FEED-A-GENE

Adapting the feed, the animal and the feeding techniques to improve the efficiency and sustainability of monogastric livestock production systems

Deliverable D1.4

Characterisation and evaluation of the bioavailability of novel feed protein ingredients for pigs and poultry

Due date of deliverable: M48

Actual submission date: M48

Start date of the project: March 1st, 2015 **Duration:** 60 months

Organisation name of lead contractor: AU

Revision: V1

Dissemination level	
Public – PU	**X**
Confidential, only for members of the consortium (including Commission Services) – CO	
Classified, as referred to in Commission Decision 2001/844/EC - CI	

Table of contents

1. Summary 3
2. Introduction 5
3. Results 6
3.1 Nutritive value of gently-processed European soybean meal in piglets (KU) 6
3.2 Nutritive value of gently-processed European soybean meal in broilers (UNEW) 14
3.3 Nutritive value of protein extracted from green biomass (AU) 17
3.4 Nutritive value of rapeseed meal (IRTA) 21
4. Conclusions 24
5. Annexes 26
Annex 1: Product and diet compositions 27
Annex 2: Basal endogenous losses and digestibility of N-free diets 33
Annex 3: Analytical procedures 34
Annex 4: Bibliographic references 39
Annex 5: Scientific outputs 41

1. Summary

Objectives

The objectives of task 1.4 is to evaluate the nutritional value of upgraded rapeseed protein concentrate, European grown and gently-processed soybean meals, and protein extracted from green biomass produced in tasks 1.1, 1.2, and 1.3.

Rationale:

Nutritive value of European soybean meal in weaned piglets
The nutritive value of four different European grown and gently-processed soybean meals (SBM) produced in task 1.1 (i.e., flaking-cooking-pressing (FCP) and extrusion-pressing process (EP) of dehulled (DH) and whole beans (WH)) were determined in a 28-day performance trial with a total of 70 DanBred 5-week-old barrows, followed by a 5-day N-retention study while placed in metabolic cages. Apparent and standardized ileal digestibility of the SBM were determined post-mortem to evaluate whether trypsin inhibitor activity of different soybean meals influenced the host's protease activity in jejunal digesta and pancreas. Growth performance and N-retention was compared to a commercially available good-quality SBM, while in the ileal digestibility study, casein was used as a reference protein source due to its expected almost complete digestion. Except for the N-free diet used to determine endogenous N and amino acid losses, the diets were iso-nitrogenous (180 g CP per kg feed) and iso-caloric on a NE basis (10.9 MJ/kg). The SBM and casein were the sole protein source in each experimental feed but with supplementation of methionine to ensure a realistic feed evaluation in the performance and N-retention studies. During the performance study, animals received a coarse meal diet and water *ad libitum*. In the N-retention study, feed intake was restricted to 90% of *ad libitum*.

Nutritive value of European soybean meal in weaned in broilers
The same four processed European soybean products were used in a study with 288 male Ross 308 day-old chicks housed in 24 pens of 1.7 m^2 with *ad libitum* access to feed and water throughout the trial. Starter (0-14 d) and grower (15-28 d) mash diets were formulated using the four SBM products. Synthetic amino acids were added to cover limiting essential amino acid requirements at the same level on a digestible amino acid basis for all starter or grower diets. Differences in AME of the four dietary treatments were accounted for by supplementation of soybean oil. Each treatment was replicated in six pens with 12 birds per pen. Performance was measured by pen body weight measured at arrival and pen body weight and average daily feed intake at the end of the starter and grower period. For collection of intestinal contents, three randomly selected chickens with a body weight close to the pen average were weighed individually at 14 and 28 days of age. They were euthanized and intestinal tissue collected from one of the three birds. From the birds killed at d28, a subsample of pooled ileal digesta from the upper 1/3 of the ileum was also collected for determination of intestinal viscosity. An additional bird per pen was selected at d28 for morphometric measurements.

Nutritive value of protein extracted from green biomass
Two trials with ileum-cannulated growing pigs were performed to determine protein quality of protein concentrate from green biomass. Both trials were performed with four diets each, containing one protein concentrate as the only protein source and a N-free diet used to correct for basal endogenous N and amino acid losses. Each diet was fed to the pigs for 1 week, and all diets fed to all pigs (n=5) using a Latin Square design. Ileal content was collected for 8 h on day 5 and day 7 and pooled for analysis. In trial 1, four iso-nitrogenous diets were formulated with 30% of the diet coming from protein concentrate produced from a harvest of ryegrass and red clover with or without the use of cell wall degrading enzymes during the first large pilot scale extraction in 2016. In trial 2, three diets containing either concentrates of ryegrass, red clover, or lucerne harvested in 2017 without the use of cell wall degrading enzymes during processing were compared to conventional SBM. For comparison within and between studies, the protein level of the diets was fixed at 10% of dry matter and the animals fed at a level of 8% of $BW^{0.75}$.

Nutritive value of conventional and upgraded rapeseed meal for growing pigs
A trial was conducted to compare the efficacy of conventional rapeseed meal (RSM) grown in the EU and the same RSM upgraded by mechanical processing at Bühler to increase its crude protein content. Growth performance, feed intake, feed conversion ratio, and total tract apparent digestibility of major nutrients were used as response criteria. The trial was conducted following a 2 x 2 x 2 factorial arrangement with eight experimental treatments. These corresponded to two diets based on conventional RSM or upgraded RSM in combination with pelleting conditions at the feed mill. The pelleting conditions tested were the die size (4x40 or 4x60 mm long) and the utilization of steam (with or without) to produce feeds. The mixed cereal-based diets were formulated according to the nutrient requirements of growing pigs. A similar amount (22.5%) of conventional RSM was replaced by upgraded RSM, and no adjustment by the addition of synthetic amino acids was done. Therefore, crude protein, lysine, and other amino acid contents were higher in diets containing upgraded RSM. Pigs were fed the experimental diets *ad libitum* over the whole experimental period, which lasted 49 days. One-hundred forty-four crossed Pietrain x (Large White x Landrace) pigs initially weighing 27.6 kg were allocated at two pigs per pen for a total of nine pens per treatment. One male and one female were penned together, so that 72 males and 72 females were used. They were grouped in nine blocks of live weight at the beginning of the trial.

Teams involved:

KU, UNEW, IRTA, AU

Delivery of material from IFIP, Bühler, DuPont Industrial Biosciences

Species and production systems considered:

Conventional pig and broiler production systems are considered. The processes concern dry fractionation and wet extraction without the use of enzyme or organic solvent technology and can also be used in organic farming systems.

2. Introduction

Soybean meal (SBM) represents 61% of the protein sources used to feed livestock, whilst the European Union (EU) has a self-sufficiency of 3% for its soybeans and SBM needs, which is considered unsustainable for a different reasons (Leinonen *et al.*, 2013; Boerema, *et al*., 2016; Zander, *et al*., 2016). The increased demand for imported soybean, especially by the non-ruminant feed industry, is attributed to its high crude protein (CP) content and amino acid profile, which is complementary to that of cereals, and its high amino acid digestibility (de Visser 2014; Ravindran, *et al*., 2014).

Increasing EU production of domestic soybeans and processing at medium plants from local, non-GMO crops may contribute to the reduction of imported SBM (Martin, 2015). The classical processing of soybeans involves dehulling, defatting with hexane, treatment with equate ethanol under vacuum to eliminate soluble carbohydrates, and drying with ethanol. However, hexane is extremely flammable and non-renewable, and is regulated as a hazardous air pollutant (O'Quinn, *et al*., 1997). In addition, solvent extraction requires a considerable capital investment, a consistent supply of soybeans to operate continuously, and involves a high energy consumption (Pacheco *et al*., 2013). Although expeller extraction of soybeans is an alternative to the use of hexane, performance responses to expeller extracted SBM are inconsistent and suggest variations in the nutritional value depending on the seed source and the production process (Karr-Lilienthal *et al*., 2006; Opapeju *et al*, 2006). Sustainability is an important aspect of the evaluation of alternative sources of protein for non-ruminant farm animals. Partially defatted SBM could have an additional economic advantage over imported SBM related to its non-GMO character and its local origin (Le Cadre *et al*, 2015). In task 1.1, alternative processes for the production of expeller SBM from European grown soybeans were explored. Following grinding, soybeans were dehulled or not and then either extruded or flaked and cooked, and finally pressed to extract the oil, resulting in four SBM products. These four products have been evaluated regarding their nutritional quality for piglets and broilers in task 1.4.

With high yields of dry matter (DM) and crude protein (CP) per hectare at temperate climates, green crops like grasses and leguminous plants (*Fabaceae*) such as clover and lucerne have the potential to become an alternative source of protein for non-ruminants. These forages have an amino acid composition similar to SBM. To be used for monogastrics, the protein needs to be extracted from the fibre rich matrix. In task 1.2, protein concentrates from green biomass have been produced in a newly developed pilot plant at Aarhus University. Apart from being sufficiently efficient to be sustainable, a crucial step for implementing protein from green biomass is the evaluation of the nutritional value. Depending on source of biomass and processing conditions, the protein concentration and quality may vary (see D1.2). In task 1.4, the nutritional value of protein concentrates from 2 years of harvest were evaluated. The sources used were perennial ryegrass, red clover, and lucerne. To enhance extraction yield and possibly thereby the digestibility, cell wall degrading enzymes were tested as a technical aid during the first years production.

The use of cost effective protein sources can reduce the demand of protein imports in EU and increase the profitability of pig production. Rapeseeds (*Brassica napus* and *Brassica juncea*) have a high protein content and a well-balanced amino acid profile. Rapeseed meal (RSM), a by-product of oil industry after the seed´s crushing, contains high amounts of protein (approximately 35% CP), and can be used in growing-finishing diets for pigs as an alternative to SBM. The EU was the biggest

worldwide producer of rapeseed in 2016/2017, with 20.5 million metric tons, which makes this feedstuff highly recommendable to satisfy the protein demand of the animal feed industry in Europe. Despite the high protein content, RSM also contains antinutritional factors, such as glucosinolates, sinapine, and phytate and has a high fibre content that limit its use in animal feed (Ndou *et al*., 2018). Glucosinolates, after being hydrolysed, can limit feed intake due to its bitterness and goitrogenic toxic effect. Sinapine can induce a bitter taste in the feed, reducing its palatability (Woyengo *et al*., 2017). The high fibre and phytate phosphorus contents can reduce the dietary digestibility of minerals, CP, and amino acids (Mejicanos *et al*., 2018; Hansen *et al*., 2017). The high fibre content in RSM is due to the high hull content in rapeseed expeller, and a dehulling process can enhance the nutritive value of RSM. In terms of improvement of the nutritional value of RSM, the dehulling process is the most studied and disclosed method, although it is currently an economically unfeasible procedure for the oil industry due to the loss of oil present in the hulls (Mejicanos *et al*., 2018). Under non-disclosed methods, some companies (e.g., Hamlet Protein A/S, Denmark; Bühler AG, Uzwil, Switzerland) have produced low-glucosinolates RSM, with a higher CP content and reduced contents of fibre and other antinutritional factors.

3. Results

3.1 Nutritive value of gently-processed European soybean meal in piglets (KU)

Figure 1 shows an overview of the trial performed to evaluate the nutritive value of gently-processed European SBM in weaned piglets. Chemical composition of the ingredients produced in task 1.1 is shown in Annex 1, Table A1.1. Feed formulation and composition of experimental diets for the study with piglets is shown in Annex 1, Table A1.2.

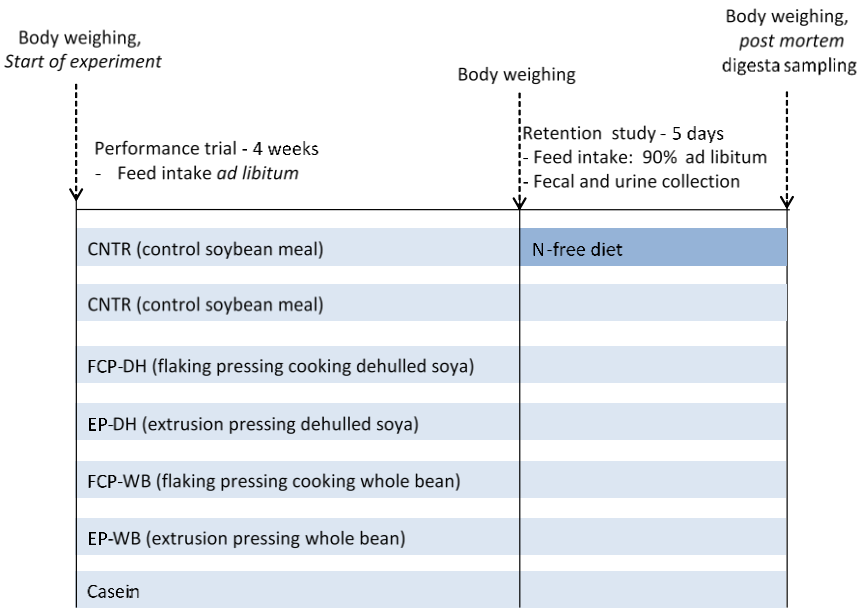

Figure 1. *Experimental design and time line for trial with commercially and gently-processed European grown soybean meals in piglets.*

3.1.1. Performance and N-retention

The growth performance of the pigs regarding body weight, average daily gain, daily feed intake, and feed conversion ratio in each week and in the total 4-week trial are shown in Table 1, except for the Casein group. Unfortunately, animals in the Casein group had significant feed refusals and reduced growth during weeks 2 and 3. Therefore, the feed was changed to the control SBM diet for the rest of the experiment. During the N-retention study (i.e., the last 5 days of the experiment), animals of the Casein group were fed with the casein-based diet to determine the digestibility of amino acids using casein as a reference. Since the arginine content of the casein-based feed was much lower than that in the other feeds, the casein diet was supplemented with 1 g/kg arginine in the second repetition. The arginine supplementation resulted in the same arginine content as that in the experimental diets, but the problem of feed refusals occurred in the second replicate as well. The pigs recovered when a Ca injection was given, but their performance data were excluded from the statistical analysis.

Table 1. Effect of different processed soybean meals on the growth performance of weaned pigs.

