1. GENERAL INTRODUCTION AND WORK HYPOTHESIS

1.1. INTRODUCTION

In the European Union (EU), soybeans are the most important protein source included in pig diets (Bertheau et al., 2011). More than 60 to 70% of soybeans used in the EU are imported from oversea countries, amounting to nearly 30 million tons in 2015 (USDA, 2016a). For sustainability in the future, protein feedstuffs that are cultivated in parts of Europe can be used for livestock feeding including pigs. Until 2015, the production of home-grown European soybeans has been gradually increased in several central and eastern European countries along Danube river amounting to 7.5 million tons (Krön and Bittner, 2015), which is still below the production of rapeseed in the EU amounting to 25 million tons (USDA, 2016b). Therefore, the reduction of soybean imports can be steadily compensated by increased availability of home-grown European soybean and rapeseed products.

In diet formulation for pigs, the determination of crude protein (CP) and amino acid (AA) digestibility in feed ingredients at the ileal level has proven to be a more suitable approach for meeting animals' CP and AA requirements rather than fecal digestibility (Mosenthin et al., 2000). Over the last decade, standardized ileal digestibility (SID) of CP and AA has been widely accepted as an accurate estimate for bioavailability of AA in feed ingredients (Stein et al., 2007). However, there is a scarcity of information on the SID of CP and AA in home-grown European soybean and rapeseed products, and these values may be different from published values due to the differences both in environmental and processing conditions.

With regard to anti-nutritional factors (ANF), the presence of trypsin inhibitor in soybean products has been reported to adversely affect SID of CP and AA (Goebel and Stein, 2011), whereas high glucosinolates (GSL) in rapeseed products have been shown to negatively impact feed intake and growth performance (Bell, 1984; Mawson et al., 1994). Thermal treatment can efficiently reduce trypsin inhibitor activity (TIA) in soybean products (e.g. Kaankuka et al., 1996; Messerschmidt et al., 2012) as well as GSL contents in rapeseed products (Eklund et al., 2015). However, an excessive heat treatment may have a negative impact on the stability of AA due to the formation of Maillard reaction products

(Pahm et al., 2008), which is reflected in lower SID of CP and AA in soybean meal (SBM; Messerschmidt et al., 2012) and rapeseed meal (RSM; Eklund et al., 2015).

Due to legislative regulations within the EU (EC directive 1804/1999), low-protein diets can be used to reduce surplus nitrogen excretion to the environment (Ferket et al., 2002; Adebiyi et al., 2015). Reducing the dietary CP content has also been shown to influence microbial composition in the gut (Rist et al., 2014) resulting in lower production of harmful microbial metabolites (Htoo et al., 2007). In contrast, increasing dietary CP content may stimulate microbial fermentation of undigested CP, and encourage proliferation of pathogenic bacteria (Ball and Aherne, 1987). Furthermore, dietary supplementation of probiotics such as *Bacillus* spp. may result in positive effects on microbial activity, thereby improving gut health and growth performance (Chen et al., 2006; Meng et al., 2010). Recently, weaned pigs fed diets supplemented with *Bacillus* spp. showed slight improvements in ileal digestibility of some AA (Kim et al., 2015), which has been associated with enhanced production of digestive enzymes induced by *Bacillus* spp. (Ferrari, 1993; Davis et al., 2008). However, the combined effect of supplementation with *Bacillus* spp. to low- and high-protein diets on ileal CP and AA digestibility and microbiota composition in growing pigs has not yet been determined.

1.2. Home-grown European soybean and rapeseed producing countries

1.2.1. Soybean production

The world production of soybean was 320 million tons in 2015 (USDA, 2016b). The top three soybean producing countries in 2015 were the United States, Brazil and Argentina, with a production of more than 265 million tons (USDA, 2016b) compared to the European soybean production of 7.5 million tons (Krön and Bittner, 2015). However, approximately 5-7 million tons of soybeans used in the EU are genetically modified organisms-free (GMO-free), and one of third (1.5-2.0 million tons) of the demand can be currently supplied by European cultivation (Krön and Bittner, 2015).

In the EU, GMO-free soybeans have been cultivated mainly in central and eastern European countries including Southern Germany and Austria (Krön and Bittner, 2015). The GMO-free soybean production increased rapidly from 1.6 million tons in 2013 to 2.5 million tons in 2015. The proportion of individual European countries to the whole soybean production in Europe is shown in Figure 1. Furthermore, it is predicted that the

GMO-free soybean production will increase by 5.6 million tons in 2025 (Krön and Bittner, 2015). Therefore, home-grown European soybeans may steadily substitute imported soybean products, especially GMO-free, from oversea countries to ensure sustainability in the future.

