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Original

Chemical properties and sensory characteristics of wild boar meat (*Sus scrofa scrofa*) fed with acorns (*Quercus robur*)

Propiedades químicas y características sensoriales de la carne de jabalí (*Sus scrofa scrofa*) alimentada con bellotas (*Quercus robur*)

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ABSTRACT

Backround: The wild boar (*Sus scrofa scrofa*) has favourable meat characteristics compared to the domestic pig particularly regarding the fat and cholesterol content. The present study aimed to determine whether the meat of wild boar fed with 20% or 40% acorns in their diet had different chemical properties and sensory meat characteristics.

Methods: A group of six wild boar (*Sus scrofa* s.) with an initial live weight of 40 kg, received fresh whole *Quercus robur* acorns at 20% w/w of their daily feedstuff for 60 days, a second

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group received 40% w/w and a third group consumed commercial feed without acorns (control group). Proximal chemical analysis was performed on the feedstuff, acorns, and meat. Fatty acids and cholesterol in the *Longissimus lumborum* muscle were measured via gas chromatography.
Results: The meat from the control fatty acid group had a higher composition of C16: 0 fatty acids and cholesterol, and also had a lower proportion of monounsaturated and polyunsaturated fatty acids, and of the n6: n3, P/S ratio, than the acorn-fed wild boar groups.
Conclusions: The meat of wild board fed with acorn had better characteristics in terms of nutritional factors such as the level of monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol, and sensory characteristics.

Key words: animal nutrition; animal feed; essential fatty acids; food composition; meat industry (*Source: UNBIS*)

RESUMEN

Antecedentes: El jabalí (*Sus scrofa scrofa*) tiene características de carne favorables en comparación con el cerdo doméstico, especialmente en lo que respecta al contenido de grasa y colesterol. El presente estudio tuvo como objetivo determinar si la carne de jabalí alimentada con 20% o 40% de bellotas en su dieta tenía diferentes propiedades químicas y características sensoriales de la carne.
Métodos: Un grupo de seis jabalíes (*Sus scrofa* s.), con un peso vivo inicial de 40 kg, recibieron bellotas frescas (Q*uercus robur)* al 20% p / p de su alimento diario durante 60 días, un segundo grupo recibió 40% p / p y un tercer grupo consumió ración comercial sin bellotas (grupo control). Se realizaron análisis químicos proximales a la ración, bellota y carne. Los ácidos grasos y el colesterol en el músculo *Longissimus lumborum* se midieron mediante cromatografía de gases.
Resultados: La carne del grupo control mostraron una mayor composición de ácido graso C 16: 0 y colesterol, y menor proporción de ácidos grasos monoinsaturados, poliinsaturados y de la relación n6: n3 y P/S, que los grupos de jabalíes alimentados con bellotas.
Conclusiónes: La carne de jabalíes alimentados con bellota presentó mejores características en cuanto a factores nutricionales como el nivel de ácidos grasos monoinsaturados, ácidos grasos poliinsaturados, colesterol y características sensoriales.

Palabras clave: ácidos grasos esenciales, alimentación animal, composición de los alimentos, industria de la carne, nutrición animal *(Source: UNBIS)*

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INTRODUCTION

The wild boar (*Sus scrofa scrofa*) has favourable meat characteristics compared to the domestic pig particularly regarding the fat and cholesterol content (Morán *et al*., 2019; Russo *et al*., 2017).

The high quality of meat from wild boars is the consequence of several factors, including genetics, crossbreeding, rearing systems and processing conditions (Morán *et al*., 2019). Within the factors encompassed by the rearing system, feed composition seems to be key to influencing wild boar product quality (Morán *et al*., 2019).

