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Home-grown oilseed rape meal and oil seed rape products as protein sources for pigs and poultry

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CONTENTS

1. Abstract

The aim of this program of work was to revisit nutritional value and recommended upper inclusion levels of oilseed rape (OSR) co-products from UK OSR varieties for pigs and poultry, in order to increase confidence in using OSR meal in pig and poultry diets as home-grown alternatives for soya bean meal. OSR co-products were prepared from OSR batches of known variety, and were cold-pressed rapeseed expeller (RSE), and hexane extracted rapeseed meal under reduced (soft) heat intensity (sRSM) and under standard heat intensity (RSM). Animal studies assessed their standardised ileal digestible amino acids, apparent metabolizable energy and P bioavailability, and were underpinned with a systematic biochemical investigation into OSR co-product composition. The latter aimed to identify variation in glucosinolates, tannins, sinapine, phytic acid and fibre, and whether nutritional value was negatively correlated to these biochemical parameters, traditionally known as anti-nutritional factors. Digestibility studies were followed by growth performance trials with broilers and fattening pigs to assess upper inclusion levels of RSM as soya bean meal replacer.

The large amount of data collated over three harvest seasons from almost 30 varieties of OSR indicated that meals from modern OSR varieties continue to display significant variation in nutritional factors (levels amino acid and residual oil) but also in tannins, phytic acid, glucosinolate, sinapine and fibre. However, variation in protein quality in terms of amino acids composition was rather small. We also observed significant variation between OSR varieties in terms of amino acid digestibility, energy metabolisability and P bioavailability. However, with the exception of fibre, OSR co-product biochemistry did not negatively correlate with amino acid or energy digestibility, indicating that between-variety variation in tannin, phytic acid, glucosinolate and sinapine in modern OSR varieties is likely below a threshold to negatively impact nutritional value. Therefore, amino acid and energy availability largely depends on their content in OSR co-products, although reducing fibre levels in OSR meals would be expected to improve nutritional quality for pigs. The comparison between RSE, sRSM and RSM confirmed that nutritional value reduces as heat intensity increases during processing. Inclusion of RSM reduced feed intake in broilers and growing pigs, most clearly above a threshold of 100 g/kg for broilers and 50 g/kg for growing pigs, but not for finishing pigs fed RSM up to 250 g/kg diet. However, both broilers and pigs all performed above breed and commercial targets at any RSM inclusion level.

The overall implications of our work are that there is opportunity to improve nutritional value of OSR meals through amending oil extraction processes and variety selection, with key informants being amino acid, residual oil and fibre levels, as classically considered key plant secondary metabolites did not inform on digestibility within currently available and tested varieties. Intake constraints remain for the more sensitive stock, indicating upper limits for broilers to be ~100 g/kg and for growing pigs between 50 and 150 g/kg. However, for finishing pigs, upper limit may be well above 250 g/kg, which could contribute greatly to a reduced reliance on soya bean meal.

2. Introduction

Oilseed rape (OSR) is part of the Brassica family, with two major species, i.e. *B. napus* and *B. campestris* or *B. rapa*. The negative association of the word "rape" in North America has resulted in the adoption of the name canola, derived from Canadian oil, low acid (Hazzledine, 2008), though in order to be classified as canola, the oil of rapeseed must contain less than 2% erucic acid, while the total glucosinolates level of the resulting OSR meal (RSM) must be less than 30 µmoles/g (Thacker, 1990). The early cultivars had total glucosinolate level in excess of 100 µmoles/g of seed. By 1984, breeding progress was such that a European standard of 35 µmoles/g could be introduced. This was as part of a new double-low standard for erucic acid and glucosinolate content in varieties eligible for addition to the National List and use on farms. Subsequently, the glucosinolate standard operated for variety testing in the UK has been further reduced to 25 µmoles/g and then to 18 µmoles/g, based on the average glucosinolates recorded in seed samples from two years' of National List trials, with up to 10 trials per year.

The RSM is the co-product from OSR processing for oil extraction through a combination of crushing and solvent extraction, and it is this form in which OSR is mostly available for animal feeding. However, other OSR products are used in animal nutrition, including the use of full fat OSR, as is, or in blends with e.g. pulses, and rapeseed expeller (RSE), where oil is removed through e.g. cold pressing alone. RSM is an attractive feedstuff for pigs and poultry due to its relatively high level of crude protein (CP, ~34%; Hazzledine, 2008) and good amino acid (AA) composition, with similar to higher ileal digestible methionine, threonine and tryptophan to lysine ratios than those in soya bean meal (SBM). Earlier work indicated that an AA availability of about 92% for 16 AA in RSM (Nwokolo et al., 1976), though especially arginine may be limiting. Its digestible energy (DE) levels are moderate at ~11.9 DE MJ/kg (Hazzledine, 2008). However, apparent metabolizable energy (AME) values for RSM determined in the early literature differ widely (4.05 to 9.62 MJ/kg), perhaps because of earlier methodology issues rather than actual energy availability issues (Rao and Clandinin, 1970; Sell, 1966). There is no current data on AME of RSM and in view of the development in OSR breeding programs, it is doubtful if the values currently available in the literature are reliable. The RSM's decent CP level and beneficial AA profile provides *a priori* good opportunities to explore using RSM as home-grown alternative for SBM, provided sufficient amounts can be included in pig and poultry diets, and specific AA deficiency can be avoided. There is, however, no recent data available to explore whether varietal differences in AA digestibility can be exploited to achieve this, and thus to increase confidence in RSM as SBM alternative. Although RSM has high phytate content, it is also a very good source of P because it may have up to 0.7% inorganic P, compared to 0.2% inorganic P in SBM (Clandini et al., 1972). Moreover, Leinonen et al. (2012) showed in their resource-use analyses that the global warming potential of RSM is only ~50% of that of SBM.

Upper limits of dietary inclusion levels of any feedstuff in animal diets, including RSM, are dictated by factors like palatability, anti-nutritional factors and impact on feed and end-product

quality. Prior to the general adoption of the new cultivars of canola, the presence of high levels of glucosinolates was considered the major factor limiting the use of RSM in pig and poultry diets (Bell, 1984); total glucosinolates levels in traditional RSM were 120 to 150 μmoles/g. In addition, high fibre levels have also been indicated as limiting factor for pig and poultry feeds, because it is inversely related to OSR meal energy content (Downey and Bell, 1990).

Glucosinolates are secondary metabolites of *Brassica* spp, where their main functions are in the plant's defence system against insects and micro-organisms (Rask et al, 2000, Kliebenstein et al, 2005). Their presence determines flavour and bitterness of the plant tissues and they are widely discussed for their anti-nutritional properties in livestock (EFSA, 2008). Glucosinolates themselves are relatively harmless but can be broken down by plant enzymes (during processing) or microbial enzymes (during digestion). The breakdown products are a mixture of different thiocyanates (3-butenyl, 4-pentenyl, 2-OH-3-butenyl, CH3-thiobutenyl, phenylethyl, 3-CH3-indolyl and 4-OH-3-CH3-indolyl; Landero et al., 2011), which are very bitter tasting and goitrogenic. The latter can to some extent be compensated through iodine supplementation (Tripathi and Misra, 2008). However, the former can severely limit intake. Therefore, RSM inclusion has long been based on keeping total glucosinolate levels in the total diet below a threshold.

Nutritional value of OSR meal has resulted in depressed broiler growth performance or layer egg production (Summers et al., 1971a; McNeill et al., 2004), enlarged thyroid gland, perosis etc., all of which have been attributed to high glucosinolate levels. Indeed, low glucosinolate RSM may not result in growth depression, even at inclusion rates of 20%, when balanced with appropriate AA to overcome AA limitations (Summers et al., 1971b; Leslie et al., 1976; Fenwick and Curtis, 1980). RSM nutritional value as a good protein source was further demonstrated when produced under low temperature processing conditions (Clandinin, 1967; Leslie and Summer, 1975). A more recent HGCA study assessing RSM blends in poultry diets reached similar conclusions (Gordon, 2005).

The upper limit of glucosinolate levels in the total diet has been proposed to be 2.0 μmoles/g (Hazzledine, 2008), though this varies considerably between studies (Tripathi and Mishra, 2007). Based on glucosinolate levels of typical UK varieties between 1992 and 2001 averaging around 16 μmoles/g (NIAB, 2001), this level is the equivalent to 12.5% OSR meal, consistent with the commercially used upper limit in finishing pigs diets, whilst a 5% upper level is advised for grower pig diets (Hazzledine, 2008). This may be conservative as studies elsewhere show greater maximal inclusion levels (Tripathi and Mishra, 2007), and UK feed industry anecdotally use an upper limit of 5 and 15% for grower and finisher pig diets, respectively. Moreover, total glucosinolates levels in more recent AHDB Recommended List varieties have averaged around 8 μmoles/g, suggesting that based on glucosinolate levels alone, higher than currently used inclusion levels could be attained in UK pig diets. The environmental control of glucosinolate content is not well documented but Chalmers (1989) reported that levels were elevated in response to increasing rates of nitrogen (N) fertilisation. This is a matter of some concern as there are currently indications that oilseed crop

yields would benefit from higher N rates which might thus reverse the current trend towards lower glucosinolate contents.

There are no recent studies in poultry and pigs in the UK to support the view that greater than commercially accepted upper inclusion levels of RSM may be used without penalties on growth performance. However, two recent Canadian studies concluded that 20% dietary inclusion of RSE (Landero et al., 2012) and RSM (Landero et al., 2011) may completely replace SBM in diets fed to weaned pigs without detrimental effects on growth performance. It should be noted that these diets were formulated to equal net energy and SID AA content. We used the same modern feed formulation basis in recent studies to demonstrate that 30% inclusion of peas and faba beans can completely replace SBM in nutritionally balanced grower and finisher pig diets without detrimentally affecting performance (Smith et al., 2013; White et al., 2015; Houdijk et al., 2013).

Therefore, the aim of the series of studies reported here was to revisit nutritional value and recommended upper inclusion levels of current varieties of OSR meal under UK conditions for pigs and poultry. The animal studies carried out to establish levels of standardised ileal digestibility (SID) AA, digestible energy and P bioavailability were underpinned with a systematic investigation into RSM composition and variation with respect to anti-nutritional factors, including glucosinolates, tannins, sinapine, phytic acid and fibre, with an aim to identify whether nutritional value may be correlated to these biochemical parameters.

3.   Materials and methods

3.1.   ORS varieties used and processing

Twenty-two varieties, from each of five locations (Appendix 1a) from the 2012 UK National List (APHA, 2009) variety trials program (Harvest 2012), were selected to provide maximum diversity using known data on oil content, whole-seed glucosinolate content, breeding type (hybrid/open pollinated) and origin (breeding program). This provided common origin material from a range of environments. Seed samples were milled and de-fatted by cold-hexane extraction to prepare meal samples as per standard quality control protocols. Using standard laboratory methodologies (see below), samples were then analysed for CP and AA composition, total glucosinolate content and composition, tannin, phytic acid and sinapine.

Informed by analyses from these Harvest 2012 samples but constrained by commercial crop availability, a total of 16 OSR samples of known variety were obtained in 300 kg batches during Harvest 2013 (Appendix 1b) from farm grain stores in 1-tonne tunnel-lift bulk bags. Each bulk was sampled for biochemical analysis (as above) at the point of intake. At the inception of the project and in the absence of any small-scale crushing plants with hexane extraction capability in the UK, it had been agreed that the meals from the bulks would be prepared by cold pressing. However, upon project implementation, it was concluded that such meals would not be representative of commercial RSM products, particularly with respect to residual oil content, glucosinolate content and protein

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solubility. A pilot crushing and hexane extraction plant was identified in Pessac, France (Centre de Recherche et d'Expérimentation sur les OLéagineux et les protéagineux, CREOL), and contracted to prepare experimental RSM samples. A total of 12 of the 16 collected bulk samples were shipped to CREOL. To avoid possible masking of varietal differences in feed value by industrial intensity of oil extraction, under advice of CREOL (P Carré and A3 Quinsac, personal communications), the 12 OSR bulks were extracted under a reduced-heat processing regime (soft processing), in order to maintain a good level of differentiation between the samples, in terms of their biochemical characterisation, in the final meals (see section 3.1.1 for soft processing conditions). The resulting 12 soft-extracted meals (sRSM) were re-sampled for further biochemical analysis and comparison with the original bench hexane extraction analyses. The remaining 4 bulks were cold-pressed into RSE, to provide a comparison with this relatively minor but growing market source for the feed market (see section 3.1.2 for cold pressing conditions).

At Harvest 2014, two popular commercial varieties, the hybrid PR46W21 and the open-pollinated line DK Cabernet, were selected for the Year-3 growth studies. These had exhibited consistent differences in glucosinolate content and indications of differences in digestibility results in poultry, especially for AA digestibility. Four tonnes of each variety was purchased, sampled for analysis and shipped to CREOL for processing. On this occasion, the batches were split, so that meals could prepared from both varieties by both soft processing and standard processing, with steam conditioning (see section 3.1.3), thus resulting in sRSM and RSM samples, respectively. A detailed report on the preparation of these test meals is provided in Appendix 2.

3.1.1.   Hexane extracted under "soft" processing conditions

The seeds were dried to a moisture content of approximately 70 g/kg in a static dryer with movable containers of 1.6×1.2 m surface connected to a warm air generator using air at 70°C. The dried seeds were then cold-pressed at a rate of 250 kg/h using a MBU 75 press (La Mécanique Moderne, France) with a gap between pressing each batch 20 min, in order to avoid mixing the varieties. The expeller meal was then pelletized in 6 mm pellets to prevent possible differences in percolation during the extraction. Pellets were transferred immediately into the extractor. Continuous extraction was undertaken in a belt diffuser (Desmet Ballestra, Belgium). The expeller was leached by a counter flow of hexane in 6 stages. The flow of hexane at 50–55°C was 230 L/h, resulting in the meal extraction at the rate 140 kg/h (standard deviation, SD: 12 kg/h). Subsequently, by a semi-continuous mode, the meal was forwarded to the desolventisation unit using a 6 tray continuous desolventiser (Desmet Ballestra, Belgium). The flow rate was 180 kg/h and direct steam was injected at 25 kg/h by the bottom tray with the temperature 102.5°C to the mass of the de-oiled meal. Recorded residence time was 80 min for the following rapeseed varieties: Avatar, Compass, Incentive, Palmedor, PR46W21, Quartz, and DK Cabernet-1. The variety of Ability, DK Cabernet-2, V2750L and Excalibur had a residence time of 65, 86, 90, and 110 min, respectively.

3.1.2.   Cold-press expeller

The cold-pressing was performed at a local plant in Norfolk (Crush Foods, UK). The seeds were crushed at rate of 50 kg/h by a KernKraft KK40 press (Egon Keller Gmbh, Remscheid, Germany). The rate of pressing led to an increased temperature of exiting RSE to 55°C. The RSE was expelled through a 10 mm sieve plate, as pellets, briefly experiencing up to 70°C when passing though the press head.

3.1.3.   Hexane extracted under standard conditions

The hexane extraction under standard conditions, i.e. reflected those under commercial RSM production, was also performed at CREOL, and followed the same procedure as under 3.1.1 with modifications. The exception was that dried seeds were cooked at 90 °C for a period of 44±1.5 min, following warm pressing at 79±2.3 °C by a MBU 75 press (La Mécanique Moderne, France).

3.2.   Biochemistry

3.2.1.   Glucosinolates

Glucosinolate analysis of de-fatted samples was performed by HPLC in accordance with a documented in-house method at NIAB, conforming to BS 4325 Part 12 (ISO 9167-1:1992). Analysis of whole seed glucosinolate levels was performed by X-ray fluorescence (XRF), according to ISO 9167-2:1994 (Rapeseed Determination of glucosinolates content Part 2: Method using X-ray fluorescence).

3.2.2.   Tannins

Tannins were analysed using the vanillin HCl assay (Butler et al., 1982; Hagerman, 2011). Condensed tannins (proanthocyanidins) are flavanoid-based tannins. The vanillin reaction is widely used to estimate the condensed tannin content of plants. However, the assay is not specific for condensed tannins as any appropriately substituted flavanol can react in the assay. The vanillin reaction involves reaction of vanillin (an aromatic aldehyde) with the meta-substituted ring of flavanols to yield a red adduct.

3.2.3.   Sinapin

Sinapin levels were quantified through an in-house method combining extraction and HPLC conditions as detailed previously (Cai and Arntfield, 2001; Li and Rassi, 2002).

3.2.4.   Crude protein and amino acid profile

The level of CP (N x 6.25) in meals and diets were either analysed through Dumas Gas Analysis (ISO 16634-2: 2009) or classical Kjeldahl sulphuric acid digestion methodology followed

by steam distillation using the Gerhardt Vapodest system. The AA analysis was done through ion exchange chromatography with post-column derivitisation with ninhydrin (Commission Directive, 1998; 2000; Masey-O'Neill et al., 2012). Here, AA are oxidised with performic acid, which is neutralised with sodium metabisulphite (Llames and Fontaine, 1994), liberated from the protein by hydrolysis with 6N HCL for 24 hours at 110 °C and quantified with the internal standard method by measuring absorption of reaction products with ninhydrin at 570 nm. Tryptophan is determined by HPLC with fluorescence detection after alkaline hydrolysis (extinction 280 nm, emission 356 nm) with barium hydroxide octahydrate for 16 to 20 h at 110 °C in order to prevent decomposition of this AA (Commission Directive, 2000).

3.2.5. Phytic acid

Phytic acid was analysed measured as phosphorus released by phytase and alkaline phosphatase, using commercially available kits (Megazyme assay procedure; K-PHYT kit).

3.2.6. Neutral detergent fibre

The level of fibre, i.e. neutral detergent fibre (NDF), was measured using the fibre bag method according to ISO methodology (EN ISO, 2006).

3.2.7. Dry matter

Dry matter (DM) of rapeseed co-products and experimental feeds was measured through drying at 100 °C in a forced air convection oven to constant weight, whilst ileal digesta DM was measured through freeze-drying.

3.2.8. Oil extraction through cold-hexane (sample preparation)

Bench based cold hexane extraction took place on whole OSR samples arriving at NIAB laboratory as part of their standard quality assurance procedure. Seeds were milled, and resulting meals were steeped in hexane, with hexane changed 5 times. Hexane was then removed and resulting meal left to evaporate off the remaining hexane till dry.

3.2.9. Oil

The level of oil in the meals and oilseeds was determined through continuous-wave low resolution nuclear magnetic resonance spectroscopy as per ISO 5511:1992 (Rapid method).

3.2.10. Gross energy

Gross energy is assessed through classical bomb calorimetry, which in its simplest form consists of a strong metal chamber (the bomb) resting in an insulated tank of water. The test sample

is places in the bomb, and the oxygen admitted under pressure. The temp of the water is taken and the sample is then ignited electronically. The heat produced by the oxidation is absorbed by the surrounding water and is taken again. The quantity of heat produced is then calculated from the rise in temp and the weights and specific heats of the water and bomb.

3.2.11.   Acid hydrolysed ether extract (Oil B)

Oil levels in animal feeds were analysed through first analysis of crude fat (diethyl ether extraction) and followed by resulting residue being hydrolysed with hydrochloric acid and then re-extracted with petroleum ether. This was done as per AOAC Official Method 2003.05.

3.2.12.   Ash

Ash levels in animal feeds were determined through muffle furnace ashing for 4 hours at 500 °C, based on Method 6 Total Ash in plant material (MAFF/ADAS RB427).

3.2.13.   Titanium dioxide

The content of titanium dioxide (TiO_2) in feeds and excreta/digesta was determined according to a method of Short et al. (1996).

3.2.14.   Phosphorus

Phosphorus was analysed using spectrophotometric analysis as per AOAC (2006).

3.3.   Nutritional value

3.3.1.   Apparent metabolizable energy in poultry (Harvest 2013 and 2014)

A total of 357 Ross 308 male broilers at 14 days old were allocated to 17 treatments to assess AME from the Harvest 2013 samples. Each treatment had 7 replicates and three birds per replicate. The treatments consisted of a maize-SBM reference diet and 16 test diets in which 12 sRSM and 4 RSE samples (at 100g/kg diet) proportionally replaced all the energy-yielding ingredients in the reference diet; diet composition and analysis is presented in Appendix 3a. Birds received the experimental diets between days 14 and 21 and excreta were collected on days 20 and 21. Apparent metabolisable energy (AME) of the test diets was determined using the index method and the AME of the 12 sRSM and 4 RSE samples was determined using the difference method.

A total of 168 Ross 308 male broilers were used to assess AME for the Harvest 2014 samples. As above, the birds were allocated to one of seven dietary treatments on day 14 of age. The treatments consisted of a maize-SBM reference diet, and an additional 4 diets in which the sRSM and RSM form DK Cabernet and PR46W were added at 300 g/kg to proportionally replace the energy yielding components of the reference diet; diet composition and analysis is presented in

as for Harvest 2013. Furthermore, diets 6 and 7 had the unprocessed seeds of DK Cabernet and PR46W added at the rate 300 g/kg to the reference diet. Each of the treatments had 8 replicate cages with 3 birds per replicate cage. Excreta were collected on days 19 to 21. The AME and AMEn of the sRSM, RSM and OSR were calculated using the difference method.

3.3.2. P availability in poultry (Harvest 2014)

A total of 450 Ross 308 male broilers at 11 days old were allocated to 15 treatments in a randomised complete block design. The birds were previously raised from 0 to day 11 on broiler starter diet formulated to meet all the nutrients requirements. On day 11, the birds were allocated to 15 treatments, each treatment had 6 replicate pens and each pen had 5 birds. Birds and feed were weighed on days 11 and 21. On day 21, the birds were euthanised by cervical dislocation and the left tibia bones were collected from 2 randomly selected birds per pen and the bones were later defatted and ashed.

The 15 treatments included a basal diet (diet 1) that was formulated to be adequate in all nutrients and energy and deficient in non-phytate P. SBM was the only source of P in the basal diet and provided 2.9 g/kg total P. Diets 2 and 3 were similar to the basal diet except that mono-sodium phosphate (MSP), was added at the rates of 4.8 g/kg (diet 2) or 9.3 g/kg to increase dietary total P levels to 4.0 g/kg or 5.0 g/kg for diets 2 and 3, respectively. The remaining 12 diets had two levels each of sRSM, RSM or OSR of DK Cabernet or PR46W. The sRSM and RSM were added at the rates of 110 or 220 g/kg to the basal diet to provide dietary total P levels of 3.9 or 4.9 g/kg, respectively. The OSR were added at the rates of 190 or 360 g/kg to provide dietary total P levels of 3.9 or 4.7 g/kg, respectively; diet composition and analysis is presented in Appendix 3b.

3.3.3. Standardized ileal digestibility of AA in poultry (Harvest 2013 and 2014)

Harvest 2013 samples (12 sRSM and 4 RSE) and Harvest 2014 samples (2 sRSM and 2 RSM) were analysed for SID AA following the same protocol for the two years. Test samples were ground (4 mm screen) and formulated in semi-synthetic diets at 500g/kg; diet composition and analysis is presented in Appendix 3c. Day-old Ross 308 male broilers were fed conventional diets for 14 days followed by test diets for 8 days (n=12). Birds were then culled by CO_2 asphyxiation and cervical dislocation to confirm death. Ileal digesta were collected and freeze dried. AA digestibility was estimated by quantification of AA and inert marker (TiO_2) in diets and ileal digesta. The SID of AA was calculated by correcting apparent ileal digestibility for basal ileal endogenous losses (John Htoo, Evonik, personal communication).

3.3.4. Standardized ileal digestibility of AA in pigs (Harvest 2013 and 2014)

This experiment was conducted to analyse selected Harvest 2013 RSE and sRSM samples, and each Harvest 2014 sRSM and RSM sample.   A total of 48 large white Duroc Landrace males

pigs weighing 41±2.8 kg were used to assess SID AA for the eight rapeseed co-products. The pigs were allocated to these treatments in a completely randomised design with six pigs allocated to each diet over six time periods. Pigs were individually housed in pens (2.45 m x 5.6 m) without any bedding but with rubber matting. Feed and water were provided *ad libitum*. A standard commercial pig diet was fed for five days during adaptation to the experimental conditions, followed by feeding the experimental diets for seven days; diet composition and analysis is presented in Appendix 3d. On day eight, the pigs were culled following sedation by an intramuscularly injection of midazolam and administration of an intravascular injection of pentobarbital followed by exsanguination. The ileal digesta was then collected from the ileum, measured as 1 m from the ileal caecal colonic junction towards the jejunum, and stored at -20 °C.

Coefficients of AID and SID of CP and AA in the diets were calculated by previously described equations (Toghyani et al., 2015). The basal ileal endogenous CP and AA losses (g/kg dry matter intake) were corrected by published values (Jansman et al., 2002): CP 11.82, lysine 0.40, methionine and cysteine 0.32, threonine 0.61, isoleucine 0.38, leucine 0.49, valine 0.54, histidine 0.19, arginine 0.39, phenylalanine 0.34 and tryptophan 0.14 g/kg DMI. The lysine to CP ratio was calculated as an indicator of heat damage in the each rapeseed co-product material.

3.4. Growth performance and upper inclusion levels

3.4.1. Growth performance in broilers (Harvest 2014)

For the broiler growth trial, a total of 1,350 Ross 308 male broilers at zero day old were allocated to 10 dietary treatments. Each of the treatments had 10 replicate pens with 15 birds per replicate pen. The treatments included a wheat-SBM basal diet, which was formulated to meet the nutrient recommendation for the birds. Diets 2 to 5 had DK Cabernet RSM (Harvest 2014), added at the rates of 50, 100, 150 or 200 g/kg for diets 2, 3, 4, and 5, respectively to partly replace wheat and SBM in the basal diets. Diets 6 to 9 had PR46W21 RSM (Harvest 2014) added at the rates of 50, 100, 150 or 200 g/kg in the same way. Replacement of SBM and wheat was on the basis of previously determined SID AA and AME contents, respectively. A final diet, diet 10, was used to explore impact of unprocessed OSR. Here, unprocessed DK Cabernet (Harvest 2014) seeds were added at the rate of 80 g/kg to partly replace wheat and SBM in the basal diet; diet composition and analysis is presented in Appendix 3e.

The diets were fed as crumbed pellets from day 0 to 7, and as pellets for the remainder of the experiment. The diets were fed in two phases with the starter phase (day 0 to 21) and the finisher phase (day 21 to 42). Feed, feed refusal and birds were weighed on days 0, 21 and 42 to determine the growth performance.

3.4.2. Growth performance in pigs (Harvest 2014)

For the pig growth trial, RSM from oilseed rape varieties DK Cabernet and PR46W21 were incorporated at 0, 50, 150 and 250 g/kg, gradually replacing SBM and a proportion of wheat in nutritionally complete grower and finisher pig diets, with modified oil and pure AA levels to formulated for net energy levels of 9.5 and 9.3 MJ/kg, and standardized ileal digestible lysine levels of 9.8 and 8.9 g/kg, respectively; diet composition and analysis is presented in Appendix 3f. Residual levels of SBM in grower and finisher diets were 40 and 0 g/kg, respectively. After one week of adaptation week, and for separate batches of grower pigs, with initial body weight of 39±0.5 kg, and finisher pigs, with initial body weight of 62±0.9 kg, RSM containing diets were fed *ad libitum* to 2 groups of 3 male and of 3 female pigs for 3 weeks; control diets were fed to 4 groups of 3 males and of 3 females. Weekly live weights for individual pigs, and pen feed intakes were taken to assess body weight gain, average daily feed intake and feed conversion ratio.

4. Results

4.1. Biochemistry

4.1.1. Initial screening (Harvest 2012)

Results of the analyses of the 22 variety cold hexane-extracted meal samples, from each of five locations, for CP, glucosinolate content, tannin, phytic acid and sinapine content are summarised in Table 1. Oil content and glucosinolate content of the whole seed are also given. More detailed site-by-site results for each of the 22 entries are provided in Appendix 4a.

Average CP content (22 varieties x 5 locations) was 34.5 %, with a maximum range (across varieties and sites) from 28.9 to 37.8 %, with site and varieties effects contributing equally to this. Glucosinolate content averaged 20.4 µmol/g with a wide overall range, i.e. 10.8 to 52.5 µmol/g. This variation came principally from variety effects. This range was exaggerated by the inclusion of a new variety type with an altered oil profile and relatively high glucosinolate content. Excluding this variety reduced the overall mean value to 19.4 µmol/g and the upper range limit to 36.1 µmol/g. The individual glucosinolate components showed relatively little variation, with most coming from progoitrin and 4OH-glucobrassicin. Tannins averaged at 1.59 mg/g catechin equivalents but also exhibited considerable variation (0.28 to 3.21 mg/g), largely from site effects. Phytic acid averaged 2.83 g/100g and varied less (1.32 to 3.78 g/100g), with the main variation again coming from sites. Sinapine averaged at 7.58 mg/g (5.10 to 9.30 mg/g), with similar variation observed for sites and varieties.

The AA profile was very consistent between varieties. Figure 1 shows the AA profile for one specific site (Lincolnshire). Results for the Lincolnshire site showed that leucine, arginine and lysine were predominant and present in very similar proportions, together comprising almost 43% of total amino acids, averaged across varieties.

In contrast to AA composition, glucosinolate composition was much more variable between varieties. Figure 2 shows the glucosinolate composition of meals from the 22 cultivars of oilseed rape used in Harvest 2012, as a mean of 5 trial sites; individual site data is presented in Appendix 4b. A common pattern was observed over the five trial sites, with progoitrin being the principal glucosinolate, averaging 9.5 µmoles/g, or approximately 40% of the total glucosinolate. Other glucosinolate types were present in a generally consistent pattern of declining proportions of the total, with relative small variation between varieties. The most marked outlier was entry number 22, bred for its modified high oleic-low linolenic oil profile. This was characterised by a greater than average progoitrin, low levels of 4OH-glucobrassicin and relatively great gluconapin levels.

4.1.2. OSR variety and co-product type biochemistry (Harvest 2013)

Tables 2a and 2b present a summary of the analyses done on the 16 bulk sample of oilseed rape acquired for digestibility studies (Harvest 2013). Table 2a shows levels of oil, CP, fibre (NDF) and impact on CP solubility; Table 2b shows levels of glucosinolates, tannin, phytic acid and sinapine. The cold-hexane, bench extraction (P1) allowed comparability with the analytical results from Harvest 2012. Overall, the CP content of these 16 variety samples, from diverse geographic locations, was 37.2% of the meal, by weight, compared with a mean of 34.5% from the 22 varieties across the five locations from Harvest 2012.

Glucosinolate content of Harvest 2013 samples was considerably greater than the Harvest 2012 mean, i.e. 32 µmoles/g compared with 20.4 µmoles/g. Tannin levels were similar, 1.3 mg/g catechin equivalents compared with 1.59 mg/g, sinapine levels were slightly lower at 6.1 mg/g, compared with 7.58 mg/g in Harvest 2012, whilst the phytic acid mean was 1.3 g/100g during Harvest 2013, which was less than half the Harvest 2012 value of 2.83 g/100g.

The CREOL semi-industrial crushing and hexane extraction process (P2) was, as expected, clearly more efficient than the bench extraction with lower residual oil levels, i.e. 3.2%, compared with 8.7% from bench extraction for the 12 comparable seed samples. Likewise, and as expected, glucosinolate differed considerably; glucosinolate content was approximately 50% lower in the P2 meals compared with P1. Both tannin and phytic acid levels were elevated in the P2 process, both approximately doubling the P1 values, while sinapine levels were very similar. The fibre analyses showed that NDF averaged 265 g/kg and a range of 226 to 283 g/kg for P2 samples.

The commercially cold-pressed meal samples (P3) had high residual oil contents, averaging 25.7% of the dry matter. The CP content was 6% lower than from the P2 process, as would be expected from the reduced oil extraction efficiency. Tannin and sinapine levels were slightly below those of the P1 process for this small sample set, while phytic acid was slightly elevated. The fibre analyses showed that NDF values for P3 were marginally smaller than for P2, at 245 g/kg, with a range of 239 to 251 g/kg.

In addition to UK analyses, CREOL provided solubility data for the 12 meal batches that they processed (Table 3a). The range of values of samples 2-12 was within normal limits but sample 1 was perhaps slightly anomalous, suggesting a degree of under-processing.

The variability of analytical results between varieties and between sites observed for the Harvest 2013 samples was in general agreement with observations from Harvest 2012. While the analyses of the two samples of the variety Compass (3/12 and 1/4) collected from the same heap of grain gave directly comparable values, the 3 samples of DK Cabernet (4/12, 5/12 and 2/4) provided some insight into the between site variation for that specific variety.

Detailed glucosinolate analyses for the 16 bulk samples were generally in line with those of the 22 lines investigated in Harvest 2012 (Tables 3a, 3b and 3c). However, there were two exceptions. Firstly, there appeared to be a reversal in the relative abundance of gluconapin and 4OH-glucobrassicin, and secondly, in four of the samples (Ability, Avatar, PR46W21 and V275OL) glucoalyssin appeared, which had not been previously observed. The difference in glucosinolate level and composition between bench-extracted seeds and plant extracted seeds is also shown in Figure 3.

Using the glucosinolate data in seeds (through cold hexane extraction) and meals (from either mild hexane extraction or cold pressing), we calculated the relative stabilities of the individual glucosinolates in response to the processing. This is summarised in Table 4. The two predominant glucosinolates, progoitrin and gluconapin showed losses of around 45% in the mild hexane extracted meals and over 50% in the cold pressed meals. 4OH-glucobrassicin decreased by 85.6% in the mild hexane extracted meals but only 21.5% when cold pressed. Glucoalyssin, although only present at low levels, appeared to be very stable, with losses of only 1 and 2% respectively, from the two treatments. Other individual glucosinolates were present at such low levels that sampling and analytical accuracy leaves further interpretation outside the scope of this study.

4.1.3. OSR variety and processing intensity biochemistry (Harvest 2014)

Four sub-samples from each of the two 4-tonne bulks were analysed before despatch to the CREOL crushing and hexane extraction plant in France (Table 5). At intake the two varieties were very similar in dry matter content and oil content. DK Cabernet had 3.3 μmoles lower glucosinolate content but this was less of a difference between the two varieties in previous tests. After cold hexane extraction DK Cabernet retained 0.7% more oil than PR46W2, was 3.7% lower in CP and 4.6μmoles lower in glucosinolate content. Sinapine and phytic acid contents were very similar but, though quite variable, the tannin content of DK Cabernet was over twice the level in PR46W21.

Analytical results for the rapeseed meals returned from crushing and hexane extraction in France are given in Table 6. The standard processing method, using steam and higher temperatures to condition and crush the seed resulted in a small increase in oil extraction and a consequent increase in crude protein in the meal. These changes were greater for the DK Cabernet meals than for those produced from PR46W21. Tannin, sinapine and phytic acid levels were relatively

unaffected by processing conditions but standard processing resulted in lower levels of glucosinolates in the resulting meals, at approximately half the levels achieved from the mild processing method (Table 6).

Further insight into the fate of individual glucosinolates is given in Figures 4 and 5 which compare the content of individual glucosinolates in the original seed batches with the meals from both mild and soft processing. As in previous analyses, progoitrin was the most abundant constituent in all 8 samples, with gluconapin, 4OH-glucobrassicin, glucobrassinapin and glucoraphanin the only other individual glucosinolates present at 1.0 or more µmole/g in the seed, before processing. Of these, 4OH-glucobrassicin was the most sensitive to processing and was not detected in any of the meals.

Variation in depression of detectable total and individual glucosinolates levels was observed, both between varieties and between treatments, after processing. In the case of DK Cabernet, total glucosinolates fell by 83.6% in the soft process meal and 87.1% in the standard process meal. The meals from PR46W21, with higher initial levels in the seed, showed glucosinolate reductions of 71.9% from soft processing and 82% from standard processing. Progoitrin, present at the high levels in the seed, showed a relatively high stability to remain the dominant constituent in all four meals, while 4OH-glucobrassicin proved to be highly sensitive to processing and was not detected in any of the meals. Gluconaturtin, found in small quantities in DK Cabernet only, also showed high sensitivity to processing and was removed by standard processing.

4.2. Nutritional value

4.2.1. OSR variety and co-product type poultry SID AA (Harvest 2013)

Table 7 shows the levels of DM, CP, NDF, total AA and Lys to CP ratio, whilst Table 8 shows the individual essential AA of the RSE and sRSM samples used in the Harvest 2013 broiler AA digestibility study. Some of this data was also presented in Table 2, but is repeated here for completeness. The sRSM samples had a greater content of CP (419 to 560 g/kg DM) compared to RSE samples (293 to 340 g/kg DM), and a slightly lower Lys to CP ratio.

Table 9 shows the AID for CP as well as the SID for the essential AA analysed. Significant differences between varieties were observed within the sRSM samples but not within the RSE samples. For Compass, we had both RSE and sRSM, which allowed for avoiding confounding comparisons between variety and type of processing. The SID of lysine, arginine, histidine and threonine were greater in Compass RSE compared to its sRSM counterpart (P<0.05). However, SID of AA did not differ in both DK Cabernet sRSM used, which were cultivated in two different farms (P>0.05). The SID of lysine was on average 0.03 units greater (P<0.001) in RSE than in sRSM. The sRSM produced from variety PR46W21 showed similar or greater SID of most individual AA than the RSE from four other rapeseed varieties, and was as such observed to be a superior variety in terms of SID AA coefficient.

4.2.2. OSR variety and processing intensity poultry SID AA (Harvest 2014)

Table 10 shows the chemical composition of the sRSM and RSM used from Harvest 2014 for broiler AA digestibility. The protein solubility and NDF levels were remarkably different for the DK Cabernet RSM sample compared to its sRSM counterpart, whilst these were rather similar for the sRSM and RSM PR46W21 samples. Table 11 shows the SID of CP and individual AA. The variety PR46W21 showed a greater SID of CP, arginine, leucine, methionine, cysteine, phenylalanine, valine and lysine in RSM compared to the DK Cabernet RSM (P<0.05). The soft processing increased SID of CP, histidine and lysine in SRSM of PR46W21 and DK Cabernet compared to their RSM counterparts (P<0.05). An interaction between variety and processing was only observed for SID of tryptophan (P<0.001), as only in PR46W21 RSM the tryptophan SID was reduced compared to its sRSM counterpart.

4.2.3. OSR variety and co-product type poultry AME (Harvest 2013)

The energy retention coefficient (EM), AME and AMEn of sRSM and RSE are shown in Table 12. The RSE samples showed a greater energy retention coefficient, AME and AMEn than the RSM samples but the margin of difference in AME and AMEn between RSM and RSE was larger than the margin of difference in retention coefficient. Energy retention coefficient of RSM was greater (P<0.05) for V275OL than all the other varieties, which had statistically similar energy retention coefficients. In addition, both AME and AMEn were greater for Ability than all the other varieties.

For RSE, Compass had greater (P < 0.05) energy retention coefficient, AME and AMEn than the other varieties, whereas AME and AMEn were lower for Sesame compared with the other varieties. DK Cabernet and NK Grandia both had similar AME and AMEn.

4.2.4. OSR variety and processing intensity poultry AME (Harvest 2014)

The energy retention coefficient (EM), AME and AMEn of sRSM and RSM from Harvest 2014 are shown in Table 13. There were no significant effect of variety on energy retention (EM) coefficient, AME or AMEn of the two RSM varieties (DK Cabernet and PR46W21) tested (Table 13). However, there were significant effects (P<0.01) of processing intensity on AME, AMEn and EM and tendency for interaction for AME and AMEn (P<0.10). Generally, AME and AMEn were greater (P<0.01) for RSM processed by mild processing technique. The tendency for interaction was shown by lower (P<0.05) AME and AMEn for DK Cabernet processed by the mild technique compared to standard process, whereas AME and AMEn tended (P<0.10) to be similar for PR46W21 processed by the two techniques.

4.2.5. OSR variety and processing intensity P availability (Harvest 2014)

There were linear effects (P < 0.05) of supplemental Na_2PO_4 and RSM on weight gain and feed intake and linear effect on Na_2PO_4 on bone ash only (Table 14). There was a tendency for a linear increase (P < 0.10) in weight gain in response to increased dietary level of PR46W21 processed using the mild technique. There were no significant quadratic treatment effects except for feed intake response (P<0.05) to increasing level of DK Cabernet processed using the standard technique or FCR response to increasing level of DK Cabernet processed using the mild technique (P<0.01).

On the basis of the regression equation, percentage relative P bioavailability in the RSM varieties, based on weight gain or tibia ash are presented in Table 15. Phosphorus relative bioavailability values, using weight gain or tibia ash responses, were generally greater in RSM processed using the mild technique, except for DK Cabernet when tibia ash was used as the response criterion.

