

INFLUENCE OF ACORN INTAKE ON BLOOD LIPID PROFILE AND LONGISSIMUS MUSCLE CHARACTERISTICS OF BLACK SLAVONIAN PIG

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ABSTRACT

The beneficial effect of acorn on blood lipid level could be linked with presence of both high tannin content (70.10 g/kg DM) and high amounts of unsaturated fatty acids (more than 80%) such as linoleic (43.38%), oleic (30.52%) and alpha linolenic (4.58%) acid. In this study the influence of *ad libitum* acorn intake on blood lipid profile and longissimus muscle (MLD) composition of Black Slavonian pig were investigated and compared with the effects of commercial corn based diet. Acorn consumption in last three weeks of fattening period had a decreasing effect on triglyceride and total cholesterol concentration in blood without adverse effect on liver function. Compared with the intake of corn based diet, the intake of acorn resulted in 11% (0.22 mmol/l) lower total cholesterol ($P < 0.05$) concentration and 48% lower (0.20 mmol/l) triglycerides concentration ($P < 0.01$). In addition, there was no observed statistically significant differences ($P > 0.05$) among finishing diets for any histochemical characteristics, except for diameter of intermediate fast-twitch oxidative-glycolytic (FOG) fiber types (58.10 μm and 51.67 μm in acorn and corn based fed pigs, respectively; $P < 0.01$). The trend toward lower proportion (10.80 vs. 12.36%) and smaller diameter (59.36 vs. 64.57%) of red slow-twitch oxidative (SO) fiber types ($P < 0.08$) was observed in acorn than in corn-based fed pigs.

Key words: pigs / breeds / Black Slavonian pig / animal nutrition / acorn / *Quercus robur* / histochemical characteristics / blood lipids / *longissimus dorsi*

VPLIV ZAUŽIVANJA DOBA NA MAŠČOBE V KRVI IN ZNAČILNOSTI DOLGE HRBTNE MIŠICE PRI ČRNEM SLAVONSKEM PRAŠIČU

IZVLEČEK

Ugoden vpliv doba na nivo maščob v krvi je lahko povezan s prisotnostjo velike vsebnosti tanina (70,10 g/kg SS) in velike količine nenasičenih maščobnih kislin (več kot 80 %), kot so linolna (43,38 %), oleinska (30,52 %) in alfa linolenska (4,58 %). V raziskavi smo preučevali in primerjali vpliv *ad libitum* zauživanja doba na profil lipidov v krvi in na sestavo dolge hrbtna mišice (MLD) pri prašičih črne slavonske pasme z vplivom komercialne krmne mešanice. Z zauživanjem doba v zadnjih treh tednih pitanja se je zmanjšala vsebnost trigliceridov in skupnega holesterola v krvi brez škodljivega vpliva na delovanje jeter. V primerjavi z zauživanjem komercialne krmne mešanice je zauživanje doba povzročilo 11 % (0,22 mmol/l) manjšo koncentracijo skupnega holesterola ($P < 0,05$) in 48 % (0,20 mmol/l) manjšo koncentracijo trigliceridov ($P < 0,01$). Pri histokemičnih lastnostih med krmama ni bilo opaziti nobenih statistično značilnih razlik ($P > 0,05$), razen za lastnost premer vmesnih hitrih oksidativno glikolitičnih (HOG) mišičnih vlaken (58,10 μm pri prašičih, krmljenimi z dobom in 51,67 μm pri prašičih, krmljenimi s komercialno krmo; $P < 0,01$). Pri rdečih počasnih oksidativnih (PO) mišičnih vlaknih smo opazili trend ($P < 0,08$) k manjšemu deležu (10,80 proti

12,36 %) in manjšemu premeru (59,36 proti 64,57 %) pri prašičih, krmljenimi z dobom, v primerjavi s prašiči, krmljenimi s komercialno krmo.

Ključne besede: prašiči / pasme / črni slavonski prašič / prehrana živali / dob / *Quercus robur* / histokemične lastnosti / krvne maščobe / *longissimus dorsi*

