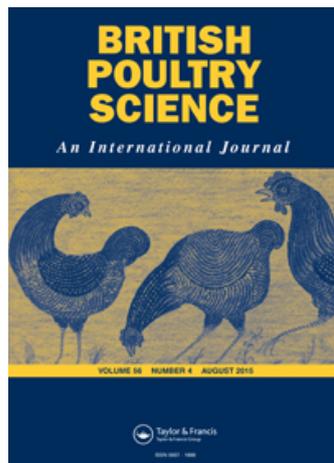


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The effects of grape seed in the diet of the Penedes chicken, on growth and on the chemical composition and sensory profile of meat

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Abstract 1. Effect of a diet with 5% grape seed inclusion, substituting for maize compared to a standard diet, was studied in the Penedes chicken.
2. A total of 128 chickens were used, half from each sex. Individual weights and feed intake were controlled weekly from the first d to 5th week and fortnightly until the 15th week. On the 16th week, chemical analyses of meat from 16 thighs from each diet and sex were carried out, as well as a sensory analysis of meat from 24 thighs. Differences between diet and sex were analysed using live body weight, feed intake, feed conversion rate (FCR), chemical composition and sensory attributes of the meat.
3. At the end of the experiment, no significant differences were observed on live body weight, feed intake and FCR due to diet.
4. Meat showed no differences due to diet in the percentages of protein, lipid and ash.
5. Meat from the grape seed diet showed a higher percentage of unsaturated fatty acids due to linoleic acid. It also showed a more nutty smell, a more metallic flavour and more stringiness. There was, also, less of a pork crackling odour and flavour, a less sweet flavour and less of a broiler meat flavour.

INTRODUCTION

From the earliest times, farm by-products have been used in animal feed, although in some cases their use has been discontinued, being replaced by other products that offer better guarantees for optimum production. In assessing the production of local meat products, raised on local raw ingredients as was done historically, we are forced to reconsider the use of these by-products and compare their productive potential with that of other raw ingredients which have become established as traditional and which, in turn, permit production with Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI) status.

Spain is the world's third highest wine-producing country. Within Spain, Catalonia is the second biggest wine producer of the autonomous regions, with the Penedes DO region devoting

most hectares of its lands to viticulture, 40% of the total of all of the Catalan vineyards.

The Penedes region also produces a local chicken breed known as *Penedesenca*. This breed is documented back to the early twentieth century. At the end of the century, recuperation and conservation initiatives were established to protect the breed (Francesch and Jordà, 1988). At the same time, genetic breeding programmes Francesch (2002) had led to development of a black-feathered chicken, with white skin and black feet. It offered a higher meat yield (Francesch *et al.*, 1999) but maintained the typical sensory characteristics at the same age (Francesch *et al.*, 2001). Other works on their rearing and on the meat's chemical composition were published by Escoda *et al.* (2001), Villalba *et al.* (2001), Tor *et al.* (2005) and Francesch and Escoda (2010). The present production figures relating to this breed in Catalonia, known locally as "*Gall del*

Penedès”, or Penedes chicken, are around 60 000 units per year. Normally this chicken is free-range reared.

Penedes producers, with the aim of achieving a PGI, have proposed that it should be reared as it was in the past, when farmers included grape by-products in its diet. Grape by-products such as the pomace, skins or seeds are used in feed for rabbits, ruminants and swine, but are not considered adequate for use in poultry nutrition (FEDNA, 2003). The inclusion of grape seed in poultry diets has been poorly documented. Nevertheless, Rotava *et al.* (2009), in experiments with broilers, added 2.35% of grape seed to the diet of one-day-old broiler chickens to study the effect of tannins present in grape by-products as growth promoters. In their study, the inclusion of the grape by-products did not have any influence on growth rate or any growth promoter activity.

Other authors (Goñi *et al.*, 2007) used grape pomace as a source of dietary fibre and polyphenols in order to study the effect of the diet on the growth rate of broilers. A group of broilers were fed for 21 d on a diet with 3% of grape pomace and was compared to another group, fed on a basal diet. No differences were observed between groups. Likewise, no differences were observed in broilers up to 42 d old between a control group and a group fed on a diet with 3.6 g kg⁻¹ of grape seed extract (GSE) in the study of Brenes *et al.* (2010).

