## **CHAPTER 1**

### **GENERAL INTRODUCTION AND WORK HYPOTHESIS**

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#### **1.1.** INTRODUCTION

Feed costs represent at least 55 to 65 % of variable costs in swine production, and play a major role in determining the profitability of swine enterprises (Urbaityte et al., 2009a). The ban of meat and bone meal (European Community directive 999/2001) increases the need for alternative protein sources. A popular protein-rich feed ingredient used in pig diets is soybean meal (SBM) which has to be imported from countries outside the European Union (EU) due to suboptimal growing conditions in the EU. Moreover, legislative regulations within the EU challenge nutritionists to formulate diets as close as possible to animals' crude protein (CP) and amino acid (AA) requirements, thus limiting surplus nitrogen (N) excretion to the environment (EC directive 1804/1999; Eklund, 2007). Over the past years, standardized ileal digestibility (SID) of CP and AA has been introduced as worldwide standard in diet formulation for pigs. The key issue in the determination of SID of CP and AA in feedstuffs is the measurement of apparent ileal digestibilities (AID) of CP and AA as well as basal ileal endogenous losses (IAAL<sub>B</sub>) which represent the AA losses that are related to the dry matter (DM) intake of the animal and thus are not influenced by the diet (Eklund, 2007). Until now, many studies have been conducted in growing pigs to determine SID of CP and AA in different protein-rich feed ingredients such as soybeans (SB), peas, faba beans and lupins (e.g. Jezierny et al., 2011). However, it is questionable if tabulated SID values originally determined in grower-finisher pigs can be used in diet formulation for newly weaned piglets (Urbaityte et al., 2009b) as experimental conditions in digestibility trials with grower finisher pigs may vary from those in studies with weaned pigs (e.g. level of feed intake). Furthermore, piglets in comparison to grower-finisher pigs seem to be more susceptible to dietary antinutritive factors (ANF) like fiber or trypsin inhibitors (TI) which may have a negative impact on AID of CP and AA (e.g. Goebel and Stein, 2011). Moreover, Karr-Lilienthal et al. (2004a) reported variations for SID of CP and AA in SBM of different origins fed to growing pigs. However, there are so far no studies on the effect of SBM origin on SID of CP and AA in piglets. Additionally, there is evidence that the determination of SID of CP and AA may also be confounded by age and (or) body weight (BW) of the animals (Caine et al., 1997a,b) or the level of feed intake (Moter and Stein, 2004; Diebold et al., 2005).

#### **1.2.** SOYBEAN PRODUCTION COUNTRIES

During the last decade, SB production within the EU varied between 1.4 million tonnes in 2001 and 0.7 million tonnes in 2008 (Figure 1; FAO, 2012). Between 2008 and 2010, SB production in the EU increased to almost 1.1 million tonnes. Within the EU, Italy had highest SB production amounting to almost 0.6 million tonnes in 2010. Further SB producing EU countries and their respective SB production in 2010 are listed in Table 1.



Figure 1: Soybean production (in million tonnes) in the European Union from 2000 to 2010 (FAO, 2012)

However, the amount of SB produced in the EU is far below the demand for soy products used in human and animal nutrition. Accordingly, high imports of SB and soybean cake (FAO only refers to soybean cake, but the amount probably also includes SBM) amounting to 12.6 and 20.7 million tonnes, respectively, were needed to meet the requirement of the EU for soy products in 2009 (FAO, 2012). Countries with a SB production of more than 1 million tonnes in 2010 were the United States (US), Brazil, Argentina, China, India, Paraguay, Canada, Uruguay, Ukraine, Bolivia and the Russian Federation. In 2010, more than 80 % of the SB world production originated from 3 countries (Figure 2; SoyStats, 2011): the US, Brazil and Argentina. According to the FAO, the SB production of these countries amounted to 90.6, 68.5 and 52.7 million tonnes, respectively, in 2010. However, US grown SB remain in the US to meet the country's own demands, thereby reducing the amounts available for export (Figure 2). These exports include both, SB and fully processed SBM.

Country	Soybean production (tonnes)			
Austria	94544			
Bulgaria	1700			
Czech Republic	16100			
France	139959			
Germany	1000			
Greece	4000			
Hungary	85400			
Italy	552500			
Poland	245			
Romania	149940			
Slovakia	24045			
Slovenia	290			
Spain	1700			

Table 1: Soybean production in countries of the European Union in 2010 (FAO, 2012)



Figure 2: Worldwide production of soybeans and export of soybean meal in 2010 (SoyStats, 2011)

