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The Effect of Green Oak Acorn (*Quercus ilex*) Based Diet on Growth Performance and Meat Fatty Acid Composition of Broilers

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ABSTRACT : This experiment was conducted to compare the effects of oak acorns and corn on broilers growth performance, carcass characteristics and meat fatty acid (FA) composition according to different ages of broilers. Two separate groups of 400 male broilers, reared for six weeks, were fed diets containing 33.5% green oak acorns (GO) and 67% of corn (C), respectively. At day 35, the body weight of the chickens fed the GO diet was 12% lower ($p<0.05$) compared with controls (C). However, at day 56 birds reached similar final weights. Abdominal adipose tissue (AAT) was 78% higher in the controls compared to those fed the oak acorn-based diet. The total lipids (TL) of thigh muscle were higher in the controls compared to dietary treatment GO (3.1 against 2.5 g/100 g of muscle). There were significant differences in fatty acid (FA) composition of the muscle between two groups. The broilers fed oak acorn exhibited more C18:2, as well as a higher proportion of polyunsaturated fatty acid (PUFA) and PUFA:SFA (saturated fatty acid) ratio than those on the control diet, but no significant differences were observed among groups for the age of birds. There was a higher proportion of linolenic acid in broilers fed the oak acorn compared to the controls (0.8 vs. 0.6%). Furthermore, linolenic acid content decreased with age ($p<0.001$) in both groups. The results indicated no significant differences in FA with the age of birds. In conclusion, the meat fatty acids provided by the GO diet appeared to improve the nutritional quality preferred by the consumer. (**Key Words :** Broilers, Oak Acorn, Fatty Acid, Meat)

INTRODUCTION

In North Africa, large areas of unexploited oak acorn forest have raised the interest of scientists mainly due to their large availability and their resistance to drought. The idea appeared in most Mediterranean countries to exploit them for livestock feeding as an energy source (47 to 60% starch, Kekos and Kaukios, 1985); 7 to 14.4% lipids, some unsaturated fatty acids (UFA) and for oil extraction because of similarities to olive oil (Ofcarick and Burns, 1971). Ofcarick and Burns (1971), Dodd et al. (1993), Cantos et al. (2003) and Lopes and Bernardo-Gil (2005) reported that the major fatty acids (FA) in acorns were oleic (48-63%), linoleic (16.5-17%), palmitic (12.1-13%), stearic (3-6%), linolenic (1-5%), and myristic, palmitoleic, arachidic, 11-eicosenoic and behenic acids which were found in amounts below 0.5% or in traces. The amount of cholesterol was low (0.1%). The concentrations of α-tocopherol in *Quercus ilex*, were 131 mg/kg of dry matter (Cantos et al., 2003).

In addition to this biochemical composition, oak acorns contain bioactive anti-nutritive factors (Shimada, 2001), including tannins (7%) which possess antioxidant activity (Rakié et al., 2007).

It is well known that diet is one of the most important factors that can modify the quantity and the quality of lipids stored in chicken tissues. The FA composition of muscular and adipose tissues is modified by the composition of dietary lipids: for example, tallow fat in the diet increases the proportion of C16:0 and C18:0 (Lopez-Ferrer et al., 2001), whereas dietary vegetable oil or seeds increases PUFA content (Loria and Padgett, 1997; Li et al., 1999; Mantzioris et al., 2000; Ayerza et al., 2002).

Lipids of oak acorns contain linoleic acid, an ω-3 polyunsaturated fatty acid which is important in eicosanoid synthesis (Petrovié et al., 2004), and may contribute to enriching meat with polyunsaturated fatty acid (PUFA) via their use in broiler feeding. Thus, oak acorn can be used as an ingredient in animal diets. In North Africa countries, it is used as a substitution for corn in broiler diets (Bouderoua and Selselet-Attou, 2003) and effects on growth performance, carcass characteristics and FA composition of

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Table 1. Composition of the experimental diets

Diet	C	GO
Ingredients (%)		
Corn	67.0	33.5
Green oak acorn	-	33.5
Soya bean meal	27.0	27.0
Wheat bran	4.0	4.0
Vit-min premix	1.0	1.0
Calcium	0.5	0.5
Phosphorus	0.5	0.5
Calculated composition		
ME (Kcal/kg)	2,960	2,995
Moisture (%)	11.0	12.3
Calcium (%)	1.0	1.0
Phosphorus (%)	0.3	0.4
Lysine (%)	1.1	1.1
Sulphur amino acid (%)	0.8	0.7
Analysed composition (%)		
Crude protein	21.0	19.1
Lipids	3.4	3.9
Total phenol (gallic acid equivalents)	ND	1.1
Ash	5.1	4.5
FA analysis (% of the identified FA)		
C14:0	0.10	0.18
C16:0	13.05	13.07
C16:1(n7)	0.12	0.20
C18:0	2.53	2.95
C18:1(n9)	46.01	33.65
C18:2(n6)	33.70	43.24
C20:0	0.36	0.36
C18:3(n3)	2.82	5.44
C20:1(n9)	0.46	0.45
SFA	16.04	16.56
MUFA	46.59	34.30
PUFA	36.52	48.68

