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# Gene Expression of Peroxisome Proliferator-Activated Receptor γ in Mangalica and Large White Pigs

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The expression of peroxisome proliferator-activated receptor γ gene (PPARγ) – a major regulator of adipogenesis and intramuscular fat deposition in pigs – was analysed in different tissues of Blonde Mangalica (M) and Hungarian Large White (LW) by means of quantitative reverse transcription PCR. In total, 20 experimental animals were raised and fed under identical conditions, and transported to the abattoir at similar body weight (124.7±10.3 kg; $p>0.05$). In both breeds highest PPARγ expression was observed in backfat ($p<0.001$), whereas lower levels were measured in muscle samples. The PPARγ mRNA levels in loin (m. longissimus dorsi) and semimembranosus muscle samples did not differ ($p>0.05$). Elevated PPARγ expression was observed in M backfat compared to LW ($p<0.05$). Loin and semimembranosus muscle samples of M showed higher PPARγ mRNA levels than LW ($P<0.001$), as well. Loin muscle marbling scores significantly correlated with β-actin normalized PPARγ expression ($r=0.63$; $p<0.001$) indicating the involvement of PPARγ in the regulation of intramuscular fat development. In conclusion, the differential expression of PPARγ in backfat and muscle samples can contribute to the remarkable differences in the constitution and intramuscular fat accumulation of fat-type M and lean-type commercial LW. Muscle PPARγ mRNA levels may be used as an indicator of marbling characteristics in pigs.

## 1. Introduction

European pork production vastly relies on a limited number of efficient meat-type commercial breeds; however, historical indigenous pig breeds may provide sustainable and high-quality alternatives to intensive large-scale pork production. Furthermore, native breeds are crucial sources of genetic variability that is required for the adaptation to the changing environment. Overall, approximately 29 % of known pig breeds are either extinct or endangered worldwide, according to the assessment of the Food and Agriculture Organization of the United Nations (FAO, 2015). Notably, 107 pig breeds are now extinct of a total of 709 pig breeds of the world, while a further 26 breeds are in critical condition, 9 breeds received critical-maintained status, 42 breeds are endangered, and 20 breeds are endangered-maintained. The number of local, indigenous pig breeds has rapidly declined over the last century (Lukic et al., 2020) due to the appearance and fast dissemination of economically efficient lean-type cosmopolitan breeds that produce pork with favourably high lean meat content for commercial markets and clearly surpass historical breeds regarding reproductive performance, as well. The importance of local breeds is apparent in the preservation of genetic resources and diversity of the species, while in addition, native breeds improve the sustainability of the pork supply chain on both local and global scales, contribute to the maintenance of local traditions and facilitate the marketing of high-quality traditional pork products (Dadousis et al., 2022). In addition, commercial large-scale pork production poses potential environmental risks and challenges through considerable manure (Rodriguez et al., 2017) and wastewater production (Ngilangil and Quinquito, 2020), whereas extensive low-input farming with local breeds is associated with more sustainable waste management and a holistic approach.

In several countries worldwide, the application of local autochthonous breeds under free-range conditions receives growing attention due to the increasing consumers' preference toward organically raised livestock and the enhanced awareness for improved animal welfare requirements. Indigenous pig breeds provide viable

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alternatives for pork production under poor environmental and nutritional conditions due to an improved tolerance towards various diseases and centuries-long adaptation to local climatic challenges. Climate change-induced temperature increase is expected to negatively affect large-scale commercial pork production, even in confined housing systems, regarding overall animal performance, e.g., feed intake, feed conversion ratio, body mass gain, and conception rate (Hörtenhuber et al., 2020).

In Hungary, the native fat-type Mangalica breed has been gaining attention in the last decades primarily due to its superior meat quality. The Mangalica breed group consists of four Mangalica breeds, namely the Blonde Mangalica, the Red Mangalica, the Swallow-bellied Mangalica, and the Black Mangalica that has practically been resurrected in recent years by conscious breeding efforts via the discovery of sporadically emerging black Mangalica individuals and the selection of rare black piglets from Swallow-bellied Mangalica litters. Mangalica was developed in Hungary in the first decades of the 19$^{th}$ century from old local breeds (e.g., Bakony, Alföld and Szalonta varieties) and with further influences from the Serbian Sumadija breed (Zsolnai et al., 2006); the former local Hungarian varieties that contributed to the development of Mangalica have long since disappeared. Mangalica is one the fattiest pig breeds in the world, with fat content in the carcass reaching up to 60 %, while lean meat content rarely surpasses 40 % (Rátky et al., 2013), and the average daily gain of Mangalica lags behind commercial breeds (Tempfli et al., 2016). Due to its excessive intramuscular fat (IMF) content, Mangalica pork is optimal for high-quality branded dry-cured ham production, which renders the breed popular for worldwide exportation with high demands from Spain, France, Singapore, the USA, Hong Kong and Japan (Gaál, 2019). The IMF content of pork is essential for an improved flavour, juiciness and tenderness, while reducing IMF might be associated with pork sensory and processing characteristics (Jankowiak et al., 2019). Fat-type pig breeds also provide extremely valuable animal models for human obesity and metabolic syndrome research due to extensive anatomical and physiological similarities between the two species (Cluzel et al., 2022). A better understanding of lipid metabolism pathways is also essential for the improvement of commercial production and modern pig breeds and hybrids.

