the maximum difference of 2 percentage points (Asp, His) is negligible.

Table 4. Digestibility (%) of the four basal diets used in the digestibility trials with triticale, rye, corn, and wheat

Basal diet	Triticale trial	Rye trial	Corn trial	Wheat trial
Animal group	1	1	1	2
Weeks of age	45−64	32−54	55−73	55−76
Number of birds	23	24	22	21
Arg	92	91	91	91
Ala	85	84	84	84
Asp	82	82	81	80
Cys	88	88	88	87
Glu	97	97	97	97
His	87	88	87	86
Ile	92	92	92	92
Leu	93	93	93	93
Lys	90	90	90	89
Met	95	95	95	95
Phe	94	94	94	94
Pro	96	96	96	96
Ser	89	89	89	88
Thr	81	81	81	80
Trp	87	87	87	86
Tyr	91	91	91	91
Val	90	89	90	89

Furthermore, the feeding procedure and the type of experimental diets are also known to affect AA digestibility. In some digestibility assays based on quantitative excreta collection, the birds were force-fed the test ingredients (Sibbald, 1979; Green et al., 1987; Adedokun et al., 2009). This approach was mainly used with roosters in the so called “precision-fed cecectomized rooster assay” (Sibbald, 1986). However, this procedure has been repeatedly criticized because it does not reflect the normal feeding behavior of the birds. Therefore, the natural feeding of a complete diet, as in the present thesis, seems preferable. When excreta are to be collected quantitatively, without using an indigestible marker, it is also necessary to quantify the feed intake of the birds. This is a challenge in feeding experiments with poultry, and can be alleviated by pelleting the experimental diets. Another advantage of using pelleted diets is the prevention of a selective feed intake by the hens. This is of crucial importance in the assay procedure used herein since the AA intake of the hens was calculated as the feed intake multiplied by the respective AA concentration of the complete diet. Although, the feeding of

pelleted diets is not a common practice in commercial laying hen husbandry, this form of feed preparation was necessary for the reasons mentioned above. However, the fact that diet preparation has an influence on the chemical and physical characteristics of the feed cannot be ignored (Pettersson et al., 1991). Especially the pelleting process, which is associated with an increase in temperature, needs to be discussed critically. Although not measured in the present study, it is imaginable that during the pelleting process, the temperature of the BD increases more than the cereal diets due to the high corn starch concentration (500 g/kg) in the former. A different increase in temperature might affect the properties of the basic mix (mixture without corn starch or cereal) or of the pellets. Abdollahi et al. (2010) showed that an increase in pelleting temperature raised pellet hardness. A different level of pellet hardness might, in turn, affect the AA digestibility of the basic mix, which was generally assumed to be identical for all experimental diets of this thesis. However, as mentioned above, pellet temperature and hardness were not measured in this work and a definite conclusion cannot be drawn as to whether diet pelleting affected the results. Nevertheless, the influence of different increases in temperature on the diet types caused by the pelleting process is considered rather minor, since very small amounts were prepared for each experimental diet and the increase in temperature was probably not high.

Another important aspect in digestibility trials with poultry is joint voiding of feces and urine. When non-colostomized birds are sampled by collecting their excreta, the renal AA contribution is ignored in the calculation of AA digestibility (McNab, 1995). This assumption is mainly based on the results of several previous studies (O'Dell et al., 1960; Bragg et al., 1969; Yamazaki, 1983; Whittow, 2000), which found the AA concentration in chicken urine to be negligibly low. Furthermore, Jirjis et al. (1997) determined that the AA concentration in turkeys is independent of the CP concentration in the diet.

The digestion process implies inevitable losses of AA (secretion of digestive enzymes, desquamation of cells, mucus, etc.). Several reviews of procedures to measure the endogenous losses of AA have been published (Sibbald, 1987; Nyachoti et al., 1997; Ravindran and Bryden, 1999). One possibility is the measurement of AA excretion of starved birds. It is obvious that this situation is abnormal for the bird and probably does not reflect the endogenous AA losses occurring under common feeding situations. Moreover, starvation can cause severe health problems in young growing birds, and can increase the incidence of shell-less eggs in laying hens (Sibbald, 1986), which, in turn, can contaminate the excreta. Another way is to use a N-free diet (Kim et al., 2012; Adedokun et al., 2015). However, according to the assessment of the author, this approach is likewise unsuitable for reflecting a natural feeding situation.

Moreover, Kong and Adeola (2013) showed that a variation in the proportion of cornstarch and dextrose in N-free diets might affect endogenous AA losses. Probably the biggest disadvantage of the methods mentioned above is that the values for endogenous AA losses are determined under specific conditions and then applied to others in which the endogenous AA loss might be different. This probably leads to wrong digestibility coefficients since Parsons et al. (1983) clearly demonstrated that diet composition affects the excretion of endogenous AA. Ravindran and Hendriks (2004) measured the amount and composition of endogenous AA at the terminal ileum of broilers when the birds were 14 and 42 days old. They found that the endogenous flow of AA increased with age. Moreover, age affected the composition of the endogenous AA flow in the study of Ravindran and Hendriks (2004), too. Therefore, the regression approach, as Rodehutscord et al. (2004) suggested, is a sophisticated alternative since it allows the consideration of endogenous AA losses without the need for extra diets and measurements. Furthermore, this approach considers the endogenous AA losses under the conditions of the trial and is, thus, more accurate than the abovementioned alternative approaches. A further advantage of the regression method is that it only corrects the basal endogenous losses, whereas feed-specific endogenous losses are part of the estimated digestibility (Rodehutscord et al., 2004).

Although the assay procedure used in this thesis is based on several assumptions, it overcomes the limitations of the other procedures. An aspect mentioned above is the comparatively low number of birds needed. Considering the increasingly stringent animal welfare legislations and the growing public claim to animal trials, this could help to justify feeding trials, which are still unavoidable in poultry nutrition science.

3.2.2 Non-starch polysaccharides

In the present thesis, the AA digestibility varied considerably within and among cereal grains though the crops were examined in a strictly standardized assay procedure. Thus, feed-related factors, including the chemical composition and morphological characteristics of the grains, must have caused the differences in AA digestibility. Due to the reasons mentioned in chapter 1, it was hypothesized that especially the concentration of various NSP fractions might negatively affect the AA digestibility of the cereal grains. To examine the relationship between AA digestibility and NSP concentration, the 20 samples of triticale, rye and wheat were comprehensively analyzed for their carbohydrate composition (Rodehutscord et al., 2016). Since the NSP concentration and the solubility of the polymers are generally low in corn grains

(Bach Knudsen, 1997; 2014), those were not analyzed for NSP. The following statements consequently refer to triticale, rye, and wheat grains. As summarized in Table 5, the concentration of total NSP as well as that of AX and β-glucan was highest in rye grains.