Quercus spp. acorns form an important part of the diet of free-living wild boar (*Sus scrofa s.*) in their native European habitat (Mikulka *et al*., 2018). Meat products obtained under these ecological conditions command higher prices in markets due to high nutritional value and particular sensory properties desired by consumers, and they are considered a significant source of healthy food (Russo *et al*., 2017). Some studies of Iberian pigs fed on grass and acorns reported increased meat tenderness and taste (Rodríguez-Estévez *et al*., 2008). These quality characteristics are mainly attributed to the high consumption of acorns, which are rich in oleic acid (Rey *et al*., 2006; Tejerina *et al*., 2018).

In Chile, wild boars are raised for commercial purposes in confinement conditions and these animals are mainly fed diets based on corn, soybean meal and wheat (Skewes & Morales, 2006). The final meat products are also distributed to higher priced markets due to their believed attributes similar to those explained above, even when their chemical characteristics have not been documented.

Quercus genus trees can also be found in Chile; however, their fruits have not been reported to be part of the wild boar production diet, and thus there is not much information on what effects this acorn intake could have on wild boar meat characteristics.

The present study was designed to test the hypothesis that the addition of acorns to the diet of wild boars increases the level of monounsaturated and polyunsaturated fatty acids in their meat, as well as the improvement of cholesterol and sensory characteristics.

MATERIALS AND METHODS

Location and facilities

Healthy and pure wild boars (male and females) were selected from a Chilean commercial farm, located in the county of Chillan, 36°34’S 72°06’W, 118 m above sea level, with an annual mean precipitation of 1034 mm and annual mean temperature of 14 °C, with a maximum mean for the hottest month (January) of 28.8 °C and a minimum mean for the coldest month (July) of 3.5 °C. Animals were kept in outdoor pens with 8 m^2 per animal, wooden feeders on the ground (providing linear access of 0.3 m per animal), a sucking drinker for every 15 animals, a soil-covered floor, a hay bed for refuge, and community housing of 6 m^2.

Feeding and management

The animals were initially subjected to similar nutritional and health management until reaching a live weight of 40 kg. The amount of feed given daily to experimental animals was equivalent to 4.1% of their live weight and was adjusted every 15 days. The feedstuff contained corn, soybean meal and wheat, and its nutritional value was determined based on proximal chemical analysis and fatty acid composition using gas chromatography.

Animals were randomly assigned to three experimental groups of six animals (4♀ and 2♂), which were housed together for five days until the start of the trial. Each group received the following feedstuff daily for 60 days:

- Control group: commercial ration (4.1% live weight)

- 20% group: 80% w/w commercial feed+ 20% w/w fresh acorns.

- 40% group: 60% w/w commercial feed + 40% w/w fresh acorns.

The acorns were collected directly from the ground under *Q. robur* trees from the county of Chillan and then kept dry at room temperature in a barn.

Animals were stunned and slaughtered at a local slaughterhouse at the end of the trial at a live weight of 55.3 kg (±2.57 kg). In the slaughterhouse, carcass weight (CW) and backfat thickness (BT) at the level of the last rib were taken. Samples of *Longissimus lumborum* muscle (LL) at the level of the L1 to L4 lumbar vertebra were taken at cutting (24 hours after slaughter), weighed, vacuum-packed in low-oxygen permeable bags and kept frozen at –18 °C ± 2 °C until analysis. Analyses were carried out within three weeks of slaughter.

Chemical analysis

Chemical analysis were performed on 100 g samples of the feedstuff, acorns, and meat (n=3). The moisture of the samples was immediately determined by drying at 103 ºC ± 2 ºC until constant weight. The total intramuscular fat was measured by Soxhlet extraction, Crude protein was measured by the Kjeldahl method, Fibre was analysed by Weende method, and the Ash was measured gravimetrically by burning samples (AOAC, 1990).