Lau and King (2003) used a diet with 5.18% of GSE to 4-d-old chicks, which grew less than the control group. The authors suggested that it was probably due to the tannins present in GSE because tannins can bind proteins and reduce their digestibility (Butler *et al.*, 1984).

The aim of this work was to study the effects of the inclusion of grape seed to substitute some of the maize in the diet of the Penedes chicken. The objectives were to study, under the chosen PGI diet, the viability in terms of growth performance, and conversion indices, and also to carry out a comparative study of the chemical composition and sensory qualities of the chicken meat as affected by the diet.

MATERIALS AND METHODS

Biological material

A total of 128 one-day-old chickens (64 males and 64 females) were used. Each chick was identified with a numbered tag inserted into its wing membrane.

Location and environmental conditions

The chickens were separated into 8 runs, 4 runs with 32 males and 4 runs with 32 females. Two

diets were randomly allocated. So, the 8 runs were divided into diet and sex (2 diets × 2 sexes × 2 replicates). For rearing, each group of 32 chickens was placed in a 6 m² run, the ground of which was covered with woodchips, and having a gas warmer, a trough and two feed-hoppers. Food and water were provided *ad libitum*. Lighting was continuous for the first 24 h and then modified to 11 h of light and 13 h of darkness every day. For the first 2 d, the temperature was 31°C. For the next 5 d, the temperature was reduced to 29°C, then to 27°C for 10 d. From d 17 until the end of the experiment, the temperature had fluctuations determined by the weather and averaged 25°C.

Diets

Two different diets were used, diet A and diet B, the composition and chemical analysis of which are presented in Table 1. It should be noted that diet A included no grape seed supplement, while

Table 1. *Ingredients, chemical composition and nutrient composition of experimental diets without (A) and with grape seed (B)*

Ingredients (g/k)	A	B
Maize	628	578
Soy 48	164.16	164.16
Soy full fat	173	173
Grape seed	0	50
Calcium carbonate	11.6	11.6
Dicalcium phosphate	15.6	15.6
Sodium chloride	3.4	3.4
L-lysine	0.23	0.23
DL-methionine	0.51	0.51
Vitamins and minerals ¹	3	3
Maxiban (coccidiostat)	0.5	0.5
<i>Chemical analysis</i>		
ME (MJ/kg)	12.70	12.18
Protein	18.91	18.82
Fibre	2.98	5.14
Lipids	5.52	6.34
Lysine	0.94	0.95
Methionine	0.30	0.30
Met+cystine	0.50	0.50
<i>Fatty acids</i>		
Palmitic	11.84	12.36
Stearic	3.53	3.41
Myristic	0.04	0.03
<i>Saturated</i>	16.41	16.63
Oleic	22.05	20.37
Palmitoleic	0.09	0.08
Vaccenic	0.93	0.91
<i>Monounsaturated</i>	23.31	21.79
Linoleic	54.42	55.89
Linolenic	5.51	4.98
<i>Polyunsaturated</i>	60.28	61.57
<i>Unsaturated</i>	83.59	83.36

¹Vitamins and mineral mix supplied the following per kg of diet: vitamin A (retinol acetate), 4.64 mg; vitamin D₃ (cholecalciferol), 0.12 mg; vitamin E (d-alpha-tocopherol), 45 mg; vitamin B₁ (thiamine mononitrate), 3 mg; vitamin B₂ (riboflavin), 9 mg; vitamin B₆ (pyridoxine chlorhydrate), 4.5 mg; vitamin B₁₂ (cyanocobalamin), 0.017 mg; vitamin K₃ (menadione), 3 mg; Ca pantothenate, 16.5 mg; nicotinic acid, 51 mg; folic acid, 1.8 mg; biotin, 0.03 mg; Fe, 54 mg; I, 1.2 mg; Co, 0.6 mg; Cu, 12 mg; Mn, 90 mg; Zn, 66 mg; Se, 0.18 mg; Mb, 1.2 mg.

diet B included 5% grape seed supplement. Inclusion was done substituting 5% of maize. Diet B resulted in having $0.527 < \text{MJ/kg EM}$ less than diet A.

