Evaluation of extruded or unextruded double-low rapeseed meal and multienzyme preparation in pigs nutrition during the finishing phase of production

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Abstract

The aim of this study was to compare the effects of extruded or non-extruded double-low rapeseed meal (DL-RSM) and multienzyme preparation on performance, nutrient digestibility, immune function and antioxidant status of the finishing pigs. Forty-eight pigs (Duroc×Landrace×Yorkshire) with an average weight of 62 kg were randomly divided into four groups with three replicates according to gender. Four diets were formulated to meet NRC (1998) nutrient requirements. Diet 1, based on the corn and soybean meal (SBM), was used as control. Diet 2 used 13% DL-RSM instead of the 11% SBM used in Diet 1. Diet 3 used 13% extruded double-low rapeseed meal (E-DL-RSM) instead of 11% SBM, and Diet 4 0.03% multienzyme preparation was supplemented in Diet 3. The results showed that replacement of 11% SBM by 13% DL-RSM had no negative effects on performance and nutrient digestibility for finishing pigs. Extrusion and multienzyme preparation also had no beneficial effects on pig performance. However, the concentration of immunoglobulin G (IgG) in serum of DL-RSM treatment was lower (P<0.05) than that of the control, but it increased to the level of control in the treatment of extrusion combined with multienzyme preparation. The diet containing E-DL-RSM combined with multienzyme preparation significantly decreased the malondialdehyde (MDA) content (P<0.05) in serum. It was concluded that DL-RSM was an acceptable alternative to SBM as part of a protein supplement for finishing pig diets. In addition, extrusion and multienzyme preparation were not economically applied in the current study because of their extra cost.

Introduction

Rapeseed meal with approximately 35% crude protein and a good amino acid profile (Bell et al., 1991, 1998) has been considered an important alternative resource for supplementary protein for livestock. Over the last twenty years in China, the content of anti-nutritional factors in rapeseed, such as glucosinolates and erucic acid, has been greatly reduced by the introduction of double low rapeseed. It has been proved that the protein biological value of double low rapeseed meal (DL-RSM) is comparable to that of soybean meal (SBM) (Campbell et al., 1981; Nasi, 1991). Previous studies on poultry (Hickling, 2001; Taylor et al., 2004), swine (Baidoo et al., 1987; Siljander-Rasi et al., 1996) and fish (Davies et al., 1990) have shown that DL-RSM was a feasible protein source substitute for SBM. However, results from studies on pig diets using DL-RSM vary considerably. This may be due to the different content of anti-nutritional factors, meal processing, and the age and weight of the pigs used in the experiments.

Extrusion involves heat treatment, pressure and shear force, which has been found to have beneficial effects on the nutritional value of rapeseed meal and other feedstuffs (Pongmaneerat and Watanabe, 1993; Burel et al., 2000; Allan and Booth, 2004). It has been reported that diets with less than 9% extruded rapeseed meal instead of SBM have no negative effect on the feed intake or weight gain of weaning piglets, and 5% extruded rapeseed meal can even improve the productive performance of growing pigs (Yang et al., 2004a, 2004b). Furthermore, adding commercial enzymes is one of the methods used to improve the nutritional quality of rapeseed meal. Exogenous commercial enzymes have been shown to improve the nutritional value of feedstuffs in animals (Bedford, 1995; Classen, 1996; Gdala et al., 1997).

To our knowledge, there is still some controversy concerning the effect of extrusion and enzyme supplementation on the dietary quality of DL-RSM for finishing pigs. Therefore, the aim of this study was to make a comprehensive evaluation of the effects of extruded DL-RSM and multienzyme preparation in the diets of finishing pigs on growth performance, nutrient digestibility, serum thyroid hormone level, immune function, antioxidant status and conventional biochemical index.

Materials and methods

Experimental design and feeding management

The experiment was conducted in accordance with the Chinese guidelines for animal welfare and was approved by the animal welfare committee of the Animal Science College, Zhejiang University.

