Comparison of Growth Performance and Meat Quality Traits of Commercial Cross-bred Pig versus Large Black Pig Breeds

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Abstract

**Background**: The meat quality of different pig breeds is associated with their different muscle tissue physiological processes, which involves a large variety of genes related with muscle fat and energy metabolism. Understanding the differences of biological processes of muscle after slaughter is helpful to reveal the meat quality development of different breeds. Therefore, 8 domestic Large Black pigs (BP), a domestic breed with high fat contents in meat, and 7 Cross-bred commercial pig (CP), which has a high feed efficiency with high lean meat, was used to investigate the differences of their meat quality and genotype.

**Results**: The average daily gain (ADG) and hot carcass weight (HCW) of CP were higher than BP, but the back-fat thickness of BP was higher than CP ($P < 0.05$). The CP had higher a* but lower h value than BP ($P < 0.05$). The metmyoglobin (MMb) percentage of CP was higher ($P < 0.05$) than BP. The fat content and oxygen consumption of longissimus dorsi (LD) muscle in BP were higher ($P < 0.05$) than CP. BP had higher SFA and MUFA content, but CP had higher PUFA content ($P < 0.05$). The RNA-seq was applied to compare the genome differences between the two pig breeds. The RNA-seq data highlighted 201 genes differentially expressed between breeds ($P < 0.05$), with 75 up-regulated and 126 down-regulated genes in BP compared with CP. The real-time PCR was used to validate the results of RNA-seq for 8 genes, and the genes related with lipid and energy metabolism were highly expressed in BP ($P < 0.05$).

**Conclusions**: Based on the results, BP had superior general meat quality to CP, while the growth performance of CP was better, and the genotype differences between these two breeds may cause the meat quality and growth performance variance.

**Keywords**

Commercial cross-bred pigs; British Large Black pigs; Meat quality; Breeds; Intramuscular fat; RNA-seq; Gene expression

**Background**

Increasing carcass weight and leanness of pigs have been a strong emphasis on production efficiency in swine breeding programs for many years. The researchers concentrated on improving pork production such as growth rate and feed conversion ratio, and they also aimed at decreasing carcass
fat content and backfat thickness. However, pork quality has received a prime focus on pig production as consumers demand better meat quality, for example, tenderness, marbling, color, and water holding capacity (WHC) [1]. The interactive effects of pig breeds, environmental situations, pre-harvest management and post-harvest process result in different meat quality [2]. However, the effects of genotype on meat eating quality, generally, are higher than external feed conditions [3]. It was reported that meat quality was also related to postmortem meat metabolic, and oxygen consumption of muscle tissue is one of its phenotype [4]. In addition, a large variety of genes related to both muscle structures and metabolic substances could affect the postmortem physiological developments inside the muscle cells. Comparing the transcriptome expression profile differences between different breeds could help us to understand the principles of genes related with meat quality and muscle biological processes.

Commercial cross-bred pigs (CP) are widely used in the world pig industry for its great feed efficiency and higher average daily gain (ADG), with high productivity of lean meat and less fat content. In contrast, the British Large Black pigs (BP) are British domestic pigs with long and deep-bodied and are well known for its high-fat contents and high meat quality characteristics [5, 6].

The aim of this experiment was to investigate the carcass, meat quality traits of British Large Black Pigs (BP) and Commercial Cross-bred pigs (CP) in relation to their transcriptome profiles, and consequently clarify the phenotypic and genotypic differences between these two breeds. The outcome of the study provided new sights into the adoption of British Large Black breed in pig industry. In order to investigate the genome organization's complexity and the transcriptional landscape of tissues and cells between different breeds, comparative transcriptome analysis is freshly used [7]. However, in the past, general gene expression was determined by microarray hybridization technology [8]. Recently, sequencing technology offers more benefits than microarrays, because of it rapidly advance. For instances, RNA-seq concentrates more on the reconstruction of the entire transcriptome [9], and provides a complete comprehension of genotype items [10]. On the base of RNA-seq, functional analysis of Gene Ontology (GO) biological process (BP) terms can be used to highlight the genes association and the networks of relevant biological
pathways. The real-time PCR is a verification of RNA-seq data. Hence, RNA-seq and real-time PCR could be useful tools to investigate the reasons attributed to carcass and meat quality traits between BP and CP.