Fatty acids

Samples of muscle were divided into three equal portions (n=18) for each experimental group. The fat extraction was performed according to Bligh & Dyer (1959) and Lumley & Colwell (1991). In brief, 20 g of sample were extracted by a solvent composed of methanol, chloroform and water (80:50:32). The fat was concentrated in the chloroform layer. The chloroform phase was removed by evaporation and 500 µL of n-hexane was added to the extract, which was then stored at –18 ºC ± 2 °C until analysis. Transmethylation was carried out according to ISO: 5509:1978. This was calculated for 1 g oil or fat in 10 ml of hexane, to which 0.5 ml 2 mol^{-1} Na_2OH_4 in methanol was added and stirred for 20 s. After phase separation, the supernatant was collected for subsequent gas chromatography analysis. The fatty acid profile was determined in a gas chromatograph (Clarus 600 Perkin Elmer®, USA) equipped with a flame ionization detector (FID). A capillary column SP-2560™ (Sigma-Aldrich Co., USA) of 100 m × 0.25 mm × 0.25 µm

film were used. Helium was used as the carrier gas at 1.0 ml/min and inlet pressure of 15 psi, and the method of injection was split (100:1). The injector temperature was fixed at 250 ºC and the detector was fixed at 260 ºC. The sample volume injected was 1.0 µl. The oven temperature was programmed to increase from 140 ºC (held for 5 min) to 240 ºC (held for 15 min) at 4 ºC/min. Fatty acids were identified by comparing the retention times of the chromatograph peaks with those of the methyl esters from a mixture prepared with a standard 37 component FAME Mix (Sigma-Aldrich Co., USA).

Cholesterol

Cholesterol extraction was performed by direct saponification of KOH 2% in ethanol (Mazalli Saldanha & Bragagnolo *et al*., 2003) with subsequent chromatographic analysis (n=18) in the following conditions: the injector temperature was fixed at 270 ºC and the detector was fixed at 300 ºC. The sample volume injected was 1.0 µl. The oven temperature was programmed to increase from 160 ºC (held for 1 min) to 300 ºC (held for 7 min) at 10 ºC/min. Cholesterol was quantified by the standard internal method using 5 alpha cholestane (Supelco Analytical, Bellefonte, PA, USA) as the reference.

Sensory panel

The day before the test, the samples were thawed at 4 °C ± 2 °C over 24 h. Subsequently, each sample was sealed with aluminium foil and was cooked in a pre-heated oven (FAGOR Innovation Class A) at 200 °C to achieve an internal temperature of 70 ºC. Subsequently, the specimens were wrapped in aluminium foil to avoid drying and were used for the sensory analysis. A panel of six members was selected and trained according to ISO 3972:1991 and ISO 8586-1:1993 standards. A total of four tasting sessions were performed in which each assessor evaluated the six samples coded using random three-digit numbers. The order of presentation of the samples and the first-order and carry-over effect were blocked (MacFie *et al*., 1989). The assessors conducted a descriptive sensory analysis to evaluate the flavour, juiciness and tenderness in the cooked meat using a hybrid hedonic scale described by Villanueva *et al*. (2005).

Analyses were performed in the Animal Nutrition and Meat quality laboratory of the Agricultural Research Institute (INIA Remehue), Osorno, Chile.

Statistical analysis

The data were analysed as a completely randomised design using the general linear model (GLM) procedure contained in SAS Statistical Package v 8.01 (SAS Institute, 2001). Before the statistical analysis, percentage of fatty acids was arcsin transformed when necessary to fulfil the population normality and homogeneity assumptions. The comparative analysis between means was conducted using the Duncan test.

RESULTS AND DISCUSSION

Carcass, chemical and fatty acids composition of diets

With respect to type of diet consumed, no differences for carcass weight (CW) and backfat thickness (BT) were observed between the control group and the wild boars fed with 20% acorns. The CW found were 37.6, 40.1 and 44.6 kg, and BT of 11.8, 14.0 and 15.6 mm for wild boars fed with commercial feed, 20% acorns, and 40% acorns respectively. These values were higher than those reported by Daza *et al*. (2007) in Iberian pigs, which is probably due to higher crude fat content found in acorns during this experiment. A significant correlation coefficient between carcass weight and backfat thickness was found ($r = 0.69$; $P \leq 0.01$).