Table 5. Concentrations of selected NSP fractions (mean and range, in g/kg DM, unless otherwise stated) and respective coefficients of the variation of cereal grains grown under standardized environmental and agronomic conditions[#]

	Triticale (n = 20)	Rye (n = 20)	Wheat (n = 20)
Total NSP	103 (92−115)	139 (122−158)	98 (90−113)
CV (%)	7	7	6
Soluble NSP	21 (15−31)	41 (33−53)	19 (11−30)
CV (%)	18	12	24
% soluble of total NSP	20 (16−27)	30 (25−35)	19 (11−27)
CV (%)	17	9	21
Total AX	55 (40−74)	85 (74−96)	64 (59−74)
CV (%)	22	7	7
Soluble AX	13 (8−17)	31 (24−40)	14 (8−22)
CV (%)	20	13	25
% soluble of total AX	23 (17−33)	36 (32−42)	22 (14−31)
CV (%)	16	8	21
A:X (total)	0.67 (0.55−0.78)	0.69 (0.65−0.75)	0.64 (0.59−0.70)
CV (%)	8	4	4
A:X (soluble)	0.70 (0.56−0.83)	0.69 (0.62−0.75)	0.65 (0.53−0.87)
CV (%)	12	4	10
Total β-glucan	7 (5−8)	20 (17−26)	6 (5−8)
CV (%)	10	14	15
Soluble β-glucan	1 (0−2)	7 (5−9)	2 (1−3)
CV (%)	75	16	26
% soluble of total β-glucan	13 (0−25)	33 (26−41)	32 (22−43)
CV (%)	76	14	18
Cellulose	19 (12−27)	12 (6−18)	14 (12−16)
CV (%)	25	23	10

[#] Rodehutscord et al. (2016) reported a comprehensive analytical description of the cereals.

These values are in accordance with the data on NSP concentrations in poultry feedstuff published by Bach Knudsen (2014). Moreover, rye grains showed the lowest mean digestibility of AA including Lys (49%) and Met (67%), compared with the grains of triticale (74% for Lys and 83% for Met) and wheat (80% for Lys and 84% for Met) (see chapters 4.1, 4.2, and 4.4). Triticale and wheat grains had a similar level of AA digestibility as well as similar concentrations of NSP fractions. Thus, it can be hypothesized that the lower AA digestibility of rye grains might have been caused by their higher concentration of NSP.However, the examination of the relationship between the concentration of NSP and AA digestibility across the three cereal species would be misleading because the concentrations of different NSP fractions were unevenly distributed among the cereal species and the regression lines run through two point clouds. Moreover, other analyzed fractions were different among the grains, and the NSP concentration alone may not have caused the differences in AA digestibility.

Against the initial hypothesis, none of the analyzed NSP fractions, alone or in combination, was suitable to explain the variability of AA digestibility within the cereal species (chapters 4.1, 4.2, 4.4). However, as discussed in chapter 4.1, this observation does not necessarily imply that the cereal NSP might not have had an effect on AA digestibility in the present thesis. This assumption is mainly based on the structural heterogeneity of cereal NSP and the related influence on the physicochemical properties of the polymers. As described in chapters 4.1 and 4.4, the analytical detection of the NSP fractions of the three cereals followed a hydrolysis of the polymers and subsequently, a quantification of the constituent sugar monomers. Hereby, the sum of arabinose and xylose is interpreted as AX, whereas the sum of glucose (after removal of starch and cellulose) is considered as β-glucan. Consequently, differences in the fine structure of the polymers are not revealed. However, the physicochemical properties of the polymers, and consequently their behavior in the gastrointestinal tract depend primarily on the fine structure and the structural arrangement in the cell walls (Bengtsson et al., 1992). Numerous reviews of the structural diversity of AX (Fincher and Stone, 1986, Izydorczyk and Biliaderis, 1995; Saulnier et al., 2007; Vinkx and Delcour, 1996; Fincher and Stone, 2004; Bach Knudsen, 2014) and β-glucan (Lazaridou and Biliaderis, 2007; Cui and Wang, 2009) in cereal grains have been published. The extent and complexity of this topic makes it impossible to address all the details in this thesis. However, a brief overview of the structural heterogeneity and the resulting properties will be presented herein. Readers will be referred to the relevant literature for further information.

Arabinoxylans

Arabinoxylans are the main NSP polymers in the cell walls of triticale, rye, and wheat grains (Bach Knudsen, 2014). As shown in Table 5, this was confirmed for samples of triticale, rye, and wheat grains studied in this thesis. Arabinoxylans consist of a linear backbone of D-xylopyranosyl residues connected by β-(1→4)-glycosidc linkages (Bach Knudsen, 2014). The xylan backbone is substituted with α-L-arabinofuranosyl residues to varying degrees. Hereby, the xylose units can be unsubstituted, mono-substituted on the O-2 or O-3 position, or di-substituted on the O-2 and O-3 position, resulting in four structural elements (Izydorczyk and Biliaderis, 1995; Saulnier et al. 2009; Bach Knudsen, 2014). In addition to the arabinose residues, other substituents including hexoses, hexuronic acids, phenolic acids, ferulic acids, and proteins, can be linked to the xylan backbone (Fincher and Stone, 1986; Choct, 1997; Dervilly-Pinel et al., 2001). Figure 2 shows an exemplary structure of an AX polymer.

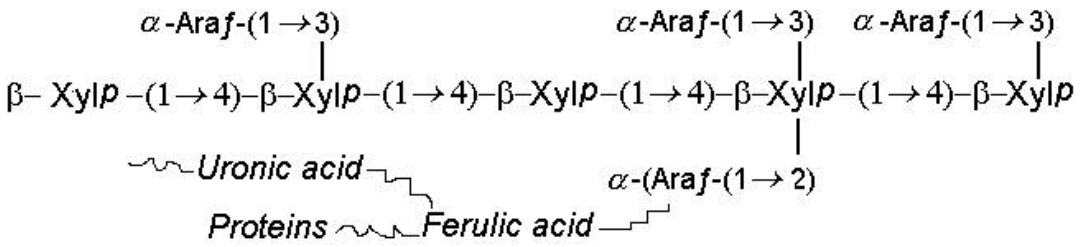

Figure 2. The structural feature of arabinoxylans from cereal grains (Choct [1997])

According to Bengtsson et al. (1992) and Burton and Fincher (2012), the substitution pattern of the xylan backbone affects the physical properties of the polymer. A heavy substitution with arabinose will make the AX polymer more soluble, because the arabinose residues inhibit an intermolecular alignment of individual molecules and prevent aggregation and precipitation. The observations made by Ordaz-Ortiz et al. (2005) and Saulnier et al. (2007) are of particular importance with regard to the present thesis. They found that the substitution pattern varies among different cultivars of the same cereal species. Moreover, Dervilly-Pinel et al. (2001) observed that the soluble AX from rye gave stronger gels than the AX from wheat and rye through the cross-linking of AX chains via substituted ferulic acids. Ferulic acid residues also provide some potential for AX-protein interactions (Fincher and Stone, 1986). This highlights the importance of minor components associated with the AX polymer for their physicochemical properties, though their quantities might be low.