**Material and methods**

**Animals**

Before the initiation of research, the University of Arkansas’s Institutional Animal Care and Use Committee approved all procedures of the experiment involving animals during the study. Eight British Large Black pigs and seven commercial Cross-bred pigs were allocated to BP group and CP group, and their initial mean body weights were tabulated (23.31±1.93 kg for BP group and 18.82±1.41 kg for CP group). They were fed *ad libitum* and kept individually in digestibility pens for 101 days. The final body weight of CP groups was determined at the end of 101-day experiment. However, due to low growth performance of the BP, the average body weight of BP group was 115.4 kg, lower than the average market live weight which was 127.9 kg ([https://www.pork.org/facts/stats/consumption-and-expenditures/typical-market-pig-today](https://www.pork.org/facts/stats/consumption-and-expenditures/typical-market-pig-today)). The BP group was kept for 108 days. The difference value between initial and final body weight was used to calculate ADG. Animals were off feed 12 h prior to slaughter, with access to water. Then, they were rendered unconscious by a nonpenetration stunning method.

**Carcass characteristics and sampling**

Immediately after stunning and completion of exsanguination, hot carcass weight (HCW) and backfat thickness (midline, between the 4th and 5th lumbar vertebra level) were determined. Then, muscles from *longissimus dorsi* (LD) of left carcass was removed. One piece of samples was stored at -80°C for RNA isolation process. The carcasses were chilled at -9°C for two hours and then kept at 4°C for 24h. Another piece from LD muscle was obtained (between the 12th and 13th rib) and transported under refrigeration until being processed for meat quality. The muscles were cut into small pieces, vacuum-packed and stored at -20°C before analyzing the fatty acid profile.

**Meat quality**

The intramuscular fat (IMF) content of *longissimus dorsi* muscle was determined by using Soxhlet
apparatus to extract ether without previous acid hydrolysis [11]. A 2 cm 100g thick slice cut from LD muscle was placed into a polypropylene bag then stored in a vacuum package for 24 h at 4℃, and the weight difference of samples were regarded as drip loss showed as percentage [12]. In order to ensure stable data of color measurements, sample were bloomed for 20 min before experiment. The L, a*, and b* color values were determined by a CR-400 Chroma Meter. Then, the hue angle (h=arctan(b*/ a*)) and chroma (C*=( (a*)²+(b*)²)0.5) was calculated by the L, a*, and b* values.

Fatty acid composition
The fatty acid composition of LD muscle IMF was determined by fat extract [13]. Ten grams minced meat were homogenized at 3000 rpm for 1.5 min by UltraTurrax using 0.003% butylhydroxytoluene (BHT) with 200 ml Folch solution (chloroform-methanol mix 2:1). After paper-filtering (Whatman No. 1) the homogenized liquid, Folch solution (50ml) was added. After filtered, the solution was poured out into a decantation infundibulum mixed with 8% sodium chloride (80ml) for 24 h. The solvent, collected from lipidic phase, was evaporated. After evaporation, the fatty acids composition was analysis using a gas chromatography (Agilent 6890 N Network GC System). As a carrier gas, helium was used at a division ratio of 1:50 with a 3.2 ml per minute flow rate. The undecanoic acid methyl ester was used as an internal standard to quantify the methyl esters of fatty acids.

RNA extraction and cDNA Synthesis
The total RNA of longissimus dorsi muscle was extracted by TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). The total RNA, extracted from the muscle, was then treated with DNase I (Promega, Madison, WI, USA) to eliminate the contamination of genomic DNA, following the directions of manufacturer. The integrity of total RNA was assessed by NanoDrop (Agilent Technologies, CA, USA). The decontaminated RNA samples were performed to reverse-transcription using Takara PrimeScript™ RT reagent Kit, and then prepared for cDNA synthesis according to the instructions of Takara PrimeScript™ RT.

Real-time PCR
According to the recorded sequences showed in GenBank, the primers of SLC26A7, TKTL2, ACBD7, THRSP, SLPI, FADS1, ACSL6, FOS and GAPDH were designed using Oligo 6.0 Software (Table 3).
The cDNA was used to perform Real-time PCR in order to obtain the expression level of SLC26A7, TKTL2, ACBD7, THRSP, SLPI, FADS1, ACSL6, FOS. Real-time PCR was performed using 15 μL reaction system: 7.5 μL 2× Real Master Mix; 0.75 μL upstream and 0.75 downstream primer (10 pmol/L); 3 μL cDNA; and 3 μL water. The reaction liquid was added on iCycler IQ5 (Bio-Rad, USA). The reactive conditions and primers were presented in Table 3. Relative expression levels were normalized to GAPDH gene and expressed as fold change [14].