The acorns had low protein content, and were relatively high in oleic acid content (C18:1 *n*-9). The commercial feed had a relatively high proportion of linoleic acid (C18:2*n*-6) in accordance with data from Rey *et al*. (2006) and a higher amount of protein than that provided by the fresh acorns (15.6% vs. 5.6% respectively). Acorns have a higher content of crude fat, net energy, and metabolizable energy when compared with commercial feeds. The fresh acorns had the highest MUFA, PUFA, and *n*6:*n*3 ratio (**Table 1**).

Table 1. Analysed composition of feed (g/100g) and fatty acid (mg/100 mg) composition of the experimental diet groups

Nutrient composition	Feedstuff	Fresh acorns	*P**
Moisture (g/100 g)	14[b]	32.4[a]	0.0017
Crude protein (g/100 g)	15.6[a]	5.6[b]	0.0001
Crude fat (g/100 g)	3.3[b]	5[a]	0.002
Fibre (g/100 g)	6.8[a]	5.6[b]	0.013
Ash (g/100 g)	3.7[a]	2.7[b]	0.010
Net energy (Mcal/kg)	1.73[b]	1.85[a]	0.09
Metabolisable energy (Mcal/kg)	2.9[b]	3.13[a]	0.011
Value D	80[b]	88[a]	0.0012
Fatty acid profile mg/100 mg			
C 14:0	18.16[a]	0.12[b]	0.0032
C16:0		17.48	ns[A]
C 16:1*n*-9	1.18	1.7	ns
C 16:1*n*-7		0.16	
C 17:0		0.05	
C18:0		1.72	
C18:1*n*-9	18.39[b]	27.3[a]	0.0016
C18:1*n*-7	0.75[b]	5.84[a]	0.0031
C18:2*n*-6	57.7[a]	39.1[b]	0.009
C 20:0	3.51[b]	4.58[a]	0.0001
C18:3 *n*-3		1.65	
C 20:2	19.75[a]	0.06[b]	0.0001
SFA[B]		23.9	
MUFA[C]	19.1[b]	35.01[a]	0.0001
PUFA[D]	61.2[a]	40.8[b]	0.0001
*n*6:*n*3[E]	16.4[b]	23.7[a]	0.001
P:S[F]	3.1[a]	1.7[b]	0.001
[A]ns: not significant			
[B]Σ Saturated fatty acids: C14+C16+C17+C18+C20			
[C]Σ Monounsaturated fatty acids: C16:1+C18:1*n*-9+C18:1*n*-7			
[D]Σ Polyunsaturated fatty acids: C18:2*n*-6+C18:3*n*-3+C20:2			
[E]*n*-6:*n*-3: Fatty acids ratio			
[F]P:S: Polyunsaturated: Saturated fatty acids ratio			

*The p-value correspond to Student t test between feedstuff and fresh acorn

Fatty acid profile in muscle

Wild boars fed with acorns had a significantly lower proportion (*P*= 0.0001) of C 16:0 than those fed only with commercial feed (Table 2). However, no differences in C 14:0, C 16:1*n*-9, C 16:1*n*-7, and C 20:3*n*-3 were detected among the groups. Similar results have been described before in Iberian pigs fed with acorns and grass (Carrapiso *et al*., 2020; Rey et al., 2006). However, there is no information available about the effects of acorns or combinations of acorn and commercial feed diets in confinement. According to the results of the present study, acorn intake significantly modified the proportion of saturated fatty acids (SFA) of the muscle. Wild boars raised with acorns had significantly (*P*= 0.0001) higher proportions of C18:1 *n*-9 in *Longissimus lumborum* (LL) muscle than the control group. Similar results were found in C18:2 *n*-6, C18:3 *n*-3, and MUFA. This effect is more marked in diets with 40% acorns. Consequently, the group fed with 40% acorns was a better indicator of the feeding background. It has been reported that during winter commercial pigs reared outdoors (5 °C) had a reduced proportion of SFA when compared with pigs fed indoors (22°C) (Bee Guex & Herzog, 2004). This differs from the present study, where the average of temperatures between outdoors and indoors was not as marked (17°C). Other studies showed that grass intake also increases the proportion of C18:3 *n*-3 in the backfat and intramuscular neural lipids in pigs fed with acorns and grass (Rey *et al*., 2006). In this study, the proportion of C18:3 *n*-3 in LL can be attributed to the high content of linolenic acid in acorns. According to the results of this experiment, it seems that the content of fatty acids in the muscle is very sensitive to small variations in dietary fat composition. This evidence indicates the importance of the acorn diets on the quality of final products in wild boar meat. Ayuso *et al*. (2020) found that eating acorns produced a higher C18:3 *n*-3 compared with commercial feed in Iberian pigs, and Karolyi *et al*. (2007) found similar results in Black Slovenian pigs.