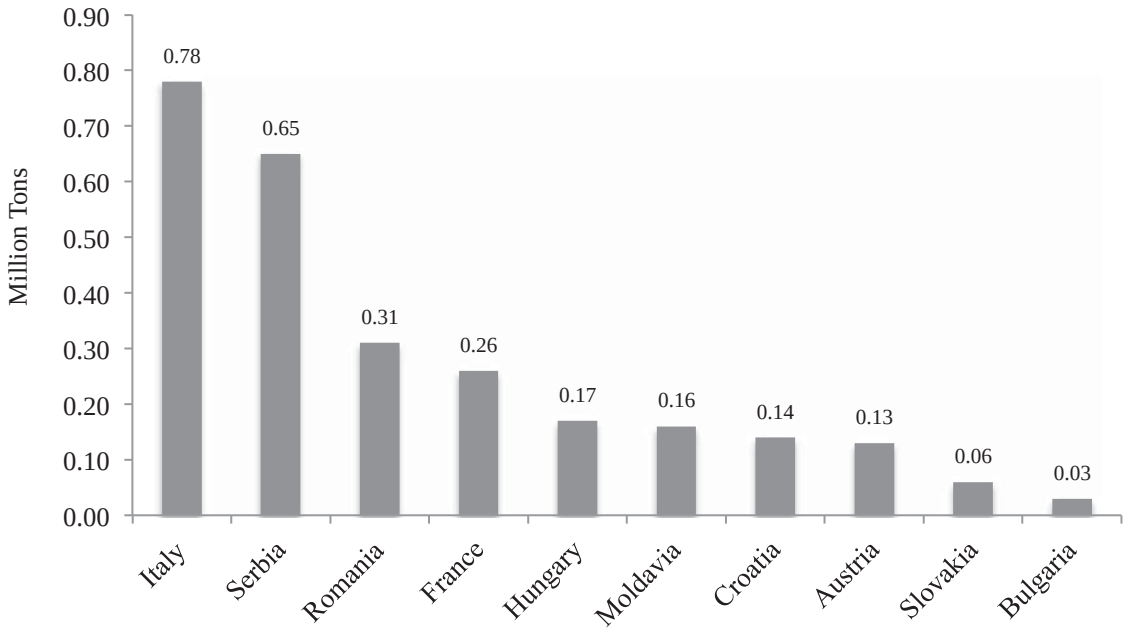

Figure 1: Soybean production in countries of the European Union in 2015 (Krön and Bittner, 2015)

1.2.2. RAPESEED PRODUCTION

Rapeseeds rank second in terms of world oilseed production behind soybeans, amounting to 71 million tons (USDA, 2016b). The leading producers in the world include the EU, Canada, China and India with a total production of approximately 61 million tons in 2015. In the EU, France exhibits highest production, followed by Germany and Poland, amounting to 5.29, 5.02 and 2.70 million tons in 2015, respectively (Eurostat, 2016). The top ten rapeseed producing countries in the EU are displayed in Figure 2.

