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# **Comparison of Meat Quality, Including Fatty Acid Content and Amino Acid Profile, and Transcriptome Profile Among Jeju Black Cattle, Korean Black Cattle, and Hanwoo**

## **Abstract**

This study aimed to compare the meat quality, including fatty acid content, amino acid profile, and transcriptome profile, among three Korean cattle breeds: Hanwoo, Korean black cattle (KBC), and Jeju black cattle (JBC). We analyzed fatty acid compositions, revealing that Hanwoo had higher levels of saturated fatty acids such as pentadecanoic acid, palmitic acid, and margaric acid than other cattle breeds. In contrast, KBC showed higher levels of linolenic acid, which is one of the omega-3 polyunsaturated fatty acids. Free amino acid profiles showed that Hanwoo and JBC had significantly higher levels of glutamic acid, glycine, and phenylalanine compared to KBC. KBC had a significantly higher arginine content, while Hanwoo had a significantly higher serine content compared to the other two breeds. Regarding constituent amino acid content, JBC had a higher glutamic acid content, which is associated with umami, and exhibited a lower level of valine, arginine, isoleucine, and phenylalanine compared to the other breeds. RNA transcriptome analysis identified key differentially expressed genes (DEGs) involved in lipid metabolism and energy homeostasis, including MOGAT1, ANGPTL8, and SLC38A4. Network analysis highlighted substantial differences in muscle system processes, fat cell differentiation, and other pathways between the breeds. These findings provide foundational data for genetic selection programs aimed at enhancing meat quality and offer valuable insights into preserving the unique characteristics of Korean indigenous cattle.

**Keywords:** Korean indigenous cattle, Meat quality, Fatty acid composition, Amino acid profile, Transcriptome Profile (RNA-seq)

## **Introduction**

Korean cattle have a long history and tradition. Four major breeds include Korean brown cattle (Hanwoo), Korean black cattle (KBC), Jeju black cattle (JBC), and Korean brindle cattle (Chikso) (Lee et al., 2014). The well-known Hanwoo cattle breed boasts the largest population in South Korea. In contrast, the populations of the other three breeds are relatively minor.

Specifically, Jeju black cattle are exclusively found on Jeju Island (Han et al., 2011). Among these, Hanwoo is renowned for its superior meat quality, which is characterized by its marbling and rich flavor, making it highly popular among consumers (Jo et al., 2012). KBC refers to black cattle raised in regions outside Jeju Island and exhibit distinct characteristics from JBC.

Although comparative studies between KBC and other native Korean cattle breeds are limited, recent research has reported that JBC showed enhanced flavor and umami due to its higher oleic acid content and flavor-related compounds than KBC (Hoa et al., 2024). According to historical records, JBC was renowned for being presented as a tribute to the king. The unique taste and quality of JBC enriched the royal table, serving as a historical testament to the breed's excellence during the Joseon Dynasty. This tradition continues to elevate the value and reputation of JBC today.

Beef is an important animal food source that provides essential nutrients, including essential amino acids, unsaturated fatty acids, minerals, and various vitamins (Rabia Shabir et al., 2018). The nutritional value of meat has garnered significant attention in recent years. Fatty acid composition is a crucial indicator of beef quality, influencing flavor, juiciness, tenderness, and overall taste, which in turn greatly affects consumer preferences (Davis et al., 2022). Numerous studies have shown that diets rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) have significant health benefits, such as reducing the risk of cardiovascular diseases, diabetes, and premature death.

The accumulation of fatty acids is regulated by various biological processes such as fatty acid synthesis, transport, degradation, and beta-oxidation (Rabia Shabir et al., 2018). Several studies have previously been conducted to identify candidate variants and genes related to fatty acid composition in cattle (Wang et al., 2019; Zhang et al., 2022). Furthermore, many studies have explored the genetic basis of fatty acids in muscles using multi-omics approaches, including transcriptomic, proteomic, and metabolomic analyses. For example, transcriptome analysis of bovine *longissimus dorsi* and skeletal muscle revealed that differentially expressed genes (DEGs) are involved in various pathways, such as fatty acid degradation and beta-oxidation, as well as PPAR and AMPK signaling pathways (Fernyhough et al., 2007; Underwood et al., 2008). In addition to the role of fatty acids, the amino acid composition of beef is a fundamental determinant of its quality, sensory attributes, and nutritional efficacy. Amino acids such as glutamic acid and alanine are pivotal in imparting umami and sweet flavors, while serine, glycine, and methionine modulate taste profiles, influencing sweetness and bitterness perceptions (Kirimura et al., 1969; Lindemann, 1996; Nelson et al., 2002; Shallenberger and Acree, 1967). Beyond their organoleptic contributions, amino acids are indispensable for human health, facilitating vital processes such as protein synthesis and enzymatic functions and serving as precursors for bioactive compounds (Chakrabarti et al., 2018). Consequently, the amino acid profile augments beef's palatability and nutritional value, thereby shaping consumer preferences and influencing the marketability of various beef breeds.

Recently, a series of comparative studies have been performed to evaluate the meat quality of native Korean cattle breeds. Notably, investigations have focused on the carcass and meat quality and the sensory attributes of Hanwoo and Chikso (Hoa et al., 2023a; Hoa et al., 2023b; Lee et al., 2023; Utama et al., 2018). In summary, comparative studies on the meat quality of Korean cattle breeds suggest that Hanwoo is notable for its high intramuscular fat content, tender meat quality, and significantly elevated levels of umami-related amino acids. However, the perception of

umami can also be affected by cooking methods, specific meat cuts, and individual taste preferences.

Despite these findings, there remains a gap in understanding the specific genetic factors that contribute to the distinct meat quality and flavor of different Korean cattle breeds. Therefore, a comprehensive transcriptomic analysis of candidate genes for different beef cuts using high-throughput RNA sequencing can help elucidate potential gene expression regulation related to fatty acid composition.

The purpose of this study is to analyze the free amino acids, constituent amino acids, and fatty acids of Hanwoo, JBC, and KBC and to investigate their meat quality characteristics. Particularly, we compared the expression levels of flavor-related genes among the breeds through RNA transcriptome analysis. JBC, with its high oleic acid content, possesses a unique flavor, which may be closely related to the expression of specific genes. This research aims to elucidate how genetic differences among cattle breeds affect the taste and nutritional components of the meat, providing crucial foundational data for developing genetic selection programs aimed at improving meat quality. Additionally, this study provides important insights to preserve the unique characteristics of Korean indigenous cattle and enhance their competitiveness in the global market.

## **Materials and Methods**

### **Animal and Sample preparation**

The *Longissimus lumborum* was utilized in this study. For the analysis, samples were collected from five JBC, five Hanwoo cattle, and five KBC, all obtained from a local meat supplier in Jeju, 24 hours post-slaughter. All experimental animals were raised on various farms across Jeju Island. The animals were all castrated males, with slaughter ages of  $34.4 \pm 0.9$  months for KBC ( $487.0 \pm 12.9$  kg carcass weight),  $40.1 \pm 7.9$  months for JBC ( $438.8 \pm 70.9$  kg carcass weight),

and  $31 \pm 1.4$  months for Hanwoo ( $453.3 \pm 30.7$  kg carcass weight). All animals were fed commercially available feed. The cattle used in this study were all graded as the highest quality(1++) according to the standards of the Korean Institute for Animal Products Quality Evaluation (KAPE). The detailed beef grading criteria of Korea are presented in Table 1.

### **Proximate analysis**

The proximate analysis of the *Longissimus lumborum* samples was conducted using Association of Official Analytical Chemists(AOAC) methods specific for moisture, protein, fat, ash, and carbohydrate content determination (AOAC, 2019).

### **Temperature, pH and color measurement**

The temperature at 24h postmortem and pH of the *Longissimus lumborum* were measured using a spear-type pH meter (206-pH2, Testo, Germany). The meat color was measured using a Minolta chromameter (CR-300, Minolta Camera Co., Japan) after exposing the meat surface to air for 30 min. The average of three measurements was recorded, and the results were expressed as CIE L\*, a\*, and b\* values.

### **Cooking loss**

The *Longissimus lumborum* was cut into a rectangular shape (2 cm × 4 cm × 6 cm), tightly wrapped in a polyethylene bag, and heated to 85°C in a water bath (KMC-1205W1, Vision Co., USA). Once the core temperature reached 75°C, the samples were cooled in cold water for 20 min. After removing surface moisture, cooking loss was calculated as the percentage difference between the initial and final weights relative to the initial weight.

## **Shear force**

*Longissimus lumborum* pieces, 3 cm in thickness, were sealed in polyethylene bags and heated to 85°C in a water bath (KMC-1205W1, Vision Co.). Once the core temperature reached 80°C, the samples were cooled in cold water for 20 min. After cooling, a 13 mm-diameter steel borer (Cork borer No. 6, Sigma-Aldrich, USA) was used to collect cylindrical samples. These samples were then sliced into 1 cm-thick sections perpendicular to the muscle fibers and subjected to shearing using a texture analyzer (Texture Analyzer CT3, Brookfield, USA). Defined as the peak force exerted by the machine to shear the sample, the shear force, indicating the toughness of the meat, was measured in Newtons (N). The conditions for shear force measurement with the CT3 Texture Analyzer were a target displacement of 20 mm, a trigger load of 10 g, and a test speed of 1 mm/s. The measurement was carried out in a single cycle with a TA-52 probe and a TA-SBA fixture.

## **Texture profile analysis (TPA)**

A texture analyzer (CT3, Brookfield, USA) was used to perform TPA, which included the measurement of hardness, adhesiveness, resilience, cohesiveness, springiness, gumminess, and chewiness. This test simulates the chewing process with a two-cycle compression (Barbut, 2015). *Longissimus lumborum* samples were cut into 3 cm-thick pieces, tightly wrapped in polyethylene bags, and cooked in a water bath at 85°C. Once the core temperature reached 75°C, the samples were cooled in cold water for 20 min. After cooling, six subsamples, each measuring 2.5 cm × 2.5 cm × 2.5 cm, were prepared for TPA. The probe moved at 2.0 mm/s (pre-test), 1.0 mm/s (test), and 4.5 mm/s (post-test). It compressed the samples to 75% of their thickness, retracted to the initial contact point, paused for 2 s, and then initiated the second compression cycle.



### **Free amino acid analysis**

For the analysis of free amino acids, 200 mg of *Longissimus lumborum* sample was homogenized in 10 mL of 70% ethanol and sonicated for 30 minutes. After sonication, the mixture was centrifuged at 9000 rpm for 15 minutes. The supernatant was carefully collected and filtered through a 0.45- $\mu$ m aqueous syringe filter to remove any particulates. A 20  $\mu$ L aliquot of the filtered extract was then mixed with 20  $\mu$ L of an internal standard solution containing 50  $\mu$ M of norvaline (Sigma-Aldrich, USA). The resulting mixture was injected into the gas chromatography system with flame ionization detection (GC-FID) for analysis. The column used was a ZB-AAA (Phenomenex, Torrance, USA), measuring 10 meters by 0.25 mm. Nitrogen (N<sub>2</sub>) was used as the carrier gas with a flow rate of 1.5 mL/min. The injector temperature was set to 250°C, and the detector temperature to 320°C, with a split ratio of 5:1. Identification and quantification of the free amino acids were performed by comparing the retention times and peak areas with those of a standard amino acid solution (Phenomenex, Torrance, USA)

### **Constituent amino acid analysis**

For the constituent amino acid analysis, 200 mg of *Longissimus lumborum* sample was hydrolyzed with 30 mL of 6 N HCl at 110°C for 24 h and filtered through a 0.45- $\mu$ m aqueous syringe filter. A mixture of 20  $\mu$ L of the hydrolyzed sample and 20  $\mu$ L of diluent was then analyzed using GC-FID. The column used was a ZB-AAA (Phenomenex, Torrance, USA), measuring 10 meters by 0.25 mm. The carrier gas was N<sub>2</sub> at a flow rate of 1.5 mL/min, with the injector set to 250°C, the detector set to 320°C, and a split ratio of 5:1. The identification and quantification of amino acids were achieved by comparing the retention times and peak areas with those of a standard amino acid solution (Phenomenex, Torrance, USA).