In addition, it is of interest to observe that the proportion of polar lipids such as C 22:6*n*-3 in LL was also increased (*P*= 0.0001) by the acorns, but not by the commercial feed. This fatty acid, C 22:6*n*-3, can reduce the risk of human cardiovascular disease (Morán *et al*., 2019).

The P/S ratio (Table 2) in LL ranged from 0.33 in the control group to 0.39 in 40% acorn diet (*P*= 0.0001). For optimal health, the P/S ratio of meat should be at least 0.4 (Batorska *et al*., 2018). Studies of meat from the wild boars recorded the P/S ratio at a level of 0.50 (Batorska *et al*., 2018). The study concluded that the P/S ratio was related to a high content of C18:2 *n*-6 (15.5–24.7 g 100 g^{-1}), the origin of which is associated with chestnuts, acorns, corn, and potatoes (Batorska *et al*., 2018).

Cholesterol in muscle

The cholesterol content showed significant differences between groups (Table 2), being lower for the wild boars fed with acorns, and with the highest value found in the control group fed with commercial feed (*P*= 0.0001). Lui *et al*. (2007) reported a low content of cholesterol in wild boars (29.6 mg/100 g), which was far from the 80 mg/100 g found in domestic pigs by Sudom *et al*. (2001). The cholesterol contents of the present study are low compared to those reported for wild boars (48.1 to 63.8 mg/100 g) by Russo *et al*. (2017) or by other studies on wild boars in

Chile (Skewes *et al*., 2009). These results coincide with those of other researchers who associated the consumption of diets rich in MUFA with reductions in cholesterol and triglycerides (Márquez Contreras *et al*., 2018).

Sensory characteristics

In this study, the hedonic scale reflected a higher perception by consumers of meat sensory characteristics. The sensory panel detected differences ($P \geq 0.05$) in the flavour, tenderness and juiciness between groups (**Table 2**). A positive relationship was observed between the percentages of MUFA and PUFA with the attributes flavour, tenderness and juiciness. This is explained by the fact that meat from animals fed with acorns was perceived as juicier and more tender by a sensory panel. Tartrakoon *et al*. (2016) reported that SFA and MUFA have a positive influence on the flavour, tenderness and juiciness of the meat. Similarly, Aaslyng & Meinert (2017) reported a strong relationship between sensory characteristics such as flavour with the type and amount of fatty acids in meat, especially C18:1 *n-9*, and in Iberian dry-cured loins, Soto *et al*. (2008) found a significant difference in flavour in pigs reared with free access to acorns (*Quercus spp*.) and grass. These studies are all in agreement with the acorn impact observed in the present experiment.

Table 2. Fatty acid composition (mg/100 mg), cholesterol and sensory characteristics of *L. lumborum* muscle from wild boars fed the experimental diets.