Vit-min premix: provided (in mg per kg of diet); vitamin E, 6; vitamin K_3, 0.80; vitamin B_1, 1; vitamin B_2, 3; Pantothenate of Ca, 6; vitamin B_6, 1.5; vitamin B_{12}, 0.006; folic acid, 0.2; nicotinic acid, 12; copper, 5; cobalt, 0.65; manganese, 65; zinc, 65; selenium, 0.25; iron, 50; iode, 0.8; magnesium, 100.
EM = Metabolisable energy, ND = Not determined, C = Control diet, GO = Green oak acorn diet, SFA = Saturated fatty acids, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acids.

Iberian pigs have been studied (Rey et al., 2005).

The purpose of this trial was to investigate the possible effects of green oak acorns (*Quercus ilex*) as a natural product-based diet on growth performance, carcass characteristics and meat fatty acid composition according to different age of broilers.

MATERIALS AND METHODS

Animals and diets

Eight hundred 1-d-old, male Hubbard-ISA broilers were obtained from a commercial hatchery and placed in 20 floor pens of 2×2 meters with 40 birds per pen. All chicks were fed until 14 days of age on a standard starter diet (3,100 kcal/kg, 22% protein) and were allowed free access to water and food. Birds had an initial body weight (BW) of 298±42 g at 14 days of age. The experimental diets, which were based on green oak acorn (GO) and a control corn-based diet (C), were fed from 15 to 56 days of age. Acorns consisted of the whole fruit collected in December 2006. To ensure safer and longer storage of the experimental diet, acorn was incorporated into the diet after being air-dried in the shade for 20 days and ground using a local commercial grain mill fitted with a 3 mm pore size sieve. Ingredients and nutrient composition of the diets are shown in Table 1. The weights of birds and feed consumption for each pen were recorded on days 14, 21, 28, 35, 49 and 56 of age.

Measurements at slaughter

On days 35, 49 and 56, ten birds from each diet were selected, killed, processed, and eviscerated in a local commercial slaughterhouse. After evisceration, the birds were apportioned by hand. Samples (100 g) from the left thigh were obtained. Meat samples from 10 birds per treatment were placed in plastic bags and frozen at -20°C until analysis. Liver and abdominal adipose tissue (AAT) were also removed and weighed individually.

Laboratory analysis

Analysis of diets : Samples of diets were dried and stored for subsequent analyses. Mineral content was determined by ashing at 600°C for 8 h. Nitrogen was determined by the Kjeldahl method (CP = N×6.25). Total phenol content was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). The extract was diluted 5-10 times by methanol (ether extracts) to obtain a final absorbance below 0.5. A 2.5 ml aliquot of Folin-Ciocalteu reagent (diluted 10 times by water) and 2 ml of a sodium carbonate (75 g/L) were added to 0.5 ml of the diluted extract. Sodium carbonate was added 30 s to 8 min after Folin-Ciocalteu reagent. The assay tubes were kept for 5 min in a water bath at 50°C and then transferred to cold water. After cooling, absorbance was measured at 760 nm. Results were expressed in gallic acid equivalents per amount of diet extracted. Calibration was achieved with an aqueous solution of gallic acid (8-80 μg/ml).

Analysis of meats : The total lipids (TL) of each sample (diet or meat) were extracted by chloroform-methanol (2:1) according to the method of Folch et al. (1957). FA of lipids were freed by saponification (NaOH), and then methylated by methanol-BF_3 (Morisson and Smith, 1964). The methyl esters of FA were separated and quantified by gas chromatograph (Perkin-Elmer AutoSystem XL) equipped with flame ionization detector and a capillary column (30 m×0.25 mm internal diameter). The operating conditions of the gas chromatograph were as follows: injector and

Table 2. Influence of experimental diet on growth performance of broilers (accumulative results)

