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In Vivo and *in Vitro* Studies with Growing Pigs on Standardised Ileal Amino Acid Digestibilities in Grain Legumes

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IN VIVO AND *IN VITRO* STUDIES WITH GROWING PIGS ON STANDARDISED ILEAL AMINO ACID DIGESTIBILITIES IN GRAIN LEGUMES

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
AA	Amino acid
ADF	Acid detergent fibre
ADL	Acid detergent lignin
AID	Apparent ileal digestibilities
ANF	Antinutritional factor
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists` Society
BW	Body weight
CO_2	Carbon dioxide
CP	Crude protein
CV	Coefficient of variation
cv.	Cultivar
CVB	Centraal Veevoederbureau
d	Day
DE	Digestible energy
DLG	Deutsche Landwirtschaftsgesellschaft
DM	Dry matter
DMI	Dry matter intake
DN	Digested nitrogen
EC	European Commission
EE	Ether extract
EU	European Union
g	Gram
GfE	Gesellschaft für Ernährungsphysiologie
h	Hour
H_2O_2	Hydrogen peroxide
HCl	Hydrogen chloride
$IAAL_B$	Basal ileal endogenous crude protein and amino acid losses
kg	Kilogram
LSmeans	Least square means
m	Meter
M	Molar
ME	Metabolisable energy
mg	Milligram

MJ	Megajoule
ml	Milliliter
mm	Millimeter
µm	Micrometer
N	Nitrogen
n, *n*	Number of observations
NaOH	Sodium hydroxide
$NaHCO_3$	Sodium hydrogen carbonate
NE	net energy
NDF	Neutral detergent fibre
NH_3	Ammonia
NRC	National Research Council
NSP	Non-starch polysaccharides
p, P, *P*	Probability
pH	Potentia hydrogenii
r^2	Coefficient of determination
SAS	Statistical Analysis System
SBM	Soybean meal
SCFA	Short chain fatty acids
SE	Standard error
SEM	Standard error of mean
SD	Standard deviation
SID	Standardised ileal digestibilities
spp.	Species
TI	Trypsin inhibitor
TIA	Trypsin inhibitor activity
TID	True ileal digestibilities
TiO_2	Titanium dioxide
TIU	Trypsin inhibitor unit
UDM	Undigested dry matter
UFOP	Union zur Förderung von Oel- und Proteinpflanzen e.V.
vs.	Versus
wt/vol	Weight per volume

Amino acids

Arg	Arginine
His	Histidine
Ile	Isoleucine

Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Thr	Threonine
Trp	Tryptophan
Val	Valine
Ala	Alanine
Asp	Aspartic acid
Cys	Cystine
Glu	Glutamic acid
Gly	Glycine
Pro	Proline
Ser	Serine
Tyr	Tyrosine

CHAPTER 1

GENERAL INTRODUCTION:

THE USE OF GRAIN LEGUMES AS A PROTEIN SOURCE IN PIG NUTRITION

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1 General Introduction: The Use of Grain Legumes as a Protein Source in Pig Nutrition

1.1 Summary

Grain legumes are valuable sources of protein and energy for monogastric animals. Grain legumes, such as faba beans, peas and lupins, can partially or even totally replace traditional protein sources of animal origin such as meat and bone meal or fish meal. Moreover, they represent an alternative protein-rich feed ingredient for soybean meal (SBM) and other oilseed meals. However, the presence of secondary plant metabolites, also referred to as antinutritional factors, such as protease inhibitors, saponins, pyrimidine glycosides, lectins, tannins, and alkaloids, has restricted the use of grain legumes in pig feeding. Furthermore, a high proportion of α-galactosides present in some grain legumes may lead to excessive fermentation and diarrhoea, while high levels of non-starch-polysaccharides (NSP) may have a negative impact on energy utilisation. Among different processing methods designed to further improve the nutritive value through reductions in content of secondary plant metabolites, recent progress in plant breeding has contributed to the commercial release of cultivars with improved feeding value in association with lower contents of secondary plant metabolites. This review focuses on the evaluation of the nutritional value of currently available cultivars of faba beans, peas and lupins, and their use in pig diets. Special interest is directed to nutritional composition, energy and amino acid (AA) digestibility of faba beans, peas and lupins, but also to their contents of secondary plant metabolites, including the threshold levels to be accounted for in diet formulation for pigs. Furthermore, feed processing technologies developed to improve the nutritive value of grain legumes are introduced.

1.2 Introduction

In 2001, the use of meat and bone meal and its by-products in diets for livestock was banned by the European Commission (EC directive 999/2001) to assure consumer safety on animal products. Soybean meal (SBM) is the most commonly used protein supplement of plant origin in pig diets, and is generally known as protein source with a high and consistent product quality. As SBM is the major by-product of oil extraction from soybeans, costs and availability of SBM are strongly correlated with the price development of

agricultural commodities on the world market. Factors which may influence world market prices include variations in population and economic growth, changes in consumer's product preferences, but world market prices are also dependent on weather conditions (Gill, 1997; Trostle, 2008). Therefore, price and availability of SBM on global markets may change rapidly, thereby stimulating swine producer's interest to maximise the use of locally produced feed ingredients including grain legumes. Furthermore, organic farming does not accept the use of processed oilseed products, when subjected to solvent extraction processes (e.g. hexane), or the use of genetically modified feed ingredients (e.g. soybeans), and in addition, the supplementation with crystalline amino acids (AA) to balance pigs' diet according to their AA requirement is prohibited (IFOAM, 2005). Therefore, it is crucial to develop suitable alternatives to meet the animals' protein and AA requirement in organic production systems according to IFOAM standards (2005). However, in the European Union, over 20 million tons of protein feeds are annually used in compound feeds for livestock, but only six million tons of protein feeds are produced within the European Union (Blair, 2007).

The search for alternative protein sources has led to an increasing interest in the use of grain legumes, as they supply an important source of plant protein. Moreover, grain legumes are grown as a nitrogen-fixation crop in rotation systems (López-Bellido et al., 2005). When grown in rotation with other crops, the use of nitrogen fertiliser can be reduced (Peoples et al., 1995) and soil fertility may be improved, whilst incidence of weeds, diseases and pests may be lowered (Peoples et al., 1995; Mwanamwenge et al., 1998). However, the use of grain legumes in animal nutrition has been hampered due to partially high concentrations of secondary plant metabolites, also referred to as antinutritional factors (ANF), including condensed tannins, protease inhibitors, alkaloids, lectins, pyrimidine glycosides and saponins. Possible negative effects of these secondary plant metabolites include, for example, feed refusals (tannins, alkaloids), reduced nutrient digestibilities (tannins, protease inhibitors, lectins) or even toxic effects (alkaloids) (e.g. Lallès and Jansman, 1998; Huisman and Tolman, 2001). Furthermore, a high proportion of α-galactosides as a part of the carbohydrate fraction in some grain legumes (Mul and Perry, 1994) may cause antinutritional effects in pigs, such as excessive fermentation, flatulence and diarrhoea (Benno et al., 1987; Fishbein et al., 1988). Moreover, considerable amounts of non-starch-polysaccharides (NSP) are present in several grain legumes such as lupins (Bach Knudsen, 1997), These NSP may exert negative effects in growing pigs, including reduced digesta passage rate which, in turn, may result in a

lowered feed intake and decreased growth performance (Dunshea at al., 2001; Ferguson et al., 2003). Due to considerable progress in plant breeding, the level of secondary plant metabolites present in grain legumes has been notably decreased, resulting e.g. in the development of zero-tannin faba bean cultivars (Duc et al., 1999) and sweet lupins (Petterson, 1998). Furthermore, progress in plant breeding offers possibilities for growing grain legumes with higher protein content but also better protein quality (Monti and Grillo, 1983; Clarke and Wiseman, 2000). On the other hand, it has to be emphasised that growing and harvesting conditions may also affect these characteristics (Mossé and Baudet, 1983; Simon and Köhn, 2004). Finally, several processing methods are available to lower the amount of secondary plant metabolites and NSP in grain legumes, thereby improving their feeding value. Thus, there is a need to re-evaluate the nutritional characteristics of currently available cultivars from faba beans, peas and lupins to optimise their utilisation as a locally produced feed component of high nutritional value in diets for pigs.

1.3 GRAIN LEGUMES

The botanical family of grain legumes is known as fabaceae, also referred to as leguminosae. Grain legumes are cultivated primarily for their seeds which are harvested at maturity, and which are rich in protein and energy. The mature dry seeds of grain legumes are used either as animal feed ingredient or for human consumption (Singh et al., 2007). Generally, legumes are characterised by their ability to use atmospheric nitrogen as a nutrient due to the symbiosis with nitrogen-fixing bacteria from the *Rhizobium* species (Sprent and Thomas, 1984; Zahran, 1999). Therefore, unlike other cultivated plants, legume crops need less nitrogen fertiliser for optimal growth, and the use of legumes in crop rotation systems reduces the need of nitrogen fertiliser in subsequent crops (Rochester et al., 1998). Nitrogen benefits in legume-cereal rotation systems have been attributed not only to the transfer of biologically fixed nitrogen (Chalk, 1998; Evans et al., 2001), but also to lower immobilisation of nitrate in the soil during the decomposition of legumes compared to cereal residues (Green and Blackmer, 1995), also termed as nitrogen-sparing effect. Thus, nitrogen benefits may result from a combination of legume nitrogen-sparing effects and the bacterial nitrogen fixation (e.g. Chalk et al., 1993; Herridge et al., 1995). In addition, crop rotation and intercropping with legumes may provide successful strategies for weed suppression (Liebman and Dyck, 1993; Bulson et al., 1997). Weed growth and development may be disrupted due to varying cultivation

conditions prevailing for the different crops used (e.g. fertiliser requirements, planting or maturation dates), thereby preventing domination of only a few weed species (Froud-Williams, 1988; Liebman and Janke, 1990). Due to these pioneer crop effects, cultivation of grain legumes is an important part of crop rotation, particularly in organic farming (Poetsch, 2006; Badgley et al., 2007). In animal nutrition, grain legumes are mainly used as protein supplements, but also as a valuable energy source, due to their partly high contents of starch (faba bean, peas) and lipids (lupins) (Gatel, 1994; Bach Knudsen, 1997; Salgado et al., 2002). In Europe, amongst others, the major grain legumes cultivated are peas (*Pisum sativum*), faba beans (*Vicia faba*) and lupins (*Lupinus* spp.), whereas in Argentina, Brazil, China, India, and the United States soybeans dominate (Karr-Lilienthal et al., 2004).

1.3.1 FABA BEANS (*VICIA FABA*)

Faba beans, also known as field beans, horse beans, broad beans or tick beans, represent an annual legume that is well adapted to cool climates, and thus is preferably cultivated in regions with mild winters and adequate summer rainfall (Blair, 2007). According to their seed size, *Vicia faba* can be classified in three subspecies: *Vicia faba minor* (small seeded), *Vicia faba major* (large seeded) and *Vicia faba equina* (intermediate seed size) (Hegi, 1964). For livestock, usually the small and intermediate seeded cultivars of *Vicia faba minor* and *Vicia faba equina* are grown, which have typical seed weights of up to 50 g/100 seeds and up to 100 g/100 seeds, respectively (Cubero and Suso, 1981; Duc, 1997). Furthermore, coloured flowered and white flowered cultivars are available, although the coloured flowered cultivars have been proven to be more disease-resistant than the white flowered ones, therefore the coloured flowered cultivars dominate (Duc, 1997). Faba beans represent a well established ingredient in diets for horses and ruminants (Blair, 2007). Recently, they have been receiving growing attention as protein supplement in diets for pigs, particularly in Europe, due to the low production of protein feed ingredients within the European Union (Blair, 2007).

1.3.2 PEAS (*PISUM SATIVUM*)

Cultivation of peas provides a good cool-season alternative for regions not suited for growing soybeans due to their climate conditions, as peas are less frost sensitive and thus may tolerate low temperatures for germination and growth (Miller et al., 2002). Two subspecies of peas are grown in Europe, namely *Pisum sativum hortense* and *Pisum sativum arvense*. *Pisum sativum hortense* is characterised by white flowers, whereas *Pisum sativum arvense* shows dark-coloured flowers (Gatel and Grosjean, 1990). *Pisum sativum hortense* is classified according to its grain characteristics into white peas, marrowfats, small blues and large blues (Gatel and Grosjean, 1990). In contrast to *Pisum sativum arvense* ("coloured" or "fodder" peas), white flowered peas (*Pisum sativum hortense)* do not contain any tannins, and are therefore usually grown in Europe for livestock feeding (Peyronnet et al., 1996). For both subspecies, seed weights in the range from 15 to 28 g/100 seeds have been reported (Peyronnet et al., 1996; Zdunczyk et al., 1997; Bastianelli et al., 1998). Peas are primarily grown for human consumption, however, over the last years, they have been increasingly used in pig nutrition as well, especially in Canada, the Northern United States and Australia (Blair, 2007).

1.3.3 LUPINS (*LUPINUS ALBUS, LUPINUS ANGUSTIFOLIUS, LUPINUS LUTEUS*)

Cultivated species of lupins used as feed ingredient for pigs, ruminants and poultry mainly include *Lupinus albus*, *Lupinus angustifolius* and *Lupinus luteus*, and they all originate from the Mediterranean area (van Barneveld, 1999; Kim et al., 2007). The European white lupin *(L. albus)* shows white or violet-blue flowers, and typically seed weights are of about 35 g/100 seeds, although some European cultivars may have weights of about 50 to 60 g/100 seeds (Petterson, 2000). On the other hand, flowers of the narrow-leafed lupin or Australian sweet lupin *(L. angustifolius)* are normally blue, thus sometimes being referred to as blue lupin (e.g. Salgado et al., 2002; Sujak et al., 2006), with a typical seed weight of about 15 g/100 seeds (Petterson, 2000). In contrast, the yellow lupin *(L. luteus)* has golden yellow flowers and a typical seed weight of about 12 g/100 seeds (Petterson, 2000).

1.4 Nutritional Composition

1.4.1 Crude Protein and Amino Acids

Contents of crude protein (CP) and AA in grain legumes in comparison to SBM are presented in Table 1. Within the grain legumes, lupins have highest amounts of CP (324 to 381 g/kg dry matter (DM)), compared to faba beans (301 g/kg DM) and peas (246 g/kg DM) (Degussa, 2006). Jezierny et al. (2007) reported similar contents of CP in different batches of lupins, faba beans and peas averaging 387, 308 and 249 g/kg DM, respectively. In comparison to SBM, faba beans and peas contain between 45 to 55% and lupins (*L. albus*) even up to 70% of its CP content (Degussa, 2006).

Table 1. The crude protein and amino acid contents of grain legumes compared to soybean meal (g/kg DM)[a]

	Vicia faba	*Pisum sativum*	*Lupinus albus*	*Lupinus angustifolius*	*Lupinus luteus*	SBM
CP	301	246	381	324	361	541
Indispensable AA						
Arginine	26.4	21.0	39.3	33.5	38.0	39.7
Histidine	7.8	6.1	9.3	8.8	9.7	14.4
Isoleucine	11.8	10.0	15.3	12.7	14.2	24.3
Leucine	21.4	17.4	27.5	21.5	24.1	40.9
Lysine	18.4	17.3	18.2	15.0	16.3	33.1
Methionine	2.2	2.2	2.5	2.0	2.0	7.3
Phenylalanine	12.6	11.7	14.9	12.5	13.6	27.2
Threonine	10.5	9.1	13.3	10.9	11.9	21.3
Tryptophan	2.6	2.2	3.0	2.6	3.0	7.4
Valine	13.3	11.4	14.5	12.5	13.6	25.5
Dispensable AA						
Alanine	11.9	10.5	12.5	10.9	11.8	23.3
Aspartic acid	31.6	28.2	38.5	31.5	35.1	62.0
Cystine	3.5	3.5	6.7	4.3	4.8	8.0
Glutamic acid	46.9	40.0	79.3	65.6	72.5	97.6
Glycine	12.2	10.6	15.0	13.4	14.3	23.0
Proline	11.8	10.2	15.3	13.5	14.3	27.5
Serine	14.1	11.5	19.0	15.3	17.0	27.3

[a] Degussa (2006).
SBM, Soybean meal.
CP, Crude protein.
AA, Amino acids.

The protein of grain legumes consists mainly of globulins, with this fraction being higher in lupins and soybeans than in faba beans or peas (Table 2). The globulins

themselves are composed of two major proteins characterised by their sedimentation coefficients, namely the 7S and the 11S globulins (Casey et al., 1986). In faba beans and peas, these globulins are called vicillin (7S) and legumin (11S) (Guéguen, 1983). The ratio between these two globulins differs from one species to another, e.g. in soybeans and lupins the 7S-like protein is found in a higher proportion than the 11S-like protein (Guéguen, 1983). Contrary, 11S-like protein (legumin) dominates in peas and faba beans (Guéguen, 1983).

Table 2. Distribution of protein fractions in grain legumes compard to soybean meal (g/kg CP)[a]

	Albumin	Globulin	Glutelins	7S : 11S ratio[b]
Faba bean	200	600	150	1:1.6–1:3.7
Pea	210	660	120	1:1.3–1:4.2
Lupin	100–200	800–900	0	1.3:1
Soybean	100	900	0	1.6:1

[a] adapted from Guéguen (1983).
[b] 7S, 11S, Major proteins of globulin.

The protein of faba beans and peas contains similar or even higher proportions of lysine (70 and 80 g/kg CP, respectively), when compared to protein from SBM (69 g/kg CP) or lupins (51 to 54 g/kg CP) (Degussa, 2006). The proportion of threonine in grain legume protein (38 to 42 g/kg CP) is similar to that in SBM (45 g/kg CP) (Degussa, 2006), however, there is a severe deficiency in the sulphur containing AA methionine+cystine, while tryptophan is marginally deficient to fulfil nutrient requirements for pigs (20 to 50 kg body weight) (NRC, 1998; Degussa, 2006). In fact, apart from *L. albus*, the seeds of faba beans, peas and lupins contain less than 50% of these AA in comparison to SBM (Table 1), thus constraining the use of grain legumes as sole protein source in pig diets (Gatel and Grosjean, 1990; Gatel, 1994; Mekbungwan, 2007). Therefore, pig diets formulated to contain grain legumes as protein source need to be supplemented with alternative protein sources, such as SBM or crystalline AA, to fulfil the animals' requirement both for the sulphur containing AA and tryptophan (NRC, 1998).

1.4.2 Crude Fat and Lipids

The crude fat content (ether extract) in peas and faba beans is generally rather low compared to lupins (DLG, 1999; Jezierny et al., 2007). For example, crude fat contents of faba beans and peas range from 15 to 20 g/kg DM (DLG, 1999; Jezierny et al., 2007), thus

being in a similar range as values for SBM (15 to 28 g/kg DM) (DLG, 1999; Jezierny et al., 2007). In lupins, the crude fat content varies between species, with values of about 57 g/kg DM (*L. luteus*, *L. angustifolius*) to 88 g/kg DM (*L. albus*) (DLG, 1999). However, there is general agreement that the ether extract or crude fat content in grain legumes represents an inadequate measure for the lipid contents in these feed ingredients, as these compounds comprise significant amounts of non-nutritive lipids (e.g. waxes, cutin) while lipids of high nutritional value (triacylglycerol, including fatty acids) are often incompletely extracted (Palmquist and Jenkins, 2003). Alternatively, by means of gas chromatography, detailed information on fatty acid content and composition can be obtained (Coxon and Wright, 1985; Palmquist and Jenkins, 2003). Using this method, linoleic acid (480 mg/g of total lipids) and oleic acid (260 mg/g of total lipids) were identified as predominant fatty acids in peas, whereas the total lipid content (sum of fatty acids) amounted to 18 g/kg DM (Bastianelli et al., 1998). In faba beans, palmitic and oleic acid were found to be the major fatty acids (170 and 150 mg/g of total lipids, respectively), while the total lipid content (sum of fatty acids) was of about 39 g/kg (air-dry basis) (Akpinar et al., 2001). In contrast, Duc et al. (1999), determined a lower total lipid content in faba beans (18 g/kg DM; sum of fatty acids), with linoleic and oleic acid being the predominant fatty acids (52 and 28 mg/g of total lipids, respectively). The predominant fatty acids of lupins are oleic acid (210 to 530 mg/g of total lipids) and linoleic acid (172 to 473 mg/g of total lipids), but the ratio of these fatty acids may vary between different lupin species (Petterson, 2000).

With regard to the fatty acid composition, the rather high proportion of essential unsaturated fatty acids of some grain legumes, e.g. some *Vicia* species (Akpinar et al., 2001) or *L. albus* (Erbaş et al., 2005) may be attractive both from the human and animal nutrition perspective (Bézard et al., 1994), while adverse effects of unsaturated fatty acids on meat quality should be taken into account (Wood et al., 2003). For example, in faba beans a ratio of saturated to unsaturated fatty acids of 40 to 60 has been reported (Akpinar et al., 2001), whereas in *L. albus*, a ratio of saturated, monounsaturated and polyunsaturated fatty acids of 13.5 to 55.4 to 31.1 has been established (Erbaş et al., 2005).

1.4.3 CARBOHYDRATES

The carbohydrate composition of various grain legumes, in comparison to SBM, is presented in Table 3. The carbohydrate fraction includes the low molecular-weight sugars,

starch and various NSP (Bach Knudsen, 1997). The NSP and lignin are the principal components of cell walls and are commonly referred to as dietary fibre (Theander et al., 1989; Canibe and Bach Knudsen, 2002). Generally, faba beans and peas are rich in starch (422 to 451 and 478 to 534 g/kg DM, respectively) (DLG, 1999; Jezierny et al., submitted to Livestock Science, Chapter 2), whereas lupins have comparatively low levels of starch (42 to 101 g/kg DM) (DLG, 1999; Jezierny et al., submitted to Livestock Science, Chapter 2). On the other hand, faba beans and peas contain rather low amounts of fibre fractions in comparison to lupins (Bach Knudsen, 1997; Jezierny et al., submitted to Livestock Science, Chapter 2), and, with regard to lignin content, faba beans and *L. angustifolius* have similar amounts of lignin (1 to 7 and 6 to 9 g/kg DM, respectively), whereas the lignin content in peas is of minor importance (0.4 to 3 g/kg DM) (Salgado et al., 2002; Jezierny et al., submitted to Livestock Science, Chapter 2). The NSP fraction of faba beans consists mainly of cellulose (100 to 134 g/kg DM), with lower levels of hemicellulose (16 to 65 g/kg DM) (Salgado et al., 2002; Jezierny et al., submitted to Livestock Science, Chapter 2). Hemicellulose contents in peas range from 23 to 95 g/kg DM and cellulose contents range from 59 to 84 g/kg DM (Salgado et al., 2002; Jezierny et al., submitted to Livestock Science, Chapter 2). Lupins contain high levels of NSP, with contents of cellulose generally being higher than hemicellulose (131 to 222 *vs.* 31 to 66 g/kg DM) (Bach Knudsen, 1997; Salgado et al., 2002; Jezierny et al., submitted to Livestock Science, Chapter 2), and they also have considerable amounts of oligosaccharides (Bach Knudsen, 1997; Salgado et al., 2002) (Table 3). The oligosaccharides of the raffinose family, also referred to as galacto-oligosaccharides or α-galactosides, are soluble low-molecular weight oligosaccharides, such as raffinose, stachyose, and verbascose (Dey, 1985), and they are considered to be important constituents of a wide variety of grain legumes. They vary in their concentration among species as well as in cultivars of different grain legumes (Mohamed and Rayas-Duarte, 1995). In comparison to peas, faba beans and SBM, lupins contain relatively high levels of stachyose and raffinose (Table 3). Among different lupin species, higher total contents of α-galactosides have been reported in *L. albus* than in *L. angustifolius* (Cherrière et al., 2003). *L. albus* shows higher levels of stachyose than *L. angustifolius*, but both species contain similar amounts of sucrose, raffinose and verbascose (Table 3). Ferguson et al. (2003), who compared oligosaccharide contents in the kernels and hulls of *L. albus* and *L. angustifolius*, determined highest concentrations in the kernels of both species with somewhat higher levels for *L. albus* (49.1 g/kg DM) compared to *L. angustifolius* (44.5 g/kg DM).

Generally, oligosaccharides appear to be indigestible in the upper intestinal tract of monogastric animals due to the lack of appropriate enzymes in the intestinal mucosa (Mul and Perry, 1994; Pires et al., 2007). They are readily fermented by the intestinal bacteria in the lower intestinal tract, resulting in the production of various gases and short-chain fatty acids (SCFA) (Williams et al., 2001), although for some oligosaccharides considerable microbial fermentation has been shown in the small intestine as well (Houdijk, 1998). In general, oligosaccharides are known to exert "prebiotic" effects by promoting the beneficial activity of specific members of the intestinal microflora, thus improving gut health, when given in appropriate amounts (Gibson and Roberfroid, 1995; Williams et al., 2001). For example, studies in adult humans revealed a daily supply of 2.5 to 10 g short-chain fructo-oligosaccharides as optimal and well-tolerated dose which leads to a significant increase in colonic *Bifidobacteria* (Bouhnik et al., 1999; Bouhnik et al., 2006). On the other hand, excessive consumption of fermentable carbohydrates such as oligosaccharides (e.g. α-galactosides), may also lead to detrimental conditions in the large intestine of mammals, if the amount of carbohydrates entering the colon exceeds the fermentative capacity resulting in an increased rate of passage and soft faeces, or even diarrhoea (Benno et al., 1987; Fishbein et al., 1988; Saini, 1989; Mul and Perry, 1994). According to several studies (Mariscal-Landín et al., 2002; Ferguson et al., 2003), incidence of flatulence due to intestinal gas production was increased when pigs received diets containing whole or dehulled lupins (*L. albus*). In these studies, further symptoms such as loss of appetite, abdominal pain (Ferguson et al., 2003), feed refusal, death from stomach distension and rupture have also been observed (Mariscal-Landín et al., 2002). Such adverse effects of the α-galactosides on e.g. growth performance and feed intake in growing pigs suggest their antinutritional status in diets for monogastric animals (Ferguson et al., 2003). Furthermore, although SCFA produced from carbohydrate fermentation are generally known for their trophic effects (Roediger, 1982), there is evidence that excessive production of SCFA may inhibit feed intake and/or increase digesta retention time in pigs fed lupins (Dunshea et al., 2001). Van Barneveld (1999), by reviewing nutritional value of different lupin species for livestock, concluded that high levels of α-galactosides may have a number of negative effects on the nutritional value of lupins. These include interference with the digestion of other nutrients in the small intestine (e.g. AA), a decrease in dietary net energy (NE) content due to a higher proportion of hindgut fermentation, and osmotic effects (e.g. viscosity of ileal digesta) of α-galactosides in the small intestine resulting in an increased rate of passage.

Table 3. Carbohydrate contents of grain legumes compared to soybean meal (g/kg DM)[a]

	Vicia faba	*Pisum sativum*	*Lupinus albus*	*Lupinus angustifolius*	*Lupinus luteus*	SBM
Starch	422–451	478–534	74	68–101	42–49	51–69
Sucrose	20–27	17–34	24	35	–	57–70
α–Galactosides						
Raffinose	1–4	5–6	10	11	–	8–10
Stachyose	8–16	23–26	53	43	–	47–49
Verbascose	25–34	22–34	14	20	–	0–3
Fibre	89	67	130	163	168	67
NDF	126–173	104–154	–	220–261	252	114–162
ADF	101–137	59–86	–	154–230	208	74–81
ADL	1–8	0–3	–	6–9	11	1–3
Hemicellulose	16–65	23–95	–	31–66	44	40–81
Cellulose	100–134	59–84	131	148–222	197	71–80
Total NSP	190	180/178[b]	405	–	–	217

[a] Compiled from Bach Knudsen (1997), DLG (1999), Salgado et al. (2002), Jezierny et al. (submitted to Livestock Science, Chapter 2).
[b] White/coloured flowered cultivar.
–, No value reported.
NDF, Neutral detergent fibre.
ADF, Acid detergent fibre.
ADL, Acid detergent lignin.
NSP, Non-starch polysaccharides.
SBM, Soybean meal.