Gene expression studies provide a novel way of discovering various benefits and special characteristics of old indigenous pig breeds. Only by fundamental research can these forgotten animals find the way to contemporary intensive breeding applications where specific and well-researched traits might be consciously introduced to modern breeds and hybrids. Native fat-type pigs are capable of improving the meat quality of commercial populations. For example, Mangalica sows can be crossed with Duroc boars – as often seen in practice in Hungary – in order to provide higher IMF besides the excellent growth rate and muscle development of the Duroc breed. On a considerably long list of fat metabolism-associated genes, proliferator-activated receptor γ (*PPARγ*) is a key regulator of adipogenesis and IMF accumulation (Gu et al., 2021). PPARs are nuclear receptors that belong to the steroid/thyroid/retinoid receptor superfamily (Omi et al., 2005), are activated by lipids and enhance the expression of various genes (e.g., lipoprotein lipase, fatty-acid binding proteins, perilipin) playing central roles in lipid metabolism, fat deposition, transport, storage and catabolism (Gu et al., 2021). The central role of the gene in the processes of adipogenesis was greatly demonstrated when *PPARγ* knock-out mouse models were unable to accumulate adipose tissue (Barak et al., 1999). Other pathways related to *PPARγ* may contribute to differences in body composition, as well: Nunez et al. (2021) reported by the means of loin transcriptome analysis in Mangalica that *PPARγ* coactivator 1-beta (*PPARGC1B*) can be a potential regulator of breed-specific differences in fat metabolism between fat-type pigs and modern breeds.

The *PPARγ* regulatory pathway is well-described in several meat-type pig breeds; however, relevant literature is scarce regarding historical fat-type pigs, such as the Hungarian Mangalica.

## 2. Materials and methods

### 2.1 Experimental animals, recorded traits and sample collection

Ten Blonde Mangalica and ten Hungarian Large White gilts were raised under identical feeding and housing conditions in order to analyse potential differences in production traits between an indigenous pig breed and a popular commercial breed. The experimental animals were transported to a local abattoir at similar ($p>0.05$) body weight. A standard electrical stunning procedure was applied before bleeding the animals in both groups. Loin diameter and backfat thickness were recorded at the last thoracic vertebra. Average daily gain was calculated for the fattening stage by dividing total weight gain (g) by the number of days spent from the start to the end of the fattening period.

Marbling scores between 1 and 5 (where 1 represented minimal IMF and 5 represented most IMF coverage) were recorded on 2 cm wide loin (longissimus dorsi) slices by two persons in order to evaluate intramuscular fat accumulation. Tissue (backfat and loin) sampling was done at the fourth vertebra. Collected tissue samples (approximately 0.5-1 g) were stored in 2 mL RNase-free cryoprotective tubes (Merck, Darmstadt, Germany) and submerged in liquid nitrogen in order to prevent RNA degradation. Tissue samples were transported and stored in liquid nitrogen containers until further processing.

Throughout this study, animal handling was conducted in accordance with the standards recommended by the 2010/63/EU Directive.

### 2.2 Ribonucleic acid isolation, complementary deoxyribonucleic acid production and gene expression analysis

Total ribonucleic acid (RNA) was isolated from the collected samples in the laboratories of the Széchenyi István University, Department of Animal Sciences by means of TRIzol Reagent (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and 1-bromo-3-chloropropane (VWR International GmbH, Wien, Austria) based on the protocol developed by Chomczynski and Sacchi (2006). Prior to isolation, 300 mg tissue samples were homogenized in a TissueLyser LT (QIAGEN GmbH, Hilden, Germany) bead mill using 2 mm ZR BashingBead Lysis Tubes (Zymo Research Corp., Irvine, California, USA).
Following isolation, RNA quantity was assessed by the means of a NanoDrop 2000 (Thermo Fisher Scientific) spectrophotometer. For each sample, isolated RNA was treated by dsDNase (Thermo Fisher Scientific) according to the manufacturer's recommendations in order to eliminate potential DNA contamination during quantitative polymerase chain reaction (qPCR). The integrity of total RNA was evaluated by 1 % agarose gel electrophoresis in a RunOne Unit (EmbiTec, San Diego, California, USA), and only samples showing clear rRNA bands were subjected to further processing.
One µg total RNA was reverse transcribed by the application of the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) following the manufacturer's instructions. The produced complementary deoxyribonucleic acid (cDNA) samples were used for quantitative gene expression analysis on the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, California, USA) by the means of Maxima SYBR Green Master Mixes (Thermo Fisher Scientific). The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was applied to measure the relative *PPARγ* mRNA levels in samples with *β-actin* used as the internal reference gene. Each sample was run in duplicates regarding both *PPARγ* and the reference gene. Oligonucleotide primers with the following sequences (in 5'–3' direction) were used for the amplification of *PPARγ* (Li et al., 2012): F-GAT TTC TCC AGC ATT TCC A; R-GCT CTT CGT GAG GTT TGT T; and *β-actin* (Luo et al., 2009): F-CCA GGT CAT CAC CAT CGG; R-CCG TGT TGG CGT AGA GGT. The amplicon sizes were 184 and 158 bp for *PPARγ* and *β-actin*, respectively.