To characterize the substitution pattern of AX even further, the ratio of arabinose to xylose is used occasionally (Izydorczyk and Biliaderis, 1995). Hence, this ratio was calculated for the total and the soluble fractions of arabinose and xylose in the present thesis (Table 5). However, the AX ratio was not suitable to explain the variation in AA digestibility for any of the cereals examined in the present work. Interestingly, the coefficient of variation for the ratio of soluble arabinose to soluble xylose was smallest in rye compared with wheat and triticale, though the variability in AA digestibility was highest in rye grains. This indicates that the AX ratio alone probably did not cause the variability in AA digestibility within the cereal grains. Hence, the AX ratio provides only little additional information about the differences in the fine structure and other factors seem to be important too. Andrewartha et al. (1979) reported in this context that the distribution of arabinosyl substituents along the xylan backbone might be of greater relevance for the properties of the AX than the degree of substitution, since it affects the conformation and interaction with other cell wall components. This supports the observations made in the present work that the AX ratio alone is not suitable to explain differences in AA digestibility within and among cereal samples.

β-glucan

The concentration of β-glucan is lower than that of AX in the grains of triticale, rye, and wheat (Bach Knudsen, 2014). Cereal β-glucans typically consist of glucose residues linked mostly via two to three consecutive β-(1→4) linkages. A single β-(1→3) linkage separates the resulting trisaccharide and tetrasaccharide units (Bengtsson et al., 1990; Lazaridou and Biliaderis, 2007). Figure 3 shows a typical structure of a cereal β-glucan. The presence of the β-(1→3) linkages results in irregularities and makes the β-glucan more soluble than cellulose (Theander et al., 1993).

According to Cui and Wang (2009) more than 90% of the β-glucans is composed of trisaccharide and tetrasaccharide units, and the remaining 10% is mainly composed of longer cellulose-like structures with 5−14 glucose residues connected with β-(1→4) linkages. It was suggested that the occurrence of trisaccharide and tetrasaccharide units, as well as the occurrence of longer cellulose-like segments, was random (Ebringerova, 2006; Cui and Wang, 2009). The incorporation of β-(1→3) linkages breaks the regular structure and prevents an extensive inter-molecular packing. Thus, the molecules are relatively soluble in water, despite a degree of polymerization of 1,000 and more (Choct, 1997). Nevertheless, inter-molecular

association among β-glucan molecules may occur through hydrogen bonding. These associations provide greater stiffness to the polymer chain (Cui and Wang, 2009).

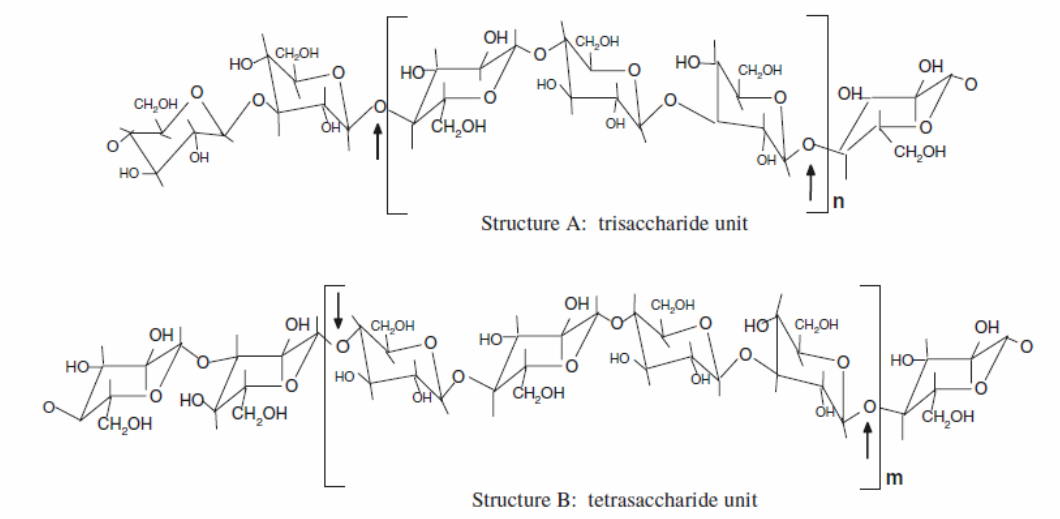

Figure 3. Structural features mixed linkage (1→3;1→4)-β-glucans from cereal grains (Cui and Wang [2009])

Similar to the A/X ratio, the ratio of trisaccharide and tetrasaccharide units, can be used to characterize cereal β-glucan in more detail. A higher trisaccharide/tetrasaccharide ratio favors gel formation, fastens the gelation process, and ultimately gives stronger gels (Cui and Wang, 2009). Furthermore, as Cui and Wang (2009) suggested, the distribution of trisaccharide and tetrasaccharide building blocks is random. However, no information on the trisaccharide/tetrasaccharide ratio is available for the cereal samples examined in the present thesis. Lazaridou and Biliaderis, (2007) summarized in their review the structural features of cereal β-glucans from previous investigations and revealed a considerable variation in the trisaccharide/tetrasaccharide ratio within and among the cereal species. Thus, the different samples within a cereal species from the present work could likewise have differed with regard to the trisaccharide/tetrasaccharide ratio and the distribution of these two building blocks along the polymer. Moreover, the molecular weight of the polymers affects the physicochemical properties of cereal β-glucan (Cui and Wang, 2009). However, no information about the differences in the molecular weight or the fine structure of the polymers between samples of the same cereal species is available from the analysis in the present work.

It can be summarized, therefore, that cereal NSP are heterogeneous group of components with considerable differences in chemistry within and among the polymers. Differences in the fine structure affect the physicochemical properties of the polymers and probably their behavior in the gastrointestinal tract of poultry. As Rakha et al. (2012) showed, structural features of AX and β-glucan vary within and among different cultivars of the same cereal species. This might have affected AA digestibility in the present work. The experimental approach used herein, did not allow for viscosity measurements. Thus, it remains unknown whether cereal samples with a similar NSP concentration affected digesta viscosity to a different extent. However, according to Rakha et al. (2011), the modern triticale grain has a dietary fiber profile and molecular weight distribution of extractable dietary fiber components that are more similar to wheat than rye. This supports the findings of the present thesis that triticale grain is rather similar to wheat grain than rye grain with regard to NSP concentration and AA digestibility.

