Elisa Strang (Autor)

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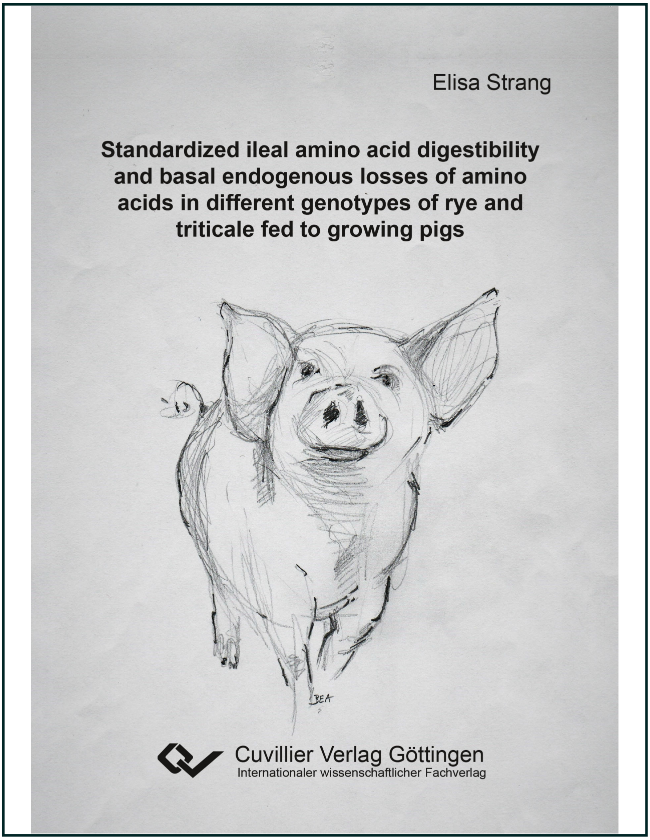

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1 GENERAL INTRODUCTION AND WORK HYPOTHESIS

1.1 INTRODUCTION

Cereal grains, such as rye, and triticale are a major source of energy in pig nutrition. The crude protein (CP) content in cereal grains is rather low, with values ranging from 130 g/kg DM in rye to 163 g/kg DM in wheat, compared to protein ingredients such as soybean meal (SBM), which is the most commonly used protein supplement of plant origin in pig diets (Jezierny, 2009; NRC, 2012). However, as SBM is the major by-product of oil extraction from soybeans, costs and availability are strongly correlated with the price development of agricultural commodities on the world market. Therefore, swine producers will make increasing efforts to use protein from locally produced cereal grains more efficiently to reduce feed costs. Due to their high inclusion level in pig diets, cereal grains can supply up to 60% of the pigs' total amino acid (AA) requirement (Myrie et al., 2008).

Rye, with an annual production of about 9 million tons in the European Union, is less used in livestock feeding compared to wheat and barley with a production of 157 and 60 million tons, respectively (FAOSTAT, 2014). Moreover, the average CP content in rye is relatively low when compared to protein ingredients such as SBM with 47.7% (NRC, 2012). But with its largely beneficial cost factor compared to other cereal grains due to the significantly higher yield increment, especially of hybrid rye, it will continue to be an important crop for farmers in many countries (Budzyński et al., 2003; Meyer et al., 2012). Furthermore, high protein content, combined with a low percentage of fiber, make new rye genotypes an attractive choice for pig feeding (Schwarz et al., 2015). To combine the tolerance to non-optimal growing environments of rye and the yield potential of wheat, triticale was bred to pool the advantages of both parental species (Ammar et al., 2004). The protein content of triticale grain is similar to that of wheat, and the lysine content in the triticale protein is higher than that in wheat protein (Rodehutscord et al., 2016). Thus, triticale is considered to be a suitable feed source for poultry, pigs and ruminants.