	Day						
	14	21	28	35	42	49	56
BW (g)							
Green oak (GO)	298[a]	561[a]	903[a]	1,440[a]	1,651[a]	2,111[a]	2,204[a]
Control (C)	298[a]	591[a]	986[b]	1,622[b]	1,793[b]	2,189[a]	2,260[a]
SEM	5.61	9.69	7.42	14.23	16.52	14.63	16.11
Dietary effect	NS	NS	<0.05	<0.05	<0.05	NS	NS
FC							
GO	2.1[a]	2.1[a]	2.4[a]	2.0[a]	2.7[a]	1.9[a]	1.7[a]
C	1.6[b]	1.7[b]	1.5[b]	2.0[a]	2.3[a]	2.0[a]	1.6[a]
SEM	0.72	0.61	0.81	0.74	0.85	0.76	0.57
Dietary effect	<0.05	<0.05	<0.05	NS	NS	NS	NS

For each group n = 30. Results expressed as mean and standard error of the mean (SEM).
Means in the same column with different superscripts are significantly different. BW = Body weight; FC = Feed conversion; NS = Not significant.

detector temperature of 220 and 280°C, respectively; the oven temperature was programmed to increase from 45 to 240°C at 20 to 35°C per minute; aliquots of 1 µl were injected with bicyanopropyl phenyl silicone as a stationary phase; hydrogen was used as conductor gas; FA peaks were identified by comparison with retention times of methyl fatty acid standards; quantification was made by reference to an internal standard (C17:0).

Statistical analyses

Statistical analyses of performance parameters, meat quality and FA composition was conducted by analysis of variance using SAS Software (GLM procedure, SAS institute, 1989).

RESULTS

Productive performance

BW and feed conversion (FC) values are presented in Table 2. At day 35, the GO diet reduced chicken growth by about 12% compared to the control diet (p<0.05). However, at day 56 birds on both diets reached similar final weights. A similar observation was made for FC which was significantly (p<0.05) poorer until day 28 of age, but the negative effect of GO diet on FC appeared to have been reduced from day 49 and no significant differences (p>0.05) in FC were recorded between C and GO diets at day 56.

Carcass parameters

The carcass parameters are shown in Table 3. Eviscerated weight (EW) of birds fed on GO reached similar final values to the controls. At day 56, the AAT proportion was roughly 78% higher (p<0.01) in C broilers than in those fed GO. The C birds exhibited a heavier (p<0.05) thigh weight at days 35 and 49 compared to the GO diet, but at day 56 no significant differences (p>0.05) were detected. There were no significant differences in liver weight between the two diets.

Table 3. Influence of experimental diet on carcass characteristics of broilers

	Day	C	GO	SEM	Dietary effect
EW (g)	35	834[a]	759[b]	9.27	<0.05
	49	1,497[a]	1,461[a]	13.07	NS
	56	1,764[a]	1,773[a]	12.98	NS
AAT weight (g)	35	14[a]	15[a]	2.12	NS
	49	28[a]	25[b]	2.82	<0.05
	56	41[a]	32[b]	2.54	<0.05
AAT (% of EW)	35	1.71[a]	2.01[b]	0.71	<0.05
	49	1.91[a]	1.90[a]	0.81	NS
	56	2.30[a]	1.80[b]	0.59	<0.01
Thigh weight (g)	35	111[a]	103[b]	3.67	<0.05
	49	210[a]	185[b]	4.94	<0.05
	56	246[a]	236[a]	0.59	NS
Liver weight (g)	35	43[a]	38[a]	1.41	NS
	49	55[a]	53[a]	1.27	NS
	56	58[a]	55[a]	1.43	NS

For each group n = 10. Results expressed as mean and standard error of the mean (SEM).
Means in the same line with different superscripts are significantly different.
EW = Eviscerated weight; AAT = Abdominal adipose tissue; NS = Not significant.

Fatty acid composition

Total lipids and FA composition of the thigh muscle are shown in Table 4. The level of TL was significantly higher ($p<0.001$) in C fed chickens than in those fed GO (2.5 vs. 3.1 g/100g of muscle) and no significant effect with age of chickens was detected. Palmitic and stearic acid contents were lower ($p<0.001$) in meat of chickens fed oak acorn. However, no significant effect of age of birds was recorded. The same tendency was noted for total SFA expressed as a percentage of FA.

Among FA, the monounsaturated fatty acids (MUFA) showed the greatest level in meat for both types of diet, with oleic acid being the predominant constituent. However, meat produced by animals fed GO had significantly higher ($p<0.001$) MUFA levels compared with the control diet, with no significant difference between ages of chickens.

Linoleic acid was significantly higher ($p<0.001$) in the meat of broilers fed the GO diet than in those fed the C diet (18.2 vs. 14.5% at day 56, Table 4). No age effect was detected.