3.2.3 Protein fractions

Digesta viscosity of poultry is primarily increased by the presence of soluble NSP in cereal grains (Choct and Annison, 1992a; Bach Knudsen, 2014). However, according to Scheele et al. (1995), the soluble proportions of the wheat storage protein fractions gliadin and glutenin, which together form gluten, can also have viscosity-increasing properties. In this context, Weipert (1997) reported that the water solubility of rye proteins is higher than that of wheat proteins. This might explain the observations made in the present thesis, that the AA digestibility of rye grains was considerably lower than that of wheat grains. Since the proportions of different protein fractions were not determined in the framework of the thesis, the relationship among their proportion and AA digestibility can only be examined indirectly by means of the AA concentration of the cereal samples. Gliadin and glutenin are both rich in Glu and Pro (Wieser, 2007), and a higher concentration of these two AA in the protein indicates higher proportions of these protein fractions. However, for none of the four cereal species tested was the concentration of single or several AA consistently related to the digestibility of AA. In wheat grains, the concentration of Glu and Pro (expressed as g/16 g N) was significantly correlated to the digestibility of any AA (chapter 4.4), whereas in triticale grains (chapter 4.1) and rye grains (chapter 4.2), significantly positive correlations were determined for the digestibility of a few AA. Thus, a higher or lower AA digestibility of the cereal grains was not continuously associated with a higher or lower concentration of certain AA in the protein. Moreover, the signs of the few significant correlation coefficients were against that what would

be expected with regard the viscosity-increasing properties of gluten proteins reported by Scheele et al. (1995). However, the chemistry of cereal proteins is very complex, and the information on the AA concentration in the grain or in the protein is probably not sufficiently precise to a draw a conclusion on the influence of various protein fractions on AA digestibility. Shewry and Halford (2002) reviewed the chemistry and properties of cereal seed storage proteins and reported that the prolamins of wheat and rye consist of three broad groups: sulfur-rich, sulfur-poor and high-molecular-weight prolamins. Similarly, the authors reported that several groups of proteins (α, β, γ, δ-zeins) comprise the prolamins of corn. The amino acid composition differs considerably among these subgroups, which probably affects their properties too. As mentioned above, no information about the proportions of various protein fractions or their composition is available for the cereal samples used herein. Thus, their potential influence is only speculative. Nevertheless, as discussed about the effect of the fine structure in NSP (chapter 3.2.2), a similar concentration of protein in the grains might have affected viscosity to a different extent. Since no consistent relationship between the concentration of certain AA and AA digestibility was found within and among the cereal species, it seems unlikely that differences in the proportion of the protein fractions alone caused the differences in AA digestibility in the present work. Nevertheless, in combination with other factors, differences in the proportions of protein fractions and related properties might have contributed to the variability in AA digestibility.

3.3 Predictability of amino acid digestibility

Previous studies have clearly demonstrated that a diet formulation based on digestible AA is superior to total AA with regard to the efficiency of protein utilization (Fernandez et al., 1995; Rostagno et al., 1995; Wang and Parsons, 1998; Douglas and Parsons, 1999). The considerable differences in AA digestibility within and among cereal grains determined in the present thesis (chapters 4.1−4.4) underline the potential of such a feed formulation system to reduce safety margins in diets, and hence, N emissions from laying hen farms. The two approaches to predict AA digestibility examined in the present thesis, regression equations based on physical and chemical characteristics on the one hand and the *in vitro* solubility of N on the other hand, were not suitable to predict AA digestibility with an acceptable accuracy for practical application. A correlation analysis between AA digestibility and the *in vitro* solubility of N showed inconsistent results across the four cereal species examined. While two significantly negative correlations were found in triticale grain (chapter 4.1), and five

significantly positive correlations were found in rye grains (chapter 4.2), no significant relationship was found in corn and wheat grains (chapters 4.3 and 4.4). As discussed in chapter 4.1, by using porcine pepsin and pancreatin, this approach is probably too species-specific to be applicable to feed evaluation in poultry. Moreover, the incubation time applied in this *in vitro* approach is too long to reflect the digestive conditions in the gastrointestinal tract of laying hens. Similarly, alternative *in vitro* approaches to predict AA digestibility likewise suffer from a low level of accuracy (Rochell et al., 2013). However, the availability of suitable *in vitro* approaches to predict AA digestibility in poultry is of fundamental importance for the development of calibrations for near infrared reflectance spectroscopy (**NIRS**). The NIRS technique depends on a large number of samples with known digestibility values for the calibration of the equipment. However, the determination of AA digestibility of a sufficiently large number of samples for NIRS calibration directly with laying hens is hardly feasible. The trials conducted in the present work to determine the AA digestibility of 20 samples per cereal species lasted nearly two years. In addition, the workload during the digestibility trial and the analytical effort and costs cannot be ignored. Moreover, the NIRS calibrations have to be extended for samples of new feedstuff. Thus, an appropriate *in vitro* approach to predict AA digestibility in the birds would be an important intermediate step to provide large data sets for NIRS calibrations. Therefore, the development of suitable *in vitro* approaches to predict AA digestibility in laying hens is suggested for future research. The application of prediction equations is only useful for practical application if easily determinable variables enter the regression equation. However, such equations were shown to be unsuitable in the present thesis due to their low prediction accuracy. It remains to be tested whether characteristics of the grains other than those determined in the GrainUp project are suitable to calculate prediction equations with an acceptable accuracy. However, due to the analytical effort and complexity, it is questionable whether these equations will be useful for practical application during feed formulation.

3.4 Conclusions and perspectives for future research

The cereal grains examined in the present thesis were grown under identical (triticale, rye, wheat) or similar (corn) agronomic and environmental conditions. Therefore, the variability in the chemical composition within and among the grains was comparatively low. However, a greater variability in the chemical composition of the grains and thus, in their properties, might be expected when the crops are grown under a broad range of conditions. Nevertheless, the

present study revealed a considerable variability of AA digestibility within and among cereal grains in laying hen. This clearly demonstrates the potential of a feed formulation system based on digestible AA to increase the efficiency of protein utilization. Moreover, it can be assumed that a higher variability in the chemical composition of the grains, caused by varying growing conditions, will cause even greater differences in AA digestibility than those observed in this thesis. This, in turn, would increase the potential of a feed formulation system based on digestible AA to increase the efficiency of protein utilization further. Thus, the determination of AA digestibility from cereal grains grown in a broad range of environmental and agronomic conditions is suggested for future research. Furthermore, a higher variability in the chemical composition of the grains and their AA digestibility might reveal significant relationships more clearly.