Currently available feed tables on the chemical composition and nutritional value of rye and triticale have in common that they hardly consider the impact of recent advances in plant breeding such as introduction of new cultivars, on the nutritive value of rye and triticale. In an effort to further optimize swine production, diet composition should accurately meet pigs` CP and AA requirement. Therefore, a precise evaluation of feed ingredients, with regard to their capacity to supply AA for growth and maintenance, is warranted (Williams, 1995). For diet formulation of pigs, the determination of CP and AA digestibility in feed ingredients at the ileal level has proven to be the most suitable approach to reflect animals' CP and AA requirement (Mosenthin et al., 2000). Furthermore, there is general agreement to transform apparent ileal digestibility (AID) values into their corresponding standardized ileal digestibility (SID) values by correcting for basal ileal endogenous losses (IAA_{end}) of CP and AA to avoid an underestimation of the AID. Standardized ileal digestibility of CP and AA in rye and triticale have been published in several feed tables (e.g. NRC, 2012; Evonik, 2016), but there is scarce information on SID values for newly introduced rye and triticale genotypes.

1.2 RYE

Rye is a member of the grass family *Gramineae* and the genus *Secale*, of which *S. cereale* is the most commonly cultivated species (Bushuk, 2001). It is mostly grown as a winter cereal and is primarily produced for human consumption. Spring rye is superior in extremely cold areas with a long lasting snow cover (Geiger and Miedaner, 2009). However, it has become increasingly important as animal feedstuff primarily in the feeding of cattle and pigs (Meyer et al., 2012; Stein et al., 2016). Rye is mainly cultivated in Europe, with about 75% of the global production in Russia, Poland, Germany, Belarus, and Ukraine, where the grain forms a substantial portion of human and animal diets (Hübner et al, 2013). While production of rye has declined considerably during the 1990s and 2000s (Figure 1), production of wheat, rice, and maize has increased (Bushuk, 2001). However, due to the best overwintering ability and the highest tolerance to drought, salt, or aluminum stress from all small grain cereals, rye may receive more attention in the future. Its current use is highly diversified, especially in hybrid breeding, due to different breeding goals (Miedaner and Hübner, 2011; Wang et al., 2014). The flour is used for bread making, while the whole grain is used for livestock feeding, and to supply as renewable energy source the growing demand for ethanol production (Bushuk, 2001; Geiger and Miedaner, 2009). For baking, pentosane content and thus viscosity should be high, whereas protein content plays not an important role. In contrast, for feeding viscosity should be low and protein content as high as possible. For ethanol production, both low pentosane and protein contents are preferred (Miedaner and Hübner, 2011). Rye flour can be used alone to produce so called "black" bread, which is consumed extensively in Eastern Europe and parts of Asia (Bushuk, 2001). Consumption of whole grain has been shown in epidemiological studies to reduce the risk of many chronic diseases, such as diabetes, cardiovascular disease and certain cancers (Pietinen et al. 1996; Juntunen et al. 2003; McIntosh et al. 2003).

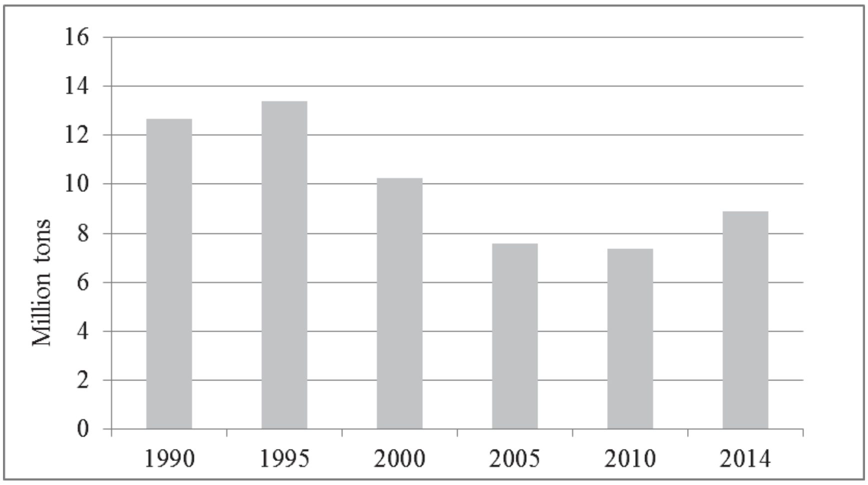

Figure 1: Rye production in the European Union over the last decades (FAOSTAT, 2014).

