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Expression analysis of m^{6}A-related genes in various tissues of Meishan pigs at different developmental stages

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ABSTRACT - To characterize the N6-methyladenosine (m^{6}A)-related gene expression profiles in various tissues of Meishan pigs at different stages, m^{6}A modification-related genes (*METTL3*, *METTL14*, *METTL16*, *WTAP*, *RBM15*, and *FTO*) were detected from newborn to physical maturity of Meishan pigs at eight important developmental stages (1, 7, 14, 21, 28, 35, 134, and 158 days old). The expression of m^{6}A-related genes was tissue-specific. Furthermore, the level of *METTL3* messenger RNA (mRNA) was higher on day 35 than in other stages in most tissues, and the expression of *METTL14* increased after day 35, and *FTO* exhibited a peak on day 14 in muscle, intestine, lymph nodes, thymus, and kidney. This study provided a reference for an in-depth study of the expression patterns of m^{6}A modification-related genes in Meishan pigs.

Keywords: *FTO*, *METTL3*, tissue expression profile, weaned piglets

1. Introduction

The Chinese Meishan pig breed is well known as one of the most prolific breeds in the world. Meishan pig has an excellent high reproductive performance, including more litters, strong lactation ability, higher conception rate, and early maturity (Ellis et al., 1995; Désautés et al., 1999; Groenen et al., 2012; Zheng et al., 2020). Besides the great reproductive performance, the Meishan pig breed also has the advantage of tolerating rough feeds and environments (Christenson et al., 1993; Haley and Lee, 1993; Zheng et al., 2020). Hence, it is necessary to discover the reason why the Meishan pig breed possesses the superior phenotypes.

Several studies have focused on the identification of genetic diversity and population structure to unravel functional genes and found that the harbored gene insulin like growth factor 1 receptor (*IGF1R*) may contribute to the higher fertility of Meishan pigs. Nuclear factor-kappaB (*NF-κB*) as the key gene of *NF-κB* signaling, positively regulates the hyaluronan biosynthesis, which may explain the wrinkled skin and face of Meishan pigs (Zhao et al., 2018; Zhou et al., 2021). Previous research studying the protein expression patterns in endometrial tissue from Meishan and Duroc sows revealed that there were 114 differentially expressed proteins identified at day 49 and 98 differentially expressed proteins were identified at day 72 during pregnancy, suggesting that the differentially expressed proteins may be a major factor influencing the differences in embryonic loss between Meishan and Duroc sows during mid-gestation (Wang et al., 2019a). However, given the high complexity of reproduction performance and related phenotypes, the genetic basis of superior characteristics in Meishan pigs remains largely unknown.

In eukaryotes, N6-methyladenosine (m^{6}A) is the most abundant form of methylation modification and is critical for various physiological and pathological processes. The m^{6}A methylation is a dynamic process, which is added by methyltransferases (writers) or removed by demethylases (erasers) and exerts its function by m^{6}A binding proteins (readers) (Wang et al., 2020a). Methyltransferases are a complex that contains methyltransferase-like protein 3 (*METTL3*) and *METTL14*. Other methyltransferases also have been identified, such as wilms tumor 1 associated protein (*WTAP*; Ping et al., 2014), Virilizer (*KIAA1429*; Jiang et al., 2021a), and RNA binding motif protein 15 (*RBM15*; Zhang et al., 2015). Conversely, two demethylases, fat mass and obesity-associated protein (*FTO*; Jia et al., 2011) and AlkB homolog 5 (*ALKBH5*; Zheng et al., 2013) have the function to remove the m^{6}A modification. Additionally, the specific readers mediate the function of m^{6}A modification on target mRNA. Members of the YTH family have been identified to specifically bind to m^{6}A-containing precursor RNA. The members of YTH family contain *YTHDF1*, *YTHDF2*, and *YTHDF3*, which are located mainly in the cytoplasm (Zaccara and Jaffrey, 2020).

Research has shown that m^{6}A modification plays critical roles in numerous biological functions such as the differentiation of stem cells (Zhang et al., 2016; Chen et al., 2019), maturation of egg cells (Kasowitz et al., 2018), and development of brain and metabolism of adipose (Wang et al., 2020b). *METTL3* plays multiple roles in biological process, tumorigenesis, and cell proliferation (Li et al., 2017b; Liu et al., 2019). Additionally, *METTL3* also has the function to modulate virus replication (Hao et al., 2019). *METTL14* was identified as the other writer complex component and provides a platform with *METTL3* for RNA recognition (Wang et al., 2017). Knocking down *METTL14* leads to decreased m^{6}A levels in human cell lines (Schwartz et al., 2014). Besides, knockdown of *METTL14* inhibits the differentiation and promotes the proliferation of C2C12 myoblast cells (Zhang et al., 2020). *FTO*, a demethylase, except for being associated with obesity (Loos and Yeo, 2014), is a crucial component of m^{6}A modification. *FTO* plays a critical role in occurrence, progression and treatment of various cancers, and even acts as a cancer oncogene in acute myeloid leukemia (Chen and Du, 2019). Previous study found that *FTO* level is increased in human melanoma and enhances melanoma tumorigenesis in mice, and that *FTO* regulates melanoma tumorigenicity and response to anti-PD-1 blockade (Yang et al., 2019). Another study also reported that m^{6}A modification regulates the expression of the growth arrest and DNA damage-inducible 45B (*GADD45B*) gene by activation of P38/MAPK signaling pathway, in which is involved in myogenic differentiation (Deng et al., 2021). Recent study compared the differences in m^{6}A methylation pattern between fat and lean broilers and found that the high m^{6}A methylated genes (fat birds vs. lean birds) are primarily involved in fatty acid biosynthesis and fatty acid metabolism, while the low m^{6}A methylated genes are mainly participated in processes associated with development (Cheng et al., 2021). Additionally, the m^{6}A methylation regulates the embryo viability and germline development in mouse. The nuclear m^{6}A reader YTHDC1is required for spermatogonial development in males and for oocyte growth and maturation in females (Kasowitz et al., 2018). However, there are few studies about the expression pattern of m^{6}A modification-related genes in various tissues of Meishan pigs at different developmental stages.

