High-fiber rapeseed co-product diet for Norwegian Landrace pigs: Effect on digestibility

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A B S T R A C T

The effect of partially replacing soybean meal (SBM) and wheat with high-fiber rapeseed (RS) co-products on the nutrient and energy digestibility of 40 Norwegian Landrace pigs (17.8 ± 2.7 kg initial BW) was investigated. Pigs were fed a pelleted diet containing 200 g/kg of a coarse fraction of air-classified rapeseed meal (RSM) and 40 g/kg of RS hulls or a SBM control diet (20 pigs/dietary treatment) for 3 wk to estimate apparent ileal (AID) or total tract (ATTD) digestibility of energy and nutrients, organ weight, intestinal histomorphology, and digestive enzyme activities of individual pigs. Feeding high-fiber RS co-products increased ($P = 0.004$) the thyroid to body weight ratio and reduced ($P < 0.05$) the AID and ATTD of energy, dry matter, organic matter, crude protein (CP), neutral detergent fiber, acid detergent fiber, P, and most of the amino acids (AA) and monosaccharides. The reduction in digestibility was not associated with morphological changes in ileum or colon. The reduced AID of CP and AA coincided with a decrease ($P = 0.030$) in trypsin activity in the jejunum. The AID of starch was not affected by the dietary treatment, which also coincided with similar amylase and maltase activities in the jejunum. Variation in nutrient digestibility was observed among individual pigs within each dietary treatment. In conclusion, feeding high-fiber RS co-products to pigs enlarged the thyroid gland and reduced the AID and ATTD of most nutrients and energy. The reduction in digestibility was not associated with changes in intestinal morphology, but correlated with digestive enzyme activities.

Keywords: Digestibility; Feed efficiency; Fiber; Landrace pigs; Rapeseed meal; Soybean meal

1. Introduction

The European pig industry is heavily dependent on imported feed ingredients, especially soybean meal (SBM) as a protein source in commercial diets (FEFAC, 2015). Increased and more efficient use of local protein sources, such as rapeseed (RS), could improve the sustainability and self-sufficiency of pig

production in Europe. European RS production is rapidly increasing, mainly because of higher demands from the biofuel industry (Messerschmidt et al., 2014). Rapeseed meal (RSM), a co-product from RS processing for oil and biofuel production, has potential to replace a significant proportion of SBM in pig diets (Weightman et al. 2014). Life cycle assessment studies of the environmental impacts of feed production have shown that global warming potential decreased up to 10% when SBM is replaced with RSM in diets for pigs (van Zanten et al., 2015). However, the use of RSM in pig diets is associated with reduced feed intake, growth rate, and nutrient utilization (Landero et al., 2011; Seneviratne et al., 2011; Torres-Pitarch et al., 2014). These effects have been attributed to the high dietary fiber (DF) content and the presence of several antinutritional factors (ANF) in RSM, including glucosinolates (Mejicanos et al., 2016).

High content of DF in pig diets is associated with impaired nutrient utilization and reduced net energy values (Noblet and Le Goff, 2001), although the magnitude of such negative impacts will be determined by the fiber source, relative solubility and fermentability, and the age and genotype of the animal (Lindberg, 2014). Indigenous pig breeds have been shown to digest fiber better than exotic breeds genetically selected for improved growth performance (Len et al., 2009a, 2009b; Urriola and Stein, 2012). Improvements in feed efficiency (FE) are crucial for a more economically and environmentally sustainable pork production. According to Herd and Arthur (2009), nutrient digestibility is one of the factors contributing to variation in FE. Fiber digestibility is more variable and lower than that of other main nutrients (Jha and Berrocoso, 2015), and thus the use of high-fiber diets may affect the progress of selection for improved FE. The Norwegian Landrace breed has a history of selection for improved FE beginning in the 1960s (Kolstad and Vangen, 1996). However, genetic selection has emphasized traits such as average daily gain and carcass lean of pigs fed high-quality diets (Kolstad and Vangen, 1996), which indirectly affect FE. Thus, the Norwegian Landrace pigs may have great potential for a high FE when fed high-quality diets, but this may not be applicable when fed diets with higher fiber content and ANF. Genetic variability of organic matter (OM), N and energy digestibility in Large White pigs fed a

high-fiber diet has been reported (Noblet et al., 2013). We hypothesized that replacing SBM with high-fiber RS co-products affects the capacity of Norwegian Landrace pigs to digest nutrients and energy and that there is individual variation in digestibility among pigs fed such diets. Therefore, the objectives of this study were to evaluate nutrient and energy digestibility of Norwegian Landrace weanling pigs fed standard or a high-fiber RS diet, and to identify underlying biological mechanisms associated with variation in digestibility.

2. Materials and methods

The research protocol was reviewed and approved by the Norwegian Food Safety Authority. The trial was conducted at the experimental farm of the Norwegian University of Life Sciences (NMBU), Ås, Norway.

2.1. Animals, housing and allotment

The experiment was conducted as a randomized complete block design with 2 periods, 2 dietary treatments and 10 replicates per treatment per period (total of 20 replicates per treatment). Each experimental period lasted 3 wk, and consisted of a 2-wk adaptation period followed by a 1-wk period of feces and data collection. For each period, 20 castrated male pigs (Norwegian Landrace; Norsvin, Hamar, Norway) with an average initial BW of 16.1 ± 2.2 and 19.6 ± 1.8 kg, respectively, were obtained from 2 different multiplier herds. In total, 40 pigs from 14 different litters with 2 or 4 pigs per litter were used. Upon arrival, pigs were allocated into 10 metabolic crates (1.2 × 1.4 m) in pairs, based on similar BW and litter. Pigs within the same crate were assigned randomly to 1 of the 2 dietary treatments. Each crate was equipped with a self-feeder and a low-pressure nipple drinker and had a partially covered rubber slatted flooring. Pigs were provided ad libitum access to water except at the time of feeding. Pigs were offered toys according to the Norwegian animal welfare legislation (Lovdata, 2003). The room temperature was

kept at 21 ± 4°C and a 12-h light/12-h dark cycle was provided for the duration of the experiment. The clinical health status of the pigs was monitored daily.