	Treatments[1]					RMSE	*P*-value	
	CNTR	FCP-DH	EP-DH	FCP-WH	EP-WH		Trt	R
Body weight (kg)								
initial	11.62	11.59	11.55	11.50	11.45	1.53	ns	ns
week1	15.05^{a}	13.04^{b}	14.12^{ab}	13.99^{ab}	14.05^{ab}	1.55	ns	ns
week2	19.31^{a}	14.92^{b}	18.65^{a}	17.91^{a}	18.35^{a}	2.13	0.0005	ns
week3	24.97^{a}	17.98^{b}	24.45^{a}	22.92^{a}	23.67^{a}	2.49	<0.0001	ns
week4	29.01^{a}	20.63^{b}	28.79^{a}	27.52^{a}	28.47^{a}	2.70	<0.0001	ns
Average daily gain (g/d)								
week1	490^{a}	206^{c}	366^{b}	355^{b}	372^{b}	88.50	<0.0001	ns
week2	609^{a}	303^{b}	647^{a}	561^{a}	614^{a}	150.00	<0.0001	0.006
week3	809^{a}	438^{b}	829^{a}	716^{a}	761^{a}	118.60	<0.0001	0.07
week4	577^{a}	379^{b}	620^{a}	657^{a}	686^{a}	105	<0.0001	0.06
total	621^{a}	323^{b}	616^{a}	572^{a}	608^{a}	62.60	<0.0001	0.02
Average daily feed intake (g/d)								
week1	704^{a}	597^{c}	670^{ab}	655^{ab}	637^{bc}	44.5	<0.0001	ns
week2	1008^{a}	772^{b}	949^{a}	905^{ab}	955^{a}	112.8	<0.0001	ns
week3	1183^{a}	920^{b}	1181^{a}	1078^{a}	1133^{a}	108.3	<0.0001	ns
week4	1214^{a}	885^{b}	1175^{a}	1161^{a}	1192^{a}	117.3	<0.0001	ns
total	1027^{a}	794^{b}	994^{a}	950^{a}	979^{a}	78.7	<0.0001	ns
Feed conversion ratio (kg feed/kg gain)								
week1	1.49^{a}	2.99^{b}	1.86^{a}	1.86^{a}	1.97^{a}	0.711	0.0009	ns
week2	1.76^{a}	2.93^{b}	1.52^{a}	1.78^{a}	1.66^{a}	0.599	<0.0001	0.007
week3	1.48^{a}	2.42^{b}	1.44^{a}	1.52^{a}	1.51^{a}	0.429	<0.0001	0.01
week4	2.15^{ab}	2.52^{b}	1.93^{a}	1.79^{a}	1.77^{a}	0.393	0.0005	0.08
total	1.66^{a}	2.56^{b}	1.62^{a}	1.66^{a}	1.61^{a}	0.241	<0.0001	ns

[1] Soybean meal were good quality commercial SBM (CNTR), flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processed whole SBM (FCP-WH), and extrusion-pressing processed whole SBM (EP-WH). Trt, treatment; R, raw material.

There was a significant difference in body weight and average daily gain of pigs for the different dietary treatments from the first week and throughout the experiment. Growth performance of the pigs receiving the control SBM was the best in the first week, and that of FCP-DH group was the worst throughout the trial. The difference in body weight and body weight gain was attributed at least partly to the low feed intake in FCP-DH pigs that resulted in a high feed conversion ratio. The control

SBM was superior during the first week and it seemed that the piglets needed some time to get used to the SBM processed with new technologies (i.e., EP-DH, FCP-WH, and EP-WH). However, in the last week of the trial, the growth rate and the feed conversion was the best for pigs fed the new technology SBM (i.e., FCP-DH vs. EP-DH, FCP-WH, EP-WH; $P<0.05$). The control SBM fed group was not significantly different from the worst group (FCP-DH) for the feed conversion ratio.
The results of the N-retention study are shown in Table 2. Since the performance trial was carried out before the retention study and the diets resulted in differences in growth rate of the pigs, the initial body weight of the pigs allocated in the trial groups was different in the retention study (see Table 1). In line with the performance trial, the N-intake was significantly lower in treatment FCP-DH compared to other treatments. Faecal nitrogen excretion was the lowest in pigs fed the EP-DH diet, intermediate for the EP-WH and FCP-WH diet ($P>0.05$), and the highest for pigs fed the FCP-DH and CNTR diets. Faecal digestibility of crude protein (N) was 86% in the FCP-DH group, which was significantly lower compared to the other diets (N digestibility averaged 91.3% for CNTR, EP-DH, FCP-WH, and EP-WH). The urinary N excretion and the total N excretion were the lowest in the FCP-DH group. There was no significant difference among treatments regarding the efficiency of N retention relative to total or digestible N intake.

Table 2. Effect of different processed soybean meals on N-retention of weaned pigs[1].

	Diets[1]					RMSE	*P*-value	
	CNTR	FCP-DH	EP-DH	FCP-WH	EP-WH		Trt	R
Intake, g/d	32.8^{a}	25.2^{b}	30.8^{a}	33.0^{a}	31.9^{a}	2.34	<0.0001	ns
Faecal excretion, g/d	3.27^{ab}	3.56^{a}	2.31^{c}	2.97^{abc}	2.71^{bc}	0.668	0.0014	ns
Urinary excretion, g/d	6.60^{a}	3.90^{b}	6.43^{a}	6.22^{a}	6.46^{a}	1.111	<0.0001	ns
Total N excretion, g/d	9.87^{a}	7.47^{b}	8.74^{ab}	9.19^{a}	9.16^{a}	1.296	0.0029	0.07
N-retention, g/d	23.0^{a}	17.7^{b}	22.1^{a}	23.8^{a}	22.8^{a}	2.08	<0.0001	0.006
Faecal digestibility, %	90.0^{a}	85.9^{b}	92.5^{a}	91.0^{a}	91.5^{a}	2.15	<0.0001	0.034
N-retention, % of N-intake	69.8	70.3	71.5	72.1	71.3	3.79	ns	0.003
N-retention,% of digested N	77.5	81.9	77.3	79.2	78.0	3.98	0.08	0.032

[1] Soybean meal were good quality commercial SBM (CNTR), flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processed whole SBM (FCP-WH), and extrusion-pressing processed whole SBM (EP-WH). Trt, treatment; R, raw material.

The mean body weight of the pigs in treatments CNRT, FCP-DH, EP-DH, FCP-WH, and EP-WH was 34.6, 24.6, 34.7, 33.0, and 33.8 kg, respectively at the end of the retention study.

The results suggest that the European SBM processed by different technologies, particularly the extrusion-pressing and flacking-cooking-pressing technologies without dehulling the bean, is suitable to weaned pigs as a protein source. The diet formulated with flacking-cooking-pressing dehulled SBM as a sole protein source had a lower faecal CP digestibility. However, the utilization of dietary N was the same as in the other SBM used in the trial.

3.1.2. Ileal digestibility

The apparent ileal digestibility (AID) of amino acids in the experimental feeds are shown in Table 3. The SBM or casein were the sole protein source of the mixed feeds. Therefore the amino acid digestibility reflects the apparent ileal amino acid digestibility of the protein sources. The results show that there was significant difference in AID of amino acids among the protein sources except for tryptophan. In line with the faecal CP digestibility data, the amino acid digestibility was the lowest in FCP-DH SBM for all amino acids. The AID of threonine, glycine, methionine, tyrosine, lysine, and

tryptophan in the control SBM were not statistically different from those in FCP-DH SBM. The AID of threonine, alanine, methionine, cysteine, tyrosine, and particularly of lysine of the control SBM were significantly lower than of the other three new technology processed, such as EP-DH, FCP-WH, and EP-WH SBM. There was no difference between the AID of amino acids of those three protein sources. As expected, the ileal digestibility of amino acids was the highest in casein for all amino acids, except for cysteine. The cysteine content of casein is low and, based on our results, it has a very low AID value (11.5%), which is probably underestimation. The basal endogenous amino acid loss was determined with the "N-free method" (Annex 2, Table A2.1) and used to calculate the standardized ileal digestibility (SID) of amino acids in each protein source (Table 4). The SID values were the highest for casein, reflecting to a complete digestion and absorption of amino acids. The fact that the SID values of amino acids were close to 100% in casein is feasible. However, the very high SID value for glycine (140%) seems to be either an overestimation of the real value for glycine or, less likely, indicating a significant specific endogenous glycine loss. The difference in SID digestibility showed the same tendency as in AID. The FCP-DH SBM had the worst protein quality, since the SID of amino acids were the lowest among the SBM. The control SBM was somewhat a better protein source than the FCP-DH SBM, since its total amino acid digestibility was higher (P>0.05). However, SID of most of the limiting amino acids was the same, and the digestibility was significantly higher only for asparagine, serine, glutamine, valine, leucine, phenylalanine, histidine, and arginine in CONTR compared to the FCP-DH SBM. The EP-DH, EP-WH, and FCP-WH products had high SID and in case of threonine, serine, alanine, valine, methionine, leucine, phenylalanine, lysine, and total amino acids, the SID was significantly higher than that in control SBM. It has to be noted that methionine supplementation was used in all diets to be sure that methionine would not be limiting growth. The total, AID, and SID amino acid composition of the European SBM processed by new technologies are shown in Table 5. The digestible methionine content is slightly overestimated since synthetic methionine supplementation was used in the trial feeds.

3.1.3. Protease activity

Pancreatic protease and jejunal protease activities were determined to check if trypsin inhibitor activity of different SBM had an impact on the total protease activity. Due to technical problems, only samples from the first repetition were analysed for this. The results are shown in Table 6. Due to the very high standard deviation within a group, there was no (reliable) difference in the total protease activity of pancreatic and jejunal digesta samples from the pigs received different SBM.

Table 3. Apparent ileal digestibility of the control soybean meal, European soybean meals processed by different technologies, and casein determined in weaned pigs.

	Treatments[1]						RMSE	*P*-value		
	CNTR n=10	FCP-DH n=8	EP-DH n=10	FCP-WH n=10	EP-WH n=8	Casein n=6		Trt	R	Trt x R
Asp	0.777^{a}	0.667^{b}	0.835^{a}	0.850^{a}	0.815^{a}	0.807^{a}	0.0543	<0.0001	ns	0.025
Thr	0.696^{cd}	0.647^{d}	0.796^{ab}	0.811^{ab}	0.750^{bc}	0.830^{a}	0.0538	<0.0001	ns	<0.0001
Ser	0.773^{b}	0.671^{c}	0.855^{a}	0.857^{a}	0.811^{ab}	0.830^{ab}	0.0528	<0.0001	ns	0.002
Glu	0.827^{a}	0.676^{b}	0.895^{a}	0.884^{a}	0.845^{a}	0.873^{a}	0.0652	<0.0001	ns	ns
Pro	0.790^{a}	0.462^{b}	0.826^{a}	0.817^{a}	0.782^{a}	0.900^{a}	0.1125	<0.0001	ns	0.025
Gly	0.663^{ab}	0.555^{b}	0.775^{a}	0.783^{a}	0.658^{ab}	0.625^{b}	0.0945	<0.0001	ns	0.093
Ala	0.648^{b}	0.629^{b}	0.818^{a}	0.818^{a}	0.757^{a}	0.788^{a}	0.0564	<0.0001	ns	<0.0001
Cys	0.569^{b}	0.558^{b}	0.736^{a}	0.758^{a}	0.652^{ab}	0.115^{c}	0.0680	<0.0001	0.0005	0.0003
Val	0.703^{c}	0.628^{d}	0.848^{ab}	0.830^{ab}	0.805^{b}	0.895^{a}	0.0452	<0.0001	ns	<0.0001
Met	0.765^{b}	0.790^{b}	0.920^{a}	0.906^{a}	0.901^{a}	0.953^{a}	0.0356	<0.0001	0.0004	<0.0001
Ile	0.694^{b}	0.612^{c}	0.850^{a}	0.828^{a}	0.796^{a}	0.862^{a}	0.0524	<0.0001	ns	<0.0001
Leu	0.744^{c}	0.636^{d}	0.874^{ab}	0.853^{ab}	0.827^{b}	0.901^{a}	0.0431	<0.0001	ns	<0.0001
Tyr	0.762^{cd}	0.658^{d}	0.889^{ab}	0.838^{bc}	0.842^{bc}	0.954^{a}	0.0713	<0.0001	0.015	0.007
Phe	0.766^{c}	0.659^{d}	0.881^{ab}	0.859^{b}	0.843^{b}	0.927^{a}	0.0405	<0.0001	ns	<0.0001
His	0.786^{b}	0.662^{c}	0.842^{ab}	0.829^{ab}	0.793^{b}	0.867^{a}	0.0435	<0.0001	0.0099	0.0008
Lys	0.761^{c}	0.725^{c}	0.886 ^{ab}	0.878^{ab}	0.843^{b}	0.903^{a}	0.0376	<0.0001	ns	<0.0001
Arg	0.865^{c}	0.771^{d}	0.932^{a}	0.921^{ab}	0.907^{abc}	0.871^{bc}	0.0349	<0.0001	0.058	0.0005
Trp	0.903^{a}	0.821^{a}	0.916^{a}	0.864^{a}	0.874^{a}	.	0.0851	ns	0.0015	ns
Amino acids	0.763^{b}	0.648^{c}	0.861^{a}	0.854^{a}	0.807^{ab}	0.868^{a}	0.0514	<0.0001	ns	0.0002

[1] Soybean meal were good quality commercial SBM (CNTR), flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processes whole SBM (FCP-WH), extrusion-pressing processes whole SBM (EP-WH). Trt, treatment; R, raw material.

Table 4. Standardized ileal digestibility of the control soybean meal, European soybean meal processed by different technologies, and casein determined in weaned pigs.

	Treatments[1]						RMSE	*P*-value		
	CNTR n=10	FCP-DH n=8	EP-DH n=10	FCP-WH n=10	EP-WH n=8	Casein n=6		Trt	R	Trt x R
Asp	0.861^{b}	0.752^{c}	0.927^{ab}	0.925^{ab}	0.909^{ab}	0.981^{a}	0.0543	<0.0001	ns	0.014
Thr	0.862^{cd}	0.820^{d}	0.985^{ab}	0.967^{ab}	0.930^{bc}	1.019^{a}	0.0538	<0.0001	ns	<0.0001
Ser	0.904^{b}	0.805^{c}	1.000^{a}	0.977^{a}	0.954^{ab}	0.970^{ab}	0.0528	<0.0001	ns	0.0015
Glu	0.889^{a}	0.740^{b}	0.959^{a}	0.939^{a}	0.914^{a}	0.927^{a}	0.0652	<0.0001	ns	ns
Pro	1.022^{a}	0.694^{b}	1.081^{a}	1.030^{a}	0.930^{a}	1.021^{a}	0.1125	<0.0001	ns	0.025
Gly	0.993^{bc}	0.894^{c}	1.138^{b}	1.095^{bc}	1.037^{bc}	1.405^{a}	0.1650	<0.0001	ns	Ns
Ala	0.789^{c}	0.777^{c}	0.972^{ab}	0.951^{ab}	0.911^{b}	1.040^{a}	0.0564	<0.0001	ns	<0.0001
Cys	0.913^{ab}	0.829^{b}	1.004^{ab}	1.026^{a}	0.902^{ab}	1.097^{a}	0.1292	0.02	0.0005	0.06
Val	0.818^{c}	0.747^{d}	0.970^{ab}	0.939^{ab}	0.932^{b}	1.007^{a}	0.0452	<0.0001	ns	<0.0001
Met	0.843^{b}	0.867^{b}	0.992^{a}	0.988^{a}	0.980^{a}	0.993^{a}	0.0356	<0.0001	ns	<0.0001
Ile	0.820^{b}	0.742^{b}	0.984^{ab}	0.949^{ab}	0.932^{b}	1.013^{a}	0.0524	<0.0001	ns	<0.0001
Leu	0.842^{c}	0.736^{d}	0.977^{a}	0.944^{a}	0.937^{a}	1.002^{a}	0.0431	<0.0001	0.08	<0.0001
Tyr	0.828^{bc}	0.731^{c}	0.969^{a}	0.903^{ab}	0.932^{ab}	1.016^{a}	0.0713	<0.0001	0.07	0.002
Phe	0.844^{c}	0.738^{d}	0.962^{ab}	0.930^{b}	0.934^{b}	1.033^{a}	0.0405	<0.0001	ns	<0.0001
His	0.913^{c}	0.791^{d}	0.982^{ab}	0.947^{abc}	0.935^{bc}	1.022^{a}	0.0435	<0.0001	0.047	0.0006
Lys	0.849^{b}	0.814^{b}	0.980^{a}	0.959^{a}	0.942^{a}	0.988^{a}	0.0376	<0.0001	ns	<0.0001
Arg	0.934^{c}	0.832^{d}	1.001^{ab}	0.979^{bc}	0.975^{bc}	1.047^{a}	0.0349	<0.0001	ns	0.0001
Trp	0.998^{a}	0.916^{a}	1.002^{a}	0.959^{a}	0.977^{a}	.	0.0851	ns	0.0017	ns
Amino acids	0.877^{b}	0.764^{c}	0.982^{a}	0.959^{a}	0.930^{ab}	0.997^{a}	0.0514	<0.0001	ns	0.0001

[1] Soybean meal were good quality commercial SBM (CNTR), flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processes whole SBM (FCP-WH), extrusion-pressing processes whole SBM (EP-WH). Trt, treatment; R, raw material.