1.5 Secondary Plant Metabolites

Grain legumes contain a number of secondary plant metabolites, also referred to as bioactive substances (Champ, 2002), which may exert a wide range of different effects on the animals that consume them (Champ, 2002). These effects have been described as positive, negative or both (Champ, 2002; Jamroz and Kubizna, 2008). Most secondary plant metabolites, however, such as condensed tannins, protease inhibitors, alkaloids, lectins, pyrimidine glycosides and saponins have been classified as ANF (e.g. Liener, 1989; D´Mello, 1995), since they may impair growth performance, fertility and health status of livestock due to a variety of underlying mechanisms (Huisman and Tolman, 2001; Brenes et al., 2004; Pusztai et al., 2004). These secondary plant metabolites may be divided into two major categories: a heat-labile group, such as protease inhibitors and lectins, which is sensitive to temperatures eventually occurring during feed processing, and a heat-stable group including condensed tannins, alkaloids, pyrimidine glycosides and saponins which is stable under processing temperatures (D´Mello, 1995). Contents of secondary plant metabolites in batches of faba beans, peas and lupins in comparison to SBM are summarised in Table 4.

Table 4. Contents of secondary plant metabolites in different cultivars of faba beans, peas and lupins compared to soybean meal (g/kg DM)[a]

	Condensed tannins	Vicine	Convicine	Alkaloids	TIA (mg TI/g CP)
Vicia faba					
Aurelia[b]	ND	7.2	2.8	–	3.9
Divine[c,d]	2.1	0.3	0.0	–	1.4
Gloria[b]	ND	6.4	3.2	–	3.3
Limbo[c]	7.0	5.6	3.1	–	<0.2
Fuego[c]	7.4	7.0	3.7	–	<0.2
Espresso[c]	4.2	6.7	3.7	–	<0.2
Pisum sativum					
Santana[b]	ND	–	–	–	2.4
Jutta[b]	ND	–	–	–	1.8
Phönix[b]	ND	–	–	–	5.0
Harnas[b]	ND	–	–	–	<0.2
Rocket[b]	ND	–	–	–	3.9
Hardy[b]	ND	–	–	–	4.5
Lupinus angustifolius					
Probor	ND	–	–	0.07	2.9
Boregine	ND	–	–	0.04	<0.2
Boruta	ND	–	–	0.28	<0.2
Idefix	ND	–	–	0.18	<0.2
Lupinus luteus					
Bornal	ND	–	–	0.26	<0.2
SBM	ND	–	–	–	5.8

[a] Jezierny et al. (submitted to Livestock Science, Chapter 2).
[b] White flowered cultivar.
[c] Coloured flowered cultivar.
[d] Low vicine/convicine genotype.
ND, Not detectable.
TIA, Trypsininhibitor activity.
TI, Trypsininhibitor.
CP, Crude protein.
SBM, Soybean meal.

1.5.1 TANNINS

Tannins are water-soluble polyphenolic compounds occurring in different plants, including faba beans, peas, sorghum, barley, clover, and lucerne (Jansman, 1993). They can be separated in two tannin sub-groups, referred to as hydrolysable tannins and non-hydrolysable or condensed tannins (proanthocyanidins) (Jansman, 1993). Tannins may have a considerable influence on the nutritive value of grain legumes, partly by decreasing palatability due to their astringent properties, which are caused by formation of complexes between tannins and salivary glycoproteins (Reed, 1995). Also attributed to this protein-binding capacity of tannins is the prevention of enzymatic digestion (Jansman, 1993), which may lead to an increasing secretion of endogenous proteins (Marquardt, 1989).

Thus, tannins reduce the apparent ileal digestibility (AID) of CP (nitrogen×6.25) (e.g. Jansman et al., 1993; van der Poel et al., 1992; Grosjean et al., 1998), AA (Jansman et al., 1993), and, but to a lesser extent, energy (Grosjean et al., 1998).

Condensed tannins are the predominant phenolic compounds present in faba beans and peas (Marquardt et al., 1977; Bastianelli et al., 1998), while lupins are devoid of any phenolic compounds (Huisman and Tolman, 2001). Their presence in the seed-coats of faba beans and peas is restricted to coloured flowered cultivars (Griffiths, 1981), with contents ranging from 15.7 to 35.4 g/kg (air-dry basis) (Makkar et al., 1997). Distinctly lower tannin contents in the range of 2.1 to 7.4 g/kg DM were recently determined in four batches of coloured flowered faba beans (Jezierny et al., 2008). However, no condensed tannins could be detected in white flowered cultivars (Makkar et al., 1997; Jezierny et al., 2008). Similarly, condensed tannins clearly dominate in coloured flowered pea cultivars, with levels being 100 times higher than in white flowered peas (5.49 *vs*. 0.07 g/kg DM, air-dry basis) (Bastianelli et al., 1998). Through plant breeding measures tannins can be removed in faba beans by introducing cultivars with any of the two complementary zero-tannin genes, *zt-1* and *zt-2*, which are characteristic for white-flowered plants (Picard, 1976). The allele *zt-2* has been shown to be superior in feeding value when compared to *zt-1*, as it seems to be associated with increased protein levels of the seeds and a decreased fibre content, due to a reduction of the seed coat (Duc et al., 1999).

Jansman et al. (1993) investigated in piglets the effects of diets containing 300 g/kg of different faba bean cultivars. The authors obtained significant lower AID of CP and most AA for diets containing coloured flowered faba bean cultivars, with contents of condensed tannins ranging from 1.0 to 2.3 g/kg diet, when compared to a white flowered and tannin free faba bean cultivar. On the other hand, Flis et al. (1999) found no detrimental effect on growth rate of pigs (25 to 63 kg body weight) fed a faba bean diet containing 0.59 g/kg diet condensed tannins in comparison to pigs fed diets with condensed tannin contents below 0.07 g/kg diet.

1.5.2 Protease Inhibitors

Generally, protease inhibitors are widely distributed in plant seeds (Liener and Kakade, 1980), and most grain legumes contain considerable amounts of these inhibitors

(Huisman and Tolman, 2001). Traditionally, protease inhibitors belong to two major classes, the Kunitz trypsin inhibitor which is mainly present in soybeans, and the family of Bowman-Birk trypsin/chymotrypsin inhibitors, which widely occurs in grain legumes (Huisman and Tolman, 2001; Pusztai et al., 2004). Generally, trypsin inhibitor activity (TIA) is used as measure to determine protease inhibitor activity (e.g. Struthers et al., 1983; Le Guen et al., 1995; Grosjean et al., 2000), which can be expressed as mg or g trypsin inhibited (mg or g TI) or as trypsin inhibitor unit (TIU), assuming that 1.90 TIU/mg is equivalent to 1.0 mg TI/g (Kakade et al., 1974). Additionally, TIA can be related to CP (nitrogen×6.25) content (Smith et al., 1980).

The primary mode of action of protease inhibitors is described as inhibition of the proteolytic pancreatic enzymes trypsin and chymotrypsin secreted into the intestinal lumen, by forming stable inactive complexes (Liener and Kakade, 1980; Lallès and Jansman, 1998). Furthermore, as a secondary effect, protease inhibitors may act by suppressing negative feedback regulation of pancreatic secretion through an increased release of the hormone cholecystokinin from the intestinal mucosa (Liener, 1989). However, as reviewed by Lallès and Jansman (1998), the feedback consequences on pancreatic function differ considerably among animal species and extrapolation of results from one species to another appears to be rather difficult, as different species respond differently to trypsin inhibitor containing diets (Struthers et al., 1983). Moreover, an increased pancreatic secretion of trypsin and chymotrypsin due to TIA may lead to an enhanced loss of endogenous methionine and cystine, as these pancreatic enzymes are rich in the sulphur containing AA (Gatel, 1994). As a result, losses of endogenous methionine and cystine via enhanced secretion of trypsin and chymotrypsin, may increase growth depression as grain legumes are already deficient in sulphur containing AA (Belitz and Weder, 1990; Liener, 1994).

According to Jezierny et al. (2007), grain legumes contain rather low TIA, ranging between <0.2 to 3.9, <0.2 to 5.0 and <0.2 to 2.9 mg TI/g CP, in different batches of faba beans, peas and lupins, respectively. These values were below those obtained for SBM (5.8 mg TI/g CP) (Table 4). Also, the authors found lower TIA in coloured flowered faba bean cultivars compared to white flowered cultivars. This is in agreement with reports by Makkar et al. (1997), according to which TIA in coloured flowered faba bean cultivars was only about 64% of that in white flowered cultivars, when expressed in mg TI/g CP (6.72 to 7.35 *vs.* 9.01 to 12.74 mg TI/g CP). However, TIA up to 3.2 mg TI/g diet did not affect

pancreatic secretion of nitrogen or protein or pancreatic chymotrypsin activity in young pigs (Gabert et al., 1996). Similarly, according to Batterham et al. (1993), growing pigs may tolerate dietary levels of at least 4.7 mg TI/g, without significant negative effects on performance criteria such as growth rate, feed intake or feed conversion ratio. Contrary to Batterham et al. (1993), a lower dietary maximum tolerance level for fattening pigs of approximately 0.5 mg TI/g was recommended by Huisman and Tolman (2001). However, depending on the dietary inclusion level of the respective grain legumes, it can be assumed that these threshold levels are unlikely to be exceeded in conventional pig diets containing grain legumes.

1.5.3 ALKALOIDS

Alkaloids are naturally-occurring toxic amines produced by plants mainly as defence mechanism to protect themselves against herbivores (Wink, 1988; Kim et al., 2007). Within grain legumes, mainly lupins are known to contain alkaloids in considerable amounts (Wink et al., 1995), while faba beans and peas are devoid of alkaloids (Huisman and Tolman, 2001). The alkaloids of lupins are commonly bicyclic (e.g. lupinine), tricyclic (e.g. angustifoline) or tetracyclic (e.g. sparteine, lupanine) derivatives of quinolizidine (Wink et al., 1995). An important exception is the simple indole alkaloid gramine, which is found in some cultivars of *L. luteus* (Wink et al., 1995). The main toxic effects of alkaloids result in disturbances of the central nervous system, digestive processes, reproduction and the immune system (Lallès and Jansman, 1998). Anti-palatability effects of lupine alkaloids might be mediated in part by neurological effects (Cheeke and Kelly, 1989).

Wink et al. (1995) determined the alkaloid composition in different lupin species. The major alkaloids of *L. albus* are lupanine (700 mg/g total alkaloids), albine (150 mg/g total alkaloids) and 13α-hydroxylupanine (80 mg/g total alkaloids). In *L. angustifolius,* the authors found mainly lupanine (70 mg/g total alkaloids), 13α-hydroxylupanine (120 mg/g total alkaloids), and angustifoline (100 mg/g total alkaloids), while predominant alkaloids of *L. luteus* were identified as lupanine (600 mg/g total alkaloids) and sparteine (300 mg/g total alkaloids). The average alkaloid content of cultivars of low-alkaloid lupins, also referred to as sweet lupins, is below 0.28 g/kg for *L. angustifolius* and *L. albus* (van Barneveld, 1999; Jezierny et al., submitted to Livestock Science, Chapter 2) and below 0.26 g/kg DM for *L. luteus* (Roth-Maier and Paulicks, 2004; Jezierny et al., submitted to Livestock Science, Chapter 2).

It has been shown that pigs are susceptible to the presence of alkaloids in their diets (Godfrey et al., 1985), however, current data pertaining to the use of lupins in pig nutrition are controversial (Kim et al., 2007). For example, decreased growth and feed intake were observed in pigs fed a diet containing 150 to 430 g/kg *L. albus* seeds (Batterham, 1992; Zettl et al., 1995). Van Nevel et al. (2000) and King et al. (2000) described growth depression, decreased feed intake and reduced feed conversion rate when 300 g/kg of *L. albus* were included in the diet. In contrast, Gdala et al. (1996) did not find any growth depression in young pigs fed a diet supplemented with *L. angustifolius* (410 g/kg of diet) or *L. luteus* (320 g/kg diet) compared to barley and soybean based diets. The tolerated level of lupins in diets for pigs is dependant both on the total amount of dietary alkaloids and their origin (Kim et al., 2007). For example, according to Buraczewska et al. (1993), pigs do not tolerate more than 0.12 g/kg of alkaloids of *L. albus*, whereas diets containing up to 0.45 g/kg of alkaloids from *L. luteus* were consumed without any negative effect on feed intake. Therefore, differences in alkaloid composition between lupin species may explain, at least in part, the variation in feed intake response. According to Godfrey et al. (1985) and Allen (1998) total alkaloid contents in diets for growing pigs fed *ad libitum* should not exceed 0.2, and 0.33 g/kg, respectively. As alkaloid levels in present cultivars of sweet lupins have been proven to be very low (van Barneveld, 1999; Roth-Maier and Paulicks, 2004; Jezierny et al., submitted to Livestock Science, Chapter 2), these recommended threshold levels would not be exceeded when using these lupin cultivars as a component in commercial pig diets. However, alkaloid contents in lupins may vary, depending on growing conditions, such as drought stress (Christiansen et al., 1997) or phosphor-potassium-fertilisation (Gremigni et al., 2003). Therefore, it is necessary to monitor alkaloid levels in lupins, especially with respect to new cultivars and the environment in which they have been grown (van Barneveld, 1999).

1.5.4 LECTINS

Lectins, also referred to as phytohaemagglutinins, are glycoprotein compounds which have been shown to agglutinate red blood cells *in vitro* (Gatel, 1994), a feature which may be used to identify and detect lectins in feed ingredients (D´Mello, 1995). *In vivo*, lectins can bind to receptors of epithelial cells of the intestinal mucosa and disturb digestive processes (Gatel, 1994). Further effects of feeding lectin containing diets include changes in gut immune function, reduced production of endocrine cells and gut hormones,

interference with the bacterial ecology in the gut lumen, and damage to mucosal cells (King et al., 1983).

Lectins are found in a wide range of grain legumes including faba beans, peas, soybeans and lupins (Valdebouze et al., 1980; Gupta, 1987; Huisman and Tolman, 2001). However, literature data on lectin contents in legumes seeds, generally expressed as lectin activity or haemagglutinin activity (e.g. Valdebouze et al., 1980; Gatel, 1994; Makkar et al., 1997), are not very precise and sometimes difficult to compare due to the different analytical methods used for measuring lectin activity (Valdebouze et al., 1980; Gatel, 1994; Champ, 2002). Thus, a direct comparison between lectin activities in grain legumes, determined in different studies is not possible. Peas have about one tenth and faba beans about one fiftieth of the lectin activity of raw defatted soybeans (Valdebouze et al., 1980). Lectin activities in other grain legumes (e.g. black beans, kidney beans) are about 25 times higher than in faba beans and peas (Gupta, 1987), while lupins contain less than one percent of the lectin activity of faba beans and peas (Valdebouze et al., 1980). Also Makkar et al. (1997) determined low lectin activities in six white and six coloured flowered faba bean cultivars with no significant difference between white and coloured flowered cultivars. Generally, lectin activity of all analysed faba bean cultivars in this study was lower than that of SBM, except of one white flowered cultivar, which was similar to SBM. Furthermore, there is some evidence that low-alkaloid lupins may be completely devoid of lectins (Schoeneberger et al., 1983).

1.5.5 Pyrimidine Glycosides

Vicine and convicine are generally present in *Vicia faba* and belong to the group of pyrimidine glycosides, which are composed of one molecule glucose linked to one pyrimidine nucleoside (Champ, 2002). In contrast, other grain legumes (e.g. *Phaseolus vulgaris, Pisum sativum*) contain only negligible amounts as compared to faba beans (Jamalian et al., 1977; Saini, 1993). Vicine and convicine act by reducing glutathione and glucose-6-phosphate dehydrogenase activity, which may result in haemolytic anaemia due to biochemical abnormalities of blood cells (Gupta, 1987). For example, vicine and convicine are known to be responsible for haemolytic anaemia (favism) in humans (Duc, 1997).

Recently, Jezierny et al. (2007) reported vicine and convicine contents in five vicine and convicine containing cultivars of faba beans ranging from 5.6 to 7.2 g/kg DM and 2.8 to 3.7 g/kg DM, respectively (Table 4). Accordingly, Grosjean et al. (2001) determined vicine and convicine contents in different faba bean cultivars, ranging from 3.4 to 10.4 g/kg DM for vicine and from 1.7 to 4.3 g/kg DM for convicine. As a result of plant breeding activities, faba bean cultivars with a reduced content of vicine and convicine due to their low vicine-convicine gene *vc−* have been introduced (Duc et al., 1999). Jezierny et al. (2007) determined vicine and convicine contents in one low vicine and convicine genotype amounting to 0.3 and 0.0 g/kg DM, respectively (Table 4). Accordingly, Grosjean et al. (2001) reported negligible contents of vicine and convicine in these faba bean genotypes (0.2 to 0.6 g/kg DM and 0.1 to 0.2 g/kg DM, respectively).

Previously, Olaboro et al. (1981) and Muduuli et al. (1982) demonstrated that 10 g vicine/kg diet fed to laying hens may impair feed intake, egg weight, fertility and hatchability of eggs, packed cell volume and erythrocyte haemoglobin levels and, in addition, may lead to increased liver weights, liver glutathione levels, liver and plasma lipid levels, plasma lipid peroxide levels and erythrocyte haemolysis *in vitro*. However, in a study of Grosjean et al. (2001), faba beans were fed to pigs at a dietary inclusion level of 500 g/kg, which corresponded to vicine and convicine contents up to 10.4 and 4.3 g/kg DM, respectively. In this study, only small effects of vicine and convicine on protein and energy digestibility were found.

1.5.6 SAPONINS

Saponins are glycosides and present in many plants. They are characterised by an astringent taste, and their antinutritional effect is likely due to an increased permeability of the small intestinal mucosa cells which, in turn, may lead to an inhibition of active nutrient transport across the intestinal wall (Johnsson et al., 1982). Furthermore, they are poorly absorbed from the intestine, because they form not only insoluble complexes with 3-β-hydroxysteroids, but also large mixed micelles with bile acids and cholesterol (Pusztai et al., 2004).

Saponin levels in faba beans determined by Makkar et al. (1997) were almost twice as high in coloured flowered cultivars (25.5 to 39.3 g/kg) compared to white flowered

cultivars, but did not significantly differ (16 to 18 g/kg) within the white flowered cultivars analysed. Notably, the saponin content of SBM is higher than of faba beans amounting to 49.4 g/kg (Makkar et al., 1997). Cuadrado et al. (1995) reported levels of saponins in *L. albus* below the detection limit of 0.012 g/kg, whereas *L. luteus* contained 0.055 g/kg total saponins. Saponin levels in *L. angustifolius* ranged from 0.379 to 0.740 g/kg (Ruiz et al., 1995). According to Cuadrado et al. (1995), saponin contents are positively correlated with the alkaloid content of lupins. In general, despite of their bitter taste, saponins are supposed not to restrict the feed intake of monogastric animals as their levels in most common feed ingredients including grain legumes, are rather low (Huisman and Tolman, 2001).

1.6 Feeding Value

1.6.1 Energy and Amino Acid Digestibility

Energy. Faba beans, peas and lupins are used as feed components for non-ruminant and ruminant animals, primarily because of their high CP and AA content (Bach Knudsen, 1997). Besides, the carbohydrate fraction present in grain legumes, including low molecular-weight sugars, starch and various NSP, provides an important energy source (Bach Knudsen, 1997). The ether extract and carbohydrate content (g/kg DM) and energy content (MJ/kg DM) in grain legumes in comparison to cereals and SBM are presented in Table 5. The composition of the carbohydrate fraction of grain legumes differs considerably from that of cereals (Bach Knudsen, 1997). Cereals contain significant higher quantities of starch than faba beans, peas and lupins, whereas these grain legumes have higher amounts of total sugars (total of monosaccharides, sucrose, raffinose, stachyose and verbascose) and NSP (Bach Knudsen, 1997).

The digestible energy (DE) contents of faba beans, peas and lupins are similar to those of cereals (Table 5). The higher crude fat content of *L. albus* (88 g/kg DM) in comparison to *L. angustifolius* (57 g/kg DM) (DLG, 1999) is reflected in a higher DE content in comparison to *L. angustifolius* (King et al., 2000). In pigs, increasing levels of lupins in the diet, DE content did not change, but the proportion of energy digested by the end of the small intestine (which will influence NE content) significantly decreased (van Barneveld et al., 1995) due to the high amounts of NSP and α-galactosides present in

lupins (van Barneveld, 1999). Approximately 40% of the DE in lupins is known to originate from microbial fermentation in the large intestine (Taverner et al., 1983), thus explaining lower NE values of lupin seeds compared to cereals (Table 5). However, there is evidence, that α-galactosides contents may vary between lupin subspecies and cultivars (Martínez-Villaluenga et al., 2005), which, in turn, may explain in part the wide range of NE values determined in *L. albus* (3.23 to 10.89 MJ/kg DM) and *L. angustifolius* (6.15 to 10.54 MJ/kg DM).

Table 5. Ether extract and carbohydrate content (g/kg DM) and energy content (MJ/kg DM) in grain legumes compared to soybean meal and cereals[a]

	Vicia faba	*Pisum sativum*	*Lupinus albus*	*Lupinus angustifolius*	SBM	Maize	Wheat	Rye	Barley
Ether extract	15.0–15.9	14.8–20.0	87.5	56.8	14.8–28.0	43.8	22.7	18.2	21.3
Carbohydrates									
Total sugars[b]	86	88/102[c]	104	–	137	20	19	32	21
Starch	407	454/407[c]	14	–	27	690	651	613	587
Total NSP	190	180/178[c]	405	–	217	97	119	152	186
Energy									
GE	–	15.06–18.54	21.11	19.82	–	–	–	–	–
DE	15.62	16.53	17.42–18.11	15.66–17.35	16.42–18.00	16.58	16.01	15.56	14.35
Ileal DE	–	–	10.94	9.08	12.80	–	–	–	–
ME	14.65	15.10	15.55–16.51	14.71	14.96–16.26	16.09	15.27	14.56	13.69
NE	9.62	9.16–10.33	3.23–10.89	6.15–10.54	9.10–10.60	11.27	10.59	10.94	11.01

[a] Compiled from Bach Knudsen (1997), Noblet (1997), Grosjean et al. (1998), NRC (1998), DLG (1999), King et al. (2000), Jezierny et al. (2007).
[b] Sum of monosaccharides, sucrose, raffinose, stachyose and verbascose.
[c] White/coloured flowered cultivars.
–, No value reported.
NSP, Non-starch polysaccharides.
GE, Gross energy.
DE, Digestible energy.
ME, Metabolisable energy.
NE, Net energy.
SBM, Soybean meal.

Amino acids. The major constraint when using faba beans, peas and lupins as a source of protein in pig diets is their low level of the sulphur containing AA methionine and cystine and, in addition, of tryptophan when compared to SBM (Gatel and Grosjean, 1990; Gatel, 1994; Mekbungwan, 2007). However, if adequately complemented with cereal grains and supplemented with the limiting indispensable AA, faba beans, peas or lupins can be included in diets for growing pigs without any negative effects on growth performance (Partanen et al., 2003; Stein et al., 2004; Zraly et al., 2007). Faba beans can be included in diets for grower and finisher pigs up to 150 and 250 g/kg, respectively (UFOP, 2004a). However, white flowered cultivars should be preferred, due to their low tannin contents (UFOP, 2004a). For peas, an inclusion level of up to 400 g/kg in diets for grower and finisher pigs has been recommended, with low tannin white flowered cultivars also being superior to the coloured cultivars (UFOP, 2004b). The recommended inclusion level for lupins in diets for grower and finisher pigs is set at 200 g/kg, however, only up to 150 g/kg of *L. albus* should be included in diets for growing pigs (30 to 60 kg body weight) (UFOP, 2004c).

Jezierny et al. (submitted to Livestock Science, Chapter 2) determined in growing pigs standardised ileal digestibility (SID) of CP and AA in different cultivars of faba beans, peas and lupins, and for comparison, in SBM. The authors reported that within grain legumes, SID of CP and AA were lowest in faba beans, intermediate in peas and highest in lupins (Table 6) with significant differences for cystine, glycine and proline. The SID of CP and AA of lupins are similar to those of SBM, and except for lysine, arginine, histidine, aspartic acid, glutamic acid and glycine, values in SBM were higher than in peas (Jezierny et al., submitted to Livestock Science, Chapter 2). The SID of CP from different cultivars of faba beans and peas reported in this study, were up to 11 percentage units lower in comparison to data from Kasprowicz and Frankiewicz (2004). In both studies, SID values for CP and AA in faba beans, peas and lupins were in general agreement with tabulated values published by Rademacher et al. (2009) and AmiPig (2000). Furthermore, SID of methionine, cystine and tryptophan in these grain legumes were relatively low when compared to SID of the other AA (Kasprowicz and Frankiewicz, 2004; Jezierny et al., submitted to Livestock Science, Chapter 2). These low SID values may be due to methodological draw backs when the difference method is used to calculate SID values (Knabe et al., 1989). The relatively low contents of methionine, cystine and tryptophan in faba beans, peas and lupins, in comparison to the other dispensable and indispensable AA, result in a low contribution level of these AA to the total contents of these AA in the

experimental diets. Therefore, any change in SID of these AA in the assay diets results in a considerable change in the digestibility of these AA in the assay feed ingredient (Knabe et al., 1989), thus in part low SID of these AA in faba beans, peas and lupins might be a reflection of an experimental error.

Table 6. Coefficients of standardised ileal digestibilities of crude protein and amino acids in grain legumes compared to soybean meal[a]

	Faba beans	Peas	Lupins	SBM
CP	0.76	0.79	0.87	0.87
Indispensable AA				
Arginine	0.87	0.89	0.95	0.94
Histidine	0.78	0.81	0.88	0.89
Isoleucine	0.80	0.81	0.89	0.91
Leucine	0.79	0.80	0.88	0.89
Lysine	0.82	0.85	0.87	0.90
Methionine	0.67	0.76	0.81	0.91
Phenylalanine	0.79	0.82	0.88	0.90
Threonine	0.74	0.75	0.84	0.85
Tryptophan	0.61	0.67	0.82	0.85
Valine	0.76	0.78	0.85	0.88
Dispensable AA				
Alanine	0.75	0.76	0.82	0.87
Aspartic acid	0.81	0.83	0.89	0.89
Cystine	0.57	0.67	0.84	0.82
Glutamic acid	0.86	0.87	0.93	0.91
Glycine	0.68	0.73	0.83	0.82
Proline	0.74	0.80	0.89	0.91
Serine	0.81	0.81	0.89	0.90

[a] Jezierny et al. (submitted to Livestock Science, Chapter 2).
SBM, Soybean meal.
CP, Crude protein.
AA, Amino acids.

Variations in CP and AA digestibility of grain legumes seem to be multifactorial (Salgado et al., 2002; Salgado et al., 2003). For example, high levels of secondary plant metabolites or fibre may result in lower CP and AA digestibilities in faba beans, peas and lupins (Salgado et al., 2003), as they increase endogenous CP and AA losses (e.g. Jansman et al., 1995; Grala et al., 1999). Differences in SID of CP and AA within faba bean and pea cultivars have been linked to variations in their chemical composition, in particular to varying contents of secondary plant metabolites inherent to different cultivars (Mariscal-Landín et al., 2002; Kasprowicz and Frankiewicz, 2004; Jezierny et al., submitted to Livestock Science, Chapter 2). For example, the SID of CP and AA in faba beans and peas from white flowered cultivars are higher than those in coloured flowered

cultivars due to their higher tannin contents (Mariscal-Landín et al., 2002; Kasprowicz and Frankiewicz, 2004; Jezierny et al., submitted to Livestock Science, Chapter 2). Within peas, the SID of CP and AA in spring pea cultivars are superior to those determined in winter pea cultivars, which has been associated with higher TIA in winter than in spring pea cultivars (Mariscal-Landín et al., 2002). Higher ileal CP and AA digestibilities in lupins compared to faba beans or peas (Jezierny et al., submitted to Livestock Science, Chapter 2) may be partly explained by lower levels of neutral detergent fibre (NDF)-bound protein in lupins (Kim et al., 2007). Indeed, the level of NDF-bound protein in lupins is rather low (29 mg/g of total CP), when compared to peas (78 mg/g of total CP) or faba beans (85 mg/g of total CP) (Gdala, 1998). An higher dietary NDF content due to supplemental purified NDF source, resulted in higher endogenous and exogenous losses of nitrogen in ileal digesta of growing pigs (Schulze et al., 1994). The total amount being lost may depend both on the level and the composition of dietary fibre (Schulze et al., 1994; Schulze et al., 1995).

