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USAGE OF FOOD INDUSTRY BY PRODUCTS (FLAXSEED AND GRAPESEED MEAL) IN FATTENING PIGS' DIET

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Abstract

The 4 weeks study was conducted on 12 pigs divided in 2 groups (C, E). The average initial body weight for control group C was 66.42 kg±10.27 and 66.25 kg±9.88 for experimental group E, respectively. The C diet based on corn and soybean meal was characterized by: 17.46% crude protein and 3232 kcal/kg ME. Compared to diet C, the E diet had included 7.5% flaxseed meal and 1% grapeseed meal (17.60% CP, and 3200 kcal/kg ME). At the end of the experiment there were no significant differences (P≥0.05) among groups concerning the performances. All carcasses were assessed in E category, with no statistical differences between groups. Regarding meat quality, best results were noticed at E group for α-linolenic acid concentration (omega 3 fatty acid) of ham (1.29±0.04 g/100g total fatty acids) compare to C (0.51±0.01 g/100 g total fatty acids). Also α-linolenic acid concentration of tenderloin (1.12±0.03 g/100 g total fatty acids) in group E was higher compare to C group (0.6±0.06 g/100g total fatty acids), but without significant differences.

Key words*: pigs, flaxseed, grapeseed, carcass, meat, quality.*

INTRODUCTION

Flaxseed or Linseed (*Linum usitatissimmum*), represents one of the richest vegetarian source of α-linolenic acid (omega 3 fatty acid) and soluble mucilage, a novel high quality source of nutrition (Ganorkar and Jain, 2012).

The omega-3s and lignan phytoestrogens of flaxseed are in focus for their benefits for a wide range of health conditions and may possess chemo-protective properties in animals and humans, making flaxseed an important functional food ingredient (Singh et al., 2011). In general, the fatty acid profile of meat directly reflects the fatty acid profile in the pig diet (Eastwood, 2008). Also the content of insoluble fiber of flax helps improve laxation and prevent constipation, mainly by increasing fecal bulk and reducing bowel transit time (Greenwald et al., 2001) and water-soluble fiber helps in maintaining blood glucose levels and lowering the blood cholesterol levels (Kristensen et al., 2012).

Nowadays, consumers are interested in a higher content of PUFA by reason of healthier diet (in modern human diets, the ratio of n-6/n-3 PUFA is very high, 10- 15:1 and optimum is 4:1), while the increase in the PUFA brings producers complications in the durability of meat and fat (Warnants et al., 1999). Therefore, the dietary inclusion of a potentially antioxidant and low-cost ingredient such as grapeseed meal, concomitantly with flaxseed meal could improve the oxidative stability of omega-3 fatty acid. Grape skin and seed extracts exert strong free radical scavenging and chelating activities and inhibit lipid oxidation in various food and cell models *in vitro*. Also, grapeseed meal is seen as a potential source of protein (Fantozzi, 1981) improving protein digestibility significantly by removing the polyphenols.The objective of this study was to investigate the hypothesis that inclusion of flaxseed and grapeseed meal in fattening pigs' diet could enrich the Omega -3 fatty acids content of the pork meat in while improving the oxidative stability as well as obtaining the best carcass classification.

MATERIALS AND METHODS

The experimental protocol was approved by the Ethical Committee of the National Research-Development Institute for Animal Nutrition

and Biology, Balotesti, Romania (Ethical Committee no. 52/2014). The experiment was conducted on 12 crossbreed TOPIG hybrid [(Landrace × Large White)×(Duroc x Pietrain)] pigs with an average body weight of 68.92 kg±12.82, randomly assigned to two experimental groups (6 pigs per group) for a 30 days experimental period. The 9.80 m^2 concrete-floored pens were equipped with nipple drinkers. Feed and water were provided *ad libitum* during the experiment. The experiment was designed for two diets: a control diet based on corn, wheat, soybean meal and an experimental diet which included 7.5% of flaxseed meal and 1% grapeseed meal added to the basal diet (Table 1). The diets were formulated according to the requirements of TOPIGS guide management for fattening-finishing category pigs. The animals were cared for in accordance with the Romanian Law 43/2014 for handling and protection of animals used for experimental purposes and the EU Council Directive 98/58/EC concerning the protection of farmed animals.The animals were individually weighed at the start and at the end of the experiment. The main productive parameters: the average daily gain, average daily feed intake and feed conversion ratio were calculated per pen. The amount of feed given was weighed daily, as well as the leftovers (collected and weighed each morning). No veterinary interventions or pharmaceutical treatments were applied during the experiment. At the end of the experimental trial, pigs were slaughtered in an authorized slaughterhouse in according with EU legislation. The samples of ham and tenderloin (200 g weight) for laboratory analysis of fatty acid content were collected immediately after slaughtering, put in bags and frozen for further analyses.