#### **1.2.1.** CHEMICAL COMPOSITION OF SOYBEANS

Soybeans contain 345 to 428 g CP/kg (as fed) and up to 210 g ether extract (EE)/kg (as fed) (Urbaityte et al., 2009a; NRC 2012). The storage protein of SB consists of a mixture of proteins including conglycinin, glycinin and globulins (Wolf, 1970; Urbaityte et al., 2009a). The major protein fraction in SB is made of globulins (60 to 90 %) which are storage proteins, rich in Lys, Arg, Glu, Asp and their amides (Urbaityte et al., 2009a). However, storage

proteins are poor in sulphur AA (Friedman and Brandon, 2001; Urbaityte et al., 2009a), thus SB contain low concentrations of essential Met and Cys. Additionally, SB contain 10 to 20 % albumins with higher Thr and Trp concentrations than globulins (Friedman and Brandon, 2001; Urbaityte et al., 2009a). Furthermore, SB contain bioactive proteins such as lectin, cytochrome and TI. Moreover, several enzymes such as  $\alpha$ -amylase, lipoxygenase and urease secondary metabolites including isoflavones, saponins, well as phytic acid, as oligosaccharides and goitrogens (Liener, 1994; Friedman and Brandon, 2001) are present in SB. Beneficial effects of SB compounds include e.g. decrease of cholesterol levels, anticarcinogenic effects and protective effects against obesity, diabetes, irritants of the digestive tract, and bone and kidney diseases (Friedman and Brandon, 2001). In contrast, adverse effects of SB compounds include poor digestibility and allergy to soy proteins such as glycinin or ß-conglycinin (Friedman and Brandon, 2001). The main growth inhibiting factors of SB and SBM are Bowman-Birk and Kunitz TI, which reduce the activity of chymotrypsin and trypsin. This reduction is associated with poor digestibility and delayed absorption of CP and AA (Huisman and Jansman, 1991; Nitsan, 1991). In addition, specific ileal endogenous AA losses (mainly Met and Cys) are induced by dietary factors such as ANF or fiber (Walker et al., 1986; Nitsan, 1991; Fan et al., 1995). To improve the nutritional quality of SB products, heat treatment during processing aims to inactivate heat labile ANF, e.g. lectins and Kunitz TI (Friedman and Brandon, 2001).

#### **1.2.2.** SOYBEAN PROCESSING AND CHANGES IN CHEMICAL COMPOSITION

Different methods of SB processing (e.g. extrusion/expelling and solvent extraction), resulting in different SB products are shown in Figure 3. In animal nutrition SBM, soy protein concentrate and soy protein isolate play an important role in diet formulation (e.g. for pigs and poultry), due to the high protein content of these products. The chemical composition of different SB products is summarized in Table 2.



Figure 3: Products from soybean processing (Soya & Oilseed Bluebook, 2004)

	SB <sup>a</sup>	SBM <sup>b</sup>	SBM <sup>a</sup>	SBM <sup>a</sup>	Soy protein	Soy protein	
	heat treated	extruded- expelled	solvent extracted	solvent extracted without hulls	concentrate <sup>a</sup>	isolate <sup>a</sup>	
Dry matter (%)	90	92	89	90	90	92	
Digestible energy	4140	-	3490	3685	4100	4150	
(kcal/kg)							
Crude protein (%)	35	47	44	48	64	86	
Ether extract (%)	18.0	1.5	1.5	3.0	3.0	0.6	

Table 2: Chemical composition of selected soybean products (as fed)

SB= soybean; SBM= soybean meal

<sup>a</sup> NRC (1998)

<sup>b</sup> Amipig (2000)

#### Soybean meal:

Soybean meal is obtained after removal of most of the oil from dehulled SB by means of a solvent extraction process (Kellems and Church, 2002; Urbaityte et al., 2009a). Thereafter, defatted flakes are subjected to steam heating, also referred to as toasting, where the residuals of the solvent are removed and, in addition, ANF (mainly TI, lectins) are partial inactivated (O'Quinn et al., 1997; Urbaityte et al., 2009a). High-protein SBM contain between 450 and 500 g/kg CP (as fed) and not more than 30 g/kg crude fiber (Van Eys et al., 2004; Urbaityte et al., 2009a), whereas low protein SBM contain 360 up to 440 g/kg CP (Urbaityte et al., 2009a). The crude fiber content in SBM depends on the amount of sov hulls that are added to the product after the extraction process has been completed (Peisker, 2001; Urbaityte et al., 2009a). Growth performance and ileal CP and AA digestibilities in SBM are dependent on the residual levels of ANF (TI, lectins) and oligosaccharides present in SBM after processing (Walker et al., 1986; Sohn et al., 1994; Fan et al., 1995; Smiricky et al., 2002; Urbaityte et al., 2009a). For example, TI contents in raw SB range from 20.1 up to 32.6 mg trypsin inhibitor activity (TIA)/g product (Huisman and Jansman, 1991; Qin et al., 1998). After toasting, TI contents in SBM may be reduced to levels ranging from 0.2 to 5.4 mg TIA/g product (Van Kempen et al., 2006; Urbaityte et al., 2009a). Accordingly, levels of oligosaccharides, mainly raffinose and stachyose, are reduced to concentrations ranging from 0.6 to 4 % (Van Kempen et al., 2006; Urbaityte et al., 2009a) and from 5.2 to 6 % (Smiricky et al., 2002; Van Kempen et al., 2006; Urbaityte et al., 2009a), respectively.

