July 2014

Research Review No. 80

Nutritional value of oilseed rape and its co-products for pig and poultry feed: potential improvements and implications for plant breeders

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This review was produced as the final report of a 13 month project (RD-2012-3811) which started in January 2013. The work was funded by a contract for £20,051 from HGCA.

HGCA is the cereals and oilseeds division of the Agriculture and Horticulture Development Board.

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1. Abstract

Oilseed rape (OSR) produces a seed with high energy content, and rape meal, its co-product following oil extraction, is a valuable mid-protein animal feed. Both rape seed and meal have value in poultry diets, but at present only rape meal is used in pig diets. This review considers the potential for improving the nutritional value of OSR seed and meal for non-ruminants, and seeks to inform plant breeders about potential targets for variety development for the feed market. Four key elements were considered: (1) A literature search focusing on the limiting factors affecting utilisation of OSR seed and meal, to identify potential traits for improvement; (2) Potential changes to OSR through genetic modification (GM) to improve seed quality; (3) Review of UK databases to quantify available levels of variation in the key traits; (4) A feed formulation exercise carried out to assess the value of a hypothetical new rape seed (and meal derived from it) based on an 'improved' genotype of *Brassica napus*.

While there is a wealth of information on anti-nutritional factors (ANFs) in the literature, e.g. fibre, glucosinolates (GSL) phytate, polyphenols, sinapine and tannins, it is difficult to quantify the financial benefits in terms of improvements to animal feed quality of reducing any ANF, other than GSL or fibre in OSR. Moreover, it is concluded that the traits improved by GM routes e.g. low phytate/high phytase and low sinapine, would be of little financial value, as there are technical solutions to addressing these ANFs already employed by the feed industry. Nevertheless, it is possible to envisage an improved rape seed genotype which, as well as low GSL, would have a thinner seed coat with resulting higher oil and protein and less fibre, having a real value in the feed market. Moreover, if such a genotype was also derived from yellow seed types, it would be possible to also reduce polyphenols in the seed coat, which would improve the attractiveness of rape seed and meal as feed ingredients and thereby increase their uptake. In general, the datasets studied contained information on oil, protein and GSL concentrations, and in some, seed yield, but lacked information on other ANF and relevant seed quality traits.

The value of an improved variety containing an additional 4% oil + protein (93%DM basis) with a thinner, yellow-seed coat was evaluated using a least cost ration formulation system for broilers, layers and pigs. For whole seed, improved seed types would be favoured in broiler diets where feed is pelleted and hence disruption of the seed coat and cotyledon occurs. The new (improved) meal appears to have the highest value in pig rather than poultry feeds. Within the poultry sector, the improved rape meal would appear to warrant a higher value in layer diets than broiler. To relate these improvements to a value to the industry, broadly speaking the improved feeding values are worth an extra £22.50/t to £30/t of rape seed, with the value of conventional rapeseed estimated at £345/t at the time of writing the report. This value could easily be eroded if the financial scenarios changed by lower prices of competing commodities, higher transport and/or energy costs, but support a general target of breeding for oil plus protein. Areas for further research are highlighted, particularly the need to evaluate the newer yellow seeded types of *B. napus* recently developed in Canada and France, to confirm performance in UK feeding systems.

2. Introduction

2.1. Aims and objectives

Oilseed rape (OSR) is a high oil and mid-concentration protein crop with the potential to replace a significant proportion of soya in pig and poultry feed. Although its high protein quality makes it valuable for livestock feed, there are concerns within the industry about its suitability for inclusion into poultry and pig diets, in part due to the presence of anti-nutritional factors. This means the industry is conservative in its use. This project was designed to review the potential for improving the nutritional value of OSR co-products for pig and poultry, and to inform plant breeders regarding targets for new variety development.

The objectives were to:

1. Review the potential changes to quality traits in oilseed rape which would impact on quality, based on known variation in key traits, and other reported GM approaches;
2. Quantify the likely economic costs and benefits of such changes to quality traits using a least cost ration formulation approach, and thereby guide plant breeders as to the likely value of incorporating such trait improvement into breeding programmes.

2.2. Approach and sources of data

2.2.1. Literature review

The preparation for this report has involved an extensive literature search focused on the pig and poultry requirements when feeding oilseed rape and rapeseed meal. Attention has been focused on potential GM traits and the seed composition for each species of commercial oilseed rape developed around the world (*B. napus, B. rapa* and *B. juncea*) – including: anti-nutritional factors, seed colour, phytates, sinapine, protein, fibre, oil, tannin, starch and glucosinolates (GSL). Search terms also included: full fat rapeseed, extracted rapeseed meal and rape expeller.

2.2.2. Information on potential GM modifications

Data was collated from public databases CERA (Centre for Environmental Risk Assessment, http://www.cera-gmc.org/) and BCH (Biosafety Clearing House, http://bch.cbd.int/). The aim was to identify potential modifications to OSR which had been reported and submitted for registration (worldwide). Input traits like gluphosinate and glyphosate resistance were discounted as they are assumed to exhibit 'substantial equivalence' to conventional OSR, i.e. no fundamental difference to the quality or composition of the product(s). For the purposes of this review, the focus was on novel traits, those defined as those relating to complex traits (e.g. abiotic stress tolerance) which might affect composition, or those where the intended modification is a positive change to an output or quality trait.

2.2.3. Databases of quality traits

Data were accessed from six different sources. In general, the data contained information on oil, protein and GSL content, and in some, seed yield (and oil yield derived from primary data) but lacked information on traits such as seed colour, tannins, fibre, phytic acid, hull thickness, seed size, protein content or protein subunit composition. The datasets were as follows:

1. LINK Project LK0979 ‘Breeding oilseed rape with a low requirement for N fertiliser’ measured seed quality traits on 29 elite varieties grown in six experiments during 2006/7 and 2007/8,
2. Breeders’ data was accessed: Seed quality data collected from 61 UK varieties grown in 2011/12 which are either recommended or close to recommendation are summarised,
3. Tapidor x Victor substitution lines; a population of 75 Tapidor x Victor substitution lines were grown in 3 experiments during the 2008/9 and 2009/10 seasons in LINK project LK0979,
4. A population of 86 Rocket x Capitol doubled haploid (DH) lines were grown in 4 experiments during the 2008/9 and 2009/10 seasons within LINK project LK0979,
5. A *Brassica napus* diversity panel developed by the John Innes Centre (JIC) was grown by KWS in 2009/10 with 60 accessions, and by the JIC in 2012 (105 accessions). This included a very wide range of *B. napus* germplasm, ranging from elite UK varieties, older UK varieties and non UK germplasm,
6. The OREGIN project (Defra project IF0144) developed a collection of fixed lines that represents the diversity within *Brassica napus* Diversity Fixed Foundation Set; this set of lines was tested for oil and protein contents in 2007.

2.3. Historical overview – composition and meeting livestock requirements

The aim of this section is not to review in detail the research into inclusion levels of different rapeseed products for livestock. Much of the historic data are now outdated and related to older (higher erucic acid, high glucosinolate varieties). Moreover, typical maximum inclusion rates for rape seed meal (RSM) have been reviewed recently by Cottrill *et al.* (2005). The purpose of this section is to draw attention to the main anti-nutritional factors (ANFs) in the seed, and indicate their nutritional significance, before considering the opportunities for their improvement through plant breeding.

Oilseed rape (generally *Brassica napus* L. is an important source of oil and is grown widely across the UK. Rapeseed meal is a by-product of the oil extraction process and can be used as a valuable, ‘mid protein’ animal feed containing 37% crude protein, that is, sitting between a low protein feed like wheat at 11.5%, and soya meal and its derivatives at >45% crude protein on a dry basis. However, ANFs contained within rapeseed meal can have toxic effects on the animal, and as a minimum can result in reduced digestibility when included in livestock diets at high levels.

ANFs are present in many plant species and protect the plant from attack by micro or macro organisms, with the highest concentrations often being in the seeds. In non-ruminants, ANFs can inhibit the digestion of nutrients from the feed or cause deleterious effects to the animal that consumes them. Other seed components which are not strictly ANFs, such as dietary fibre, also limit the use of OSR and rape meal in non-ruminant livestock diets through their effects on apparent digestibility of protein and energy.

2.3.1. Nutrient composition (OSR and rapemeal co-products)

The economically important oil fraction of OSR, is relatively rich in oleic acid (58–62% of total fatty acids), with only soya bean oil having higher oleic acid content. Some new OSR varieties developed for specific food and industrial sectors have high oleic (HO-) content which means oleic acid contents in the oil some 11% higher than conventional OSR. There is some environmental variation, with HO lines varying from 76 to 81% in oleic acid content (Weightman et al. 2011), but this is not considered further in this as based on the authors experience, there is no perceived value to the livestock industry of HO lines.

The whole seed contains 18–24% protein and after oil extraction the residue meal contains about 32–40% protein. Rapeseed meal therefore is a ‘mid-protein’ feed for livestock (compared to an ingredient like soya bean meal; SBM).

The original varieties of rapeseed had particular problems with GSL, as their metabolites are toxic to a number of livestock species. By introducing double low varieties (low in both GSL and erucic acid), new opportunities for feeding rapeseed were introduced. Henkal and Mosenthin (1989) reported that double zero cultivars could be the sole source of supplementary protein in livestock diets, and since that time much progress has been made in reducing GSL levels in rapeseed. In Canada, Khajali and Slominski (2012) report that the GSL level of double low rapeseed (canola) is about one twelfth of that of older varieties of rapeseed, confirming that substantial progress has been made through plant breeding.

When compared to soya bean meal, rapeseed meal is a richer source of minerals (Clandinin and Robblee, 1981, Nwokolo and Bragg, 1977); however, because of the presence of phytic acid and indigestible fibre, rapeseed meal can have lower availabilities of P, Ca, Mg and Zn. Khajali and Slominski (2012) point out that RSM contains less Na and K than SBM, and so the dietary electrolyte balance is lower, which should be corrected for by adding Na to the diet. High sulphur content can be a problem with feeding RSM to poultry, but this is less of a problem as GSL contents have dropped (much of the non-protein sulphur being present in GSL) and high S levels can also be corrected at feeding, by inclusion of additional Ca in the diet. The levels of individual minerals therefore have little relevance in terms of plant breeding targets for livestock feed, as mineral and vitamin supplements are added to all livestock rations to remove any deficiencies. With the exception of phytase and its impact on mineral availability, minerals are not considered further in this review.

2.4. Current rapeseed products used in livestock rations

2.4.1. Full fat rapeseed

According to the literature, broilers may be fed full fat, low GSL rapeseed at dietary levels up to 200 g/kg, whilst layers can be fed 100 g/kg without affecting production (Leeson et al., 1978). Full fat rapeseed is now firmly established as a key ingredient used in the form of whole seeds in broiler diets, at inclusion levels of up to 8%. Moreover, it sometimes is used as a component of a cooked rape/pulse blend in a 50/50 mix.

Although experimentation has not always indicated the need for heat treatment of the seed, it was suggested that this be carried out as a precautionary measure (Clandinin & Robblee, 1981). In commercial practice, blends tend to be cooked via extrusion, otherwise whole seed is subjected to normal conditioning during the pelleting process for making broiler feed, which involves some degree of heat treatment.

Henkal and Mosenthin (1989) noted that even at low levels of inclusion, full fat rapeseed can adversely affect structure and fatty acid composition of lipids in meat and eggs. In fact whole rapeseed is not used in UK layer diets as these are fed in mash form, and do not undergo the necessary physical disruption which occurs when pelletting broiler feed. This is discussed further in Section 6.

2.4.2. Extracted rapeseed meal

Feeding meal from early varieties of rapeseed to poultry was shown to lower the rate of egg production, cause perosis, off-flavours in eggs and even liver damage, as well as poorer growth rates and thyroid enlargement (Tookey, van Etten and Daxenbichler, 1980). Of most importance practically, a fishy odour was reported in brown eggs (Hawrysh *et al.*, 1975). This fishy odour has been attributed to the inability of some strains of birds (particularly the Rhode Island Red) to oxidise trimethylamine (TMA) to form the odourless TMA-oxide at a rate sufficient to prevent TMA being deposited in the eggs (Clandinin and Robblee., 1981; Fenwick *et al*., 1989). The source of this TMA in rapeseed is sinapine, an alkaloidal amine (a choline ester of sinapic acid) found in brassica seeds. Consequently a maximum inclusion level of (1g Sinapine/kg) is recommended to prevent egg taint. (Goh *et al.*, 1979). In practice, the industry adapted to this restriction by moving to white bird varieties, and more recently, the breeding companies have “bred out” the tainters in their strains and the UK layer flock now comprises 95%+ brown birds. Most brown strains can therefore now be fed rapeseed/meal without fishy taint being a problem. There are exceptions but these are recognised and rapeseed meal use is modified accordingly.

