South African Journal of Animal Science 2012, 42 (No. 2)

Fatty acid profile and cholesterol content of *m. longissimus* of free-range and conventionally reared Mangalitsa pigs

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Abstract

This study investigated the effects of different feeding systems (free-range versus conventional rearing) on carcass characteristics, chemical composition, fatty acid profile and cholesterol content of the *musculus longissimus lumborum et thoracis (MLLT)* of Mangalitsa pigs. Depending on the rearing system employed and live weight observed, we found statistically significant differences in the weight of the warm and cold Mangalitsa carcasses. Furthermore, we observed that conventionally reared Mangalitsa pigs weighed more. Measurements showed that the free-range-fed pigs had a lower total backfat thickness in comparison with the group reared in the conventional system, but that these differences were not significant. Outdoor rearing of the pigs led to higher protein, ash and water contents, and to a decrease in total fat content and pH values of the *MLLT*. The choice of rearing system did not significantly affect the cholesterol content. The fat of the free-range pigs had a higher concentration of *n*-3 and *n*-6 polyunsaturated fatty acids (PUFA), while the *n*-6/*n*-3 PUFA ratio was significantly lower than in conventionally reared pigs. The proportion of PUFA/SFA (saturated fatty acids) was not significantly different, whereas the proportion of monounsaturated fatty acids (MUFA/SFA) was significantly lower in the free-range group. It is concluded that the rearing system affects the carcass properties and chemical characteristics of Mangalitsa meat; it does so in particular by improving the fatty acid composition in free-range pigs.

Keywords: Rearing system, pork quality, indigenous breed, health food
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Introduction

A new generation of consumers not only choose meat products according to perceived eating quality and affordable pricing, but also consider the nutritional value and the ethical quality of the meat, animal welfare issues, and the degree of impact on the environment caused by the production system. Another reason for choosing ecologically or non-intensively produced meat is the belief that the taste and nutritional value of this type of meat are superior to those of conventionally produced meat (Nilzén *et al.*, 2001; Edwards, 2005; Alfaia *et al.*, 2007; 2009; Muchenje *et al.*, 2009a; Mapiye *et al.*, 2011). These are the reasons that in the last few years there has been an increase in demand for foodstuffs obtained from so-called organic, natural or biological livestock production systems. Meat from these biological production systems is thought to be more nutritious and more appealing to the senses (Nilzén *et al.*, 2001; Sundrum, 2001; Edwards, 2005).

There is not much information on the effects of grass intake on pork meat characteristics. Some studies of improved genotypes fed on grass showed no effects on meat quality (Danielsen *et al.*, 2000). On the other hand, the effect of outdoor rearing has been studied in light pig breeds, but in most cases resulted in lack of improvement in pig performance, pork loin or muscle characteristics (Gentry *et al.*, 2002; Bee Guex & Herzog, 2004).

It is important to investigate the free-range rearing system and its implications on access to pastures and pork quality. Previous trials indicated that compared with more conventional regimens, feeding on fresh

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 222-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v42i2.2

grass and herbs would alter the intramuscular fatty acid profiles in pigs, resulting in a polyunsaturated fatty acid/saturated fatty acid (PUFA/ SFA) ratio that is more beneficial to human health (Jakobsen, 1995; Ahn *et al.*, 1996; Mapiye *et al.*, 2011).

The fatty acid composition of foodstuffs is of great importance for healthy human nutrition. Nutritionists recommend a reduction in total fat intake, particularly of SFA and trans fatty acids, which are associated with an increased risk of cardiovascular disease and some cancers (Burlingame *et al.*, 2009; Brouwer *et al.*, 2010; USDA and HHS, 2010; Mapiye *et al.*, 2011). Besides advocating that they reduce their fat intake, nutritionists urge consumers to increase their intake of PUFA, particularly *n*-3 PUFA, at the expense of *n*-6 PUFA (Simopoulos, 2004; Griffin, 2008; Harris *et al.*, 2009; Mapiye *et al.*, 2011). The PUFA/SFA and *n*-6/*n*-3 PUFA ratios have therefore become some of the most important parameters in evaluating the nutritional value and healthiness of foods (Aldai *et al.*, 2005; Alfaia *et al.*, 2007; Riediger *et al.*, 2009; Mapiye *et al*., 2011). Nevertheless, in recent years, red meat consumption has been discredited as a result of its causal relationships with coronary heart disease (CHD) and cancer (Forman, 1999).

In monogastrics such as pigs, there is a reasonable possibility that by varying the structure of the diet, the producer may influence the body composition of the animals and the composition of food products obtained from them (Bee & Wenk, 1994; Klingenberg *et al.*, 1995; Overland *et al.*, 1996).

