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Effect of the Dietary By-Product Grape Seed Cake on Performances and Carcass Quality of TOPIGS Pigs

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Abstract. Grape seed cake (GSC) is a valuable by-product for pigs. The aim of this study was to evaluate the effects of GSC in the diet of finishing pigs on performances (body weight, BW; feed intake, FI; average daily gain, ADG; feed efficiency, FE; feed conversion, FC), carcass quality (fat thickness, FT; eye muscle area, EMA; lean meat proportion in carcass, LMP) and classes of quality (CC). Twenty four pigs during 24 days (75.63±1.41, BW) were assigned to 2 groups (control, C and experimental, GC). Group C received a classical compound feed whereas in the diet of group GC 3% corn and 2% protein sources were replaced by 5% GSC. PIGLOG 105 apparatus was used to measure the carcass quality. Muscle pH and temperature (T) at different periods of time (45', 24 h and 48 h) was assessed by pH-meter (Hanna HI 99163). The BW (C-97.83kg, GC-102.00 kg; P>0.05), FI (C-3.41 kg/day; GC-3.42 kg/day; P>0.05), ADG (C-0.848 kg/day; GC-0.848 kg/day; P>0.05), FE (C-4.02 kg feed/gain; GC-4.03 kg feed/gain kg; P>0.05), FC (C-0.25 kg gain/feed kg; GC-0.25 kg gain/feed kg) showed that the GSC maintaining the physiological state of the animals. The LMP slightly increased in the carcass of GC comparing with C. The muscle pH and T (45' and 24 h) wasn't affected by (P>0.05) GCS. All carcass were included in E class. In conclusion feeding finishing pigs with GSC, not significantly affect the animal performances or carcass quality.

Keywords: grape cake, by-product, performances, carcass quality, pigs

INTRODUCTION

In the last decade, there has been increasing interest in converting vegetable sources such as corn, rape, soy, *camelina* into biofuels (Cottrill *et al.*, 2007). The survival of pig industry depends on these sources of energy and protein. The possible increased utilization of these raw materials in other fields of activity will disturb their market prices and availability. This changes, necessitate looking for some nonconventional feed sources available and suitable for pigs whose utilization will not affect productivity and the quality of animal food products.

On the other hand, some other industries (wine making, for instance) are growing. The pressing of grapes produces a juice (must of grapes) and leaves behind a by-product, the grape pomace. This by-product represent un accounts for about of the 20% of the weight of the grape processed into wine (Schieber *et al.*, 2001). Grape pomace is actually a blend of separated solids must (marc) or wine (fermented pomace) made of peels (55-65%), seeds (18-25%), and traces of grape clusters and wine extracted from pressing (Pomohaci *et al.*, 2000). Recently, for nutritional reasons the cold pressed grape pomace produced grape oil and grape seed cake as by-product. Thus, large amounts of grape cake seed will be available.

Grape seed cake it is a noteworthy and valuable by-product with possible benefits for pig industry due to the its availability and price competitiveness. From the nutritional point of view, beside the main active substance stored in the GSC such as polyphenols with antioxidant properties, residual oil (6.42 %) has an interesting fatty acids (FA) composition. A higher amount of unsaturated fatty acid (UFA, 84.66 % total FAME), especially C18:2-n6 (62.26 % total FAME) and C18:3-n3 acids (2.32 % total FAME), and a lower amount of saturated fatty acid (SFA, 14.85 % total FAME). Those aspects convert this by-product into an alternative feeding source of PUFA for pigs with possible economic benefits for the grapes seed oil producers. Moreover, such an approach is necessary due to that, feedstocks belonging to these categories presumably do not compete with food production and thus are very important for animal production. And, also, for environmental considerations, this need to be suitably processed and utilized.

The aim of this study was to investigate the effects of replacing 3% corn and 2% protein sources with a polyphenols and C18:3-n3-rich by-product, GSM on the performances and carcass characteristics determined on lived animals.

MATERIALS AND METHODS

Animals and diets. Animals were treated in accordance with the Romanian Law 305/2006 for handling and protection of animals used for experimental purposes. This study protocol was approved by the Ethical Committee of The National Research –Development Institute for Animal Nutrition and Biology–Balotesti, Romania.