This study focused on the specific expression of m^{6}A modification-related genes in different tissues at the different stages of Meishan pigs and aimed to explore the relationship between m^{6}A modification and the development of Meishan pigs at different stages.

2. Material and Methods

2.1. Animals and collection of samples

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Animal Experiments Ethics Committee, China (SYXK (Su) 2016-0019). All experimental procedures were carried out in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals. The Meishan pigs were obtained from the Meishan Pig National Breeding Farm (Taicang City, Jiangsu Province). Pigs were chosen based on the similarity of fur color,

body shape, and weight from five litters at the various developmental stages (1, 7, 14, 21, 28, 35, 134, and 158 days old). Forty pigs (five pigs per group) were used; the weight information of pigs was listed in Table 1. All pigs were raised in the same condition, fed commercial fodder, and had free access to water.

Table 1 - Weight information of pigs

Number of pigs in group	Sex	Age (days)	Weight (kg)
5	2 females and 3 males	1	1.04±0.21
5	3 females and 2 males	7	1.77±0.43
5	1 female and 4 males	14	3.23±0.41
5	3 females and 2 males	21	4.36±0.73
5	2 females and 3 males	28	5.80±0.49
5	3 females and 2 males	35	7.94±0.51
5	4 females and 1male	134	35.44+3.50
5	3 females and 2 males	158	68.10±4.05

Tissue samples (muscle, heart, duodenum, jejunum, ileum, stomach, liver, spleen, thymus, lymph node, kidney, and lung tissues) at the same position from different pigs were rapidly excised, the residual blood was washed with normal saline, and the tissue was dried with filter paper to remove surface liquid. The samples were then placed in 1.5 mL nuclease-free Eppendorf tubes, and immediately placed into liquid nitrogen, and stored at −80 °C until RNA extraction.

2.2. Primers design

The primers (Table 2) were designed using software Primer Premier 5.0 referenced to the sequence from Genebank data.

Table 2 - Primer sequence of genes for qPCR

Gene	Sequence of the primer	Length of products (bp)
METTL3	F: 5'- CCCTATGGGACCCTGACAGA- 3' R: 5'- TGACACCAACCAAGCAGTGT- 3'	250
METTL14	F: 5'- GGGAGAGTGTGTTTACGCAAG- 3' R: 5'- TGAAGTCCCCGTCTGTGCTA- 3'	184
METTL16	F: 5'- ATTAAGAAAGAGGTTCTGGAGAGGC- 3' R: 5'- AGGGCATGCCTTCAATCATCA- 3'	126
WTAP	F: 5'- TCCATTCGTCTTTCCTCTCCG- 3' R: 5'- GCCTCACTCAGTCGAACCTTT- 3'	130
RBM15	F: 5'- CATTGTCCGTGCAAAACTGGT- 3' R: 5'- ACTTAAAACACCGGCATTGGC- 3'	211
FTO	F: 5'- GATCTCAATGCCACCCACCA- 3' R: 5'- CCACTCAAACTCGACCTCGT- 3'	237
GAPDH	F: 5'- ACATCATCCCTGCTTCTACTGG- 3' R: 5'- CTCGGACGCCTGCTTCAC- 3'	187

2.3. qRT-PCR

Total RNA was extracted by RNAiso Plus (Takara, Japan) according to the manufacturer's instructions. Briefly, tissues were homogenized by adding 1 mL of RNAiso Plus, then 200 µL of chloroform was mixed until the solution became milky, and was kept at room temperature for 5 min, then centrifuged

at 12000 *g* for 15 mins at 4 °C. The upper layer was transferred to a new centrifuge tube and equal volume of isopropanol was added, and the mixture was kept at room temperature for 10 min and centrifuged at 12000 *g* for 20 mins at 4 °C. The supernatant was carefully removed and washed with 75% ethanol twice, the supernatant was then discarded. Finally, the precipitated RNA was dried and dissolved with appropriate amount DPEC-treated water. Reverse transcription of the RNA was performed by using reverse transcriptase (R312-01, Vazyme, China). Two microliter cDNA templates were added to the reaction system (Q111-02, Vazyme, China). The qRT-PCR were programmed as denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing/elongation at 65 °C for 30 s. From three independent experiments, different gene expressions were calculated by using the comparative Ct method and normalized against *GAPDH* level.

2.4. Statistical analysis

The results are expressed as means ± standard error of mean (SEM) from at least three independent experiments. Statistical analysis was performed by SPSS version 16.0. The difference among different groups was analyzed by Student's t-test and One-way Analysis of Variance (ANOVA), with *P<0.05 and **P<0.01.