### **Fatty acid composition**

The fatty acid composition was analyzed by gas chromatography with flame ionization detection (GC-FID). The GC column used was an SPR® -2560 (Sigma-Aldrich), measuring 100 m in length, with an internal diameter of 0.25 mm and a film thickness of 0.2 µm. For the analysis, 25 mg of *Longissimus lumborum* sample was extracted and methylated by adding 14% BF<sub>3</sub> in methanol, then diluted with 1 mL of isooctane. The GC operating conditions included an N<sub>2</sub> gas flow rate of 0.8 mL/min, an injector temperature of 240°C, a detector temperature of 285°C, and a split ratio of 100:1. Fatty acid peaks were identified by comparing retention times with those of a standard fatty acid mixture (Supelco 37 Component FAME Mix, Sigma-Aldrich) under the same conditions.

### **Statistical Analysis**

A completely randomized design was adopted to analyze the main effect of the experimental factor. The significance of the model was determined using analysis of variance (ANOVA), and Duncan's multiple range test was performed when the main factors were significant ( $p < 0.05$ ). All statistical analyses were conducted using SAS software v.9.4 (SAS Institute Inc., Cary, NC, USA).

### **RNA isolation and library construction**

The *Longissimus lumborum* samples were stored in a -80°C ultra-low temperature freezer until RNA extraction. RNA extraction and library construction were outsourced to MacroGen, Inc (Seoul, Republic of Korea).

## **Reads mapping, quantification of gene expression level, and differential expression analysis**

The quality of the raw data was assessed for total bases, read count, GC (%), AT (%), Q20 (%), and Q30 (%) using the FastQC program (version 0.12.0) (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Subsequently, low-quality reads were filtered out using the Trimmomatic program (version 0.39). The trimmed reads were then re-evaluated with the FastQC program to ensure quality. Next, the trimmed data were aligned to the cattle reference genome using HISAT2 software (version 2.2.1). For this alignment, the reference genome assembly used was *Bos taurus* (GCA\_002263795.4) from the National Center for Biotechnology Information (NCBI). The resulting SAM format data files were converted to BAM files using SAMtools software (version 1.17). The number of reads mapping to each gene or gene group was quantified using the featureCounts software (subread-2.0.6) on the BAM files. For featureCounts, the reference annotation file used was NCBI's *B. taurus* (GCF\_002263795.3). The calculated gene read counts were then utilized for DEG analysis using R software and associated packages. DEGs were identified based on criteria of false discovery rate (FDR < 0.05) and p-value (<0.05).

## **Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, and network analysis of DEGs**

GO and KEGG pathway enrichment analysis of DEGs were performed using the DAVID web tool (<https://david.ncifcrf.gov/>). The functional enrichment of the gene list was also assessed using DAVID, and functions and pathways related to cultivar biological differences were identified. In DAVID, the *Sus scrofa* species was used as the basis for GO and KEGG databases. After that, GO terms and KEGG pathways with FDR < 0.05 were identified. In addition, the DEG results were analyzed using the ClueGO (v2.5.10) tool in the Cytoscape 3.10.1

environment to identify major groups with  $p$ -value  $< 0.05$  in the correlated pathways and functional networks.

## Results and Discussion

### The proximate composition and meat quality characteristics

Table 2 compares the proximate composition and meat quality attributes among JBC, Hanwoo cattle, and KBC. The differences in parameters of the proximate composition among the groups were not statistically significant. For pH, temperature, meat color, and cooking loss, these parameters also showed no significant differences among the groups. According to previous research, the elevated intramuscular lipid content observed in Aberdeen Angus, Highland, and Jersey breeds may partially account for their enhanced flavor profiles, while the reduced intramuscular fat content in Limousin cattle might contribute to their comparatively subdued beef flavor (Gagaoua et al., 2016). Variations in lipid content, intramuscular collagen, and fiber type are considered significant factors influencing the sensory quality of beef (Chrïki et al., 2012; Chrïki et al., 2013). However, it is essential to note that these differences are inconsistently associated with muscle type, animal breed, and the breed's primary production function, whether dairy or meat-oriented.

Regarding meat color, several studies indicate that *Bos indicus* breeds exhibit darker meat color and higher pH values, particularly when pasture-fed than *Bos taurus* (Cafe et al., 2011; Gama et al., 2013). Furthermore, the increased fat deposition observed in the meat of *Bos taurus* cattle contributes to a lighter muscle coloration than that of *Bos indicus* cattle. However, research on the relationship between meat color and detail commercial meat cattle breeds is limited. Moreover, these results should consider individual nutritional status and production systems. In our study, when selecting samples, we ensured that all samples were of the same grade according

to the evaluation criteria of KAPE, which likely explains the lack of significant differences in the meat quality attributes.

### **Shear force and TPA**

In the case of shear force (Table 3), the value was significantly higher in JBC ( $8591.98 \pm 1128.72$ ) compared to the other groups ( $p < 0.05$ ). This difference can be attributed to the fact that breeds differ in muscle growth potential and muscle characteristics, which affect meat texture. Although previously extending the aging period of beef appears to mitigate differences in sensory quality across breeds, in our study, since samples were used 24 hours post-mortem, the differences in tenderness among breeds due to aging are considered to have a minimal impact on the shear force results. Notably, after one week of aging, fast-growing breeds such as Pirenaica and Rubia Gallega exhibited superior tenderness scores compared to the double-muscled Spanish breed (Asturiana de los Valles), the dual-purpose breed (Brown Swiss), and rustic breeds (Avelana-Negra Iberica, Morucha, and Retinta). However, these differences in tenderness were no longer statistically significant following 21 days of aging (Campo et al., 1999). Additionally, greater collagen content of tissue has been observed in the *longissimus muscle* of Angus steers compared to Limousin steers (Chambaz et al., 2003), and in Limousin steers compared to double-muscled Belgian Blue cattle (Raes et al., 2003). This implies that differences in collagen content among breeds can affect meat tenderness. Furthermore, the shear force is positively correlated with intramuscular collagen content, the degree of cross-linked collagen development, and the amount of insoluble collagen, all of which increase with age (Bruce and Roy, 2019). Additionally, it was previously reported that type IIX muscle fibers positively correlated with increased shear force (Cho et al., 2024). Although this study did not investigate the muscle fiber characteristics of cattle groups' muscle samples, our result of shear force aligns with Cho et al.'s research (2024), which found that the *Longissimus lumborum* of JBC exhibits higher shear force

than Hanwoo cattle. Moreover, JBC, which was slaughtered at a relatively older age compared to Hanwoo and KBC in this study, is suggested to have higher collagen content, more cross-linked collagen, and a more significant amount of insoluble collagen than other breeds. These factors are considered to explain the high shear force observed in JBC in this study.

TPA is extensively used to assess the texture of meat and meat products, encompassing parameters such as hardness, springiness, adhesiveness, cohesiveness, gumminess, chewiness, and resilience (Novaković and Tomašević, 2017). This study performed TPA to test the cooked texture attributes of meat from three cattle breeds (Table 3). Excluding resilience, no statistical differences were observed in the texture parameters among the groups. As mentioned above, the resilience values of JBC and Hanwoo were significantly higher when compared to KBC ( $p < 0.01$ ). Generally, among the TPA parameters, hardness and shear force are accepted as indicators for estimating meat tenderness (Caine et al., 2003). While both TPA and shear force evaluate aspects related to the tenderness of meat texture, they do so through different mechanisms: TPA through compression and Warner-Bratzler Shear force (WBS) through shearing. These differences in measurement can lead to variations in results, especially concerning aspects of texture not directly related to tenderness (Novaković and Tomašević, 2017). For this reason, both measurements were presented in this study to provide a more comprehensive evaluation. In our study, the differences in shear force among breeds, but not in hardness, are considered to be due to methodological differences.

### **Analysis of the free amino acids and constituent amino acids contents**

The analysis of the free amino acid contents in the *Longissimus lumborum* of different cattle groups is exhibited in Table 4. Compared to KBC, the two other cattle groups (JBC and Hanwoo cattle) exhibited significantly higher levels of glutamic acid, glycine, and phenylalanine, whereas KBC had a significantly higher arginine content compared to the other cattle breeds. For alanine,

Hanwoo had a significantly higher content than KBC. In the case of serine and threonine, Hanwoo showed significantly higher serine values than the other two cattle breeds and had a significantly higher threonine content than JBC.

In analysis of the constituent amino acids contents (Table 5), lysine, valine, arginine, isoleucine, and phenylalanine were observed to be significantly lower in JBC than in the other cattle breeds. On the other hand, KBC had significantly lower levels of glutamic acid compared to JBC and Hanwoo. According to previous research, an analysis of amino acids in the *longissimus dorsi* muscle of Hanwoo and JBC under identical rearing conditions showed that Hanwoo had a higher glutamic acid content than JBC (Lee et al., 2019). Our study does not provide feeding information; therefore, this study suggests that the differences in amino acid profiles are likely due to distinct metabolic pathways and dietary factors among the cattle groups. Higher levels of specific amino acids, such as arginine and glutamic acid, could have significant implications for the nutritional and flavor profiles of the *Longissimus lumborum* (Chen and Liu, 2004; Khan et al., 2015). For instance, arginine is known for its role in protein synthesis and metabolic processes (Wu et al., 2009), and glutamic acid is a key contributor to the umami taste, enhancing the meat's flavor (Kurihara, 2015).

In our study, the analysis of the constituent amino acids in *longissimus lumborum* revealed significant differences among the cattle groups. JBC showed a lower content of arginine, which is involved in protein synthesis and metabolic processes, compared to other cattle groups. Meanwhile, JBC and Hanwoo exhibited significantly higher levels of glutamic acid, which can influence umami and meat flavor, compared to KBC. These findings have significant implications for both the nutritional value and sensory attributes of meat, providing valuable information for consumers, producers, and the meat industry. Future research could further explore the factors contributing to these differences and their potential impacts on meat quality and health benefits.