#### Refined SBM products:

Further processing of SBM or soy flakes results in the production of feeds and foods referred to as soy protein, soy protein concentrate and soy protein isolate (Urbaityte et al., 2009a). In general, these products contain higher CP contents and have lower levels of ANF than SBM (Urbaityte et al., 2009a). Due to higher CP and lower ANF concentrations these highly purified feed ingredients can be used in milk replacers for piglets (Urbaityte et al., 2009a). However, the use of highly purified ingredients (e.g. soy protein concentrate) in animal nutrition may not be cost efficient due to higher processing expenditures (Peisker, 2001; Lakemond and Vereijken, 2003; Urbaityte et al., 2009a).

# **1.2.3.** IMPACT OF GEOGRAPHIC ORIGIN ON NUTRITIONAL VALUE OF SOYBEANS AND SOYBEAN PRODUCTS

#### Soybeans:

There is considerable variation in nutrient contents of SB depending on climate and environmental conditions during growth, genotype and on cultivation practice. For example, the CP content is known to be relatively constant at average temperatures below 28°C, however, with temperatures greater than 28°C during seed fill and maturation CP levels increase linearly (Gibson and Mullen, 1996). As a result, in northern regions of SB production CP contents are generally lower due to climatic conditions such as low temperatures and high amounts of precipitation (Vollmann et al., 2000). In contrast, oil contents increase linearly with temperature up to 25 to 28°C and decline at temperatures above 28°C (Gibson and Mullen, 1996). Furthermore, isoflavone levels in SB decline at high temperatures during growth (Tsukamoto et al., 1995), thus isoflavone and CP contents are negatively correlated (Chiari et al., 2004). Moreover, independent of temperature level, a negative correlation between CP and oil content on the one hand and between CP and carbohydrate content, e.g. stachyose, on the other hand, has been documented (Wilcox and Shibles, 2001). According to Kumar et al. (2010), SB oligosaccharides, i.e. sucrose, stachyose and raffinose contents, are also affected by cultivation region and SB variety. In regions with cooler environmental temperatures of about 15 to 25°C, SB sucrose content was significantly increased compared to regions with temperatures of about 24 to 32°C. Conversely, Kumar et al. (2010) did not find an effect of cultivation region on raffinose and stachyose content in SB, but there was an interaction of genotype and cultivation region.

Several studies have shown considerable differences in nutrient content of SB within and among geographic regions of the world. Different growing conditions (e.g. temperature, humidity, cultivation practice) and the use of various SB genotypes in these regions may lead to variations in the contents of CP, AA, oil (e.g. Piper and Boote, 1999; Vollmann et al., 2000), oligosaccharides (Kumar et al., 2010) and protease inhibitors (Goebel and Stein, 2011). According to a survey on the composition of SB grown in different states of the US in the years 1986, 1987 and 1988, there were consistent regional differences in CP and oil content (Hurburgh et al., 1990). Hurburgh et al. (1990) reported that SB from northern and western SB-growing states (North Dakota, South Dakota, Minnesota, Iowa, Wisconsin) contained 1.5 to 2 % less CP and 0.2 to 0.5 % more oil than SB from the southern states (Texas, Arkansas, Lousiana, Mississippi, Tennessee, Kentucky, Alabama, Georgia, South Carolina, North Carolina). Grieshop and Fahey (2001) compared the chemical composition of Brazilian, Chinese and US SB. The authors reported CP contents of 40.9, 42.1 and 41.6 % (DM basis) for Brazilian, Chinese and US SB, respectively, with significant higher CP contents in Chinese compared to Brazilian SB (Grieshop and Fahey, 2001). Oil content was 18.7, 17.3 and 18.7 % (DM basis) in Brazilian, Chinese and US SB, respectively, and was significantly lower in Chinese SB compared to both other origins (Grieshop and Fahey, 2001). Furthermore, US SB contained highest amounts of both total essential and nonessential AA (Grieshop and Fahey, 2001). Moreover, there existed compositional differences within SB collected from 5 Brazilian states, 6 Chinese provinces or 7 US maturity zones, and it was shown that AA concentrations within US SB were more consistent than those from Brazil or China (Grieshop and Fahey, 2001). Karr-Lilienthal et al. (2004b) reported CP contents of 33, 39, 45 and 37 % in high quality SB originating from Argentina, Brazil, China and the US, respectively.