3. Results

3.1. *METTL3* mRNA levels in different tissues of Meishan pigs at different developmental stages

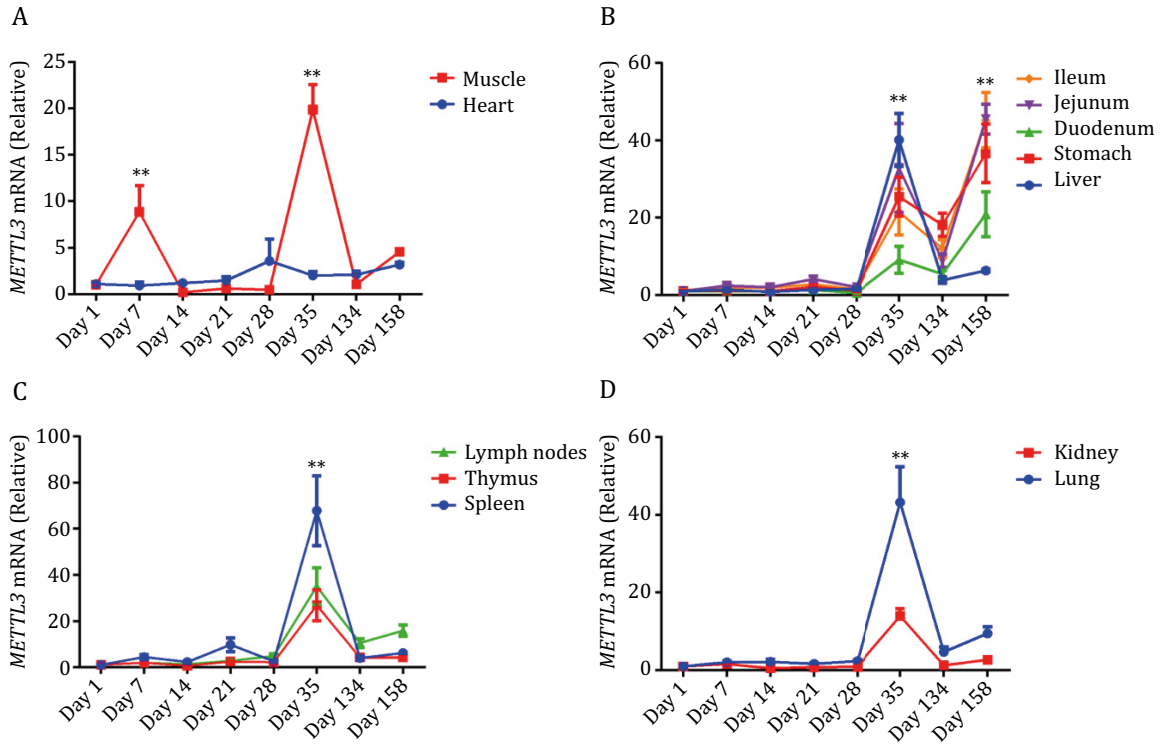
METTL3 is the core element of m^{6}A modification. The levels of *METTL3* mRNA in different tissues of Meishan pigs at different developmental stages were detected by qRT-PCR. The organs were classified according to their functions. *METTL3* displayed a similar expression pattern in different tissues, and the results showed that the expression of *METTL3* peaked at 35 days of age in almost all organs except the heart (Figure 1). In the digestive system, the level of *METTL3* in the liver was higher on day 35 and increased after day 134 in ileum, jejunum, stomach, and liver (Figure 1B). As the largest immune organ, it was worth mentioning that the *METTL3* gene was highly expressed in the spleen at the weaning stage (Figure 1C). Regardless of the developmental stage, the level of *METTL3* mRNA is much lower in heart and muscle tissue compared with other tissues.

3.2. *METTL14* mRNA levels in different tissues of Meishan pigs at different developmental stages

METTL14 can form a stable heterodimer with *METTL3*, and both co-localize in the nucleus and can maintain the stability of each other's protein. The mRNA level of *METTL14* was also detected and displayed a different expression pattern from *METTL3*, and the expression of *METTL14* in the heart at different stages remained low (Figure 2A). The changing trends of each part were consistent in intestinal tissues, and the expression of *METTL14* in liver increased after day 35 (Figure 2B). Besides, a peak expression appeared in lymph nodes at seven days of age, and the mRNA level increased in lymphatic organs from 35 days old (Figure 2C). Additionally, the expression of *METTL14* in the kidney and lung was up-regulated after day 134 (Figure 2D).