Table 5. Total amino acid content, apparent and standardized ileal digestible amino acid content of European soy bean meals processed by novel technologies used in the present trial[1].

	Total AA content				AID AA content				SID AA content			
	FCP-DH	EP-DH	FCP-WH	EP-WH	FCP-DH	EP-DH	FCP-WH	EP-WH	FCP-DH	EP-DH	FCP-WH	EP-WH
Asp	5.74	5.95	5.30	5.70	3.83	4.79	4.86	4.67	4.32	5.32	5.33	5.17
Thr	1.96	2.03	1.81	1.94	1.27	1.56	1.58	1.47	1.61	1.94	1.90	1.81
Ser	2.54	2.62	2.33	2.51	1.70	2.17	2.17	2.06	2.05	2.55	2.49	2.41
Glu	9.01	9.34	8.31	8.95	6.09	8.07	7.93	7.63	6.67	8.66	8.45	8.19
Pro	2.47	2.56	2.28	2.46	0.85	2.04	2.01	1.72	1.41	2.67	2.55	2.30
Gly	2.11	2.19	1.94	2.09	1.17	1.63	1.64	1.40	1.89	2.41	2.31	2.12
Ala	2.21	2.29	2.04	2.20	1.39	1.81	1.80	1.69	1.72	2.16	2.11	2.02
Cys	0.73	0.76	0.67	0.73	0.41	0.54	0.55	0.48	0.61	0.73	0.76	0.68
Val	2.44	2.53	2.25	2.42	1.53	2.07	2.02	1.97	1.82	2.38	2.29	2.26
Met	0.70	0.73	0.65	0.70	0.55	0.64	0.63	0.63	0.61	0.70	0.69	0.69
Ile	2.32	2.41	2.14	2.31	1.42	1.97	1.91	1.85	1.72	2.30	2.20	2.16
Leu	3.71	3.85	3.42	3.69	2.36	3.24	3.15	3.08	2.73	3.65	3.51	3.47
Tyr	1.68	1.74	1.55	1.67	1.10	1.49	1.39	1.42	1.23	1.64	1.51	1.55
Phe	2.54	2.63	2.34	2.52	1.67	2.24	2.17	2.15	1.87	2.45	2.36	2.35
His	1.34	1.39	1.24	1.33	0.89	1.13	1.11	1.07	1.06	1.32	1.27	1.24
Lys	3.08	3.20	2.84	3.06	2.23	2.73	2.70	2.61	2.51	3.03	2.96	2.90
Arg	3.74	3.88	3.45	3.71	2.88	3.49	3.44	3.41	3.11	3.75	3.66	3.65
Trp	0.66	0.68	0.61	0.65	0.54	0.60	0.57	0.58	0.60	0.67	0.63	0.64

[1] Soybean meal were good quality commercial SBM (CNTR), flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processes whole SBM (FCP-WH), extrusion-pressing processes whole SBM (EP-WH).

Table 6. Total protease activity in pancreas and jejunal digesta in pigs fed different soybean meals.

	Treatments[1]									
	CNTR		FCP-DH		EP-DH		FCP-WH		EP-WH	
Trypsin inhibitor activity in SBM (TIU/mg)	2.9		7.6		3.5		3.6		2.6	
	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
Pancreas										
mg azocasein decomposed/ml sample	0.397	0.215	0.232	0.177	0.397	0.242	0.206	0.169	0.420	0.406
mg azocasein decomposed/g sample/h	15.60	8.51	8.75	6.57	15.39	9.41	8.07	6.63	15.81	15.01
mg azocasein decomposed/g DM/h	168.60	77.91	88.81	66.98	151.61	134.70	57.94	31.26	156.10	125.27
Jejunal digesta										
mg azocasein decomposed/ml sample	0.303	0.297	0.293	0.288	0.382	0.290	0.195	0.146	0.154	0.097
mg azocasein decomposed/g sample/h	11.35	10.88	11.08	10.89	14.28	10.62	7.60	5.90	5.97	3.82
mg azocasein decomposed/g DM/h	54.52	53.79	52.50	54.10	60.72	44.99	33.39	26.74	24.67	15.51

[1] Soybean meal were good quality commercial SBM (CNTR), flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processes whole SBM (FCP-WH), extrusion-pressing processes whole SBM (EP-WH).

3.2 Nutritive value of gently-processed European soybean meal in broilers (UNEW)

Feed formulation and chemical composition of the diets used in the study with broilers is shown in Annex 1, Table A1.3.

3.2.1 Performance

Effects of processing method, hulling method, and their interaction on performance variables are presented in Table 7. There was no effect of processing method on any of the performance variables. The hulling method affected feed intake (*P*=0.032) during the starter period, which was significantly higher for birds when hulls were present in the diet rather than not. However, the hulling method did not affect performance neither during the grower period nor over the whole growing period. The processing method and the hulling method interacted for average daily gain during the starter period (*P*=0.004), being significantly lower (*P*<0.05) for EP-DH and FCP-WH, compared to EP-WH SBM. Furthermore, the processing method and the hulling method interacted for feed intake during the starter period, which was significantly lower for the EP-DH than the EP-WH SBM.

Table 7. Effect of soybean meal processing and hulling methods on performance variables[1] of broilers over the starter (d1-14) and grower periods (d15-28).

	BW (g)	ADFI (g/d)			ADG (g/d)			FCR		
Days of age	**0**	**1-14**	**15-28**	**1-28**	**1-14**	**15-28**	**1-28**	**1-14**	**15-28**	**1-28**
Processing (P)[2]										
EP	37.1	37.8	110.9	74.3	28.3	66.2	47.3	1.33	1.63	1.57
FCP	36.7	37.6	113.1	75.4	28.2	68.0	48.1	1.34	1.63	1.57
Hulling (H)[3]										
DH	37.1	37.2	111.3	74.2	28.2	66.4	47.3	1.32	1.65	1.57
WH	36.6	38.3	112.7	75.5	28.4	67.9	48.1	1.35	1.62	1.57
SEM	0.32	0.34	0.98	0.54	0.30	0.77	0.41	0.013	0.018	0.015
P × H[2]										
EP-DH	37.2	36.7[a]	110.3	73.5	27.5[a]	65.5	46.5	1.33	1.65	1.58
EP-WH	37.0	38.9[b]	111.4	75.1	29.1[b]	66.9	48.0	1.33	1.61	1.57
FCP-DH	37.0	37.6[ab]	112.2	74.9	28.8[ab]	67.2	48.0	1.30	1.64	1.56
FCP-WH	36.3	37.7[ab]	114.1	75.9	27.6[a]	68.9	48.2	1.37	1.62	1.57
SEM	0.45	0.48	1.39	0.76	0.43	1.09	0.59	0.019	0.025	0.021
P-values										
Processing (P)	0.341	0.705	0.116	0.184	0.832	0.114	0.157	0.783	0.965	0.804
Hulling (H)	0.298	0.032	0.295	0.104	0.660	0.186	0.164	0.094	0.279	0.975
P × H	0.571	0.043	0.758	0.698	0.004	0.913	0.293	0.090	0.709	0.513

[a,b] Means within a row with different letters differ significantly (*P*<0.05).
[1] Body weight (BW), average daily feed intake (ADFI), average daily gain (ADG), feed conversion ratio (FCR).
[2] Soybean meal were flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processes whole SBM (FCP-WH), extrusion-pressing processes whole SBM (EP-WH).

3.2.2 Ileal digestibility and digesta viscosity

The effects of the processing method, the hulling method, and their interaction on apparent ileal CP and DM digestibilities and ileal digesta viscosity are presented in Table 8. There was no significant effect of the processing method, the hulling method, or their interaction on any parameter.

Table 8. Effect of soybean meal treatments, produced through the combination of different processing and hulling methods, on the coefficient of apparent ileal dry matter (DM) and crude protein (CP) digestibility at the end of the starter (d14) and grower period (d28) and on ileal viscosity at the end of the grower period.

	DM		CP		Viscosity (Cps)
Days of age	**14**	**28**	**14**	**28**	**28**
Processing (P)[1]					
EP	0.709	0.727	0.842	0.858	5.93
FCP	0.711	0.723	0.824	0.837	5.48
Hulling (H)[1]					
DH	0.720	0.724	0.834	0.842	5.68
WH	0.700	0.726	0.832	0.852	5.73
SEM	0.0107	0.116	0.064	0.091	0.24
P x H[1]					
EP-DH	0.723	0.728	0.843	0.855	5.76
EP-WH	0.695	0.727	0.840	0.860	6.10
FCP-DH	0.717	0.720	0.825	0.830	5.60
FCP-WH	0.705	0.726	0.823	0.843	5.37
SEM	0.0151	0.0164	0.0090	0.0130	0.33
P-values					
Processing	0.906	0.785	0.069	0.118	0.203
Hulling	0.201	0.877	0.778	0.486	0.881
P × H	0.615	0.861	0.958	0.751	0.414

[1] Soybean meal were flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processes whole SBM (FCP-WH), extrusion-pressing processes whole SBM (EP-WH).

3.2.3 Carcass and carcass part yields

The effects of the processing method, the hulling method, and their interaction on carcass and carcass part yields are presented in Table 9. There was no significant effect of the processing method, the hulling method, or their interaction on carcass part yield at d28. However, hull presence significantly reduced carcass yield ($P<0.05$).

Table 9. Effect of soybean meal treatments, produced through the combination of different processing and hulling methods on carcass yield and carcass part yield (% of carcass weight) at d28 of age.

	Carcass yield (%)	Breast meat (%)	Wing (%)	Thigh (%)
Processing (P)[1]				
EP	67.1	33.6	11.7	28.2
FCP	67.8	34.1	11.7	27.5
Hulling (H)[1]				
DH	68.9	33.8	11.5	27.4
WH	66.0	33.9	11.9	28.2
SEM	0.84	0.64	0.27	0.48
PxH[1]				
EP-DH	68.9	34.0	11.5	27.5
EP-WH	65.3	33.2	11.9	28.8
FCP-DH	68.9	33.6	11.5	27.3
FCP-WH	66.7	34.6	11.8	27.6
SEM	1.12	0.90	0.38	0.68
P-values				
Processing (P)	0.616	0.540	0.936	0.294
Hulling (H)	0.023	0.909	0.342	0.260
P × H	0.550	0.342	0.963	0.483

[1] Soybean meal were flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processes whole SBM (FCP-WH), extrusion-pressing processes whole SBM (EP-WH).

3.2.4 Histology

The effects of the processing method, the hulling method, and their interaction on villus height, crypt depth and villus height-to-crypt depth ratio are presented in Table 10. There was no effect of the processing method, the hulling method or their interaction on villus height, crypt depth, and villus height-to-crypt-depth ratio.

Table 10. Effect of soybean meal treatments, produced through the combination of different processing and hulling methods on jejunal histomorphometric features at the end of the starter (d14) and grower period (d28).

Days of age	14			28		
Histomorphometry[1]	VH (µm)	CD (µm)	VCR	VH (µm)	CD (µm)	VCR
Processing (P)[2]						
EP	494	75.5	6.80	748	99.8	7.85
FCP	502	78.6	6.65	773	101.9	7.89
Hulling (H)[2]						
DH	509	77.3	6.85	776	104.9	7.78
WH	487	76.8	6.60	745	96.8	7.95
SEM	21.2	2.52	0.237	29.6	4.64	0.198
P × H[2]						
EP-DH	491	77.4	6.67	775	102.1	8.03
EP-WH	497	73.7	6.92	722	97.5	7.67
FCP-DH	528	77.3	7.03	778	107.6	7.53
FCP-WH	477	79.9	6.28	768	96.1	8.24
SEM	30.0	3.56	0.335	41.8	6.6	0.280
P-values						
Processing (P)	0.786	0.404	0.676	0.569	0.757	0.896
Hulling (H)	0.476	0.886	0.462	0.457	0.235	0.546
P × H	0.354	0.389	0.153	0.608	0.609	0.068

[1] Villus height (VH), crypt depth (CD), villus height-to-crypt depth ratio (VCR).
[2] Soybean meal were flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processes whole SBM (FCP-WH), extrusion-pressing processes whole SBM (EP-WH).

3.2.5 Gastrointestinal tract development

The effects of processing method, hulling method, and their interaction on the gastrointestinal tract and organ weight and on small intestinal segment lengths are presented in Table 11. Processing method did not affect gastrointestinal tract and organ measurements. This was also the case for the interaction between processing method and hulling method. However, hull presence significantly increased gizzard, proventriculus and jejunal weights ($P<0.05$).

Table 11. Effect of soybean meal treatment, produced through the combination of different processing and hulling methods on intestinal segment length and weight relative to eviscerated carcass weight at the end of the grower period (d28) (cm/kg and g/kg of carcass weight, respectively) [1].