#### Soybean meal:

Variations in the chemical composition of SB as well as processing conditions may create differences in nutrient content of SBM within and among geographic regions of the world (Baize, 2000; Grieshop et al., 2003). According to Qin et al. (1998), each batch of SB requires its own optimum processing procedure to produce SB products of high nutritional quality. Van Kempen et al. (2002) evaluated variations in nutritional value of SBM processed in the same processing plant over a 45-d period, and variations that exist among various production locations (4 locations in the US and 1 location in the Netherlands). Although there were small differences in nutrient content among locations, the authors concluded that the processing

conditions among the tested locations were rather consistent. On the other hand, as reviewed in Table 3, regional variations in growing and processing conditions of SB can be, at least in part, held responsible for variations in CP and AA contents of SBM.

	Argentina	Brazil	Canada	China	India	Marocco	Paraguay	Spain	USA
$n^1$	64	150	12	16	29	12	13	22	367
CP <sup>2</sup>	46.1	47.6	46.9	45.2	47.1	46.0	48.9	46.9	46.9
	41.7-50.1	42.2-51.7	44.3-49.0	42.1-48.2	42.2-52.1	43.3-47.7	48.0-49.5	42.1-50.5	39.8-51.2
Lys	2.81	2.89	2.86	2.71	2.89	2.83	2.91	2.84	2.89
	2.47-3.15	2.62-3.17	2.68-3.01	2.33-2.86	2.50-3.16	2.73-2.94	2.87-3.06	2.54-3.04	2.32-3.13
Met	0.62	0.62	0.63	0.62	0.62	0.62	0.66	0.62	0.65
	0.54-0.72	0.54-0.69	0.56-0.66	0.58-0.66	0.55-0.68	0.56-0.67	0.65-0.68	0.53-0.66	0.54-0.73
Cys	0.65	0.67	0.68	0.65	0.63	0.68	0.74	0.67	0.68
	0.57-0.76	0.57-0.74	0.63-0.75	0.59-0.70	0.51-0.72	0.64-0.72	0.72-0.78	0.60-0.71	0.51-0.78
Thr	1.80	1.85	1.85	1.76	1.83	1.80	1.91	1.82	1.85
	1.61-2.00	1.64-2.07	1.68-1.93	1.62-1.89	1.60-2.02	1.70-1.92	1.85-1.97	1.59-1.98	1.54-2.08
Trp	0.62	0.64	0.66	0.61	0.61	Not	0.64	0.62	0.63
	0.60-0.68	0.57-0.68	0.65-0.69	0.57-0.63	0.54-0.69	analyzed	0.62-0.65	0.58-0.67	0.52-0.67
Arg	3.34	3.50	3.45	3.27	3.48	3.39	3.50	3.41	3.40
	2.92-3.80	3.05-3.85	3.18-3.69	2.95-3.52	3.05-3.89	3.19-3.59	3.47-3.65	3.01-3.77	2.80-3.77
Ile	2.09	2.18	2.09	2.03	2.14	2.08	2.24	2.14	2.11
	1.88-2.29	1.92-2.44	1.92-2.21	1.86-2.17	1.86-2.40	1.93-2.16	2.17-2.26	1.86-2.36	1.70-2.38
Leu	3.51	3.63	3.35	3.42	3.62	3.49	3.68	3.56	3.55
	3.17-3.85	3.28-3.99	3.27-3.70	3.10-3.66	3.22-4.03	3.32-3.65	3.58-3.74	3.10-3.87	3.03-3.95
Val	2.20	2.25	2.17	2.13	2.23	2.16	2.34	2.23	2.21
	1.91-2.41	1.94-2.48	2.03-2.32	2.00-2.25	1.97-2.51	1.96-2.30	2.29 <b>-</b> 2.36	1.96-2.42	1.81-2.49

Table 3: Average contents and range of CP and indispensable AA (%) in soybean meal (88 % DM) of different origin (adapted from AminoDat 4.0, 2010)

 $^{\rm 1}$  number of soybean meal samples which were analyzed for the different AA, except for Trp  $^{\rm 2}$  crude protein

Finally, variations in nutrient digestibility of various SBM fed to pigs may reflect differences in their chemical composition (Smiricky-Tjardes et al., 2003; Karr-Lilienthal et al., 2005). For example, higher amounts of SB galactooligosaccharides either present in SBM or being added to a SB protein concentrate reduced digesta transit time in weanling piglets (Zhang et al., 2001). As a result, ileal and total tract digestibility of DM, organic matter and CP in growing pigs decreased (Smiricky-Tjardes et al., 2003). However, according to the results of another study with growing pigs, there were only minor effects of oligosaccharides in SBM on SID of