INTRODUCTION

Recent study showed that the meat and meat products (dry-cured products) obtained from pigs reared traditionally in outdoor production system have a higher technological and nutritional value. Its has been characterized by high tocopherol content in tissues as a result of the intake of pasture (Cava *et al.*, 2000), high content of monounsaturated fatty acids (MUFA) from acorn (Cava *et al.*, 1997), high content of total iron and higher oxidative capacity of the muscles as a results of physical exercise performed by these animals in extensive system (Lawrie, 1998). In the past, acorn grazing was a cost-effective energy source in fattening of Black Slavonian pigs, and grazing in oak woodland was constituent part of traditional production system. In the recent years with popularization of free range and/or organic pig production, acorn grazing is again a common practice. Previous studies reported that in spite of their relatively high carbohydrates content, acorns contain low crude protein (CP ranged from 30 to 40 g/kg DM) and variable amounts of lipids and various sterols (EE ranged from 15 to 60 g/kg DM; Leon-Camacho *et al.*, 2004; Lopes and Bernardo-Gil, 2005; Margaletić, 2001). Acorn is a rich source of cholesterol-neutral oleic acid (Estevez *et al.*, 2004; Cava *et al.*, 1997) and contain a noticeable amount of alpha linolenic acid (Karolyi *et al.*, 2007; Petrović *et al.*, 2004), an n-3 polyunsaturated fatty acid (PUFA), which is important in eicosanoid synthesis and has beneficial effects on serum triglyceride and HDL-cholesterol level (Simopoulos, 1991). In addition acorns contain various biologically active phenolic compounds such as tannins, gallic and ellagic acid, and different galloyl and hexahydroxydiphenoyl derivatives (Cantos *et al.*, 2003; Rakić *et al.*, 2006) which are known for their role in regulation of blood lipid level. Specifically, they can reduce total plasma cholesterol level as well as LDL-cholesterol level decreasing the activity of 3-hydroxi-3-methylglutaryl coenzyme A (HMG-CoA) reductase and Acyl CoA-cholesterol-o-acyltransferase (ACAT), the key enzymes in cholesterol biosynthesis and their esterification in the blood. Furthermore, tannins can inhibit the accumulation of macrophage-lipid complex on the endothelium wall of artery and may prevent atherosclerosis (Chung *et al.*, 1998). Influence of finishing diet of acorn or corn based diet on fatty acid profile of longissimus muscle of Black Slavonian pigs was presented previously (Karolyi *et al.*, 2007). In this study, the influence of acorn intake on blood lipid profile and longissimus muscle (MLD) composition were investigated and compared with the effects of commercial corn based diet.

MATERIAL AND METHODS

Animals and diets

The study was performed on 10 pigs of the local Black Slavonian (BS) breed. All animals were reared in outdoor production system and maintained under same management and feeding programme. The examined pigs were reared on pasture utilizing nature resources with the addition about of 0.5 kg of concentrate daily per pig. In addition, last two mounts pigs were fattened in piggery with corn based mixture (Table 1.). Three weeks before slaughter pigs are randomly divided into two groups, the experimental (E) group were fed acorn from Slavonian oak (*Quercus robur* L.) *Ad libitum* and second one (Control group, C) were continued to be fed mixture diets, about 2 kg daily per pig. At the slaughter pigs were on average 154 ± 22 kg and 18

mounts of age. The chemical composition of finishing diets (acorn and concentrate) was determined with standard procedures according A.O.A.C. (1990) methods.

Table 1. Chemical composition and fatty acid profile of finishing diets

	Finishing diets	
	Acorn	Concentrate
% Dry matter ^a (DM)	61.5	88.2
% Crude protein ^a (CP)	3.4	16.2
% Ether extract ^a (EE)	1.8	3.2
% Crude fiber ^a (CF)	8.9	2.4
% Nitrogen free extract ^a (NFE)	84.7	73.1
% Ash ^a	1.2	5.1
% FAMES ^b		
C14	0.15	0.14
C16	18.09	17.54
C16:1	0.29	0.95
C17	0.12	1.05
C18	1.32	2.36
C18:1 (n-9)	30.52	24.85
C18:2 (n-6)	43.38	50.18
C18:3 (n-3)	4.58	1.92
C20:1 (n-9)	0.37	0.27
% Tannins ^a	7.01	-

^a Expressed as % of dry matter; ^b Expressed as % of fatty acid methyl esters

Collection and analysis of blood samples

Blood samples were collected from each of animal from experimental (E) group and control (C) group before exsanguinations. Samples were taken from the jugular vein using commercial vacutainer system and serum collection tube for blood lipid profile analyses. Serum triglyceride, total cholesterol, HDL-cholesterol and LDL-cholesterol concentration were measured using the automated biochemistry analyzer Olympus AU 400 (Olympus Diagnostica GmbH, Hamburg, Germany) and Olympus AU[®] Series Reagents.

Meat quality, histochemical characteristics and fatty acid analysis

On the *Longissimus dorsi* muscle the initial (pH_i) and the ultimate (pH_u) pH were taken at the last rib level using Testo 230 portable pH meter (TESTO, Germany) with penetration electrode (type 13) 45 min and 24 h post mortem. On the same place, after 24 h muscle colour (CIE L* a* b*) was measured on the fresh cut surface of cross section after approximately 5 minutes of blooming time using a Minolta Chromameter CR-410 (Minolta Camera Co. Ltd. Japan). In the addition, 30–45 min after slaughtering muscle sample for histochemical analysis was taken from middle portions of m. longissimus dorsi (MLD) at the last rib level of each animal. The histochemical profile was determined by staining 8 µm transverse serial sections for SDH activity and with myosin ATPase method at different pH. Diameter and proportions of muscle fibre types were analysed on two different pieces of each muscle samples and more than 200 muscle fibres per sample were analysed. The muscle fibre diameter was measured on cross-sections of individual muscle fibres using a Nikon microscope and a Leitz microscale with 10 µm divisions. Muscle sampling and fatty acid profile analysis of MLD was described previously by Karolyi *et al.* (2007).