2.2. Dietary treatments and feeding

The dietary treatments were: 1) a Norwegian commercial diet based on wheat, barley and SBM (Control) and 2) a diet in which wheat and SBM were partially replaced by 20% coarse RSM and 4% pure RS hulls (RSF). The coarse RSM was produced by air-classification of a commercial hexane-extracted RSM (Bunge, Warsaw, Poland), which separated a coarse high-fiber fraction and a fine low-fiber fraction with a higher protein content (Hansen et al., 2017). The parent RSM was jet-milled (JMX-200; Comex AS, Rud, Norway) to an average particle size of 35 µm. The RSM fractions were obtained after a multiple separation process using air-classification at 3 different rotor speeds (2,200, 1,900, and 1,700 rpm), where the lower bulk density fractions were separated, and the coarse fraction was used for further separation (ACX-200; Comex AS). After 3 fractionation steps, the remaining coarse fraction had a particle size of 74 µm and a yield of 56.4% of the parent RSM. The RS hulls were obtained by grinding whole seeds (Askim Frukt- og Bærpresseri AS; Askim, Norway) with a roller mill (DT900-12; CPM-Roskamp, Waterloo, IA, United States), and separating the low bulk density hulls with a laboratory air-classifier. The chemical composition of the coarse RSM fraction and the RS hulls used in this experiment is shown in the footnote of Table 1.

The diets were formulated to meet or exceed the requirements for indispensable amino acids and all other nutrients and energy for pigs of this age (NRC, 2012), and were subsequently mixed and pelleted at the NMBU Center for Feed Technology (Ås, Norway). Yttrium (III) oxide was included (0.01%) as an inert marker for digestibility calculations. The composition and chemical contents of the diets are presented in Table 1 and Table 2, respectively.

Upon arrival at the research facility, pigs were offered a commercial weaner diet, which was gradually replaced (over 3 d) by 1 of the 2 experimental diets. The animals were fed equal meals twice

daily at 0800 and 1500 h, with the total amount of feed corresponding to 3.5% of their BW per day. Pigs within the same crate were separated for 15 min by a physical barrier at every meal to allow individual feeding. Water was added to the feed immediately before feeding at a ratio of 2:1 (w/w). All pigs were weighed weekly, and daily feed allowance was adjusted accordingly.

2.3. Sample collection and processing

During the collection period, feces were obtained daily by grab sampling directly from pen floors or by rectal stimulation. Feces from each pig over the 7-d period were pooled and frozen at -20°C. Upon completion of the trial, feces were freeze-dried, ground through a 1-mm screen in an ultra centrifugal mill (ZM 100; Retsch, Haan, Germany) and mixed before chemical analyses to determine apparent total tract digestibility (ATTD) of energy and nutrients. Feces and ileal digesta were also ground through a 0.5-mm screen for determination of starch and amino acid content. Cumulative samples of the diets were also obtained during the collection period and ground through a 1 or 0.5-mm screen before chemical analyses, as explained for the feces.

Blood samples were collected from all pigs at the start of the experiment and 1 d before slaughter. Approximately 10 mL pre-prandial blood was collected into EDTA and lithium heparin-coated tubes (Vacuette; Greiner Bio-One, Kremsmünster, Austria) via venipuncture in the jugular vein/bijugular trunk. After collection, plasma was harvested by centrifugation at 2,000 × g for 10 min at 4°C and kept on ice until transport, along with EDTA whole blood, to the laboratory for analysis of standard clinical chemistry and hematology variables.

At the end of each experimental period, pigs were euthanized using a captive bolt pistol followed by exsanguination. All pigs received a normal morning meal 2.5 to 3 h before slaughter to ensure the presence of digesta along the gastrointestinal tract (GIT). An incision was made to expose the abdominal cavity, and the entire GIT was immediately removed. Approximately 25 cm sections of the duodenum (25 cm from the pyloric sphincter), mid-jejunum, ileum (20 cm anterior to the ileocecal valve), cecal apex,

and the central flexure of the spiral colon were tied off with cotton string, excised and placed in aluminum trays on ice. Tissues from distal jejunal lymph node, liver, thyroid gland, kidney, spleen, heart and lungs were sampled from standardized locations. The livers and thyroid glands were weighed before samples of these organs were taken. Each animal and its organs were evaluated macroscopically by a trained pathologist. Tissues and digesta from the different intestinal segments were further sampled for microbial investigation, histology, enzyme activity, transcriptomics, and metabolomics analyses. Only the results from histology and enzyme activity are included in the present paper. The digesta samples were snap-frozen in liquid nitrogen and the tissue samples were rinsed with PBS and put on dry ice. All samples were stored at -80°C until analysis. Digesta from the last 2 meters of the small intestine were collected, stored at -20°C, and processed as previously discussed for the feces before chemical analyses to determine apparent ileal digestibility (AID). Samples for histology were fixed in 10% buffered formalin solution for 48 h, embedded in paraffin and sectioned in an automatic microtome. Two micron thick sections were mounted on glass slides and stained with hematoxylin-eosin for histological evaluation.

2.4. Histopathology

The morphological evaluation was performed blindly and all assessments were conducted by the same trained pathologist. Histological sections of all the harvested organs were examined in a microscope (Axio Imager Z2; ZEISS, Jena, Germany), and digital images were obtained using a color camera (Axiocam 506 color; ZEISS). The evaluation of intestinal morphology was performed following a modified version of the protocol described by Day et al. (2008), made to fit normal intestinal morphology in the pig. The morphological assessment included evaluation of epithelial damage, formation of crypt dilation and crypt abscesses, mucosa fibrosis, lacteal dilation, follicle atrophy, number of intraepithelial lymphocytes (IELs), presence of infectious agents, and infiltration of leucocytes (i.e., lymphocytes, plasma cells, eosinophils, neutrophils, and macrophages). The results were recorded semi-quantitatively as normal (0), mild (1), moderate (2), and severe (3) changes.

All micrographs were captured with the same ×10 objective magnification in 6 different locations of each intestinal section (i.e., base, middle, and apex areas of intestinal plicas, both from plicas with and without Peyer's Patches). Villi height (VH) and crypt depth (CD) were measured in pixels/inch using the software program Image J-Fiji (Schindelin et al., 2012), and the VH to CD ratio was calculated by dividing VH with CD. One rarely encounters many villi in full length and all perpendicular to the lumen in a row in one section. Therefore, only the longest villi were selected and measured from the base of the crypt to the tip of the villus. Between 3 and 6 villi were measured in each of the 6 different areas of every section. Crypts were selected when the crypt epithelium was visible from the *Lamina muscularis mucosae* and measured from this point to the crypt-villus junction.

