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ANTONIO DIEGO BRANDÃO MELO

EVALIATION OF FEED TECHNOLOGIES IN RAPESEED MEAL AND THEIR EFFECTS ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF GROWING PIGS

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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal, área de concentração Saúde, Tecnologia e Produção Animal, da Escola de Ciências da Vida da Pontifícia Universidade Católica do Paraná, para obtenção do título de Doutor em Saúde, Tecnologia e Produção Animal Integrada.

Orientador: Prof. Dr. Leandro Batista Costa

Coorientador: Dr. Rosil Lizardo

CURITIBA

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TERMO DE APROVAÇÃO

Pontifícia Universidade Católica do Paraná
Programa de Pós-Graduação em Ciência Animal
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PUCPR
GRUPO MARISTA

ATA Nº 0002 E PARECER FINAL DA DEFESA DE TESE DE DOUTORADO EM CIÊNCIA ANIMAL DO ALUNO ANTONIO DIEGO BRANDÃO MELO

Aos dez dias do mês de maio do ano de dois mil e dezenove, às 8horas, realizou-se na sala Tele Saúde, 1º andar, Bloco Verde, da Pontifícia Universidade Católica do Paraná, localizada no Campus de Curitiba, Rua Imaculada Conceição, nº 1155, Prado Velho – Curitiba – PR, a sessão fechada de defesa da tese do doutorando Antonio Diego Brandão Melo, intitulada: **"EVALUATION OF FEED TECHNOLOGIES IN RAPESEED MEAL AND THEIR EFFECTS ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY OF GROWING PIGS"**. O doutorando concluiu os créditos exigidos para obtenção do título de Doutor em Ciência Animal, segundo os registros constantes na secretaria do Programa. Os trabalhos foram conduzidos pelo Professor orientador e Presidente da banca, Dr. Leandro Batista Costa (PUCPR), auxiliado pelos Professores Doutores Paulo Levi de Oliveira Carvalho (UNIOESTE), Maicon Sbardella (UFMT), Aline Mondini Calil Racanicci (UNB) e a Doutora Carla de Andrade. Procedeu-se à exposição da tese, seguida de sua argüição pública e defesa. Encerrada a fase, os examinadores expediram o parecer final sobre a tese, que foi considerada aprovada.

MEMBROS	ASSINATURA
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Drª Carla de Andrade	Carla de Andrade

Proclamado o resultado, o Presidente da Banca Examinadora encerrou os trabalhos, e para que tudo conste, eu Caroline Nocera Bertton, confiro e assino a presente ata juntamente com os membros da Banca Examinadora.

Curitiba, 10 de maio de 2019.

Caroline Nocera Bertton

Caroline Nocera Bertton
Secretária do Programa de Pós-Graduação em Ciência Animal

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TABLE OF CONTENTS

“**Meu discurso é igual ao meu percurso**”

Elly Brandão Gomes

Dedico esta tese ao meu irmão Elly Brandão Gomes (*in memorian*); a sua resiliência sempre será lembrada por mim.

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PREFACE

This thesis is composed of 3 chapters. Chapter 1 presents a general introduction, the context of the theme or justification, if any, and the study objectives. Chapter 2 is a complete scientific article, containing references, and formatted according to the journal instructions for which it will be submitted for peer-review. Chapter 3 is a complete scientific article, containing references, and formatted according to the journal instructions for which it will be submitted for peer-review. The experiments developed in chapters 2 and 3 are part of the EU-funded Feed-a-Gene project. Due to the participation of foreign members in the research team that developed these experiments, the thesis was written in English.

GENERAL ABSTRACT

Rapeseed meal (RSM) is the most representative protein source produced in EU. Improvement of its nutritional value is a potential strategy to valorize its use in animal feed and to reduce EU dependency on protein feed imports. RSM is an industry by-product then solvent oil extraction that contains large amounts of protein but also contains a high fiber content that limit its use to pigs. In this scrutiny, the use of feed technologies can become RSM more attractive for animal feeding. After tail-end dehulling process or fermentation, conventional RSM (RSM-) can present higher protein content and reduced fiber concentration (RSM+) and improving protein and amino acids (AA) digestibility. Furthermore, in view of the high content of fiber fractions, including non-starch polysaccharides (NSPs), a more efficient energy and protein utilization could be promoted by the inclusion of exogenous enzymes able to hydrolyze indigestible fractions. Also, pelleting process (agglomerating particles) could efficiently contribute to improve feed efficiency, enhancing RSM use in diets of pigs. However, factors such as feed ingredients, die specification and steam conditioning have effect on pellety quality and there is a lack of information of the influence of these factors on diets based on different types of RSM. In this way, it was performed two experiments using growing pigs to evaluate the *in vivo* value of RSM+ compared to RSM- and their response to feed technologies (i.e. use of exogenous enzymes and pelleting conditions). The first experiment evaluated the potential benefits of RSM+ in combination with exogenous protease and/or NSPase enzymes in diets for growing pigs on growth performance and nutrient digestibility. The second one evaluated the growth performance and nutrient digestibility of growing pig´s diet based on RSM+, combined with the effect of die size and steam from pelleting process. In the both experiments RSM+ presented a greater feed efficacy due to an improved feed conversion ratio. Furthermore, RSM+ promoted a greater body weight gain when included in the same levels of RSM- without a balanced supplementation of indispensable amino acids. The addition of exogenous enzymes did not affect animal growth performance, neither improved the digestibility of protein, energy, or NSPs. Due to the higher nutritional value of RSM+ compared to RSM-, RSM+ diets were able to improve feed efficacy and growth performance of growing pigs. Steam conditioning and ideal specification of die size are essential to produce high-quality pellets that can positively affect feed efficacy and nutrient digestibility. Mineral availability was improved by the combination of RSM- plus enzymes, without affecting animal growth performance. RSM+ diets had a better feed efficacy for growing pigs making this feedstuff more attractive to monogastric animals. A higher nutrient content and availability such as found in diets based on RSM+ is crucial to improve animal performance and it was enhanced depending the pelleting condition used to feed agglomeration.

Keywords: dehulling, enzymes, monogastric, nutrition, pelleting, protein, rapeseed meal.

CHAPTER 1

GENERAL INTRODUCTION

In the worldwide, the bioethanol is the major produced biofuel, while the biodiesel is the main biofuel produced in European Union (OECD/FAO, 2016). Biodiesel is a natural fuel composed by methyl esters of long chain fatty acids produced from renewable sources, such as animal fats and vegetables oils (Dworakowska et al., 2011). A stable biodiesel can be achieved thought the form of pure methyl esters of long chain fatty acids from bio-components or containing up to 30% of bio-components in a mixture with diesel fuel.

The main oilseeds produced worldwide intended to biodiesel production are soya bean and rapeseed. It is clear that if the production of biofuels tends to increase in European Union (EU), the production of rapeseed residues also tends to increase, and an adequate fate to these residues is very important to avoid environmental concerns. The oil is extracted from the seeds by hexane solvent, and a cake is produced as a remained residue. The cake remained is processed to originate soya bean (SBM) and rapeseed (RSM) meals, that are the main protein sources used to fed animals, being the RSM largely produced in EU.

The consumer demand for meat tends to increase worldwide in the next years, especially in development countries, which will result in the increase demand of protein supplies for animal feed. Currently, the animal production in EU relies on protein imports to satisfy the demand of the animal feed industry (FEFAC, 2015), in which about half of oilseed annually imported are used to fed animals (European Commission, 2018). These imports are subject of increasing concerns and the use of alternative protein sources, especially grown in EU, is strongly recommended. Furthermore, it has been emphasized the potential contribution of new technologies, research and innovation for the viable development of protein plant in EU (European Commission, 2018).

The greater features observed in soybean meal to fed animals is the large amount and availability of protein provided, the superior amino acid profile to the animal and the reduced number of antinutritional factor contained in this feedstuff. RSM also contains large amounts of protein content available for animals, but also contains a relatively high concentration of antinutritional factors (ANFs) that limit its use to non-ruminant animals (Kracht et al., 2004).

In general, glucosinolates are the main ANF contained in rapeseed, which present potent goitrogenic effects that can constraint the productive performance of the animals

(Labalette et al., 2011). In the case of soya bean, it presents trypsin inhibitor as main ANF, which can affect protein availability to the animals.

Contrarily for glucosinolates contained in rapeseed, the content of trypsin inhibitor in soya bean can decrease by thermal treatments such as steam cooking, once glucosinolates are less heat-sensitive than trypsin inhibitor (Barba et al., 2016; Csapó and Albert, 2018). For reduce this concern in rapeseed, non-genetic modified plant breeding in EU has produced double-zero varieties of rapeseed containing low content of glucosinolates.

Although producing seeds with low content of glucosinolates, the high levels of fiber fraction can compromise the availability of nutrients in RSM, especially high costly nutrient such as amino acids, energy and phosphorus. To become this feedstuff more attractive to animal feed industry, some technologies have been approached and investigated aiming improve nutritive value of RSM, such as dehulling meal, solid state fermentation by microorganisms, and the use of exogenous enzymes (Mc Alpine et al., 2012; Ahmed et al., 2014; Mejicanos et al., 2016).

The reduction of fiber fraction by dehulling RSM has been pointed to improve protein, AA and phosphorus digestibility (Hansen et al., 2017; Mejicanos et al., 2018). Exogenous enzymes are a technological tool that can aid the use of co-products in pig´s diet improving nutrient digestibility possible compromised with elevated dietary fiber and antinutritional factors (Mc Alpine et al., 2012). Solid state fermentation of canola meal by microorganisms (*Aspergillus ficuum*, *Rhizopus oligosporus*, *Lactobacillus salivaris*) has been reported to reduce these undesirable factors (GLS, fiber, phytic acid) and increase protein content (Nair et al., 1990; Vig and Walia, 2001; Ahmed et al., 2014). Enzymes such as carbohydrases may hydrolyze non-starch polysaccharide and improve energy use by the pig. In addition, the inclusion of proteases could also aid the digestion of some proteins that, in this way, are not digested by endogenous enzymes into the intestine (O´Shea et al., 2014).

Besides RSM, animal diets are formulated using other ingredients with varied chemical structure and complexity, especially in terms of fibrous fractions (Liu et al., 2016), which can alter the response of each diet to the processing. In this way, there is a lack of information if nutrients of diets based on rich-fiber feedstuff (i.e. RSM, wheat) can be better availed by pigs after pelletizing.

The present study evaluated potential benefits of processed high-protein RSM in combination with protease and/or NSPase enzymes in diets for growing pigs on growth performance and nutrient digestibility. Furthermore, under the same responsive criteria, it was evaluated the influence of pelleting process (die size and steam) in diets based on conventional or high protein rapeseed meal.