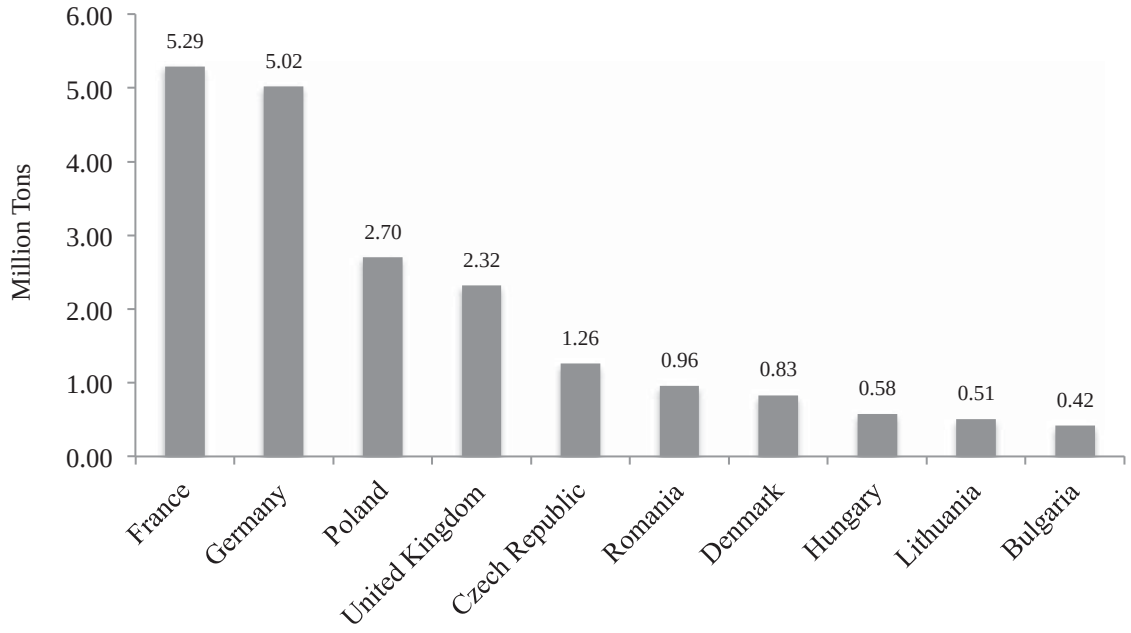

Figure 2: The production of rapeseeds in the top ten countries of the European Union in 2015 (Eurostat, 2016)

1.3. CHEMICAL COMPOSITION OF SOYBEAN AND RAPESEED PRODUCTS

Soybeans are an excellent source of AA that can be fed to pigs as FFSB or defatted soybean products (Baker et al., 2010). The FFSB contain approximately 41% CP and 22% ether extract (EE) on dry matter basis (DM; NRC, 2012). However, the use of soybean products in diets for monogastric animals is often limited due to the presence of heat-labile trypsin inhibitors (Goebel and Stein, 2011). Different methods of heat processing (e.g. roasting, expelling and solvent extraction) have proven to be capable of lowering TIA in soybean products (Qin et al., 1996; Baker et al., 2009). An overview of the processing of soybeans to obtain different soybean products is shown in Figure 3. For processing of FFSB, several heat treatment procedures such as roasting, autoclaving and extrusion have been applied to reduce TIA resulting in improved daily gain and feed efficiency (Herkelman et al., 1992; Marty and Chavez, 1993). Furthermore, FFSB can be defatted to produce soybean cakes (SBC; Woodworth et al., 2001) and SBM (Baker et al., 2010). The removal of oil can be accomplished using the mechanical oil press for the production of SBC or the solvent extraction method for manufacturing of SBM (Berk, 1992). On DM basis, solvent extraction results in less than 2.0% oil in SBM, whereas up to 7% oil remains in SBC after screw pressing or expelling (NRC, 2012). As a result, the CP content is greater in SBM than in SBC (NRC, 2012).