Linolenic acid was significantly higher ($p<0.01$) in the thigh meat of broilers fed GO, compared to the C diet (1.0 vs. 0.6% of the identified FA). Furthermore, linolenic acid content decreased with broiler age ($p<0.001$) on both diets. For the content of other FA, no differences with age were detected. The PUFA:SFA ratio was higher ($p<0.001$) in animals fed GO than the controls (0.7 vs. 0.5) and no age effect was detected. The n-6:n-3 ratio was also higher ($p<0.001$) in broilers fed the GO diet.

DISCUSSION

The low BW and FC obtained with an acorn based diet, especially at days 35 and 49, agrees with observations made when unsaturated lipid-rich sources were added to broiler diets as pepper seed oil meal (An et al., 2007), *chia* seed (Ayerza et al., 2002), flaxseed (Hrdinka et al., 1996) and fish products (Hulan et al., 1988). It was also suggested that tannin, a major anti-nutritional factor in acorns (Short, 1976), makes amino acids less available for chickens (Leclercq et al., 1984). Several studies have reported that feeding diets containing tannin to broiler chicks negatively affects performance in terms of growth, feed intake, and feed efficiency (Rostagno et al., 1973; Armstrong et al., 1974). The anti-nutritive effects of feeding tannin-containing GO to poultry appear to decline with age and agrees with observations made by Doughs et al. (1993), Hur et al. (2006) on turkeys and goats fed sorghum tannin. They suggested that birds may adapt to tannin-containing diets and/or the digestive system may develop functions to overcome the anti-nutritional effects of tannins (Ozkan et al., 2006). These observations agree with our results which showed decreased BW at 35 days of age which became similar to the controls at 56 days of age. Furthermore, this adverse effect of tannins can be reduced by thermal treatments of the acorns (Rakié et al., 2007).

Birds fed the corn-based control diet had abdominal fat deposition similar to reported levels (Pikul, 1985; Legrand et al., 1987). The lower AAT in birds fed GO was similar to

Table 4. Total lipid content (%) and fatty acid composition (% of identified FA) of *Sartorius* muscle of broilers fed green oak acorn and control diets

Day	C			GO			SEM	Dietary effect	Age effect
	35	49	56	35	49	56			
TL (%)	3.51^{a}	3.62^{a}	3.10^{a}	2.51^{b}	2.60^{b}	2.61^{b}	1.01	p<0.001	NS
C14:0	0.61^{a}	0.60^{a}	0.65^{a}	0.46^{b}	0.53^{b}	0.55^{b}	0.23	<0.001	NS
C16:0	27.2^{a}	26.23^{a}	28.82^{a}	22.17^{b}	22.88^{b}	22.70^{b}	1.11	<0.001	NS
C16:1	8.38^{a}	7.63^{a}	7.38^{a}	5.50^{b}	4.57^{b}	5.32^{b}	0.87	<0.001	NS
C18:0	8.02^{a}	8.11^{a}	8.69^{a}	6.52^{b}	6.93^{b}	5.66^{b}	0.99	<0.001	NS
C18:1 (n-9)	35.5^{a}	37.71^{a}	35.42^{a}	42.81^{b}	42.61^{b}	43.92^{b}	1.54	<0.001	NS
CI8:2 (n-6)	15.9^{a}	14.92^{a}	14.51^{a}	18.40^{b}	18.60^{b}	18.20^{b}	1.07	<0.001	NS
C20:0	0.24^{a}	0.24^{a}	0.24^{a}	0.24^{a}	0.24^{a}	0.33^{a}	0.21	NS	NS
CI8:3 (n-3)	0.80^{c}	0.71^{ad}	0.60^{ae}	1.11^{bc}	1.02^{bd}	0.84^{be}	0.29	<0.001	<0.001
C20:1 (n-9)	0.52^{a}	0.51^{a}	0.57^{a}	0.48^{b}	0.45^{b}	0.35^{b}	0.44	<0.001	NS
C20:4 (n-6)	1.61^{a}	2.11^{a}	1.89^{a}	1.31^{b}	1.41^{b}	1.25^{b}	0.85	<0.001	NS
C24:1	0.12^{a}	0.14^{a}	0.12^{a}	0.15^{a}	0.10^{a}	0.08^{a}	0.26	NS	NS
C20:5 (n-3)	0.07^{a}	0.06^{a}	0.07^{a}	0.05^{b}	0.02^{b}	0.04^{b}	0.18	<0.001	NS
C22:5 (n-3)	0.12^{a}	0.10^{a}	0.18^{a}	0.11^{a}	0.16^{a}	0.12^{a}	0.19	NS	NS
C22:6 (n-3)	0.12^{a}	0.10^{a}	0.18^{a}	0.10^{a}	0.16^{a}	0.11^{a}	0.25	NS	NS
SFA	36.0^{a}	35.19^{a}	38.40^{a}	29.39^{b}	29.30^{b}	29.25^{b}	1.35	<0.001	NS
MUFA	45.2^{a}	45.91^{a}	43.50^{a}	48.92^{b}	47.71^{b}	49.71^{b}	1.64	<0.001	NS
PUFA	18.5^{a}	18.45^{a}	17.94^{a}	21.58^{b}	21.57^{b}	20.82^{b}	1.42	<0.001	NS
PUFA:SFA	0.62^{a}	0.55^{a}	0.55^{a}	0.79^{b}	0.71^{b}	0.71^{b}	0.18	<0.001	NS
n-6:n -3	14.2^{a}	14.75^{a}	13.89^{a}	13.91^{a}	18.57^{b}	16.27^{c}	1.02	<0.001	<0.001