Data on the lean percentage, muscle depth and back fat thickness were obtained from the slaughterhouse, where carcasses were classified according to EUROP, using an optical measurement system (device series A7509).

All the analyses concerning the meat samples, feed ingredients and compound feed were analyzed within the Laboratory of Chemistry and Animal Physiology of the National Research-Development Institute for Animal Nutrition and Biology, Balotesti.

Table 1. Formulation and chemical composition of compound feeds used for hybrid Topigs piglets

Ingredients (g/Kg as feed bases)	C	E
	(%)	
Corn	32.85	39.18
Flaxseed meal	-	**7.5**
Grapeseed meal	-	**1**
Rice bran	10	6.96
Wheat	30	23.07
Rapeseeed meal	12	12
Soybean meal	11.93	6.59
Monocalcium phosphate	0.82	0.9
Calcium carbonate	0.63	0.51
Salt	0.43	0.43
Methinonine	-	0.04
Lysine	0.26	0.49
Choline	0.08	0.08
Premix *	1.00	1.00
Biotronic SE^+	-	0.1
Biomin IMBO		0.15
Total	100	100
Calculated nutrients (g/kg feed)		
ME (Kcal/kg DM)	3232	3200
Dry matter (DMr)	88.21	90.28
Organic matter (OM)	83.03	84.90
Crude protein (g)	17.46	17.60
Crude fat (%)	3.07	4.30
Crude fibre (%)	5.18	6.48
Ash (%)	5.19	5.37
Non-protein nitrogen (or NPN)	57.31	56.52
Calcium (%)	0.80	0.80
Phosphorus (%)	0.65	0.65
Acid linolenic α C18:3n3	2.73	14.02

**1 kg premix content: 1500000 UI/g vit.A; 500000 UI/g vit.D3; 500 UI/kg vit.E; 200 mg/kg Vit.K; 200 mg/kg Vit.B1; 480 mg/kg Vit.B2; 1485 mg/kg Acid panthotenic; 2700 mg/kg acid nicotinic; 300 mg/kg Vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium.*

The following determinations were performed: dry matter; crude protein; ether extractives; fatty acids; crude fiber; gross ash, expressed by 100 g dry matter. Samples of the studied products (flaxseed meal, grapeseed meal) and of the compound feeds were collected and assayed for the basic chemical composition: dry matter, crude protein, crude fibre and ash, using the chemical methods from Regulation (CE) nr. 152/2009 (Methods of sampling and analysis for the official inspection of feeds). The collected samples were prepared within the laboratory using standardized methods complying with ISO standards. The crude protein of the meat was determined using a semiautomatic classical Kjeldahl method using a Kjeltek auto 1030 – Tecator (SR ISO 973, 2007). The meat fat was extracted using an improved version of the classical method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal (SR ISO 1444, 2008). The meat ash was determined by calcinations at 550°C (SR

ISO 936, 2009). The meat fatty acids composition was determined by gas chromatography. After lipid extraction from the samples, the fatty acids were transformed into methyl esters by transmethylation, and the components were separated in the capillary chromatograph column. The fatty acids were identified by comparison with blank chromatograms and were subsequently determined quantitatively as percent for 100 g fat. The analytical data were compared by variance analysis (ANOVA) using STATVIEW for Windows (SAS, version 6.0). The difference between the means was considered significant at $P<0.05$. The results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSIONS

For the protein content value of flaxseed meal (Table 2), Panaite et al. (2016) reported 89.25% DM, 32.99% CP, 9.42% EE, 4.65% Ash and 11.99% CF and Petit (2003) reported 90.8% DM, 24% CP and 10.5% CF. Flax fibers include both soluble and insoluble dietary fibers.

Panaite et al. (2016) studied basic chemical composition of some Romanian by-products, including grapeseed meal, reporting 89.16% DM, 11.91% CP, 2.93% Ash, and 35.68% CF while Olteanu et al. (2017) reported for the same by-product: 88.44% DM, 10.64% CP and 40.66% CF.

It has been suggested that linseed meal could be used to best advantage at a level of up to 50% of the protein supplement (Seerley, 1991).