Rapeseed meal has therefore often been excluded from UK layer and broiler diets. When included, it is only at low concentrations in broiler finisher rations due to concerns about the potential adverse effects of ANF to growth of young birds and to meat and egg quality as described above. Henkal and Mosenthin (1989) noted that even at low levels of inclusion, full fat or partially defatted low GSL rapeseed can adversely affect the rates, structure and fatty acid composition of meat or eggs. It was thought that young poultry should receive no more than 2–3% of their diet as rapeseed meal, whereas up to 5 % may be fed to older birds.

In pigs, the rate of inclusion is governed by ANFs (principally glucosinolates) and the balance of nutrients (particularly ileal digestible amino acids and net energy). The palatability of rapeseed is believed to limit its inclusion in piglets diets and *ad libitum* feeding of grower-finishing pigs, but not when feeding is restricted (Henkal and Mosenthin, 1989). Factors influencing palatability and feed intake are complex, involving dry matter intake, viscosity of feed components, as well as more obvious taste and the impacts of specific odour components (including sinapine), and are poorly understood. Factors controlling palatability *per se* is outside the scope of this review.

Although not an objective of this review, it is worth noting that rape meal has been widely used in the diets of ruminant livestock. For ruminant livestock, an important characteristic of rapeseed meal is the rate of which it degrades in the rumen, as this directly affects the supply of nitrogen to rumen micro-organisms, and the extent to which the protein by-passes the rumen. There are a range of reports measuring rumen protein degradability. Effective degradability of rapeseed meal protein varies from 0.52 to 0.68 (Ha and Kennelly, 1984, Kendall *et al.,* 1991 and Hvelplund and Madsen 1990) and this range of results can be attributed to the variety of cultivars used in ruminant feed.

2.4.3. Rape expeller meal

One method of extracting rapeseed oil is by using an expeller press (a process for extraction without solvents) but this method is less efficient at removing the oil (Bourdon and Aumaitre, 1990; Spragg and Mailer, 2007). As a result, expeller-pressed rapeseed meal has an oil content of 10% to 15% (Leming and Lember, 2005) and can still be a valuable amino acid source in pig diets. The metabolisable energy in expelled meal is therefore greater than that of solvent-extracted meal (Smulikowska *et al*., 1997, 2011).

Individually housed grower-finisher pigs may tolerate 10% to 18% expeller meal in their diets without detrimental effects on growth performance and only minor effects on carcass characteristics (Brand *et al*., 2001). An increased dietary inclusion of expeller meal has however been found to reduce pork quality (Whitney *et al*., 2006) because the remaining oil which is rich in unsaturated fatty acids, may soften carcass fat (Rowghani *et al*., 2007).

2.4.4. Other products, e.g. Extrupro/Beano/Beamix

There are products available to the compound feed industry that include whole rapeseed blended with other materials as noted above. The purpose of the blending is partly to give a material with better handling characteristics and to facilitate the cooking via extrusion of the whole seed, which on its own has too high an oil content to allow frictional heat to increase temperatures to allow cooking of both components.

The typical product is a 50/50 blend of whole rapeseed and field beans although on occasions the non-rape element has been peas or a blend of peas and beans. Historically there have also been products with different levels of rapeseed meal blended with whole rapeseed. Current products are rapeseed/bean mixes.

The processing of the material must yield nutritional advantages to give the resultant cooked blend a higher value than the sum of the ingredient costs to cover additional haulage and processing. This is certainly the case and the nutritional value ascribed to the products is significantly higher than the combined value of the ingredients and poultry performance is not adversely affected by using the higher values. Interestingly, several studies have been conducted to try and elucidate the energy and digestible protein value of the products in chick/cockerel studies but, so far, there is no satisfactory explanation for their benefits.

3. Candidate traits for improvement in OSR

This section reviews the main components of the seed which impact on animal nutrition, and describe their biology as a pre-requisite to understanding which of these might be amenable traits for improvement by breeding.

3.1. Glucosinolates and myrosinase

3.1.1. Introduction

About thirty different GSL have been identified in *B.napus* (Sorensen, 1990). Glucosinolates are not generally considered to be harmful in themselves, but form toxic breakdown products following hydrolysis by the enzyme myrosinase (Huisman and Tolman, 1992). Bjerg *et al.* (1987) regarded intact GSL as anti-nutritional factors, but noted that their degradation through the action of myrosinase enhanced their toxicity. Hydrolysis by myrosinase occurs whenever raw plant material is wetted (Tookey van Etten and Daxenbichler, 1980) and causes breakdown to potentially toxic thiocyanates and nitriles.

Myrosinase, an iso-enzyme of thioglucoside glucohydrolase (Buchwaldt *et al*., 1986), shows activity over a range of pH values and is stable up to 60°C with optimum activity around 50°C (Fenwick *et al*., 1989). The resulting degradation of GSL produces various flavours, off flavours, anti-nutritive and toxic components (Bille et al., 1983; Bjerg et al., 1987). The nature of breakdown products is dependent on the pH, structure of the parent GSL and a number of co-factors which include presence of ferrous ions (Fenwick et al., 1989).

Threshold levels for GSL were set by the EC in 1985 and those cultivars below the threshold became eligible for a bonus. Consequently, ‘double low’ cultivators were bred containing low levels of erucic acids and GSL.

3.1.2. The role of GSL/myrosinase in plants

GSLs are natural plant products derived from amino acids (Fenwick, Heaney and Mawson, 1989). GSLs are present throughout the whole plant but their concentrations are usually greatest in the seed (Tookey et al., 1980). Their role is to protect the plant from insect and animal attack.

3.1.3. Biochemical properties

Glucosinolates are natural plant products, with most lying on a common biosynthetic pathway (Fenwick, Heaney and Mawson, 1989). GSL may be divided into three main types depending on the toxic hydrolysis products generated (Pusztai, 1989b):

1. Isothiocyanate
2. Oxazolidone-2-thione
3. Thiocyanate

In nearly all known double zero cultivators of rapeseed, 4-hydroxyglucobrassicin is the GSL present in the largest quantities (Bjerg et al., 1987). This is also one of the more unstable GSL and is easily oxidised, particularly during oil extraction procures based on traditional techniques. In some cases all the 4-hydroxyglucobrassicin is destroyed (Sorensen, 1990), while the variation in degradation of other GSLs can vary from 30-70% (Daun, 1986).

3.1.4. GSL metabolism

The breakdown of GSL can occur either in the seed prior to consumption, or in the gut of the bird through the action of bacterial thioglucosidases. Thus, even if the endogenous myrosinase is eliminated by pre-treating the seed by heating (Naczk *et al*., 1998), the breakdown of GSL in the bird cannot be avoided. Therefore, the best practical target to reduce toxicity from GSL is to reduce their concentration in the seed.

3.1.5. Levels of glucosinolates in OSR

Older (high GSL) rapeseed cultivars had GSL concentrations greater than 120 µmol/g (Fenwick *et al*., 1989). GSL concentrations in the seed are now typically around 10 µmol/g as reported in recent scientific studies (Khajali and Slominski (2012) and average 11.9 µmol/g in UK elite germplasm (HGCA Recommended List winter OSR East/West region 2012/13), underlining the considerable progress which has been made by plant breeders. Following oil extraction, Slominski *et al* (1999) reported GSL contents of between 11 and 22 µmol/g in the oil-free meal, implying very low levels in the seed of canola types.

GSL concentration is dependent on several factors, the most obvious being genetic variation, which was exploited in developing double zero cultivars. There are also botanical influences which cause seeds from the top siliques of the lowest branches to have the highest GSL concentrations (Rahman, Stolen and Sorensen, 1986) and use of sulphur-based fertilizers increases the GSL content in the seed. Stress, for example induced by drought or high plant density, has been suggested to increase GSL and the effect of sowing date has also been reported to influence GSL level (Fenwick et al., 1989).

3.2. Sinapine

3.2.1. Introduction

Sinapine is an alkaloidal amine, considered to be the choline ester of sinapic acid (Kozlowska *et al*, 1990), and which is reported to account for 80% of the total phenolic acids in rapeseed meal (Shahidi and Naczk, 1992). Sinapine is thought to contribute to the dark colour, bitter, sour, and astringent tastes of rapeseed meal (Naczk *et al*., 1998; Qaio and Classen, 2003). As mentioned earlier in genetically pre-disposed brown laying hens, the incomplete metabolism of sinapine results in a fishy egg taint (Hobson-Frohock *et al*., 1973 and Fenwick *et al*., 1984). Sinapine acts as a reserve of choline and sinapic acid for young plants. Choline is an important nutritional component of the diet, and it is added as a synthetic to poultry rations, thus it may not be wholly desirable to remove sinapine for this reason.

3.2.2. Levels of sinapine in OSR and meal

The content of sinapine is at its highest between the final stage of the green seeds and the beginning of their browning, reaching a stable level at seed ripeness (Naczk *et al.,* 1998). Levels of sinapine in *B. napus* have been reported to be significantly higher than those found in *B.campestris* cultivars (Mueller et al., 1978) although other studies have shown the sinapine content of *B.napus* to range from 11.7–18.4 g/kg (Fenwick *et al*, 1984). Levels as low as 9.4 and 9.6 g/kg have been measured for *B.napus* and *B. campestris*, respectively (Austin and Wolff, 1968).

The content of sinapine in de-fatted rapeseed was found to be 26.7 g/kg (Blair and Reichert, 1984) and in rapeseed meal can range from 6 - 30 g/kg depending on cultivar, growing conditions and location (Krygier *et al.,* 1982 and Lacki and Duvnjak, 1996).

3.2.3. Properties

Phenolic acids are capable of forming complexes with proteins, thus lowering their nutritional value (Kozlowska et al., 1990). However, there do not appear to be any studies which relate this to animal performance. Sinapines are converted in the large intestine to trimethylamine (TMA) which is subsequently absorbed, deaminated, and then excreted, causing the fishy taint in eggs mentioned above in early studies, when sinapine levels in the diet exceeded 0.8 mg/kg (Fenwick, 1982). This taint, however, is managed within the industry and modern strains of birds are not susceptible to taint problems. Other ANF's are implicated in the production of TMA in the yolk as tannins and goitrin (from progoitrin) can inhibit the enzymatic oxidation of TMA into TMA oxide. Birds with the Rhode Island genes lack the ability to deaminate this compound, which builds up in the blood, and it is transferred to the egg. Jensen *et al.* (1991) reported that heating reduced the sinapine content of rapeseed meal, but it was accompanied by an increase in the content of lignin-type products.

3.2.4. Current status

The layer industry does use some rapeseed meal, its limitation is now purely based on economics, as a protein source with respect to soya. The British Egg Industry Council (BEIC) have relaxed their constraint on rape; it used to be specifically excluded from diets under the Lion Code but now the code only requires feeds to not contain materials which might cause a taint. Retailer limits are sometimes less flexible. It is probably more important for HGCA to demonstrate to the layer industry through knowledge transfer, that sinapine levels are not a problem with current flock genetics.

Nair et al. (1999) reported that efforts to develop low-sinapine varieties of *B. napus* had not been successful. Velasco and Mollers (1998) analyzed a collection of 1,487 accessions from 21 Brassica species and 1,361 samples of *B. napus* breeding lines for sinapic acid ester (SAE) content. The lowest SAE content was at 1.7 g/kg in *B. tournefortii* Gouan and at 5.0 g/kg in a *B. napus* breeding line where as the average SAE content was at 8.0 g/kg. The lack of robust screening methods suitable for identifying any low-sinapine Brassica breeding lines and the amphidiploid nature of *B. napus* made progress through conventional breeding difficult at that time.

It is possible that with newer screening tools, more progress could now be made, if the market were to value a low sinapine type. However, given that choline is required by both plant and animal, that the move to birds with the correct genetics has removed the problem of taint in eggs, that palatability is unlikely to be a problem in broilers, and its importance regarding palatability is unquantified in pigs, it is unlikely that reduction in sinapine will be a valuable target for plant breeders. Nevertheless there have been attempts to reduce sinapine levels using GM technology, and these are considered later for completeness (Section 5).

3.3. Phytate, phosphorus and mineral nutrition

3.3.1. Introduction

Phytic acid is major reserve of phosphorus in the seed. In rapeseed meal, over 80% of the total phosphorus is found in phytic acid and is assumed to be unavailable to the animal (Thompson, 1990; Segueilha et al., 1992). Naczk et al., (1998) reported that phytic acid can bind mono and divalent metal ions to form complex phytates, thus reducing their bioavailability (citing Erdman, 1979; Cheryan, 1980; Cosgrove, 1980, Magna, 1982; Morris, 1986; Hallberg, 1987; Thompson, 1990). A reduction in the bioavailability of several metals, notably zinc and iron and to a lesser extent, calcium and magnesium in the presence of phytic acid has been reported (Erdman, 1979; Jones, 1979; Cheryan, 1980; Magna, 1982). Phytates have a low digestibility in monogastric animals, and are also reported to form complexes with basic amino acids (Atwal et al., 1980; Shah *et al.*, 1979).