Although the grains used in this thesis were comprehensively analyzed, their chemical and physical characteristics could not explain the variation in their AA digestibility. As discussed in chapters 3.2.2 and 3.2.3, mainly two approaches of explanation seem plausible for this observation. It cannot be denied that differences beyond those analyzed in the present work affected AA digestibility. Therefore, a more detailed characterization of the grains with regard to the fine structure of NSP and protein fractions might be helpful to explain differences in AA digestibility. In this context, the effect of different cereal samples on the digesta viscosity is of special interest. The experimental approach used in this thesis did not allow for digesta viscosity measurements. Hence, the possibility of viscosity measurements in the excreta of cecectomized birds, as an alternative to the measurements made in the digesta, should be examined. Had viscosity measurements made in excreta been an appropriate procedure to reflect the viscosity conditions in the ileum, this would further clarify the effects of different cereal samples in laying hens. Consequently, the question of whether similar concentrations of NSP or protein can cause different viscosities in the gastrointestinal tract of the birds could be answered. Another approach to identify the factors that affect AA digestibility might be the supplementation of enzymes with a well-defined activity. Reduced differences in AA digestibility caused by a certain enzyme supplementation would provide an indication to the factors responsible for the differences in AA digestibility.

Alternatively, characteristics not determined in the framework of this thesis might have caused or contributed to the differences in AA digestibility between the cereal samples. In this context, alkylresorcinols (Tlušik, 1978; Nyström et al., 2008), pectic polysaccharides (Chateigner-Boutin et al., 2014) and trypsin inhibitors (Chang and Tsen, 1981, Johnson and Eason, 1988) should be mentioned. However, there is no evidence of their presence or quantity

in the cereal samples used herein. Nevertheless, since the abovementioned studies demonstrated their occurrence in cereal grains, their role in digestibility measurements with poultry should be examined further.

In addition, as discussed in chapter 3.3, the development of suitable *in vitro* approaches to predict AA digestibility is suggested. However, these *in vitro* approaches need to be validated with digestibility values that were determined directly in laying hens. Thus, the data base of digestibility values should be extended until a suitable assay procedure is established. Furthermore, it cannot be ruled out that different *in vitro* approaches are necessary for different classes of feedstuff. Thus, a comprehensive investigation of the variability of AA digestibility of protein feedstuff in laying hens is also suggested. For these studies, the experimental approach used in this thesis is recommended to minimize the methodological influence on the AA digestibility coefficients.

To conclude, AA digestibility differed significantly within and among the four cereal species investigated in this thesis. However, neither single nor several chemical and physical characteristics were suitable to explain the differences in AA digestibility between cereal samples. Based on the findings of this work, it seems unlikely that one factor alone caused considerable differences in AA digestibility among laying hens. The reasons, therefore, seem rather multifactorial.

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4 Included Studies

4.1 Manuscript I

Variability in amino acid digestibility of triticale grain from diverse genotypes as studied in cecectomized laying hens

T. Zuber[1], H.P. Maurer[2], J. Möhring[3], N. Nautscher[1], W. Siegert[1], P. Rosenfelder[1], M. Rodehutscord[1]

[1] Institut für Nutztierwissenschaften, Universität Hohenheim, 70599 Stuttgart, Germany

[2] Landessaatzuchtanstalt, Universität Hohenheim, 70599 Stuttgart, Germany

[3] Institut für Kulturpflanzenwissenschaften, Universität Hohenheim, 70599 Stuttgart, Germany

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ABSTRACT

Triticale, the man-made cross between wheat and rye, is increasing in importance as a feed grain for laying hens. However, the knowledge of its nutritional qualities and their impact on bird performance is limited and prevents the optimization of its use. Thus, it was the objective of the present study to examine the variability of amino acid (**AA**) digestibility of triticale grain in laying hens. Additionally, relationships between AA digestibility and chemical and physical characteristics of the grains were investigated. Twenty genotypes of triticale that were grown under standardized agronomic and environmental conditions were comprehensively characterized according to their physical properties (thousand seed weight, test weight, falling number, extract viscoelasticity), chemical composition (proximate nutrients, non-starch polysaccharides, AA, minerals, inositol phosphates), gross energy concentration and the *in vitro* solubility of nitrogen. The feeding trial comprised four Latin Squares (6×6) that were distributed among two runs. A total of twelve cecectomized LSL-Classic hens were individually housed in metabolism cages and either fed a basal diet containing 500 g/kg cornstarch or one of 20 triticale diets, each replacing the cornstarch with one triticale genotype, for eight days. During the last four days, feed intake was recorded and excreta were collected quantitatively. The digestibility of AA of the triticale genotypes was calculated using a linear regression approach. The 20 genotypes differed significantly in the digestibility of all AA, including Lys (digestibility range 68–80%), Met (77–86%), Thr (68–78%) and Trp (74–83%). However, AA digestibility only correlated with characteristics of the grains in few cases. Moreover, no consistent pattern among AA was observed. Prediction equations for AA digestibility based on the grain's physical and chemical characteristics were calculated by multiple linear regression. The explanatory power (adjusted R^2) of these prediction equations was below 0.7 for most AA and thus not sufficiently precise to be suitable for practical application. To conlude, AA digestibility of triticale grain is generally on a high level in laying hens but varies significantly between different crop genotypes. Physical and chemical characteristics of the grains could not well explain this variation.

4.2 Manuscript II

Amino acid digestibility of different rye genotypes in caecectomised laying hens

T. Zuber[1], T. Miedaner[2], P. Rosenfelder[1], M. Rodehutscord[1]

[1] Institut für Nutztierwissenschaften, Universität Hohenheim, 70599 Stuttgart, Germany

[2] Landessaatzuchtanstalt, Universität Hohenheim, 70599 Stuttgart, Germany

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ABSTRACT

It was the objective of this study to determine the variability of amino acid (**AA**) digestibility of rye grains in laying hens and to study relationships between AA digestibility and characteristics of the grains. Twenty genotypes of rye were grown under standardized agronomic and environmental conditions as part of a collaborative research project known as "GrainUp" and comprehensively characterized based on their physical properties (thousand seed weight, test weight, falling number, and extract viscoelasticity), chemical composition (proximate nutrients, non-starch polysaccharides, AA, minerals, and inositol phosphates), gross energy concentration and the *in vitro* solubility of nitrogen (**N**). Each rye genotype was added to a basal diet at 500 g/kg at the expense of cornstarch to produce 20 rye diets. The animal trial comprised four Latin Squares (6x6) distributed over two subsequent runs, resulting in 12 experimental periods. Cecectomized LSL-Classic hens were individually housed in metabolism cages and fed the respective experimental diets for eight days per period. During the last four days excreta were collected quantitatively and the feed intake was recorded. Amino acid digestibility of the rye genotypes was determined using linear regression. The digestibility of AA of the rye grains was generally low and varied significantly between the 20 rye genotypes. Especially the digestibility of Lys (digestibility range 35–59%), Met (57–75%), Thr (34–54%), and Trp (36–71%) differed considerably between the rye genotypes. Nevertheless, physical and chemical characteristics as well as the *in vitro* solubility of N correlated with AA digestibility only in few cases. Multiple linear regressions were calculated to develop equations to predict AA digestibility based on the analyzed characteristics. However, explanatory power of the equations, as judged by the adjusted R^2, was below 0.6 for most AA, and thus not sufficiently precise for practical application. To conclude, the AA digestibility of rye grain is low overall and varies significantly between crop genotypes. Predictions equations based on the physical and chemical characteristics of the rye grains were not sufficiently precise to be useful for practical feed formulation.