The experiment used twenty-two hybrid finishing Topigs [(Landrace×Large White)×(Duroc×Pietrain)] with an average initial weight of 75.63 kg±1.41, for a period of 24 days. The animals were assigned to 2 experimental groups of 12 pigs each, control (C) and experimental (GC) groups. The diet of group C received a classical compound feed, while in the diet for group GC 3% corn and 2% protein sources were replaced by 5% GSC (rich in polyphenols and 18:3, n-3 FA). The two experimental diets (*Tab. 1*) were isoenergetic and isoproteic and pigs had *ad libitum* access to feed and water (nipple drinkers) during the entire experimental period. The feed intake was recorded on a daily basis.

Since GSM is a by-product its nutritional value varies between the cultivars or manufacturing process, thereby it is necessary to evaluate permanently chemical composition (Tab. 2), polyphones (data not shown) and fatty acids content (Tab. 3) in order to establish its nutritional value.

Chemical composition of the raw ingredients and feed compound. The chemical analyses of the raw feed ingredients were performed within the laboratory of chemistry and nutrition physiology of INCDBNA. The standard analytical methods were used according to working protocols in agreement with the similar international protocols.

We determined the content of dry matter (DM), crude protein (CP), ether extractives (EE), fatty acids (FA), crude fibre (CF), ash (Ash) per 100 g DM. The protein content were determined using the classical semiautomatic method of Kjeldahl, using the Kjeltek auto 1030 – Tecator analyser. The ether extractives were extracted using the improved classical method by continuous extraction in solvent, followed by solvent drying and fat measurement using a Soxhlet.

The fibre content was determined using the classical semiautomatic method Fibertec-Tecator, and the ash was measured by burning at 550^0C until constant mass was obtained.

The nitrogen-free extractives were calculated using the following formula: NFE = DM - (CP + EE + CF + Ash).

The metabolisable energy was calculated using a regression equation developed by the „Oskar Kellner" Institute: ME = 5.01 x DP + 8.93 EE + 3.44 CF + 4.08 DNFE (Stoica and Stoica, 2001).

Tab. 1

Experimental diets composition and calculated nutrient analyses
of finishing TOPIGS pigs

Items	Dietary treatment finishing pigs	
	C	GSC
Corn	56.84	52.83
Rice bran	15.00	15.00
Soybean meal	9.00	10.00
Sunflower meal	13.00	10.00
Sunflower oil	2.00	3.00
Grape seed meal	-	5.00
DL-methionine	-	0.04
L-Lysine	0.28	0.27
Calcium carbonate	2.03	1.90
Monocalcium phosphate	0.35	0.46
Salt	0.40	0.40
Choline premix	0.10	0.10
Vitamin-mineral premix P3+4	1.00	1.00
Calculated analysis		
ME Kcal /Kg	3073	3089
CP (%)	15.29	15.05
Lys. B (%)	0.88	0.88
Met.+cys. B (%)	0.59	0.59
Fiber (%)	5.68	7.03
Calcium (%)	0.90	0.87
Total P (%)	0.60	0.59

Note: C-conventional diet; GC- 5% grape seed cake

The detailed chemical composition in fatty acids was determined by gas chromatography. The principle of the method consists in transforming the sample fatty acids in methyl esters, followed by separation of the components in the chromatographic column, their identification by comparison with standard chromatograms and the quantitative determination of the fatty acids (expressed as % total FAME). Sample collection and preservation was done in agreement with PSL–18 procedure. The sample was kept under conditions which prevented any change of composition. The reference material (CRM) was a standard solution of methyl fatty acids SUPELCO 37 Component FAME Mix; 10 mg/mL, Soybean Oil and Sunflower Oil; SUPELCO. We used a Perkin Elmer-Clarus 500 chromatograph with capillary injection system (splitting ratio 1:100), with flame ion detection (FID) and programmed heated capillary oven; the separation capillary column had a high polarity stationary phase (TR-Fame, 60m × 0.25mm inner diameter, 0.25µm film); or high polarity cyanopril phase, which give a similar resolution for different geometrical isomers THERMO TR-Fame 120m × 0.25mm ID x 0.25µm film. Hydrogen was the carrier gas.