3.3.2. The role of phytate in the plant

Phytic acid is the primary phosphorus and myoinositol reserve in the seed and hence is also a store of energy (Cosgrove, 1980; Greenwood, 1990). The phosphorus is mobilised during seed germination and growth by phytase enzymes within the plant. Phytate is also believed to protect plants against oxidative damage during storage and from moulds by binding the zinc required for their growth (Graf et al., 1987).

3.3.3. Levels in seed and meal

Phytic acid levels for whole seeds and in de-fatted meals at levels were originally reported to be greater than those found in many other oilseeds and oilseed products (Thompson, 1990). However, the level in whole seed is lower than that for soyabean meal, and for meal it is similar to that for wheat feed/middlings/bran, therefore particularly with the use of phytase enzymes, this is not seen to be a major constraint on use of rape products in feed. The standard values used by the industry now, are 0.41% phytate P in whole rapeseed, and 0.78% in rape meal.

3.3.4. Current status

Collation of information on the phytic acid content in a number of varieties of rapeseed has been carried out and the variation within a variety was nearly as high as within a population of varieties of the same species (Uppström and Svensson, 1980). The results imply that the phytic acid content of rapeseed is mostly influenced by environmental factors such as the availability of phosphorus in the soil, and its heritability is likely to be low, and therefore a poor target for breeding. Ravindran *et al*. (1999) reported that the enzyme phytase, when added to a wheat-casein diet, improved amino acid utilisation in broilers, and Smulikowska et al. (2006), have shown benefits with rapemeal.

Since the late 1990's, the commercial use of phytase has been widely adopted by the industry and is currently used in all commercial UK pig and poultry diets. Phytase for poultry feeds costs 50 to 75p/t of treated feed in December 2013, and is therefore a relatively cheap option to improve P digestibility in the animal, acting potentially on phytate arising from all components of the diet. This means there is little incentive currently to search for a single low phytate feed ingredient such as OSR, from a feed industry point of view.

For completeness, GM options for introducing phytase into the seed are considered in Section 5.

3.4. Condensed tannins and other polyphenols

3.4.1. Introduction

Tannins are complex phenolic compounds present in most plant materials (Larbier and Leclercq, 1994; Naczk *et al*., 1998). Amongst phenolic substances, tannins are the compounds with the greatest anti-nutritional activity (Larbier and Leclercq, 1994). Condensed tannins are particularly abundant in the hulls of black-seeded rapeseed, and can precipitate proteins, including digestive enzymes. It is believed that condensed tannins affect the nutritional value of feed in a number of ways:

- Formation of complexes with dietary proteins (Griffiths, 1979; van Sumere *et al*, 1975),

- Formation of complexes with carbohydrates and other macromolecules (Swain, 1965; Haslam, 1979),
- Inhibition of the activity of various digestive enzymes (Griffiths, 1979; Huisman and van der Poel,1987),
- Formation of complexes with divalent metal ions (Srikantia, 1976),
- Erosion of gut epithelial cells (Bernays et al.,1989).

It is thought that phenol-protein complexation results from the formation of hydrogen bonds and hydrophobic interactions (Hagerman and Butler, 1980).

Detailed examination of condensed tannins shows that their anti-nutritional importance varies between types. Condensed tannins are particularly abundant in the hull of rapeseed and can lead to a general reduction in digestibility of amino acids (Green and Kiener, 1989) - primarily of proteins, and to a lesser extent, starch, presumably through their action on amylase enzymes. The condensed tannins present in the hulls may contribute to the astringent taste of rapeseed meals (Naczk *et al*, 1994; Reed, 1995).

3.4.2. The role of tannins in plants

Swain (1965) regarded tannins as water-soluble phenolic compounds having molecular weights of between 500 and 5000 Da, which have the ability to precipitate alkaloids, enzymes, carbohydrates and metal ions. In the seed coat, tannins probably inhibit attack by pathogenic microorganisms, by complexing the hydrolytic enzymes used to break down the plant cell walls, and thereby slowing the rate of attack. When the crop is fed to livestock, the same anti-nutritional properties are also associated with lower digestibility, and ultimately a reduction in performance (Martin-Tanguy et al., 1977; Ward, Marquardt and Campbell 1977).

3.4.3. Levels of condensed tannins in rapeseed

Naczk et al. (1994) found differences in tannin contents between rapeseed cultivars due to environmental growing conditions, with total tannins in the hulls ranging between 1.9 and 6.2%.

3.4.4. Levels of lignin in rapeseed

Lignin is another related but water-insoluble polyphenol complex, which has much higher molecular weight than tannins, and is generally assumed to be indigestible to monogastric animals, and hence reduced the energy density of rapeseed and meal. Khajali and Slominski (2012) reported that canola meal contains 10.4% lignin plus polyphenols, compared to only 2.6% in SBM. A lower fibre content in yellow-seeded rapeseed tends to be associated with lower lignin and polyphenol contents (3.7% vs 7.1% DM compared to black-seeded rapeseed; Slominski et al., 2011). The association with fibre is discussed further below.

3.4.5. Current status

There has been little work selecting for tannin content *per se*, but given the marked differences in tannin contents between yellow and black-seeded OSR, selection for a yellow-seed coat is likely to be the cheapest and quickest method of making progress, and has been pursued outside the UK, most notably in Canada. At the same time, this would tend to reduce the amount of indigestible cell wall material through reducing the overall lignin content of the seed (Badani et al. 2006). This is discussed further below.

3.5. Fibre

3.5.1. Introduction

Fibre is made of non-starch polysaccharides and lignin which comprise the plant cell walls. They are poorly digested by non-ruminant animals. A high fibre content reduces metabolisable energy (ME) content, protein digestibility and bioavailability of minerals. The hull of a rape seed is largely fibre and all of it remains in the meal after oil extraction. However, hulls can vary widely in digestibility, particularly for pigs. There has, therefore, been an interest in developing low-fibre/high energy fractions from rapeseed to be suitable for inclusion in rations for monogastric animals, particularly poultry.

The simplest practical approach to improving the quality of rape meal involves dehulling prior to oil extraction. This is because the low-hull fraction contains significantly lower levels of both fibre and tannins, with a digestibility and ME similar to that of soya bean meal. However, this is not generally carried out in practice as it is reported that there is a significant loss of oil during the dehulling process, and subsequent oil extraction from the dehulled cotyledons is less efficient.

3.5.2. The role of fibre in the oilseed rape seed

Fibre exists in two main forms in the oilseed rape seed. Firstly, the cotyledons have primary cell walls containing cellulose, pectins and arabinogalactans, whereas the testa (hulls) contain secondary cell walls, with more cellulose, other insoluble polysaccharides and lignin. The cell walls of the cotyledon have evolved to be modified quickly during germination, and due to their greater concentrations of soluble non-starch polysaccharides, can be broken down more easily to release cell contents (proteins and lipid) than the hulls. Cellulose cannot be digested directly by non-ruminant animals but can be fermented to a certain extent by bacteria in the large bowel of pigs, and are therefore considered to be more digestible than lignin. Lignin is considered indigestible to non-ruminant animals, and is only degraded very slowly by fungi. The hull (testa), therefore, functions as a tough barrier for the seed, which protects it from fungal and insect attack in the soil.

3.5.3. Chemical composition and levels of fibre in OSR

The rapeseed hull traditionally comprises ca. 16% of the seed by mass, and about 25-30% of the meal (the proportion probably varies as a result of seed size) and about half of this is poorly digested fibre (Pusztai, 1989). The fibre contains approximately 14.5% cellulose, 5.0% hemicellulose and 8.3% lignin. This all results in a crude fibre (CF) content of 10.6% for commercial rapeseed meal (Mwachireya *et al*., 1999) and 26% neutral detergent fibre (NDF) (Khajali and Slominski, 2012). The relatively low digestible and metabolisable energy values of rapeseed meal are to a large extent associated with this high level of fibre. De-hulled rapeseed meal contains over 20% non-detergent fibre (NDF).

Breeding for reduced fibre content based on selection of specific genes or targeting particular polysaccharide fractions has not been attempted. Even with GM approaches this is likely to be a challenging target, because cell wall polysaccharides are secondary plant products involving complex synthetic pathways with multiple membrane-bound enzymes situated within the cell organelles, and a site of assembly outside the plasma membrane. They are therefore not under simple genetic control, as would synthesis of an enzyme or storage protein. However other simpler approaches based on seed size and the thickness of the coat, have shown some potential to reduce the seed fibre content.

Following a Swedish report (Jonsson and Bengtsson 1970) that yellow-seed *B. campestris* had a lower percentage seed coat than brown seed types, Stringam *et al*. (1974) examined yellow and brown seeds segregating from several *B. campestris* lines and found that yellow hulls contained about one-third less crude fibre, more protein and more oil than brown hulls. Subsequent research by Bell and Shires (1982) confirmed these lower fibre levels of yellow hulls. It was also evident that while fibre was lower in yellow hulls, all measures of fibre were higher in the embryos of yellow-seeds than in brown seeds, thus the beneficial effect of reduction in hull fibre was partially offset by increased fibre and lignin within the whole seed (Slominski and Campbell, 1990). Badani et al. (2006) also report that selecting for yellow-seeded varieties reduces the levels of fibre (ADF; acid detergent fibre, representing essentially cellulose plus lignin). Progress in breeding for the seed colour trait is considered further in Section 3.8.

3.6. Starch

Starch is present in oilseed rape seed, but only in small quantities (0.1–0.2%) and only in the cotyledons (Blair and Reichert, 1984). Initially, the developing embryos of oilseed rape accumulate starch, but starch levels decline as the rates of storage lipid and protein synthesis increase (Hills, 2004; Andriotis *et al*., 2010a). At maturity, lipids account for up to 45% of the seed weight (O'Neill *et al*., 2003; Andriotis *et al*., 2010b, Hills, 2004; Andriotis *et al*., 2010a).

From a livestock nutrition point of view, there would be little point in increasing the concentration of starch, as it would be most likely to simply reduce the concentration of oil in the seed, even if more variation existed in wider germplasm. Existing breeding targets for the high oil trait are of over-riding importance economically, and so breeding for starch content can effectively be ignored.

3.7. Protein

3.7.1. Introduction

The digestibility of rapeseed meal proteins and amino acids in pigs was investigated by Furuya et al. (1988). The coefficient of true digestibility of all amino acids was found to be 0.817, with crude protein at 0.798 and for the individual amino acids lysine, threonine, and methionine the coefficients of true digestibility at the terminal ileum was 0.826, 0.781, and 0.837, respectively. The NDF content of rapeseed meal had a strong negative effect on the apparent digestibility of crude protein in pigs (Bell and Keith, 1987). Protein quality in rapeseed may be defined in several ways. Studies with chickens and pigs emphasise the need for processing conditions that provide only the minimum heat necessary to inactivate enzymes (especially myrosinase) and to avoid denaturation of the storage proteins. Excessive heat leads to undesirable reactions and to reduced availability of amino acids, especially lysine (Clandinin *et al*. 1959). Any reduction in lysine availability will seriously affect the competitive position of rape meal for monogastric use.

3.7.2. Amino acid and protein levels in the seed

There is little information on variation in seed protein content or composition. Slominski et al. (2012) showed that yellow-seeded *B. napus* contained more total amino acids (+2.6 g per 16 g N) than black-seeded *B. napus*, resulting from higher levels of arginine, phenylalanine, asparagine and glutamic acid. However, lysine and methionine contents of yellow-seeded *B. napus* were lower, meaning on balance, that the yellow-seeded type had slightly poorer AA profile.

Berry and Roques (2011) have shown that seed protein content increases by between ca. 1.4% for each 100 kg/ha of N applied in nitrogen response trials. This change in protein broadly matches the change in oil content (-1.33% per 100 kg N/ha applied). In contrast to wheat therefore, applied N has a much smaller effect on seed protein content in OSR. Germination does not seem to be affected by seed protein content over the range 25% to 19% (Stokes et al., 2000).

Protein content was negatively associated with high yields at low levels of N supply in two doubled haploid (DH) populations (Nyikako, 2003). It is possible that relocation of N from the pod walls is slower in low protein lines and this extends seed filling. Alternatively, this relationship may be the result of other traits improving yield and diluting the protein. Protein content has been observed to vary by 40% amongst 64 oilseed rape varieties (Malabat et al., 2003) in a study which showed that breeding to increase oil content had reduced protein content, mainly by reducing the seed storage proteins napin and cruciferin. It is not known whether these changes have affected amino acid composition, but in theory, changing seed storage composition could be used as one approach to increase protein quality.

3.8. Seed coat

3.8.1. Seed coat characteristics

As noted in the earlier discussion, the seed coat (testa or hull) is very important when considering the use of rapeseed for animal feed (Bell and Shires, 1982; Shahidi and Naczk, 1989). Yellow-seeds in *Brassica* species are associated with higher oil and protein content and a lower fibre content (Badani et al., 2006; Rahman and McVetty, 2011; Slominski et al., 2012). Such seeds are yellow because the underlying embryo is visible through a translucent seed coat.