1.3 TRITICALE

Triticale (× *Triticosecale* sp. Wittmack) is the first reported man made cereal, and was bred in 1875 by crossing female parent wheat (*Triticum* sp) with male parent rye (*Secale* sp). It belongs to the family of Poaceae and is facultative self-pollinated. Annual global production of triticale is about 17 million tons, more than 90% of which is grown in Europe (FAOSTAT, 2014). Winter and spring triticale genotypes have been grown in more than 30 countries, including Germany, Poland, Belarus, and France (Mergoum et al., 2004; FAOSTAT, 2014). Triticale is one of the new and successful species of cereals, developed aiming to combine positive characteristics of both parents into a single plant (Ammar et al., 2004). These positive characteristics include high yields and short straw of wheat on the one hand, and the tolerance to non-optimal growing environments, low demands of climate and soil quality of rye on the other hand (Djekic et al., 2011; McGoverin et al. 2011). Triticale accumulates more nitrogen than wheat in heading period, thus triticale is considered an appropriate culture for cultivation on nitrogen poor soils. High ability of nitrogen accumulation results in decreased requirement for nitrogen fertilizers (Djekic, 2011). Moreover, new triticale varieties may reach the yield of the primary wheat cultivars, while they surpass varieties of rye, barley, and oat (Djekic et al., 2011). Jondreville et al. (2007) confirmed the usefulness of the introduction of triticale in chicken diets due to high phosphorus availability leading to a reduction of mineral supplementation and consequently to lower phosphorus excretion by the birds. Consequently, interest in triticale cultivation has steadily grown in Europe (Figure 2), mainly due to higher yields, associated with lower production inputs and potentially less impact on the environment (Fohner and Hernández Sierra, 2004; Mcgoverin et al., 2011). Although it is currently not widely cultivated in the United States and Canada (FAOSTAT, 2014), areas of triticale cultivation might increase worldwide as climate conditions change and the use of adaptive crops become increasingly important.

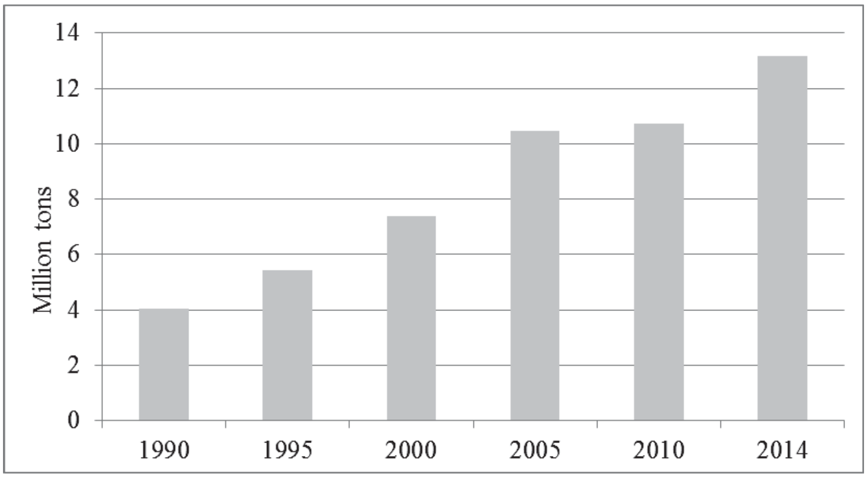

Figure 2: Triticale production in the European Union over the last decades (FAOSTAT, 2014).

1.4 NUTRITIONAL COMPOSITION OF RYE AND TRITICALE

Contents of proximate nutrients and energy of rye and triticale are presented in Table 1. Average CP content is higher in triticale than in rye (130 *vs*. 112 g/kg DM). Regarding chemical composition, triticale is very similar to wheat, except for sugar content which is higher than in wheat and closer to the content in rye. The wheat-like composition of triticale is due to the fact that triticale receives two genomes from wheat and only one from rye (Varughese et al., 1996). Within different cultivars of rye and triticale, there exists a large variation in CP content, ranging from 89 to 130 and from 102 to 183 g/kg DM for rye and triticale, respectively. This large variation may particularly reflect the effect of varying climatic conditions on the protein content of the kernel. In this context, genetic and environmental influences on CP content, yield and test weight of triticale have been investigated (Metayer et al., 1993; Barnett et al., 2006). Barnett et al. (2006) concluded that triticale exhibited considerable genetic diversity and environmental stability for yield and test weight. Persson et al. (2006) showed that the environment markedly influences genetic variation in rye genotypes. Rye and triticale have mean gross energy (GE) content of 17.6 and 17.7 MJ/kg DM, respectively. The CP fraction of wheat is low in lysine, as first limiting AA in pig nutrition, and other essential AA, such as methionine and threonine. In contrast, triticale and rye grains contain higher levels of lysine averaging 4.3 and 4.2 compared to 3.8 g/kg DM in wheat genotypes (Heger and Eggum 1991, Fernandez-Figares et al., 2000; NRC, 2012; Rosenfelder et al., 2013; Schwarz et al., 2015).

