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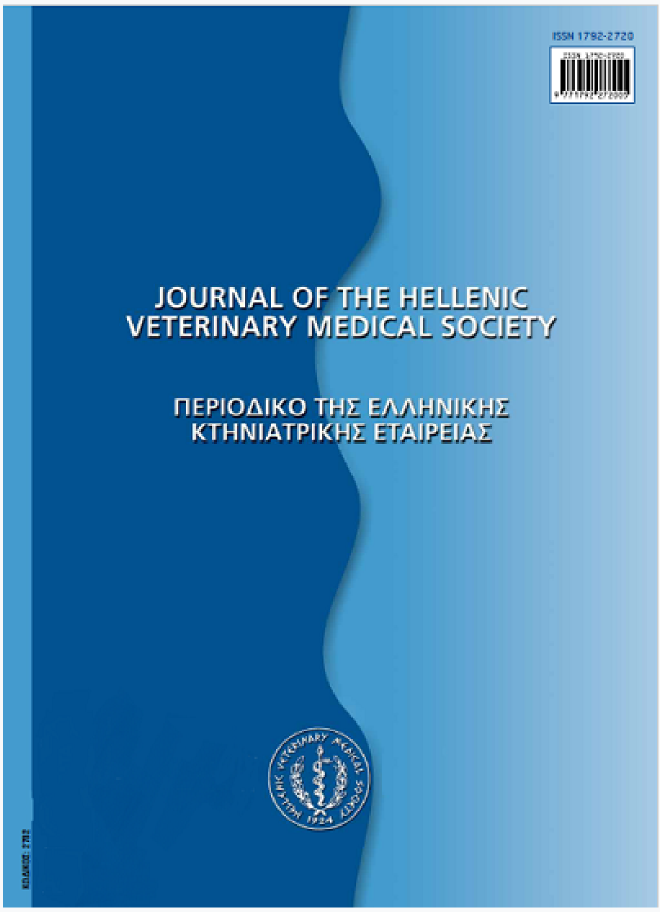

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Digestibility and nitrogen balance of pig diets containing gradually increasing levels of rapeseed meal

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ABSTRACT. Rapeseed meal (RSM), a by-product of extracted rapeseed oil production, is a potential protein source for use in pig diets. To determine the best levels of RSM inclusion in diets of fattening pigs as well as the digestibility and energy value of diets containing gradually increasing levels of RSM, a digestibility trial was conducted. The trial was performed according to a 4x4 Latin square experimental design, with 4 Large White x Landrace castrated male pigs weighing on average 47.3kg. The animals were housed in metabolism cages during the whole period the duration of which was a total of 66 days. The experiment was divided into 5 periods, an adjustment period and 4 experimental periods, during which pigs consumed 4 experimental diets. The diet C (control) was a basal diet based on corn and soybean meal, while the other experimental diets included RSM at levels 90 (low- L), 180 (medium- M) and 270 (high- H) $g.kg^{-1}$ of feed, respectively. Each experimental period consisted of 10 preliminary days during which the animals were adapted to the diets, followed by a 4 days period for collection of faeces, urine and feed refusal. The gradual increase in the level of RSM in the experimental diets resulted in a significant ($P<0.05$) reduction in the apparent digestibility of CP, but only for the diet with the highest level of RSM, compared to the control diet and this one with the lowest level of RSM . Any increase in the level of RSM in the experimental diets resulted in significant ($P<0.05$) increase on the apparent digestibility coefficient (ADC) of CF and very significant ($P<0.01$) increase in diets M and H, compared to the control and diet L. Similar were the effects of RSM levels in the diets on the apparent digestibility coefficient of NDF, ADF and cellulose. As conclusion, the gradual increase in the level of RSM in the fattening pigs' diets did not significantly affect ADC of DM and gross energy, caused a significant reduction in the ADC of CP, and a significant improvement in the ADC of CF as well.