In recent years, livestock breeders worldwide have joined forces to save indigenous and traditional domestic livestock breeds from extermination. The best strategy for preventing the disappearance of such breeds is to strive to maintain their genetic diversity (Csapó *et al.*, 2002). The future of the Mangalitsa, one of the European indigenous breeds, is dependent largely on whether products derived from them can be utilized effectively and whether long-term market opportunities can be secured. The Mangalitsa pig is currently enjoying a renaissance, owing to attempts to preserve traditional breeds. Its meat is of outstanding quality; it has high dry-matter content, and its red colour corresponds with current requirements. The distinctive palatable flavour is derived from the fat surrounding the muscle tissue (Csapó *et al.*, 2002).

Certain findings have been published recently in connection with the fatty acid composition and cholesterol content of the meat and backfat of the Mangalitsa pig. It has been claimed that its fat is softer and easier to digest than that of modern pigs. Its softer, granular consistency is attributable to its different and healthier fatty acid composition (Csapó *et al.*, 2002).

The aim of this study was to investigate differences between carcass properties, chemical and fatty acid composition and the cholesterol content of *m. longissimus lumborum et thoracis (MLLT)* in free-range and conventionally reared Mangalitsa pigs.

Material and Methods

Twenty-two castrated male Mangalitsa pigs were selected from a herd in a breeding programme. These pigs had been reared at a research station on a small farm near the town of Bela Crkva, Serbia, that was equipped appropriately to meet the requirements of the experiments. The experimental pigs were reared in late spring and early summer. The trial started when the animals reached the 70-day age threshold. Twelve Mangalitsas were raised conventionally – six pigs per cage, allowing 4 m^2 living space for each animal. This pen formed part of a group of pens located inside a pig farmer's shed, which was enclosed by walls and covered with a roof. The airflow was controlled manually by opening or closing the windows. The floor of the pen was concrete, and one third had concrete slats above a faeces and urine drainage channel. The other 10 Mangalitsa pigs were allowed to range freely over an area of 10 000 m^2. In other words, the free-range group were given regular access to fresh pasture, acorns and grass.

After reaching a live weight of 60 kg, both groups of pigs were fed a conventional slaughter-pig feeding mixture that was distributed *ad libitum*. The animals were fed a compound feed according to the recipe: 70% maize, 14% meal (wheat feed flour, barley, wheat, oats, dehydrated lucerne flour), 9% soybean meal (soya press cake, soya protein concentrate with fish oil), 4% sunflower meal, 1% chalk, 1% DCP (dicalcium phosphate) and a 0.5% sodium chloride, lysine, methionine and threonine supplement.

By the end of the trial, at live weights of between 75 kg and 120 kg, the Mangalitsa pigs were transported to an abattoir (8 km away) and left in crates for approximately four hours. The animals were slaughtered conventionally. Standard commercial procedures were followed, consisting of electrical stunning (250 V AC, ear to ear for 3 - 5 s) and sticking within 30 seconds. The pigs were subsequently eviscerated and inspected by the appropriate government health official. Each carcass was weighed warm and after cooling (4 °C for 24 h). Forty-five minutes *post mortem*, the initial pH was taken (pH_{45}) and then, after a 24 h cooling period, the final pH (pH_{24}). The pH measurements were taken on the *MLLT* with a penetrating glass electrode on a hand-held Testo 205 pH meter (± 0.02 pH; ± 0.4 °C; Germany, 2007). The pH meter was rinsed with distilled water after each reading and re-calibrated after every fourth reading.

After 24 hours of cooling at 4 °C, backfat measurements were taken with a ruler above the *m. gluteus medius* at the carcass split-line, at the following positions: at the beginning (P1); at the highest spot of the *m. gluteus medius* (P2); and at the end of the muscle (P3). Carcass length was measured from the cranial edge of the *symphisis pubis* to the anterior edge of the atlas vertebrae. During the routine splitting and cutting, samples of the *MLLT* were taken between the 13th and 14th thoracic vertebrae and stored in a freezer for further analyses. Prior to laboratory analysis, all samples were vacuum-packaged and kept frozen at approximately –20 °C.

The following measurements were taken of the chemical composition of the *MLLT* of the trial pigs: protein, water, total fat, ash, total fatty acid and cholesterol concentrations. Chemical composition was determined by the methods defined by the Association of Official Analytical Chemists (AOAC, 1990). Cholesterol content was measured with a HPLC/PDA on the HPLC Waters 2695 Separations Module, with a Waters 2996 Photo Diode Array Detector, as defined by Maraschiello *et al*. (1996). Chromatographic separation was achieved with a Phenomenex Luna $C_{18(2)}$ column (150 mm x 3.0 mm, 5 µm) with adequate pre-column, isocratically, with a mobile phase of isopropanol-acetonitrile 20% : 80% v/v. Injection volume was 10 µL. Cholesterol was determined by absorption at a wavelength of 210 nm. Analytical yield (recovery) for given quantities was between 66.3% and 74.8%. External calibration was used for calculating the cholesterol content. Empower Pro software was employed for system control and data gathering and processing.