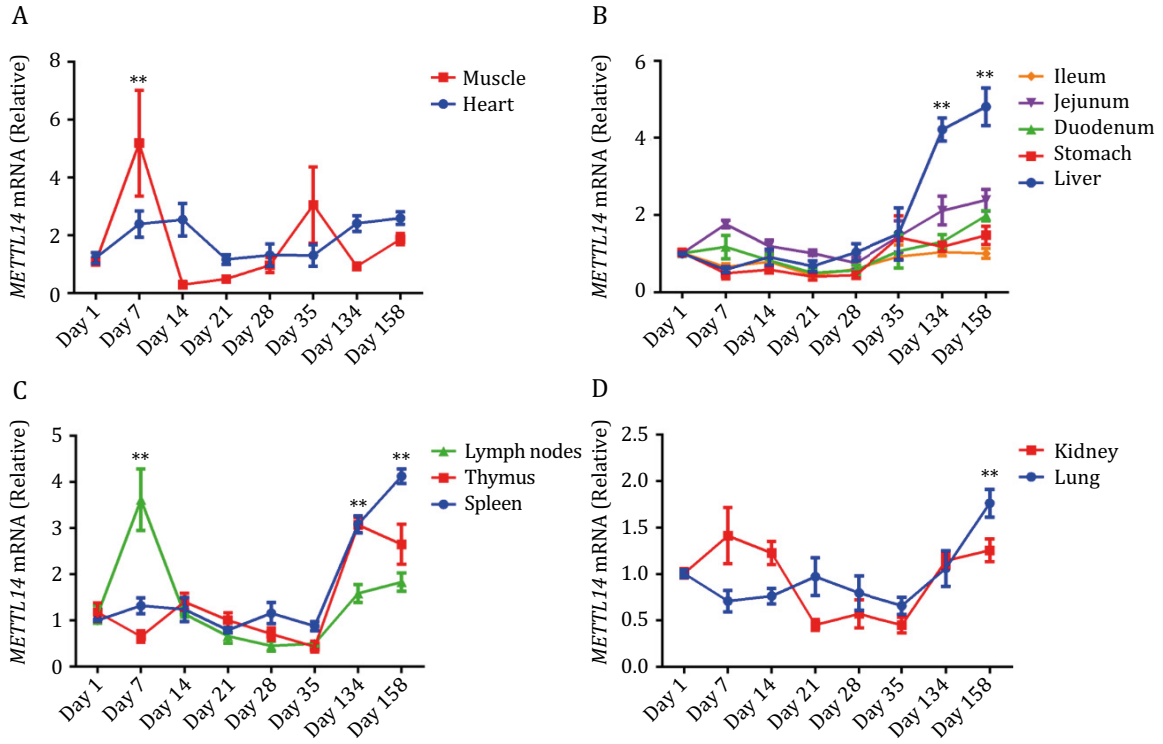
3.3. *METTL16* mRNA levels in different tissues of Meishan pigs at different development stages

The expression of *METTL16* was higher in muscle at 35 days of age (Figure 3A). In addition, the expression of *METTL16* was lower before 134 days old and became high at 158 days old in sections of small intestine and stomach (Figure 3B). The expression of *METTL16* was higher on day 28 in the thymus than in other developmental stages (Figure 3C). However, the expression of *METTL16* in the kidney presented a trend of first increasing and then decreasing, while in the lung, it fluctuated (Figure 3D).



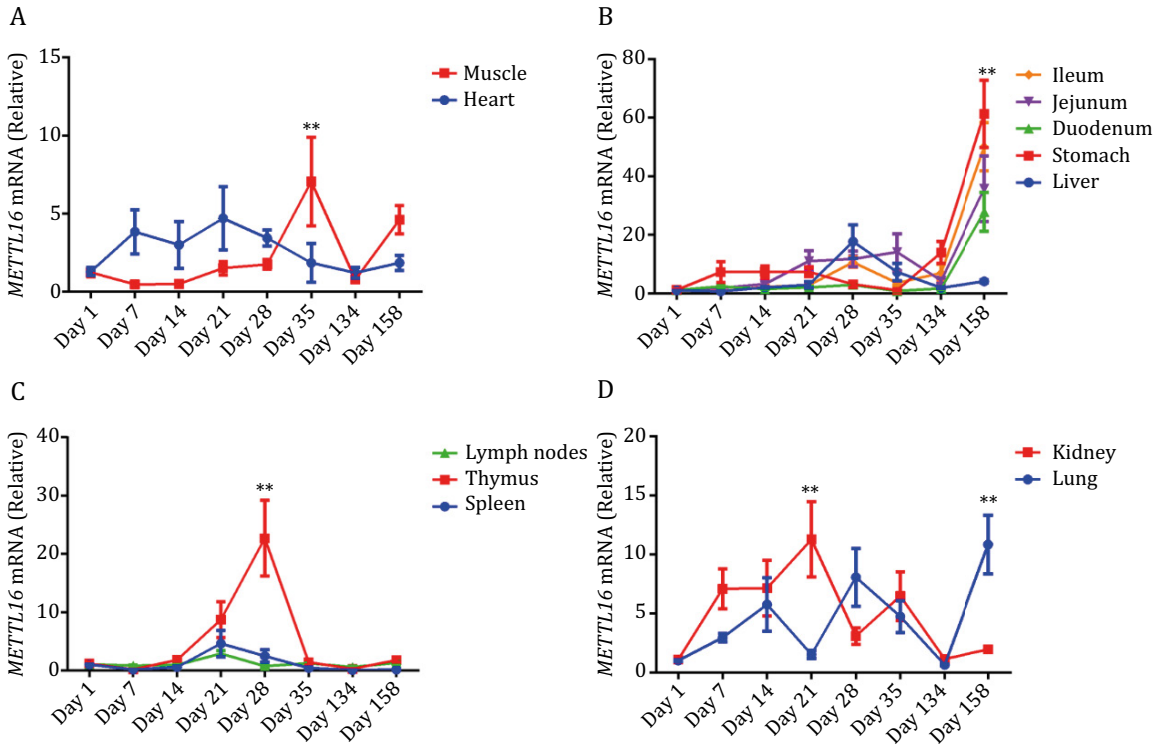
mRNA level of *METTL3* in heart and muscle (A); ileum, jejunum, duodenum, stomach, and liver (B); lymph nodes, thymus, and spleen (C); kidney and lung (D).
*P<0.05, **P<0.01.