4.3 Manuscript III

Variability in amino acid digestibility and metabolizable energy of corn studied in cecectomized laying hens

T. Zuber and M. Rodehutscord

Institut für Nutztierwissenschaften, Universität Hohenheim, 70599 Stuttgart, Germany

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ABSTRACT

The consideration of differences in amino acid (**AA**) digestibility and metabolizable energy between different corn samples during feed formulation is a useful approach to optimize the use of this crop in laying hen feeding. However, differences between corn samples are largely unknown. Thus, it was the objective of the present study to investigate the variability of AA digestibility and AME_n concentration of 20 corn samples in cecectomized laying hens. The corn grains were comprehensively characterized based on their physical properties (thousand seed weight, test weight, grain density, and extract viscoelasticity), chemical composition (proximate nutrients, AA, minerals, and inositol phosphates), gross energy concentration, and *in vitro* solubility of nitrogen to study any relationship with AA digestibility or AME_n. The experimental design comprised four Latin squares (6×6) distributed among two subsequent runs. Cecectomized laying hens (LSL-Classic) were individually housed in metabolism cages from 55 to 73 weeks of age and fed either a basal diet containing 500 g/kg cornstarch or one of 20 corn diets, each replacing the cornstarch with one corn batch, for eight days. During the last four days, feed intake was recorded and excreta were collected quantitatively. The AA digestibility of the corn samples was calculated using linear regression. The digestibility of all AA differed significantly between the 20 corn samples, including Lys (digestibility range 64–85%), Met (86–94%), Thr (72–89%), and Trp (21–88%). Similarly, the AME_n differed considerably between the corn samples with values ranging from 15.7 to 17.1 MJ/kg DM. However, AA digestibility and AME_n correlated with the physical and chemical characteristics of the grains only in few cases. Moreover, equations to predict AA digestibility and AME_n based on the physical and chemical characteristics of the grains were calculated by multiple linear regressions. However, the explanatory power of the prediction equations, as judged by the adjusted R^2, was below 0.6 for most AA and AME_n. Thus, the calculated equations were not sufficiently precise for practical application. Possible explanations for the observed variation in AA digestibility and AME_n beyond the characteristics that were determined in the present study are discussed. In conclusion, AA digestibility and AME_n of corn grain is generally high in laying hens, but varies between different corn samples. Neither physical nor chemical characteristics were suitable to explain these variations.

4.4 Manuscript IV

Variability in amino acid digestibility of wheat grains from diverse genotypes examined in caecectomised laying hens

T. Zuber and M. Rodehutscord

Institut für Nutztierwissenschaften, Universität Hohenheim, 70599 Stuttgart, Germany

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ABSTRACT

For the practical application of a feed formulation system that is based on digestible amino acids (**AA**) rather than total AA, thorough information about the level and variation of AA digestibility, is necessary. Moreover, approaches to rapidly predict AA digestibility under practical conditions are needed. Wheat grains are commonly used at high levels in the diets of laying hens. Consequently, they are an important source of AA for the birds. Nevertheless, systematic studies investigating the AA digestibility of wheat grain in laying hens are limited. Therefore, it was the objective of the present study to determine the AA digestibility of 20 wheat genotypes, and to examine possible relationships between AA digestibility and characteristics of the grains. The wheat genotypes were grown under standardized conditions within the collaborative research project known as "GrainUp", and analyzed based on their physical and chemical characteristics. Additionally, the *in vitro* solubility of nitrogen (**N**) of the wheat grains was determined. The wheat genotypes were added to a basal diet at the expense of cornstarch at 500 g/kg to produce 20 wheat diets. The animal trial comprised four Latin Squares (6×6), which were equally distributed over two subsequent runs (resulting in 12 experimental periods). Twelve cecectomized laying hens (LSL-Classic) were individually housed in metabolism cages, and fed the respective experimental diets for eight days per period. During the last four days, excreta were collected quantitatively and feed intake was recorded. Amino acid digestibility of the wheat genotypes was calculated by linear regression. The digestibility of all AA varied significantly between wheat genotypes, but digestibility was generally on a high level. The largest differences between extreme genotypes were observed in Lys (23 percentage points) and Met (18 percentage points). Chemical and physical characteristics as well as the *in vitro* solubility of N significantly correlated with AA digestibility only in a few cases. Multiple linear regression analyses were used to develop prediction equations for AA digestibility based on the grain characteristics. However, the explanatory power (adjusted R^2) of the equations was below 0.7 for the majority of AA across the variable pools. Thus, the precision of the prediction equations does not warrant their use during practical feed formulation. Several explanatory approaches for the missing relationships are presented and discussed in the present paper.

5 Summary

Cereal grains are the major ingredients in the diets of laying hens and primarily serve as a source of energy. However, when included in high levels in the diet, they also contribute significantly to the amino acid **(AA**) supply to the hens. An approach to increase the efficiency of protein utilization in laying hens is the consideration of AA digestibility during feed formulation. However, for the practical application of a feed formulation system based on digestible AA, thorough knowledge of the variability of AA digestibility within and among the feed ingredients is necessary. Furthermore, approaches to predict AA digestibility rapidly and reliably are needed. However, the variability of AA digestibility of cereal grains in laying hens is largely unknown and the few studies conducted on this subject have examined only a few samples. Moreover, these studies used a multitude of different assay procedures, which impairs the comparability of results.

A common feature of cereal grains, apart from corn, is the generally high concentration of non-starch polysaccharides (**NSP**). It is well known that some NSP, especially the soluble fraction, can negatively affect digestive processes in poultry, by increasing the viscosity of digesta, entrapping nutrients and thus reducing their digestibility. To date, relationships between AA digestibility and fractions of NSP or other chemical constituents of cereal grains have not been investigated in laying hens.

Thus, it was the objective of this doctoral thesis to generate a comprehensive data set of AA digestibility values of cereal grains in laying hens by using a strictly standardized assay procedure. Additionally, the suitability of two approaches to predict AA digestibility was examined. For this purpose, 80 genotypes of triticale, rye, corn, and wheat grains (n = 20 each) were grown as part of the “GrainUp” project. Apart from corn, the cereal species were grown under identical environmental conditions. The grain samples were comprehensively analyzed according to their physical properties (thousand seed weight, test weight, kernel density, falling number, extract viscoelasticity), chemical composition (proximate nutrients, non-starch polysaccharides [apart from corn grains], AA, minerals, inositol phosphates) and gross energy concentration. The concentration of crude protein in the grain samples of triticale, rye, corn, and wheat was in the range of 113−138, 108−127, 78−112, and 125−162 g/kg dry matter, respectively. Additionally, the *in vitro* solubility of nitrogen (**N**) was determined in the grains after pretreatment with porcine pepsin and pancreatin.