Early Canadian breeding programmes developed yellow-seeded varieties of *B. rapa* (*campestris*) and demonstrated that the seeds contained more oil and protein and less fibre than conventional black-seeded types. In contrast, in Europe, OSR breeding programmes have focused principally on *B. napus*, rather than *B. rapa* types because of their focus on autumn-sown varieties. *Brassica rapa* is generally faster maturing (an advantage in Canada, and potentially for spring varieties in the UK) but of lower yield potential than *B. napus* (conveying a *ca*. 10% yield disadvantage independently of the spring sowing effect). Around twenty years ago, some UK spring rapeseed varieties were introduced based on *B. rapa* but the varieties produced in the 1990's were also associated with a greater risk of seed shedding in the field, poorer seed vigour and a larger volunteer problem in following crops.

Since this HGCA project was commissioned, the first report has been presented describing work in France, to develop yellow seeded OSR varieties (Quinsac *et al*., 2013). Data from Quinsac *et al*. (2013) suggest that rape meal from a black-seeded Canadian variety has protein and cell wall contents of 44% and 31%, respectively, compared to rape meal from French winter OSR varieties with protein and cell wall contents of 36 - 38% (DM basis; see Table 1). Inspection of the papers by Quinsac *et al*. (2013) and Slominski *et al*. (2012), show that they both examined the same Canadian yellow-seeded variety YN01-429, which in Slominski's paper is listed as a *B. napus* variety (not a *B. rapa* variety). Therefore, it seems that over the past twenty years, efforts in Canada have also focused on breeding a yellow-seeded B. *napus* (Tang *et al*. 1997; Badani *et al*. 2006; Rahman 2001; Rahman *et al*. 2001) even though they appear to differ fundamentally in composition from the European *B. napus* types. Dietary fibre content in these different seed types vary from 27-35%, with the lowest value representing the yellow-seeded *B. napus* and the highest, the commercial meal from black-seeded rapeseed.

Early studies suggested that birds fed the yellow-seeded rapeseed showed the lowest feed to weight gain ratio. Yellow-seeded rapeseed was found to contain more protein and sucrose, with less fibre and similar amounts of oligosaccharides and minerals. The fibre content was negatively correlated with protein content. Evaluation of *Brassica* seed meals in some studies showed only minor differences between the yellow and brown-seeded samples with regard to the contents of digestible protein, water-soluble fibre or soluble phenolics (Simbaya *et al*., 1997), whereas others showed that yellow-seeded *Brassica* lines have less tannins and a lower fibre content in the seed meal, which significantly improved the meal quality (Slominski *et al*. 1994). Slominski *et al*. (1999) showed yellow-seeded rapeseed to be superior to the black-seeded counterpart or other yellow-seeded species i.e*. B. juncea, B. rapa*), with regard to protein and fibre contents, true metabolisable energy content and overall nutrient utilization when determined by feed to gain ratio in broiler chickens. Badani et al. (2006) showed variation in ADF content ranging from 6.6 -11% and 7.8 - 12.2% in two contrasting mapping populations in Germany, although this purely genetic exercise and did not involve any nutritional testing.

The most recent nutritional data from Canada supporting a higher quality of yellow-seeded rapeseed genotypes has been presented by Jia et al. (2012), Slominski et al (2012) and reviewed by Khajali and Slominski (2012). The meal from yellow-seeded *B. napus* had higher protein (50 vs 46% DM) and lower fibre (24 vs 27%) than black-seeded types. When fed to birds yellow-seeded types had higher AMEn contents; 9.17 vs 7.97 MJ/kg in broilers and 9.07 vs 8.40 MJ/kg in turkeys, compared to black seeded types (Jia et al., 2012). Recent data from France (Quinsac et al., 2013) reports low levels of protein overall in French winter OSR varieties than in Canadian spring types: There were lower protein levels in the meal and smaller difference in protein content between black and yellow seeded types of winter OSR (assume *B. napus*; 37 vs 41%), and lower levels of protein in the meal of black and yellow seed Canadian spring rape types tested in France (both seed colour types 44% protein). None of the samples in Quinsac's study demonstrated protein contents in rape meal as high as the 50% reported by Slominski *et al*., (2012). Nevertheless, differences in energy content (AMEn, broilers) were noted between rape meal of black and yellow seeded types as summarised in Table 1.

Quinsac *et al*., 2013 note that introducing the yellow seed coat is almost equivalent to the de-hulling effect (for data on effects of de-hulling, see Annex). We have no data for nutritional characteristics of yellow seed rape meal fed to pigs, but Bell (1993) reported that DE of rape meal for pigs increased from 14 to 16.5 MJ/kg by de-hulling. Therefore, in simple terms we might assume that this would be the upper limit achievable to improve the energy content for pigs, and this is used for the economic illustrations in Chapter 6.

Table 1. Meal composition and nutritional value for poultry for different rapeseed types (*B. napus*) tested in France by Quinsac *et al*. (2013), and in Canada by Slominski *et al*. (2012) and Jia *et al*. (2012).

Study	**France**				**Canada**	
Country of seed origin	France	France-INRA	Canada-AARFC	Canada-AARFC	Canada-AARFC	Canada-AARFC
Phenotype (coat colour)	Black	Yellow	Black N89-53 *	Yellow YN01-0429*¶	Black n=1†	Yellow YN01-0429†¶
Analyte (DM basis)						
Gross energy (kcal/kg)	4,569	4,532	4,766	4,728	-	-
Gross energy (MJ/kg)	19.1	19.0	20.0	19.8		
Protein (%)	37.1	40.6	44.1	44.1	43.8	49.8
Fat (%DM)	1.16	1.38	1.92	2.43	1.80	1.60
Ash (%DM)	7.56	8.36	6.70	6.57	7.30	7.00
Glucosinolate (umol/g)	43.1	83.7	34.4	20.0	27.1	17.1
Cell walls (%)	37.2	31.6	31.1	28.8	-	-
Dietary fibre (%)	-	-	-	-	30.1	24.1
Crude fibre (%)	15.4	10.6	16.0	11.0	-	-
NDF (%)	30.8	20.2	27.6	21.0	-	-
ADF (%)	23.2	12.6	20.5	12.5	-	-
ADL (%)	11.1	3.0	10.8	3.3	-	-
NDF-ADF (%)	7.6	7.6	7.1	8.5	-	-
ADF-ADL (%)	12.2	9.6	9.7	9.2	-	-
AMEn (kcal/kg)	2,007	2,204	2,377	2,418	1,904	2,190
AMEn (MJ/kg)	8.40	9.23	9.95	10.12	7.97	9.17
N digestibility (%)	76.9	84.2	77.1	76.2	-	-

*, for full data set for Quinsac *et al*., see Annex

¶, note same yellow seed *B. napus* variety used in both French and Canadian studies

†, no of observations for proximate analysis 2-4 dependent on analyte of interest. For nutritional study AMEn, one variety tested for each seed type reported

3.8.2. Other aspects of seed coat thickness

Yellow-seeds have more oil in part because their seed coats are thinner and their embryos are larger (Abraham and Bhatia, 1986). In addition to the positive effect on animal feed quality, this also means that it is a potential breeding target, to increase oil content. On the negative side, seeds with thinner seed coats suffer more damage during combining, and are thought to be less resistant to plant pathogens in the field (although it has been noted that this could also be an advantage as it should reduce the tendency of the crop to produce volunteers in following crops).

An alternative approach to changing the thickness of the testa is to reduce the amount of mucilage in the seed. Rerie et al. (1994) reported that the *GLABRA2* (*GL2*) which modifies trichome, root hair development and oil production in *Arabidopsis* also affected seed mucilage production. This holds the possibility therefore that an increase in oil content could be accommodated by reducing mucilage production in the seed, while maintaining the integrity of the seed coat. Research work has continued at Pioneer HiBred and at research institutes in China, but the results regarding reductions in mucilage content are inconclusive and have not been applied to commercial species (Shen et al., 2006; Chai et al., 2010). More research work is needed in this area, and if rapid screens were available, it may be worth looking for variation in mucilage levels in seed samples contained in public germplasm collections.

3.9. UK Breeders views

The breeders consulted indicated that breeding for yellow-seeded types had been tried in the 1990's. This gave higher oil and better digestibility of the meal, but the disadvantages (poor establishment and high level of harvest damage) outweighed the advantages. The general feeling was that the genetics of breeding for seed colour was complex (double recessive trait) and difficult to achieve. It was noted that allegedly yellow-seeded types in fact were often a mix of yellow and black seeds, and that the trait was not thought to be wholly stable between generations. In *Brassica* species, the maternal genotype mainly controls seed coat colour. However, interplay between the maternal parent and the endosperm and/or embryonic genotype may also affect seed coat colour (Chen and Heneen 1992; Rahman *et al*. 2001). One paper (Gustafson et al, 2007) noted that there were both common and separate QTLs for seed coat colour and fibre content. To some extent, lower seed coat % and hence lower fibre could be achieved by breeding for larger seeds, but other factors influence seed size, for instance the type of breeding system used for hybrid production, making this more complicated.

It was also recognised that yellow-seeded types could be difficult to harvest, with significant damage to the seed occurring, and sometimes appeared to generate "meal rather than seeds coming out of the combine". All the breeders consulted thought that thinner seed coats/lower fibre were attractive, and would be interested in research projects in this area, but all the work in the literature suggests that this trait is not easily separated from the yellow-seed character, which is associated with the problems noted above. The research into *GLABRA2* mutants and seed mucilage production in *Arabidopsis* mentioned above may be relevant in this context.

An interest was expressed in breeding for lower tannin content, but nothing had been done on this point. It was assumed that at present, crushers won't pay for subtle changes in seed quality and therefore there is no advantage in selecting for such traits. In any case, it is likely that the main route to reducing tannins will be through selecting for yellow seed coats, and as noted above, this is not an attractive route at present. Even in the case of glucosinolates, crushers can take high GSL batches, and blend with low GSL batches to produce meal of acceptable quality, so there is little advantage in pushing for very low GSL levels, when yield and oil content are key. Moreover, pushing further for lower GSL contents will only slow progress in yield improvement. There was little interest in breeding for lower sinapine levels.

4. Natural variation in oilseed rape germplasm in UK collections and mapping populations

4.1. Elite varieties: LINK Project LK0979

LINK Project LK0979 ‘Breeding oilseed rape with a low requirement for N fertiliser’ measured seed quality traits on 29 elite varieties grown in six experiments during 2006/7 and 2007/8. The varieties included varieties currently on the HGCA Recommended List (RL), varieties which have recently been dropped from the RL (but entered the RL after double low varieties were introduced), an old variety introduced into the RL in 1987 (Tapidor) and National List germplasm. Varieties included a mixture of hybrid (H) and open pollinated varieties: Borneo (H), Canberra, Canti, Castille, Catalina, Disco (H), ES Betty (H), Escort, Excalibur (H), Excel (H), FD502, Grizzly, Lioness, Mendel (H), Mohican, NK Bravour, NK Grace, NSL/04/120, Ontario, Recital, RG2509, RNX3504 (H), Royal (H), Tapidor (H), Temple and Winner.

There was a wide range of glucosinolate values between the varieties from 11.7 umol/g (NK Bravour) to 26.5 µmol/g (ES Betty) (Table 2). The range of values from the 10^{th} to 90^{th} percentile was only slightly smaller at 12.5 to 22.3 µmol/g. The ranges of the oil content and protein content were smaller at 47.6% to 51.1% and 17.5% to 19.8%, respectively (Table 1). The average values for the hybrid and open pollinated varieties were similar for oil content, protein content and yield, but different for glucosinolates for which the hybrid varieties averaged 18.6 µmol/g compared with 15.1 µmol/g for the open pollinated varieties. Low glucosinolates were correlated with greater oil content, and there was a negative correlation between oil content and protein content (Table 3). Neither glucosinolates, oil nor protein were correlated with date of flowering, date of maturity or plant height at flowering.

Table 2. Summary of data for LK0979 elite variety dataset (mean of 6 experiments)

	Glucosinolate (µmol/g)	Oil (% seed dm)	Protein (% seed dm)	Seed yield (t/ha @ 91% dm)	Oil yield (t/ha @ 100% dm)
Mean	16.5	48.8	18.4	4.25	1.90
Minimum	11.7	47.6	17.5	3.51	1.57
Maximum	26.5	51.1	19.8	4.64	2.11
10 percentile	12.5	47.8	17.8	3.90	1.76
90 percentile	22.3	49.6	19.0	4.55	2.06
Sed (28 df)	0.49	0.27	0.27	0.099	0.047

Table 3. Correlation coefficient for LK0979 elite variety dataset (mean of 6 experiments). Figures in red indicate significant (P<0.05) correlations.