Extruded and non-extruded double-low rapeseed meal and multienzymes preparation (cellulase 10,000 U/g, xylanase 6000 U/g, β-glucanase 5000 U/g, protease 12,000 U/g) were all provided by Zhejiang Xinxin Feed Co., Ltd. in China. The physical conditions for extrusion are: diameter of die nozzle 14 mm, temperature 115°C, steam pressure 0.3 MPa, screw speed 55 r/min. The chemical composition of double-low rapeseed meal and extruded double-low rapeseed meal is shown in Table 1.

Forty-eight healthy fattening pigs (Duroc×Landrace×Yorkshire), 110 days of age, were randomly divided into four groups according to gender. Each group had three replicates with 4 pigs (2 males and 2 females) per replicate, and each replicate was kept in its own pen. Average initial weight of the pigs was approximately 62 kg. Basal diets with different dietary treatments were formulated in equal energy and digestible amino acids to meet...
NRC (1998) nutrient requirements (Table 2). Diet 1, based on corn and soybean meal (SBM), was used as control, Diet 2 (DL-RSM group) used 13% DL-RSM instead of the 11% SBM in the control diet, Diet 3 (E-DL-RSM group) used 13% extruded double-low rapeseed meal (E-DL-RSM) to replace 11% soybean meal, and Diet 4 (E-DL-RSM + enzyme group) contained 0.03% multi-enzyme preparation on the basis of the E-DL-RSM group diet. The entire study lasted 37 days; a 7-day acclimation and a 30-day experimental period. According to the average price of ingredients in the diet in the first six months of 2011 on the local market (Zhejiang province, China), costs of the diets studied were as follows. Diet 1: 2728 yuan/t; Diet 2: 2607 yuan/t; Diet 3: 2636 yuan/t; Diet 4: 2667 yuan/t.

The feeding trial was carried out at the Zhejiang Xinxin Breeding Co., Ltd. in China. Pigs were provided with feed and water ad libitum, and individually weighed at the beginning and the end of the study. Feed intake was recorded daily for each pen. Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated from these data.

Sampling and biochemical analyses

Faeces were collected from each pen at three time points (06:00 h, 13:00 h and 20:00 h) on Days 35, 36 and 37. All faeces samples were freeze-dried (20°C) and finely ground before analysis. At the end of the trial, 2 pigs (one male and one female) from each replicate were held in a supine position and blood samples were obtained by anterior vena cava puncture using heparinized and plain vacutainer tubes after 12 h fasting. The samples were then centrifuged at 2000 g for 15 min at 4°C, serum was stored at -80°C until hormone levels, immune and antioxidant parameters, and other biochemical indexes were measured.

Serum thyroxine (T4), free thyroxine (FT4), triiodothyronine (T3) and free triiodothyronine (FT3) were measured on a microplate reader (SpectraMax MS, Molecular Devices, Sunnyvale, CA, USA) using sandwich ELISA assay kits (ICN Pharmaceuticals, Orangeburg, NY, USA) according to the manufacturer’s instructions. The concentration of thyroid hormone level in serum was expressed as nanomole per liter (nmol/L). Levels of immunoglobulins (IgA, IgG, IgM) and complements (C3, C4) were analyzed by indirect enzyme-linked immunosorbent assays (Tiemann et al., 2006), and were all expressed as gram per litre (g/L). The GSH-Px, superoxide dismutase (SOD), and malondialdehyde (MDA), were determined using the method of

Table 1. Chemical composition of double-low rapeseed meal and extruded double-low rapeseed meal.