To determine the concentration of fatty acids, total lipids were extracted by the rapid extraction method, using solvents on the Dionex ASE 200. A homogenized sample, mixed with diatomaceous earth, was extracted with a mixture of hexane and isopropanol (60 : 40 v/v) in a 33 mL extraction cell at a temperature of 100 °C and under nitrogen pressure of 10.3 MPa. The extract thus obtained was steamed in a nitrogen flow at a temperature of 50 °C until dry fat remains were obtained (Spirić *et al*., 2010).

Fatty acids as methyl esters were detected by capillary gas chromatography with a flame ionization detector. A predetermined quantity of lipid extracts, obtained by the rapid extraction method, was dissolved in tert-butyl methyl ether. Fatty acids were converted to fatty acids methyl esters (FAME) with trimethylsulphonium hydroxide, according to the SRPS EN ISO 5509:2007 method. FAMEs were analysed with the GC-FID Shimadzu 2010 device (Kyoto, Japan) on a cyanopropyl-aryl column HP-88 (column length 100, internal diameter 0.25 mm, film thickness 0.20 µm). The injected volume was 1 µL. Temperatures of the injector and detector were 250 °C and 280 °C, respectively. Nitrogen was used as a carrier gas, 1.33 mL/min, with a split ratio of 1 : 50, while hydrogen and air were used as detector gases. The temperature of the column furnace was programmed to range between 120 °C and 230 °C. The total duration of analysis was 50.5 min. Methyl esters of acids were identified according to their retention times, which were compared with those of the mixture of methyl esters of fatty acids in the standard Supelco 37 Component FAME Mix (Spirić *et al*., 2010).

The effect of the rearing system was tested by ANOVA. Experimental data were statistically processed and analysed with the least-squares method by applying the GLM procedure implemented in SAS 9.1.3 software package (SAS, 2002–2003). The model used for the analyses of slaughter properties included rearing system as a fixed effect and live weight as a co-variable. Live weight did not significantly affect other investigated factors and was therefore removed from the final model.

Data shown in tables represents the least squares means (LSM) with their respective standard errors of the mean (SEM) and significance levels.

Results and Discussion

The live weight and carcass characteristics of the Mangalitsa pigs on the free-range and conventionally reared systems are shown in Table 1. No significant differences were observed between the final live weights of the two experimental groups. The rearing system and live weight of pigs had a significant effect (P <0.001) on the weights of the warm and cold carcasses. At the same time, conventionally reared pigs demonstrated a tendency to be heavier. The rearing system had no effect on the carcass length of the pigs, but live weight did have a significant influence (P <0.001). Moreover, statistical differences were observed between the two systems of rearing in cold carcass yield of (P <0.001) and live weight of the pigs (P <0.01). The cold carcass yield of pigs kept in the conventional rearing system was higher, by 3.49%.

Table 1 Comparison of the least squares means ± (SEM) for the slaughter traits of free-range and conventional reared Mangalitsa pigs

Item	Rearing system		Significance level[1]	
	Conv (n = 12)	FR (n = 10)	RS	LW
Live weight (kg)	102.6 ± 3.70	98.6 ± 4.06	NS	/
Warm carcass weight (kg)	80.0 ± 0.43	76.8 ± 0.47	***	***
Cold carcass weight (kg)	78.1 ± 0.46	74.7 ± 0.49	***	***
Cold carcass yield (%)	77.4 ± 0.46	73.9 ± 0.51	***	**
Carcass length (cm)	89.3 ± 0.63	89.2 ± 0.69	NS	***
Thickness of backfat P_1 (mm)	61.9 ± 1.54	58.4 ± 1.69	NS	***
Thickness of backfat P_2 (mm)	54.6 ± 1.99	51.8 ± 2.18	NS	***
Thickness of backfat P_3 (mm)	60.1 ± 1.90	56.5 ± 2.08	NS	***

n – number of samples.
[1] Significance level for rearing system (RS) and live weight (LW).
P_1 – sacral point 1; P_2 – sacral point 2; P_3 – sacral point 3.
Conv – conventionally reared pigs; FR – free-range reared pigs.
NS – not significant; * P <0.05; ** P <0.01; *** P <0.001.