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CHAPTER 2

EFFECT OF HIGH PROTEIN RAPESEED MEAL AND ENZYME SUPPLEMENTATION IN DIETS FOR GROWING PIGS ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY

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Abstract

This study evaluated the influence of rapeseed meal (RSM) with higher protein content on the growth performance and apparent total tract digestibility (ATTD) of nutrients in growing pigs, and compared it with a conventional RSM, with or without enzyme supplementation. One hundred and forty-four pigs were used in the study. Each pen was considered an experimental unit to which two individuals (one entire male and one female) were allocated. The animals were randomly distributed into 12 blocks, in accordance to its initial body weight (33.3 ± 0.57 kg), resulting in 12 replicates per treatment. A 2 × 3 factorial arrangement of diets containing conventional (35% Crude Protein (CP), RSM-) or high-protein (40% CP, RSM+) RSM with or without supplementation of protease and/or carbohydrases was carried out. Diets were formulated to provide similar energy content and standardized ileal digestibility of lysine. The trial lasted 5 weeks and feces samples were collected for 3 days, during the fifth week. Growth performance and ATTD of nutrients were analyzed using the GLM procedure of SAS, adopting $P < 0.05$ as the significance level. Feed conversion ratio (FCR) was significantly improved ($P < 0.01$) in pigs that were fed RSM+ diets in consequence of a lower ($P < 0.01$) feed intake. No interaction ($P > 0.05$) was observed between RSM type and exogenous enzymes on the growth performance parameters. ATTD of ether extract, ash, and P was higher ($P < 0.05$) for RSM+ compared with RSM-, while arabinose digestibility was higher ($P = 0.04$) for RSM- compared with RSM+. There was interaction between the RSM type and enzyme supplementation for the ATTD of ether extract ($P = 0.03$), ash ($P = 0.01$), P ($P = 0.02$), and Ca ($P = 0.006$); the ATTD of these parameters were greater when RSM- was supplemented with enzymes (Protease or Protease+NSPase). There was no interaction between RSM type and enzyme supplementation on the ATTD of dry matter, energy, nitrogen, crude fiber, N-free extract, and other non-starch polysaccharides that were evaluated. In addition, neither the RSM type nor enzyme supplementations had any effect on these parameters ($P > 0.05$). The inclusion of high-protein RSM was effective in increasing the ether extract, ash, and P availability, resulting in better feed utilization. Thus, RSM+ improves feed efficiency of growing pigs making this a more attractive feed for pigs.

Keywords: monogastric, protease, NSPase, nutrition, swine.

Introduction

The contribution of soybean meal imports to the protein ensemble used in animal production in Europe (EU) represents 61% of the total rich-protein feed material used for this industry (de Visser et al., 2014). Therefore, the use of cost-effective protein sources can reduce the demand for protein imports in EU and increase the profitability of pig production. The increase in European oilseed crop production, such as rapeseed (*Brassica napus*), of which the EU is the world's leading producer, could therefore help reduce the dependence of these imports (Zander, et al., 2016).

Rapeseed meal (RSM), a by-product generated after crushing seeds for oil extraction by solvents, contains approximately 35% crude protein (CP) and a well-balanced amino acid profile (Mejicanos et al., 2016), which could be an alternative to soybean meal as feed for growing-finishing pigs (McDonnel et al., 2010). However, RSM also presents relatively high dietary fiber content (around 350 g/kg of fiber, 220 g/kg of non-starch polysaccharides (NSPs), and 130 g/kg of lignin (% of dry matter (DM)); Back-Knudsen, 2014). Its content of fiber is high especially due the high hull content outer seeds around 120-200 g/kg (Mińkowski, 2002; Kracht et al., 2004). Besides, RSM also contains some other anti-nutritional factors namely, glucosinolates (GLS) and phytic acid (Messerschmidt et al., 2014; Mejicanos et al., 2016).

The relatively high dietary fiber and phytic-P content (about 60% of the total P; NRC, 2012) in RSM can negatively interfere in the release and absorption of minerals, protein, and amino acids (Hansen et al., 2017). On the other hand, undigested NSPs carbohydrates are natural unused sugar source, which its absorption could contribute to improve monogastric energy utilization. Seeds presenting < 30 µmol/g are considered to contain low levels of GLS and surveys from France have reported a mean of 10 µmol/g of GLS in RSM between the years 2005 and 2010 (Labalette et al., 2011). Although GLS is present in low concentrations, depending on the product formed from its hydrolysis, it may promote a bitter taste in RSM and affect the functioning of the thyroid gland (Tripathi and Mishra, 2007). Together, anti-nutritional factors present in RSM can reduce feed intake, feed efficiency, and animal weight gain.

In view that, few studies had addressed some technologies to improve the content of CP and reduce fiber and other ANFs in order to make this feedstuff more attractive for EU´s production industry. For example, solid state fermentation of canola meal by microorganisms (*Aspergillus ficuum, Rhizopus oligosporus,* and *Lactobacillus salivarius*) has been reported to reduce these undesirable factors (GLS, fiber, and phytic acid) and increase the protein content (Nair et al., 1990; Vig and Walia, 2001; Ahmed et al., 2014). Moreover, supplementation of diet with exogenous enzymes is a technological tool that can aid in the use of co-products to fed pigs, once that pigs lack several enzymes to digest or degrade specific feed components or anti-nutritional fractions, respectively. IN this way, enzymes can improve nutrients digestibility in these co-products that are possibly compromised due to elevated dietary fiber and phytate (Mc Alpine et al., 2012). Further, a more efficient energy and protein utilization could be promoted by the inclusion of exogenous enzymes that are able to hydrolyze these fractions when non digestible in digestive tract of pigs (Zijlstra et al., 2010; de Vries et al., 2012; O´Shea et al., 2014). Development of able technologies to improve nutritive value of RSM to further use in animal industry as alternative to conventional ingredients could positively contribute to the quality of rich-protein meal produced in EU. We hypothesized that processing RSM increases its nutritional value and the supplementation with exogenous enzymes improves nutrient degradability. Therefore, to validate this hypothesis, the present study evaluated the potential benefits of a processed RSM in combination with protease and/or NSPase enzymes in diets for growing pigs on growth performance and nutrient digestibility.

Materials and methods

Animal care

This study was carried out at the Experimental farm of the "Institut de Recerca i Tecnologia Agroalimentàries - IRTA", and the assay protocols followed procedures recommended by ethical committees on animal experimentation.

Animals and housing

A total of one hundred and forty-four growing pigs (Pietrain × Large White × Landrace (Hypor); 72 entire males and 72 females; Body weight (BW): 38.8 ± 0.57 kg) from IRTA´s farm was housed in a facility with 72 pens. The animals were randomly distributed into 12 blocks in accordance to their initial body weight, resulting in 12 replicates per treatment. One entire male and one female pig were allocated to each pen, and each pen was considered an experimental unit. The animals had *ad libitum* access to pellet feed and water during the entire experimental period of 5 weeks. Pigs were weighed individually and feed consumption per pen was recorded at the end of the study.

Experimental diets

RSMs produced from non-GMO rapeseeds grown in Europe were selected from the main oilseed mills of Saipol Avril group (France) based on its nutritional content and shipped to an animal feed manufacturer (Hamlet Protein, Denmark). These batches of RSM were processed to improve the CP content from 35% to 40%, approximately. The conventional (RSM-, 35% CP) and the processed high protein (RSM+, 40% CP) batches of RSM were used to manufacture the experimental diets. The diets were formulated to be isocaloric and isonitrogenous, based on the metabolizable energy (ME) and ideal protein concept (Table 1) according to the nutritional requirements for growing pigs (NRC, 2012). Diets were based on barley-wheat-maize, and conventional or high protein RSM were included at 24 and 22%, respectively. The ME content was calculated as 0.79 of the gross energy (GE) values according to INRA (2002). Titanium dioxide (0.5%) was included as an indigestible marker for digestibility evaluation.

Experimental design and procedures

The activity of novel proteinase and/or NSPase enzymes (DuPont, Copenhagen, Denmark) was evaluated on *in vitro* tests to quantify the solubility of protein, concentration of free glucose and total reducing sugars from rapeseed meal. Based on the results of nutrient released from the substrate on *in vitro* tests, a proteinase (FaG-004) and/or a mixture of NSPases (FaG-013) were used *in vivo*. Experimental treatments corresponded to a 2 × 3 factorial arrangement of conventional or high-protein rapeseed meal (RSM- and RSM+), with or without supplementation of protease (66 g/MT, FaG-004, Danisco, Denmark) or protease plus a blend of NSPases (xylanase and β-glucanase, 250 g/MT, FaG-013, Danisco, Denmark). The enzymes were kept frozen until the day they were added into the feeds by a post-pellet liquid application. After applied on pellets, the activity of enzymes was weekly evaluated.

Samples of RSM- and RSM+ were collected for analysis of chemical composition before mixing the diet´s ingredients. The fecal samples were collected for 3 days in the fifth week of the experiment and oven-dried for 72 h at 60 °C before the nutrient digestibility was determined in the laboratory.

Table 1. Composition of diets based on conventional (RSM-) and high protein rapeseed meal (RSM+) for growing pigs

Ingredients (g/kg, as fed)	RSM-[1]	RSM+[2]
Barley	200.00	200.00
Wheat	136.40	213.40
Rice broken	140.00	140.00
Maize	130.10	85.80
Rapeseed meal (RSM-, 35% CP)	240.00	---
Rapeseed meal (RSM+, 40% CP)	---	219.70
Soybean meal	14.20	---
Starch	60.00	60.00
Fat	42.10	43.10
Monocalcium phosphate	8.50	8.90
Salt	1.80	1.20
Calcium carbonate	6.30	6.60
Sodium bicarbonate	3.50	4.40
L-Lysine-HCL	4.70	4.70
DL-Methionine	0.30	0.20
L-Threonine	1.4	1.3
L-Tryptophan	0.30	0.20
L-Valine	0.10	0.20
Noxyfeed®[3]	0.20	0.20
Post-pelleting solution[4]	6.00	6.00
Vitamins/minerals premix[5]	4.00	4.00
Calculated composition g/kg, as fed		
ME, Mcal/kg[6]	3181	3190
CP	152.5	157.5
NDF	144.3	142.3
ADF	68.2	64.7
CF	46.6	44.4
Ca	7.00	7.00
P	6.37	6.26
dig P	2.80	2.80
total Lys	10.45	10.53
SID Lys	8.90	8.90
SID Thr	5.79	5.79
SID Met	2.76	2.76
SID Trp	1.69	1.69
g dLys/MJ NE	0.945	0.936

[1]RSM-: diet based on conventional unprocessed rapeseed meal with 35% of crude protein. [2]RSM+: diet based on processed rapeseed meal by Hamlet Protein to improve crude protein (40% of CP). [3]Noxyfeed® (Itpsa, Barcelona, Spain): Synergistic combinations of antioxidants (BHT, BHA, propyl gallate, and synergistic substances). Products intended for protection of raw materials, feed, premixes, fats, and animal by-products. [4]Enzymes: solution of enzymes with protease (66 g/MT, FAG-004, Danisco, Denmark) or protease plus a blend of NSPases enzyme complex (250 g/MT, FAG-013, Danisco, Denmark). [5]Premix supplementation per kg of feed: vitamin A (E-672) 5,500 IU; vitamin D_3 (E-671) 1100 IU; vitamin E (alpha-tocopherol) 7 mg; vitamin B_1 0.5 mg; vitamin B_2 1.4 mg; vitamin B_6 1 mg; vitamin B_{12} 8 µg; vitamin K_3 0.5 mg; calcium pantothenate 5.6 mg; nicotinic acid 8 mg; choline 120 mg; Fe (E-1) 80 mg from $FeSO_4 + 7\ H_2O$; I (E-1) 0.5 mg from $Ca(I_2O_3)_2$); Co (E-3) 0.4 mg from $2\ CoCO_3 + 3\ Co(OH)_2 + H_2O$; Cu (E-4) 5 mg from $CuSO_4 + 5\ H_2O$; Cu (E-4) from amino acid-chelated minerals; Mn (E-5) 40 mg from MnO; Zn (E-6) 100 mg from ZnO; Se (E-8) 0.25 mg from Na_2SeO_3). [6]Metabolizable energy (ME) content was calculated as 0.79 of the gross energy (GE) values. CP: crude protein. NDF: neutral detergent fiber. ADF: acid detergent fiber. CF: crude fiber.