Figure 1 - Expression of *METTL3* in different tissues at different stages by using the comparative Ct method.



mRNA level of *METTL14* in heart and muscle (A); ileum, jejunum, duodenum, stomach, and liver (B); lymph nodes, thymus, and spleen (C); kidney and lung (D).
*P<0.05, **P<0.01.

Figure 2 - Expression of *METTL14* in different tissues at different stages by using the comparative Ct method.



mRNA level of *METTL16* in heart and muscle (A); ileum, jejunum, duodenum, stomach, and liver (B); lymph nodes, thymus, and spleen (C); kidney and lung (D).
*P<0.05, **P<0.01.

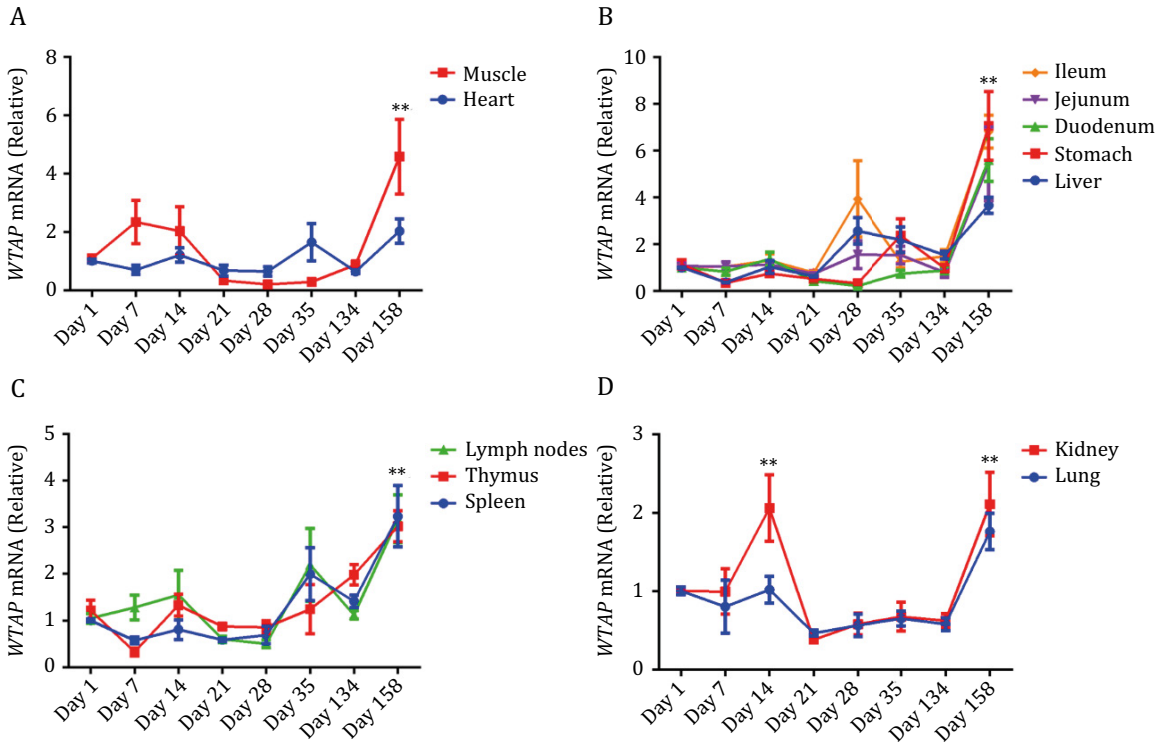
Figure 3 - Expression of *METTL16* in different tissues at different stages by using the comparative Ct method.

3.4. *WTAP* mRNA levels in different tissues of Meishan pigs at different development stages

WTAP is a conserved nuclear protein as the partner of Wilms' tumor 1, and removal of *WTAP* has been approved to be embryonically lethal. *WTAP* is a member of methylases and involves in multiple biological functions. We found that the expression of *WTAP* escalated in most organs after day 134 (Figure 4A-4D). In addition, the expression in the immune organs, such as spleen, lymph nodes, and thymus, exhibited a small peak on day 35, indicating that the change in methylation level of the immune system may be caused by weaning of piglets (Figure 4C). Moreover, the expression of *WTAP* showed a peak in kidney on day 14 (Figure 4D).