RNA sequencing and functional analysis

The RNA-seq data were aligned, using the STAR aligner, to the most current pig genome (sus scrofa 11.1_v91). Then, the Edge R package in SeqMonk (v.1.37.1) was used for differential gene expression analysis. Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to implement the enrichment analysis for specific GO terms [15, 16]. The gene lists were uploaded using the ENSEMBL gene ID (http://www.ensembl.org).

Oxygen consumption and myoglobin calculation

Approximately 20 mg of tissue from longissimus dorsi muscle from each sample was put into a respiration buffer (1.1 mM sodium pyruvate, 25 mM glucose in PBS and 2% BSA). The Oxygen consumption rate (OCR) of every sample was measured by an Orion Dissolved Oxygen platform (Scientific) about 25 minutes, repeat 3 times. A full reflectance spectral analysis was also taken on each muscle and wavelength ratios were used to calculate relative deoxymyoglobin (474 nm:525 nm), oxymyoglobin (610 nm : 525 nm), and metmyoglobin (572nm : 525nm) concentrations [17].

Statistical analysis

All the data analysis was performed by SPSS version 19.0 software (SPSS Inc., Chicago, IL). The variance between these two pig breeds was carried out using t-test of independent samples. The results were given as means and SD in the text, and the differences was considered significant when \( P < 0.05 \).

Results and discussion

Growth performance and carcass measurements
The growth performances in terms of initial and final body weight and the quality of carcass are presented in Table 1. The initial body weights of CP and BP groups showed no significant differences ($P > 0.05$). However, CP group had higher final body weight ($P < 0.05$), ADG ($P < 0.05$) and hot carcass weight ($P < 0.01$) compared with BP group. According to R Bessa, R Hughes, E Jeronimo, O Moreira, J Prates and OJ Joas Doran [18], the fat deposition of the domestic pig is higher than the crossbreed pig. Correspondingly, the BP group showed a strong fat deposition ability with a significantly higher ($P < 0.01$) back fat thickness values compared to CP.

Meat quality

Meat quality traits of CP and BP groups are summarized in Table 2. Drip loss is one of the important characteristics of water holding capacity (WHC). There is no significant difference showed in drip loss between CP and BP ($P > 0.05$). For the meat color, $L^*$ indicates lightness, $a^*$ is about red/green coordinate, $b^*$ shows the yellow/blue coordinate, $C^*$ means chroma, and $h$ is the hue angle [19]. There was no significant difference of $L^*$, $b^*$ and $C^*$ between these two groups ($P > 0.05$). In contrast, the $a^*$ value of CP is higher ($P < 0.01$) than BP, but the $h$ value of BP is higher ($P < 0.05$) than CP.

The IMF content of these two breeds was significantly different ($P < 0.05$), that BP is higher than CP. However, the different fat content did not affect the drip loss. G Watanabe, M Motoyama, I Nakajima and KJA-Ajoas Sasaki [20] pointed out that pork IMF content was not correlated with drip loss, and our result had the same trend. The fat content of CP is similar to other reports about white pig breeds [21, 22], and the fat deposition of BP is much higher than these commercial pigs. The research compared Iberian pigs and commercial cross-bred pigs showed a strong correlation between IMF and backfat thickness [23]. Our data showed higher IMF and backfat deposition in the British Large Black pigs, which demonstrate the similarity fat deposition pattern in local domestic pigs that is different from commercial cross-bred pigs.

Oxygen consumption

The OCR is positively correlated with mitochondrial concentration [24]. In addition, mitochondria present in the postmortem muscle can remain active and impact on the meat color through oxygen
consumption [25]. The OCR of BP was higher than in CP ($P < 0.01$; Fig 1), so there should be more mitochondria in LD muscle of BP. It was reported than muscle with more $\beta$-red fibers tended to have higher OCR than muscle with more $\alpha$-white fibers [26]. However, the relationship between oxygen consumption and meat quality needs to be further evaluated to understand color variations between the two breeds. The oxygen consumption also represents the activation of mitochondrial metabolism. A study of the muscle fiber type of Korean native pig reported higher portion of type I muscle fibers than that of the commercial cross-bred pigs [27]. The type I slow twitch fiber has more content of mitochondria for oxidative phosphorylation. This report was consistent with what we found that the local domestic pigs have higher mitochondrial metabolism correlated with the higher content of type I muscle fiber.