2.5. Digestive enzyme activity

Tissue samples from the jejunum were thawed and the mucosa was scraped carefully with a glass slide. Approximately 40 mg of the mucosal scrapings and 70 mg of digesta were homogenized in 1.5 mL of ice-cold water (Milli-Q) using a bead mill (TissueLyser; Qiagen Retsch, Haan, Germany) and sonicated in an ice-cold bath (T 460/H; Elma Schmidbauer GmbH, Ransbach-Baumbach, Germany). The homogenate was centrifuged at 21,100 × g for 10 min at 4°C. The supernatant was analyzed for protein concentration and enzyme activities. The total protein concentration was determined (Bradford, 1976). The activities of trypsin and amylase in jejunal digesta were measured using commercial kits (Abcam, Cambridge, UK). The activity of maltase in jejunal mucosa was assayed following an adaptation of the method described by Dahlqvist (1967) and using a commercial glucose assay kit (Sigma-Aldrich, Saint Louis, MO, United States).

2.6. Chemical analyses

All chemical analyses on ileal digesta and feces were performed in duplicate on freeze-dried samples. Triplicate analyses were conducted with feed samples. Samples of dietary ingredients, diets,

ileal digesta, and feces were analyzed for dry matter (DM) by drying to constant weight at 104°C (EC, 1971b), ash by incineration at 550°C (EC, 1971a), crude protein (CP) by Kjeldahl N × 6.25 (EC, 1993), starch according to the method described by McCleary et al., (1994), acid detergent fiber (ADF) and neutral detergent fiber (NDF) using a fiber analyzer system (Ankom200; ANKOM Technologies, Fairport, NY, United States) with filter bags (Ankom F58; ANKOM Technologies). Gross energy content was determined with an adiabatic bomb calorimeter (Parr 1281; Parr Instruments, Moline, IL, United States) according to ISO (1998). Ether extract (EE) was determined in diets and feces after extraction with petroleum ether and acetone (70/30) using an accelerated solvent extractor (Dionex ASE 200; Dionex Corp., Sunnyvale, CA, United States). Amino acid (AA) analysis (except tryptophan) of diets and ileal digesta was performed according to EC (2009) on an amino acid analyzer (Biochrom 30; Biochrom Ltd., Cambridge, United Kingdom). Tryptophan was analyzed according to EC (2009) on a high performance liquid chromatography system (Dionex UltiMate 3000; Dionex Softron GmbH, Germering, Germany) with a fluorescence detector (Shimadzu RF-535; Shimadzu Corp., Kyoto, Japan). Monosaccharides in feces and diets were analyzed on a high performance anion exchange chromatography system (Dionex ICS3000; Dionex Corp.) with pulsed amperometric detection and equipped with a CarboPac PA1 column (2 × 250 mm) connected to a guard of the same type (2 × 50 mm), after hydrolysis of the samples in trifluoroacetic acid (TFA), essentially as described by Manns et al. (2014) but with the following modifications. A flow rate of 0.25 mL/min was used with a “reversed” gradient developing from 26 to 0 mM KOH in 9.5 min, kept at 0 mM for 2.5 min and elevated to 100 mM for the next 2 min using a chromatography management system (Dionex Chromeleon; Dionex Corp.). The samples were analyzed for oligosaccharides after TFA hydrolysis and no oligosaccharides were detected, indicating that the degradation of polymeric constituents by TFA was complete. Total glucosinolate analysis of the diets was performed according to EC (1990). For the determination of Y and P concentrations in feed, ileal digesta, and feces, samples were first digested with concentrated ultrapure HNO_3 at 250°C using a microwave (Milestone UltraClave III; Milestone, Sorisole, Italy). Samples were then diluted (to 10% HNO_3 concentration), and Y and P were analyzed with an inductively coupled

plasma mass spectroscopy system (Agilent 8800 Triple Quadrupole; Agilent Technologies, Santa Clara, CA, United States). Standard clinical chemistry and hematology panels for pigs were analyzed in plasma and whole blood, respectively, according to certified procedures at the Central Laboratory of NMBU School of Veterinary Medicine.

2.7. Calculations and statistical analyses

The AID and ATTD of nutrients and energy was calculated by the indirect method, as described by Maynard and Loosli (1969), using Y_2O_3 as the inert marker (Austreng et al., 2000). Hindgut disappearance of nutrients and energy was calculated as the difference between the ATTD and AID of nutrients and energy (Urriola and Stein, 2012). Liver and thyroid to body weight (BW) ratios were calculated by dividing the weight of the organ with the BW.

The AID, ATTD, hindgut disappearance, blood and plasma measurements and organ index data were subjected to two-way analysis of variance using the general linear model procedure (SAS, 1990). According to a randomized complete block design, the fixed effects of diet (n = 2) and litter (n = 14) were included in the main model. The ileal data from 2 pigs fed the control diet were excluded because of little presence and very liquid ileal digesta. Treatment means were separated using the least-squares means test. For the histology data, mean results for each of the locations were plotted in a statistical software (GraphPad Prism 7.0; GraphPad Software, Inc., La Jolla, CA, United States) and a one-way analysis of variance test or t-test was performed. Differences between dietary treatments were considered significant if $P < 0.05$, and were considered a trend if the *P*-value was between 0.05 and 0.10. Results are presented as the LS-means for each treatment, and variance is expressed as the standard deviation or the pooled standard error of the mean. Pigs were considered the experimental unit for all analyses.

3. Results

3.1. Diets

The analyzed composition of the 2 experimental diets was consistent with calculated values from ingredient composition and inclusion rates used in the formulation of the experimental diets. Overall, the CP, GE and AA concentrations were similar for the 2 diets. The largest differences in the AA content were observed for methionine, cysteine, and threonine, which were slightly higher in the RSF diet compared with the control diet. The RSF diet contained slightly more ash and EE than the control diet, and starch content decreased when replacing SBM and wheat with RS co-products (40.2 vs. 37.1% in control and RSF diets, respectively). Inversely, NDF and ADF increased with increasing inclusion of RS co-products (11.3 vs. 15.5% for NDF and 4.2 vs. 8.2% for ADF, in the control and RSF diets, respectively). For monosaccharide content, the RSF diet had more arabinose, glucosamine, and rhamnose, and less total glucose than the control diet.