		1	2	3	4	5
1	Glucosinolate (µmol/g)	1.00				
2	Oil (% seed dry matter)	-0.40	1.00			
3	Protein (% seed dry matter)	0.15	-0.45	1.00		
4	Seed yield (t/ha)	0.07	0.16	-0.17	1.00	
5	Oil yield (t/ha)	-0.03	0.41	-0.29	0.96	1.00

4.2. Elite varieties: Plant breeder dataset

Seed quality data collected from 61 UK varieties grown in 2011/12 which are either recommended or close to recommendation are summarised in Tables 4 and 5. Glucosinolate levels ranged from 7.3 µmol/g to 16.9 µmol/g and oil content ranged from 40.6% to 45.2%. Glucosinolate levels were negatively correlated with oil content (Table 5). There was also a positive correlation between plant height at flowering and glucosinolate levels (r = 0.41). Neither glucosinolate nor oil content were correlated with date of flowering or date of maturity.

Table 4. Summary of data for plant breeder elite variety dataset (1 experiment)

	Glucosinolate (µmol/g)	Oil (% seed @ 91%dm)	Seed yield (t/ha @ 91% dm)	Oil yield (t/ha @ 91% dm)
Mean	12.55	43.0	4.03	4.22
Minimum	7.30	40.6	3.46	3.50
Maximum	16.90	45.2	4.86	5.09
10 percentile	9.62	41.9	3.77	3.93
90 percentile	16.09	44.1	4.31	4.49
LSD	1.35	0.72	0.256	0.285

Table 5. Correlation coefficient for plant breeder elite variety dataset (1 experiment). Figures in red indicate significant (P<0.05) correlations.

		1	2	3	4
1	Glucosinolate (µmol/g)	1.00			
2	Oil (% seed dry matter)	-0.60	1.00		
3	Seed yield (t/ha)	-0.22	0.29	1.00	
4	Oil yield (t/ha)	-0.32	0.46	0.98	1.00

4.3. Mapping populations: Tapidor x Victor substitution lines

A population of 75 Tapidor x Victor substitution lines were grown in 3 experiments during the 2008/9 and 2009/10 seasons in LINK project LK0979. Each substitution line was predominantly variety Tapidor with part of one chromosome substituted by the corresponding piece of chromosome from variety Victor. There was a very wide range of glucosinolate levels from 11.2 µmol/g to 57.8 µmol/g, although the 90th percentile value was only 23.2 umol/g (Table 6). The range of oil and protein contents were moderate at 45.1 to 48.9% for oil content and 20.6% to 22.3% for protein content. Glucosinolates were negatively correlated with oil content and positively correlated with protein content (Table 7). There was a strong negative correlation between oil content and protein content, and there was a negative correlation between protein content and seed yield. There were no strong correlations between glucosinolates, oil content or protein content with plant height, date of flowering and date of maturity.

Table 6. Summary of data for LK0979 Tapidor x Victor substitution line dataset (mean of 3 experiments).

	Glucosinolate (µmol/g)	Oil (% seed dm)	Protein (% seed dm)	Seed yield (t/ha @ 91% dm)	Oil yield (t/ha @ 100% dm)
Mean	17.6	46.8	21.3	3.73	1.64
Minimum	11.2	45.1	20.6	3.18	1.32
Maximum	57.9	48.9	22.3	4.42	1.97
10 percentile	13.9	46.3	20.9	3.44	1.49
90 percentile	23.2	47.4	21.7	4.04	1.79

Table 7. Correlation coefficient for LK0979 elite variety dataset (mean of 3 experiments). Figures in red indicate significant (P<0.05) correlations.

		1	2	3	4	5
1	Glucosinolate (µmol/g)	1.00				
2	Oil (% seed dry matter)	-0.29	1.00			
3	Protein (% seed dry matter)	0.40	-0.68	1.00		
4	Seed yield (t/ha)	-0.08	0.28	-0.41	1.00	
5	Oil yield (t/ha)	-0.14	0.43	-0.51	0.96	1.00

4.4. Mapping populations: Rocket x Capitol doubled haploid population

A population of 86 Rocket x Capitol doubled haploid (DH) lines were grown in 4 experiments during the 2008/9 and 2009/10 seasons within LINK project LK0979. There were a wide range of glucosinolate levels from 10.8 µmol/g to 19.2 µmol/g (Table 8). The range of oil and protein contents were moderate at 45.9 to 50.3% for oil content and 17.6% to 20.9% for protein content. Glucosinolates were negatively correlated with seed yield and oil yield and positively correlated with protein content (Table 9). There was a strong negative correlation between oil content and protein content, and there was a negative correlation between protein content and seed yield.

Table 8. Summary of data for LK0979 Rocket x Capitol doubled haploid line dataset (mean of 4 experiments).

	Glucosinolate (µmol/g)	Oil (% seed dm)	Protein (% seed dm)	Seed yield (t/ha @ 91% dm)	Oil yield (t/ha @ 100% dm)
Mean	14.8	48.3	19.1	3.09	1.33
Minimum	10.8	45.9	17.6	1.93	0.87
Maximum	19.8	50.3	20.9	3.95	1.70
10 percentile	12.6	47.3	18.4	2.58	1.13
90 percentile	17.2	49.3	19.9	3.57	1.53

Table 9. Correlation coefficient for LK0979 elite variety dataset (mean of 4 experiments). Figures in red indicate significant ($P<0.05$) correlations.

		1	2	3	4	5
1	Glucosinolate (µmol/g)	1.00				
2	Oil (% seed dry matter)	-0.25	1.00			
3	Protein (% seed dry matter)	0.56	-0.58	1.00		
4	Seed yield (t/ha)	-0.49	0.27	-0.57	1.00	
5	Oil yield (t/ha)	-0.40	0.29	-0.52	0.89	1.00

4.5. Non-elite *Brassica napus* germplasm: John Innes Centre diversity panels

Brassica napus diversity panels were grown at the John Innes Centre, Norwich in 2009/10 (60 accessions) and in 2012 (105 accessions). These included a very wide range of *B. napus* germplasm ranging from elite UK varieties such as DK Cabernet, older UK varieties such as Apex (introduced in 1993), and non UK germplasm such as the Chinese spring variety Ningyou 7. In 2010 only oil and protein concentrations were measured, with glucosinolate measurement added in 2012.

There were very wide ranges of oil and protein contents (Tables 10, 11) in both seasons and glucosinolate contents in 2012 (Table 11). This was probably predominantly due to the wide range of germplasm in these panels, although the panel grown in 2010, despite containing fewer accessions, showed a greater range of oil contents (33.6% to 54.6%) than the 2012 experiment (27.1% to 44.8%). There were strong negative correlations between oil content and protein content in both seasons ($r = -0.75$ in 2010; $r = -0.57$ in 2012). However, in contrast to the previous datasets there was a positive correlation between glucosinolate content and oil content (Figure 1; $r = 0.52$) and a negative correlation between glucosinolate content and protein content ($r = -0.55$) in 2012.

Table 10. Summary of data for *Brassica napus* diversity panel 2010 (1 experiment).

	Glucosinolate (µmol/g)	Oil (% seed dm)	Protein (% seed dm)
Mean	-	46.4	21.9
Minimum	-	33.6	15.8
Maximum	-	54.6	29.1
10 percentile	-	42.3	18.5
90 percentile	-	51.4	26.6

Table 11. Summary of data for *Brassica napus* diversity panel 2012 (1 experiment).

	Glucosinolate (µmol/g)	Oil (% seed dm)	Protein (% seed dm)
Mean	89.3	39.4	17.8
Minimum	14.8	27.1	9.0
Maximum	175.7	44.8	26.9
10 percentile	24.2	35.4	12.4
90 percentile	141.4	43.7	22.7

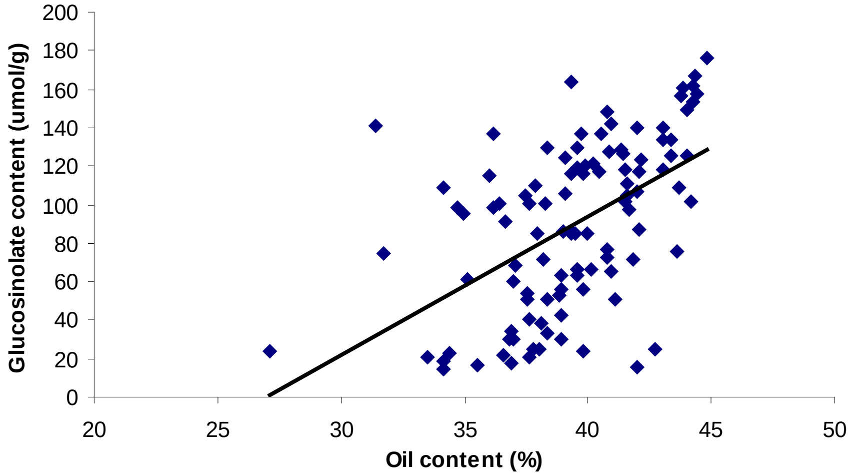

Figure 1. Relationship between Glucosinolate content (µmol/g) and oil content (%) in *Brassica napus* diversity panel 2012.

4.6. Non-elite *Brassica napus* germplasm: OREGIN Diversity Fixed Foundation Set

The OREGIN project (Defra project IF0144) developed a collection of fixed lines that represents the diversity within *Brassica napus* (Diversity Fixed Foundation Set; DFFS). This set of lines was tested for oil and protein contents in 2007. The data presented below are predictions (Best Linear Unbiased Estimates; BLUE) generated using REML (Residual Maximum Likelihood) analysis using accession as fixed effects.

The average BLUE of oil contents for this set of lines was low (38.4%) but had a large range (38.4% to 46.5%; Table 12). The average protein content was relatively high (24.6%) and there was a significant negative correlation between the oil and protein contents (r = -0.71).

Table 12. Summary of data (Best Linear Unbiased Predictions) for OREGIN DFFS.

	Glucosinolate (µmol/g)	Oil (% seed dm)	Protein (% seed dm)
Mean	-	38.4	24.6
Minimum	-	29.9	16.9
Maximum	-	46.5	30.2
10 percentile	-	34.2	21.1
90 percentile	-	42.3	27.8

4.7. Natural genetic variation: Summary

There were no data in any of the datasets studied on seed coat thickness, hull percentage, coat colour or levels of phytate, tannins, lignin, fibre or polyphenols. As would be expected, oil and protein contents of elite varieties and mapping populations were similar within experiments with ranges of less than 5%, much less variable than the non-elite germplasm. This was generally true of the glucosinolate data also (range usually <15 µmol/g). The range found in the Tapidor x Victor substitution lines was larger at 47 µmol/g, although this was still a lot less than the range found in the John Innes Centre 2012 data (161 µmol/g). In all the elite variety and mapping population experiments, there were significant negative relationships between glucosinolate content and oil content. This may simply be reflecting the fact that commercial breeders have been successful in selecting for both high oil and low GSL, rather than a genetic linkage between the traits. In contrast, in the *Brassica napus* diversity panel grown in 2012 the relationship between glucosinolate and oil was positive and, although very scattered data, was highly significant ($P<0.001$). This showed the influence of the non-elite germplasm.

5. GM approaches to modifying candidate traits

The value of rapeseed for food and feed uses can be further improved by increasing desirable traits, e.g. the oil content and by reducing undesired characteristics like the fibre content or anti-nutritional compounds. Alternatives for industrial processing and non-food purposes are, for example, high-erucic acid, low-glucosinolate rapeseed varieties or laurate Canola.

5.1. Fibre

Reducing the fibre content of rapeseed by breeding or processing could increase its dietary energy value and lead to greater inclusion levels in feed as discussed above. However polysaccharides are secondary plant products, and have complex genetic pathways, involving glycosyl transferases. There have been no instances of GM modification of non-starch polysaccharides or lignin in OSR as far as we are aware, and few in other plant species.

5.2. Starch

There is a large knowledge about the modification of enzymes controlling starch synthesis in plants. However given the low levels of starch in the seed and the greater importance of oil content economically, it is highly unlikely that a breeder would try and modify this trait by a GM approach.

5.3. Oil

There have been a number of approaches proposed to modify specific fatty acids, i.e. impacting on oil quality, as can be seen in six entries in Table 13 below. These are mainly traits to improve cooking/processing properties like high temperature stability, and have not been considered in the context of their nutritional value and essential fatty acids. However no commercial examples of modifying oil content *per se* were picked up in literature searches.

There is little starch in the seed as discussed elsewhere, so to increase oil content further would only mean reducing some of the other sinks for carbon (hull, cotyledon cell walls and to a lesser extent the carbon skeleton of amino acids), or conversely just increasing the amount of carbon which is partitioned to the seed during pod filling. Recent work by Shen et al. (2006) and Chai et al. (2010) have attempted to generate *GLABRA2* (*GL2*) mutants with reduced seed mucilage and high oil in *Arabidopsis*. In some lines it was possible to increase seed oil content by 3.5-5% but the results were inconclusive regarding any parallel changes in seed mucilage content. Therefore it is not clear whether there were any other effects on seed morphology or physiology, which would affect the practical introduction of this trait into commercial Brassica species.