Live weight and feed intake controls

The one-day-old chickens were individually weighed and subsequently weighed weekly until 5 weeks of age, then every 2 weeks until the end of the experiment at the age of 15 weeks. Their feed intake was monitored weekly until the 5th week and once every fortnight from then on, until the end of the experiment at the 15th week, to ascertain the quantity that had been consumed. By analysing the relationship between weekly consumption and live weight, we were able to estimate feed conversion rate (FCR), a measure of an animal's efficiency in the conversion of food into body mass.

Slaughter

The chickens were slaughtered at 16 weeks old, coinciding with the normal commercial slaughter age. The previous day 12 chickens from each run had been randomly selected. They were identified with a numbered ring on each foot (the number being related to their tag number) to avoid losing information due to the loss of rings. The chickens were caged for an hour before being slaughtered. Transportation was in cages (94 cm L \times 55 cm W \times 40 cm H) with 8 chickens per cage.

Slaughtering was by electric stunning, followed by cutting the neck with a knife. After that, the chickens were plucked and eviscerated. Each thigh of each animal was placed in a different bag. Left thighs were for sensory analysis, and right thighs for chemical analysis. Samples designated for sensory analysis were stored for 72 h at 1°C until the moment of use, whereas samples to be submitted for chemical analyses were stored at -20°C.

Sensory analysis

Sensory analysis was done according to Guàrdia *et al.* (2010). A total of 24 thighs from each diet and sex were used. The specific training process, selection and definition of each attribute and the quantification of the resultant sensory profile were carried out by 6 tasters from the IRTA Process in Food Industry Subprogram, specifically trained for testing different varieties of fresh meat, each with a minimum of 3 years' experience.

In order to ensure that all sensory parameters would be recorded, a consensus was reached amongst the tasters during two sessions, providing a specific descriptive sensory profile for this study.

Furthermore, two specific 90-minute training sessions were held, each focusing on different selected attributes. All of these sessions were undertaken using the same samples and under the same conditions as those in the final evaluation. Table 2 shows the selected attributes, their definition and the evaluation process. The attribute score was marked on an unstructured scale from 0 (without attribute) to 10 (high intensity).

The fresh meat samples were stored at 1°C. With a scalpel, a 6 \times 3 cm piece of meat was removed from the *biceps femoris* muscle of each thigh, and placed in an aluminium container with a lid. They were codified with random numbers and then cooked in a dry-heat convection oven for 20 minutes at 240°C, with no additives that could modify the results of the sensory test. The evaluation was carried out with hot samples (55–60°C) in a tasting room, under controlled conditions. The tasters were provided with unsalted toast and water to cleanse the palate between samples.

To evaluate the sensory tests of samples, a complete block design was employed, meaning that, in each of the 4 tasting sessions, each tester had the opportunity to try all 4 categories (two diets and two sexes). In all cases, the presentation of the samples was balanced according to Macfie *et al.* (1989).

Chemical analysis of diets and meat

A total of 64 thighs were used, 16 thighs from each diet and sex chosen at random. Thighs were deboned, and meat was minced without skin. Moisture levels were determined according to A.O.A.C. (2010; method 934.01) and crude protein by Dumas method using a nitrogen analyser FP528 Leco (AOAC, 2010; method 992.15) that allows total protein in a sample to be calculated from the total content of nitrogen. Lipids were extracted with chloroform-methanol according to Folch *et al.* (1957). Lipids were transmethylated with BF₃ and methanolic KOH (Morrison, and Smith, 1964). Fatty acids were determined by gas chromatography (Agilent Technologies 6890 N, Little Falls Delaware, USA) using a capillary column DB23 and flame ionisation detector. The ashes were determined according to A.O.A.C. (2010).

Statistical analysis

Live weight, feed consumption and FCR analysis

At the age of 15 weeks, coinciding with the end of the experiment, an analysis was carried out on live weight, feed intake and FCR in order to ascertain whether there were any differences based on the diet given and sex.