3.2. Health status and growth

All animals appeared healthy and active throughout the experiment, except for one outbreak of a mild to moderate diarrhea in 8 pigs that lasted 1 to 5 d during the collection week of the second period. The diarrhea incidence was independent of dietary treatment, and the affected pigs immediately received a probiotic treatment (ZooLac Propaste; VESO AS, Oslo, Norway) following veterinary recommendations. Feces from affected animals were only collected when the fecal consistency (data not shown) was normalized to avoid interference with the digestibility values. Pigs fed both diets consumed their feed readily and grew normally. The initial and final BW averaged 17.8 ± 2.8 and 28.3 ± 4.3 kg for pigs fed the control diet, and 17.9 ± 2.6 and 28.0 ± 4.0 kg for pigs fed RSF diet. The average daily feed intake was 728.7 ± 106.8 and 731.9 ± 102.8 g/d for pigs fed the control and RSF diets, respectively. The liver to BW ratio (data not shown) did not differ between the control and RSF groups, while an increased thyroid to BW ratio was observed in pigs fed the RSF diet (0.09 vs. 0.07; $P = 0.004$). There were no differences in the hematology and most of the clinical chemistry variables (data not shown) between the dietary treatments, except for plasma urea and creatinine levels, which were increased ($P < 0.01$) in pigs

fed RSF compared with pigs fed the control diet. The levels of these metabolites were within the normal range for this age pigs.

3.3. Digestibility of dietary components

The AID of DM, OM, CP, NDF, ADF, energy, and P was affected by diet ($P < 0.01$), and all values were lower for pigs fed RSF compared with those fed the control diet (Fig. 1 and Table 3). There was no dietary effect on the AID of starch. Feeding the RSF diet also resulted in a reduction ($P < 0.01$) in ATTD of DM, OM, CP, NDF, ADF, energy and P. The ATTD of EE and starch was similar among pigs fed both diets. There was no effect of diet on the hindgut disappearance of DM, OM, CP, starch, NDF, ADF, energy and P. The AID of total AA and all individual AA (except for methionine) was reduced ($P < 0.05$) in pigs fed the RSF diet (Table 4). Similarly to the ATTD of NDF and ADF, the ATTD of fucose, galactose, and total glucose was reduced ($P < 0.001$) in the pigs fed RSF compared with pigs fed the control diet (Table 4). In contrast, pigs fed the RSF diet had greater ($P < 0.001$) ATTD of arabinose, rhamnose, and glucosamine than pigs fed the control diet, while ATTD of xylose and mannose was not affected by diet.

Individual variation in digestibility among pigs within the same dietary treatment was observed in the terminal ileum and in the total tract (Fig. 1). The AID of CP in pigs fed the control diet ranged from 74.7 to 84.7%, with an average of 80.9%. The AID of CP in the pigs fed RSF ranged from 64.4 to 79.8%, with an average of 73.2%. Similarly, the ATTD of NDF ranged from 45.0 to 56.9% (average 52.8%) and from 23.0 to 44.5% (average 37.0%) in pigs fed control and RSF diets, respectively. Individual pig variation in fermentation capacity was also observed among pigs within the same dietary treatment (Table 3). Hindgut disappearance of CP in pigs fed the control diet ranged from 0.4 to 10.3% (average 3.9%) and from -3.1 to 13.6% (average 4.6%) in pigs fed the RSF diet. Hindgut disappearance of NDF ranged from -2.8 to 60.9% (average 33.5%) and from 1.5 to 57.4% (average 30.2%) in pigs fed control and RSF diets, respectively.

3.4. Macroscopic and histopathological evaluation

Data from the macroscopic evaluation and the histological assessment, except for VH, CD, and VH:CP, are not presented. Macroscopic evaluation of the intestines of all pigs showed no pathological conditions. Similarly, histological assessment of the various intestinal segments did not show epithelial damage in any of the pigs, and there was no sign of crypt dilation, crypt abscesses, mucosa fibrosis or follicle atrophy. No differences in VH, CD and VH:CD in ileum and CD in colon segments were observed between pigs fed the different diets (Fig. 2), nor were there differences in these measurements between pigs from the 2 different experimental periods (data not shown). A very mild to moderate multifocal infiltration of neutrophils was observed in the lamina propria and in the epithelium of the colon of some pigs. This very mild degree of inflammation was observed in 13 pigs fed the control diet and 8 pigs fed RSF. Generally there were none to a minimal number of neutrophils in the lamina propria in all other intestinal segments. *Cryptosporidium* sp. was observed in the enterocytic brush border in the jejunum and ileum of 2 pigs (no other pathology was identified in the intestines of these pigs). There was a consistent increase of IELs in all intestinal segments of the pigs from the second period, regardless of dietary treatment and diarrhea incidence. Histological evaluation of the lungs showed less severe (P = 0.060) pneumonic lesions in pigs fed RSF diet compared to the pigs fed the control diet (data not shown). No histopathological changes were observed when evaluating myocardium, liver, kidney and thyroid gland from pigs in either dietary treatment.

3.5. Digestive enzyme activity

Amylase activity in jejunal digesta and maltase activity in jejunal mucosa did not differ between dietary treatments while feeding the RSF diet reduced (P = 0.030) trypsin activity in the jejunal digesta of the pigs (Table 5).

4. Discussion

In Europe, the heavy reliance on imported SBM as a protein source in pig diets is questionable from a food security point of view, making it necessary to search for alternative protein sources and develop robust genotype pigs that perform well when fed such diets. Therefore, our objectives were to evaluate the energy and nutrient digestibility of Norwegian Landrace weanling pigs when switched from a conventional SBM-based to a RS-based diet and to identify biological mechanisms associated with differences in digestibility.

Fiber content in RSM is considerably higher than in SBM because of the greater proportion of hulls relative to seed mass (Mejicanos et al., 2016). Over 70% of the RS fiber is concentrated in the hulls, which serves as the main reservoir for non-starch polysaccharides and lignin (Carré et al., 2016). A coarse fraction of air-classified RSM and pure RS hulls were therefore used in the present experiment to increase the contrast in fiber level and fiber composition between treatments and accentuate the specific effects of the RS fiber on digestibility. Consequently, the ADF and NDF concentrations in the diet increased when replacing wheat and SBM with the high-fiber RS co-products, which was the major difference between the dietary treatments. Similarly, methionine and cysteine contents were greater in the RSF diet, as RSM contains more sulphur AA when compared to SBM (Newkirk et al., 2003).