Keywords: fattening pig; rapeseed meal extraction; apparent digestibility coefficient

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1. INTRODUCTION

Because of the increasing demand in protein for livestock feeding, the interest in using rapeseed meal (RSM) in pig diets is also increasing. Rapeseed meal extraction (RSM), a by-product of extracted rapeseed oil production, is a potential source of vegetable protein for use in pig diets. Rapeseed contains less than 2% erucic acid in the oil and less than 30 μmol/g glucosinolates (GSL) in the meal (Newkirk, 2009; Diederichsen and McVetty, 2011). After oil extraction, rapeseed meal has a high content of CP (33.7-37.5%, Sauvant et al., 2004) and a higher NDF content than soybean meal (26.0% vs 8.21%, respectively; NRC, 2012), because hulls are not eliminated and represent about 30% of the meal (Barthet and Daun, 2011); this causes a lower digestible energy content than that of soybean meal (11.59 vs 15.15 $Mj.kg^{-1}$; Sauvant et al., 2004). Among the components of RSM that may reduce palatability or nutrient availability in pig diets, GSL and degradation products, as well as the fibre content, play a key role (Bellostas et al., 2007). Royer and Gaudré (2008), recorded no adverse effect on piglet performance when including 12% RSM with GSL content up to 15.4μmol/g DM in fattening pigs diets. RSM is a protein source which can be used in growing pigs (King et al., 2001; McDonnell et al., 2010). The chemical composition of rapeseed is influenced by various environmental factors (Barthet and Daun, 2011; Newkirk, 2011). RSM use is supported by thorough knowledge of energy and nutrients digestibility. However, the nutritional value of RSM in Greece is expected to be different compared with that of other countries due to the variation in cultivars, seed quality, soil conditions and processing methods. Due to the fact that RSM has a high crude fibre (CF) content and high levels of CF significantly affect nutrients utilization in pigs, the conduct of this study was considered necessary.

2. MATERIALS AND METHODS

This experiment was conducted in the farm of School of Agriculture, Aristotle University of Thessaloniki, Greece, in accordance with the EU Directive 2010/63/EU for animal experiments.

2.1. Pigs and Experimental Design

The experiment was performed according to a 4x4 Latin square arrangement design of 4 dietary treatments, with 4 Large White x Landrace castrated male pigs weighing on average 47.3 kg. The animals were housed in metabolism cages during the whole period. The metabolism cages were built to allow the quantitative collection of faeces and urine. The metabolism cages were installed in an open stable.

The experiment was divided into five periods, an adaptation period (lasting 10 days) and four experimental. Each experimental period consisted of 10 preliminary days during which the animals were adapted to the diets followed by another period of 4 days for collection of faeces, urine and feed refusals. Thus, total experimental duration was 66 days, during which pigs consumed 4 experimental diets (C; L; M&H), containing low (L), medium (M) and high (H) level of RSM or 90, 180 and $270g.kg^{-1}$ of feed. During four experimental periods the pigs consumed daily 1500g of the diet. The daily allowance of the diet was given in 2 equal quantities at 08:00 and 14:00. Water consumption was *ad libitum*. The temperature for the duration of the experiment ranged at 22.2 ± 2,0 °C. RSM was obtained from a batch of seeds processed by the Greek enterprise "ELVI AVEE" factory. Every 14 days from the start of the experiment or the completion of each experimental period, pigs were weighed and exercised before their return in the experimental cages. The composition and chemical analysis of the experimental diets are given in Table 1. Chemical analysis of RSM showed in Table 2. As it is clear from the data in Table 1, RSM mainly replaced soybean meal, while taking care so that all the diets remained isonitrogenous and isoenergetic by using the needed amounts of essential amino acids and rapeseed oil.

2.2. Measurements and Analytical Methods

For each diet, a sample of feed was collected and measured for its DM content and subsequently used for chemical analyses. Faeces were collected into nylon bags (one bag per pig) as soon as they appeared in the metabolism crates. Samples were collected daily and their weights were recorded during the sample collection period. Each day, acidified (with H_2SO_4 to reach a pH below than 4.0) urine was collected. Daily urine and faeces collection were cumulated and stored at -20°C. Then, collected faeces were homogenized and subsampled for DM analysis and freeze-dried for

Table 1. Composition, chemical and calculated analysis of experimental diets.