Myoglobin calculation
The color of meat after slaughter is primarily affected by myoglobin which is a sarcoplasmic heme protein [28]. This protein can exist as deoxymyoglobin (DMb), oxymyoglobin (OMb) and metmyoglobin (MMb) in fresh meat [25]. Our results showed differences in the percentage of myoglobin between CP and BP breeds (Figure 2). The MMb (metmyoglobin) percentage of CP was higher ($P < 0.05$) than BP, but there were no significant differences between CP and BP in DMb (deoxymyoglobin) and OMb (oxymyoglobin). The chemistry and functions of myoglobin in live muscles and meat may be different, but the functions of myoglobin as the oxygen binder and oxygen deliverer to keep the physiological functions of mitochondria continues normally [29]. In addition, myoglobin is related to red meat color, which is a main meat purchasing factor for consumers [30].

Fatty acid composition
The fatty acid composition of LD muscles from CP and BP breeds are listed in Table 4. There was a significant fatty acid composition difference between CP and BP in most comparisons ($P < 0.05$). The total polyunsaturated fatty acids content of CP was higher ($P < 0.05$) than BP, such as C18:2n-6, C18:3n-3, C20:2, C20:3n-6, C20:4n-6, and C22:5. However, BP had higher total monounsaturated fatty acid content along with higher C:10, C:20, C18:1, and C20:0 contents compared to CP. The overall saturated and unsaturated fatty acids contents of CP and BP had no significant differences ($P > 0.05$).
The fatty acid composition has a strong relationship with the IMF content and backfat thickness [31]. It was reported that intramuscular and backfat would increase the percentage of saturated, especially monounsaturated fatty acids, and the data of our experiment is in agreement with their findings [32]. The fatty acid composition variations in CP and BP are probably attributed to the difference in fat deposition between these two breeds. The SFA level of intramuscular fat in loin has negative correlation with meat sensory quality, such as acid flavor [33]. However, there was no significant differences of SFA between BP and CP. It was reported that monounsaturated fatty acids are the major fatty acids component in the Mediterranean diet which benefits lowering the risk of cardiovascular disease [34], considering the IMF content in BP LD muscle is about 1.6 times higher than CP (10.02 ± 1.20:6.24 ± 1.53, $P < 0.05$. Table 2), which might indicate that BP pork products have higher healthy value.

Differentially expressed gene analysis

High-throughput sequencing is a powerful way to identify the differences in gene expression, which is recently used in the study of different breeds to compared the difference of genes expression related with meat quality [35]. By comparing LD muscle transcriptome differences between BP and CP, we found that there was a total of 384 differentially expressed genes found between these two breeds, in which 201 are highly differentially expressed (log2 Fold change ≥1 or ≤-1; $P$-value <0.05). Compared with CP, BP had 75 up-regulated and 126 down-regulated genes (Fig.3.). The functional category of these 201 differentially expressed genes, of which 75 are highly expressed in BP and 126 are highly expressed in CP, were determined by querying associated gene ontologies, and they were classified into biological process, cellular component, and molecular function (Fig.4-6), by using DAVID bioinformatic resources. GO analysis showed the functional enrichment of these differentially expressed genes, and the different genes expression may cause the diversities of carcass characteristics and meat quality between these two breeds. For biological process, 5 highly expressed genes in BP were related with fat cell differentiation (Fig.4). This might have led to a higher IMF content of LD muscle in BP, compared with CP. In molecular function, we found that 6 genes, which were highly expressed in CP, were responsible for oxidoreductase activity, and these genes’ higher expression may cause the different OCR of the LD muscle between BP and CP.
In order to verify our RNA-seq expression profile data, we utilized real-time quantitative RT-PCR to determine eight genes related to lipid deposition or metabolism, and the results (shown in Table 7.) validated the transcriptome profiles of RNA-seq. Results are presented as numerical relative gene expression values (using GAPDH as a housekeeping calibrator). The expression of lipid and energy metabolic related genes (TKTL2, ACBD7, ACSL6, and FOS) were higher in CP than in BP (P < 0.05). In addition, CP exhibited higher (P < 0.05) mRNA abundance of THRSP gene which is related to medium-length fatty acid chains [36]. However, the gene expression of SLPI which involved in epithelial immunity was higher in BP (P < 0.05) is consistent with the characteristic of stronger environmental tolerance in BP as a local domestic pig breed compared to CP [37]. The gene related to energy metabolism and unsaturated fatty acids composition (SLC26A7 and FADS1) were higher expressed in CP. Pigs of different breeds always have the different condition of metabolism and lipid deposition. SLC26A7 is strongly related to energy metabolism [38]. TKTL2 is related with lipid transport and metabolism, and the genetic distance increased from local pig breeds after selective seep caused by the selection of energy-rich pork production [39]. ACBD7 is one of the gene family related with intracellular lipid-binding proteins [40]. FADS1 gene is associated with the unsaturated fatty acid composition [41]. It was reported that ACSL6 contributes to lipid synthesis [42]. G Reiner, L Heinricy, E Müller, H Geldermann and V Dzapo [43] reported that FOS is an important gene correlated with skeletal muscle fiber and metabolism. Above all, the differential of gene expression related to lipid and energy metabolism might cause the different fat deposition ability of CP and BP.