5.4. Phytate level and phosphorus availability

Engineering the plant to express a phytase enzyme is an approach which has been attempted by including a fungal phytase (BASF, Table 13). Another approach would be to reduce levels of phytate in the seed, although this modification has not been reported. However given the wide availability of exogenous phytase enzymes for addition to animal feed, and the fact that rape and rape meal are now considered to be no worse than other key ingredients in terms of their phytate P content, it would be difficult to justify breeding for this as an ‘added value’ trait unless there were some other benefit, for instance addressing a legislative driver relating to environmental (P) pollution.

5.5. Glucosinolate level and myrosinase activity

There has been huge progress in reducing GSL content in OSR varieties by UK plant breeders. Fenwick et al. (1983) considered it unlikely that the complete removal of GSLs would be a major goal for plant breeders and future improvements of the crops would be mainly associated with increasing content of oil, reducing seed coat thickness and introducing light coloured seed. Thus to take another approach to reduce GSL may be expensive and unnecessary. In the survey carried out, there was little evidence of activity in trying to reduce GSL content by a GM approach.

5.6. Other seed components

There do not appear to have been any attempts to modify protein, seed coat colour, tannins, or glucosinolate levels using GM approaches. There has been one event reported looking at reducing sinapine (UPD-glucose:sinapate glucosyl transferase; Table 13). A number of other approaches to reducing sinapine have been reported in the scientific literature including suppression of the gene coding for ferulic acid hydroxylase (Nair et al., 1999), over-expression of sinapine esterase (Clauss et al., 2011), silencing of the genes coding for1-O-sinapyl-β-glucose:choline sinapoyl transferase (Weier et al., 2008), and suppression of genes coding for UDP-glucose:sinapate glucosyl transferase (Hüsken et al., 2005).

Table 13. Reported examples of commercial applications for output (quality) trait genetic modifications in oilseed rape (*B. napus*).

Genes added or modified	Effect	Event Name	Breeder	Approval Status	Source
Inserting 12:0 ACP Thioesterase encoding gene from California bay laurel (*Umberllularia californica*)	High laurate and myristate	23-18-17, 23-198	Monsanto	Short/medium term. Risk assessed in Canada for environmental, food and feed (1996) and the USA for environmental and food and/or feed (1994)	CERA
Fatty Acid Desaturase mutant	Modified seed fatty acid content (high oleic acid, low linolenic acid)	45A37, 46A40	Pioneer Hi-Bred	Short/medium term	CERA
Chemical mutagenesis of FAD2 gene	Modified seed fatty acid content (high oliec acid)	46A12, 46A16	Pioneer Hi-Bred	Short/medium term. Risk assessed in Canada for food (1996)	CERA
phyA to produce a fungal 3-phytase	Increased breakdown of plant phytates which bind phosphorous - improved phosphorous availability	Multiple	BASF	Long term. Risk assessed in the USA for import/use (2004)	BCH
Acyl-acyl carrier thioesterase (ClFatB4)	Modifed fatty acid content	NBM99-ClFatB4	BAZ, Institut für landwirtschaftliche Kulturen	Long term. Risk assessed Germany, environmental (2010)	BCH
Stilbene synthase	Synthesis of antioxidant/flavonoids - resveratrol (antifungal agent)	pPSty5	FINAB	Long term. Risk assessed Germany, environmental (2011)	BCH
UPD-glucose:sinapate glucosyltransferase	Reduced concentration of sinapine	pLH-BnSGT-GUS	FINAB	Long term. Risk assessed Germany, environmental (2011)	BCH
Thioesterase (*Ulbellularia californica*), neomycin phosphotransferase (*E. coli*)	Modified seed fatty acid content (high laurate levels and myristic acid production)	23-18-17, 23-198	Monsanto	USA (1994)	CERA
Fatty acid desaturase FAD*2*	Modified seed fatty acid content (high oleic acid, low linolenic acid)	45A37, 46A40, 46A12, 46A16	Pioneer Hi-Bred	Canada (1996)	CERA

6. Potential to improve seed quality

6.1. Summary of the ideal rape seed and rape meal for the feed industry

Feed quality depends on both the quality of the rape seed where it is fed whole, and the quality of the meal after oil extraction. From the previous three chapters, it is clear that if plant breeders are to further improve OSR seed quality beyond high oil, low GSL and low erucic acid content, then from an animal feed perspective, the key traits are likely to be:

- High protein
- Low tannins and/or low polyphenols
- Low lignin and fibres, with a parallel increase in protein content

The following traits would also be beneficial although there is little indication at present that they would be financially valuable to the industry:

- Low phytate (or high phytase possibly via GM)
- Low sinapine (possibly via GM)

The first three traits should be addressed by selecting for seed coat thickness and colour traits i.e. thin seed coat, yellow seed-types, while at the same time selecting positively for oil and protein content. This approach has been modelled in Section 6.2.

6.1.1. Rapeseed

The basic parameters for conventional rapeseed, commercially available at the present time, would be crude protein 18.5% and Oil B[1] at 46% (both on a 93% DM basis). Since high protein and high oil contents, to supply amino acids and energy are desirable, particularly in whole rapeseed fed to broilers, the 'improved' rapeseed would have 21.1% crude protein (90^{th} percentile from JIC 2012 dataset; Table 11 corrected to 93% DM) and 47.4% oil B (90^{th} percentile from JIC 2010 data set; Table 10 corrected to 93% DM). Thus the increase in total oil+protein in improved rapeseed would be +4 %.

An oil content of 47.4% in the improved line, while high compared against current commercial seed standards, is often seen in nil-N plots in N yield response experiments therefore is believed to be a realistic target. The protein content of 21.1% of the improved line is somewhat lower than that seen in two of the datasets reported here based on the 90^{th} percentile: 24.7% in JIC 2010 (Table 10), and 25.9% in OREGIN data (Table 12); data adjusted to 93%DM. However such high values might

[1] Oil B is a measure of oil content in a feed using 'EU procedure B': In animal feed testing, oil (or 'crude fat') is extracted by the EU procedure A with petroleum ether (40-60 ºC) and the dried residue weighed, whereas in EU procedure B acid hydrolysis is used as a pre-treatment prior to extraction.

be unrealistic because in the JIC 2010 study, the agronomic conditions are believed to be somewhat extreme, and in the OREGIN dataset, there is a very wide range of genetic material, much of it far removed from elite lines of commercial *B. napus*.

It should be noted that the earlier analysis suggested that in the seed of elite lines, protein and oil content are inversely related. However, the inverse relationship is only moderately strong and it is clear there is scope to maximise both oil and protein together. Additionally, it appears that in some rape breeding programmes, e.g. in France, oil+protein is actually a current target. Moreover, by assuming that total oil+protein can be increased, this sets an upper limit on the value of those seed traits for animal feed. However it is a given that all breeders select for oil content (or oil yield) primarily, and this is very unlikely to change.

6.1.2. Rape meal

If such an improved rape seed was extracted with typical efficiently by commercial processors, leaving 3.6% oil in the rape meal (91% DM basis), then the predicted protein content would be 33.8% in the current commercial meal, and 39% in the improved meal, and hence an increase in oil+protein of +5.2%. These values may appear conservative compared with the upper limits of 40.0% at 91% DM (44% protein at 100% DM) reported by Quinsac *et al*. (2013), and 45.5% at 91% DM (50% at 100% DM) reported by Slominski *et al*. (2012). However Quinsac and Slominski used Canadian varieties which are believed to differ from European types, and also extracted more oil from the meal (1.1% residual oil rather than 3.6% in UK commercial rape meal on a 91% DM basis). The changes to improved rape meal would therefore be as follows (values on 91%DM basis):

- Protein content in meal increased from 33.5 to 39%[1] with no net change in amino acid composition,
- Residual oil content of the meal fixed at 3.6%,
- The higher protein effectively compensates for reduced fibre; total fibre decreased from 38 to 32.5%, of which NDF decreased from 30 to 25.5% and CF decreased from 10 to 4%[2],
- Lignin and polyphenols decreased from 7.7 to 4.0%[1],
- Sinapine reduced to zero,
- GSL low compared as for current elite varieties at <12 μmol/g.

If all these traits could be improved, an economic value placed on such an improvement based on an assessment of using ‘new’ rapeseed and ‘new’ rape meal product through a least cost ration formulation approach. This sets a boundary on the value of aiming for such a trait combination by

[2] Based on values for black vs yellow *B. napus* rape meal in Quinsac et al. (2013)

breeders, which can be balanced against the extra cost of selecting for these traits in a commercial breeding programme.

6.2. Feed formulation exercise

6.2.1. Poultry formulation exercise

The potential exists for enhanced characteristics in selected rapeseed cultivars to be expressed through breeding programmes. These enhanced characteristics will potentially improve the nutritional value of the primary seed and of protein meals remaining after oil extraction.

To estimate financial values for the estimated higher feeding value a number of formulation exercises were conducted comparing existing rapeseed and rapeseed meal with the enhanced "New" outputs that could be developed. Diets for broiler chickens and laying hens were considered, with the following nutritional values for existing and new raw materials (Table 14)

Table 14. Nutritional values for existing and postulated rape products used in the formulation exercise.

	Units	**Existing Whole Rapeseed EU 2013**	**Existing Rapeseed Meal EU 2013**	**New Whole Rapeseed**	**New Rapeseed Meal**
Dry Matter	%	93.0	91.0	93.0	91.0
Oil A[1]	%	44.0	2.5	45.4	2.3
Oil B[2]	%	46.0	3.6	47.4	3.6
Protein	%	18.5	33.5	21.1	39.0
C.Fibre	%	8.0	13.0	6.0	10.4
Ash	%	5.0	6.5	5.0	6.5
Energy[3]	MJ/kg	19.20	7.0	20.0	7.45
Digestible Lysine	%	0.89	1.36	1.02	1.58
Digestible Methionine	%	0.32	0.57	0.37	0.66

[1] ether extract oil

[2] acid hydrolysis before ether extraction

[3] Poultry metabolisable energy values based on Premier Nutrition database (see text for further explanation)

Values for existing seed and meal compiled from analysis of materials delivered into UK feedmills during 2013, some material derived from imported seed and/or meal. Full amino acid profiles for the "new" materials are based on g amino acid/g protein and therefore are proportionally the same. Metabolisable energy (ME) of the improved products for poultry was estimated using the equation

$$ME_{Rapeseed} = 12.62 \times \text{protein} + 38.05 \times \text{OilA} + 3.81 \times \text{Nitrogen free extract}$$

$$ME_{RSMl} = 13.71 \times \text{protein} + 27.18 \times \text{OilA} + 5.543 \times \text{Nitrogen free extract}$$

The difference (improvement) in estimated ME between existing and the "new" products using these equations was then added to the standard ME value in the Premier Nutrition Database. It should be noted that the nutritional values assessed in the poultry formulations below are based only on changes in the nutrients and energy defined in Table 14. No further improvements have been assumed based on reductions in tannins, lignin or GSL. It has been assumed that in the long term these will improve the confidence in using OSR seed and meal and may encourage higher inclusions, but this is difficult to quantify at present.

Broiler diet evaluations

Raw materials costs relevant to the period December 2013 were used for all materials included in the initial formulation, rapeseed was priced at £345/t, rapeseed meal £210/t. Wheat, soya bean meal (48% protein) and maize were priced at £166/t, £370/t and £172/t respectively.

The inclusions of rapeseed and rapeseed meal in diets are constrained for reasons of pellet physical quality and existing knowledge on anti-nutritive factors. These constraints are apparent in Table 15. Four diets were used; a starter, grower, finisher and withdrawal (WDL) diet which are close to current commercial practice. The diets are based on the Aviagen guidelines for the Ross 308 broiler. The vitamin and mineral premix was used to convey both NSP and phytase enzymes and therefore has nutrient values allocated to it, to reflect the conservative contributions expected for these additions as per commercial practice.

It should be borne in mind that typically a broiler starter feed comprises less than 10% of total feed consumption; it is a high protein feed and in the absence of fish meal, soya levels tend to be constrained for reasons of litter quality. Alternative protein sources tend to have disproportionately higher values in the starter ration. Some of the values determined for rape products in the starter feeds should be considered in that light. The exercise for broilers consists of 5 scenarios

Broiler scenario 1

Table 15 shows the baseline set of formulations using current costs and raw materials, and sets out the raw material cost for the four diets ('Diet cost – baseline 1'). As can be seen, the current whole rapeseed is selected by the computer formulation software to be included at maximum levels, whilst the rapeseed meal only features in the starter diet, but at the maximal level.

The formulation software was then used to evaluate the price at which the current rapeseed meal would feature in the 3 diets that it did not appear in, and the % inclusion that would occur at these prices.

Table 15. Baseline formulations using prices for existing rapeseed of £345/t, and rapemeal at £210/t.