## **The analysis of the fatty acids compositions**

The analysis of the composition of fatty acids in the *longissimus lumborum* of different cattle groups revealed significant differences in the proportions of several fatty acids (Table 6). Pentadecanoic acid (C15:0) was present at significantly higher levels in Hanwoo than in JBC and KBC. In terms of palmitic acid (C16:0), Hanwoo exhibits the highest concentrations, followed by KBC and JBC. Although Hanwoo also shows a higher concentration of margaric acid (C17:0) than the other two groups, margaric acid accounted for a relatively low proportion of the total fatty acids, ranging from 0.58% to 0.96% in all cattle groups. For eicosenoic acid (C20:1, n-9), a MUFA, both JBC and Hanwoo display elevated levels compared to KBC. Additionally, linolenic acid (C18:3, n-3) was found in the highest concentrations in KBC, followed by Hanwoo and JBC. Oleic acid and linoleic acid are key fatty acids commonly found in beef, as identified in previous studies (Dryden and Maechello, 1970; Enser et al., 1996). Oleic acid has been reported to contribute significantly to the desirable flavor and savory taste of meat (Dryden and Maechello, 1970). These sensory attributes are highly valued by meat consumers, indicating that a high oleic acid content can enhance the overall palatability of beef. However, in this study, there were no significant differences in these two fatty acids among the cattle groups. Conversely, it was reported that saturated fatty acids (SFA) and MUFA were positively associated with eating quality traits, while PUFA negatively correlated with eating quality (Cameron and Enser, 1991). Although the levels of certain SFA such as pentadecanoic acid, palmitic acid, and margaric acid were significantly higher in Hanwoo, and KBC had the lowest levels of the unsaturated fatty acid eicosenoic acid, there were no differences in the overall ratios of total SFA, MUFA, and PUFA among the cattle groups in this study. Overall, the distinct fatty acid compositions among the cattle groups are likely influenced by genetic factors, diet, and environmental conditions. These factors, in turn, can significantly impact the nutritional and



sensory qualities of the meat. Therefore, additional in-depth research on fatty acid composition is necessary to understand its impact on meat quality and health benefits.

### **Transcriptome comparative analysis**

The transcriptomes of the *Longissimus lumborum* of a total of nine cattle (3 cattle per group) were identified. An average of about 12.1 Gb of raw reads were generated from a total of 9 samples, and the averages of the GC and AT contents of the generated transcripts were 49.3% and 50.6% (ranges were 48.2-50.8% and 49.3-51.8%, respectively). The Q20 and Q30 percentages were 98.6-99.0% and 95.7-96.9%, respectively. The raw data statistics of the paired-end data of all cattle breeds analyzed in this study are provided in Supplementary Table S1. The generated sequences were mapped to the reference genome, and 97.8-98.3% of the total sequences were mapped to each sample. Principal component analysis (PCA) is a widely used statistical technique for dimensionality reduction in genomic studies. It transforms high-dimensional data into a lower-dimensional form while retaining most of the variance present in the original data (Lever et al., 2017).

This study performed PCA to analyze sequencing data, aiming to identify major sources of variance and visualize the relationships among cattle groups. The results confirmed clear differences among cattle groups. Principal component 1 (PC1) and principal component 2 (PC2) together explained 39.48% of the total variance, accounting for 20.51% and 18.97%, respectively. This indicates that these two components capture the most critical variations within the data. Fig. 1 summarizes the high-dimensional sequencing data, revealing distinct clusters and separations among the cattle groups. By elucidating the primary sources of variance within the dataset, PCA facilitates a comprehensive understanding of the underlying biological and experimental factors. This foundational insight paves the way for more in-depth investigations and robust interpretations of the observed data variability. This study conducted an analysis of

differential gene expression between different cattle breeds, specifically comparing JBC with Hanwoo and KBC. Volcano plots were constructed to visualize the significant changes in gene expression levels, highlighting the genes that are most significantly up- or downregulated in each comparison (Fig 2). Volcano plots are a key tool in genomics for visualizing differential expression data. They plot significance (p-value and FDR) against fold-change, allowing for the identification of genes that show both statistical significance and biologically meaningful changes in expression.

By constructing volcano plots to compare the gene expression profiles of JBC with Hanwoo and KBC, this study aimed to uncover critical genetic differences that may underpin phenotypic variations (Chen et al., 2016; Love et al., 2014; Soneson and Delorenzi, 2013). The volcano plots (Fig. 2a and b) revealed distinct patterns of differential gene expression. In the comparison between JBC and KBC, the volcano plot showed several genes that were significantly upregulated and downregulated in JBC compared to Hanwoo. Notable genes that exhibit high fold-changes and low p-values and FDR are highlighted (red dots), indicating their potential role in distinguishing the two breeds. Conversely, in the volcano plot analysis comparing JBC and Hanwoo, no genes satisfied the thresholds for both FDR and P-value.

### **Differential expression and clustering analysis of transcriptomes**

In the transcriptome analysis performed to identify the DEGs between JBC and KBC, 192 and 335 genes were upregulated and downregulated, respectively, based on an  $FDR < 0.05$  (Fig. 3). Among the 527 DEGs, the top 10 upregulated and top 10 downregulated genes were selected based on the highest absolute values of  $\log_2$  fold change. Genes with unknown or uncharacterized functions were excluded from this analysis. The top 10 upregulated genes included A Disintegrin And Metalloproteinase With Thrombospondin Motifs 8 (*ADAMTS8*), SH3 Domain Binding Kinase Family Member 3 (*SBK3*), Monoacylglycerol *O*-Acyltransferase 1

(*MOGAT1*), Histone H1.6 (*H1-6*), Heat Shock Protein Family A (Hsp70) member 6 (*HSPA6*), Natriuretic Peptide C (*NPPC*), Carbohydrate Sulfotransferase 8 (*CHST8*), Leucine Rich Repeat Containing 15 (*LRRC15*), and Regulator of G-protein Signaling 16 (*RGS16*) were selected. The top 10 downregulated genes were Glutamate Transporter 1 Domain Containing 1 (*GLT1D1*), ADP Ribosylation Factor Like GTPase 5C (*ARL5C*), WD Repeat Domain 72 (*WDR72*), Angiotensin Like 8 (*ANGPTL8*), BPI Fold Containing Family B Member 5 (*BPIFB5*), Family With Sequence Similarity 205 Member C (*FAM205C*), NOP2/Sun RNA Methyltransferase Family Member 7 (*NSUN7*), DIRAS Family GTPase 3 (*DIRAS3*), Ectodysplasin A2 Receptor (*EDA2R*), and Solute Carrier Family 38 Member 4 (*SLC38A4*) were identified. Among these genes, *MOGAT1* is involved in lipid metabolism, specifically the synthesis of diacylglycerol from monoacylglycerol, and it also plays a critical role in energy storage and lipid homeostasis (Lee and Kim, 2017; Yen et al., 2002). *ANGPTL8* is important in lipid metabolism and glucose homeostasis and regulates triglyceride levels and insulin sensitivity (Luo and Peng, 2018; Zhang, 2012). *SLC38A4* is considered to be involved in metabolic processes as an amino acid transporter, playing a role in the uptake and regulation of amino acids in various tissues (Mackenzie and Erickson, 2004). The identified genes may contribute to phenotypic differences between JBC and KBC, such as growth rate, fat deposition, or adaptability to environmental conditions. The differential expression of genes exhibiting these biological functions may contribute to variations in meat quality, as well as the composition of amino acids and fatty acids. These findings suggest that genetic differences could underlie the meat quality traits observed among different cattle breeds. Further research is warranted to explore these associations and to elucidate the precise mechanisms by which these genes influence meat quality and composition.

The heatmap in Fig. 4a shows gene expression patterns between JBC and KBC, highlighting distinct clusters of samples and genes. This clustering suggests co-regulated genes or genes with

related functions that are differentially expressed between these breeds. Genes with higher expression in JBC are grouped, indicating upregulation, while those with lower expression form separate clusters, reflecting downregulation. The distinct grouping of JBC and KBC samples underscores the genetic and phenotypic differences between the breeds, supporting the differential expression analysis. The color gradient in the heatmap visualizes gene expression levels, with brighter colors for higher expression and darker for lower, enhancing understanding of expression dynamics.

Fig. 4b presents the heatmap of DEGs between Hanwoo and KBC. Rows correspond to genes and columns to samples, with the color scale indicating normalized expression levels—red for high and blue for low. Hierarchical clustering dendrograms show sample and gene groupings based on expression pattern similarities, revealing distinct profiles between HW and KBC. This color intensity aids in identifying genes significantly upregulated or downregulated, illustrating genetic and phenotypic differences between the breeds.

### **Function annotation and pathway analysis of the DEGs**

The functional analysis results of the identified 594 DEGs (JBC vs. KBC) were classified into three groups: biological process (BP), cellular component (CC), and molecular function (MF). The DEGs were confirmed to be related to 129 BPs, 40 CCs, and 23 MFs through the DAVID website ( $p < 0.05$ ). The detailed annotation results are shown in Fig. 5(a). Functional annotations discovered for CC included collagen trimer (GO:0000005581,  $FDR < 0.5$ ).

KEGG pathway analysis was performed to explore the molecular interaction network and the biological functions of DEGs (Fig. 5(b)). The nine KEGG pathways ( $FDR < 0.1$ ) encompass cGMP-PKG signaling pathway (bta04022), gastric acid secretion (bta04971), regulation of lipolysis in adipocytes (bta04923), focal adhesion (bta04510), AMPK signaling pathway (bta04152), circadian rhythm (bta04710), ECM-receptor interaction (bta04512), adrenergic

signaling in cardiomyocytes (bta04261), and endocrine resistance (bta01522), pathways.

Notably, both the AMPK signaling pathway and the regulation of lipolysis in adipocytes exhibit interconnected roles in orchestrating energy metabolism and lipid composition within muscle tissues. Their synergistic actions are pivotal for the development of high-quality meat, characterized by desirable attributes such as marbling, tenderness, and a favorable fatty acid profile. Conversely, in the GO and KEGG pathway analyses based on FDR criteria for JBC and HW, no significant terms or pathways were identified. Consequently, terms and pathways were selected based on P-values (Supplementary Fig 2). The number of terms and pathways identified for JBC vs. HW was relatively fewer compared to those identified for JBC vs. KBC. This suggests that the variability in DEGs for JBC vs. HW is relatively smaller than that for JBC vs. KBC.

### **Network analysis**

The network analysis revealed significant differences in GO terms and KEGG pathway interactions between JBC and KBC. The analysis identified substantial network in muscle system processes, fat cell differentiation, system development, muscle tissue development, intrinsic apoptotic signaling pathways mediated by the p53 class, and the positive regulation of biological processes (Fig. 6). Furthermore, the network analysis elucidated the interrelationships between the identified terms and pathways. Additionally, a network analysis was conducted between KBC and Hanwoo. The network relationships observed in this comparison differed from those identified in the analysis between JBC and KBC. Significant functional terms and pathways, including cytokine receptor activity, release of sequestered calcium ion into the cytosol, regulation of G-protein coupled receptor protein signaling pathway, and regulation of inflammatory response, were identified (Supplementary Fig. 2). While further research is necessary to elucidate the biological characteristics and meat quality factors influencing between

KBC and Hanwoo breeds, it is evident that this network analysis has limitations in fully representing the network relationships involving fatty acids, amino acids, and other meat quality-related factors between these two breeds.