4.2.6. OSR variety, co-product type and processing intensity pig SID AA (Harvest 2013 and 2014)

Table 16 shows the DM, CP, NDF, Lys to CP ratio and glucosinolates of the oilseed rape co-products used for assessment of SID AA in pigs (Harvest 2013 and Harvest 2014), whilst Table 17 shows the content of essential AA. The RSM contained less lysine per unit crude protein compared to SRSM, reducing the lysine to CP ratio from 0.061 to 0.050, and from 0.055 to 0.051 for Harvest 2014 DK Cabernet and PR46W21, respectively. Tables 18 and 19 show the outcome of the SID CP and AA assessment in pigs. The SID of CP, arginine, histidine, isoleucine, leucine, lysine, methionine and cysteine, phenylalanine, threonine, valine and tryptophan did not significantly vary between Harvest 2013 and 2014 for both DK Cabernet and PR46W21 SRSM. Among Harvest 2014, soft processing led to an increased SID of CP, arginine, histidine, lysine, methionine and cysteine, threonine and valine in DK Cabernet SRSM, whereas PR46W21 SRSM had only greater lysine and tryptophan SID compared to its RSM counterpart (P<0.05).

4.3. Linking biochemistry with nutritional value

One of the objectives of this work was to identify whether variation in rapeseed meal biochemistry could inform on meal nutritional value. To this effect, a series of linear regressions were carried out between meal levels of total glucosinolates, NDF, residual oil, phytic acid, sinapine, tannins and protein solubility on the one hand, and indicators for broiler nutritional value on the other hand, for the 12 SRSM samples from Harvest 2013. The nutritional value indicators used here were dietary levels, SID coefficients and SID levels for the essential amino acids (lysine, methionine, threonine, valine, histidine, isoleucine, leucine and phenylalanine), the conditionally essential

arginine, as well as energy (i.e. gross energy, apparent energy digestibility coefficient, and resulting sRSM AME content).

Table 20 shows that, perhaps unexpectedly, positive relationships were observed between total glucosinolates and AA level, with the exception of threonine. This was largely due to positive relationships with levels of progoitrin, 4OH-glucobrassicin, glucoalyssin and gluconapin (Table 21). No consistent relationships were found with NDF and residual oil content, whilst there were no significant relations for any AA for phytic acid, sinapine and tannins. A positive relationship was also observed between protein solubility and AA levels, with the exception of methionine.

Total GS levels did not correlate with SID amino acid coefficients (Table 22). However, some GS components positively correlated with SID coefficients of selected amino acids, whilst the most consistent observation was a negative correlation between glucoberin and SID coefficient of most AA assessed (Table 23). However, none of the other biochemistry assessed was correlated with the SID coefficient (Tables 22 and 23). The combination of correlation with AA level (Tables 20 and 21) and digestibility coefficient on SID levels in the sRSM products (Tables 22 and 23) therefore, largely reflected the observed correlations on AA levels *per se* (Tables 24 and 25) rather than being modified by correlations between biochemistry and digestibility coefficients.

Table 26 shows the outcome of linear regression between sRSM biochemistry and energy nutritional value parameters, i.e. gross energy, energy digestibility, and AME level. Level of GE was, as expected, positively correlated with oil content, but was also positively correlated with protein solubility and tended to be negatively correlated with phytic acid level. Energy digestibility was positively correlated with glucosinolate levels, and tended to be positively correlated with levels of CP and oil. There were no negative correlations. Lastly, AME, effectively the combination of gross energy and digestibility, was significantly positively correlated with levels of oil and protein solubility, whilst tended to be positively correlated with CP levels.

Table 27 shows the outcome of linear regression between sRSM individual glucosinolate levels on the one hand, and gross energy, energy digestibility, and AME level on the other hand. Gross energy levels were positively correlated with the levels of 4OH-glucobrassicin and glucoalyssin. Energy digestibility was positively correlated with several types of glucosinolates, i.e. glucoalyssin, glucoberin, gluconapin, glucoraphanin and progoitrin. Finally, in agreement with gross energy, the level of AME was also positively correlated with 4OH-glucobrassicin and glucoalyssin.

Table 28 shows the outcome of linear regression between NDF and SID of CP and AA in pigs, using the eight RSM co-products used, i.e. one RSE, five sRSM and two RSM. In contrast to the relationships observed in broilers, the SID of CP and AA were all highly significantly negatively correlated with the content of NDF, with a coefficient of determination for the linear relationship (r^2) ranging from 0.82 for SID lysine to 0.95 for SID arginine.

4.4.   Growth performance and upper inclusion level

4.4.1.   Broiler growth performance and RSM variety and level

The growth performance responses of broilers to dietary inclusion of graded levels of RSM to wheat-soybean meal diets are presented in Table 29. Body weight gain linearly reduced with increasing levels of RSM whilst feed conversion ratio linearly increased during the grower phase. Whilst there was a significant ($P < 0.05$) RSM variety × inclusion level interaction on weight gain during the finisher period, arising from a smaller reduction in gain with increasing PR46W21 levels compared to DK Cabernet levels, this effect was no longer significant over the whole experiment. The inclusion of 8% whole seeds significantly reduced performance throughout.

4.4.2.   Pig growth performance and RSM variety and level

Table 30 shows the outcome of DK Cabernet and PR46W21 feeding to grower and finisher pigs. Significant interactions between sex and feeding treatments were not observed; therefore effects of feeding treatments only are presented. RSM did not affect grower pig feed conversion ratio but reduced feed intake ($P=0.016$) and body weight gain ($P=0.064$) at RSM inclusion levels of 15 and 25%, with smaller feed intake for PR46W21 than DK Cabernet ($P=0.008$). In finisher pigs, RSM did not affect feed intake or weight gain. However, male finishers grew faster than female finishers ($P=0.022$), although there was a tendency that this was only the case in the absence of RSM ($P=0.066$). Males had better feed conversion than females, both for growers ($P=0.023$) and finishers ($P<0.001$), whilst finisher feed conversion improved at 25% RSM inclusion ($P=0.04$).

5.   Discussion

5.1.   Biochemistry

The principal objectives of the biochemistry component of this work were to survey meals produced from the seed of commercial lines of oilseed rape and to provide sufficient seed of a representative selection of these for use in feeding studies with pigs and poultry. A supplementary objective was to conduct field experiments to begin to investigate the degree to which crop management practice can influence the composition of rapeseed meal. Seed samples were sourced and analysed as scheduled and bulks of meal were supplied for use in animal nutrition studies. Analyses of Harvest 2012 samples, from 22 cultivars, harvested from each of 5 common locations across the country, indicated that meal composition, in all respects, was highly variable, both as a result of the variety sampled and the location and thus by inference, as result of soil type, weather and seasonal effects. Field experiments confirmed studies from the early days of oilseed rape growing in this country. Oil content was depressed and protein and glucosinolate content were elevated as a result of increased nitrogen fertiliser application rates (Appendix 5), arguably the most influential management tool under the control of the grower. Differences in composition at around

the 180 kg/ha rate, the national application average, were slight however. Other components of the grain, present at much lower levels can be assumed to vary very little over the range of fertiliser application in current management practice. Other sources of variation are likely to be dominated by weather conditions, including soil moisture availability throughout the spring/summer growing season and sunshine and temperature patterns during the pod-fill period and the speed of ripening during the final senescence period in particular. This final senescence period is usually controlled by growers, who either swathe or desiccate their crops, to achieve uniform maturity and minimise shedding losses. These interventions and the timing of harvesting the crop are like to impact on the final grain composition but it is beyond the scope of this study to pursue this topic further.

The scale of operation at the main UK crushing plants makes it is largely irrelevant to consider the fate of individual crops, their variety and management, because of the degree of mixing, once delivered. Although there have been periods in time when individual varieties have achieved over 50% of the market share, and under those conditions, variety effects will impact on meal the quality of meals blended into animal rations. Whilst the current data indicate a large degree of differences between varieties in terms of biochemistry, with data being consistent to those presented elsewhere (Woyengo et al., 2010; Parr et al., 2015), its correlations with nutritional value were limited, and governed largely by level of AA and residual levels of oil. Thus, our data would support the view that bringing in the complete ration variable levels of glucosinolate, tannins, sinapine and phytic acid from RSM is not very likely to influence the nutritional value of the ration. Currently, the market is highly fragmented however, and the most recent AHDB planting survey reports 5 varieties with 7 or 8% shares and 64% of the seed market taken up by varieties with 5% or less. This, of course, provides a very high level of damping of any undesirable quality traits in individual varieties but also presents a barrier to the useful introduction of any new desirable variety characteristics.

The fate of glucosinolates is of particular interest as they have be the main cause for concern over inclusion rates for monogastric feeding rations. It is apparent that the initial levels of glucosinolates in the meal were 16.9 and 21.5 µmoles/g of meal, after bench cold hexane extraction, for DK Cabernet and PR46W21, respectively. Glucosinolates remain intact until they are exposed to water and the enzyme myrosinase during the crushing process (Kliebenstein et al., 2005). They can remain in the meal if the enzyme is destroyed by steam heating prior to flaking. Glucosinolates are degraded by heat, as observed here from the difference between bench extractions and industrially processed samples, as well as from variation between cold-pressing, soft hexane extraction and standard hexane extraction. If they are hydrolysed, they form isothiocyanates which are oil soluble. These isothiocyanates are largely volatile and are lost during refining. Measuring glucosinolate in processed meal is misleading because breakdown products retained in the meal are difficult to detect. Whilst the degrees to which enzyme and heat degradation occur within different processing plants differ, and are as such further sources of meal quality variation (Labelette et al, 2011; Peremans et al, 2006; Quinsac et al, 2015), assessing glucosinolate breakdown products in addition

to the glucosinolates themselves may provide additional insight in linking meal quality and nutritional value.

5.2. Amino acid digestibility

All Harvest 2013 and 2014 rapeseed varieties used were grown in similar climatic condition in the South of Great Britain. This was supported by the very similar AA content in DK Cabernet RSM1 and DK Cabernet RSM2 within Harvest 2013. The magnitude of variation in nutritional composition of Harvest 2013 and 2014 samples was rather similar compared to the Harvest 2012, though the absolute levels may be sensitive to type of sample preparation (i.e. cold-hexane extractions in the lab vs soft or standard hexane extraction in the pilot plant).

The effect of processing and variety caused substantial changes not only in AA but also in oil content, which accords with earlier observations (Bell, 1993; Newkirk et al., 2003a). For Harvest 2013 samples, AA content almost doubled in the Compass sRSM compared to Compass RSE, as well as averaged across sRSM vs averaged across RSE. This change in AA content would have been due to a large extent to a greater removal of oil during the hexane extraction processing. Nevertheless, the resulting AA content in sRSM and RSE (Harvest 2013) and sRSM and RSM (Harvest 2014) were within the wide range reported through other studies (Maison and Stein, 2014; Seneviratne et al, 2011a; Seneviratne et al, 2011b; Bell and Keith, 1991; Fan et al. 1996; Landero et al., 2011; Liu et al., 2014; Eklund et al., 2015; Rezvani et al., 2012; Messerschmidt et al., 2014).

For the Harvest 2013 samples, we used relatively mild processing conditions in order to minimise the possibility of overriding varietal effects on RSM quality. This mild temperature process, both for the soft processed hexane extraction and the cold pressing, did not markedly change individual AA as a percentage of total AA in RSM compared to RSE, resulting in the proportion of Lys of 5.5-5.8% and 6.0-6.2% in total AA content in RSM and RSE, respectively. Furthermore, the proportion of the other AA in the rapeseed protein was almost identical in RSM and RSE. This implies that both the soft processing and cold pressing used might have preserved AA in the resulting rapeseed co-products. Indeed, in contrast, Harvest 2014 samples indicated that standard processing reduced the proportion of Lys compared to soft processing, with varietal differences still being observed. Whilst this may indicate that the deliberately imposed soft processing conditions for the Harvest 2013 samples may not have been needed to observe varietal differences, the implication is that selection of a superior rapeseed variety through such studies is expected to continue to show its merits under standard hexane extraction processing.

To the best of our knowledge, this is the first report showing the effect of modern Western rapeseed variety and processing on ileal digestibility of AA in rapeseed co-products. Such paucity of data may have arisen from difficulty of variety collection, as although many rapeseed varieties are collected by industry, they are mixed to producing RSM, with consequently varying AA content and digestibility. Generally, the heat treatment during the rapeseed processing, along with the glycoproteins associated with the cell wall structure, are responsible for a decrease in AID and SID

of individual AA (such as Lys) in rapeseed co-product-rich diets when fed to broiler chickens (Khajali and Slominski, 2012). Variation in digestibility might also arise from level of rapeseed meal inclusion in the diet, with digestibility reduced at greater inclusion levels (Woyengo et al., 2010; Newkirk et al., 2003b).

The reduction in SID for some AA observed in sRSM relative to RSE (Harvest 2013) is in agreement with other data on impact of heat treatments (Newkirk et al., 2003a; Villanea, 2017), and was consistent between the poultry and the pig studies undertaken here. Moreover, values for the SID of AA for the RSE, sRSM and RSM samples tested are in agreement with previously published values for rapeseed meal, processed under varying conditions (Adedokun et al., 2008; Stein et al., 2005; Almeida et al., 2014; Li et al., 2015). There were significant variations in AID and SID of individual AA due to the effect of rapeseed variety within the sRSM samples. As such, PR46W21 sRSM emerged as having the greatest SID AA among the sRSM samples tested, which was as high as or even greater than SID AA in RSE from the four rapeseed varieties used. For Harvest 2014, PR46W21 was similarly superior in SID AA compared to DK Cabernet. The variation in AA digestibility between varieties and processing was unexpectedly not correlated with the significant variation in glucosinolate content observed. This is especially illustrated by AA digestibility in V2750L sRSM, which despite having the greatest level of glucosinolate (47.4 µmol/g DM) also had one of the greatest levels of SID AA among all meals tested, for both pig and poultry studies. Overall, these outcomes would support the view that variation in glucosinolate from current OSR varieties is unlikely to be limiting nutritional value of resulting RSM co-products. For Harvest 2013, we also observed that SID did not differ between DK Cabernet RSM1 and DK Cabernet RSM2. Taken together, this implies that both type of processing and rapeseed variety influence the digestibility of individual AA in the rapeseed co-products, but that site of seed production may be of minor influence, at least within the currently used locations.

The AA content varied between Harvest 2013 and Harvest 2014 samples for both DK Cabernet and PR46W21, but the effect of harvest year on SID AA was not significant. Although based on a small number of varieties, such data would support the view that the impact of prolonged storage under adequate conditions on digestibility of RSM AA is limited. A recent increase in small- and medium oil plants focusing on production of high quality virgin oil (Ghazani et al., 2014) is giving new perspectives to parallel delivery a rapeseed co-product with high quality rapeseed protein, potentially derived from a singular rapeseed variety. The consistent selection of rapeseed variety and processing is important to decrease the variation in chemical composition of co-products as well as deliver a product with a consistent nutritional value.

An effective way to improve the nutritional value of protein is denaturation of native protein. However, extensive heating may cause AA damage (Gonzalez-Vega et al., 2011). During the production of RSM, the thermal treatment is applied from the beginning of conditioning the seed, through seed crushing until hexane extraction and desolventisation. Such prolonged exposure to heat leads to occurrence of Maillard reactions, which causes binding of the protein-bound lysine and

reducing sugars, and forms deoxyketosyl-lysine derivatives as lactulosyl-lysine (Hurrell, 1990). Purcell and Walter (1982) showed that besides the loss of lysine, thermal treatment can also reduce the content of tryptophan, as in the case of heat-treated sweet potatoes. Also, variations in thermal conditions in the oil extraction methods can also result in changes in the content of crude fat and NDF in the meal (Keith and Bell, 1991; Spragg and Mailer, 2007; Li et al., 2015). This might overall contribute to override the effect of cultivation, environment or rapeseed variety on the chemical composition of rapeseed co-products.

In agreement with the above, the standard processing used for Harvest 2014 samples caused a reduction in lysine in both RSM, but also substantially decreased the tryptophan content, especially for PR46W21 RSM compared to its sRSM counterpart. As degradation of lysine and tryptophan may occur in the cooking step and/or seed crushing prior to hexane extraction and desolventisation, the application of soft processing might prevent partially the loss of AA in the final meal. The variety of PR46W21 showed a greater content of most AA compared to DK Cabernet. This further implies that the selection of oil seed rape variety has the potential to enhance the chemical composition of the resulting defatted meal.

The PR46W21 variety showed a very similar content of NDF in both meals when processed by both soft and standard method. However, the NDF content increased in RSM compared to DK Cabernet sRSM. This was possibly due to a greater thermal treatment of DK Cabernet RSM (desolventisation temperature ~116 °C), which may have led to a reduction in CP, protein solubility and increased the NDF content. The latter is consistent with other data (Almeida et al 2014) and may have arisen from increased levels of protein that upon heat treatment bind to fibre and is recovered in the NDF fraction (Nia and Ingalls, 1992). The fibre fraction in OSR co-products mainly originates from the OSR hulls that contain pectin, cellulose, hemicellulose and β-glucan (Bell, 1984). These fibre components might also entrap the components and/or elevate the viscosity of ileal digesta (Kasprzak et al., 2012), which can possibly reduce the digestion of dietary AA. Indeed, strong negative relationships between the content of NDF and SID of AA in rapeseed co-products were observed. As such, NDF content in rapeseed co-products might be a good predictor for AA digestibility, although this was in our studies only in the case for pigs and not for poultry.

Rapeseed cooking and heat supply during crushing are crucial steps in rapeseed processing, as they improve de-oiling process making the oil extraction more efficient and cost effective but also reduce glucosinolate levels as observed in our studies. Similarly to variations in chemical composition in the meals, the standard processing of oil extraction simultaneously reduced the digestibility value of the meal, as observed in both poultry and pig studies. This reduction, however, was more pronounced for DK Cabernet than for PR46W21 in the Harvest 2014 samples. This further suggests that varieties such as PR46W21 may result in RSM co-products of superior nutritional value in terms of AA digestibility, especially if heat intensity during de-oiling can be reduced. However, because the latter will inevitably result in reduced oil recovery, a sector wide cost-benefit analysis would be needed to underpin such change.

5.3. Energy digestibility

The average AME and AMEn of the RSM assayed in the current study were 8.77 and 7.97 MJ/kg, respectively. This level of AME represents approximately 45% of the gross energy in the meal. The AME of RSM determined in the current study is similar to values reported earlier (Bell, 1993; Mandal et al., 2005; Woyengo et al., 2010). Although energy metabolisability was similar between sRSM and RSE, the greater AME content of RSE was due to its higher oil content, which was generally more than twice the oil content of sRSM (Harvest 2013). Availability of energy in feedstuffs is dependent on the balance of the energy yielding constituents in the feedstuff and factors that impede their utilisation. The generally low energy availability in our sRSM, RSM and RSE samples could also be due to the presence of such factors as pectic oligosaccharides and insoluble fibres (Khajali and Slominski, 2012), which may have negative effects on oil digestibility. De-hulling and consequent reduction in fibre content has been reported to increase AME of RSM but efforts to mitigate the negative effect of the fibres on AME by using exogenous enzymes have not be very successful (Slominski et al., 1994; Mandal et al., 2005).

There was similarity in energy retention, AME and AMEn contents in the sRSM and RSM varieties assayed in the current study. Although the varieties had high variability from a biochemical point of view, especially in their contents of phytic acid and glucosinolate, correlation analysis showed that these components were not associated with variability in AME content, although unexpectedly, a positive correlation was found between glucosinolate level and energy metabolisability. The main drivers of energy availability in RSM were their oil and GE contents which have correlation coefficient greater than 0.88. Consequently, it appears that the variation in the commonly considered anti-nutritional factors (such as tannin, phytic acid, glucosinolate, and sinapine) in modern varieties of RSM is unlikely to be constraining its energy nutritional value, which accords with aforementioned similar conclusions with regards to AA nutritional value. Therefore, energy availability will largely depend on content and ease of hydrolysis of the energy yielding fractions of the rapeseed meal, as similarly observed by Lee et al. (1995).

Whilst AME and AMEn did not greatly differ between varieties, they were markedly influenced by processing intensity. Although Aljuobori et al. (2014) showed that extruded canola meal had greater ileal digestible energy compared with un-extruded meal, the difference appeared to emanate from differences in gross energy and fibre contents rather than the effect of processing *per se*. Chemical analysis showed that the difference in processing influenced the oil content of the meal. Generally, the meals that underwent the conventional processing had at least 20% less oil than the counterpart with milder processing. Because the cooking step occurs during the preparation of the seeds for extraction, and not during the oil extraction phase, it appears that the application of heat led to enhanced ability to completely extract oil from the seed and hence, reducing the value of the meal as an energy source. Nevertheless, although oil is the major contributor to gross energy content of the meal, there is also negative effect of additional heat treatment on energy metabolisability, the

latter being the greatest in our RSE samples, reduced in our sRSM samples and the lowest in our RSM samples. Consequently, it is the combination of the effects of the processing on oil content and energy metabolisability that ultimately influenced the AME content of the meals; in this case, cold processing resulting in greater AME compared to soft processing, which in turn, was greater than for standard processing.

5.4.   Relative bioavailability of phosphorus

There is a considerable amount of P in RSM and when it is added at high levels in the diets, can contribute a sizeable amount of P. However, as with other plant feedstuffs, half or more of the total P is the in the form of phytate P (Bell, 1993; Olukosi et al., 2015). Because P is a critical mineral for growth, the provision of extra P by inclusion of incremental levels of RSM resulted in enhanced growth performance and tibia ash, relative to the control treatment. The bioavailable P content was generally greater for sRSM compared to RSM, whilst this difference was wider for DK Cabernet than for PR46W21. Olukosi et al. (2015) reported that the coefficient of true P digestibility of P was 0.425 for conventionally processed RSM of DK Cabernet variety. The digestible content was calculated to be 4.39 g/kg. In the current study, the bioavailable P content for DK Cabernet processed using the mild processing technique was 3.88 g/kg. Because not all the digestible P will be ultimately available, the value calculated for bioavailable P is in line with expectation from its digestible P content and is similar to values reported by Khajali and Slominski (2012).

Processing can impact on P bioavailability. In a study with barley and wheat, Carlson and Poulsen (2003) observed that heat treatment inactivated the plant phytase and this negatively affect P availability although plant phytase in rapeseed is generally low. On the other hand, heat treatment has been shown to improve P bioavailability in maize-DDGS (Amezcua et al. 2004; Amezcua and Parsons, 2007). Heat treatment generally decreased phytate P (Khan et al. 1991) but heat application can also reduce P extractability as demonstrated in autoclaved soybean meal (Chompreeda and Fields, 1984). The reduced extractability was suggested to be due to possible complex formation with P leading to reduced P availability. It has also been shown that heat treatment decreased phytate P digestibility in other animals (Park et al., 2000). It can be expected that the effect of heat treatment on P availability is feedstuff-dependent but negative effect of additional heat application during processing was evident in P bioavailability of RSM used in the current study.

5.5.   Growth performance and upper inclusion limits

In this program of work, two growth performance trials were carried out, one with broiler chickens and one with growing pigs. The aim was to identify the upper limit of RSM inclusion whilst replacing SBM. Striking differences were observed between these two classes of animals with respect to their response to increasing levels of RSM derived from DK Cabernet and PR46W21. For

these studies, we used meals that were de-oiled under standard conditions, so that outcomes would be most applicable to current industry standards, where RSM are produced with heat treatments throughout the extraction process.

The broiler trial showed that weight gain decreased and FCR increased in a linear fashion with addition of RSM in wheat-SBM based diets. There was 8.9g loss in body weight gain with every 1 g/kg inclusion of RSM from PR46W21 and a greater loss for DK Cabernet, at 11.2g loss in body weight gain with every 1 g/kg inclusion. Similar depression in broiler growth performance following inclusion of RSM in broiler diets has been observed by others (Woyengo et al., 2011; Aljuobori et al., 2014). Woyengo et al. (2011) observed deterioration of growth performance and FCR with increased supplementation of expeller extracted canola meal in their study. Others have suggested that factors such as high glucosinolates content of RSM may be a factor in reduced growth performance. However, in view of the fact that the glucosinolate content is much less in modern varieties, and we failed to observe strong negative correlations between glucosinolate and both AA and energy digestibility, the impact of this class of compounds alone is likely to be very small if at all (Khajali and Slominski, 2012).

At face value, the above results could support the view that RSM is not well tolerated by broiler chickens, and should be avoided. However, it must be noted that in the starter phase of the current study, broiler chickens receiving 50 and 100 g/kg RSM or 80 g/kg unprocessed rape seed in their diets performed at, or above, Ross 308 target performance. Consequently, significant decrease in growth performance was more pronounced at dietary inclusion of 150 and 200 g/kg RSM. Furthermore, all broilers performed well above breed target during the finisher phase and consequently during the overall experiments, and arguably those fed PR46W21 performing slightly better than those fed DK Cabernet during the finisher phase. This indicates that the birds were able to tolerate very well the components in their diet, provided diets were fed that meet their nutrient requirement. This proposition needs to be tested under more challenging environments, including through field trials. In the current study, all the diets were formulated on the basis of SID AA and were iso-energetic. Part of the reduction in growth performance may have been due to the decrease in feed intake which may influence intake of nutrient and thus depress growth performance especially during the early growing phase.

The pig study showed that grower pig performance was significantly reduced at 15 and 25% RSM inclusion. This is in agreement with a series of other studies suggesting that replacing more than 50% of the SBM with RSM would be detrimental for performance in grower pigs (see Mejicanos et al (2016) for a recent review). In our study, it was clear that the reduced performance was directly related to the reduction in intake, as impact on FCR was not significant. The latter supports the view that nutrient digestibility of the whole meal was not affected by RSM inclusion. It is of interest to note that the reduction in intake was greater for PR46W21 than for DK Cabernet, with the former having a greater glucosinolate level than the latter. Thus, whilst our digestibility studies show no real effect of glucosinolate, intake depression is still a key concern for younger pigs, as well as for poultry. It

should also be noted that our grower pig trial started at a mean body weight of ~40 kg rather than the 30 kg originally planned. It might be expected that had the latter been achieved, the negative impact on intake could have been even greater. We do not know, however, where between 5 and 15% RSM inclusion the threshold might be.

In contrast to the younger pigs, the finisher pigs, with initial body weight of about 60 kg, did not respond to SBM replacement with RSM. There was no effect on intake, weight gain and FCR, and if anything, there was a suggestion that at greatest inclusion level of RSM (25%, and in the absence of SBM), FCR was improved. The recent review of Mejicanos et al (2016) supports the view that finisher pigs may indeed be less sensitive to RSM inclusion. It should also be noted that the diets for the grower and finisher pigs were formulated using analysed AA levels, and using book values for digestibility (Hazzledine, 2008), the slightly greater levels of AA in PR46W21 thus attracted less pure AA to balance for ideal protein. Had we been able to use our own pig digestibility data, which was not available yet at the time of the pig performance trial, our feeds would have attracted even less pure AA to balance for ideal protein. Taking this work into larger trials, including replicated field trials, would allow to translate these findings into novel commercial guidelines on upper use of RSM inclusions for fattening pigs.

The significant difference in SID AA digestibility between DK Cabernet and PR46W21 observed in our digestibility studies did not result in variation in FCR in the pig growth trial, though there were some indications that FCR in poultry was better on PR46W21 than on DK Cabernet. This accords with the findings of a recent study on the impact of primary and secondary processing on RSM nutritional quality (Villanea, 2017), which supports the view that pelleting can to a large extent reverse the heat-induced reduction in AA digestibility for pigs. Consistent with this position, our digestibility studies employed meals whilst the performance studies used pellets. Future work may be directed in identifying whether varietal differences in digestibility remain post pelleting, and in this respect poultry may be more sensitive to detect such variation that pigs.

An increased rate of RSM feeding to pigs could, in some circumstances, increase erucic acid intake. Breeding effort to reduce the erucic acid content in rapeseed oil was stimulated by historic health concerns. In 1981, hundreds of deaths and many more poisonings of people in Spain were linked to consumption of rapeseed oil (McMichael, 1981) and erucic acid was implicated as the causative agent (James, 1994). This view was discredited when samples of the oil subsequently showed that it was a mix of vegetable oils and animal fats, treated with aniline for industrial purposes (Tabuenca, 1981; Gollob, 1981). However, clinical studies have demonstrated an association between dietary erucic acid and myocardial lipidosis in a number of species, which is reported to reduce the contractile force of heart muscle. For pigs, a no-observable-effect-limit (NOEL) of 750 mg per kg body weight per day is considered appropriate, based on the occurrence of myocardial lipidosis at 900 mg per kg body weight per day (Kramer and Sauer, 1983). The current erucic acid content standard in the oil of food-use varieties is 2% or less, compared with 50% in the early types and many new varieties are tested with less than 0.1% erucic acid. Assuming rapeseed oil has an

averaged 0.50% erucic acid (Premier Nutrition, 2008) and 5% residual oil, as observed in our RSM samples, meal erucic acid levels could be ~0.25 g/kg. This would indicate our 40 kg grower pigs and 60 kg finisher pigs would have needed to ingest ~25 times more RSM than they did in our experiments to approach erucic acid NOEL. However, a small proportion of the OSR crop (<5%) retains a high erucic fatty acid profile in the oil, which is used for a number of industrial applications, with modern varieties used for this purpose having erucic acid contents in the 50-55% range. Consequently, erucic acid content of the resulting meal could exceed 25g/kg and at intakes observed in our study, NOEL would have been reached. Since RSM used in practice is derived from a combination of many varieties, impact of a small proportion of high erucic acid varieties will have been diluted. However, erucic acid in vegetable oil and as a residual presence in RSM remains topical, with a number of reports of apparent contamination of double low rape crops with an, as yet unidentified, source, or sources of erucic acid in Harvest 2015. Erucic acid content is not routinely assessed in variety trials because of the degree to which erucic acid content is influenced by cross pollination from neighbouring plots. However, it might be useful to consider developing an erucic assay as part of a RSM quality assurance program to safeguard pig health from greater utilisation of RSM as indicated from this program of work.

5.6.   Overall conclusions and implications

This program of work has provided detailed information on the sensitivity of OSR co-products biochemistry and nutritional value to OSR variety, processing conditions and their interaction. The main conclusions are summarized here as a number of bullet points.

- OSR meals from modern varieties of OSR continue to display significant variation in nutritional factors (e.g. levels amino acid and residual oil) and commonly suggested anti-nutritional factors (tannins, phytic acid, glucosinolate and sinapine). Variation in protein quality in terms of amino acids composition is rather small.

- Selection of rapeseed varieties and extraction methods has a potential to deliver high protein dietary ingredients with a good digestibility value.

- The data support the view that modification of thermal treatment during hexane-based oil extraction might improve nutritional value of rapeseed meals, in terms of amino acid, energy and phosphorus availability.

- The between-variety variation in tannin, phytic acid, glucosinolate and sinapine in modern OSR varieties is below a threshold to negatively impact on amino acid and energy availability. Therefore, amino acid and energy availability largely depends on their content in OSR co-products. However, reducing fibre levels in OSR meals would be expected to improve nutritional quality.

- Inclusion of RSM reduced feed intake in broilers and growing pigs, most clearly above a threshold of 100 g/kg for broilers and 50 g/kg for growing pigs, but not for finishing pigs fed

RSM up to 250 g/kg diet. However, both broilers and pigs all performed above breed and commercial targets at any RSM inclusion level.

The overall implications of our work are that there is certainly room to improve nutritional value of OSR co-products through amending oil extraction processes and variety selection, with key informants being amino acid, residual oil and fibre levels as classically considered key plant secondary metabolites did not inform on digestibility within currently available and tested varieties. Intake constraints remain for the more sensitive stock, indicating upper limits for broilers to be ~100 g/kg and for growing pigs between 50 and 150 g/kg. However, for finishing pigs, upper limit may be well above 250 g/kg. Future work may extend candidate biochemical markers to include glucosinolate breakdown products and erucic acid as predictors for nutritional value and meal safety, to assess impact of secondary processing on heat-induced reduction in nutritional value, to extend performance trials under more challenging conditions, including through replicated field trials, and to undertake a sector-wide cost-benefit analysis on scenarios that trade-off oil yield for nutritional value of OSR co-products.

6. References

Adedokun SA, Adeola O, Parsons CM, Lilburn MS, Applegate TJ, 2008. Standardized ileal amino acid digestibility of plant feedstuffs in broiler chickens and turkey poults using a nitrogen-free or casein diet. Poult Sci 87, 2535-2548.

Aljuobori A, Zulkifli I, Farjam AS, Abdullah N, Liang JB, 2014. Extrusion enhances metabolizable energy and ileal amino acids digestibility of canola meal for broiler chickens. It J Anim Sci 13, 3032 – 3035.

Almeida FN, Htoo JK, Thomson J, Stein HH, 2014. Effects of heat treatment on the apparent and standardized ileal digestibility of amino acids in canola meal fed to growing pigs. Anim Feed Sci Tech 187, 44-52.

Amezcua CM, Parsons CM, 2007. Effect of heat processing and particle size on phosphorus bioavailability in corn distillers dried grains with solubles. Poultry Sci 86, 331 - 337.

Amezcua CM, Parsons CM, Noll SL, 2004. Content and relative bioavailablity of phosphorus in distillers dried grains with solubles in chicks. Poultry Sci 83, 971 – 976.

APHA (2009) United Kingdom National List Trials: Protocol for official examination of value for cultivation and use (VCU) – Oilseed Rape.

Bell JM, Keith MO, 1991. A survey of variation in the chemical composition of commercial canola-meal produced in Western Canadian crushing plants. Can J Anim Sci 71, 469-480.

Bell JM, 1984. Nutrients and toxicants in rapeseed meal: A review. J Anim Sci 58, 996-1010.

Bell JM, 1993. Factors affecting the nutritional value of canola meal: A review. Can J Anim Sci 73, 679-697.

Butler LG, Price ML, Brotherton JE, 1982. Vanillin assay for proanthocyanidins (condensed tannins) - Modification of the solvent for estimation of the degree of polymerization. J Agri Food Chem 30, 1087-1089.

Cai R, Arntfield SD, 2001. A rapid high-performance liquid chromatographic method for the determination of sinapine and sinapic acid in canola seed and meal. JAOCS 78, 903-910.

Carlson D, Poulsen HD, 2003. Phytate degradation in soaked and fermented liquid feed – effect of diet, time of soaking, heat treatment, phytase activity, pH and temperature. Anim Feed Sci Technol 103, 141–154.

Chalmers AG, 1989. Autumn and spring nitrogen requirements for winter oilseed rape. Aspects Appl Biol 38, 125-134.

Chompreeda PT, Fields ML, 1984. Effect of heat and fermentation on the extractability of minerals from soybean meal and corn meal blends. J Food Sci 49, 566-568.

Clandini DR, Slinger SJ, Bell JM, 1972. Composition of Canadian rapeseed meal. Rapeseed Association Canada, Publ. 16, 8-15.

Clandini DR, 1967. Nutrient composition of expeller prepress-solvent and solvent processed rapeseed meal. Poultry Sci 46, 1596-1597.

Commission Directive, 2000. Establishing community methods for the determination of vitamin A, vitamin E and tryptophan, annex part C. Determination of Tryptophan. Off J Eur Comm L174, 45–50.

Downey RK, Bell JM, 1990. New developments in canola research. In Canola and Rapeseed – Production, Chemistry, Nutrition and Processing Technology (ed F Shahidi). Van Nosytand Reinhold, New York, NY, USA. pp 37-46.

EFSA, 2008. Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission on glucosinolates as undesirable substances in animal feed, The EFSA J 590, 1-76.

Eklund M, Sauer N, Schone F, Messerschmidt U, Rosenfelder P, Htoo JK, Mosenthin R, 2015. Effect of processing of rapeseed under defined conditions in a pilot plant on chemical composition and standardized ileal amino acid digestibility in rapeseed meal for pigs. J Anim Sci 93, 2813-2825.

Fan MZ, Sauer WC, Gabert VM, 1996. Variability of apparent ileal amino acid digestibility in canola meal for growing-finishing pigs. Can J Anim Sci 76, 563-569.

Fenwick RC, Curtis RF, 1980. Rapeseed meal and its use in poultry diets: A review. Anim Feed Sci Technol 5, 255-298.

Ghazani, SM, Garcia-Llatas G, Marangoni AG, 2014. Micronutrient content of cold-pressed, hot-pressed, solvent extracted and RBD canola oil: Implications for nutrition and quality. Eur J Lipid Sci Tech 116, 380-387.

Gollob D, 1981. Hydrolytic enzyme activities, mainly from lysosomal localization, in sera from patients who ingested a toxic oil. Lancet 2, 1102.

Gonzalez-Vega JC, Kim BG, Htoo JK, Lemme A, Stein HH, 2011. Amino acid digestibility in heated soybean meal fed to growing pigs. J Anim Sci 89, 3617-3625.

Gordon, 2005. Optimising the use of home-grown oilseeds and pulses as protein sources in feeds for table chickens. HGCA Project 2365; Project Report No. 360; 219 p.

Hagerman AE, 2011. The Tannin Handbook.

Hazzledine M, 2008. Premier Atlas Ingredients Matrix. Rugeley: Premier Nutrition Products Ltd.

Houdijk JGM, Smith LA, Tarsitano D, Tolkamp BJ, Topp CEF, Masey-O'Neill H, White G, Wiseman J, Kightley S, Kyriazakis I, 2013. Peas and faba beans as home-grown alternatives for soya bean meal in grower and finisher pig diets. In: Rec Adv Anim Nutr 2013, Edited by P.C. Garnsworthy and J. Wiseman. Nottingham University Press, Nottingham, pp. 145-175.

Hurrell RF, 1990. Influence of the Maillard reaction on the nutritional value of foods. In: The Maillard reaction in food processing, human nutrition and physiology. Eds Finot PA, Aeschbacker HU, Hurrell RF, Liardon R. Birkhauser Verlag, Boston. pp 245-258.

James TN, 1994. The toxic oil syndrome. Clin cardiol 17, 463-470.

Jansman AJM, Smink W, van Leeuwen P, Rademacher M, 2002. Evaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. Anim Feed Sci Tech 98, 49-60.

Kasprzak MM, Laerke HN, Knudsen KEB, 2012. Effects of isolated and complex dietary fiber matrices in breads on carbohydrate digestibility and physicochemical properties of ileal effluent from pigs. J Agri Food Chem 60, 12469-12476.

Keith MO, Bell JM, 1991. Composition and digestibility of canola press cake as a feedstuff for use in swine diets. Can J Anim Sci 71, 879-885.

Khajali F, Slominski BA, 2012. Factors that affect the nutritive value of canola meal for poultry. Poultry Sci. 91, 2564-2575.

Khan N, Zaman R., Elahi M, 1991. Effect of heat treatments on the phytic acid content of maize products. J Sci Food Agric 54, 153-156.

Kliebenstein DJ, Kroymann J, Mitchell-Olds T, 2005. The glucosinolate-myrosinase system in an ecological and evolutionary context. Curr Opinion Plant Biol 8, 264-271.

Kramer JKG, Sauer FD, 1983. Results obtained with feeding low erucic acid rapeseed oils and other vegetable oils to rats and other species. In: High and low erucic acid rapeseed oils. Production, usage, chemistry, and toxicological examination. (Eds Kramer JKG, Sauer FD, Pigden WJ). Academic Press, Toronto, Canada, pp 413–474.

Landero JL, Beltranena E, Cervantes M, Morales A, Zijlstra RT, 2011. The effect of feeding solvent-extracted canola meal on growth performance and diet nutrient digestibility in weaned pigs. Anim Feed Sci Tech 170, 136-140.

Landero JL, Beltranena E, Cervantes M, Airaza AB, Zijlstra RT, 2012. The effect of feeding expeller-pressed canola meal on growth performance and diet nutrient digestibility in weaned pigs. Anim Feed Sci Technol 171, 240-245.

Lee K, Qi G, Sim JS, 1995. Metabolizable energy and amino acid availability of full fat seeds, meals and oils of flax and canola. Poultry Sci 74, 1341-1348.

Leinonen I, Williams AG, Wiseman J, Guy J, Kyriazakis I, 2012. Predicting the environmental impacts of chicken systems in the United Kingdom through a life cycle assessment: broiler production system. Poultry Sci 91, 8-25.

Leslie AJ, Summer JD, 1975. Amino acid balance of rapeseed meal. Poultry Sci 54, 532 – 538.

Leslie AJ, Summers JD, Grandhi R, Leeson S, 1976. Arginine-lysine relationship in rapeseed meal. Poultry Sci 55, 631-637.

Li J, El Rassi Z, 2002. High performance liquid chromatography of phenolic choline ester fragments derived by chemical and enzymatic fragmentation processes: Analysis of sinapine in rape seed. J Agri Food Chem 50, 1368-1373.