|                        | Double-low rapeseed meal | Extruded double-low rapeseed meal |
|------------------------|--------------------------|----------------------------------|
| Dry matter, %          | 91.93                    | 91.21                            |
| Crude protein, %       | 35.62                    | 35.44                            |
| Ether extract, %       | 4.37                     | 3.58                             |
| Crude fibre, %         | 5.69                     | 5.42                             |
| ADP, %                 | 13.06                    | 12.53                            |
| NDF, %                 | 20.34                    | 19.47                            |
| Ash, %                 | 6.87                     | 6.68                             |
| Amino acids            |                          |                                  |
| Aspartic acid, %       | 2.57                     | 2.50                             |
| Threonine, %           | 1.57                     | 1.60                             |
| Serine, %              | 1.62                     | 1.66                             |
| Glutamic acid, %       | 8.33                     | 8.27                             |
| Proline, %             | 1.90                     | 1.97                             |
| Glycine, %             | 1.81                     | 1.80                             |
| Alanine, %             | 1.47                     | 1.62                             |
| Cystine, %             | 0.34                     | 0.36                             |
| Valine, %              | 1.56                     | 1.57                             |
| Methionine, %          | 0.51                     | 0.57                             |
| Isoleucine, %          | 1.23                     | 1.27                             |
| Leucine, %             | 2.37                     | 2.57                             |
| Tyrosine, %            | 0.95                     | 0.95                             |
| Phenylalanine, %       | 1.60                     | 1.62                             |
| Lysine, %              | 2.20                     | 2.13                             |
| Histidine, %           | 0.92                     | 0.93                             |
| Arginine, %            | 1.70                     | 1.69                             |
| Total amino acids      | 32.65                    | 33.08                            |
| Calcium, %             | 0.62                     | 0.59                             |
| Total phosphorus, %    | 1.53                     | 1.62                             |
| Glucosinolate, mol/g   | 25.67                    | 24.04                            |
| Erucic acid, %         | 2.15                     | 1.86                             |
| Tannin, %              | 1.28                     | 1.03                             |

ADF, acid detergent fibre; NDF, neutral detergent fibre. All components were measured wet.

Table 2. Ingredients and nutrient composition of diets.

| Ingredients       | Control group | Test group |
|-------------------|---------------|------------|
|                   |               |            |
| Ingredients       |               |            |
| Corn, %           | 68.0          | 68.0       |
| Soybean meal, %   | 21.5          | 10.5       |
| Wheat, %          | 5             | 5          |
| DL-RSM, %         | 13            |            |
| CaHPO4, %         | 0.5           | 0.4        |
| Limestone, %      | 1.0           | 1.0        |
| NaCl, %           | 0.3           | 0.3        |
| Rice bran, %      | 1.5           |            |
| Zeolite powder, % | 1.2           | 0.8        |
| Premix*, %        | 1.0           | 1.0        |
| Calculated content |               |            |
| DE, MJ/kg         | 13.56         | 13.23      |
| CP, %             | 15.72         | 15.66      |
| Ca, %             | 0.56          | 0.58       |
| P, %              | 0.46          | 0.47       |
| Digestible Lys, % | 0.67          | 0.65       |
| Digestible Met+Cys, % | 0.46      | 0.48       |
| Digestible Thr, % | 0.47          | 0.46       |

DL-RSM, double-low rapeseed meal; °provided per kg of diet: vitamin A, 6000 U; vitamin D3, 1200 U; vitamin E, 20 U; vitamin K3, 1.5 mg; vitamin B1, 1.5 mg; vitamin B2, 3 mg; vitamin B3, 1.6 mg; pantethenic acid, 15 mg; nicotinic acid, 5 mg; manganese, 5 mg; iron, 84 mg; zinc, 36 mg; copper, 100 mg; iodine, 0.15 mg; cobalt, 0.15 mg; selenium, 0.15 mg. #Calculated values expressed as dry matter. DE, digestible energy; CP, crude protein; Ca, calcium; P, phosphorus; Lys, lysine; Met, methionine; Cys, cystine; Thr, threonine.
mixed equally, and then immediately stored at toluene to every 100 g faeces sample. This was hydrochloric acid solution and a few drops of day for three days. We added 20 mL 10% of samples in the four groups were collected each weeks of the feeding experiment, the faeces the endogenous marker method. After four

**Digestibility trial**

The nutrient digestibility was determined by the endogenous marker method. After four weeks of the feeding experiment, the faeces samples in the four groups were collected each day for three days. We added 20 mL 10% of hydrochloric acid solution and a few drops of toluene to every 100 g faeces sample. This was mixed equally, and then immediately stored at

| Table 3. Effects of different treatment diets on the growth performance of finishing pigs. | Diet° | SEM |
|---------------------------------|-------|-----|
|                                  | Control | DL-RSM | E-DL-RSM | E-DL-RSM+ enzymes |
| IW, kg                          | 62.70   | 62.12  | 62.50    | 62.77            | 2.23 |
| FW, kg                          | 86.60   | 85.93  | 85.96    | 87.40            | 4.04 |
| ADG, g                          | 797     | 794    | 782      | 821              | 69   |
| ADFI, kg                        | 2.22    | 2.13   | 2.15     | 2.24             | 0.63 |
| FCR                             | 2.79    | 2.68   | 2.77     | 2.72             | 0.21 |