**Conclusion**

In conclusion, the growth performance of CP was higher than BP, but the meat quality traits of BP was better than CP. The difference in intramuscular fat content, oxygen consumption and myoglobin calculation of LD muscle between these two breeds were also high. The RNA-seq and gene expression data proved an efficient sight about the differences of transcriptome profiles and genotype of these two breeds. Comparing BP with CP, 201 significantly differentially expressed genes in LD muscle were identified.
List of Abbreviation

BP    Black pigs
CP    Cross-bred commercial pig
ADG   Average daily gain
HCW   Hot carcass weight
MMb   Metmyoglobin
DMb   Deoxymyoglobin
OMb   Oxymyoglobin
LD    Longissimus dorsi
SFA   Saturated fatty acid
UFA   Unsaturated fatty acid
MUFA  Monounsaturated fatty acid
PUFA  Polyunsaturated fatty acid
WHC   Water holding capacity
GO    Gene Ontology
PCR   Polymerase chain reaction
IMF   Intramuscular fat
BHT   Butylhydroxytoluene
RNA   Ribose nucleic acid
cDNA  Complementary deoxyribonucleic acid
SLC26A7 Solute carrier family 26 member 7
TKTL2 Transketolase like 2
ACBD7 Acyl-CoA binding domain containing 7
THRSP Thyroid hormone responsive
SLPI  Secretory leukocyte peptidase inhibitor
FADS1 Fatty acid desaturase 1
ACSL6 Acyl-CoA synthetase long chain family member 6
FOS   Fos proto-oncogene, ap-1 transcription factor subunit
GAPDH Glyceraldehyde-3-phosphate dehydrogenase
DAVID Database for annotation, visualization and integrated discovery
OCR   Oxygen consumption rate
Declarations:

Ethics approval
the University of Arkansas’s Institutional Animal Care and Use Committee approved all procedures of the experiment involving animals during the study.

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests

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Authors' contributions
YW, SS, JC analyzed the data. YW, SS, YH took measurements and collected samples. KT conducted RNA sequencing and functional analysis. YW, SS wrote the manuscript. All authors read an approved the final manuscript.
Table 1. Effect of breeds on the carcass characteristics (CP: Cross-bred commercial pigs, BP: British Large Black pigs)

*ADG: Average daily gain

b NS: not significant/(*), (**) Significant $P<.05$, 0.01, 0.001 respectively

Table 2. Effect of breeds on the meat quality of pork (*longissimus dorsi*) (CP: Cross-bred commercial pigs, BP: British Large Black pigs).