## **Conclusions**

This study conducted a comparative analysis of Hanwoo, KBC, and JBC cattle, identifying significant differences in meat quality, fatty acid and amino acid compositions, and transcriptome profiles. Although there were no differences in most meat quality parameters, JBC exhibited increased toughness, while KBC demonstrated lowered resilience. Within the constituent amino acids, glutamic acid, associated with umami, was measured at the highest proportion, with JBC and Hanwoo having higher levels than KBC. KBC had higher levels of aspartic acid, the second highest proportion among the constituent amino acids. DEGs related to lipid metabolism and energy homeostasis, such as *MOGAT1*, *ANGPTL8*, and *SLC38A4*. Network analysis highlighted differences in GO terms and KEGG pathways between JBC and KBC, indicating the significance of genetic factors in meat quality. However, the network analysis between KBC and Hanwoo showed different patterns, suggesting limitations in fully capturing the relationships involving meat quality factors. While this study acknowledges certain limitations in experimental design related to the rearing methods and feeding practices of the test animals, it nonetheless underscores the promising potential of genetic selection and breeding programs to enhance meat quality. It advocates further research into the genetic mechanisms that influence these traits.

## References

- AOAC. 2019. Official Methods of Analysis of AOAC INTERNATIONAL. 21st Edition. AOAC INTERNATIONAL, Gaithersburg, MD, USA.
- Barbut S. 2015. Chapter 16-evaluating texture and sensory attributes. The science of poultry and meat processing. In: Floor 2021. Texture methods for evaluating meat and meat analogue structures: A review.
- Bruce HL, Roy BC. 2019. Meat science and muscle biology symposium: Biological influencers of meat palatability: Production factors affecting the contribution of collagen to beef toughness<sup>1,2</sup>. *J Anim Sci* 97:2270-2278.
- Cafe LM, Robinson DL, Ferguson DM, McIntyre BL, Geesink GH, Greenwood PL. 2011. Cattle temperament: Persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. *J Anim Sci* 89:1452-1465.
- Caine WR, Aalhus JL, Best DR, Dugan MER, Jeremiah LE. 2003. Relationship of texture profile analysis and warner-bratzler shear force with sensory characteristics of beef rib steaks. *Meat Science* 64:333-339.
- Cameron ND, Enser MB. 1991. Fatty acid composition of lipid in longissimus dorsi muscle of duroc and british landrace pigs and its relationship with eating quality. *Meat Sci* 29:295-307.
- Campo MM, Sañudo C, Panea B, Alberti P, Santolaria P. 1999. Breed type and ageing time effects on sensory characteristics of beef strip loin steaks. *Meat Science* 51:383-390.
- Chakrabarti S, Guha S, Majumder K. 2018. Food-derived bioactive peptides in human health: Challenges and opportunities. *Nutrients* 10.
- Chambaz A, Scheeder MRL, Kreuzer M, Dufey PA. 2003. Meat quality of angus, simmental, charolais and limousin steers compared at the same intramuscular fat content. *Meat Science* 63:491-500.

- Chen G, Liu M. 2004. Analysis of muscle nutrition characteristics of wild pig hybrids. *Pig breeding* 1:24-27.
- Chen Y, Lun AT, Smyth GK. 2016. From reads to genes to pathways: Differential expression analysis of rna-seq experiments using rsubread and the edger quasi-likelihood pipeline. *F1000Res* 5:1438.
- Cho S-H, Hoa V-B, Song D-H, Kim DK, Kim Y-S, Kim H-W, Bae I-S, Sung PN, Park J, Song S, Cheng H, Du L, Im C, Kim G-D. 2024. Comparison of muscle fiber and meat quality characteristics of beef strip loin, tenderloin, and round cuts among jeju black cattle, hanwoo, and their crossbreeds. *Food Science of Animal Resources* 44:1181-1194.
- Chriki S, Gardner GE, Jurie C, Picard B, Micol D, Brun J-P, Journaux L, Hocquette J-F. 2012. Cluster analysis application identifies muscle characteristics of importance for beef tenderness. *BMC Biochemistry* 13:29.
- Chriki S, Renand G, Picard B, Micol D, Journaux L, Hocquette JF. 2013. Meta-analysis of the relationships between beef tenderness and muscle characteristics. *Livestock Science* 155:424-434.
- Davis H, Magistrali A, Butler G, Stergiadis S. 2022. Nutritional benefits from fatty acids in organic and grass-fed beef. *Foods* 11.
- Dryden FD, Maechello JA. 1970. Influence of total lipid and fatty acid composition upon the palatability of three bovine muscles. *Journal of Animal Science* 31:36-41.
- Enser M, Hallett K, Hewitt B, Fursey GaJ, Wood JD. 1996. Fatty acid content and composition of english beef, lamb and pork at retail. *Meat Science* 42:443-456.
- Fernyhough ME, Okine E, Hausman G, Vierck JL, Dodson MV. 2007. Ppar $\gamma$  and glut-4 expression as developmental regulators/markers for preadipocyte differentiation into an adipocyte. *Domestic Animal Endocrinology* 33:367-378.



- Gagaoua M, Terlouw C, Micol D, D., Hocquette J-F, J.-F., Moloney AP, Nuernberg K, Bauchart D, Boudjellal A, Scollan N, Richardson RI, Picard B, B. 2016. Sensory quality of meat from eight different types of cattle in relation with their biochemical characteristics. *Journal of Integrative Agriculture* 15:1550–1563.
- Gama LT, Bressan MC, Rodrigues EC, Rossato LV, Moreira OC, Alves SP, Bessa RJB. 2013. Heterosis for meat quality and fatty acid profiles in crosses among *bos indicus* and *bos taurus* finished on pasture or grain. *Meat Science* 93:98-104.
- Han S-H, Cho I-C, Kim J-H, Ko M-S, Kim Y-H, Kim E-Y, Park S-P, Lee S-S. 2011. Coat color patterns and genotypes of extension and agouti in hanwoo and jeju black cattle. *Journal of Life Science* 21:494-501.
- Hoa V-B, Song D-H, Seol K-H, Kang S-M, Kim H-W, Bae I-S, Kim E-S, Park Y-S, Cho S-H. 2023a. A comparative study on the carcass and meat chemical composition, and lipid-metabolism-related gene expression in korean hanwoo and brindle chikso cattle. *Current Issues in Molecular Biology* 45:3279-3290.
- Hoa V-B, Song D-H, Seol K-H, Kang S-M, Kim H-W, Bae I-S, Kim E-S, Park Y-S, Cho S-H. 2023b. A comparative study on the meat quality, taste and aroma related compounds between korean hanwoo and chikso cattle. *Foods* 12:805.
- Hoa V-B, Kim DG, Song DH, Ko JH, Kim HW, Bae IS, Kim YS, Cho SH. 2024. Quality properties and flavor-related components of beef longissimus lumborum muscle from four korean native cattle breeds. *Food Sci Anim Resour* 44:832-848.
- Jo C, Cho SH, Chang J, Nam KC. 2012. Keys to production and processing of hanwoo beef: A perspective of tradition and science. *Animal Frontiers* 2:32-38.
- Khan MI, Jo C, Tariq MR. 2015. Meat flavor precursors and factors influencing flavor precursors—a systematic review. *Meat Science* 110:278-284.
- Kirimura J, Shimizu A, Kimizuka A, Ninomiya T, Katsuya N. 1969. Contribution of peptides and

- amino acids to the taste of foods. *Journal of Agricultural and Food Chemistry* 17:689-695.
- Kurihara K. 2015. Umami the fifth basic taste: History of studies on receptor mechanisms and role as a food flavor. *Biomed Res Int* 2015:189402.
- Lee D, Kim HJ, Ismail A, Kim SS, Yim DG, Jo C. 2023. Evaluation of the physicochemical, metabolomic, and sensory characteristics of chikso and hanwoo beef during wet aging. *Anim Biosci* 36:1101-1119.
- Lee S-H, Park B-H, Sharma A, Dang C-G, Lee S-S, Choi T-J, Choy Y-H, Kim H-C, Jeon K-J, Kim S-D, Yeon S-H, Park S-B, Kang H-S. 2014. Hanwoo cattle: Origin, domestication, breeding strategies and genomic selection. *Journal of Animal Science and Technology* 56:2.
- Lee SH, Kim CN, Ko KB, Park SP, Kim HK, Kim JM, Ryu YC. 2019. Comparisons of beef fatty acid and amino acid characteristics between jeju black cattle, hanwoo, and wagyu breeds. *Food Sci Anim Resour* 39:402-409.
- Lee YJ, Kim JW. 2017. Monoacylglycerol o-acyltransferase 1 (mgat1) localizes to the er and lipid droplets promoting triacylglycerol synthesis. *BMB Rep* 50:367-372.
- Lever J, Krzywinski M, Altman N. 2017. Principal component analysis. *Nature Methods* 14:641-642.
- Lindemann B. 1996. Taste reception. *Physiol Rev* 76:719-766.
- Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for rna-seq data with deseq2. *Genome Biology* 15:550.
- Luo M, Peng D. 2018. Angptl8: An important regulator in metabolic disorders. *Front Endocrinol (Lausanne)* 9:169.
- Mackenzie B, Erickson JD. 2004. Sodium-coupled neutral amino acid (system n/a) transporters of the slc38 gene family. *Pflügers Archiv* 447:784-795.

- Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, Zuker CS. 2002. An amino-acid taste receptor. *Nature* 416:199-202.
- Novaković S, Tomašević I. 2017. A comparison between warner-bratzler shear force measurement and texture profile analysis of meat and meat products: A review. *IOP Conference Series: Earth and Environmental Science* 85:012063.
- Rabia Shabir A, Ali I, Muhammad Bilal H. 2018. Nutritional composition of meat. In *Meat science and nutrition*. Muhammad Sajid A (ed.). IntechOpen, Rijeka.
- Raes K, Balcaen A, Dirinck P, De Winne A, Claeys E, Demeyer D, De Smet S. 2003. Meat quality, fatty acid composition and flavour analysis in belgian retail beef. *Meat Science* 65:1237-1246.
- SAS Institute Inc. 2023. *SAS/STAT® 15.3 User's Guide*. Cary, NC: SAS Institute Inc.
- Shallenberger RS, Acree TE. 1967. Molecular theory of sweet taste. *Nature* 216:480-482.
- Soneson C, Delorenzi M. 2013. A comparison of methods for differential expression analysis of rna-seq data. *BMC Bioinformatics* 14:91.
- Underwood KR, Means WJ, Zhu MJ, Ford SP, Hess BW, Du M. 2008. Amp-activated protein kinase is negatively associated with intramuscular fat content in longissimus dorsi muscle of beef cattle. *Meat Science* 79:394-402.
- Utama DT, Lee CW, Park YS, Jang A, Lee SK. 2018. Comparison of meat quality, fatty acid composition and aroma volatiles of chikso and hanwoo beef. *Asian-Australas J Anim Sci* 31:1500-1506.
- Wang Z, Zhu B, Niu H, Zhang W, Xu L, Xu L, Chen Y, Zhang L, Gao X, Gao H, Zhang S, Xu L, Li J. 2019. Genome wide association study identifies snps associated with fatty acid composition in chinese wagyu cattle. *Journal of Animal Science and Biotechnology* 10:27.
- Wu G, Bazer FW, Davis TA, Kim SW, Li P, Marc Rhoads J, Carey Satterfield M, Smith SB,

Spencer TE, Yin Y. 2009. Arginine metabolism and nutrition in growth, health and disease. *Amino Acids* 37:153-168.

Yen CL, Stone SJ, Cases S, Zhou P, Farese RV, Jr. 2002. Identification of a gene encoding mgat1, a monoacylglycerol acyltransferase. *Proc Natl Acad Sci U S A* 99:8512-8517.