Animal performance. In the beginning and at the end of the experiment, the animals were weighed individually, to determine the following parameters: body weight, average daily gain, daily consumption of feed compound, feed efficiency and feed conversion (FC).

Carcass characteristics. After 24 days we determined carcass quality for all live animals using ultrasonic equipment PIGLOG 105, SFK-Technology, Denmark. Thus, for the evaluation of meat production on the live animal, the dorsal fat layer was measured at two distinct points, between lumbar vertebrae 3 and 4, at 7 cm from the median line, and between ribs 3 and 4, at 7 cm from the median line. The muscle eye area was measured between ribs 3 and 4, at 7 cm from the median line. Using the body weight, the information was processed by the software of the instrument in order to estimate the proportion of muscle tissue in the carcass then classes of quality according to EUROP system.

Twelve hours before slaughter the access of animals to feed was restricted. All pigs (N=24), with an average weight of 99.92 ± 7.87 kg, were transferred (30 minutes travel) to an authorised slaughter facility, where they were stunned and bled.

Muscle pH and T. The pH and temperature (T) of pork muscle samples were measured using a portable pH-metre specific for meat products, model HI 99163 Hanna, Romania. The muscle was kept under refrigeration at 4^0C.

Statistical analyses. The effects of GSC on pigs performances and carcass quality were analysed by one-way ANOVA StatView version 5.0. The results were expressed at mean values and standard deviation. The differences among treatments were considered significant at P<0.05.

RESULTS AND DISCUSSIONS

The present study was performed in order to obtain preliminary information about the effects of dietary 5% GSC on the bioproductive performances and carcass quality of finishing pigs. Grape seed cake consist of about 2268 kcal/kg metabolisable energy (ME), 10.64% crude protein, 5.97% ether extractives and about 37.91 crude fibre, on dry matter bases (*Tab. 2*).

Tab. 2

Weende analysis of grape seed cake

Items (g/100g sample)	Chemical composition of the grape seed cake used in the experimental diet
	Grape seed cake
DM (%)	90.74
ME (Kcal/kg)	2268
CP (%)	10.64
CF (%)	37.91
EE (%)	5.97
Ash (%)	2.97
NDF (%)	66.22
ADF (%)	59.95
Carbohydrates (%)	71.16

The main concern regarding the use of GSC in pig feeds is the degree of variation in composition which can further affect the digestibility of nutrients. Variation can arises from differences in nutrient analyses of the incoming raw material and technology of wine/oil making (Schieber *et al.*, 2001).

The biological value of EE is given by the content of fatty acids. According to our results presented in *Table 3* GSC was found to be rich in unsaturated fatty acids (UFA) especially polyunsaturated fatty acid (PUFA). GSC also contained monounsaturated fatty acids (MUFA). Among MUFA, oleic acid (17.05% total FAME) and eicosenoic acid (1.78% total FAME) were the most representative. Palmitic and stearic acid (10.05% total FAME; 3.67% total FAME) were the predominant among the SFA while linoleic (62.26% total FAME) and linolenic (2.32% total FAME) among the PUFA in the GSC. Some of this fatty acids such as linoleic and especially linolenic are essential for pig nutrition since they are exclusively of exogenous origin and, so these fatty acids must be consumed in the diets.

Tab. 3

Fatty acids analyses of the grape seed cake

Fatty acids (g/100 g EE)	Grape seed cake
Carpic (10:0)	0.05
Lauric (12:0)	0.11
Miristic (14:0)	0.16
Pentadecanoic (15:0)	0.43
Palmitic (16:0)	10.05
Heptadecanoic (17:0)	0.07
Stearic (18:0)	3.67
Arachidic (20:0)	0.26
Behenic (22:0)	0.05
Pentadecenoic (15:1)	0.29
Palmitoleic (16:1)	0.28
Oleic (18:1)	17.05
Eicosenoic (20:1)	1.78
Erucic (22:1)	0.26
Linoleic (18:2 n-6)	62.26
Eicosadienoic (20:2 n-6)	0.10
Eicosatrienoic (20:3 n-6)	0.13
Docosatetraenoic (22:4 n-6)	0.04
Arachidonic (20:4 n-6)	0.08
Linolenic (18:3 n-3)	2.32
Eicosatrienoic (20:3 n-3)	0.07
Other FA	0.49
∑ SFA	14.85
∑ UFA	84.66
∑ MUFA	19.66
∑ PUFA	65.00
∑ SFA/∑ UFA	0.18
∑ MUFA/∑ PUFA	0.30
∑ n-6	62.71
∑ n-3	2.39
∑ n-6/ ∑ n-3	26.24
18:2 n-6/18:3 n-3	0.04