Furthermore, it can be derived from *in vitro* and *in vivo* studies that digestion of native grain legume storage proteins is limited because of the structure and conformation of these proteins (Chang and Satterlee, 1981; Jivotovskaya et al., 1996). For example, globulins from soybean (glycinin and β-conglycinin) were well digested *in vitro* (Nielsen et al., 1988), whereas *in vivo* digestibility of phaseolin, a globulin from kidney bean protein was reduced in pigs and rats (Begbie and Ross, 1993; Santoro et al., 1999). Moreover, *in vivo* studies revealed that proteins of the globulin fraction from peas (vicillin and legumin) could be degraded by proteolytic enzymes,, whereas the albumin proteins (lectin, PA1b, and PA2) have proven to be resistant in porcine stomach and small intestine (Salgado et al., 2003; Le Gall et al., 2005). Thus, inherent structural properties of the major storage globulins may be important factors limiting digestion of these proteins during passage through the gastrointestinal tract (Carbonaro et al., 2000).

1.6.2 Processing of Grain Legumes

Some secondary plant metabolites with known antinutritional effects, such as alkaloids, tannins and pyrimidine glycosides may be eliminated or reduced by means of plant breeding technology (Petterson, 1998; Duc et al., 1999). Furthermore, contents of secondary plant metabolites and α-galactosides may be reduced or eliminated by different

processing methods, such as physical treatments (e.g. dehulling, soaking), heat treatments (e.g. extrusion, cooking) or biological methods (e.g. germination, enzyme supplementation). According to several studies (e.g. Owusu-Asiedu et al., 2002; Stein and Bohlke, 2007), processing of grain legumes has been proven to efficiently improve starch and protein digestibility in pigs, which can be attributed, at least in part, to a reduction of secondary plant metabolites. However, the choice of processing technology always depends on the availability of facilities and also economic considerations, thus their use may differ in feed industry (Enneking and Wink, 2000; Mekbungwan, 2007).

Physical treatments. Since tannins are mainly concentrated in the seed coats (hulls), dehulling is known to be an adequate method to minimise their antinutritional effects (Petterson, 2000). For example, Alonso et al. (2000) yielded a 92% reduction of tannin content in faba beans after dehulling. According to Flis et al. (1999), condensed tannin contents of coloured flowered faba bean seeds could be reduced from 2.28 to 0.29 g/kg DM by using dehulling technology. Van der Poel et al. (1992) reported that dehulling of faba beans increased AID of DM and CP from 0.63 to 0.72 and from 0.67 to 0.75, respectively. Dehulling of lupins is known to increase nutrient density (i.e. higher digestible nutrient contents in association with lower indigestible fibre content), and may hence increase feeding value for pigs (Kim et al., 2007). Furthermore, inclusion of dehulled lupins in diets for pigs tended to increase feed intake and feed-conversion ratio compared to whole seeds (Dunshea et al., 2001). Contrary, Kim et al. (2007) recommended a restriction of inclusion level of dehulled lupins in pig diets, since dehulling of lupins also increased concentrations of fermentable fibre and oligosaccharides (King et al., 2000), which may be responsible for detrimental conditions in the large intestine (e.g. flatulences, diarrhoea) (e.g. Saini, 1989).

Heat treatments. Heat treatment procedures have been shown to be adequate methods for reducing contents or activity of several secondary plant metabolites (Alonso et al., 2000; O´Doherty and Keady, 2000; Jiménez-Martínez et al., 2001), especially those of the heat-labile group (protease inhibitors, lectins), but also tannins. Furthermore, as shown in peas, heat treatment technologies are assumed to induce conformational changes in storage proteins, which may render them more accessible to digestive enzymes, and thus may increase AA digestibility (van der Poel et al., 1991; Canibe and Eggum, 1997).

A further method to reduce secondary plant metabolites contents is extrusion, where feeds are treated under varying conditions of high temperature and high pressure. Extrusion of faba beans at 152 to 156°C significantly reduced the level of condensed tannins of about 54%, and led to a decrease of TIA of about 53% (Alonso et al., 2000). It has been suggested that reduction of TIA and condensed tannin content may contribute to the improved AID and SID values in extruded peas (O´Doherty and Keady, 2000; Mariscal-Landín et al., 2002). For example, extrusion of peas at a temperature of about 115°C increased AID and SID of CP, AA, and also starch and energy digestibilities in growing pigs (Stein and Bohlke, 2007). Similarly, Owusu-Asiedu et al. (2002) reported an improved AID and SID of CP and most AA when extruded (135°C) peas were fed to weaned piglets. Furthermore, feeding of peas extruded at 130°C for 30 s significantly increased growth rate and improved feed conversion ratio in grower and finisher pigs in comparison to pigs fed untreated peas (O´Doherty and Keady, 2001).

Cooking in deionised water for 1 h (after soaking for 18 h) decreased the condensed tannin content of coloured flowered peas on average from 8.46 to 5.51 g/kg DM (Pastuszewska et al., 2004). Armour et al. (1998) reported complete inactivation of soy lectin and protease inhibitory activity by aqueous heat treatment of fully imbibed soy seeds at 100°C for 10 min. Hydratation and heating at boiling temperature for 6 h in water and in a 0.5% sodium bicarbonate solution reduced alkaloid contents in *Lupinus campestris* (a wild lupin species) from 27 g/kg to 0.3 g/kg and to 0.02 g/kg, respectively (Jimenéz-Martínez et al., 2001). Furthermore, due to these treatments the content of mono-, di- and oligosaccharides including the α-galactosides stachyose, raffinose and verbascose was reduced between 70 (water treatment) and 90% (alkaline treatment) (Jiménez-Martínez et al., 2001).

Biological treatments. For several grain legumes, germination has been shown to be an effective method to reduce content of secondary plant metabolites. For example, germination at 25°C over periods of 24 h, 48 h and 72 h significantly decreased the levels of condensed tannins in faba beans by 56, 58 and 60%, respectively (Alonso et al., 2000). Also, germination for 48 h and 72 h reduced TIA in faba beans by 11 and 12%, respectively (Alonso et al., 2000). Germination at 16 to 17°C for 7 d (after overnight soaking) reduced vicine and convicine content in faba beans by 84 and 100%, respectively, and a further reduction of vicine content by 92% could be achieved, when faba beans were treated with a 3% hydrogen peroxide solution for 1 h following

germination (Jamalian, 1999). Moreover,, contents of oligosaccharides in lupins may also be lowered due to germination. For example, De la Cuadra et al. (1994) found a 100% reduction of α-galactosides (raffinose, stachyose and verbascose) in the seeds of *L. albus* and *L. luteus* after 48 h of germination, while sucrose content increased.

Supplementation of the enzyme α-galactosidase to lupin-based diets increased digestibility of α-galactosides, AA, and energy (Gdala et al., 1997), enhanced N retention and improved performance of growing pigs (Froidmont et al., 2005). Supplementation of cereal-soybean-pea based diets with α-galactosidase, improved growth performance, of grower and finisher pigs, associated with an increase in protein digestibility (Baucells et al., 2000).

1.7 Conclusions

It can be concluded that the use of grain legumes as a protein source in pig diets may be limited due to rather low concentrations of sulphur containing AA and tryptophan, when compared to other plant protein sources such as SBM. Therefore, legume-containing diets should be adequately complemented with cereal grains and/or supplemented with these AA in crystalline form. A further limitation, which needs to be considered when grain legumes are used in pig diets, are the contents of secondary plant metabolites, such as condensed tannins, protease inhibitors, and alkaloids, as well as considerably high amounts of α-galactosides. However, grain legume cultivars with negligible low contents of several secondary plant metabolites are commercially available now and should be favoured in pig nutrition. Furthermore, different processing methods, such as physical treatments, heat treatments or enzyme supplementations can be applied to further improve the feeding value of grain legumes for pigs.

1.8 Scope and Objective of the Thesis

Since there is a general ban on meat and bone meal and its by-products in diets for livestock in the EU, it is crucial to focus on alternative protein feed ingredients to be used in pig nutrition. Beside soybean meal as a commonly used plant protein supplement, grain legumes may also be used as alternative protein sources in diets for pigs. Current protein evaluation system for feed ingredients for pigs are based on the concept of standardised ileal digestibilities (SID) of crude protein (CP) and amino acids (AA), but there is a scarcity of information on SID of CP and AA in grain legumes grown in Central Europe.

In Chapter 2, the chemical composition and the SID of CP and AA in seed-grade cultivars of faba beans (*Vicia faba*), peas (*Pisum sativum*) and lupins (*Lupinus* spp.) were determined in growing pigs using the difference method. Furthermore, to obtain SID of CP and AA, the basal ileal endogenous CP and AA losses in growing pigs were estimated by means of regression analysis from apparent ileal digestible and total dietary contents of CP and AA.

In Chapter 3, *in vitro* prediction of SID of CP and AA in faba bean, pea and lupin cultivars by means of a two-step enzymatic method with subsequent pepsin and pancreatin incubations was validated by comparison of these SID values with those obtained under *in vivo* conditions in Chapter 2.

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CHAPTER 2

CHEMICAL COMPOSITION AND STANDARDISED ILEAL DIGESTIBILITIES OF CRUDE PROTEIN AND AMINO ACIDS IN GRAIN LEGUMES FOR GROWING PIGS

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2 Chemical Composition and Standardised Ileal Digestibilities of Crude Protein and Amino Acids in Grain Legumes for Growing Pigs

2.1 Summary

The study was conducted to determine chemical composition and standardised ileal digestibilities (SID) of crude protein (CP) and amino acids (AA) in currently available grain legume cultivars for growing pigs. Three consecutive experiments were conducted with six barrows each, fitted with simple ileal T-cannulas. In total, 18 assay diets including six different cultivars of faba beans (*Vicia faba*) and peas (*Pisum sativum*), respectively, five different cultivars of lupins (*Lupinus luteus*, *Lupinus angustifolius*) and one assay diet with a commercial soybean meal (SBM, 490 g kg^{-1} CP as-is) were fed according to a row-column-design. The assay feed ingredients were added to a corn starch casein-based basal diet at the expense of corn starch. Each diet was formulated to supply approximately 50% of CP and AA from the assay feed ingredient and casein, respectively. Furthermore, the basal ileal endogenous losses and SID of CP and AA in casein were estimated by regression analysis from apparent ileal digestible and total dietary contents of CP and AA. The SID of CP and AA in the grain legumes were determined in difference to SID of CP and AA originating from casein, after correcting the apparent ileal digestibilities (AID) of CP and AA in the assay diets for basal ileal endogenous CP and AA losses. The average SID of CP in faba bean cultivars (76%) and pea cultivars (79%) were lower compared to lupin cultivars (87%) and SBM (87%) (P<0.05). The SID of all AA in faba bean cultivars were lower compared to lupin cultivars and SBM (P<0.05). Digestibility values measured in the present study provide further information to accurately formulate diets for pigs based on standardised ileal digestible contents of CP and AA in currently available grain legumes for growing pigs.

2.2 Introduction

Since there is a general ban on meat and bone meal and its by-products in diets for livestock in the European Union (EC directive 999/2001), it is crucial to focus on alternative protein feed ingredients as components in diets for pigs. Beside soybeans and their by-products as commonly used plant protein supplements in diets for pigs, other grain legumes grown in Central Europe may be used as alternative protein sources as well.

Currently, protein evaluation systems for feed ingredients for pigs (e.g. NRC, 1998; AmiPig, 2000; CVB, 2003; Degussa, 2006; GfE, 2008) are based on the concept of standardised ileal digestibilities (SID) of crude protein (CP) and amino acids (AA). These feed tables have in common that there is a scarcity of information on SID values in grain legumes other than soybeans and their by-products. For example, SID values for CP and individual AA in soybean meal published by GfE (2008) have been extracted from up to 77 literature sources, whereas the corresponding values in faba beans, peas and lupins are based on up to 10, 25 and 7 reports, respectively. Moreover, published feed tables (e.g. NRC, 1998; AmiPig, 2000; CVB, 2003; Degussa, 2006; GfE, 2008) hardly take into account the variations in SID values that may occur due to recent progress in breeding of grain legumes, including the introduction of new cultivars with varying nutrient contents, in particular with respect to lower levels of antinutritional factors (ANF). The level of ANF present in grain legumes has been notably decreased, resulting in the development of zero-tannin faba bean cultivars (Duc et al., 1999) and sweet lupins with very low alkaloid contents (Petterson, 1998). Furthermore, progress in plant breeding is often associated with higher protein content but also improved protein quality of grain legumes (Monti and Grillo, 1983; Clarke and Wiseman, 2000). Moreover, growing and harvesting conditions in Central Europe, compared to other regions in the world, may affect the contents of nutrients and ANF in grain legumes (Mossé and Baudet, 1983; Simon and Köhn, 2004). Thus, the objective of this study was to determine the chemical composition and, in addition, to measure in experiments with growing pigs SID of CP and AA in currently available cultivars of faba beans (*Vicia faba*), peas (*Pisum sativum*) and lupins (*Lupinus luteus*, *Lupinus angustifolius*) grown in Central Europe.

2.3 Materials and Methods

2.3.1 Animals, Housing and Surgical Procedures

A total of three consecutive experiments with six barrows (German Landrace×Piétrain) each were conducted. The pigs were obtained from the University of Hohenheim Research Station. They were housed individually in metabolic crates (0.80 m×1.50 m), and the temperature in the research unit was adjusted automatically to 22°C. Each crate was equipped with a low pressure drinking nipple, which allowed free access to water. The pigs were surgically fitted with simple T-cannulas at the distal ileum according to the procedures described by Li et al. (1993). The cannulas were prepared from high

molecular weight polyethylene. The internal diameter of the barrel of the cannulas was 17 mm, the length of the barrel was 80 mm and each of the two curved flanges was 55 mm in length. The washer had 70 mm in diameter and screw caps were used to seal the cannulas. The research protocol was approved by the German Ethical Commission for Animal Welfare. Care of the animals used in this experiment was in accordance with the EEC directive 86/609 (1986).

2.3.2 Experimental Design and Dietary Treatments to Determine SID of CP and AA in Grain Legumes

The SID of CP and AA were determined in a total of 18 assay feed ingredients including six seed-grade cultivars of faba beans (*Vicia faba*) and peas (*Pisum sativum*), respectively, five seed-grade cultivars of lupins (*Lupinus* spp.*),* and one commercially available soybean meal (SBM, 490 g kg^{-1} CP as-is) as a reference feed ingredient. The faba bean cultivars included the two white flowered cultivars Aurelia and Gloria (Saatzucht Gleisdorf, Austria), and the four coloured flowered cultivars Divine (Agri Obtentions, France), Limbo (Lochow-Petkus GmbH, Germany), Fuego and Espresso (Norddeutsche Pflanzenzucht, Germany). All faba bean cultivars were harvested in 2004, except from cultivar Divine, which was harvested in 2005. The pea cultivars Santana (Lochow-Petkus GmbH, Germany), Jutta, Harnas, Hardy (Norddeutsche Pflanzenzucht, Germany), Phönix and Rocket (Südwestsaat GbR, Germany) were white flowered. The cultivars Santana, Jutta, Harnas and Hardy were harvested in 2004, cultivar Phönix was harvested in 2001 and cultivar Rocket was harvested in 2005. The lupin cultivars Probor, Boregine, Boruta (Saatzucht Steinach GmbH, Germany) and Idefix (Südwestsaat GbR, Germany) were *L. angustifolius* cultivars, also referred to as blue lupins, and the cultivar Bornal (Saatzucht Steinach GmbH, Germany) was a *L. luteus* cultivar, also referred to as yellow lupin. All lupins were harvested in 2005 and were low-alkaloid cultivars, also referred to as sweet lupins.

The experiment was arranged as a row-column design (John and Williams, 1995). Each of the three experiments included six pigs (rows) and six periods (columns). Within each of the three experiments, the animals were randomly allocated to the 18 assay feed ingredients in periods 1 to 3 and 4 to 6, respectively, resulting in two replications per experiment and a total of six observations per assay feed ingredient throughout all three experiments. The pigs received their assay diets at a daily level of 30 g kg^{-1} (as-fed) of

their individual body weight (BW), determined on day one of each experimental period to keep the daily feed intake constant in relation to the animal's BW during the whole experiment. The average initial and final body weight of the experimental animals was 23.2±1.9 kg and 45.3±4.1 kg, respectively. The assay diets (Table 7) were formulated to contain approximately 50% of CP and AA of the assay feed ingredient and casein, respectively. To reach the dietary threshold level for CP and each AA in the assay diet (Fan et al., 1994), casein and crystalline lysine, cystine and threonine were added to the diets. Casein was preferred as a highly digestible source of protein with a high and balanced AA profile and free of ANF to exclude potential negative effects of ANF on the determination of SID of CP and AA in the assay feed ingredients. Casein and the assay feed ingredients were included in the assay diets at the expense of corn starch. The assay diets were supplemented with 100 g kg^{-1} (as-is) dextrose to improve palatability of the diets and with 50 g kg^{-1} (as-is) cellulose as a source of fibre. Rapeseed oil at a level of 20 g kg^{-1} (as-is) was included to reduce the dustiness of the diets. DL-alpha-tocopherol acetate (Lutavit® E 50%, BASF, Ludwigshafen, Germany) was added as an antioxidant at a level of 0.1 g kg^{-1} ether extract content in the assay diets according to the manufacturer's recommendation. All diets were supplemented with 30 g kg^{-1} (as-fed) of a commercial mineral and vitamin premix and, if necessary, sodium chloride and monocalcium phosphate were added to fulfil NRC (1998) nutrient requirements for pigs from 20 to 50 kg BW. Titanium dioxide was used as a digestibility marker (5 g kg^{-1}, as-fed). The diets were fed in meal form and mixed with water (1/1 w/v).

2.3.3 Experimental Design and Dietary Treatments to Estimate Basal Ileal Endogenous Losses and SID of CP and AA in Casein

After the conclusion of each of the three experiments designed for the determination of SID of CP and AA in grain legumes, the pigs were used to estimate basal ileal endogenous losses of CP and AA ($IAAL_B$) and SID of CP and AA in casein. The basal ileal endogenous losses of CP and AA ($IAAL_B$) and SID of CP and AA in casein were estimated by regression analysis from apparent ileal digestible and total dietary contents of CP and AA (Fan et al., 1995; Eklund et al., 2008). The average initial and final BW of the experimental animals was 45.3±4.1 kg and 47.2±4.0 kg, respectively. The pigs received three semi-synthetic corn starch based diets with graded inclusion levels of casein, added to the assay diets at the expense of corn starch, and resulting in CP contents of 75, 150, 225 g kg^{-1} (as-fed). The experimental animals were randomly allocated to one of three

assay diets (Table 8) resulting in a total of six observations per assay diet. The pigs were fed their assay diets at a daily level of 30 g kg^{-1} (as-fed) of their individual BW.

Table 7. Composition of the assay diets[1] for determination of standardised ileal digestibilities of crude protein and amino acids in grain legumes (g kg^{-1} as-fed)

	Assay diets																	
Ingredient	B1	B2	B3	B4	B5	B6	P1	P2	P3	P4	P5	P6	L1	L2	L3	L4	L5	S
Assay feed ingredient	390.0	405.0	359.0	392.0	408.0	425.0	430.0	460.0	455.0	460.0	445.0	500.0	350.0	300.0	360.0	355.0	341.0	230.0
Casein[2]	91.0	94.5	89.5	92.0	95.0	93.0	97.0	97.0	107.0	97.0	96.0	107.0	82.0	90.0	89.0	88.0	84.5	120.0
Corn starch[3]	307.2	289.2	339.4	304.1	285.8	271.2	259.9	230.0	227.1	230.0	246.1	182.5	349.6	394.7	333.7	339.6	356.7	438.3
Dextrose[3]	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Cellulose[4]	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Rapeseed oil	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
L–Cystine	0.9	0.9	0.9	0.9	0.9	0.9	1.0	1.1	1.0	1.1	0.8	1.1	1.4	1.8	1.3	1.3	1.1	1.1
L–Threonine	0.5	0.6	0.5	0.6	0.5	0.5	0.3	0.5	–	0.5	0.3	–	1.4	0.8	0.9	1.0	1.1	–
L–Lysine HCl	–	–	–	–	–	–	1.0	1.0	–	1.0	1.0	–	–	–	–	–	–	–
Vitamin and mineral premix[5]	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Vitamin E[6]	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.3
Monocalcium phosphate	4.3	3.8	4.7	4.3	3.8	3.3	4.7	4.3	3.8	4.3	4.7	3.3	9.0	6.2	8.5	8.5	9.0	4.3
NaCl	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.2	1.2	1.2	1.2	1.2	1.0
Titanium dioxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

[1] Assay diets: B1=*Vicia faba cv.* Aurelia; B2=*Vicia faba cv*. Divine; B3=*Vicia faba cv.* Gloria; B4=*Vicia faba cv*. Limbo; B5=*Vicia faba cv.* Fuego; B6=*Vicia faba cv*. Espresso; P1=*P. sativum cv*. Santana; P2=*P. sativum cv*. Jutta; P3=*P. sativum cv*. Phönix; P4=*P. sativum cv*. Harnas; P5=*P. sativum cv*. Rocket; P6=*P. sativum cv.* Hardy; L1=*L. angustifolius cv.* Probor; L2=*L. luteus cv*. Bornal; L3=*L. angustifolius cv*. Boregine; L4=*L. angustifolius cv*. Boruta; L5=*L. angustifolius cv*. Idefix; S=Soybean meal (490 g kg^{-1} CP, as-is).
[2] Meggle AG, Wasserburg, Germany.
[3] Roquette GmbH, Frankfurt, Germany.
[4] Rettenmaier & Soehne, GmbH+Co.KG, Rosenberg, Germany.
[5] Supplied per kg of diet: Ca, 7.35 g; P, 1.5 g; Na, 1.65 g; Mg, 300 mg; Fe, 120 mg; Cu, 15 mg; Mn, 80 mg; Zn, 100 mg; J, 2 mg; Se, 0.4 mg; Co, 0.8 mg; Vitamin A, 1,200 IU; Vitamin D3, 180 IU; Vitamin E, 75 mg; Vitamin B1, 1.5 mg; Vitamin B2, 4 mg; Vitamin B6, 3 mg; Vitamin B12, 30 µg; Vitamin K3, 3 mg; Niacin, 18 mg; Ca-pantothenat, 11 mg; Folic acid, 0.7 mg; Cholin chlorid, 225 mg.
[6] Lutavit® E 50%, BASF, Ludwigshafen, Germany.

Table 8. Composition of the assay diets for determination of basal ileal endogenous losses and standardised ileal digestibilities of crude protein and amino acids in casein (g kg^{-1} as-fed)

	Dietary CP level		
Ingredient	75	150	225
Casein[1]	82.6	165.3	248.0
Corn starch[2]	697.2	616.7	535.5
Dextrose[2]	50.0	50.0	50.0
Cellulose[3]	50.0	50.0	50.0
Lactose[4]	50.0	50.0	50.0
Rapeseed oil	20.0	20.0	20.0
L–Cystine	0.3	1.6	3.0
L–Threonine	–	–	–
L–Lysine HCl	–	–	–
Vitamin and mineral premix[5]	30.0	30.0	30.0
Vitamin E[6]	0.2	0.2	0.2
Monocalcium phosphate	13.3	10.0	7.1
NaCl	1.4	1.2	1.2
Titanium dioxide	5.0	5.0	5.0

[1] Meggle AG, Wasserburg, Germany.
[2] Roquette GmbH, Frankfurt, Germany.
[3] Rettenmaier & Soehne, GmbH+Co.KG, Rosenberg, Germany.
[4] Peter Kölln KGaA, Elmshorn, Germany.
[5] Supplied per kg of diet: Ca, 7.35 g; P, 1.5 g; Na, 1.65 g; Mg, 300 mg; Fe, 120 mg; Cu, 15 mg; Mn, 80 mg; Zn, 100 mg; J, 2 mg; Se, 0.4 mg; Co, 0.8 mg; Vitamin A, 1,200 IU; Vitamin D3, 180 IU; Vitamin E, 75 mg; Vitamin B1, 1.5 mg; Vitamin B2, 4 mg; Vitamin B6, 3 mg; Vitamin B12, 30 µg; Vitamin K3, 3 mg; Niacin, 18 mg; Ca-pantothenat, 11 mg; Folic acid, 0.7mg; Cholin chlorid, 225 mg.
[6] Lutavit® E 50%, BASF, Ludwigshafen, Germany.

2.3.4 Experimental Procedure

After a 7-day recuperation period from surgery, the pigs were fed twice daily their assay diets in two equal meals. During each experimental period, the pigs were allowed to adapt to their daily feed allowance for 5 days before ileal digesta were collected for a total of 24 h from 19:00 to 07:00 on day 5 and from 07:00 to 19:00 on day 6. Digesta collection procedure was adapted from Li et al. (1993) using soft plastic bags attached to the barrel of the cannula by elastic bands. The bags were changed at least every 20 min and frozen immediately at –18°C. During collection, 2 ml of 2.5 M formic acid were added to the sampling bags to minimise further bacterial fermentation. The individual samples of digesta of each pig were pooled separately after each sampling period, freeze-dried and ground to 0.5 mm prior to analyses.

2.3.5 Analytical Procedure

All samples of grain legumes were analysed for proximate nutrient (Naumann and Bassler, 1997) and fibre contents (van Soest et al., 1991). Trypsin inhibitor activities (TIA) in grain legumes were determined according to the American Oil Chemists´ Society official method Ba 12-75 (AOCS, 1997). Measurements of condensed tannins in grain legumes were performed as described by Makkar et al. (1993). Furthermore, alkaloid contents of lupin samples were measured by means of gas liquid chromatography mass spectrometry procedures according to Tei and Wink (1999). Vicine and convicine contents were analysed with high performance liquid chromatography as described by Marquardt and Fröhlich (1981). The nitrogen (N) contents in the assay feed ingredients, the assay diets and in ileal digesta samples were analysed using a gas combustion method (AOAC, 2000; FP-2000, Leco Corp. St. Joseph, MI, USA). Ethylenediaminetetraacetic acid was used as a reference standard before and after all N analyses. Amino acid analyses in the assay feed ingredients, the assay diets and in ileal digesta samples were performed according to the procedures as outlined by Llames and Fontaine (1994). Tyrosine was not determined. The titanium dioxide concentrations in the assay diets and ileal digesta samples were determined according to the method described by Brandt and Allam (1987).

2.3.6 Calculations

The apparent ileal digestibilities (AID) of CP and AA and the apparent ileal digestible dietary CP and AA contents in the grain legume and casein containing assay diets were calculated using the following equations:

$$D_{Ai}=100\%-[(I_{Di}\times A_{Fi})/(A_{Di}\times I_{Fi})]\times 100\% \quad (1)$$

$$D_{Ai}=A_{Di}-(I_{Di}\times A_{Fi})/I_{Fi} \quad (2)$$

where D_{Ai}=AID of CP and AA in the i^{th} assay diet (%) in equation 1 and apparent ileal digestible content of CP and AA in the i^{th} assay diet (g kg^{-1} dry matter intake, DMI) in equation 2, I_{Di}=marker concentration in the i^{th} assay diet (g kg^{-1} DM), A_{Di}=content of CP and AA in the i^{th} assay diet (g kg^{-1} DMI), A_{Fi}=recovery of CP and AA in ileal digesta of the

i^{th} assay diet (g kg^{-1} DMI) and I_{Fi}=marker concentration in digesta of the i^{th} assay diet (g kg^{-1} DM).