Table 2. Description of different attributes and evaluation methods of meat samples

Sensory attribute	Description	Evaluation method	
<i>Odour</i>			
Farm animal	Characteristic smell of chicken farms, reminiscent of feather odour	Open the lid of the container at one end and breathe in 3 or 4 times. Cover the container and repeat the process as many times as you need before sample gets cool	
Fruity	Has a touch of dried fruit; like raisins or dried figs		
Oily	Smells like heated animal fat		
Pork crackling	Characteristic smell of pork crackling snacks, like roasted skin		
Dried nuts	Smells like dried nuts, particularly peanuts	Assess the attributes on one piece of the sample before it gets cool	
Stale	Pungent smell like aged olive oil		
<i>Flavour</i>			
<i>Sweet</i>			
	Basic flavour like aqueous solution of saccharose	Assess the next attributes according to its appearance on the sensory profile panel on the other piece of sample	
Metallic	Metallic flavour like a ferrous sulphate solution, reminiscent of fresh liver or blood		
Farm animal	Flavour reminiscent of chicken farms and also can be associated with feather flavour		
Sweet dried fruit	Has a touch of dried fruit, like raisins or dried figs		
Broiler	Taste associated with broiler meat and like commercial chicken broths	Assess the next attributes according to its appearance on the sensory profile panel on the other piece of sample	
Pork crackling	Taste associated with pork crackling snacks, like roasted skin		
Dried nuts	Taste like dried nuts, particularly peanuts		
Stale	Slightly pungent and irritant flavour like aged olive oil		
<i>Texture</i>			
Toughness	Resistance of the sample at the first bite, related to the ease of chewing	Assess the next attributes according to its appearance on the sensory profile panel on the other piece of sample	
Stringiness	Detection of long and parallel fibres during meat chewing		
Doughy	Pasty sensation in the mouth while chewing, like a mix of water and flour		
Stickiness	Perception of leftovers stuck to the teeth or the molars after chewing the meat		

(Continued)

Table 2. (Continued)

Sensory attribute	Description	Evaluation method
Acceptability	Global subjective assessment of the value of the final product. This value does not have to coincide with the opinion of the end product consumers	Integrate all values for the sample in a single value that quantifies overall quality of the sample

In all three cases, a dual-factor model (Equation 1) was utilised: the dietary treatment and sex, together with their interaction. In the live weight test, replications were the animals, while in the feed intake and FCR, replications were based on the runs.

$$Y_{ijkz} = \mu + D_i + S_j + (DS)_{ij} + e_{ijk} \quad (1)$$

where μ is the general average, D_i is the diet with two levels, S_j is the sex with two levels, DS_{ij} is the interaction between diet and sex and e_{ijk} is the random error.

Model (1) was resolved by general linear model (GLM) procedures of SAS Institute (2002). The model was modified when interaction was non-significant, and another GLM procedure was run without it.

Sensory evaluation analysis

The data obtained were analysed according to model (2), which included the sample chickens' diet and sex, the taster and session hierarchical to the taster as fixed effects. Means were compared by Tukey's test to determine significance at $P \leq 0.05$.

$$Y_{ijkz} = D_i + S_j + C_k + R_z(k) + e_{ijkz} \quad (2)$$

where D_i is the diet with two levels, S_j is the sex with two levels, T_k is the taster with 6 levels, $R_z(k)$ is the session hierarchical to taster and e_{ijkz} is the random error.

Model (2) was resolved by GLM procedures of SAS Institute (2002).

Meat chemical analysis

In order to study the chemical characteristics of the meat, model (1) was used and it was resolved in the same way. In this case, replications were the samples.

RESULTS AND DISCUSSION

Live weight, feed intake and FCR

Table 3 shows live weight, accumulated feed intake and FCR of the chickens at 15 weeks of age for each diet. No significant differences were observed between diets in any parameter. Differences were only noted between sexes. The interaction between diet and sex was not significant. Females weighed the order of 1180 g less than males, consumed around 3 kg less and their FCR (feed/gain) was higher: 0.41 kg/kg live weight.