	Duo L	Jej L	Ile L	Duo W	Jej W	Ile W	Pro W	Giz W	Liv W	Pan W	Cec W
Processing (P)[2]											
EP	27.9	70.3	72.8	11.2	20.4	16.7	6.90	28.1	35.1	3.54	16.4
FCP	30.6	73.6	77.2	10.6	19.8	16.5	7.27	31.0	31.8	3.50	16.1
Hulling (H)[2]											
DH	28.4	70.6	74.4	10.4	18.8	16.7	6.55	27.9	32.8	3.58	15.9
WH	30.1	73.3	75.7	11.4	21.5	16.5	7.61	31.6	34.1	3.46	16.5
SEM	1.47	3.03	3.01	0.47	0.68	0.69	0.302	1.20	1.47	0.20	0.74
P × H[2]											
EP-DH	27.5	69.0	72.8	10.1	18.6	15.8	6.47	27.2	33.0	3.33	15.2
EP-WH	28.4	71.6	72.9	12.3	22.2	17.5	7.32	29.8	36.2	3.75	17.6
FCP-DH	29.4	72.2	76.0	10.6	19.0	17.5	6.62	28.6	32.6	3.83	16.6
FCP-WH	31.7	75.0	78.5	10.6	20.7	15.4	7.91	33.4	31.0	3.17	15.5
SEM	2.09	4.29	5.15	0.67	0.96	0.99	0.427	1.70	2.09	0.287	1.04
P-values											
Processing	0.225	0.447	0.315	0.387	0.564	0.852	0.394	0.156	0.127	0.670	0.809
Hulling	0.449	0.532	0.762	0.123	0.012	0.858	0.021	0.041	0.536	0.479	0.591
P × H	0.732	0.985	0.784	0.127	0.346	0.067	0.606	0.509	0.179	0.129	0.103

[1] Duodenum (Duo), jejunum (Jej), ileum (Ile), length (L), weight (W), proventriculus (Pro), gizzard (Giz), liver (Liv), pancreas (Pan), ceca (Cec).
[2] Soybean meal were flaking-pressing-cooking processed dehulled SBM (FCP-DH), extrusion-pressing processed dehulled SBM (EP-DH), flaking-pressing-cooking processes whole SBM (FCP-WH), extrusion-pressing processes whole SBM (EP-WH).

3.3 Nutritive value of protein extracted from green biomass (AU)

Details on processing conditions and composition of the concentrates are given in deliverable D1.2. Shortly, for trial 1, ryegrass harvested at the end of August 2016 and was screw-pressed to separate juice from pulp with or without a cocktail of cell wall degrading enzymes added during screw-pressing. Red clover was also harvested in August 2016 and processed with or without addition of the same cocktail of cell wall degrading enzymes as for ryegrass. Due to shortage of protein concentrate of red clover with enzyme processing, another batch was produced at the end of September 2016. Hence, the protein concentrate of red clover with enzyme processing consisted of a 26:74 ratio of batch 1 and 2. Initially, the soluble proteins were precipitated by heat in a heat exchanger (i.e., for ryegrass with or without enzyme), but due to technical problems, precipitation was changed to acid precipitation by spontaneous fermentation (i.e., for red clover with and without enzyme). In trial 2, concentrates produced from juice from ryegrass, red clover, and lucerne by acid precipitation in the growing season 2017 was compared to conventional SBM.

3.3.1 Ileal digestibility of N and amino acids of concentrates from 2016 (Trial 1).

Due to limitations in amounts of available biomass, and to avoid having too important feed refusals at higher inclusion levels, the diets were formulated to contain 30% concentrate from green biomass corresponding to 10% CP on a DM basis. The diets were well accepted by the pigs, and although gain was reduced due the feed restriction and the low protein level in the diet, the pigs appeared to be healthy.

Table 12 shows the apparent ileal digestibility (AID) for nitrogen (N) and amino acids of the concentrates produced from the two green protein sources with or without the addition of

enzymes during processing. Except from Met and Ala, where the digestibility was higher for protein from ryegrass with enzyme treatment than both sources without enzyme, there was no difference between the sources.

Table 12. Apparent ileal digestibility of green protein extracted from ryegrass or red clover without or with cell wall degrading enzymes during processing (Trial 1).

Protein source	Ryegrass		Red clover			
Precipitation	Heat		Fermentation			
Enzyme	-	+	-	+	SEM	*P*-value
N	38.0	39.1	30.2	39.3	6.21	0.1003
Arg	56.9	58.7	50.0	57.7	6.04	0.0882
His	60.0	63.5	57.1	59.7	2.33	0.1690
Ile	67.9	71.6	64.9	68.3	1.87	0.1528
Leu	70.9	74.7	68.0	70.2	1.85	0.1461
Lys	67.8	70.2	65.1	65.0	1.77	0.0510
Met	71.4^{ab}	76.7^{a}	69.1^{b}	70.7^{ab}	1.70	0.0428
Phe	67.4	71.6	64.5	66.3	2.01	0.1481
Thr	55.7	58.1	51.3	54.7	2.66	0.3326
Trp	60.2	64.7	57.4	59.5	2.51	0.2148
Val	65.6	68.6	62.3	65.9	1.97	0.2103
Ala	57.0^{ab}	61.8^{a}	51.6^{b}	60.8^{ab}	3.25	0.0166
Asp	62.6	64.1	59.0	60.3	2.26	0.2605
Cys	5.6	16.1	-3.4	-0.2	5.29	0.0820
Glu	61.3	63.5	56.7	59.1	2.40	0.1867
Gly	8.9	11.1	-4.2	14.5	12.86	0.0714
Ser	46.4	48.0	42.1	46.3	3.51	0.3924
Tyr	55.5	58.4	51.9	54.3	2.89	0.3912

Generally, the digestibility was higher for the indispensable amino acids than the dispensable amino acids. In general, the AID was quite low, which can partly be explained by the relatively high contribution of endogenous N and amino acids to ileal outflow at a low inclusion level (Annex 5.2, Table A2.2). Hence, the calculated standardized ileal digestibilities, were 4.6 to 26.3% units higher, and for Gly even almost 50 % units higher (Table 13).

The SID values were still 22 to 30% units lower for CP and 5 to 24% units lower for the essential amino acids than table values for solvent extracted SBM (NRC, 2012). It is worth noting that the SID of Cys was extremely low (21.8 to 36.9%). This points towards proteins being cross-linked by oxidation through the action of polyphenoloxidase during processing (see also D1.2).

Table 13. Standardized ileal digestibility of green protein extracted from ryegrass or red clover without or with cell wall degrading enzymes during processing (Trial 1).

Protein source	Ryegrass		Red clover			
Precipitation	Heat		Fermentation			
Enzyme	-	+	-	+	SEM	*P*-value
N	61.3	62.0	54.6	62.6	6.21	0.1724
Arg	77.8	79.6	72.2	80.1	6.04	0.1242
His	69.7	73.0	67.3	70.0	2.33	0.2498
Ile	74.1	77.8	71.4	74.5	1.87	0.1808
Leu	76.6	80.5	74.1	76.3	1.85	0.1707
Lys	74.2	76.5	71.9	71.3	1.77	0.0668
Met	76.1^{ab}	81.4^{a}	73.8^{b}	75.2^{ab}	1.70	0.0400
Phe	75.7	79.9	73.3	75.2	2.01	0.1885
Thr	69.8	72.2	66.1	69.5	2.66	0.4132
Trp	69.1	73.4	66.5	68.6	2.51	0.2475
Val	72.9	75.9	70.1	73.4	1.97	0.2619
Ala	70.8^{ab}	75.5^{a}	65.6^{b}	72.5^{ab}	3.25	0.0281
Asp	72.3	73.9	69.3	70.3	2.26	0.3430
Cys	29.3	36.9	21.8	26.1	5.29	0.2271
Glu	70.6	72.9	66.5	68.7	2.40	0.2178
Gly	58.1	60.2	46.6	63.9	12.86	0.1031
Ser	64.3	66.0	60.8	65.3	3.51	0.4581
Tyr	69.5	71.8	67.3	69.4	2.89	0.6854

3.3.2. Ileal digestibility of concentrates of N and amino acids from 2017 (Trial 2).

In trial 2, concentrates made by acid precipitation of protein from ryegrass, red clover, and lucerne harvested in 2017 were compared to conventional SBM. To compare results with that of the previous study, the CP content of the diets were set at the same level, so differences in inclusion level were only caused by differences in CP content of the concentrates (see D1.2).

Table 14. Apparent ileal digestibility of green protein extracted from ryegrass, red clover, or lucerne compared to a good quality SBM (Trial 2).

Protein source	Ryegrass	Red clover	Lucerne	SBM	SEM	*P*-value
N	36.4^{c}	44.8^{b}	38.8^{bc}	56.8^{a}	4.32	0.0008
Arg	55.8^{b}	59.5^{b}	58.1^{b}	71.6^{a}	4.45	0.0044
His	56.6^{b}	61.7^{b}	58.8^{b}	77.9^{a}	2.20	<0.0001
Ile	61.0^{b}	65.5^{b}	62.2^{b}	76.9^{a}	2.51	0.0004
Leu	65.5^{b}	69.8^{b}	66.8^{b}	77.7^{a}	2.33	0.0015
Lys	62.6^{b}	66.1^{b}	64.2^{b}	78.3^{a}	2.20	0.0003
Met	68.9^{b}	71.4^{b}	69.7^{b}	80.4^{a}	2.41	0.0019
Phe	64.2^{b}	68.0^{b}	64.5^{b}	77.0^{a}	1.98	0.0004
Thr	51.5^{b}	56.1^{b}	49.3^{b}	63.5^{a}	2.32	0.0008
Trp	56.0^{b}	60.4^{b}	54.9^{b}	71.9^{a}	2.72	0.0003
Val	60.9^{b}	65.2^{b}	62.4^{b}	74.4^{a}	2.37	0.0007
Ala	54.0	57.8^{b}	56.7	63.1	3.48	0.1295
Asp	58.2^{b}	63.3^{b}	59.8^{b}	72.5^{a}	2.41	0.0009
Cys	-9.7^{b}	0.3^{b}	-0.4^{b}	63.6^{a}	4.00	<0.0001
Glu	56.0^{b}	61.6^{b}	58.2^{b}	80.0^{a}	2.71	<0.0001
Gly	15.7	20.9	14.5	16.3	8.44	0.7631
Ser	45.3^{b}	48.5^{b}	42.8^{b}	68.8^{a}	2.61	<0.0001
Tyr	51.2	56.1	50.9	67.6	2.38	0.0008

The AID (Table 14) and SID values (Table 15) were quite similar to the values obtained for the 2016 harvest despite attempts to improve the quality. They were all significantly lower than the reference SBM.

Table 15. Standardized ileal digestibility of green protein extracted from ryegrass, red clover, or lucerne compared to a good quality SBM (Trial 2).

Protein source	Ryegrass	Red clover	Lucerne	SBM	SEM	*P*-value
N	57.6^{b}	64.2^{b}	58.6^{b}	78.3^{a}	4.32	0.0006
Arg	74.6^{b}	77.6^{b}	76.6^{b}	86.6^{a}	4.45	0.0227
His	65.3^{b}	69.6^{b}	66.4^{b}	85.3^{a}	2.20	<0.0001
Ile	67.8^{b}	71.9^{b}	68.5^{b}	83.6^{a}	2.51	0.0004
Leu	70.9^{b}	75.0^{b}	71.9^{b}	83.5^{a}	2.33	0.0011
Lys	68.6^{b}	71.8^{b}	70.0^{b}	84.2^{a}	2.20	0.0003
Met	72.4^{b}	75.7^{b}	73.4^{b}	86.2^{a}	2.41	0.0006
Phe	70.9^{b}	74.6^{b}	70.8^{b}	84.2^{a}	1.98	0.0003
Thr	64.6^{b}	68.6^{b}	61.9^{b}	78.3^{a}	2.32	0.0003
Trp	63.1^{b}	67.7^{b}	61.8^{b}	82.7^{a}	2.72	<0.0001
Val	67.6^{b}	71.5^{b}	68.7^{b}	82.1^{a}	2.37	0.0004
Ala	64.9^{b}	69.7^{b}	67.7^{b}	79.0^{a}	3.48	0.0158
Asp	67.1^{b}	71.4^{b}	68.2^{b}	79.6^{a}	2.41	0.0021
Cys	12.8^{b}	21.8^{b}	20.1^{b}	76.2^{a}	4.00	<0.0001
Glu	64.3^{b}	69.7^{b}	66.4^{b}	84.8^{a}	2.71	0.0002
Gly	58.8	64.6	57.0	70.2	8.44	0.2261
Ser	61.7^{b}	64.2^{b}	58.5^{b}	81.9^{a}	2.61	<0.0001
Tyr	63.6^{b}	66.7^{b}	62.2^{b}	80.8^{a}	2.38	0.0004

It should be noted that also the SID values of SBM were quite low compared to tabulated values of SBM. We speculate that the low inclusion level may lead to an underestimation of the digestibility.

3.3.3. Total tract digestibility of concentrates (Trials 1 and 2).

Based on faecal grap samples analysed for marker, ash, and N, the total tract digestibility of the experimental diets was calculated and, after correction for the digestibility of the N-free diet, also the DM, organic matter, and N digestibility of the concentrates (Tables 16 and 17).

Table 16. Apparent total tract digestibility of dry matter (DM) and organic matter (OM) and apparent and standardized total tract digestibility (STTD) of N in trial 1 with protein concentrates extracted from ryegrass and red clover without or with cell wall degrading enzymes.

Protein source	Ryegrass		Red clover		SEM	*P*-value
Enzyme	-	+	-	+		
DM, diet	71.8^{b}	69.7^{c}	71.9^{b}	77.6^{a}	0.43	<0.0001
OM, diet	80.5^{b}	80.7^{b}	79.1^{c}	83.0^{a}	0.42	0.0011
DM, concentrate	26.7^{b}	19.6^{c}	27.0^{b}	45.7^{a}	1.42	<0.0001
OM, concentrate	40.1^{b}	36.9^{b}	36.3^{b}	53.4^{a}	1.58	0.0002
N, concentrate	56.6^{bc}	60.2^{ab}	54.9^{c}	62.7^{a}	1.47	0.0214
N, STTD	65.7^{b}	69.2^{ab}	64.5^{b}	71.8^{a}	1.47	0.0276

In trial 1, red clover protein extracted with enzyme had higher DM, OM, and N digestibilities compared to products extracted without enzyme. No significant difference was found for ryegrass with or without enzyme. However, effects allocated to enzyme treatments should be interpreted with caution as the pilot plant was in its start-up phase and it was not possible to obtain biomass from the same cut for treatments with and without enzyme. However, there was no positive effect of enzyme treatment on either yield (D1.2) or digestibility.

In trial 2, all protein concentrates from green biomass had similar faecal DM, OM, and N digestibilities (Table 17) and all were far below the digestibility of SBM. As mentioned in D1.2,

this is mainly caused by the high ash and fibre contents, presumably resulting in an impaired N digestibility due to cross-linking during processing.

Table 17. Apparent total tract digestibility of dry matter (DM) and organic matter (OM) and apparent and standardized total tract digestibility (STTD) of N in trial 2 with protein concentrates extracted from ryegrass, red clover, and lucerne compared to SBM.

Protein source	Ryegrass	Red clover	Lucerne	SBM	SEM	*P*-value
DM, diet	76.4^{bc}	73.6^{c}	78.0^{b}	90.1^{a}	1.36	<0.0001
OM, diet	81.9^{b}	82.8^{b}	83.2^{b}	93.2^{a}	1.07	<0.0001
DM, concentrate	35.5^{b}	31.2^{b}	38.2^{b}	80.1^{a}	4.58	<0.0001
OM, concentrate	44.0^{bc}	54.3^{b}	36.4^{c}	84.2^{a}	3.87	<0.0001
N, concentrate	60.2^{b}	63.1^{b}	62.1^{b}	80.1^{a}	2.45	0.0005
N, STTD	68.9^{b}	71.0^{b}	70.3^{b}	88.9^{a}	2.45	0.0004

Based on the chemical composition of the protein concentrates (D1.2) and the obtained digestibility values, the content of metabolizable energy of the concentrates was calculated to be 9.19 MJ/kg DM.