Table 2. Analyzed composition of conventional (RSM-) and high-protein RSM (RSM+)

Analyzed composition	RSM-[1]	RSM+[2]
DM, %	88.2	94.4
Ash, %	6.38	7.29
CP, %	35.0	40.0
CF, %	12.0	12.4
phytic P, %	3.45	1.79
ME, kcal/kg[3]	2439	2515
Glucosinolates, µmol/g	13.7	16.9

[1]RSM-: conventional unprocessed rapeseed meal with 35% of crude protein. [2]RSM+: processed rapeseed meal by Hamlet Protein to improve crude protein (40% of CP). [3]Metabolizable energy (ME) content was calculated as 0.79 of the gross energy (GE) values. DM: dry matter. CP: crude protein. CF: crude fiber.

Composition of RSM, and diets, and digestibility analysis

RSM- and RSM+ were analyzed for DM, ash, CP, crude fiber (CF), phytic P, GE, and GLS. Diets and feces samples were analyzed for DM, GE, nitrogen, ether extract, CF, ash, P, Ca, and NSPs according to AOAC (2000). Ca, P, and Ti contents from ash samples previously obtained were analyzed after acid digestion by inductively coupled plasma mass spectrometry (ICP-MS, LC Agilent® 1290 Infinity). Total starch was analyzed by colorimetry, as a result of glucose release after enzymatic activity, according to the procedure described by Willamil et al. (2012). Total NSPs were extracted from samples according to Englyst et al. (1994).

Table 3. Analyzed composition of diets based on conventional (RSM-) and high protein rapeseed meal (RSM+) for growing pigs

RSM type[1] **Enzymes**[2]	**RSM-** **No**	**RSM+** **No**	**RSM-** **Prt**	**RSM+** **Prt**	**RSM-** **Prt+ NSPase**	**RSM+** **Prt + NSPase**
Item (% wt/wt DM)						
DM	90.21	90.65	90.20	90.65	90.26	90.65
CP	14.61	15.61	15.03	15.51	14.98	15.87
CF	3.77	3.56	3.86	3.71	3.67	3.95
Ash	4.90	5.01	4.92	5.02	4.97	5.05
Ca	0.73	0.73	0.71	0.77	0.74	0.80
Total P	0.54	0.56	0.53	0.56	0.56	0.57
phytic P	0.26	0.21	0.27	0.21	0.27	0.22
ME, Mcal/kg[2]	3248	3269	3254	3263	3243	3276
Total NSPs	11.81	11.77	11.69	11.09	11.20	11.64
Ara, mol%	14.00	13.42	12.92	13.90	13.44	13.21
Xyl, mol%	20.30	20.10	19.29	20.81	19.92	19.65
Man, mol%	13.41	15.17	13.84	15.32	14.09	14.90
Gal, mol%	7.17	6.77	7.03	7.00	7.18	7.00
Glu, mol%	45.19	44.54	46.96	42.97	45.38	45.24
Protease activity U/kg	< 50	< 50	529	595	408	449
NSPase activity[3]	0.89	1.02	0.98	0.95	0.26	0.24

[1]RSM-: diet based on conventional unprocessed rapeseed meal with 35% of crude protein. RSM+: diet based on processed rapeseed meal by Hamlet Protein to improve crude protein (40% of crude protein). [2]ME: metabolizable energy content was calculated as 0.79 of the gross energy (GE) values, according to INRA (2002). DM: dry matter. CP: crude protein. CF: crude fiber. NSPs: Non-starch polysaccharides. Ara: arabinose. Xyl: xylose. Man: mannose. Gal: galactose. Glu: glucose. [3]NSPase activity was determined as disappearance of substrate after enzyme action.

Calculation and statistical analysis

Apparent total tract digestibility (ATTD) of the major nutrients was calculated according to the following equation: TTAD = $(1-(X_{feces}/X_{diet}) \times (M_{diet}/M_{feces})) \times 100$, where X_{feces} and X_{diet} are the nutrient concentrations in feces and the diet, respectively, and M_{diet} and M_{feces} are the marker (Ti) concentrations in the diet and feces, respectively.

Data per pen were analyzed using the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA), following the randomized block design previously reported. Means were subjected to Tukey's test to account for multiple comparisons. P values < 0.05 were considered statistically significant.

Results

Chemical composition of RSM, diets, and enzyme activity

The chemical composition of RSM- and RSM+ is presented in Table 2. The dry matter content averaged at 88.2% for RSM- and 94.4% for RSM+. The CF content was about 12.0% for both RSM- and RSM+. The concentration of phytic P was almost twice higher in the RSM+ than RSM- (3.45 vs. 1.79 g/kg). Based on the DM, the concentration of total GLS was slightly similar for RSM- (13.7 µmol/g) and RSM+ (16.9 µmol/g). The analyzed composition of diets based on conventional (RSM-) and high protein rapeseed meal (RSM+) for growing pigs is presented in Table 3, as well as the enzyme activity in the supplemented and non-supplemented diets. Protease and NSPase presented greater activity in all weekly analysis.

Pig´s growth performance

Table 4 presents the effects of RSM- or RSM+ supplemented with exogenous enzymes on growth performance of growing pigs. At the start of the trial, the pigs weighed around 38.88 kg and after 35 days reached 65.76 kg, showing an average daily weight gain (ADWG) of 768 g/d. However, no differences were observed on the ADWG or BW due to the type of RSM ($P > 0.05$) used or the type of enzymes ($P > 0.05$) included in the diets. The type of RSM showed a significant effect on the average daily feed intake (ADFI; $P < 0.01$) and feed conversion ratio (FCR; $P < 0.002$): pigs that were fed RSM+ diets showed a 70 g lower ADFI, improving their FCR by about 100 units relative to their count partners that were fed RSM- diets. Enzyme supplementation did not affect ($P > 0.05$) either of these growth performance parameters. Furthermore, there were no interactions ($P > 0.05$) between the type of RSM and enzyme supplementation on the growth performance or feed efficiency.

Table 4. Influence of high protein rapeseed meal (HPRSM) supplemented with digestive enzymes on growth performance of growing pigs

	Rapeseed meal[1]		Enzymes[2]			Statistical analysis[3]			
Treatments	**RSM-**	**RSM+**	**No**	**Prt**	**Prt+NSP**	**RSM**	**ENZ**	**R*EZ**	**RSD**
BW, Kg	38.88	38.89	38.75	38.80	39.10	0.97	0.75	0.62	1.71
ADFI, Kg	1.66	1.59	1.619	1.631	1.615	0.01	0.89	0.71	0.12
ADG, g	767	769	757	779	768	0.92	0.60	0.97	78.1
F:G	2.17	2.07	2.15	2.10	2.11	0.002	0.36	0.79	0.12
BW(35d), kg	65.73	65.81	65.24	66.80	65.98	0.93	0.67	0.88	3.54

[1]RSM-: effect of diet based on conventional unprocessed rapeseed meal with 35% of crude protein on the evaluated parameters. RSM+: effect of diet based on processed rapeseed meal by Hamlet Protein to improve crude protein (40% of CP). [2]Enzyme supplementation: No enzymes: RSM type without enzyme supplementation; Prt: RSM type and protease supplementation; Prt+NSP: RSM type and protease plus NSPase supplementation. [3]RSM: P values for the effect of rapeseed meal type (RSM- vs. RSM+); ENZ: P values for the effect of enzymes supplementation (No, Prt, and Prt+NSPase); RSM*ENZ: P values for the interaction between RSM (rapeseed meal type, RSM- and RSM+) and ENZ (enzyme supplementation, No, Prt, and Prt+NSPase), and; RSD: Root standard deviation. *P* values<0.05 were considered statistically significant. BW: body weight. ADFI: average daily feed intake. ADWG: average daily weight gain. F:G: feed conversion ratio.

Apparent total tract digestibility

Table 5 presents the ATTD of nutrients of diets based on RSM- or RSM+ supplemented with exogenous enzymes. ATTD of EE (80.4 vs. 81,5%, P = 0.02), ash (45.2 vs. 46.9%, P=0.04), and P (42.0 vs. 47.4%, P = 0,01) was higher for diets based on RSM+ compared to diets based on RSM-. Digestibility of arabinose was higher (P = 0.04) for RSM- compared with RSM+ (74.7 vs. 73.1%). Effects of enzymes supplementation were no observed on the ATTD of nutrients. There was interaction (P < 0.05) between the RSM type and enzyme supplementation for the ATTD of EE (P = 0.03), ash (P = 0.01), P (P = 0.02), and Ca (P = 0.006): the ATTD of these parameters were greater when RSM- was supplemented with enzymes (Prt or Prt+NSPase). Neither the RSM type nor enzyme supplementations showed any effect on the digestibility of DM, energy, nitrogen, CF, N-free extract (NFE), and most NSPs evaluated. In addition, there was no interaction between the RSM type and enzyme supplementation on the digestibility of these parameters (P > 0.05).

Table 5. Influence of RSM treatment and enzyme inclusion on main nutrient total tract digestibility of growing pigs

	Rapeseed meal[1]		Enzymes[2]			Statistical analysis[3]			
Treatments	**RSM-**	**RSM+**	**No**	**Prt**	**Prt+NSP**	**RSM**	**ENZ**	**R*EZ**	**RSD**
DM	84.04	84.06	83.97	84.07	84.14	0.92	0.90	0.08	1.29
OM	86.36	86.24	86.23	86.29	86.38	0.71	0.90	0.10	1.19
Energy	85.29	85.11	85.14	85.20	85.27	0.57	0.94	0.08	1.30
Protein	82.92	82.30	82.36	82.64	82.83	0.25	0.75	0.12	2.11
Ether extract	80.36	81.53	80.71	81.01	81.12	0.02	0.84	0.03	2.39
Crude fiber	39.93	39.20	39.93	39.39	39.37	0.57	0.92	0.35	5.18
NFE	91.61	91.60	91.57	91.59	91.66	0.95	0.90	0.15	0.76
Ash	45.18	46.89	45.63	46.46	46.01	0.04	0.72	0.01	3.37
Phosphorus	42.03	47.40	44.50	45.03	44.62	0.0001	0.91	0.02	4.23
Calcium	46.36	48.15	45.99	47.72	48.05	0.17	0.36	0.006	5.13
total NSP	65.22	64.16	65.37	64.28	64.41	0.29	0.62	0.53	3.86
Arabinose	74.70	73.17	74.53	73.27	73.99	0.04	0.38	0.54	2.90
Xylose	54.92	55.61	55.78	54.47	55.56	0.63	0.72	0.70	5.66
Mannose	87.71	87.41	87.88	87.50	87.29	0.48	0.49	0.33	1.66
Galactose	71.08	70.86	71.55	70.26	71.10	0.79	0.47	0.14	3.41
Glucose	58.08	56.35	58.14	56.67	56.83	0.15	0.53	0.22	4.69

[1]RSM-: effect of diet based on conventional unprocessed rapeseed meal with 35% of crude protein on the evaluated parameters. RSM+: effect of diet based on processed rapeseed meal by Hamlet Protein to improve crude protein (40% of CP). [2]Enzyme supplementation: No enzymes: RSM type without enzyme supplementation; Prt: RSM type and protease supplementation; Prt+NSP: RSM type and protease plus NSPase supplementation. [3]RSM: P values for the effect of rapeseed meal type (RSM- vs. RSM+); ENZ: P values for the effect of enzymes supplementation (No, Prt, and Prt+NSPase); R*EZ: P values for the interaction between RSM (rapeseed meal type, RSM- and RSM+) and ENZ (enzyme supplementation, No, Prt, and Prt+NSPase), and; RSD: Root standard deviation. *P* values<0.05 were considered statistically significant. DM: dry matter. OM: organic matter. GE: gross energy. CP: crude protein. EE: ether extract. CF: crude fiber. NFE: N-free extract. Total NSPs: total non-starch polysaccharides.