Table 2. Chemical composition of flaxseed and grapeseed meal (g/100 g DM)

Specification	Flaxseed meal	Grapeseed meal
Proximal chemical analysis		
Dry matter (DM), %	92.47	92.98
Organic matter (OM), %	87.38	90.13
Crude protein (CP) , %	31.95	13.26
Crude fat (EE), %	15.52	8.00
Crude fibre (CF), %	12.94	34.04
Non-protein nitrogen (NPN),%	26.98	34.82
Ash, %	5.09	2.85
Gross energy (GE), kcal/kg	1515.23	1763.55
Polyunsaturated fatty acid concentration (PUFA) from fat		
Alpha linolenic acid (C 18:3 omega 3), g FAME/100g total FAME	60.80	0.18
Fat degradation index		
Peroxide index, mlThiosulfate 0,01 Ng/gr	0.350	0.445
Fat acidity, mg KOH	9.10	11.24
KREISS reaction	negative	Negative

Flaxseed is the richest plant source of the ω-3 fatty acid i.e. α-linolenic acid (ALA) (Gebauer et al., 2006) and this is proven by data obtained in table 2 where α-linolenic acid is noticed having the highest percent among others PUFA, The bioavailability of ALA is dependent on the type of flax ingested (ALA has greater bioavailability in oil than in milled seed, and has greater bioavailability in oil and milled seed than in whole seed) (Austria et al., 2008). After manufacturing the mixed fodder, samples of combined feed were collected (aprox. 500 g/samples) and analysed from chemical and microbiological point of view.

Concerning the gross chemical composition of the compound feed (Table 1), we mention that crude protein ranged between 17.46% CP (C group) and 17.60% CP (E group). A higher content of fat were observed for group E diet (4.30%) compared to group C diet (3.07%). This increase is due to the use of flaxseed meal, high-fat ingredient of 16.33% EE.

In terms of fatty acid content, the experimental diet contains 14.02 g of α-linolenic acid/100 g of FAME compared to the control group containing 2.73 g of α-linolenic acid/100 g of FAME.Fat degradation indices were within the maximum admissible limits for compound feed, for both storage periods, 14 days and 28 days, respectively (Table 3).

Table 3. Fat degradation indices of feed compound

Specification	C	E
Peroxide index, ml Thiosulfate 0,01 Ng/gr		
Initial	0.389	0.446
At 14 days	0.465	0.504
At 28 days	0.764	0.836
Fat acidity, mg KOH		
Initial	9.12	8.47
At 14 days	13	14.1
At 28 days	19.44	22.02
KREISS reaction, %		
Initial	Negative	Negative
At 14 days	Negative	Negative
At 28 days	Negative	Negative

Neither the Kreiss Reaction, 28 days after manufacture, did not show any differences regarding lipid peroxidation in feed.

Initial average weight was similarly between the groups, 66.42±10.27 kg for C group and 66.25±9.88 kg for E group.

Table 4. Productive performances (average values/group)

Specification	C	E
Initial body weight (kg)	66.42 ±10.27	66.25 ±9.88
Final body weight (kg)	98.50 ±11.62	98.33 ±12.99
Average daily gain (kg/day)	0.972±0.06	0.972±0.103
Average feed intake (kg CF/head/day)	3.09	3.16
Feed consumption ratio (kg feed: kg gain)	3.46	3.54

Final average weight was 98.50±11.62 kg for C group, 98.33±12.99 kg for E group. The average daily gain was the same for both groups. The average feed intake and the feed efficiency were higher in group E compared to C group but the differences obtained were not significant (P>0.05) (Table 4). Romans et al. (1995a) fed ground flax at 0%, 5%, 10% and 15% of the diet to 48 barrows and noted no differences in ADG, hot carcass weight or percentage lean. In a companion study, Romans et al. (1995b) fed 15% ground flax to growing barrows and gilts for seven, 14, 21 or 28 days and again found no differences in performance or carcass traits. Kouba et al. (2003) fed growing pigs 6% crushed flax for 20, 60 or 100 days and found no differences in animal growth or carcass composition when compared with hogs fed wheat and soybean meal. It has been reported that an inclusion rate of 5% reduced efficiency of feed in growing pigs (Bell et al., 1993). However, flaxseed meal could be used at levels between 5 and 10% in diets for growing-finishing pigs provided that the diet has been balanced for digestible amino acids (Defa Li et al., 2000). Same author observed that the performances of growing-finishing pigs registered a liner decline in growth rate as the level of flaxseed in the diet increased, especially at the 15% inclusion. In the same trend, the feed intake showed a decrease concomitantly with the increasing levels of flaxseed. Defa Li et al. (2000) noticed also that an inclusion rate of 10% or greater of flaxseed meal produces declines of 5.7% and 20.0% in feed conversion, whereas the daily gain is reduced 18.9% for the 15% flaxseed meal inclusion. It is known that flaxseed meal contains some anti-nutritional factors so this could be the reason why the productive performances are so poor, when introducing high levels of flaxseed meal.