Data analysis

The Student t-test for independent variables (PROC TTEST; SAS, 1999) was used to compare the blood lipid data and histo-chemical characteristics of MLD from two finishing diet groups.

RESULTS AND DISCUSSION

The influence of acorn (*Quercus robur* L.) consumption on blood lipid profile and some blood constituents are shown in Table 2.

Table 2. Influence of acorn (*Quercus robur* L.) intake on blood lipid level and some blood constituents

	Finishing diets		Significance
	Concentrate □ (SD)	Acorn □ (SD)	
Triglycerides (mmol/l)	0.42 (0.07)	0.22 (0.03)	0.001
Total cholesterol (mmol/l)	2.01 (0.13)	1.79 (0.08)	0.05
HDL-cholesterol (mmol/l)	0.83 (0.12)	0.77 (0.15)	NS
LDL-cholesterol (mmol/l)	1.20 (0.12)	1.14 (0.09)	NS
Glucose (mmol/l)	5.37(0.72)	5.06 (0.61)	NS
ALT (U/L)	59.60 (2.51)	43.25 (4.11)	0.01
AST (U/L)	46.20 (7.66)	50 (11.17)	NS
GGT (U/L)	21.20 (6.02)	25.00 (4.24)	NS

NS = Non Significant (P > 0.05)

As shown in Table 2. *Ad libitum* acorn consumption during the three weeks before slaughter resulted in 11% (0.22 mmol/l) lower total cholesterol (P < 0.05) concentration and 48% lower (0.20 mmol/l) triglycerides concentration (P < 0.01) compared with the intake of corn based diet. The blood lipids level, especially cholesterol and triglycerides, are the most related to types of lipids in feed and presence of biologically active compounds with strong influence on lipid metabolism such as tannins (Park *et al.*, 2002; Yugarani *et al.*, 1992). Tannins are phenolic compounds commonly found in fruits of many plant and non-toxic when are orally administrated to a mouse and rats at a dose of less than 5 000 mg/kg or in humans at dose of 500 mg/kg of body weight. The analyses of *Quercus robur* L. acorn from 43 locations in Croatia (Margaletić, 2001) suggest that acorn is tannin reach fruits with variation between 45 and 85 g/kg DM. Acorn used in our study contained a 70.10 g tannin /kg DM, and we presume that at *Ad libitum* consumption (> 2 kg/pigs/day) of acorn, the tannin daily intake was more than 500 mg/kg body weight. At this tannin intake we could not expected some adversely effect on liver function what is supported with results of serum enzymes activity of ALT, AST and GGT which was within physiological range for pigs (Kaneko, 1997). The previous study (Park *et al.*, 2002) reported that tannin supplementation decrease plasma total cholesterol and triglyceride level and increase HDL-cholesterol/total-cholesterol ratio. The same authors were found lower activity of 3-hydroxy3-methyl-glutaryl-coenzyme A (HMG-CoA) and acyl-coenzyme A cholesterol acyltransferase (ACAT), the key enzymes involved in cholesterol synthesis and their esterification in the blood (Bocan *et al.*, 1998). Yugarani *et al.* (1992) noted that tannin is able to reduce LDL-cholesterol and triglyceride without affecting the HDL-cholesterol level. In this study, acorn intake did not influence HDL-cholesterol or LDL-cholesterol level. In addition, the beneficial effect of acorn on blood lipid profile could be linked with fatty acid composition of acorn. General is accepted that unsaturated fatty acids have beneficial effect on blood lipid level, especially n-3 fatty acids which have been linked to a wide-range of health benefits, including reduced risk of elevated