Control diet, based on corn and soybean meal, DL-RSM (double-low rapeseed meal), used 13% double-low rapeseed meal instead of 11% soybean meal in the control diet; E-DL-RSM, used 13% extruded double-low rapeseed meal to replace 11% soybean meal; E-DL-RSM+ enzymes, contained 0.03% multienzymes preparation (cellulase enzyme 10,000 U/g, xylanase 6000 U/g, β-glucanase 5000 U/g, protease 12,000 U/g) on the basis of E-DL-RSM group diet. Data were not statistically significant (P>0.05). IW, initial weight; FW, final weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio. Values are expressed as individual data.

| Table 4. Effects of different treatment diets on the apparent nutrient digestibility of finishing pigs. | Diet° | SEM |
|---------------------------------|-------|-----|
|                                  | Control | DL-RSM | E-DL-RSM | E-DL-RSM+ enzymes |
| CP                              | 78.30   | 76.91  | 77.21    | 78.36            | 1.70 |
| EE                              | 72.51   | 70.52  | 70.95    | 72.92            | 0.93 |
| Ash                             | 48.11   | 46.51  | 46.82    | 47.97            | 1.67 |
| CF                              | 46.79   | 45.85  | 44.40    | 47.08            | 1.30 |
| Ca                              | 56.00   | 53.72  | 56.50    | 58.33            | 2.27 |
| P                               | 46.00   | 43.39  | 44.08    | 46.08            | 1.42 |

Control diet, based on corn and soybean meal; DL-RSM (double-low rapeseed meal), used 13% double-low rapeseed meal instead of 11% soybean meal in the control diet; E-DL-RSM, used 13% extruded double-low rapeseed meal to replace 11% soybean meal; E-DL-RSM+ enzymes, contained 0.03% multienzymes preparation (cellulase enzyme 10,000 U/g, xylanase 6000 U/g, β-glucanase 5000 U/g, protease 12,000 U/g) on the basis of E-DL-RSM group diet. Data were not statistically significant (P>0.05). CP, crude protein; EE, ether extract; CF, crude fibre; Ca, calcium; P, phosphorus. Values are expressed per pen.

| Table 5. Effects of different treatment diets on the serum thyroid hormone level of finishing pigs. | Diet° | SEM |
|---------------------------------|-------|-----|
|                                  | Control | DL-RSM | E-DL-RSM | E-DL-RSM+ enzymes |
| T3(nmol/L)                      | 1.65    | 1.75   | 1.89     | 1.66             | 0.17 |
| T4(nmol/L)                      | 43.07   | 41.54  | 42.24    | 42.42            | 0.81 |
| FT3(nmol/L)                     | 0.13    | 0.14   | 0.14     | 0.13             | 0.01 |
| FT4(nmol/L)                     | 0.77    | 0.81   | 0.78     | 0.75             | 0.06 |

Control diet, based on corn and soybean meal; DL-RSM (double-low rapeseed meal), used 13% double-low rapeseed meal instead of 11% soybean meal in the control diet; E-DL-RSM, used 13% extruded double-low rapeseed meal to replace 11% soybean meal; E-DL-RSM+ enzymes, contained 0.03% multienzymes preparation (cellulase enzyme 10,000 U/g, xylanase 6000 U/g, β-glucanase 5000 U/g, protease 12,000 U/g) on the basis of E-DL-RSM group diet. Data were not statistically significant (P>0.05). T3, triiodothyronine; T4, thyroxine; FT3, free triiodothyronine; FT4, free thyroxine.
Table 6. Effects of different treatment diets on the immunity of finishing pigs.