Note: SFA - saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Therefore, dietary fatty acids can influence the endogenous sintese of PUFA and thus the level of those fatty acids in pork tissues (Wood *et al.,* 1999).

The literature has a lot of reports on the effect of dietary grape pomace on broiler (Brenes *et al.,* 2008; Goñiet *et al.,* 2007; Sáyago-Ayerdi *et al.,* 2009; Viveros *et al.,* 2011) and ruminants (Abarghuei *et al.,* 2010; Ahn *et al.,* 2002; Zalikarenab *et al.,* 2007), while the reports on pigs are few (Sehm *et al.,* 2007; Yi and Kim, 2011). Moreover, the vast majority are conducted on the effect of grape pomace as natural source of bioactive metabolites with antioxidant proprieties (Ahn *et al.,* 2002; Brenes *et al.,* 2008; Goñiet *et al.,* 2007; Sáyago-Ayerdi *et al.,* 2009; Sehm *et al.,* 2007; Viveros *et al.,* 2011) while just a fraction of the investigations looked into the use of this by-product as source for dietary PUFA for pigs (Yi *et al.,* 2009). Thus, given the fatty acid composition with larger proportions of PUFA (60.50% total FAME) and lower content of SFA (14.85% total FAME) we hypothesized that this by-product could be a promising sources of valuable PUFA for finishing pigs nutrition. Therefore, further measurements are required to confirm these hypothesis.

Animal performance. The effect of GSC on growth performance in finishing pigs was showed in *Table 4*. Initial average body weight of the experimental animals was 75.63±7.07. After 24 experimental days, the animals were weighed individually and the results were processed statistically and used to calculate other parameters such as the average daily gain (kg/day), feed efficiency (kg feed:kg gain) and feed conversion (kg gain:kg feed).

The BW of pigs fed GSC diet were slightly increased (+1%) comparing to control group, (P>0.05). There weren't significant differences (P>0.05) in ADG between the controls and GSC fed pigs during experimental period. We didn't observed any significant differences regarding FI, FE or FC of pigs fed GSC diet comparing to C diet.

Tab. 4

Effects of using grape seed meal on productive performance of finishing TOPIGS pigs

Items	C	GC
No. of pigs, animals/group	12	12
Finishing period, days	24	24
Body weight: Initial, kg	75.75 ± 6.37	75.50 ± 7.78
Final, kg	97.83 ± 7.90	102.00 ± 7.83
Average daily gain at: 24 day, g/day	0.848 ± 0.10	0.848 ± 0.14
Feed intake, kg/day:	3.41	3.42
Feed efficiency, kg feed/kg gain	4.02	4.03
Feed conversion, kg gain/kg feed	0.25	0.25

Note: Different letters between dietary treatments denote significant differences (ANOVA; p < 0.05).

Similar results have been described in literature. Thus, Yan and Kim (2011) reported that there was no negative effect on growth performance in growing-finishing pigs when the diets contained 30 g/kg fermented grape pomace product. Brenes *et al.* (2008) and Goñi *et al.* (2007) reported that grape pomace can be included at up to 60 g/kg of the diet of broilers without detrimentally affecting growth performance. However, Hughes *et al.* (2007) and Viveros *et al.* (2010) observed that inclusion of grape seed extract significantly reduced growth performances, feed intake and feed efficiency of broilers chicks. According to Yan and Kim (2011) the reduction of bioproductive performances could be due to the specific concentrations of polyphenols contained by grape seed extract which hinder the efficient use of the nutrients and affect animal health. In our study we didn't observed any adverse effects on bioproductive performances when we added 5% GSC in finishing pigs diets.