Diet:		Broiler Starter	Broiler Grower	Broiler Finisher	Broiler WDL
	Raw material price (£/t)	Inclusion level or diet cost			
Baseline formulations					
Existing rapeseed[1] (%)	*345*	4	5	6	8
Existing rapemeal[2] (%)	*210*	2	0	0	0
Diet Cost – baseline 1 (£/t)		278.63	270.34	255.47	252.17
Broiler scenario 1					
Lower cost rapemeal (£/t)			181.89	181.89	199.53
New inclusion level of existing rapemeal (%)			0.07	4	0.36

[1], Rapeseed EU 2013, whole, 00 18.5%/44%P/O

[2], Rapeseed EU 2013, ext, 00

Broiler scenario 2 – New (improved) rapeseed, used with existing rapemeal

This scenario assumes that the whole seed is so attractive for inclusion in the diet such that none would go for crushing, and the only rapemeal which remains on the market is from the existing varieties, which as noted earlier, only feature in starter diets.

New rapeseed was therefore offered alongside existing rapeseed meal (Table 16). For each diet, the cost of the new rape seed was adjusted so that the final formulated cost was equal to that of the base line formulations in Table 15. In this scenario, the formulation software is effectively calculating what the maximum price of the new seed could be under current cost conditions, such that it features significantly without adding cost to the diets.

The increase in value over current rapeseed is estimated at between £32.50 and £34.50/tonne. There seems to be a higher value in the starter feed of +£40/tonne but this is exaggerated by the fact that soya bean meal in the starter ration is constrained to a maximum of 32% for reasons of controlling wet litter. For similar reasons as in the baseline formulations, current rapeseed meal has featured only in the starter diet, where it has a higher value, because soya bean meal is restricted in this diet.

Table 16. Valuation of new (improved) rapeseed, estimated by offering the product alongside existing rape meal in a least cost ration formulation while matching the baseline diet price.

Diet:		**Broiler Starter**	**Broiler Grower**	**Broiler Finisher**	**Broiler WDL**
	Raw material price (£/t)	Inclusion level or seed cost			
Broiler scenario 2					
Existing rapemeal[1] (%)	*210*	2	0	0	0
New whole rapeseed (%)		4	5	6	8
Price of new whole seed (£/t) to match base formulation cost in Table 15		385.00	377.50	378.50	379.50

[1], Rapeseed EU 2013, ext, 00

Broiler scenario 3 – Evaluation of existing rapemeal used alone

This exercise acts as a base line for evaluation of new rapeseed meal. Whole seed is not offered and higher levels of current meal are made available. In this situation more rapemeal was taken in the starter feed as a soya substitute, nothing in the grower diet, 1.1% in the finisher and 4.17% in the withdrawal feed. The diet costs were increased over those determined in baseline by £1.46, £1.18, £1.69 and £2.90/t. These increases are effectively a reflection of the value of whole seed which was excluded.

Table 17. Valuation of existing rapeseed meal, estimated by offering the product alone (no whole seed) in a least cost ration formulation.

Diet:		**Broiler Starter**	**Broiler Grower**	**Broiler Finisher**	**Broiler WDL**
	Raw material price (£/t)	Inclusion level or diet cost			
Broiler scenario 3					
Existing rapemeal[1] (%)	*210*	4	0	1.1	4.17
Diet Cost – baseline 2 (£/t)		280.15	271.52	257.16	255.06

[1], Rapeseed EU 2013, ext, 00

Broiler scenario 4 – Evaluation of new rapemeal used alone

Following on from Scenario 3 the new (improved) rape meal was offered to each diet at prices so that the total diet cost was equal to that achieved in Scenario 3. The value of the new rape meal varied between £225 and £263 and at the prices used, slightly more was taken by the formulation than was for the existing rape meal (Table 18).

Adjusting the prices downwards to achieve maximum inclusion levels suggested the mean value in this range of diets is somewhere in the region of £222 per tonne (ignoring the higher value in the starter diet). At these lower prices and higher inclusions diet cost is reduced below that for Scenario 3 (baseline 2) but are not equal to the baseline 1 formulations.

Table 18. Valuation of new (improved) rapeseed meal, estimated by offering the product alone (no whole seed) in a least cost ration formulation.

Diet:	Broiler Starter	Broiler Grower	Broiler Finisher	Broiler WDL
	Inclusion level or ingredient/diet cost			
Broiler scenario 4				
New Rapemeal (%)	3.8	0.144	0.9	3.36
Price of meal to match Scenario 3 diet cost (£/t)	263	225	255	258
Price of meal to maximise inclusion level (£/t)	260.5	221	222.5	222.5
Ref above, inclusion level (%)	4	5	6	8
Diet cost (£/t)	280.05	271.49	256.68	253.71

Broiler scenario 5 – Evaluation of new whole seed and new rapemeal

It was assumed that in the event of a new (improved) variety of rapeseed being available which has a higher oil content it might be more attractive to the crushers, such that its availability for feed manufacture became restricted. However in this scenario, new (improved) higher protein meal might also be more available. Hence formulations were run with lower new whole seed maxima, but higher meal inclusions allowed. On this occasion (Table 19), prices for the materials were first taken from previous scenarios such that seed was priced at £377.5 and meal at £225 (lowest costs from Scenarios 2 and 4 for the respective materials to feature).

Whole seed was selected at maximum values whilst meal featured only in the starter and withdrawal diets. Other than the starter diet, the remaining diet costs were higher for the later phase diets, compared to scenario 1, by £0.58 to £0.75/tonne of feed.

This scenario then shows the cost at which the rape meal would feature in the diets at maximum levels where it is currently not taken (bottom two rows in Table 19). These apply to both the grower and finisher diets, and a slight decrease in cost to £221.60 or £222.80 (compared to the applied cost of £225/t) resulted in maximum use.

For the starter and withdrawal feeds where it was selected, the prices shown are the higher values at which new rapemeal will still feature. For starter this is £260.50 (with reference to earlier comments regarding starter diets this is not a broad valuation) and in withdrawal £244.00.

Table 19. Valuation of new (improved) rapeseed and rapemeal, estimated using a least cost ration formulation, while matching the baseline diet price.

Diet:		**Broiler Starter**	**Broiler Grower**	**Broiler Finisher**	**Broiler WDL**
	Raw material price (£/t)	Inclusion level or ingredient/diet cost			
Broiler scenario 5					
Diet Cost (£/t)		277.78	270.92	256.05	252.92
New whole rapeseed (%)	*377.50*	2	2.5	3	4
New Rapemeal (%)	*225.00*	3	0	0	1.81
		Inclusion level or raw material cost			
Highest price for new meal to feature (£/t)		260.5	221.6	222.80	244
Level of inclusion (%)		2.73	5.0	6.0	1.15

The higher protein of the new (improved) seed and resultant meal is of value, as indicated by new rapeseed meal which has an approximate value of +£11.00 to £12.50/t above that for current rapeseed meal. However, the value of current rapeseed will reflect its place in the total animal feed market and it is conceivable that from a poultry perspective it is, at the time of writing, overvalued at £210/t due to demand from other livestock sectors.

It appears that the new whole seed would earn a greater premium due to its higher protein content and its useful energy value. This is approximately £33.00/t of whole seed; this value could be regarded as a genuine "poultry" value since whole seed is not widely used in animal feeds other than for poultry.

Any improvements in anti-nutritive characteristics could also encourage nutritionists to move towards higher inclusion levels and therefore increase uptake by the poultry industry, but have not been valued here. If the changes to fibre, oil and protein levels assumed above are achieved through a move to thinner yellow seed coats, then the reduction in tannins and polyphenols would occur anyway. It is a given also that GSL levels would be as low as possible (i.e. below the average of 12 µmol/g in the current RL varieties).

Whilst it is appealing to consider that rapeseed and rapemeal could replace imported vegetable protein in poultry diets it should be considered that at a maximal inclusion of 8% new rapeseed meal with a higher protein content of 39.0% compared to current 33.5% the additional protein delivered into the feed is 0.44% which could be supplied by <1% soyabean meal. It would be more correct to look at it in terms of digestible amino acids and taking lysine as an example the benefits from the improved rapeseed equates to 0.64% soya meal. Therefore substitution of soya will be achieved more by higher rape inclusions than the improved protein content of the rape products. However this will make the material more attractive and aids in achieving this aim.

The whole seed is effectively competing against both protein sources and vegetable oils; in the formulation exercise soyabean oil is priced at £675.00/t and variations in the cost of this key ingredient (primarily an energy source) will affect the value of the whole seed, as much as the price of soyabean meal (a protein source).

6.2.2. Laying Hen Diet Evaluation

Diet specifications were based on the Hy-Line guidelines for hens in the early stages of production (high density) and those for hens at the late stage of production (low density), feed intakes of 103g/b/d and 110 g/b/d were assumed.

As for the broiler diets a number of costing scenarios were used. In the first instance a base diet cost where no rape products were used was established. With the current rape seed and rape meal the inclusions and diet cost implications were different.

Layer Scenario 1 – basal diet with no rape products, and introduction of existing rapeseed

For the whole seed at current market price the high density diet would take it to the maximum permitted level of 5% and a diet cost saving of £1.24/tonne is seen (Table 20). For the low density diet this material was not attractive until the price was reduced to £343.42 and then only 0.14% was taken.

Table 20. Base layer diet formulations, and effect of introducing existing wholeseed rape into the diet

		High Density Layer	**Low Density Layer**
	Raw material price (£/t)	Inclusion level or seed cost	
Base Diet, no rape		225.66	190.13
Diet cost (£/t)			
Layer scenario 1			
Existing rape seed [1]	*345*		
Level taken (%)		5.0 (maximum)	0
Diet cost (£/t)		224.42	190.13
Whole seed price to feature (£/t)			343.42
Inclusion at above price (%)			0.14

[1], Rapeseed EU 2013, whole, 00 18.5%/44%P/O

Layer scenario 2 – inclusion of existing rape meal in layer diets

The current rapemeal needs to be priced at £199.53/t to feature at 1.26% in the high density diet. However, in the lower density ration it featured, albeit at a low level of 0.59%, and saved £0.08/t in diet costs.

For the low density diet, if the price of the rapemeal were to be reduced to £206.37 inclusion level increases to 3.12% and diet cost marginally lower to £0.10/tonne less than the base diet..

Table 21. Effect of introducing existing rapemeal into the layer diets

		High Density Layer	**Low Density Layer**
	Raw material price (£/t)	Inclusion level or ingredient/diet cost	
Layer scenario 2			
Existing rapemeal [1]	*210*		
Level taken (%)		0	0.59
Diet cost (£/t)		225.66	190.05
Rapemeal price to feature (£/t)	*199.53*		
Inclusion at above price (%)		1.26	

[1], Rapeseed EU 2013, ext, 00

Layer scenario 3 – Introduction of new rapeseed

New (improved) whole seed was offered to both diets in the absence of current rape products at a price of £378.50 (Table 22). In the high density ration, this was taken to the maximum level and reduced diet cost to a similar value as that achieved with the current whole seed. In the low density diet the new whole seed was not selected at the price used, a lower cost of £371.41 being needed to get a minimal inclusion of 0.12%.

This indicates a price premium for new rape seed of £33.50/t is achievable for high density feeds, but for lower density rations it is nearer £26.00/t.

Table 22. Effect of introducing new (improved) whole rapeseed into the layer diets

		High Density Layer	**Low Density Layer**
	Raw material price (£/t)	Inclusion level or seed/diet cost	
Layer scenario 3			
New rapeseed	*378.50*		
Level taken (%)		5.0 (maximum)	0
Diet cost (£/t)		224.46	
Whole seed price to feature (£/t)	*371.41*		
Inclusion at above price (%)			0.12

Layer scenario 4 – Introduction of new rapemeal

For the new rape seed meal a price of £231/t saw an inclusion of 1.02% in the high density diet and a lower diet cost slightly lower than that achieved with the current meal. At £244/t the inclusion remained the same, and the diet cost was matched to that where current meal is used (Table 23).

In the low density feed the price of the meal to match diet cost where current meal in used is £245.00, this gives a low level of inclusion at 0.5%. Dropping the price to £240.00 increases inclusion to 3.77% and gave a marginal diet cost saving.

These data suggests that the whole seed is more attractive in high density feeds whilst the place for the meal at current prices is in low density rations. On the face of it the new whole seed appears to have a value of approximately £26.40 to £33.50, and for the new meal £21.00 to £30.00.