	Total mixed diets			
	RSM[1]			
Ingredients (g.kg^{-1})	C	L	M	H
Corn grain	723.0	665.0	588.0	545.0
Wheat bran	25.0	56.0	100.0	120.0
Soybean meal 44%	210.0	140.0	70.0	-
Rapeseed meal (RSM)	-	90.0	180.0	270.0
Rapeseed oil	6.0	15.0	29.0	34.2
Limestone	8.0	8.0	7.5	7.0
$CaHPO_4$	8.0	6.0	5.0	3.0
Vitamin and trace elements premix[2]	15.0	15.0	15.0	15.0
Salt	5.0	5.0	5.0	5.0
Lysine	-	-	0.5	0.8
Chemical composition (%)				
Dry matter	89.50	89.60	89.70	89.80
Crude protein	16.00	16.0	16.00	16.00
Crude fat	3.20	3.30	3.40	3.50
Crude fibre	3.20	4.00	4.80	5.50
Ash	4.30	4.50	4.60	4.70
Nitrogen free extract	73.3	72.20	71.20	70.30
Ca*	0.50	0.50	0.50	0.50
P available*	0.22	0.21	0.23	0.22
Lysine*	0.78	0.75	0.77	0.75
M+C*	0.54	0.58	0.61	0.66
ME (Mj.kg^{-1})*	13.60	13.60	13.60	13.60
NDF-NDF	13.50	14.70	15.90	17.10
ADF-ADF	4.50	5.60	6.64	7.71
Cellulose	3.80	4.20	4.50	5.00
Hemicelluloses	9.00	9.10	9.30	9.40
Lignin (sa)	0.70	1.40	2.10	2.70
Glucosinolates, GSL (μmol.g^{-1})	-	1.34	2.68	4.02

**NRC Tables (1998).*
[1] Rate of inclusion of RSM in pig diets (L- low, M- medium and H- high level or 90, 180 and 270g.kg^{-1} of diet, respectively.
[2] Premix that provided per kilogram of growing pigs diet: Vitamin A 13,000 IU, D_3 5,000 IU, E 100mg, K_3 4.0 mg, B_1 2.6g, B_2 8mg, B_6 3mg, B_{12} 0.015mg, C 10mg, Choline-Cl 500mg, Niacin 85mg, Viotine 0.2mg, Pantothenic Acid 20mg, Folic acid 2mg, Cu 20.0mg, I 1.0mg, Fe 40mg, Mn 120mg, Se 0.3mg, Zn 100.0mg, Co 0.2mg.

Table 2. Chemical composition of RSM (%).

Dry matter	89.80
Crude protein (Nx6,25)	33.50
Crude fat	2.73
Crude fibre	12.00
Ash	6.50
Nitrogen free extract	45.27
Ca1	0.68
P available1	0.26
NDF-NDF	26.90
ADF-ADF	16.40
Lignin (sa)	8.10
Glucosinolates (μmol.g-1)2	14.90
Lysine1	1.71
Methionine1	0.72
Cystine1	0.85

[1] *NRC Tables (1998).*

[2] *Kargopoulos et al. (2014).*

further chemical analyses at the end of the collection period.

The calculations of dry matter (DM) by drying at 102°C for 16h in a forced air oven, and for crude protein (CP), crude fat, crude fibre (CF), and ash were performed according to methods 976.06, 920.39, 978.10 and 942.05 respectively, of AOAC (1990). The NDF and ADF were determined according to the methods of Van Soest et al. (1991). Hemicelluloses were calculated as NDF-ADF difference and cellulose as ADF-ADL one (Rinne et al., 1997). The GSL in the RSM calculated with the HPLC method (ISO 9167-1) by the use of Hewlett Packard chromatograph- Type 1050 apparatus (Spinks et al., 1984). Samples of rapeseed meal, diets, faeces and urine were analysed for gross energy (GE) via an adiabatic bomb calorimeter (Parr Instruments, Moline, IL, UAS).