Li PL, Wu F, Chen YF, Wang JR, Guo PP, Li ZC, Liu L, Lai CH, 2015a. Determination of the energy content and amino acid digestibility of double-low rapeseed cakes fed to growing pigs. Anim Feed Sci Tech 210, 243-253.

Li PL, Wang FL, Wu F, Wang JR, Liu L, Lai CH, 2015b. Chemical composition, energy and amino acid digestibility in double-low rapeseed meal fed to growing pigs. JASB 6, 1-10.

Liu Y, Song M, Maison T, Stein HH, 2014. Effects of protein concentration and heat treatment on concentration of digestible and metabolizable energy and on amino acid digestibility in four sources of canola meal fed to growing pigs. J Anim Sci 92, 4466-4477.

Llames CR, Fontaine J, 1994. Determination of amino acids in feeds: collaborative study. J AOAC Int 77, 1362–1402.

Maison T, Stein HH, 2014. Digestibility by growing pigs of amino acids in canola meal from North America and 00-rapeseed meal and 00-rapeseed expellers from Europe. J Anim Sci 92, 3502-3514.

Mandal AB, Elangovan AV, Tyagi PK, Tyagi PK, Johri AK, Kaur S, 2005. Effect of enzyme supplementation on the metabolisable energy content of solvent-extracted rapeseed and sunflower seed meals for chicken, guinea fowl and quail. Br Poultry Sci 46, 75-79.

Masey-O'Neill HV, Rademacher M, Mueller-Harvey I, Stringano E, Kightley S, Wiseman J, 2012. Standardised ileal digestibility of crude protein and amino acids of UK-grown peas and faba beans by broilers. Anim Feed Sci Tech 175, 158-167.

Masey-O'Neill, HV, White GA, Li D, Bedford MR, Htoo JK, Wiseman J, 2014. Influence of the in vivo method and basal dietary ingredients employed in the determination of the amino acid digestibility of wheat distillers dried grains with solubles in broilers. Poultry Sci 93, 1178-1185.

McNeill L, Bernard K, Macleod MG, 2004. Food intake, growth rate, food conversion and food choice in broilers fed on diets high in rapeseed meal and pea meal with observations of the resulting poultry meat. Br Poultry Sci 45, 519-523.

Mejicanos G, Sanjayan N, Kim IH, Nyachoti CM, 2016. Recent advances in canola meal utilization in swine nutrition. J Anim Sci Technol 58, 7.

Messerschmidt U, Eklund M, Sauer N, Rist VTS, Rosenfelder P, Spindler HK, Htoo JK, Schone F, Mosenthin R, 2014. Chemical composition and standardized ileal amino acid digestibility in rapeseed meals sourced from German oil mills for growing pigs. Anim Feed Sci Tech. 187, 68-76.

Newkirk RW, Classen HL, Edney MJ, 2003a. Effects of prepress-solvent extraction on the nutritional value of canola meal for broiler chickens. Anim Feed Sci Tech 104, 111-119.

Newkirk RW, Classen, HL, Scott, TA, Edney, MJ, 2003b. The digestibility and content of amino acids in toasted and non-toasted canola meals. Can J Anim Sci 83, 131-139.

Nia SAM, Ingalls JR, 1992. Effect of heating on canola meal protein degradation in the rumen and digestion in the lower gastrointestinal tract of steers. Can J Anim Sci 72, 83-88.

NIAB, 2001. Glucosinolate content of oilseed rape seed. Oilseeds Trials Advisory Committee. Paper No. 197.

Nwokolo E, Bragg DB, Kitts WD, 1976. The availability of amino acids from palm kernel, soybean, cottonseed and rapeseed meal for the growing chick. Poultry Sci 55, 2300-2304.

Olukosi OA, Combemorel C., Kightley S, Wiseman J, Houdijk JGM, 2015. True digestibility of phosphorus determined by regression method for double zero rapeseed meal. Livest Sci 182, 8-10.

Park WY, Matsui T, Yano F, Yano H, 2000. Heat treatment of rapeseed meal increases phytate flow into the duodenum of sheep. Anim Feed Sci Technol 88, 31 – 37.

Parr CK, Liu Y, Parsons CM, Stein HH, 2015. Effects of high-protein or conventional canola meal on growth performance, organ weights, bone ash, and blood characteristics of weanling pigs. J Anim Sci 93, 2165-2173.

Purcell AE, Walter WM, 1982. Stability of amino acids during cooking and processing of sweet potatoes. J Agri Food Chem 30, 443-444.

Quinsac A, Carre P, Fauconnier M-L, Garrioux J, Peronnet C, Pignolet C, Trisman D, Wathelet J-P, 2015. Glucosinolates and by-products in rapeseed meal related to hydrothermal processing. 14th International Rapeseed Congress, Saskatoon.

Rao PV, Clandinin DR, 1970. Effect of method of determination on the metabolisable energy vale of rapeseed meal. Poultry Sci 49, 1069-1074.

Rask L, Andreasson E, Ekbom B, Errikson S, Pontoppidan B, Meijer J, 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol Biol 42, 93-113.

Rezvani M, Kluth H, Bulang M, Rodehutscord M, 2012. Variation in amino acid digestibility of rapeseed meal studied in caecectomised laying hens and relationship with chemical constituents. Brit Poultry Sci 53, 665-674.

Sell JL, 1966. Metabolizable energy of rapeseed meal for the laying hen. Poultry Sci 45, 854-856.

Seneviratne RW, Beltranena E, Goonewardene LA, Zijlstra RT, 2011a. Effect of crude glycerol combined with solvent-extracted or expeller-pressed canola meal on growth performance and diet nutrient digestibility of weaned pigs. Anim Feed Sci Tech 170, 105-110.

Seneviratne RW, Beltranena E, Newkirk RW, Goonewardene LA, Zijlstra RT, 2011b. Processing conditions affect nutrient digestibility of cold-pressed canola cake for grower pigs. J Anim Sci 89, 2452-2461.

Short FJ, Gordon P, Wiseman J, Boorman KN, 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim Feed Sci Tech 59, 215-221.

Slominski BA, Campbell LD, Guenter W, 1994. Oligosaccharides in canola meal and their effect on nonstarch polysaccharide digestibility and true metabolisable energy in poultry. Poultry Sci. 73, 156 – 162.

Smith LA, Houdijk JGM, Homer D, Kyriazakis I, 2013. Effects of dietary inclusion of peas and faba beans as a replacement for soybean meal on grower and finisher pig performance and carcass quality. J Anim Sci 91, 3733-3741.

Spragg J, Mailer R, 2007. Canola meal value chain quality improvement. Australian Oilseed Federation and Pork CRC.

Stein HH, Pedersen C, Wirt AR, Bohke RA, 2005. Additivity of values for apparent and standardized ileal digestibility of amino acids in mixed diets fed to growing pigs. J Anim Sci 83, 2387-2395.

Summers JD, Rajaratnam G, Pepper WF, 1971a. Evaluation of rapeseed meal as a protein supplement for laying hen diets. Poultry Sci 50, 1382-1386.

Summers JD, Pepper WF, Wood AS, 1971b. The value of rapeseed meal in broiler breeder diets. Poultry Sci 50, 1387-1391.

Tabuenca JM, 1981. Toxi-allergic syndrome caused by ingestion of rapeseed oil denatured with aniline. Lancet 2, 567–568.

Thacker PA, 1990. Non-traditional Feed Sources for Use in Swine Production. Butterworths, NY.

Tripathi MK, Mishra AS, 2007. Glucosinolates in animal nutrition: A review. Anim Feed Sci Technol 132, 1-27.

Toghyani M, Rodgers N, Iji PA, Swick RA, 2015. Standardized ileal amino acid digestibility of expeller-extracted canola meal subjected to different processing conditions for starter and grower broiler chickens. Poultry Sci 94, 992-1002.

Villanea S, 2017. Of proteins and processing. Mechanisms of protein damage upon rapeseed processing and their effects on nutritional value. PhD Thesis, Wageningen University.

White G, Smith LA, Houdijk JGM, Kyriazakis I, Wiseman J, 2015. Replacement of soya bean meal with peas and faba beans in growing / finishing pig diets: effect on performance, carcass composition and nutrient excretion. Anim Feed Sci Technol 209, 202-210.

Woyengo TA, Kiarie E, Nyachoti CM, 2010. Metabolizable energy and standardized ileal digestible amino acid contents of expeller-extracted canola meal fed to broiler chicks. Poultry Sci 89, 1182-1189.

Woyengo TA, Kiarie E, Nyachoti CM, 2011. Growth performance, organ weights, and blood parameters of broilers fed diets containing expeller-extracted canola meal. Poultry Sci 90, 2520 – 2527.

7. Tables

7.1. Seed biochemical analyses Harvest 2012

Table 1: Summary of biochemical analyses of 22 cultivars of oilseed rape meals and whole seeds, from each of 5 UK trial locations (Harvest 2012).

	Overall mean	L.S.D.[2]	Range between site (mean of 22 varieties)		Range between variety (mean of 5 sites)		Overall range (22 varieties x 5 sites)	
			Min	Max	Min	Max	Min	Max
Meal content analysis[1]								
Protein (g/100g)	34.50	1.44	31.80	35.50	32.69	36.66	28.88	37.83
Total glucosinolate (µmol/g)	20.40	3.93	18.10	22.30	12.53	42.49	10.80	52.63
Tannin (mg/g)	1.59	0.42	1.05	2.24	1.03	1.90	0.28	3.21
Phytic acid (g/100g)	2.83	0.33	1.85	3.29	2.53	3.15	1.32	3.78
Sinapine (mg/g)	7.58	0.81	6.20	8.40	6.67	8.24	5.10	9.93
Whole seed analysis								
Oil content (%)	44.30	0.78	42.05	45.98	43.10	46.38	39.90	48.10
Total glucosinolate (µmol/g)	11.55	1.26	9.20	12.67	6.66	21.20	3.50	26.70

[1]On a dry matter basis

[2]Least Significant Difference (P<0.05).

7.2. Seed and meal biochemical analysis Harvest 2013

Table 2a. Analysis of whole seed received (WS) and meal samples prepared by Process 1 (bench cold hexane extraction of the milled seed - P1), Process 2 (French (CREOL) crushed and hexane extracted using mild heating - P2) or Process 3 (Commercial cold pressing - P3) for oil, crude protein and fibre, and crude protein solubility for P2 samples

ID	Variety	Oil content (g/kg)[1]			Crude protein (g/kg)[1]		Fibre (NDF) (g/kg)[1]	CP solubility (%)
		WS	P 1	P 2	P 1	P 2	P 2	P2
1/12	Ability	482	106	43	437	439	266	71.6
2/12	Avatar	469	93	35	379	392	255	58.5
3/12	Compass	502	69	28	331	387	283	53.1
4/12	DK Cabernet	480	127	28	348	367	279	50.9
5/12	DK Cabernet	457	57	28	348	371	281	49.6
6/12	Excalibur	457	73	27	377	398	260	54.0
7/12	Incentive	489	68	32	400	418	226	56.5
8/12	Palmedor	510	86	26	409	436	269	56.8
9/12	PR46W21	472	51	32	380	409	252	59.5
10/12	Quartz	483	129	29	366	390	266	50.0
11/12	Trinity	505	117	31	419	369	271	47.1
12/12	V2750L	461	66	40	388	414	271	59.6
	P1/P2 subset mean	481	87	32	382	399	265	55.6

ID	Variety	Oil content (g/kg)			Crude protein (g/kg)		Fibre (NDF) (g/kg)	
		WS	P 1	P 3	P 1	P 3	P 3	
1/4	Compass	501	72	242	332	309	239	
2/4	DK Cabernet	476	62	266	351	353	251	
3/4	NK Grandia	463	50	247	344	340	240	
4/4	Sesame	461	76	272	348	350	249	
	P1/P3 subset mean	475	65	257	344	338	245	
	P1 full set mean	479	81		372		260	

[1] All constituents expressed as dry matter basis.

Table 2b. Analysis of whole seed received (WS) and meal samples prepared by Process 1 (bench cold hexane extraction of the milled seed - P1), Process 2 (French (CREOL) crushed and hexane extracted using mild heating - P2) or Process 3 (Commercial cold pressing - P3) for glucosinolates, tannin, sinapine and phytic acid.

ID	Variety	Glucosinolate (µmoles/g)[1]			Tannin (mg/g CE)[1,2]		Sinapine (mg/g)[1]		Phytic acid (g/100g)[1]	
		WS	P 1	P 2	P 1	P 2	P 1	P 2	P 1	P 2
1/12	Ability	19.4	23.4	14.2	1.04	2.31	5.03	5.79	0.61	1.10
2/12	Avatar	17.6	30.0	11.3	1.04	2.28	5.03	8.05	0.71	1.31
3/12	Compass	10.1	13.7	7.4	1.94	2.25	6.97	6.59	1.21	2.59
4/12	DK Cabernet	16.7	25.3	14.4	0.98	2.29	4.58	6.41	0.50	1.62
5/12	DK Cabernet	18.4	35.4	12.7	1.32	2.40	6.19	5.37	0.64	1.71
6/12	Excalibur	22.0	49.0	21.6	0.93	2.54	5.49	5.69	1.03	2.40
7/12	Incentive	13.8	29.6	13.9	1.15	2.47	6.42	5.90	2.20	3.67
8/12	Palmedor	16.3	27.2	15.3	0.59	2.35	5.65	5.44	1.30	2.56
9/12	PR46W21	21.2	42.8	25.8	1.04	2.62	6.97	5.78	1.05	2.34
10/12	Quartz	16.3	26.5	10.0	1.26	2.55	4.75	4.61	1.48	2.38
11/12	Trinity	12.4	18.1	8.3	0.48	1.40	4.67	5.44	1.14	2.32
12/12	V2750L	33.6	70.4	47.4	1.65	1.38	6.48	5.18	1.14	1.73
	P1/P2 subset mean	18.2	32.6	16.9	1.12	2.24	5.69	5.86	1.09	2.14

ID	Variety	Glucosinolate (µmoles/g)			Tannin (mg/g cat eq)		Sinapine (mg/g)		Phytic acid (g/100g)	
		WS	P 1	P 3	P 1	P 3	P 1	P 3	P 1	P 3
1/4	Compass	9.4	12.8	11.1	2.22	1.30	8.89	5.24	2.07	1.81
2/4	DK Cabernet	15.5	29.7	14.8	1.38	1.18	7.08	5.53	2.22	1.98
3/4	NK Grandia	18.5	41.5	23.6	1.88	1.29	6.88	5.73	0.96	1.84
4/4	Sesame	19.9	37.1	20.5	1.60	1.23	6.98	5.59	0.78	2.23
	P1/P3 subset mean	15.8	30.3	17.5	1.77	1.25	7.46	5.52	1.51	1.97
	P1 full set mean	17.6	32.0		1.28		6.13		1.19	

[1] All constituents expressed as dry matter basis

[2] Catechin equivalents

7.3. Detailed glucosinolate analysis Harvest 2013

Table 3a. Analysis of meal samples prepared by Process 1 (bench cold hexane extraction of the milled whole seed, P1), Process 2 (French (CREOL) crushed and hexane extracted using mild heating, P2) or Process 3 (Commercial cold pressing, P3) for selected types of glucosinolates: progroitrin, gluconapin, 4OH-glucobrassicin and glucoalyssin.

ID	Variety	Progoitrin (µmoles/g)		Gluconapin (µmoles/g)		4OHglucobrassicin (µmoles/g)		Glucoalyssin (µmoles/g)	
		P 1	P 2	P 1	P 2	P 1	P 2	P 1	P 2
1/12	Ability	5.1	3.1	2.5	1.7	5.3	1.6	6.6	4.4
2/12	Avatar	11.3	4.1	5.1	2.0	4.4	0.2	2.0	2.2
3/12	Compass	4.7	3.1	2.5	1.4	3.8	0.6	0.0	0.0
4/12	DK Cabernet	9.8	6.2	5.6	3.6	3.9	0.8	0.0	0.0
5/12	DK Cabernet	15.8	6.4	6.8	2.9	4.9	0.2	0.0	0.2
6/12	Excalibur	22.1	10.9	10.5	5.6	4.4	0.4	0.0	0.0
7/12	Incentive	11.8	6.2	6.6	3.6	3.7	0.3	0.0	0.4
8/12	Palmedor	10.9	7.5	4.9	3.3	3.9	0.7	0.0	0.0
9/12	PR46W21	16.4	10.2	7.0	4.9	5.8	0.9	4.9	4.2
10/12	Quartz	10.7	5.0	8.2	1.9	1.9	0.2	0.0	0.0
11/12	Trinity	6.4	3.6	3.7	1.9	3.5	0.4	0.0	0.0
12/12	V2750L	24.5	16.2	12.3	8.1	5.2	1.1	10.5	12.2
	P1/P2 subset mean	12.5	6.9	6.3	3.4	4.2	0.6	2.0	2.0

ID	Variety	Progoitrin (µmoles/g)		Gluconapin (µmoles/g)		4OHglucobrassicin (µmoles/g)		Glucoalyssin (µmoles/g)	
		P 1	P 3	P 1	P 3	P 1	P 3	P 1	P 3
1/4	Compass	2.6	2.8	2.4	1.5	4.7	4.0	0.0	0.0
2/4	DK Cabernet	11.4	4.3	5.3	2.3	5.2	4.2	0.0	0.5
3/4	NK Grandia	15.4	5.9	6.0	2.7	6.5	4.6	6.0	5.2
4/4	Sesame	14.1	6.1	4.8	2.4	5.3	4.3	2.0	2.2
	P1/P3 subset mean	10.9	4.8	4.6	2.2	5.4	4.3	2.0	2.0
	P1 full set mean	12.1		5.9		4.5		2.0	

Table 3b. Analysis of meal samples prepared by Process 1 (bench cold hexane extraction of the milled whole seed, P1), Process 2 (French (CREOL) crushed and hexane extracted using mild heating, P2) or Process 3 (Commercial cold pressing, P3) for selected types of glucosinolates: glucobrassinapin, glucoraphanin, epi-progoitrin and glucobrassicin.

ID	Variety	Glucobrassinapin (µmoles/g)		Glucoraphanin (µmoles/g)		Epi-progoitrin (µmoles/g)		Glucobrassicin (µmoles/g)	
		P 1	P 2	P 1	P 2	P 1	P 2	P 1	P 2
1/12	Ability	0.0	0.0	0.0	0.3	0.0	0.0	0.2	0.1
2/12	Avatar	0.8	0.0	0.8	0.3	0.0	0.0	0.2	0.1
3/12	Compass	0.0	0.0	0.0	0.3	0.0	0.2	0.4	0.1
4/12	DK Cabernet	1.0	0.0	0.9	0.6	0.0	0.0	0.2	0.1
5/12	DK Cabernet	1.1	0.0	0.9	0.4	0.0	0.2	0.1	0.0
6/12	Excalibur	2.4	0.0	1.3	0.7	0.0	0.0	0.0	0.0
7/12	Incentive	0.6	0.0	0.8	0.4	1.2	0.0	0.3	0.1
8/12	Palmedor	2.0	0.0	0.9	0.5	0.0	0.3	0.3	0.1
9/12	PR46W21	1.0	0.0	0.8	0.5	0.0	0.4	0.2	0.1
10/12	Quartz	0.0	0.0	1.2	0.6	0.0	0.0	0.0	0.0
11/12	Trinity	0.7	0.0	0.5	0.2	0.0	0.0	0.1	0.1
12/12	V2750L	2.9	0.0	1.5	1.0	1.8	0.0	0.2	0.0
P1/P2 subset mean		1.0	0.0	0.8	0.5	0.2	0.1	0.2	0.1

ID	Variety	Glucobrassinapin (µmoles/g)		Glucoraphanin (µmoles/g)		Epi-progoitrin (µmoles/g)		Glucobrassicin (µmoles/g)	
		P 1	P 3	P 1	P 3	P 1	P 3	P 1	P 3
1/4	Compass	0.5	0.5	0.3	0.0	0.0	0.0	0.2	0.2
2/4	DK Cabernet	1.7	0.6	1.3	0.4	0.0	0.0	0.2	0.2
3/4	NK Grandia	0.9	0.0	0.0	0.0	0.0	0.0	0.4	0.2
4/4	Sesame	2.2	1.1	2.2	0.0	0.0	0.0	0.2	0.2
P1/P3 subset mean		1.3	0.6	1.0	0.1	0.0	0.0	0.3	0.2
P1 full set mean		1.1		0.8		0.2		0.2	

Table 3c. Analysis of meal samples prepared by Process 1 (bench cold hexane extraction of the milled whole seed, P1), Process 2 (French (CREOL) crushed and hexane extracted using mild heating, P2) or Process 3 (Commercial cold pressing, P3) for selected types of glucosinolates: gluconasturtiin, neoglucobrassicin, glucoberin and gluconapoliferin.

ID	Variety	Gluconasturtiin (µmoles/g)		Neoglucobrassicin (µmoles/g)		Glucoberin (µmoles/g)		Gluconapoliferin (µmoles/g)	
		P 1	P 2	P 1	P 2	P 1	P 2	P 1	P 2
1/12	Ability	0.0	0.2	0.0	0.0	0.0	0.3	0.0	0.0
2/12	Avatar	0.4	0.1	0.4	0.0	0.0	0.4	0.0	0.0
3/12	Compass	0.0	0.1	0.0	0.0	0.3	0.4	0.0	0.0
4/12	DK Cabernet	0.0	0.0	0.1	0.0	0.0	0.3	0.0	0.0
5/12	DK Cabernet	0.0	0.0	0.2	0.0	0.0	0.4	0.0	0.0
6/12	Excalibur	0.6	0.2	0.1	0.0	0.0	0.3	0.0	0.0
7/12	Incentive	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0
8/12	Palmedor	0.0	0.0	0.1	0.0	0.0	0.3	0.0	0.0
9/12	PR46W21	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
10/12	Quartz	0.5	0.2	0.0	0.0	0.0	0.5	0.0	0.0
11/12	Trinity	0.0	0.2	0.0	0.0	0.4	0.4	0.0	0.0
12/12	V2750L	0.8	0.0	0.0	0.0	0.0	0.6	0.0	0.0
P1/P2 subset mean		0.2	0.1	0.1	0.0	0.1	0.4	0.0	0.0

ID	Variety	Gluconasturtiin (µmoles/g)		Neoglucobrassicin (µmoles/g)		Glucoberin (µmoles/g)		Gluconapoliferin (µmoles/g)	
		P 1	P 3	P 1	P 3	P 1	P 3	P 1	P 3
1/4	Compass	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.4
2/4	DK Cabernet	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3/4	NK Grandia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9
4/4	Sesame	0.0	0.0	0.5	0.3	0.0	0.0	0.0	0.8
P1/P3 subset mean		0.0	0.0	0.2	0.1	0.0	0.0	0.0	0.5
P1 full set mean		0.1		0.1		0.0		0.0	

7.4. Loss of glucosinolates due to processing

Table 4: Average content of individual glucosinolate for the variety sample sets, before and after processing to produce meals from the hexane extraction and cold processing methods.

	Seeds			Meals		Percent loss	
	Cold hexane extracted			Mild hexane extracted	Cold pressed	Mild hexane extracted	Cold pressed
	All	P2	P3	P2	P3	P2	P3
Progoitrin	12.08	12.46	10.85	6.87	4.76	45	56
Gluconapin	5.91	6.30	4.62	3.42	2.21	46	52
4OHglucobrassicin	4.51	4.23	5.43	0.61	4.26	86	22
Glucoalyssin	1.99	1.99	2.01	1.97	1.96	1	2
Glucobrassinapin	1.10	1.04	1.31	0.00	0.56	100	57
Glucoraphanin	0.84	0.80	0.97	0.49	0.09	39	90
Epi-progoitrin	0.19	0.25	0.00	0.08	0.00	66	na
Glucobrassicin	0.21	0.19	0.27	0.07	0.21	65	21
Gluconasturtiin	0.14	0.19	0.00	0.08	0.00	57	na
Neoglucobrassicin	0.12	0.10	0.17	0.00	0.08	100	51
Glucoberin	0.04	0.06	0.00	0.35	0.00	-521	na
Gluconapoliferin	0.00	0.00	0.00	0.00	0.51	na	na

7.5. Seed biochemistry Harvest 2014 samples

Table 5: Analysis[1] of bulk samples of varieties DK Cabernet and PR46W21 (four replicates), sourced for meal preparation for growth studies (Harvest 2014). Values expressed on dry matter basis.

Source		Whole seed			Cold hexane-extracted milled seed					
		DM	Oil	GS	Oil	CP	GS	Sinapine	Tannin2	PA
		%	%	µmole/g	%	%	µmole/g	mg/g	mg/g	g/100g
DK Cabernet	1	92.1	51.1	7.7	6.1	34.1	16.8	3.45	1.40	1.71
	2	92.5	51.0	8.5	6.5	34.5	16.7	3.32	0.66	1.62
	3	92.6	51.2	8.0	7.5	33.9	17.0	3.33	1.59	1.44
	4	91.9	51.3	8.3	6.7	34.3	17.0	4.82	0.85	0.53
Mean		92.3	51.1	8.1	6.7	34.2	16.9	3.73	1.13	1.33
PR46W21	1	92.4	51.3	11.6	6.3	37.6	22.9	3.81	0.02	1.71
	2	92.7	51.8	11.0	6.2	37.9	22.1	3.67	0.85	0.90
	3	92.5	51.4	11.4	5.6	37.9	20.4	3.47	0.94	0.67
	4	92.8	51.2	11.7	5.7	38.0	20.6	3.43	0.20	2.15
Mean		92.6	51.4	11.4	6.0	37.9	21.5	3.59	0.50	1.35

[1]DM: dry matter; GS: total glucosinolates; CP: crude protein; PA: phytic acid.
[2]Catechin equivalents

7.6. Meal biochemistry Harvest 2014 samples

Table 6: Analysis[1] of meal samples from bulks of two oilseed rape varieties, DK Cabernet and PR46W21 supplied for nutritional studies. Whole seed analysis dry matter, oil and glucosinolate provided for reference. Values presented as dry matter basis.

	DM %	Oil %	GS µmole/g	CP %	Tannin2 mg/g	Sinapine mg/g	PA g/100g
DK Cabernet							
Whole seed	92.3	51.1	8.09				
sRSM	95.8	7.8	5.53	33.4	2.04	3.10	1.24
RSM	93.9	5.0	2.32	36.0	1.88	2.68	1.54
PR46W21							
Whole seed	92.6	51.4	11.39				
sRSM	89.5	5.8	5.96	39.0	2.04	3.22	2.18
RSM	93.0	4.6	3.91	40.1	2.15	3.32	2.16

[1]DM: dry matter; GS: total glucosinolates; CP: crude protein; PA: phytic acid.
[2]Catechin equivalents

7.7. Meal biochemistry Harvest 2013 for SID AA study

Table 7: Contents of dry matter, neutral detergent fibre, glucosinolates, crude protein, total amino acids and lysine to crude protein ratio in rapeseed cake and soft rapeseed meal (g/kg DM as not stated otherwise) of oilseed rape co-products used in boiler AA digestibility studies.

Variety	DM	NDF	GLS*	CP	TAA	Lys:CP**
RSE						
Compass	899	239	11.1	293	256	5.2
Sesame	890	249	20.5	332	293	5.5
NK Grandia	892	240	23.6	335	303	5.4
DK Cabernet	881	251	14.8	340	305	5.6
Average	890	245	17.5	325	289	5.6
SEM	3.6	3.2	2.81	10.7	11.4	0.83
sRSM						
DK Cabernet[1]	866	279	14.4	419	396	5.5
DK Cabernet[2]	864	281	12.7	457	411	5.2
Quartz	866	266	10.0	430	400	5.5
Trinity	868	271	8.3	443	399	5.3
Compass	848	283	7.4	468	386	4.9
Incentive	853	226	13.9	469	440	5.2
Excalibur	833	260	21.6	495	430	5.1
Avatar	856	255	11.3	495	410	4.9
PR46W21	822	252	25.8	507	453	5.4
Palmedor	859	269	15.3	517	451	5.1
V2750L	838	271	47.4	521	444	5.1
Ability	821	266	14.2	560	457	4.5
Average	849	265	16.9	482	423	5.1
SEM	5.0	4.5	3.16	12.0	7.3	0.84

CP, crude protein; DM, dry matter; Lys, lysine; NDF, neutral detergent fibre SEM, standard error of the difference mean; TAA, total amino acids; *GLS, glucosinolates expressed as µmol/g DM; **Lys:CP ratio expressed as %.

7.8.   Meal AA levels Harvest 2013 for SID AA study

Table 8: Contents of essential amino acids in rapeseed cake and soft rapeseed meal (g/kg DM) of oilseed rape co-products used in boiler AA digestibility studies.

Variety	Arg	His	Ile	Leu	Lys	M+C	Phe	Thr	Val
RSE									
Compass	16.3	7.2	10.9	19.7	15.3	16.2	11.4	12.6	14.5
Sesame	18.4	8.6	12.4	22.1	18.3	20.6	12.7	13.9	17.1
NK Grandia	19.6	8.6	13.0	22.3	18.0	21.1	12.9	13.9	16.8
DK Cabernet	19.2	9.5	13.6	23.1	18.9	23.3	12.8	13.8	18.0
Average	18.4	8.5	12.5	21.8	17.6	20.3	12.5	13.5	16.6
SEM	0.73	0.47	0.57	0.74	0.81	1.50	0.35	0.31	0.75
sRSM									
DK Cabernet[1]	24.9	12.0	18.7	31.8	22.9	27.8	17.6	18.2	25.0
DK Cabernet[2]	25.9	12.2	17.7	32.1	24.0	28.3	17.5	19.5	23.1
Quartz	25.5	11.9	17.9	31.6	23.6	27.9	17.6	19.1	23.5
Trinity	25.8	11.7	18.3	31.2	23.7	28.7	17.4	18.5	23.9
Compass	25.0	11.9	16.8	31.3	23.0	24.5	18.6	19.4	23.2
Incentive	29.5	12.7	20.8	35.6	24.5	28.0	19.2	20.6	27.0
Excalibur	27.7	12.7	19.4	33.7	25.0	30.6	18.9	20.2	25.6
Avatar	26.1	12.9	18.7	32.9	24.3	28.2	19.3	19.7	25.4
PR46W21	30.0	13.7	19.8	35.2	27.4	33.6	19.5	21.0	25.8
Palmedor	29.9	14.5	20.9	36.4	26.6	30.8	19.9	21.1	27.8
V2750L	29.2	13.9	20.9	35.9	26.3	30.5	20.3	20.2	27.9
Ability	30.7	14.0	20.4	37.1	25.1	30.7	20.7	21.1	26.9
Average	27.5	12.8	19.2	33.7	24.7	29.1	18.9	19.9	25.4
SEM	0.64	0.28	0.40	0.63	0.42	0.66	0.33	0.28	0.50

Arg, arginine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; M+C, methionine and cysteine; Phe, phenylalanine; SEM, standard error of the difference mean; Val, valine

7.9. Protein and amino acid Apparent Ileal Digestibility Harvest 2013

Table 9: AID of CP and SID of amino acids in rapeseed co-products for broiler chickens

Samples	CP	Arg	His	Ile	Leu	Lys	M+C	Phe	Thr	Val
RSE										
Compass	0.79	0.92	0.93	0.85	0.86	0.85	0.79	0.88	0.82	0.81
Sesame	0.77	0.91	0.91	0.83	0.84	0.83	0.79	0.87	0.76	0.77
NK Grandia	0.80	0.93	0.93	0.88	0.88	0.87	0.83	0.90	0.82	0.83
DK Cabernet	0.80	0.92	0.92	0.86	0.87	0.85	0.83	0.87	0.80	0.82
Average	0.79	0.92	0.92	0.86	0.86	0.85	0.81	0.88	0.80	0.81
SEM	0.018	0.011	0.010	0.020	0.016	0.016	0.030	0.016	0.024	0.023
p value	0.426	0.451	0.166	0.084	0.174	0.256	0.339	0.319	0.079	0.112
sRSM										
DK Cabernet[1]	0.77[def]	0.89[bc]	0.89[bc]	0.85[bcd]	0.86[ab]	0.79[cd]	0.78[bc]	0.86[abc]	0.78[bc]	0.82[bc]
DK Cabernet[2]	0.78[cde]	0.89[bc]	0.90[abc]	0.85[bcd]	0.87[ab]	0.81[bc]	0.78[bc]	0.86[abc]	0.80[ab]	0.81[bcd]
Quartz	0.74[f]	0.86[d]	0.86[d]	0.82[d]	0.84[b]	0.77[d]	0.75[c]	0.83[c]	0.75[c]	0.78[d]
Trinity	0.79[bcde]	0.91[ab]	0.90[abc]	0.87[ab]	0.88[a]	0.82[bc]	0.81[ab]	0.88[ab]	0.79[bc]	0.83[abc]
Compass	0.79[bcde]	0.89[bc]	0.89[bc]	0.84[bcd]	0.86[ab]	0.80[bcd]	0.78[bc]	0.87[ab]	0.78[bc]	0.80[cd]
Incentive	0.76[ef]	0.90[ab]	0.88[cd]	0.85[bcd]	0.86[ab]	0.80[bcd]	0.77[bc]	0.86[abc]	0.78[bc]	0.82[bc]
Excalibur	0.80[bcd]	0.90[ab]	0.90[abc]	0.85[bcd]	0.87[ab]	0.82[bc]	0.79[bc]	0.87[ab]	0.80[ab]	0.83[abc]
Avatar	0.79[bcde]	0.87[cd]	0.88[cd]	0.83[cd]	0.84[b]	0.79[cd]	0.77[bc]	0.85[bc]	0.77[bc]	0.80[cd]
PR46W21	0.84[a]	0.92[a]	0.92[a]	0.89[a]	0.89[a]	0.87[a]	0.85[a]	0.89[a]	0.84[a]	0.86[a]
Palmedor	0.81[abc]	0.91[ab]	0.91[ab]	0.87[ab]	0.88[a]	0.83[b]	0.82[ab]	0.87[ab]	0.80[ab]	0.84[ab]
V2750L	0.81[abc]	0.90[ab]	0.90[abc]	0.86[abc]	0.87[ab]	0.83[b]	0.79[bc]	0.87[ab]	0.79[bc]	0.84[ab]
Ability	0.82[ab]	0.90[ab]	0.90[abc]	0.85[bcd]	0.87[ab]	0.82[bc]	0.80[abc]	0.87[ab]	0.80[ab]	0.82[bc]
Average	0.79	0.90	0.90	0.85	0.87	0.82	0.80	0.87	0.80	0.82
SEM	0.017	0.012	0.012	0.017	0.014	0.017	0.026	0.014	0.020	0.016
p value	<0.001	<0.001	0.001	0.005	0.014	<0.001	0.034	0.021	0.008	0.003

7.10. Meal biochemistry Harvest 2014 for SID AA study

Table 10. Chemical composition of rapeseed co-products used in Harvest 2014 broiler AA study (g/kg DM).

Variety	PR46W21		DK Cabernet	
Processing	soft	standard	soft	standard
Sample name	SRSM	RSM	SRSM	RSM
DM	899	932	922	924
CP	439	411	391	378
Arg	25.4	23.4	22.7	20.1
His	12.3	10.7	11.5	9.4
Ile	16.5	17.0	16.1	15.6
Leu	29.2	28.4	27.1	25.4
Lys	24.1	21.0	23.7	18.8
Met	8.1	7.5	7.4	6.9
Cys	19.4	19.7	17.1	16.6
Met+Cys	27.5	27.2	24.4	23.4
Phe	14.8	15.3	14.9	13.9
Thr	18.0	17.1	16.9	15.8
Val	21.4	21.5	20.7	19.9
Trp	4.5	2.0	4.1	3.9
Lys:CP(%)*	5.5	5.1	6.1	5.0
Protein solubility (%)	48.8	43.5	44.6	35.8
Oil content	5.8	4.6	7.8	5.0
NDF	325	321	330	433
Tannin catechnin equivalent	2.5	2.5	2.3	2.2
Phytic acid	26.7	25.4	14.2	18.0
Sinapin	3.9	3.9	3.6	3.1
Total glucosinolates**	7.3	4.6	6.3	2.7

Arg, arginine; CP, crude protein; Cys, cysteine; DM, dry matter; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; NDF, neutral detergent fibre; Phe, phenylalanine; RSM, rapeseed meal; SEM, standard error of the difference mean; SRSM, soft rapeseed meal; Trp, tryptophan; Val, valine. *Lys:CP ratio expressed as %. ** Total glucosinolates expressed as (µmol/g DM).

7.11. Protein and amino acid Apparent Ileal Digestibility Harvest 2014

Table 11. Effects of rapeseed variety and processing on standardised ileal digestibility of CP and AA in rapeseed co-products (Harvest 2014) in broiler chickens.

| Variety | PR46W21 | | DK Cabernet | | | P value | | |
| Processing | soft | standard | soft | standard | | | | |
Sample name	sRSM	RSM	sRSM	RSM	SEM	Var	Process	Var. x Proc.
CP	0.83[a]	0.79[b]	0.80[ab]	0.75[c]	0.009	<0.001	<0.001	0.830
Arg	0.89[a]	0.86[ab]	0.85[bc]	0.82[c]	0.010	0.002	0.005	0.863
His	0.88[a]	0.83[bc]	0.86[ab]	0.79[c]	0.013	0.014	<0.001	0.538
Ile	0.84[a]	0.82[ab]	0.80[ab]	0.78[b]	0.013	0.005	0.114	0.780
Leu	0.84[a]	0.83[a]	0.81[ab]	0.78[b]	0.014	0.004	0.080	0.723
Lys	0.80[a]	0.72[b]	0.77[a]	0.66[c]	0.013	0.004	<0.001	0.166
Met	0.86[a]	0.84[a]	0.83[ab]	0.80[b]	0.010	<0.001	0.022	0.548
Cys	0.72[ab]	0.73[a]	0.68[ab]	0.67[b]	0.020	0.018	0.929	0.603
M+C	0.77[a]	0.77[a]	0.73[ab]	0.71[b]	0.016	0.009	0.510	0.588
Phe	0.84[a]	0.83[a]	0.81[ab]	0.78[b]	0.013	0.007	0.193	0.242
Thr	0.77[a]	0.74[ab]	0.74[ab]	0.69[b]	0.017	0.014	0.028	0.418
Val	0.79[a]	0.77[a]	0.76[ab]	0.72[b]	0.015	0.010	0.088	0.499
Trp	0.85[a]	0.61[c]	0.82[ab]	0.80[b]	0.010	<0.001	<0.001	<0.001

Arg, arginine; CP, crude protein; Cys, cysteine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; RSM, rapeseed meal; SEM, standard error of the difference mean; SRSM, soft rapeseed meal; Trp, tryptophan; Val, valine. Values in the same row followed by different letters are significantly different (p < 0.05).

7.12. Energy digestibility Harvest 2013 samples

Table 12: Apparent metabolisable (and nitrogen corrected) energy of sRSM and RSE for broilers

Varieties	Coefficient of EM	AME, MJ/kg	AMEn, MJ/kg
sRSM			
Ability	0.4564[bc]	9.03[a]	8.13[a]
Avatar	0.455[bc]	8.74[cd]	7.95[cd]
Compass	0.455[bc]	8.77[c]	8.00[d]
DK Cabernet[1]	0.455[bc]	8.72[de]	7.96[de]
DK Cabernet[2]	0.456[bc]	8.67[de]	7.91[de]
Excalibur	0.455[bc]	8.67[e]	7.86[e]
Incentive	0.457[bc]	8.71[de]	7.83[de]
Palmedor	0.456[bc]	8.73[cd]	7.82[cd]
PR46W21	0.454[c]	8.73[cd]	7.93[cd]
Quartz	0.455[bc]	8.77[c]	7.99[c]
Trinity	0.455[bc]	8.73[cd]	8.04[cd]
V275OL	0.459[a]	8.97[b]	8.16[b]
Pooled SEM	0.00090	0.018	0.024
P-values	0.003	< 0.001	< 0.001
RSE			
Compass	0.470[a]	11.5[a]	10.86[a]
DK Cabernet	0.463[b]	10.8[b]	10.12[b]
NK Grandia	0.463[b]	10.8[b]	10.09[b]
Sesame	0.463[b]	10.6[c]	9.91[c]
Pooled SEM	0.001	0.033	0.039
P-values	0.003	< 0.001	< 0.001

[a-e] Means in the same column, within a group, but with different superscripts are different (P < 0.05)

7.13.   Energy digestibility Harvest 2014 samples

Table 13: Apparent metabolisable energy (AME) and nitrogen corrected AME of oilseed rape meal from two oilseed rape varieties subjected to mild or harsh processing techniques.