Zhang R. 2012. Lipasin, a novel nutritionally-regulated liver-enriched factor that regulates serum triglyceride levels. *Biochem Biophys Res Commun* 424:786-792.

Zhang T, Niu Q, Wang T, Zheng X, Li H, Gao X, Chen Y, Gao H, Zhang L, Liu GE, Li J, Xu L. 2022. Comparative transcriptomic analysis reveals diverse expression pattern underlying fatty acid composition among different beef cuts. *Foods* 11.

ACCEPTED

Table 1. Korean Hanwoo Beef Grading Standards

Grade	Marbling Score <sup>1</sup>	Meat Color <sup>2</sup>	Fat Color <sup>3</sup>	Texture <sup>4</sup>	Maturity <sup>5</sup>
	(1 to 9)	(1 to 7)	(1 to 7)	(1 to 5)	(1 to 9)
1++	7, 8, 9	3, 4, 5	1, 2, 3, 4	1	8, 9
1+	6	3, 4, 5	1, 2, 3, 4	1	8, 9
1	4, 5	3, 4, 5	1, 2, 3, 4	1	Normal (1-7)
2	2, 3	3, 4, 5	1, 2, 3, 4	1	Normal (1-7)
3	1	Other	Other	5	Normal (1-7)

<sup>1</sup> Marbling Score (1 to 9): Evaluates the distribution of intramuscular fat with higher scores indicating greater marbling. Higher grades are given to beef with higher marbling, and marbling scores of 7, 8, or 9 correspond to grade 1++.

<sup>2</sup> Meat Color (1 to 7): Evaluates the color of the meat, with overly pale or overly dark colors being undesirable. Grades 3 to 5 are considered ideal for high-quality beef.

<sup>3</sup> Fat Color (1 to 7): Assesses the color of fat within the muscle and the surrounding fat. Grades 1 to 4 are rated highest for fat quality and are categorized as 1++.

<sup>4</sup> Texture (1 to 5): Assesses the fineness and elasticity of the muscle fibers. The best texture (grade 1) is rated as 1++.

<sup>5</sup> Maturity (1 to 9): Refers to the degree of aging (related to the animal's age). Higher scores (8 to 9) indicate advanced maturity without significant quality degradation. Maturity is judged based on connective tissue presence.

Table 2. Characteristics of meat quality of *Longissimus lumborum* among cattle groups.

	JBC	Hanwoo	KBC	LS
Water contents (%)	59.67 ± 5.60 <sup>1)</sup>	57.81 ± 1.52	59.81 ± 3.10	NS
Crude protein (%)	18.39 ± 2.42	18.45 ± 0.98	17.61 ± 1.04	NS
Crude fat (%)	20.13 ± 4.22	20.56 ± 2.90	19.63 ± 3.51	NS
Crude ash (%)	0.75 ± 0.19	0.89 ± 0.11	0.79 ± 0.08	NS
Temperature (°C)	4.60 ± 0.67	5.67 ± 1.10	4.54 ± 0.75	NS
pH	5.59 ± 0.16	5.71 ± 0.23	5.43 ± 0.13	NS
L*	39.10 ± 4.29	41.36 ± 2.35	43.18 ± 4.06	NS
a*	19.94 ± 0.79	20.74 ± 1.02	20.36 ± 2.00	NS
b	9.52 ± 0.56	10.67 ± 1.02	10.41 ± 1.02	NS
Cooking loss (%)	18.49 ± 1.61	17.67 ± 1.29	17.06 ± 4.10	NS

Abbreviations: JBC, Jeju black cattle; KBC, Korean black Cattle; LS, level of significance; L\*, lightness; a\*, redness; b\*, yellowness.

<sup>1)</sup> Mean ± standard deviation

Level of significance: NS, not significant.

Table 3. Texture profile analysis Shear force on sirloin among cattle groups.

	JBC	Hanwoo	KBC	LS
Shear force (g)	8591.98 ± 1128.72 <sup>a</sup>	5656.50 ± 1692.84 <sup>b</sup>	6422.62 ± 650.93 <sup>b</sup>	*
Hardness (g)	2938.03 ± 291.21	2714.35 ± 523.22	2816.29 ± 190.24	NS
Adhesiveness (mJ)	1.10 ± 0.75	1.16 ± 0.18	0.93 ± 0.43	NS
Resilience	0.11 ± 0.01 <sup>a</sup>	0.10 ± 0.01 <sup>a</sup>	0.07 ± 0.01 <sup>b</sup>	**
Cohesiveness	0.33 ± 0.01	0.34 ± 0.03	0.30 ± 0.05	NS
Springiness (mm)	5.81 ± 0.29	6.75 ± 0.34	6.07 ± 0.30	NS
Gumminess (g)	969.62 ± 105.09	883.98 ± 117.44	790.94 ± 108.36	NS
Chewiness (mJ)	56.43 ± 5.54	54.21 ± 5.05	51.24 ± 12.54	NS

Abbreviations: JBC, Jeju black cattle; KBC, Korean black Cattle; LS, level of significance; L\*, lightness; a\*, redness; b\*, yellowness.

<sup>1)</sup> Mean ± standard deviation

<sup>a,b</sup> Means with different superscripts in the same row significantly differ.

Level of significance: NS, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$

Table 4. Analysis of the free amino acid in *Longissimus lumborum* among cattle groups

(%)	JBC	Hanwoo	KBC	LS
1-methyl-L-histidine	-	-	-	-
3-methyl-L-histidine	-	-	-	-
Alanine	6.49 ± 0.36 <sup>ab, 1)</sup>	7.15 ± 1.33 <sup>a</sup>	5.14 ± 1.19 <sup>b</sup>	*
Arginine	7.38 ± 2.83 <sup>b</sup>	4.68 ± 1.92 <sup>b</sup>	24.05 ± 12.87 <sup>a</sup>	*
Asparagine	-	-	2.01 ± 0.19	-
Aspartic acid	-	1.19 ± 0.1	-	-
Carnosine	-	2.63 ± 1.12	-	-
Cystine	-	-	-	-
Glutamic acid	7.18 ± 1.13 <sup>a</sup>	6.93 ± 2.51 <sup>a</sup>	3.14 ± 2.06 <sup>b</sup>	*
Glycine	1.51 ± 0.26 <sup>a</sup>	1.65 ± 0.41 <sup>a</sup>	0.8 ± 0.4 <sup>b</sup>	*
Histidine	1.47 ± 1.09	0.92 ± 0.04	0.63 ± 0.1	NS
Hydroxy-proline	-	-	-	-
Lysine	1.59 ± 0.75	1.33 ± 0.67	-	NS
Methionine	0.77 ± 0.57	1.34 ± 0.3	1.28 ± 0.55	NS
O-Phosphoethanolamine	8.71 ± 4.48	5.6 ± 0.39	5.99 ± 2.45	NS
Ornithine	-	1.27 ± 0.03	0.67 ± 0.43	NS
Phenylalanine	2.98 ± 1.1 <sup>a</sup>	2.85 ± 0.66 <sup>a</sup>	1.08 ± 0.45 <sup>b</sup>	**
Phospho-L-serine	0.54 ± 0.4	2.82 ± 4.07	-	NS
Proline	-	0.95 ± 0.22	-	-
Serine	0.68 ± 0.09 <sup>b</sup>	1.77 ± 0.2 <sup>a</sup>	0.72 ± 0.25 <sup>b</sup>	***
Threonine	0.60 ± 0.15 <sup>b</sup>	1.18 ± 0.02 <sup>a</sup>	0.93 ± 0.12 <sup>ab</sup>	*
Tryptophan	54.47 ± 5.33	50.52 ± 3.32	47.85 ± 9.25	NS



Tyrosine	1.94 ± 0.44	2.33 ± 0.54	1.47 ± 0.52	NS
Urea	0.66 ± 0.47	-	0.38 ± 0.12	NS
Valine	0.81 ± 0.53	1.51 ± 0.22	0.86 ± 0.17	NS
Citrulline	-	-	-	-
Isoleucine	1.95 ± 0.65	1.77 ± 0.23	1.2 ± 0.25	NS
α-Amino-n-butyric acid	-	-	-	-
α-Aminoadipic acid	-	-	-	-
β-Aminoisobutyric acid	1.75 ± 1.02	-	-	-
γ-Amino-n-butyric acid	1.75 ± 1.31	1.15 ± 0.12	0.68 ± 0.25	NS
Leucine	3.91 ± 1.56	2.84 ± 1.23	2.32 ± 0.38	NS
Taurine	-	-	-	-
β-Alanine	-	-	-	-

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Abbreviations: JBC, Jeju black cattle; KBC, Korean black Cattle; LS, level of significance.

<sup>1)</sup> Mean ± standard deviation

- When observations are not available, or a statistical mean cannot be determined.

<sup>a,b</sup> Means with different superscripts in the same row significantly differ.

Level of significance: NS, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Table 5. Analysis of the constituent amino acid contents in *Longissimus lumborum* among cattle groups

(%)	JBC	Hanwoo	KBC	LS
Tyrosine	3.46 ± 0.23	3.62 ± 0.26	3.68 ± 0.45	NS
Glycine	4.49 ± 0.11	4.6 ± 0.09	4.76 ± 0.21	NS
Serine	4.13 ± 0.49	3.93 ± 0.15	3.67 ± 0.03	NS
Alanine	5.78 ± 0.5	5.78 ± 0.54	5.69 ± 0.03	NS
Aspartic Acid	10.92 ± 0.54 <sup>b</sup>	10.29 ± 0.62 <sup>b</sup>	13.32 ± 0.2 <sup>a</sup>	***
Glutamic Acid	17.33 ± 1.37 <sup>a</sup>	16.29 ± 1.04 <sup>a</sup>	14.31 ± 0.18 <sup>b</sup>	**
Lysine	9.00 ± 0.22 <sup>b</sup>	9.58 ± 0.25 <sup>a</sup>	9.81 ± 0.09 <sup>a</sup>	***
Leucine	7.68 ± 0.32	8.25 ± 0.5	7.9 ± 0.14	NS
Methionine	0.82 ± 0.08	1.82 ± 1.12	0.6 ± 0.15	NS
Valine	3.74 ± 0.56 <sup>b</sup>	4.68 ± 0.29 <sup>a</sup>	4.37 ± 0.1 <sup>a</sup>	*
Arginine	5.77 ± 0.18 <sup>b</sup>	6.45 ± 0.47 <sup>a</sup>	6.29 ± 0.07 <sup>a</sup>	**
Isoleucine	3.87 ± 0.77 <sup>b</sup>	4.88 ± 0.3 <sup>a</sup>	5.03 ± 0.16 <sup>a</sup>	*
Threonine	4.55 ± 0.13 <sup>b</sup>	4.68 ± 0.11 <sup>b</sup>	4.94 ± 0.07 <sup>a</sup>	***
Phenylalanine	3.56 ± 0.36 <sup>b</sup>	4.19 ± 0.27 <sup>a</sup>	4.06 ± 0.08 <sup>a</sup>	*
Proline	3.08 ± 0.51	3.1 ± 0.3	3.53 ± 0.17	NS
Histidine	5.68 ± 0.83	5.02 ± 0.43	5.14 ± 0.11	NS
Cystine	3.4 ± 4.17	1.77 ± 2.21	1.02 ± 0.09	NS
Tryptophan	3.23 ± 1.89	1.05 ± 0.05	1.94 ± 0.49	NS

Abbreviations: JBC, Jeju black cattle; KBC, Korean black Cattle; LS, level of significance.