2.3. Calculations

The determination of apparent digestibility of the nutrients of the experimental diets was done according to the standard method (McDonald et al., 1988) and according to the equation: ADC= (Nd-Nf)/Nd, where ADC= apparent digestibility coefficient, Nd = nutrient in the diet, Nf = nutrient in the faeces. The DE of the diets was calculated from the Morgan et al. (1975) equations:

ME = 16.13-9.5 NDF+16 OIL + 23 CP x NDF - 138 ASH x NDF

DE= ME/(0.997-0.000189 CP)

Nitrogen balance of the experimental diets based on the equation:

Nr = Nd- (Nf + Nu), (g/day) where Nr = N remained, Nd= N of the diet, Nf= N of the faeces and Nu= N of the urine.

2.4. Statistical Analysis

Data were analysed as a 4x4 Latin square experimental design with diet, period and pig as factors, using the general linear models procedure of SAS (1998). The model used was the following:

$Y_{ijkl} = \mu + T_i + P_j + p_k + \varepsilon_{ijkl}$ where: Y_{ijkl} = observation, μ = population mean, T_i = diet effect (i = 1 to 4), P_j = period effect (j = 1 to 4), p_k = pig effect (k = 1 to 4) and ε_{ijkl} = residual error. Significant differences among treatment means were tested using Duncan's multiple range test at the 0.05 probability level (Steel and Torrie, 1980).

3. RESULTS

3.1. Apparent Digestibility Coefficients of Nutrients

Apparent digestibility coefficients (ADC) for the experimental diets showed in the Table 3. ADC of CP tended to decrease with the gradual increase in the level of RSM in the experimental diets, but this trend was statistically significant (P<0.05) only in the diet with the highest level of RSM (H) compared to the control diet and this one with the lowest level of RSM (L), but not in comparison to the diet with the intermediate level of RSM (M). ADC of CF significantly (P<0.05) increased with every increase of RSM in the experimental diets. ADC of NDF showed a gradual improving trend, which was statistically significant (P<0.05) only in the diet with the high (H) level of RSM compared to the control. ADC of ADF significantly (P<0.05) increased with every increase in the level of RSM in the diets. Similarly, the ADC of cellulose, showed a significant (P<0.05) increase for

Table 4. N balance[1] of experimental pig diets (g/g).

Treatments[2]	N faeces	N urinary	N retained
	N intake		
C	0,154[a]	0,385[a]	0,461[a]
L	0,180[ab]	0,339[ab]	0,482[a]
M	0,195[ab]	0,297[bc]	0,508[ab]
H	0,198[b]	0,260[c]	0,542[b]
SEM	0.03	0.03	0.03
P	0,038	0,027	0,310

[1] *Means and standard error of the means.*
[2] *Diets containing RSM in low (L), medium (M) and high (H) level or 90, 180 and 270g.kg^{-1} of diet, respectively.*
[a,b,c] *Means in the same column sharing a different superscript are significantly different (P<0.05).*

Table 3. Apparent digestibility coefficients of experimental pig diets[1].

	Diets[2]					
	C	L	M	H	SEM	P
Dry matter	0.826	0.813	0.805	0.793	0.02	0,094
Crude protein	0.778[a]	0.765[a]	0.752[ab]	0.739[b]	0.01	0,043
Crude fat	0.660	0.662	0.663	0.664	0.03	0,098
Crude fibre	0.433[a]	0.453[ab]	0.473[bc]	0.493[c]	0.02	0,036
NDF-NDF	0.699[a]	0.714[ab]	0.749[ab]	0.753[b]	0.03	0,026
ADF-ADF	0.285[a]	0.413[b]	0.509[c]	0.590[d]	0.04	0,021
Cellulose	0.409[a]	0.582[b]	0.668[c]	0.742[d]	0.03	0,019
Hemicelluloses	0.778	0.792	0.808	0.821	0.03	0,073
N	0.846[a]	0.820[a]	0.805[ab]	0.802[b]	0.02	0,038
GE	0.792	0.782	0.779	0.773	0.02	0,084

[1] *Means and standard error of the means.*
[2] *Diets containing RSM in low (L), medium (M) and high (H) level or 90, 180 and 270g.kg^{-1} of diet, respectively.*
[a,b,c] *Means in the same row sharing a different superscript are significantly different (P<0.05).*

every increase of the level of RSM in the experimental diets. The increase of RSM in the experimental diets did not significantly affect the ADC of energy.