Norwegian Landrace pigs have a high FE when fed standard diets (Kolstad and Vangen, 1996). In this paper, we refer to standard diets as high-energy density and -protein diets based on conventionally used ingredients. Energy and nutrient digestion of feed has been reported as one of the physiological processes contributing to variation in the FE of an animal (Herd and Arthur, 2009). Therefore, it was important to assess differences in the capacity of Norwegian Landrace pigs to digest energy and nutrients in the diet. The observed reduction in AID and ATTD of most nutrients and energy, including most AA and monosaccharides, by the RSF diet may be attributed to the higher fiber content. It is well known that high fiber content reduces the digestibility of energy and dietary components both at the ileum and total tract level (Len et al., 2009a, 2009b; Wilfart et al., 2007a; Yin et al., 2000). Furthermore, the decreased

346 nutrient and energy digestibility observed in the present study is consistent with previous research where
347 increasing dietary levels of up to 20% solvent-extracted (Landero et al., 2011) or expeller-pressed canola
348 meal (Landero et al., 2012), to replace SBM, linearly reduced ATTD of energy, DM and CP in weanling
349 pigs. Similarly, Sanjayan et al. (2014) reported that increasing dietary inclusion of 20 to 25% of 2 types of
350 canola meal (*Brassica juncea* yellow and *Brassica napus* black) led to a reduction in ATTD of DM, CP
351 and energy in weanling pigs. These authors attributed this effect to an increased NDF content, which was
352 lower than in the present experiment.

353 In addition to the level of fiber, the fiber type or source has specific effects on the digestion and
354 absorption processes (Wenk, 2001). Fiber in different feedstuffs vary in solubility, degree of lignification
355 and fermentability, that will in turn affect the physico-chemical properties of the diet, which are important
356 for its utilization in pigs (Bach Knudsen and Jørgensen, 2001). The ADF content was almost twice as
357 high in the RSF diet compared to the control diet, indicating that the fiber fraction in this diet was more
358 insoluble than in the control diet. Lignin is highly resistant to degradation and is known to cause a
359 considerable reduction in digestive processes (Wenk, 2001). A larger amount of lignin was expected in
360 the RSF diet because lignin concentration in RSM is considerably greater than in SBM, especially
361 because of the high degree of lignification of the RS hulls compared with soybean hulls (26.2 vs. 2.1% of
362 DM, Bach Knudsen, 2014), and our RSF diet contained a coarse RSM fraction and pure RS hulls.
363 Consequently, the inclusion of RS co-products may have resulted in a more insoluble and indigestible DF
364 fraction, which may explain the reduced digestibility of ADF and NDF of the pigs fed the RSF diet, as
365 previously shown in several studies (Urriola and Stein, 2010; Wilfart et al., 2007a). In fact, the low ADF
366 and NDF digestibility in pigs fed the RSF diet indicates that the fiber fraction in this diet is highly
367 resistant to digestion. The negative AID of ADF obtained in pigs fed the RSF diet may be an artifact
368 because of endogenous losses, as significant amounts of non-dietary material may be co-analyzed with
369 fiber in ileal digesta and feces (Montoya et al., 2016), but it indicates that this fraction is predominantly
370 indigestible in the small intestine. Insoluble fiber increases passage rate and decreases mean retention

time in the small and large intestine of pigs (Wilfart et al., 2007b). Thus, feeding the RSF diet may have reduced the time that the digesta was exposed to enzymatic degradation and microbial fermentation, which may have contributed to the reduced AID and ATTD of nutrients and energy in pigs fed this diet. Rapeseed meal also has a rigid lignin-cellulose matrix (Pustjens et al., 2013) that can further hinder the accessibility and action of digestive enzymes (Hansen, 1986), and may have contributed to the reduced nutrient digestibility observed in the ileum. The reduction in digestibility by the RSF may also be partially explained by the low digestibility of the protein and AA present in the RS hulls. The hull fraction represents about 30% of the RSM (Mejicanos et al., 2016) and the RS hulls used in the present experiment contained 13.2% of CP. The high lignification of RS hulls may create a complex fiber matrix that binds and encapsulates protein and AA, preventing the action of digestive enzymes (Bach Knudsen, 2014), and thus reducing their digestibility. Lindberg (2014) reported an increased digesta bulk caused by insoluble fiber and this may be an additional factor reducing the digestibility of the RSF diet in the present experiment. Insoluble fiber appears to increase endogenous losses through physical abrasion, scraping the mucin from the intestinal mucosa (Montagne et al., 2003), and may have contributed to the reduction in the apparent digestibility of CP and AA by the RSF diet.

Majority of the starch in the diets originated from cereal ingredients, with minimal contribution from SBM or RS co-products. As a consequence, starch digestibility of both diets was similar. The greater ATTD of arabinose and rhamnose in pigs fed the RSF diet may be attributed to the higher content of these monosaccharides in the RSF diet. The negative ATTD of glucosamine is most likely because of the low concentration of glucosamine in the diets and its presence in endogenous losses, as glucosamine is one of the main carbohydrates in mucin (Montagne et al., 2003) and bacterial cell walls (Ward, 1973). Noblet et al. (2013) observed within-population genetic variability of nutrient and energy digestibility in Large White growing pigs from 4 different sires fed a high-fiber diet. These authors suggested that this genetic variability in digestive efficiency may be heritable. The design of the present experiment did not allow for the evaluation of a heritable effect on digestibility. However, the individual variation in AID of

CP, ATTD of NDF and in hindgut fermentation of CP and NDF indicates that there are differences in the digestion and fermentation processes among pigs. Whether this variation is heritable should be estimated to investigate the possibility to select pigs for an increased ileal and total tract digestibility when fed high-fiber RS diets. Noblet et al. (2013) hypothesized that differences in N absorption between different half-sib families may have occurred at the hindgut level. In the present experiment, the difference in CP digestibility among pigs was also observed at the ileum level. We acknowledge the limitations of the slaughter procedure for collecting representative ileal digesta samples, which may contribute to the variation observed in the AID data.