Processing	Variety	AME, MJ/kg	AMEn, MJ/kg	Coefficients
		Means for simple effects		
Harsh	PR46W21	7.59[bc]	6.78[bc]	0.397[bc]
Harsh	DK Cabernet	6.79[c]	6.06[c]	0.357[c]
Mild	PR46W21	8.36[ab]	7.55[ab]	0.435[ab]
Mild	DK Cabernet	8.67[a]	7.92[a]	0.442[a]
Pooled SEM		0.296	0.302	0.015
Variety × Processing		0.082	0.097	0.151
		Means for main effect of processing		
Harsh		7.22	6.46	0.379
Mild		8.50	7.73	0.437
Pooled SEM		0.200	0.205	0.010
Processing effect		< 0.001	< 0.001	< 0.001
		Means for main effect of RSM variety		
	PR46W21	7.89	7.14	0.412
	DK Cabernet	7.87	7.09	0.406
Pooled SEM		0.207	0.209	0.010
Variety effect		0.408	0.563	0.275

[a-b] Means in the same column, within a group, but with different superscripts are different (P < 0.05)

7.14. Growth, bone mineralisation and P digestibility Harvest 2014

Table 14. Growth performance, bone mineralisation and ileal P digestibility of broilers receiving graded levels of dietary phosphorus supplied by sodium phosphate or oilseed rape produced by two processing methods

Treatment	Diet total P, %	Weight gain[a], g	FI, g	FCR	Tibia ash[b], %
Basal (B)	0.34	471.7	439.8	0.935	15.08
B + Na_2PO_4	0.45	607	539.1	0.889	19.07
B + Na_2PO_4	0.55	646.3	557.4	0.863	18.55
B + PRH	0.49	510.1	459.9	0.902	15.53
B + PRH	0.61	523.1	501.9	0.962	16.32
B + PRS	0.42	492.4	433.6	0.88	16.6
B + PRS	0.54	514.7	488.4	0.949	16.33
B + DKCH	0.46	504.7	447	0.886	16.67
B + DKCH	0.57	544.2	493.9	0.909	16.73
B + DKCS	0.44	522	447.6	0.858	14.3
B + DKCS	0.53	540.6	519.8	0.961	16.1
Pooled SEM		13.2	12.5	0.02	0.791
		P-values for linear and quadratic contrasts			
Linear – Na_2PO_4		< 0.001	< 0.001	0.021	0.029
Quadratic – Na_2PO_4		0.072	0.060	0.686	0.084
Linear – PRH		0.015	0.024	0.500	0.318
Quadratic – PRH		0.423	0.602	0.206	0.873
Linear – PRS		0.053	0.033	0.602	0.235
Quadratic – PRS		0.967	0.104	0.024	0.323
Linear – DKCH		0.001	< 0.001	0.393	0.235
Quadratic – DKCH		0.824	0.039	0.174	0.518
Linear – DKCS		0.002	0.001	0.393	0.433
Quadratic – DKCS		0.310	0.064	0.006	0.258

PRH and PRS- PR46W21 oilseed rape meal derived from harsh or mild processing techniques, respectively; DKCH and DKCS – DK Cabernet oilseed rape meal derived from harsh or mild processing technique, respectively.

[a] Multiple regression of weight gain (Y, g) on supplemental P intake (g) from Na_2PO_4 or PRH or PRS yielded the equation: $Y = 486 + 88.3MSP + 19.7 PRH + 27.0 PRS$ (r^2 = 0.70) whereas the equation for DKCH and DKCS yielded the equation $Y = 487 + 87.5MSP + 11.9DKCH + 28.2DKCS$ (r^2 = 0.72).

[b] Multiple regression of tibia ash (Y, %) on supplemental P intake (g) from Na_2PO_4, PRH or PRS yielded the equation: $Y = 14.1 + 3.82MSP + 1.38 PRH + 1.29 PRS$ (r^2 = 0.66) whereas the equation for DKCH and DKCS yielded the equation $Y = 13.9 + 3.9MSP + 0.57DKCH + 1.49DKCS$ (r^2 = 0.60).

7.15. Relative P bioavailability Harvest 2014 samples

Table 15: Relative P bioavailability, total P and bioavailable P content of the oilseed rape meals

Variety	Processing	RB[a], %[a]	Total P, %	Bioavailable P content[b], %
		Weight gain		
PR46W21	Harsh	22.3	0.931	0.208
PR46W21	Mild	30.6	0.942	0.288
DK Cabernet	Harsh	13.6	1.144	0.156
DK Cabernet	Mild	32.2	1.206	0.388
		Tibia ash		
PR46W21	Harsh	36.1	0.931	0.336
PR46W21	Mild	33.8	0.942	0.318
DK Cabernet	Harsh	46.6	1.144	0.533
DK Cabernet	Mild	21.2	1.206	0.256

[a] RB: Relative bioavailability; bioavailability of the P in oilseed rape meals relative to Na_2PO_4. Calculated by the common intercept slope ratio using the multiple regression equations in the footnote of Table 14.

[b] Bioavailable P content was derived as the product of bioavailability coefficient and the total P in the oilseed rape meals.

7.16. Biochemistry Harvest 2013 and 2014 samples for pig SID AA

Table 16. Chemical composition of rapeseed co-products used for pig AA digestibility (g/kg DM).

Variety	Year	Sample	DM	GSL	NDF	CP	Lys:CP
DK Cabernet	2013	RSE	894	14.8	254	344	0.059
DK Cabernet	2013	SRSM	975	14.4	351	435	0.048
V2750L	2013	SRSM	958	47.4	298	473	0.054
PR46W21	2013	SRSM	961	25.8	295	428	0.050
DK Cabernet	2014	SRSM	922	6.3	330	391	0.061
DK Cabernet	2014	RSM	924	2.7	433	378	0.050
PR46W21	2014	SRSM	899	7.3	325	439	0.055
PR46W21	2014	RSM	932	4.6	321	411	0.051

CP, crude protein; DM, dry matter; GSL, glucosinolates (µmol/g); NDF, neutral detergent fibre; S-HE and HE, soft and standard hexane extraction

7.17. Amino acids in Harvest 2013 and 2014 samples for pig SID AA

Table 17. Essential amino acid composition of rapeseed co-products (g/kg DM)

Variety	Year	Sample	Arg	His	Ile	Leu	Lys	C+M	Phe	Thr	Val	Tryp
DK Cabernet	2013	RSE	19	9.5	12.9	21.9	20.3	22.7	11.2	13.9	16.6	3.6
DK Cabernet	2013	SRSM	21.3	9.8	15.2	25.5	20.8	23.2	14.7	15.8	19.6	4.2
V2750L	2013	SRSM	28.2	13.3	18.7	31.5	25.5	29.1	16.6	18.4	24.3	5
PR46W21	2013	SRSM	22.1	10.3	15.0	26.1	21.6	24.6	14.9	15.9	19.1	4.5
DK Cabernet	2014	SRSM	22.7	11.5	16.1	27.1	23.7	24.4	14.9	16.9	20.7	4.1
DK Cabernet	2014	RSM	20.1	9.4	15.6	25.4	18.8	23.4	13.9	15.8	19.9	3.9
PR46W21	2014	SRSM	25.4	12.3	16.5	29.2	24.1	27.5	14.8	18	21.4	4.5
PR46W21	2014	RSM	23.4	10.7	17	28.4	21.0	27.2	15.3	17.1	21.5	2.0

Arg, arginine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; M+C, methionine and cysteine; Phe, phenylalanine; S-HE and HE, soft and standard hexane extraction; Trp, tryptophan; Val, valine.

7.18. Amino acid digestibility Harvest 2013 and 2014 in pigs (1)

Table 18. Coefficients of standardised ileal digestibility of crude protein and selected amino acids (lysine, methionine+cysteine, threonine and tryptophan) in rapeseed rich diets fed to pigs.

Variety	Year	Sample	CP	Lys	M+C	Thr	Trp
DK Cabernet	2013	RSE	0.80[a]	0.85[a]	0.84[a]	0.77[a]	0.79[a]
DK Cabernet	2013	SRSM	0.72[ab]	0.69[bc]	0.71[b]	0.69[ab]	0.77[a]
V2750L	2013	SRSM	0.75[ab]	0.77[ab]	0.79[ab]	0.76[a]	0.80[a]
PR46W21	2013	SRSM	0.81[a]	0.76[ab]	0.72[b]	0.74[a]	0.79[a]
DK Cabernet	2014	SRSM	0.69[b]	0.72[b]	0.72[b]	0.71[a]	0.76[ab]
DK Cabernet	2014	RSM	0.55[c]	0.54[d]	0.60[c]	0.59[b]	0.65[b]
PR46W21	2014	SRSM	0.71[ab]	0.73[b]	0.70[bc]	0.73[a]	0.76[ab]
PR46W21	2014	RSM	0.67[b]	0.62[cd]	0.69[bc]	0.68[ab]	0.45[c]
SEM			0.05	0.05	0.05	0.05	0.05
p value			<0.001	<0.001	0.001	0.038	<0.001

Data were analysed by one way ANOVA. Data in the same columns with different superscripts are significantly different. Arg, arginine; CP, crude protein; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; M+C, methionine and cysteine; Phe, phenylalanine; S-HE and HE, soft and standard hexane extraction; Trp, tryptophan; Val, valine.

7.19. Amino acid digestibility Harvest 2013 and 2014 in pigs (2)

Table 19. Coefficients of standardised ileal digestibility of crude protein and selected amino acids (arginine, histidine, isoleucine, leucine, phenylalanine and valine) in rapeseed rich diets fed to pigs.

Variety	Year	Sample	CP	Arg	His	Ile	Leu	Phe	Val
DK Cabernet	2013	RSE	0.80[a]	0.90[a]	0.90[a]	0.86[a]	0.83	0.84	0.77[a]
DK Cabernet	2013	SRSM	0.72[ab]	0.82[c]	0.81[cd]	0.79[ab]	0.78	0.80	0.73[a]
V2750L	2013	SRSM	0.75[ab]	0.89[ab]	0.88[ab]	0.84[a]	0.83	0.83	0.79[a]
PR46W21	2013	SRSM	0.81[a]	0.87[abc]	0.86[abc]	0.83[a]	0.81	0.83	0.75[a]
DK Cabernet	2014	SRSM	0.69[b]	0.85[abc]	0.83[bcd]	0.79[ab]	0.79	0.79	0.73[a]
DK Cabernet	2014	RSM	0.55[c]	0.73[d]	0.71[e]	0.72[b]	0.70	0.71	0.63[b]
PR46W21	2014	SRSM	0.71[ab]	0.85[abc]	0.84[abcd]	0.80[ab]	0.80	0.78	0.73[a]
PR46W21	2014	RSM	0.67[b]	0.84[bc]	0.78[d]	0.78[ab]	0.78	0.78	0.72[ab]
SEM			0.05	0.03	0.03	0.04	0.04	0.04	0.05
p value			<0.001	<0.001	<0.001	0.043	0.051	0.081	0.040

Data were analysed by one way ANOVA. Data in the same columns with different superscripts are significantly different. Arg, arginine; CP, crude protein; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; M+C, methionine and cysteine; Phe, phenylalanine; S-HE and HE, soft and standard hexane extraction; Trp, tryptophan; Val, valine.

7.20. Linear regression RSM chemistry on RSM amino acids

Table 20. Linear regression of RSM chemistry on RSM amino acid content

		Amino acid								
		Lys	Met	Thr	Arg	Val	His	Ile	Leu	Phe
GS	Intercept	23.26	8.67	19.37	25.88	23.85	12.03	32.05	17.96	17.98
	s.e.	0.63	0.27	0.53	1.10	0.79	0.46	1.08	0.64	0.56
	Slope	0.097	0.032	0.035	0.110	0.107	0.054	0.113	0.082	0.060
	s.e.	0.036	0.015	0.030	0.063	0.045	0.026	0.061	0.036	0.032
	P-value	0.021	0.063	0.277	0.111	0.039	0.065	0.094	0.048	0.091
NDF	Intercept	32.86	13.39	28.04	46.80	38.82	16.50	50.60	32.01	25.59
	s.e.	7.36	2.73	4.63	10.20	8.30	5.08	10.50	6.21	5.80
	Slope	-0.031	-0.016	-0.031	-0.073	-0.051	-0.014	-0.064	-0.048	-0.025
	s.e.	0.028	0.010	0.018	0.038	0.031	0.019	0.040	0.023	0.022
	P-value	0.293	0.150	0.108	0.088	0.137	0.487	0.137	0.065	0.273
Oil	Intercept	22.28	8.86	17.85	21.24	20.74	10.25	27.01	15.58	14.63
	s.e.	2.58	1.05	1.71	3.58	2.84	1.60	3.44	2.29	1.64
	Slope	0.770	0.089	0.644	1.990	1.489	0.822	2.130	1.142	1.347
	s.e.	0.807	0.327	0.536	1.120	0.890	0.500	1.080	0.715	0.513
	P-value	0.362	0.790	0.257	0.106	0.125	0.131	0.076	0.141	0.025
Phytic Acid	Intercept	24.51	8.90	19.43	26.74	25.28	13.36	33.64	18.73	19.34
	s.e.	1.48	0.57	1.00	2.24	1.77	0.98	2.23	1.40	1.16
	Slope	0.090	0.112	0.213	0.365	0.075	-0.242	0.047	0.211	-0.219
	s.e.	0.659	0.254	0.443	0.998	0.787	0.434	0.994	0.623	0.517
	P-value	0.894	0.670	0.641	0.722	0.926	0.590	0.963	0.742	0.681
Sinapine	Intercept	27.35	10.84	20.76	31.49	26.06	13.49	36.24	21.09	17.99
	s.e.	3.14	1.15	2.19	4.79	3.88	2.16	4.84	3.03	2.56
	Slope	-0.510	-0.327	-0.168	-0.766	-0.120	-0.124	-0.483	-0.368	0.170
	s.e.	0.600	0.219	0.420	0.916	0.742	0.414	0.925	0.580	0.489
	P-value	0.416	0.166	0.697	0.423	0.875	0.770	0.613	0.540	0.736
Tannin	Intercept	24.52	8.86	18.23	26.67	26.85	12.81	33.08	19.91	19.02
	s.e.	2.60	1.01	1.69	3.96	3.08	1.74	3.92	2.46	2.06
	Slope	0.090	0.143	0.836	0.430	-0.710	0.015	0.340	-0.370	-0.070
	s.e.	1.300	0.503	0.841	1.970	1.530	0.866	1.950	1.230	1.020
	P-value	0.944	0.781	0.344	0.832	0.652	0.987	0.867	0.770	0.944
Psol	Intercept	18.11	8.03	13.47	13.07	15.48	6.41	18.39	11.78	9.97
	s.e.	3.33	1.49	1.73	3.85	3.47	1.66	3.38	2.91	1.29
	Slope	0.119	0.020	0.115	0.260	0.179	0.116	0.276	0.133	0.160
	s.e.	0.060	0.027	0.031	0.069	0.062	0.030	0.060	0.052	0.023
	P-value	0.074	0.469	0.004	0.004	0.016	0.003	0.001	0.028	<.001

7.21. Linear regression glucosinolates on RSM amino acids

Table 21: Linear regression of glucosinolate compounds on RSM amino acid content

		Essential amino acid								
		Lys	Met	Thr	Arg	Val	His	Ile	Leu	Phe
4OH-gluco-brassicin	Intercept	23.80	9.06	19.23	25.65	24.08	11.96	31.76	18.20	17.75
	s.e.	0.71	0.30	0.47	0.96	0.78	0.40	0.91	0.65	0.45
	Slope	1.481	0.137	1.066	3.070	2.230	1.441	3.250	1.600	1.830
	s.e.	0.957	0.413	0.641	1.300	1.060	0.547	1.240	0.877	0.613
	P-value	0.153	0.747	0.127	0.040	0.062	0.025	0.025	0.098	0.014
Epi-progoitrin	Intercept	24.22	9.03	19.62	27.16	25.44	12.63	33.49	19.17	18.78
	s.e.	0.45	0.20	0.32	0.77	0.63	0.33	0.78	0.50	0.41
	Slope	5.710	1.280	3.100	4.300	0.030	2.550	2.910	0.180	1.150
	s.e.	2.870	1.260	2.090	4.990	4.050	2.120	5.040	3.230	2.660
	P-value	0.075	0.332	0.168	0.409	0.993	0.257	0.576	0.956	0.674
Gluco-alyssin	Intercept	24.27	9.04	19.70	26.94	24.91	12.54	33.12	18.80	18.48
	s.e.	0.42	0.19	0.32	0.68	0.50	0.28	0.65	0.41	0.31
	Slope	0.223	0.053	0.095	0.295	0.269	0.152	0.317	0.196	0.198
	s.e.	0.106	0.047	0.081	0.170	0.126	0.070	0.163	0.104	0.079
	P-value	0.062	0.284	0.269	0.112	0.059	0.054	0.082	0.088	0.031
Gluco-berin	Intercept	26.20	9.56	20.84	29.16	25.66	13.46	34.86	19.55	19.27
	s.e.	1.12	0.46	0.77	1.79	1.47	0.79	1.81	1.16	0.96
	Slope	-4.200	-1.180	-2.690	-4.620	-0.630	-1.750	-3.150	-1.050	-1.120
	s.e.	2.950	1.200	2.030	4.710	3.860	2.090	4.770	3.060	2.530
	P-value	0.185	0.351	0.214	0.350	0.873	0.422	0.525	0.739	0.669
Gluco-brassicin	Intercept	24.27	9.18	19.26	26.27	24.81	12.30	32.49	18.74	18.23
	s.e.	0.73	0.29	0.45	1.04	0.87	0.46	1.03	0.70	0.54
	Slope	6.550	-0.550	9.360	18.700	9.400	8.150	18.700	6.590	9.650
	s.e.	8.920	3.560	5.480	12.600	10.500	5.550	12.500	8.450	6.570
	P-value	0.479	0.880	0.118	0.170	0.395	0.173	0.164	0.454	0.173
Gluco-napin	Intercept	23.19	8.55	19.41	25.93	23.75	12.12	32.13	17.83	18.16
	s.e.	0.73	0.28	0.60	1.26	0.90	0.55	1.25	0.72	0.67
	Slope	0.442	0.174	0.139	0.466	0.496	0.212	0.471	0.395	0.208
	s.e.	0.187	0.073	0.153	0.324	0.231	0.141	0.320	0.184	0.173
	P-value	0.040	0.037	0.385	0.181	0.058	0.165	0.171	0.057	0.256
Gluco-nasturtiin	Intercept	25.17	9.35	20.08	28.03	25.99	13.13	34.36	19.62	19.02
	s.e.	0.57	0.22	0.40	0.90	0.68	0.39	0.87	0.54	0.48
	Slope	-5.770	-2.540	-2.440	-6.330	-6.760	-3.560	-7.670	-5.400	-1.790
	s.e.	4.880	1.870	3.460	7.690	5.850	3.300	7.480	4.650	4.090
	P-value	0.264	0.204	0.496	0.429	0.275	0.306	0.329	0.273	0.671
Gluco-raphanin	Intercept	23.43	8.50	19.68	26.44	23.56	12.13	32.32	17.79	18.25
	s.e.	1.06	0.39	0.77	1.70	1.20	0.72	1.65	0.98	0.88
	Slope	2.590	1.305	0.420	2.200	3.810	1.440	2.890	2.830	1.260
	s.e.	1.980	0.726	1.450	3.190	2.260	1.360	3.100	1.830	1.650
	P-value	0.220	0.102	0.776	0.507	0.122	0.314	0.373	0.153	0.462
Progoitrin	Intercept	23.05	8.50	19.36	25.95	23.86	12.06	32.17	17.91	18.17
	s.e.	0.70	0.27	0.60	1.28	0.94	0.55	1.27	0.75	0.68
	Slope	0.241	0.094	0.076	0.229	0.230	0.114	0.228	0.185	0.102
	s.e.	0.090	0.035	0.076	0.164	0.120	0.070	0.162	0.096	0.087
	P-value	0.023	0.023	0.343	0.193	0.084	0.135	0.190	0.082	0.269

7.22. Linear regression RSM chemistry on RSM amino acids digestibility

Table 22. Linear regression of RSM chemistry on RSM amino acid standardised ileal digestibility coefficient.

		Essential amino acid								
		Lys	Met	Thr	Arg	Val	His	Ile	Leu	Phe
GS	Intercept	0.77	0.92	0.75	0.86	0.77	0.85	0.84	0.82	0.84
	s.e.	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.01	0.01
	Slope	0.001	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000
	s.e.	0.001	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	P-value	0.300	0.423	0.372	0.606	0.761	0.464	0.649	0.892	0.585
NDF	Intercept	0.97	0.96	0.88	0.83	0.87	1.07	0.84	0.83	0.94
	s.e.	0.19	0.06	0.13	0.12	0.15	0.21	0.09	0.14	0.11
	Slope	-0.001	0.000	0.000	0.000	0.000	-0.001	0.000	0.000	0.000
	s.e.	0.001	0.000	0.001	0.000	0.001	0.001	0.000	0.001	0.000
	P-value	0.346	0.530	0.405	0.721	0.553	0.350	0.950	0.958	0.388
Oil	Intercept	0.74	0.93	0.74	0.88	0.81	0.82	0.86	0.86	0.85
	s.e.	0.07	0.02	0.05	0.04	0.05	0.08	0.03	0.05	0.04
	Slope	0.016	-0.001	0.009	-0.004	-0.011	0.013	-0.005	-0.011	-0.002
	s.e.	0.021	0.006	0.015	0.013	0.016	0.024	0.010	0.015	0.012
	P-value	0.448	0.826	0.568	0.743	0.502	0.580	0.589	0.460	0.891
PhyticAcid	Intercept	0.77	0.91	0.76	0.86	0.75	0.83	0.83	0.80	0.82
	s.e.	0.04	0.01	0.03	0.02	0.03	0.04	0.02	0.03	0.02
	Slope	0.009	0.005	0.003	0.003	0.015	0.013	0.005	0.011	0.012
	s.e.	0.016	0.005	0.012	0.010	0.012	0.018	0.008	0.011	0.009
	P-value	0.595	0.343	0.819	0.808	0.237	0.486	0.516	0.339	0.192
Sinapine	Intercept	0.79	0.94	0.75	0.92	0.87	0.85	0.88	0.91	0.87
	s.e.	0.08	0.02	0.06	0.05	0.05	0.09	0.04	0.05	0.05
	Slope	-0.001	-0.003	0.002	-0.010	-0.017	0.003	-0.008	-0.017	-0.005
	s.e.	0.016	0.004	0.011	0.009	0.010	0.018	0.007	0.010	0.009
	P-value	0.950	0.502	0.849	0.300	0.124	0.854	0.269	0.125	0.547
Tannin	Intercept	0.82	0.93	0.76	0.88	0.75	0.90	0.84	0.82	0.85
	s.e.	0.07	0.02	0.05	0.04	0.05	0.07	0.03	0.05	0.04
	Slope	-0.014	-0.003	-0.001	-0.004	0.012	-0.019	-0.001	0.003	-0.004
	s.e.	0.032	0.009	0.023	0.020	0.024	0.036	0.015	0.023	0.019
	P-value	0.680	0.759	0.974	0.847	0.638	0.615	0.975	0.901	0.819
Psol	Intercept	0.66	0.91	0.66	0.88	0.79	0.74	0.84	0.85	0.81
	s.e.	0.09	0.03	0.06	0.06	0.07	0.11	0.05	0.07	0.06
	Slope	0.002	0.000	0.002	0.000	0.000	0.002	0.000	-0.001	0.001
	s.e.	0.002	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001
	P-value	0.171	0.688	0.119	0.924	0.817	0.280	0.932	0.663	0.565

7.23. Linear regression glucosinolates on RSM amino acids digestibility

Table 23. Linear regression of glucosinolate compounds on RSM essential AA SID coefficient

		Lys	Met	Thr	Arg	Val	His	Ile	Leu	Phe
					Essential amino acid					
4OHgluco-Brassicin	Intercept	0.76	0.92	0.74	0.87	0.77	0.84	0.83	0.82	0.83
	s.e.	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
	Slope	0.047	0.010	0.035	0.007	0.008	0.046	0.011	0.008	0.021
	s.e.	0.022	0.007	0.015	0.016	0.020	0.027	0.012	0.019	0.014
	P-value	0.060	0.191	0.046	0.674	0.683	0.117	0.364	0.672	0.166
Epi-Progoitrin	Intercept	0.78	0.92	0.76	0.86	0.77	0.86	0.83	0.81	0.84
	s.e.	0.01	0.00	0.01	0.01	0.01	0.01	0.00	0.01	0.01
	Slope	0.054	0.021	0.074	0.088	0.093	0.011	0.076	0.101	0.045
	s.e.	0.084	0.024	0.056	0.044	0.057	0.096	0.032	0.052	0.047
	P-value	0.532	0.392	0.215	0.073	0.133	0.911	0.038	0.080	0.360
Gluco-Alyssin	Intercept	0.78	0.92	0.76	0.87	0.78	0.86	0.84	0.82	0.84
	s.e.	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Slope	0.003	0.000	0.002	0.000	-0.001	0.002	0.000	-0.001	0.000
	s.e.	0.003	0.001	0.002	0.002	0.002	0.004	0.002	0.002	0.002
	P-value	0.343	0.838	0.475	0.943	0.693	0.499	0.911	0.668	0.905
Gluco-Berin	Intercept	0.83	0.94	0.80	0.89	0.82	0.89	0.86	0.85	0.87
	s.e.	0.03	0.01	0.02	0.02	0.02	0.03	0.01	0.02	0.02
	Slope	-0.103	-0.035	-0.113	-0.060	-0.112	-0.070	-0.072	-0.091	-0.064
	s.e.	0.075	0.021	0.045	0.046	0.050	0.089	0.031	0.051	0.042
	P-value	0.201	0.128	0.031	0.223	0.049	0.449	0.042	0.101	0.159
Gluco-Brassicin	Intercept	0.77	0.92	0.75	0.86	0.77	0.85	0.83	0.81	0.83
	s.e.	0.02	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
	Slope	0.251	0.059	0.259	0.091	0.106	0.240	0.128	0.134	0.166
	s.e.	0.217	0.064	0.141	0.138	0.170	0.248	0.100	0.159	0.121
	P-value	0.275	0.381	0.097	0.524	0.547	0.356	0.230	0.419	0.200
Gluco-Napin	Intercept	0.77	0.92	0.75	0.86	0.77	0.85	0.83	0.82	0.84
	s.e.	0.02	0.01	0.02	0.01	0.02	0.03	0.01	0.02	0.01
	Slope	0.006	0.002	0.004	0.003	0.003	0.004	0.002	0.002	0.003
	s.e.	0.006	0.002	0.004	0.003	0.004	0.006	0.003	0.004	0.003
	P-value	0.352	0.265	0.370	0.447	0.445	0.505	0.450	0.622	0.439
Gluco-Nasturtiin	Intercept	0.78	0.92	0.76	0.88	0.78	0.86	0.84	0.83	0.85
	s.e.	0.02	0.00	0.01	0.01	0.01	0.02	0.01	0.01	0.01
	Slope	0.059	0.000	0.002	-0.077	-0.050	0.091	-0.043	-0.073	-0.003
	s.e.	0.130	0.038	0.093	0.076	0.097	0.145	0.060	0.091	0.075
	P-value	0.662	1.000	0.980	0.334	0.622	0.544	0.488	0.442	0.968
Gluco-Raphanin	Intercept	0.78	0.92	0.76	0.87	0.78	0.85	0.84	0.83	0.84
	s.e.	0.03	0.01	0.02	0.02	0.02	0.03	0.01	0.02	0.02
	Slope	0.018	0.007	0.002	-0.011	-0.008	0.025	-0.007	-0.018	0.004
	s.e.	0.054	0.016	0.038	0.033	0.041	0.060	0.025	0.038	0.031
	P-value	0.744	0.643	0.964	0.746	0.857	0.693	0.799	0.642	0.904
Progoitrin	Intercept	0.77	0.92	0.75	0.86	0.77	0.85	0.83	0.82	0.84
	s.e.	0.02	0.01	0.02	0.01	0.02	0.03	0.01	0.02	0.01
	Slope	0.002	0.001	0.002	0.001	0.002	0.002	0.001	0.001	0.001
	s.e.	0.003	0.001	0.002	0.002	0.002	0.003	0.001	0.002	0.002
	P-value	0.422	0.345	0.448	0.451	0.491	0.612	0.473	0.657	0.551

7.24. Linear regression RSM chemistry on RSM SID AA content

Table 24. Linear regression of RSM chemistry on RSM SID AA content.

		Lys	Met	Thr	Arg	Val	His	Ile	Leu	Phe
					Essential amino acid					
GS	Intercept	17.90	7.97	14.56	22.36	18.44	10.20	26.78	14.75	15.10
	s.e.	0.86	0.27	0.60	1.10	0.73	0.56	1.04	0.63	0.57
	Slope	0.108	0.032	0.042	0.106	0.089	0.060	0.103	0.069	0.058
	s.e.	0.049	0.015	0.034	0.062	0.041	0.032	0.059	0.036	0.032
	P-value	0.051	0.063	0.249	0.121	0.057	0.086	0.110	0.082	0.105
NDF	Intercept	30.60	12.72	23.73	39.60	32.52	16.83	42.70	26.59	23.42
	s.e.	9.23	2.73	5.40	10.60	7.21	5.83	10.20	6.01	5.69
	Slope	-0.042	-0.016	-0.032	-0.059	-0.048	-0.022	-0.054	-0.041	-0.028
	s.e.	0.035	0.010	0.020	0.040	0.027	0.022	0.038	0.023	0.021
	P-value	0.257	0.149	0.144	0.170	0.107	0.347	0.187	0.102	0.218
Oil	Intercept	16.34	8.24	13.08	18.86	17.05	8.31	23.29	13.53	12.50
	s.e.	3.26	1.05	1.97	3.71	2.72	1.91	3.50	2.28	1.81
	Slope	1.010	0.068	0.666	1.610	0.862	0.881	1.590	0.712	1.098
	s.e.	1.020	0.329	0.617	1.160	0.850	0.598	1.100	0.713	0.567
	P-value	0.347	0.839	0.306	0.195	0.334	0.171	0.176	0.341	0.082
Sinapine	Intercept	21.78	10.16	15.65	28.75	22.52	11.49	31.82	18.94	15.82
	s.e.	4.05	1.15	2.51	4.64	3.33	2.54	4.52	2.70	2.57
	Slope	-0.437	-0.330	-0.092	-0.927	-0.532	-0.077	-0.675	-0.610	0.027
	s.e.	0.775	0.219	0.480	0.888	0.637	0.486	0.864	0.517	0.491
	P-value	0.585	0.164	0.852	0.321	0.423	0.877	0.453	0.265	0.957
Tannin	Intercept	19.93	8.23	13.88	23.33	20.15	11.48	27.69	16.18	16.18
	s.e.	3.29	1.01	1.97	3.91	2.75	2.03	3.72	2.30	2.05
	Slope	-0.210	0.111	0.654	0.310	-0.190	-0.200	0.320	-0.200	-0.110
	s.e.	1.640	0.505	0.981	1.950	1.370	1.010	1.850	1.150	1.020
	P-value	0.901	0.830	0.520	0.877	0.890	0.849	0.866	0.863	0.917
Psol	Intercept	10.99	7.31	8.15	11.49	12.48	3.98	15.27	10.26	7.81
	s.e.	4.18	1.49	2.07	4.39	3.46	2.09	3.81	3.02	1.71
	Slope	0.153	0.021	0.127	0.224	0.131	0.128	0.235	0.099	0.147
	s.e.	0.075	0.027	0.037	0.079	0.062	0.037	0.068	0.054	0.031
	P-value	0.067	0.458	0.007	0.017	0.060	0.006	0.006	0.096	<.001

7.25. Linear regression RSM glucosinolates on RSM SID AA content

Table 25. Linear regression of glucosinolate compounds on RSM SID essential AA content.

| | | Essential amino acid | | | | | | | | |
		Lys	Met	Thr	Arg	Val	His	Ile	Leu	Phe
4OH-gluco-Brassicin	Intercept	18.07	8.32	14.25	22.19	18.58	9.97	26.42	14.88	14.78
	s.e.	0.83	0.30	0.49	0.97	0.70	0.44	0.86	0.61	0.43
	Slope	2.360	0.219	1.526	2.870	1.934	1.831	3.110	1.470	1.934
	s.e.	1.120	0.410	0.668	1.320	0.948	0.599	1.170	0.822	0.577
	P-value	0.062	0.605	0.046	0.055	0.069	0.012	0.024	0.104	0.007
Epi-Progoitrin	Intercept	19.00	8.33	14.84	23.42	19.56	10.88	27.89	15.59	15.80
	s.e.	0.59	0.19	0.36	0.73	0.54	0.39	0.71	0.45	0.40
	Slope	6.120	1.410	3.940	6.190	2.450	2.460	5.120	2.130	1.900
	s.e.	3.840	1.250	2.310	4.710	3.510	2.540	4.580	2.930	2.610
	P-value	0.142	0.287	0.120	0.218	0.501	0.356	0.290	0.484	0.484
Gluco-Alyssin	Intercept	19.02	8.35	14.97	23.43	19.41	10.77	27.81	15.50	15.62
	s.e.	0.56	0.19	0.37	0.69	0.49	0.34	0.65	0.41	0.34
	Slope	0.253	0.051	0.106	0.261	0.180	0.164	0.259	0.140	0.171
	s.e.	0.140	0.047	0.093	0.172	0.122	0.085	0.163	0.104	0.085
	P-value	0.100	0.307	0.281	0.161	0.170	0.082	0.143	0.206	0.071
Gluco-Berin	Intercept	21.68	8.96	16.74	25.98	20.97	11.97	30.14	16.73	16.74
	s.e.	1.37	0.45	0.79	1.71	1.24	0.92	1.65	1.04	0.93
	Slope	-6.100	-1.430	-4.400	-5.750	-3.400	-2.470	-5.140	-2.680	-2.190
	s.e.	3.610	1.180	2.080	4.510	3.250	2.410	4.330	2.740	2.460
	P-value	0.122	0.255	0.060	0.231	0.320	0.330	0.263	0.351	0.394
Gluco-Brassicin	Intercept	18.74	8.45	14.35	22.68	19.09	10.41	26.97	15.23	15.20
	s.e.	0.90	0.29	0.49	1.02	0.75	0.53	0.94	0.63	0.52
	Slope	11.600	0.060	12.410	18.800	10.130	10.190	20.200	8.130	11.310
	s.e.	11.000	3.580	5.900	12.400	9.150	6.400	11.400	7.700	6.280
	P-value	0.318	0.986	0.062	0.160	0.294	0.142	0.109	0.316	0.102
Gluco-Napin	Intercept	17.84	7.84	14.56	22.30	18.18	10.27	26.74	14.54	15.19
	s.e.	0.98	0.28	0.67	1.24	0.78	0.65	1.17	0.67	0.66
	Slope	0.491	0.179	0.181	0.479	0.465	0.242	0.463	0.362	0.225
	s.e.	0.252	0.072	0.172	0.317	0.199	0.166	0.301	0.173	0.170
	P-value	0.080	0.032	0.318	0.162	0.041	0.177	0.155	0.063	0.215
Gluco-Nasturtiin	Intercept	19.79	8.64	15.33	24.55	20.29	11.26	28.96	16.24	16.09
	s.e.	0.76	0.22	0.47	0.87	0.60	0.47	0.82	0.50	0.48
	Slope	-3.370	-2.360	-1.870	-7.590	-6.480	-2.060	-7.930	-5.800	-1.670
	s.e.	6.510	1.890	3.990	7.460	5.130	4.030	7.030	4.240	4.080
	P-value	0.616	0.241	0.650	0.333	0.235	0.620	0.286	0.201	0.692
Gluco-Raphanin	Intercept	18.28	7.83	15.00	23.14	18.42	10.32	27.24	14.82	15.40
	s.e.	1.38	0.39	0.88	1.69	1.12	0.85	1.59	0.96	0.88
	Slope	2.500	1.272	0.360	1.620	2.730	1.560	2.200	1.950	1.140
	s.e.	2.600	0.734	1.650	3.180	2.100	1.600	2.990	1.800	1.650
	P-value	0.358	0.114	0.834	0.621	0.222	0.352	0.479	0.303	0.507
Progoitrin	Intercept	17.77	7.80	14.56	22.31	18.28	10.26	26.78	14.62	15.23
	s.e.	0.98	0.27	0.68	1.25	0.82	0.65	1.20	0.70	0.68
	Slope	0.253	0.094	0.091	0.237	0.216	0.122	0.225	0.168	0.106
	s.e.	0.126	0.035	0.087	0.160	0.104	0.084	0.153	0.090	0.087
	P-value	0.071	0.022	0.320	0.171	0.066	0.176	0.172	0.090	0.249

7.26.   Linear regression RSM biochemistry on energy parameters

Table 26. Linear regression of sRSM biochemistry with gross energy, energy digestibility and AME in broilers.

| | | Energy parameter | | |
	`	GE	Digestibility	AME
CP	Intercept	17.60	66.67	11.71
	s.e.	0.99	0.91	0.74
	Slope	0.041	0.042	0.036
	s.e.	0.025	0.023	0.018
	P-value	0.129	0.093	0.077
GS	Intercept	19.13	68.15	13.04
	s.e.	0.12	0.09	0.09
	Slope	0.007	0.014	0.008
	s.e.	0.007	0.005	0.005
	P-value	0.310	0.016	0.148
NDF	Intercept	18.59	68.76	12.78
	s.e.	1.16	1.10	0.90
	Slope	0.002	-0.002	0.001
	s.e.	0.004	0.004	0.003
	P-value	0.584	0.722	0.688
Oil	Intercept	18.17	67.73	12.30
	s.e.	0.21	0.33	0.15
	Slope	0.341	0.199	0.272
	s.e.	0.067	0.102	0.047
	P-value	<.001	0.080	<.001
PhyticAcid	Intercept	19.60	68.36	13.40
	s.e.	0.19	0.21	0.15
	Slope	-0.168	0.000	-0.115
	s.e.	0.084	0.094	0.068
	P-value	0.072	0.998	0.118
Sinapine	Intercept	19.35	68.78	13.31
	s.e.	0.49	0.44	0.38
	Slope	-0.021	-0.081	-0.030
	s.e.	0.093	0.085	0.072
	P-value	0.824	0.362	0.682
Tannin	Intercept	19.64	68.90	13.53
	s.e.	0.37	0.33	0.28
	Slope	-0.199	-0.274	-0.190
	s.e.	0.184	0.163	0.138
	P-value	0.306	0.123	0.201
Psol	Intercept	17.89	67.69	12.10
	s.e.	0.41	0.52	0.31
	Slope	0.024	0.012	0.019
	s.e.	0.007	0.009	0.006
	P-value	0.007	0.220	0.007

7.27. Linear regression RSM glucosinolates on energy parameters

Table 27. Linear regression of sRSM individual glucosinolate levels with gross energy, energy digestibility and AME in broilers.

| | | Energy parameter | | |
	`	GE	Digestibility	AME
4OH-gluco-brassicin	Intercept	18.99	68.26	12.96
	s.e.	0.07	0.11	0.06
	Slope	0.409	0.168	0.312
	s.e.	0.095	0.142	0.075
	P-value	0.002	0.263	0.002
Epi-progoitrin	Intercept	19.27	68.39	13.18
	s.e.	0.08	0.07	0.06
	Slope	-0.388	-0.353	-0.334
	s.e.	0.496	0.470	0.381
	P-value	0.452	0.469	0.401
Gluco-alyssin	Intercept	19.16	68.28	13.09
	s.e.	0.06	0.05	0.04
	Slope	0.039	0.040	0.035
	s.e.	0.015	0.013	0.010
	P-value	0.023	0.011	0.006
Gluco-berin	Intercept	19.12	68.07	13.02
	s.e.	0.18	0.14	0.14
	Slope	0.333	0.827	0.388
	s.e.	0.475	0.379	0.356
	P-value	0.499	0.054	0.301
Gluco-brassicin	Intercept	19.18	68.40	13.12
	s.e.	0.11	0.11	0.09
	Slope	0.910	-0.570	0.510
	s.e.	1.350	1.290	1.050
	P-value	0.516	0.668	0.640
Gluco-Napin	Intercept	19.24	68.16	13.11
	s.e.	0.14	0.11	0.11
	Slope	0.002	0.060	0.013
	s.e.	0.035	0.027	0.027
	P-value	0.963	0.054	0.645
Gluco-nasturtiin	Intercept	19.19	68.44	13.13
	s.e.	0.09	0.08	0.07
	Slope	0.603	-0.928	0.233
	s.e.	0.761	0.681	0.602
	P-value	0.447	0.203	0.707
Gluco-raphanin	Intercept	19.21	68.09	13.08
	s.e.	0.17	0.13	0.13
	Slope	0.059	0.548	0.147
	s.e.	0.322	0.251	0.245
	P-value	0.858	0.054	0.561
Progoitrin	Intercept	19.24	68.16	13.11
	s.e.	0.14	0.11	0.11
	Slope	0.001	0.029	0.006
	s.e.	0.018	0.014	0.014
	P-value	0.964	0.063	0.655

7.28. Linear regression RSM NDF on SID AA in pigs

Table 28. Linear regression of NDF with SID for CP and essential amino acids in pigs, using a combination of RSE, sRSM and RSM samples.