The relationship between SID and AID of CP and AA in the assay diets containing graded levels of CP from casein can be expressed by equation 3. After further mathematical derivation, this relationship can also be expressed by equation 4:

$$D_S=[(D_{Ai}/A_{Di})+(A_L/A_{Di})]\times 100\% \quad (3)$$

$$D_{Ai}=-A_L+[(D_S/100)\times A_{Di}] \quad (4)$$

where D_S=SID of CP and AA (percentage), D_{Ai}=apparent ileal digestible content of CP and AA in the i^{th} assay diet (g kg^{-1} DMI), determined using equation 2, A_{Di}=content of CP and AA in the i^{th} assay diet (g kg^{-1} DM), A_L=$IAAL_B$ in ileal digesta (g kg^{-1} DMI).

Equation 4 represents a simple linear regression model in which D_{Ai} and A_{Di} are the dependent and independent variables, respectively. A_L and D_S are the regression coefficients and are estimated by fitting the linear regression model. If there are linear relationships between apparent ileal digestible and total dietary contents of CP and AA, then the $IAAL_B$ can be determined by extrapolating the dietary intakes of CP and AA to zero, namely, obtaining intercepts of the linear regression equations. The slopes of this linear regression model represent SID of CP and AA in casein.

The SID of CP and AA in the grain legumes containing assay diets were calculated by correcting AID of CP and AA for $IAAL_B$, expressed as g kg^{-1} DMI.

$$SID_D=AID_D+(IAAL_B/CP_D \text{ or } AA_D)\times 100\% \quad (5)$$

where SID_D=SID of CP or AA in the assay diets (percentage), AID_D=AID of CP and AA in the assay diets (percentage), $IAAL_B$=basal ileal endogenous losses of CP or AA (g kg^{-1} DMI) and CP_D or AA_D=CP or AA content in the assay diets (g kg^{-1} DM).

Crystalline AA (L-Lys-HCl, DL-Met, L-Cys and L-Thr) were assumed to be completely digestible at the end of the ileum. The SID of CP and AA in the assay feed ingredients were calculated according to Equation 6:

$$SID_A=(SID_D–SID_B×C_B)/C_A \quad (6)$$

where C_B=contribution level of CP or AA from casein to the assay diet (percentage) and C_A=contribution level of CP or AA from the assay feed ingredient to the assay diet (percentage).

2.3.7 Statistical Analyses

Estimates of $IAAL_B$ and determination of SID of CP and AA in casein from apparent ileal digestible and total dietary contents of CP and AA were obtained by a linear regression on CP and AA levels, respectively, using the MIXED procedure of SAS (2003). The linear model included the random effect of experiment. Moreover, the effects of chemical composition and ANF content on SID of CP and AA in grain legumes seeds were modelled by a linear regression analysis on chemical composition and ANF content. In addition, a lack-of-fit test for departure from linearity was performed. The lack-of-fit test for departure from linearity was non-significant for CP and most AA. The significance level for all Wald-type F-tests was set at α=0.05. The SID of CP and AA were subjected to a mixed model analysis using the MIXED procedure of SAS (2003). The linear model included the fixed effects of grain legume species, experiment, grain legume species×cultivar, experiment×animal, experiment×replication and the random effects experiment×replicate×period and experiment×replicate×animal. Multiple comparisons among all pairs of treatments were performed using a t-test with degrees of freedom determined by the Kenward-Roger method (Kenward and Roger, 1997). Significant differences between treatments were indicated by different superscript letters using the algorithm for letter-based representation of all pair-wise comparisons according to Piepho (2004).

2.4 Results and Discussion

2.4.1 Chemical Composition of the Assay Feed Ingredients

The chemical composition of the assay feed ingredients including contents of proximate nutrients, sugar, starch, fibre fractions, AA and ANF is presented in Tables 9 to 12. Within grain legumes, lupin cultivars had the highest average CP contents (387±52.5 g kg^{-1} DM), compared to faba bean (308±19.2 g kg^{-1} DM) and pea (249±12.8 g kg^{-1} DM) cultivars. The average CP contents in pea, faba bean and lupin cultivars were 54, 43 and 28% lower than in the SBM (541 g kg^{-1} DM) assayed in the present study, respectively. The average Met contents in the faba bean, pea and lupin cultivars were distinctly lower than in the SBM assayed in the present study (2.0, 2.3 and 2.2 g kg^{-1} DM, respectively, *vs.* 6.7 g kg^{-1} DM). The average Cys contents in the lupin cultivars (5.7 g kg^{-1} DM) were higher than in faba bean and pea cultivars (3.7 and 3.5 g kg^{-1} DM, respectively), but lower compared to the SBM assayed in the present study (7.4 g kg^{-1} DM). Moreover, the faba bean, pea and lupin cultivars contained distinctly lower amounts of Trp compared to the SBM assayed in the present study (2.6, 2.3 and 3.2 g kg^{-1} DM, respectively, *vs.* 6.9 g kg^{-1} DM). The average Thr contents in the faba bean, pea and lupin cultivars (10.6, 9.0 and 12.8 g kg^{-1} DM, respectively) were about half of the Thr content in the SBM assayed in the present study (20.0 g kg^{-1} DM). The faba bean, pea and lupin cultivars had lower average Lys contents when compared to the SBM assayed in the present study (19.0, 17.9 and 17.8 g kg^{-1} DM, respectively, *vs.* 31.8 g kg^{-1} DM), but distinctly higher contents of Lys when compared to cereals (Degussa, 2006). However, on average, the protein in the faba bean and pea cultivars contained similar or even higher levels of Lys (61.9 and 72.0 g kg^{-1} CP, respectively) when compared to SBM protein (58.8 g kg^{-1} CP) or lupin protein (46.2 g kg^{-1} CP). The average contents of Thr and Cys in the faba bean, lupin and pea protein ranged from 33.3 to 36.2 and 12.0 to 14.6 g kg^{-1} CP, respectively, and were similar to that in the SBM assayed in the present study (37.0 and 13.7 g kg^{-1} CP, respectively). The protein of faba bean, pea and lupin cultivars contained considerably lower amounts of Met (on average 6.6, 9.1 and 5.7 g kg^{-1} CP, respectively) and Trp (on average 8.6, 9.3 and 8.3 g kg^{-1} CP, respectively) in comparison to the SBM assayed in the present study (12.4 and 12.8 g kg^{-1} CP, respectively). The protein of grain legumes contains mainly two storage proteins, globulins and albumins, with a higher content of globulins in lupins and soybeans compared to faba beans or peas, whereas the content of albumins is similar or higher in faba beans and peas compared to lupins or soybean meal (Guéguen, 1983). Albumins are

relatively rich in Thr, Trp and sulphur containing AA, whereas globulins have lower contents of sulphur containing AA (Casey et al., 1986).

The average content of ether extracts (EE) in pea and faba bean cultivars was lower compared to the lupin cultivars (on average 15 and 20 g kg^{-1} DM, respectively, *vs.* 60 g kg^{-1} DM). The faba bean and pea cultivars were rich in starch (on average 438 and 505 g kg^{-1} DM, respectively), whereas lupin cultivars contained comparatively low levels of starch (on average 79 g kg^{-1} DM). Average sugar contents amounted to 28.5 g kg^{-1} DM in the faba bean cultivars, 45 g kg^{-1} DM in pea cultivars and 59 g kg^{-1} DM in lupin cultivars. All assayed grain legumes contained similar amounts of ash. With regard to fibre contents, the average contents of neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose (NDF–ADF) and cellulose (ADF–ADL) were lower in faba bean and pea cultivars and SBM, compared to lupin cultivars. In lupin cultivars, cellulose contents were higher than hemicellulose contents (on average 199 *vs*. 41 g kg^{-1} DM), which is in accordance with other reports (Bach Knudsen, 1997; Reddy et al., 1984; Salgado et al., 2002). In the present study, faba bean fibres consisted mainly of cellulose (on average 115 g kg^{-1} DM), with some hemicellulose (on average 21 g kg^{-1} DM) whereas pea fibres contained a smaller proportion of hemicellulose (on average 32 g kg^{-1} DM) than cellulose (on average 76 g kg^{-1} DM). In contrast to the pea cultivars in the present study, Salgado et al. (2002) assayed higher hemicellulose than cellulose contents in peas (95 *vs.* 59 g kg^{-1} DM). The differences between both studies may result from the use of different batches of peas. Although proximate nutrient contents of peas in the present study and that by Salgado et al. (2002) were similar, there were considerable differences in the starch contents (505 *vs.* 356 g kg^{-1} DM) between both studies.

In the present study, the CP or AA content was not affected by the chemical composition, including contents of proximate nutrients, fibre fractions and ANF of the grain legumes ($P>0.05$, data not shown). On average, analysed chemical composition of faba bean, pea and lupin cultivars were in general agreement with published feed tables (AmiPig, 2000; Degussa, 2006; NRC, 1998). However, the individual values of chemical composition in grain legumes may vary greatly, both between and within grain legume species (Castell et al., 1996; Gatel and Grosjean, 1990; Petterson, 2000). Among faba bean cultivars, CP contents ranged from 285 g kg^{-1} DM (*cv*. Espresso) to 337 g kg^{-1} DM (*cv.* Gloria), which is up to 28 g kg^{-1} lower (*cv*. Espresso) compared to values published by AmiPig (2000) and up to 45 g kg^{-1} higher (*cv*. Gloria) compared to values published by

NRC (1998). Among pea cultivars, the CP content was the lowest in the cultivar Hardy (224 g kg^{-1} DM), and ranged from 247 to 260 g kg^{-1} DM for the remaining pea cultivars Phönix, Santana, Rocket, Harnas and Jutta. These values are up to 32 g kg^{-1} lower (*cv*. Hardy) compared to values published by NRC (1998) and up to 25 g kg^{-1} higher (*cv*. Jutta) compared to values published by Degussa (2006). Among lupin cultivars, CP contents of the *L. angustifolius* cultivars (Boruta, Boregine, Probor and Idefix) ranged from 339 to 383 g kg^{-1} DM, whereas the *L. luteus* cultivar Bornal had a distinctly higher CP content (476 g kg^{-1} DM). In contrast to the present study, most published feed tables (e.g. AmiPig, 2000; NRC, 1998), except for Degussa (2006), include data on *L. albus* only, but not on *L. angustifolius* and *L. luteus* species. In the present study, the average CP content in *L. angustifolius* (339 to 383 g kg^{-1}) was similar to values reported by AmiPig (2000) for *L. albus* (343 g kg^{-1}) but lower compared to values reported by NRC (1998) for white lupins (392 g kg^{-1}). The CP contents of *L. angustifolius* cultivars in the present study were similar to values published by Degussa (2006) for blue lupins. Similarly to the present study, Degussa (2006) reported higher CP levels for *L. luteus* compared to *L. angustifolius*. However, the CP content of the *L. luteus* cultivar Bornal was 95 g kg^{-1} DM higher in the present study compared to data published by Degussa (2006) for yellow lupins. Thus, there may be a need to differentiate in feed tables between lupin subspecies.

Table 9. The analysed chemical composition of assay feed ingredients (g kg^{-1} DM)

	Cultivar	DM	CP	EE	Ash	Starch	Sugar	NDF	ADF	ADL	HC	Cellulose
Vicia faba	Aurelia[1]	877.5	314.2	16.4	38.8	448.6	26.4	125.8	101.0	1.2	24.8	99.8
	Divine[2]	882.5	300.3	14.2	38.1	450.5	31.0	127.5	111.9	7.9	15.6	104.0
	Gloria[1]	886.1	336.9	13.1	43.0	432.2	30.2	127.0	111.3	1.3	15.7	110.0
	Limbo[2]	890.4	318.8	16.5	34.0	435.9	27.2	137.6	115.9	4.8	21.7	111.1
	Fuego[2]	875.7	291.6	14.7	39.8	425.3	29.5	165.0	137.0	3.1	28.0	133.9
	Espresso[2]	871.6	285.2	15.7	34.5	422.0	26.8	155.6	134.4	3.9	21.2	130.5
	Mean±SD	*880.6±6.99*	*307.8±19.17*	*15.1±1.34*	*38.0±3.38*	*438.0±11.78*	*28.5±1.96*	*139.8±16.74*	*118.6±14.17*	*3.7±2.50*	*21.2±4.92*	*114.9±14.07*
P. sativum	Santana[1]	880.9	252.2	18.4	32.2	498.4	45.5	104.7	80.5	2.2	24.2	78.3
	Jutta[1]	869.2	260.0	19.7	33.8	501.4	42.4	104.3	78.9	1.9	25.4	77.0
	Phönix[1]	876.7	246.7	22.8	31.2	510.1	47.3	106.0	68.0	3.1	38.0	64.9
	Harnas[1]	871.0	254.7	19.8	31.6	492.4	47.1	108.9	86.2	1.9	22.7	84.3
	Rocket[1]	874.4	254.0	19.6	34.9	490.3	44.4	126.3	82.9	2.3	43.4	80.6
	Hardy[1]	872.0	223.9	19.3	32.5	534.2	41.1	114.9	75.0	2.2	39.9	72.8
	Mean±SD	*874.0±4.27*	*248.6±12.83*	*19.9±1.49*	*32.7±1.40*	*504.5±16.18*	*44.6±2.51*	*110.9±8.52*	*78.6±6.41*	*2.3±0.44*	*32.3±9.15*	*76.3±6.77*
Lupinus spp.	Probor[3]	902.4	377.0	62.7	37.5	93.4	61.4	223.5	185.0	8.6	38.5	176.4
	Bornal[4]	892.3	475.5	57.2	49.3	41.9	64.2	252.2	208.1	10.9	44.1	197.2
	Boregine[3]	908.6	358.7	59.3	35.7	99.8	66.0	247.1	195.0	8.6	52.1	186.4
	Boruta[3]	901.3	339.2	53.7	41.2	93.0	50.7	260.5	229.7	7.4	30.8	222.3
	Idefix[3]	905.3	382.5	68.1	41.5	68.4	50.7	258.3	218.9	8.6	39.4	210.3
	Mean±SD	*902.0±6.11*	*386.6±52.53*	*60.2±5.49*	*41.0±5.23*	*79.3±24.11*	*58.6±7.4*	*248.3±14.84*	*207.3±17.93*	*8.8±1.27*	*41.0±7.84*	*198.5±18.32*
SBM		905.4	540.8	27.7	73.9	50.6	105.8	113.9	74.4	3.3	39.5	71.1
Casein		900.5	989.1	–	–	–	–	–	–	–	–	–

[1] White flowered cultivar; [2] Coloured flowered cultivar.
[3] *L. angustifolius;* [4] *L. luteus.*
–, Not determined.
SD, Standard deviation.
SBM, Soybean meal (490 g kg^{-1} CP, as-is).
EE, Ether extract; NDF, Neutral detergent fibre; ADF, Acid detergent fibre; ADL, Acid detergent lignin; HC, Hemicellulose=NDF–ADF; Cellulose=ADF–ADL.

Table 10. The analysed contents of indispensable amino acids in the assay feed ingredients (g kg^{-1} DM)

	Cultivar	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Vicia faba	Aurelia[1]	30.6	8.0	12.7	22.4	18.9	2.0	13.0	10.5	2.7	14.0
	Divine[2]	30.8	7.5	11.8	22.0	18.5	2.0	12.7	10.8	2.6	13.1
	Gloria[1]	32.8	8.5	13.9	24.1	20.7	2.2	14.0	11.1	2.7	14.9
	Limbo[2]	29.7	8.2	12.3	22.3	19.3	2.0	13.0	10.8	2.7	13.6
	Fuego[2]	24.9	7.7	11.8	20.8	18.5	2.0	12.5	10.2	2.6	13.1
	Espresso[2]	25.2	7.6	11.5	20.6	18.3	1.9	12.4	9.9	2.5	13.0
	Mean±SD	*29.0±3.22*	*7.9±0.39*	*12.3±0.88*	*22.0±1.27*	*19.0±0.89*	*2.0±0.10*	*12.9±0.58*	*10.6±0.44*	*2.6±0.08*	*13.6±0.74*
P. sativum	Santana[1]	22.8	6.4	10.8	18.1	18.5	2.3	12.0	9.1	2.4	12.0
	Jutta[1]	25.2	6.3	10.2	17.8	17.8	2.3	11.6	9.3	2.4	11.6
	Phönix[1]	20.8	6.3	10.7	17.7	18.1	2.2	12.0	8.9	2.3	11.7
	Harnas[1]	23.2	6.3	10.3	18.0	18.1	2.3	11.6	9.4	2.4	11.7
	Rocket[1]	23.2	5.7	10.6	18.1	18.5	2.2	12.2	8.9	2.2	11.8
	Hardy[1]	19.0	5.6	9.4	15.7	16.3	2.2	10.6	8.4	2.2	10.6
	Mean±SD	*22.4±2.16*	*6.1±0.35*	*10.3±0.51*	*17.6±0.93*	*17.9±0.82*	*2.3±0.05*	*11.7±0.58*	*9.0±0.36*	*2.3±0.10*	*11.6±0.49*
Lupinus spp.	Probor[3]	48.8	10.3	15.4	26.6	17.6	2.0	15.7	13.1	3.2	14.4
	Bornal[4]	49.6	11.5	16.6	31.3	21.6	2.6	17.1	14.3	3.3	15.6
	Boregine[3]	38.9	9.2	13.6	23.3	16.1	2.1	13.5	11.8	3.1	13.6
	Boruta[3]	41.2	9.4	14.1	24.3	16.5	2.1	14.1	12.3	3.0	13.9
	Idefix[3]	41.1	9.5	15.1	24.7	17.3	2.1	14.9	12.5	3.2	14.4
	Mean±SD	*43.9±4.91*	*10.0±0.95*	*15.0±1.17*	*26.0±3.17*	*17.8±2.2*	*2.2±0.24*	*15.1±1.41*	*12.8±0.96*	*3.2±0.11*	*14.4±0.76*
SBM		39.8	14.6	24.7	40.2	31.8	6.7	26.1	20.0	6.9	26.4
Casein		36.0	29.7	50.1	91.8	78.4	28.2	50.7	41.2	12.6	65.5

[1] White flowered cultivar.
[2] Coloured flowered cultivar.
[3] *L. angustifolius.*
[4] *L. luteus.*
SD, Standard deviation.
SBM, Soybean meal (490 g kg^{-1} CP, as-is).

Table 11. The analysed contents of dispensable amino acids in the assay feed ingredients (g kg^{-1} DM)

	Cultivar	Ala	Asp	Cys	Glu	Gly	Pro	Ser
Vicia faba	Aurelia[1]	12.4	33.4	3.8	49.8	13.0	12.4	14.2
	Divine[2]	11.8	32.2	3.5	50.5	12.5	13.0	14.7
	Gloria[1]	13.0	36.6	4.0	53.6	13.8	13.0	15.5
	Limbo[2]	12.4	33.6	3.8	50.9	12.8	12.0	14.9
	Fuego[2]	11.8	31.0	3.6	47.3	12.3	11.4	13.6
	Espresso[2]	11.6	31.1	3.4	47.4	11.9	11.4	13.3
	Mean±SD	*12.2±0.53*	*33.0±2.08*	*3.7±0.22*	*49.9±2.37*	*12.7±0.66*	*12.2±0.73*	*14.4±0.83*
P. sativum	Santana[1]	10.8	29.8	3.6	42.2	10.9	10.4	11.4
	Jutta[1]	10.7	29.3	3.6	42.4	10.7	10.5	11.6
	Phönix[1]	10.5	29.4	3.5	40.8	10.8	10.6	11.1
	Harnas[1]	10.7	29.2	3.6	42.0	10.7	10.2	11.7
	Rocket[1]	10.4	28.4	3.0	42.5	10.4	10.3	11.7
	Hardy[1]	9.8	25.7	3.4	36.6	9.8	9.1	10.3
	Mean±SD	*10.5±0.37*	*28.6±1.51*	*3.5±0.23*	*41.1±2.28*	*10.6±0.40*	*10.2±0.55*	*11.3±0.54*
Lupinus spp.	Probor[3]	12.5	39.3	5.4	89.4	15.8	16.4	19.8
	Bornal[4]	14.3	43.4	8.2	100.8	16.9	16.6	21.1
	Boregine[3]	11.4	33.1	5.2	74.1	14.3	13.2	16.1
	Boruta[3]	12.2	35.1	5.2	83.8	15.0	14.8	17.2
	Idefix[3]	12.0	37.4	4.5	80.4	15.4	14.7	18.0
	Mean±SD	*12.5±1.09*	*37.7±3.97*	*5.7±1.44*	*85.7±10.1*	*15.5±0.97*	*15.1±1.40*	*18.4±2.01*
SBM		23.2	60.4	7.4	94.1	22.4	27.3	25.8
Casein		29.6	69.4	3.3	206.5	17.8	101.7	55.3

[1] White flowered cultivar.
[2] Coloured flowered cultivar.
[3] *L. angustifolius.*
[4] *L. luteus.*
SD, Standard deviation.
SBM, Soybean meal (490 g kg^{-1} CP as-is).

Table 12. The analysed contents of antinutritional factors in the assay feed ingredients (g kg^{-1} DM)

	Cultivar	TIA (mg TI/g CP)	Condensed tannins	Alkaloids	Vicine	Convicine
Vicia faba	Aurelia[1]	3.9	ND	–	7.2	2.8
	Divine[2]	1.4	2.1	–	0.3	0.0
	Gloria[1]	3.3	ND	–	6.4	3.2
	Limbo[2]	<0.2	7.0	–	5.6	3.1
	Fuego[2]	<0.2	7.4	–	7.0	3.7
	Espresso[2]	<0.2	4.2	–	6.7	3.7
P. sativum	Santana[1]	2.4	ND	–	–	–
	Jutta[1]	1.8	ND	–	–	–
	Phönix[1]	5.0	ND	–	–	–
	Harnas[1]	<0.2	ND	–	–	–
	Rocket[1]	3.9	ND	–	–	–
	Hardy[1]	4.5	ND	–	–	–
Lupinus spp.	Probor[3]	2.9	ND	0.07	–	–
	Bornal[4]	<0.2	ND	0.26	–	–
	Boregine[3]	<0.2	ND	0.04	–	–
	Boruta[3]	<0.2	ND	0.28	–	–
	Idefix[3]	<0.2	ND	0.18	–	–
SBM		5.8	ND	–	–	–

[1] White flowered cultivar.
[2] Coloured flowered cultivar.
[3] *L. angustifolius.*
[4] *L. luteus.*
–, not determined.
TI, Trypsin inhibitor; TIA, Trypsin inhibitor activity; ND, Not detectable; SBM, Soybean meal (490 g kg^{-1} CP, as-is).

2.4.2 Basal Ileal Endogenous Losses and SID of CP and AA in Casein

Three graded levels of CP in the assay diets were obtained by including casein at levels of 83, 165 and 248 g kg^{-1} (as-fed) in these diets. The analysed contents of CP and AA in the assay diets are presented in Table 13, whereas the contents of apparent ileal digestible CP and AA are shown in Table 14. In response to the increase in the dietary CP level from 150 to 225 g kg^{-1} (as-fed), linear increases (P<0.05) in the contents of apparent ileal digestible CP and AA were obtained. Linear relationships between apparent ileal digestible and total dietary contents of CP and AA were established. The slopes of these linear regressions represent estimates for SID of CP and AA originating from casein. By extrapolating the dietary CP and AA levels to zero intake, estimates of $IAAL_B$ can be obtained. The SID of CP and AA and the $IAAL_B$ i.e. the intercepts of the linear relationships with their corresponding standard errors are shown in Table 15. There were significant linear relationships between apparent ileal digestible and total dietary contents of CP and AA, and estimates of $IAAL_B$ were significantly different from zero, as well. Estimates of SID of CP and AA from casein were close to 100% with values ranging from 99.3 (P<0.001) to 101.3% (P<0.001) for Cys and Gly, respectively, which is in accordance with previous reports in grower finisher pigs, determined either by regression analysis (Jorgensen and Gabert, 2001) or by means of the homoarginine method (Nyachoti et al., 1997b; Yin et al., 2004). In the present study, $IAAL_B$ amounted to 16.1 g kg^{-1} DMI for CP and ranged from 0.2 g kg^{-1} DMI for Trp and Met to 2.1 g kg^{-1} DMI for Glu. These estimates of $IAAL_B$ are in general agreement with those obtained by regression analysis in grower finisher pigs fed diets with graded levels of CP and AA, originating from soybean meal (Fan et al., 1995).

Table 13. The analysed chemical composition of the assay diets for determination of basal ileal endogenous losses and standardised ileal digestibilities of crude protein and amino acids in casein (g kg^{-1} DM)

	Dietary CP level[1]		
	75	150	225
DM	903.6	904.8	894.7
CP	89.2	174.5	270.1
Indispensable AA			
Arg	3.4	6.5	9.9
His	2.8	5.4	8.0
Ile	4.7	9.3	13.9
Leu	8.9	17.1	25.8
Lys	7.5	14.6	21.8
Met	2.5	5.0	7.6
Phe	4.8	9.3	14.0
Thr	4.1	7.6	11.5
Trp	1.1	2.2	3.3
Val	6.1	12.2	18.0
Dispensable AA			
Ala	3.0	5.5	8.2
Asp	6.8	12.9	19.4
Cys	0.6	1.7	3.0
Glu	20.9	40.1	60.2
Gly	1.8	3.3	5.0
Pro	11.1	22.4	33.3
Ser	5.5	10.2	15.5

[1] g kg^{-1} as-fed.

Table 14. The contents (g kg^{-1} DMI) of apparent ileal digestible crude protein and amino acids in the assay diets for the determination of basal ileal endogenous losses and standardised ileal digestibilities of crude protein and amino acids in casein

	Dietary CP level[1]				
Item	75	150	225	Pooled SEM	*P*-values[2]
n	6	6	6		
CP	73.7	160.4	256.2	1.06	<0.001
Indispensable AA					
Arg	3.0	6.1	9.4	0.03	<0.001
His	2.5	5.2	7.7	0.02	<0.001
Ile	4.0	8.8	13.3	0.04	<0.001
Leu	8.1	16.4	25.1	0.05	<0.001
Lys	6.9	14.0	21.2	0.04	<0.001
Met	2.3	4.9	7.4	0.01	<0.001
Phe	4.4	8.8	13.6	0.03	<0.001
Thr	3.2	6.9	10.7	0.07	<0.001
Trp	0.9	2.0	3.1	0.01	<0.001
Val	5.3	11.5	17.3	0.05	<0.001
Dispensable AA					
Ala	2.3	4.9	7.7	0.04	<0.001
Asp	5.7	11.8	18.4	0.06	<0.001
Cys	0.3	1.5	2.7	0.04	<0.001
Glu	18.9	38.3	58.5	0.14	<0.001
Gly	1.2	2.7	4.3	0.04	<0.001
Pro	10.3	21.7	32.4	0.07	<0.001
Ser	4.4	9.3	14.6	0.06	<0.001

[1] g kg^{-1} as-fed.
[2] *P*-values for linear effect of dietary CP level.
n, number of observations.
SEM, Standard error of means.

Table 15. The estimates of the linear regression between dietary contents of apparent ileal digestible and total crude protein and amino acids

	Standardised ileal digestibility (%)		Endogenous loss (g kg^{-1} DM intake)	
Item	Slope±SE	*P* slope[1]	Intercept±SE	*P* intercept[2]
CP	100.9±0.6	<0.001	16.1±1.33	<0.001
Indispensable AA				
Arg	100.7±0.6	<0.001	0.5±0.04	<0.001
His	99.8±0.6	<0.001	0.3±0.03	<0.001
Ile	101.1±0.5	<0.001	0.7±0.05	<0.001
Leu	100.3±0.4	<0.001	0.8±0.08	<0.001
Lys	100.4±0.4	<0.001	0.6±0.06	<0.001
Met	100.5±0.2	<0.001	0.2±0.01	<0.001
Phe	100.3±0.4	<0.001	0.5±0.04	<0.001
Thr	100.7±0.7	<0.001	0.8±0.08	<0.001
Trp	100.8±0.8	<0.001	0.2±0.02	<0.001
Val	100.5±0.5	<0.001	0.8±0.07	<0.001
Dispensable AA				
Ala	101.1±0.9	<0.001	0.7±0.06	<0.001
Asp	101.0±0.6	<0.001	1.2±0.09	<0.001
Cys	99.3±0.7	<0.001	0.3±0.04	0.012
Glu	100.7±0.3	<0.001	2.1±0.18	<0.001
Gly	101.3±0.2	<0.001	0.7±0.06	<0.001
Pro	99.6±0.4	<0.001	0.7±0.10	<0.001
Ser	101.2±0.8	<0.001	1.1±0.09	<0.001

[1] *P*-values of the estimates for the slopes of the regression equations.
[2] *P*-values of the estimates for the intercepts of the regression equations.
SE, Standard error.