Discussion

Due to the increasing interest in oil production for biodiesel generation, rapeseed has been widely produced in EU. Rapeseed meal, the by-product resulting after oil extraction is extensively used as protein source in diets for pigs. However, in comparison with soybean meal, RSM presents lower CP content (35% vs. 47% CP) and higher CF content (11.0% vs. 3.5% CF) (Zduńczyk et al., 2013). In this way, an increase in the CP content and a reduction in the fiber fraction could make RSM more attractive to the animal feed industry. A process for improve nutritional value of RSM is solid state fermentation by microorganisms (Mejicanos et al., 2016), which produce suitable enzymes to release and degrade specific components in a solid matrix (Martins et al., 2011), producing peptides and amino acids, releasing complexed P, converting complex carbohydrates to monosaccharides and degrading GLS.

In terms of improvement in nutritional value, the processed RSM+ used in the current experiment presented 50.0 g/kg more CP content. In addition, considering the methods of analysis and standard deviation, GLS concentration in RSM was not affected by the procedures maybe due to a short-term degradation of glucose and sulfur fractions by microbial enzymes. Nevertheless, Ahmed et al. (2014) reported increased CP (41.2% vs. 42.2% CP) and reduced GLS (22 vs. 13.6 µmol/g GLS) in fermented canola meal by *Lactobacillus salivarius* compared to non-fermented canola meal. The effect of solid-state fermentation by strains of *Rhizopus oligosporus* on rapeseed meal after 10 days also reduced GLS (6.3% vs. 3.6% GLS) and phytic acid (2.9% vs. 1.7%), while it increased the CP (27.1 vs. 44.8%) (Vig and Walia, 2001). Phytic P was reduced in RSM+ corroborating with results reported for fermented RSM (Vig and Walia, 2001). Although the NSPs content in RSM types had not been evaluated, the content of NSPs in the diets was not different.

The diets based on RSM+ presented improved FCR due to a reduction in feed intake. Voluntary feed intake is often dependent on the energy concentration, protein and amino acid content, and availability of nutrients (Li and Patience, 2017). Even in diets that were formulated to be isonitrogenous and isocaloric, a slightly higher level of CP for diets based on RSM+ is observed. In addition, improved RSM has been reported to have a greater digestibility of amino acids (Hansen et al., 2017). It is possible that RSM+ diets presenting a slightly improved content of CP and a greater digestibility of amino acids could, in part, contribute to the greater feed efficiency observed in the present study. Furthermore, due to the different concentrations of ingredients in the diets, the breakdown of the diets could result in the release of different nutrients, triggering a feedback mechanism in the functioning of glucostatic or aminostatic response, which might increase feelings of satiety (Mc Alpine et al., 2012). Although presenting similar quantity of NSPs in the diets, it is possible that some types of soluble NSP in RSM+ diets interacted with the intestinal mucus and resulted in the production of a layer that delayed the transport of breakdown products from lipid digestion (Back-Knudsen et al., 2016). Such delays in nutrient transport potentially may also occur with protein-based compounds, and by rising the time of digesta in the gut, may increase feeling of satiety mediated by gastrointestinal hormones. Considering the improved feed convention ratio for diets based on RSM+, the process applied on RSM efficiently improved diet utilization by growing pigs.

GLS are plant secondary metabolites that are composed of sulfur groups linked to glucose and an alkyl, aralkyl, or indolyl side chain (Barba et al., 2016). The hydrolyzed products of GLS from RSM might enhance the bitter taste of diets and impair thyroid functioning, affecting diet palatability and animal metabolism, respectively. The level of GLS can differ due to the technological tool applied to concentrate CP in the RSM+ (mechanical or biological) or due to an intense heat treatment on RSM- that can reduce its content by affecting myrosinase activation (Messerschmidt et al., 2014; Barba et al., 2016). It was reported that the growth performance of pigs is not affected by *B. napus* based diet containing GLS levels up to 2.5 µmol/g (Woyengo et al., 2016). Further, in another study, despite the inclusion of RSM in the diet, the growth performance of growing pigs was not affected when a diet with similar GLS levels (4.09 µmol/g) was provided during the first 6 weeks of the study (Choi et al., 2015). In addition, RSM diet with 6.29 µmol/g GLS, did not reduce ADWG, ADFI, FCR, or the final BW of growing pigs fed for 35 days when compared to pigs fed soybean-based diet (Little et al., 2015). These studies have shown that even presenting similar or higher concentrations of GLS (4.09 to 6.29 µmol/g) than the diets provided in the present experiment (around 3.5 µmol/g GLS in the diet) did not affect the growth performance of the animals after 5 weeks of feeding. Further the concentration of GLS in the diets and their harmful effect is also dependent on the hydrolyzed products formed after myrosinase action. Given that in the present study, the GLS level in RSM- and RSM+ did not affect the feed efficiency of the diets provided, the maximum concentration of GLS to include RSM in the diets is difficult to calculate based on literature alone.

The use of exogenous enzymes in the diets is expected to provide additional energy, protein, and amino acids resulting in cost-effective and a more sustainable pork production system (Zijlstra et al., 2010; de Vries et al., 2012; O'Shea et al., 2014). The common feature of the feedstuffs (barley, wheat, rye, and rapeseed) used to compose the diets in the present study is the high content of NSPs and lignin, which resulted in the diets being higher in these content when compared to diets based on corn and soybean (Back-Knudsen, 2014). Nevertheless, the fiber fractions of these feedstuffs present a complex chemical and structural composition and organization, resulting in poor intestinal fermentation in pigs. In view of the challenge to improve nutrient utilization in these diets, the effect of processed RSM and endogenous enzymes was evaluated. RSM- diet plus enzymes showed a beneficial impact on the ATTD of EE, ash, P, and Ca. It is not clear if proteases and NSPase enzymes can directly improve the availability of P and Ca in the diets but may be suggested that enzymes can act in reducing digesta viscosity contributing to improving the digestibility of macronutrients. No interaction between RSM type and enzyme supplementation was observed to affect the protein or NSP digestibility.

Growing-finishing pigs present a completely mature digestive system and the pancreas or small intestine segregate enough endogenous enzymes to hydrolyze the substrates, especially proteins or polypeptides, and the action of exogenous enzymes could be less effective in these stages of pig production. On the contrary, it also may be suggested that exogenous enzymes contributed to spare the activity of endogenous enzymes and hence reduced the requirement for endogenous enzyme activity. Also, the lack of effect of proteases on the ATTD of crude protein may be a consequence of having formulated diets with the same content of digestible CP and AA. Moreover, the efficiency of the use of NSPases depends enormously on the structural organization and degree of lignification of the fibers (Back-Knudsen, 1997; 2014). These factors are related to feedstuffs used to compose the experimental diets and, when taken together, can explain why NSPs and protein digestibility were not enhanced by exogenous enzymes.

The NSPs present in germ and cotyledon of rapeseed are potential targets to exogenous NSPases, while hulls, due its rigid structure, are very resistant to degradation. Processing RSM by biological (fermentation) procedures could reduce complex structures present in the meal, thus aiding the enzyme action. The process applied on RSM increased the availability of EE, ash, and P while exogenous enzymes promoted similar effect on these parameters for RSM-. However, as previously explained, NSPs and protein in diets based on RSM- or RSM+, irrespective of supplementation with exogenous enzymes, were equally digested by growing pigs. The increased digestibility of EE, ash, P, and Ca promoted by the

RSM- and enzyme supplementation point out that the ATTD of these parameters resembling the results observed to RSM+ diet. Thus, an increase in the nutrient content in the diet was observed as a result of adding enzymes to diets with reduced nutritional value.

Conclusion

Processing RSM to increase protein content improves feed efficiency of growing pigs and can positively contribute to reducing the protein imports of the European Union for feeding animals. The processing applied to obtain a high-protein RSM was effective in increasing EE, ash, and P availability, resulting in better feed utilization. Exogenous protease and NSPases supplementation did not result in improved nutrient availability in diets based on RSM; however, interaction between RSM- and enzymes had improved ATTD of EE, ash, P, and Ca. The tested enzymes were not efficient in increasing the growth performance of the pigs or the ATTD of protein and NSPs in the diets based on conventional or processed RSM. Further studies could focus on the use of proteases and NSPases on RSM based diets with reduced nutrient content, aiming a compensatory effect of the enzymes to optimize nutrient utilization and a more cost-effective diet.

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CHAPTER THREE

TAIL-END DEHULLED RAPESEED MEAL IMPROVES GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN GROWING PIGS

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Abstract

A trial was designed to investigate the effect of pellet processing (steam and die size) on diets based on RSM- grown in the EU and the same RSM- after tail-end dehulling process in order to increase its crude protein (CP) content. The experiment consisted of a 2x2x2 factorial design of two types of RSM (RSM-, 35% of CP or RSM+, 40% of CP), two dies with different length sizes (4x40 or 4x60 mm), and two steam conditions (no/yes). One-hundred forty-four growing pigs were randomly distributed by two (one entire male and one female) per pen into 9 blocks (9 replicates/treatment), in accordance to initial body weight (27.6 ± 0.60 Kg), fed by 7 weeks. Growth performance of growing pigs and nutrient digestibility were used as responsive criteria. There was interaction (*P*=0.04) between die size and steam; steaming pellets improved feed conversion ratio (FCR) on 4x40 die size. Average daily weight gain (ADWG) and final body weight (BW) were higher (P<0.01) for RSM+, as well as for 4x60 mm die size (P<0.01). Steamed pellets presented lower (P<0.05) average daily feed intake (ADFI), without affecting ADWG. In general, interaction was observed between RSM type, die size, and steam for the ATTD of fiber fractions and minerals; ADF (P=0.01), lignin (P=0.03), cellulose (P=0.03), ash (P=0.03), P (P=0.04), and Ca (P=0.002). Pellets from the diets based on RSM- presented improved digestibility of fiber fraction when conditioned with steam and 4x40 mm die. Pelletizing the diets based on RSM- without steam conditioning and 4x40 mm die reduced ATTD of ash and P, while no effect was observed suiting the die to the 4x60 mm. ATTD of Ca was improved for steamed pellets with 4x60 mm in diets based on RSM+. There was not interaction for the ATTD of amino acids between the tested pelleting conditions on diets based on RSM. In conclusion, feed efficiency of diets based on RSM can ameliorate when conditioned with steam and pelleted with 4x60 mm die and this pelleting condition was beneficial to enhance the digestibility of fiber fractions, ash and P in RSM- diets; animal growth, feed efficiency, and nutrient digestibility were longer better for diets based on RSM+ without influence of applied pelleting conditions.

Keywords: die size; feed processing; monogastric; nutrition; protein source

Introduction

Brassica napus, a representative oilseed grown in European Union (EU), has in its defatted byproduct the production of rapeseed meal (RSM), which is an important protein source fated to animal feed industry (Maison and Stein, 2014). RSM presents a relative high fiber content that negatively can affect its nutritional value and reduce inclusion levels when used for fed monogastric animals (Messerschmidt et al., 2014). Thus, the varieties of *B. napus*, presenting low levels of erucic acid and glucosinolates, has been processed to improve its protein content and reduce fiber fraction in the meal (Kracht et al., 2004; Zhou et al., 2013, 2015).

The selection of seeds of *Brassica napus* with reduced concentration of fiber and high content of protein has been an efficient method to produce improved high protein canola meal, approximating its nutritive value to the found in soybean meal (Liu et al., 2014; 2016). However, soybean meal, the main worldwide protein source for pig's nutrition, presents superior values and digestibility of protein and amino acids than meal from rapeseed meal. Thus, the development of techniques able to improve nutritional value of this feedstuff in a commercial scale is a potential strategy to valorize RSM for animal production and to reduce EU dependency on protein feed imports (FEFAC, 2015). The tail-end dehulling by fractionation has been reported as an efficient method to reduce fiber content in the meal, while concentrates the content of crude protein besides contributing to improve nutrient digestibility (Mejicanos et al., 2016; Hansen et al., 2017).