The carcass weight and cold carcass yield percentage were lower in the free-range reared pigs than in those fed conventionally. This could possibly be because the intake of grass fibres led to a better developed digestive system (mainly the large intestine). In fact, Roskosz *et al.* (1990) reported that wild pigs, fed a high-cellulose diet, developed a longer large intestine than pigs kept on an animal protein-based diet, which developed a longer small intestine. Pigs in confinement, fed either acorns or acorns and grass, did not show any variation in carcass weight compared with other groups. However, the carcass yield was higher in the group fed acorns and grass while in confinement than in those raised extensively, which is compatible with a higher development of the muscular system. Moreover, pigs raised free-range had the lightest lean meat cuts (*longissimus dorsi* and *psoas major* muscles, hams and shoulders), which could be explained by the higher content of fat and a lower development of muscle in the pigs raised free-range (Rey *et al.*, 2006).

Backfat thickness measurements at three control points showed differences (P1 = 3.49 mm, P2 = 2.76 mm, P3 = 3.60 mm). There was a difference in backfat thickness (P <0.001), depending on the live weight of pigs, but no difference with regard to the rearing system (P >0.05).

Free-range-reared Mangalitsa pigs measured a lower total backfat thickness in comparison with the group reared in the conventional system with a formulated diet. Owing to the high fat content of acorns and grass (Rey *et al.*, 1997), one would expect to find a higher backfat thickness in pigs fed on them. However, total backfat measurements of pigs fed either acorns or acorns and grass while in confinement had not been different from the other groups (P >0.05). Our experiment demonstrates that free-range feeding had no significant effect on accumulation of subcutaneous fat. Lewis *et al.* (1989) and Andersson *et al.* (1990) determined that outdoor exercise reduced backfat thickness and intramuscular fat. Rey *et al.* (2006) found that outdoor feeding and grass intake led to a slight increase in the fat thickness of the inner layer measured at the medial edge of the muscle *longissimus dorsi*, from the superior edge of the *longissimus dorsi* to the inferior edge of the outer layer. The differences were not proved to be significant in any of the cases. Hoffman *et al.* (2003) reported that carcasses of conventionally housed pigs had higher (P = 0.051) P_2 fat values and subsequently lower (P ≤0.05) calculated lean meat percentages (69%) than free-range pigs (70%). Warriss *et al.* (1983), Enfält *et al.* (1997) and Sather *et al.* (1997) noted that free-range pigs had lower percentages of backfat thickness (lower P_2 fat values) in comparison with indoor housed pigs.

The effects of outdoor/free-range rearing on pork quality have been reviewed by Edwards (2005) and Lebret (2008). Depending on the climate, increased and decreased carcass fatness and intramuscular fat contents have been reported. Increased exercise results in a higher glycogen store, lower ultimate pH and decreased technological yield in ham, whereas the loin is usually unaffected (Gandemer *et al.*, 1990; Bee *et al.*, 2004).

Comparisons of the means for the proximate chemical composition of the *MLLT* derived from the free-range and conventionally reared Mangalitsa pigs are presented in Table 2. No significant differences ($P > 0.05$) were observed in the *MLLT* water content depending on rearing system. Rahelić (1984) found 71.9% of water in *MLLT* of the same breed of pigs. Smaller differences were found while investigating published results and the work of Holló *et al.* (2003), who established that the water content in Mangalitsa pigs of different body masses varied between 68.8% and 69.0%. Hoffman *et al.* (2003) compared the average water content in the *MLLT* of commercial pigs reared in a free-range (74.8%) and a conventional system (74.5%) and did not find any significant differences ($P > 0.05$).

Table 2 Comparison of the least square means ± (SEM) for the chemical composition and pH of *m. longissimus thoracis et lumborum* of free-range and conventionally reared Mangalitsa pigs

	Rearing system		Significance level
	Conv (n = 12)	FR (n = 10)	
Water content (%)	61.7 ± 1.36	65.2 ± 1.49	NS
Protein content (%)	19.0 ± 0.59	21.7 ± 0.65	**
Total fat content (%)	18.2 ± 1.91	12.1 ± 2.10	*
Ash content (%)	0.86 ± 0.03	0.98 ± 0.03	**
pH_{45} value	6.12 ± 0.05	5.89 ± 0.06	**
pH_{24} value	5.80 ± 0.06	5.41 ± 0.06	***