2.4.3 Standardised Ileal Digestibility of CP and AA in Grain Legumes

The analysed chemical composition of the assay diets for determination of SID of CP and AA in grain legumes is presented in Table 16. The CP and AA contents in the assay diets were in good agreement with those calculated from the single ingredients. All pigs recovered well from surgery, seemed healthy and readily consumed their daily feed allowances. Observations of animals with ileal DM digestibility in the assay diets having a Cook´s D greater than 2.5 were considered as influential and hence deleted from further analysis. Therefore, the observation of one animal for the lupin cultivar Idefix (experiment 3, period 2) with an 18 percentage units lower ileal DM digestibility compared to the average of all treatments was removed from the data set. Thus, there were only five observations for the lupin cultivar Idefix.

Table 16. The analysed chemical composition of the assay diets for determination of standardised ileal digestibilities of crude protein amino acids in grain legumes (g kg^{-1} DM)[1]

	Assay diets																	
	B1	B2	B3	B4	B5	B6	P1	P2	P3	P4	P5	P6	L1	L2	L3	L4	L5	S
DM	895.1	897.1	891.2	897.5	892.4	896.3	895.0	897.2	885.6	895.6	894.4	895.5	903.8	900.3	900.0	902.1	902.5	897.5
CP	224.6	218.4	222.2	219.1	217.6	221.7	213.8	220.6	225.3	221.4	213.4	220.0	229.5	237.8	223.6	224.0	223.0	262.8
Indispensable AA																		
Arg	15.8	14.8	15.6	15.0	13.5	14.0	13.6	14.7	13.2	14.3	13.9	13.3	18.8	19.2	16.7	16.9	17.5	14.5
His	6.5	6.2	6.2	6.2	6.2	6.2	6.0	6.0	6.4	6.0	5.8	6.2	6.6	6.7	6.5	6.4	6.2	7.4
Ile	10.2	10.1	10.2	9.9	9.9	9.8	10.0	9.9	10.6	10.1	10.0	10.4	10.1	10.1	10.1	9.9	9.8	12.6
Leu	18.7	18.4	18.5	18.3	18.0	18.3	17.9	17.8	19.0	18.0	17.8	18.4	18.0	19.1	18.0	17.7	17.1	22.4
Lys	15.7	15.4	15.4	15.5	15.4	15.6	17.2	17.0	17.2	17.3	17.0	16.8	13.4	14.6	13.8	13.4	13.1	18.3
Met	3.7	3.7	3.6	3.6	3.7	3.7	4.0	3.9	4.3	4.0	3.8	4.2	3.3	3.6	3.5	3.4	3.2	5.3
Phe	10.3	10.1	10.1	10.2	10.1	10.2	10.6	10.3	11.3	10.5	10.4	10.9	9.8	10.1	9.8	9.6	9.5	13.3
Thr	9.2	9.1	8.9	9.1	8.9	8.9	8.9	9.3	9.1	9.3	8.7	9.0	9.8	9.5	9.4	9.4	9.2	10.6
Trp	2.3	2.3	2.2	2.2	2.3	2.3	2.3	2.3	2.5	2.3	2.2	2.4	2.2	2.3	2.4	2.3	2.2	3.2
Val	12.3	12.2	12.1	12.1	12.0	12.2	12.2	12.1	12.8	12.2	12.0	12.7	11.1	11.3	11.6	11.2	10.9	14.8
Indispensable AA																		
Ala	8.1	8.0	7.9	8.0	7.9	7.9	7.9	8.0	8.3	8.1	7.8	8.3	7.2	7.6	7.3	7.3	7.0	9.6
Asp	20.5	19.8	20.3	20.0	19.3	19.6	20.1	20.3	21.0	20.5	19.4	20.3	19.7	20.1	19.0	19.0	19.1	24.2
Cys	2.8	2.7	2.7	2.8	2.8	2.7	2.9	2.9	3.1	2.9	2.5	3.1	3.7	4.8	3.7	3.6	3.1	3.5
Glu	42.5	41.8	41.8	42.2	41.1	41.8	41.8	41.7	44.1	42.4	41.0	43.0	49.4	51.4	47.8	47.9	46.6	52.5
Gly	7.0	6.9	6.9	7.0	6.8	6.8	6.6	6.7	7.0	6.9	6.5	6.9	7.2	7.1	7.1	7.1	7.0	7.9
Pro	18.8	17.6	17.6	17.8	17.0	17.1	17.5	17.0	19.2	17.3	16.8	19.0	17.3	17.5	17.9	16.9	16.9	23.3
Ser	11.7	11.4	11.4	11.5	11.1	11.2	11.2	11.0	11.9	11.3	10.9	11.4	11.8	12.1	11.3	11.4	11.2	14.0

[1] Assay diets: B1=*Vicia faba cv.* Aurelia; B2=*Vicia faba cv.* Divine; B3=*Vicia faba cv.* Gloria; B4=*Vicia faba cv.* Limbo; B5=*Vicia faba cv.* Fuego; B6=*Vicia faba cv.* Espresso; P1=*P. sativum cv.* Santana; P2=*P. sativum cv.* Jutta; P3=*P. sativum cv.* Phönix; P4=*P. sativum cv.* Harnas; P5=*P. sativum cv.* Rocket; P6=*P. sativum cv.* Hardy; L1=*L. angustifolius cv.* Probor; L2=*L. luteus cv.* Bornal; L3=*L. angustifolius cv.* Boregine; L4=*L. angustifolius cv.* Boruta; L5=*L. angustifolius cv.* Idefix; S=Soybean meal (490 g kg^{-1} CP, as-is).

The average SID of CP and AA in faba beans (*n*=6 cultivars, 36 observations), peas (*n*=6 cultivars, 36 observations), lupins (*n*=5 cultivars, 29 observations) and SBM (*n*=1, 6 observations) are shown in Table 17. The SID of indispensable AA in SBM were consistently high with values ranging from 85% for Thr and Trp to 94% for Arg, and were similar to SID values for soybean meal reported in currently used feed tables (e.g. AmiPig, 2000; Rademacher et al., 2009).

The average SID of CP and most AA did not differ ($P<0.05$) between lupin cultivars and SBM, but SID of CP and most AA were lower ($P<0.05$) in pea and faba bean cultivars compared to lupin cultivars and SBM. The average SID of CP and most AA in faba bean and pea cultivars were 5 to 25 percentage units lower compared to lupin cultivars and SBM ($P<0.05$). The greatest differences in SID of AA in grain legumes was found for Met, Cys and Trp, which is in agreement with values published by Rademacher et al. (2009). The relatively low ileal digestibility of Met, Cys and Trp in faba beans, peas and lupins can be attributed, at least in part, to the higher albumin content in these grain legumes compared to SBM (Guéguen, 1983). Although albumin has a high concentration of Met, Cys and Trp, AA originating from albumin are assumed to have a relatively low digestibility compared to other protein fractions, such as globulins (Le Guen et al., 1995a,b). Another factor explaining the low SID of Met, Cys and Trp in faba beans, peas and lupins is their low concentration in these grain legumes, resulting in a low contribution level of these AA to their total contents in the assay diet when the difference method is used. Due to the low contribution level, any change in SID of these AA in the assay diets results in a considerable change in the digestibility of these AA in the assay feed ingredient (Knabe et al., 1989). Therefore, low SID of Met, Cys and Trp in faba beans, peas and lupins might, at least in part, be simply a reflection of an experimental error. As a result, relatively low contents and SID of these AA may limit the use of grain legumes as a sole protein source in diet formulation for pigs (Gatel and Grosjean, 1990; Gatel, 1994; Mekbungwan, 2007).

Table 17. Average standardised ileal digestibilities (%) of crude protein and amino acids in faba beans, peas, lupins and soybean meal[1]

Species	Faba beans	Peas	Lupins	SBM	*P*-value[2]	*P*-value[2]
Cultivars	6	6	5	1	species	species×cultivar
n	36	36	29	18		
CP	76±1.4[a]	79±1.4[a]	87±1.5[b]	87±3.5[b]	<0.001	<0.001
Indispensable AA						
Arg	87±0.9[a]	89±0.9[ab]	95±0.9[c]	94±2.1[bc]	<0.001	<0.001
His	78±1.4[a]	81±1.4[ab]	88±1.6[c]	89±3.5[bc]	0.002	<0.001
Ile	80±1.4[a]	81±1.4[a]	89±1.5[b]	91±3.4[b]	0.001	<0.001
Leu	79±1.3[a]	80±1.3[a]	88±1.4[b]	89±3.2[b]	0.001	<0.001
Lys	82±1.0[a]	85±1.0[ab]	87±1.1[b]	90±2.6[b]	0.011	0.003
Met	67±2.4[a]	76±2.4[b]	81±2.7[bc]	91±6.0[c]	0.003	<0.001
Phe	79±1.2[a]	82±1.2[a]	88±1.4[b]	90±3.0[b]	0.002	<0.001
Thr	74±1.6[a]	75±1.6[a]	84±1.8[b]	85±4.1[b]	0.003	<0.001
Trp	61±2.3[a]	67±2.3[a]	82±2.6[b]	85±5.8[b]	<0.001	<0.001
Val	76±1.5[a]	78±1.5[a]	85±1.6[b]	88±3.7[b]	0.003	<0.001
Dispensable AA						
Ala	75±1.5[a]	76±1.5[a]	82±1.7[b]	87±1.5[b]	0.010	<0.001
Asp	81±1.2[a]	83±1.2[ab]	89±1.3[c]	89±2.8[bc]	0.003	<0.001
Cys	57±2.3[a]	67±2.3[b]	84±2.5[c]	82±5.7[c]	<0.001	<0.001
Glu	86±0.9[a]	87±0.9[ab]	93±1.1[c]	91±2.2[bc]	<0.001	0.027
Gly	68±1.6[a]	73±1.6[b]	83±1.8[c]	82±3.9[bc]	<0.001	<0.001
Pro	74±2.1[a]	80±2.1[b]	89±2.3[c]	91±5.1[bc]	0.001	<0.001
Ser	81±1.5[a]	81±1.5[a]	89±1.6[b]	90±3.7[b]	0.003	<0.001

[1] LSmeans±SEM (standard error of means).
[2] *P*-values for Wald–type F–tests for treatment differences.
SBM, Soybean meal (490 g kg^{-1}CP, as-is).
n, Number of observations.
[a,b,c] Within a row LSmeans with a common superscript are not significantly different at (α=0.05).

The SID of CP and AA in the individual cultivars of faba beans, peas and lupins are shown in Tables 18 to 20. Among faba bean cultivars, the SID of CP ranged from 70% (*cv*. Espresso) to 81% (*cv*. Aurelia and Divine). The SID of indispensable AA ranged from 53% for Trp (*cv*. Fuego and Espresso) to 91% for Arg (*cv*. Aurelia). In general, highest SID in faba bean cultivars were obtained for Arg (84 to 91%) and lowest for Cys, Trp, Met and Gly (48 to 65, 53 to 71, 58 to 77 and 59 to 74%, respectively). Except for Lys and Glu, SID values for the white flowered cultivars Aurelia, Gloria and the coloured flowered cultivar Divine were 3 to 19 percentage units higher (P<0.05) than those in the coloured flowered cultivars Limbo, Espresso and Fuego. The SID of CP and AA in cultivars Aurelia, Gloria and Divine were 2 to 14 percentage units lower than those in SBM, whereas SID of Lys,

Thr, Asp and Glu in cultivars Aurelia and Gloria was similar to those in SBM (P>0.05). The SID of CP and AA in cultivars Limbo, Fuego, Espresso were 7 to 26 percentage units lower than in SBM (P<0.05).

The lower SID values in faba bean cultivars compared to lupin cultivars or SBM may be, at least in part, due to differences in their chemical composition including the presence of several ANF in faba beans (e.g. Lallès and Jansman, 1998; van der Poel et al., 1992) (Table 12). The two white flowered faba bean cultivars Aurelia and Gloria were free of tannins. The coloured flowered cultivar Divine contained small amounts of condensed tannins (2.1 g kg^{-1} DM), whereas contents in the other coloured flowered faba bean cultivars Espresso, Limbo and Fuego ranged from 4.2 to 7.4 g kg^{-1} DM. As a result, contents of condensed tannins amounted to 0, 0, 0.9, 1.8, 2.7, and 3.0 g kg^{-1} DM in the assay diets containing the cultivars Aurelia, Gloria, Divine, Limbo, Fuego and Espresso, respectively. For the faba bean containing assay diets, there were linear decreases in SID of CP and AA (P<0.05) as the contents of condensed tannins in the assay diets increased (Table 21). Therefore, lower SID values in the coloured flowered faba bean cultivars Limbo, Fuego and Espresso may result from their higher tannin contents. Condensed tannins are the predominant phenolic compounds found in faba beans (Bastianelli et al., 1998; Marquardt et al., 1977). Condensed tannins are known to exert particularly negative effects on protein digestibility, such as they may form complexes with both dietary proteins and digestive enzymes (Jansman, 1993). As a result, the activity of proteinaceous digestive enzyme may be inhibited or the secretion of endogenous proteins may be stimulated (Marquardt, 1989). In agreement with the results of the present study, Jansman et al. (1993) and Mariscal-Landín et al. (2002) reported lower digestibility of CP and AA as the tannin contents in faba beans increased. Tannin levels in the coloured flowered faba beans in the present study correspond well with values published by Kasprowicz and Frankiewicz (2004), whereas considerably higher tannin levels (15.7 to 35.4 g kg^{-1}, air-dry basis) have been reported in earlier studies by Makkar et al. (1997). This reduction in condensed tannin levels in coloured flowered faba beans in the present study reflects progress in plant breeding towards removal of tannins by using cultivars with any of the two complementary zero-tannin genes, *zt-1* and *zt-2*, which are also present in tannin free, white flowered plants (Picard, 1976). As a result, higher SID values were obtained in white flowered tannin free faba bean cultivars compared to coloured flowered cultivars (Mariscal-Landín et al., 2002). Furthermore, the allele *zt-2* seems to be associated with decreased fibre content, due to a reduction of the seed coat (Duc et al., 1999). In the present study,

the four coloured flowered, tannin containing faba bean cultivars had higher NDF and ADF contents compared to the two white flowered tannin free cultivars. Ileal AA digestibility have been shown to decrease as the fibre contents, such as NDF and ADF, increase, because endogenous CP and AA losses are enhanced at higher dietary fibre levels (e.g. Jansman et al., 1995; Grala et al., 1999; Nyachoti et al., 1997a). Moreover, in faba beans, the NDF-bound protein is rather high (85 mg g^{-1} of total CP) (Gdala, 1998) and poorly digestible. In the present study, NDF and ADF levels in the faba bean cultivars ranged from 126 to 165 and 101 to 137 g kg^{-1} DM, respectively. Thus, NDF and ADF levels, originating from faba beans, in the assay diets ranged from 46 to 67 and 39 to 57 g kg^{-1} DM, respectively. However, the relationships between NDF or ADF contents and SID values in the assay diets were not significant (P>0.05, data not shown) in contrast to those obtained between SID values and contents of condensed tannins in the faba bean containing assay diets (P<0.05, Table 21). Since both, coloured flowered, tannin containing, and white flowered tannin free faba beans are used in pig diets, differences in SID values between these faba beans need to be accounted for in feed tables.

Other than tannins, the faba beans contained a variety of ANF including TIA, vicine and convicine (Table 12). The TIA in faba beans ranged from <0.02 to 3.9 mg TI g^{-1} CP. The white flowered faba bean cultivars Gloria and Aurelia exhibited higher TIA (3.3 and 3.9 mg TI g^{-1} CP) in comparison to the coloured flowered faba bean cultivars Divine, Limbo, Fuego and Espresso (<0.2 to 1.4 mg TI g^{-1} CP). Similarly, Makkar et al. (1997) reported higher TIA in white flowered compared to coloured flowered faba beans, however TIA (6.6 to 12.7 mg TI g^{-1} CP) was considerably higher compared to the results of the present study. The TIA values of faba beans used in this study were distinctly lower than those determined in SBM (5.8 TI g^{-1} CP), thus no effect of TIA level in the assay diets on SID values in the assay diets could be observed (P>0.05, data not shown).

Vicine and convicine belong to the group of pyrimidine glycosides, that are generally present in faba beans (Champ, 2002). Vicine and convicine contents in the faba bean cultivars Limbo, Gloria, Espresso, Fuego and Aurelia ranged from 5.6 to 7.2 and 2.8 to 3.7 g kg^{-1} DM, respectively, which corresponds well with other reports (Grosjean et al., 2001; Simon, 2004). In the present study, the faba bean cultivar Divine exhibited very low vicine and convicine contents (0.3 and 0.0 g kg^{-1} DM, respectively) due to its low vicine-convicine gene *vc*− (Duc et al., 1999), indicating further progress in plant breeding. Vicine and convicine containing diets are known to have negative effects in laying hens, such as they

depressed feed consumption, egg weight, fertility and hatchability of eggs (Muduuli et al., 1982; Olaboro et al., 1981). However, in agreement with other reports (Grosjean et al., 2001), there was no effect of vicine/convicine levels in the assay diet on SID of CP and AA in growing pigs (P>0.05, data not shown).

In summary, there were strong linear relationships between tannin levels and SID values in faba beans. However, it remains open if lower SID of CP and AA in faba beans compared to lupins are due to associated effects of other ANF present in faba beans, in association with a poorly digestible fibre-bound protein fraction.

Table 18. Standardised ileal digestibility (%) of crude protein and amino acids in faba bean cultivars and soybean meal

	Aurelia	Divine	Gloria	Limbo	Fuego	Espresso	SBM
CP	81^{b}	81^{b}	80^{b}	73^{a}	71^{a}	70^{a}	87^{c}
Indispensable AA							
Arg	91^{c}	89^{b}	90^{bc}	86^{a}	84^{a}	84^{a}	94^{d}
His	85^{c}	81^{b}	84^{bc}	76^{a}	73^{a}	73^{a}	89^{d}
Ile	85^{b}	82^{b}	85^{b}	75^{a}	76^{a}	74^{a}	91^{c}
Leu	84^{b}	82^{b}	84^{b}	76^{a}	75^{a}	74^{a}	89^{c}
Lys	87^{cd}	84^{bc}	84^{bc}	80^{ab}	79^{a}	79^{a}	90^{d}
Met	77^{c}	68^{b}	77^{c}	61^{a}	62^{a}	58^{a}	91^{d}
Phe	84^{b}	82^{b}	85^{b}	76^{a}	75^{a}	74^{a}	90^{c}
Thr	80^{bc}	78^{b}	80^{bc}	71^{a}	68^{a}	67^{a}	85^{c}
Trp	69^{b}	66^{b}	71^{b}	54^{a}	53^{a}	53^{a}	85^{c}
Val	82^{b}	79^{b}	82^{b}	72^{a}	71^{a}	71^{a}	88^{c}
Dispensable AA							
Ala	81^{b}	78^{b}	80^{b}	72^{a}	70^{a}	70^{a}	87^{c}
Asp	86^{bc}	83^{b}	86^{bc}	79^{a}	77^{a}	77^{a}	89^{c}
Cys	65^{b}	63^{b}	64^{b}	51^{a}	52^{a}	48^{a}	82^{c}
Glu	89^{c}	88^{bc}	87^{abc}	84^{ab}	84^{ab}	83^{a}	91^{c}
Gly	73^{b}	74^{b}	73^{b}	63^{a}	64^{a}	59^{a}	82^{c}
Pro	83^{c}	77^{b}	82^{bc}	69^{a}	65^{a}	67^{a}	91^{d}
Ser	85^{b}	85^{b}	86^{bc}	77^{a}	77^{a}	77^{a}	90^{c}

SBM, Soybean meal (490 g kg^{-1}CP, as-is).
^{a,b,c} Within a row LSmeans with a common superscript are not significantly different at (α=0.05).

The SID of CP and AA in the individual cultivars of peas are shown in Table 19. Among pea cultivars, the SID of CP ranged from 76% (*cv.* Hardy) to 81% (*cv.* Santana). The SID of CP and most AA were higher in the cultivar Santana compared to the cultivar

Hardy (P<0.05). The SID of indispensable AA ranged from 62% for Trp (*cv*. Hardy) to 91% for Arg (*cv*. Jutta). In general, highest SID in pea cultivars were obtained for Arg (88 to 91%) and lowest for Trp and Cys (62 to 69 and 62 to 71%, respectively). Similarly, Stein et al. (2004) obtained in peas highest SID values for Arg and lowest SID values for Trp and Cys. In all pea cultivars, SID values, except for Glu, were lower compared to those in SBM (P<0.05). In contrast, Stein et al. (2004) determined similar or even higher SID values in peas compared with soybean meal. In the present study, similar to reports by Mariscal-Landín et al. (2002), there were only small variations in SID values among the assayed pea cultivars. In contrast, large variations in SID values have been reported in previous studies with white flowered pea cultivars, due to large variations in the TIA (Grosjean et al., 2000). In the present study, the white flowered pea cultivars had a similar chemical composition and ANF content including TIA. Thus, it may be concluded that there are only small variations in SID values in pea cultivars in the present study due to similar chemical composition and ANF contents. However, up to 10 percentage units higher SID values were determined in peas compared to the results of the present study (Kasprowicz and Frankiewicz, 2004). These differences may be, at least in part, due to ANF present in peas (e.g. Lallès and Jansman, 1998; Le Guen et al., 1995a) (Table 12). The TIA levels in peas, assayed in the present study, ranged from <0.2 mg TI g^{-1} CP (*cv.* Harnas) to 5.0 mg TI g^{-1} CP (*cv.* Phönix), which was similar or even lower compared to TIA in other pea cultivars assayed by Mariscal-Landín et al. (2002). The corresponding TIA levels in the assay diets ranged from 0.56 and 0.72 g TI kg^{-1} DM and did not affect SID values of the pea assay diets (P>0.05, data not shown). It has to be emphasised that TIA up to 3.2 g TI kg^{-1} diet (as-fed) did not affect pancreatic secretion of nitrogen or protein in young pigs (Gabert et al., 1996). Similarly, results from Batterham et al. (1993) indicated that growing pigs may tolerate dietary levels of at least 4.7 g TI kg^{-1} (as-fed) from chickpeas without significant negative effects on parameters such as growth rate, feed intake or feed conversion ratio. In the present study, NDF and ADF levels in the pea cultivars ranged from 104 to 126 and 68 to 86 g kg^{-1} DM, respectively. Thus, NDF and ADF levels, originating from peas in the assay diets ranged from 45 to 56 and 31 to 40 g kg^{-1} DM, respectively. However, although the fibre content in peas is rather low, peas, similar to faba beans may contain considerable amounts of NDF-bound protein (78 mg/g of total CP), which is poorly digestible. Moreover, the water holding capacity in pea inner fibre has been shown to be high (Canibe and Bach Knudsen, 2002), resulting in increased ileal excretion of endogenous proteins (Leterme et al., 1996). Thus, it may be speculated if lower SID of CP

and AA in peas compared to lupins are due to associated effects of other ANF present in peas.

Table 19. Standardised ileal digestibility (%) of crude protein and amino acids in pea cultivars and soybean meal

	Santana	Jutta	Phönix	Harnas	Rocket	Hardy	SBM
CP	81^{b}	81^{ab}	79^{ab}	79^{ab}	78^{ab}	76^{a}	87^{c}
Indispensable AA							
Arg	90^{b}	91^{b}	88^{a}	90^{ab}	89^{ab}	88^{a}	94^{c}
His	84^{c}	82^{abc}	83^{bc}	82^{bc}	80^{ab}	78^{a}	89^{d}
Ile	84^{b}	83^{b}	81^{b}	82^{b}	80^{ab}	78^{a}	91^{c}
Leu	82^{b}	81^{b}	80^{b}	81^{b}	80^{b}	77^{a}	89^{c}
Lys	87^{a}	85^{a}	85^{a}	85^{a}	84^{a}	83^{a}	90^{b}
Met	79^{a}	79^{a}	75^{a}	77^{a}	75^{a}	74^{a}	91^{b}
Phe	83^{b}	81^{ab}	81^{ab}	82^{ab}	82^{ab}	79^{a}	90^{c}
Thr	77^{a}	77^{a}	76^{a}	75^{a}	75^{a}	72^{a}	85^{b}
Trp	69^{b}	69^{ab}	67^{ab}	69^{b}	65^{ab}	62^{a}	85^{c}
Val	80^{b}	78^{ab}	78^{ab}	78^{ab}	78^{ab}	74^{a}	88^{c}
Dispensable AA							
Ala	79^{b}	78^{ab}	77^{ab}	77^{ab}	75^{ab}	73^{a}	87^{c}
Asp	85^{b}	84^{b}	83^{ab}	83^{b}	81^{ab}	80^{a}	89^{c}
Cys	71^{b}	71^{ab}	69^{ab}	66^{ab}	62^{a}	63^{a}	82^{c}
Glu	89^{cd}	89^{bcd}	88^{bcd}	86^{abc}	84^{ab}	84^{a}	91^{d}
Gly	75^{a}	74^{a}	74^{a}	74^{a}	72^{a}	71^{a}	82^{b}
Pro	81^{a}	81^{a}	81^{a}	80^{a}	79^{a}	78^{a}	91^{b}
Ser	84^{b}	83^{b}	82^{b}	82^{b}	80^{ab}	75^{a}	90^{c}

SBM, Soybean meal (490 g kg^{-1} CP, as-is).
^{a,b,c} Within a row LSmeans with a common superscript are not significantly different at (α=0.05).

In Table 20, the SID of CP and AA in the individual cultivars of lupins are presented. The SID of CP in the different cultivars ranged from 84% (*cv*. Boruta and *cv.* Idefix) to 90% (*cv.* Probor). The SID of indispensable AA ranged from 71% for Met (*cv*. Idefix) to 97% for Arg (*cv*. Probor). In general, highest SID in lupin cultivars were obtained for Arg (93 to 97%) and lowest for Met and Trp (71 to 84 and 77 to 88%, respectively). The SID of CP and most AA were up to 13 percentage units lower (P<0.05) in the cultivar Idefix compared to cultivar Probor. In all lupin cultivars, SID of CP were similar to those in SBM (P>0.05). In support of the observed high SID values in lupins reported herein, Mariscal-Landín et al. (2002) reported SID values in the range from 83 to 103% in a *L. angustifolius* cultivar. The earlier reported low SID values in lupins have been associated with their alkaloid contents (Sujak et al., 2006; Wink et al., 1995), whereas the lupin cultivars used in the present

study have proven to be low-alkaloid cultivars with total alkaloid levels ranging between 0.04 and 0.28 g kg^{-1} DM (Table 12). The alkaloid levels resulted in a maximal alkaloid content of 0.1 g kg^{-1} DM in the assay diets. The level of alkaloids in the assay diet did not affect SID values in the assay diet (P>0.05, data not shown). This observation supports that dietary alkaloid levels below 0.20 g kg^{-1} diet (as-fed) (Godfrey et al., 1985) or 0.33 g kg^{-1} DM (Allen, 1998) are not detrimental to SID. The high SID values in lupins in the present study compared with faba bean or peas may be attributed to the low level of ANF present in the assayed lupin cultivars. The level of TIA was considerably lower compared to most of the faba bean and pea cultivars. Moreover, lupins in contrast to faba beans are free of tannins. The NDF and ADF contents in lupin cultivars ranged from 224 to 261 and 185 to 230 g kg^{-1} DM, respectively, resulting in NDF and ADF levels originating from lupins in the assay diets from 76 to 89 and 62 to 82 g kg^{-1} DM, respectively. Despite their high fibre levels, SID values of CP and AA in lupin cultivars were not affected by NDF or ADF contents in lupins (P>0.05). The higher SID of CP and AA digestibility in lupin cultivars compared to faba bean or pea cultivars in the present study may be partly explained by lower levels of NDF-bound protein in lupins compared to faba beans or peas (Gdala, 1998; Kim et al., 2007).