After processing seeds to produce a high-quality meal, other technological tool recurrently used in animal feed industry is pelletizing process then grinding and mixing feed ingredients (Sallazar-Villanea et al., 2018). Feeding animals with pelleted diets results in lower feed wastage than mash form and eliminates ingredient´s segregation (van der Poel et al., 2018), which can reduce production cost. In the pelleting processing, thermal and pressure treatments are applied to the mash ingredients to form a durable pellet (Abdollahi et al., 2013). Different die size and steam conditions can be applied to forming durable pellets, and it can affect nutrient digestibility and animal consumption (Rojas and Stein, 2017). Few studies have been carried out to determine the impact of pelleting process on nutrient digestibility and productive performance of growing pigs fed with diets based on processed RSM. This investigation is important once the use of byproducts in animal diets have been markedly increased, considering the economical and sustainability point of view.

In general, animal diets are formulated using different ingredients with varied chemical structure and complexity, especially in terms of fibrous fractions (Liu et al., 2016), which can alter the response of each diet to the processing (Jha et al., 2011). In this way, there is a lack of information if nutrients of diets based on rich-fiber feedstuffs (i.e. RSM) can be better availed by pigs after pelletizing. Therefore, the present study evaluated the effect of pellet processing (die size and steam) on diets based on conventional or tail-end dehulled RSM in growing pigs on growth performance and apparent total tract digestibility (ATTD).

Material and Methods

Selected batches of conventional RSM (CRSM) produced in Europe (Saipol S/A, France), were processed (fractioned) by Bühler S/A (Uzwil, Switzerland) aiming to improve the content of crude protein from 35% to 40%, approximately, and reducing fiber content. For that, raw conventional RSM (RSM-, 35% of crude protein (CP)) from selected seeds were fractioned after solvent defatted procedures (tail-end dehulling, RSM+, 40% of CP), which reduce hull´s seed content. To fractionate it, the raw RSM- was ground with a roller mill and separated with a shaking sieve with 300 µm mesh size and 3 min sifting time in a fine (throughs) and coarse (overs) fraction. Raw RSM- and fine RSM+ were used as only protein source in the experimental diets for growing pigs evaluated in this study.

The Animal Ethics Committee of the Institute of Agrifood Research and Technology (IRTA) approved all experimental procedure for this trial. One hundred forty-four growing pigs from IRTA´s sow herds ([Large White × Landrace] (Hypor) × Pietrain) were allotted in a room with 72 pens (2 animals in each pen, one entire male and one female). The animals were randomly distributed into 9 blocks (9 replicates per diet) in accordance to initial body weight (27.6 ± 0.60 Kg) and raised for 7 weeks. Each pen was considered an experimental unit. The animals were fed 1 of 8 diets, in a 2x2x2 factorial arrangement of RSM- or RSM+ based diet, 4x40 or 4x60 mm die size, with (Yes) or without (No) steam.

Diets were formulated based on growing pig requirement´s (NRC, 2012). Inclusion of RSM- or RSM+ in diets was based on a similar amount and no adjustment of synthetic AA was done. Feeds were produced at IRTA’s feed mill plant, according to a multi-step manufacturing schedule. Major feed ingredients were ground through a 25 HP hammer mill (Rosal VR-30, Barcelona, Spain) until the particles passed through a 3 mm sieve and then sent to a 500 kg horizontal mixer (Rosal, Barcelona, Spain) and mixed during 5 min. For each batch of feed, a 10 kg sample of SBM (48%, CP) was taken mixed with a mineral and vitamin premix, amino acids, macro-minerals. Titanium dioxide (5 g/Kg) was included on top of the diets as an indigestible marker to allow calculation of nutrient digestibility. Then, this mixture was incorporated to the horizontal mixer (500 kg mixer) and homogenized, prior to the addition of fat/oil. Mash feed was then sent to the pelleting system: conditioner, pelleting press and vertical cooler (Mabrik PV-30, Barcelona, Spain). Steam flux and feed entry were regulated automatically by the system (Mipps 210, Mangra SA, Barcelona, Spain). The steamed pellets were conditioned at a standard temperature of 80 °C for 20 s before into 4x40 or 4x60 mm die. To form pellets without steam conditioning the process reached a temperature of 60 °C due the heat produced for the mechanical process.

The experimental diet´s composition and calculated nutrient are presented in Table 1. The RSM- and RSM+ were used at fixed 22.5% inclusion in the diets for growing pigs. The animals had *ad libitum* access for pellet feed and water for all the experimental period. Analyzed chemical composition of conventional (RSM-) and high-protein (RSM+) rapeseed meal was presented in Table 2. One set of feed samples was analyzed for the main nutrient categories, namely dry matter (DM), ash, gross energy (GE), crude protein (CP), fat, crude fiber (CF), Van soest fiber fractions, starch, total P, Ca, Cu, Zn, and amino acid (AA) contents (Table 3).

Pellet durability index (PDI) was evaluated using Pfost equipment. In brief, 1000g of feed were collected and sieved to eliminate fines. Then 500 g of the screened pellets were placed in a metal box and rotated at 50 rpm for 10 minutes, after the pellets were removed and screened. PDI is defined as the percentage of pellets surviving the test and retained on the screen (American Feed Manufacturers' Association, 1976).

The animal growth performance was recorded at the end of the study and average daily weight gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated. Fecal samples were collected for 3 days and frozen until lyophilized and grounded (<0.5 mm) prior to analysis. Diets and fecal samples were analyzed for DM, OM, NFE, GE, N, starch, ash, fat, CF, NDF, ADF, lignin, hemicellulose, cellulose, minerals and AA content. All the methods and procedures were described and accepted by AOAC (2000). Nitrogen was quantified by the Dumas procedure, by means of Nitrogen/protein FP-528 analyzer (LECO corp., St Joseph, Mo, USA) and CF using an ANKOM 200/220 Fiber Analyzer (Macedon, NY).

Amino acids were determined by HPLC of protein hydrolysates from acid hydrolysis by precolumn derivatization with *o*-phthalaldehyde for primary AA and 9-fluorenylmethyl chloroformate for secondary AA. For methionine and cysteine determination, a peroxidation was done prior to hydrolysis. GE was determined in an adiabatic calorimeter (model C4000 adiabatic calorimeter, IKA Werke GmbH & Co. KG, Staufen, Germany), using benzoic acid as a standard calibrator, while metabolizable and net energy (ME and NE, respectively) contents were calculated as 0.79 and 0.58 from GE values, according to INRA (2002). Calcium, P, Cu, Zn and Ti contents from ash samples previously obtained were analyzed after acid digestion by inductively coupled plasma mass spectrometry (ICP-MS).

The apparent total tract digestibility of major nutrients was calculated according to the following equation:

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

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

The effects of diets on growth performance and nutrient digestibility were analyzed using the general linear model (1) procedure of SAS (SAS Institute Inc., Cary, NC), following the randomized block design previously reported. Each pen was considered an experimental unit. Outliers were analyzed and removed of the final data. Means were subjected to Tukey's test to account multiple comparisons. *P* values<0.05 were considered statistically significant.

$$(1) \quad Y_{ijk}= A+ Bv+ C + AB + AC + BC + ABC + \varepsilon ijk$$

A = RSM type: i = 2

B = Die size: i = 2

C = Steam: i = 2

i = j = k = 2

Table 1. Composition of the experimental diets based on conventional or high protein RSM used in trial with growing pigs

Ingredients (%)	RSM-[1]	RSM+[2]
Maize	23.448	23.448
Barley	22.500	22.500
Wheat	22.000	22.000
RSM-	22.500	---
RSM+	---	22.500
Soybean meal	2.000	2.000
Fat 3/5 Grefacsa	4.718	4.718
Monocalcium phosphate	0.628	0.628
Calcium carbonate	0.748	0.748
Salt	0.086	0.086
Sodium bicarbonate	0.482	0.482
L-Lysine-HCl	0.375	0.375
DL-Methionine	0.004	0.004
L-Threonine	0.081	0.081
L-Tryptophan	0.012	0.012
Noxyfeed[3]	0.020	0.020
Minerals & vitamins[4]	0.400	0.400
Nutrients		
ME[5], Mcal/kg	3.180	3.180
CP, %	15.14	16.47
CF, %	5.08	3.89
Fat, %	6.77	6.60
Ash, %	4.92	5.15
Ca, g/kg)	7.00	7.00
Total P, g/kg	6.19	6.19
Digestible P, g/kg	2.80	2.80
Total Lysine, g/kg	9.60	10.27
SID-Lysine, g/kg	8.11	8.60
SID-Threonine, g/kg	5.19	5.59
SID-Methionine, g/kg	2.44	2.67
SID-Met+Cys	5.32	5.81
SID-Tryptophan, g/kg	1.51	1.63
SID-Valine, g/kg	5.80	6.29
SID-Isoleucine, g/kg	4.63	5.03

[1]RSM-: diet based on conventional unprocessed rapeseed meal with 35% of crude protein. [2]RSM+: diet based on RSM- processed to improve crude protein (40% of CP). [3]Noxyfeed® (Itpsa, Barcelona, Spain): Synergistic combinations of antioxidants (BHT, BHA, propyl gallate, and synergistic substances). Products intended for protection of raw materials, feed, premixes, fats and animal by-products. [4]Premix supplementation per Kg of feed: vitamin A (E-672) 5,500 IU; vitamin D_3 (E-671) 1,100 IU; vitamin E (alpha-tocopherol) 7 mg; vitamin B_1 0.5 mg; vitamin B_2 1.4 mg; vitamin B_6 1 mg; vitamin B_{12} 8 µg; vitamin K_3 0.5 mg; calcium pantothenate 5.6 mg; nicotinic acid 8 mg; choline 120 mg; Fe (E-1) 80 mg from $FeSO_4+7H_2O$; I (E-1) 0.5 mg from $Ca(I_2O_3)_2$); Co (E-3) 0.4 mg from $2CoCO_3+3Co(OH)_2+H_2O$; Cu (E-4) 5 mg from $CuSO_4+5H_2O$; Cu (E-4) from amino acid-chelated minerals; Mn (E-5) 40 mg from MnO; Zn (E-6) 100 mg from ZnO; Se (E-8) 0.25 mg from Na_2SeO_3).

[5]ME and Ne: Metabolizable and net energy contents were calculated as 0.79 and 0.58 of the gross energy (GE) values. DM: dry matter. OM: organic matter. CP: crude protein. EE: ether extract. NFE: N-free extract. CF. crude fiber. Hmcell: hemicellulose. Cell: cellulose.

Table 2. Analyzed chemical composition of conventional (RSM-) and high-protein (RSM+) rapeseed meal

Analyzed composition (%)	**RSM-[1]**	**RSM+[2]**
Dry matter (%)	89.91	89.81
Crude protein (%)	34.26	40.16
Ether extract (%)	1.77	1.01
Ash (%)	7.13	8.19
ME (cal/g)[3]	3314	3292
Crude fiber (%)	13.54	8.26
NDF (%)	28.96	19.39
ADF (%)	21.27	11.75
Lignin	16.9	13.7
Starch (%)	0.34	0.38
P total (%)	5.63	7.46
Phytate P (g/kg)	3.14	3.53
Non phytate P	2.49	3.93
Ca (%)	4.00	3.88
Essential Amino acids (%, as-is basis)		
Arg	1.86	2.31
His	0.79	1.00
Ile	1.10	1.37
Leu	2.23	2.75
Lys	2.19	2.25
Met	0.70	0.83
Phe	1.24	1.52
Thr	1.43	1.66
Val	1.32	1.63
Non-essential amino acids (%, as-is basis)		
Ala	1.39	1.70
Asp	2.35	2.81
Cys	0.74	0.97
Glu	5.54	6.78
Gly	1.64	2.01
Pro	2.06	2.43
Ser	1.42	1.71
Tyr	0.93	1.12

[1]RSM-: diet based on conventional unprocessed rapeseed meal with 35% of crude protein. [2]RSM+: diet based on RSM- processed to improve crude protein (40% of CP). [3]ME: Metabolizable energy contents was calculated as 0.79 of the gross energy (GE) values.