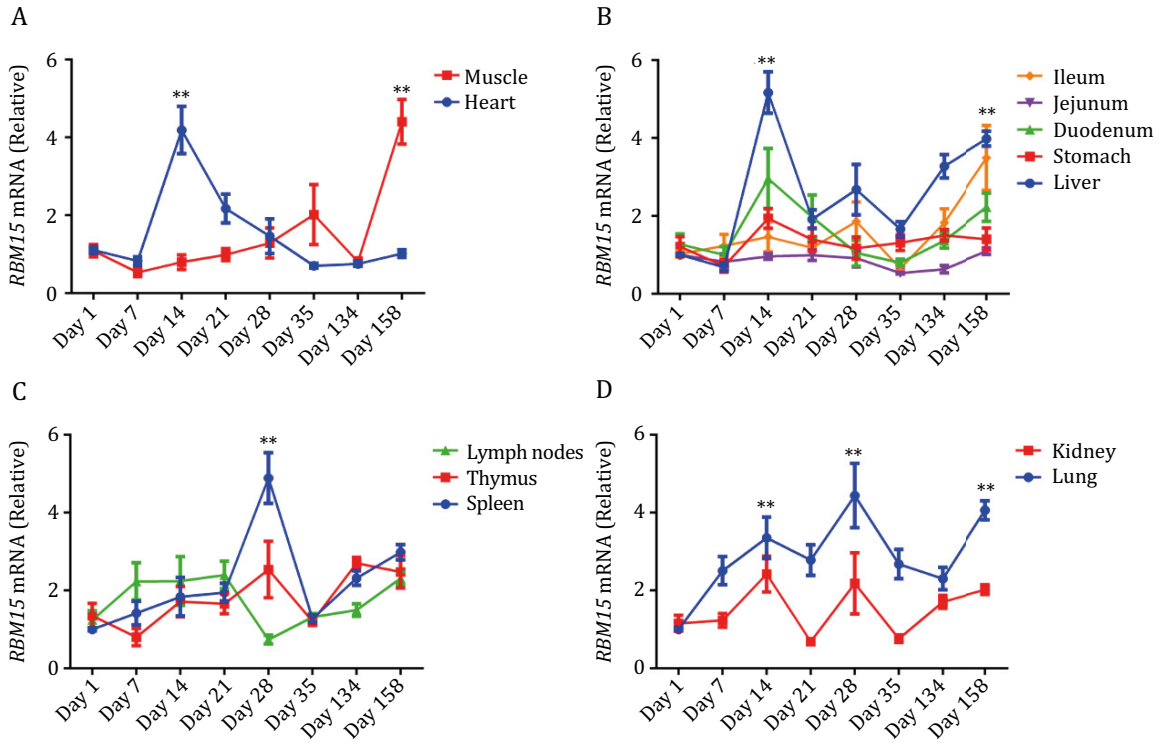
3.5. *RBM15* mRNA levels in different tissues of Meishan pigs at different developmental stages

RBM15 is an RNA-binding protein that is part of the *WTAP-METTL3* m^{6}A methyltransferase complex. *RBM15* and its paralogue *RBM15B* interact with *WTAP* to recruit the complex to target mRNA and are essential for the long non-coding RNA X-inactive specific transcript (XIST)-mediated gene silencing (Patil et al., 2016). In this study, the results showed that the expression of *RBM15* is higher on day 14 in the heart, and then gradually decreased, whereas the *RBM15* in muscle maintained a lower expression before day 35 and increased after day 134 (Figure 5A). The expression of *RBM15* was relatively higher on day 14 in liver, duodenum, and stomach (Figure 5B). Besides, *RBM15* mRNA level showed a short rise on day 28 in the thymus and spleen (Figure 5C). The *RBM15* in the kidney and lung displayed a similar expression pattern at different developmental stages (Figure 5D). These results indicated that *RBM15* showed a tissue-specific expression.



mRNA level of *WTAP* in heart and muscle (A); ileum, jejunum, duodenum, stomach, and liver (B); lymph nodes, thymus, and spleen (C); kidney and lung (D).
*P<0.05, **P<0.01.

Figure 4 - Expression of *WTAP* in different tissues at different stages by using the comparative Ct method.