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Dependent variable	Prediction equation	SE		p value		Model	
		Intercept	Estimate	Intercept	Estimate	r^2	p value
CP SID	1.1816-0.0014×NDF	0.0862	0.0003	0.001	0.002	0.83	0.002
Arg SID	1.1727-0.0010×NDF	0.0307	0.0001	0.001	0.001	0.95	0.001
His SID	1.1934-0.0011×NDF	0.0586	0.0002	0.001	0.001	0.87	0.001
Ile SID	1.0596-0.0008×NDF	0.0322	0.0001	0.001	0.001	0.92	0.001
Leu SID	1.0371-0.0008×NDF	0.0312	0.0001	0.001	0.001	0.91	0.001
Lys SID	1.2495-0.0017×NDF	0.1040	0.0003	0.001	0.002	0.82	0.002
M+C SID	1.1235-0.0012×NDF	0.0756	0.0002	0.001	0.002	0.83	0.002
Phe SID	1.0378-0.0007×NDF	0.0391	0.0001	0.001	0.001	0.87	0.001
Thr SID	1.0424-0.0010×NDF	0.0437	0.0001	0.001	0.001	0.91	0.001
Val SID	1.0017-0.0008×NDF	0.0492	0.0001	0.001	0.001	0.84	0.001

AA, amino acid; Arg, arginine; CP, crude protein; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; M+C, methionine and cysteine; NDF, neutral detergent fibre; Phe, phenylalanine; r^2, coefficient of determination; SE, standard error; SID, standardised ileal digestibility; Val, valine.

7.29. Growth trial on RSM in broilers Harvest 2014

Table 29. Simple effect means for growth performance response of broilers to increasing dietary levels of PR46W21 and DK Cabernet RSMs and whole seed inclusion.

| Variety	Level, g/kg	Starter BWG, g	Starter FCR	Finisher BWG, g	Finisher FCR	Overall BWG, g	Overall FCR
Basal diet	0	1015	1.241	2593	1.554	3608	1.466
PR46W21	50	981	1.278	2490	1.597	3471	1.506
PR46W21	100	971	1.265	2427	1.595	3398	1.500
PR46W21	150	894	1.316	2414	1.679	3308	1.581
PR46W21	200	899	1.327	2352	1.646	3252	1.558
DK Cabernet	50	958	1.275	2453	1.624	3411	1.525
DK Cabernet	100	956	1.288	2496	1.608	3451	1.520
DK Cabernet	150	894	1.309	2375	1.655	3270	1.560
DK Cabernet	200	893	1.348	2265	1.718	3158	1.613
DK Cabernet seed	80	963	1.259	2351	1.624	3314	1.518
Pooled SEM							
P-values for main effects and interaction							
Variety		0.375	0.346	0.408	0.072	0.001	0.497
Level		< 0.001	< 0.001	< 0.001	0.005	< 0.001	<0.001
Variety × Level		0.755	0.491	0.024	0.140	0.054	0.170
P-values for contrasts							
Linear - PR46W21		< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001
Quadratic - PR46W21		0.726	0.822	0.288	0.428	0.255	0.612
Linear - DK Cabernet		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Quadratic - DK Cabernet		0.173	0.796	0.442	0.558	0.741	0.169
Basal vs. DK Cabernet seed		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

7.30. Growth trial on RSM in growing and finish pigs Harvest 2014

Table 30. Growth performance response of grower and finisher pigs to increasing dietary levels of PR46W21 and DK Cabernet RSMs.

		Growers			Finishers		
RSM Variety	RSM level	ADFI	BWG	FCR	ADFI	BWG	FCR
	(g/kg)	(g/day)	(g/day)		(g/day)	(g/day)	
None	0	2112	1062	1.984	2684	1150	2.33
DK Cabernet	50	2106	1079	2.030	2807	1112	2.46
	150	2057	969	2.078	2647	1157	2.31
	250	2046	982	2.112	2601	1168	2.22
PR46W21	50	2113	1035	2.019	2706	1197	2.31
	150	1948	929	2.006	2921	1160	2.43
	250	1827	946	2.041	2702	1254	2.29
	s.e.d.	64	65	0.096	120	60	0.07
P-values							
Diet effects	RSM	0.016	0.064	0.331	0.562	0.546	0.881
	Variety	0.010	0.291	0.542	0.269	0.228	0.800
	Level	0.001	0.050	0.599	0.299	0.321	0.040
	Interaction	0.023	0.932	0.597	0.317	0.963	0.070

8. Figures

8.1. Amino acids in Harvest 2012 samples (Lincolnshire)
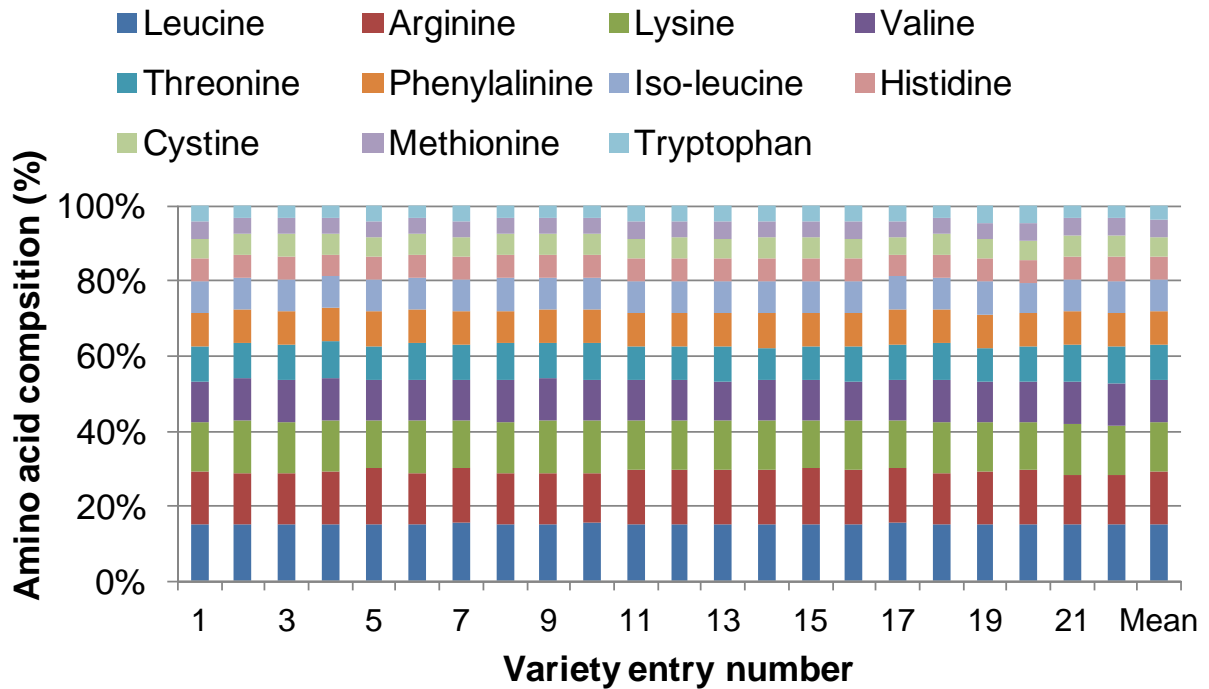
Figure 1. The AA profiles for 22 cultivars of bench prepared defatted oilseed rape meal from a single trial site (Lincolnshire).
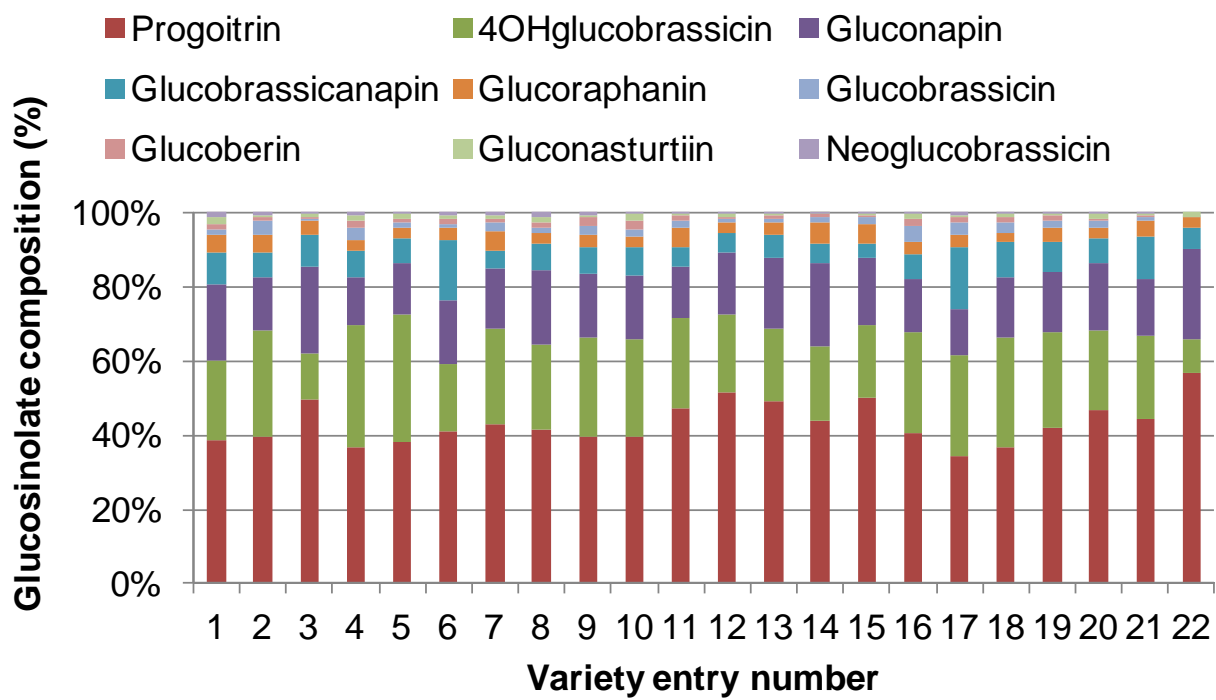
Figure 2. Glucosinolate composition of 22 cultivars of bench prepared defatted oilseed rape meal as a mean of 5 trial sites.

Glucosinolates before and after processing Harvest 2013
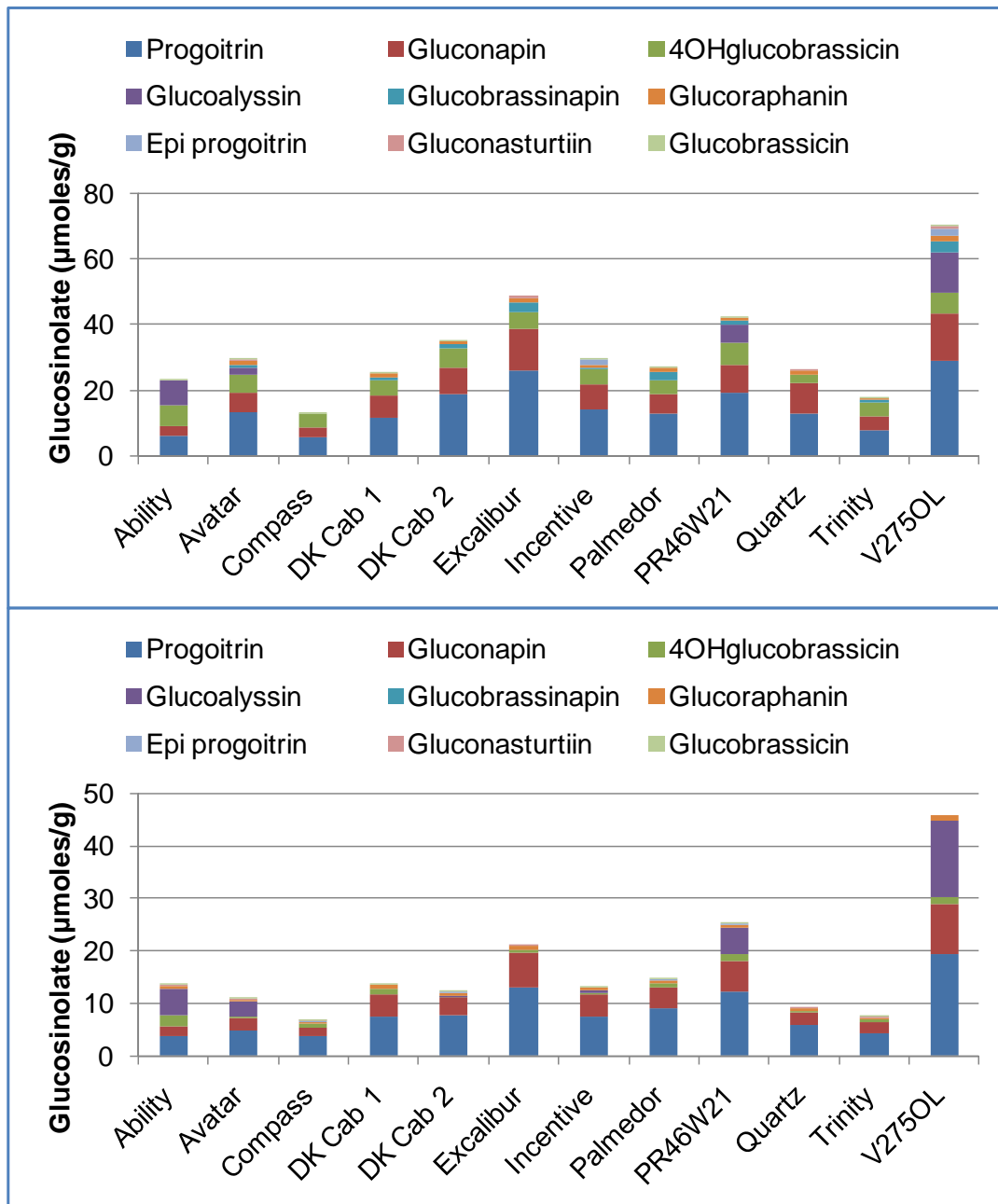
Figure 3. Glucosinolate composition of 12 cultivars of oilseed rape before and after processing with the semi-industrial 'mild' process at CREOL, France.

8.4.   Glucosinolates before and after processing Harvest 2014
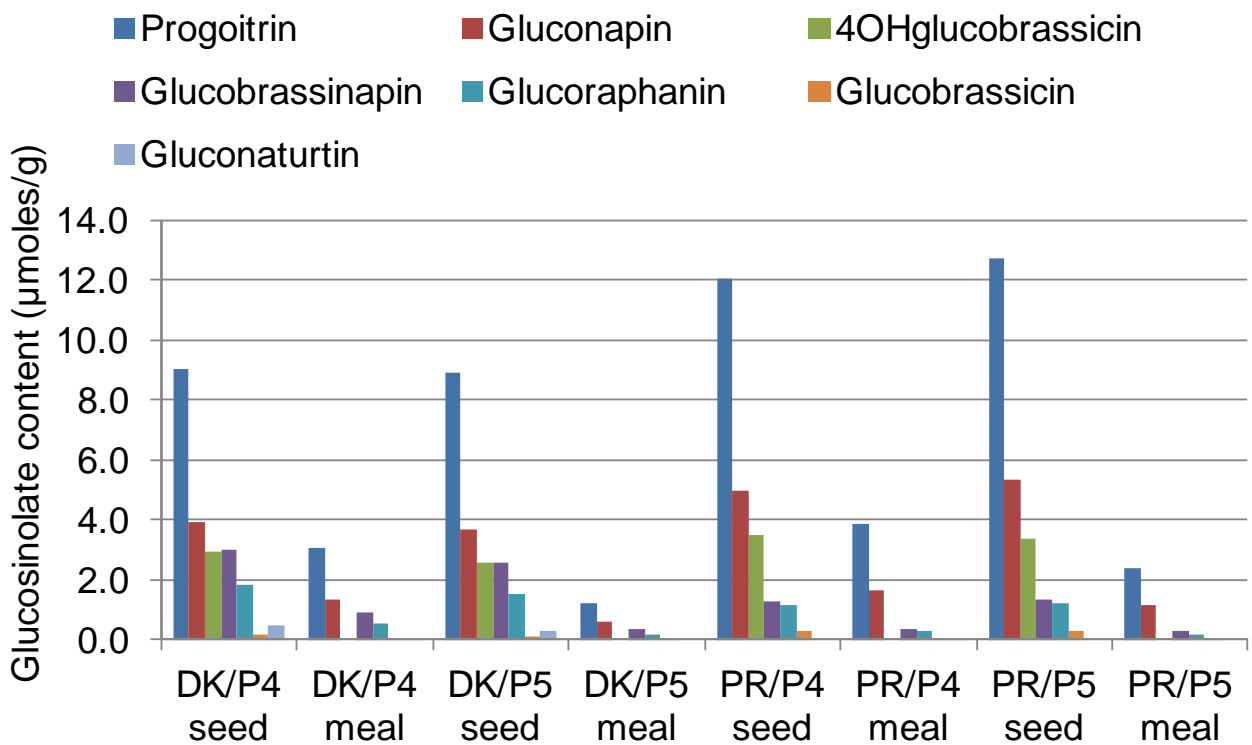
Figure 4. Composition of glucosinolates in the seed supplied and the meals obtained from mild processing (P4) and hard processing (P5) for the varieties DK Cabernet (DK) and PR46W21 (PR).

8.5. Glucosinolates loss during processing Harvest 2014
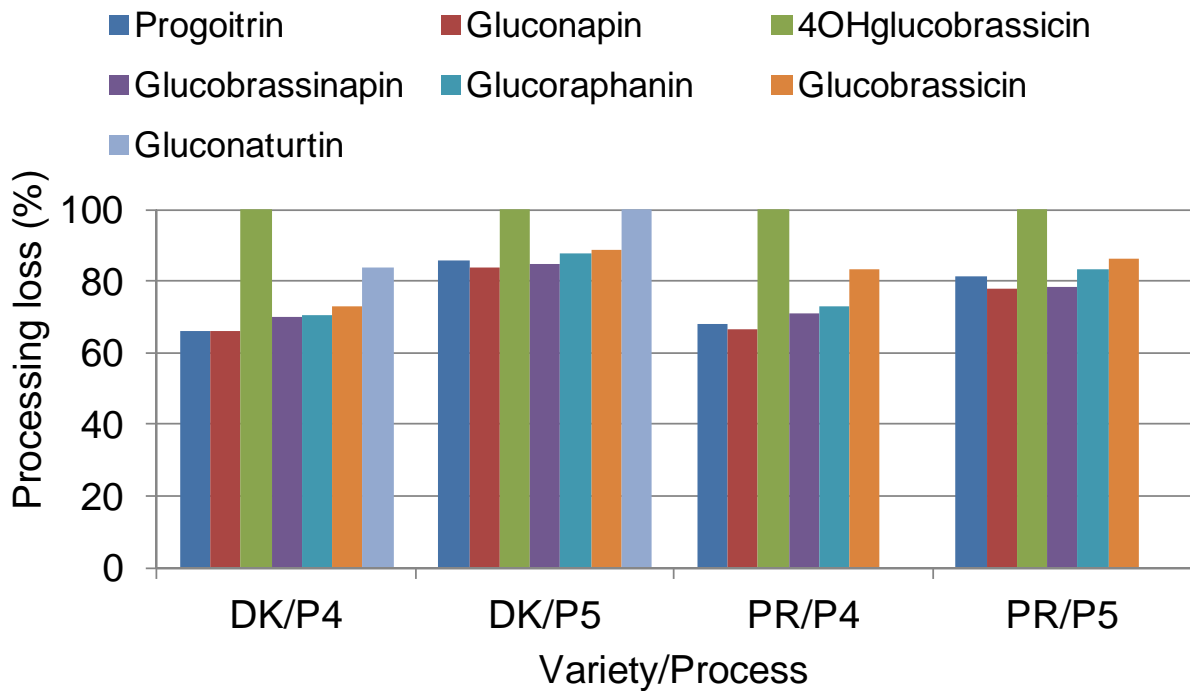
Figure 5. Reduction of individual glucosinolates in the meals obtained from mild processing (P4) and hard processing (P5) for the varieties DK Cabernet (DK) and PR46W21 (PR), compared with analysis of the seed, extracted by cold hexane extraction.

9. Appendices

9.1. Appendix 1a: Varieties used from Harvest 2012

Twenty-two varieties of oilseed rape selected to represent a wide range of breeding type, performance, quality, disease resistance and morphological characteristics from National List trials at Harvest 2012, for biochemical assays for project year 1. Trial sites used were: Elsoms, Notts; DSV, Oxon; Limagrain, Lincs; Scottish Agronomy, Angus; and NIAB, Kent.

Variety		Status	Breeding	Descriptive notes
No. (/22)	Name	(Harvest 2012)	type	(relative to current varieties in test)
1	DK Cabernet	Control	Open pollinated	Relatively low glucosinolate content. Late flowering. Market leader
2	Vision	Control	Open pollinated	Mid-range glucosinolate content. Relatively low yield and oil content.
3	Excalibur	Control	Hybrid	Very early flowering. Relatively low yield and oil content.
4	*	NL2	Hybrid	Very high glucosinolate content. Low oil content.
5	Charger	NL2	Open pollinated	Very high yield. Low oil content. Very early flowering. Poor disease resistance.
6	Ventura	NL2	Open pollinated	Moderate yield. Mid-range glucosinolate content.
7	Incentive	NL2	Open pollinated	Very high yield. Mid-range glucosinolate content.
8	Trinity	NL2	Open pollinated	High yield. Mid-range glucosinolate content.
9	Fletcher	NL2	Hybrid	High yield. Very good stem canker resistance. Mid-range glucosinolate content.
10	PX109	NL2	Hybrid semi-dwarf	Very short. Moderate yield. Mid-range glucosinolate content.
11	Ginfizz	NL2	Hybrid	Moderate yield
12	PT299CL	NL2	Clearfield™ Hybrid	Tolerant to Cleranada herbicide. Low yield. Mid-range glucosinolate content.
13	DK Sentinel	NL2	Hybrid semi-dwarf	Short. Low oil. Low yield. Mid-range glucosinolate content.
14	*	NL2	Hybrid	Relatively high glucosinolate content. Low oil content. Low yield.
15	*	NL2	Open pollinated	Relatively high glucosinolate content. Low oil content. Low yield.
16	*	NL1	Open pollinated	Very low glucosinolate content.
17	Popular	NL1	Hybrid	Very high yield. Very high oil content. Mid-range glucosinolate content.
18	Marble	NL1	Hybrid semi-dwarf	Very short. High yield. Very high oil content. Mid-range glucosinolate content.
19	SY Medal	NL1	Hybrid	High yield. Mid-range glucosinolate content.
20	Picto	NL1	Open pollinated	High yield. Mid-range glucosinolate content.
21	Attletick	NL1	Open pollinated	Hybrid. High yield. Mid-range glucosinolate content.
22	*	NL1	Hybrid HOLL	High glucosinolate content. Low oil content. High oleic/low linolenic oil profile.

*These varieties did not progress to National List and their anonymity was requested.

9.2. Appendix 1b: Varieties used from Harvest 2013

Six-teen bulks of OSR of known variety were sourced for from Harvest 2013 for digestibility studies in project Year 2.

Sample No	Variety	Origin	Processing sRSM	Processing RSE	Type[1]
1/12	Ability	Eltisley, Cambs	X		SOR, O.P.
2/12	Avatar	Eltisley, Cambs	X		WOR, hybrid
3/12	Compass[2]	Wymondham, Norfolk	X		WOR, hybrid
4/12	DK Cabernet	Coton, Cambs.	X		WOR, O.P.
5/12	DK Cabernet	Eltisley, Cambs	X		WOR, O.P.
6/12	Excalibur	Aby, Lincs.	X		WOR, hybrid
7/12	Incentive	Norfolk	X		WOR, hybrid
8/12	Palmedor	Suffolk	X		WOR, HEAR
9/12	PR46W21	Coton, Cambs.	X		WOR, hybrid
10/12	Quartz	Granchester, Cambs.	X		WOR, O.P.
11/12	Trinity	Spalding, Lincs.	X		WOR, O.P.
12/12	V275OL	Welborn, Lincs.	X		WOR, HOLL
1/4	Compass	Wymondham, Norfolk		X	WOR, hybrid
2/4	DK Cabernet	Grantchester, Cambs		X	WOR, O.P.
3/4	NK Grandia	Longstanton, Cambs.		X	WOR, O.P.
4/4	Sesame	Grantchester, Cambs		X	WOR, O.P.

[1]WOR: Winter rape; SOR: Spring rape; HEAR: high erucic; O.P.: Open pollinated; HOLL: high oleic / low linolenic

[2]A double bulk sample was acquired to allow processing by both methods

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9.3. Appendix 2: Preparation of test meals at CREOL

Objective

The aim of the production was to supply 4 rapeseed meals resulting from the regular extraction of Flaked and Warm Pressed seeds (FWP) for Cabernet and PR46W21 (3500 kg each), and Cold Pressed seeds (CP): Cabernet and PR46W21 (500 kg each).

Materials and methods

Seeds

Two types of rapeseeds: Cabernet and PR46W21 were delivered at Pessac. 4796 kg of Cabernet were received and sorted; 4750 kg were kept. 4616 kg of PR46W21 were received and sorted; 4525 kg were kept. Moisture content was around 7.8-7.9% (NIR).

Equipment

Flaker (Damman Croes): Seeds or cracked seeds are flaked by passing through two contra-rotating smooth cylinders of 500 mm in diameter. The space between the cylinders can be adjusted and a couple of hydraulic jacks hold the mobile cylinder against the still one. The rotating speed of the rolls is 350 rpm, both rolls have the same rotating speed. The motor has power is 22 kW but this one is connected in 3x220V, the power when running empty is 5.8 kW.

Hydraulic jack (5MPa)
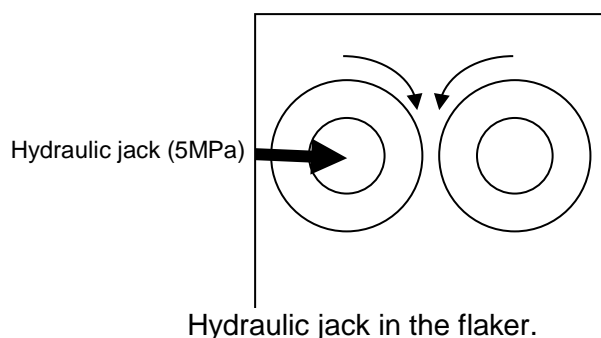
Hydraulic jack in the flaker.

Horizontal cooker (La Mécanique Moderne): This cooker is made up of two superposed horizontal cylinders of 900 mm in diameter and 2000 mm in length. The walls of these cookers are heated by a thermal fluid heated itself by 4 electrical resistances of 4 kW and circulated by a centrifugal pump. The convection of heat in the material is forced by continuous stirring provided by a helical ribbon. The feeding of the upper cylinder is provided by a volumetric feeder fitted with an anti-bridging agitator. The discharge is operated by sliding gates located on the extremity of the cylinders at half height. These gates are commanded by a detector located in the hopper of the discharging screw. As soon as the detector is covered, the gates are closed and reciprocally they are opened when the material in the hopper disappears. Residence time can be adjusted from 20 to 240 min and temperature from 20 to 110°C. The second stage is connected to a fan that can extract the mist steaming from the drying material. One can send water or steam to control the final moisture of the cooked product.
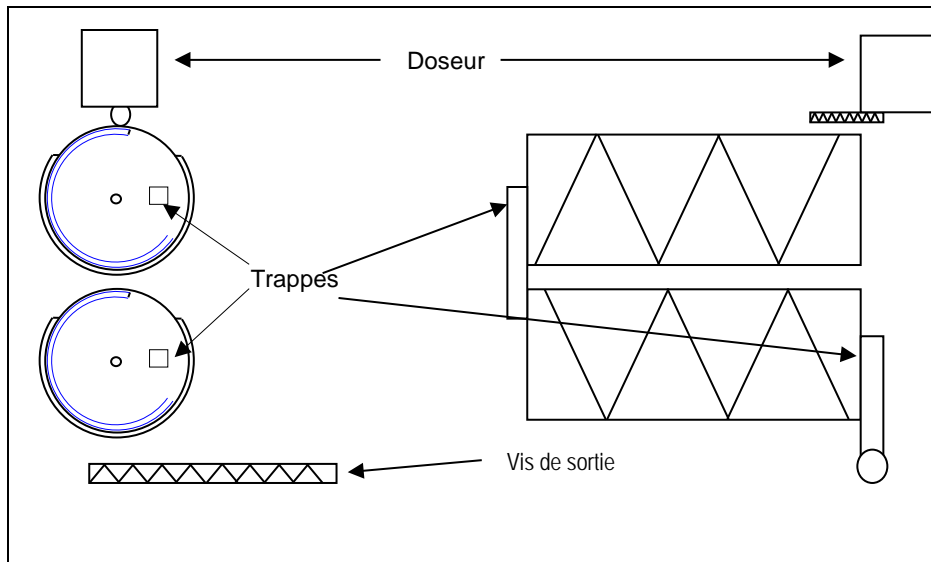
Horizontal cooker.

Screw press (La Mécanique Moderne MBU 75): The material is compressed in a screw that generates smaller volume along the progression toward the discharging choke. The reduction of volume comes from a progressive increasing of the diameter of the shaft and a reduction of the pitch. The flight is not continuous, smooth rings separate elements of screw allowing the accumulation of material in the press. In some places, these rings are conical allowing a stronger resistance to the progression of the cake. This screw is enclosed in a cage formed by longitudinal bars hold by a heavy frame. The bars are separated by metallic spacers in such a way that oil can leave while the solid material is kept inside. The screw elements are removable so that it is possible to adapt the screw profile to the material to be processed. The screw has a rotating speed from 10 to 30 rpm. The motor has a power of 18 kW and the flow can vary from 50 to 600 kg/h according to the nature of seeds and their preparation.

Screw press photo.

13323 13789 13789 13791 22035 22036 16116 16113 13787

18293 19856 19857 10839 13792 15912 15913 15914 22145

13911 16112 15916

x1.33 x1.6 x1.7 x1.20 x1.33 x1.25

Pas 100 à 120 120 90 100 80 80 60 60

18305 18816 18817

Ø 110 Ø 122 Ø 127 Ø 127 Ø 127 Ø 127 Ø 133 Ø 132 8 Ø 136

40 270 120 70 120 70 150 90 110 85 110 85 110 85 35 110 58 210
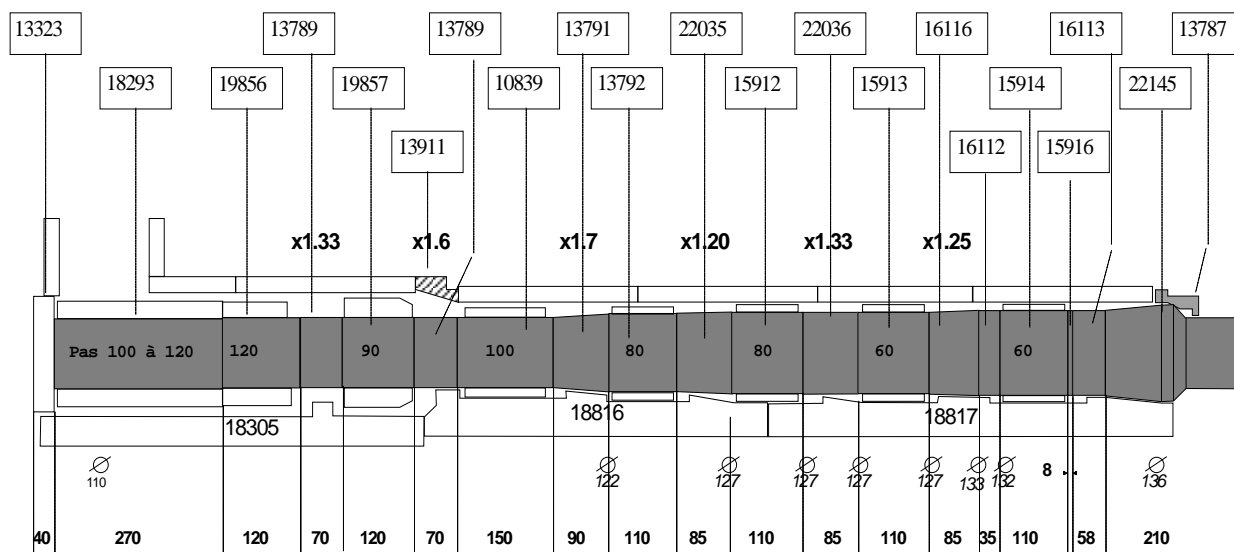
MBU75-25 screw profile with arrangement 13811-1.

Oil screening: Vibrating screen (Chauvin). Directly at the outlet of the press, crude oil is screened to remove the sediments. The screen has 800 mm of diameter and the cloth a porosity of 0.8 mm.

Oil filtration: filter press (Amafilter). Crude oil is filtered after being heated to 80°C in a filter press. The filtrating cloth is made up of polypropylene and has a porosity of 10-20 µm. Its total surface is of 4 square meters; it is composed of 8 plates and may hold around 12 kg of cake.

Oil dryer: Oil is heated to 80°C then pulverized in a vacuum. The drier has a holding capacity of 100 l, the vacuum is provided by a liquid ring pump that allows absolute pressure of 50 mbar (5 kPa).

Continuous belt extractor *(Desmet Ballestra):* The continuous extractor is a belt of 0.4 m in width, 4 m in length bearing a layer of 0.4 m of material to be extracted. The counter flow extraction is carried out by 6 loops of miscella recirculation. The solvent percolates through the material and dissolves the oil according to the gradient of concentration encountered: the richer material at the entry is soaked in miscella with the higher oil concentration while the lesser fatty residue near the outlet is washed by pure hexane to remove the residual oil. The temperature of the miscella is maintained between 50 and 55°C to maximize the de-oiling. The speed of the belt can be adjusted from 1.15 to 5.6 m / hour. In practice, the feeding rate of the extractor can vary from 100 to 300 kg/h according to the extractability of the material or its ability to be desolventized. The feeding is secured by a belt and a vibrating feeder. These features allow the structure of the material to be preserved during the transfer and may avoid problems of percolation with fragile pellets or press-cake. The percolation can be observed through the top of the extractor sealed by a lid of glass.
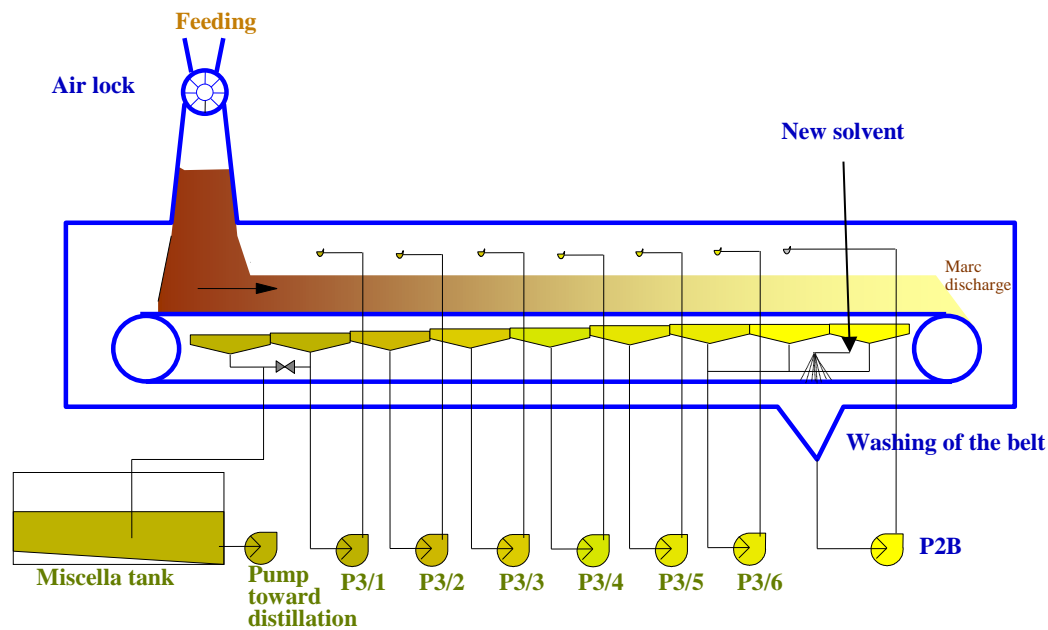
Counter-current extractor.

Desolventizer *(Desmet Schumacher type):* Residue going out of extraction has between 20 and 35 % hexane. This solvent must be removed and recovered for new processing. This operation is carried out in a desolventizer where the residue is heated and stripped by counter-flow steam injection. A chain conveyor brings the residue in the upper part of the apparatus through an air lock. The material is pre-desolventized by passing on the three higher trays heated by indirect steam. The pressure of the indirect steam can be adjusted between 1 and 10 bars (0.1 to 1 MPa). Under the effect of heating, the solvent changes of phase to become gaseous. These vapors are carried by a duct to a scrubber to get rid of dust, then to a condenser where they pass again to the liquid form. By going down, the pre-desolventized meal meets the steam going upward. Because the temperature of pre-desolventized meal is around 70°C, the steam injected through the bottom tray condenses on the meal increasing its water content up to 25 %. This moistening provokes the release of hexane residues adsorbed on the meal and allows a good solvent recovery. In the lower stages of the desolventizer, the meal dries while its temperature increases up to 105°C. Moreover, the steam that crosses the layers of meal displace the phase equilibriums to complete the solvent desorption. At the bottom of the desolventizer, a screw conveyor with variable speed carries the meal outward where it is cooled down by air ventilation.
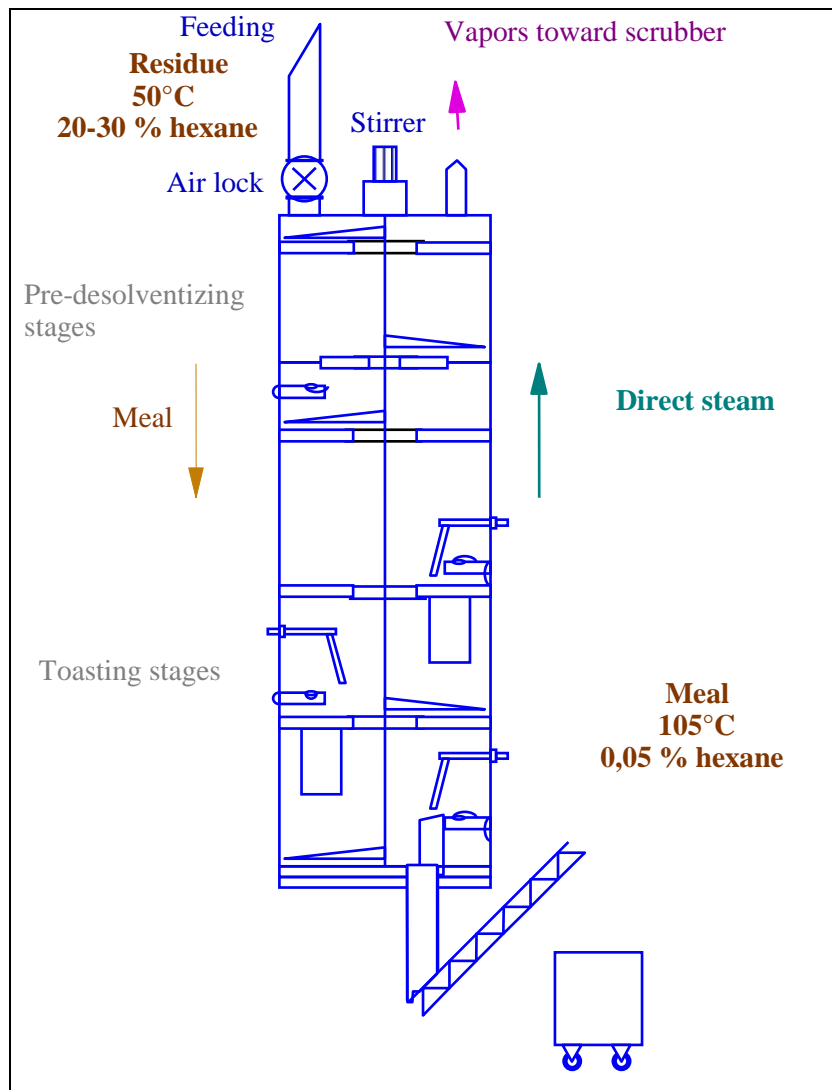
Desolventizer.