Table 20. Standardised ileal digestibility (%) of crude protein and amino acids in lupin cultivars and soybean meal

	Probor	Bornal	Boregine	Boruta	Idefix	SBM
CP	90^{b}	88^{ab}	86^{ab}	84^{a}	84^{a}	87^{ab}
Indispensable AA						
Arg	97^{b}	95^{ab}	95^{ab}	94^{a}	93^{a}	94^{b}
His	91^{b}	88^{ab}	88^{ab}	87^{a}	86^{a}	89^{ab}
Ile	92^{d}	90^{bcd}	88^{abc}	88^{ab}	85^{a}	91^{cd}
Leu	91^{c}	89^{bc}	87^{b}	87^{ab}	84^{a}	89^{bc}
Lys	90^{bc}	88^{bc}	88^{bc}	86^{ab}	83^{a}	90^{c}
Met	84^{b}	84^{b}	82^{b}	82^{b}	71^{a}	91^{c}
Phe	91^{b}	87^{a}	88^{ab}	87^{a}	84^{a}	90^{bc}
Thr	88^{b}	86^{b}	83^{ab}	83^{ab}	78^{a}	85^{b}
Trp	88^{bc}	83^{abc}	80^{ab}	79^{ab}	77^{a}	85^{bc}
Val	88^{b}	85^{ab}	84^{ab}	84^{ab}	81^{a}	88^{b}
Dispensable AA						
Ala	86^{b}	84^{b}	82^{b}	83^{b}	76^{a}	87^{b}
Asp	91^{c}	89^{bc}	88^{abc}	88^{ab}	86^{a}	89^{abc}
Cys	87^{bc}	91^{c}	83^{abc}	81^{ab}	78^{a}	82^{abc}
Glu	94	94	94	91	92	91
Gly	85	84	82	81	81	82
Pro	95^{b}	89^{a}	87^{a}	85^{a}	85^{a}	91^{bc}
Ser	93^{b}	91^{ab}	87^{a}	87^{a}	86^{a}	90^{ab}

SBM, Soybean meal (490 g kg^{-1} CP, as-is).
^{a,b,c} Within a row LSmeans with a common superscript are not significantly different at (α=0.05).

Table 21. The estimates of the linear regression analysis between standardised ileal digestibilities of crude protein and amino acids in the faba bean containing assay diets and dietary contents of condensed tannins

Item	Slope±SE	*P* slope[1]	Intercept±SE	*P* intercept[2]
CP	–23.6±2.9	<0.001	90.7±0.3	<0.001
Indispensable AA				
Arg	–21.3±2.4	<0.001	93.9±0.3	<0.001
His	–22.5±2.2	<0.001	92.1±0.3	<0.001
Ile	–18.7±2.4	<0.001	93.2±0.3	<0.001
Leu	–17.6±2.2	<0.001	92.5±0.3	<0.001
Lys	–14.4±2.1	<0.001	93.6±0.2	<0.001
Met	–14.8±1.8	<0.001	95.4±0.2	<0.001
Phe	–18.0±2.2	<0.001	92.3±0.3	<0.001
Thr	–19.6±3.1	<0.001	90.6±0.3	<0.001
Trp	–36.6±4.2	<0.001	88.2±0.5	<0.001
Val	–18.8±2.4	<0.001	92.1±0.3	<0.001
Dispensable AA				
Ala	–23.3±3.4	<0.001	88.1±0.4	<0.001
Asp	–19.9±2.6	<0.001	91.0±0.3	<0.001
Cys	–46.9±6.3	<0.001	86.2±0.7	<0.001
Glu	–10.1±2.1	<0.001	94.9±0.2	<0.001
Gly	–42.0±5.3	<0.001	83.2±0.6	<0.001
Pro	–21.3±2.0	<0.001	94.5±0.2	<0.001
Ser	–17.5±3.0	<0.001	93.2±0.4	<0.001

[1] *P*-values of the estimates for the slopes of the regression equations.
[2] *P*-values of the estimates of the intercepts of the regression equations.
SE, Standard error.

2.5 CONCLUSIONS

The chemical composition of faba beans, peas and lupins was in agreement with tabulated values. The SID of CP and most AA in lupins were significantly higher than those in peas or faba beans. The SID of CP and AA in lupins were similar to those in SBM. In general, SID values of CP and AA determined in faba beans, peas and lupins were in agreement with tabulated values. The SID of CP and AA in grain legume species were unaffected by their contents of vicine/convicine, alkaloids or TIA. Due to considerable progress in plant breeding, these ANF contents in grain legumes were negligible, however, some ANF should be still concerned. Differences in SID of CP and AA within pea and lupin cultivars, as well as lower SID values in peas compared to lupins, are partly due to differences in fibre composition. Differences in SID values between faba bean cultivars

can be attributed to the presence of condensed tannins. Therefore, tannin free white flowered or even low-tannin coloured flowered faba bean cultivars should be preferred in diet formulation for pigs. However, since both tannin containing and tannin free faba beans are used in pig nutrition, differences in SID values need to be accounted for in feed tables.

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CHAPTER 3

IN VITRO PREDICTION OF STANDARDISED ILEAL CRUDE PROTEIN AND AMINO ACID DIGETIBILITIES IN GRAIN LEGUMES FOR GROWING PIGS

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3 *In Vitro* Prediction of Standardised Ileal Crude Protein and Amino Acid Digestibilities in Grain Legumes for Growing Pigs

3.1 Summary

The study was conducted to validate *in vitro* prediction of standardised ileal digestibilities (SID) of crude protein (CP) and amino acids (AA) in grain legumes for growing pigs using six different cultivars of faba beans (*Vicia faba*) and peas (*Pisum sativum*), respectively, and five different cultivars of lupins (*Lupinus* spp.). The SID for CP and AA were predicted from *in vitro* analysis by means of a two-step enzymatic method with subsequent pepsin and pancreatin incubations. *In vitro* predicted SID values of CP and AA were generally higher than the corresponding SID values measured *in vivo*. There were strong linear relationships ($r^2 = 0.73$ for Lys to $r^2 = 0.91$ for Cys and Trp) between *in vivo* and *in vitro* predicted SID values in the assay feed ingredients if grain legume species (i.e. faba beans, peas and lupins) was included as a covariate in multiple linear regression analysis. However, to rapidly and accurately predict SID of CP and AA in individual batches of various feed ingredients, further studies are warranted.

3.2 Implications

Close linear relationships between *in vivo* standardised ileal digestibilities (SID) and *in vitro* predicted SID values for crude protein (CP) and amino acids (AA) in faba beans, peas and lupins were obtained. However, probably due to the presence of antinutritional factors (ANF), differences between *in vitro* predicted SID and *in vivo* SID values varied between and within these grain legume species. Thus, further validation of the *in vitro* assay based on results of *in vivo* SID studies is required before being used for prediction of SID of CP and AA in feedstuffs and diets for pigs.

3.3 Introduction

Grain legumes are frequently used as feed ingredients in pig diets due to their high contents of CP, AA and energy (Mekbungwan, 2007). Progress in plant breeding may improve both, quality and protein contents in grain legumes (Monti and Grillo, 1983; Clarke and Wiseman, 2000), thus there may be a need to re-evaluate the nutritional value of currently available grain legume cultivars. At present, feed protein evaluation systems for

pigs are based on SID of CP and AA, which have been published for different feed ingredients in various feed tables (e.g. NRC, 1998; AmiPig, 2000; Degussa, 2006; GfE, 2008). Data in these feed tables originate from measurements of *in vivo* apparent ileal digestibilities (AID) of CP and AA in growing pigs, which were subsequently corrected for estimates of basal ileal endogenous CP and AA losses to obtain SID values (e.g. Jansman *et al.*, 2002; GfE, 2008). However, there is a scarcity of information on SID of CP and AA in grain legumes grown in Central Europe. Moreover, published feed tables (e.g. NRC, 1998; AmiPig, 2000; Degussa, 2006; GfE, 2008) do not take into account any variations that may result from progress in breeding of new cultivars of grain legumes (Jezierny *et al.*, submitted to Livestock Science, Chapter 2). For example, the level of ANF has been notably decreased through breeding of zero-tannin faba bean cultivars (Duc *et al.*; 1999, Jezierny *et al.*, submitted to Livestock Science, Chapter 2) or low alkaloid lupin cultivars (Petterson, 1998; Jezierny *et al.*, submitted to Livestock Science, Chapter 2). Furthermore, chemical composition of grain legumes may also be affected by variable growing and harvesting conditions in Central Europe compared to other regions in the world (Mossé and Baudet, 1983; Simon and Köhn, 2004). *In vivo* ileal digestibility experiments are time consuming, labour intensive and expensive and, in addition, require the use of surgically modified pigs for collection of ileal digesta (e.g. Zimmermann and Mosenthin, 2002). Thus, the feed industry would most likely benefit from an evaluation system that could provide rapidly and accurately SID of CP and AA in individual batches of grain legumes used for feed manufacturing. As an alternative to *in vivo* digestibility assays, several *in vitro* methods, simulating the digestive processes along the digestive tract have been developed to predict energy and nutrient digestibilities in feed ingredients for monogastrics (Boisen and Eggum, 1991). Multi-enzyme systems, such as one-step or two-step systems, aim to reflect digestive processes in the stomach and the small intestine and appear to be effective to predict ileal CP and AA digestibilities in pigs (Boisen and Eggum, 1991). Boisen and Fernández (1991) introduced a two-step method for prediction of ileal CP and AA digestibilities based on pepsin and pancreatin digestion with further modifications as described by Boisen and Fernández (1995). However, there is still a lack of validation studies that confirm the suitability of this method for *in vitro* prediction of SID of CP and AA in feed ingredients for growing pigs.

The aim of this study was to predict SID of CP and AA in different legume seeds according to the *in vitro* procedures as proposed by Boisen and Fernández (1995) and Boisen (2007), and to compare these SID values with those obtained previously under *in*

vivo conditions with growing pigs (Jezierny *et al.*, submitted to Livestock Science, Chapter 2). The SID values of CP and AA were predicted from *in vitro* degradation of DM and nitrogen in different grain legumes including six cultivars of either faba beans (*Vicia faba*) or peas (*Pisum sativum*) and five cultivars of lupins (*Lupinus luteus*, *Lupinus angustifolius*).

3.4 Materials and Methods

3.4.1 Grain Legume Samples

In total, six seed-grade cultivars of either faba beans (*Vicia faba*) or peas (*Pisum sativum*) and five seed-grade cultivars of lupins (*Lupinus* spp.) were assayed. The faba bean cultivars included the two white flowered cultivars Aurelia and Gloria (Saatzucht Gleisdorf, Austria), and the four coloured flowered cultivars Divine (Agri Obtentions, France), Limbo (Lochow-Petkus GmbH, Germany), Fuego and Espresso (Norddeutsche Pflanzenzucht, Germany). All faba bean cultivars were harvested in 2004, except from cultivar Divine, which was harvested in 2005. The pea cultivars Santana (Lochow-Petkus GmbH, Germany), Jutta, Harnas, Hardy (Norddeutsche Pflanzenzucht, Germany), Phönix and Rocket (Südwestsaat GbR, Germany) were white flowered. The cultivars Santana, Jutta, Harnas and Hardy were harvested in 2004, cultivar Phönix was harvested in 2001 and cultivar Rocket was harvested in 2005. The lupins used in this study were harvested in 2005 and were low-alkaloid cultivars, also referred to as sweet lupins. The cultivars Probor, Boregine, Boruta (Saatzucht Steinach GmbH, Germany) and Idefix (Südwestsaat GbR, Germany) were *L. angustifolius* cultivars, also referred to as blue lupins, wheras the cultivar Bornal (Saatzucht Steinach GmbH, Germany) was a *L. luteus* cultivar, also referred to as yellow lupin. All subsamples of grain legumes were drawn from reference samples of material, which had been previously used for *in vivo* digestibility studies with growing pigs (Jezierny *et al.*, submitted to Livestock Science, Chapter 2). These grain legume subsamples were ground to pass a 0.5 mm mesh sieve prior to chemical analyses for proximate nutrients (Naumann and Bassler, 1997) and fibre contents (van Soest *et al.*, 1991). Measurements of condensed tannins in grain legumes were performed as described by Makkar *et al.* (1993). Trypsin inhibitor activities (TIA) in grain legumes were determined according to the American Oil Chemists´ Society official method Ba 12-75 (AOCS, 1997). Amino acid analyses in grain legumes were performed according to the procedures as outlined by Llames and Fontaine (1994).

3.4.2 *IN VITRO* DIGESTION METHOD

The *in vitro* digestion method proposed by Boisen and Fernández (1995) and Boisen (2007) was applied to predict SID of CP and AA in faba beans ($n = 6$), peas ($n = 6$) and lupins ($n = 5$). This *in vitro* approach included consecutive incubations of the assay feed samples with solutions of pepsin and pancreatin according to the slightly modified two-step procedure by Boisen and Fernández (1995).

Step 1. A series of two samples with approximately 0.5 g of finely ground material (<1 mm) were weighed to an accuracy of ±0.02 mg in 100 ml conical flasks. In each of the series, a blank was included. To each sample, 25 ml of a 0.1M phosphate buffer (pH 6.0) were added and mixed thereafter through magnetic stirring. To this mixture, 10 ml of a 0.2M HCl solution was added, and pH was adjusted to pH 2.0 by means of a 1M HCl or a 1M NaOH solution. Subsequently, 1 ml of a freshly prepared pepsin solution was added containing 0.1 g porcine pepsin (2000 FIP U/g, Merck No 7190) per 10 ml of a 0.2M HCl solution. Bacterial contamination was prevented by adding 0.1 ml of a chloramphenicol solution, containing 0.1 g chloramphenicol (Sigma No C-0378) per 100 ml ethanol to each sample. Thereafter, the flasks were closed with rubber plugs and placed on multipoint magnetic stirrers (H+P Labortechnik AG, Oberschleissheim, Germany). These stirrers were located in a drying oven (WTC Binder, Tuttlingen, Germany) at 40±1°C, and the samples were stirred continuously for 6 h.

Step 2. To each sample, 5 ml of 0.6M NaOH solution and 10 ml of a 0.2M phosphate buffer (pH 6.8) was added. The pH was then adjusted to pH 6.8 with a 1M HCl or a 1M NaOH solution. To this mixture, 1 ml of a freshly prepared pancreatin solution was added, containing 0.5 mg pancreatin from porcine pancreas (Sigma No p-1750) per 10 ml of a 0.2M phosphate buffer (pH 6.8). After closure with rubber plugs, the flasks were incubated under continuous magnetic stirring at 40±1°C for 18 h. Thereafter, 5 ml of 20% sulphosalicylic acid were added to every sample. Solubilised but not digested proteins were precipitated under continuous magnetic stirring during 30 min incubation at room temperature. The undigested residues were then collected in a filtration unit (Fibertec™ system, Tecator FOSS, Rellingen, Germany) by using dried and pre-weighed glass filter crucibles (diameter, 3 cm; pore size, P2, 40 to 90 µm) containing about 0.4 g Celite® 545 (Roth No 11.2) as filter aid. All material was then transferred with 1% sulphosalicylic acid to the crucibles. After consecutive washings with approximately 10 ml of ethanol and

acetone, respectively, the undigested residues were dried at 103°C for 4 h. The *in vitro* digestibility of DM in the sample was calculated by difference between DM content in the sample and the undigested residue after correction for DM in the blank. The undigested material together with the Celite was wrapped into a piece of nitrogen-free paper, and undigested nitrogen was measured by the Kjeldahl method (Naumann and Bassler, 1997). The *in vitro* digestibility of nitrogen was calculated as difference between nitrogen content in the sample and the undigested nitrogen residue after correction for nitrogen in the blank.

Calculations. Since *in vitro* nitrogen digestibility is not influenced by basal and specific ileal endogenous nitrogen losses during digestion, this value reflects true ileal digestibility (TID) of dietary nitrogen (Boisen and Moughan, 1996a and 1996b). *In vivo*, ileal endogenous nitrogen and AA losses can be separated into basal losses, which are not influenced by feed ingredient composition, and specific losses, which are induced by feed ingredient characteristics, such as levels and types of fibre and ANF. Correcting apparent ileal digestibility (AID) values for both, basal and specific endogenous CP and AA losses, yields TID values, whereas correction of AID values for basal endogenous losses yields SID values for CP and AA (Stein *et al.*, 2007). Thus, SID of CP (nitrogen × 6.25) and AA can be predicted from *in vitro* enzyme digestibility of CP (nitrogen × 6.25) after correction for the specific endogenous CP losses (nitrogen × 6.25) of the assay feed ingredient (Equations 1 to 4), which have been shown to correspond linearly to indigestible DM content (Boisen and Fernández, 1995; Boisen, 1998). For practical feed evaluation, the AA composition of the endogenous protein can be considered as constant (Boisen and Moughan, 1996b). Thus, specific endogenous losses of the individual AA can be calculated by using conversion factors from nitrogen to the individual AA in a standardised composition of endogenous protein (Boisen, 1998) (Equation 6). Therefore, the SID of CP (nitrogen × 6.25) and AA can be predicted from *in vitro* enzyme digestibility of CP (nitrogen × 6.25) according to the following equations by Boisen (2007).

$$TD_{CP} = CP_A \times DN / 1000 \quad (1)$$

where TD_{CP} = true digestible CP (g/kg DM); CP_A = CP content of the assay feed ingredient (g/kg DM), DN = *in vitro* digested nitrogen (g/kg DM).

$$SEL_{CP} = 0.066 \times UDM \quad (2)$$

where SEL_{CP} = specific endogenous losses of CP (g/kg DMI), UDM = *in vitro* undigested DM (g/kg).

$$CP_{SID} = TD_{CP} - SEL_{CP} \quad (3)$$

where CP_{SID} = standardised ileal digestible CP (g/kg DM).

$$SID_{CP} = CP_{SID} / CP_A \times 100\% \quad (4)$$

where SID_{CP} = standardised ileal digestibility of CP (%).

$$TD_{AA} = AA_A \times DN / 1000 \quad (5)$$

where TD_{AA} = true digestible AA (g/kg DM), AA_A = AA content in the assay feed ingredient (g/kg DM)

$$SEL_{AA} = CF \times SEL_{CP} / 6.25 \quad (6)$$

where SEL_{AA} = specific endogenous AA loss (g/kg DMI); CF = conversion factor from nitrogen to the individual AA is for Lys (0.188), Thr (0.281), Met (0.063), Cys (0.100), Trp (0.075), Ile (0.156), Leu (0.250), Lys (0.219), His (0.094), Phe (0.188), and Tyr (0.125).

$$AA_{SID} = TD_{AA} - SEL_{AA} \quad (7)$$

where AA_{SID} = standardised digestible AA (g/kg DM).

$$SID_{AA} = AA_{SID} / AA_A \times 100\% \quad (8)$$

where SID_{AA} = standardised digestibility of AA (%).

3.4.3 Statistical Analyses

The experimental data were subjected to analysis of variance using the GLM procedure of SAS (2003). The factor grain legume species was included as a fixed effect in the model. Significant differences between grain legume species were determined using the PDIFF option. The significance level was set at $P<0.05$. Simple linear relationships between *in vivo* and *in vitro* predicted SID values were established, using the GLM procedure. The model was Y = aX + b, where Y = *in vivo* SID of CP and AA, a = slope, X = *in vitro* predicted SID of CP and AA, b = intercept. Homogeneity of the slopes and the intercepts of the linear regression were tested for the grain legume species. The corresponding model for multiple linear regression analysis included grain legume species as a covariate. The effect of condensed tannins in faba beans on the *in vitro* predicted SID values was modelled by linear regressions on condensed tannin level.

3.4.4 Results and Discussion

Nutrient composition and ANF contents. The nutrient composition and ANF contents in faba bean (*Vicia faba*), pea (*Pisum sativum*) and lupin (*Lupinus* spp.) cultivars as shown in Table 22 and 23, were in good agreement with values published in current feed tables (NRC, 1998; AmiPig, 2000; Degussa, 2006). The average CP level was highest in lupin (387 g/kg DM), intermediate in faba bean (308 g/kg DM) and lowest in pea (249 g/kg DM) cultivars. Among different faba bean cultivars, CP contents ranged from 285 g/kg DM in the coloured flowered cultivar Espresso to 337 g/kg DM in the white flowered cultivar Gloria. Average contents of Met, Trp, Cys and Lys in faba bean cultivars amounted to 2.0, 2.6, 3.7 and 19.0 g/kg DM. Highest AA contents were observed in the white flowered faba bean cultivar Gloria and lowest in the coloured flowered faba bean cultivar Espresso. Lowest CP content among peas was determined in cultivar Hardy (223 g/kg DM), whereas the CP content of the remaining pea cultivars ranged from 246 to 260 g/kg DM. Average contents of Met, Trp, Cys and Lys in all pea cultivars amounted to 2.3, 2.3, 3.5 and 17.9 g/kg DM, respectively, with lowest contents of most AA in cultivar Hardy. Among lupin cultivars, the *L. luteus* cultivar Bornal had the highest CP (476 g/kg DM) and AA contents. The CP contents of the *L. angustifolius* cultivars (Probor, Boregine, Boruta and Idefix) ranged from 339 to 383 g/kg DM and the contents of Met, Trp, Cys and Lys ranged from 2.0 to 2.1, 3.0 to 3.2, 4.5 to 5.4, and 16.1 to 17.3 g/kg DM, respectively.

In agreement with reports by Salgado *et al.* (2002), the lupin cultivars assayed herein had higher average NDF and ADF contents (248 and 207 g/kg DM, respectively) compared to faba bean and pea cultivars. In the present study, coloured flowered faba bean cultivars (Divine, Limbo, Fuego, Espresso) had higher NDF and ADF contents compared to white flowered cultivars (Aurelia, Gloria), which may be associated with a thicker seed coat in coloured flowered faba beans (Duc *et al.*, 1999). Similarly, Bach Knudsen (1997) and Bastianelli *et al.* (1998) found higher NDF and ADF levels in coloured than in white flowered pea cultivars. Faba bean and pea cultivars were rich in starch (on average 436 and 505 g/kg DM, respectively), whereas lupin cultivars contained comparatively low levels of starch (on average 77 g/kg DM).

The level of TIA in faba bean, pea and lupin cultivars used in the present study (Table 22) was distinctly lower than that reported in soybean meal (AmiPig, 2000; Jezierny *et al.*, submitted to Livestock Science, Chapter 2). In agreement with previous reports, the white flowered faba bean and pea cultivars used in the present study were devoid of condensed tannins, because condensed tannins are only present in coloured flowered faba beans and peas (Griffiths, 1981; Makkar *et al.*, 1997). In the present study, contents of condensed tannins in the coloured flowered faba bean cultivars Divine, Limbo, Fuego and Espresso ranged from 2.1 to 7.4 g/kg DM.

Table 22. The analysed nutrient composition and contents of antinutritional factors in the assay feed ingredients (g/kg DM)

Seed	Cultivar	DM	CP	EE	Ash	NDF	ADF	Starch	Sugar	TIA	CT
Vicia faba	Aurelia[1]	877.5	314.2	16.4	38.8	125.8	101.0	448.6	26.4	3.9	ND
	Divine[2]	882.5	300.3	14.2	38.1	127.5	111.9	450.5	31.0	1.4	2.1
	Gloria[1]	886.1	336.9	13.1	43.0	127.0	111.3	432.2	30.2	3.3	ND
	Limbo[2]	890.4	318.8	16.5	34.0	137.6	115.9	435.9	27.2	<0.2	7.0
	Fuego[2]	875.7	291.6	14.7	39.8	165.0	137.0	425.3	29.5	<0.2	7.4
	Espresso[2]	871.6	285.2	15.7	34.5	155.6	134.4	422.0	26.8	<0.2	4.2
	Mean±SD	*880.6±6.99*	*307.8±19.17*	*15.1±1.34*	*38.0±3.38*	*139.8±16.74*	*118.6±14.17*	*435.8±11.78*	*28.5±1.96*	–	–
	CV (%)	*0.8*	*6.2*	*8.9*	*8.9*	*12.0*	*11.9*	*2.7*	*6.9*	–	–
Pisum sativum	Santana[1]	880.9	252.2	18.4	32.2	104.7	80.5	498.4	45.5	2.4	ND
	Jutta[1]	869.2	260.0	19.7	33.8	104.3	78.9	501.4	42.4	1.8	ND
	Phönix[1]	876.7	246.7	22.8	31.2	106.0	68.0	510.1	47.3	5.0	ND
	Harnas[1]	871.0	254.7	19.8	31.6	108.9	86.2	492.4	47.1	<0.2	ND
	Rocket[1]	874.4	254.0	19.6	34.9	126.3	82.9	490.3	44.4	3.9	ND
	Hardy[1]	872.0	223.9	19.3	32.5	114.9	75.0	534.2	41.1	4.5	ND
	Mean±SD	*874.0±4.27*	*248.6±12.83*	*19.9±1.49*	*32.7±1.40*	*110.9±8.52*	*78.6±6.41*	*504.5±16.18*	*44.6±2.51*	–	–
	CV (%)	*0.5*	*5.2*	*7.5*	*4.3*	*7.7*	*8.2*	*3.2*	*5.6*	–	–
Lupinus spp.	Probor[3]	902.4	377.0	62.7	37.5	223.5	185.0	93.4	61.4	2.9	ND
	Bornal[4]	892.3	475.5	57.2	49.3	252.2	208.1	41.9	64.2	<0.2	ND
	Boregine[3]	908.6	358.7	59.3	35.7	247.1	195.0	99.8	66.0	<0.2	ND
	Boruta[3]	901.3	339.2	53.7	41.2	260.5	229.7	83.0	50.7	<0.2	ND
	Idefix[3]	905.3	382.5	68.1	41.5	258.3	218.9	68.4	50.7	<0.2	ND
	Mean±SD	*902.0±6.11*	*386.6±52.53*	*60.2±5.49*	*41.0±5.23*	*248.3±14.84*	*207.3±17.93*	*77.3±23.08*	*58.6±7.4*	–	–
	CV (%)	*0.7*	*13.6*	*9.1*	*12.7*	*6.0*	*8.6*	*29.9*	*12.6*	–	–

[1] White flowered cultivar.
[2] Coloured flowered cultivar.
[3] *L. angustifolius.*
[4] *L. luteus.*
DM = dry matter; EE = ether extract; TIA = trypsininhibitor activity (mg TI/g CP); CT = condensed tannins; ND = not detectable.