3.4 Nutritive value of rapeseed meal (IRTA)

Feed formulation and chemical composition of the diets used in the study with growing pigs is shown in Annex 1, Tables A1.6 and A1.7.

3.4.1 Growth performance and feed efficiency

The effects of RSM and pelleting conditions on the durability index of feeds and on growth performance are presented in Table 18. On average, pigs gained 734 g/d, ingested 1.545 kg/d and had a feed conversion ratio (FCR) of 2.135. No significant interactions between the type of RSM and die size or the use of steam were observed. Utilisation of upgraded RSM significantly improved average daily gain (ADG) from 704 to 763 g/d (P<0.01), and the body weight at the end of the trial from 62.0 to 65.0 kg (P<0.01). The average daily feed intake was not affected by the type of RSM used, whilst the FCR was improved by the use of upgraded RSM (P<0.001). Die size also affected ADG, final body weight, and FCR (P<0.01). Pigs fed with pellets produced with the long-sized die grew faster and showed a better feed efficacy. Animals fed with pellets produced with steam had a lower feed intake (P<0.05) with no effect on ADG. Consequently their FCR was significantly reduced (P<0.001). No significant interaction between the type of RSM and die size or the use of steam was observed. However, a tendency of interactions between die size and steam were observed for ADG, final body weight, and FCR (P<0.10). The use of steam on die size 4x40mm increased ADG and body weight, while the contrary was observed when using steam on die size 4x60mm. Finally, the use of steam reduced FCR on both die sizes (P<0.04).

Table 18. Influence of rape seed meal (RSM) and pelleting conditions (die size and steam) on durability index of feeds and on performance of growing pigs.

	RSM			Die size			Steam			
	Ctrl	Bühler	*P*	4x40	4x60	*P*	No	Yes	*P*	RMSE
Durability index, %	40.0	69.4		51.7	61.4		25.8	87.3		
Body weight, kg	27.5	27.6	0.65	27.6	27.5	0.52	27.5	27.6	0.83	0.60
Feed intake, kg/d	1.55	1.54	0.98	1.52	1.57	0.14	1.58	1.51	0.03	0.15
ADG, g/d	704	763	0.003	707	760	0.006	730	737	0.71	79.0
FCR	2.20	2.03	0.001	2.16	2.07	0.002	2.18	2.05	0.001	0.11
Body weight (49 d)	62.0	65.0	0.002	62.2	64.8	0.01	63.3	63.7	0.69	4.02

The interaction Die x Steam was significant for FCR (*P*=0.04), indicated a tendency for ADG (*P*=0.07) and final body weight (*P*=0.08), and was not significant for feed intake. The interactions RSM x Die, RSM x Steam, and RSM x Die x Steam were not significant for all parameters. The block effect was significant for all parameters (*P*<0.05), except for feed efficiency.

3.4.2 Total tract apparent digestibility

The effects of RSM and pelleting conditions on total tract apparent digestibility of major nutrients and amino acids are presented in Tables 19 and 20.

Table 19. Influence of rape seed meal (RSM) and pelleting conditions (die size and steam) on total tract apparent digestibility (%) of major nutrient components for growing pigs.

	Rapeseed meal			Die size			Steam			
	Ctrl	Bühler	*P*	4x40	4x60	*P*	No	Yes	*P*	RMSE
DM	83.8	85.4	0.0001	84.6	84.6	0.98	84.9	84.2	0.02	1.25
OM	86.0	87.8	0.0001	86.9	86.9	0.90	87.3	86.5	0.007	1.154
Gross energy	84.6	86.4	0.0001	85.5	85.6	0.68	86.1	85.0	0.001	1.28
CP	78.4	80.5	0.0001	79.4	79.4	0.99	80.4	78.4	0.0002	2.12
Fat	84.7	86.3	0.012	85.3	85.6	0.63	86.8	84.2	0.001	2.47
NFE	90.5	91.8	0.0001	91.1	91.2	0.73	91.3	91.0	0.198	0.838
NDF	62.0	64.7	0.005	63.2	63.3	0.94	63.0	63.5	0.59	3.15
ADF	50.0	58.0	0.0001	54.3	53.7	0.65	54.7	53.3	0.29	5.72
Lignin	24.5	33.7	0.001	29.8	28.4	0.62	31.1	27.1	0.14	11.90
Hemicellulose	75.7	70.1	0.0001	72.5	73.4	0.37	71.7	74.1	0.02	3.63
Cellulose	61.7	66.2	0.0001	64.2	63.7	0.66	64.1	63.9	0.88	4.08
Ash	46.5	45.5	0.33	46.2	45.7	0.60	45.9	46.0	0.92	4.07
P	42.4	39.7	0.004	41.9	40.2	0.06	40.9	41.3	0.66	3.74
Ca	45.2	44.3	0.43	44.5	45.0	0.66	43.9	45.6	0.14	4.71

The interaction RSM x Die was significant for total tract apparent digestibility of DM, OM, CP, NDF, ADF, cellulose, ash, Ca, and P (*P*<0.05), and indicated a tendency for gross energy (*P*=0.06) and lignin (*P*=0.09). Interactions for RSM x Steam, Die x Steam were statistically significant for Ca and P (*P*<0.05) and not significant for the other nutrients. The interaction RSM x Die x Steam was significant for total tract apparent digestibility of DM, ADF, lignin, cellulose, ash, and P (*P*<0.05), and indicated a tendency for OM and CP (*P*<0.10). The block effect was significant for all nutrients (*P*<0.05).

Digestibility of dry matter (DM), organic matter (OM), gross energy (GE), crude protein (CP), fat, fibre fractions (i.e., NDF, ADF, lignin) and amino acids was significantly improved (*P*<0.01) when the upgraded RSM were included in the diets. However, the size of the die did not affect the digestibility of nutrients (*P*>0.10). The use of steam reduced the digestibility of DM, OM, GE, fat, CP and, with exception of lysine, the total tract apparent digestibility of most amino acids (*P*<0.01). An interaction between the type of RSM and die size was observed for the digestibility of DM, OM, CP, NDF, and ADF (*P*<0.05) and for gross energy (*P*=0.06). In general, the digestibility of upgraded RSM increased with the die size whereas the contrary was observed on conventional RSM. This interaction was not observed on the digestibility of amino acids. The interactions between the type of RSM and use of steam, or die size and steam were not significant for any of the parameters evaluated.

Table 20. Influence of RSM and pelleting conditions (die size and steam) on total tract apparent digestibility of amino acids (%) for growing pigs.

	Rapeseed meal			Die size			Steam			
	Ctrl	Bühler	*P*	4x40	4x60	*P*	No	Yes	*P*	RMSE
Lys	88.5	90.2	0.002	89.5	89.2	0.52	89.6	89.1	0.32	2.26
Arg	86.2	88.3	0.0001	87.2	87.4	0.64	87.9	86.6	0.0006	1.41
Thr	80.7	82.7	0.0001	81.8	81.5	0.55	82.4	80.9	0.002	1.89
Met	79.9	81.4	0.005	80.6	80.6	0.92	81.8	77.4	0.0001	2.12
Cys	86.1	88.4	0.0001	87.5	87.0	0.18	87.7	86.9	0.05	1.64
Val	79.5	81.8	0.0001	80.7	80.6	0.82	81.6	79.6	0.001	2.24
Leu	83.1	84.4	0.006	83.8	83.7	0.80	84.4	83.0	0.003	1.80
Ile	78.4	80.7	0.0003	79.6	79.5	0.77	80.5	78.6	0.001	2.45
His	88.7	90.0	0.0008	89.3	89.3	0.97	89.8	88.9	0.02	1.51
Ser	81.5	83.7	0.0001	82.8	82.4	0.33	83.3	81.9	0.005	2.05
Ala	75.8	78.0	0.001	76.9	76.8	0.90	78.1	75.7	0.0005	2.63
Tyr	79.4	81.8	0.0001	80.7	80.5	0.82	81.5	79.7	0.0007	2.13
Phe	82.5	84.2	0.0002	83.4	83.3	0.81	84.1	82.6	0.0009	1.81
Gly	80.7	83.4	0.0001	82.2	82.0	0.66	82.7	81.4	0.005	1.86
Pro	89.4	91.3	0.0001	90.4	90.3	0.52	90.6	90.1	0.03	0.13
Asp	84.2	86.1	0.0001	78.3	78.1	0.74	79.3	77.1	0.0008	2.43
Glu	90.0	91.1	0.0001	90.6	90.5	0.60	90.1	90.0	0.0002	1.07

The interactions RSM x Die, RSM x Steam, Die x Steam, and RSM x Die x Steam were significant for all parameters. The block effect was significant for all parameters ($P<0.05$).

4. Conclusions

The nutritive value of gently-processed European soybean meal in piglets

The European SBM processed by new technologies, particularly extrusion-pressing and the flacking-cooking-pressing technology without dehulling the bean have a high nutritive value considering the superior ileal digestibility of amino acids and excellent biological value. The apparent and standardized ileal digestibility of amino acids were consequently higher than the high quality commercial SBM used in the trial. The lower digestibility of amino acids and protein in SBM produced by the flacking-cooking-pressing technology without dehulling was likely due to the relative high trypsin inhibitor activity in that SBM.

The nutritive value of gently-processed European soybean meal in broilers

Regardless of trypsin inhibitor levels, dietary treatments did not differ in terms of small intestinal architecture or pancreas weight. The crude protein and dry matter digestibilities were not affected by the presence of hulls, which may be attributed to the fact that diets were wheat-based rather than corn-based, to reflect European feeding practices, and should be further investigated in wheat-based diets with xylanase inclusion. The use of hulled SBM led to higher proventriculus and gizzard development, which could be ascribed to their increased total NSP content, primarily in the form of insoluble NSP. Although effects on proventriculus and gizzard development were only assessed at 28 days of age, they may have been present earlier, thus accounting for the absence of adverse effects of soy hull inclusion on dry matter and crude protein digestibility at the end of the starter period. An increase in relative jejunum empty weight was observed as well as a reduced carcass yield, which reflects a higher gastrointestinal tract development in birds offered hulled SBM. As far as performance is concerned, EP-WH showed ~6% higher feed intake compared to the EP-DH treatment, as well as ~5.5% higher daily gain in comparison to both FCP-WH and EP-DH SBM over the starter period. However, these differences were absent by the end of the grower period and over the growing period as a whole. In conclusion, both processing methods of locally produced SBM resulted in products which equally promoted broiler performance over the grower and overall growing period. Although there were some differences in KOH solubility and trypsin inhibitor levels, these were poor predictors of dry matter and ileal crude protein digestibility. Hulled products resulted in similar performance, which could be ascribed to their effect on the development of the proximal gastrointestinal tract. Nonetheless, this was achieved at an additional cost. Slightly higher proportions of the hulled products were used to achieve similar crude protein content.

Nutritive value of protein extracted from green biomass

The results of the digestibility studies with ileal-cannulated pigs illustrate that protein concentrates from green biomass produced in a pilot scale (D1.2) could be fed to pigs without a negative impact on palatability. However, the quality of the concentrates needs further improvement in terms of concentration and digestibility to be an attractive alternative source of protein for pigs. Actions have already been taken to reduce the content of ash by changing the harvest and precipitation method, and results from the production in 2018 have shown that it is possible to increase the protein content of the concentrate (see D1.2). It has not been possible to determine SID of the protein from the 2018 harvest within the timeframe of the Feed-a-Gene project, but a feeding trial within a national project indicates good performance. Previous studies in rats at Aarhus University have shown a strong relationship between protein concentration and faecal N-digestibility. Particularly the low digestibility of cysteine, which is

indicative of cross-linking and complex formation initiated by the action of polyphenoloxidase during processing warrant actions to be taken to limit this process taking place during production. Previous studies have indicated that precipitation by fermentation is inferior to precipitation by heat regarding quantity and/or quality (Pirie, 1987). The implementation of facilities for steam precipitation is expected to be a step forward in the application of green protein as a source of protein for monogastric animals.

Conventional and upgraded EU rapeseed meal in growing pigs

The conventional EU RSM meal and upgraded RSM by mechanical processing by Bühler to increase its protein content were used at a 22.5% inclusion level as the only protein source in diets for growing pigs. Replacement of RSM was based on a similar amount and without adjustment of the amino acid profile through the use of synthetic amino acids. Consequently, upgraded RSM diets contained more protein and essential amino acids, which could explain the improvement of performance observed. Nonetheless, an increase in the nutrient digestibility was also observed, which may indicate a higher nutritional value of upgraded RSM for pigs. The size of die has an impact on performance and feed efficiency, the longer the die the better the results, with no impact on feed intake. Nevertheless, the die size did not have an effect on nutrient digestibility. The use of steam to pelletize feeds did not show any impact on growth performance. However, feed intake was reduced in steam-produced pellets either due to a better utilization or less feed wastage, thereby improving feed efficiency. However, nutrient digestibility was decreased with use of steam. This result was unexpected and deserves further attention. In conclusion, EU RSM and upgraded RSM by mechanical processing showed an adequate nutritional value to be extensively used in diets for growing pigs.

5. Annexes

Annex 1: Product and diet compositions
Annex 2: Basal endogenous losses and digestibility of N-free diets
Annex 3: Analytical procedures
Annex 4: Bibliographic references
Annex 5: Scientific outputs

Annex 1: Product and diet compositions

Table A1.1. Chemical composition of the ingredients produced in task 1.1 and used for weaned piglets (KU).

Proximate analysis (%)	Raw soybeans	Extrusion-pressing		Flaking-pressing-cooking	
		Dehulled	With hulls	Dehulled	With hulls
Dry matter	86.6	93.85	94.2	92.3	91.3
Crude fat	17.8	4.8	4.6	5.9	7.8
Crude protein	38.36	52.3	50.1	50.5	46.6
Soluble proteins (% of CP)	36.5	39.7	35.2	44.9	38.2
Protein solubility KOH (%)	95	75.9	70.2	88.8	82
Crude fibre	4.8	2.9	5.53	3.19	5.06
Trypsin inhibitors (TIU/mg)[1]	25	3.5	2.6	7.6	3.6
Soluble NSP[2] (% DM)	-	3.8	3.9	2.2	4.4
Insoluble NSP[2] (% DM)	-	12.6	17.2	15.3	16.9
Total NSP[2] (% DM)	-	16.4	21.1	17.5	21.3

[1] Good commercial SBM used as control in the study with piglets contained 2.9 TIU/mg.
[2] Non starch polysaccharides (NSP).

Table A1.2. Composition and analysed nutrient content of the experimental feeds (g/kg) used for weaned piglets (KU).