### 2.3 Statistical analysis

Production traits (e.g., body weight, average daily gain, ham weight, backfat thickness, loin diameter, marbling score) of the experimental groups M and LW were analysed in SPSS for Windows v.16 (SPSS Inc., Chicago, Illinois, USA) by independent samples t-tests, whereas relative gene expression values were compared by Mann–Whitney tests. Normal distribution was examined by Kolmogorov–Smirnov tests. Pearson correlation coefficients were calculated between loin muscle *PPARγ* relative gene expression values and marbling scores.

## 3. Results and discussion

### 3.1 Production traits

The comparison between the production traits of M and LW pigs highlights remarkable differences regarding body composition and pork quality (IMF or marbling score) of the local indigenous breed and the commercial breed (Table 1). The experimental groups did not differ ($p>0.05$) concerning live body weight at the end of the finishing period; however, significant ($p<0.05$) differences were detected for average daily gain, left ham weight, backfat thickness, loin diameter, and IMF scores. Based on the results presented in Table 1, it is evident that at a similar finishing weight, M accumulates more fat, whereas LW is capable of faster growth and can be characterised as more efficient concerning commercial pork production. On the other hand, a higher marbling score for M indicates improved pork quality.
In a comparison of Mangalica and Mangalica×Slovak Large White crossbred pigs Lipova et al. (2019) made similar observations regarding the IMF content of m. longissimus dorsi, as M loin, contained significantly ($p<0.05$) higher IMF compared to the samples of crossbred animals. Loin diameter values of the experimental LW group were comparable to those measured in a population reflecting the Polish large-scale pig sector with 60.6±8.3 mm average (Winarski et al., 2004).
Poklukar et al. (2020) reviewed the literature on the performance of local pig breeds and modern lean-type pigs and concluded that local breeds generally accumulate larger amounts of fat, exhibit larger adipocyte size and higher activity of lipogenic enzymes. In previous studies, the backfat thickness of LW and LW crossbred pigs varied between 15 mm (Madeira et al., 2013) and 24 mm (Lebret et al., 2014).

*Table 1: Production and carcass traits of M and LW gilts (mean±standard deviation)*

| Traits | Mangalica | Large White |
|---|---|---|
| Body weight (kg) | 126.8±11.7 | 122.6±9.1 |
| Average daily gain (g/day) | 587±64[b] | 773±68[a] |
| Left ham weight (kg) | 9.5±1.7[b] | 12.2±1.4[a] |
| Backfat thickness (mm) | 51±7[a] | 32±4[b] |
| Loin diameter (mm) | 46±6[b] | 58±7[a] |
| Intramuscular fat (score) | 4.2±0.8[a] | 2.3±0.7[b] |

[a,b] Different superscripts within the same row indicate significant (p<0.05) difference

### 3.2 Peroxisome proliferator-activated receptor gamma gene expression

In both breeds, *PPARγ* was predominantly (p<0.001) expressed in backfat samples. Relative *PPARγ* expression was significantly (p<0.05) higher in M backfat compared to that in LW samples (Figure 1); furthermore, in both loin (m. longissimus dorsi) and ham (m. semimembranosus) muscles *PPARγ* expression was significantly (p<0.001) higher in M compared to LW.