*a* L*: Lightening; a*: red-green; b*: yellow-blue; C*: bright-dull; h: hue

b NS: not significant/(*), (**), (***) Significant $P<.05$, 0.01, 0.001 respectively

Fig. 1. Effect of breeds on the oxygen consumption (*longissimus dorsi*) (CP: Cross-bred commercial pigs; BP: British Large Black pigs; NS: not significant/(*), (**), (***) Significant $P<.05$, 0.01, 0.001, respectively)

Fig. 2. Effect of breeds on the myoglobin calculation (*longissimus dorsi*) (CP: Cross-bred commercial pigs, BP: British Large Black pigs; MMb: Metmyoglobin, DMb: Deoxymyoglobin, OMb: Oxymyoglobin; NS: not significant/(*), (**), (***) Significant $P<.05$, 0.01, 0.001, respectively)

Fig. 3. Volcano figure of RNA-seq (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs; Up: up regulated in BP compared with CP; Normal: No significant differences; Down: down regulated in BP compared with CP)

Fig. 4. Gene ontology (GO) analysis of differentially expressed genes highly express in BP (A) and CP (B). The identified differentially expressed genes were classified in to Biological Process. The numbers of genes the GO term is shown above (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs)

Fig. 5. Gene ontology (GO) analysis of differentially expressed genes highly express in BP (A) and
The identified differentially expressed genes were classified into Cellular Component. The numbers of genes the GO term is shown above (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs).

Fig. 6. Gene ontology (GO) analysis of differentially expressed genes highly express in BP (A) and CP (B). The identified differentially expressed genes were classified into Molecular Function. The numbers of genes the GO term is shown above (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs).

Fig. 7. Effect of breeds on the related gene expression (BP vs. CP; CP: Cross-bred commercial pigs, BP: British Large Black pigs) (*) Significant $P < .05$.

Table 3. Primer information for gene chosen for confirmation of expression using quantitative real-time PCR.

Table 4. Effect of breeds on the fatty acid profile of intramuscular fat (Longissimus dorsi) (CP: Cross-bred commercial pigs, BP: British Large Black pigs)

NS: not significant/(*) , (**), (*** ) Significant $P < .05, 0.01, 0.001$, respectively
Table 1.

| Parameters                        | CP       | BP       | P-Value<sup>b</sup> |
|----------------------------------|----------|----------|---------------------|
| Mean sd                          | Mean sd  | Mean sd  |                     |
| Initial body weight (kg)         | 18.82 1.41 | 23.31 1.93 | NS                  |
| Final body weight (kg)           | 130.04 8.16 | 121.17 2.80 | *                   |
| ADG<sup>a</sup> (kg)             | 1.10 0.05  | 0.91 0.01  | *                   |
| Hot carcass weight (kg)          | 77.95 9.81 | 63.63 0.95 | **                  |
| Back fat thickness (cm)          | 0.72 0.07  | 1.42 0.22  | ***                 |
| Parameters          | CP     | BP     | P-Value<sup>b</sup> |
|--------------------|--------|--------|---------------------|
|        Mean | sd | Mean | sd |          |
| Drip loss (%)      | 3.38  | 0.11  | 3.41  | 0.17 | NS      |
| L<sup>a</sup>      | 57.78 | 4.52  | 60.24 | 2.43 | NS      |
| a<sup>a</sup>      | 16.72 | 1.73  | 14.58 | 1.09 | **      |
| b<sup>a</sup>      | 14.31 | 1.68  | 13.73 | 0.67 | NS      |
| C<sup>a</sup>      | 22.02 | 2.28  | 19.99 | 1.09 | NS      |
| h<sup>a</sup>      | 40.49 | 2.13  | 43.47 | 1.64 | *       |

*Proximal composition:*

| Total fat (%) | CP | BP | P-Value<sup>b</sup> |
|--------------|----|----|---------------------|
| Mean | sd | Mean | sd |          |
| 6.24 | 1.53 | 10.02 | 1.20 | *       |
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.

A

Enriched Go Terms (Biological Process) in BP

- Positive regulation of osteoblast differentiation
- Circadian rhythm
- Negative regulation of cell death
- Face morphogenesis
- Multicellular organism growth
- Fat cell differentiation
- Skeletal muscle cell differentiation

B

Enriched Go Terms (Biological Process) in CP

- Positive regulation of gene expression
- Regulation of actin cytoskeleton organization
- Positive regulation of tyrosine phosphorylation of Stat3 protein
- Bicarbonate transport
- Pigmentation
- Chondrocyte development
- Visual perception
- Megakaryocyte development
- Axonogenesis
- Detection of mechanical stimulus involved in sensory perception of sound
Fig. 5.

A  Enriched Go Terms (Cellular Component) in BP

- Transcription factor complex
- Nucleus

B  Enriched Go Terms (Cellular Component) in CP

- Apical plasma membrane
- Male germ cell nucleus
- External side of plasma membrane
- Extracellular space
- Cell surface

Number
Fig. 6.