	CNTR	FCP-DH	EP-DH	FCP-WH	EP-WH	Casein	N-free[2]
Corn starch	482.7	542.6	555.2	508.9	533.7	624.1	805.9
Sugar	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Soybean meal[1]	378.0	355.0	343.0	386.6	358.0	0	0
Casein	0	0	0	0	0	214.3	0
Arbocel	0	0	0	0	0	50.0	50.0
Sunflower oil	45.0	9.0	8.0	12.0	15.0	15.0	42.0
MCP	15.5	15.1	15.5	14.2	15.0	19.5	25.5
Limestone	8.5	8.0	8.0	8.0	8.0	8.0	7.3
NaCl	4.3	4.3	4.3	4.3	4.3	4.3	4.3
DL-Methionine	1.0	1.0	1.0	1.0	1.0	1.0	
Vitamin and mineral premix 1.0%	10.0	10. 0	10. 0	10. 0	10. 0	10.0	10.0
Ti-dioxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Total	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**
NE[2]	10.9	10.9	10.9	10.9	10.9	10.9	10.9
Dry matter	912	916	918	916	919	927	
Crude protein	176	174	166	183.5	173	171.5	5.1
Ether extract	50.0	36.5	26.0	44.0	32.5	14.5	49.0
Crude fibre	15.5	12.0	9.0	21.5	18.5	29.5	32.0
Crude ash	54.5	50.5	49.5	52.5	51.5	47.0	42.5
Lys[3]	1.04	1.03	0.96	1.09	1.00	1.20	
Met[3]	0.29	0.29	0.30	0.27	0.30	0.53	
Thr[3]	0.70	0.67	0.63	0.71	0.67	0.67	
Trp[3]	0.20	0.20	0.20	0.20	0.20	0.20	
Arg[2]	1.22	1.39	1.22	1.42	1.31	0.94	
Ile[3]	0.76	0.74	0.71	0.77	0.73	0.73	
Val[3]	0.82	0.80	0.77	0.84	0.80	0.99	
Ca	7.3	6.6	6.3	6.7	6.7	7.4	7.4
P	5.8	5.4	5.4	5.6	5.5	6.1	5.7

[1] Soybean meal were good quality commercial SBM (CNTR), flaking-pressing-cooking processed dehulled (FCP-DH), extrusion-pressing processed dehulled (EP-DH), flaking-pressing-cooking processes whole bean (FCP-WH), extrusion-pressing processes whole soybean meal (EP-WH).
[2] Composition of N-free diet fed 5 days prior to slaughter (g/kg).
[3] Calculated values.

Table A1.3. Composition of the four diets produced through the combination of different processing and hulling methods, offered to broilers over the starter (0-14 d) and grower periods (15-28 d) (UNEW).

	Starter (d1-14)				Grower (d15-28)			
Processing Method [1]	**EP**		**FCP**		**EP**		**FCP**	
Hulling Method [1]	**DH**	**WH**	**DH**	**WH**	**DH**	**WH**	**DH**	**WH**
Ingredient (%)								
Maize	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Wheat	52.9	51.7	53.2	50.7	56.9	55.3	56.1	53.4
EP-DH	29.0				25.0			
EP-WH		30.0				26.5		
FCP-DH			29.0				26.0	
FCP-WH				32.0				29.0
Soybean oil	3.00	3.20	2.70	2.20	3.50	3.65	3.35	3.00
Limestone	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Monocalcium phosphate	1.25	1.25	1.50	1.50	1.25	1.25	1.25	1.25
Vitamin and mineral premix[2]	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Titanium dioxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysine HCL	0.40	0.41	0.44	0.43	0.30	0.30	0.30	0.30
DL-Methionine	0.35	0.35	0.41	0.41	0.35	0.35	0.35	0.35
L-Threonine	0.15	0.16	0.17	0.17	0.15	0.15	0.15	0.15
Sodium bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Coccidiostat	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Calculated composition (%)								
AME_n (MJ/kg)[4]	12.79	12.80	12.80	12.80	13.02	13.01	13.04	13.05
Calcium	0.97	0.98	0.98	0.98	0.92	0.92	0.92	0.93
Total phosphorous	0.72	0.72	0.72	0.73	0.64	0.65	0.65	0.66
Available phosphorous	0.48	0.48	0.48	0.49	0.42	0.42	0.42	0.42
Digestible Lys	1.31	1.31	1.32	1.32	1.13	1.13	1.13	1.14
Digestible Met + Cys	1.00	1.00	1.00	1.00	0.91	0.91	0.91	0.91
Digestible Thr	0.83	0.83	0.83	0.83	0.76	0.77	0.76	0.77
Digestible Trp	0.24	0.23	0.23	0.23	0.21	0.21	0.22	0.22
Analysed composition (%)								
Dry matter	90	90	89.4	89.4	89.1	89.3	89.1	88.9
Crude protein	23.1	23	22.9	22.8	21.6	21.1	21.8	21.6
Crude fibre	2.3	2.8	2.2	2.5	2.2	2.7	2.3	2.6
Ash	7.6	7.2	6.8	6.6	6.2	6.4	7	6.4
NDF[4]	7.8	9.3	7.8	8.8	7.7	8.8	7.8	8.5
ADF[4]	3.1	3.7	3.1	3.5	2.7	3.6	3.1	3.5
Crude fat	6.5	6.2	6.0	5.5	5.9	6.2	7.2	7.1
Total oil	7.7	7.4	6.9	6.8	7.0	7.5	8.1	8.0
Titanium dioxide	0.610	0.631	0.549	0.551	0.530	0.500	0.581	0.531

[1] Soybean meal were good quality commercial SBM (CNTR), flaking-pressing-cooking processed dehulled (FCP-DH), extrusion-pressing processed dehulled (EP-DH), flaking-pressing-cooking processes whole bean (FCP-WH), extrusion-pressing processes whole soybean meal (EP-WH).

[2] The premix supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.30 mg; cholecalciferol, 5000 IU/kg µg; folic acid, 2.2 mg; menadione, 3.2 mg; niacin, 60 mg; pyridoxine, 5.4 mg; trans-retinol, 13.000IU/kg; riboflavin, 8.6 mg; thiamine, 3.2 mg; dl-α-tocopheryl acetate 80IU/kg; choline chloride, 1700 mg;; Cu, 16 mg; Fe, 20 mg; I, 1.25 mg; Mn, 120 mg; Mo, 0.5 mg; Se, 300 µg; Zn, 110 mg.

[4] AMEn: apparent metabolisable energy, ADF: acid detergent fibre, NDF: neutral detergent fibre.

Table A1.4. Composition of the experimental diets used in trial 1 with ileum-cannulated pigs fed protein extracted from green biomass (AU).

	Ryegrass		Red clover		N-free
Enzyme during processing	**No**	**Yes**	**No**	**Yes**	**diet**
Ingredients, g/kg					
Concentrate from ryegrass without enzyme	300				
Concentrate from ryegrass with enzyme		300			
Concentrate from red clover without enzyme			300		
Concentrate from red clover with enzyme				300	
Native maize starch	465	465	465	465	765
Sucrose	100	100	100	100	100
Maize oil	50	50	50	50	50
Cellulose powder	30	30	30	30	30
Calcium carbonate	17	17	17	17	17
Mono calcium phosphate	25	25	25	25	25
Sodium chloride	3	3	3	3	3
Mineral/Vitamin premix	2	2	2	2	2
Titanium dioxide	3	3	3	3	3
Coloured maize grits	5	5	5	5	5
Analysed composition					
DM, g/kg	938	937	935	938	917
Organic matter, g/kg DM	884	859	896	913	954
Ash g/kg DM	116	141	104	87	46
Crude protein, g/kg DM (N x 6.25)	107	109	102	107	5
Amino acids, g/kg DM					
Arg	5.85	5.83	5.48	5.44	
His	2.36	2.43	2.25	2.24	
Ile	5.15	5.19	4.91	5.10	
Leu	8.67	8.68	8.26	8.20	
Lys	6.04	6.17	5.67	6.07	
Met	1.87	1.84	1.86	1.91	
Phe	5.64	5.68	5.38	5.33	
Thr	4.70	4.73	4.52	4.51	
Trp	2.04	2.08	2.01	1.98	
Val	6.35	6.42	6.02	6.20	
Ala	6.06	6.08	5.97	7.08	
Asp	9.74	9.67	9.17	9.40	
Cys	0.85	0.97	0.80	0.77	
Glu	10.74	10.51	10.21	10.40	
Gly	5.39	5.40	5.22	5.36	
Pro	4.55	4.54	4.34	4.30	
Ser	4.59	4.56	4.39	4.32	
Tyr	3.75	3.94	3.41	3.48	
Titanium dioxide g/kg DM	3.88	4.05	3.78	3.49	3.33

Table A1.5. Composition of the experimental diets used in trial 2 with ileum-cannulated pigs fed protein extracted from green biomass and soybean meal (AU).

	Ryegrass	Red clover	Lucerne	SBM	N-free diet
Ingredients, g/kg					
Concentrate from ryegrass	282.5	0	0	0	0
Concentrate from red clover	0	307.7	0	0	0
Concentrate from lucerne	0	0	266.2	0	0
SBM (Hamlet)	0	0	0	194.1	0
Native maize starch	488	462	504	576	770
Sucrose	100	100	100	100	100
Maize oil	50	50	50	50	50
Cellulose powder	30	30	30	30	30
Calcium carbonate	17	17	17	17	17
Mono calcium phosphate	25	25	25	25	25
Sodium chloride	3	3	3	3	3
Mineral/Vitamin premix	2	2	2	2	2
Titanium dioxide	3	3	3	3	3
Analysed composition					
Dry matter, g/kg	937	940	935	916	911
Organic matter, g/kg DM	898	858	909	933	949
Ash, g/kg DM	102	142	91	67	51
Crude protein, g/kg DM (N x 6.25)	106	116	113	104	4
Amino acids, g/kg DM					
Arg	5.83	6.05	5.93	7.34	
His	2.32	2.57	2.64	2.74	
Ile	5.13	5.42	5.58	5.22	
Leu	8.75	9.15	9.29	8.14	
Lys	6.11	6.44	6.25	6.25	
Met	2.31	1.90	2.20	1.41	
Phe	5.78	5.88	6.21	5.39	
Thr	4.66	4.88	4.84	4.12	
Trp	2.18	2.13	2.26	1.44	
Val	6.33	6.65	6.66	5.48	
Ala	6.75	6.21	6.71	4.66	
Asp	9.45	10.33	10.12	12.01	
Cys	0.82	0.86	0.90	1.46	
Glu	10.81	11.07	10.91	18.85	
Gly	5.61	5.53	5.69	4.48	
Pro	4.62	4.71	4.59	5.34	
Ser	4.48	4.67	4.67	5.59	
Tyr	3.43	3.98	3.76	3.19	
Titanium dioxide, g/kg DM	3.43	3.72	3.33	3.04	2.95

Table A1.6. Composition of the experimental diets of normal and upgraded RSM used in trial with growing pigs (IRTA).

Ingredients (%)	Normal RSM	Upgraded RSM
Maize	23.448	23.448
Barley	22.500	22.500
Wheat	22.000	22.000
RSM from TerresInovia	22.500	
RSM upgraded by Bühler		22.500
Soybean meal	2.000	2.000
Fat 3/5 Grefacsa	4.718	4.718
Monocalcium phosphate	0.628	0.628
Calcium carbonate	0.748	0.748
Salt	0.086	0.086
Sodium bicarbonate	0.482	0.482
L-Lysine-HCl	0.375	0.375
DL-Methionine	0.004	0.004
L-Threonine	0.081	0.081
L-Tryptophan	0.012	0.012
Noxyfeed	0.020	0.020
Minerals & vitamins*	0.400	0.400
Nutrients		
Metabolizable energy (Mcal/kg)	3.180	3.180
Net energy (Mcal/kg)	2.418	2.418
Crude protein (%)	15.14	16.47
Crude fibre (%)	5.08	3.89
Fat (%)	6.77	6.60
Ash (%)	4.92	5.15
Calcium (g/kg)	7.00	7.00
Total P (g/kg)	6.19	6.19
Digestible P (g/kg)	2.80	2.80
Total Lysine (g/kg)	9.60	10.27
SID Lysine (g/kg)	8.11	8.60
SID Threonine (g/kg)	5.19	5.59
SID Methionine (g/kg)	2.44	2.67
SID Met+Cys	5.32	5.81
SID Tryptophan (g/kg)	1.51	1.63
SID Valine (g/kg)	5.80	6.29
SID Isoleucine (g/kg)	4.63	5.03

* Provides per kg feed: vitamin A (E-672) 5500 UI; vitamin D_3 (E-671) 1100 UI; vitamin E (alfa-tocopherol) 7 mg; vitamin B_1 0.5 mg; vitamin B_2 1.4 mg; vitamin B_6 1 mg; vitamin B_{12} 8 µg; vitamin K_3 0.5 mg; calcium panthotenate 5.6 mg; nicotinic acid 8 mg; choline 120 mg; Fe (E-1) (from $FeSO_4 \cdot 7H_2O$) 80 mg; I (E-2) (from $Ca(I_2O_3)_2$) 0.5 mg; Co (E-3) (from $2CoCO_3 \cdot 3Co(OH)_2 \cdot H_2O$) 0.4 mg; Cu (E-4) (from $CuSO_4 \cdot 5H_2O$) 5 mg; Cu (E-4) (from amino acids quelate) 5 mg; Mn (E-5) (from MnO) 40 mg; Zn (E-6) (from ZnO) 100 mg; Se (E-8) (from Na_2SeO_3) 0.25 mg.

Table A1.7. Chemical composition of the experimental diets used in trial with normal and upgraded RSM in growing pigs (IRTA).