<sup>1)</sup> Mean ± standard deviation

<sup>a,b</sup> Means with different superscripts in the same row significantly differ.

Level of significance: NS, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Table 6. Analysis of the fatty acids composition in *Longissimus lumborum* among cattle groups

%	JBC	Hanwoo	KBC	LS
Capric acid (C10:0)	0.07 ± 0.04 <sup>1)</sup>	0.05 ± 0.01	0.05 ± 0.01	NS
Lauric acid (C12:0)	0.11 ± 0.05	0.1 ± 0.04	0.09 ± 0.02	NS
Myristic acid (C14:0)	2.9 ± 1.04	3.42 ± 0.48	3.03 ± 0.33	NS
Tetradecanoic acid (C14:0)	1.15 ± 0.62	0.79 ± 0.22	1.13 ± 0.22	NS
Pentadecanoic acid (C15:0)	0.23 ± 0.06 <sup>b</sup>	0.37 ± 0.04 <sup>a</sup>	0.23 ± 0.03 <sup>b</sup>	**
Palmitic acid (C16:0)	24.41 ± 3.12 <sup>b</sup>	29.37 ± 1.89 <sup>a</sup>	26.96 ± 0.95 <sup>b</sup>	*
Hexadecanoic acid (C16:0)	3.05 ± 3.32	2.11 ± 2.13	5.7 ± 0.6	NS
Margaric acid (C17:0)	0.62 ± 0.12 <sup>b</sup>	0.92 ± 0.32 <sup>a</sup>	0.58 ± 0.05 <sup>b</sup>	*
Stearic acid (C18:0)	9.44 ± 1.25	11.28 ± 0.39	9.9 ± 1.15	NS
Elaidic acid (C18:1, trans)	1.76 ± 0.55	2.41 ± 1.63	1.39 ± 0.22	NS
Oleic acid (C18:1, n-9)	52.98 ± 7.03	49.16 ± 5.88	47.76 ± 1.37	NS
Linoleic acid (C18:2, n-6)	2.3 ± 0.55	2.84 ± 0.51	2.29 ± 0.35	NS
Arachidic acid (C20:0)	0.14 ± 0.17	0.08 ± 0.04	0.05 ± 0.01	NS
Eicosenoic acid (C20:1, n-9)	0.31 ± 0.1 <sup>a</sup>	0.29 ± 0.11 <sup>a</sup>	0.15 ± 0.04 <sup>b</sup>	*
Linolenic acid (C18:3, n-3)	0.11 ± 0.04 <sup>b</sup>	0.24 ± 0.14 <sup>ab</sup>	0.27 ± 0.07 <sup>a</sup>	*
Eicosatrienoic acid (C20:3, n-6)	0.15 ± 0.05	0.15 ± 0.07	0.13 ± 0.04	NS
Tricosanoic acid (C23:0)	0.29 ± 0.08	0.3 ± 0.08	0.23 ± 0.06	NS
Saturated fatty acid	42.38 ± 6.96	44.9 ± 7.5	47.92 ± 1.32	NS
Monounsaturated fatty acids	55.05 ± 6.99	51.87 ± 7.08	49.31 ± 1.32	NS
Polyunsaturated fatty acids	2.57 ± 0.62	3.24 ± 0.49	2.77 ± 0.45	NS
Unsaturated fatty acids	57.62 ± 6.96	55.1 ± 7.52	52.08 ± 1.31	NS

Abbreviations: JBC, Jeju black cattle; KBC, Korean black Cattle; LS, level of significance.

<sup>1)</sup> Mean  $\pm$  standard deviation

<sup>a,b</sup> Means with different superscripts in the same row significantly differ.

Level of significance: NS, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

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Supplementary Table S1

Sample	Total read bases (bp)	Total reads	GC (%)	AT (%)	Q20 (%)	Q30 (%)
JBC1	10208732560	101076560	49.5	50.5	98.9	96.5
JBC2	13157838832	130275632	48.2	51.8	98.9	96.6
JBC3	13364442614	132321214	48.7	51.3	98.9	96.4
Hanwoo1	11019065054	109099654	49.8	50.2	98.6	95.7
Hanwoo2	11062483944	109529544	49.4	50.6	98.7	95.9
Hanwoo3	13324970602	131930402	49.2	50.8	99	96.9
KBC1	12695083698	125693898	49.3	50.7	98.9	96.4
KBC2	11705929088	115900288	50.7	49.3	98.8	96.2
KBC3	11561256284	114467884	49.7	50.3	98.6	95.6

Abbreviations: JBC, Jeju black cattle; KBC, Korean black cattle; GC, Guanine-Cytosine content;

AT, Adenine-Thymine content; Q20, Quality Score 20; Q30, Quality Score 30

## Figure legend

Fig 1. Principal component analysis (PCA) of genetic variation among Hanwoo, Jeju black cattle (JBC), and Korean black cattle (KBC). The PCA plot illustrates the clustering of individual samples from each cattle breed based on their genetic variation. Principal Component 1 (PC1) and Principal Component 2 (PC2) explain 20.51% and 18.97% of the total variance, respectively. Hanwoo samples are represented by red circles, KBC samples by blue circles, and JBC samples by green circles.

Fig 2.

Volcano plot of differentially expressed genes (DEGs). (a) Jeju black cattle (JBC) versus Korean black cattle (KBC). (b) KBC versus Hanwoo (HW). The volcano plot displays the  $\log_2$  fold change ( $\log_2FC$ ) on the x-axis and the  $-\log_{10}$  p-value on the y-axis for each gene. Genes with significant differential expression ( $FDR < 0.05$ ) are highlighted. Red dots represent up- or down-regulated genes in JBC compared to KBC. Genes that do not meet the significance criteria are shown in gray.

Fig 3.

Number of up and down regulated differentially expressed genes (DEGs). Jeju black cattle (JBC), Korean black cattle (KBC), Hanwoo (HW).  $FDR < 0.05$ .

Fig 4.

Heatmap of differentially expressed genes (DEGs) (a) Jeju black cattle (JBC) versus Korean black cattle (KBC). (b) Hanwoo (HW) versus KBC. The heatmap represents the expression levels of DEGs across the cattle groups. Each row corresponds to a gene, and each column represents an individual sample. The color scale indicates the normalized expression levels, with red representing high expression and blue representing low expression. Clustering dendrograms on the top and left of the heatmap indicate the hierarchical clustering of samples and genes, respectively.

Fig 5.

Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis of differentially expressed genes (DEGs) between Jeju black Cattle (JBC) and Korean black cattle (KBC). (a) GO enrichment analysis: Bar plot showing the top enriched GO terms categorized into Biological Process, Molecular Function, and Cellular Component. The Y-axis represents the GO terms, and the X-axis represents the number of related genes. (b) KEGG pathway analysis: Bar plot illustrating the top enriched KEGG pathways. The x-axis represents the number of related genes and the y-axis lists the KEGG pathways. The color gradient indicates the adjusted p-value.

Fig 6.

The network analysis of significant differences in gene ontology terms and Kyoto encyclopedia of genes and genomes pathway interactions between Jeju black cattle and Korean black cattle.

SFig.1

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of differentially expressed genes (DEGs) between Korean black cattle (KBC) and Hanwoo (HW). (a) GO enrichment analysis: Bar plot showing the top enriched GO terms categorized into Biological Process, Molecular Function, and Cellular Component. The Y-axis represents the GO terms, and the X-axis represents the number of related genes. (b) KEGG pathway analysis: Bar plot illustrating the top enriched KEGG pathways. The x-axis represents the number of related genes and the y-axis lists the KEGG pathways. The color gradient indicates the adjusted p-value.

SFig.2

The network analysis of significant differences in gene ontology terms and Kyoto encyclopedia of genes and genomes pathway interactions between Korean black cattle and Hanwoo.