3.2. Nitrogen Balance

Nitrogen balance showed in the Table 4. Nitrogen retained (Nr), as a percentage of N intake (Ni), increased depending on the level of RSM in the diets and was significantly ($P<0.05$) higher for the highest level of RSM (H) compared to the control. In particular gradual increase in the level of RSM in the diet, resulted to increased losses of N in faeces and lower losses of N in the urine. However, the reduction of N loss in urine was higher than the size of the increase of the losses of N in faeces.

4. DISCUSSION

4.1. Apparent Digestibility Coefficient

In this work, GSL content of C, L, M and H diets was 0.0, 1.34, 2.68 and 4.02μmol.g[1] as fed basis, respectively. Crude fibre content of the diets was 32, 40, 48 and 55 g.kg^{-1} and NDF-NDF content was 135, 147, 159 and 171g.kg^{-1}, respectively. The predictable undesirable effect of GSL in treatments M & H was not observed, which could be attributed both to the limited feeding, given that pigs consumed daily only79% of the amount of *ad libitum* feed intake (NRC, 1998) and the limited duration of each experimental period (14 days).

The addition of RSM in the experimental diets did not cause any adverse effect on ADC of DM and DE. Contrary were the results of other researchers (Landero et al., 2011; Moset et al., 2012) who observed that the addition of RSM in pigs' diets causes a significant reduction in the digestibility of nutrients, which is attributed to the increase in the proportion of CF in the faeces, and secondly because of increased water retention capability of the CF (Wilfart et al., 2007; Moset et al., 2012). This contradictory result is probably due to the limited feed intake in the present study.

The RSM is well known high CF feed (NRC 2012; Kargopoulos et al., 2014) with high protein content. According to Souffrant (2001) fibre is a heterogeneous mixture of polysaccharides (structural and non-structural) and lignin that is not digested by endogenous secretions of the digestive tract. Hansen et al. (2007), had explained that CF diets influence N excretion due to N repartitioning from urine to faeces, which invariably affects apparent digestibility coefficient of CP. NRC (2012) reports that fibre provides substrate for microbial fermentation in the large intestine, which could also improve the apparent digestibility coefficient of dietary CF of up to 92% for growing pig diets.

Increasing CF concentration in diets of growing - fattening pigs is associated with significant reduction in the digestibility of energy and CP of diets. In particular, the reduction of digestibility of energy potential is attributed to the reduction of digestibility of dry and organic matter of diets (Wilfart et al., 2007) or to the reduction of the digestibility of carbohydrates in diets (Le Gall et al., 2009), due to acidification of the digesta, which results in limited absorption of nutrients of the digesta by the epithelial cells of the intestinal tract (Rainbird et al., 1984). The decreased digestibility of CP and amino acids is possibly attributed to the restriction of their absorption and / or to increased concentration of endogenous N and thus to the loss of amino acids (Mosenthin et al., 1994). The presence of CF in the diet of growing-fattening pigs causes transformation of bacterial fermentation in the colon, resulting N produced from the fermentation of undigested CP of feed and N of endogenous origin to be used to form microbial protein (Zervas and Zijlstra, 2002). Consequently there is a significant reduction in the quantity of N which is absorbed into the bloodstream and is used in the liver for synthesis of urea (Mroz et al., 2000; Zervas and Zijlstra, 2002). As a result, the N amount in urine is limited.