The fact that grape seed inclusion did not affect the live weight, feed intake and FCR agrees with the results of Rotava *et al.* (2009), who included 2.35% of grape seed in broiler diets to 21 d of age, and the same was observed by Goñi *et al.* (2007) and Brenes *et al.* (2008, 2010), who worked respectively with broilers to 21 d old, fed on diets containing 3% of grape pomace or with broilers to 42 d old, fed on diets including 6% of grape pomace concentrate. However, Hughes *et al.* (2005) and Lau and King (2003) observed growth depression in chickens fed until 42 d old on 3% and 5.18% of grape seed extract inclusion, which according to Goñi *et al.* (2007) and Brenes *et al.* (2010) may have been provoked by the higher concentration of polyphenols.

Sensory analysis on cooked meat

Mean sensory scores for odour, flavour and texture attributes of the cooked meat are given in Table 4. Statistical analysis showed differences between diets and/or between sexes in some attributes, but the interaction between diet and sex was not significant in any case.

The results can be divided into three groups, depending on the score achieved in each attribute. Twelve attributes were detected only very slightly in comparison to other animal meats. Within this group were all odour attributes and also some flavours such as farm animal, sweet dried fruit, pork crackling, dried nuts and stale flavour, and finally, a doughy texture. Of the 12 attributes, significant differences were detected in pork crackling odour, stronger in the diet without

grape seed; and in dried nuts odour, stronger in the diet with 5% grape seed. Furthermore, there are also differences in farm animal flavour, these depending on sex, the cocks tasting more like a farm animal.

On the other hand, there were 7 attributes that were detected moderately: sweet flavour, metallic flavour and broiler flavour, while texture attributes included toughness, juiciness, stringiness and stickiness. Sweet flavour was significantly lower in the meat from chickens in the 5% grape seed diet, having a notably more metallic flavour that can be explained by a substantial higher quantity of iron, copper and manganese in grapes compared to maize (FEDNA, 2003). The males were also found to have a more metallic flavour than the females probably because the muscles of males, due to a higher level of locomotor activity of males, contain more blood than female muscles, and have more haemoglobin and therefore more iron. On the other hand, the meat from chickens fed without grape seed had a more discernible broiler flavour. Significant differences were observed for stringiness, chickens fed on the 5% of grape seed diet being noticeably stringier than those without it.

Finally, the acceptability score was high, with no discernible differences between males and females, or between diets.

No other references were found in relation to grape seed inclusion in chicken diets and the sensory characteristics of the meat.

Chemical analysis of meat*Moisture, ash, protein and lipids*

No significant differences were observed between diets and sexes with regard to moisture, ashes, protein and lipids. The average percentage values were 75.75, 1.01, 20.03 and 3.21, respectively. These results, especially with respect to lipids, do not coincide with the literature which usually shows that the males have fewer lipids than females (Tougan *et al.*, 2013). Baeza *et al.* (2010), who worked with similar strains and similar slaughter ages (84 and 120 d), also found sex differences in the amount of fat in the thigh and breast meat. Nevertheless, Eleroğlu *et al.* (2013)

Table 3. Live weight (LW) (g), feed conversion rate (FCR) and total feed intake (kg) (FI) of Penedes chicken in the 15th week, on diets with 0% (A) or 5% (B) of grape seed (LS means \pm standard error)

	Diet		Sex		P-value	
	A	B	Males	Females	Diet	Sex
LW	3079.72 \pm 25.95	3046.27 \pm 26.28	3652.43 \pm 25.39	2473.56 \pm 26.82	ns	***
FI	10.99 \pm 0.22	11.07 \pm 0.22	12.57 \pm 0.22	9.50 \pm 0.22	ns	***
FCR	3.60 \pm 0.09	3.68 \pm 0.09	3.44 \pm 0.09	3.85 \pm 0.09	ns	**

ns, non-significant $P > 0.05$; **significant $P \leq 0.01$. ***significant $P \leq 0.001$.