EC Regulations No. 3220/84 and No. 2967/85 require that classification of pigs has to be based on objective measurements that enable estimation of the lean meat percentage. This is calculated after dissections of the left carcass sides according to the EC reference method, which means complete separation of the muscles, including those of the head, as far as possible by knife. A lean meat percentage above 55 classifies it to E category, very important aspect taking into consideration that producers are being paid according to the lean meat percentage in pigs. All the pigs within the experimental trial satisfied the conditions for class „E".

Table 5. Carcass classification scale according to EUROPE

Specification	C	E
Carcass final weight warm (kg)	72.400±0.283	72.750±1.485
Fat thickness (mm)	13.350±1.202	12.950±0.636
Lean meat depth (mm)	48.300±1.697	50.00±0.424
Average lean meat percentage (%)	58.200±0.707	58.800±0.566
Quality carcass clasification according to EUROP scale	E	E

Data of table 5 show that there were no statistical differences (P>0.05) registered between the 2 groups concerning carcass final weight warm (kg), fat thickness (mm), lean meat depth (mm), average lean meat percentage (%) and quality carcass classification according to EUROP scale. The same result was published by Wiseman et al. (2006).

Table 6. Gross chemical composition of meat samples (average values/group)

Specification		C	E
Ham	Dry matter, %	30.39±1.047	33.67±2.135
	Crude protein , %	20.25±0.502	21.29±1.563
	Ether extract , %	8.05±0.29	8.99±3.642
	Ash, %	0.86±0.028	0.74±0.071
Tenderloin	Dry matter (DM), %	35.11±0.785	36.82±2.609
	Crude protein (PB), %	18.86±2.503	18.60±0.707
	Ether extract (EE), %	12.86±0.035	13.83±1.457
	Ash, %	0.84±0.12	0.74±0.255

Both, ham and tenderloin samples registered no differences between their nutrients (Table 6).
The values obtained for nutrients content of the edible parts mentioned above were according with those found by Gerber (2007).
The chemical composition of meat was found to be fairly constant, containing 62 to 75 %

water, 19 to 25 % protein and around 1 % ash, which is comparable with data reported by other food composition tables (Souci, Fachmann and Kraut, 2000).

Feeding flaxseed meal to pigs we noticed that the concentration of α-linolenic acid had increasing tendency for the edible parts analysed (Table 7).

Table 7. Fatty acids composition of ham and tenderloin samples (g/100g total fatty acids)

Specification		Ham		Tenderloin	
		C	E	C	E
Capric	C10:0	0.19±0.01	0.13±0.01	0.14±0.01	0.18±0.01
Lauric	C12:0	0.20±0.03	0.13±0.02	0.21±0.11	0.15±0.04
Miristic	C 14:0	1.51±0.04	1.43±0.01	1.72±0.09	1.86±0.09
Pentadecanoic	C 15:0	0.16±0.01	0.12±0.03	0.24±0.01	0.30±0.09
Pentadecenoic	C 15:1	0.42±0.07	0.71±0.01	0.22±0.03	0.26±0.01
Palmitic	C 16:0	23.73±0.94	23.67±0.10	26.73±0.21	26.53±0.02
Palmitoleic	C 16:1	3.10±0.02	3.04±0.03	2.66±0.06	2.59±0.08
Heptadecanoic	C 17:0	0.46±0.03	0.34±0.02	0.35±0.14	0.50±0.01
Heptadecenoic	C 17:1	0.47±0.03	0.33±0.02	0.30±0.03	0.30±0.02
Stearic	C 18:0	13.23±1.40	11.40±0.08	13.87±0.40	13.89±0.15
Oleic cis	C 18:1n9c	44.05±0.96	41.38±0.09	38.87±0.20	37.89±0.20
Linoleic cis	C 18:2n6	9.94±0.09	12.83±0.29	11.72±0.66	12.06±0.04
Linolenic α	C 18:3n3	0.51±0.01	1.29±0.04	1.06±0.06	1.12±0.03
Octadecatetraenoic	C 18:4n3	0.76±0.11	0.59±0.01	0.57±0.01	0.60±0.02
Eicosadienoic	C 20:2n6	0.41±0.03	0.52±0.07	0.41±0.01	0.50±0.14
Eicosatrienoic	C 20:3n6	nd	0.19±0.01	0.09±0.01	0.07±0.01
Eicosatrienoic	C 20:3n3	0.88±0.44	1.12±0.09	0.37±0.01	0.40±0.06
Arachidonic	C20:(4n6)	nd	0.05±0.01	0.05±0.01	0.07±0.01
Other fatty acids		nd	0.77±0.20	0.46±0.08	0.51±0.21
Total fatty acids determined		100	100	100	100