For each group n = 10. TL: Total lipid. Results expressed as mean and standard error of the mean (SEM).
Means in the same line with different superscripts are significantly different; NS = Not significant.

when fed sunflower and linseed oils (Pan and Storlien, 1993; Crespo Sanz et al., 2000; Esteve-Garcia, 2001) which suggested an effect of diet on fat deposition. Thus, previous reports indicated that broilers chickens fed diets enriched with PUFA have less abdominal lipid content (Sanz et al., 1999; Crespo and Esteve-Garcia, 2001).

The type of diet had no effect on the final weight of thigh. However, thigh from birds fed oak acorn diet showed lower lipid content compared to those fed a corn diet (Choi et al., 2008), although dietary lipids had no influence on muscular lipids (Ajuyah et al., 1991).

Oleic acid was the predominant FA in thigh muscle of both groups. In the experiments reported by Ajuyah et al. (1991) and Crespo and Esteva-Garcia (2001), oleic acid was the major FA found in carcass and muscle fats. This could be explained by the fact that oleic acid was the predominant FA in all diets (Ajuyah, et al., 1991) and all the analyzed tissues were from animals fed diets with soybean oil containing a high level of linoleic acid (Hrdinka et al., 1996). In bird liver, SFA are synthesized from dietary starch, then stearic acid is desaturated into oleic acid (Legrand et al., 1987). The linoleic acid content was higher in birds fed GO than in those fed a C diet, namely 18.2 vs. 14.5%, respectively, at day 56. This result appeared to be similar to that reported in ostrich meat (18-19%; Girolami et al., 2003) and higher than for broilers fed with fish products (14.2%, Ajuyah et al., 1991) but lower than those fed flaxseeds (Ratnayakeet et al., 1989). In fact, our findings could be explained by an increase in lipogenesis in birds fed on corn compared to acorn. Undoubtedly, broilers fed the GO diet received less digestible starch than those fed corn and therefore had a weak lipogenic activity of liver (Mourot and Hermier, 2001). Thus, dietary linoleic acid would be directly deposited in tissues. Linolenic acid was the only FA that decreased with age on both diets. The same result was obtained by Girolami et al. (2003) who reported that meat from older ostriches showed a decrease of C18:3ω3 α-linolenic acid. However, this FA appeared in low proportions in thigh tissue of chickens fed the experimental diet compared to those fed different levels of fish products at ratio of 1.63 to 2.98%, (Lòpez-Ferrer et al., 2001). However, our results showed that birds fed GO had enriched their muscle with a higher PUFA proportion compared to those fed the control diet (20.82 vs. 17.94%). Ayerza et al. (2002) reported that 18.8% of PUFA was deposited in muscular tissues in broilers fed on *chia* seed. This observation was consistent with an increase in the PUFA: SFA ratio on GO compared to the control diet (0.7 vs. 0.5%). Raes et al. (2004) suggested a PUFA:SFA ratio of 0.7 or higher; Wood and Enser (1997) recommended a ratio of 0.45 as the minimum. The PUFA levels and PUFA:SFA ratio of broilers fed GO were similar to the recommended ratio and therefore the meat should be considered acceptable nutritionally as a component of a healthy consumer diet. Feeding the GO diet to broilers increased the n-6:n-3 ratio of the meat produced which compared favorably with nutritional recommendations for reducing the risk of coronary heart disease (Food and Agriculture organization, 1994).

In conclusion, results from this experiment suggest that the starch and FA of the GO diet play an important role in lipid deposition. Lower AAT of birds fed GO suggests the fatty acids and starch quality of oak acorns could cause an inhibition of lipogenesis. Finally, the inclusion of this feed resource in the diet of growing chickens is possible.

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