Distillation – continuous ensemble of 3 vacuum loops. The miscella stemming from the extraction contains 10 to 30 % of oil. The boiling point of mixtures of hexane and vegetable oil increases with the lower hexane concentration. Decreasing the pressure gives lower boiling point but it is not sufficient to reach satisfactory solvent recovery at temperatures below 100°C. It is necessary to carry out a steam stripping to bring the residues to an acceptable level. Thus, the solvent is eliminated in two steps: in the first loop which works at 0.4 bar and 80°C, the hexane concentration passes from 70-90 % to 8 %, in the second loop under 0.13 bar and 90°C, stripping with steam secures the elimination of residues to a maximum concentration of 0.1 %. The role of the last loop which works at 0.07 bar is to remove water which could have been trapped by phospholipids during stripping. Each loop comprises a pump, a heat exchanger, an evaporator and a cyclonical separator that stops the droplets of oil carried by the gas. The exchangers have been designed in order to minimize the differential of temperatures between the oil and the calorific fluid. They have oversized areas of exchange and they can be heated by low pressure steam (0.5 to 1 bar).

Protocol

A first preliminary trial was performed in December 2014 in order to estimate the desolventizer residence time. Two different time durations were tested (twice): 80 and 100 min. The corresponding protein solubility were both satisfactory (between 45 and 50 %) and quite similar.

Process 1 FWP seeds processing: 3500 kg of each type of seeds were pressed; DK Cabernet and PR46W21 seeds were processed on 6 and 8 January 2015, respectively. The cooking and press temperature was supposed to be around 90°C. The residence time targeted was 45 min, combined with a constant throughput of 320 kg/h. The aim was to get 20 % oil in the cake, so the press speed rotation had to be adjusted to achieve a 32% oil content coming out of the press. The press has 7 screw elements separated by smooth rings. The volume generated by the screw rotation is reduced each time that the material passes from one screw element to the following. The number preceded by x on the figure indicates the volume reduction ratio R = $\frac{V_n}{V_{n+1}}$, with V_n = volume generated by the rotation of the screw number n.

The total compression ratio of the press was 7.2. Extraction was carried out at constant throughputs (200 kg/h for the cake and 230-250 l/ h for the solvent). The temperature was fixed at 50-55°C. The desolventization step was performed during 90 min at 105°C with an indirect steam pressure of 3.5 bars and a direct steam of 15 kg/h.

Process 2 CP seeds process: 511 and 512 kg of Cabernet and PR46W21 seeds were dried (water content < 7 %) and pressed, respectively at a constant throughput of 250 kg/h; this processing took place on 7 January 2015. The extraction was at a constant throughput 180 kg/h for the cake and 220-240 L/h for the solvent, temperature 50-55°C. The desolventisation time was 80 min, temperature 105°C, indirect steam pressure of 1 bar and direct steam of 25 kg/h. This was on 7 January for DK Cabernet and 9 January for PR46W21.

Results

Process 1: FWP seeds processing for DK Cabernet

1) Cooking and pressing for DK Cabernet:
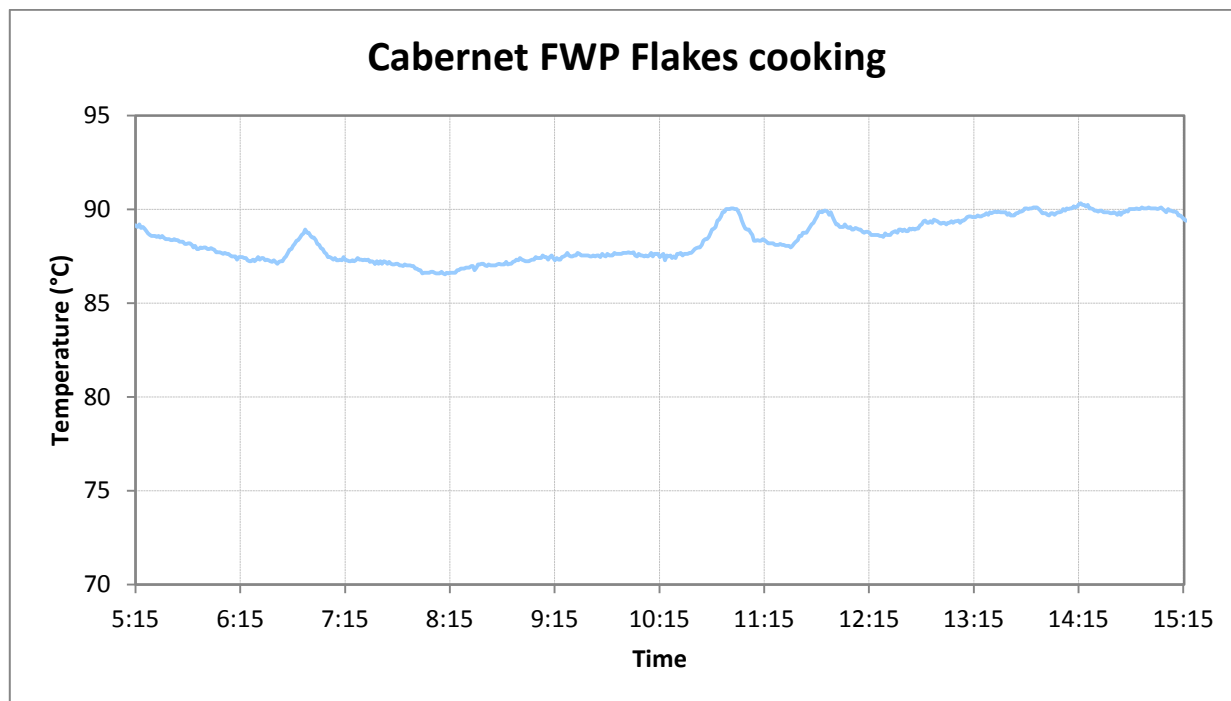
Cooking temperature for DK Cabernet (stabilized period).
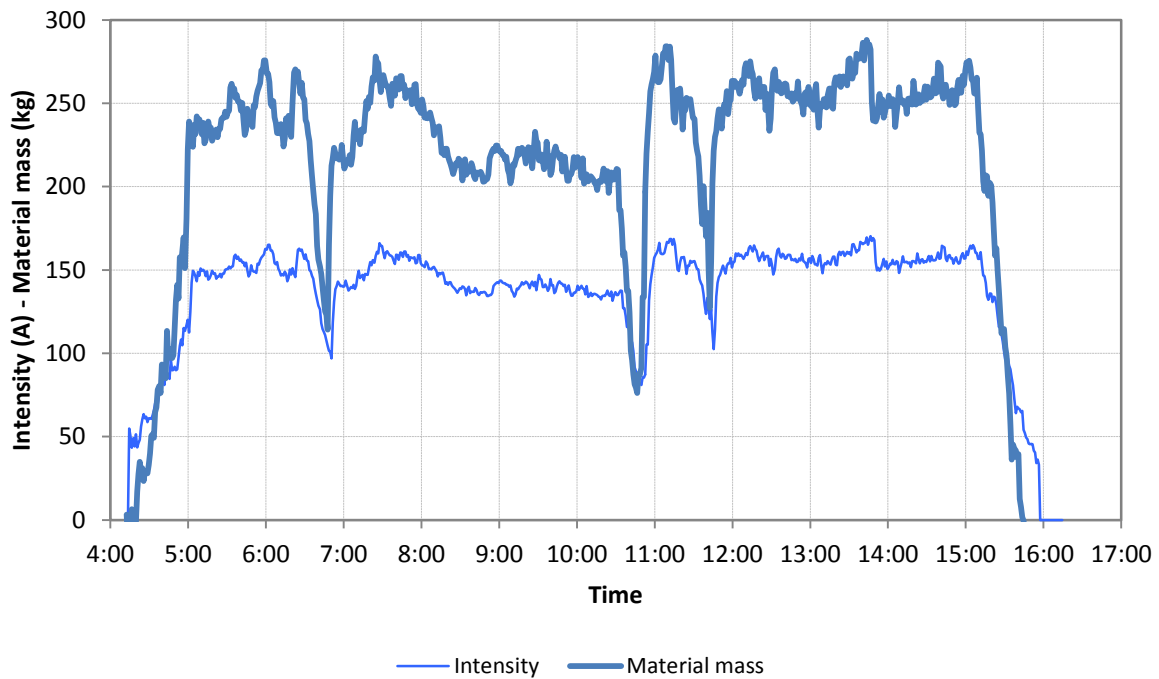
Intensity recorded in the cooker and material mass estimated for DK Cabernet

Material residence time in the cooker can be assessed by compiling intensity and throughputs measurements. Indeed, the cooker is charged with 234.9 kg of seeds on average (SD 34.0 kg) and the throughputs is 325 kg/h (SD 4.8 kg/h), so the residence time corresponding is 43.4 min (SD 6.3 min).

Characterization of the cooking step for DK Cabernet seeds.

	Average	Standard deviation
Temperature	90.1°C	3.6°C
Feeding throughput	325 kg/h	4.8 kg/h
Residence time	43.4 min	6.3 min
Moisture content of the cooked flakes	4.09 %	0.39 %
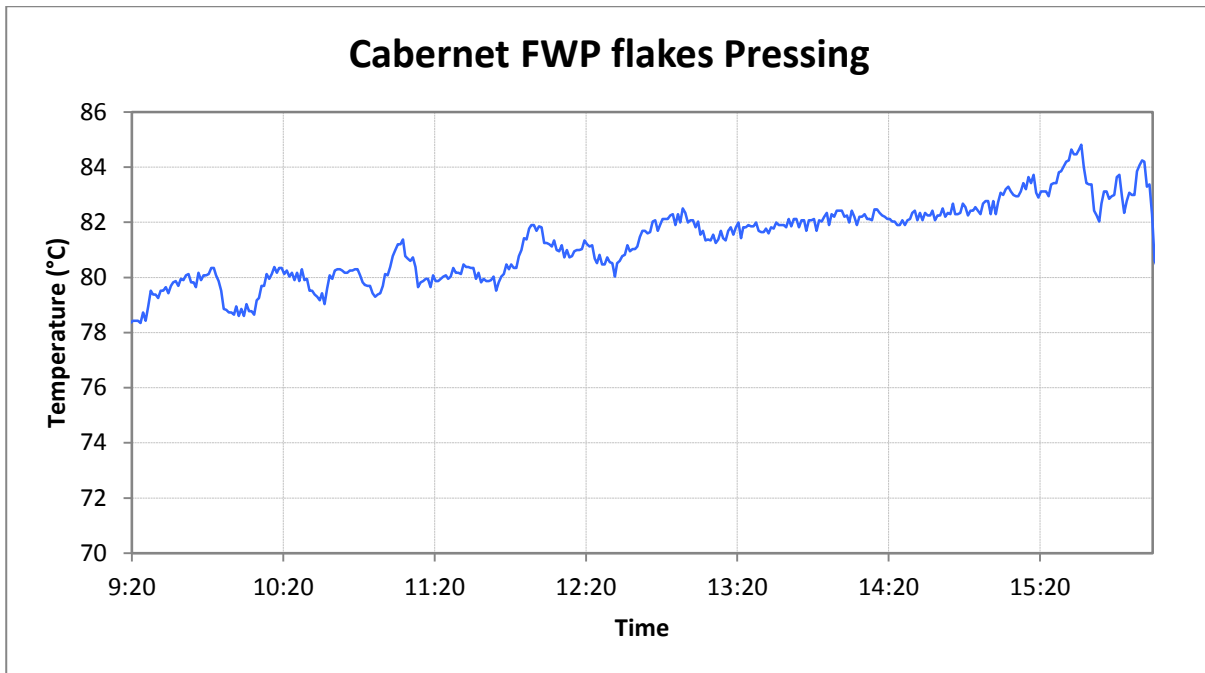
Press temperature for DK Cabernet (stabilized period).
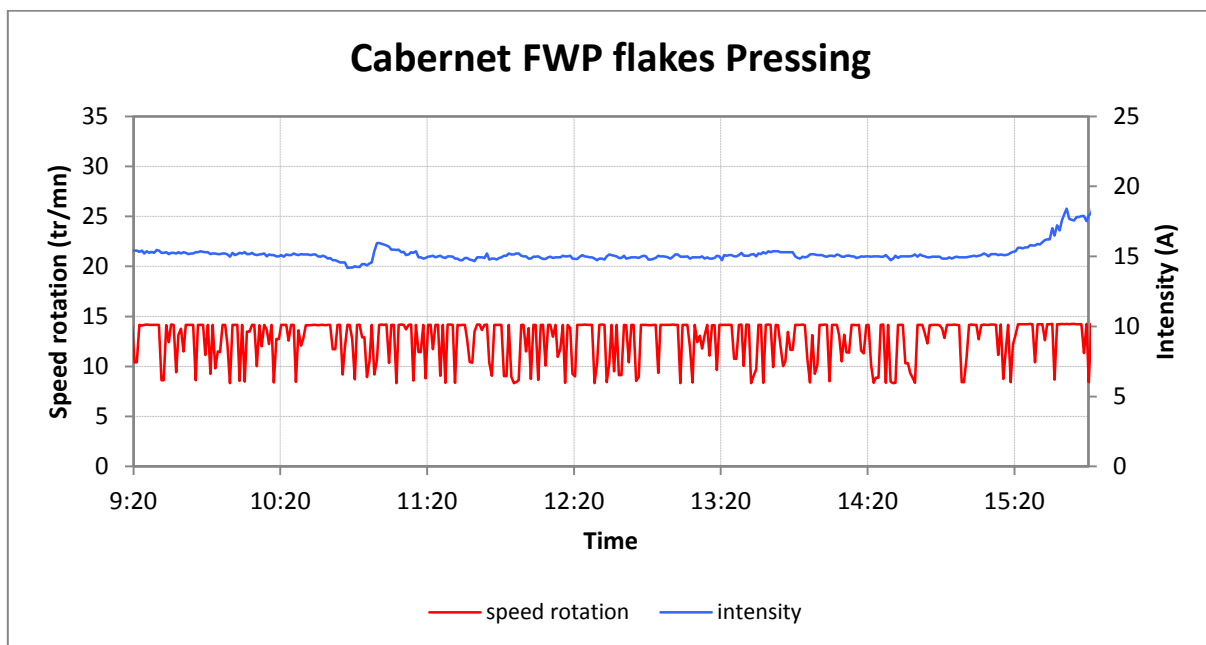
Speed rotation and intensity of the press for DK Cabernet (stabilized period)

Characterization of the pressing for DK Cabernet

	Average	Standard deviation
Press temperature	80.3°C	2.1°C
Total output	323.4 kg/h	9.3 kg/h
% of oil throughput	38.8 %	2.4 %
% of sediments (foots)	0.8 %	0.3 %
Intensity of the press	15.1 A	0.2 A

2) Extraction and desolventization of the cake for DK Cabernet:

1984 kg of cake were introduced into the extractor.

Cabernet FWP Extraction (1)

Extraction temperature for DK Cabernet (1).
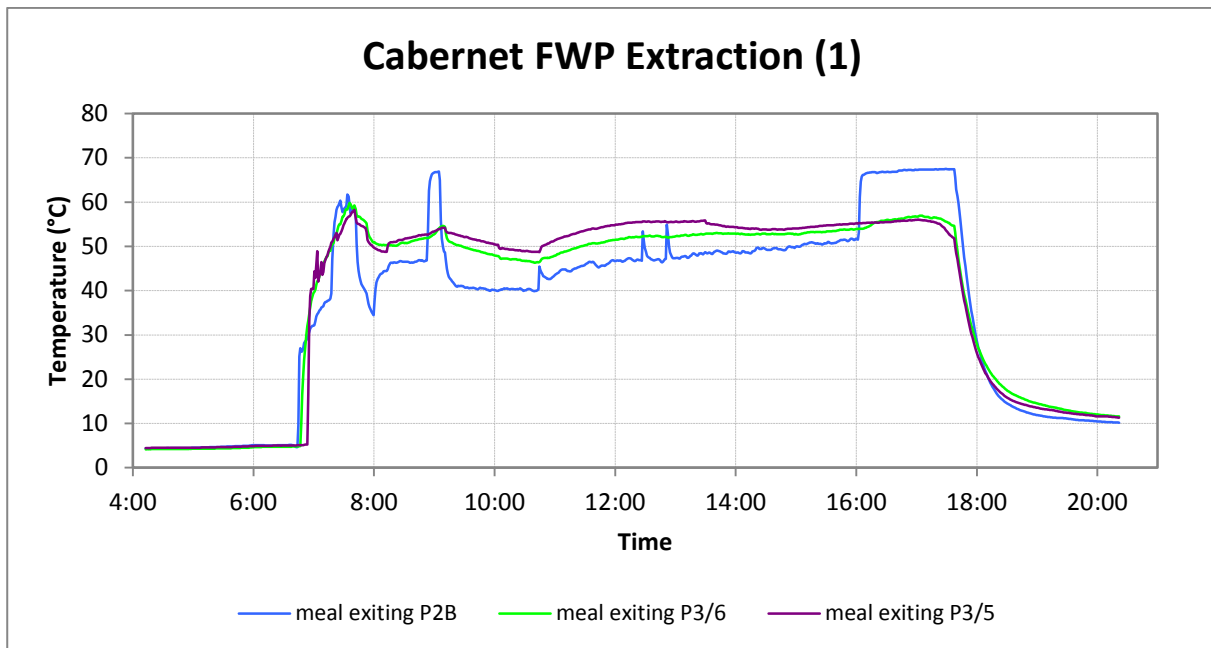
Cabernet FWP Extraction (2)
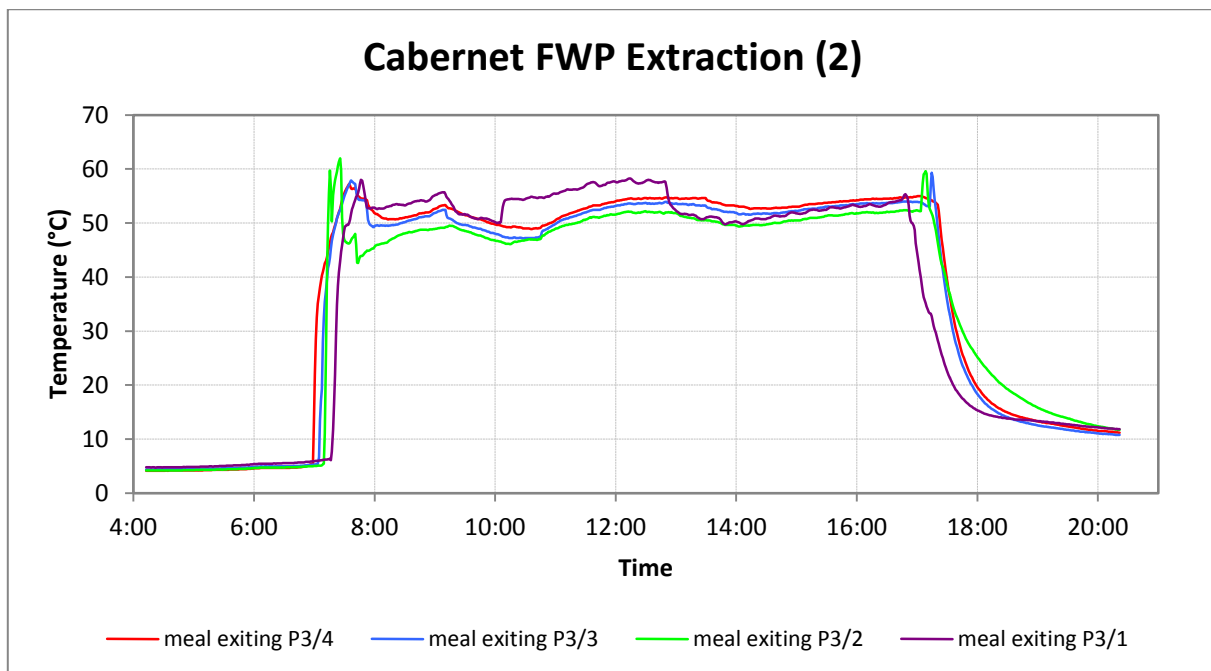
Extraction temperature for DK Cabernet (2).

Notes for extraction temperature graphs:
- P2B is the pump sending the new solvent in the extractor
- P3/6 is the pump moving the poorer miscella (= miscella from stage 6)
- P3/5 ➔ P3/1 are pumps moving miscellas with increasing concentrations:
  stage 5 ➔ stage 1

Cabernet FWP miscella concentration at different stages
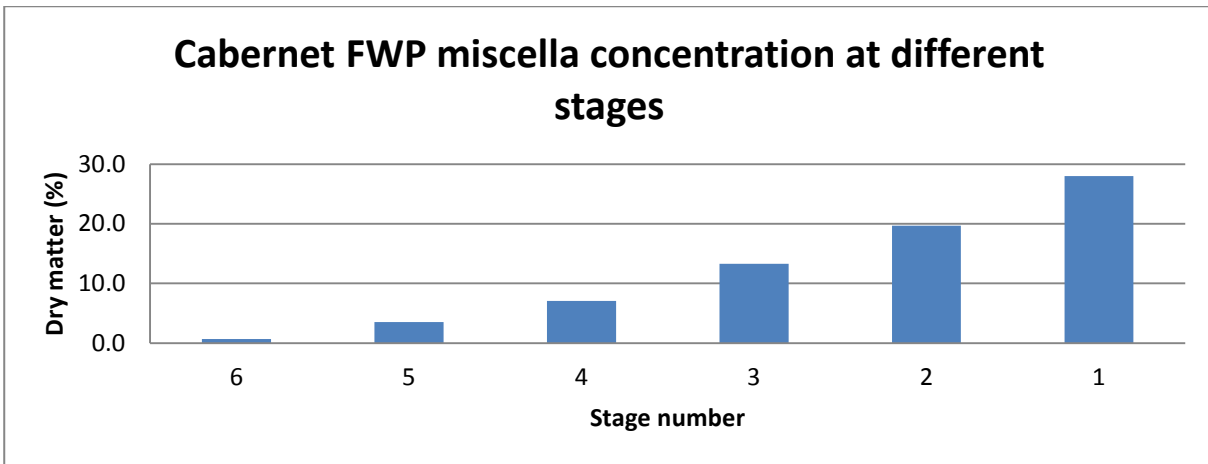
Miscella dry matter concentration for DK Cabernet as a function of the stage number.

Cabernet FWP Desolventization
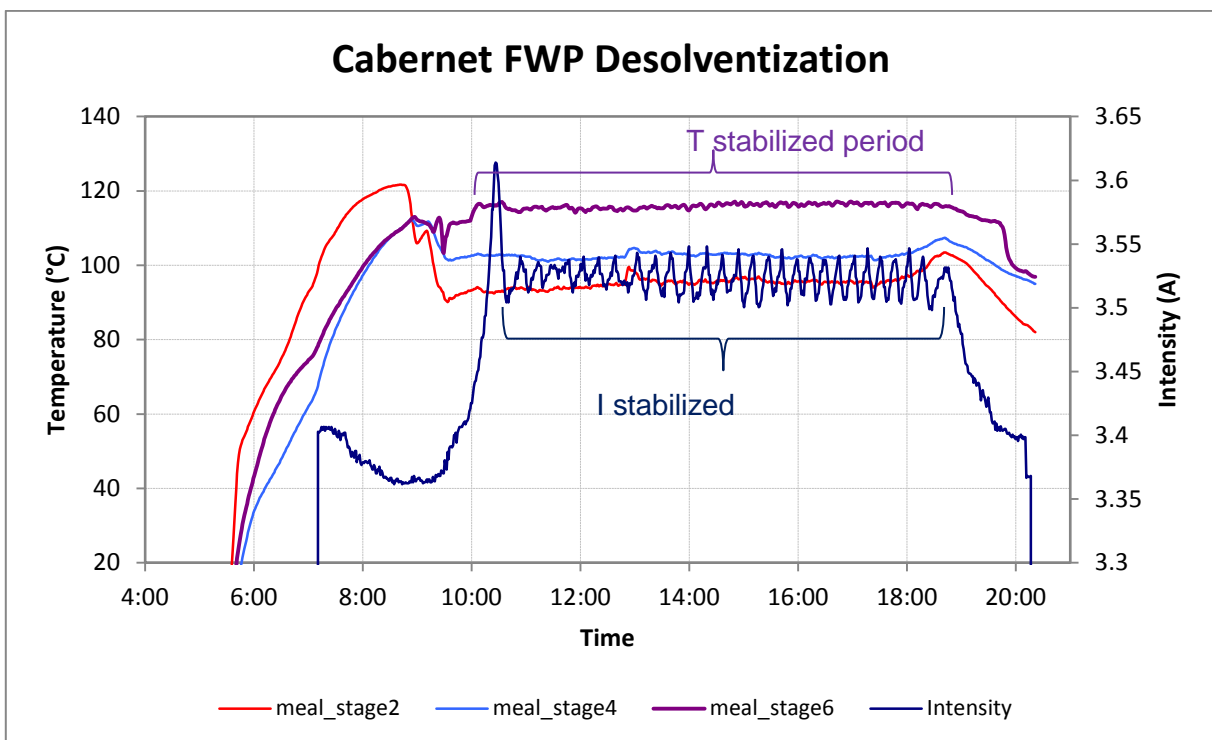
Desolventization temperature and intensity for DK Cabernet

Characterization of the desolventisation for DK Cabernet.

	Average	Standard deviation
Temperature (stabilized period)	115.9°C	0.8°C
Intensity (stabilized period)	3.523 A	0.011 A
Indirect steam pressure	3.5 bars	0.0 bars
Direct steam throughput	25 kg/h	0.0 kg/h

Process 1: FWP seeds processing for PR46W21

1) Cooking and pressing for PR46W21.

PR46W21 FWP flakes Cooking
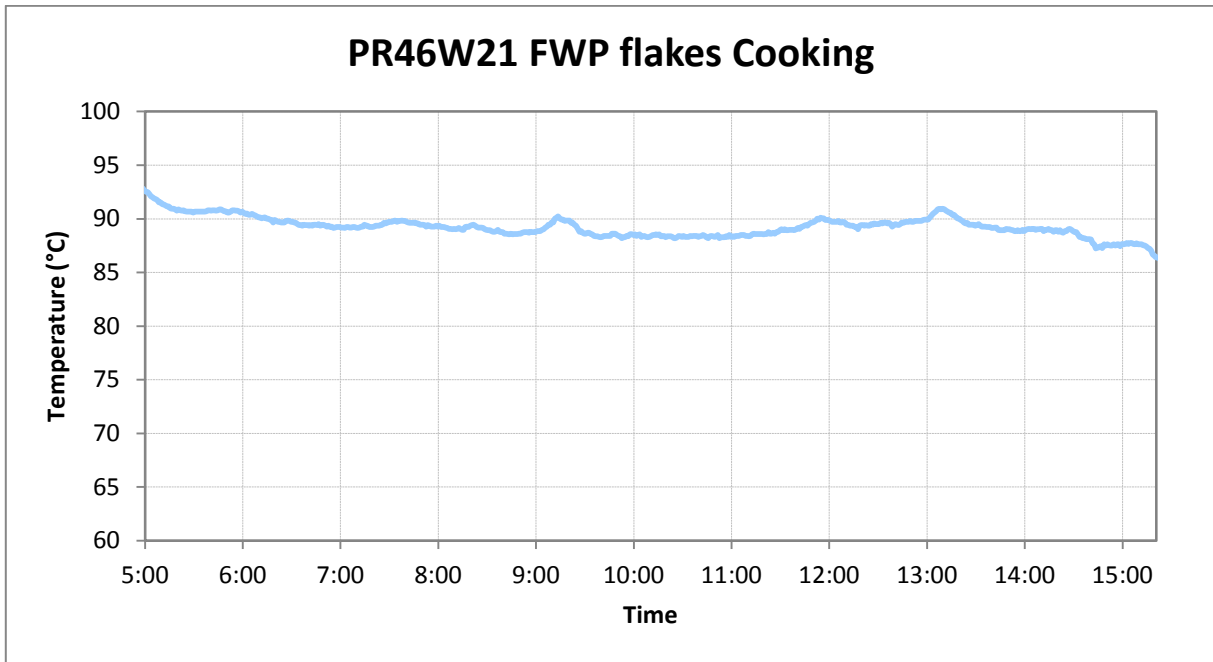
Cooking temperature for PR46W21 (stabilized period).

Intensity and material mass in the cooker for PR46W21 FWP
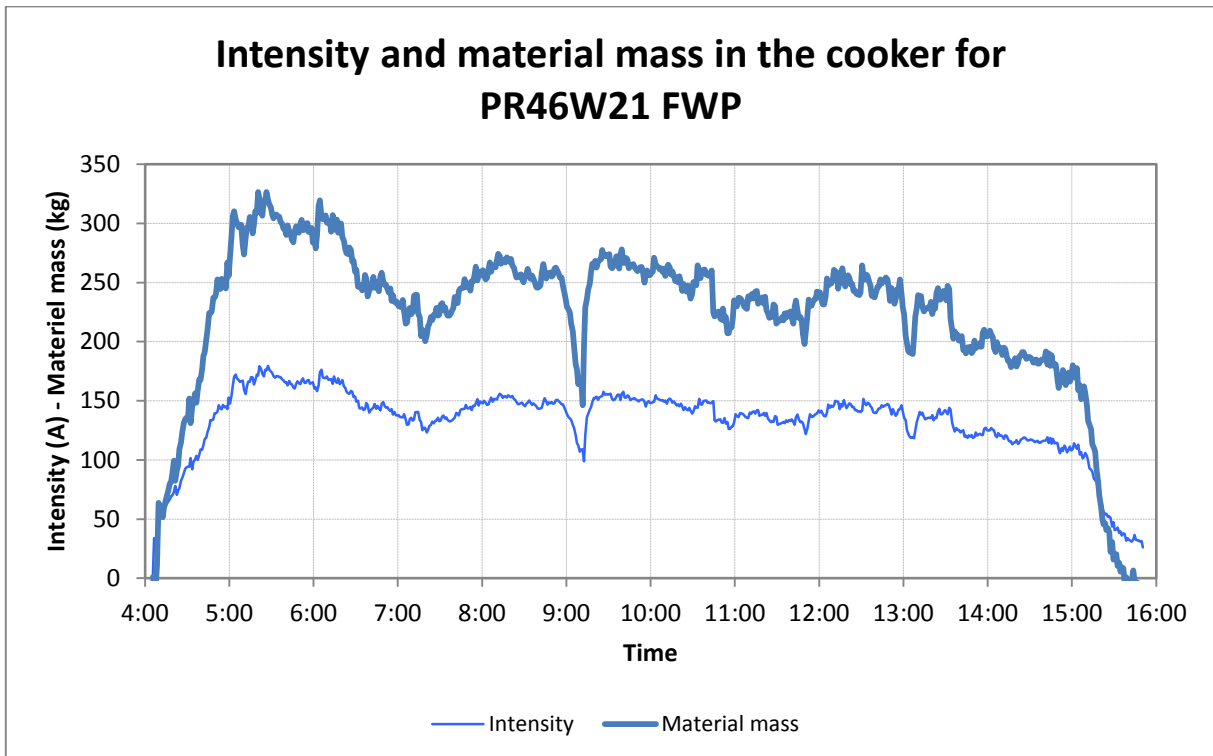
Intensity recorded in the cooker and material mass estimated for PR46W21

As explained before, the cooker is charged with 241.8 kg of seeds on average (SD 35.0 kg) and the throughputs is 319 kg/h (SD 14.1 kg/h), so the residence time corresponding is 45.5 min (SD 6.6 min).

Characterization of the cooking step for PR46W21

	Average	Standard deviation
Temperature	89.6°C	1.1°C
Residence time	45.5 min	6.6 min
Feeding throughput	319 kg/h	14.1 kg/h
Moisture content of the cooked flakes	3.79 %	0.83 %
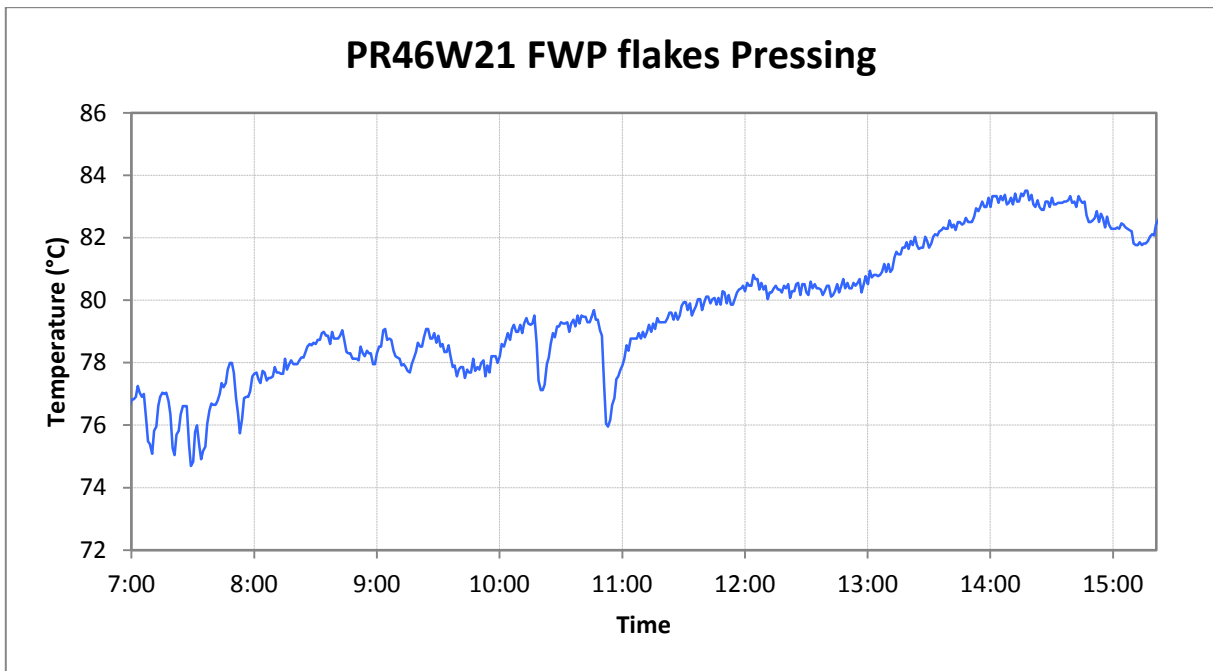
Temperature of the press for PR46W21 (stabilized period).
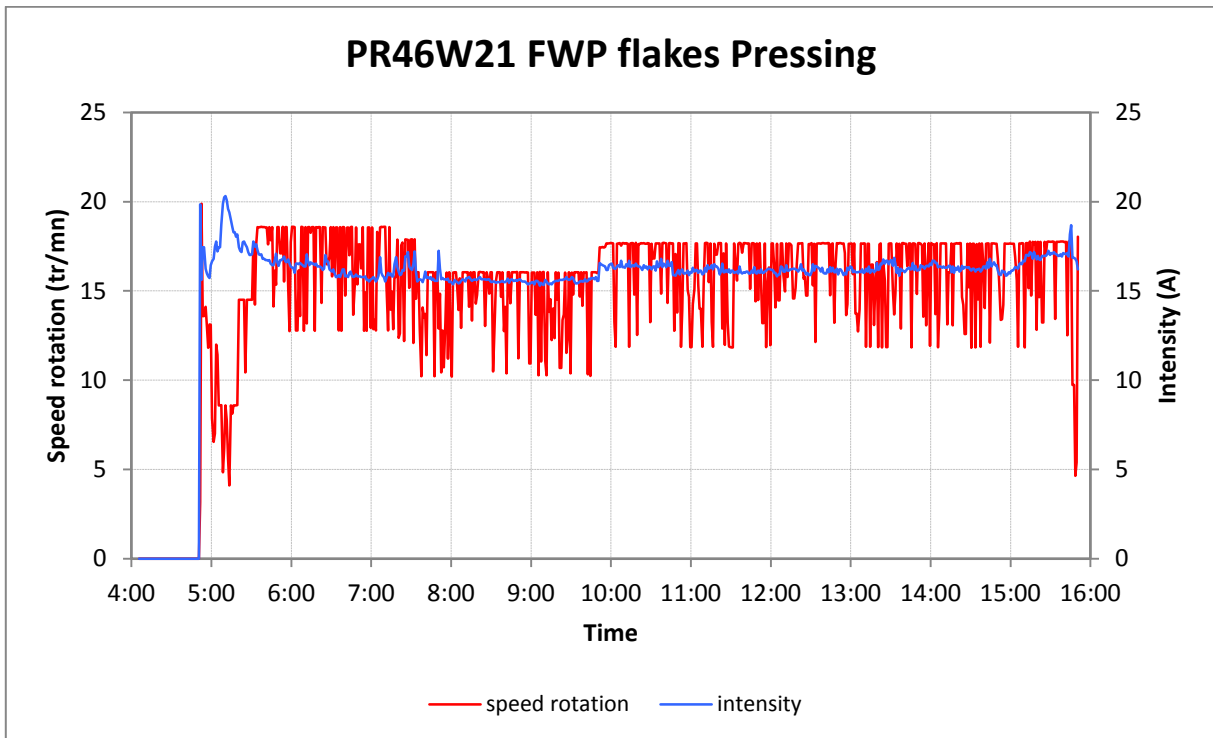
Speed rotation and intensity of the press for PR46W21 (stabilized period).

Characterization of the pressing for PR46W21

	Average	Standard deviation
Press temperature	77.1°C	2.1°C
Total output	294.8 kg/h	18.8 kg/h
% of oil throughput	37.2 %	0.9 %
% of sediments (foots)	0.8 %	0.3 %
Intensity of the press	15.9 A	0.1 A

2) Extraction and desolventization of the cake for PR46W21
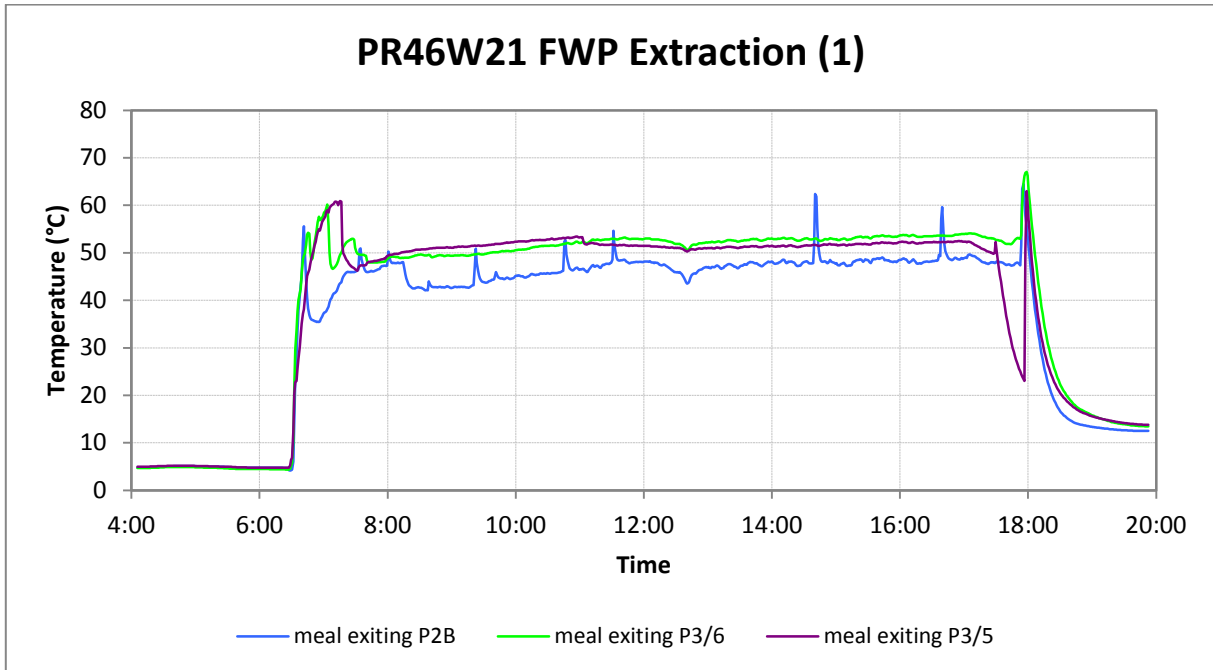
Extraction temperature for PR46W21 (1).

Extraction temperature for PR46W21 (2).