Determinations of carcass quality. In our study when GSC with approximately 6% residual oil rich in PUFA is included in finishing TOPIGS diets, there is a decrease in FT (-1.11%, P>0.05), whilst EMA and LMP recorded important increases (+1.03%, P>0.05; +1.03%, P>0.05) comparing to C group (*Tab.5*).

This effect could be attributed to the increasing level of PUFA containing more than 18 carbon atoms which make animals leaner, most likely due to the reduction of lipogenesis rate (Heckart *et al.* 1999). In the literature, we found similar results on carcass treats when 30 g/kg fermented grape pomace product was add in pig diet (Yan and Kim 2011).

Addition of by-product increased the yield at slaughtering by (+1.09%, P>0.05) comparing to C.

The proportion of muscular tissue in carcass weight exceeded 55%, thus all carcass were included in E classes of quality (*Tab. 5*).

Tab. 5

Effects of using grape seed cake in diets of finishing TOPIGS pigs on qualitative parameters of carcass

Carcass quality	C	GC
Determination on live pigs*:		
- fat thickness**, mm	12.20 ± 1.30	11.00 ± 1.70
- eye muscle area***, cm^2	44.00 ± 7.07	45.17 ± 4.17
- lean meat proportion in carcass, %	57.52 ± 2.22	59.20 ± 1.93
- yield at slaughtering (%)	77.88 ± 7.99	83.11 ± 7.31
Classes of quality	E	E

Note: Different letters between dietary treatments denote significant differences (ANOVA; $p < 0.05$); *Measuring done with PIGLOG 105; ** Fat thickness measured between ribs 3 and 4, at 7 cm from the median line; ***Eye muscle area measured between ribs 3 and 4, at 7 cm from the median line.

The degree of reduction of muscle pH and T after slaughter has a significant effect on the quality of the resulting meat and also on the storage life of meat (Geay *et al.*, 2001; Hăbeanu *et al.*, 2011). Table 6 shows the muscle pH and T at different time intervals, respective 45 minute, 24 and 48 hours after slaughter. Thus, there are not significant differences between groups of animals (P>0.05) regarding muscle pH values at 45’ and 24 h after slaughter. Significant difference (p<0.05) was only observed on the pH muscle at 48 h after slaughter (4.00 *vs*. 3.33), which was decreased by GSC diet compared with the C group.

Tab. 6

Effects of using grape seed cake in diets of finishing TOPIGS pigs on pH and T

Muscle pH and T	C	GC
pH at 45’	6.17 ± 0.75	6.00 ± 1.10
pH at 24 h	5.00 ± 0.00	5.00 ± 0.00
pH at 48 h	$4.00^a \pm 0.00$	$3.33^b \pm 0.52$
T at 45'	18.50 ± 3.02	18.33 ± 2.16
T at 24 h	15.83 ± 0.75	15.18 ± 0.41
T at 48 h	19.00 ± 0.00	19.00 ± 0.00

Note: Different letters between dietary treatments denote significant differences (ANOVA; $p < 0.05$);

Garrido *et al.* (2011) obtained similar acidification of the pH values of the three types of pork burgers packed under aerobic conditions in days 3 of storage with grape pomace extract. The authors explain these values by the high phenolic acid level of the extracts.

In our experiment, the decrease of pH value 48 h after slaughter is probably due to the higher muscle content of polyunsaturated lipids, susceptible to disturbance by oxidative reactions. Even if GSC has a high content of polyphenols, much of them may get lost in the digestive tract and/or 5% GSC may not be enough to ensure an efficient antioxidant protection. No difference (P>0.05) was observed in muscle T values between two groups.

CONCLUSION

In the present research work, we demonstrated that the addition of 5% GSC in finishing pigs diets did not affect the bio productive performances of the animals. It seems that this by-product improved the indices of carcass. Furthermore, the addition of GSC contributed to the maintenance of an acidic pH in raw pig muscle.

Also, due to the content in PUFA this vegetable by-product is a promising sources of valuable essential fatty acids and polyphenols for finishing pigs nutrition. More detailed studies regarding this particular aspects are required.

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