n – number of samples.
(pH_{45} – pH value 45 min after slaughter; pH_{24} – pH value 24 hours after slaughter.
Conv – conventionally reared pigs; FR – free-range reared pigs.
NS – not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The differences in main protein values between the groups were significant ($P < 0.01$). In our research, indoor-bred Mangalitsa pigs had lower protein content in the *MLLT* compared with the results obtained by Holló *et al.* (2003) and Petrović *et al.* (2009). Rearing pigs of the same genotype (Italian local breed) in an open system, instead of a closed one, while providing them with a diet based on commercial mixtures, outdoor pigs showed higher percentages of intramuscular fat (4.04% vs. 3.29%) and crude protein (23.5% vs. 22.8%, respectively) (Pugliese *et al.*, 2005). We established that the *MLLT* originating from Mangalitsa pigs reared in an open system contained a 2.74% higher protein content than Mangalitsa pigs kept in a closed system. The protein content in *MLLT* of both groups, as calculated in our research, was lower than those established by Pugliese *et al.* (2005) and Rey *et al.* (2004).

Total fat content was 6.19% higher in conventionally reared Mangalitsa pigs than in the free-range group. Consequently, obtained differences in average values have been found to be significant ($P < 0.05$). Rahelić (1984), Holló *et al.* (2003) and Petrović *et al.* (2009) established that *MLLT* from Mangalitsa pigs contained between 4.91% and 9.04% pure fat. Our research determined these percentages to be significantly higher.

The ash content in the *MLLT* of the free-range Mangalitsa pigs was higher than in the conventionally reared group. The statistical difference in ash content between these groups was found to be significant ($P < 0.01$). Rahelić (1984) spotted a slight difference in ash content between the Mangalitsa and Swedish Landrace (1.21% and 1.18%, respectively). Our research found these differences to be greater. Holló *et al.*

(2003) established no significant difference in average ash content in the *MLLT* in three trial groups of Mangalitsa pigs.

The obtained average pH_{45} and pH_{24} values in the *MLLT* of these groups have been statistically different, depending on the choice of rearing system. Initial muscle pH in the free-range Mangalitsa pigs had lower values than that of conventionally reared ones ($P < 0.01$). The final pH measurements showed that the free-range pigs had lower pH values than pigs reared indoors ($P < 0.001$). Hoffman *et al.* (2003) concluded that muscle pH values (pH_{45} and pH_{24}) were not influenced by the two housing systems. Sather *et al.* (1997) found that initial muscle pH of free-range housed pigs tended to be lower than that of conventionally housed pigs. The results of Barton Gade & Blaabjerg (1989) and Enfält *et al.* (1997) showed that free-range pigs had lower final pH measurements than the indoor housed pigs. These researchers reasoned that free-range pigs had higher levels of muscle glycogen than their pen-housed counterparts, which resulted in lower pH readings.

The fatty acid composition of the *MLLT* in the Mangalitsa pigs reared free-range and conventionally, are shown in Table 3. In both rearing systems palmitic acid (C16:0) was the most abundant SFA, oleic acid (C18:1 *n*-9) the most abundant MUFA and linoleic acid (C18:2 *n*-6) the most abundant PUFA in the *MLLT* of the pigs. The free-range pigs showed a higher PUFA content in the *MLLT* than pigs reared indoors and fed conventionally. These differences were produced mainly by an almost four times higher total *n*-3 PUFA content in the *MLLT* of the free-reared pigs ($P < 0.001$), and also by slightly higher levels of total *n*-6 PUFA ($P > 0.05$). These led to significantly lower *n*-6/*n*-3 ratios in the *MLLT* of the pigs reared outdoors and fed on acorns and free pasture ($P < 0.001$). Therefore, although the *n*-6/*n*-3 ratio was higher than dietary recommendations in all cases (British Nutrition Foundation, 1994), free-rearing appears to be an interesting way to reduce this ratio in porcine animals. In their research, Mapiye *et al.* (2011) concluded that the PUFA and total *n*-3 fatty acid proportions were significantly higher in meat from steers on an *Acacia karroo* leaf diet and control diets with no supplement than those receiving the sunflower cake diet. Meat from steers given the sunflower cake diet had lower PUFA/MUFA and PUFA/SFA ratios than those on the *Acacia karroo* diet. The lowest *n*-6/*n*-3 ratio was recorded in meat from steers that received the *Acacia karroo* diet ($P < 0.05$).

Total *n*-3 PUFA content was significantly affected by the rearing system ($P < 0.001$). Animals reared outdoors showed a higher content in the *MLLT* than those reared indoors on concentrates. The same effect has been even more pronounced in muscles from outdoor-bred animals, having approximately a 75% higher *n*-3 PUFA concentration than the indoor-bred ones. These differences are probably caused by a higher C18:3 *n*-3 content in pasture (around 50% of total fatty acids). Forage-fed beef can exhibit an improved *n*-6 to *n*-3 fatty acid ratio, which has a positive cardiovascular impact (Baublits *et al*., 2006; Razminowicz *et al*., 2006; Muchenje *et al*., 2007). Realini *et al.* 2004 and Muchenje *et al.* (2009a) pointed out that pasture-fed animals have higher concentrations of PUFA, stearic (18:0), linoleic (LA), linolenic (LNA), arachidonic (20:4 *n*-6, AA), eicosapentaenoic (20:5 *n*-3, EPA), and docosapentaenoic (22:5 *n*-3, DPA) acids in their fat than animals fed on protein concentrates.