The animal trial comprised 16 Latin Squares (6x6), distributed among six subsequent runs. Thus, each run contained two to three Latin Squares. Cecectomized laying hens

(LSL-classic) were individually housed in metabolism cages and fed either on a basal diet containing 500 g/kg cornstarch or one of the 80 cereal diets, with the cornstarch being replaced with a grain sample, for eight days. During the last four days, feed intake was recorded and excreta were collected quantitatively twice daily. After each collection period, the hens were group-housed in a floor pen on litter for two days and offered a conventional layer diet. Amino acid digestibility of the grain samples was calculated using a linear regression approach. Relationships between AA digestibility and single analyzed fractions or the *in vitro* solubility of N of the cereal grains were examined by calculating Pearson product-moment correlation coefficients. Prediction equations to estimate AA digestibility were calculated by multiple regression analysis using a stepwise selection approach. Therefore, the variables were pooled according to their characteristics, and the prediction equations were calculated for the digestibility of each AA using each pool. The variables were offered in a linear or linear plus quadratic fashion and classified as significant predictors at $P \leq 0.10$. The calculated equations were assessed based on the adjusted R^2 and the root-mean-square error.

The AA digestibility varied widely within and among the cereal species. The mean digestibility of lysine was 74% (digestibility range: 68−80%), 49% (35−59%), 79% (64−85%), and 80% (69−87%) for triticale, rye, corn, and wheat grains, respectively. A similar ranking was observed for methionine with a mean digestibility of 83% (digestibility range: 77−86%), 67% (57−75%), 91% (86−94%) and 84% (70−93%) for triticale, rye, corn, and wheat grains, respectively. Correlation analysis showed inconsistent results within and across the cereal species. Among the physical characteristics, significant correlations were detected for the thousand seed weight and the digestibility of a few AA in wheat, and for the test weight and the digestibility of a few AA in rye and corn. Significant correlations between NSP fractions and the digestibility of essential AA were detected only for rye grains. In this crop, the concentration of arabinoxylans and total NSP in the grains was negatively correlated with the digestibility of arginine, leucine, phenylalanine, and threonine. The concentration of crude protein in corn grains was positively correlated with the digestibility of essential AA, except isoleucine, tryptophan, and valine. In contrast, only a few significant positive correlations between crude protein concentration and essential AA digestibility were found for triticale and rye grains. No significant correlations were found for wheat grains in this regard. The *in vitro* solubility of N was negatively and positively correlated with the digestibility of a few AA in triticale and rye grains, respectively. The accuracy of the predictive equations was generally low (adjusted R^2 below 0.7 in most cases), and varied considerably between both pools of variables for the same AA and the same pool of variables for different AA. Thus, single or several physical or chemical

characteristics (including NSP) could not explain the variation in AA digestibility in laying hens and the development of prediction equations sufficiently precise for the practical application was not possible.

However, factors beyond the chemical analyses carried out might have caused or contributed to the variability of AA digestibility in the grains. The effects of the fine structure of cereal NSP, as well as their structural arrangement in the cell wall, have been discussed as they affect the properties of the polymers, and thus, their behavior in the gut. The visco-elastic properties of certain protein fractions also might have affected AA digestibility in the cereals. It was suggested that this is investigated in follow-up projects.

6 Zusammenfassung

Getreidekörner stellen die Hauptkomponente in Rationen für Legehennen dar und dienen in erster Linie als Energiequelle. Bei hohen Mischungsanteilen in der Ration tragen diese jedoch ebenfalls erheblich zur Versorung der Hennen mit Aminosäuren (**AS**) bei. Ein Ansatz die Effizienz der Proteinausnutzung bei Legehennen zu erhöhen, ist die Berücksichtigung der AS-Verdaulichkeit bei der Rationsforumlierung. Für die praktische Anwedung einer Rationsformulierung auf Basis der verdaulichen AS ist jedoch eine umfassende Kenntnis der Variabilität der AS-Verdaulichkeit innerhalb und zwischen den Futterkomponenten notwendig. Darüber hinaus werden Verfahren benötigt, mit denen die AS-Verdaulichkeit schnell und zuverlässig vorhergesagt werden kann. Die Variabilität der AS-Verdaulichkeit von Getreidekörnern bei Legehennen ist jedoch weitgehend unbekannt und die wenigen Studien, die zu diesem Thema durchgeführt wurden, haben nur wenige Proben untersucht. Darüberhinaus wurden in diesen Studien eine Vielzahl unterschiedlicher Verfahren angewendet, wodurch die Vergleichbarkeit der Ergebnisse beeinträchtigt ist.

Eine gemeinsame Eigenschaft von Getreidekörnern, mit Ausnahme von Maiskörnern, ist die generell hohe Konzentration an Nicht-Stärke-Polysacchariden (**NSP**). Bekanntermaßen können einige NSP, insbesondere die lösliche Fraktion, die Verdauungsprozesse des Geflügels negativ beeinflussen, indem die Chymusviskosität erhöht, Nährstoffe eingeschlossen und folglich deren Verdaulichkeit reduziert wird. Bislang wurden die Beziehungen zwischen der AS-Verdaulichkeit und den NSP-Fraktionen oder anderen chemischen Bestandteilen der Getreidekörner nicht bei Legehennen untersucht.

Demnach war es das Ziel dieser Doktorarbeit einen umfassenden Datensatz zur AS-Verdaulichkeit von Getreidekörnern bei Legehennen zu erstellen, indem ein streng standardisiertes Testverfahren angewendet wurde. Darüber hinaus wurde die Eignung zweier Ansätze zur Vorhersage der AS-Verdaulichkeit geprüft. Zu diesem Zweck wurden 80 Genotypen von Triticale, Roggen, Mais und Weizen (jeweils n = 20) im Rahmen des “GrainUp” Projektes angebaut. Mit Ausnahme des Maises wurden die Kulturarten unter identischen Umweltbedingungen angebaut. Die Getreideproben wurden umfangreich hinsichtlich ihrer physikalischen Eigenschaften (Tausendkorngewicht, Hektolitergewicht, Körner-Dichte, Fallzahl, Extraktviskoelastizität), chemischen Zusammensetzung (Rohnährstoffe, Nicht-Stärke-Polysaccharide [außer Mais], AS, Mineralstoffe, Inositol-phosphate) und Bruttoenergie-Konzentration, analysiert. Die Konzentration an Rohprotein in den Triticale-, Roggen-, Mais- und Weizenproben lag jeweils im Bereich von

113–138, 108–127, 78–112 und 125–162 g/kg TM. Zusätzliche wurde die *in vitro* Löslichkeit des Stickstoffs (**N**) der Getreidekörner nach Vorbehandlung mit porcinem Pepsin und Pankreatin bestimmt.