A  
Enriched Go Terms (Molecular Function) in BP

Transcriptional activator activity

Transcription factor activity

B  
Enriched Go Terms (Molecular Function) in CP

Copper ion binding

Oxidoreductase activity
| Gene   | Direction | Primer sequence | GenBank Accession No. |
|--------|-----------|-----------------|-----------------------|
| GAPDH  | Forward   | 5'-TCGGAGTGAAACGGATTTGGC -3' | NM_00120659.1 |
|        | Reverse   | 5'-TGACAAGCTCCCTTCCGTTCTCC -3' | |
|        | Forward   | 5'-GAAAATGCCAGCAATCAGCCA -3' | XM_021089130.1 |
|        | Reverse   | 5'-AGGGCCACAGTTCCCATTG -3' | |
| SLC26A7| Forward   | 5'-CTGGCCTTTTCATCCCCTA -3' | XM_013978706.2 |
|        | Reverse   | 5'-GTATCCATGCAGTGCGCAAG -3' | |
| TKTL2  | Forward   | 5'-GGAAGATGCCATGAGTGCCT -3' | XM_003357745.4 |
|        | Reverse   | 5'-CTGAGGGCTTTCAAAGGCAAA -3' | |
| ACBD7  | Forward   | 5'-GTAGCCCTCGGACTCTAGGCA -3' | NM_001244376.1 |
|        | Reverse   | 5'-CTGCAGGTCCAGGTCTTTCT -3' | |
| THRSP  | Forward   | 5'-CAAGTGCACAATGACTGACG -3' | NM_213870.1 |
|        | Reverse   | 5'-GGCCATAGACCATGGACAC -3' | |
| SLPI   | Forward   | 5'-GTCACTGCCTGGCCTATTCT -3' | NM_001113041.1 |
|        | Reverse   | 5'-AGGTGGTTCACGTTAGGCTG -3' | |
| FADS1  | Forward   | 5'-GAATACTGGGACACTCTGTGGC -3' | XM_021084743.1 |
|        | Reverse   | 5'-CTTAGGACCCAGTTGCAGC -3' | |
| ACSL6  | Forward   | 5'-GACTGCTATTCGACCAGGCC -3' | NM_001123113.1 |
|        | Reverse   | 5'-CTGGCATGGTTCCAGACT -3' | |
| Fatty acid (%) | CP Mean | cp sd | BP Mean | bp sd | P-Value |
|---------------|---------|-------|---------|-------|---------|
| C10:0         | 0.08    | 0.01  | 0.10    | 0.01  | **      |
| C12:0         | 0.06    | 0.01  | 0.07    | 0.01  | NS      |
| C14:0         | 1.14    | 0.17  | 1.20    | 0.08  | NS      |
| C16:0         | 23.76   | 0.66  | 24.21   | 0.73  | NS      |
| C16:1         | 3.06    | 0.46  | 3.32    | 2.41  | NS      |
| C17:0         | 0.23    | 0.03  | 0.24    | 0.02  | NS      |
| C18:0         | 11.74   | 0.79  | 11.95   | 0.73  | NS      |
| C18:1         | 41.81   | 3.20  | 46.21   | 1.05  | **      |
| C18:2n-6      | 12.67   | 2.74  | 8.74    | 1.40  | **      |
| C18:3n-3      | 0.27    | 0.02  | 0.23    | 0.04  | *       |
| C20:0         | 0.13    | 0.01  | 0.19    | 0.02  | ***     |
| C20:1         | 0.51    | 0.10  | 0.70    | 0.07  | ***     |
| C20:2         | 0.29    | 0.04  | 0.23    | 0.02  | **      |
| C20:3n-6      | 0.35    | 0.11  | 0.22    | 0.05  | **      |
| C20:4n-6      | 2.82    | 0.87  | 1.53    | 0.48  | **      |
| C22:5         | 0.28    | 0.09  | 0.14    | 0.04  | **      |
| SFA           | 37.07   | 1.09  | 37.96   | 1.40  | NS      |
| UFA           | 62.04   | 1.05  | 61.25   | 1.34  | NS      |
| MUFA          | 45.32   | 3.61  | 50.18   | 1.21  | ***     |
| PUFA          | 3.75    | 1.06  | 2.11    | 0.55  | ***     |
| n6:n3         | 27.59   | 3.63  | 28.35   | 3.08  | NS      |
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