In our study, C18:3 *n*-3 concentration was significantly higher in the pigs reared outdoors and fed on acorns and pasture than in the animals fed indoors the conventional way ($P < 0.001$). Other researchers have also found a higher total *n*-3 PUFA concentration in the muscle phospholipids of animals fed a diet high in C18:3 *n*-3 (Ahn *et al*., 1996; Specht-Overholt *et al*., 1997), and increasing levels of C18:3 *n*-3 that are mainly responsible for a higher total *n*-3 PUFA. A higher C18:3 *n*-3 diet content led to increased amounts of certain fatty acids of the *n*-3 pathway, especially EPA (C20:5 *n*-3) and C22:5 *n*-3, though not DHA (C22:6 *n*-3). In Muriel *et al.*'s (2002) study, all individual *n*-3 PUFAs, including EPA, DHA and C22:5 *n*-3, were significantly higher in animals reared outdoors and fed on acorns and pasture than in indoor animals fed concentrates.

The role of EPA and DHA in easing the symptoms of a number of diseases, including coronary heart disease, has been well recognized (British Nutrition Foundation, 1994). An increasing EPA and DHA content and a decreasing *n*-6/*n*-3 ratio, together with high MUFA levels, indicate a potentially beneficial effect of feeding animals on pasture and support a "healthy" image of "organic" pork. In fact, nutritional studies have already related the inclusion in the diet of meat products from Iberian pigs reared outdoors to the improvement of plasmatic indicators of coronary and vascular diseases (García *et al*., 1998). A positive feature of grass feeding is that levels of the nutritionally important long chain *n*-3 PUFA are increased EPA (20:5 *n*-3) and DHA (22:6 *n*-3) concentrations. Future research should focus on increasing *n*-3 PUFA proportions in lean carcasses and on the use of biodiverse pastures and conservation processes that retain the benefits of fresh leafy grass. The varying fatty acid compositions of adipose tissue and muscle have profound effects on meat quality (Wood *et al.*, 2008).

Table 3 Comparison of the least squares mean ± (SEM) for the fatty acids composition (%) and cholesterol content (mg/100 g) of *m. longissimus thoracis et lumborum* from a free-range and conventional reared Mangalitsa pigs

Fatty acids	Rearing system		Significance level
	Conv (n = 12)	FR (n = 10)	
C14:0	1.12 ± 0.017	1.24 ± 0.019	***
C16:0	23.2 ± 0.196	24.6 ± 0.215	***
C17:0	0.195 ± 0.013	0.235 ± 0.014	*
C18:0	9.27 ± 0.226	9.24 ± 0.248	NS
C20:0	0.123 ± 0.005	0.136 ± 0.006	NS
C16:1	3.95 ± 0.166	4.86 ± 0.182	**
C17:1	0.213 ± 0.011	0.245 ± 0.012	NS
C18:1 *cis*-9	47.0 ± 0.430	44.3 ± 0.472	***
C18:1 *trans*-9	0.549 ± 0.031	0.543 ± 0.034	NS
C18:*1 cis*-11	4.77 ± 0.162	4.84 ± 0.178	NS
C18:2 cis n-6	4.73 ± 0.399	4.90 ± 0.437	NS
C18:3 *n*-3	0.152 ± 0.036	0.536 ± 0.039	***
C20:1 *n*-9	0.732 ± 0.021	0.733 ± 0.023	NS
C20:2 *n*-6	0.323 ± 0.024	0.295 ± 0.026	NS
C20:3 *n*-6	0.503 ± 0.038	0.415 ± 0.041	NS
C22:1+C 20:4	0.225 ± 0.013	0.191 ± 0.014	NS
C22:5 *n*-3	0.00 ± 0.006	0.105 ± 0.006	***
SFA	33.9 ± 0.342	35.5 ± 0.375	**
MUFA	57.2 ± 0.622	55.5 ± 0.681	NS
PUFA	5.93 ± 0.458	6.45 ± 0.502	NS
USFA	63.1 ± 0.334	62.0 ± 0.366	*
Total *n*-3 PUFA	0.152 ± 0.040	0.641 ± 0.043	***
Total *n*-6 PUFA	5.55 ± 0.429	5.61 ± 0.470	NS
MUFA/PUFA	10.38 ± 0.708	8.96 ± 0.774	NS
MUFA/SFA	1.69 ± 0.032	1.57 ± 0.035	*
PUFA/SFA	0.175 ± 0.013	0.183 ± 0.015	NS
n-6/*n*-3 PUFA	37.3 ± 1.31	9.2 ± 1.44	***
Cholesterol	63.1 ± 1.93	61.7 ± 2.11	NS