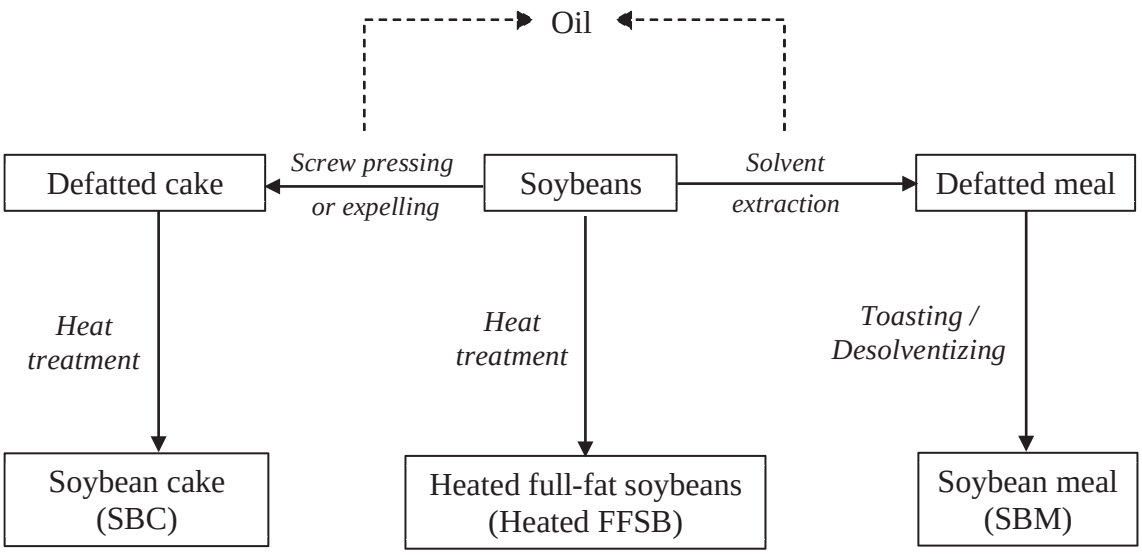

Figure 3: Processing of soybeans into soybean products

Rapeseed co-products upon the removal of oil such as rapeseed cake (RSC) and RSM can be used as an alternative protein source for pig diets (Maison and Stein, 2014). However, if GSL contents in rapeseed products exceed certain threshold levels, pigs' feed intake may be impaired due to bitter taste (Spiegel and Blum, 1993; Schöne et al., 2001) resulting in lower growth performance (Bell, 1984; Mawson et al., 1994). For production of RSC, oil is partially removed by using different pressing equipment at low temperature (Li et al., 2002), whereas RSM is the co-product from solvent-extraction with high temperature in the desolventizer/toaster (Eklund et al., 2015). As a result, CP and EE content in RSC amounts to approximately 36 and 8% on a DM basis, respectively, whereas RSM contains approximately 43% CP and 2% EE, on a DM basis (CVB, 2011). Furthermore, defatted rapeseed products are rich in methionine and cystine when compared to defatted soybean products (Jondreville et al., 2000; NRC, 2012). However, the presence of rapeseed hulls is associated with greater specific endogenous losses of CP and AA (Grala et al., 1998; Jondreville et al., 2000) resulting in lower SID of CP and AA in rapeseed products when compared to soybean products (CVB, 2011; NRC, 2012). The chemical composition of different soybean and rapeseed products is shown in Table 1.

With regard to pig farming systems in the EU, organic farming is primarily based on home-grown European feedstuffs, and excludes the use of oilseed meals such as SBM and RSM as these protein supplements are exposed to chemical solvent-extraction treatment during manufacturing (IFOAM, 2005). Therefore, FFSB, SBC and RSC are the

major protein sources to be used in diets for pigs kept in organic farming systems (Sundrum et al., 2005).

Table 1: Chemical composition of soybean and rapeseed products (dry matter basis)

	Soybeans[1]			Rapeseeds[2]	
	Full-fat soybeans	Soybean cake	Soybean meal	Rapeseed cake	Rapeseed meal
Crude protein (%)	40.7	47.5	49.4	36.2	42.8
Ether extract (%)	21.9	6.1	1.4	8.4	1.8
Neutral detergent fiber (%)	10.8	14.8	11.1	23.4	26.2
Acid detergent fiber (%)	6.7	7.8	7.5	17.9	20.1
Indispensable amino acids (%)					
Arginine	2.65	3.34	3.57	2.21	2.62
Histidine	0.95	1.25	1.42	1.02	1.20
Isoleucine	1.73	2.10	2.21	1.41	1.67
Leucine	2.89	3.51	3.86	2.54	3.00
Lysine	2.41	3.04	3.11	1.99	2.35
Methionine	0.60	0.60	0.68	0.73	0.86
Phenylalanine	1.88	2.33	2.55	1.49	1.75
Threonine	1.54	1.84	1.98	1.60	1.89
Tryptophan	0.53	0.71	0.66	0.47	0.55
Valine	1.87	2.19	2.17	1.85	2.19
Dispensable amino acids (%)					
Alanine	1.72	2.01	2.16	1.63	1.93
Aspartic acid	4.21	5.16	5.50	2.72	3.21
Cystine	0.64	0.75	0.77	0.91	1.07
Glutamic acid	6.55	8.06	8.86	6.13	7.24
Glycine	1.65	2.01	2.13	1.88	2.23
Proline	1.79	2.30	2.74	2.17	2.57
Serine	1.81	2.25	2.41	1.60	1.89

[1]NRC (2012)
[2]CVB (2011)

1.4. HEAT TREATMENTS OF OILSEED PRODUCTS

Heat treatment is an effective method for inactivating ANF such as TIA in soybean (Marty and Chavez, 1993) and GSL in rapeseed products (Eklund et al., 2015). Dry heating and wet (moisture) heating represent the major heating techniques. In general, dry heating refers to the use of hot air only, while wet heating (WH) is includes application of steam resulting in a hot and humid environment during processing (Newkirk, 2010). Wet heating is a more efficient heat treatment than dry heating, as greatest decrease in soluble CP and non-protein nitrogen content was observed in FFSB under wet heating compared to dry heating conditions (Samadi and Yu, 2011). Furthermore, there exist alternative heat treatments including roasting (Zollitsch et al., 1993), toasting (Qin et al., 1996), expansion (Fancher et al., 1996), extrusion (Liu et al., 2013) and autoclaving (AC; Herkelman et al., 1992).