Table 1: Content of proximate nutrients, energy, and amino acids in rye and triticale.

Item	Rye 1, 2, 3, 4, 5, 6, 10, 12, 13					Triticale 1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 14, 15				
	Mean	SD	min	max	*n*	Mean	SD	min	max	*n*
DM (g/kg DM)	891	7.3	880	895	4	882	14.7	851	897	6
Proximate nutrients(g/kg DM)										
CP	112	15.0	89	130	6	130	26.7	102	183	7
CA	18	1.3	16	20	7	20	1.9	16	23	8
EE	21	4.9	15	27	7	20	5.1	11	27	8
Energy content (MJ/kg DM)										
GE	17.6	1.16	16.3	18.4	6	17.7	1.57	14.2	18.8	7
DE	14.0	0.44	13.7	14.3	2	14.5	0.96	13.9	15.7	3
ME	13.5	0.25	13.4	13.7	2	13.8	0.42	13.6	14.3	3
NE	10.2	0.14	10.1	10.3	2	10.2	0.42	9.9	10.5	2
Indispensable AA (g/kg DM)										
Arginine	6.1	0.91	5.1	7.8	6	7.3	2.03	4.3	11.0	8
Histidine	2.6	0.33	2.2	3.0	6	3.1	0.62	1.9	4.1	8
Isoleucine	3.6	0.39	3.2	4.3	6	4.5	1.11	2.7	6.4	8
Leucine	6.9	0.75	6.0	7.8	6	8.9	2.22	5.2	12.7	8
Lysine	4.3	0.40	3.8	4.8	6	4.2	1.04	2.7	5.6	8
Methionine	1.8	0.14	1.6	1.9	6	2.4	0.53	1.5	3.0	8
Phenylalanine	5.1	0.56	4.3	5.6	6	5.9	1.54	3.5	9.0	8
Threonine	3.6	0.31	3.3	4.1	6	4.3	1.07	2.6	6.0	8
Tryptophan	1.1	0.07	1.0	1.1	6	1.5	0.34	0.9	2.0	8
Valine	5.1	0.47	4.6	5.8	6	6.0	1.50	3.6	8.7	8
Dispensable AA (g/kg DM)										
Alanine	4.7	0.34	4.2	5.0	6	5.7	1.46	3.5	7.7	7
Aspartic acid	7.9	0.58	7.1	8.6	6	8.4	1.87	5.2	10.8	7
Cystine	2.3	0.20	2.0	2.5	6	3.1	0.88	1.9	4.6	7
Glutamic acid	26.2	3.16	21.0	29.4	6	37.7	10.3	20.7	52.4	7
Glycine	4.8	0.39	4.3	5.4	6	5.9	1.49	3.5	8.0	7
Proline	12.8	3.74	8.6	17.6	4	12.8	1.86	10.6	15.6	5
Serine	4.6	0.46	4.2	4.9	6	6.5	1.61	3.7	8.8	7
Tyrosine	2.5	0.32	2.1	2.4	4	3.6	1.12	1.8	5.0	7

AA, amino acids; CA, crude ash; CP, crude protein; DE, digestible energy; DM, dry matter; EE, ether extracts; GE, gross energy; Max, maximum; ME, metabolizable energy; Min, minimum; NE, net energy; SD, standard deviation.