Table 4. Odour, flavour and texture attributes of the Penedes chicken meat on diets with 0% (A) or 5% (B) of grape seed inclusion and sex (LS means \pm standard error)

Attribute	Diet		Sex		P-value	
	A	B	Males	Females	Diet	Sex
<i>Odour</i>						
Farm animal	2.06 \pm 0.22	1.71 \pm 0.22	1.78 \pm 0.21	1.9 \pm 0.22	ns	ns
Fruity	1.41 \pm 0.22	1.89 \pm 0.22	1.84 \pm 0.21	1.45 \pm 0.22	ns	ns
Oily	1.85 \pm 0.14	1.66 \pm 0.14	1.89 \pm 0.14	1.62 \pm 0.14	ns	ns
Pork crackling	1.57 \pm 0.14	1.13 \pm 0.14	1.30 \pm 0.14	1.40 \pm 0.15	*	ns
Dried nuts	0.63 \pm 0.14	1.17 \pm 0.14	0.88 \pm 0.13	0.93 \pm 0.14	**	ns
Stale	0.21 \pm 0.11	0.45 \pm 0.11	0.27 \pm 0.11	0.39 \pm 0.11	ns	ns
<i>Flavour</i>						
Sweet	3.09 \pm 0.18	2.48 \pm 0.18	2.67 \pm 0.18	2.89 \pm 0.18	*	ns
Metallic	2.08 \pm 0.20	2.88 \pm 0.20	2.97 \pm 0.20	1.97 \pm 0.20	**	**
Farm animal	1.17 \pm 0.17	1.47 \pm 0.17	1.59 \pm 0.17	1.04 \pm 0.17	ns	*
Sweet dried fruit	0.81 \pm 0.18	0.86 \pm 0.18	0.74 \pm 0.18	0.94 \pm 0.18	ns	ns
Broiler	3.26 \pm 0.09	3.00 \pm 0.09	3.14 \pm 0.09	3.13 \pm 0.09	*	ns
Pork crackling	0.41 \pm 0.09	0.31 \pm 0.09	0.35 \pm 0.09	0.37 \pm 0.09	ns	ns
Dried nuts	0.41 \pm 0.10	0.49 \pm 0.10	0.38 \pm 0.10	0.53 \pm 0.10	ns	ns
Stale	0.21 \pm 0.11	0.45 \pm 0.11	0.27 \pm 0.11	0.39 \pm 0.11	ns	ns
<i>Texture</i>						
Toughness	4.47 \pm 0.14	4.78 \pm 0.14	4.78 \pm 0.14	4.47 \pm 0.14	ns	ns
Juiciness	4.99 \pm 0.14	4.66 \pm 0.14	4.74 \pm 0.14	4.91 \pm 0.14	ns	ns
Stringiness	4.41 \pm 0.15	4.83 \pm 0.15	4.78 \pm 0.15	4.45 \pm 0.15	*	ns
Doughy	1.03 \pm 0.07	0.96 \pm 0.07	0.99 \pm 0.07	1.00 \pm 0.07	ns	ns
Stickiness	3.64 \pm 0.12	3.54 \pm 0.12	3.64 \pm 0.12	3.54 \pm 0.12	ns	ns
Acceptability	6.74 \pm 0.13	6.68 \pm 0.13	6.69 \pm 0.13	6.73 \pm 0.13	ns	ns

ns, non-significant $P > 0.05$; *significant $P \leq 0.05$; **significant $P \leq 0.01$.

also worked with other slow-growing chickens and at 98 d of age did not find sex differences in the amount of fat in the breast meat; neither did De Marchi *et al.* (2005) in the Padovana breed, with very slow growing, at 150 and 180 d of age. Perhaps differences between sexes in the quantity of lipids in the meat depend on the genotype, as was observed by Sunday *et al.* (2010) in a work on Nigerian native chickens, where they found an interaction between sex and genotype.

Fatty acid profile

Fatty acid profile differed between diets, but not between sexes. No significant interaction between diet and sex was found. These results in the case of sex, considering all saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), coincide with that observed in breast meat of Padovana chickens by De Marchi *et al.* (2005) at 150 and 180 d of age. On the other hand, Eleroğlu *et al.* (2013), in breast meat at 98 d of age, found higher concentrations of SFAs in females than in males and more PUFAs in males than in females. Baeza *et al.* (2010) found age-dependent differences between sexes in the meat of thighs and breast. At 84 d of age, they found differences between sexes both in MUFAs and PUFAs, in thigh as well as breast meat. The quantity of MUFAs was higher in the females than in the males, and the reverse

was true of PUFAs. At 120 d of age, differences between sexes appeared also in SFAs, but only in breast meat, being greater in males than in females. At this age, the differences between sexes in the MUFAs and PUFAs had the same order as at 84 d. In contrast to literature, sex differences were not uniform in the present study regarding meat fatty acid profile, so this subject needs more research to better elucidate the effects of genotype, age and sex.