Roasting is commonly accomplished by passing the soybeans through a flame in a continuous flow system, which provides a high temperature for a short time (Agulanna et al., 2013). The heat can be generated by oven, gas flame or electricity. Roasting temperature must be carefully controlled to ensure that the oilseeds are heated sufficiently to eliminate the ANF, but not too excessive to avoid negative effects on AA digestibility (Agulanna et al., 2013). Previous studies (Marty and Chavez, 1993; Zollitsch et al., 1993) demonstrated that optimum roasting was achieved when soybeans were heated at 110-130°C for 2-5 min.

Toasting is a heat processing technique, which uses lower temperature when compared to roasting (Zollitsch et al., 1993). The raw soybeans are commonly toasted at 100-105°C for 30-45 min (Zollitsch et al., 1993; Qin et al., 1996). This process is mainly used to remove the solvent held in oilseed meals as desolventizing process after oil extraction (Messerschmidt et al., 2012). Under typical commercial processing conditions, SBM is toasted at 100-105°C for 15-30 min (Witte, 1995), whereas RSM is toasted at 100°C for 30 min (Jensen et al., 1995).

Extrusion is a complex process, which utilizes friction as a heat source, together with the use of pressure and attrition (Marty and Chavez, 1993). This technology has been used in processing of soybeans to reduce TIA resulting in improved pig performance (Hines et al., 1990). Heat and pressure are generated by passing materials through a barrel

by means of a screw with increasing resistance and finally discharging the product into the atmosphere (Marty and Chavez, 1993). One or two screws are available. Temperature range from 80 to 200°C, and retention time ranges from 30 to 150 seconds depending on the use of dry or wet extrusion techniques.

Expansion is similar in design to a single screw extrusion, but expansion could reduce energy consumption and increase production rate due to the lower temperature and shorter time than extrusion (Fancher et al., 1996). The temperature ranges from 93 to 127°C, and retention time ranges from 10 to 25 seconds (Liu et al., 2013).

Autoclaving is an effective wet heating process for decreasing TIA in soybean products (Batal et al., 2000), as this method is associated with pressure, moisture and high temperature (González-Vega et al., 2011). The heating temperature for FFSB is 110°C for 20-40 min (Herkelman et al., 1992).

The efficiency of heat treatment to reduce ANF may vary considerably with processing types and operating conditions (Marty and Chavez, 1993; Qin et al., 1996). For FFSB processing, roasting has proven to be popular for farmers who raise livestock (Agulanna et al., 2013). According to a previous study, roasted FFSB exhibits lower TIA when compared to toasted FFSB and extruded FFSB (Zollitsch et al., 2013). However, the lower TIA in roasted FFSB may indicate over-heating of FFSB resulting in destruction of AA (Wöhlbier and Fangauf, 1983; Frank, 1988).

1.5. Feed processing and Maillard reaction products

The thermal treatment during feed processing can induce reactions between reducing sugars and free amino groups, resulting in the formation of Maillard reaction products (Faist and Erbersdobler, 2001). A series of Maillard reactions including initial, intermediate and final stages is shown in Figure 4. At the initial stage, reducing sugars (such as glucose and lactose) react with free amino groups of AA or protein (Ledl and Schleicher, 1990; Ames, 1992), giving rise to so called Amadori products (Tamanna and Mahmood, 2015). At the intermediate stage, dehydration and fragmentation take place in the sugar molecules, in addition to potential degradation of AA at this stage (Tamanna and Mahmood, 2015). The derivatives from this stage are referred to as advanced Maillard reaction products, acting as melanoidin precursors and premelanoidins (Finot et al., 1981; 1989). The color of these products has been described as light yellow or colorless

(Tamanna and Mahmood, 2015). At the final stage of the Maillard reaction, aldol condensation occurs, and finally heterocyclic nitrogen compounds form melanoidins exhibiting an intense brown color (Hodge, 1953).