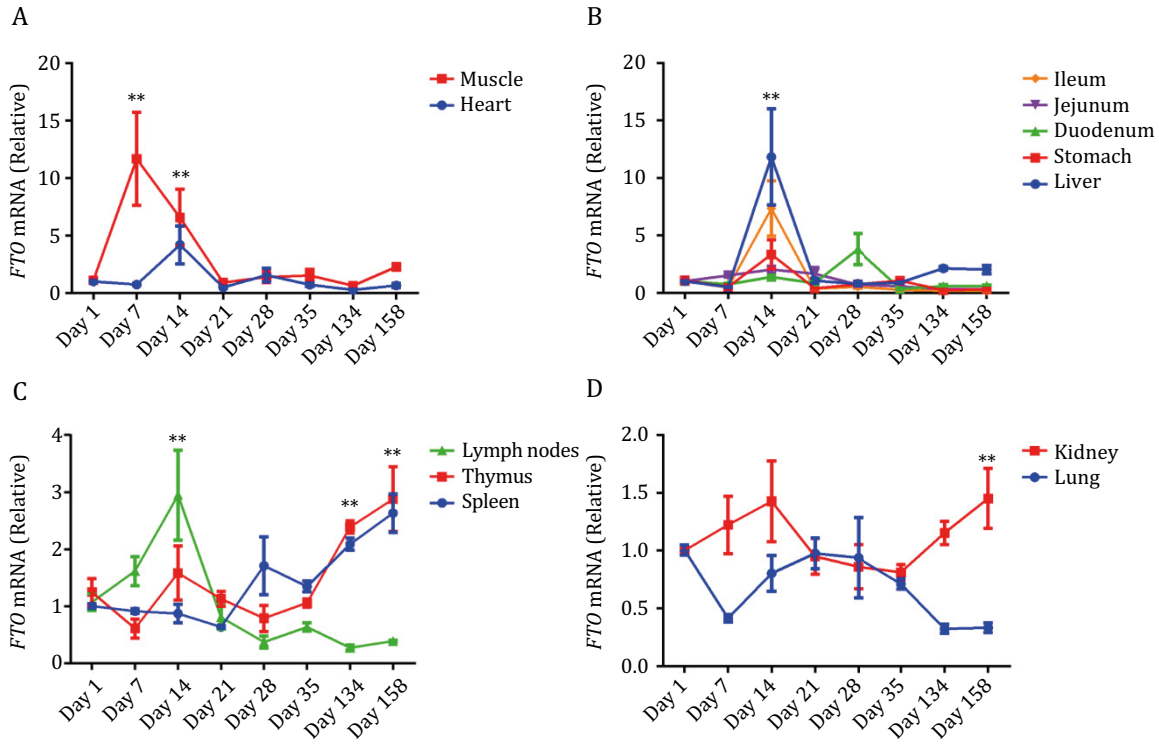


mRNA level of *RBM15* in heart and muscle (A); ileum, jejunum, duodenum, stomach, and liver (B); lymph nodes, thymus, and spleen (C); kidney and lung (D).
*P<0.05, **P<0.01.

Figure 5 - Expression of *RBM15* in different tissues at different stages by using the comparative Ct method.

3.6. *FTO* mRNA levels in different tissues of Meishan pigs at different developmental stages

The *FTO* mRNA level was low after day 21 in muscle and heart (Figure 6A) and showed a higher expression than other stages on day 14 in various parts of the intestine (Figure 6B), lymph nodes, and thymus (Figure 6C). Additionally, we also found that the expression was up-regulated after weaning on day 35 in the lymph nodes, spleen, and kidney (Figure 6C and 6D). Intriguingly, the *FTO* mRNA level in the kidney and lung appeared in a converse situation (Figure 6D).



mRNA level of *FTO* in heart and muscle (A); ileum, jejunum, duodenum, stomach, and liver (B); lymph nodes, thymus, and spleen (C); kidney and lung (D).
*P<0.05, **P<0.01.

Figure 6 - Expression of *FTO* in different tissues at different stages by using the comparative Ct method.

4. Discussion

Meishan pig breed is one of the most famous Chinese domestic pig breeds, which is renowned for the high prolificacy, high reproductive performance, great meat physical properties, and tolerance for rough feed (Zhao et al., 2018). To reveal the mechanisms of Meishan pigs with excellent economic traits, researchers have used the technique of molecular genetics to explore why Meishan pig breed has good productive performance. The m^{6}A modification has been found to be involved in regulating a variety of physiological activities (Cao et al., 2016), but compared with the studies about humans and mice, there are few studies underscoring the influences of m^{6}A modification on the productive performance of Meishan pigs.

In this study, we performed the expression changes of m^{6}A modification-related genes by qRT-PCR in various tissues of Meishan pigs at different developmental stages. The results showed obvious tissue specificity and preference for the expression of m^{6}A-related genes of Meishan pigs. Importantly, we found that the expression of *METTL3* showed a similar trend in different organs at different developmental stages, which was higher on day 35. Moreover, *METTL14* and *WTAP* mRNA levels were

increased after day 134. As one of the demethylases, the expression of *FTO* was up-regulated in the heart, intestine, lymph nodes, thymus, kidney and lung on day 14.

The m^{6}A methylation plays important roles in the development of organisms, which can modulate stem cell specification and cell development and cell fate (Zhang et al., 2017). m^{6}A is installed by m^{6}A methyltransferases, removed by m^{6}A demethylases and recognized by reader proteins. m^{6}A methylation regulates RNA metabolism including translation, splicing, export, degradation, and microRNA processing (He et al., 2019). Additionally, the m^{6}A methylation regulates the growth and development of ovaries and testes. Jiang et al. (2021b) found that *FTO*-knockdown granulosa cells show faster aging-related phenotypes and *FTO* retards FOS-dependent ovarian aging. Study also found that the *METTL3*-mediated mRNA N^6-methylademosine plays important roles in oocyte and follicle development of mice (Mu et al., 2021). Moreover, the m^{6}A modification is essential for oogenesis, and researchers proved that there was significant enrichment of m^{6}A methylation-related genes in several signaling pathways associated with the development of ovary. Besides, study also has confirmed that *METTL3*, *METTL14*, *YTHDF1*, *YTHDF2*, *YTHDF3*, and *KIAA1429* were involved in ovulation (Chen et al., 2022). *METTL3/METTL14* mediated mRNA N6-methyladenosine participates in murine spermatogenesis (Lin et al., 2017). Sun et al. (2020) also investigated the dynamic status of m^{6}A during the development of ovary and testis, and found that the m^{6}A level increases with age in both females and males. These studies all indicated that the m^{6}A modification plays a vital role in the growth and development of animals.

In this study, we focused on the dynamic m^{6}A-related genes expression patterns of Meishan pigs at different developmental stages. The expression levels of m^{6}A-related genes were compared based on functions and cell composition of tissues. Interestingly, the different tissues exhibited distinct expression patterns of m^{6}A-related genes, and we found that the expression of m^{6}A-related genes in muscle and heart was low than in other tissues, maybe because these two organs are made up with muscle cells.