Table 3. Analysed chemical composition of the experimental diets used in trial with conventional (RSM-) and high protein (RSM+) rapeseed meal in growing pigs

Rapeseed meal[1]	RSM-	RSM+	RSM-	RSM+	RSM-	RSM+	RSM-	RSM+
Die size, mm	4x40	4x40	4x40	4x40	4x60	4x60	4x60	4x60
Steam	No	No	Yes	Yes	No	No	Yes	Yes
ME[2], Kcal/kg	3.55	3.51	3.57	3.57	3.51	3.52	3.56	3.66
DM, %	90.74	90.87	89.93	89.84	91.06	91.07	89.83	89.89
OM, %	94.68	94.21	94.46	94.14	94.33	94.25	94.33	94.4
CP, %	16.49	18.26	16.69	17.85	16.96	18.10	16.80	17.23
EE, %	7.31	6.98	7.03	6.85	7.24	6.96	7.21	9.14
NFE, %	66.67	65.46	66.04	66.18	66.23	66.11	65.63	64.52
Starch, %	45.59	44.68	44.15	44.66	43.82	43.66	43.51	43.42
CF, %	4.21	3.52	4.70	3.26	3.91	3.09	4.69	3.50
NDF, %	15.67	15.57	17.10	16.06	16.82	13.89	18.79	15.02
ADF, %	8.82	6.77	8.94	7.72	9.30	6.87	9.45	7.00
Lignin, %	2.74	1.36	2.94	1.68	2.68	1.72	3.14	2.19
Hmcell, %	6.86	8.80	8.16	8.34	7.52	7.02	9.34	8.02
Cell, %	6.07	5.40	6.00	6.04	6.62	5.15	6.31	4.81
Ash, %	5.32	5.79	5.54	5.86	5.67	5.75	5.67	5.60
Ca, %	0.73	0.75	0.75	0.75	0.79	0.73	0.74	0.69
P, %	0.66	0.78	0.68	0.77	0.69	0.76	0.71	0.69
Zn, ppm	70.6	76.9	72.2	86.0	89.6	89.9	78.6	74.2
Cu, ppm	15.4	14.4	12.2	14.0	13.2	12.4	14.8	13.7
Lys, %	1.07	1.41	1.21	1.16	1.12	1.08	1.04	0.99
Arg, %	0.81	0.92	0.85	0.97	0.84	0.90	0.85	0.89
Thr, %	0.66	0.71	0.67	0.72	0.68	0.69	0.67	0.67
Met, %	0.32	0.35	0.34	0.35	0.34	0.35	0.32	0.34
Cys, %	0.31	0.35	0.35	0.38	0.33	0.35	0.35	0.33
Val, %	0.60	0.67	0.67	0.75	0.63	0.67	0.65	0.65
Leu, %	1.09	1.22	1.13	1.26	1.12	1.21	1.13	1.17
Ile, %	0.48	0.53	0.53	0.61	0.5	0.54	0.51	0.52
His, %	0.37	0.4	0.38	0.43	0.37	0.41	0.36	0.37
Ser, %	0.64	0.72	0.65	0.69	0.67	0.7	0.67	0.69
Ala, %	0.65	0.74	0.67	0.74	0.68	0.71	0.67	0.7
Tyr, %	0.46	0.5	0.48	0.51	0.47	0.5	0.48	0.49
Phe, %	0.62	0.69	0.65	0.72	0.65	0.69	0.65	0.67
Gly, %	0.67	0.77	0.69	0.79	0.69	0.74	0.69	0.72
Pro, %	1.13	1.18	1.13	1.25	1.22	1.14	1.22	1.35
Asp, %	1.03	1.16	1.06	1.18	1.07	1.13	1.07	1.10
Glu, %	2.91	3.28	3.01	3.31	3.03	3.23	3.02	3.14

[1]RSM-: diet based on conventional unprocessed rapeseed meal with 35% of crude protein. RSM+: diet based on RSM- processed to improve crude protein (40% of CP). [2]ME and Ne: Metabolizable and net energy contents were calculated as 0.79 and 0.58 of the gross energy (GE) values. DM: dry matter. OM: organic matter. CP: crude protein. EE: ether extract. NFE: N-free extract. CF: crude fiber. Hmcell: hemicellulose. Cell: cellulose.

Results

The Table 2 presents the analyzed composition of RSM- (raw RSM) and RSM+ (dehulled RSM). RSM+ is the result of a fraction with improved nutritional content by raised concentration of CP (34.26% to 40.16%), great profile of essential and non-essential amino acids, and total P (5.63% to 7.46%), and with reduced content of fiber fraction (13.54% to 8.26% CF, 28.96% to 19.39% NDF, 21.27% to 11.75% ADF, 16.9% to 13.7% Lignin) compared to the conventional RSM. Dehulling process was efficient to improve the nutritional content of RSM.

The PDI was analyzed and steam conditioning was an essential factor to reach high pellety quality, as viewed in the follow: PDI of 18.0% for T1 (RSM-, 4x40 mm, no steam); 24.6% (RSM+, 4x40 mm, no steam), 91.9% for T3 (RSM-, 4x40 mm, with steam), 92.4% for T4 (RSM+, 4x40 mm, with steam), 30.0 % for T5 (RSM-, 4x60 mm, no steam), 30.5% for T6 (RSM+, 4x60 mm, no steam), 93.9% for (RSM-, 4x60 mm, with steam), and 91.1% for T8 (RSM+, 4x60 mm, with steam). These data report that steamed pellets from RSM based diets, independently of tested die size presented a better pellet quality due to a higher PDI.

The Table 4 presents the effects of RSM- and RSM+ based diets and pelleting conditioning (die size and steam) on animal growth performance. There was no interaction between RSM type, die size, and the use of steam for none of the evaluated growth performance parameters. However, there was interaction between die size and steam for FCR (P=0.04), meaning that animals fed with steamed pellets based on RSM diets formed using a 4x40 mm die size presented improved FCR than the animals fed with no steamed pellets with 4x40 mm die size. Animals fed with steamed pellets showed a lower ADFI ($P<0.03$), compared with animals fed with no steamed pellets (1,510 kg/d vs 1,580 kg/d), which reach a reduction of 70 g/d of average feed intake. ADWG of pigs fed with RSM+ based diet increased ($P<0.01$) from 704 to 763 g/d and, consequently, BW at the end of the trial was also improved ($P<0.01$) from 62.1 to 64.1 kg compared to pigs fed with RSM- based diets. Die size of 4x60 mm positively affected the ADWG (708 g/d vs. 761 g/d) and final BW (62.3 kg vs 64.9 kg) compared to 4x40 mm die.

The influence of pelleting condition on nutrients digestibility in diets based on RSM type for growing pigs were presented in Table 5. The effect of the factor levels RSM type, die size and steam conditioning on the ATTD of ADF (P=0.01), Lignin (P=0.04), cell (P=0.03), ash (P=0.03), P (P=0.04) and Ca (P=0.002) shown significant interaction. Pellets based on RSM-, 4x40 mm, with steam presented improved ATTD of ADF (38.3% vs. 47%), Lignin

(3.1% vs. 16.4%), and cell (53.8% vs. 60.4%) than pellets based on RSM-, 4x60 mm, with steam. Whilst the ATTD of ash (39.2% vs. 46.3), P (35.5% vs. 41.1), and Ca (41.2% vs. 52.3%) was improved due the interactive effect of RSM+, 4x60 mm, and steam conditioning compared to RSM+, 4x40 mm, and steam conditioning. In general, the beneficial effect of pelleting process was observed to the digestibility of fiber fractions of diets based on RSM-, 4x40 mm die size, conditioned with steam, while for mineral matter a better digestibility was observed on diets based RSM+, 4x60 mm die size, conditioned with steam. There was significant interaction between the factor levels RSM type and die size on the ATTD of DM (*P*=0.03), OM (*P*=0.04), CP (*P*=0.03), and NDF (*P*=0.04). The ATTD of DM (84.3% vs 85.8%), OM (86.9% vs. 88.1%), CP (77.4% vs. 80.3%), and NDF (67.0% vs. 69.9%) was improved for pellets based on RSM+, 4x60 mm die size, conditioned with steam compared to RSM+, 4x40 mm die size, conditioned with steam. These results indicate that all the beneficial effect from the interactions RSM type and die size were observed in pellets conditioned with steam.

Furthermore, no interactive effects were observed between the tested pelleting process condition (4x40/4x60 mm die size and no/yes steam) on diets based on RSM- or RSM+ for the ATTD of fat, hemicellulose, N-free extracts, and energy digestibility. However, pellets based on RSM+ showed higher ATTD of fat (*P*=0.01, 84.6% vs. 85.7%), NFE (*P*=0.01, 90.5% vs. 91.9%), and energy (*P*=0.01, 84.6% vs. 86.4%) than pellets based on RSM-. The ATTD of hemicell was higher for pellets based on RSM- (*P*=0.01, 73.6% vs. 78.8%) than pellets based on RSM+. Also, steamed pellets presented higher ATTD of N-free extracts (*P*=0.01, 75.3% vs. 77.9%) and energy (*P*=0.01, 85.2% vs. 86.1%) than no steamed pellets, while no steam pellets presented higher ATTD of fat (*P*=0.01, 83.8% vs. 85.6%) than steamed pellets.

The influence of pelleting condition on ATTD of amino acids in diets based on RSM type for growing pigs were presented in Table 6. No interactive effects were observed between the tested pelleting process condition (4x40/4x60 mm die size and no/yes steam) on diets based on RSM- or RSM+ for the ATTD of amino acids. All analyzed amino acids presented higher (*P*=0.01) ATTD for diets based on RSM+ compared to diets based on RSM-, while no effect (*P*>0.05) of die size was observed for ATTD of amino acids. Except to the ATTD of lysine that was not affected (*P*=0.32) by steam conditioning, all the analyzed AAs showed lower ATTD due the effect of steam (*P*<0.05).