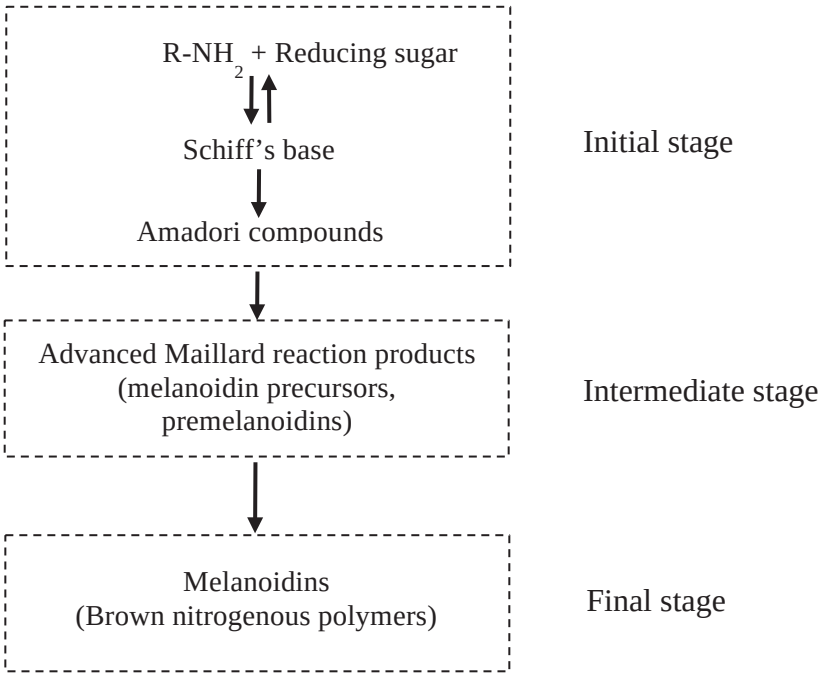

Figure 4: Overview of Maillard reactions (adapted from Faist and Erbersdobler, 2001)

Amadori compounds may be absorbed and excreted in feces (Erbersdobler et al., 1981). According to Erbersdobler et al. (1970), the absorbed Amadori compounds cannot be used for protein synthesis as these compounds are excreted in urine. However, Finot and Magnenat (1981) reported that Amadori compounds may accumulate in different tissues of the body such as kidneys, liver and pancreas. For advanced Maillard reaction products, previous studies (Finot and Magnenat, 1981; Homma and Fujimaki, 1981; Nair et al., 1981; Finot, 1990; Lee et al., 1992) indicated that only small amounts were absorbed through the gut wall, whereas most of them are excreted in feces. The advanced Maillard reaction products are in parts biologically unavailable for protein deposition (Finot and Magnenat, 1981; Hurrell and Carpenter, 1981), but according to Faist and Erbersdobler (2001), absorbed advanced Maillard reaction products such as malanoidins may be retained in the kidneys.

1.6. ILEAL CRUDE PROTEIN AND AMINO ACID DIGESTIBILITY

The use of ileal digestibility is more accurate to describe CP and AA bioavailability than fecal digestibility as dietary CP and AA are absorbed from the small intestine only (Sauer and Ozimek, 1986). Digestibility values can be expressed as apparent, true and standardized CP and AA digestibility, depending on the consideration of endogenous CP and AA losses (Stein et al., 2007).

1.6.1. APPARENT ILEAL DIGESTIBILITY (AID)

The AID of CP and AA is calculated as the percentage of CP and AA intake that does not appear in ileal digesta (Mosenthin et al., 2000). Values for AID are calculated according to the following equation:

AID (%) = [(CP or AA intake – ileal CP or AA outflow)/CP or AA intake] × 100

However, the use of AID is limited in feed formulation due to the lack of additivity of AID in mixture of feed ingredients. This has been largely attributed to the effect of dietary CP and AA level on AID values due to changes in the relative contribution of endogenous CP and AA losses to total CP and AA output (Stein et al., 2007). This is in agreement with Fan et al. (1994, 1995), where AID of CP and AA showed a quadratic response with plateau relationship as the dietary CP content increases from 4 to 24% CP (as-fed basis). Therefore, estimates of AID values are considered to be crucial, in particular, when feed ingredients with low CP and AA levels are included in the pig diets (Jansman et al., 2002; Stein et al., 2005).

Thus, “true” or “standardized” ileal digestibility is considered as alternative to AID of CP and AA in pigs. The transformation is associated with the correction of two main fractions in ileal endogenous losses (Stein et al., 2007) that originate from various sources such as saliva, pancreatic and bile secretions, sloughed off epithelial cells and from mucus (Souffrant, 1991).

1.6.2. ILEAL ENDOGENOUS LOSSES

The total ileal endogenous losses can be divided into basal (non-specific) and specific endogenous losses (Mosenthin et al., 2000). The sources of CP and AA at the terminal ileum are shown in Figure 5.