Fig. 1

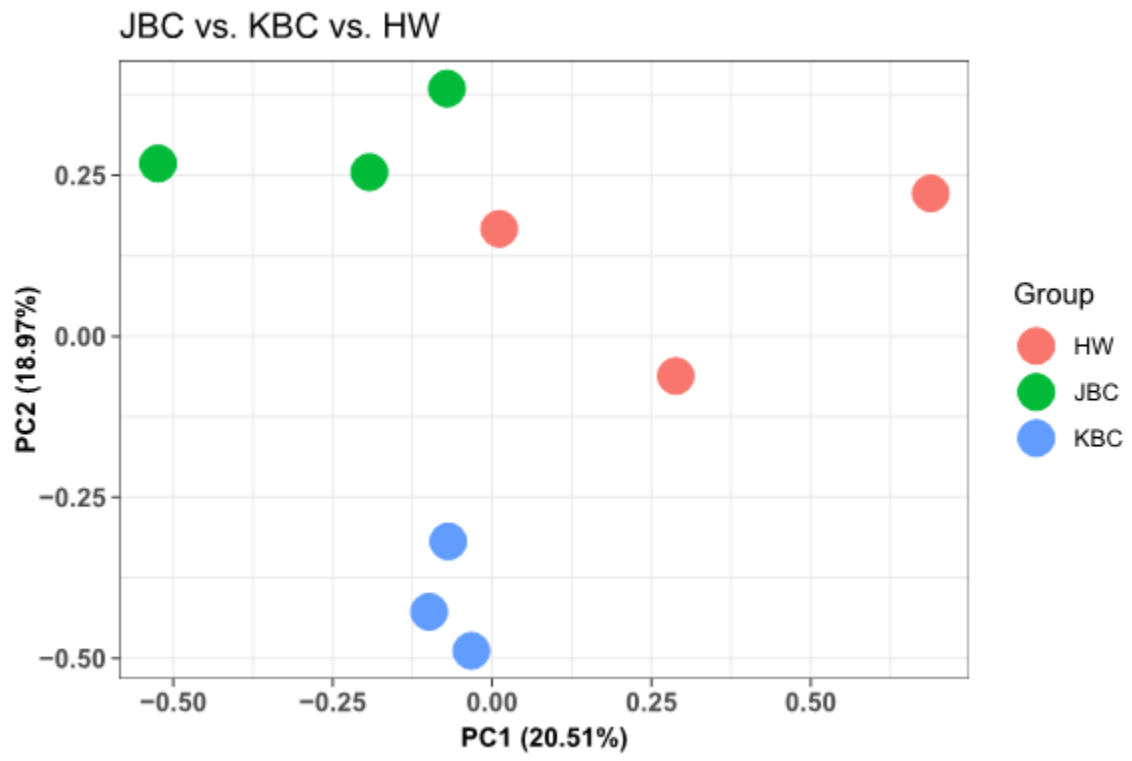
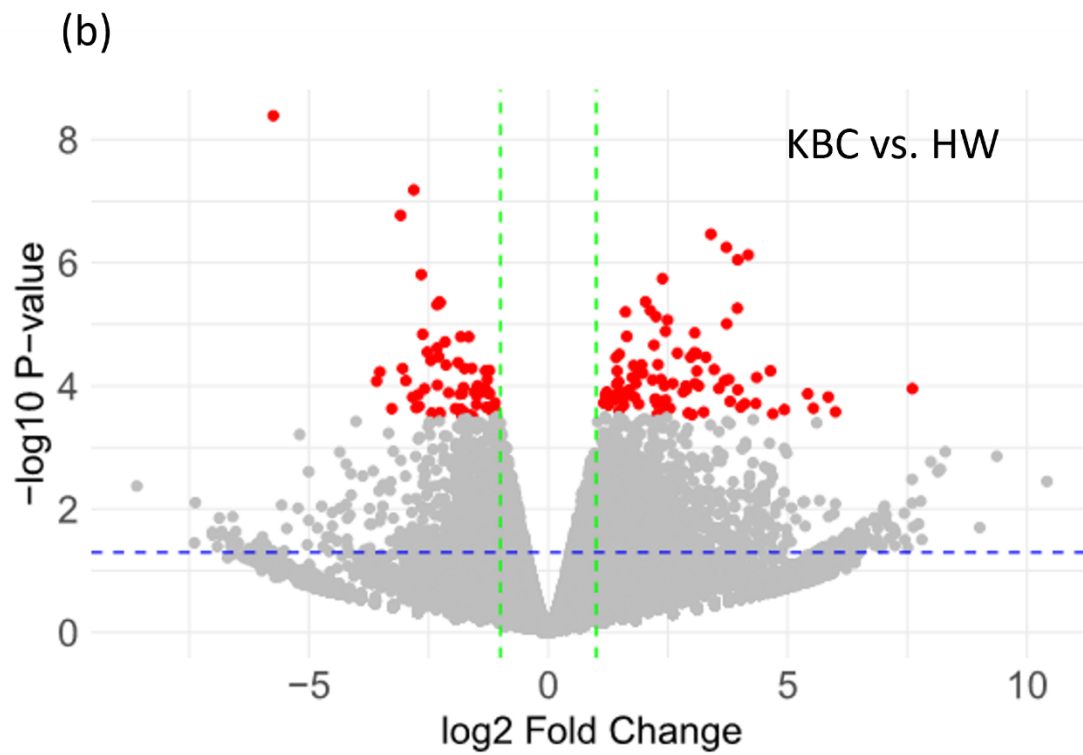
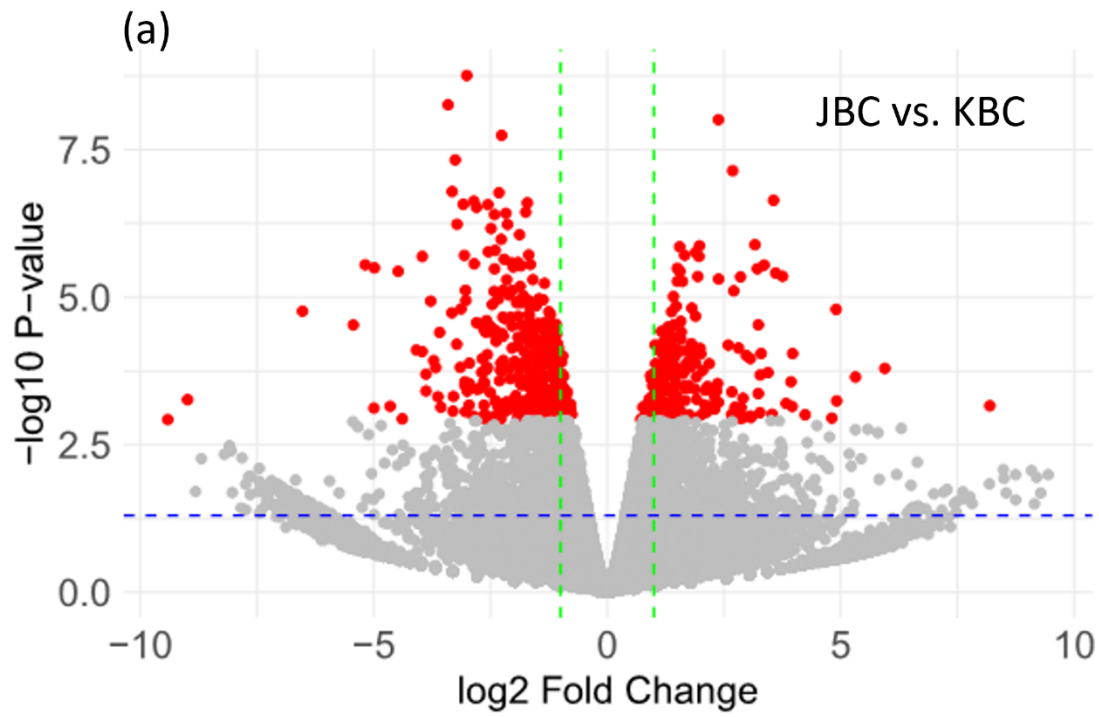
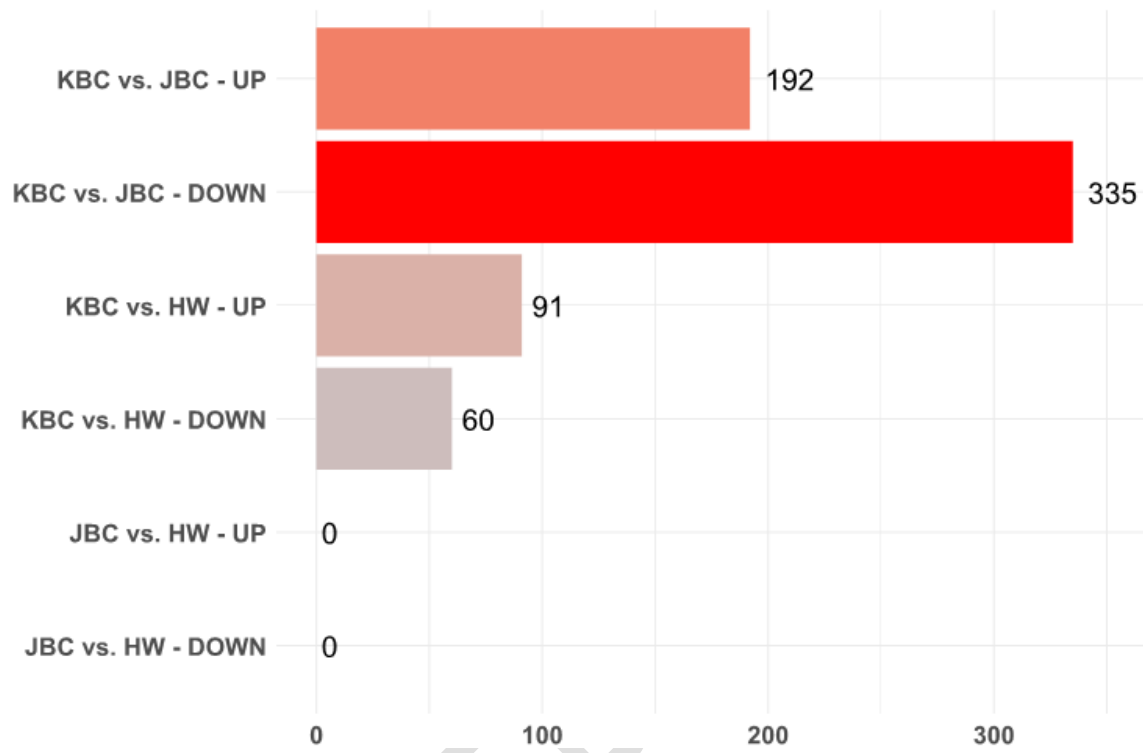