Significant differences were detected regarding the ADC of CP. The ADC of CP was significantly lower for the diet with the high (H) level of RSM as compared to the other three treatments of the experiment. Findings of other research (McDonnell et al., 2011), suggest that this is probably due to the high content of RSM in CF and furthermore to ability of CF binding CP resulting to limiting its digestibility. According to Grundy et al. (2016), the presence of water-soluble dietary fibre in the digesta could minimise the interactions between enzymes and substrates because of the increase in viscosity and subsequent reduced mixing in the GI tract. In addition, dietary fibre present as solubilised polysaccharide chains may reduce macronutrient hydrolysis by direct binding to digestive enzymes and/

or by physical interaction (binding) with hydrophilic substrate surfaces. Moreover, it has been proved that the increased content of CF in pig diets causes a significant increase of bacterial N in the faeces which affects the ADC of CP (Bindelle et al., 2009; Moset et al., 2012; Sanjayan et al., 2014). In this experiment daily feed intake of pigs was 1500g, representing 78.9% of their *ad libitum* feed intake (NRC, 1998). Under restricted feeding, microflora of growing-fattening pigs' digestive tract may have been adapted well to the high fibre levels diets (Low, 1980; Dotas, 1986; Nikolakakis, 1995; Amaefule et al., 2009).

In this work, ADC of CF increased significantly ($P<0.05$) in the diets with the medium (M) and the high (H) level of RSM compared to the control. Similar were the results of other researches (Goedhart, 1990; Anguita et al., 2006). Improved digestibility of CF is probably due to increased microbial activity, which occurs when consumed diets with high content in CF increases the indigestible quantity of fibrous substrate and other substances that reach the colon (Mellange et al., 1992; He, 2004; Anguita et al., 2006). Contradictory are the results of other researchers (Wilfart et al., 2007), showing that the increase of the level of CF in pig diets causes no positive effect on their digestibility. These results may be due to the different source of origin and content of CF used (Stanogias and Pearce, 1983; deVriesa et al., 2012; Zhang et al., 2013).

ADC of NDF tended to improve with increased levels of RSM in the experimental diets, but this effect was statistically significant ($P<0.05$) only for pigs consumed the high level of RSM compared to the control diet. ADC of ADF, dramatically improved, with increasing levels of RSM in the experimental diets. Statistically significant ($P<0.05$) differences were observed regarding the ADC of ADF among the treatments with low (L) and intermediate (M) level of RSM. Significant ($P<0.05$) was the improvement on the ADC of cellulose with every increase in the level of RSM in the experimental diets.

The high mean values in ADC of cellulose and hemicelluloses of diets with high content of RSM, possibly indicates a low degree of lignification and therefore a relatively easier use by the digestive tract of fattening pigs, covering a part of their energy needs.

4.2. Effect of RSM Level on N-Balance

The mean daily N retention for the diets in which RSM was added was 19.6g, was combined with a consumption of 20.4Mj ME per day and pig. These values are in agreement with corresponding values of other researchers (Dunkin et al., 1984) given that in this experiment, the daily feed consumption was reduced. It has been shown (Dunkin et al., 1984; Urynek and Buraczewska, 2003) that there is a positive linear relationship between ME intake and N retained, assuming that the diet contains the required CP level to achieve the maximum N retention. The presence of CF in the pig diets, favours the formation of microbial protein in the colon (Zervas and Zijlstra, 2002), and limits the amount of ammonia absorbed into the bloodstream and is used by the liver for biosynthesis of urea (Mroz et al., 2000; Zervas and Zijlstra, 2002). As a result, the amount of urine N is limited (Bindelle et al., 2009).

5. CONCLUSIONS

The gradual replacement of soybean meal in the diets of fattening pigs by RSM, caused a significant reduction in the digestibility of CP. However, the digestibility of CP remained high, even in the diet in which the exclusive protein source was RSM, which probably could be attributed to improved nitrogen retention capability of pigs. At the same time, a gradual improvement in digestibility of CF observed, which probably indicates that pig's digestive tract is adapted in the best way to diets with higher than usual CF levels, in an effort to meet their requirements of energy and nutrients.

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