References: (1) Stein et al. (2016), (2) Rodehutscord et al. (2016), (3) Jondreville et al (2007), (4) Józefiak et al. (2007), (5) Schwarz et al. (2015), (6) Glitsø et al. (1998), (7) Haydon and Hobbs (1991), (8) Perttilä et al. (2002), (9) Çiftci et al. (2003), (10) NRC (2012), (11) Leterme (1991), (12) Evonik Degussa (2016), (13) Cervantes-Pahm et al. (2014), (14) Sullivan et al. (2007), (15) Hale et al. (1985).

Carbohydrate contents of rye and triticale are presented in Table 2. The carbohydrates, which include the low molecular-weight sugars, starch and various cell wall and storage non-starch polysaccharides (NSP), are the most important energy sources for non-ruminant and ruminant animals (Bach Knudsen, 1997). Generally high levels of NSP, such as β-glucan and arabinoxylan (AX), can be found in the cell walls of cereal grains, particularly in soluble form (Bach Knudsen, 2014). The major NSP in triticale and rye are AX, which are also considered to be the main anti-nutritional substances in wheat (Bach Knudsen, 1997; McCann et al. 2006). Soluble AX negatively influence feed consumption, nutrient digestibility and overall growth performance due to high viscosity and water retention properties especially in broilers (Mcgoverin et al., 2011; Bach Knudsen, 2014). On the other hand, dietary inclusion of fermentable carbohydrates such as AX may favor proliferation of beneficial microbiota e.g. members of the *Clostridium* cluster IV and *Roseburia* spp. in feces of pigs (Nielsen et al., 2014). Triticale is more similar to wheat than rye in terms of NSP content. Non-starch polysaccharides content can vary from 120 g/kg DM in triticale to 146 g/kg DM in rye, and accordingly, AX content from 69 to 92 g/kg DM. Variation in content and composition of NSP are influenced considerably by genotype, environmental conditions and harvest year (Hansen et al., 2003).

Starch is the main component of cereal grains, averaging 621 g/kg DM in rye and 663 g/kg DM in triticale. The digestibility of starch mainly depends on its physical structure, which determines the accessibility of intestinal enzymes and also on the ratio between amylose and amylopectin (Svihus et al., 2005). In monogastrics, most of the starch is readily digested and absorbed until the end of the small intestine (Bach Knudsen and Hansen, 1991; Svihus et al., 2005).

Table 2: Carbohydrate content of rye and triticale (g/kg DM)

	Rye 1, 2, 3, 4, 5, 6, 7					Triticale 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13				
	Mean	SD	Min	Max	*n*	Mean	SD	Min	Max	*n*
Starch	621	52.7	519	663	6	663	44.6	564	726	8
Cellulose	14	1.7	12	14.0	4	20	1.2	19	21	2
β-glucan	18	1.8	16	20.1	4	8	2.9	6	14	6
Arabinoxylan	92	15.2	71	112	5	69	10.7	55	85	5
Total NSP	146	13.5	128	164	5	120	13.5	103	131	4
NDF	136	9.5	123	146	4	124	17.8	106	148	5
ADF	33	12	25	51.4	4	35	3.6	32	39	5
ADL	9	0.1	9	9	3	9	0.1	9	9	3

ADF, acid detergent fiber; ADL, acid detergent lignin; Max, maximum; Min, minimum; NDF, neutral detergent fiber; NSP, non-starch polysaccharides; SD, standard deviation.

References: (1) Bach Knudsen (1997), (2) Bach Knudsen (2014), (3) Jondreville et al (2007), (4) Rodehutscord et al. (2016), (5) Glitsø et al. (1998), (6) Stein et al. (2016), (7) NRC (2012), (8) Metayer (1993), (9) Çiftci et al. (2003), (10) Jozefiak 2007, (11) Fernández-Fígares (2008), (12) McGoverin (2011), (13) Leterme et al. (1991).