Der Tierversuch umfasste 16 Lateinische Quadrate (6x6), die auf 6 aufeinander folgende Durchgänge verteilt waren. Dementsprechend beinhaltete jeder Durchgang zwei bis drei Lateinische Quadrate. Caecectomierte Legehennen (LSL-classic) wurden einzeln in Stoffwechselkäfigen gehalten und erhielten für acht Tage entweder eine Basalration, die zu 500 g/kg aus Maisstärke bestand, oder eine von 80 Getreiderationen, bei denen die Maisstärke jeweils durch eine Getreideprobe ersetzt wurde. Während der letzten vier Tage wurde die Futteraufnahme erfasst und die Exkremente wurden zweimal täglich quantitativ gesammelt. Nach jeder Sammelperiode wurden die Hennen für zwei Tage in Gruppenhaltung in einem Bodenabteil auf Einstreu untergebracht und bekamen eine konventionelle Legehennenmischung vorgelegt. Die AS-Verdaulichkeit der Getreideproben wurde mittels eines linearen Regressionsansatzes berechnet. Beziehungen zwischen der AS-Verdaulichkeit und einzelnen analysierten Fraktionen oder der *in vitro*-Löslichkeit des N wurden durch die Berechnung der Pearsonschen Produkt-Moment-Korrelationskoeffizienten untersucht. Schätzgleichungen zur Vorhersage der AS-Verdaulichkeit wurden mittels multipler Regressionsanalyse berechnet, wobei eine Variablenselektion mittels Stepwise-selection zum Einsatz kam. Hierfür wurden die Variablen entsprechend ihrer Eigenschaften in Pools zusammengefasst und Schätzgleichungen für die AS-Verdaulichkeit wurden mit jedem Pool berechnet. Die Variablen wurden linear und als Kombination linear plus quadratisch angeboten und bei $P \leq 0{,}10$ als signfikanten Prädiktor eingestuft. Die berechneten Gleichungen wurden anhand des adjustierten R^2 und des mittleren quadratischen Fehlers hinsichtlich ihrer Güte beurteilt.

Die AS-Verdaulichkeit variierte stark innerhalb und zwischen den Kulturarten. Die mittlere Verdaulichkeit des Lysins lag jeweils bei 74% (Spanne der Verdaulichkeit: 68–80%), 49% (35–59%), 79% (64%–85%) und 80% (69–87%) für Triticale, Roggen, Mais und Weizen. Für Methionin wurde mit einer mittlerern Verdaulichkeit von 83% (Spanne der Verdaulichkeit: 77–86%), 67% (57−75%), 91% (86−94%) und 84% (70−93%) für Triticale, Roggen, Mais und Weizen eine ähnliche Rangierung festgestellt. Die Korrelationsanalyse zeigte sowohl innerhalb der Kulturarten also auch über die Kulturarten hinweg uneinheitliche Ergebnisse. Bei den physikalischen Eigenschaften wurden signifikante Korrelationen zwischen dem Tausenkorngewicht und der Verdaulichkeit weniger AS des Weizens, sowie zwischen dem Hektolitergewicht und der Verdaulichkeit weniger AS des Roggens und des Maises, nachgewiesen. Signifikante Korrelationen zwischen NSP Fraktionen und der Verdaulichkeit

essentieller AS traten ausschließlich beim Roggen auf. Bei dieser Kulturart war die Konzentration an Arabinoxylanen und gesamten NSP in den Körnern negativ mit der Verdaulichkeit von Arginin, Leucin, Phenylalanin und Threonin korreliert. Die Konzentration an Rohprotein in den Maiskörnern war positiv korreliert mit der Verdaulichkeit der essentiellen AS, ausgenommen Isoleucin, Tryptophan und Valin. Im Gegensatz dazu wurden nur wenige signifikant positive Korrelationen zwischen der Konzentration an Rohprotein und der Verdaulichkeit der essentiellen AS bei Triticale- und Roggenkörnern gefunden. Für den Weizen konnten in diesem Zusammenhang keine signifikanten Korrelationen nachgewiesen werden. Die *in vitro*-Löslichkeit des N war negativ und positiv mit der Verdaulichkeit weniger AS in Triticale- bzw. Roggenkörnern korreliert. Die Genauigkeit der Schätzgleichungen war generell niedrig (adjustiertes R^2 unter 0,7 in den meisten Fällen), und variierte beachtlich sowohl zwischen den Variablenpools für die selbe AS, als auch für den selben Variablenpool bei unterschiedlichen AS. Folglich konnten einzelne oder mehrere physikalische oder chemische Eigenschaften (einschließlich der NSP) die Variation in der AS-Verdaulichkeit bei Legehennen nicht erklären und die Entwicklung von ausreichend genauen Schätzgleichungen für die praktische Anwendung war nicht möglich.

Jedoch könnten Faktoren, die nicht mit den chemischen Analysen erfasst wurden, die Variabilität in der AS-Verdaulichkeit des Getreides verursacht oder einen Teil dazu beigetragen haben. Die Effekte der Feinstruktur der NSP, sowie die strukturelle Anordnung der NSP in der Zellwand wurden diskutiert, da diese Faktoren die Eigenschaften der Polymere und folglich deren Verhalten im Darm, beeinflussen. Die visko-elastischen Eigenschaften bestimmter Proteinfraktionen könnte ebenfalls die AS-Verdaulichkeit des Getreides beeinflusst haben. Es wurde empfohlen diesen Fragestellungen in Folgeprojekten nachzugehen.

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Curriculum Vitae

Personal Details

Name:	Tobias Zuber
Date of Birth:	09.11.1985
Place of Birth:	Heidelberg, Germany

Education

09/1992 – 07/1996	Grundschule Aglasterhausen, Germany
09/1996 – 06/2005	Auguste-Pattberg-Gymnasium Mosbach-Neckarelz, Germany
10/2005 – 07/2006	Diploma Study in Agricultural Biology, University of Hohenheim, Stuttgart, Germany
09/2006 – 05/2007	Community Service at Johannes-Diakonie, Mosbach, Germany
10/2007 – 09/2011	Resumption of Diploma Study in Agricultural Biology, University of Hohenheim, Stuttgart, Germany Degree: Dipl.-Agr. Biol.
Since 10/2011	Research for PhD, Institute of Animal Science, Animal Nutrition Group, University of Hohenheim, Stuttgart, Germany

Practical Experiences

06/2007 – 07/2007	Agricultural internship at the farm of Marc Lucht, Pellworm, Germany
03/2009 – 09/2010	Student Assistant at the Institute of Animal Nutrition, University of Hohenheim, Stuttgart, Germany
04/2011 – 06/2011	Internship at Skretting ARC, Stavanger, Norway
07/2011 – 08/2011	Internship at BASF SE, Global Business Unit Animal Nutrition, Lampertheim, Germany