PR46W21 FWP miscella concentration at different stages
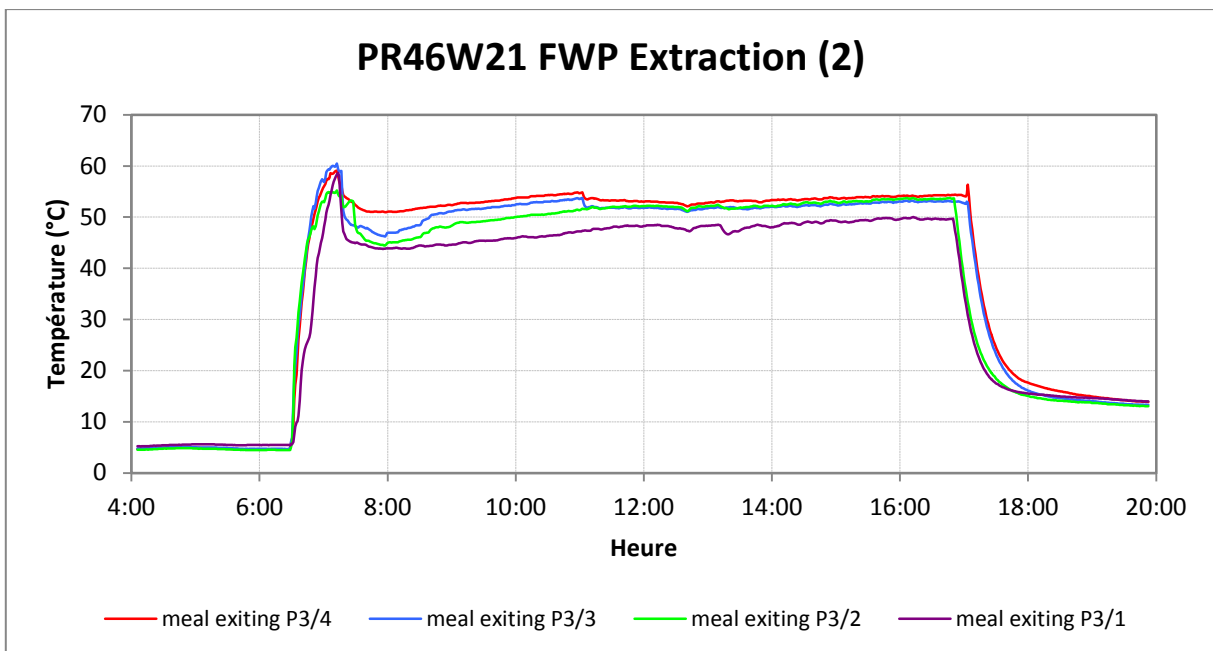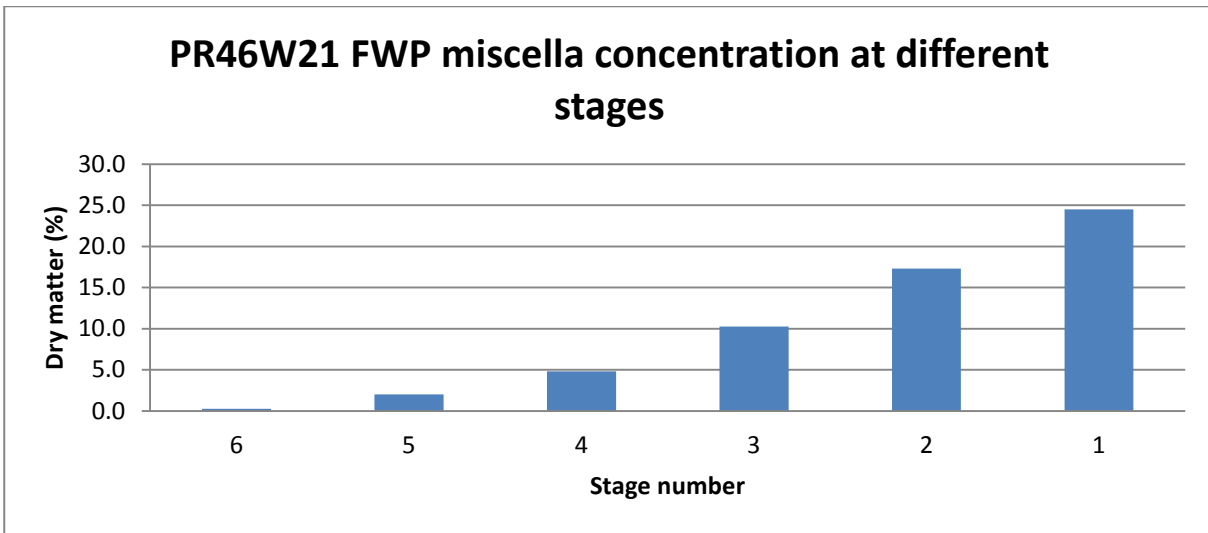
Miscella dry matter concentration for PR46W21 as a function of the stage number.
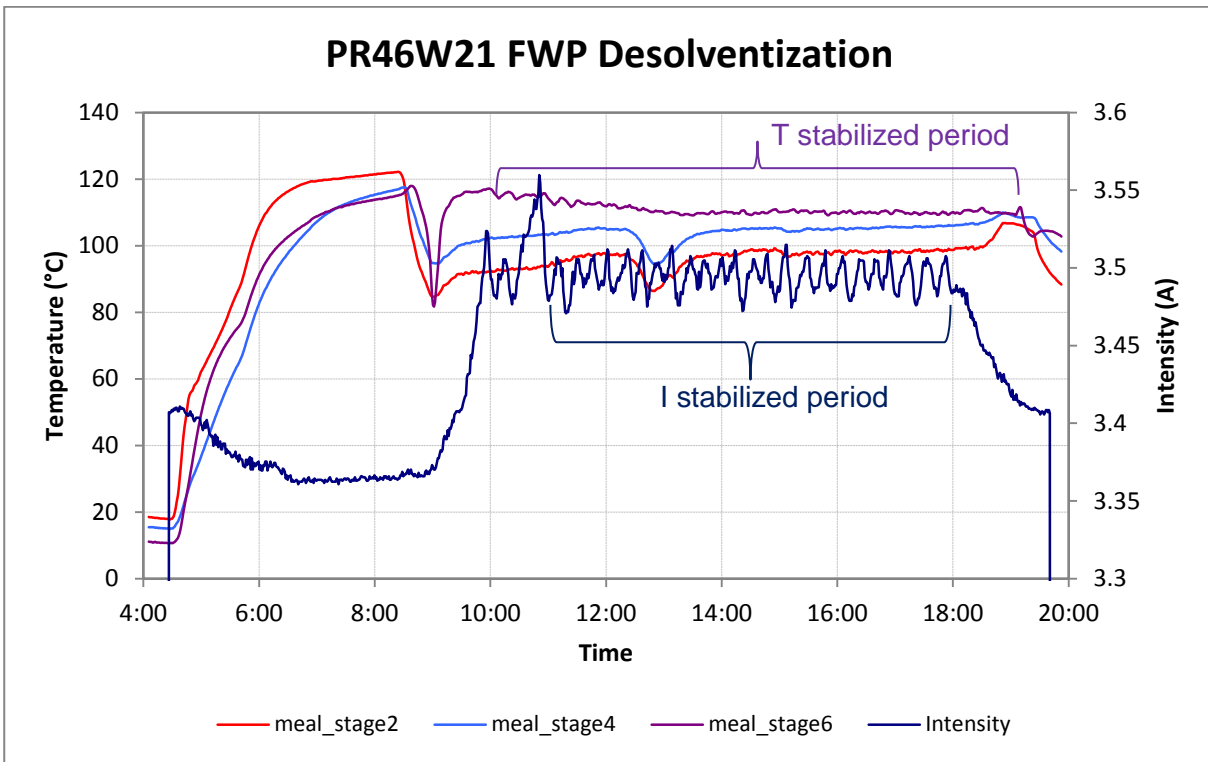
Desolventization temperature for PR46W21

Characterization of the desolventisation for PR46W21

	Average	Standard deviation
Temperature (stabilized period)	110.5°C	1.1°C
Intensity (stabilized period)	3.493 A	0.020 A
Indirect steam pressure	3.5 bars	-
Direct steam throughput	25 kg/h	-

Process 1 FWP seeds process: seed balance

FWP seeds material balance

	Mass unit	Cabernet FWP		PR46W21 FWP	
		In	Out	In	Out
Seeds used		3500	-	3500	-
Seeds non used		750	-	490	-
Cake	kg	-	1984	-	2019
Oil (press)		-	1241	-	1238
Meal		-	1609	-	1631

Process 2: CP seeds processing

1) Cabernet and PR46W21 Flaking + cold pressing
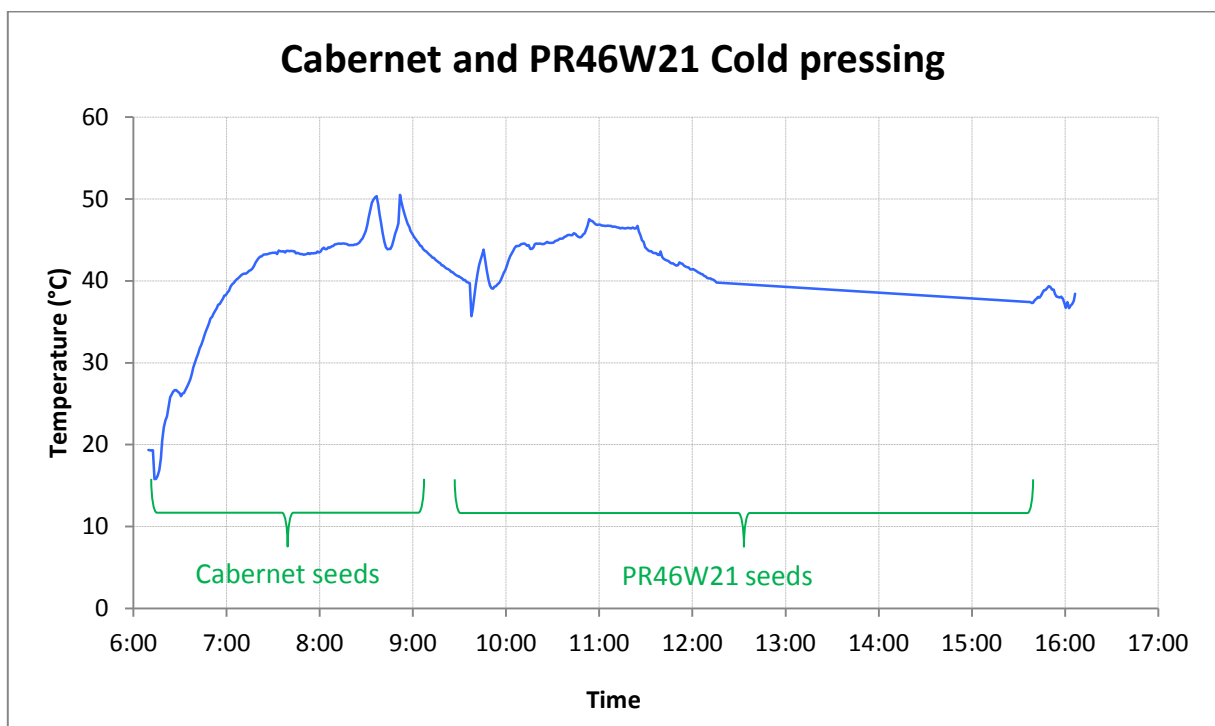
Cold pressing temperature for DK Cabernet and PR46W21

Characterization of the cold pressing for DK Cabernet and PR46W21

	Cabernet seeds		PR46W21 seeds	
	Average	Standard deviation	Average	Standard deviation
Press temperature	39.0°C	7.1°C	45.5°C	0,7°C
Total output	223.7 kg/h	19.0 kg/h	265.0 kg/h	15.6 kg/h
% of oil throughput	34.1 %	3.7 %	32.3 %	2.7 %
% of sediments (foots)	7.0 %	0.9 %	6.1 %	0.9 %
Intensity of the press	25.5 A	2.1 A	25.6 A	2.3 A
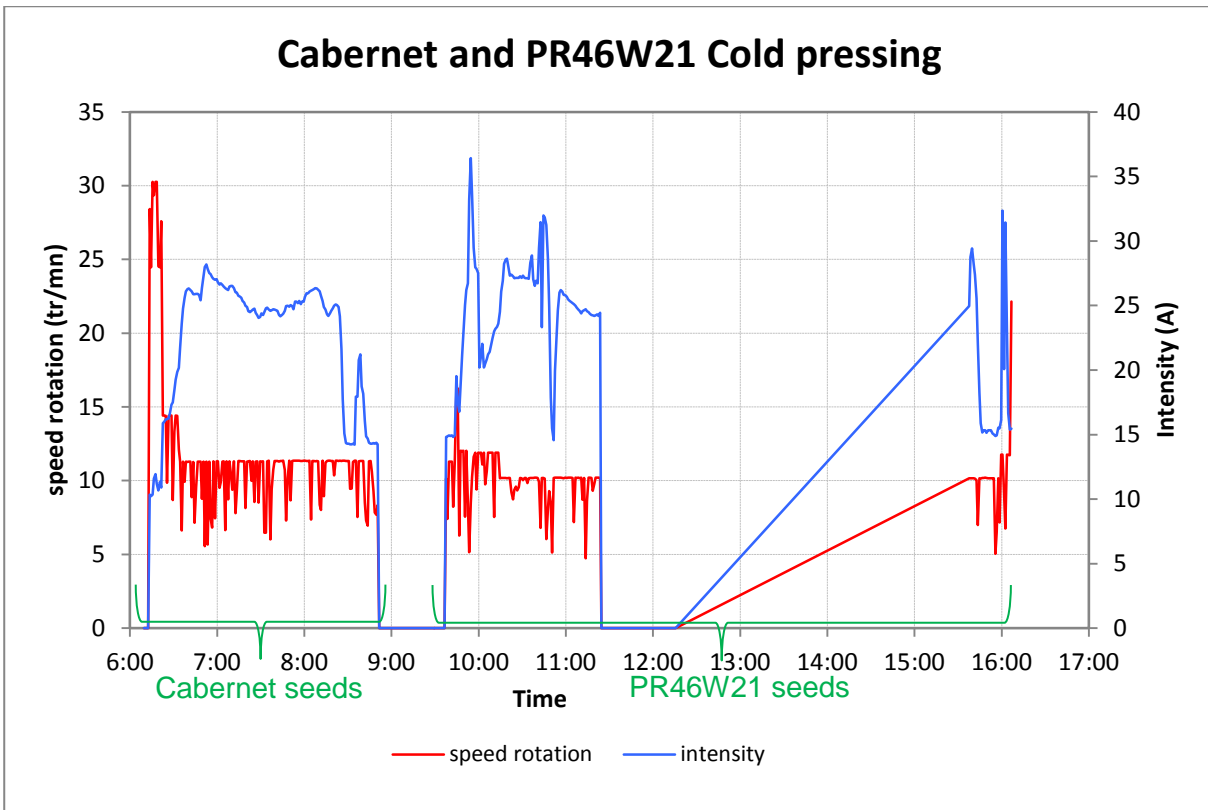
Cold pressing speed rotation and intensity of the press for DK Cabernet and PR46W21

2) CP DK Cabernet seeds extraction and desolventization
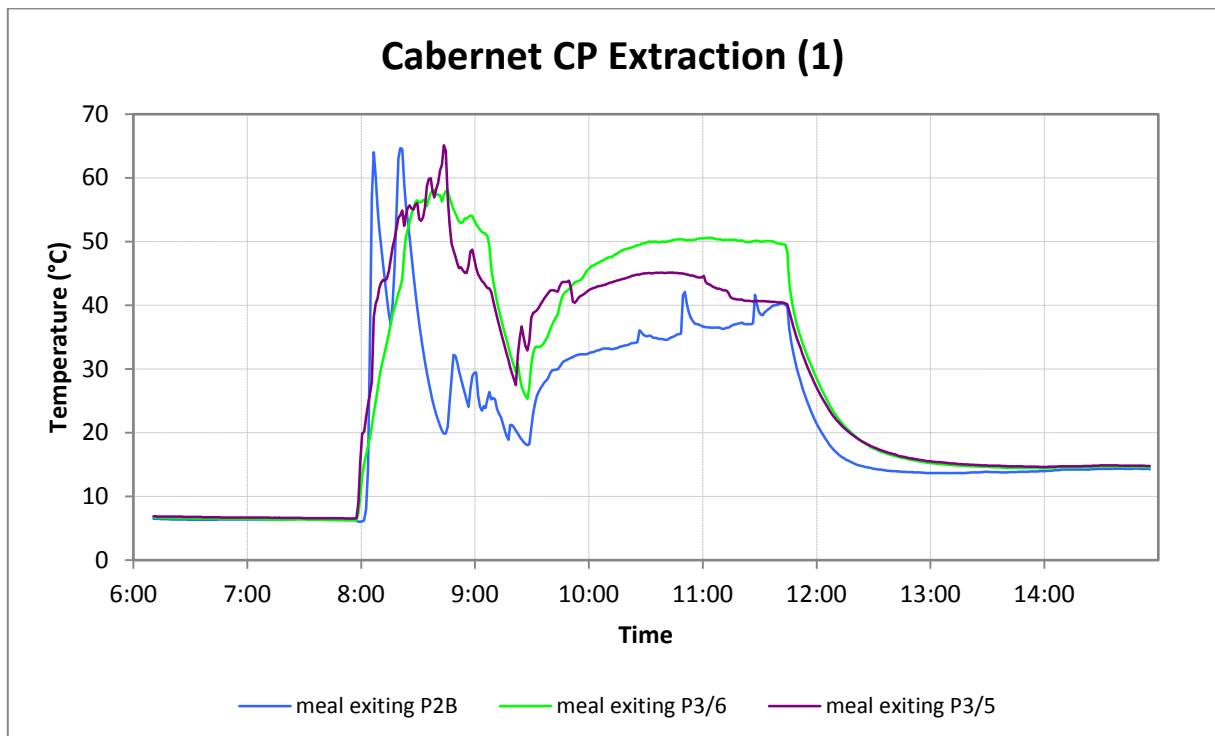
Extraction temperature for CP DK Cabernet (1)
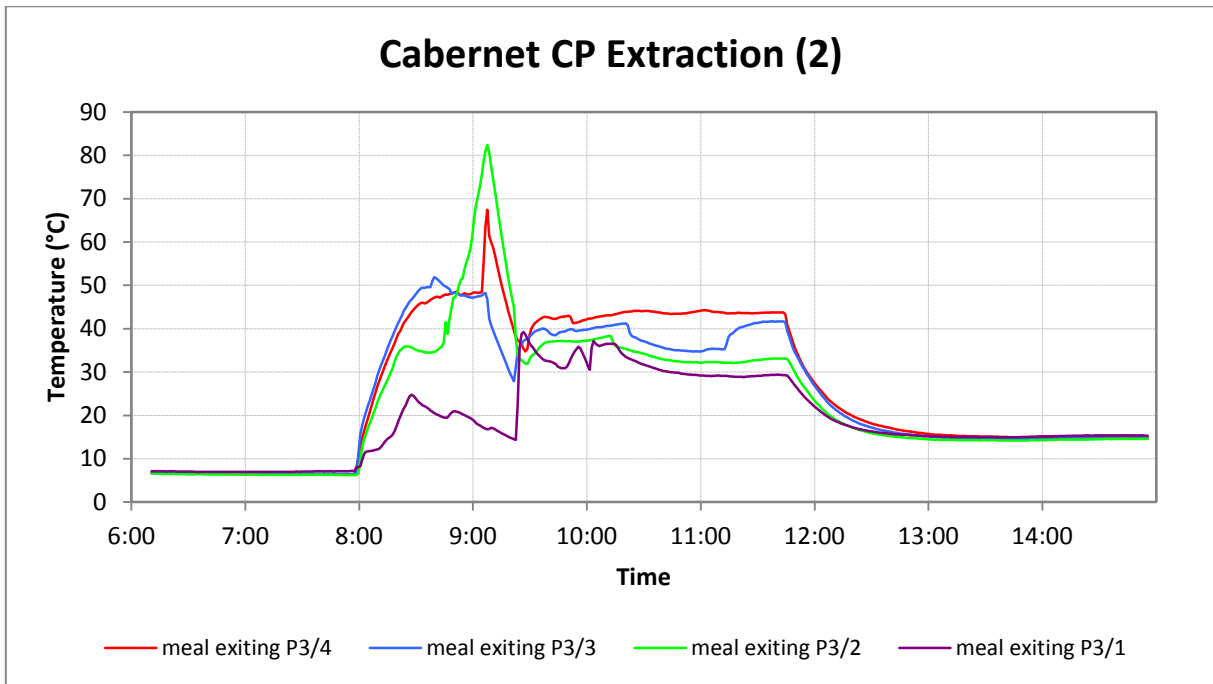
Extraction temperature for CP DK Cabernet (2)
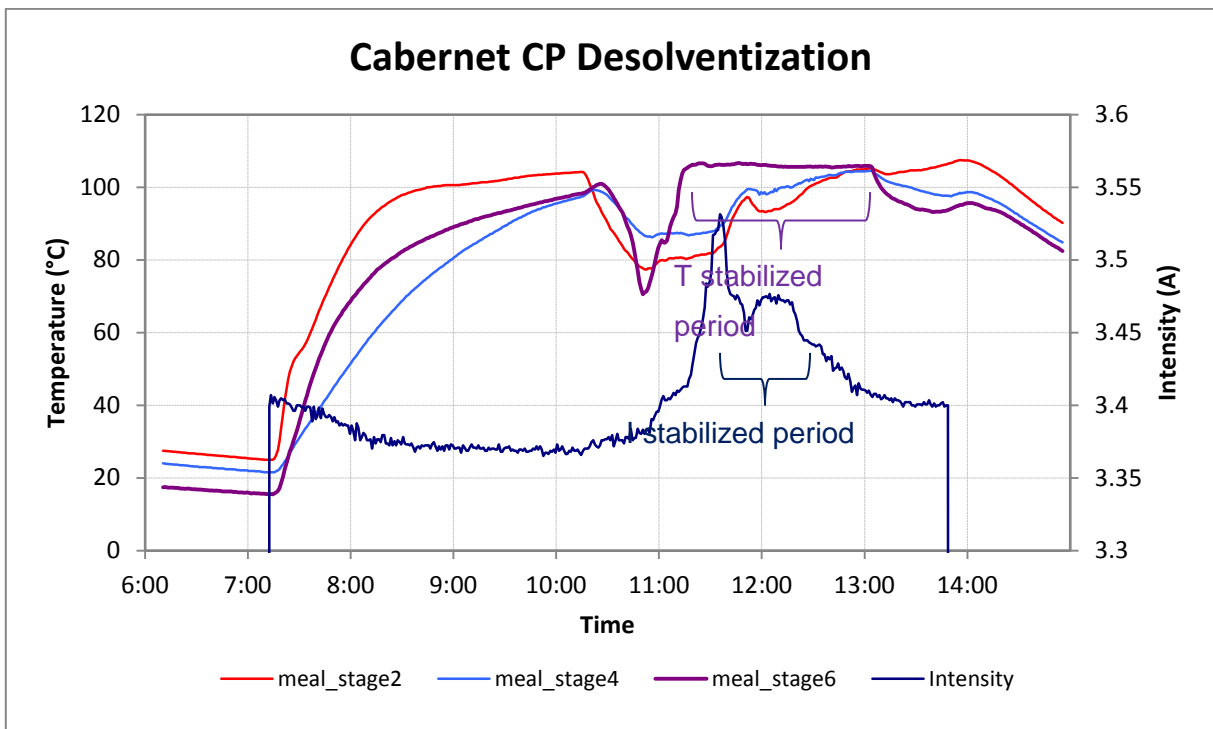

Desolventization temperature for CP DK Cabernet

Characterization of the desolventisation for CP DK Cabernet

	Average	Standard deviation
Temperature (stabilized period)	105.9°C	0.3°C
Intensity (stabilized period)	3.469 A	0.007 A
Indirect steam pressure	1.75 bars	-
Direct steam throughput	25 g/h	-

3) CP PR46W21 seeds extraction and desolventization
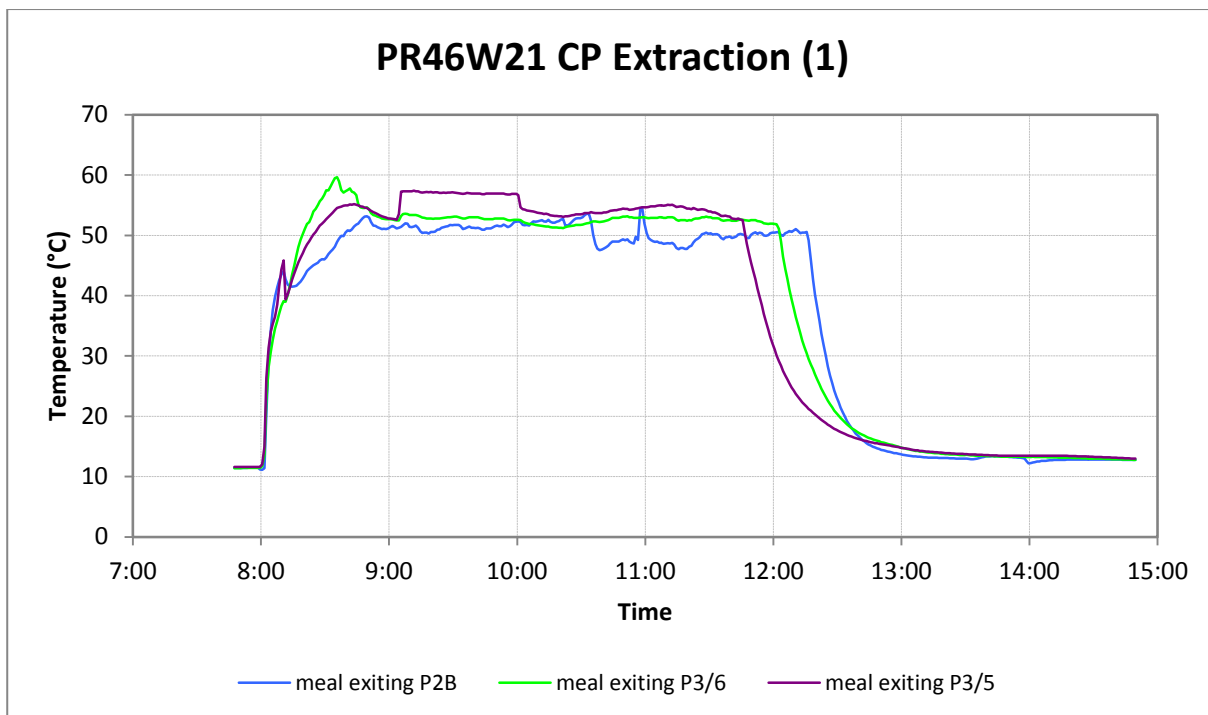
Extraction temperature for CP PR46W21 (1)
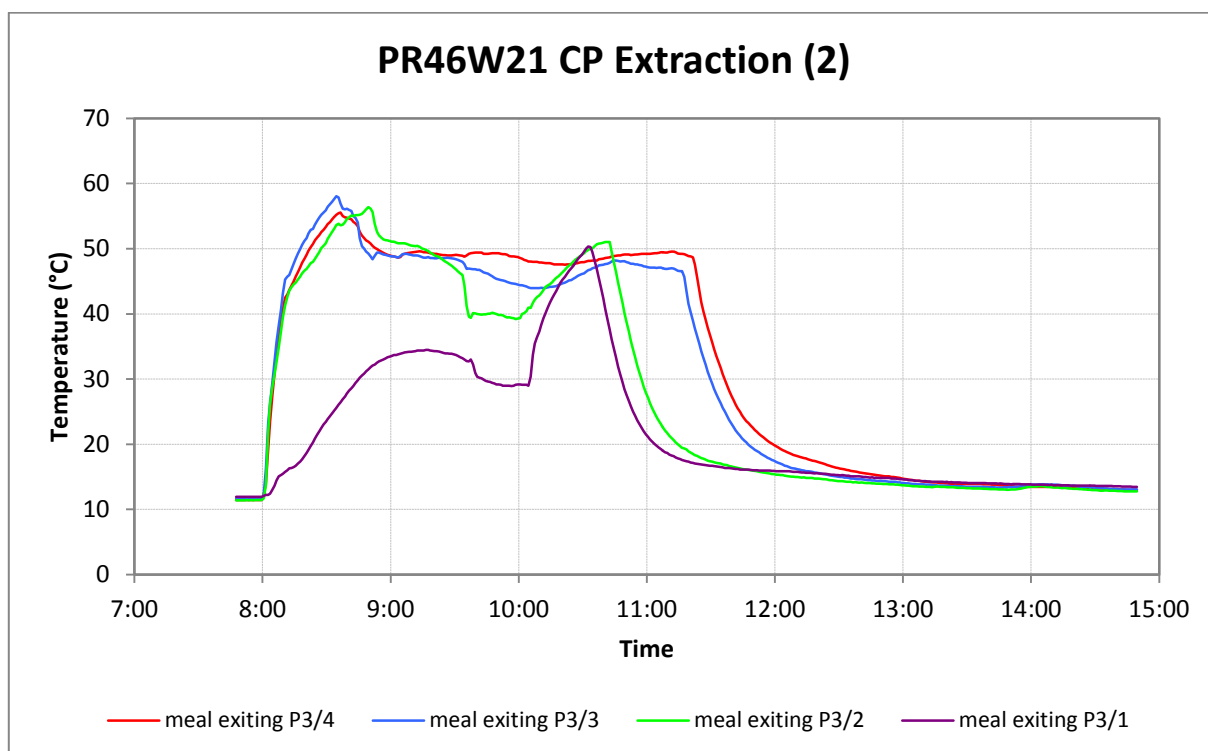
Extraction temperature for CP PR46W21 (2).
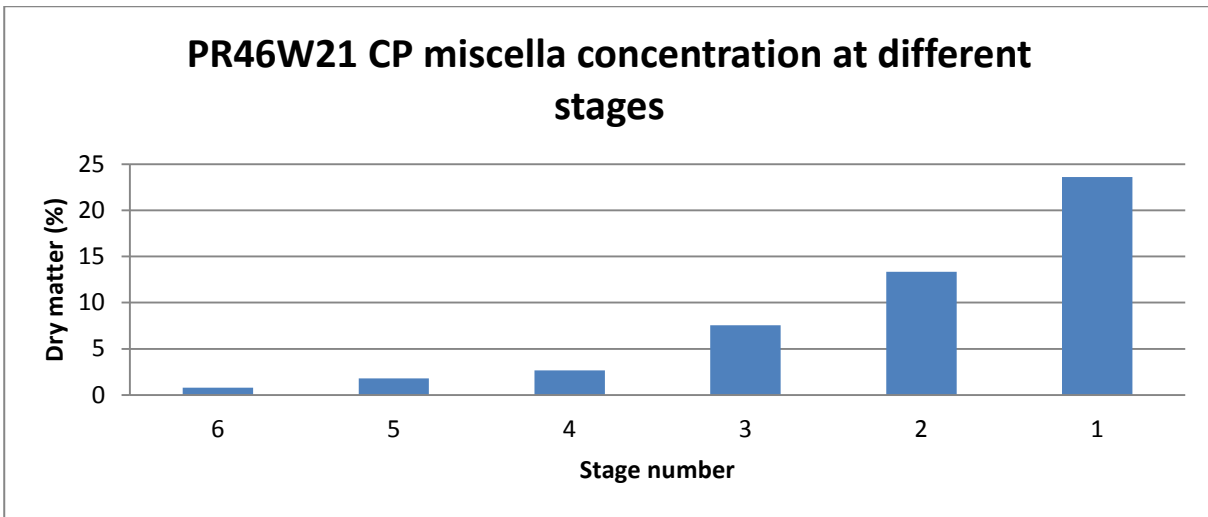
Miscella concentration as a function of stage number for CP PR46W21
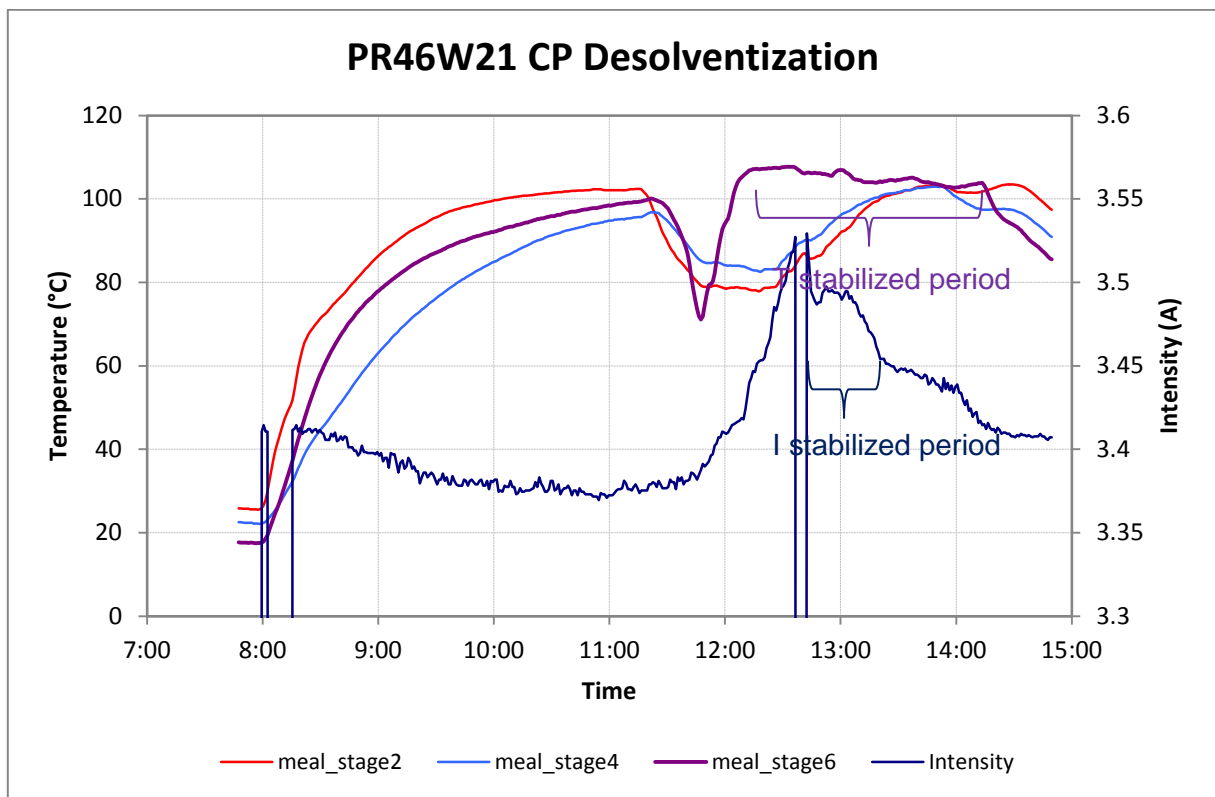
Desolventization temperature for CP PR46W21

Characterization of the desolventisation for CP PR46W21

	Average	Standard deviation
Temperature (stabilized period)	105.3°C	1.6°C
Intensity (stabilized period)	3.488 A	0.012 A
Indirect steam pressure	1.5 bars	-
Direct steam throughput	25 kh/h	-

Process 2 FCP seeds process: seed balance

Material balance of cold FCP seeds

	Mass unit	Cabernet		PR46W21	
		In	Out	In	Out
Seeds used	kg	511	-	512	-
Cake		-	317	-	280
Oil (press)		-	167	-	179
Meal		-	269	-	244

Process 1 and 2: Residence time and processing temperature comparison

		FWP process		CP process	
		Cabernet	**PR46W21**	**Cabernet**	**PR46W21**
Cooker	Temperature	90°C	89.6°C	-	-
	Residence time	43.4 min	45.5 min	-	-
	Loss in the water content	3.70 %	4.01 %	-	-
New Press	Temperature	80.3°C	77.1°C	39.0°C	45.5°C
Desolventizer	Temperature	115.9°C	110.5°C	105.9°C	105.3°C
	Intensity	3.523 A	3.493 A	3.469 A	3.488 A

Process 1 and 2: Analytical results

	Original Seeds		FWP processed seeds				CP processed seeds			
			Cake		Meal		Cake		Meal	
	Cab	**PR**	**Cab**	**PR**	**Cab**	**PR**	**Cab**	**PR**	**Cab**	**PR**
Dry matter (%)	94.6	94.6	94.3	94.2	93.4	92.5	91.2	91.2	95.1	89.1
Oil content (%)	50.4	51.3	19.8	20.5	3.3	3.2	19.4	19.6	5.7	4.1
Protein Solubility (%)					35.8	43.5			44.6	48.8

The pressing step was satisfactory: defatting, temperature and residence time in the cooker (for FWP seeds) are quite the same for both seeds. However, the relatively poor de-oiling performance (3 and 5 % of oil in the final meal) can be explained by the absence of granulation.

The reproducibility of the protein solubility is not as good as expected concerning the Cabernet FWP processed seeds. This degradation could be explained by the fact that, during the desolventization step, the material has been exposed to higher temperature (115.9°C vs 110.5°C). This difference could be linked with the higher intensity measured at the stirrer level. The difference is slight (30 mA) but considering that the empty desolventizer was calling 3.337 A, it represents a 16 % difference between the batches. On the other hand, the standard deviation of the intensity was 20 mA, therefore, this indicator is not entirely reliable.

Although cold pressing leads to a slight better protein solubility than warm pressing (if FWP Cabernet data is discarded), these solubility were compared to Harvest 2013 samples. The difference is explained by the method of controlling the residence time. During the processing of Harvest 2013 samples, the gates between the trays of the DT were opened of closed by mean of cables handled from outside the apparatus in order to manage the succession of the processed batches without mixing. Therefore, it was not a really continuous processing because the material was arriving on the trays abruptly when the gates were opened. This time, the amount of meal to produce being higher, this potentially resulting in the variations observed.

9.4. Appendix 3a: Experimental diets AME study Harvest 2013 and 2014

	Reference diet	Test diet
Corn	535.2	366.0
Soybean meal	367.0	251.0
Soybean oil	47.0	32.2
Dicalcium phosphate	17.5	17.5
Limestone	14.0	14.0
Titanium dioxide	5.0	5.0
Test feedstuff	-	300
Vitamin-mineral premix*	5.0	5.0
Methionine	1.9	1.9
Lysine	3.6	3.6
Threonine	0.7	0.7
Salt (NaCl)	3.1	3.1
Total	1000.0	1000.0
Calculated Nutrients & Energy		
Protein, g/kg	220	267
ME, kcal/kg	3006	3079
Ca, g/kg	9.9	11.6
P, g/kg	7.0	8.5
Available P, g/kg	4.5	5
Na	1.4	1.4
Cl	2.1	2.1
Total amino acids, g/kg		
Arg	14.8	13.2
His	5.9	5.3
Ile	9.3	8.3
Leu	19.1	17.1
Lys	15.1	13.8
Met	5.3	5.0
Cys	3.6	3.2
Phe	10.6	9.5
Tyr	8.8	7.8
Thr	9.1	8.2
Trp	3.0	2.7

[1]Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate,2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 250 µg.

9.5. Appendix 3b: Experimental diets P bioavailability study Harvest 2014

Items	Basal diet	Na_2PO_4		RSM	
Corn starch	308	304	302	236	163
Dextrose	151.25	150.45	149.05	113.25	76.25
Monosodium phosphate (Na_2PO_4)	-	4.8	9.3	-	-
Rapeseed meal (RSM)	-	-	-	110	220
Soybean meal	474	474	474	474	474
Soybean oil	35	35	35	35	35
Limestone	12	12	12	12	12
Titanium dioxide	5	5	5	5	5
Salt	4	4	2.9	4	4
Vitamin-mineral premix[1]	5	5	5	5	5
DL-Methionine	2.5	2.5	2.5	2.5	2.5
L-Threonine	1.3	1.3	1.3	1.3	1.3
L-Lysine.HCl	2	2	2	2	2
Total	1000	1000	1000	1000	1000
	Calculated Nutrients & Energy, g/kg				
Protein	225	225	225	268	311
ME, kcal/kg	3146	3127	3115	3091	3036
Ca	5.8	5.8	5.8	6.6	7.3
P[2]	2.9 (3.0)	4.0 (4.0)	5.0 (5.0)	3.9 (4.4)	4.9 (5.5)
P from Na_2PO_4 or RSM	-	1.0	2.0	1.0	2.0
Na	1.6	2.3	2.6	1.6	1.6
Cl	2.4	2.4	1.7	2.4	2.4
	Total amino acids, g/kg				
Arg	16.5	16.5	16.5	18.8	21.1
His	6.1	6.1	6.1	7.1	8.1
Ile	10	10	10	11.6	13.1
Leu	17.7	17.7	17.7	20.4	23.2
Lys	15.6	15.6	15.6	17.7	19.9
Met	5.7	5.7	5.7	6.5	7.2

[1]Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 250 µg.