Table 23. The analysed contents of amino acids in the assay feed ingredients (g/kg DM)

Seed	Cultivar	Met	Cys	Lys	Thr	Trp	Ile	Leu	Val	His	Phe
Vicia faba	Aurelia[1]	2.0	3.8	18.9	10.5	2.7	12.7	22.4	14.0	8.0	13.0
	Divine[2]	2.0	3.5	18.5	10.8	2.6	11.8	22.0	13.1	7.5	12.7
	Gloria[1]	2.2	4.0	20.7	11.1	2.7	13.9	24.1	14.9	8.5	14.0
	Limbo[2]	2.0	3.8	19.3	10.8	2.7	12.3	22.3	13.6	8.2	13.0
	Fuego[2]	2.0	3.6	18.5	10.2	2.6	11.8	20.8	13.1	7.7	12.5
	Espresso[2]	1.9	3.4	18.3	9.9	2.5	11.5	20.6	13.0	7.6	12.4
	Mean±SD	*2.0±0.10*	*3.7±0.22*	*19.0±0.89*	*10.6±0.44*	*2.6±0.08*	*12.3±0.88*	*22.0±1.27*	*13.6±0.74*	*7.9±0.39*	*12.9±0.58*
	CV (%)	*4.9*	*6.1*	*4.7*	*4.2*	*3.1*	*7.1*	*5.8*	*5.4*	*4.9*	*4.5*
Pisum sativum	Santana[1]	2.3	3.6	18.5	9.1	2.4	10.8	18.1	12.0	6.4	12.0
	Jutta[1]	2.3	3.6	17.8	9.3	2.4	10.2	17.8	11.6	6.3	11.6
	Phönix[1]	2.2	3.5	18.1	8.9	2.3	10.7	17.7	11.7	6.3	12.0
	Harnas[1]	2.3	3.6	18.1	9.4	2.4	10.3	18.0	11.7	6.3	11.6
	Rocket[1]	2.2	3.0	18.5	8.9	2.2	10.6	18.1	11.8	5.7	12.2
	Hardy[1]	2.2	3.4	16.3	8.4	2.2	9.4	15.7	10.6	5.6	10.6
	Mean±SD	*2.3±0.05*	*3.5±0.23*	*17.9±0.82*	*9.0±0.36*	*2.3±0.10*	*10.3±0.51*	*17.6±0.93*	*11.6±0.49*	*6.1±0.35*	*11.7±0.58*
	CV (%)	*2.4*	*6.8*	*4.6*	*4.0*	*4.2*	*5.0*	*5.3*	*4.3*	*5.8*	*4.9*
Lupinus spp.	Probor[3]	2.0	5.4	17.6	13.1	3.2	15.4	26.6	14.4	10.3	15.7
	Bornal[4]	2.6	8.2	21.6	14.3	3.3	16.6	31.3	15.6	11.5	17.1
	Boregine[3]	2.1	5.2	16.1	11.8	3.1	13.6	23.3	13.6	9.2	13.5
	Boruta[3]	2.1	5.2	16.5	12.3	3.0	14.1	24.3	13.9	9.4	14.1
	Idefix[3]	2.1	4.5	17.3	12.5	3.2	15.1	24.7	14.4	9.5	14.9
	Mean±SD	*2.2±0.24*	*5.7±1.44*	*17.8±2.20*	*12.8±0.96*	*3.2±0.11*	*15.0±1.17*	*26.0±3.17*	*14.4±0.76*	*10.0±0.95*	*15.1±1.41*
	CV (%)	*11.0*	*25.2*	*12.3*	*7.5*	*3.6*	*7.8*	*12.2*	*5.3*	*9.5*	*9.4*

[1] White flowered cultivar.
[2] Coloured flowered cultivar.
[3] *L. angustifolius.*
[4] *L. luteus.*

In vivo and in vitro predicted SID of CP and AA. For comparison of *in vivo* and *in vitro* predicted SID of CP and AA in grain legumes, the corresponding *in vivo* data were extracted from a previous study with growing pigs (Jezierny *et al.*, submitted to Livestock Science, Chapter 2).

The analysed values for *in vitro* digestibilities of nitrogen and *in vitro* undigested DM are shown in Table 24. The *in vivo* SID of CP and AA in grain legumes, as determined in a previous study with growing pigs, are summarised in Table 25 (Jezierny *et al*., submitted to Livestock Science, Chapter 2), whereas the corresponding *in vitro* predicted SID values for CP and AA are shown in Table 26. *In vitro* predicted SID of CP in faba bean, pea and lupin cultivars averaged 84, 91 and 91%, respectively. The corresponding mean values for *in vitro* predicted SID of AA ranged from 81% (Met) to 87% (Lys, Leu), and from 90% (Met, Cys, Thr, Trp) to 94% (Lys), and from 86% (Met) to 93% (Lys, Ile, Leu, His) in faba bean, pea and lupin cultivars, respectively. The largest variations in *in vitro* predicted SID values were observed in faba bean cultivars (CV = 7.5% for Lys to CV = 9.1% for Met), followed by lupins (CV = 1.3% for His to CV = 2.5% for Met and Cys) whereas smallest variation was obtained in peas (CV = 1.1% for Cys to CV = 1.6% for Lys). The *in vivo* SID values for CP and AA were in general agreement with tabulated values published by Degussa (2006) or AmiPig (2000). Similar to *in vitro* predicted SID, largest variations in *in vivo* SID values for CP and AA were observed in faba bean cultivars (CV = 4.0% for Lys to CV = 14.0% for Trp), whereas the variation was rather low in pea (CV = 1.4% for Lys to CV = 6.0% for Cys) and lupin cultivars (CV = 2.2% for His to CV = 6.8% for Met). In agreement with *in vivo* SID values (Jezierny *et al.*, submitted to Livestock Science, Chapter 2), *in vitro* predicted SID values in the white flowered faba bean cultivars Aurelia and Gloria were higher compared to the coloured flowered cultivars Divine, Fuego, Limbo and Espresso. The large variation in *in vivo* SID values in faba beans resulted from a linear decrease in *in vivo* SID as the contents of condensed tannins increased (Jezierny *et al*., submitted to Livestock Science, Chapter 2). Similarly, *in vitro* predicted SID values decreased linearly ($P<0.05$) as the contents of condensed tannins in the faba bean cultivars increased (r^2 = 0.77 for His to r^2 = 0.80 for Met and Thr) (Table 27). In agreement with this observation, Smulikowska *et al.* (2001) reported a detrimental effect of increasing levels of condensed tannins on CP digestibility in peas under *in vitro* conditions. Condensed tannins are known to exert negative effects on protein digestibility, such as they may form complexes with both dietary proteins and digestive enzymes (Jansman, 1993) which, in turn may inhibit

the activity of proteolytic enzymes and/or increase the secretion of endogenous proteins (Marquardt, 1989).

Table 24. *In vitro* enzyme digested nitrogen (DN, g/kg DM) and *in vitro* enzyme undigested dry matter (UDM, g/kg DM) in grain legumes

		In vitro	
Seed	Seed/Cultivar	DN	UDM
Vicia faba	Aurelia[1]	984	223
	Divine[2]	870	269
	Gloria[1]	969	228
	Limbo[2]	860	296
	Fuego[2]	842	314
	Espresso[2]	863	259
Pisum sativum	Santana[1]	967	191
	Jutta[1]	956	193
	Phönix[1]	967	182
	Harnas[1]	961	194
	Rocket[1]	990	246
	Hardy[1]	943	184
Lupinus spp.	Probor[3]	976	318
	Bornal[4]	968	315
	Boregine[3]	973	343
	Boruta[3]	955	390
	Idefix[3]	968	388

[1] White flowered cultivar.
[2] Coloured flowered cultivar.
[3] *L. angustifolius*.
[4] *L. luteus*.

Table 25. *In vivo* standardised ileal digestibilities of crude protein and amino acids in grain legumes (%)[1]

Seed	Cultivar	CP	Met	Cys	Lys	Thr	Trp	Ile	Leu	Val	His	Phe
Vicia faba	Aurelia[2]	81	77	65	87	80	69	85	84	82	85	84
	Divine[3]	81	68	63	84	78	66	82	82	79	81	82
	Gloria[2]	80	77	64	84	80	71	85	84	82	84	85
	Limbo[3]	73	61	51	80	71	54	75	76	72	76	76
	Fuego[3]	71	62	52	79	68	53	76	75	71	73	75
	Espresso[3]	70	58	48	79	67	53	74	74	71	73	74
	Mean±SD	*76±5.2*[a]	*67±8.4*[a]	*57±7.4*[a]	*82±3.4*[a]	*74±6.0*[a]	*61±8.6*[a]	*80±4.9*[a]	*79±4.7*[a]	*76±5.6*[a]	*79±5.3*[a]	*79±4.7*[a]
	CV (%)	*6.8*	*12.5*	*13.0*	*4.0*	*8.1*	*14.0*	*6.2*	*6.0*	*7.3*	*6.8*	*6.0*
Pisum sativum	Santana[2]	81	79	71	87	77	69	84	82	80	84	83
	Jutta[2]	81	79	71	85	77	69	83	81	78	82	81
	Phönix[2]	79	75	69	85	76	67	81	80	78	83	81
	Harnas[2]	79	77	66	85	75	69	82	81	78	82	82
	Rocket[2]	78	75	62	84	75	65	80	80	78	80	82
	Hardy[2]	76	74	63	83	72	62	78	77	74	78	79
	Mean±SD	*79±1.8*[a]	*76±2.0*[b]	*67±4.1*[b]	*85±1.2*[ab]	*75±1.9*[a]	*67±3.0*[a]	*81±2.2*[a]	*80±1.7*[a]	*78±2.1*[a]	*81±2.0*[a]	*82±1.3*[a]
	CV (%)	*2.3*	*2.7*	*6.0*	*1.4*	*2.5*	*4.4*	*2.7*	*2.2*	*2.7*	*2.5*	*1.6*
Lupinus spp.	Probor[4]	90	84	87	90	88	88	92	91	88	91	91
	Bornal[5]	88	84	91	88	86	83	90	89	85	88	87
	Boregine[4]	86	82	83	88	83	80	88	87	84	88	88
	Boruta[4]	84	71	78	83	78	77	85	84	81	86	84
	Idefix[4]	84	82	81	86	83	79	88	87	84	87	87
	Mean±SD	*86±2.4*[b]	*81±5.5*[b]	*84±5.1*[c]	*87±2.5*[b]	*83±3.6*[b]	*81±4.1*[b]	*89±2.6*[b]	*88±2.7*[b]	*85±2.8*[b]	*88±2.0*[b]	*87±2.3*[b]
	CV (%)	*2.8*	*6.8*	*6.1*	*2.9*	*4.4*	*5.0*	*2.9*	*3.0*	*3.3*	*2.2*	*2.6*

[1] Jezierny *et al*. (submitted to Livestock Science, Chapter 2).
[2] White flowered cultivar; [3] Coloured flowered cultivar.
[4] *L. angustifolius;* [5] *L. luteus.*
[a,b,c] Within a same column means with a common superscript are not significantly different (α = 0.05).

Table 26. *In vitro* predicted standardised ileal digestibilities of crude protein and amino acids in grain legumes (%)

Seed	Cultivar	CP	Met	Cys	Lys	Thr	Trp	Ile	Leu	Val	His	Phe
Vicia faba	Aurelia[1]	94	91	92	96	92	92	95	96	95	96	95
	Divine[2]	81	78	79	84	80	79	83	84	82	83	83
	Gloria[1]	93	90	91	95	91	90	94	84	93	94	94
	Limbo[2]	80	76	78	83	78	77	82	82	81	82	81
	Fuego[2]	77	74	75	81	75	75	80	80	79	80	79
	Espresso[2]	80	77	78	83	78	78	83	83	82	83	82
	Mean±SD	*84±7.1*^{a}	*81±7.4*^{a}	*82±7.4*^{a}	*87±6.6*^{a}	*82±7.2*^{a}	*82±7.3*^{a}	*86±6.8*^{a}	*87±6.7*^{a}	*85±6.9*^{a}	*86±6.7*^{a}	*86±6.8*^{a}
	CV (%)	*8.5*	*9.1*	*9.0*	*7.5*	*8.8*	*8.9*	*7.9*	*7.7*	*8.1*	*7.7*	*8.0*
Pisum sativum	Santana[1]	92	91	91	95	91	90	94	94	93	94	94
	Jutta[1]	91	90	90	93	89	89	93	93	92	93	92
	Phönix[1]	92	91	91	95	91	90	94	94	93	94	94
	Harnas[1]	91	90	90	94	90	90	93	93	92	93	93
	Rocket[1]	93	91	90	96	91	90	95	95	94	95	95
	Hardy[1]	89	89	89	92	88	88	91	91	90	91	91
	Mean±SD	*91±1.3*^{b}	*90±1.1*^{b}	*90±1.0*^{b}	*94±1.5*^{b}	*90±1.1*^{b}	*90±1.1*^{b}	*93±1.4*^{b}	*93±1.4*^{b}	*92±1.3*^{b}	*93±1.3*^{b}	*93±1.4*^{b}
	CV (%)	*1.4*	*1.2*	*1.1*	*1.6*	*1.2*	*1.2*	*1.5*	*1.5*	*1.4*	*1.4*	*1.5*
Lupinus spp.	Probor[3]	92	87	91	94	90	90	94	94	93	95	94
	Bornal[4]	93	89	93	94	90	89	94	94	92	94	93
	Boregine[3]	91	86	90	93	89	89	93	93	92	94	92
	Boruta[3]	88	83	88	91	86	85	91	91	89	91	90
	Idefix[3]	90	85	88	92	88	87	93	93	91	93	92
	Mean±SD	*91±1.8*^{b}	*86±2.1*^{ab}	*90±2.3*^{b}	*93±1.4*^{b}	*89±1.8*^{b}	*88±1.8*^{b}	*93±1.3*^{b}	*93±1.3*^{b}	*91±1.4*^{b}	*93±1.3*^{b}	*92±1.4*^{b}
	CV (%)	*2.0*	*2.5*	*2.5*	*1.5*	*2.1*	*2.1*	*1.4*	*1.4*	*1.5*	*1.3*	*1.5*

[1] White flowered cultivar; [2] Coloured flowered cultivar.
[3] *L. angustifolius;* [4] *L. luteus.*
[a,b] Within a same column means with a common superscript are not significantly different ($\alpha = 0.05$).

Table 27. The estimates of the linear regression analysis between *in vitro* predicted standardised ileal digestibilities of crude protein and amino acids and contents of condensed tannins in faba beans

Item	Slope±SE	*P* slope[1]	Intercept±SE	*P* intercept[2]	r^2
CP	-19.1±5.1	0.020	90.7±2.3	<0.001	0.78
Met	-20.1±5.0	0.016	88.1±2.3	<0.001	0.80
Cys	-19.8±5.3	0.020	89.0±2.4	<0.001	0.78
Lys	-17.6±4.7	0.020	93.1±2.1	<0.001	0.78
Thr	-19.6±4.9	0.016	89.1±2.2	<0.001	0.80
Trp	-19.7±5.1	0.018	88.7±2.3	<0.001	0.79
Ile	-18.1±4.9	0.020	92.5±2.2	<0.001	0.78
Leu	-17.9±4.8	0.020	92.8±2.2	<0.001	0.78
Val	-18.5±4.9	0.020	91.7±2.3	<0.001	0.78
His	-17.8±4.8	0.021	92.6±2.2	<0.001	0.77
Phe	-18.3±4.8	0.020	92.0±2.2	<0.001	0.78

[1] The *P*-values of the estimates for the slopes of the regression equations.
[2] The *P*-values of the estimates of the intercepts of the regression equations.

In vitro predicted SID values, however, do not provide direct estimates of *in vivo* SID of CP and AA in the assayed grain legumes. In fact, *in vitro* predicted SID values were higher than measured *in vivo* SID values, which were also reported by Cone and van der Poel (1993) for AID values. *In vitro* methods lack interactions with the digestive tract of the animal such as absorption of nutrients, secretion of endogenous compounds into the gut lumen, but also digesta transit and microbial activity (Cone and van der Poel, 1993; Moughan, 1999). Thus, several methodological factors may interfere with the prediction of digestibility values based on *in vitro* measurements as was reviewed by Moughan (1999). For example, most of the differences between *in vivo* SID and *in vitro* predicted SID values may result from the presence of ANF. Trypsin inhibitors, for example, may increase endogenous CP and AA losses and depress nutrient digestibility *in vivo,* but have much smaller effects on *in vitro* digestibility (Boisen and Eggum, 1991; Cone and van der Poel, 1993). Trypsin inhibitors act by inhibiting the proteolytic pancreatic enzymes trypsin and chymotrypsin, by forming stable inactive complexes *in vivo* (Lallès and Jansman, 1998) whereas *in vitro* predicted SID values in raw soybeans were not affected by the presence of trypsin inhibitors (Święch *et al.*, 2004). Under *in vitro* conditions, a surplus of digestive enzymes may alleviate the negative effects of trypsin inhibitors (Boisen and Eggum, 1991; Cone and van der Poel, 1993). However, in the present study, differences between *in vivo* SID and *in vitro* predicted SID values in faba beans, as well as in pea or lupin cultivars

cannot be exclusively attributed to differences in the level of ANF. Furthermore, some of the differences between *in vivo* SID and *in vitro* predicted SID values may result from the presence of microbial enzymes in the small intestine, thereby affecting ileal digestibility of organic matter, but which are devoid in *in vitro* assays (Moughan, 1999). Moreover, part of the feed proteins may be digested in the small intestine without being absorbed due to various physical conditions, such as viscosity and osmolarity of digesta (Moughan, 1999). *In vivo*, this digested but not absorbed nitrogen fraction is recognised as undigested protein, whereas *in vitro* all solubilised nitrogen appears as being digested (Cone and van der Poel, 1993).

Table 28. Differences[1] between *in vitro* predicted and *in vivo* standardised ileal digestibilities of crude protein and amino acids in grain legumes (percentage units)

Seed	Cultivar	CP	Met	Cys	Lys	Thr	Trp	Ile	Leu	Val	His	Phe
Vicia faba	Aurelia[2]	13	14	27	9	12	23	10	12	12	11	11
	Divine[3]	1	10	16	1	2	13	2	2	3	3	1
	Gloria[2]	13	13	27	11	11	20	9	10	11	11	9
	Limbo[3]	7	15	26	3	7	24	7	6	9	7	5
	Fuego[3]	6	12	23	2	7	21	3	6	8	7	4
	Espresso[3]	11	19	30	5	11	26	8	9	11	10	8
	Mean±SD	*8±4.7^{ab}*	*14±3.1^{a}*	*25±4.9^{a}*	*5±4.1^{a}*	*8±4.0^{a}*	*21±4.4^{a}*	*7±3.4^{a}*	*7±3.6^{a}*	*9±3.4^{a}*	*8±2.9^{a}*	*6±3.7^{a}*
	CV (%)	*57.6*	*22.4*	*19.7*	*82.1*	*48.4*	*21.1*	*52.0*	*48.6*	*37.8*	*36.6*	*58.8*
Pisum sativum	Santana[2]	10	13	20	8	13	21	10	12	13	10	11
	Jutta[2]	10	11	19	8	13	21	10	12	14	11	11
	Phönix[2]	13	16	22	10	15	23	13	14	15	11	12
	Harnas[2]	12	14	24	9	15	21	11	13	14	11	11
	Rocket[2]	14	17	28	13	16	25	15	15	17	15	13
	Hardy[2]	13	15	26	9	16	26	14	14	16	13	12
	Mean±SD	*12±1.6^{a}*	*14±2.1^{a}*	*23±3.5^{a}*	*10±1.8^{b}*	*15±1.4^{b}*	*23±2.3^{a}*	*12±2.0^{b}*	*13±1.3^{b}*	*15±1.6^{b}*	*12±1.8^{b}*	*12±0.9^{b}*
	CV (%)	*13.1*	*14.9*	*15.0*	*18.5*	*9.7*	*10.0*	*17.0*	*9.8*	*10.9*	*15.2*	*7.7*
Lupinus spp.	Probor[4]	2	3	4	4	3	2	2	3	4	3	3
	Boregine[4]	5	5	2	6	5	6	4	6	7	6	6
	Bornal[5]	5	5	7	5	6	8	5	6	7	6	5
	Boruta[4]	4	12	10	8	8	8	6	7	9	5	6
	Idefix[4]	6	2	7	7	5	8	5	6	6	5	5
	Mean±SD	*4±1.3^{b}*	*5±4.0^{b}*	*6±3.0^{b}*	*6±1.2^{a}*	*5±2.0^{a}*	*7±2.7^{b}*	*4±1.4^{a}*	*6±1.5^{a}*	*7±1.6^{a}*	*5±1.1^{c}*	*5.0±1.3^{a}*
	CV (%)	*30.9*	*75.6*	*47.5*	*21.3*	*37.9*	*40.6*	*32.7*	*26.3*	*24.0*	*21.4*	*26.5*

[1] = *in vitro* predicted SID values–*in vivo* SID values.
[2] White flowered cultivar; [3] Coloured flowered cultivar.
[4] *L. angustifolius;* [5] *L. luteus.*
[a,b,c] Within a same column means with a common superscript are not significantly different (α = 0.05).

In the present study, simple linear regression analysis showed only poor linear relationships between *in vivo* SID and *in vitro* predicted SID values for the 17 feed ingredients assayed herein (r^2 = 0.39 for Thr to r^2 = 0.60 for His, data not shown). Therefore, the homogeneity of the slopes and the intercepts between faba bean, pea and lupin cultivars in the linear regression model between *in vivo* SID and *in vitro* predicted SID values were tested. The slopes of the linear relationship between *in vivo* SID and *in vitro* predicted SID did not differ between faba beans, peas and lupins (P>0.05), whereas the corresponding intercepts differed (P<0.05). Different intercepts for each grain legume species can be attributed to dissimilar differences (P<0.05) between *in vitro* predicted SID and *in vivo* SID of CP and AA and, in addition, to different coefficients of variation between grain legume species (Table 28). For example, the differences between *in vitro* predicted SID and *in vivo* SID of CP averaged 12, 8 and 4 percentage units for pea, faba bean and lupin cultivars, respectively. The differences between *in vitro* predicted SID and *in vivo* SID of CP in peas ranged from 10 to 14 percentage units, corresponding to a CV of 13%. In faba bean cultivars, in contrast to pea cultivars, differences between *in vitro* predicted SID and *in vivo* SID of CP varied largely and ranged from 1 to 13 percentage units, corresponding to a CV of 58%. In lupin cultivars the differences between *in vitro* predicted SID and *in vivo* SID of CP ranged only from 2 to 6 percentage units. However, as differences between *in vitro* predicted SID and *in vivo* SID of CP and AA were rather small, a CV of 31% was calculated. Thus, even small variations in absolute differences between *in vitro* predicted SID and *in vivo* SID of CP and AA resulted in high CV-values. To account for the different intercepts and CV values between grain legume species, multiple linear regression analysis between *in vivo* SID and *in vitro* predicted SID values, which included grain legume species as covariate, was conducted. As a result, there were strong linear relationships (P<0.05) between *in vivo* SID and *in vitro* predicted SID values (Table 29). The coefficient of linear determination for CP was 0.83 and coefficients of linear determination for AA ranged from 0.73 for Lys to 0.91 for Cys and Trp. The relationships between *in vivo* SID and *in vitro* predicted SID values were closer for Cys (r^2 = 0.91), Trp (r^2 = 0.91) and His (r^2 = 0.89), than for Phe (r^2 = 0.85), Leu (r^2 = 0.85), Met (r^2 = 0.84), Trp (r^2 = 0.84), Val (r^2 = 0.82), Thr (r^2 = 0.81) and Lys (r^2 = 0.73). The inclusion of grain legume species as a covariate accounts for the significant effect of legume species on the differences between *in vivo* SID and *in vitro* predicted SID values and the different CV-values between legume species.

Table 29. The estimates of the multiple linear regression analyses between *in vivo* standardised ileal digestibilities of crude protein and amino acids and *in vitro* predicted standardised ileal digestibilities of crude protein and amino acids in grain legumes

			Faba beans		Peas		Lupins		
Item	Slope±SE	*P* slope[1]	Intercept±SE	*P* intercept[2]	Intercept±SE	*P* intercept[3]	Intercept±SE	*P* intercept[4]	r²
CP	0.58±0.15	<0.002	27.2±12.9	0.056	26.4±14.0	0.082	33.9±13.9	0.030	0.83
Met	1.06±0.21	<0.001	-18.6±16.7	0.286	-19.4±18.6	0.315	-10.3±17.7	0.571	0.84
Cys	0.91±0.24	0.002	-17.4±19.5	0.389	-15.1±21.4	0.493	2.0±21.4	0.927	0.91
Lys	0.45±0.12	0.002	43.1±10.3	0.001	42.6±11.1	0.002	45.5±10.9	0.001	0.73
Thr	0.73±0.16	<0.001	14.2±13.6	0.316	10.1±14.8	0.507	19.1±14.6	0.214	0.81
Trp	1.05±0.21	<0.001	25.2±16.9	0.158	-27.5±18.4	0.159	-11.2±18.1	0.546	0.91
Ile	0.66±0.14	<0.001	23.0±12.3	0.085	20.0±13.3	0.159	27.4±13.3	0.060	0.84
Leu	0.64±0.14	<0.001	24.3±11.9	0.062	20.7±12.8	0.130	28.4±12.8	0.045	0.85
Val	0.73±0.15	<0.001	14.3±12.7	0.279	10.6±13.7	0.454	18.2±13.5	0.202	0.82
His	0.74±0.13	<0.001	14.9±11.1	0.201	12.8±11.9	0.302	19.6±11.9	0.125	0.89
Phe	0.60±0.12	<0.001	27.6±10.5	0.021	25.3±11.4	0.045	31.7±11.3	0.015	0.85

[1] The *P*-values of the estimates for the slopes of the regression equations.
[2] The *P*-values of the estimates for the intercepts of the regression equations for faba beans.
[3] The *P*-values of the estimates for the intercepts of the regression equations for peas.
[4] The *P*-values of the estimates for the intercepts of the regression equations for lupins.

In conclusion, the present study reveals close linear relationships between *in vivo* SID and *in vitro* predicted SID values for CP and AA in different cultivars of faba beans, peas and lupins. However, differences between *in vitro* predicted SID and *in vivo* SID values varied between and within grain legume species, probably due to the ANF contents in grain legumes, which are known to depress nutrient digestibility *in vivo,* but have much smaller effects on *in vitro* digestibility. There exist, however, up to now, only few studies directed to *in vitro* prediction of SID values in feed ingredients used in diet formulation for pigs. In consequence, further application of the *in vitro* procedure, as originally being proposed by Boisen and Fernández (1995) and Boisen (2007), warrants combined *in vitro* and *in vivo* studies to further validate this method for *in vitro* prediction of SID of CP and AA in feedstuffs and diets used in pig nutrition.

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3.6 REFERENCES

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CHAPTER 4

GENERAL DISCUSSION

4 General Discussion

4.1 Introduction

Research on plant protein sources has increased as a result of the European Union ban on the inclusion of meat and bone meal in diets for livestock (Diaz et al., 2006). Furthermore, with regard to organic food production, the use of genetically modified feed ingredients (e.g. soybeans) or the use of processed oilseed products, when subjected to solvent extraction processes (e.g. soybean meal, SBM) is not permitted (IFOAM, 2005). Also, with respect to organic food production, supplementation with crystalline amino acids (AA) to balance pigs' diet according to their AA requirement is prohibited (IFOAM, 2005). Furthermore, in the European Union over 20 million tons of protein feeds are annually used in compound feeds for livestock, but only six million tons of protein feeds are produced within the European Union (Blair, 2007). Therefore, interest has increased in locally produced suitable alternative feed ingredients to meet the animals' protein and AA requirement.

Grain legumes, such as faba beans (*Vicia faba*), peas (*Pisum sativum*) and lupins (*Lupinus albus*, *Lupinus angustifolius*, *Lupinus luteus*) have been proven to be appropriate protein and energy containing feed ingredients in monogastric (e.g. Perez-Maldonado et al., 1999; Salgado et al., 2002; Diaz et al., 2006), and ruminant nutrition as well (e.g. Yu et al., 2002). However, their use as feed component may be limited, as several types of secondary plant metabolites, such as condensed tannins, trypsin inhibitors, alkaloids, glycosides, saponins and lectins may occur in grain legumes, in addition to sometimes high amounts of oligosaccharides (Chapter 1). Possible effects of secondary plant metabolites and oligosaccharides have been described as negative, positive or both (Champ, 2002). Altogether, composition and digestibility of nutrients in grain legumes, and the content of secondary plant metabolites and oligosaccharides have to be considered when used in diet formulation both for monogastrics and ruminants.