Considering that differences between sexes were not significant, Table 5 only shows the values achieved for each fatty acid, depending on the diet with or without grape seed. Within each group (SFAs, MUFAs and PUFAs), only fatty acids with high concentrations and the total values of each group are shown. The values refer to the total percentage of fatty acids achieved.

The total SFAs, contributing between 26% and 27% of total fatty acids, demonstrated significant differences between diets, being higher in meat from the no grape diet, may be because the feed without grape seed also contains significant amounts of palmitic and myristic acids (Table 5). Anyway what was most responsible for these differences was palmitic acid.

Regarding total MUFAs, contributing between 28% and 31% of total fatty acids, there were significant differences between diets, with higher values in the diet without grape seed included. Oleic acid was detected at the highest

Table 5. Fatty acids (%) in Penedes chicken meat on diets with 0% (A) or 5% (B) of grape seed inclusion (LS means \pm standard error)

Fatty acids	Diet A	Diet B	P-value
<i>Saturated</i>			
Palmitic (C16:0)	18.44 \pm 0.21	17.28 \pm 0.21	***
Stearic (C18:0)	8.56 \pm 0.18	8.45 \pm 0.21	ns
Myristic (C14:0)	0.43 \pm 0.01	0.39 \pm 0.01	**
Total saturated	27.73 \pm 0.32	26.51 \pm 0.32	**
<i>Monounsaturated</i>			
Oleic (C18:1n-9 cis)	24.30 \pm 0.40	22.37 \pm 0.40	***
Palmitoleic (C16:1)	3.14 \pm 0.12	2.88 \pm 0.12	ns
Pentadecanoic (C15:1)	1.81 \pm 0.10	2.00 \pm 0.10	ns
Vaccenic (C18:1n-7)	1.53 \pm 0.04	1.48 \pm 0.04	ns
Total monounsaturated	31.13 \pm 0.49	28.97 \pm 0.49	**
<i>Polyunsaturated</i>			
Linoleic (C18:2n-6 cis)	26.68 \pm 0.76	29.62 \pm 0.76	*
Arachidonic (C20:4n-6)	7.29 \pm 0.32	7.85 \pm 0.32	ns
α -Linolenic (C18:3n-3 cis)	1.97 \pm 0.03	1.89 \pm 0.03	ns
Docosatetraenoic (C22:4n-6)	1.04 \pm 0.05	1.18 \pm 0.05	ns
Docosapentaenoic (C22:5n-3)	1.10 \pm 0.07	1.06 \pm 0.07	ns
Total polyunsaturated	41.03 \pm 0.63	44.51 \pm 0.63	***
Total unsaturated	72.16 \pm 0.32	73.49 \pm 0.32	**
<i>Omega fatty acids</i>			
n-3	4.55 \pm 0.16	4.38 \pm 0.16	ns
n-6	28.80 \pm 0.73	31.90 \pm 0.73	**

ns, non-significant $P > 0.05$; *significant $P \leq 0.05$; **significant $P \leq 0.01$.
***significant $P \leq 0.005$.

content in the diet without grape seed, and also showed the highest value of significance. So the meat of *Gall del Penedes* given a diet without grape seed had a two-percentage-unit higher concentration of oleic acid than the meat from chickens on a diet without grape seed.

Considering PUFAs, differences between diets were also significant, but the higher values were observed in the meat from the diet with grape seed included. Mean values were between 41% and 45%. The most important acid was linoleic acid, which is responsible for the significant differences. Its concentration was three percentage units higher in the meat from chickens fed with grape seed diet.