[2]Analysed content shown in parenthesis

9.6. Appendix 3c: Experimental diet poultry SID Harvest 2013 and 2014

Items	g/kg diet
RSM/RSE[1]	500
Wheat Starch	200
Glucose (Dextrose)	195
Vitamins and Minerals Premix[2]	50
Rapeseed Oil	50
TiO_2	5

[1]RSM – rapeseed meal. RSE – rapeseed expeller.

[2] Content per kg of complete diet: 5 g phosphorous, 0.09 g magnesium, 7.5 g calcium, 1.5 g sodium, 0.6 mg copper (as copper sulphate), 160 µg selenium (as selenium BCP), 7500 IU vitamin A, 1500 IU vitamin D3, 10 IU vitamin E (as α-tocopherol acetate), 5 mg vitamin B_1, 4 mg vitamin B_2, 4 mg vitamin B_6, 10 µg vitamin B_{12}, 9 mg pantothenic acid, 1.5 mg folic acid, 150 µg biotin, 1500 mg choline.

9.7.   Appendix 3d: Experimental diet pig SID Harvest 2014

Items	g/kg
RSE/SRSM/RSM[1]	500
Maize starch	254.5
Dextrose	160
Soya oil	60
Dicalcium phosphate	12
Salt	4
Titanium dioxide	5
Vitamin and minerals[2]	3.3
Limestone flour	1.2

[1]RSE, rapeseed expeller; SRSM, soft rapeseed meal; RSM, rapeseed meal;

[2]Content per kg of complete diet: Fe, 100 mg; Mn , 50 mg; Cu, 20 mg; Zn , 100.6 mg; I , 1 mg; Se, 0.3 mg; retinol, 10000 IU; cholecalciferol, 2000 IU; tocopherol, 50 mg ; thiamine, 2 mg; riboflavin, 3 mg; pyridoxine, 2 mg; cyanocobalamin, 30 mg; menadione, 1 mg; nicotinic acid, 20 mg; pantothenic acid, 10 mg.

9.8. Appendix 3e: Experimental diets poultry growth Harvest 2014

Items	Starter phase			Finisher phase		
	Control	RSM50	RSM200	Control	RSM50	RSM200
Wheat	370.0	430.1	399.0	378.0	435.0	404.3
Corn	190	130	130	190	130	130
Soybean meal	345	298	175	345	298	175
Soybean oil	45.0	43.0	50.0	42.0	43.0	50.0
Monocalcium phosphate	15.0	14.4	13.0	13.0	12.5	10.5
Limestone	16.0	15.5	14.0	13.0	12.5	11.2
RSM[1]	0.0	50.0	200.0	0.0	50.0	200.0
Vitamin-mineral premix[2]	5.0	5.0	5.0	5.0	5.0	5.0
DL- Methionine	4.0	4.0	4.0	4.0	4.0	4.0
L-Lysine	5.0	5.0	5.0	5.0	5.0	5.0
Sodium bicarbonate	3.0	3.0	3.0	3.0	3.0	3.0
Salt NaCl	2.0	2.0	2.0	2.0	2.0	2.0
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
			Calculated analysis			
Protein, g/kg	227	227	226	228	227	226
ME, kcal/kg	2998	2944	2886	2995	2959	2902
Ca, g/kg	10.0	10.0	10.1	8.5	8.5	8.5
P, g/kg	7.1	7.1	7.3	6.6	6.7	6.9
Available P, g/kg	4.5	4.5	4.5	4.0	4.0	4.0
			Digestible amino acids, g/kg			
Arg	13.1	12.8	12.1	13.1	12.8	12.1
His	5.1	5.0	5.1	5.1	5.1	5.1
Ile	8.2	8.0	7.6	8.2	8.0	7.6
Leu	15.0	14.6	14.0	15.1	14.6	14.0
Lys	14.3	13.9	13.2	14.3	14.0	13.2
Met	6.9	6.9	7.2	6.9	6.9	7.2
Phe	10.2	9.7	8.5	10.2	9.7	8.5
Thr	7.1	7.0	6.9	7.1	7.0	6.9
Trp	3.3	3.1	2.7	3.3	3.1	2.7
Val	8.9	8.8	8.7	8.9	8.8	8.7
TSAA	10.0	10.6	12.2	10.1	10.6	12.2
Phe+Tyr	16.8	16.8	16.6	16.9	16.8	16.6

[1]RSM is oilseed rape meals from either DK Cabernet or PR46W21 varieties, and was incorporated to the control diet at the rate of 50, 100, 150 or 200 g/kg

[2]Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate,2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 250 µg.

9.9. Appendix 3g: Experimental diets pig growth Harvest 2014

Grower pigs

	Grower pigs						
Items	Control	CAB50	PR50	CAB150	PR150	CAB250	PR250
Soybean meal	220	160	160	100	100	40	40
DK Cabernet RSM	0	50	0	150	0	250	0
Pr46W21 RSM	0	0	50	0	150	0	250
Soybean oil	11	16	16	32	32	45	45
Wheat	340.12	344.11	344.13	289.45	289.56	237.70	237.89
Barley	300	300	300	300	300	300	300
Wheat feed	100	100	100	100	100	100	100
L-Lysine	2.46	3.6	3.58	4.1	4.06	4.6	4.53
DL- Methionine	0.55	0.59	0.59	0.43	0.43	0.26	0.26
L-Threonine	0.69	1.05	1.05	0.99	0.98	0.93	0.91
L-Tryptophan	0	0	0	0.12	0.06	0.31	0.21
Dicalcium phosphate	7.7	7.7	7.7	7.15	7.15	6.65	6.65
Limestone	10.98	10.45	10.45	9.26	9.26	8.05	8.05
Salt	4	4	4	4	4	4	4
Vitamin-mineral premix[2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
				Analysis			
Dry matter, g/kg	875	878	885	893	880	886	892
Crude protein, g/kg DM	211	201	198	198	204	205	202
Oil, g/kg DM	42	36	61	78	45	61	78
Ash, g/kg DM	55	54	55	55	54	55	56
NDF, g/kg DM	125	141	178	207	146	182	205
Digestible Energy, MJ/kg DM	16.3	15.9	15.7	15.5	16.0	15.7	15.6

Finisher pigs

Items	Finisher pigs						
	Control	CAB50	PR50	CAB150	PR150	CAB250	PR250
Soybean meal	180	120	120	60	60	0	0
DK Cabernet RSM	0	50	0	150	0	250	0
Pr46W21 RSM	0	0	50	0	150	0	250
Soybean oil	13.7	17	17	31.5	31.5	45.4	45.4
Wheat	265	271	271	218	218	165	165
Barley	315	315	315	315	315	315	315
Wheat feed	200	200	200	200	200	200	200
L-Lysine	2.24	3.37	3.35	3.87	3.83	4.37	4.30
DL- Methionine	0.42	0.46	0.46	0.29	0.29	0.13	0.13
L-Threonine	0.53	0.89	0.88	0.82	0.81	0.76	0.74
L-Tryptophan	0.00	0.00	0.00	0.08	0.02	0.27	0.17
Dicalcium phosphate	6.35	6.35	6.35	5.81	5.81	5.25	5.25
Limestone	10.94	10.41	10.41	9.21	9.21	8.05	8.05
Salt	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Vitamin-mineral premix[2]	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
	Analysis						
Dry matter, g/kg	881	885	897	892	892	895	885
Crude protein, g/kg DM	189	182	173	173	181	182	182
Oil, g/kg DM	41	45	66	80	46	65	79
Ash, g/kg DM	54	54	53	51	53	53	54
NDF, g/kg DM	154	157	178	217	176	189	217
Digestible Energy, MJ/kg DM	15.7	15.6	15.6	15.4	15.4	15.5	15.3
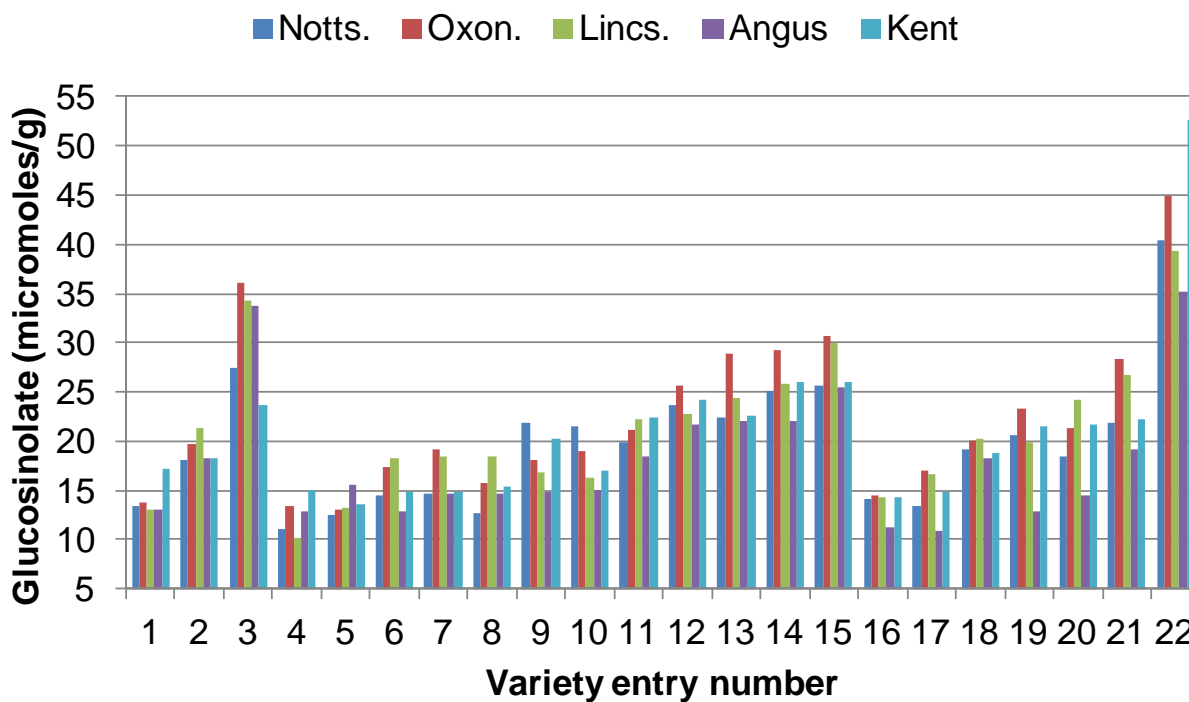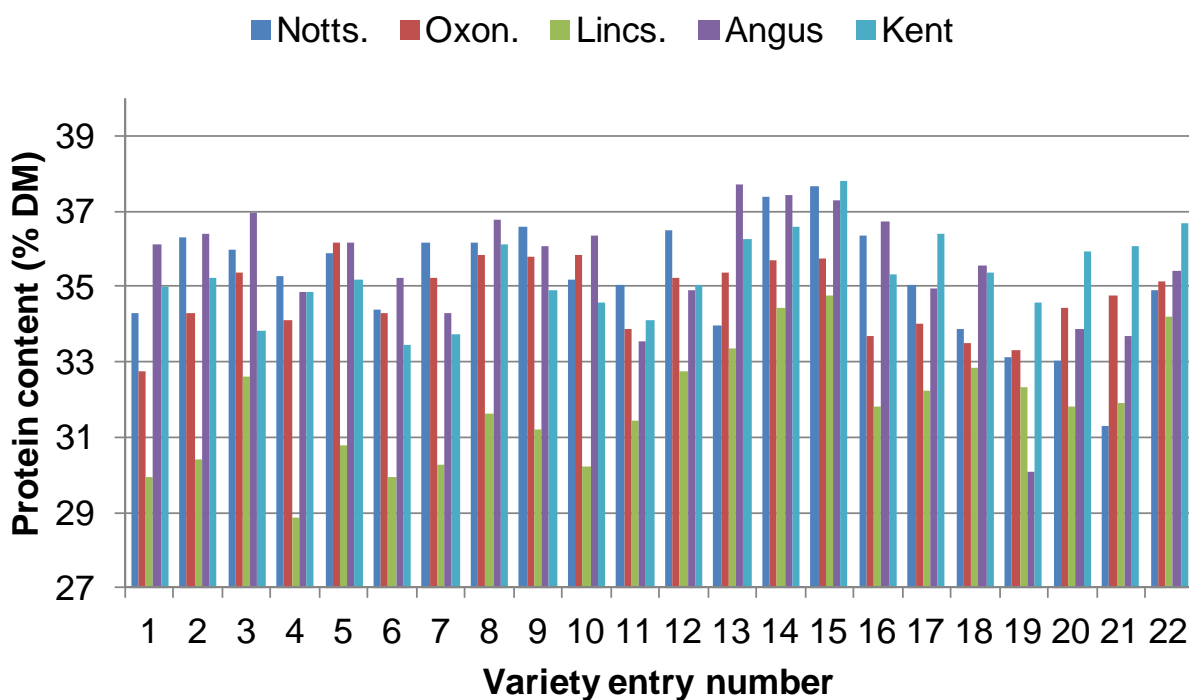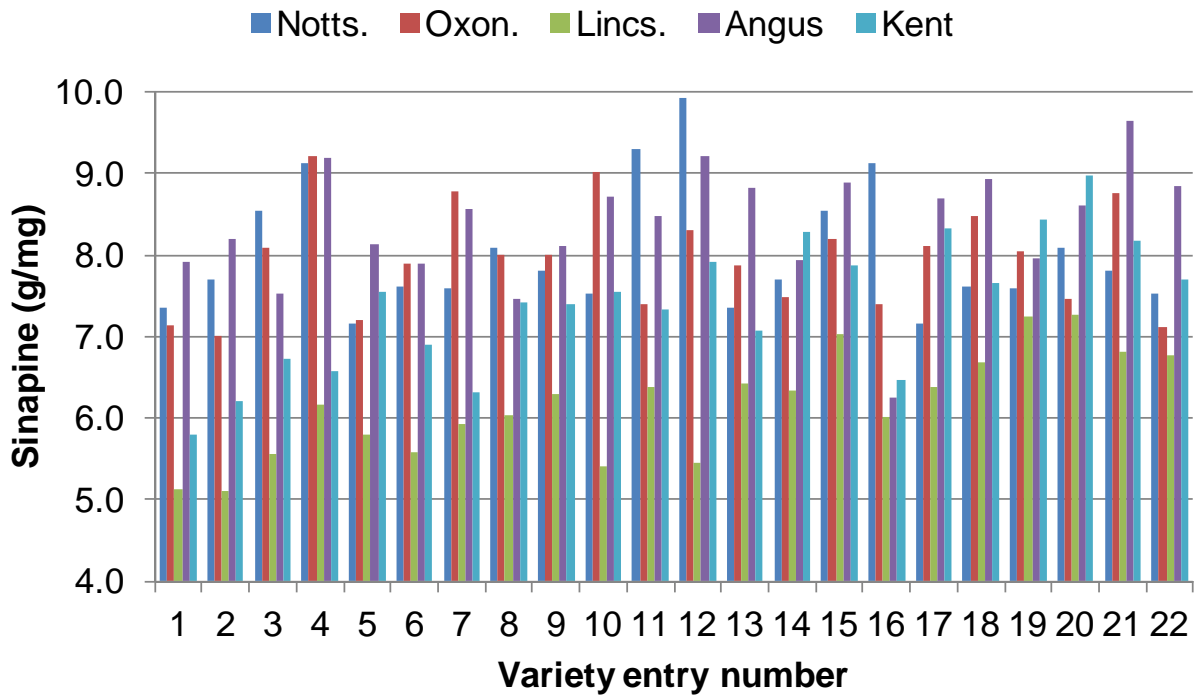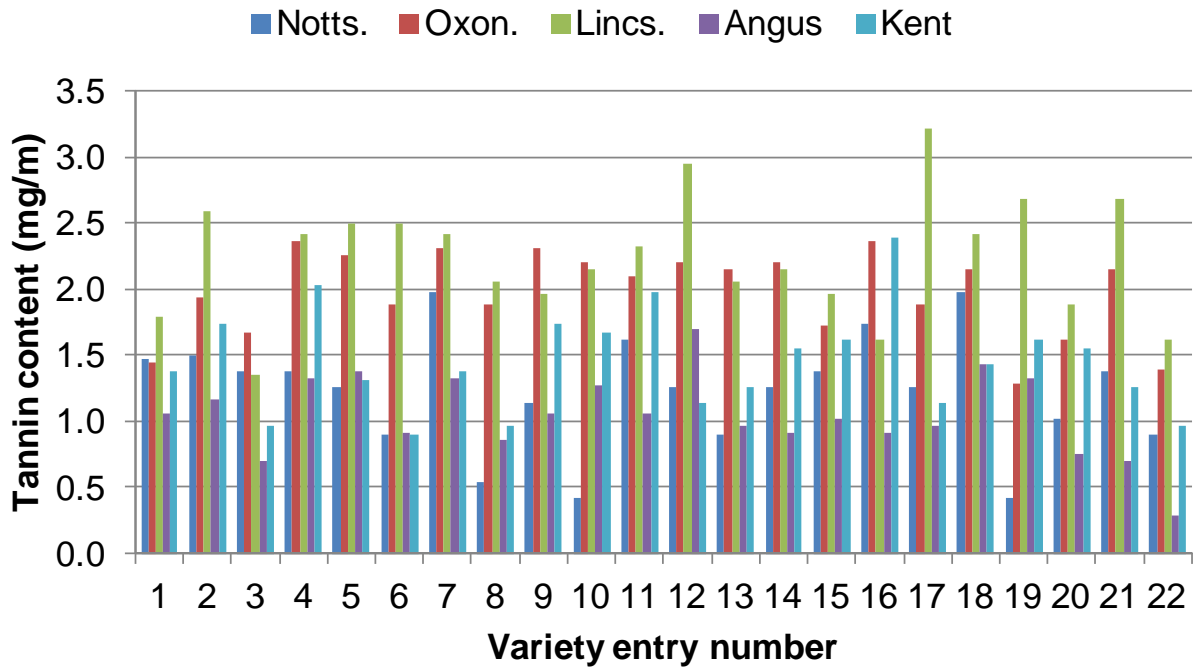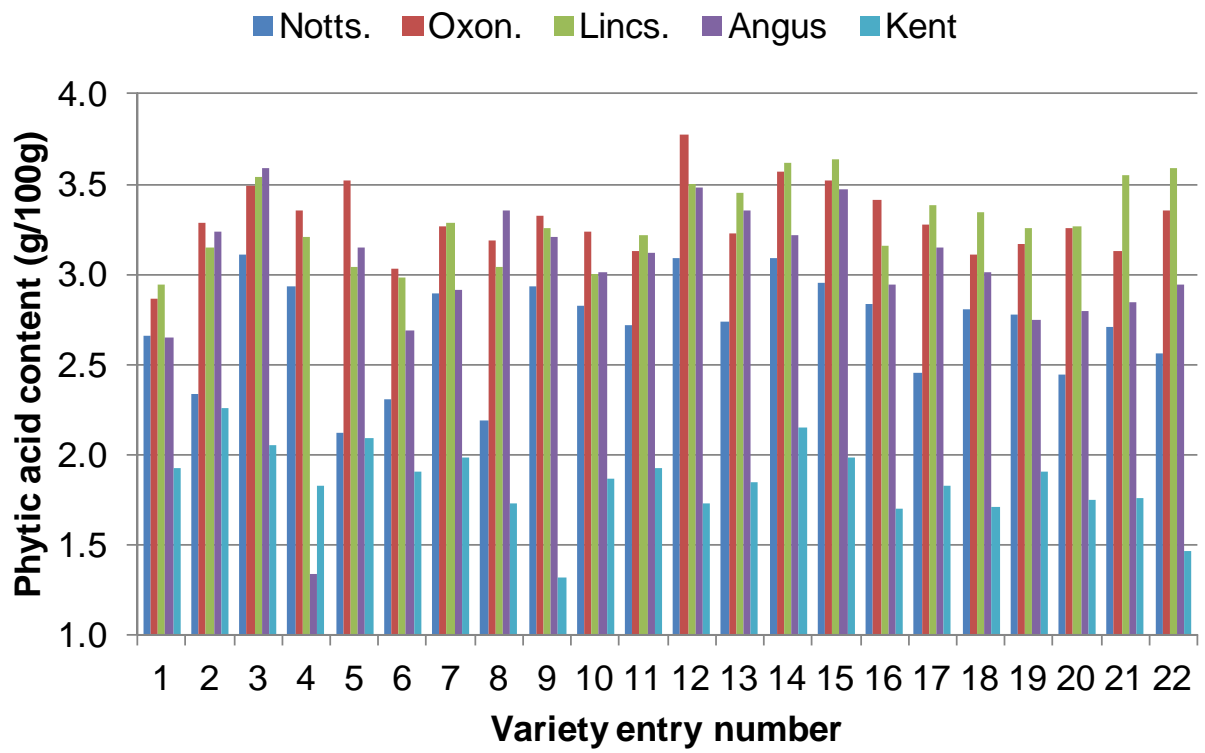
9.11. Appendix 4b: Site by site glucosinolate composition Harvest 2012

Site: Elsoms, Nottinghamshire	Entry number										
	1	2	3	4	5	6	7	8	9	10	11
Glucosinolate, µmol/g											
Glucoberin	0.4	0.6	0.0	0.4	0.0	0.5	0.0	0.0	0.9	0.7	0.7
Progoitrin	7.3	10.4	21.5	5.5	7.0	10.3	10.0	8.1	8.7	8.4	13.3
Epi-progoitrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	1.5	1.3	2.0	0.8	0.9	1.3	1.7	0.9	1.1	1.2	2.3
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	3.4	3.6	9.2	1.6	2.3	4.0	3.6	3.6	3.1	2.9	3.1
4OHglucobrassicin	3.7	6.9	4.4	4.7	6.0	3.9	5.2	3.8	5.5	5.5	6.8
Glucobrassicanapin	2.1	1.8	3.9	1.1	1.4	10.3	1.2	1.6	1.6	1.7	1.5
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.2	1.0	0.2	0.5	0.3	0.2	0.5	0.3	0.7	0.5	0.7
Gluconasturtiin	0.3	0.0	0.8	0.3	0.0	0.0	0.0	0.3	0.0	0.4	0.0
Neoglucobrassicin	0.4	0.2	0.1	0.2	0.1	0.3	0.2	0.5	0.2	0.2	0.2
Total	19.3	25.9	42.0	15.1	17.9	30.7	22.4	19.1	21.8	21.5	28.5

	Entry number										
	12	13	14	15	16	17	18	19	20	21	22
Glucosinolate, µmol/g											
Glucoberin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Progoitrin	16.8	11.2	11.1	12.8	4.6	4.1	5.5	7.3	8.1	9.4	22.3
Epi-progoitrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	1.3	1.1	1.7	1.8	0.6	0.6	0.7	1.0	1.0	1.3	2.3
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	3.3	3.8	5.3	4.0	2.0	0.0	2.3	2.7	2.9	3.0	9.1
4OHglucobrassicin	5.9	4.4	5.2	5.5	5.0	1.6	5.4	5.0	4.3	5.4	3.9
Glucobrassicanapin	1.7	1.3	1.3	0.9	1.3	6.6	1.7	1.6	1.4	2.4	2.0
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.3	0.2	0.5	0.6	0.6	0.5	0.6	0.5	0.4	0.3	0.1
Gluconasturtiin	0.6	0.4	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.7
Neoglucobrassicin	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0
Total	30.1	22.5	25.1	25.6	14.2	13.4	16.4	18.5	18.5	22.0	40.4

Site: DSV, Oxfordshire	Entry number										
	1	2	3	4	5	6	7	8	9	10	11
Glucosinolate, µmol/g											
Glucoberin	0.3	0.0	0.0	0.3	0.3	0.4	0.0	0.0	0.4	0.4	0.0
Progoitrin	5.7	7.9	17.6	5.2	4.9	8.1	8.7	6.9	7.4	7.4	11.0
Epi-progoitrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	0.0	0.9	1.5	0.0	0.0	0.3	0.5	0.0	0.0	0.0	0.8
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	3.1	3.2	8.9	2.0	1.8	3.7	3.7	3.7	3.6	4.1	3.3
4OHglucobrassicin	3.0	5.6	4.3	4.5	5.1	3.3	4.7	3.5	5.0	5.5	4.7
Glucobrassicanapin	1.4	1.4	3.5	0.9	0.8	1.4	1.1	1.4	1.3	1.2	1.0
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.1	0.7	0.2	0.4	0.2	0.1	0.4	0.2	0.4	0.3	0.4
Gluconasturtiin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neoglucobrassicin	0.0	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0
Total	13.8	19.7	36.1	13.4	13.1	17.4	19.2	15.7	18.0	19.0	21.1

	Entry number										
	12	13	14	15	16	17	18	19	20	21	22
Glucosinolate, µmol/g											
Glucoberin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Progoitrin	13.7	15.1	13.0	15.8	6.2	5.9	7.1	9.8	10.3	13.3	25.3
Epi-progoitrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	0.4	0.0	1.7	1.5	0.5	0.7	1.0	1.0	0.0	0.7	1.6
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	5.0	6.2	7.1	5.9	2.3	2.7	3.5	4.1	4.5	4.6	10.7
4OHglucobrassicin	5.1	5.5	5.5	5.8	4.0	5.2	6.0	5.9	4.6	6.1	4.1
Glucobrassicanapin	1.3	1.8	1.7	1.1	0.9	1.7	1.8	1.9	1.5	3.2	2.6
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.2	0.3	0.4	0.4	0.6	0.6	0.5	0.5	0.3	0.4	0.0
Gluconasturtiin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
Neoglucobrassicin	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0
Total	25.7	29.0	29.3	30.6	14.6	17.0	20.0	23.3	21.3	28.4	45.0

Site: Limagrain, Lincolnshire	Entry number										
	1	2	3	4	5	6	7	8	9	10	11
Glucosinolate, µmol/g											
Glucoberin	0.0	0.0	0.0	0.3	0.0	0.0	0.4	0.4	0.4	0.4	0.5
Progoitrin	5.3	8.7	17.1	3.4	5.2	7.8	7.6	7.5	6.6	6.5	10.4
Epi-progoitrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	0.6	0.8	1.0	0.2	0.0	0.7	0.9	0.5	0.5	0.5	1.2
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	3.0	3.2	8.3	1.2	1.8	3.6	3.2	3.8	3.0	2.8	3.5
4OHglucobrassicin	2.7	6.4	4.9	4.1	5.2	4.2	4.9	4.4	4.8	4.4	5.1
Glucobrassicanapin	1.3	1.5	2.9	0.8	0.9	1.7	1.1	1.6	1.3	1.3	1.2
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.1	0.5	0.0	0.2	0.1	0.1	0.2	0.1	0.3	0.2	0.2
Gluconasturtiin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neoglucobrassicin	0.1	0.1	0.2	0.0	0.0	0.1	0.1	0.1	0.0	0.1	0.1
Total	13.0	21.3	34.4	10.2	13.2	18.2	18.4	18.4	16.8	16.3	22.2

	Entry number										
	12	13	14	15	16	17	18	19	20	21	22
Glucosinolate, µmol/g											
Glucoberin	0.4	0.4	0.5	0.0	0.4	0.5	0.5	0.5	0.0	0.6	0.0
Progoitrin	11.0	11.8	10.5	14.6	6.1	5.7	8.0	8.1	11.4	11.8	21.6
Epi-progoitrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	0.9	0.8	1.3	1.6	0.5	0.7	0.4	0.8	1.1	1.5	1.4
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	4.3	4.8	6.0	6.2	2.3	2.3	3.9	3.2	4.2	4.2	9.9
4OHglucobrassicin	4.3	4.4	5.7	6.0	3.8	4.9	5.3	5.0	5.5	4.8	3.9
Glucobrassicanapin	1.8	2.0	1.5	1.2	1.0	1.9	1.7	2.0	1.7	3.8	2.5
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.2	0.1	0.3	0.3	0.4	0.3	0.3	0.3	0.2	0.2	0.0
Gluconasturtiin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neoglucobrassicin	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1	0.0	0.0
Total	22.8	24.4	25.8	29.9	14.4	16.6	20.2	19.9	24.2	26.8	39.3

Site: Scottish	Entry number										
Agronomy, Angus	1	2	3	4	5	6	7	8	9	10	11
Glucosinolate, µmol/g											
Glucoberin	0.0	0.5	0.6	0.4	0.6	0.7	0.7	0.7	0.6	0.7	0.0
Progoitrin	4.8	6.6	16.6	4.2	5.5	5.8	5.9	6.0	5.3	5.6	7.5
Epi-progoitrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	0.8	0.8	1.2	0.5	0.7	0.5	0.8	0.4	0.5	0.8	0.8
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	2.6	2.2	8.1	1.5	2.1	2.3	1.8	2.8	2.3	2.2	2.1
4OHglucobrassicin	2.6	5.1	3.4	3.9	4.7	2.1	3.9	2.7	4.0	3.1	6.2
Glucobrassicanapin	1.0	1.1	2.7	0.9	1.0	0.6	0.6	0.9	0.9	1.4	0.6
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.5	1.2	0.3	0.6	0.4	0.2	0.5	0.4	0.6	0.5	0.6
Gluconasturtiin	0.8	0.6	0.8	0.6	0.7	0.5	0.5	0.6	0.5	0.7	0.6
Neoglucobrassicin	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1
Total	13.1	18.4	33.8	12.9	15.6	12.8	14.7	14.7	14.8	15.1	18.5

	Entry number										
	12	13	14	15	16	17	18	19	20	21	22
Glucosinolate, µmol/g											
Glucoberin	0.0	0.0	0.5	0.6	0.5	0.5	0.6	0.5	0.6	0.0	0.0
Progoitrin	9.2	9.5	8.8	11.8	4.8	3.1	6.4	5.2	6.1	7.7	20.0
Epi-progoitrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	0.6	1.1	1.3	1.4	0.5	0.0	0.0	0.0	0.6	1.0	0.0
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	3.4	3.8	4.5	3.9	1.4	1.3	2.6	1.8	2.4	2.4	9.1
4OHglucobrassicin	6.2	5.4	4.7	5.2	2.1	3.8	5.5	3.7	2.9	5.3	3.3
Glucobrassicanapin	0.8	1.3	1.0	0.9	0.7	0.8	1.7	0.8	0.6	1.9	1.6
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.4	0.5	0.7	0.9	0.6	0.7	0.8	0.4	0.6	0.4	0.3
Gluconasturtiin	0.9	0.5	0.5	0.8	0.7	0.5	0.7	0.4	0.6	0.5	0.8
Neoglucobrassicin	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.1	0.0
Total	21.6	22.1	22.0	25.5	11.3	10.8	18.3	12.9	14.4	19.3	35.1

Site: NIAB,	Entry number										
Kent	1	2	3	4	5	6	7	8	9	10	11
Glucosinolate, µmol/g											
Glucoberin	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Progoitrin	6.6	7.6	11.2	6.2	5.4	6.5	6.1	6.2	8.2	7.5	11.0
Epi-progoitrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	0.7	0.8	0.9	0.6	0.4	0.6	0.8	0.5	0.9	0.0	1.3
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	3.7	3.1	5.1	2.2	2.4	2.6	2.2	2.8	3.6	3.3	3.3
4OHglucobrassicin	4.2	5.2	4.5	4.8	4.2	3.5	4.4	4.5	5.4	4.4	5.0
Glucobrassicanapin	1.0	0.9	1.7	0.9	0.7	0.9	0.8	0.8	1.6	1.2	1.5
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.2	0.5	0.1	0.2	0.1	0.1	0.2	0.1	0.3	0.2	0.2
Gluconasturtiin	0.4	0.0	0.0	0.0	0.3	0.3	0.3	0.3	0.0	0.4	0.0
Neoglucobrassicin	0.2	0.1	0.1	0.0	0.2	0.2	0.1	0.2	0.2	0.0	0.1
Total	17.3	18.3	23.7	15.1	13.6	14.8	14.9	15.4	20.2	17.0	22.4

	Entry number										
	12	13	14	15	16	17	18	19	20	21	22
Glucosinolate, µmol/g											
Glucoberin	0.0	0.4	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Progoitrin	13.2	12.0	12.7	13.6	6.4	6.0	7.6	10.0	11.1	10.6	31.2
Epi-progoitrin	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sinigrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoraphanin	0.0	0.8	1.0	1.5	0.5	0.3	0.4	0.5	0.4	0.5	1.2
Gluconapoliferin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucoalyssin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucosinalbin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gluconapin	4.9	4.7	6.1	5.1	1.9	2.6	2.9	3.5	4.4	3.9	13.3
4OHglucobrassicin	4.3	3.7	4.6	4.6	3.8	4.2	5.4	5.3	3.9	4.9	4.1
Glucobrassicanapin	1.1	1.1	1.4	1.1	0.5	1.3	1.7	1.8	1.1	2.2	2.8
Glucotropaeolin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glucobrassicin	0.1	0.2	0.2	0.2	0.5	0.4	0.4	0.4	0.3	0.2	0.0
Gluconasturtiin	0.0	0.0	0.0	0.0	0.3	0.0	0.4	0.0	0.4	0.0	0.0
Neoglucobrassicin	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	24.3	22.8	26.0	26.0	14.2	14.8	18.8	21.5	21.6	22.3	52.6

### 9.12. Appendix 5: Nitrogen fertiliser response experiments

A series of two additional studies were undertaken on the influence of nitrogen fertiliser inputs on rapeseed meal composition. One of the largest variables, within the control of most growers, is the quantity of nitrogen fertiliser applied to the oilseed rape crop and the project team undertook to perform a small series of field experiments to see if earlier studies (Chalmers, 1989) were still applicable. These would predict a decrease in oil content and an increase in glucosinolate content with increasing levels of nitrogen input.

In 2013/14, two trials were sown at Callow (Hereford) and at Cambridge. Trials comprised 4 replicates and 6 N rates from 0 to 360 kg/ha, using the open pollinated, line variety, Charger. The Cambridge trial failed to establish adequately and was abandoned. The figure below shows response curves for oil and glucosinolate content, which were directly comparable to those predicted. Data for the 240 kg/ha treatment was inexplicably anomalous and was excluded but polynomial trend lines for the remaining data indicated very high levels of significance in both cases. Over the range of N inputs oil content declined by 2.3%, while glucosinolate content increased by 1.1 µmoles/g. Given that most growers apply in the region of 180-240 kgN/ha, this study suggests a very low level of seed composition sensitivity to nitrogen fertiliser and does not explain the high degree of variation observed in the results sections reported earlier.
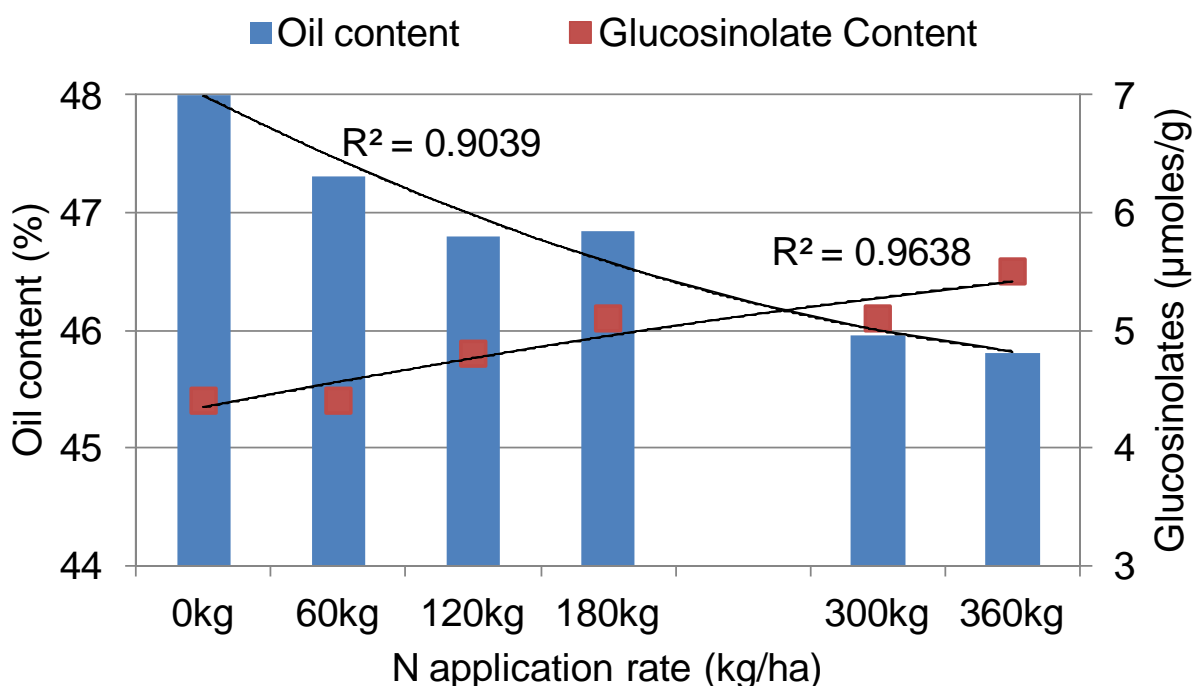
Whole-seed oil content and glucosinolate content responses
to increasing levels of nitrogen fertiliser application (Callow, Harvest 2014)

Further studies were undertaken in the 2014/15 growing year, at Sutton Scotney, Hampshire and at Morley, Norfolk. The same range (0-360 kg/ha) was used, this time with the open-pollinated variety, DK Cabernet. Here again there was only partial success and the drought-affected Hampshire trial was discarded because of very low yield and a high coefficient of variation. In the Morley trial oil content reduced by 1.72%, while protein content and glucosinolates increased by 2.32% and 2.94 μmoles respectively, over the range of N applications. This provided not only further evidence of the relatively small sensitivity of oil, protein and glucosinolate content to nitrogen fertiliser application, but also a very consistent between-year directionality of the responses, which across the two year data are in accord with those from earlier reported field trials (Chalmers, 1989).
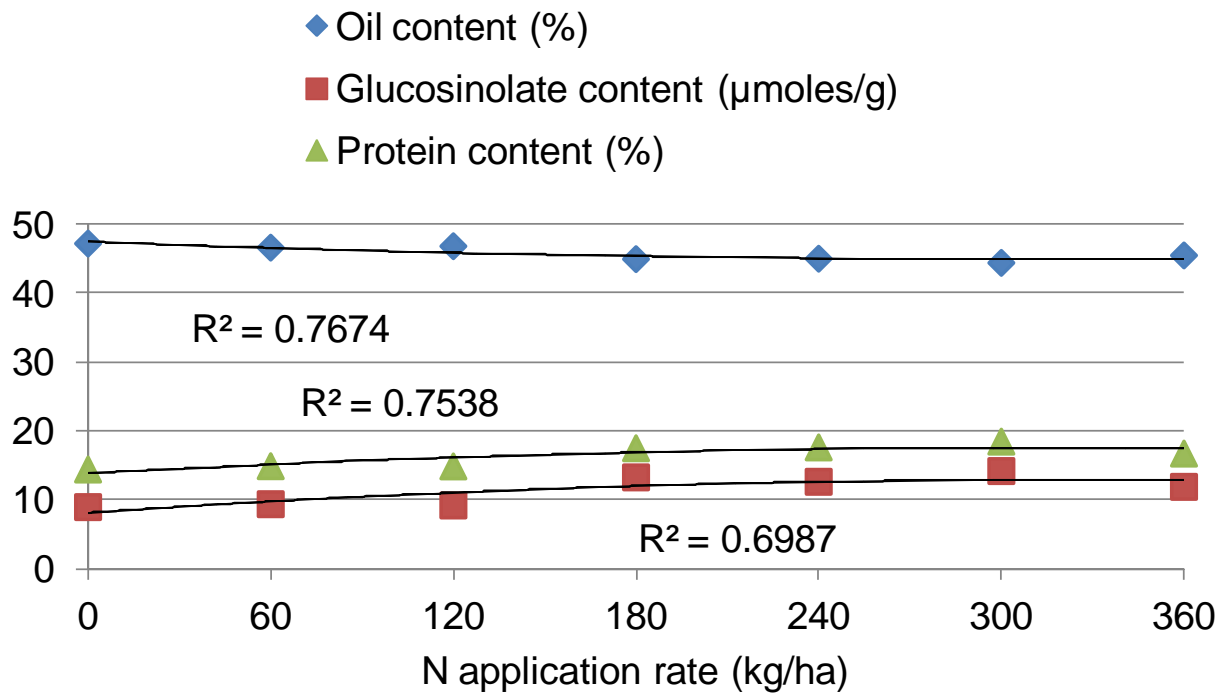
Whole-seed oil content, protein content and glucosinolate content responses
to increasing levels of nitrogen fertiliser application (Morley, Harvest 2015)