1.5 FEEDING VALUE OF RYE AND TRITICALE

1.5.1 RYE

Historically, rye was included in diets for pigs at relatively low amounts due to reduced palatability and concentrations of anti-nutritional factors such as AX, the major constituents of soluble dietary fiber fraction (Stein et al, 2016). Furthermore, rye is not recommended as a cereal component in diets for broiler chicks, due to the viscosity and high water holding capacity of rye AX (Boros et al., 1999). It can be even toxic, if rye samples contain ergot, which is one of the most serious diseases of rye. It produces dark purplish-black mycelial mass called sclerotium that replaces the kernel in the rye spikelet (Mirdita et al., 2008). However, techniques of hybrid rye breeding presently developed make it possible to create genotypes with clearly defined end-uses. Therefore, it is postulated that at least two contrasting criteria have to be taken into account in rye breeding programs: rye, developed for human consumption and ethanol production should be selected for a high AX content, whereas varieties intended for animal feed should have low concentrations of these polysaccharides (Boros et al., 2002; Geiger and Miedaner, 2009). New varieties of hybrid rye have reduced concentrations of anti-nutritional factors and are less susceptible to be contaminated with ergot compared to older varieties (Schwarz et al., 2015). Therefore, hybrid rye may be included in diets fed to weanling, growing and finishing pigs at levels of 10, 25, and 50%, respectively, without causing reductions in growth performance or carcass quality (Schwarz et al., 2015). Furthermore, due to its low production costs per ha and its high energy content, rye is particularly attractive for on-farm feed mixers.

1.5.2 TRITICALE

Triticale is primarily used as livestock feed, and may be used in all its forms including grain, forage, silage, hay, and straw (Myer et al., 2004). Furthermore, the superior lysine content of triticale has made it a crop of interest in pig nutrition. Triticale has been substituted into a maize-based diet to investigate its effect on pigs´ growth performance and pork meat quality (Brand et al., 1995, Sullivan et al., 2007). An inclusion rate of up to 67% triticale caused a decrease in average daily growth rate, and sensory and fatty acid profile evaluation indicated that triticale may be fed to pigs without compromising pork quality. Furthermore, Zofia et al. (2011) showed that feeding triticale-based diets to pigs has beneficial effects on performance and carcass characteristics, and triticale could totally replace barley in rations for fattening swine. Performance trials suggest that triticale can be included in laying hen diets at levels higher than 50% (Davidson and Davidson, 1979; Çiftci et al., 2003) without negatively affecting animals’ performance. According to Vatandoodt et al. (2007), no significant difference in milk production was observed when feeding cows triticale, barley or corn whole crop silage. Thus, triticale has proven to be a suitable feed source for poultry, pigs, and ruminants supplied as grain, forage or silage.

1.5.3 INTERACTION BETWEEN RYE AND TRITICALE AND PIGS´ MICROBIOTA

Diets for pigs containing fiber from cereal grains have proven to modulate the intestinal microbiota through changes in intestinal viscosity, selective stimulation of specific bacteria, and they may also influence the amount and composition of metabolites produced by the microbiota in the gastrointestinal tract of pigs (reviewed by Montagne et al., 2003). Glitsø et al. (1998) fed four rye-bread diets (based on whole rye, pericarp/testa, aleurone or endosperm) to growing barrows to assess the effect of total NSP and AX from rye on metabolites of intestinal fermentation. The aleurone flour contains higher amounts of total AX, and is characterized by a higher ratio of insoluble to soluble AX compared with the flour of whole rye. Insoluble AX are fermented more distally in the gastrointestinal tract compared to soluble AX (Le Gall et al., 2009), thus rye aleurone flour containing more insoluble AX than whole rye flour provides more fermentation substrate in the distal parts of the gastrointestinal tract (Glitsø et al., 1998, 1999). As a consequence, bread based on aleurone flour showed higher concentration of total short-chain fatty acids and butyrate in pigs' cecum and colon. Furthermore, feeding a rye- and wheat-based diet to pigs resulted in higher carbohydrate fermentation particularly as AX in the large intestine with the rye diet caused moister feces and significantly enhanced concentrations of butyrate both in the intestinal tract and plasma compared to the wheat-based diet (Bach Knudsen et al., 2005). Short-chain fatty acids and especially butyrate have been beneficially associated with human and animal health (Sakata and Inagaki, 2001). In the large intestine, short-chain fatty acids stimulate the re-absorption of water and sodium (Roediger and Moore, 1981; Slavin et al., 2013), thus limiting the risk of diarrhea. By creating an acidic environment, short-chain fatty acids are capable of inhibiting in pigs the growth of some intestinal bacterial pathogens such as species of *Escherichia coli* and *Clostridium difficile* (May et al., 1994; Bearson et al., 1997; McDonald et al., 2001). It appears that only little research has been done with rye, and almost none with triticale to study the influence of whole grain cereals on pigs´ microbiota.