Rapeseed meal contains several ANF, such as tannins, sinapine, glucosinolates, and phytic acid, which could interfere with proteolytic enzymes and reduce P bioavailability (Khajali and Slominski, 2012). This may partly explain the decrease in trypsin activity and further contribute to the reduced CP, AA, and P digestibility in the pigs fed the RSF diet. Breakdown products from glucosinolates in RSM have been shown to affect palatability and impair feed intake, alter thyroid function by inhibiting production of thyroid hormones, and impair liver and kidney function (Mejicanos et al., 2016). The total glucosinolate content in the RSF diet was below the recommended limit of 2.1 mmol/kg feed for pigs (EFSA, 2008). Interestingly, an increased thyroid to BW ratio was observed in pigs fed the RSF diet, indicating a possible negative impact on thyroid function even at low inclusion levels and short time of exposure. However, histological assessment of thyroid gland did not confirm any changes related to tissue damage in pigs fed RSF diet. As reviewed by Mejicanos et al., (2016), enlarged thyroid after short-term exposure to low dietary concentrations of glucosinolates indicates that young pigs are highly sensitive to these components. In contrast, liver to BW ratio was not affected by the RSF diet, which is supported by the absence of histopathological changes in the liver. However, long-term experiments are needed to further evaluate potential negative effects because enlarged livers after 13 wk of exposure to RSM has been reported (Choi et al., 2015).

The VH and VH:CD have been commonly used as general indicators of the digestibility in the small intestine, with shorter villi and reduced VH:CD being considered as detrimental for digestion and absorption processes (Montagne et al., 2003). Feeding the RSF diet did not affect VH and VH:CD in the ileum or colonic CD compared with the control diet, indicating that the reduced AID and ATTD of nutrients and energy observed in this study cannot be explained by a decrease in digestive and absorptive intestinal surfaces. Pin et al. (2012) developed a model using rodent data and determined that at least 14 d are required to observe changes in the intestinal crypt structure after a dietary modification. The lack of changes in the intestinal architecture observed in this study after 21 d indicates that changes in intestinal architecture may occur after a longer exposure to the experimental diet, as reported by Chen et al. (2015) after feeding weaned piglets with different fiber sources for 30 d. Variations in brush border enzymatic activities are often linked to morphological changes in the intestine (Kelly et al., 1991). Montagne et al. (2003) suggested that an increase in VH:CD may enhance the hydrolytic capacity of the intestinal epithelium. The lack of effect of feeding the RSF diet on maltase activity, a brush border disaccharidase, coincides with the absence of intestinal morphological changes. Pure lignin and cellulose have been shown to strongly inhibit pancreatic amylase and trypsin activities *in vitro* (Hansen, 1986). In our study, feeding the RSF diet did not affect amylase activity but reduced trypsin activity in the jejunal digesta. Overall, the reduction in AID and ATTD of most nutrients and energy by the RSF diet was not associated with changes in ileal or colonic morphology. However, the reduced AID of CP and AA coincided with a decrease in trypsin activity, and the lack of a dietary effect on AID of starch coincided with similar amylase and maltase activities in the jejunum. Chen et al. (2015) found that weaned piglets fed a diet containing 10% of wheat bran for 30 d had the lowest ATTD of GE, DM, OM and CP compared with pigs fed diets containing 10% of soybean or pea fiber, although they had greater jejunal villi length and greater digestive enzyme activities. Our results indicate that the AID and ATTD of nutrients in pigs fed the RSF diet did not correlate with the intestinal morphology features measured, but correlated with the digestive enzyme activities.

Taken together, from the observations on AID and ATTD of nutrients, enzyme activity, and intestinal morphology, the authors suggest that the decrease in nutrient digestibility in pigs fed RS co-products as compared with SBM may not be because of lack of adaptation in the GIT, but potentially caused by inhibition of enzyme activity and/or lack of enzyme-substrate interaction resulting from entrapped nutrients in the fiber matrix (Grundy et al., 2016), and/or interference with other ANF. Entrapment of nutrients seems to be commonly observed with insoluble fibers, of which RS hulls have a high content. Therefore, we speculate that selection of pig genotypes for improved digestibility when feeding high-fiber RS diets may give rise to pigs with greater ability to digest recalcitrant and insoluble fiber (Hedemann et al., 2006; Pustjens et al., 2014).

5. Conclusion

In conclusion, partial replacement of wheat and SBM with high-fiber RS co-products in pig diets increased the thyroid to BW ratio and reduced the AID and ATTD of most nutrients and energy. Individual variation in nutrient digestibility was observed among pigs within each dietary treatment. The reduction in nutrient digestibility was not associated with changes in ileal and/or colonic morphology, but correlated with a decrease in trypsin activity in the jejunum.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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644 **Figure captions**

645 **Figure 1**

646 Effects of feeding a high-fiber rapeseed diet (RSF) as compared to a soybean meal-based diet (Control)
647 for 3 wk on: (A) apparent ileal digestibility of crude protein (CP, n = 18 for Control and 20 for RSF) and
648 (B) apparent total tract digestibility of neutral detergent fiber (NDF, n = 20 for both groups) in Norwegian
649 Landrace weanling pigs.

650

651 **Figure 2**

652 Effects of feeding a high-fiber rapeseed diet (RSF) as compared to a soybean meal-based diet (Control) for
653 3 wk on: (A) ileum villi height (VH, n = 18 for Control and 20 for RSF); (B) ileum crypt depth (CD, n =
654 18 for Control and 20 for RSF); ileum VH:CD (n = 18 for Control and 20 for RSF); and (D) colon crypt
655 depth (n = 18 for Control and 20 for RSF) in Norwegian Landrace pigs. Measurements are presented in
656 pixels/inch, 11.5 pixels/inch = 500 μm.