Treatment	T1	T2	T3	T4	T5	T6	T7	T8
Rapeseed meal	Ctrl	Bühler	Ctrl	Bühler	Ctrl	Bühler	Ctrl	Bühler
Die size, mm	4x40	4x40	4x40	4x40	4x60	4x60	4x60	4x60
Steam	No	No	Yes	Yes	No	No	Yes	Yes
Met. energy(1), Kcal/kg	3.55	3.51	3.57	3.57	3.51	3.52	3.56	3.66
Net energy(1), Kcal/kg	2.6	2.58	2.62	2.62	2.57	2.59	2.61	2.69
OM, %	94.68	94.21	94.46	94.14	94.33	94.25	94.33	94.4
Protein, %	16.49	18.26	16.69	17.85	16.96	18.1	16.8	17.23
Fat, %	7.31	6.98	7.03	6.85	7.24	6.96	7.21	9.14
NFE, %	66.67	65.46	66.04	66.18	66.23	66.11	65.63	64.52
Starch, %	45.59	44.68	44.15	44.66	43.82	43.66	43.51	43.42
Fibre, %	4.21	3.52	4.7	3.26	3.91	3.09	4.69	3.5
NDF, %	15.67	15.57	17.1	16.06	16.82	13.89	18.79	15.02
ADF, %	8.82	6.77	8.94	7.72	9.3	6.87	9.45	7
Lignin, %	2.74	1.36	2.94	1.68	2.68	1.72	3.14	2.19
Hmcell, %	6.86	8.8	8.16	8.34	7.52	7.02	9.34	8.02
Cell, %	6.07	5.4	6	6.04	6.62	5.15	6.31	4.81
Ash, %	5.32	5.79	5.54	5.86	5.67	5.75	5.67	5.6
Ca, %	0.73	0.75	0.75	0.75	0.79	0.73	0.74	0.69
P, %	0.66	0.78	0.68	0.77	0.69	0.76	0.71	0.69
Zn, ppm	70.6	76.9	72.2	86	89.6	89.9	78.6	74.2
Cu, ppm	15.4	14.4	12.2	14	13.2	12.4	14.8	13.7
Lys, %	1.07	1.41	1.21	1.16	1.12	1.08	1.04	0.99
Arg, %	0.81	0.92	0.85	0.97	0.84	0.9	0.85	0.89
Thr, %	0.66	0.71	0.67	0.72	0.68	0.69	0.67	0.67
Met, %	0.32	0.35	0.34	0.35	0.34	0.35	0.32	0.34
Cys, %	0.31	0.35	0.35	0.38	0.33	0.35	0.35	0.33
Val, %	0.6	0.67	0.67	0.75	0.63	0.67	0.65	0.65
Leu, %	1.09	1.22	1.13	1.26	1.12	1.21	1.13	1.17
Ile, %	0.48	0.53	0.53	0.61	0.5	0.54	0.51	0.52
His, %	0.37	0.4	0.38	0.43	0.37	0.41	0.36	0.37
Ser, %	0.64	0.72	0.65	0.69	0.67	0.7	0.67	0.69
Ala, %	0.65	0.74	0.67	0.74	0.68	0.71	0.67	0.7
Tyr, %	0.46	0.5	0.48	0.51	0.47	0.5	0.48	0.49
Phe, %	0.62	0.69	0.65	0.72	0.65	0.69	0.65	0.67
Gly, %	0.67	0.77	0.69	0.79	0.69	0.74	0.69	0.72
Pro, %	1.13	1.18	1.13	1.25	1.22	1.14	1.22	1.35
Asp, %	1.03	1.16	1.06	1.18	1.07	1.13	1.07	1.1
Glu, %	2.91	3.28	3.01	3.31	3.03	3.23	3.02	3.14

Annex 2: Basal endogenous losses and digestibility of N-free diets

Table A2.1. Basal ileal endogenous amino acid flow measured with pigs received N-free diet in the study with weaned piglets (KU).

Amino acid	g/kg DM intake	Amino acid	g/kg DM intake
Asp	1.883	Ile	1.042
Thr	1.265	Leu	1.416
Ser	1.317	Tyr	0.400
Glu	2.283	Phe	0.764
Pro	2.277	His	0.671
Gly	2.691	Lys	1.003
Ala	1.178	Arg	0.924
Cys	0.660	Trp	0.208
Val	1.032	Total amino acids	21.564
Met	0.237	Nitrogen	2.431

Table A2.2. Basal ileal endogenous N and amino acid flow and faecal N flow (g/kg DM intake) measured with pigs received N-free diets in the studies with ileum-cannulated growing pigs (AU).

	Trial 1 (2016)		Trial 2 (2017)	
	Mean	SD	Mean	SD
N	3.97	1.07	3.60	0.41
CP (N x 6.25)	24.84	6.69	22.52	2.54
Arg	1.22	0.48	1.10	0.22
His	0.23	0.05	0.20	0.03
Ile	0.32	0.07	0.35	0.07
Leu	0.50	0.11	0.47	0.10
Lys	0.38	0.09	0.37	0.07
Met	0.09	0.02	0.08	0.02
Phe	0.47	0.09	0.39	0.07
Thr	0.67	0.15	0.61	0.10
Trp	0.18	0.04	0.16	0.03
Val	0.47	0.09	0.42	0.09
Ala	0.83	0.26	0.74	0.12
Asp	0.94	0.20	0.84	0.14
Cys	0.20	0.04	0.18	0.03
Glu	1.00	0.19	0.90	0.14
Gly	2.65	0.70	2.42	0.28
Ser	0.82	0.16	0.73	0.09
Tyr	0.53	0.10	0.42	0.07
N (faeces)	1.56	0.36	1.47	0.30

Table A2.3. Apparent ileal and total tract (faecal) digestibility of DM and OM in N-free diet in the studies with ileum-cannulated growing pigs (AU).

	Trial 1 (2016)		Trial 2 (2017)	
	Mean	SD	Mean	SD
Dry matter (ileum)	86.17	2.03	86.17	2.03
Organic matter (ileum)	90.45	1.24	90.45	1.24
Dry matter (faeces)	91.20	1.94	92.49	0.98
Organic matter (faeces)	94.76	1.50	95.32	1.13

Annex 3: Analytical procedures

5.3.1 Piglet trial (KU)

Registrations and sampling: The experiment consisted of a 28 day pre-feeding and a 5 day collection period. Each animal received the same diet during the trail except for one group, which was assigned to N-free treatment in the last 5 days. The daily feed intake was calculated based on the difference of feed volume offered and not consumed. The daily feed intake was determined with gram precision. The animals were weighted with 0.05 kg precision at the beginning of the trial and at weekly intervals. The general health of the animals and diarrhoea score were monitored and recorded daily.

During the retention study, faeces were collected quantitatively twice daily (following the morning and afternoon feeding), weighed with gram precision and stored at -18°C until further processing. At the end of the trial, the faeces were homogenized and carefully dried (65°C), ground, and prepared for laboratory analysis. Urine was collected continuously into a sealed container connected to the metabolic crate, and its volume was measured following the morning feeding. After homogenizing the daily collected urine, 15% was filtered through a N-free filter and stored at -18°C until further processing. At the end of the collection period, the urine samples were carefully thawed, homogenized again and filtered, and prepared for laboratory analysis. During collection, urine was preserved with 50% concentrated sulfuric acid. Live weight of the animals was recorded at the start and the end of the collection period. Post-mortem ileal digestibility of protein and amino acid was determined after the retention study. The euthanasia was carried out in anaesthesia by blottering via vena cava jugularis. Pigs were injected with 2.5 mg Zoletil (Virbac), 3 mg CP-Xylazin (2%, CP-Pharma Handelsges) and 6 mg Stresnil (Janssen-Cilag) per kg body weight intramuscularly 30 minutes before slaughter. During the course of bleeding, the bodies were held on a desk with the head hanging down. The abdomen was dissected and the intestine and pancreas were removed. The jejunal gut content from the mid of 10-20 cm and the ileal gut content from 10-15 cm anterior to the ileo-caeco-colonic junction was flushed with distilled water. Ileal digesta samples were used for determination of ileal digestibility, while jejunal digesta and pancreas was examined for total protease enzymes' activity.

Laboratory analysis: Ileal digesta and feacal samples were dried at 65ºC for further laboratory analysis. The nutrient contents of the diets and digesta samples (e.g., dry matter, crude protein, crude fat, crude fibre, crude ash, Ca, P, and amino acids and TiO_2) were determined in accordance with the AOAC (1989) recommendations. Dry matter and crude protein content of faeces and urine was also determined.

The total proteolytic activity was determined at the Institute of Animal Science, Prague (Czech Republic). Pancreas and jejunal digesta samples were stored under CO_2 at -80°C until analysis. Dry mater of samples was determined by heating at 105°C for 24 h. Pancreas and digesta samples were diluted with phosphate buffer (pH 7.5) according to Marounek *et al*. (1995). Azocasein solution was prepared in a concentration of 4 mg/ml in 0.1 M potassium phosphate buffer pH 7.5. For each sample, the following were prepared: four plastic 10 ml polypropylene tubes containing azocasein solution [1], azocasein solution and 25% trichloracetic acid [2], 0.1 M potassium phosphate buffer pH 7.5 [3] and 0.1 M potassium phosphate buffer pH 7.5 + 1 ml 25% trichloracetic acid [4]. All materials and reagents (azocasein, K_2HPO_4, KH_2PO_4, trichloracetic acid and NaOH) were purchased from Sigma Aldrich and Lach-Ner (Czech Republic). At t = 0, samples were added to all tubes. After 1 h incubation at 39°C, 25% trichloracetic acid was added to [1] and [3]. Tubes were transferred

to ice water and then centrifuged at 4,000 g. The supernatant was removed into another tube with 0.5 M NaOH. Absorbance was measured at 440 nm and result on absorbance was calculated from four values: A1-A2-A3+A4. Calibration was made by a 0.2 mg/ml solution of azocasein. Proteolytic activity was expressed as mg azocasein hydrolysed/h per dry matter of digesta or pancreas sample (Hoffmann *et al*., 2010).

Calculations: In the retention studies, absolute and relative N-retention (expressed as g/d and as a percentage of intake and digested N) were determined.

The basal ileal endogenous amino acid flow was calculated as follows:

$IAA_{endo} = AA_{digesta} \times (M_{diet} / M_{digesta})$

Apparent and standardized ileal digestibility of each amino acid was determined as follows:

$AID\ (\%) = (1 - AA_{digesta} / M_{digesta} \times M_{diet} / AA_{diet}) \times 100$

$SID\ (\%) = AID + (\text{basal } IAA_{endo} / AA_{diet}) \times 10$

Statistical analysis: The experimental data were analysed by two-way ANOVA (SAS, 2004). In case of a significant treatment effect, the differences among treatments were checked by a Tukey test (SAS, 2004)

5.3.2 Broiler trial (UNEW)

Animals and sampling: All procedures were approved by the Animal Welfare and Ethical Review Body (AWERB) of Newcastle University. Two-hundred and eighty-eight male Ross 308 day old chicks were obtained from a commercial hatchery and were housed in a windowless, thermostatically controlled building in 24 pens of 1.7 m^2. Each pen was equipped with a tube feeder and a bell-drinker, and wood shavings were used as litter to a depth of 5 cm. Birds had *ad libitum* access to feed and water throughout the trial. Temperature at pen level was monitored daily and maintained to meet Aviagen recommendations (Aviagen, 2014), starting at 34°C at chick placement and gradually reduced to 20°C by 25 days of age. Light intensity at pen level ranged from 80 to 100 lux, whilst a lighting schedule of 23 h light and 1 h darkness was applied for the first 7 days of age and switched to 18h light and 6 h darkness for the remainder of the trial.

Feed analyses: All diets were analysed at a UKAS accredited commercial laboratory to the internationally recognised standard for competence ISO/IEC 17025:2005 (Table 3; Sciantec Analytical Services, Cawood, UK). Briefly, DM was measured by determining the loss in weight of the feed sample after heating at 103 - 105°C for 3 h. Crude fat was determined by extraction with petroleum ether and total fat by acid hydrolysis followed by petroleum ether extraction. CP was determined with the Dumas method on a LECO FP-528 Nitrogen Analyser (Leco Instruments UK, Cheshire), Crude fibre and acid detergent fibre (ADF) using an Ankom 220 Analyser (Ankom, Technology, Fairport, NY, USA). Ash was determined gravimetrically after incinerating the sample at 510°C for 4 h. TiO_2 analysis in feed was measured according to the method described by Vogel (1961). Neutral detergent fibre (NDF) was estimated by enzymatic gravimetry.

Viscosity and chemical analyses of digesta: Frozen ileal digesta samples originating from the upper 1/3 of the ileum were thawed in a water bath at 40°C. After defrosting, the samples were centrifuged (with 12,000 × g for 10 min), 0.5 ml of supernatant was then loaded to a Brookfield Digital Viscometer (Model LDVI+CP, Brookfield, Engineering Laboratories, Stoughton, MA) with a plate-plate geometry and a gap of 2 mm appropriate for small volumes to carry out digesta viscosity measurements. The temperature of the sample was maintained at 40°C during the measurements. The results are presented as solution viscosity in centipoise (CPs). The ileal digesta samples collected from the lower 2/3 of the ileum were freeze-dried overnight

and along with samples of the diets, and were ground to pass through a 0.5 -mm sieve and stored in airtight plastic containers at −20°C pending chemical analyses. All samples were analysed for CP with the Dumas combustion method using a Leco CNS 2000 analyser (Leco Instruments UK, Cheshire), and titanium according to the methodology of Short *et al*., (1996).
Histology: Excised, formalin-fixed intestinal sections from the duodenum and jejunum were dehydrated through a series of graded ethanol baths followed by xylene in a Shandon™ Excelsior™ ES Tissue Processor (Thermo Fisher Scientific Inc., Waltham, Massachusetts), before being embedded in paraffin wax, sectioned at 4 µm and stained with haematoxylin/eosin. Histological sections were examined under a Zeiss Primostar light microscope and images were captured using ZEN imagine software (Zeiss Germany, Oberkochen, Germany). Images were viewed to measure morphometric features of the intestinal structure at 10x magnification. From sections, the villus height (VH) and the crypt depth (CD) were determined using ImageJ (NIH) software (Schneider, *et al*., 2012). The VH was estimated by measuring the vertical distance from the villus tip to the villus-crypt junction for 10 villi/section, and the CD by the vertical distance from the villus-crypt junction to the lower limit of the crypt, for 10 corresponding crypts/section. The ratio between VH and CD was calculated (VCR).
Calculations and statistical analysis: All statistical analyses were conducted in SAS 9.4 (SAS Institute, Cary, NC). For all statistical assessments, pen was considered the experimental unit and data were analysed with PROC GLM with processing method and hulling as fixed factors and their interaction. Weight of carcass parts, organs and gastrointestinal tract segments as well as length of small gastrointestinal tract segments, obtained at 28 d of age were expressed as a ratio to the sampled empty carcass weight of the slaughtered bird. When significant differences were detected, treatment means were separated and compared by the Tukey's multiple comparison test. Significance was determined at $P<0.05$. All values are expressed as model-predicted least square means with the SEM.

5.3.3. Trials with ileum-cannulated pigs (AU)
Animals and sampling: Animal experiments were conducted according to licenses obtained from the Danish Animal Experiments Inspectorate, Ministry of Food, Agriculture and Fisheries, Danish Veterinary and Food Administration and in compliance with the Danish law concerning animal experiments and care of experimental animals (Act no. 474 of 15/05/2014). For each experiment, five barrows (Duroc x Danish Landrace-Yorkshire) were surgically fitted with a permanent T-cannula at the distal ileum as described by Jørgensen *et al*. (1992). At surgery, the pigs weighed 31.5±1.8 kg in Trial 1 (2016) and 28.6+1.0 kg in Trial 2 (2017). After surgery, the pigs were housed in individual pens with partly slatted floors to recover for 10 days. Two days before the study, pigs were gradually adapted to the experimental diets. The pigs were assigned to a 5x5 Latin Square Design with 5 diets and 5 periods, each period lasting 7 days. The pigs were weighed once a week at the beginning of each experimental period. Based on estimated average weight on day 4 of each period, pigs were fed 80 g/kg$^{0.75}$/d. Meals were provided twice daily at 0730 and 1530 h. Water was available *ad libitum*. On days 5 and 7, ileal digesta were collected for 8 consecutive hours after the morning meal. Plastic bags were attached to the cannula and emptied whenever full or at least every 30 minutes and frozen immediately after collection. Two or three drops of 0.2 % Na-azide were added to each collection bag to prevent microbial activity in the bags. Ileal digesta from days 5 and 7 were pooled and stored in a freezer at -20°C until analysis. On both days of collection of ileal digesta, a fresh faecal spot sample was taken and pooled. After the end of the experiment, the pigs

were euthanized by an intramuscular injection 0.1 ml/kg of zoletil mixture containing 50 mg/mL tiletamine/zolazepam (Vibrac SA, Carros, France), 2.5 mg/mL butorphanol (Torbugesic® Vet, Scan Vet Animal Health A/S, Fredensborg, Denmark), 12.5 mg/mL ketamine (Ketaminol Vet, Intervet Danmark, Skovlunde, Denmark), and 12.5 mg/mL xylazine (Rompun, Bayer Health Care AG, Animal Health Division, Leverkusen, Germany) 15 minutes before an intracardial injection of a lethal dose of Pentobarbital Sodium (Vepidan Aps, Løgstør, Denmark) and exsanguination.