Fig. 2

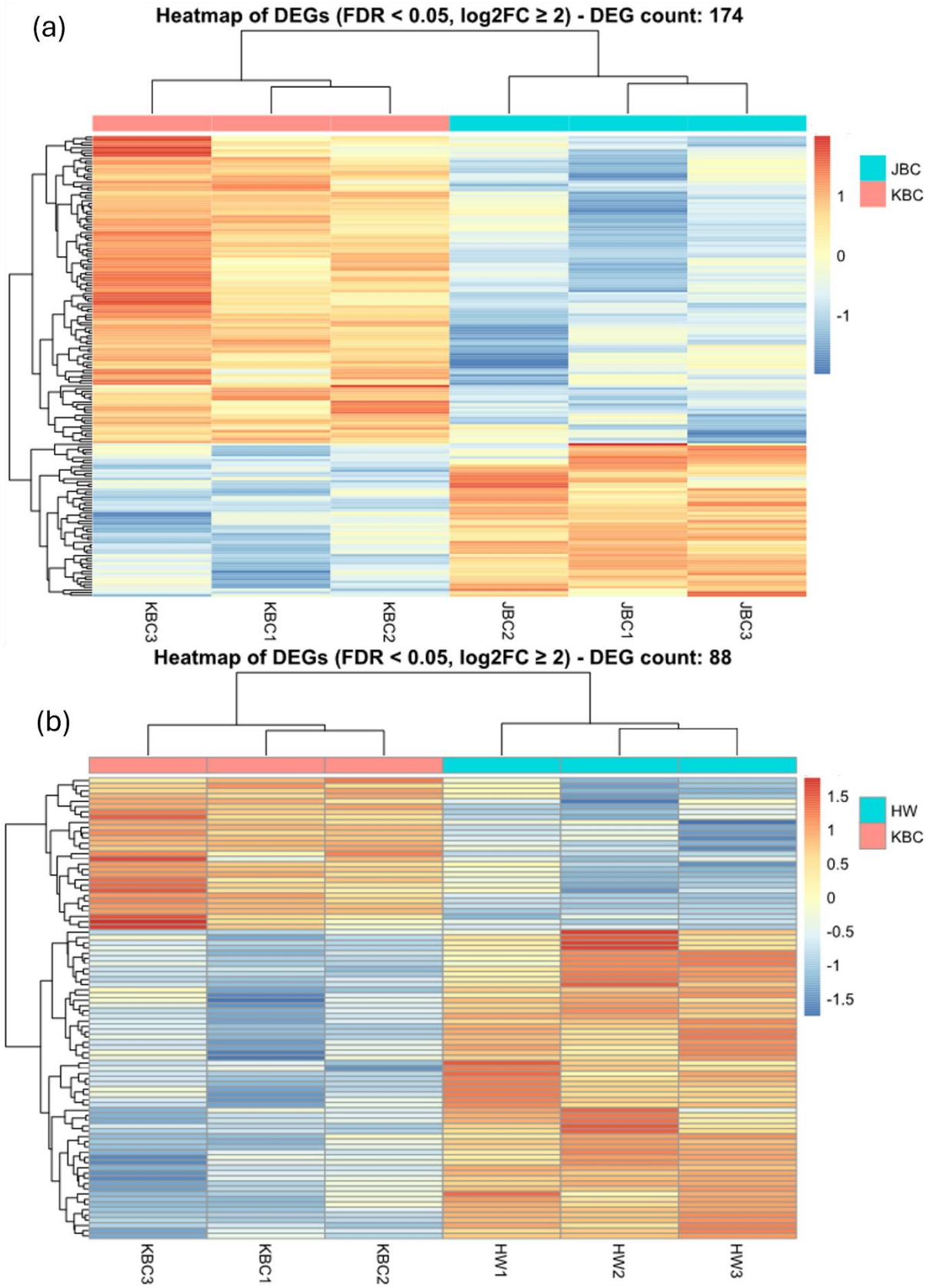


**Fig. 3**



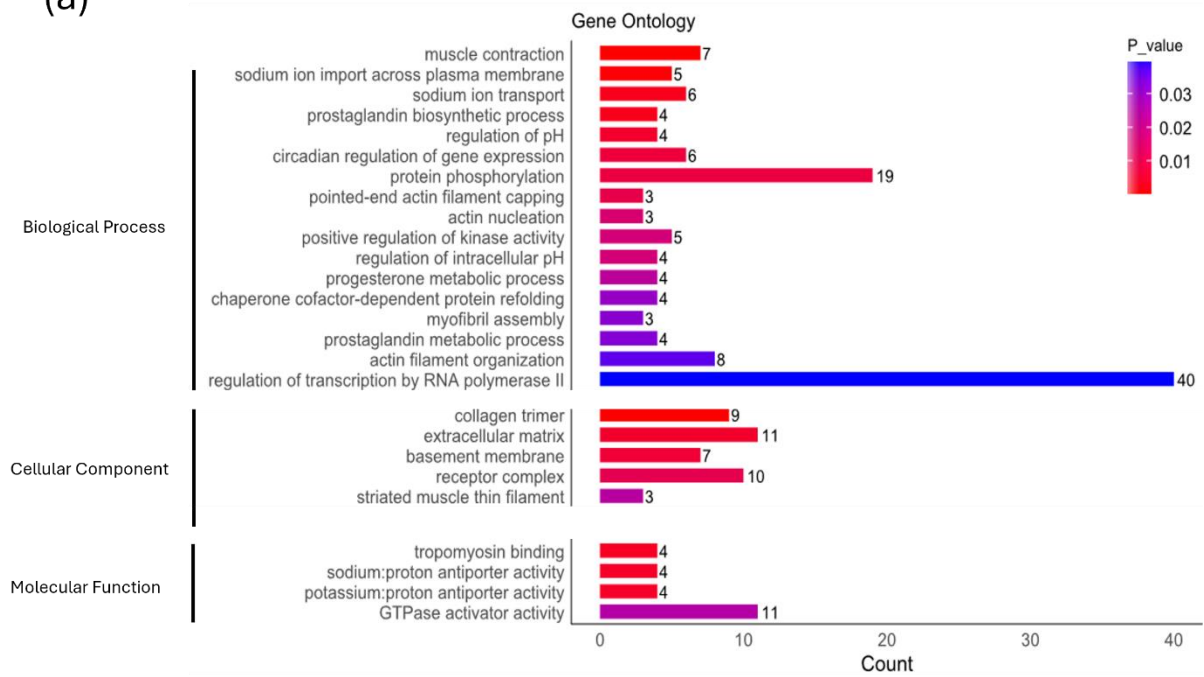
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**Fig 4.**

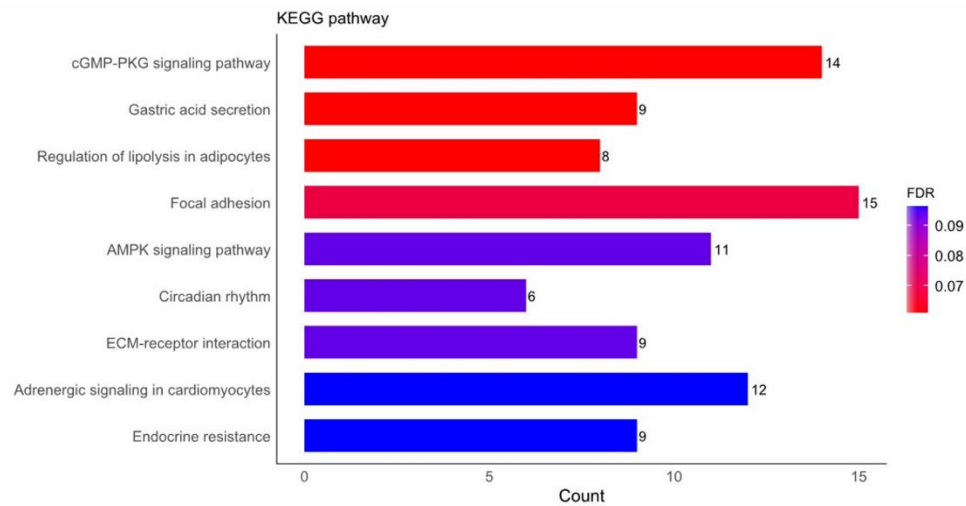


**Fig. 5**

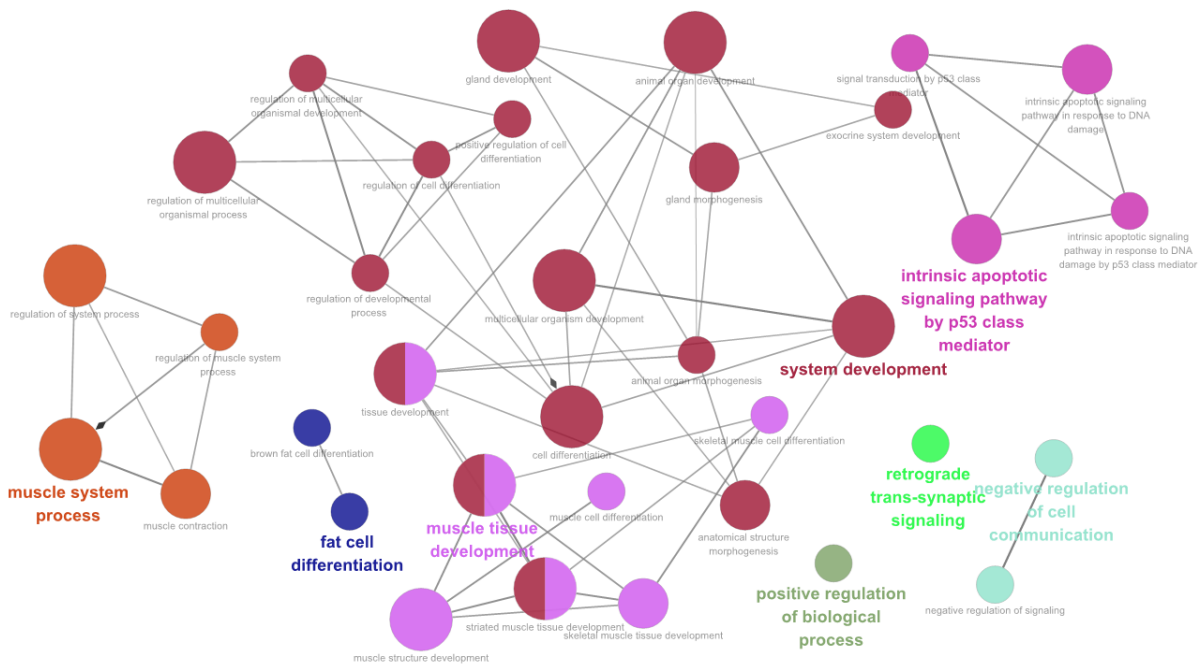
(a)



(b)

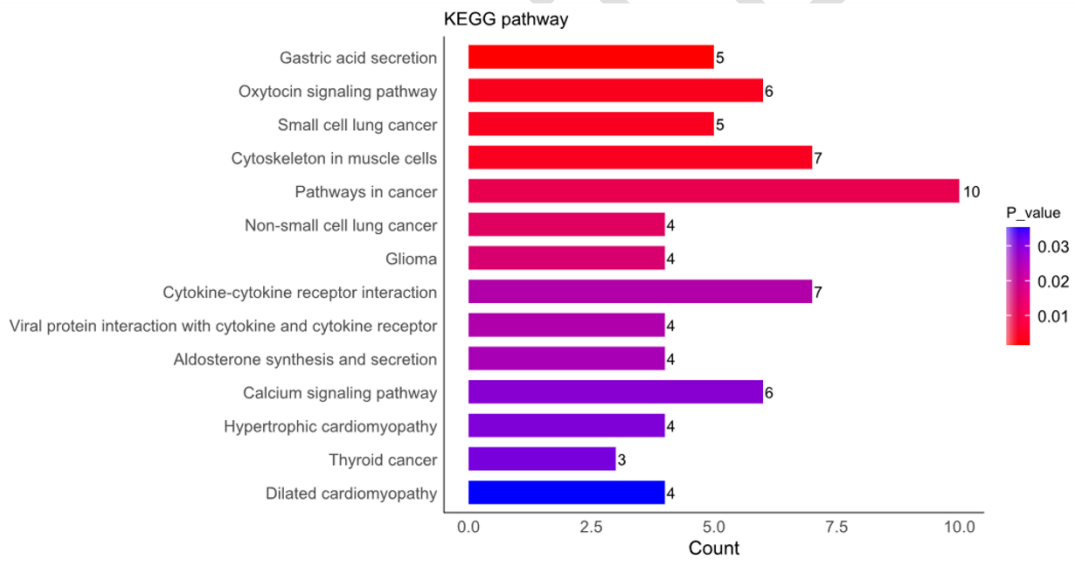
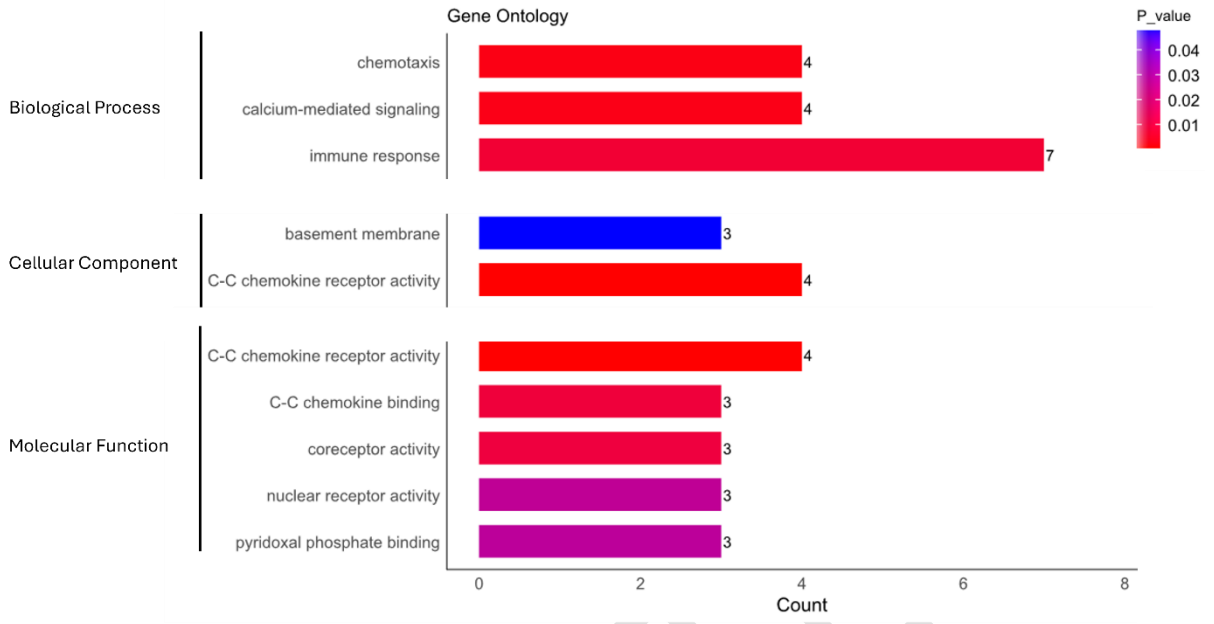


**Fig. 6**



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# Supplementary Fig S1



## Supplementary Fig S2