Moreover, previous studies indicated that the *METTL3* has the capability to promote the activation of dendritic cells and regulates immune response of the host (Wang et al., 2019b). Herein, we found that the expression patterns of *METTL3* in different tissues were similar, and the expression of *METTL3* and *METTL14* was higher on day 35, which indicated that environmental changes, including weaning stress, may lead to the changes. Besides, the expression of *WTAP* also showed similar trend in different tissues; the expression was lower on day 134 and then upregulated on day 158, and it was reported that *WTAP* may participate in fat deposition after day 134 (Heng et al., 2019). Combined with these results, we suggested that *WTAP* play an important role in finishing pigs.

FTO is a member of Fe (II)-and oxoglutarate-dependent AlkB dioxygenase family and is closely related to obesity and intellectual disability (Li et al., 2017a). Previous study found that loss of *FTO* leads to the brain size and body weight change (Li et al., 2017a). Moreover, *FTO* could regulate immune response. Studies showed that tumors use *FTO* as an epitranscriptomic regulator to escape immune surveillance (Su et al., 2020), and *FTO* promotes M1 and M2 macrophage activation (Gu et al., 2020). However, in this study, as the demethylase, the expression of *FTO* showed different trends in spleen, lymph nodes, and thymus, which was highly expressed in the lymph nodes at early stage and then decreased from day 21. Compared with that in lymph node, the *FTO* showed diverse expression patterns in spleen and thymus, which was lowly expressed in early stage and highly expressed in late stage; the composition of immune cells in different immune tissues may contribute to this phenomenon. In digestive system, *FTO* mRNA level was highly expressed on day 14 and then remained at relatively low level, which may be related to the role of *FTO* in regulating fat and weight. Additionally, although the expression of *FTO* is higher in patients with lung cancer and lower in patients with nephritis (Liu et al., 2018; Zhao et al., 2021), the diverse expression of *FTO* in lung and kidney may due to the fact that *FTO* play different roles the in kidney and lung. Apart from Meishan pigs, the m^{6}A modification takes part in the growth and development of other pig breeds. He et al. (2017) profiled transcriptome-wide m6A in porcine liver at three developmental stages of Rongchang pigs: newborn (0 day), suckling (21 days), and adult (two years) and found that one third transcribed genes are modified by m^{6}A. Wang et al. (2022)

reported the m6A-methylation patterns of lncRNA via MeRIP-seq and found that m^{6}A methylation regulates the muscle-fiber-type conversion of Duroc pigs. Taken together, the dynamic regulation of m^{6}A modification may contribute to the excellent production performance of Meishan pigs.

In this study, three important points were associated with the changes of m^{6}A-related genes, including 7 to 14 days old, 35 days old, and 134 days old. Generally, the newborn piglets are susceptible to kinds of pathogens, and the piglets are usually weaned on day 35. In those three different developmental stages, the expression of m^{6}A-related genes was usually altered. The immune protection of piglets mainly derives from two aspects. The immunity obtained from breast milk is passive immunity (Myles and Datta, 2021), and the immune protection formed by the development of the autoimmune system is active immunity (Baxter, 2014).

In this study, we noticed a phenomenon that the methyltransferase *METTL3* was lowly expressed in the thymus, spleen, and lymph nodes before 35 days of age, but highly expressed at 35 days of age. We suspected that weaning may be responsible for this phenomenon. The first two weeks after weaning is the key turning point of the early developmental stage of piglets. Moreover, weaning usually causes the interruption of maternal antibodies, while self-digestion and immune functions are not yet complete in piglets. Therefore, in this period, the metabolism of main immune organs like the thymus and spleen is very rapid. These organs are in the peak period of activity and are greatly affected by maternal factors. During the development of thymus and spleen, the high expression of methyltransferases is involved in the development of immunity to against weaning stress.

Furthermore, newborn piglets usually obtain immunoprotection from breast milk, and the immune efficacy reaches peak at seven days old. Besides, the autoimmune system of piglets works from 28 to 35 days old, which may explain the higher expression of *METTL3* on day 35. Apart from affecting the immune function, weaning stress affects intake, production performance, and development of piglets. In the intestinal tissues, the expression of *METTL3* also was upregulated on day 35. Hence, the dynamic regulation of m^{6}A modification during weaning stress stage may contribute to the function of intestine and immunity of piglets.

5. Conclusions

The expression of *METTL3* is higher in different tissues on day 35, which is usually the weaning date. The expression of *METTL14* increases after day 35. Compared with the expression of *METTL3* on day 35, the expression of *FTO* exhibits a lower level, indicating that organism regulates m^{6}A methylation in response to weaning stress. Collectively, our study provided a reference for the expression pattern of m^{6}A methylation-related genes in different tissues in Meishan pigs.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Y.N. Cao and S.L. Wu. Formal analysis: Y.N. Cao, S.S. Zhang and G.Z. Wang. Funding acquisition: S.L. Wu. Methodology: Y.N. Cao, S.S. Zhang and G.Z. Wang. Supervision: S.L. Wu. Visualization: Y.N. Cao and S.S. Zhang. Writing – original draft: Y.N. Cao and S. Zhang. Writing – review & editing: W.B. Bao and S.L. Wu.

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