n – number of samples.
SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids
USFA – monounsaturated fatty acids + polyunsaturated fatty acids.
Content of SFA, MUFA, PUFA – calculated from all recorded acids.
Conv – conventionally housed pigs; FR – free-range housed pigs.
NS – not significant; * *P* <0.05; ** *P* <0.01; *** *P* <0.001.

The *n*-6/*n*-3 ratio plays an important role in reducing the risk of coronary heart disease (American Heart Association, 2008). However, the optimal balance between these two classes of fatty acids is still a matter of debate (Simopoulos, 2002). The *n*-6 PUFAs are involved in the synthesis of eicosanoids, biologically active in very small quantities and with properties much more inflammatory than eicosanoids from the *n*-3 PUFAs (Simopoulos, 2002). Therefore, nutritional guidelines recommend reducing fat intake, especially SFA, and minimizing the intake of *n*-6 fatty acids relative to *n*-3 fatty acids (Department of Health, 1994).

The conventionally reared Mangalitsa pigs showed slightly higher levels of MUFA (P >0.05) in the *MLLT* than in pigs reared outdoors and fed on pasture and acorns. This seems strange since in other studies free-reared animals fed on pasture and acorns showed higher levels than those fed concentrates (Ruiz *et al.*, 1998; Andrés *et al.*, 2001). Nevertheless, oleic acid (C18:1 *cis*-9) levels in the *MLLT* of Mangalitsa pigs reared indoors were considerably higher than concentrations of this fatty acid in the meat of Mangalitsa pigs reared under extensive conditions. These results are consistent with research by Hansen *et al.* (2006), who demonstrated that organic pig carcasses had a higher content of PUFA and a lower content of MUFA. Similarly, there were higher concentrations of C18:2 *n*-6 and PUFA *n*-6 in pigs fed organically than in pigs fed conventionally (Högberg *et al.*, 2003). In our research, the higher C18:2 *n*-6 concentration in free-range Mangalitsa pigs contributed to their higher total PUFA concentration (6.44 ± 1.38) compared with that of the conventionally reared pigs (5.93 ± 1.74). This finding is consistent with the results of Nilzén *et al.* (2001). The higher level of this fatty acid in the free-range Mangalitsa pigs resulted in these animals having a higher calculated sum of *n*-6 fatty acids compared with the conventionally reared pigs. Table 3 also shows that the total MUFA to SFA ratios of the *MLLT* differed significantly, with a higher (P <0.05) MUFA/SFA ratio for the conventionally reared Mangalitsa pigs (1.69 ± 0.13) compared with the free-range pigs (1.57 ± 0.08). On the other hand, total PUFA to SFA ratios of the *MLLT* did not differ significantly. The difference in fatty acid composition between conventional and free-range reared pigs is probably a consequence of the different feeds. The fatty acids composition of the intramuscular fat is influenced by several factors, of which diet in general seems to be one of the most important (Nürnberg *et al.*, 1998). Similarly, a higher content of PUFA in organically produced pigs may not only be a result of the different feed, but also be caused partially by the higher lean meat percentage (Hansen *et al.*, 2006).

Holló *et al.* (2003) set MUFA values in *MLLT* from Mangalitsa pigs within a range of 56.0% and 56.1%. They established PUFA values ranging between 6.51% and 8.24% and SFA values between 35.8% and 37.4%. Loins of Iberian pork are characterized by a high concentration of MUFA in the intramuscular fat of the *MLLT*, especially where animals were reared in an open system and fed grasses and acorn (59.2%), while the other two groups of animals, reared in a closed system, regardless of diet, showed no difference in MUFA concentration (56.7% and 56.3%) (Daza *et al.*, 2007). No significant variations in PUFA concentrations were established between pig groups on different rearing systems and diets. The results of a study carried out by Sans *et al.* (2004) on the fresh meat quality in Gascon pigs that had been reared within a system connected to natural resources, and fed with acorn and limited quantities of concentrates established more MUFA (58.3%), and less SFA (36.1%) and PUFA (5.6%) in the *MLLT*. Furthermore, some statistically significant differences in their content were noted, depending on the type of muscle examined (*MLLT* and *biceps femoris*). Similar values for MUFA concentration (58.1%) in the *MLLT* of Iberian pigs reared in an open system were established by Rey *et al.* (2004).