The best results were observed in the ham samples where we registered differences concerning the concentration of omega 3 fatty acid α-linolenic (LNA) for E group, who had 1.29 ± 0.04 compared to 0.51 ± 0.01 within the C group, but these differences were not significant statistically ($P>0.05$). In tenderloin samples the concentration of cis-oleic fatty acid was higher for C group (C group-38.87±0.20 vs. E group-37.89±0.20), but without significant differences. The ability to substantially alter the n-3 fatty acid content of pork was demonstrated in 1972 by Anderson et al., feeding 20% flaxseed oil for two months to six month old pigs, increasing the fat depot concentrations of LNA from 1% to 15%. Cunnane et al. (1990) fed 5% ground flaxseed to weaned pigs for 8 weeks and found several fold increases in LNA and its elongation and desaturation products in a number of tissues. Due to the fact that the experimental diet consisted of a combined feed rich in polyunsaturated fatty acids, it was necessary to analyze the degree of lipid degradation of fat in meat samples by determining TBARS. As a major degradation product of lipid hydroperoxides, malondialdehyde (MDA) was assessed as marker (Table 8).

Table 8. Malondialdehyde (mg/kg MDA) as a lipid peroxidation marker in meat samples

Meat sample	**C**	**E**
0 days(mg/kg MDA)		
Ham	0.028±0.003	0.028±0.021
Tenderloin	0.036±0.009	0.028±0.004
7 days(mg/kg MDA)		
Ham	0.177±0.026	0.098±0.045
Tenderloin	0.100±0.013	0.059±0.009

Table 8 contains data on the concentration of malondialdehyde (mg/kg) in the ham and tenderloin. The assessment of the meat oxidative stability during the storage depends on the concentration of the degradation

products, such as, for example, the malondialdehyde concentration (as by-product). The amount of malondialdehyde increases in proportion to the amount of fatty acids in the tissues. In the fresh samples (0 days) of tenderloin from the experimental group, a smaller amount of malondialdehyde was detected compare to group C. The amount of malondialdehydeof ham was at a similar level for both groups (Table 8). O'Grady et al. (2008) reported that the dietary grape pomace had no antioxidant effect on the lipids of the meat. On the other hand Mason et al. (2005) and Lahucky et al. (2010) demonstrated positive results regarding to oxidative stability of the meat or meat products when using various plant extracts, essential oils or parts containing antioxidant compounds to the diets of pigs.

Although only the experimental group diet was enriched in polyunsaturated fatty acids (flaxseed meal), the ham and the tenderloin contained a lower amount of malondialdehyde compared to conventional diet. This is due to the influence of grapeseed meal with proven antioxidant effect, added to diet E, in the process of inhibiting the propagation of oxidative reactions within muscle tissues.

At 7 days of refrigeration, the meat samples of the E group proved to have a higher oxidation stability 80.61% for ham and 69.49% for tenderloin compare tothe same meat samples of C group (Table 8).

Table 9 shows the antioxidant capacity of meat samples determined at 0 and 7 days of refrigeration. At 0 days, in the fresh ham and tenderloin of E group, the antioxidant capacity was superior compared to the C group.

Table 9. Antioxidant capacity of the meat samples

Meat samples	**C**	**E**
0 days(mM ascorbic acid equivalent)		
Ham	18.182±0.39	18.311±1.68
Tenderloin	16.945±1.30	21.218±1.22
7 days(Mm ascorbic acid equivalent)		
Ham	8.584±0.72	11.586±0.28
Tenderloin	9.470±0.11	10.473±2.39

Also, the antioxidant activity of the ham and tenderloin of E group at 7 days after refrigeration was higher than in the case of samples from group C.

CONCLUSIONS

The bioproductive performances of fattening-finishing pigs were not influenced by the inclusion of 7.5% of flaxseed meal and 1% grapeseed meal added to the basal diet, nor the carcass classification.

During the experimental trial a slight increasing in PUFA content was noticed but without any statistical significance both in ham and tenderloin. Also, it could be observed within E group the protective effect of grapeseed meal for ham and tenderloin suggesting a possible antioxidant effect of grapeseed meal.

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