Figures

Figure 1

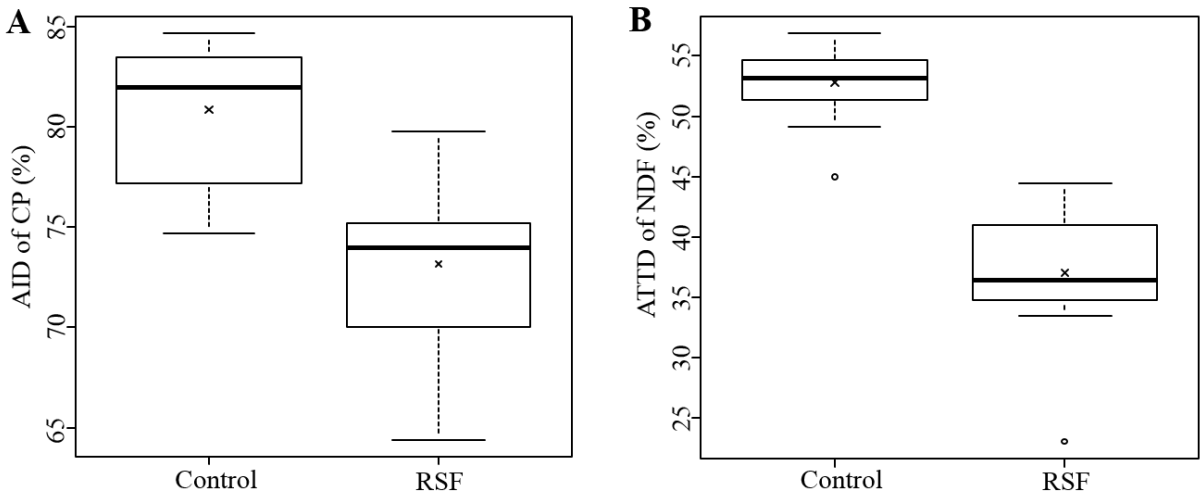
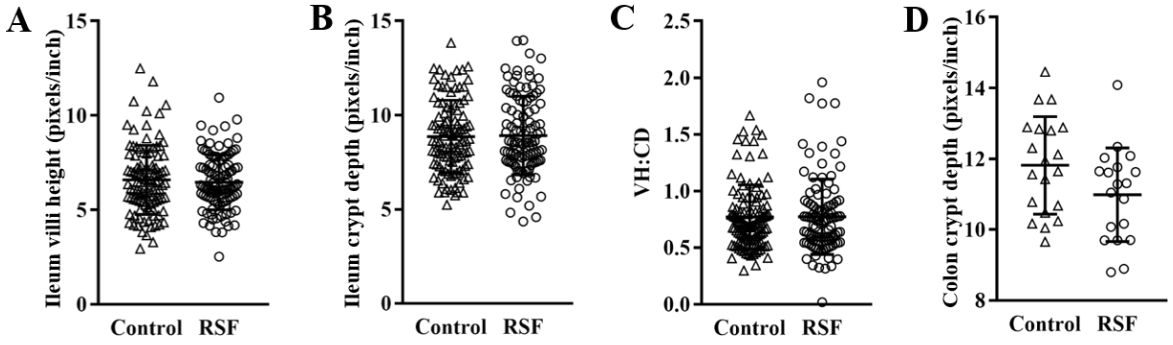


Figure 2



Tables

Table 1

Dietary composition of experimental diets.

	Dietary treatments[1]	
Ingredient, g/kg as-fed	Control	RSF
Wheat[2]	629.1	506.5
Barley[3]	100.0	100.0
Soybean meal[4]	140.0	30.0
Coarse rapeseed meal[5]	-	200.0
Rapeseed hulls[6]	-	40.0
Fish meal	40.0	40.0
Soybean oil	50.0	50.0
Monocalcium phosphate	16.4	9.1
Limestone	11.3	11.2
L-Lys·HCl	3.4	3.4
DL-Met	0.5	0.5
L-Thr	1.3	1.3
L-Trp	0.2	0.2
Sodium chloride	4.0	4.0
Vitamin-trace mineral premix[7]	3.2	3.2
Attractant[8]	0.5	0.5
Y_2O_3	0.1	0.1

[1]Control diet based on wheat and soybean meal; RSF = high-fiber rapeseed diet.

[2]Whole wheat: 86.4% dry matter (DM), 11.1% crude protein (CP), 1.6% ether extract (EE), 58.1% starch, 9.0% neutral detergent fiber (NDF), 2.2% acid detergent fiber (ADF), 1.4% ash.

[3]Barley: 86.2% DM, 7.4% CP, 1.3% EE, 53.5% starch, 16.0% NDF, 5.1% ADF, 1.6% ash.

[4]Soybean meal: 89.0% DM, 43.3% CP, 1.4% EE, 1.4% starch, 8.9% NDF, 5.7% ADF, 5.4% ash.

[5]Coarse fraction from an air-classified hexane-extracted rapeseed meal: 90.0% DM, 31.2% CP, 2.5% EE, 26.2% NDF, 18.6% ADF, 6.7% ash.

[6]Rapeseed hulls: 88.8% DM, 13.2% CP, 8.0% EE, 55.1% NDF, 48.6% ADF, 4.4% ash.

[7]Provided per kilogram of diet: 90 mg Zn (ZnO); 90 mg Fe ($FeSO_4$); 45 mg Mn (MnO); 19.5 mg Cu ($CuSO_4$); 0.45 mg I ($Ca(IO_3)_2$); 5700 IU vitamin A; 4500 IU cholecalciferol; 100.7 mg dl-α-tocopheryl acetate; 2.40 mg menadione; 9.0 mg riboflavin; 36.0 mg D-pantothenic acid; 12.0 µg cyanocobalamine; 12.0 mg niacin; 0.24 mg biotin; and 1.8 mg folic acid.

[8]Maxarome; Felleskjøpet, Kambo, Norway.

Table 2

Analyzed chemical concentrations of experimental diets.

	Dietary treatments[1]	
Item, g/kg of DM	Control	RSF
Gross energy, MJ/kg	17.6	17.8
Dry matter, g/kg	908.4	906.1
Crude protein	201.8	201.8
Ether extract	79.2	87.7
Starch	402.1	370.8
Neutral detergent fiber	113.0	154.8
Acid detergent fiber	41.8	82.3
Ash	57.0	60.8
P	9.7	8.7
Y	0.1	0.1
Amino acid		
Ala	8.9	9.1
Arg	11.5	11.1
Asp	17.1	15.3
Cys	3.7	4.5
Glu	43.6	41.3
Gly	9.5	10.1
His	5.0	5.1
Ile	8.4	8.3
Leu	14.7	14.4
Lys	12.8	13.2
Met	4.0	4.3
Phe	9.0	8.2
Pro	14.7	15.0
Ser	10.4	10.0
Thr	9.4	10.4
Trp	2.8	2.7
Tyr	5.1	5.2
Val	9.2	9.8
Total amino acids	199.8	198.0
Monosaccharides		
Arabinose	13.3	20.2
Fucose	4.2	4.2
Galactose	12.2	11.1
Glucosamine	0.4	0.7
Total glucose	588.9	457.7
Rhamnose	1.1	2.3
Xylose and Mannose	19.7	18.8
Total glucosinolates, mmol/kg	-	1.0

[1]Control diet based on wheat and soybean meal; RSF = high-fiber rapeseed diet.