In summary, both diets gave meats rich in unsaturated fatty acids, taking into account MUFAs and PUFAs. Unsaturated fatty acids values were 74.5% in the meat from the diet with grape seed included and 72.2% in meat from the diet without grape seed included, and differences between these values were significant. Linoleic acid would be responsible for these differences.

Finally, considering unsaturated fatty acids, grouped in n-3 acids and n-6 acids in Table 5, it was observed that n-3 acids did not show significant differences between diets, whereas n-6 acids showed significant differences in favour of the inclusion of grape seed in the food. The main cause of this difference was linoleic acid.

Considering the contribution of SFAs, MUFAs and PUFAs in the feed, depending on whether the grape seed was included or not, PUFAs were the most abundant, contributing between 60% and 62%, followed by MUFAs, contributing between 23% and 22%, and finally SFAs, contributing between 16% and 17%. They were found in the same order in the meat, although there was a decrease of 20 percentage units in PUFAs, this causing an increment around 10 units of percentage on MUFAs and other 10 units on SFAs. This conversion is due to oxidation. As we reported in the results of statistical analysis, oxidation was more marked in meat from the no grape diet. In the meat from the grape seed diet, SFAs were lower than in the other meat, whereas diets had the same concentrations of SFAs. This can probably be explained by the content of polyphenols in grape seed, which will reduce the oxidation of fatty acids (Goñi, *et al.*, 2007; Brenes, *et al.*, 2010).

A higher proportion of unsaturated fatty acids than of saturated, as in the present work, is what is usually found in chicken meat coinciding with De Marchi *et al.* (2005), Jaturasitha *et al.* (2008), Baeza *et al.* (2010), Miguel *et al.* (2011); with Eleroğlu *et al.* (2013) in slow-growing chickens; with Rondelli *et al.* (2003) in lines of fast-growing broilers and with Escoda *et al.* (2001) and Tor *et al.* (2005) in chickens of the Penedesenca breed, the same as used in the present work. Referring to thigh meat, which was also studied here, the total proportion of unsaturated fatty acids has been in the order of 10 percentage units higher in this study than in most of the studies mentioned above, except Baeza *et al.* (2010), which presented values around 70%. However, if we consider only PUFAs, in the present work between 8 and 13 percentage units more were observed than that given in the literature cited. It might be a genotype effect. Escoda *et al.* (2001), Jaturasitha *et al.* (2008) and Baeza *et al.* (2010) found differences among genotypes in the proportion of PUFAs in chicken meat. Specifically Escoda *et al.* (2001) found significant differences between Penedesenca and Prat breeds, with a higher proportion in Penedesenca. However, Escoda *et al.* (2001) and Tor *et al.* (2005) found in Penedesenca the lowest proportions: 29.50% and 18.96% of PUFAs respectively compared to 42.8% average in the present work. We can also think of a diet effect, but most of these authors do not report the proportions of fatty acids in the diets that were used. Only Escoda *et al.* (2001) and Tor *et al.* (2005) provide this information. Specifically, Tor *et al.* (2005) give a value of 41.08% of PUFAs in the diet, about 20 units less than in the present work, which could explain the differences in PUFAs in the meat. But Escoda *et al.* (2001) give a value of 64.63%, slightly higher than that used in

this work, so that the differences between the results of Escoda *et al.* (2001) and the results in this work could not be explained by diet. On the other hand, in the present work, we also observe an effect of diet on the proportion of PUFAs in chicken meat. The inclusion of grape seed resulted in a higher proportion of PUFAs with a decrease of MUFAs as was also observed with the addition of linseed (Shen *et al.*, 2005; Guillevic *et al.*, 2009). No other references were found in the literature reporting on the relationship between grape seed inclusion and chemical composition of chicken meat.

In conclusion, the inclusion of 5% of grape seed substituting for maize in the Penedes chicken diet did not affect live body weight, feed intake and FCR until the 15th week of age. Further, meat showed no differences due to diet in the percentages of protein, lipid and ash. Meat of chickens on the grape seed diet showed a higher percentage of unsaturated fatty acids due to linoleic acid, a more nutty smell, more metallic flavour, more stringiness, less pork crackling odour, less pork crackling flavour, less sweet flavour and less broiler meat flavour.

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