1.6 STANDARDIZED ILEAL DIGESTIBILITY AND BASAL ILEAL ENDOGENOUS LOSSES OF CRUDE PROTEIN AND AMINO ACIDS IN RYE AND TRITICALE

It is generally acknowledged that measures of AID or SID are used as estimates of AA bio-availability in pig feed ingredients (Mosenthin et al., 2007). Furthermore, there is general agreement to transform AID values into their corresponding SID values by correcting IAA_{end} to avoid an underestimation of the AID in low-protein feed ingredients such as cereal grains. This underestimation is caused by a relatively greater contribution of endogenous AA and CP in ileal digesta collected from pigs fed low-protein diets compared with pigs fed diets containing greater concentrations of CP and AA (Stein et al., 2005). The IAA_{end} are obligatory losses closely associated with the metabolic functions of the animal and are independent of diet type (Stein et al., 2007). Therefore, IAA_{end} represent the minimum losses that can be expected under any feeding situation (Nyachoti et al., 1997). The basal endogenous losses can be measured by different methods. Among other methods, feeding of N-free diets is frequently used to measure basal endogenous losses. Furthermore, they can be determined by feeding diets supplemented with highly digestible protein sources (e.g. casein and wheat gluten) assumed to be completely digestible, or by diets based on enzyme-hydrolyzed casein. Moreover, the IAA_{end} of CP and AA can be estimated by means of regression analysis (Fan et al., 1995; Jansman et al., 2002; Stein et al., 2007). Besides IAA_{end}, there exist also specific endogenous losses which are related to the composition of the feedstuff or diet (e.g. presence of inherent factors such as lectins, trypsin inhibitors and tannins) (Jansman et al., 2002). However, no routine procedures are available to determine specific endogenous losses, but it is possible to calculate these losses by estimating the total endogenous losses and then subtracting the IAA_{end}. The total endogenous losses can be estimated by using the homoarginine technique (Hagemeister and Ebersdobler, 1985; Rutherfurd and Moughan, 1990), the isotope tracer dilution technique (Krawielitzki et al., 1977; Simon et al., 1987; de Lange et al., 1990), and they can be calculated by *in vitro/ in vivo* digestibility differences (Boisen and Fernandez, 1995).

The use of methods for the determination of AA digestibility in assay feed ingredients is dependent on the inclusion level of the ingredient incorporated into the assay diet (Mosenthin et al., 2007). There are basically three methods to measure ileal AA digestibility. With the direct method, the assay feedstuff provides the only protein source in the assay diet, and the AA digestibility in the assay feed ingredient is often assumed to be equal to the corresponding value in the assay diet. An alternative procedure is the difference method, which involves the formulation of both a basal and an assay diet. The basal diet contains the basal feed ingredients, whereas the assay diet consists of a mixture of the basal and the assay feed ingredient (Fan and Sauer, 1995; Mosenthin et al., 2000; Mosenthin et al., 2007). Another method to determine SID of CP and AA is the regression method, where SID values can be directly obtained from the slope of the regression equation. For example, SID of AA can be calculated from apparent ileal digestible and total dietary AA contents, resulting in direct estimates of SID of AA (Fan et al., 1995).

Standardized ileal digestibility of CP and AA has been previously determined for commonly used feeds in pig nutrition including protein supplements and different grains, but information on SID in rye and triticale is still limited. Triticale genotypes have greater SID of CP when compared to rye, with average values of 87 and 83%, respectively (NRC, 2012). Furthermore, SID of most AA is lower in rye than in triticale (NRC, 2012, Evonik, 2016). The greater NSP content in rye compared with triticale may negatively affect the nutritive value of rye. Brestenský et al. (2013) observed significantly lower SID of most AA in rye than in SBM, barley and sorghum, which is ascribed to higher concentrations of NSP in rye genotypes. Jondreville et al. (2001) determined a decrease in digestibility of most AA in triticale as crude fiber (CF) and acid detergent fiber (ADF) contents of the diet increased. This is in agreement with previous studies with growing pigs, where CP digestibility was reduced with increased dietary fiber content (Högberg and Lindberg, 2004; Fernández-Fígares et al., 2008).