Table 4. Influence of pelleting condition in diets based on conventional or high protein RSM on growth performance of growing pigs

Die size[1]		**4x40**	**4x40**	**4x60**	**4x60**					
Steam[2]		**No**	**Yes**	**No**	**Yes**		***P* values**			
Item	**RSM**[3]					**AVG**[4]	**RSM**	**Die**	**Steam**	**RSD**[5]
BW, kg (0d)	RSM-	27.6	27.5	27.5	27.5	27.5	0.65	0.52	0.83	0.60
	RSM+	27.6	27.7	27.5	27.6	27.6				
		Die size		Steam						
		4x40	4x60	Yes	No					
	AVG	27.5	27.5	27.6	27.6					
ADFI, kg/d	RSM-	1.51	1.53	1.63	1.51	1.55	0.98	0.14	0.01	0.11
	RSM+	1.56	1.48	1.63	1.51	1.55				
		Die size		Steam						
		4x40	4x60	No	Yes					
	AVG	1.53	1.57	1.58^{a}	1.51^{b}					
ADWG, g/d	RSM-	653	712	734	718	$704^{\#}$	0.01	0.01	0.71	79.0
	RSM+	720	743	814	776	$763^{\&}$				
		Die size		Steam						
		4x40	4x60	No	Yes					
	AVG	708^{B}	761^{A}	731	738					
FCR[6]	RSM-	2.33^{aB}	2.14^{bA}	2.23^{bA}	2.11^{bA}	$2.20^{\&}$	0.01	0.01	0.01	0.10
	RSM+	2.17^{aB}	2.00^{bA}	2.00^{bA}	1.96^{bA}	$2.03^{\#}$				
		Die size		Steam						
		4x40	4x60	No	Yes					
	AVG	2.25^{A}	2.07^{B}	2.12^{a}	2.03^{b}					
BW, kg (49d)	RSM-	59.6	62.4	63.5	62.7	$62.1^{\#}$	0.01	0.01	0.69	4.02
	RSM+	62.9	64.1	67.4	65.6	$64.1^{\&}$				
		Die size		Steam						
		4x40	4x60	No	Yes					
	AVG	62.3^{b}	64.9^{a}	63.4	63.8					

[1]Die size: 4x40 mm or 4x60 mm. [2]Steam: pelletizing process without (No) or with (Yes) steam. [3]RSM-: conventional unprocessed rapeseed meal with 35% of crude protein; RSM+: RSM- processed by Bühler S/A to improve crude protein (40% of CP). [4]AVG: average means. [a,b]Means within the same row with different letter script are statistically different for steam effect ($P<0.05$).[5]RSD: root mean standard deviation. [A, B]Means within the same row with different letter script are statistically different for die size effect ($P<0.05$). [#,&]Means within the same column with different character scrip are statistically different for RSM type effect ($P<0.05$). [6]Interaction between Die size*Steam is statistically significant for FCR ($P=0.04$).

Table 5. Influence of pelleting condition on ATTD of nutrients in diets based on conventional or high protein RSM for growing pigs

Die size[1] Steam[2]		**4x40 No**	**4x40 Yes**	**4x60 No**	**4x60 Yes**		**RSM*Die* Steam**	**RSM* Die**	**RSM* Steam**	**Die* Steam**	**RSM**	**Die**	**Steam**	
ATTD, %	**RSM[3]**					**AVG**				**P values[4]**				**RSD[5]**
	RSM-	84,4$^{A\#}$	83,9$^{A\#}$	84,1$^{A\#}$	82,9$^{A\#}$	83,8								
DM	RSM+	85,7$^{A\&}$	84,3$^{A\&}$	85,6$^{A\&}$	85,8$^{B\&}$	85,3	0,05	0,03	0,67	0,45	0.01	0.98	0.02	1,25
	AVG	83,0	84,1	84,8	84,3									
	RSM-	86,5$^{A\#}$	86,1$^{A\#}$	86,4$^{A\#}$	85,1$^{A\#}$	86.0								
OM	RSM+	88,2$^{B\&}$	86,9$^{A\#}$	88,2$^{B\&}$	88,1$^{B\&}$	87.8	0,06	0,04	0,78	0,70	0.01	0.90	0.01	1.12
	AVG	87,3	86,5	87,2	86.6									
	RSM-	80,4$^{A\#}$	78,8$^{A\#}$	79,7$^{A\#}$	77,4$^{A\#}$	79.1								
CP	RSM+	81,2$^{A\#}$	77,4$^{B\#}$	80,7$^{A\#}$	80,3$^{A\&}$	79.9	0,06	0,03	0,99	0,21	0.01	0.68	0.01	2,12
	AVG	80,8	78,1	80,9	78,8									
	RSM-	61.6$^{A\#}$	64.3$^{A\#}$	61.3$^{A\#}$	61.1$^{A\#}$	62.1								
NDF	RSM+	68.5$^{B\&}$	67.0$^{A\#}$	69.0$^{B\&}$	69.9$^{B\&}$	68.6	0.11	0.04	0.35	0.96	0.01	0.73	0.59	3.15
	AVG	65.1	65.7	65.2	65.5									
	RSM-	43.9$^{aA\#}$	47.0$^{aA\#}$	43.8$^{aA\#}$	38.3$^{bB\#}$	43.2								
ADF	RSM+	64.0$^{aA\&}$	59.6$^{aA\&}$	63.6$^{aA\&}$	64.7$^{aA\&}$	63.0	0.01	0.01	0.77	0.82	0.01	0.94	0.29	5.72
	AVG	54.0	53.3	53.7	51.5									
	RSM-	13.9$^{aA\#}$	16.4$^{aA\#}$	13.1$^{aA\#}$	3.1$^{bA\#}$	11.6								
Lig	RSM+	55.7$^{aA\&}$	45.6$^{aA\&}$	53.7$^{aA\&}$	54.6$^{aA\&}$	52.4	0.03	0.09	0.75	0.88	0.01	0.65	0.14	11.9
	AVG	34.8	31.0	33.4	28.9									
	RSM-	57.1$^{bA\#}$	60.4$^{aA\#}$	57.2$^{bA\#}$	53.8$^{bB\#}$	57.1								
Cell	RSM+	67.7$^{aA\&}$	65.8$^{aA\&}$	67.9$^{aA\&}$	69.2$^{aA\&}$	67.7	0.03	0.03	0.90	0.53	0.01	0.66	0.88	4.08
	AVG	62.4	63.1	62.6	61.5									
	RSM-	47.5$^{aA\&}$	46.3$^{aA\&}$	43.9$^{aB\&}$	43.0$^{aA\&}$	45.2								
Ash	RSM+	42.6$^{aA\#}$	39.2$^{bA\#}$	40.4$^{aA\&}$	46.3$^{aB\&}$	42.1	0.03	0.01	0.27	0.02	0.33	0.60	0.92	4.07
	AVG	45.1	42.8	42.2	44.7									
	RSM-	49.2$^{aA\&}$	44.8$^{aA\&}$	42.2$^{aB\&}$	43.0$^{aA\&}$	44.8								
P	RSM+	37.1$^{aA\#}$	35.5$^{aA\#}$	33.8$^{aB\#}$	41.1$^{bB\&}$	36.9	0.04	0.01	0.02	0.01	0.01	0.06	0.66	3.74
	AVG	43.2	40.2	38.0	42.1									

continuation

Die size[1] Steam[2]		4x40 No	4x40 Yes	4x60 No	4x60 Yes		RSM*Die* Steam	RSM* Die	RSM* Steam	Die* Steam	RSM	Die	Steam	
ATTD, %	**RSM[3]**					**AVG**			**P values[4]**					**RSD[5]**
	RSM-	$45.8^{aA\#}$	$45.1^{aA\#}$	$42.9^{aA\#}$	$41.9^{aA\#}$	43.9								
Ca	RSM+	$44.0^{aA\#}$	$41.2^{aA\#}$	$40.8^{aA\#}$	$52.3^{bB\&}$	44.6	0.01	0.01	0.01	0.01	0.43	0.66	0.14	4.71
	AVG	44.9	43.2	41.9	47.1									
	RSM-	85.8	83.0	85.7	83.8	$84.6^{\#}$								
	RSM+	87.3	83.8	87.1	84.5	$85.7^{\&}$								
	AVG	86.6	83.4	84.6	84.2									
EE		Die size		Steam			0.99	0.97	0.66	0.47	0.01	0.68	0.01	2.47
		4x40	4x60	No	Yes									
	AVG	85.0	84.4	85.6^{a}	83.8^{b}									
	RSM-	77.3	79.6	76.8	81.3	$78.8^{\&}$								
	RSM+	72.5	73.6	73.8	74.5	$73.6^{\#}$								
	AVG	74.9	73.6	75.3	77.9									
Hmcell		Die size		Steam			0.48	0.79	0.16	0.62	0.01	0.37	0.01	3.63
		4x40	4x60	No	Yes									
	AVG	72.5	73.4	71.7^{b}	74.1^{a}									
	RSM-	90.7	90.5	90.8	90.1	$90.5^{\#}$								
	RSM+	91.9	91.6	92.0	92.2	$91.9^{\&}$								
	AVG	91.3	91.0	91.4	91.2									
NFE		Die size		Steam			0.21	0.15	0.38	0.99	0.01	0.73	0.20	0.83
		4x40	4x60	No	Yes									
	AVG	91.2	91.3	91.4	91.1									
	RSM-	84.4	85.3	83.7	85.1	$84.6^{\#}$								
	RSM+	85.3	86.9	87.0	86.6	$86.4^{\&}$								
	AVG	84.9	86.1	85.4	85.9									
GE		Die size		Steam			0.15	0.06	0.75	0.55	0.01	0.68	0.01	1.28
		4x40	4x60	No	Yes									
	AVG	85.5	85.7	85.2^{b}	86.1^{a}									

[1]Die size: 4x40 mm or 4x60 mm. [2]Steam: pelletizing process without (No) or with (Yes) steam. [3]RSM-: conventional unprocessed rapeseed meal with 35% of crude protein; RSM+: RSM-processed by Bühler S/A to improve crude protein (40% of CP). [4]P<0.05 for significant interaction. [5]RSD: root mean standard deviation. [a,b]Means within the same row with different letter script are statistically different for steam effect (P<0.05). [A, B]Means within the same row with different letter script are statistically different for die size effect (P<0.05). [#,&]Means within the same column with different character scrip are statistically different for RSM type effect (P<0.05). DM: dry matter. OM: organic matter. CP: crude protein. Lig: lignin. Cell: cellulose. EE: ether extract. Hmcell: hemicellulose. NFE: N-free extract. Ge: gross energy.

Table 6. Influence of pelleting condition on ATTD of amino acids in diets based on conventional or high protein RSM for growing pigs

	RSM[1]		Die size		Steam		RSM	Die size	Steam	RSD[2]
ATTD[3], %	RSM-	RSM+	4x40	4x60	No	Yes		P<0.05[4]		
Lys	88.5	90.2	89.5	89.2	89.6	89.1	0.01	0.52	0.32	2.26
Arg	86.2	88.3	87.2	87.4	87.9	86.6	0.01	0.64	0.01	1.41
Thr	80.7	82.7	81.8	81.5	82.4	80.9	0.01	0.55	0.01	1.89
Met	79.9	81.4	80.6	80.6	81.8	77.4	0.01	0.92	0.01	2.12
Cys	86.1	88.4	87.5	87.0	87.7	86.9	0.01	0.18	0.01	1.64
Val	79.5	81.8	80.7	80.6	81.6	79.6	0.01	0.82	0.01	2.24
Leu	83.1	84.4	83.8	83.7	84.4	83.0	0.01	0.80	0.01	1.80
Ile	78.4	80.7	79.6	79.5	80.5	78.6	0.01	0.77	0.01	2.45
His	88.7	90.0	89.3	89.3	89.8	88.9	0.01	0.97	0.01	1.51
Ser	81.5	83.7	82.8	82.4	83.3	81.9	0.01	0.33	0.01	2.05
Ala	75.8	78.0	76.9	76.8	78.1	75.7	0.01	0.90	0.01	2.63
Tyr	79.4	81.8	80.7	80.5	81.5	79.7	0.01	0.82	0.01	2.13
Phe	82.5	84.2	83.4	83.3	84.1	82.6	0.01	0.81	0.01	1.81
Gly	80.7	83.4	82.2	82.0	82.7	81.4	0.01	0.66	0.01	1.86
Pro	89.4	91.3	90.4	90.3	90.6	90.1	0.01	0.52	0.03	0.13
Asp	84.2	86.1	78.3	78.1	79.3	77.1	0.01	0.74	0.01	2.43
Glu	90.0	91.1	90.6	90.5	90.1	90.0	0.01	0.60	0.01	1.07

[1]RSM-: conventional unprocessed rapeseed meal with 35% of crude protein; RSM+: RSM- processed by Bühler S/A to improve crude protein (40% of CP). [2]RSD: root mean standard error. [3]ATTD: apparent total tract digestibility. [4]P values: there was no interaction between RSM, die size and steam for all the amino acids. The only interaction observed was between die size and steam for Cys (*P*=0.03).