| Items                  | Dieta | SEM          |
|------------------------|-------|--------------|
|                        | Control | DL-RSM | E-DL-RSM | E-DL-RSM+ enzymes |
| IgA, g/L               | 1.18   | 1.18    | 1.18    | 1.21             | 1.34×10^-2   |
| IgG, g/L               | 5.43b  | 5.07c   | 5.21d   | 5.65e            | 0.13         |
| IgM, g/L               | 1.05   | 0.99    | 0.97    | 1.05             | 0.22         |
| C3, g/L                | 0.65   | 0.65    | 0.65    | 0.67             | 1.30×10^-2   |
| C4, g/L                | 0.18   | 0.17    | 0.18    | 0.18             | 3.20×10^-3   |

Control diet, based on corn and soybean meal; DL-RSM (double-low rapeseed meal), used 13% double-low rapeseed meal instead of 11% soybean meal in the control diet; E-DL-RSM, used 13% extruded double-low rapeseed meal to replace 11% soybean meal; E-DL-RSM+ enzymes, contained 0.03% multienzymes preparation (cellulase enzyme 10,000 U/g, xylanase 6000 U/g, β-glucanase 5000 U/g, protease 12,000 U/g) on the basis of E-DL-RSM group diet. IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; T-AOC, total antioxidant capability; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde. *Data in the same row with different letter mean significant difference (P<0.05), while with the same and no small letter superscripts mean no significant difference (P>0.05).

Table 7. Effects of different treatment diets on serum antioxidant status of finishing pigs.

| Items         | Dieta | SEM          |
|---------------|-------|--------------|
|                | Control | DL-RSM | E-DL-RSM | E-DL-RSM+ enzymes |
| T-AOC, U/mL    | 3.54   | 3.62    | 3.67    | 3.76             | 0.50         |
| GSH-Px, U/mL   | 513.61 | 518.14  | 524.19  | 535.12           | 49.16        |
| SOD, U/mL      | 91.28  | 89.71   | 91.24   | 93.83            | 5.17         |
| MDA, nmol/mL   | 3.62   | 3.35a   | 3.32    | 2.78b            | 0.25         |

Control diet, based on corn and soybean meal; DL-RSM (double-low rapeseed meal), used 13% double-low rapeseed meal instead of 11% soybean meal in the control diet; E-DL-RSM, used 13% extruded double-low rapeseed meal to replace 11% soybean meal; E-DL-RSM+ enzymes, contained 0.03% multienzymes preparation (cellulase enzyme 10,000 U/g, xylanase 6000 U/g, β-glucanase 5000 U/g, protease 12,000 U/g) on the basis of E-DL-RSM group diet. T-AOC, total antioxidant capability; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde. *Data in the same row with different letter mean significant difference (P<0.05), while with the same and no small letter superscripts mean no significant difference (P>0.05).

Table 8. Effects of different treatment diets on conventional biochemical index of finishing pigs.

| Items         | Dieta | SEM          |
|---------------|-------|--------------|
|                | Control | DL-RSM | E-DL-RSM | E-DL-RSM+ enzymes |
| Ca, mmol/L     | 1.10   | 1.06    | 1.08    | 1.14             | 0.13         |
| P, mmol/L      | 2.56   | 2.37    | 2.42    | 2.66             | 0.16         |
| BUN, mmol/L    | 18.36  | 16.50   | 15.83   | 15.13            | 3.26         |

Control diet, based on corn and soybean meal; DL-RSM (double-low rapeseed meal), used 13% double-low rapeseed meal instead of 11% soybean meal in the control diet; E-DL-RSM, used 13% extruded double-low rapeseed meal to replace 11% soybean meal; E-DL-RSM+ enzymes, contained 0.03% multienzymes preparation (cellulase enzyme 10,000 U/g, xylanase 6000 U/g, β-glucanase 5000 U/g, protease 12,000 U/g) on the basis of E-DL-RSM group diet. Ca, calcium; P, phosphorus; BUN, blood urea nitrogen. Data were not statistically significant (P>0.05).