Table 23. Effect of introducing new (improved) rapemeal into the layer diets

		High Density Layer	**Low Density Layer**
	Raw material price (£/t)	Inclusion level or ingredient/diet cost	
Layer scenario 4			
New rapemeal	*231*		
Level taken (%)		1.01	0
Diet cost (£/t)		225.52	
New meal price to feature (£/t)	*245*		
Inclusion at above price (%)			0.5
New meal price to maximise inclusion (%)	*240*		3.77
Diet cost (£/t)			190.01
Maximum meal price to match base diet cost	*244*		
Level taken (%)		1.01	
Diet cost (£/t)		225.66	

6.2.3. General points to consider – poultry diets

Rape seed products will have to compete with other raw materials and most feed mills are limited by the number of bins that they can use and therefore the number of raw materials offered. To introduce a new material will mean the removal of an existing one and the value of a new material must exceed the cost penalty of the outgoing one. Therefore for a mill making layers feed where no rape is currently handled it is likely that a material like sunflower meal would need to be displaced. This could completely alter the costing situation.

Additionally it is unlikely that in a commercial feed mill that whole seed would be added to layers mashes unless it has been processed to break the seed coat and rupture internal cell structure. Whole seeds in the ration are likely to not be broken down by the birds gizzard making the material an expensive filler. Therefore it is probable that improved whole seed will be of greater interest to the broiler sector whilst improved meal will appeal to the layer feed manufacturers.

6.3. Pigs

Whole rapeseed is not commonly used in pig feeds. Many mills will not be able to handle it and ensure that the seed wall is fractured. Further most pig feeds contain appreciable quantities of biscuit meal or crisps which provide a cheaper source of oil than whole rape. Biscuit and crisps are not as popular in broiler because of variation in mineral content and concerns over litter quality.

The value of improved rapeseed meal was determined in pelleted feed for pigs of 30-65 kg, 65-110 kg and in lactating sows. January 2014 costs were used and major raw materials included were wheat, barley, biscuit meal, wheatfeed and soya 46. Feeds were formulated to net energy (6.1 and 7.1MJ NE/kg being used for conventional and improved rapeseed) and standardised ileal digestible amino acids. The energy content was derived from the DE using the equations of Noblet; the DE of the rape meals was determined by calculating the gross energy from proximate analysis and then the energy digestibility from the NDF content. Feeds for pigs up to 30 kg were not included as rapemeal inclusion is limited in these feeds, largely because of its low energy density. Dry sow feeds were also excluded as these are low in amino acids, and rapemeal is normally not required.

The feeds were formulated with conventional rapemeal and inclusion rates were 10, 15 and 7.5% respectively in finisher 1, finisher 2 and lactating sow. The conventional rapemeal was then excluded, the improved rapemeal offered, and the price at which the improved rapemeal gave the same overall raw material cost as the conventional was determined. It was found that the improved rapemeal had a higher value of £38/t, £30/t and £44/t respectively in the 3 feeds. On a weighted basis (weighted according to the amount of feed of each type produced) the improved rapemeal carries a premium of £34/t at the same inclusion rates in the feeds.

Table 24 shows the major ingredients used in the 65-110kg pig diet, where it is evident that improving the rapemeal reduces the inclusion rate of soya and wheat, whilst more wheatfeed is used.

Table 24. Effects of introducing new (improved) rapemeal into pig (65-110kg) diets.

Ingredient	Conventional	Improved	Diff
	Inclusion level (%)		
Barley	25	25	
Wheat	32.23	29.72	2.5
Wheatfeed	8	12.3	-4.3
Soya 46	5.06	3.35	1.7
Biscuit	10	10	
Rape meal	15	15	

The £34/t premium established above is only robust with the raw material costs used, quality control data and raw materials available, and pelleted feed specifications. Almost half of UK pig feed is produced as meal, sunflower meal is often used in pelleted feeds, and maize is currently available in many mills in the north. There are therefore many potential scenarios although the above is the most common.

6.4. Summary combining broiler, layer and pig values

Whole rapeseed does not feature in pig diets as discussed above, but has value in poultry. For whole seed the added values for improved seed types are broadly similar in the two diet groups at ~£30/t, although will be favoured in broiler diets for reasons described.

The new (improved) meal appears to have the highest value in pig feed (£34/t) rather than poultry feeds. Within the poultry sector, the improved meal would appear to warrant a higher value in layer diets (£21-30/t) than broiler (~£12/t). To relate this to a value to the grower, in simple terms, an increase in the value of the meal of £34/t would equate to an increase value of the seed from which it is derived, of ~£15/t. In addition because in this scenario the additional oil (1.4%, Table 14) also adds value to the food market, this would add £7.50/t (using current rape oil price of ca. £510/t), giving a total value of the new (improved) seed of +£22.50/t.

It is likely therefore that the value of a new improved rape seed with increased oil+protein of 4% achieved through reducing seed coat thickness and a possible move to yellow seeded varieties, would lie somewhere between £22.50 (based on higher value of improved rapemeal in pig diets, plus higher levels of oil for the food market) and £30/t (based on higher value of improved whole rapeseed in poultry, principally broiler diets).

7. Discussion and Conclusions

This report shows that there are opportunities to improve the quality of rapeseed for the animal feed sector, based on benefits which can be quantified in terms of both an improved whole seed, and also its extracted meal. The values differ between sectors within the non-ruminant livestock types, and are dependent on the prices of feed ingredients at the time of preparing this report. They underline the value of selecting for higher oil and protein in producing an improved rapeseed.

The changes modelled here in terms of improved rapeseed (+4% oil+protein) may be seen as being somewhat conservative given the range of variation seen in OSR germplasm particularly for oil (Chapter 4). However, in pushing for higher oil in particular, breeders are likely find slower progress in seed yield improvement which is of similar, or greater, importance. Hence the improvements modelled here are something of a compromise, as the authors are unable to accurately quantify the trade-offs between selecting for yield and any of the particular quality traits discussed in this report. This may require further research.

Glucosinolates will continue to be an important anti-nutritional factor to reduce in rapeseed. While it has not been included as a specific target in the feed formulation exercise (Chapter 6) because all rapeseed varieties are currently low enough to satisfy current feed standard, there is still anecdotal evidence from time to time of poor livestock performance on rape meal from specific crushers in the UK attributed to GSL, and the authors are aware of examples of this as recent as 2013. Given that there is a range in GSL between 8.5 to 17.4 µmol/g in different varieties on the RL, then a large proportion of a high GSL variety grown in one region could lead to high GSL levels in rapemeal, on a transient basis. If associated with poor performance in commercial herds or flocks, such observations of poor performance, rightly or wrongly, can be attributed to inclusion of rapemeal in the diet, and subsequently lead to a conservative use of rapemeal as a feed ingredient.

At the start of the project it was believed that sinapine was an important ANF which caused rapeseed and meal to be limited in layer diets, however industry evidence is that this is no longer a valid target for reduction due to improvement in bird genetics since use of rape meal became widespread. Hence the authors cannot see that it would be worthwhile investing in a breeding programme, either conventionally or via GM, in order to reduce sinapine. It may be that the message about sinapine no longer being a major issue has not been clearly communicated to the industry, in which case there may be a knowledge transfer requirement.

There has been research to reduce phytate and/or increase phytase in rapeseed, but to the feed industry, it is difficult to see that this would provide a commercial benefit given that phytase enzymes are available and relatively cheap and in any case an exogenous source of phytase will still be needed to break down phytate in the other (non-rape) components in a compound feed.

There is clear logic in reducing the amount of fibre, lignin and polyphenols in the seed coat but the benefits of reducing polyphenols is difficult to quantify. The value of reducing fibre is reflected in reduction in crude fibre for poultry and NDF for pigs in the formulations, but the value has actually been quantified in terms of the higher levels of protein plus oil that result from these changes. Since these changes can be driven through selecting thinner/yellow seed coat varieties, in practice these changes should occur together.

It was clear from discussions that most breeders perceive that the Canadian ‘canola’ types are quite distinct genetically from UK OSR, and are derived principally from *B. campestris*. However, recent work in France has apparently studied meal derived from yellow seeded varieties of *B. napus* (see Annex). If these varieties are indeed *B. napus* and appropriate for the UK industry, they should be tested not only as meal but as whole seed (as discussed this is a significant product for broiler rations), and in UK-type production systems.

Broadly speaking the improved feeding values (of between ca. £22.50 and £30/t) should be greater than the costs of segregation of specific varieties within the feed supply chain, but could easily be eroded if the financial scenarios changed (lower prices of competing commodities, higher transport and energy costs). More importantly however, mill managers do not like keeping separate bins for specific feed ingredients, as has been demonstrated in the past for feed wheats which were identified as having superior feed quality. Therefore it is more likely that such changes would only be supported if the whole industry were to move to a newer type of rapemeal, and this underlines the role for HGCA in supporting continued variety development through the Recommended Lists.

7.1. Recommendations for further work

- There is a need to educate the wider feed industry on the improvements in poultry production for instance better flock genetics which means that perceived problems such as taint in eggs arising from sinapine are no longer an issue,
- Screening of OSR germplasm in public programmes should ideally include traits other than oil, GSL and protein, for instance seed coat colour, thickness and polyphenol content,
- There is a need to understand the trade-offs between breeding for yield, and breeding for oil+protein,
- There is a need to understand the implications of breeding for thin seed walls and yellow seeds on agronomic qualities such as seed dormancy, seed vigour and ability to withstand damage at harvest,

- Further work is needed to clarify whether improved yellow seeded varieties produced in Canada and recently tested in France are derived from *B. campestris*, or are truly *B.napus*. types,
- Improved *B. napus* types as recently reported in France should be tested in UK conditions, ideally both as whole seed in broilers and as meal in pigs and poultry to truly quantify the values to the industry.

8. Acknowledgements

The authors would like to thank Rachel Wells and Ian Bancroft from JIC for supplying data on *B. napus* diversity panels, and the consortium members of project LK0979 for data on mapping populations and elite lines. Information on French studies was kindly supplied by Alain Quinsac, CETIOM. Comments on breeding targets from Matthew Clarke, Mark Nightingale, Richard Jennaway and Jo Bowman are also gratefully acknowledged.

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Annex. Meal composition and nutritional value for poultry for different rapeseed types (*B. napus*) tested in France by Quinsac *et al.* (2013).

Type of seeds	Winter rapeseed										Spring rapeseed	
Country	France	France	France	France	France-INRA	France-INRA	France-INRA	France-INRA	France-INRA	France-INRA	Canada-AARFC	Canada-AARFC
Phenotype	Black	Black	Black	Black	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Black	Yellow
Seed batch	Regular	Regular	Control Goeland	Control Goeland	YS-1	YS-1	YS-2	YS-2	YS-3	YS-3	N89-53	YN01-429
Dehulling	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	No
Dry matter %	90.6	91.4	93.1	93.5	91.5	92.0	91.5	92.2	91.5	92.0	90.7	90.4
Analyte (DM basis)												
Gross energy (kcal/kg)	4,587	4,676	4,551	4,564	4,507	4,551	4,579	4,605	4,509	4,568	4,766	4,728
Gross energy (MJ/kg)	19.2	19.6	19.1	19.1	18.9	19.1	19.2	19.3	18.9	19.1	20.0	19.8
Protein (%)	35.8	43.7	38.4	48.3	39.7	47.8	41.0	47.2	41.1	48.0	44.1	44.1
Fat (%)	1.44	0.86	0.88	0.43	1.26	0.72	1.81	1.64	1.06	0.67	1.92	2.43
Ash (%)	7.05	7.28	8.06	9.22	8.19	8.88	8.59	9.30	8.31	8.52	6.70	6.57
Glucosinolate (µmol/g)	23.7	35.9	62.4	79.0	28.3	33.5	56.6	58.9	166.3	216.2	34.4	20.0
Cell walls (%)	38.1	22.5	36.2	19.6	32.6	19.7	32.4	19.5	29.7	17.3	31.1	28.8
Crude fibre (%)	16.0	7.8	14.8	6.2	10.5	6.6	10.1	6.7	11.2	6.4	16.0	11.0
NDF (%)	31.4	14.1	30.1	11.5	20.2	12.3	20.2	11.5	20.1	11.2	27.6	21.0
ADF (%)	23.4	8.9	23.0	7.0	12.9	7.4	12.3	6.5	12.5	6.4	20.5	12.5
ADL (%)	10.8	2.5	11.3	1.3	2.9	1.1	2.9	0.7	3.2	0.5	10.8	3.3
NDF-ADF (%)	8.0	5.2	7.1	4.5	7.3	4.9	7.9	5.0	7.6	4.8	7.1	8.5
ADF-ADL (%)	12.6	6.4	11.7	5.7	10.0	6.3	9.4	5.8	9.3	5.9	9.7	9.2
AMEn (kcal/kg)	2,068	2,391	1,946	2,459	2,185	2,467	2,378	2,570	2,048	2,342	2,377	2,418
AMEn (MJ/kg)	8.66	10.01	8.15	10.30	9.15	10.33	9.96	10.76	8.57	9.81	9.95	10.12
Dehulling effect (MJ/kg)		1.35		2.15		1.18		0.80		1.23		
N digestibility (%)	74.3	78.8	79.4	85.3	83.4	85.5	84.8	82.9	84.4	83.5	77.1	76.2
Dehulling effect (%)		4.5		5.9		2.1		-1.9		-0.9		