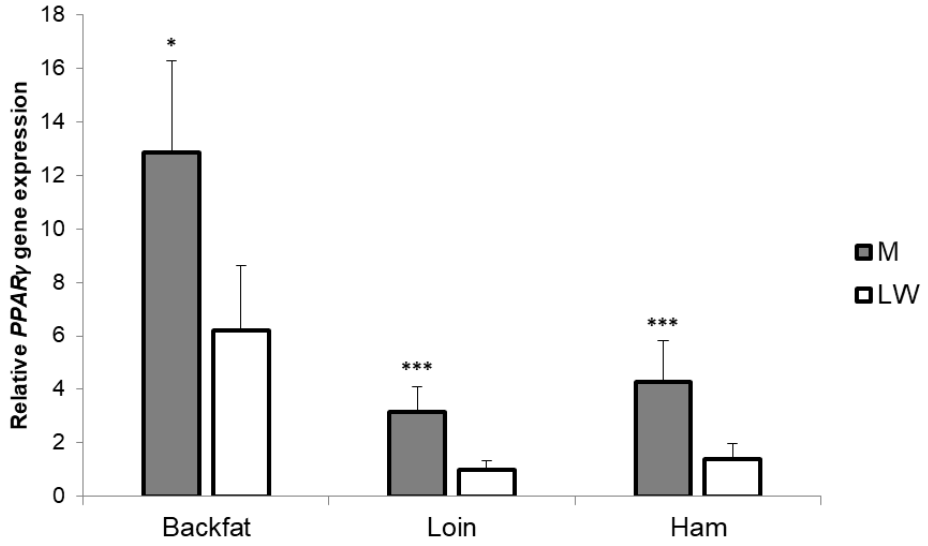


*Figure 1: Relative PPARγ gene expression in different tissues of M and LW animals*

*Asterisks * and *** indicate significant (p<0.05 and p<0.001, respectively) difference between breeds and within tissues*

For the whole population of experimental animals (n=20), *PPARγ* loin relative expression values significantly (p<0.001) correlated with marbling scores (r=0.63), which clearly indicates the involvement *PPARγ* in trait development.

Cui et al. (2016) investigated *PPARγ* expression in loin (longissimus dorsi) muscle samples from three different breeds, namely Laiwu Black (indogenous Chinese fat-type pig), Lulai Black (crossbred between Laiwu and LW), and LW. By the application of the same reference gene (*β-actin*) as in the present study, a remarkably similar *PPARγ* expression pattern was described: Laiwu produced the highest *PPARγ* levels, and was followed by Lulai, and the LW breed. In the correlation analysis *PPARγ* levels showed 0.58 correlation with IMF content (IMF was chemically determined in loin samples by the Soxhlet extraction method). Cui et al. (2016) suggested that by regulating the tissue-specific expression of *PPARγ* (e.g., by the application of muscle-specific promoters in transgenic pigs), IMF content of specific muscle groups might be modified, potentially without increasing backfat thickness.

Gu et al. (2021) successfully generated muscle-specific overexpression of *PPARγ* in CRISPR/Cas9 transgenic pigs, and confirmed that muscle IMF can be significantly (p<0.05) increased without affecting muscle production (expressed as lean meat percentage of the body). Across a wide selection of tissues, *PPARγ* expression was greatest in backfat in both the wild-type and the transgenic LW populations that did not differ significantly (p>0.05); however, *PPARγ* expression was significantly (p<0.05) higher in several types of muscle (e.g., longissimus dorsi and biceps femoris muscle) in transgenic pigs compared to wild-type animals.

In the study of Wang et al. (2020) the Chinese fat-type Laiwu showed significantly ($p<0.05$) higher *PPARγ* levels in backfat tissue compared to a modern meat-type hybrid of Duroc×Landrace×Yorkshire. Marbling scores were recorded on a 5-point scale for both experimental groups, and –similarly to the present study – Laiwu presented significantly higher values (over 4.5) than the modern hybrid (below 1.9). The higher scores for the lean-type LW presented in this study can be mainly explained by the difference in the finishing weight of the experimental animals (123±9 in the present study and 80±5 in the Chinese population) because IMF and marbling gradually increased during the finishing growth period.

In order to separately regulate the expression of *PPARγ* in subcutaneous and intramuscular adipose tissues, Wei et al. (2017) investigated the microRNA profile in both tissues and concluded that miR-130a was directly targeting *PPARγ* and its overexpression successfully inhibited *PPARγ* expression and preadipocyte differentiation. The designed targeting of *PPARγ* by microRNA might be another viable way to regulate tissue-specific fat accumulation that facilitates the improvement of pork quality without compromising muscle mass production.

## 4. Conclusions

Based on the presented performance traits it can be concluded that – at identical finishing weight – M gilts contain more fat, achieve significantly ($p<0.05$) lower average daily gain, and display higher IMF coverage on loin compared to LW pigs. The observed differences indicate that the optimal use of M remains in high-quality pork production.

Differential expression of *PPARγ* in the analysed tissues can contribute to extreme breed differences in body composition and growth performance. Measurements of *PPARγ* expression may be the indicators of IMF content of pork based on a significant ($p<0.001$) correlation between marbling scores and relative mRNA levels. Due to the unique characteristics of the indigenous Hungarian M, the breed is drawing attention in the fields of fat metabolism and obesity research, as well as in quality pork markets.

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