The amount of intramuscular fat in organic pork has been reported to be higher (Sundrum *et al.*, 2000), and the fatty acid composition to be more unsaturated compared with those of the meat from traditionally reared pigs (Hansen *et al.*, 2000). Because organic pigs had a higher proportion of *n*-3 and *n*-6 PUFA than conventional ones, lipid oxidation might have occurred in organic meat. This may result in inferior meat quality owing to enhanced lipid oxidation and the presence of soft fat (Nilzen *et al.*, 2001).

The rearing system significantly affected total SFA content in the *MLLT* (P <0.01), with free-range animals showing higher levels than indoor ones. These differences were produced mainly by higher (P <0.001) myristic acid (C14:0), palmitic acid (C16:0) and (P <0.05) margaric acid (C17:0) concentrations in the *MLLT* of animals reared outdoors. Stearic acid (C18:0) concentration, one of the major SFAs, did not differ significantly (P >0.05) between the two rearing systems. The free-range pigs had a slightly higher C18:0 concentration than the conventionally reared pigs. However, stearic acid (C18:0) is considered a neutral fatty acid that has no effect on blood cholesterol (Mahan & Escott-Stump, 2000) compared with myristic acid (C14:0) and palmitic acid (C16:0). It is known that the fatty acid composition of the porcine intramuscular fat is affected by feed composition, as reviewed by Wood and Enser (1997) and Bosi (1999).

In our research, the type of rearing system did not have a significant effect on cholesterol content in the Mangalitsa pigs. The total cholesterol concentration of the *MLLT* for pigs reared outdoors ranged from a minimum of 57.5 mg/100 g to a maximum of 70.7 mg/100 g, while the level of cholesterol concentration of indoor Mangalitsa pigs ranged from a minimum of 52.0 mg/100 g to a maximum of 76.9 mg/100 g. A number of previous studies reported lower levels of cholesterol in the *MLLT* with 57 mg/100 g (Dorado *et al.*, 1999) and 59 mg/100 g (Moss *et al.*, 1983). Similarly, Bohac & Rhee (1988) reported a cholesterol content of 55.9 mg/100 g, 53.1 mg/100 g, and 59.7 mg/100 g for the *MLLT*. On the other hand, Tu *et al.* (1967) reported that the cholesterol contents were 62 mg/100 g and 65 mg/100 g for pork *MLLT*. These values correspond well with our current data. Measurements taken in the study of Csapó *et al.* (2002) indicate that the cholesterol content of Mangalitsa pig fat varies between 71 mg/100 g and 109 mg/100 g. The authors concluded that there is no truth in reports that indicate that the fat of the Mangalitsa pigs contains less cholesterol than that of the more generally produced types of fattening pig. Mapiye *et al*. (2010) and Muchenje *et al.* (2009b) concluded that the cholesterol levels in beef were not affected by diet. The finding that diet had no substantial effects on meat cholesterol contradicts that of García & Casal (1992), who observed that beef from steers finished on pasture has lower fat and cholesterol concentrations than that from concentrate-fed ones.

More studies are needed to gain better insight into the characteristics of meat obtained from free-range-reared Mangalitsa pigs. This includes efforts in sensory analysis and technological aptitude, and also efforts aimed at clarifying the role of free-range rearing on the eating quality of this type of meat.

Conclusion

Our study leads to the conclusion that the free-range-reared Mangalitsa pigs had a lower backfat thickness than conventionally reared ones. In this investigation, the outdoor rearing of Mangalitsa pigs led to a higher protein, ash and water content and decreased total fat content and pH value in the *MLLT*. Mangalitsa pigs produced meat with higher PUFA levels as well as higher concentrations of PUFA *n*-3, *n*-6 and PUFA/SFA in the *MLLT* compared with the indoor-reared group. Rearing system choice did not have a significant effect on cholesterol content in Mangalitsa pig fats. Consequently, we conclude that free-range rearing may have negative and positive effects on the quality and nutritional value of the meat. Therefore, feeding animals outdoors on pasture appears to be an interesting approach to improving the healthy image of organic pork from the human health point of view. However, the possible effects of different amounts and varying quality of pasture intake by pigs on *n*-3 PUFA content and *n*-6/*n*-3 ratio of pork should be studied further.

Acknowledgements

This research was part of the project "Development and Application of New Biotechnology to Increase Production of Pork Quality", funded by the Ministry of Science, Republic of Serbia, record number 20087, project manager: Milica Petrovic. The authors would like to thank all participants in the project and contributors to this paper.

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