4.2 Grain Legumes in Pig Nutrition

In pig nutrition, grain legumes are primarily used as protein and energy source and dietary inclusion levels are given for each grain legume species. According to recommendations by UFOP (2004a), faba beans can be included in diets for growing and

finishing pigs up to levels of 150 and 250 g/kg, respectively. However, due to their low contents of condensed tannins, the use of white flowered cultivars should be favoured. For peas, when using white flowered cultivars, an inclusion level of even up to 400 g/kg in diets for growing finishing pigs has been recommended (UFOP, 2004b). On the other hand, lupins may be included up to levels of 150 to 200 g/kg in diets for growing and growing finishing pigs (UFOP, 2004c). Compared to SBM, faba beans, peas and lupins contain rather low levels of the sulphur containing AA methionine and cystine and, in addition, of tryptophan (Degussa, 2006; Chapter 2), which has to be considered when these grain legumes are used as protein feed ingredient for pigs diets (Gatel and Grosjean, 1990; Gatel, 1994; Mekbungwan, 2007). However, if adequately complemented with cereal grains and/or supplemented with crystalline AA, faba beans, peas or lupins can be included in diets for growing pigs without any negative effects on growth performance (Partanen et al., 2003; Stein et al., 2004; Zraly et al., 2007).

In the present work, standardised ileal digestibilities (SID) of crude protein (CP) and AA of currently available faba beans, peas and lupins were determined in growing pigs. The SID of CP in faba beans (76%) and peas (79%) were significant lower than those in lupins (87%). The SID of CP and AA in lupins were similar to those in SBM (P>0.05). Lupins had also significant higher SID of most AA, when compared to peas or faba beans. The results show, that grain legumes, especially lupins can be used as an adequate protein source for growing pigs. The SID values of the grain legumes tested in the present study were in general agreement with tabulated values for growing pigs (Rademacher et al., 2009). However, feed tables do not take into account partly low SID values in different species and/or cultivars of grain legumes, resulting from their contents of antinutritional factors. Although recent progresses in plant breeding of grain legumes have resulted in decreased contents of secondary plant metabolites, such as alkaloids, condensed tannins and trypsin inhibitors (Petterson, 1998; Duc et al., 1999), the use of several faba bean cultivars as a feed ingredient for pigs may still be limited due to their condensed tannin contents. In the present study, it has been shown that even low dietary tannin contents of 1.8 g/kg DM may exert a detrimental effect on SID values in growing pigs (Chapter 2). Furthermore, some fibre fractions present in grain legumes, such as neutral detergent fibre, acid detergent fibre, and non-starch polysaccharides including oligosaccharides or pectins, were discussed to have detrimental effects on ileal digestibility values in pigs (e.g. Mosenthin et al., 1994; van Barneveld et al., 1997). For example, the cotyledon fibres from peas have been shown to increase ileal excretion of endogenous proteins (Leterme et al.,

1996). Furthermore, as discussed in Chapter 1, high amounts of specific oligosaccharides (α-galactosides), may result in several adverse effects, such as reduced growth performance, flatulence or diarrhoea in pigs (Saini, 1989; van Barneveld, 1999; Ferguson et al., 2003).

For an assured protein evaluation of pig feed ingredients, *in vitro* measurements might be a cheap and rapid alternative. However, several methodological factors may interfere with the prediction of digestibility values based on *in vitro* measurements as was reviewed by Moughan (1999). For example, contents of secondary plant metabolites in grain legumes, which are known to depress nutrient digestibility *in vivo,* have much smaller effects on *in vitro* digestibility (Boisen and Eggum, 1991; Cone and van der Poel, 1993). This might be in part reasonable for results of the present study (Chapter 3), where prediction of SID of CP and AA with an *in vitro* method established by Boisen and Fernández (1995) and Boisen (2007) did not provide direct estimates of SID values in grain legumes. To predict SID of CP and AA further *in vivo* trials are warranted to validate *in vitro* prediction of SID values in other feed ingredients and feed mixtures.

4.3 Grain Legumes in Poultry Nutrition

In general, grain legumes are recognised as being highly digestible by poultry (Farell et al., 1999; Perez-Maldonado et al., 1999; Diaz et al., 2006). However, the mean apparent ileal digestibilities (AID) of AA in faba beans and peas (70 and 74%, respectively) were considerably lower than those for lupins (82%), whereas AID coefficients of AA in lupins were slightly lower than those in soybean meal (Ravindran et al., 2005). This ranking in digestibility between grain legumes is in general agreement with that in growing pigs (Chapter 2). Similarly, digestibility of sulphur-containing AA (methionine and cystine) and tryptophan in grain legumes has been proven to be rather low, both in poultry and pigs (Chapter 2). For example, Palander et al. (2006) determined considerably low AID of cystine (34 to 60%) in faba beans, peas and lupins in 5 and 10 weeks old turkeys, except for lupins when fed to 5 week old turkeys (76%). The AID of methionine in faba beans for 10 weeks old turkeys was also considerably low (59%). However, Pérez et al. (1993) found in peas a low AID of methionine (59%), but not for cystine (71%) when fed to growing chickens. Among grain legumes fed to broiler chickens, AID of tryptophan in lupins (*L. angustifolius, L. albus*) were higher (77% to 83%) than in faba beans (63%) and peas (63% to 75%) (Ravindran et al., 2006).

The dietary nutrient and energy requirements for chicks, broilers, laying hens and turkeys are different from those for pigs. The majority of poultry diets contains higher contents of CP and essential AA when compared to diets used in pig nutrition (Castell et al., 1996). Thus, using grain legumes in amounts being sufficient to fulfil the birds' CP and AA requirements, will result in higher dietary inclusion levels as compared to pig diets. Consequently, the presence of secondary plant metabolites and oligosaccharides may become a limiting factor in diet formulation for poultry (Castell et al., 1996). However, since genetic improvements of peas, faba beans and lupin seeds reduced the contents of these compounds, the concern over antinutrional effects of secondary plant metabolites in these ingredients has decreased (Castell et al., 1996; Petterson, 1998; Duc et al., 1999). On the other hand, similarly as described for pigs, there still is some concern directed to potential antinutritional effects of oligosaccharides in grain legumes. For example, practical use of lupins in poultry diets is limited due to the effects of lupin oligosaccharides on digesta viscosity and moisture content of excreta, resulting in health and environmental problems associated with the high moisture litter (van Barneveld, 1999; Petterson, 2000). Steenfeldt et al. (2003) observed significant higher viscosity of intestinal contents from broiler chickens fed a diet containing 200 g/kg *L. angustifolius* compared to a lupin-free control diet. In contrary, Roth-Maier and Kirchgessner (1994a) found no effects on digesta viscosity when 200 g/kg *L. albus* diet were fed to broilers. In the same study, when broilers received a diet containing 250 g/kg *L. albus*, both growth performance and faecal DM declined, resulting in sticky-wet faeces (Roth-Maier and Kirchgessner, 1994a). At present, a maximum inclusion level of about 100 g/kg lupins diet is recommended in diet formulation for poultry (van Barneveld, 1999; Petterson, 2000).

However, several methods can be applied to minimise the described negative effects of oligosaccharides in poultry nutrition, such as the dietary supplementation with specific enzymes, which is a frequently recommended method (e.g. Campbell and Bedford, 1992; Cowieson et al., 2003; Steenfeldt et al., 2003). For example, supplementation of *L. angustifolius* diets (200 g/kg diet) with a combination of β-galactanases-II and lactase decreased the intestinal viscosity, when compared to a lupin containing diet without enzyme supplementation (Steenfeldt et al., 2003). In diets for broilers containing *L. albus* up to 450 g/kg, supplementation of a mixed xylanase and endoglucanase product (Roxazyme RG™) improved growth performance, whereas faecal consistency remained unchanged (Roth-Maier and Kirchgessner, 1994b). On the other hand, Steenfeldt et al. (2003) found that substitution of SBM and maize with 200 g/kg blue lupins in broiler diets

depressed weight gain and feed conversion ratio, despite supplementation of the lupin containing diets with different enzymes (Bio-Feed® Plus, lactase, β-galactanase-I, β-galactanase-II) (Steenfeldt et al., 2003). Furthermore, in the same study AID of CP in lupins was not affected by enzyme supplementation (Steenfeldt et al., 2003). According to Annison et al. (1996), ileal protein digestibility of a broiler diet based on dehulled lupins (inclusion level 300 g/kg) was not affected by the high content of oligosaccharides from lupins, and supplementation an enzyme mixture (Bio-Feed® Plus) did not improve ileal protein digestibility. However, due to multi enzyme supplementation (Bio-Feed® Plus) the content of metabolisable energy of lupin diets was increased, probably due to higher digestibility of oligosaccharide in lupins.

4.4 Grain Legumes in Ruminant Nutrition

Grain legumes are suitable for the nutrition of monogastrics (e.g. Salgado et al., 2002; Diaz et al., 2006), but have also been introduced as soluble and rapidly degradable protein source in ruminant nutrition (Yu et al., 2002). According to van Straalen and Tamminga (1990), the protein fraction in grain legumes consists of about 85 to 100% of albumins and globulins, which are highly soluble and easily degradable in the rumen. Protein degradation of grain legumes in the rumen involves two steps, as described by Goelema et al. (1999). Firstly, the hydrolysis of peptide bonds by proteases and peptidases and, secondly, decarboxylation and/or deamination of AA, resulting in the release of peptides, AA, short-chain fatty acids, branched chain fatty acids, CO_2 and NH_3. Part of the NH_3 is reincorporated into microbial protein, but higher quantities may disappear through diffusion across the rumen wall and excreted as urea (Yu et al., 2002). Thus, a high rumen degradation rate may create an imbalance between seed protein breakdown and rate of microbial protein synthesis, resulting in preventable nitrogen loss from the rumen (Tamminga et al., 1990; van Straalen and Tamminga, 1990). In this context, according to several studies, high-temperature, high-pressure and high-moisture heat treatments (extrusion, expansion) or dry treatments (toasting) are effective in decreasing rumen degradability of protein originating from grain legumes (Goelema et al., 1998; Goelema et al.; 1999; Aufrère et al., 2001; Masoero et al., 2005). Furthermore, dietary inclusion of natural plant compounds with known ability to reduce proteolysis, such as the condensed tannins, has also been proposed (Min et al., 2003).

Condensed tannins are known to decrease protein degradability by binding feed protein, resulting in tannin-protein complexes that are less susceptible to degradation by proteolytic enzymes. Furthermore, tannins may inactivate proteolytic enzymes by binding to them, leading to a further decrease in protein degradability (Jansman, 1993). Thus, ruminants may benefit from the presence of condensed tannins in their diets. The lower nitrogen degradability due to protection of proteins by condensed tannins may increase the quantity of dietary protein including essential AA that passes into the duodenum (McMahon et al., 2000). For example, Makkar et al. (1997) observed a strong negative correlation (r=-0.92, $P<0.001$) between content of condensed tannin from coloured flowered faba beans and *in vitro* rumen protein degradability. It has been found that condensed tannins in legumes fed to ruminants markedly reduced rumen gas production which, in turn, reduced bloat incidences in grazing cattle (McMahon et al., 2000; Min et al., 2003). This effect can be attributed to the protein-binding properties of condensed tannins and, in addition, to a reduction in the counts of proteolytic bacteria in the rumen (Min et al., 2003). For example, Min et al. (2002) reported a decrease in the populations of several proteolytic rumen bacteria when a diet containing forage legumes with 32 g/kg diet condensed tannins was fed to sheep. Furthermore, it can be derived from *in vitro* results, that condensed tannins exert inhibitory effects on methanogenesis, which can be attributed to indirect effects, by reduced hydrogen production resulting from a decreased nutrient degradability in the rumen, but also to direct inhibitory effects on methanogenic rumen bacteria (Tavendale et al., 2005). Moreover, moderate concentrations of condensed tannins may result in increased weight gain, wool growth, and milk secretion (Barry and McNabb, 1999), and also have been shown to decrease the detrimental effects of gastrointestinal parasitism (Aerts et al., 1999; Athanasiadou et al., 2000; Athanasiadou et al., 2001).

4.5 Beneficial Effects of Secondary Plant Metabolites from Grain Legumes

In chapter 1, detrimental effects of secondary plant metabolites, occuring in grain legumes, such as condensed tannins, trypsin inhibitors, alkaloids, glycosides, saponins and lectins have been discussed. However, these secondary plant metabolites have also been associated with several positive pharmacological and microbiological effects.

4.5.1 PHARMACOLOGICAL EFFECTS

It can be derived from human clinical studies that apart from antinutritional effects, protease inhibitors as well as lectins may also act as anticarcinogenic agents (e.g. Champ, 2002; Messina, 1999). For example, Bowman-Birk protease inhibitors from soybean may be effective in preventing or suppressing carcinogen-induced transformation *in vitro*, as well as carcinogenesis in animals (Kennedy, 1995; Kennedy, 1998). Furthermore, a significant and dose-dependent decrease of human colorectal adenocarcinoma cells *in vitro* was observed when Bowman-Birk protease inhibitors from peas were used (Clemente et al., 2005). Moreover, lectins have been shown to inhibit tumor growth by promoting gut epithelium hyperplasia (Pryme et al., 1998; Pryme at al., 1999). For example, in a human colon cancer cell line, lectins from faba beans were able to stimulate undifferentiated cells to form gland-like structures, which are believed to slow down the progression of colon cancer and inhibit uncontrolled cellular proliferation (Jordinson et al., 1999). Beneficial effects of saponins depend on their potential in lowering blood cholesterol level, as has been reported for chicks, rats, mice and monkeys (Thompson, 1993). This might offer the possibility to reduce the incidence of heart disease in animals as well as in humans (Jamroz and Kubizna, 2008).

4.5.2 MICROBIOLOGICAL EFFECTS

Although saponins and alkaloids present in grain legumes may be detrimental when used in nutrition of animals and humans, they might be interesting feed and food additives due to their bacteriostatic and antifungal properties (Jamroz and Kubizna, 2008). For example, the alkaloid sparteine may act as an antimicrobial factor against some pathogenic funghi, including *Alternaria porri*, *Piriculata oryzae*, *Helmintosporium carbonum*, *Fusarium oxysporum* and *Aspergillus oryzae* (Jul et al., 2003). In addition to sparteine, the alkaloids lupanine and angustifoline may exert an inhibitory effect on several bacterial species, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus thuringensis*, *Bacillus subtilus* and *Staphylococcus aureus* (Jul et al., 2003). Furthermore, recent results from Verhelst et al. (2009) show that some polyphenols *in vitro* are able to prevent binding of enterotoxigenic *Escherichia coli* and its toxin to brush borders or intestinal receptors in the small intestine, suggesting antimicrobial effects of tannins against pathogenic bacteria. Enterotoxigenic *Escherichia coli* are known as important

enteropathogenes in pig production, which cause mortality, morbidity and decreased growth rates in piglets (e.g. Janke et al., 1989; Morgan et al., 1978; van Dijk et al., 2002).

4.6 Conclusions

Grain legumes, such as faba beans, peas and lupins have been shown to be an adequate protein source for pigs, poultry and ruminants. However, differences in protein digestibility between these animal species have to be considered. Furthermore, the use of grain legumes in animal nutrition might be restricted as they contain several secondary plant metabolites and/or oligosaccharides. These compounds may act as antinutritional factors, but also have been shown to possibly exert several beneficial effects. Nevertheless, since grain legume cultivars with low contents of several secondary plant metabolites are commercially available, these cultivars should be preferred in nutrition of monogastric animals. Moreover, several processing methods may further improve the feeding value of grain legumes. Further restrictions for the use of grain legumes in nutrition of monogastric animals result from their deficiency of methionine, cystine and tryptophan. Therefore, grain legume containing diets should be adequately supplemented with cereal grains and/or other protein sources (e.g. soybean meal) or crystalline AA to fulfil AA requirements in pig and poultry. Furthermore, for practical feed evaluation, cheap and rapid measurements of digestibility values might be useful, however, available *in vitro* analyses need further validation.

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CHAPTER 5

SUMMARY

5 SUMMARY

Since 2001, there is a general ban on meat and bone meal and its by-products in diets for livestock in the European Union, and it is necessary to focus on alternative protein feed ingredients to be included in diets for pigs in Central Europe. Besides soybean meal as commonly used plant protein supplement in diets for pigs, grain legume seeds may be used as alternative protein sources as well. Soybean meal is a frequently used protein supplement in diets for growing pigs due to its high and consistent product quality. However, in organic production systems, home grown grain legumes such as faba beans, peas and lupins may be preferred over soybean meal. Currently, protein evaluation systems for feed ingredients for pigs are based on the concept of standardised ileal digestibilities (SID) of crude protein (CP) and amino acids (AA). These feed tables have in common that in contrast to soybean meal, there is a scarcity of information on SID values in grain legume seeds, particular when grown in Central Europe. Moreover, published feed tables do hardly take into account the variations in SID values that may occur due to recent progress in breeding of grain legumes, including the introduction of new cultivars with varying nutrient contents, in particular with respect to lower levels of antinutritional factors (ANF). The SID values can be obtained when apparent ileal digestibility (AID) values are corrected for the so-called basal ileal endogenous CP and AA losses.

Therefore, the objective of the thesis was to investigate the chemical composition, including ANF in currently available seed-grade cultivars of faba beans (*Vicia faba*), peas (*Pisum sativum*) and lupins (*Lupinus angustifolius, Lupinus luteus*), grown in Central Europe. Furthermore, digestibility experiments with growing pigs, surgically fitted with simple ileal T-cannulas, were carried out to determine SID of CP and AA in these legume seeds. Three consecutive experiments with six barrows each were conducted. Each experiment consisted of 6 periods for ileal digesta collection. In addition, at the conclusion of each experiment, an extra-period was added, to estimate basal ileal endogenous losses and SID of CP and AA in casein by regression analysis from apparent ileal digestible and total dietary contents of CP and AA. A total of 18 assay feed ingredients was used, including six seed-grade cultivars of faba beans (*Vicia faba*) and peas (*Pisum sativum*), respectively, five seed-grade cultivars of lupins (*Lupinus angustifolius, Lupinus luteus*), and one commercially available soybean meal as reference feed ingredient. A total of 18 semi-synthetic diets based on corn starch and casein was formulated to which one of the 18 assay feed ingredients each was added. The daily feed allowance was restricted to 30

g/kg of body weight throughout all experimental periods. The SID of CP and AA in grain legumes were determined in difference to SID of CP and AA originating from casein.

A further aim of the thesis was directed to the validation of an *in vitro* procedure for estimates of SID of CP and AA in the same grain legumes. The SID for CP and AA were predicted from *in vitro* analysis by means of a two-step enzymatic method with subsequent pepsin and pancreatin incubations. Thereafter, *in vitro* predicted SID values were compared with their corresponding *in vivo* SID values.

The chemical composition and SID values of CP and AA measured in faba bean, pea and lupin cultivars generally were in good agreement with tabulated values. The average SID of CP and most AA in lupin cultivars (CP, 87%) were higher than in pea (CP, 79%) or faba bean cultivars (CP, 76%) (P<0.05). The SID of CP and AA in lupin cultivars were similar to those in SBM (P>0.05). However, all grain legume samples were in comparison to SBM deficient in methionine and cystine and, in addition, the SID of these AA were rather low, which may limit the use of legume seeds in diet formulation for growing pigs. Comparably low SID values in some faba bean cultivars can be partly explained by their contents of condensed tannins. There were linear decreases (P<0.05) in SID values in faba bean cultivars with increasing contents of condensed tannins. Even low tannin contents of 1.8 g/kg dry matter in the faba bean containing assay diets had distinct negative effects on SID values. In contrast to SID values in faba bean cultivars, there were only small variations in SID values between individual cultivars of peas or lupins.

Results of the *in vitro* experiment revealed that predicted SID of CP and AA do not provide direct estimates of SID values in grain legumes, because the *in vitro* predicted SID values were generally higher than *in vivo* SID values. Simple linear regression analysis resulted only in poor linear relationships between *in vivo* SID and *in vitro* predicted SID values. The differences between *in vitro* predicted and *in vivo* SID values were not similar between grain legume species (P<0.05), and the coefficients of variation differed considerably between grain legume species. The use of multiple linear regression analysis with grain legume species as a covariate accounted for both, the difference between *in vitro* predicted and *in vivo* SID values between the legume seeds and for their different coefficients of variation. As a result, strong linear relationships were obtained (r^2=0.73 to 0.91). Further investigations are warranted to study if these principles apply to other feed

ingredients as well. Moreover, the use of *in vitro* predicted SID values in practical diet formulations for pigs needs to be further validated.

CHAPTER 6

ZUSAMMENFASSUNG

6 ZUSAMMENFASSUNG

Mit dem von der EU-Kommission verfügten Verfütterungsverbot von Tiermehlen aus dem Jahr 2001 steigt der Bedarf an pflanzlichen Proteinfuttermitteln für Schweine. Neben Sojaextraktionsschrot als Proteinquelle von hoher und gleichbleibender Qualität können auch Körnerleguminosen als Protein- und Energieträger in Rationen für Schweine eingesetzt werden. Insbesondere in ökologisch wirtschaftenden Betrieben werden heimische, hofeigene Körnerleguminosenarten wie Ackerbohnen, Erbsen und Lupinen dem handelsüblichen Sojaextraktionsschrot vorgezogen, da zugekaufte Futtermittel nur sehr begrenzt eingesetzt werden dürfen. Die Bewertung von Proteinfuttermitteln erfolgt gegenwärtig auf Basis von standardisierten praecaecalen Verdaulichkeitswerten für das Rohprotein (XP) und die Aminosäuren (AS). Die standardisierten praecaecalen Verdaulichkeitswerte lassen sich durch eine Korrektur der scheinbaren praecaecalen Verdaulichkeitswerte berechnen, indem letztere um den Anteil an sogenannten basalen praecaecalen endogenen XP- und AS-Verlusten korrigiert werden. Die Datengrundlage für Körnerleguminosen, insbesondere solche aus mitteleuropäischem Anbau, ist jedoch in den verfügbaren Futterwerttabellen gering. Darüber hinaus sind durch züchterische Maßnahmen Veränderungen in den Gehalten an XP und AS sowie diverser antinutritiver Inhaltsstoffe erfolgt, ohne dass mögliche Auswirkungen auf die standardisierten praecaecalen Verdaulichkeitswerte bekannt sind.

Ziel der Untersuchungen war zum einen die Bestimmung der chemischen Zusammensetzung, einschließlich der Gehalte an antinutritiven Inhaltsstoffen, in derzeit verfügbaren sortenreinen Partien von Ackerbohnen (*Vicia faba*), Erbsen (*Pisum sativum*) und Lupinen (*Lupinus angustifolius, Lupinus luteus*) aus mitteleuropäischem Anbau. Des Weiteren wurden Verdaulichkeitsuntersuchungen zur Bestimmung der standardisierten praecaecalen Verdaulichkeiten des XP und der AS in den Körnerleguminosen an wachsenden Schweinen durchgeführt, die mit einfachen T-Kanülen am distalen Ileum versehen waren. Insgesamt umfassten die tierexperimentellen Untersuchungen drei Versuchsreihen mit jeweils sechs Börgen. Jeder Versuch bestand aus 6 Perioden zur Chymussammlung. Außerdem wurden am Ende eines jeden Versuches in einer zusätzlichen Periode regressionsanalytisch die basalen praecaecalen endogenen Verluste, sowie die standardisierten praecaecalen Verdaulichkeiten des XP und der AS in Kasein bestimmt. Eine halbsynthetische Ration auf der Basis von Kasein und Maisstärke wurde als Basalration verwendet und jeweils mit einer zu prüfenden Futterkomponente

ergänzt. Die insgesamt 18 zu untersuchenden Futtermittel umfassten jeweils sechs verschiedene Sorten Ackerbohnen und Erbsen, sowie fünf verschiedene Sorten Lupinen und ein handelsübliches Sojaextraktionsschrot als Referenzfuttermittel. Die tägliche Futtermengenzuteilung wurde in allen Perioden auf 30 g/kg der Lebendmasse begrenzt. Die standardisierten Verdaulichkeiten der zu untersuchenden Futtermittel wurden im Differenzversuch zu Kasein bestimmt.

Ein weiteres Ziel der Untersuchungen war die Validierung einer *in vitro* Methode zur Schätzung der standardisierten praecaecalen Verdaulichkeitswerte in den beschriebenen Körnerleguminosenarten. In zwei aufeinander folgenden Analysenschritten wurden die Futtermittelproben mit Pepsin und anschließend mit Pankreatin inkubiert. Mittels Fällungsreaktionen zur Ausfällung von gelöstem XP und anschließender Filtration wurden die standardisierten praecaecalen Verdaulichkeit des XP und der AS *in vitro* aus der Differenz zwischen dem XP-Gehalt in der Probe und dem entsprechenden nicht abgebauten Rest im Filtrationsrückstand mittels etablierter Schätzgleichungen berechnet. Die Validierung der berechneten *in vitro* Verdaulichkeiten erfolgte anhand der im Tierexperiment ermittelten *in vivo* Verdaulichkeitswerte.

Die chemische Zusammensetzung und die standardisierten praecaecalen Verdaulichkeitswerte in den untersuchten Ackerbohnen, Erbsen und Lupinen zeigten im Mittel eine gute Übereinstimmung mit bisher veröffentlichten Tabellenwerten. Die untersuchten Partien der einzelnen Ackerbohnen, Erbsen und Lupinen wiesen nur eine geringe Variation in ihrer chemischen Zusammensetzung auf. Für das XP und die meisten AS aus Lupinen (XP, 87%) und Sojaextraktionsschrot (XP, 87%) wurden deutlich höhere praecaecale Verdaulichkeitswerte als für Erbsen (XP, 79%) oder Ackerbohnen (XP, 76%) (P<0,05) ermittelt. Allerdings enthielten alle Körnerleguminosen nur vergleichsweise geringe Gehalte an schwefelhaltigen AS, deren standardisierte praecaecale Verdaulichkeitswerte zudem sehr niedrig waren, was bei der Rationsformulierung zu berücksichtigen ist. Die Unterschiede in den standardisierten praecaecalen Verdaulichkeiten von XP und AS in Ackerbohnen resultierten aus ihren unterschiedlichen Gehalten an kondensierten Tanninen. Es konnte nachgewiesen werden, dass die standardisierten praecaecalen Verdaulichkeiten des XP und der AS aus Ackerbohnen mit zunehmendem Tanningehalt der Ackerbohnen linear abnahmen (P<0,05). Offensichtlich hatten schon geringe Gehalte an kondensierten Tanninen von 1,8 g/kg Trockensubstanz in der Ration einen nachweisbaren Einfluss auf die standardisierte praecaecale

Verdaulichkeit des XP und der AS aus Ackerbohnen (P<0,05). Im Gegensatz zu den Ackerbohnen war die Variation in den standardisierten praecaecalen Verdaulichkeiten des XP und der AS innerhalb der Lupinen- und Erbsensorten sehr gering.

Die Ergebnisse der *in vitro* Untersuchungen belegen, dass eine direkte Schätzung der *in vivo* Verdaulichkeitswerte nicht möglich ist. Die *in vitro* Werte zur standardisierten praecaecalen Verdaulichkeit von XP und AS in den Körnerleguminosen lagen konstant oberhalb der entsprechenden *in vivo* Werte. Eine einfache lineare Regressionsanalyse ergab nur schwache Beziehungen zwischen den *in vitro* berechneten und den entsprechenden *in vivo* ermittelten Verdauungswerten. Darüber hinaus zeigte sich, dass sowohl die Differenzen von berechneten *in vitro* Verdaulichkeiten zu den entsprechenden *in vivo* Verdaulichkeiten (P<0,05), als auch die Varianzen dieser Differenzen zwischen den Körnerleguminosenarten unterschiedlich sind. Wurde dieser artspezifische Einfluss im Rahmen einer multiplen linearen Regressionsanalyse mit der Körnerleguminosenart als Kovariablen berücksichtigt, so konnten enge lineare Beziehungen zwischen berechneten *in vitro* Verdaulichkeiten und den entsprechenden *in vivo* Werten nachgewiesen werden (r^2=0,73 bis 0,91). Inwieweit diese Beobachtungen auf andere Futtermittelgruppen zutreffen und sich zukünftig für die Futterbewertung und Rationsformulierung nutzen lassen, bedarf weiterer Untersuchungen.

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Declaration

I declare that this thesis is a result of my personal work and no other than the indicated aids have been used for its completion. All quotations and statements that have been used are indicated.

Stuttgart-Hohenheim, June 15, 2009 Dagmar Jezierny