Table 3

Effects of feeding a high-fiber rapeseed diet for 3 wk on apparent ileal digestibility, total tract digestibility, and hindgut disappearance of nutrients, energy and phosphorous in Norwegian Landrace weanling pigs[1].

	Dietary treatments[2]		
Item	Control	RSF	*P*-value
Apparent ileal digestibility, %			
Dry matter	72.8 ± 3.8	65.7 ± 3.7	<0.001
Organic matter	75.1 ± 3.5	68.2 ± 3.5	<0.001
Gross energy	76.1 ± 3.2	69.6 ± 3.5	<0.001
Starch	95.8 ± 1.9	95.7 ± 2.3	0.941
Neutral detergent fiber	20.0 ± 12.8	7.4 ± 14.0	0.009
Acid detergent fiber	29.9 ± 13.1	-4.0 ± 12.2	<0.001
P	55.8 ± 6.6	47.1 ± 5.0	<0.001
Apparent total tract digestibility, %			
Dry matter	85.9 ± 0.8	79.2 ± 0.9	<0.001
Organic matter	87.9 ± 0.9	81.3 ± 1.0	<0.001
Gross energy	85.9 ± 1.0	79.5 ± 1.1	<0.001
Crude protein (N × 6.25)	84.4 ± 1.8	77.6 ± 1.8	<0.001
Ether extract	84.5 ± 2.1	84.2 ± 2.1	0.609
Starch	99.6 ± 0.1	99.5 ± 0.1	0.010
Acid detergent fiber	39.2 ± 6.4	10.3 ± 5.3	<0.001
P	57.2 ± 2.0	48.3 ± 2.3	<0.001
Hindgut disappearance,[3] %			
Dry matter	12.9 ± 3.9	13.6 ± 3.7	0.630
Organic matter	12.6 ± 3.7	13.1 ± 3.5	0.691
Gross energy	9.6 ± 3.5	9.8 ± 3.5	0.835
Crude protein (N × 6.25)	4.2 ± 2.7	4.7 ± 3.8	0.644
Starch	3.8 ± 2.0	3.8 ± 2.3	0.955
Neutral detergent fiber	31.6 ± 13.8	29.4 ± 15.1	0.643
Acid detergent fiber	9.0 ± 14.6	14.2 ± 13.7	0.249
P	1.5 ± 6.3	1.2 ± 5.7	0.871

[1]Values are least-squares means ± standard deviation of the mean, n = 20 except for apparent ileal digestibility and hindgut disappearance values for the control group where n = 18.

[2]Control diet based on wheat and soybean meal; RSF = high-fiber rapeseed diet.

[3]Hindgut disappearance = apparent total tract digestibility – apparent ileal digestibility of nutrients or energy.

Table 4

Effects of feeding a high-fiber rapeseed diet for 3 wk on apparent ileal digestibility of amino acids and apparent total tract digestibility of monosaccharides in Norwegian Landrace weanling pigs[1].

	Dietary treatments[2]		
Chemical constituent	Control	RSF	*P*-value
Apparent ileal digestibility, %			
Ala	78.7 ± 4.5	75.2 ± 4.9	0.028
Arg	87.2 ± 2.1	83.1 ± 3.2	< 0.001
Asp	77.1 ± 4.2	70.2 ± 4.6	< 0.001
Cys	76.5 ± 4.3	69.3 ± 5.0	< 0.001
Glu	88.7 ± 2.2	85.8 ± 2.9	0.002
Gly	69.1 ± 6.6	63.4 ± 7.7	0.018
His	84.7 ± 2.5	80.7 ± 3.1	< 0.001
Ile	84.3 ± 3.1	78.2 ± 3.6	< 0.001
Leu	84.9 ± 2.7	80.2 ± 3.6	< 0.001
Lys	87.1 ± 2.8	81.2 ± 3.6	< 0.001
Met	86.8 ± 3.8	84.8 ± 3.4	0.15
Phe	84.4 ± 2.8	79.7 ± 3.4	< 0.001
Pro	82.0 ± 5.5	73.2 ± 7.1	< 0.001
Ser	83.9 ± 2.9	77.8 ± 2.8	< 0.001
Thr	81.4 ± 3.5	75.8 ± 2.6	< 0.001
Trp	79.8 ± 3.5	72.2 ± 4.9	< 0.001
Tyr	84.9 ± 3.6	78.3 ± 3.5	< 0.001
Val	81.7 ± 3.1	75.3 ± 3.6	< 0.001
Total amino acid	83.5 ± 3.0	78.3 ± 3.6	< 0.001
Apparent total tract digestibility, %			
Arabinose	57.6[b] ± 4.4	71.8 ± 3.3	< 0.001
Fucose	94.0[a] ± 0.7	91.4 ± 1.1	< 0.001
Galactose	92.2[a] ± 0.9	83.2 ± 1.8	< 0.001
Glucosamine	-128.6 ± 33.6	-69.7 ± 25.4	< 0.001
Glucose	99.9 ± 0.1	99.7 ± 0.1	< 0.001
Rhamnose	55.5 ± 8.9	63.3 ± 5.4	< 0.001
Xylose and mannose	81.4 ± 2.9	80.7 ± 3.3	0.472

[1]Values are least-squares means ± standard deviation of the mean, n = 20 except for apparent ileal digestibility and hindgut disappearance values for the control group where n = 18.

[2]Control diet based on wheat and soybean meal; RSF = high-fiber rapeseed diet.

Table 5

Effects of feeding a high-fiber rapeseed diet for 3 wk on jejunal digestive enzyme activities in Norwegian Landrace weanling pigs[1].

	Dietary treatments[2]			
Enzyme activities, U/mg protein	Control	RSF	SEM	*P*-value
Trypsin	4,468	3,231	392	0.030
Amylase	31,241	25,942	4,469	0.396
Maltase	1.5	1.4	0.1	0.881

[1]Values are least-squares means and pooled standard error of the mean (SEM), n = 20.
[2]Control diet based on wheat and soybean meal; RSF = high-fiber rapeseed diet.