blood lipid level-related disease such as hyperlipidemia, atherosclerosis, angina pectoris, stroke and hepatic disease (Simopoulos, 1991). Previous has been reported that diet rich in oleic acid (Rebollo *et al.*, 1998) and n-3 fatty acids (alpha linolenic, DHA, EPA; Kelley *et al.*, 2007; Ni Eidhin *et al.*, 2003; Tholstrup *et al.*, 2004;) reduces plasma triglyceride, total cholesterol, and LDL-cholesterol levels and improve the HDL-cholesterol/total cholesterol ratio. In comparison to corn based finishing diet (Table 1), acorn had higher proportion of oleic (30.52 vs. 24.85%) and alpha linolenic (4.58 vs. 1.92%) acid and lower proportion of linoleic acid (43.38 vs. 50.18%). These findings could explain lower triglycerides and total cholesterol level in pigs fed acorn than in pigs fed corn based diet. In our study differences in HDL- and LDL-cholesterol concentration was not observed possible due to short fattening period and small number of animals in trial. In addition to influence on blood lipid profile, dietary fatty acid composition has a strong influence on fatty acid profile of tissues (Moloney, 2002). It has been reported that high oleic acid content in tissues from Iberian pigs raised on woodland pasture is a result of the large amounts of oleic acid in the acorn (more than 60%; Cava *et al.*, 1997; Estevez *et al.*, 2004). Furthermore, Rebollo *et al.* (1998) found reduction in the plasma levels of cholesterol, triglycerides and LDL-cholesterol in humans after consumption the fat from acorn-fed Iberian ham which have high content of oleic acid (60% of total fat). The results of the influence of acorn of Slavonian oak (*Quercus robur* L.) intake on fatty acid profile and meat quality traits of longissimus muscle has been presented previously in works by Karolyi *et al.* (2007). Feeding acorn *Ad libitum* during the last three weeks of fattening period increased the content of alpha linolenic acid (C18:3 n-3) in the *longissimus* muscle (0.37 vs. 0.12%, $P < 0.01$) and consequently decreased the n-6/n-3 ratio (24.1 vs. 69.3, $P < 0.01$) in comparison to concentrate fed pigs. Muscle pH as well lightness (L^*) and redness (a^*) were not affected by acorn consumption while the yellowness (b^*) was a higher ($P < 0.05$) in pigs fed corn-based diets than in pigs fed acorn (6.18 vs. 3.15). Generally high intramuscular fat content (Table 3.) without statistically significant differences between groups was observed in both acorn and corn based fed pigs (6.6 and 7.2%, respectively).

Table 3. Histochemical characteristics and meat quality traits of *m. longissimus dorsi* (MLD) from pigs fed acorn and mixture finishing diets

Fibre type	Finishing diets		Significance
	Concentrates □(SD)	Acorn □(SD)	
Percentage			
SO	12.36 (0.54)	10.80 (0.54)	0.08
FOG	19.00 (1.01)	19.10 (1.01)	NS
FG	68.64 (1.04)	70.10 (1.04)	NS
Diameter (µm)			
SO	64.57 (1.81)	59.36 (1.81)	0.08
FOG	58.10 (1.23)	51.67 (1.23)	0.01
FG	67.93 (1.42)	68.49 (1.42)	NS
Meat quality traits			
pH _i	6.11 (0.19)	6.25 (0.38)	NS
pH _u	5.88 (0.12)	5.86 (0.07)	NS
Lightness (L^*)	50.51 (3.93)	49.35 (6.61)	NS
Redness (a^*)	20.30 (0.70)	19.74 (1.95)	NS
Yellowness (b^*)	6.18 (2.08)	3.15 (1.07)	0.01
Dry matter, %	30.08 (2.16)	29.74 (1.42)	NS
Intramuscular fat ^a	7.19 (2.81)	6.55 (1.56)	NS

^a Expressed as percent of dry matter; Lipids profile of IMF was presented in Karolyi *et al.* (2007); NS = Non Significant ($P > 0.10$)

High potential for intramuscular fat deposition is characteristics of the many native pig breeds such as Black Slavonian (Senčić *et al.*, 2005), Mangalitza (Szente *et al.*, 2004) or Iberian pig (Lopez-Bote, 1998). Lipid composition is one of the main characteristics related to meat quality affected by muscle fiber type (Andres *et al.*, 2001). It is usually accepted that muscles showing an oxidative metabolism should have higher lipid content, due to their ability to use fatty acids as an energy source. Therefore, a higher amount of intramuscular fat content implies a higher proportion of oxidative fiber. In spite of this, the absence of differences in IMF content was reflecting on fibres type composition of MLD. As shown in Table 3 there were no significant differences ($P < 0.05$) between pigs of two finishing diets for any histochemical characteristics, except for diameter of FOG fiber type. However, the tendency to lower proportion (10.80 vs. 12.36%) and smaller diameter (59.36 vs. 64.57%) of SO fibre type ($P < 0.08$) was observed in acorn fed than in corn-based fed pigs.

CONCLUSIONS

Present study shown that *Ad libitum* consumption of acorn of Slavonian oak (*Quercus robur* L.) in the short pre-slaughter fattening period can cause positive changes in blood lipid profile such as decreasing of triglycerides and total cholesterol concentrations without adversely effect on liver function in Slavonian pigs. In addition, the considerable amounts of alpha linolenic acid in acorn could have beneficial effect on the n-6/n-3 ratio in the meat. On the other hand, three weeks of fattening period with acorn did not influence on differences in IMF or in diameter and proportion of each muscle fiber types.

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