	Control	**20%**	**40%**	**R^2**
C 14:0	1.17	1.21	1.22	0.632
C 16:0	24.56^{a}	23.11^{b}	23.51^{b}	0.553
C 16:1*n*-9	0.4	0.41	0.42	0.712
C 16:1*n*-7	2.43	2.58	2.69	0.643
C 17:0	0.25^{b}	0.32^{a}	0.33^{a}	0.783
C 17:1	0.21^{b}	0.28^{a}	0.29^{a}	0.641
C 18:0	11.73^{b}	11.9^{ab}	12.1^{a}	0.893
C 18:1*n*-9	40.3^{b}	41^{a}	41.1^{a}	0.857
C 18:1*n*-7	2.59^{b}	3.01^{a}	3.11^{a}	0.735
C 18:2*n*-6	12.5^{b}	12.9^{b}	13.1^{a}	0.884
C18:3*n*-3	0.46^{b}	0.5^{a}	0.51^{a}	0.865
C 18:4*n*-3	0.05^{c}	0.08^{b}	0.1^{a}	0.873
C 20:0	0.17^{b}	0.18^{ab}	0.19^{a}	0.842
C 20:1	0.82^{b}	0.84^{a}	0.84^{a}	0.786
C 20:2	0.53^{b}	0.53^{b}	0.57^{a}	0.768
C 20:3*n*-6	0.12^{c}	0.14^{b}	0.15^{a}	0.850
C 20:3*n*-3	0.08	0.09	0.09	0.794
C 20:4*n*-6	0.03^{c}	0.14^{b}	0.19^{a}	0.725
C 22:6*n*-3	0.1^{b}	0.11^{a}	0.12^{a}	0.639
C 23:0	0.72^{c}	0.84^{b}	1.03^{a}	0.747
C 24:1	0.09^{b}	0.1^{b}	0.16^{a}	0.759
SFA^{A}	37.54^{b}	38.13^{a}	38.77^{a}	0.825
MUFA^{B}	47.3^{b}	48.03^{a}	48.11^{a}	0.769
PUFA^{C}	14.06^{c}	14.43^{b}	14.47^{a}	0.758
*n*6: *n*3^{D}	19.96^{b}	19.92^{b}	20.76^{a}	0.807
P:S^{E}	0.33^{c}	0.38^{b}	0.39^{a}	0.811
Cholesterol	23.9^{a}	21.6^{b}	22.2^{b}	0.732
Flavour	4.99^{c}	5.04^{b}	5.33^{a}	0.620

Tenderness	4.43c	5.05[b]	5.22[a]	0.812
Juiceness	4.4[c]	4.71[b]	5[a]	0.849
[A]Σ Saturated fatty acids: C14+C16+C17+C18+C20				
[B]Σ Monounsaturated fatty acids: C16:1+C18:1*n*-9+C18:1*n*-7				
[C]Σ Polyunsaturated fatty acids: C18:2*n*-6+C18:3*n*-3+C20:2				
[D]*n*-6:*n*-3: Fatty acids ratio				
[E]P:S: Polyunsaturated: Saturated fatty acids ratio				

* Values with different superscript are significantly different using the Duncan test ($P < 0.05$).

CONCLUSIONS

The wild boar *L. lumborum* muscle from the three groups had a fatty acid profile that was quite variable, as expected in animals on a controlled diet. LL muscles of the wild boar control group showed a higher composition of C 16:0 fatty acid and cholesterol, and lower MUFA, PUFA, *n*6: *n*3, and P/S ratio than those wild boar groups fed with acorns. As a consequence, the meat of wild boars fed with acorns was superior with regard to nutritional factors and sensory characteristics. In order to understand the role of acorn diets on wild boar meat quality in Chile, further studies are recommended on specific parameters such as HDL cholesterol in the meat, because the findings of this study indicate that these could be well-received by consumers.

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AUTHOR CONTRIBUTION

Conception and design of research: PGFA, RMP, OSR; data analysis and interpretation: PGFA, RMP, OSR; redaction of the manuscript: PGFA, RMP, OSR.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.