Discussion

The fractionation of RSM is addressed to increase the content of protein and amino acids, while reduce the fiber fraction. In this study, fractionating RSM resulted in a finest fraction with improved nutritional value due the higher content of crude protein and AA and lower fiber content than found in raw RSM. This result is in keeping with previous studies that evaluated the effect of dehulling process on nutrient content in RSM meal, which it was observed increased content of crude protein ranging from 2.3 (Mejicanos et al., 2018) to 5.8 g/kg (Hansen et al., 2017), totaling 39.5% and 39.8% of CP content, respectively. Furthermore, the higher content of crude protein and amino acids in dehulled yellow *Brassica napus* meal compared to its raw meal was also reported by Mejicanos et al. (2016), and this improvement was similar or slightly higher than the comparison between RSM- and RSM+ herein observed. As well, herein it was found reduced fiber fraction in RSM+ compared to the raw RSM, which seems to be in accordance to the reported by Hansen et al. (2017). It was also reported decreased NDF (26.20% to 17.80%), ADF (19.40 to 12.10%), CF (13.70% to 11.30%), total dietary fiber (33.00% to 24.57%), and NSP (20.54% to 16.97%) by Mejicanos et al., 2018. The increased total P content in RSM+ is associated to the increased in non-phytate P content, which is a most available source of P for monogastric animals. The presence of phytate-P in seeds used to compose the diets is highly correlated with the reduction of feed efficiency (Jha et al., 2011) by chelating nutrients, and its decrease is very recommended when aiming improvements in pig´s growth performance (Torres-Pitarch et al., 2017). The most content of P in oilseeds is located within seed´s kernel (Angel et al., 2002), which can have its concentration raised after fractionating hulls and endosperm part of raw RSM. However, the effect of fiber content from seed hulls optimizing or reducing P content and availability was not evident in the study of Bournazel et al. (2018). Nutritional content of RSM+ was effectively improved by fractionation and it can make this feed commodity more attractive to animal feed industry, mainly because it can be accomplished by an improvement in nutrient digestibility in the gastrointestinal tract of pigs.

The beneficial effect of pelleting on feed efficiency is mainly due the reduction in feed wastage, although it is also expected some improved on nutrient digestibility because this process can disrupt endosperm cell wall of the grains (Ball et al., 2015). However, the benefits from pellet process on feed efficiency can be reduced if pellets have poor quality, when presenting 20 to 25% of fines (Stark et al., 1993). Herein, pellets processed without steam presented a lower quality index by being easily friable compared to steamed pellets. When pellets are easily friable the quantity of fines increases, and hence higher quantity of feed can be wastage from the feeder to the floor. Steam can have contributed to a better pellet quality improving die lubrification associated with a favorable friction force and compression on mash to cross the die, making a great condition to starch forming granules (Skoch et al., 1981). In addition, steamed pellets have a less energy consumption to forming pellets, reducing production costs (Skoch et al., 1983). The particle size is other factor that can contribute by promoting a greater binding of particles during pelletizing process (Lahaye et al., 2008). Probably, the finest fraction from dehulling process that characterizes RSM+ can also have contributed to improve forming pellets with high PDI.

No factors interaction was observed to pig´s weight gain, but there was interaction between die size and steam to feed efficacy. Improved FCR was observed for diets based on RSM that cross a 4x40 mm die size conditioned with steam. In the study of Skoch et al. (1983), the growing-finishing pigs also presented improved FCR when consumed pelleted diet based on corn-soybean meal, which higher PDI was also observed to steamed pellets. Steamed pellets improving FCR is also in agreement with the results of Wondra et al., (1995) and I´Anson et al. (2012). To discuss the effect of pelleting condition on FCR in the current study is needed to highlight the difference found herein on physical quality of pellets in function of steam conditioning. It is important because the high quantity of fines in low quality pellets that is not found in pellets with higher PDI due the greater feed agglomeration (Ball et al., 2015). If the improvement in feed efficacy promoted by pelleted processing is mainly attributed to a reduction in feed wastage (Ohh et al., 2015) by reducing fines, this study states the important impact of steam conditioning on this effect, once it is needed to promote good feed agglomeration (Abdollahi et al., 2013).

The influence of pellet length on its quality have been not usually considered (Cutlip et al., 2008), but if fines quantity and pellet durability are essential to form high quality pellets, it is expected that longer length pellets have higher quality than from lesser length. However, pellety quality was similar from 4x40 and 4x60 mm die size, being FCR improved by an interactive effect of 4x40 mm and steam conditioning. Probably, chemical aspects such as starch gelatinization and nutrient availability in feed were most efficiently promoted by the interaction 4x40 mm die size and steam conditioning than 4x60 mm steam conditioning. Thus, by changing physico-chemical aspects such as a solid form (reducing feed waste and segregation of ingredients) and starch gelatinization (improving its digestibility), respectively, heat and pressure treatment from pelleting process can improve feed efficacy provided to growing pigs (Vukmirović et al., 2017).

In view of the diets were not balanced for digestible AAs, it was expected the greater results found for RSM+ compared to RSM- in terms of animal weight gain (ADWG and BW) due its improved nutritional content (Lahaye et al., 2008) by dehulling process. The present study supports this observation, which the most adequate nutritional content in diets based on RSM+, especially due the improvement of protein and AA content and reduced concentration of fiber fraction by dehulling process, was beneficial to increase body weight gain. The lower ADFI for steamed pellets could be attributed to the lower feed wastage, as aforementioned, due the greater feed agglomeration in steamed pellets than no steamed pellets.

The nutrient digestibility is an important factor that contribute to variations on feed efficiency, which can be negatively affected by higher content of dietary fiber (Noblet and Le Goff, 2001). *Per si*, fiber fractions are poorly and differently digested by pigs due its limited enzyme capacity (Zijsltra et al., 2010), in which cellulose is less digested than hemicellulose, while lignin is almost completely undigested, pectin is almost totally digested. The fermentation of fiber fractions in pigs is largely done by gastrointestinal microbiota with a subsequent production of volatile fat acids, and its digestibility is very variable. The main physicochemical property of fibers with nutritional significance is water solubility resulting in viscous intestinal environment that imped digestion and impair normal digesta transit (Caprita et al., 2011). This viscosity effect is related to pig´s incapacity to digest water soluble cellulose, β-glucans, and arabinoxylans.

Fiber fractions can difficult nutrient absorption by physical barrier of cell wall encapsulating available nutrients (de Nanclares et al., 2017; Back-Knudsen et al., 2012), The effect of pelleting process improving nutrient availability is also a form of avoid wastage, namely nutritional feed wastage (Abdollahi et al., 2013); however, there is inconsistent evidence if pelleting process can improve fiber digestibility (van der Poel, 2018). Herein, there was interactive effect between RSM-, that contained higher content of dietary fiber than RSM+, and pelleting process; steamed pellets based on RSM- from a 4x40 mm die size have improved ATTD of ADF, lignin and cellulose than steamed pellets based on RSM- from a 4x60 mm die size. Fiber solubility is reflected in its digestibility (de Vries et al., 2012) and steamed pellets from 4x40 mm die size can have increased fiber solubility in a more digestible form than steamed pellets from 4x60 mm die size

The cell wall tissue of hull is mainly composed of insoluble, lignified, and strongly linked non-starch polysaccharides (NSPs) that are poorly digested by pigs. Thus, independently of pelleting condition, the greater degradability of fiber of RSM+ based diets compared to RSM-, might have occurred due the reduced content of fiber by dehulling process, reducing lignification degree attributed to hull´s fiber. In regard to ash, P and Ca digestibility, an interactive effect was observed between RSM+, 4x60 mm die size and steam improving their digestibility to equal levels found in diets based on RSM-. Removing hulls from RSM concentrates P in the fine fraction, and phytate-bound P is also increased (Mejicanos et al., 2018). Phytic acid can affect nutrient digestibility through binding to nutrients reducing its availability for pig´s absorption (Woyengo and Nyachoti, 2013). It is possible that the combination of 4x60 mm die size and steam from pelleting processing on diets based RSM+, applying heat treatment, has been beneficial to partially degrade phytic acid making ash, P and Ca more available for pigs (Rehman and Shah, 2005).

Pelleting processing applies heat, moisture, shear and pressure treatment to agglomerate feed, and these multiple actions change protein structure (Salazar-Villanea et al., 2016). Furthermore, the reduction of fiber fraction by dehulling RSM has been also pointed to improve protein and AA digestibility (Hansen et al., 2017; Mejicanos et al., 2018). Herein, steamed pellets from the diets based on RSM+ with a reduction of fiber fractions presented greater digestibility of DM, OM, and CP as result of the interaction between RSM+ and 4x60 mm die size. In general, a higher nutrient digestibility can be attributed to diets presenting higher nutrient content. Thus, it is important recognize the effect of pelleting process (different die size with steam condition) improving nutrient digestibility of diets based on RSM+, once these results were observed in diets formulated to present equal nutrient content. Taking together, the ideal condition to forming high quality pellets increases nutrient digestibility and improves nutritional value of ingredients compounding feed diets and these factors can contribute to improve feed efficiency of diets (Vukmirović et al., 2017).

Interactions between RSM type, die size and steam were not observed for fat, hemicellulose, N-free extracts, energy and, AA digestibility. However, all these nutrients were better digested to diets based on RSM+, except to hemicellulose digestibility. Die size doesn´t affect ATTD of these parameters, while steam conditioning reduced ATTD of fat and all analyzed AAs. The ATTD of N-free extracts was not affected by steam, while ATTD of hemicellulose and energy were improved by steam conditioning. Dehulled RSM presenting improved AA digestibility and other nutrients was also reported in previous studies, as the result of reducing fiber and concentrating AA content (Hansen et al., 2017; Mejicanos et al., 2016; 2017). For apply a multiple factor condition during feed process, it is hard predicate the effect of individual processing factor specifically affecting nutrient digestibility. However, absence of interaction between the target factors could be explained by different manner of the factors affecting positively or negatively nutrient digestibility in different diets. For example, the difference in terms of AA content in RSM- and RSM+ diets, besides the reduction of the potential recalcitrant effect of fiber find in hulls, improve the change of rising AA availability in RSM+ diets. On the other hand, the multiple effect of pelleting processing by heat, moisture, pressure, and shear can negatively affect protein structure, resulting in denaturation (van der Poel et al., 2018). Nonetheless, it was augmented that studies have failed to establish if the effect of feed processing on protein digestibility is on changes in physical or protein structure, or on elimination of antinutritive factors (Svihus and Zimonja, 2011).

Conclusion

Dehulling rapeseed meal is an efficient feed process to improve the nutritional value of conventional RSM and can positively contributes to reduce EU protein imports for animal feeding. Steam conditioning is essential factor to form high quality pellets. Feed intake was reduced for pigs fed with steamed pellets likely due a low quantity of fines that reduce feed wastage and resulted in improved feed efficiency. Body weight gain of pigs fed with diets based on RSM+ was higher than pigs fed with diets based on RSM-, probably due the higher nutritive value observed in RSM+. Nutrient digestibility was affected by pelleting process and ameliorated when pelleting feed using ideal condition for each formulated diet based on different RSM type. Fiber fractions present in RSM- diets were better digested by pigs when fed pellets formed in a 4x40 mm die size with steam conditioning. Mineral content was better digested in pigs fed with pellets formed by diets based on RSM+ in a 4x60 mm die size with steam conditioning. Thus, the present study recognizes the positive effect of dehulled RSM and pelleting process on the productive performance of growing pigs and nutrient digestibility. Furthermore, it indicates that ideal condition to forming high quality pellets for diets with different ingredients needs to be considered prior feed processing.

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