Chemical analyses: Ileal digesta and faeces were freeze dried and ground (<0.5 mm) before analysis. Dry matter was determined by oven-drying the samples to a constant weight at 103°C for 20 hours as a modification of the instructions in Commission Regulation (EC) No 152/2009 (European Commission, 2009) and ash by combustion at 525°C, modified from AOAC method 942.05 (AOAC 2007). Total N in diets and ileal contents and faeces was analysed by the Dumas method (Hansen, 1989) and crude protein (CP) calculated as total Nx6.25. Amino acids in ingredients, diets, and ileal content were analysed according to instructions from the European Commission (European Commission, 1998; European Commission 2000). The titanium dioxide concentration in diets, ileal contents and faeces were determined according to the method of Myers *et al*.(2004).

Calculations and statistical analysis: The basal endogenous loss of amino acids was calculated based on the content of amino acids in ileal digesta and the concentration of marker in the diet and ileal digesta in pigs receiving an N-free diet, and expressed as g per kg dry matter intake (Stein *et al*., 2007). The AID and SID were calculated as described in 5.3.1. A similar approach was used for total tract digestibility.

Statistical analyses: Statistical tests were performed using the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

where Y_{ij} is the response, μ is the overall mean, α_i is the effect of diet, β_j is the effect of week, and ε_{ij} is the random residual error $N(0, \sigma^2)$.The statistical analysis was performed using the MIXED procedure in SAS version 9.3 (SAS Institute, Cary, NC). The number of observations per treatment was n=5. Differences were considered significant when $P<0.05$.

5.3.4. Trials with growing pigs (IRTA)

Animal trial: IRTA has an Integrated Quality Policy and the Institute is ISO 9001:2015 certified for their R+D+T projects, contracts, and services. The experimental procedures used in this study were in accordance with ISO 9001:2015 quality criteria, and under the global SOPs PG-75 (Conducting Research studies) and IT-0602-F-011 (Quality Control of feed mill). One-hundred forty-four crossed Pietrain x (Large White x Landrace) pigs weighing 27.7 kg at beginning were used. One male and one female were assigned to each pen for a total of 9 pens per treatment. They were grouped in 9 blocks of live weight at the beginning of the trial and randomly assigned to experimental treatments. Trial lasts 49 days, and pigs were fed *ad libitum* during the trial. Pigs were weighed, and feed intake evaluated at 3, 5, and 7 weeks. Experimental treatments corresponded to 2 cereal based diets with 22.5% conventional RSM or RSM upgraded by Bühler in combination with pelleting conditions at the feed mill. These tested conditions were the size of the die (4x40 or 4x60 mm long) and the utilization of steam (with or without). Diets were formulated according to growing pig requirements (NRC, 2012) and no adjustment of synthetic amino acids was done. Feeds were produced at IRTA's feed mill plant, according to a multi-step manufacturing schedule. Major feed ingredients were ground through a 25 HP hammer mill (Rosal VR-30) until the particles passed through a 3 mm sieve and then sent to a 500-kg horizontal mixer (Rosal) and mixed during 5 min. For each

batch of feed, a 10-kg sample SBM containing 48% protein was taken and premixed with the corresponding experimental product and additives (i.e., mineral and vitamin premix, amino acids, and macro-minerals). Titanium dioxide as indigestible marker was included on top of diets. That mixture was then incorporated to the horizontal mixer (500 kg mixer) and homogenised, before the addition of fat/oil. Mash feed was then sent to the pelleting system: conditioner, pelleting press, and vertical cooler (Mabrik PV-30). Steam flux and feed entry were regulated automatically by the system (Mipps 210, Mangra SA). Temperatures observed during the pelleting /conditioning process were registered. Pelleted feed was then sent to the packaging system. At the feed mill, the feedbags were properly identified with date of mixing, treatment code, bag number, and net weight. Feeds were stored at the trial site under dry conditions at room temperature. Representative samples of each batch of feed were taken in the feed manufacturing plant following the preparation of the mixtures. Per production batch, a minimum of five sub samples of approximately 500 g each were taken at regular intervals throughout bagging, thoroughly homogenised, and final feed samples bagged in ziplock bags and labelled following protocol instructions. One set of feed samples was analysed for the main nutrient categories (i.e., DM, ash, gross energy, CP, fat, CF, van Soest fibre fractions, Ca, P, Zn, Cu, Ti, and amino acid contents). During the last week of trial, fresh faeces were collected early in morning from pigs in pens and frozen, before being processed.

Chemical analyses: Faeces were oven-dried at 65°C for 72 h and ground (<0.5 mm) before analysis. Feeds and faeces were analysed for DM, GE, N, starch, ash, fat, CF, mineral, and amino acid contents. All methods and procedures were described and accepted by AOAC (2000). Dry matter content was determined on a stove at 103 °C until reaching a constant weight, ash by incineration for 4 h in an oven at 550°C, fat by means of a Buchi Extraction System B-811 (Buchi Labortechnik AG, Flewil, Switzerland), N by the Dumas procedure by means of Nitrogen/protein FP-528 analyzer (LECO corp., St Joseph, Mo, USA), and crude fiber using a ANKOM 200/220 Fiber Analyzer. Amino acids were determined by HPLC of protein hydrolysates from acid hydrolysis by precolumn derivatisation with ophthaldehyde for primary amino acids, and 9-fluorenylmethyl chloroformate) for secondary amino acids. For methionine and cysteine determination, a peroxidation was done before hydrolysis. Gross energy was determined in an adiabatic calorimeter (IKA C-4000, IKA® - Werke GmbH, Staufen, Germany) and metabolizable energy and net energy contents were calculated as 0.79 and 0.58 of the gross energy value determined by calorimetry according to INRA (2002). Calcium, P, Cu, Zn, and Ti contents from ash samples previously obtained were analysed after acid digestion by inductively-coupled plasma mass spectrometry (ICP-MS).

Calculations and statistical analysis: the total tract apparent digestibility (TTAD) of major nutrient and individual amino acids was calculated according to the following equation:

$$TTAD = (1-(X_{faeces}/ X_{diet}) \times (M_{diet}/M_{faeces})) \times 100$$

where X_{faeces} and X_{diet} are the nutrient concentrations in faeces and the diet, respectively, and M_{diet} and M_{faeces} are the marker (Ti) concentrations in diet and faeces, respectively.

The trial was set up as a 2 x 2 x 2 factorial design 8 dietary treatments, 9 blocks of live weight or 9 replicates per treatment. All parameters were analysed using the GLM procedure of SAS. A factorial model with RSM source (Control vs upgraded), die size (4x40 vs 4x60mm), use of steam to pelletize feeds (with or without), and interaction between all factors as main effects. Means were compared using Tukey test.

Annex 4: Bibliographic references

AOAC (1989). Official methods of analysis. 13th Ed. Association of Official Analytical Chemists. Washington, DC, USA

AOAC (2000). Official methods of analysis. 17th Ed. Association of Official Analytical Chemists. Washington DC, USA.

AOAC (2007). Official methods of analysis. 18th Ed. Association of Official Analytical Chemists. Gaithersburg, MD, USA.

Aviagen (2014). Ross Broiler Management Handbook. Aviagen Ltd., Scotland, UK.

Boerema A., Peeters A., Swolfs S., Vandevenne F., Jacobs S., Staes J., Meire P. (2016). Soybean trade: Balancing environmental and socio-economic impacts of an intercontinental market. PLoS ONE 11: e0155222. doi:10.1371/journal.pone.0155222.

De Visser C.L.M., Schreuder R., Stoddard F. (2014). The EU's dependency on soya bean import for the animal feed industry and potential for EU produced alternatives. OCL – Oilseeds and Fats, Crops and Lipids 21: D407. doi:10.1051/ocl/2014021.

European Commision (2009). Commision regulation (EC) No 152/2009 of 27 January, 2009 laying down the methods of sampling and analysis for the official control of feed.

European Commision (1998). Commission Directive 98/64/EC of 3 September, 1998 establishing Community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in feedingstuffs and amending Directive 71/393/EEC. Official Journal of the European Communities Eur-Lex.

European Commision (2000). Commission Directive 2000/45/EC of 6 July 2000, establishing Community methods of analysis for the determination of vitamin A, vitamin E and tryptophan in feedingstuffs. Official Journal of the European Communities Eur-Lex.

Hansen, B. (1989). Determination of nitrogen as elementary N, an alternative to Kjeldahl. Acta Agriculturae Scandinavica 39: 113-118. doi:10.1080/00015128909438504.

Hansen J.Ø., Skrede A., Mydland L.T., Øverland M. (2017). Fractionation of rapeseed meal by milling, sieving and air classification — Effect on crude protein, amino acids and fiber content and digestibility. Animal Feed Science and Technology 230: 143–53. doi:10.1016/j.anifeedsci.2017.05.007.

Hoffmann E.M., Selje-Assmann N., Becker K., Wallace R.J., Broderick G.A. (2010). Screening for anti-proteolytic compounds. In: In vitro screening of plant resources for extra-nutritional attributes in ruminants: nuclear and relates methodologies, vol. 1, pp. 55-86 (P.E. Vercoe, H.P.S.Makker, A.C.Schlink, editors). Springer Science and Business Media B.V., Dordrecht.

INRA (2002). Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage. (D.Sauvant, J.-M. Perez, G. Tran, editors). AFZ-INRA éditions, Paris, 301 pp.

Jørgensen H., Jakobsen K., Eggum B.O. (1992). The influence of different protein, fat and fatty acids measured at the terminal ileum and in faeces of growing pigs. Acta Agriculturae Scandinavica 42: 177-184. doi:10.1080/09064709209410125.

Karr-Lilienthal L.K., Bauer L.L., Utterback P.L., Zinn K.E., Frazier R.L., Parsons C.M., Fahey G.C. (2006). Chemical composition and nutritional quality of soybean meals prepared by extruder/expeller processing for use in poultry diets. Journal of Agricultural and Food Chemistry 54: 8108-8114. doi:10.1021/jf048385i.

Le Cadre P., Pressenda F., Labalette F., Peyronnet C. (2015). Valorisation de tourteaux de soja issus d'une production locale non OGM chez les fabricants d'aliments. OCL – Oilseeds and Fats, Crops and Lipids 22: D507. doi:10.1051/ocl/2015034.

Leinonen I., Williams A.G., Waller A., Kyriazakis I. (2013). Comparing the environmental impacts of alternative protein crops in poultry diets: the consequences of uncertainty. Agricultural Systems 121: 33-42. doi:10.1002/jsfa.7202.

Marounek M., Vovk S.J., Skřivanová V. (1995). Distribution of activity of hydrolytic enzymes in the digestive tract of rabbits. British Journal of Nutrition 73: 463-469. doi:10.1079/BJN19950048.

Martin N. (2015). Domestic soybean to compensate the European protein deficit: illusion or real market opportunity? OCL – Oilseeds and Fats, Crops and Lipids 22: D502. doi:10.1051/ocl/2015032.

Mejicanos G.A., Kim J.W., Nyachoti C.M. (2018). Tail-end dehulling of canola meal improves apparent and standardized total tract digestibility of phosphorus when fed to growing pigs. Journal of Animal Science 96: 1430–1440. doi:10.1093/jas/sky040.

Myers W.D., Ludden P.A., Nayigihugu V., Hess B.W. (2004). Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. Journal of Animal Science 82: 179-183.

Ndou S.P., Kiariea E., Walsh M.C., Nyachoti C.M. (2018). Nutritive value of flaxseed meal fed to growing pigs. Animal Feed Science and Technology 238: 123-129. doi:10.1016/j.anifeedsci.2018.02.009.

NRC, 2012. Nutrient Requirements of Swine. 11th edition, National Academy Press, Washington DC, USA.

Opapeju F.O., Golian A., Nyachoti C.M., Campbell L.D. (2006). Amino acid digestibility in dry extruded-expelled soybean meal fed to pigs and poultry. Journal of Animal Science 84: 1130-1137. doi:10.2527/2006.8451130x.

O'Quinn P.R., Knabe D.A., Gregg E.J., Lusas, E.W. (1997). Nutritional value for swine of soybean meal produced by isopropyl alcohol extraction. Journal of Animal Science 75: 714-719. doi:10.2527/1997.753714x.

Pacheco W.J., Stark C.R., Ferket P.R., Brake J. (2013). Evaluation of soybean meal source and particle size on broiler performance, nutrient digestibility, and gizzard development. Poultry Science 92: 2914-2922. doi:10.3382/ps.2013-03186.

Pirie N.W. (1987). Leaf protein and its by-products in human and animal nutrition. 2nd Ed. Cambridge University Press, Cambridge, UK. doi:10.1017/S0014479700015805.

Ravindran V., Abdollahi M.R., Bootwalla S.M. (2014). Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. Poultry Science 93: 2567-2577. doi:10.3382/ps.2014-04068.

Short F.J., Gorton P., Wiseman J., Boorman, K.N. (1996). Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology 59: 215-221. doi:10.1016/0377-8401(95)00916-7.

Stein H.H., Seve B., Fuller M.F., Moughan P.J., de Lange C.F.M. (2007). Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: Terminology and application. Journal of Animal Science 85: 172-180. doi:10.2527/jas.2005-742.

Vogel A.I. (1961). A Textbook of Quantitative Inorganic Analysis 3rd Ed., pp. 788-789. Longman Scientific and Technical, New York, NY., USA.

Woyengo T.A., Beltranena E., Zijlstra R.T. (2017). Effect of anti-nutritional factors of oilseed co-products on feed intake of pigs and poultry. Animal Feed Science and Technology 233: 76-86. doi:10.1016/j.anifeedsci.2016.05.006.

Zander P., Amjath-Babu T.S., Preissel S., Reckling M., Bues A., Schläfke N., Kuhlman T., Bachinger J., Uthes S., Stoddard F., Murphy-Bokern D., Watson C. (2016). Grain legume decline and potential recovery in European agriculture: a review. Agronomy for Sustainable Development 36: 26. doi:10.1007/s13593-016-0365-y.

Annex 5: Scientific outputs

Sakkas, P., Royer, E., Smith, S., Oikeh, I. and Kyriazakis, I. (2019). Combining alternative processing methods for European soybeans to be used in broiler diets. Animal Feed Science and Technology (in press).