Results and discussion

The chemical composition of DL-RSM and E-DL-RSM is shown in Table 1. As expected, extrusion had no negative effect on nutritive value of DL-RSM. There was no significant change in DM, CP, EE, CF Ash, the amino acids, Ca and P in E-DL-RSM, but the values of some nutritive parameters, CP (35.44%), Lys (2.13%), Met (0.57%), Arg (1.69%), Ile (1.27%) and Val (1.57%) in E-DL-RSM were lower than the values reported by Woyengo et al. (2010) and Seneviratne et al. (2010), which may be due to the different strain and processing condition of rapeseed meal. Glucosinolate, erucic acid and tannin were decreased by 6.35%, 13.49% and 20.16%, respectively, due to extrusion. In the current study, replacing 11% SBM with 13% DL-RSM had no negative effect on the ADG, ADFI and FCR of finishing pigs during the entire experimental period (Table 3), and the digestibility of CP, EE, Ash, CF and TP was also not significantly affected (P>0.05) (Table 4). These findings are in agreement with previous reports (Lee et al., 1983; Thacker and Qiao, 2002; Landerer et al., 2012). Extrusion and adding exogenous enzymes could be effective ways to improve the nutritional quality and reduce the activity of anti-nutritional factors in feedstuffs (Bedford and Clasen, 1992; Choc, 1999; Friesen et al., 1993). However, in this study, extrusion and extrusion combined with multienzyme preparation in diets did not significantly increase the performance or nutrient digestibility in finishing pigs (P>0.05). Previous experiments often gave inconsistent results. The performance of pigs fed DL-RSM has been shown to be improved as result of extrusion (Froseth and Peters, 1981), and starch digestibility is increased (Bengala Freiere et al., 1991), but no beneficial effect was reported by Thacker and Qiao (2002). The reason for this variation may be due to the different content of anti-nutritional factors, meal processing and the substitute proportion. However, results from this study indicated that double-low rapeseed meal could be an alternative protein resource to soy-
bean meal for finishing pigs.

There was no significant change in hormones, T₃, T₄, FT₃, and FT₄, secreted by pig thyroid gland in serum (P>0.05) with the different dietary treatments (Table 5). There were also no significant differences between the DL-RSM group and the E-DL-RSM group (P>0.05). It has been shown that degradation of glucosinolates, isothiocyanate and oxazolines, can induce hyperthrophy of liver, kidney and thyroid and cause iodine deficiency (Schöne et al., 1997; Tripathi et al., 2001). Iodine is necessary for the synthesis of the thyroid hormones, T₃ and T₄, which regulate energy metabolism. Schöne et al. (2001, 2002) found that 15% rapeseed meal in diet can cause reduced feed intake and reduced weight gain in pigs (live weight range 24 to 104 kg), while the weight of thyroid gland and liver increased and the serum T4 concentration decreased. The present study has shown that diets containing 13% of DL-RSM did not significantly reduce the serum T4 concentration, so thyroid gland function may be not impaired. This suggests that the higher weight of pigs used in the present experiment probably (approx. 62 kg) can support a high proportion of rapeseed meal. Therefore, replacement of 11% SBM by 13% DL-RSM is appropriate for pigs during the finishing phase of production, and offers economic benefits.

Results showed that the levels of IgA, IgM, C3 and C4 in serum had not been significantly affected by different dietary treatments (P>0.05), while the level of IgG was decreased significantly by 6.62% (P<0.05) in the DL-RSM group and it returned to the level of control in E-DL-RSM + enzymes group (Table 6). So far, there has been little research examining the effects of rapeseed meal on immunoglobulin levels. The in vivo effects of extrusion and enzyme preparation were beneficial to IgG concentration. The present study has shown that exposure and enzyme preparation can not improve the nitrogen utilization of pigs during the finishing phase of production during which 11% soybean meal was replaced by 13% double-low rapeseed meal in the diet.

Conclusions

In summary, replacement of 11% soybean meal by 13% double-low rapeseed meal in finishing pig diets had no negative effects on growth performance, nutrient digestibility and biochemical indexes except IgG concentration in serum. Although extrusion and multienzyme preparation were beneficial to IgG concentration and can decrease MDA in the serum, adding DL-RSM supplements directly in the diet was still the best choice given the extra cost of extrusion and of enzyme preparation.

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